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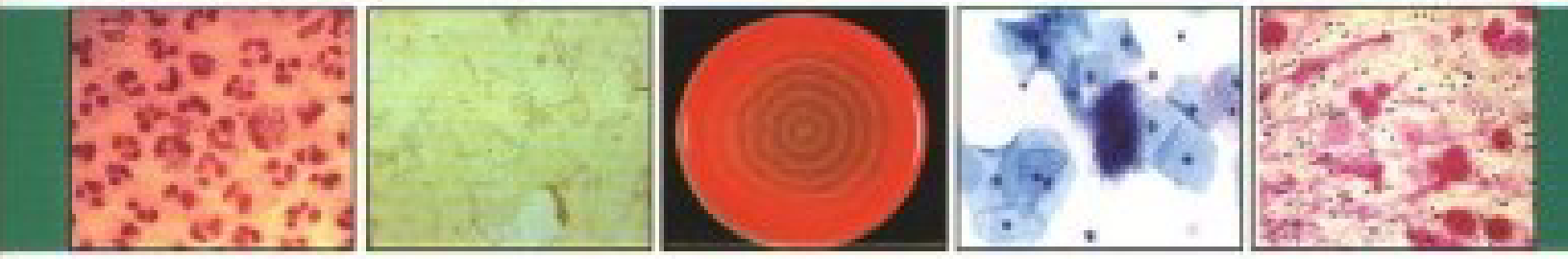

Facts and concepts from latest editions of Harrison (19th), Park (23rd), Apurba Sastry's Essentials of Medical Microbiology, Apurba Sastry's Essentials of Medical Parasitology and Ananthanarayan (9th edition).

Latest updates included such as BMW Rule 2016, Zika, H1N1, EBOLA, POLIO eradication, MERS-CoV

AIIMS (2000–2016 NOV) | PGI (2000–2016 NOV) | JIPMER (2000–2016 Nov) | ALL INDIA (2000–2012) | DNB (2000–2012)

Recent questions from other national-level examinations (2013–2016)

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Review of
Microbiology and Immunology

Sixth Edition

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Dedicated
to
Our Beloved Parents
and
Family Members

And above all, Lord Ganesha who gave us the knowledge and inspiration

Preface to the Sixth Edition

IMPORTANCE OF MICROBIOLOGY FOR PG ENTRANCES

Microbiology is one of the high-scoring sections and, hence, is the key subject for PG entrances.

- Though it might look tough before you start, but as you go through it gradually, you will surely realize that repeated revisions can take you to that level at which you can answer all the possible MCQs in any entrance.
- The beauty of this subject is if you are thorough in Microbiology, you can solve many infection-related MCQs of Medicine, PSM, Pediatrics, etc.

ENTRANCE	MICROBIOLOGY MCQs	INFECTION MCQs	ENTRANCE	MICROBIOLOGY MCQs	INFECTION MCQs
AIIMS Nov 2016	21/200	39/200	JIPMER Nov 2016	25/250	44/250
AIIMS May 2016	29/200	43/200	JIPMER May 2016	21/250	37/250
AIIMS Nov 2015	28/200	41/200	JIPMER Nov 2015	21/250	31/200
AIIMS May 2015	16/200	31/200	JIPMER May 2015	14/250	27/200
PGI Nov 2016	19/250	31/250	JIPMER Nov 2014	28/200	42/250
PGI May 2016	21/250	36/250	APPG 2015	21/200	35/200
PGI Nov 2015	15/250	25/250	TNPG 2015	25/250	39/250
PGI May 2015	18/250	32/250	MHPG 2015	38/300	52/300
ALL INDIA 2016 (According to Syllabus)	20/300		DNB 2016 (According to Syllabus)	18/300	

Each Chapter Contains

- Chapter Review: Gives a preliminary overall idea about how a chapter can be finished fast.
- MCQs with detailed explanations: Gone to the depth covering all the important aspects in detail.
- Separate section of recent 2016 entrance examination questions.
- Includes image-based MCQs at the beginning of the book.
- Biomedical waste management rules 2016 included.

Changes Done Compared to the Previous Edition

Chapter Review (Theory portion) Part

- Has been thoroughly updated from Harrison 19th edition, Park 23rd edition and Apurba Sastry's Essentials of Medical Microbiology, 1st edition and Apurba Sastry's Essentials of Medical Parasitology, 1st edition.
- All recent information, such as Zika virus, EBOLA virus, Polio eradication, Dengue vaccine, Vaccine-derived Polio Viruses (VDPVs), MERS-CoV, and updates in bacterial drug resistance etc. have been incorporated.
- Separate Annexure section containing important exam-oriented rapid-fire topics.
- Image-based question bank is further strengthened with new images (> 500 images are included).

MCQ Part

- Recent questions included from AIIMS 2016 (Nov and May), JIPMER 2016 (Nov and May), PGI 2016 (Nov and May), CMC Vellore (2016) and Recent questions from other national-level examinations.
- State entrance MCQs conducted in 2016 are also included. As no state PGMEE was carried out separately in 2017 and the same may be continued in future; so students should give more focus to MCQ pattern of All India/NEET exam; rather giving importance to previous state exam MCQs.

One Weapon-Two Targets: 2nd Year MBBS Exams and PG Entrance

This revised edition is prepared in such a manner that it will help the 2nd year MBBS students to prepare for their MBBS exam as well as for the PG entrances. The chapter review part of each chapter is revised and updated in such a way that by studying this book, the students can easily solve the long essays, short notes of MBBS exams as well as MCQs of various PG entrances.

Apurba Sankar Sastry
Sandhya Bhat K

Golden Tips for Your Exam Preparation

Study Methodology – Antegrade vs Retrograde

Students will always be in a dilemma whether to follow antegrade or retrograde methodology for preparation.

- Antegrade method is time-consuming but covers the topics in a systematic way, while retrograde method fails to cover unasked MCQs and recent updates. Long-term memory is usually poor for the followers of retrograde method.
- Our book maintains a perfect balance between both the methods.

Tips for Your Preparation

Target-oriented Labor

- Only labor: Gives you 30–40 % success
- Target-oriented labor: Provides 70% success
- That means: You should know where to read and how much to read and not to waste time reading unnecessary things which are least asked.

Repeated Revisions

Repeated revisions rather than reading extensively without any revision — Crucial Factor
Survey conducted at various coaching centers and medical colleges:

Revision	1st	2nd	3rd	4th	5th	6th	7th
Performance in Exam	25%	28%	32%	35%	50%	55%	60%
Rank in All India	Nil	Nil	5000	4700	2500	1800	< 1000

Methods to Improve Your Memory

- Try to correlate the things and remember rather than purely mugging up
- Group study or couple study
- Recalling every night
- Booster revisions: Should be done before you totally forget the matter (i.e. in short-interval)
- Mnemonics: Good but should be limited.

Regularly Assess Yourself

Most of the students assess their preparation directly at the exam hall which is absolutely worst method of assessing. You should assess your standard on daily basis and modify your preparation style according to the requirement. You can do that by:

- Group study: Comparing yourself with your friends.
- Self-assessment by recalling every night (last two hours post dinner)
- Grand test: Assess whether studying is reflected in the performance or not. You can compare yourself with students throughout India.

Role of Grand Test

- Helps in assessing yourself
- Any trials and errors can be attempted in grand tests and whichever experiment is successful can be executed in main exam
- To learn time adjustment
- To enhance guessing ability
- To improve self-confidence
- To compare your performance with others.

Weekdays (Self-study) vs Weekend Study (Coaching Center)

- Coaching institute is the place where you will be trained with the entrance-oriented important aspects of the subjects.
- However, students attending coaching institutes are getting 50 to 60 days less for their preparation as compared to the other students who prepare at home.

- You should never waste the time gap (i.e. weekdays) between classes.
- You will never get time to revise if you have not covered the last subject before the next subject starts.

Last 100 Days

- **Accelerate near the slug overs:** Last 100 days of study are very crucial—because the students' survey has shown that 80% of what you will answer correctly in the exam depends upon the last 2 to 3 months' study.
- **Never leave any subject:** Be master in your area but, at the same time, cover at least average of the uncovered area as MCQs will be asked from all the subjects.
- **Sleeping well the previous night:** Increases your efficiency at least by 10%.
- **Do not forget the importance of time:** Once lost, it can never be recycled.

Be an Early Riser

As wisely quoted by Benjamin Franklin "Early to bed and early to rise makes a man healthy, wealthy, and wise.". Remember what you read during early morning (3 am-6 am), will stimulate your memory cells maximum and will be retained longer. More so, there will be no disturbance as compared to late night reading where other friends/TV shows/parties or the whole day tiredness etc. will disturb you a lot.

My Trick to Get up Ealry

- Sleep early (10 pm)
- Keep three alarms, 5 min gap
- Keep alarm away from cot
- Brush and make tea before go to read
- Never start a fresh chapter, as you will have starting problem which will induce sleep
- Always read a chapter continued from yesterday's reading.

While Writing PG Exam ..... The Following Things are to be Kept in Mind

- Time management
- Guess ability:
  - By correlating the things
  - By ruling out the options
- Guess only in 50–50 situation (two or three options are ruled out)
- Never guess when you can rule out only one or zero options.

FINALLY, WHILE CHOOSING A PG SEAT

Students Choose a PG Seat—either 'by Choice' or 'by Chance'

- *By choice (similar to love marriage):* Take your dream PG seat (this happens only when you get a desired rank). Only a few blessed students fall under this category.
- *By chance (similar to arranged marriage):* Take the available PG seat (this happens when you get a rank, but not good enough to get your choice subject). Most of the students will fall under this category.

If you have a rank but not good enough to get your choice subject, you have two options:

- Take what is available
- Wait for the next year.

This is again a controversial situation. Many have opinions in both the categories.

- Some say that you can enjoy the subject only if it is your dream subject.
- Some say that you should not waste time in waiting as there is no guarantee that you will do better in the next exam and in the current online multisession exam pattern, the situation is highly uncertain.

Best Way to Solve this issue is

Try to see the difference between love marriage and arranged marriage.

- There is no guarantee that in love marriage, you will not fight and you will have a peaceful life.
- Love is there in arranged marriage also, but it is created after marriage.

Our advice as time is precious:

- Never wait for long to get your desired subject.
- Develop interest in the subject that you choose.
- Do justice with your subject in your three years of PG course.
- And, in turn, the subject will give you name, fame and prosperity throughout your life.
- If you dislike the subject, then it will end up in divorce (i.e. you will leave the course or will live throughout).

STRATEGY TO CHOOSE A PG SEAT

Prepare a 'priority list' of subject-college combinations, based on your rank and last-year counseling.

College Priority to be discussion Prepared

- This is equally important because whatever subject you take, you will be master of that if you do in AIIMS/PGI/JIPMER/CMC kind of college.
- Even if the subject is of low priority, it will give you more satisfaction as you will be easily famous in your locality and country.

DEMO: Who will feel more satisfied in the job and more famous in the world?

- A state-level cricket player of India, who is struggling to get a chance to play in the national-level team (e.g. doing medicine from state college); or
- Bangladesh cricket team captain (e.g. doing pathology from PGI).

Subject Priority is to be Prepared Based on the Following Facts

Based on the nature of work, time of work and your desire to earn name and money, i.e.:

- **For 9 am to 5 pm job (faculty job is the best):**
  - Subject preference should be based on job vacancy
  - All subjects will have the same salary, but night duty will be there only in a few clinical subjects.

  (A medicine faculty sometimes gets frustrated by seeing a dermatology faculty)

  For 7 am to 9 pm job (private practice is the best): Income is partly based on subject and partly on your luck. So, here you can give importance to certain subjects, like RD, Paed, Ortho, etc. But, remember, a lot of risk is involved in practice and be prepared for that.
- **Females** should keep in mind that they have to manage family in future. So, just for the infatuation sake, do not go for OBG. Be prepared that you will have duties and lot of professional responsibilities before opting for OBG.
- **If choosing RD, anesthesia:** Be prepared that there is no patient interaction. Do not cry later.
- **If choosing medicine/surgery:** Be prepared that DM/MCh is a MUST. So, a lot of hard work is expected in future and settlement is always late.
- **If choosing psychiatry/radiotherapy:** Be prepared that the patient's compliance is poor. So, you may/may not get the job satisfaction of curing a patient.
- **If choosing paraclinical and nonclinical:** Be prepared that there is no patient exposure, but one has the advantage of no duty, same salary if working as faculty and, most importantly, no need to prepare again.

Remember, For Satisfaction

- **BEING HAPPY WITH WHAT GOD HAS GIVEN IS MORE IMPORTANT THAN CRAVING FOR WHAT IS NOT GIVEN TO YOU.**
- **WHAT YOUR FAMILY WILL THINK IS MORE IMPORTANT THAN WHAT YOUR FRIENDS/NEIGHBORS WILL THINK.**

Wish You "ALL THE BEST FOR THE SUCCESS AND THE BRIGHT FUTURE AHEAD".

Keep in touch with us through the FB discussion group and personal touch via FB messanger or mail.

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Contents

Section 5: Mycology

Section 6: Parasitology

Section 7: Applied Microbiology

Annexure

POLIO VACCINE UPDATE 2016

IPV and bOPV in India

- *IPV introduced in India:* from November 2015
- *Bivalent OPV will be introduced in India:* from April 2016

IPV in India

- Government of India introduced IPV under universal immunization program
- From November 2015
- One dose of IPV
- In a phased manner
- In the first phase, IPV has been introduced in six high-risk states: Assam, Gujarat, Punjab, Bihar, Madhya Pradesh, and Uttar Pradesh
- This IPV dose is extra; given over and above the Trivalent OPV.

Indian Government Action Plan: Switchover of tOPV to bOPV and IPV

- *From November 2015 to March 2016:* Three doses of trivalent OPV plus one dose of IPV along with the third dose of OPV at 14 weeks.
- *From April 2016:* Bivalent OPV three doses plus one dose of IPV along with the third dose of bOPV at 14 weeks.

IMPORTANT NATIONAL REFERENCE CENTERS FOR INFECTIOUS DISEASES IN INDIA

- Tuberculosis:
  - TRC: Tuberculosis Research Centre, Chennai
  - LRS Institute of Tuberculosis & Respiratory Diseases, Delhi
  - National Institute of Tuberculosis, Bengaluru
  - National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra
- Leprosy: National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra
- Salmonella:
  - National salmonella reference centre for identification of unusual serotype: Central Research Institute (CRI), Kasauli
  - Reference centre for salmonella of animal origin: Indian Veterinary Institute, Izatnagar
  - Reference centre for phage typing: Lady Hardinge Medical College, Delhi
- BCG vaccine prepared in India: BCG Vaccine Laboratory, Guindy, Chennai
- Yellow fever vaccine: Central Research Institute (CRI), Kasauli
- Plague vaccine: Haffkine Institute, Mumbai
- Reference centre for phage typing of *Staph. auerus* in India: Maulana Azad Medical College, Delhi
- Cholera: National Institute of Cholera and Enteric Diseases (NICED), Kolkata
- VDRL antigen is prepared in India: Institute of Serology, Kolkata
- National Institute of Virology, Pune: Prepare diagnostic kits for Hepatitis viruses, JE, Dengue, Chikungunya and Rotavirus
- NARI (National AIDS Research Institute): Pune
- Leptospirosis Reference Laboratory: Regional Medical Research Institute (RMRI), Port Blair

INCUBATION PERIOD OF IMPORTANT ORGANISMS

MMR + 2Pox		Hepatitis viruses		Malaria		STD	
Mumps	19 d	HAV	15–45 d	P. vivax	14 d	Syphilis	9–90 d
Measles	10 d	HBV	30–180 d	P. falciparum	12 d	Chancroid	1–14 d
Rubella	14 d	HCV	15–160 d	P. malariae	28 d	Herpes	2–7 d
Smallpox	12 d	HDV	30–180 d	P. ovale	17 d	Donovaniasis	1–4 wks
Chickenpox	15 d	HEV	14–60 d	**Filariasis**	8–16 months	LGV	3 d–6 wk
Arboviruses		**Other viruses**		**Leishmaniasis**	1–4 months	**Gas gangrene**	
Dengue	5–6 d	Rabies	1–3 m	**DPT**		Cl. perfringens	10–48 hr
Chikugunya	5–6 d	Polio	7–14 d	Diphtheria	2–6 d	Cl. septicum	2–3 d
KFD	3–8 d	HIV	10 yrs (Average)	Pertussis	7–14 d	Cl. novyi	5–6 d
Japanese encephalitis	5–15 d	Infectious mononucleosis	4–8 wks	Tetanus	6–10 d	**Plague**	
Yellow fever	3–6 d	Influenza	18–72 hrs	**Leprosy**	3–5 yrs	Bubonic & Septicaemic	2–7 d
				Cholera	1–2 d	Pneumonic	1–3 d
				Typhoid	10–14 d		

ARTHROPOD-BORNE DISEASES

Mosquito	Female: anopheles: Malaria Aedes: Dengue, Chikungunya, Yellow fever Culex: JE, Filaria (Lymphatic)
Louse	Trench fever (Bartonella quintana), Epidemic relapsing fever (Borrelia recurrentis), Epidemic typhus, Pediculosis
Flea	Bubonic plague, Endemic typhus, Hymenolepis diminuta
Hard Tick	Tick typhus, Arbo (KFD, RSSE, Crimean-Congo hemorrhagic fever, Colorado tick fever), Babesia, Tularemia
Soft Tick	Endemic relapsing fever (B. duttoni), Q-fever (in animals)
Mite	Scrub typhus, Rickettsial pox, Scabies
Cyclops	Guinea worm disease, Fish tapeworm (D. latum)
Black fly	Onchocerciasis
Reduviid bug	Chaga's disease

EXAMPLES OF SPECIAL MEDIA (E—ENRICHED, EN—ENRICHMENT, S—SELECTIVE, D—DIFFERENTIAL MEDIA)

Organism	Medium
Enteric pathogens—for *Salmonella, Shigella*	Hektoen enteric agar (S) Xylose-lysin-deoxycholate agar (S) Deoxycholate citrate agar (S) Eosin methylene blue agar (S) MacConkey (D and S) Salmonella Shigella agar (S) Wilson blair for Salmonella (S) Selenite F broth (En), Tetrathionate broth (En)
Blood culture—for blood-borne pathogens	Castaneda's biphasic media (E): Brain-heart infusion agar slope and broth
Vibrio cholerae (likes alkaline growth medium)	TCBS (Thiosulfate Citrate Bile Sucrose agar) (S) Mansour's Gelatin Taurocholate Trypticase agar (S) Alkaline bile salt agar (S) APW: Alkaline Peptone Water (En)
S.aureus	Mannitol salt agar (S) Milk salt agar (S) Ludlam's medium (S)
Streptococcus	Crystal violet blood agar (S)
Neisseria	Chocolate agar (E), Thayer-Martin media (S), Modified New York medium (S)
Corynebacterium	Loeffler's serum medium (E) Potassium tellurite agar (S)
Bacillus anthracis	PLET: Polymyxin Lithium EDTA Thallous acetate (S)
Bacillus cereus	MYPA: Mannitol egg yolk phenol red polymyxin agar (S)
Anaerobes	Thioglycollate (En) Robertson cooked meat broth (En)
Listeria	PALCAM agar (S)
Pseudomonas	Cetrimide agar (S), King's media (for pigment)
Burkholderia	Ashdown's medium
Haemophilus	Blood agar with staph streak (E) Chocolate agar (E) Levinthal's medium (E), Fildes agar (E)
Bordetella	Regan low media (E) Bordet Gengou Glycerin potato blood agar (E) Lacey's DFP media (S)
Mycobacterium	Lowenstein Jensen, Dorset egg (S)
Leptospira	EMJH (E), Fletcher's (E), Korthof's (E)
Campylobacter	Skirrow's, Butzler, Campy BAP (S)
Legionella	BCYE (Buffered charcoal yeast extract) (E)
Reiter's treponema	Smith Noguchi media
Urinary pathogen	MacConkey agar (D and S) Cystein Lactose Electrolyte-deficient agar (CLED agar) (D and S)
Organism	**Transport media**
Streptococcus	Pike's media
Neisseria	Amies, Stuart's media
Vibrio	VR, Autoclaved sea water, Carry Blair
Enteric pathogen	Carry Blair medium
Shigella, Salmonella	Buffered glycerol saline
Bordetella	Modified Stuart's (with casamino acid) Mischulow's charcoal agar Dacron or calcium alginate swab used

SECTION 1

General Microbiology

CHAPTER OUTLINE

History, Taxonomy, Morphology and Physiology of Bacteria and Microbial Pathogenicity

CHAPTER 1.1

HISTORY

Louis Pasteur

Louis Pasteur is also known as *father of microbiology*. He has many contributions to microbiology:

1. He has proposed the *principles of fermentation* for preservation of food
2. He introduced *sterilization techniques* and developed steam sterilizer, hot air oven and autoclave.
3. He described the method of *pasteurization of milk*
4. He had also contributed for designing the vaccines against several diseases such as anthrax, fowl cholera and rabies
5. He disproved the theory of spontaneous generation of disease and postulated the 'germ theory of disease'. He stated that disease cannot be caused by bad air or vapor, but it is produced by the microorganisms present in air.
6. Liquid media concept- He used nutrient broth to grow microorganisms.
7. He was the founder of the Pasteur Institute, Paris.

Louis Pasteur proposed:
- Principles of fermentation
- Pasteurization of milk
- Sterilization techniques
- Germ theory of disease

Robert Koch

Robert Koch provided remarkable contributions to the field of microbiology:

1. He used of *solid media for culture* of bacteria-Eilshemius Hesse, the wife of Walther Hesse, one of Koch's assistants had suggested the use of **agar** as solidifying agent.
2. He also introduced methods for isolation of bacteria in *pure culture*.
3. Described hanging drop method for testing motility.
4. Discovered bacteria such as the anthrax bacilli, tubercle bacilli and cholera bacilli.
5. Introduced staining techniques by using aniline dye.
6. **Koch's phenomenon**: Robert Koch observed that guinea pigs already infected with tubercle bacillus developed a hypersensitivity reaction when injected with tubercle bacilli or its protein. This reaction is called Koch's phenomenon.
7. **Koch's Postulates**:

Exceptions to Koch's postulates:
- Mycobacterium leprae
- Treponema pallidum
- Neisseria gonorrhoeae

According to Koch's postulates, a microorganism can be accepted as the causative agent of an infectious disease only if the following conditions are fulfilled:

i. The microorganism should be constantly associated with the lesions of the disease.
ii. It should be possible to isolate the organism in pure culture from the lesions of the disease.
iii. The same disease must result when the isolated microorganism is inoculated into a suitable laboratory animal.
iv. It should be possible to re-isolate the organism in pure culture from the lesions produced in the experimental animals.

An additional **fifth** criterion was introduced subsequently which states that antibody to the causative organism should be demonstrable in the patient's serum.

Exceptions to Koch's postulates: It is observed that it is not always possible to apply these postulates to study all the human diseases. There are some bacteria that do not satisfy all the four criteria of Koch's postulates. Those organisms are:

- *Mycobacterium leprae* and *Treponema pallidum*: They cannot be grown *in vitro*; however can be maintained in animals.
- *Neisseria gonorrhoeae*: There is no animal model; however, bacteria can be grown *in vitro*.

Molecular Koch's postulates: It was a modification of Koch's postulates (by Stanley Falkow). He stated that gene (coding for virulence) of a microorganism should satisfy all the criteria of Koch's postulates rather than the microorganism itself.

Paul Ehrlich

1. He was the first to report the acid-fast nature of tubercle bacillus.
2. He developed techniques to stain tissues and blood cells.

Important Discoveries:
- AV Leeuwenhoek: Simple microscope
- Ernst Ruska: Electron microscope
- E.Jenner: Smallpox vaccine
- Karry B Mullis: PCR

3. He proposed a toxin antitoxin interaction called as Ehrlich phenomenon and also introduced methods of standardising toxin and antitoxin
4. He proposed the 'side chain theory for antibody production'.
5. He discovered 'salvarsan', an arsenical compound (magic bullet) for treatment of syphilis, hence known as father of chemotherapy.
6. The bacteria 'Ehrlichia'was named after him.

Other Important Contributors in Microbiology

1. **Antonie Philips van Leeuwenhoek:** Discovered single-lens microscope and named organisms as '*Little animalcules*'.
2. **Edward Jenner:** Developed the first vaccine of the world, the smallpox vaccine by using cowpox virus.
3. **Joseph Lister:** He is considered to be the *father of antiseptic surgery*. He used carbolic acid during surgery.
4. **Hans Christian Gram:** He developed 'Gram stain'.
6. **Ernst Ruska**: He was the founder of electron microscope.
7. **Alexander Fleming:** He discovered the antibiotic penicillin.
8. **Elie Metchnikoff:** He described phagocytosis and termed phagocytes.
9. **Kleinberger:** He described the existence of L forms of bacteria.
10. **Barbara McClintock:** She described transposons.
11. **Walter Gilbert and Frederick Sanger:** were the first to develop (1977) the method of DNA sequencing.
12. **Karry B Mullis:** Discovered polymerase chain reaction (PCR).

Table 1.1.1: Discovery of important microorganisms

Discoverer	Organism	Discoverer	Organism
Ogston	*Staphylococcus aureus*	Yersin and Kitasato	*Yersinia pestis*
Neisser	*Neisseria gonorrhoeae*	Schaudinn and Hoffman	*Treponema pallidum*
Weichselbaum	*Neisseria meningitidis*	Daniel Carrion	*Bartonella bacilliformis*
Loeffler	*Corynebacterium diphtheriae*	d'Herelle	Bacteriophages
Frenkel	*Streptococcus pneumoniae*	W.H. Welch	*Clostridium perfringens*
Bruce	*Brucella melitensis*	A.Epstein and Y. Barr	Epstein-Barr virus
Kitasato	*Clostridium tetani*	Hansen	*Mycobacterium leprae*

Table 1.1.2: Common names of bacteria named after the discoverers

Common name	Scientific name	Common name	Scientific name
Kleb-Loeffler bacillus	*Corynebacterium diphtheriae*	Whitmore bacillus	*Burkholderia pseudomallei*
Preisz Nocard bacillus	*Corynebacterium pseudotuberculosis*	Battey bacillus	*Mycobacterium intracellulare*
Koch Week bacillus	*Haemophilus aegyptius*	Eaton's agent	*Mycoplasma pneumoniae*
Johne's bacillus	*Mycobacterium paratuberculosis*	Pfeiffer's bacillus	*Hemophilus influenzae*
Gaffky Eberth bacillus	*Salmonella* Typhi		

BACTERIAL TAXONOMY

Cavalier-Smith's six kingdoms classification (1998) is the most recent and widely taxonomic classification. It divides organisms into 6 kingdoms Bacteria, Protozoa, Chromista, Plantae, Fungi and Animalia.

Principle used for Bacterial Classification

1. **Phylogenetic classification:** This is a hierarchical classification representing a branching tree-like arrangement; one characteristic (or trait) is being employed for division at each node of the tree

2. **Adansonian (or phonetic) classification**: To avoid the use of weighted characteristics, Michel Adanson proposed a scheme that classifies organisms based on giving equal weight to every character of the organism. This principle is used in numeric taxonomy.
3. **Molecular classification:** Based on genetic relatedness (guanine+ cytosine content) of different organisms.

Bacterial Classification:
- Phylogenetic: Based on weighted characteristics
- Adansonian (or phonetic): Based on giving equal weight to every character
- Molecular: Based on genetic relatedness

MICROSCOPY

A good microscope should have three properties:

1. **Good resolution**: Resolution power refers to the ability to produce separate images of closely placed objects so that they can be distinguished as two separate entities. The resolution power of:
   - Unaided human eye is about 0.2 mm (200 µm)
   - Light microscope is about 0.2 µm
   - Electron microscope is about 0.5 nm

   Resolution depends on refractive index. Oil has a higher refractive index than air.
2. **Good contrast:** This can be further improved by staining the specimen.
3. **Good magnification**: This is achieved by use of concave lenses.

Resolution power of:
- Unaided human eye - 0.2 mm
- Light microscope - 0.2 µm
- Electron microscope - 0.5 nm

Bright-Field or Light Microscope

The bright-field or light microscope forms a dark image against a brighter background.

Dark Field Microscope

- **Principle**: In dark field microscope, the object appears bright against a dark background. This is made possible by use of a special dark field condenser
- **Applications**: It is used to identify the living, unstained cells and thin bacteria like spirochetes which cannot be visualized by light microscopy.

- **Numerical aperture** = n sin α (n is refractive index of medium and α one-half angular aperture of the objective)
- **Resolving Power** = 0.61 × wavelength/Numerical aperture

Phase Contrast Microscope

It is used to visualize the living cells by creating difference in contrast between the cells and water. It converts slight differences in refractive index and cell density into easily detectable variations in light intensity. It is useful for studying:

- Microbial motility
- Determining the shape of living cells
- Detecting bacterial components such as endospores and inclusion bodies.

Type of Light Used in Microscopes:
- Transmitted light: Used in bright field
- Reflected light: Used in dark field
- Polarized light: Used in differential interference contrast microscope

Fluorescence Microscope

Principle: When fluorescent dyes are exposed to ultraviolet rays (UV) rays, they become excited and are said to fluoresce, i.e. they convert this invisible, short wavelength rays into light of longer wavelengths (visible light).

Applications: Epifluorescence microscope has the following applications:

- Auto fluorescence, when placed under UV lamp, e.g. *Cyclospora*
- Microbes coated with fluorescent dye, e.g. Acridine orange for malaria parasites (QBC) and Auramine phenol for *M.tuberculosis*.
- Immunofluorescence: It uses fluorescent dye tagged antibody to detect cell surface antigens or antibodies bound to cell surface antigens. There are three types: direct IF, indirect IF, and Flow cytometry.

Fluorescence microscope Applications:
- Auto fluorescence, e.g. *Cyclospora*
- Microbes coated with fluorescent dye: QBC for *Plasmodium* and Auramine phenol for *M. tuberculosis*.
- Immunofluorescence: Florescent dye tagged antibody/antigen

Electron Microscope

It was invented by Ernst Ruska in 1931. It differs from light microscope by various ways (table given below). There are two types of EM:

1. Transmission EM (MC type, examine the internal structure, resolution 0.5 nm, gives 2 dimensional view)
2. Scanning EM (examine the surfaces, resolution 7 nm, gives 3 dimensional view)

Table 1.1.3: Differences between light microscope and electron microscope

Features	Light microscope	Electron microscope
Highest practical magnification	About 1,000–1,500	Over 100,000
Best resolution	0.2 µm	0.5 nm
Radiation source	Visible light	Electron beam
Medium of travel	Air	High vacuum
Specimen mount	Glass slide	Metal grid (usually copper)
Type of lens	Glass	Electromagnet
Source of contrast	Differential light absorption	Scattering of electrons
Focusing mechanism	Adjust lens mechanically	Adjust current to the magnetic lens

Electron microscope:
- > 100,000 magnification
- 0.5 nm resolution
- Radiation source-Electron beam
- High vacuum medium
- Specimen mounted on Metal grid
- Electromagnet lens

Principle of Transmission Electron Microscope

Specimen preparation: Cells are subjected to the following steps to prepare very thin specimens (20 to 100 nm thick)

- ***Fixation*:** Cells are fixed by using glutaraldehyde or osmium tetroxide for stabilization.
- ***Dehydration***: Specimen is then dehydrated with organic solvents (e.g. acetone or ethanol).
- ***Embedding***: Specimen is embedded in plastic polymer and then, is hardened to form a solid block. Most plastic polymers are water insoluble; hence complete dehydration of specimen is must before embedding.
- ***Slicing***: Specimen is then cut into thin slices by ultramicrotome knife, and slices are mounted on a metal slide (copper).

Freeze-etching technique: It is an alternate method for specimen preparation to visualize the internal organelles within the cells. Cells are rapidly frozen then warmed → fractured by a knife exposing the internal organelles → subjected to sublimation → shadowed by coating with platinum and carbon.

Measures to increase the contrast of EM include:

- Staining by solutions of heavy metal salts like lead citrate and uranyl acetate
- Negative staining with heavy metals like phosphotungstic acid or uranyl acetate.
- Shadowing: Specimen is coated with a thin film of platinum or other heavy metal at 45° angle so that the metal strikes the microorganism on only one side

STAINING TECHNIQUES

Staining is necessary to produce color contrast and thereby increase the visibility of the object. Before staining, the fixation of the smear to the slide is done:

- **Heat fixation** is usually done for bacterial smears by gently flame heating an air-dried film of bacteria
- **Chemical fixation** such as ethanol, acetic acid, mercuric chloride, formaldehyde, methanol and glutaraldehyde. This is useful for examination of blood smears.

Staining Techniques Used in Microbiology

1. **Simple stain:** Basic dyes such as methylene blue or basic fuchsin are used as simple stains. They provide the color contrast, but impart the same color to all the bacteria in a smear.
2. **Negative staining,** e.g. India ink or nigrosin. The background gets stained black whereas unstained bacteria stand out in contrast. This is very useful in the demonstration of bacterial capsules which do not take up simple stains.
3. **Impregnation methods** (e.g. silver)**:** Used for demonstration of thin structures like bacterial flagella and spirochetes

4. **Differential stain:** Two stains are used which impart different colors which help in differentiating bacteria, e.g.
   - *Gram stain*: Differentiates bacteria into Gram-positive (appear violet) and Gram-negative (appear pink) groups
     - Primary stain by crystal violet (or gentian violet or methyl violet) for one minute.
     - Mordant by Gram's iodine for one minute.
     - Decolorization by acetone (for 1–2 sec) or ethanol (20–30 sec) with immediate wash. Decolorizer removes the primary stain from Gram-negative bacteria while the Gram-positive bacteria retain the primary stain.
     - Counter stain or Secondary stains by safranin or diluted carbol fuchsin; is added for 30 sec.
   - *Acid-fast stain*: Acid-fast organisms (table below) resist decolorization to mineral acids. This is due to presence of mycolic acids in the cell wall. (details in chapter 3.6).
   - *Albert stain*: Differentiates bacteria having metachromatic granules (e.g. *Corynebacterium diphtheriae*) from other bacteria that do not have (refer chapter 3.4).
5. **Other Special Staining Methods:**
   - *Spore staining*: Acid fast stain (using 0.25% sulfuric acid) and Malachite green stain (Schaeffer and fulton method modified by Ashby) methods are used; however, phase contrast microscope of unstained wet film is the best method.
   - *Lipids stained by*: Sudan Black stain
   - *Carbohydrate (Glycogen) stained by* Iodine stain
   - *Flagellar stain*: Tannic acid staining (Leifson method)
6. **Microscopy of Bacteria in Living State**
   - Unstained (wet) preparations: Used for:
     - Checking bacterial motility (e.g. in hanging drop and wet mount preparations)
     - For demonstration of spirochetes (e.g. in dark field or phase contrast microscopy).
   - Vital stains: Differentiate the living cells from dead cells:
     - In supravital staining, living cells that have been removed from an organism are stained; whereas intravital staining is done by injecting stain into the body.
     - Examples of vital stains are eosin, propidium iodide, trypan blue, erythrosine and neutral red.

Special Staining Methods:
- Spore staining: Acid fast stain (0.25% H_2SO_4) and Schaeffer and fulton method
- Lipids stained by: Sudan Black stain
- Carbohydrate stained by: Iodine stain
- Flagellar stain: Tannic acid (Leifson method)

Table 1.1.4: Acid fast organisms

Acid fast bacteria	Acid fast parasites
Mycobacteria –M.tuberculosis/M.leprae/NTM	*Cryptosporidium, Cyclospora* and *Isospora*
Nocardia, Rhodococcus and *Legionella micdadei*	*Tinea saginata* egg and scolex
Others: Bacterial Spore and Sperm head	Hooklets of hydatid cyst and *Schistosoma mansoni* eggs

MORPHOLOGY OF BACTERIA

Table 1.1.5: Characteristics of prokaryotes and eukaryotes

Characteristics	Prokaryotes	Eukaryotes
Major groups	Bacteria, blue green algae	Fungi, parasites, other algae, plants and animals
Nucleus	Diffuse	Well defined
Nuclear membrane	Absent	Present
Nucleolus	Absent	Present
Ribonucleoprotein	Absent	Present
Cell division	Binary fission	Mitosis, Meiosis

Contd...

Contd...

Characteristics	Prokaryotes	Eukaryotes
Chromosome	One, circular	Many, liner
Extra chromosomal DNA	Found in plasmid	Found in mitochondria
Sterols in cell membrane	Absent except in mycoplasma	Present
Cellular organelles	Absent (except ribosome)	Present
Ribosome	70s in size	80s in size
Site of respiration	Mesosome	Mitochondria
Pinocytosis	Absent	Present

Table 1.1.6: Shape of bacteria and their Gram staining property

Gram-positive cocci arranged in	
Cluster	*Staphylococcus*
Chain	*Streptococcus*
Pairs, lanceolate shaped	*Pneumococcus*
Tetrads	*Micrococcus*
Octate	*Sarcina*
Pair or in short chain, spectacle eyed shaped	*Enterococcus*
Gram-positive bacilli arranged in	
Chain (bamboo stick appearance)	*Bacillus anthracis*
Chinese letter arrangement	*C.diphtheriae*
Pallisade arrangement	Other Corynebacterium species
Branching GPB	Actinomycetes

Gram-negative cocci arranged in	
Pairs, lens shaped	*Meningococcus*
Pairs, kidney shaped	*Gonococcus*
Gram-negative bacilli arranged in	
Pleomorphic (various shapes)	*Haemophilus, Proteus*
Thumb print appearance	*Bordetella pertussis*
Curved	*Campylobacter* (Gull-wing shaped) and *Helicobacter*
Spirally coiled, rigid	*Spirillum*
Spirally coiled, flexible	Spirochetes
Comma shaped (fish in stream)	*Vibrio cholerae*
Chain	*Streptobacillus*

Gram-positive cocci arranged in:
- Cluster: *Staphylococcus*
- Chain: *Streptococcus*
- Pairs, lanceolate shaped- ***Pneumococcus***
- Tetrads: *Micrococcus*
- Octate: *Sarcina*
- Pair, spectacle eyed shaped- ***Enterococcus***

Bacterial Cell Wall

The cell wall is a tough and rigid structure, surrounding the bacterium. Apart from providing protection and conferring rigidity, certain parts of cell wall (e.g. LPS) are immunogenic and act as virulence factor.

- Peptidoglycan is main component of the cell wall which makes it rigid. It is composed of alternate units of N-acetyl muramic acid (NAM) and N-acetyl glucosamine (NAG) molecules; cross linked to each other via tetrapeptide side chains and pentaglycine bridges.
- Gram-positive bacteria has a thicker peptidoglycan and contains teichoic acid
- Gram-negative bacteria-peptidogly can layer is thin, and it contains additional parts such as (1) Outer membrane, (2) Lipopolysaccharide (LPS) which in turn consists of (i) Lipid A or endotoxin, (ii) Core polysaccharide, (iii) O side chain

Table 1.1.7: Differences between Gram-Positive and Gram-Negative cell wall

Characters	Gram-Positive cell wall	Gram-Negative cell wall
Peptidoglycan layer	Thicker (15–80 nm)	Thinner (2 nm)
At 3rd position of tetrapeptide side chain	L- Lysine present	Mesodiaminopimelic acid present
Pentaglycine bridge	Present	Absent
Lipid content	Nil or scanty (2–5%)	Present (15–20%)

Contd...

Contd...

Characters	Gram-Positive cell wall	Gram-Negative cell wall
Lipopolysaccharide	Absent	Present (endotoxin)
Teichoic acid	Present	Absent
Variety of amino acids	Few	Several
Aromatic amino acids	Absent	Present

Other Parts of Bacterial Cell

Intracytoplasmic Inclusions

They are the storage sites of nutrients/bacteria, formed in nutritional deficiency conditions:

- Organic inclusion bodies, examples include glycogen granules and poly-hydroxyl butyrate granules.
- Inorganic inclusion bodies, examples include:
  - Polymetaphosphate or volutin or metachromatic granules in *C.diphtheriae.*
  - Suflur granules found in *Actinomyces.*

Gram Positive cell wall:
- Thicker Peptidoglycan
- Absent: LPS, Aromatic AA and lipids
- Present: Teichoic acid

Nucleoid

Bacteria do not have a true nucleus; but the genetic material is located in an irregularly shaped region called the nucleoid.

- There is no nuclear membrane or nucleolus and lacks basic proteins.
- Possess a single haploid chromosome, comprising of super coiled circular ds DNA (except two chromosomes *in Vibrio*).
- Bacterial DNA divides by simple binary fission.
- The nucleoid can be seen by electron microscopy or on staining with the Feulgen stain
- Bacteria also possess extra-chromosomal DNA called *plasmids.*

Capsule and Slime Layer

Some bacteria possess a layer of amorphous viscid material lying outside the cell wall called *glycocalyx* which may be well organized (*capsule*) or unorganized loose material (*slime layer*). Some bacteria may possess both capsule and slime layer as in *Streptococcus salivarius.*

Capsulated bacteria:
- *Pneumococcus*
- *Meningococcus*
- *Haemophilus influenzae*
- *Klebsiella pneumoniae*
- *Pseudomonas aeruginosa*
- *Bacillus anthracis*

Capsulated fungus:
- *Cryptococcus*

Table 1.1.8: Examples of capsulated organisms

Capsulated bacteria	Composition
Pneumococcus	Polysaccharide
Meningococcus	Polysaccharide
Haemophilus influenzae	Polysaccharide
Klebsiella pneumoniae	Polysaccharide
Pseudomonas aeruginosa	Polysaccharide

Capsulated bacteria	Composition
Bacillus anthracis	Polypeptide (glutamate)
Streptococcus pyogenes (some stains)	Hyaluronic acid
Bacteriodes fragilis	Polysaccharide
Capsulated fungus	
Cryptococcus neoformans	Polysaccharide

The capsule has various functions

- Acts by inhibiting phagocytosis and complement-mediated lysis
- Biofilm formation and thereby helps in adherence to damaged tissues and plastic surfaces (e.g. medical devices)
- Source of nutrients and energy for the bacteria
- Capsules as vaccine, e.g. Pneumococcus, Meningococcus and *Haemophilus influenzae* serotype-b and S. Typhi Vi Vaccine.

Demonstration of capsule by

- *Negative staining* by India ink and nigrosin stain: Capsule appears as a clear refractile halo around the bacteria; whereas both the bacteria and the background appear black.

- *M'Faydean capsule stain* for *Bacillus anthracis* by using polychrome methylene blue stain.
- *Serological test*: Capsular material is antigenic and can be demonstrated by mixing it with a specific anticapsular serum:
  - Quellung reaction for *Streptococcus pneumoniae*
  - Latex agglutination test by using specific anticapsular antibodies coated on latex.

Flagella

Flagella are thread-like appendages, protruding from the cell wall, composed of protein subunits called flagellin. It has three parts: filament, kook and basal body.

- Size-They measure 5–20 µm in length and 0.01–0.02 µm in thickness
- They are organs of locomotion, confer motility to the bacteria.

Arrangement of flagella

- Monotrichous (single polar flagellum) e.g. *Vibrio cholerae, Pseuodmonas* and *Camplylobacter*
- Lophotrichous (multiple polar flagella) e.g. *Spirillum*.
- Peritrichous over the entire cell surface) e.g. *Salmonella* Typhi, *Escherichia coli*.
- Amphitrichous (single flagellum at both the ends) e.g. *Alcaligenes faecalis, Spirillum*

(***Note:*** *Spirillum* has tuff of flagella at one or both the ends (Amphi>lophotrichous)

Flagella can be demonstrated by

- Direct demonstration of flagella
  - Tannic acid staining *(Leifson's method and Ryu's method)*
  - Dark ground, phase contrast or electron microscope
- Indirect means by demonstrating the motility:
  - Cragie tube method and Hanging drop method
  - Semisolid medium, e.g. mannitol motility medium.

Types of motility:
- Tumbling: *Listeria*
- Gliding: *Mycoplasma*
- Stately: *Clostridium*
- Darting: *Vibrio, Campylobacter*
- Swarming: *Proteus, C. tetani*
- Corkscrew, lashing, flexion extension: *Spirochete*

Table 1.1.9: Various types of motility

Types of motility	Bacteria shown	Types of motility	Bacteria shown
Tumbling motility	*Listeria*	Darting motility	*Vibrio cholerae, Campylobacter*
Gliding motility	*Mycoplasma*	Swarming motility	*Proteus, Clostridium tetani*
Stately motility	*Clostridium*	Corkscrew, lashing, flexion extension	*Spirochete*

Fimbriae or Pili

Many Gram-negative and some Gram-positive bacteria possess short, fine, hair-like appendages that are thinner than flagella and not involved in motility, called fimbriae or pili. They measure 0.5 µm long and 10 nm in thickness:

- Pili are made-up of protein called pilin
- They are antigenic; however, the antibodies against fimbrial antigens are not protective.
- **Functions:** Fimbriae are called the organ of adhesion. This property enhances the virulence of bacteria.
  - Certain fimbriae called sex pili also help in bacterial gene transfer.
  - Fimbriae are not related to motility, can be found both in motile as well as in non-motile organisms.
- **Types**
  - *Common pili*: These are of six types
  - *Sex* or *F (fertility) pili*: Help in bacterial conjugation, e.g. as found in *Gonococcus*
  - Col I (colicin) pili.
- **Detection of fimbriae**
  - Direct demonstration of fimbriae by Electron microscopy
  - Indirect methods to know the presence of fimbriae are:

- Hemagglutination: Many fimbriated bacteria (e.g. *Escherichia coli, Klebsiella*) strongly agglutinate RBCs. The hemagglutination can be specifically inhibited by D-mannose.
- Surface pellicle, e.g. in some aerobic fimbriated bacteria form a thin layer at the surface of a broth culture.

Atypical forms of Bacteria

- **Involution forms:** Swollen and aberrant forms of bacteria (e.g. Gonococci and *Yersinia pestis*) in aging cultures
- **Pleomorphic bacteria:** Different shape and size of individual cells, e.g. *Proteus* and *Yersinia pestis*
- **L form or Cell wall deficient forms**: When bacteria loose cell wall, they become spherical called L form.
  - Discovered by E.Klieneberger, while studying *Streptobacillus moniliformis*
  - It is named after its place of discovery, i.e. Lister Institute, London
  - L forms mediates persistence of pyelonephritis and other chronic infections.
  - L forms in Gram-positive bacteria are called *Protoplasts* and in Gram-negative bacteria are called *Spheroplasts*.
  - *Mycoplasma* do not have a true cell wall; the peptidoglycan layer is replaced by sterol. It is postulated that *Mycoplasma* may represent stable L forms of yet to be unidentified parent bacteria. But many researchers do not consider this.

Involution forms:
- Swollen and aberrant forms of bacteria
- For example, Gonococci and *Yersinia pestis*
- Seen in aging cultures.

Bacterial Spores

Spores are highly resistant resting (or dormant) stage of the bacteria formed in unfavorable environmental conditions as a result of the depletion of exogenous nutrients.

- **Structure of a spore:** From innermost towards the outermost, the layers are core→ cortex→ coat→ exosporium
- **Sporicidal agents:** Spores are highly resistant. Only limited sterilization methods are available to kill the spores
- **Used as indicator for proper sterilization**.
  - Spores of *Geobacillus stearothermophilus* are used as sterilization control for auto-clave.
  - Spores of non-toxigenic strains of *Clostridium tetani* are used as sterilization control for hot air oven.
- Used as agents of bioterrorism; e.g. Spores of *Bacillus anthracis* in the 2001 USA attack.

PHYSIOLOGY OF BACTERIA (BACTERIAL GROWTH AND NUTRITION)

Generation Time

Time required for a bacterium to give rise to two daughter cells under optimum condition.

- For *Escherichia coli* and most of the other pathogenic bacteria, -it is 20 minutes;
- For *Mycobacterium tuberculosis*: It is 14 hours
- For *Mycobacterium leprae*: It is 12–13 days

Generation time:
- *Escherichia coli:* 20 minutes
- *Mycobacterium tuberculosis:* 14 hours
- *Mycobacterium leprae:* 12–13 days

Bacterial Count

- *Total count:* Indicates total number of bacteria (live or dead) in the specimen. This is done by counting the bacteria under microscope using counting chamber.
- *Viable count:* Measures the number of living (viable) cells in the given specimen. Viable count may be obtained by:
  - Pour plate method (best method)
  - Surface viable count by spreading method
  - Surface viable count by Miles and Misra method.

Bacterial Growth Curve

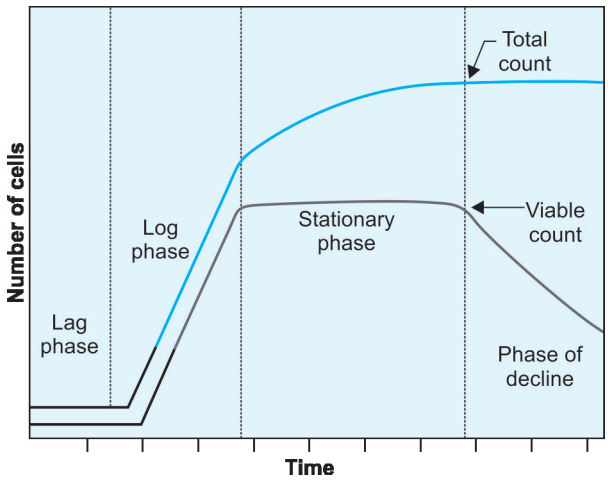


Fig. 1.1.1: Bacterial growth curve

When a bacterium is inoculated into a suitable liquid culture medium and incubated, its growth follows a definite course. When bacterial count of such culture is determined at different intervals and plotted in relation to time, a bacterial growth curve is obtained comprising of four phases (Table 1.1.10 given below).

Table 1.1.10: Phases of bacterial growth curve

	Lag phase	Log phase	Stationary phase	Decline phase
Bacteria divide	No	Yes	Yes	No
Bacterial death	No	No	Yes	Yes
Total count	Flat	Raises	Raises	Flat
Viable count	Flat	Raises	Flat	Falls
Special features	Preparatory phase Accumulation of enzymes and metabolites Attains maximum size at end of lag phase	Uniformly stained, Metabolically active Small size	Gram variable Produce: Granules, spores, exotoxin, antibiotics, bacteriocin	Produce involution forms

Bacterial growth curve:
- *Lag phase*: ↑enzymes and metabolites, Max cell size
- *Log phase*: Cells uniformly stained, active
- *Stationary phase*: Produce granules, spores, exotoxin, antibiotics, bacteriocin
- *Decline phase*: Produce involution forms

Factors Affecting Growth of Bacteria

1. **Oxygen:** On basis of their oxygen requirements bacteria are classified as:
   - *Obligate aerobes:* Grow only in the presence of oxygen (e.g. *Pseudomonas, M. tuberculosis, Bacillus, Brucella* and *Nocardia)*
   - *Facultative anaerobes:* They are aerobes that can also grow anaerobically (e.g. most of the pathogenic bacteria, e.g. *E.coli, S.aureus,* etc).
   - *Facultative aerobes:* They are anaerobes that can also grow aerobically (e.g. *Lactobacillus*)
   - *Microaerophilic bacteria*: Can grow in the presence of 5–10% of oxygen (e.g. *Campylobacter, Helicobacter, Mycobacterium bovis*).
   - *Obligate anaerobes*: Grow only in the absence of oxygen. Oxygen is lethal to these bacteria (e.g. *Clostridium)*
   - *Aerotolerant anaerobe*: They can tolerate oxygen for some time, but do not use it (*Clostridium histolyticum).*

2. **Carbon dioxide:** Capnophilic bacteria need 5–10% of CO_2. E.g.*Brucella abortus, Streptococcus pneumoniae,* etc.
3. **Temperature:** Based on optimal temperature needed for growth, bacteria can be grouped into:
   - *Psychrophiles*: Grow below 20°C, e.g. saprophytes.
   - *Mesophiles:* Grow between 25°C and 40°C, e.g. most of the pathogenic bacteria
   - *Thermophiles:* Grow above of 55°C - 80°C, e.g. *Bacillus stearothermophilus*
4. **pH:** Most pathogenic bacteria grow between pH 7.2 - pH 7.6**.** Very few bacteria (lactobacilli) can grow at acidic pH below pH 4, while bacteria such as *Vibrio cholerae* are capable of growing at alkaline pH (8.2–8.9).
5. **Light:** Bacteria (except phototrophs) grow well in dark. They are sensitive to ultraviolet rays and other radiations in light. Photochromogenic mycobacteria produce pigments only on exposure to light.
6. **Other factors**: Osmotic Effect, Mechanical and sonic Stresses, Moisture and desiccation.

MICROBIAL PATHOGENICITY

Microbial pathogenicity depends upon the sum total of several factors as described below.

1. **Route of transmission of infection**
2. **Infective dose of the organism:** Minimum inoculum size that is capable of initiating an infection. (table given below)

Low infective dose	Large infective dose
Shigella: Very low (as low as 10 bacilli) *Cryptosporidium parvum*: very low (10 to 30 oocysts) *Escherichia coli* O157: H7 (< 10 bacilli) *Entamoeba* and *Giardia*: few cysts *Campylobacter jejuni* (500 bacilli)	*Escherichia coli* (10^6 – 10^8 bacilli) *Salmonella* (10^2 – 10^5 bacilli) *Vibrio cholerae* (10^6 – 10^8 bacilli)

3. **Evasion of the local defenses**
4. **Adhesion:** By Fimbriae or pili, other adhesins, biofilm formation
5. **Invasion:** Virulence factors that help in invasion include:
   - Virulence marker antigen or invasion plasmid antigens in *Shigella*
   - Enzymes: Invasion of bacteria is enhanced by many enzymes: Hyaluronidase, Collagenase, Streptokinase (fibrinolysin) and IgA proteases
   - Antiphagocytic factors: Capsule
   - Cell wall proteins such as: Protein A of *Staphylococcus aureus* and M protein of *Streptococcus pyogenes*
   - Cytotoxins: Interfere with chemotaxis or killing of phagocytes. For example, *S. aureus* produces hemolysins and leukocidins that lyse and damage RBCs and WBCs.

Mechanism of intracellular survival	Organism
Inhibition of phagolysosome fusion	*Legionella* species, *Mycobacterium tuberculosis, Chlamydia*
Resistance to lysosomal enzymes	*Salmonella* Typhimurium, *Coxiella*, *Mycobacterium leprae*, *Leishmania*
Adaptation to cytoplasmic replication	*Listeria*, *Rickettsia* and *Francisella tularensis*

Examples of Obligate intracellular organism include:

- Bacteria: *Mycobacterium leprae*, *Rickettsia, Chlamydia* and *Coxiella*
- All viruses
- Fungi: *Pneumocystis jirovecii*
- Parasite: *Toxoplasma*, *Cryptosporidium, Plasmodium, Leishmania, Babesia, Trypanosoma cruzi*

Table 1.1.11: Differences between bacterial endotoxins and exotoxins

Endotoxins	Exotoxins
Lipopolysaccharides in nature	Protein in nature
Part of Gram-negative bacterial cell wall	Secreted both by Gram-positive and negative bacteria; diffuse into surrounding medium
Released by cell lysis, Not by secretion	Actively secreted by the bacteria
Highly stable	Heat labile destroyed at 60 °C
Mode of action – Induces ↑IL-1 and TNF	Mostly enzyme like action
Nonspecific (fever, shock, etc.)	Specific action on particular tissues
No specific affinity for tissues	Specific affinity for tissues
Only large doses are fatal	More potent, even smaller doses fatal
Poorly antigenic	Highly antigenic
Neutralization by antibodies is ineffective	Neutralized by specific antibodies
No effective vaccine is available using endotoxin	Toxoid forms are used as vaccine, e.g. tetanus toxoid

Table 1.1.12: Exotoxins and their mechanisms of action

Toxins (Exotoxins)	Mechanism
Enterotoxin and TSST of *S.aureus* Streptococcal pyrogenic exotoxin	Act as super antigen; stimulate T cell non-specifically, to release of large amounts of cytokines.
Diphtheria toxin and Exotoxin-A of *Pseudomonas*	Inhibits protein synthesis (by inhibiting elongation factor-2)
Anthrax toxin	↑cAMP in target cell, edema
α toxin of *Clostridium perfringens*	Lecithinase and phospholipase activity → causes myonecrosis
Tetanus toxin (tetanospasmin)	Decrease in neurotransmitter (GABA and glycine) release from the inhibitory neurons → Spastic paralysis
Botulinum toxin	Decrease in neurotransmitter (acetyl choline) release from neurons → Flaccid paralysis
Heat labile toxin of ETEC and Cholera toxin (*V.cholerae*)	Activation of adenylate cyclase → ↑cAMP in target cell → Secretory diarrhea
Heat stable toxin	↑cGMP in target cell → Secretory diarrhea
Verocytotoxin (EHEC) and Shiga toxin (*Shigella dysenteriae type-1)*	Inhibit protein synthesis (by inhibiting ribosome)

MULTIPLE CHOICE QUESTIONS

HISTORY

1. Syphilis discovered in which year? *(TNPG 2015)*
a. 1838 b. 1905
c. 1906 d. 1921

2. All are Koch's postulates except: *(JIPMER 2014)*
a. A microorganism should be constantly associated with the lesions of the disease
b. It should be possible to isolate the bacterium in pure culture from the lesions
c. Inoculation of such pure culture into laboratory animals should reproduce the lesions
d. Administration of broad spectrum antimicrobial agent dependably eradicates the organisms and cures the diseases

3. Corynebacterium diphtheriae is also called: *(NEET Pattern Based)*
a. Koch's bacillus b. Kleb-Loeffler bacillus
c. Roux bacillus d. Yersin bacillus

4. Leeuwenhoek is associated with: *(DNB DEC 2012)*
a. Telescope b. Microscope
c. Stains d. Immunization

5. Theory of web of causation was given by? *(DNB DEC 2012)*
a. Mc Mohan and Pugh b. Pettenkoffer
c. John snow d. Louis Pasteur

6. Louis Pasteur is associated with all EXCEPT: *(DNB DEC 2012)*
a. Vaccination of smallpox
b. Germ theory
c. Pasteurization
d. Vaccination of rabies

7. Best chemical disinfectant to disinfect stethoscope is:
a. Isopropyl alcohol *(Recent Question 2015)*
b. Ethylene oxides
c. Halogenated compound
d Steam plasma sterilization

BACTERIAL CELL BIOLOGY AND STRUCTURE

8. Which is not a prokaryote? *(JIPMER Nov 2015)*
a. Mycoplasma b. Rickettsiae
c. Shigella d. Entamoeba

9. Non motile: *(Recent MCQ 2014, NEET Pattern Based)*
a. Shigella b. E.coli
c. Proteus d. Vibrio

10. Swarming is seen in: *(NEET Pattern Based)*
a. Clostridium tetani
b. Clostridium perfringens
c. Clostridium botulinum
d. Clostridium difficile

11. Which is a eukaryote: *(PGI Dec 2008, Latest MCQ 2013)*
a. Mycoplasma b. Bacteria
c. Fungus d. Chlamydia

12. Role of bacterial capsule is: *(JIPMER May2015)*
a. Support b. Transport
c. Antiphagocytic d. Adhesion

13. Capsulated organism: *(PGI June 2003, DNB Dec 2011)*
a. Candida b. Klebsiella
c. Proteus d. Cryptococcus
e. Histoplasma

14. Which is not present in Gram-negative bacteria: *(TNPG 2014)*
a. Peptidoglycan b. Teichoic acid
c. LPS d. Porin channels

15. Craigie's tube method is used to differentiate:
a. Motile and nonmotile strains *(JIPMER 2014)*
b. Virulent and avirulent strains
c. Capsulated and noncapsulated strains
d. Rough and smooth strains

STAINING TECHNIQUES AND MICROSCOPY

16. Factor affecting electron microscope is?
a. Eyepiece *(AIIMS Nov 2016)*
b. Wavelength of the light
c. Type of glass lens used
d. Thickness of the sample

17. Resolving power of microscope depends on all except: *(AIIMS Nov 2016)*
a. Refractive index of medium
b. Wavelength
c. Diameter of aperture
d. Focal length of objective lens

18. What is the reason for decolourisation in gram-negative bacilli in Gram staining *(JIPMER May 2016)*
a. Due to the two dyes used in staining
b. Lipid content
c. Cell membrane integrity
d. Teichoic acid

19. Composition of ZN stain are all EXCEPT: *(Recent Questions 2014)*
a. Basic fuchsin b. Acid fuchsin
c. Phenol d. Alcohol

20. Acid fast bacilli – stained by: *(TNPG 2015)*
a. Ziehl–Neelsen b. Albert
c. Neisser d. Ponder

21. Correct sequence in Gram staining? *(Latest MCQ 2013)*
a. Methyl violet → Iodine → Acetone → Carbol fuchsin
b. Carbol fuchsin → Iodine → Acetone → Methyl violet
c. Methyl violet → Acetone → Iodine → Carbol fuchsin
d. Methyl violet → Carbol fuchsin → Acetone → Iodine

22. Not used in grams staining: *(DNB DEC 2012)*
a. Methylene blue b. Crystal violet
c. Iodine d. Safranin

23. Indian ink staining is positive for which of the following cocci: *(PGI Nov 2014 , TNPG 2014)*
a. Pneumococcus b. Staphylococcus
c. Meningococcus d. Gonococcus
e. Enterococcus

24. Which of the following about Gram staining is most correct: *(Latest Question 2013)*
a. Heating is done to promote entry of stain into cell wall of both Gram +ve and –ve bacteria
b. If observed staining technique and microscopy before decolorization, both Gram +ve and –ve bacteria appear same
c. Teichoic acid is present in the cell wall of Gram–ve bacteria

BACTERIAL GROWTH AND NUTRITION

25. Which event takes place lag phase of growth curve? *(Recent Question 2015)*
a. Bacterial cell number increase
b. Bacterial cell size increase
c. Sporulation

26. Which of the following is microaerophilic: *(AIIMS May 2009)*
a. Campylobacter
b. Vibrio
c. Bacteroides
d. Pseudomonas

27. The term viable not cultivable is used for: *(PGI Dec 2007)*
a. M. leprae
b. M. tuberculosis
c. Treponema pallidum
d. Salmonella
e. Staphylococcus

28. Correct sequence of bacterial growth curve: *(PGI Dec 2007)*
a. Log phase – Lag Phase – Stationary phase – Decline phase
b. Lag Phase – Log phase – Stationary phase – Decline phase
c. Stationary phase – Log phase – Lag Phase – Decline phase
d. Lag phase – Exponential phase – Log phase – Death phase
e. Exponential phase – Lag phase – Death phase – Stationary

29. Population doubling time in coliform bacilli is: *(MHPG 2014)*
a. 20 seconds b. 20 minutes
c. 20 hours d. 20 days

30. Phototropism means: *(Recent Questions 2014)*
a. Growing towards the sunlight
b. Obtaining energy from sunlight
c. Reflecting energy from light source
d. None of the above

MICROBIAL PATHOGENESIS

31. Which organism grows in alkaline pH? *(NEET Pattern Based)*
a. Klebsiella b. Vibrio
c. Pseudomonas d. E.coli

32. Obligate intracellular organism is: *(NEET Pattern Based, AI 2005)*
a. Mycoplasma b. Chlamydia
c. Cryptococcus d. Helicobacter

33. True about exotoxin: *(Recent MCQ 2014)*
a. LPS in nature b. Heat stable
c. Not antigenic d. Toxoid can be prepared

34. All the following are true regarding exotoxin except: *(JIPMER 2014)*
a. Highly antigenic
b. Heat stable
c. Neutralized by antibody
d. Active in very minute doses

35. All the following statements are true regarding endotoxins except: *(JIPMER, May2015)*
a. Neutralized by specific antibodies
b. Heat stable
c. Somatic antigen
d. Poorly antigenic

EXPLANATIONS

HISTORY

1. **Ans. (b) (1905)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p5, Ananthanarayan 9th /p371
   - Treponema pallidum, the agent of syphilis was discovered on 1905 by Schaudinn and Hoffmann.
2. **Ans. (d) Administration of broad spectrum antimicrobial agent dependably eradicates the organisms and cures the diseases** Refer text Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p4 , Ananthanarayan 9/e p5
3. **Ans. (b) (Kleb-Loeffler's...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p5, Ananthanarayan 9/e p236
   - Corynebacterium diphtheriae is also called Klebs-Loeffler bacillus
4. **Ans. (b) (Microscope)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p3, Ananthanarayan 9/e p3, 8/e p3

   Antony van Leeuwenhoek **from Holland was the first person to observe and describe microorganisms accurately by using lenses and was considered as the founder of simple microscope (1694).**
   - Zacharias Jansen and his father – described the first compound microscope
   - Ernst Ruska: Invented Electron Microscope in 1931**.**
5. **Ans. (a) (Mc Mohan and Pugh)** Ref: Park 21/e p32

   Concept of causation of disease:
   - *Spontaneous generation of life:* Life can be created by chemical reactions. It was disproved by Louis Pasteur.
   - Germ theory of life or single cause theory (Louis Pasteur) - one to one relationship between causative agent and disease, i.e. [Agent→ Man→ Disease]. It suffered critics as Not everyone exposed to an agent develops disease.
   - *Multifactorial causation:* Proposed by Pettenkofer and Munich.
   - *Theory of web of causation:* Suggested by MacMahon, Pugh, and Ipsen (1960) in their book Epidemiologic principles and methods' that disease is due to the result of complex interrelationship between all the predisposing factors with variable relative risk.
6. **Ans. (a) (Vaccination of..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p3, Ananthanarayan 9/e p4, 8/e p4
   - Vaccination of smallpox by using cowpox was proposed by Edward Jenner.
   - Louis Pasteur prepared the vaccines for – Anthrax, Rabies, Cholera (CAR)
   - **For other contributions of Louis Pasteur-** *Refer text.*
7. **Ans (a) (Isopropyl alcohol)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p42

   Low-level disinfectant such as Isopropyl alcohol should be enough as stethoscope is a noncritical item according to Spaulding's classification.

BACTERIAL CELL BIOLOGY AND STRUCTURE

8. **Ans (d) (Entamoeba)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p9

   Parasites and fungi are eukaryotes (Entamoeba is a parasite); whereas bacteria and blue green algae are prokaryotes.
9. **Ans. (a) (Shigella)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p298, Ananthanarayan 9/e p284
   - Shigella, Klebsiella are Non Motile among Gram negative Enterobacteriaceae organisms.
10. **Ans. (a) (C. tetani)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p310, Ananthanarayan 9/e p259
    - Swarming is exhibited by- Cl.tetani, Proteus, Vibrio parahemolyticus and Vibrio alginolyticus
11. **Ans. (c) (fungus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p9, Ananthanarayan 9 e/p427, 8/e p425
    - Prokaryotes – include bacteria and blue green algae
    - Eukaryotes – include fungi, algae (other than blue green), protozoa, helminths and slime moulds
    - See the table from the text for detail.
12. **Ans. (c > d) (Antiphagocytic > Adhesion)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p21
    - The main role of bacterial capsule is to escape from phagocytosis. Capsule also helps in biofilm formation and adhesion.

13. **Ans. (b) and (d) (Klebsiella and Cryptococcus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p21
    - List of capsulated bacteria-*Refer text*
14. **Ans. (b) (Teichoic acid)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p18, Ananthnarayan 9/e p15
    - Refer chapter review.
15. **Ans. (a) Motile ..** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p319 , Ananthanarayan 9/e p293

STAINING TECHNIQUES AND MICROSCOPY

16. **Ans (d) (Thickness of the sample)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p13
    - **In order to get visualized by EM, the specimen should be very thin (20 to 100 nm thickness).**
17. **Ans (d) (Focal length of objective lens)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p10
    - **Resolving power of microscope depends on wavelength and numerical aperture (the latter is in turn depends on refractive index and angular aperture).**
18. **Ans. (b) (lipid content)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p15

    Gram negative bacteria gets decolorized easily due to following factors- 1) Thin peptidoglycan layer and less cross linking, 2) Presence of lipid rich lipopolysaccharide layer which gets easily destroyed by decolorizer.
19. **Ans. (b) (Acid fuchsin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p16, Ananthanarayan 9/e p14

    ZN stain has three components:
    - Primary stain: Basic fuchsin + Phenol
    - Decolorizer: Sulfuric acid or acid-alcohol
    - Counter stain: Methylene blue or Malachite Green
20. **Ans. (a) (Ziehl–Neelsen)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p16, Ananthanarayan 9 /e p13

    Refer chapter review of chapter 1.1
21. **Ans. (a) (Methyl ..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p14-15, Ananthanarayan 9/e p13, 8/e p15

    Refer chapter review
22. **Ans. (a) (Methylene blue)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p14-15, Ananthanarayan 9/e p13
    - *Gram stain has four steps (refer chapter review)*
    - Methylene blue is used a counter stain for acid fast stain
23. **Ans. (a) (c) (Pneumococcus, Meningococcus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p21

    India ink stain is used to demonstrate the capsule. Among cocci, Pneumococcus, Meningococcus are capsulated.
24. **Ans. (b) (If observed staiming technique microscopy before decolorization, both Gram +ve and –ve bacteria appear same)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p15, Ananthanarayan 9/e p13.
    - In Gram stain:
        - Under decolorization: Everything will look gram-positive (violet)
        - Over decolorization: Everything will look gram-negative (pink)
    - Heating (Intermittent heating) is done in acid fast stain (not for gram stain)

BACTERIAL GROWTH AND NUTRITION

25. **Ans. (b) (Bacterial cell size increase)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p27
26. **Ans. (a) (Campylobacter)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p28, Ananthanarayan 9/e p24
    - Microaerophilic: Require small amount of oxygen (5%) e.g. Campylobacter, Helicobacter and Mycobacterium bovis.
    - For detail explanation: *Refer text*
27. **Ans. (a) and (c) (M. lepare and Treponema pallidum)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p4,397

    Already explained

28. **Ans. (b) (Lag- Log- Stationary- Decline)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p27
    - For detail explanation- refer text
29. **Ans. (b) (20 minutes)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p26, Ananthanarayan 9/e p22
    Population doubling time or the generation time of- E. coli- 20 min, M. tuberculosis-20 hr and M. leprae-20 days
30. **Ans. (b) (Obtaining energy...)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p28 , Ananthanarayan 9/e p23
    Refer chapter review for detail.

MICROBIAL PATHOGENESIS

31. **Ans. (b) (Vibrio)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p28, Ananthanarayan 9/e p25, 8/e p24
    - *Vibrio survives in alkaline pH of 8.6*
32. **Ans. (b) (Chlamydia)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p92
    Refer chapter review
33. **Ans (d) (Toxoid can...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p93, Ananthanarayan 9/e p75
    Refer text
34. **Ans. (b) (Heat stable)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p93 , Ananthanarayan 9/e p75
35. **Ans (a) (Neutralized by specific antibodies)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p93

CHAPTER

1.2

Sterilization and Disinfection

Definitions:
- *Sterilization:* Destroys all microbes including spore
- *Disinfection:* Destroys all microbes except spore
- *Asepsis:* Chemical agents applied to body surfaces
- *Decontamination* (or Sanitization): Makes items as safe to handle.

DEFINITIONS

Sterilization: It is the process by which all living microorganisms including viable spores, are destroyed with reduction of at least 10^6 log colony forming units (CFU) of microorganisms and their spores.

Disinfection: It refers to a process that destroys or removes most if not all pathogenic organisms but not bacterial spores with reduction of at least 10^3 log CFU of microorganism but not spores.

Asepsis: It is a process where the chemical agents (called antiseptics) applied to body surfaces (skin) will kill or inhibit the pathogenic microorganisms (and also commensals) present on skin.

Decontamination (or Sanitization): It refers to the reduction of pathogenic microbes to a level at which items are considered as safe to handle without protective attire with reduction of at least 1 log CFU of microorganism but not spores.

Factors Influencing Efficacy of Sterilant/Disinfectant

The efficiency of a sterilant/disinfectant antimicrobial agent is affected by at following factors:

1. **Organism load:** Larger microbial population requires a longer time to die than a smaller one.
2. **Nature of organisms:** It greatly influences the efficacy of the disinfectants
3. **Concentration** of the chemical agent and **temperature** of the physical agent
4. **Nature of the sterilant/disinfectant:**
   - Microbicidal ability, Rapidity of action, Residual activity.
   - Ability to act in presence of organic matter such as pus, blood, and stool.
5. **Duration of exposure:** More is the exposure time, better is the efficacy.
6. **pH:** Heat kills more readily at an acidic pH
7. **Biofilm formation:** Prevents the entry of disinfectants.

Table 1.2.1: Classification of sterilization/disinfection methods.

A. Physical methods
1. *Heat* Dry heat: Flaming, Incineration and Hot air oven Moist heat: a. Temperature < 100°C, e.g. pasteurization, water bath and inspissation b. Temperature at 100°C, e.g. boiling, steaming and tyndallization c. Temperature > 100°C, e.g. autoclave 2. *Filtration:* Depth filters and membrane filters 3. *Radiation* Ionizing radiation: Y-rays, X-rays and cosmic rays Non-ionizing radiation: Ultraviolet (UV) and infrared rays 4. *Ultrasonic vibration*
B. Chemical methods
1. *Alcohols:* Ethyl alcohol, isopropyl alcohol
2. *Aldehydes:* Formaldehyde, glutaraldehyde, Ortho-phthaladehyde
3. *Phenolic compounds:* Cresol, lysol, chlorhexidine, chloroxylenol, hexachlorophene
4. *Halogens:* Chlorine, iodine, iodophors
5. *Oxidising agents:* Hydrogen peroxide, Peracetic acid

Contd...

Contd...

B. Chemical methods
6. *Salts:* Mercuric chloride, copper salts
7. *Surface active agents:* Quaternary ammonium compounds and soaps
8. *Dyes:* Aniline dyes and acridine dyes
9. *Gas sterilization:* Low temperature steam formaldehyde, Ethylene oxide (ETO) and Beta-propiolactone (BPL)

PHYSICAL METHODS OF STERILIZATION/DISINFECTION

Heat Sterilization/Disinfection

Mechanism of Action

- Dry heat kills the organisms by **C**harring, **O**xidative damage, **D**enaturation of bacterial protein and **E**levated levels of electrolytes (CODE).
- Moist heat kills the microorganisms by denaturation and coagulation of proteins.

Mechanism of action of heat sterilization:
- Dry heat kills the organisms by (CODE):
  - Charring
  - Oxidative damage
  - Denaturation of bacterial protein
  - Elevated levels of electrolytes.
- Moist heat kills the microorganisms by denaturation and coagulation of proteins.

Dry Heat (Hot air oven)

- Holding temperature required: 160°C for 2 hours
- Materials sterilized: Hot air oven is best method for sterilization of:
  - Glassware like glass syringes, petri dishes, flasks, pipettes and test tubes.
  - Surgical instruments like scalpels, scissors, forceps, etc.
  - Chemicals such as liquid paraffin, fats, glycerol, and glove dust powder, etc.
- Sterilization control:
  - Spores (10^6) of nontoxigenic strains of *Clostridium tetani* or *Bacillus subtilis* subsp. *niger*
  - Thermocouples and Browne's tube.

Moist Heat at a Temperature below 100°C

- **Pasteurization:** Used for perishable beverages like fruit and vegetable juices, beer, and dairy products such as milk.
  - Two methods: Holder method (63°C for 30 min) and Flash method (72°C for 20 sec followed by cooling to 13°C).
  - All nonsporing pathogens are killed except Coxiella burnetii which may survive in holder method.
- **Water bath**: Used for disinfection of serum, body fluids, and vaccines (60°C for one hour)
- **Inspissation** (Fractional sterilization):
  - It is a process of heating an article on 3 successive days at 80–85°C for 30 min
  - Used for sterilization of egg based (LJ and Dorset's egg medium) and serum based media (Loeffler's serum slope).

Pasteurization: Used for beverages and dairy products such as milk:
- Holder method (63°C for 30 min)
- Flash method (72°C for 20 sec followed by cooling to 13°C).
- All nonsporing pathogens are killed except Coxiella burnetii.

Moist Heat at a Temperature of 100°C

- **Boiling:** Boiling of the items in water for 15 minutes may kill most of the vegetative forms but not the spores.
- **Steaming:** Koch's or Arnold's steam sterilizers are used to provide temperature of 100°C for 90 minutes. It is useful for those media which are decomposed at high temperature of autoclave. It kills most of the vegetative forms but not the spores.
- **Tyndallization or intermittent sterilization:** Involves steaming at 100°C for 20 min for 3 consecutive days. It is used for sterilization of gelatin and egg, serum or sugar containing media. It kills most of the vegetative forms including spores.

Moist Heat at a Temperature above 100°C (Autoclave)

- **Principle:** Autoclave functions similar to a pressure cooker. At normal pressure, water boils at 100°C but when pressure inside a closed vessel increases, the temperature at which water boils also increases.

Sterilization Conditions:
- *Hot air oven:* 160°C for 2 hours
- *Autoclave:* 121°C for 15 min at pressure of 15 psi.

- **Sterilization conditions:** 121°C for 15 min at pressure of 15 psi (most commonly used).
- **Uses of autoclave:** Autoclave is useful for surgical instruments and culture media and those materials which cannot withstand the higher temperature of hot air oven or media containing water that cannot be sterilized by dry heat.
- **Sterilization control:**
  - Biological indicator-Spores of *Geobacillus stearothermophilus* (best indicator)
  - Thermocouple and indicators like Browne's tube, Autoclave tapes:

Filtration

Filtration is an excellent way to remove the microbial population in solutions of heat-labile materials like vaccine, antibiotics, toxin, serum and sugar solution as well as for purification of air in laminar air flow systems.

There are two types of filters; depth and membrane filters.

Depth filters: They are porous filters that retain particles throughout the depth of the filter, rather than just on the surface. They are used for industrial applications such as filtration of food and beverage, and chemicals, but are not to filter bacteria. Examples include:

- Candle filters made up of diatomaceous earth (Berkefeld filters), unglazed porcelain (Chamberland filters)
- Asbestos filters (Seitz and Sterimat filters)
- Sintered glass filters

Air Filtration:
- HEPA filter removes 99.97% of particles of size ≥ 0.3 µm .
- *ULPA filters:* Removes from the air 99.999% of particles of size ≥ 0.12 µm.

Membrane filters: They are widely used filters for bacterial filtration. They are porous; retain all the particles on the surface that are smaller than their pore size.

- Made up of cellulose acetate, cellulose nitrate, polycarbonate, polyvinylidene fluoride
- Pore size: Membrane filters have an average pore diameter of **0.22 µm** (MC used)
- *Filtration of air:* Air filters are membrane filters used to deliver bacteria-free air. Examples:
  - Surgical masks (that let air in but keep microorganisms out).
  - In biological safety cabinets and laminar airflow systems; two filters are used
    - *HEPA filters* (High-efficiency particulate air filters): HEPA filter removes 99.97% of particles of size ≥ 0.3 µm.
    - *ULPA filters* (Ultra-low particulate/penetration air filters): Removes from the air 99.999% of particles of size ≥ 0.12 µm.
- ***Sterilization control*** includes *Brevundimonas diminuta* and *Serratia marcescens.*

Radiation

Ionising radiations:

- Examples include, X-rays, gamma rays (from Cobalt 60 source), and cosmic rays.
- *Mechanism:* It causes breakage of DNA without temperature rise (hence called as **cold sterilization).**
- It destroys spores and vegetative cells, but not effective against viruses. It is used for:
  - Disposable plastics, e.g. rubber or plastic syringes, infusion sets and catheters.
  - Catgut sutures, bone and tissue grafts and adhesive dressings, antibiotics and hormones.
- *Advantages* of Ionizing radiation:
  - High penetrating power,
  - Rapidity of action and
  - Temperature is not raised
- *Sterilization control:* Efficacy of ionising radiation is tested by using *Bacillus pumilus.*

Nonionizing radiation:

- Examples of nonionizing radiation include infrared and ultraviolet radiations.
- They are quite lethal but do not penetrate glass, dirt films, water.
- **Dose:** 250–300 nm wavelength for 30 min
- Used for sterilization of clean surfaces in operation theatres, laminar flow hoods as well as for water treatment.

CHEMICAL AGENTS OF STERILIZATION/DISINFECTION

Table 1.2.2: Classification of chemical disinfectants based on their efficacy

Level of disinfectant	Bacterial spores	Tubercle bacilli	Non enveloped viruses	Fungi	Enveloped viruses	Vegetative bacteria
Low level disinfectant	No	No	No	+/-	Yes	Yes
Intermediate level disinfectant	No	Yes	Yes	Yes	Yes	Yes
High level disinfectant	May be	Yes	Yes	Yes	Yes	Yes
Chemical sterilants	Yes	Yes	Yes	Yes	Yes	Yes

Alcohols

- They act on bacteria, fungi, some enveloped viru (e.g. HIV); but not spores.
- They act by denaturing proteins and possibly by dissolving membrane lipids.
- Ethyl alcohol is used as surgical spirit (70%) in hand rubs as antiseptics.
- Isopropyl alcohol: Used for clinical thermometers.

Aldehydes

They combine with nucleic acids and proteins and inactivate them, probably by crosslinking and alkylating the molecules. They are sporicidal and can be used as chemical sterilants.

1. **Formaldehyde:** It is best used for:
   - Preservation of anatomical specimen
   - Formaldehyde gas is used for fumigation of closed areas such as operation theaters
   - Preparation of toxoid from toxin. It is toxic, irritant and corrosive to metals.
2. **Glutaraldehyde** is less toxic, less irritant and less corrosive, hence is best used to sterilize endoscopes and cystoscopes:
   - It is used as 2% concentration (2% cidex) for 20 min.
   - It has to be activated by alkalinization before use. Once activated, it remains active only for 14 days.
3. **Ortho-Phthalaldehyde** (0.55%): It can also be used for sterilization of endoscopes and cystoscopes and has many advantages over glutaraldehyde:
   - It does not require activation
   - Low vapor property
   - Better odor
   - More stable during storage
   - ↑ mycobactericidal activity.

Glutaraldehyde:
- Used as 2% concentration (2% cidex) for 20 min
- It has to be activated by alkalinisation before use Once activated, it remains active only for 14 days.

Phenolic Compounds

Phenolics as disinfectants: Cresol, xylenol, Lysol and ortho-phenylphenol are used as disinfectants in laboratories and hospitals.

- All have the ability to retain activity in presence of organic matter.
- They are toxic and irritant to skin, hence used as disinfectants but not as antiseptics.

Phenolics as antiseptics: Certain phenolics are less irritant to skin, persist in skin for longer period and are widely used as antiseptics. In general they are more active against gram-positive than gram-negative bacteria.

- Chlorhexidine: It is an active ingredient of savlon (chlorhexidine and cetrimide)
- Chloroxylenol: It is an active ingredient of dettol.
- Hexachlorophane: As it can cause brain damage, hence its use as antiseptic is restricted only to a staphylococcal outbreak.

Halogens

Iodine: It is used as a skin antiseptic and kills microorganisms by oxidizing cell constituents and iodinating cell proteins, e.g. *Tincture of iodine* (2%) and *Iodophor* (iodine complexed with an organic carrier) e.g. Betadine.

Chlorine: It is the most commonly used disinfectant:

- For municipal water supplies and swimming pools and is also employed in the dairy and food industries
- As laboratory disinfectant
- As bleaching agent: to remove the stain from clothes. (Common uses of chlorine are given in table below).

- **Preparations:** It may be available as: (i) chlorine gas, (ii) sodium hypochlorite (household bleach, 5.25%), or (iii) calcium hypochlorite (bleaching powder)
- **Mechanisms:** All preparations yield hypochlorous acid (HClO) which causes oxidative destruction of vegetative bacteria and fungi, but not spores.
- **Disadvantages:**
  - Organic matter interferes with its action, hence excess chlorine always is added to water to ensure microbial destruction
  - Carcinogenic
  - Need daily preparation
  - They are not active against *Giardia* and *Cryptosporidium*,
  - Sodium hypochlorite is corrosive and should be handled cautiously.

Oxidising Agents

Hydrogen Peroxide (H_2O_2)

- Mode of action: It is a chemical sterilant, acts by liberating toxic free hydroxyl radicals which attack membrane, lipid, DNA, and other cellular components.
- Concentration of H_2O_2 3–6% is ideal, except for catalase producing organisms and spores which require 10% of H_2O_2.
- Used to disinfect ventilator, soft contact lenses, and tonometer biprisms. Vaporized H_2O_2 is used for plasma sterilization.
- Advantage: 1. It acts perfect even in presence of organic matter 2. Low toxicity 3. Environmentally safe.

Peracetic Acid

It is a chemical sterilant; often used in conjunction with H_2O_2, to disinfect hemodialyzers and in plasma sterilization. It is also used for sterilizing endoscopes. However, it may corrode steel, iron, copper, brass and bronze.

Plasma sterilization:
- Active agent: H_2O_2 and/or: peracetic acid
- Best for heat labile surgical instruments as low temp maintained (< 50°C)
- Sterilization control: *Geobacillus stearothermophilus.*

Plasma Sterilization

This is recently introduced sterilization device (e.g. Sterrad and Plazlyte) used for creating plasma state, so as to maintain a uniform vacuum inside the chamber.

- Chemical sterilants such as H_2O_2 alone or a mixture of H_2O_2 and peracetic acid
- Active agent is Ultraviolet (UV) photons and radicals (e.g., O and OH): Kill microorganisms and spores.
- Low temperature is maintained (< 50°C), So best for heat labile surgical instruments.
- Sterilization control: *Geobacillus stearothermophilus, Bacillus subtilis* subsp. *niger.*

Heavy Metal Salts

Heavy metallic salts are of limited use in certain areas:

- Silver sulfadiazine is used on burns surfaces.
- Silver nitrate (1%) solution used for eyes of infants to prevent ophthalmia neonatorum.
- Copper sulfate is an effective fungicide (algicide) in lakes and swimming pools.

- Mercury salts such as mercuric chloride, thiomersal and mercurochrome were known antiseptics in past. Thiomersal (merthiolate) is used as preservative in vaccines and sera.
- Mechanism of action: Heavy metals combine with bacterial cell proteins, often with their sulphydryl groups, and inactivate them. They may also precipitate cell proteins. Many heavy metals are more bacteriostatic than bactericidal.

Surface Active Agents

They lower the surface tension between two liquids or between a liquid and a solid. Surfactants may act as detergents, wetting agents, and emulsifiers because they have both polar hydrophilic and nonpolar hydrophobic ends.

1. **Cationic surfactants (Quaternary ammonium compounds):**
   - They disrupt microbial membranes and may also denature proteins.
   - They kill most bacteria (gram-positives are better killed than gram-negatives) but not *M. tuberculosis* or spores.
   - Nontoxic but are inactivated by acidic pH, organic matter, hard water and soap.
   - Cationic detergents are often used as disinfectants for food utensils and small instruments and as skin antiseptics.
   - Examples include:
     - Acetyl trimethyl ammonium bromide (cetavlon or cetrimide)
     - Alkyltrimethylammonium salts
     - Benzalkonium chloride and Cetylpyridinium chloride
2. **Anionic surfactants**, e.g. soaps, have strong detergent but weak antimicrobial properties. They are active at acidic pH.
3. **The amphoteric surfactants:** They have both detergent and antimicrobial activity.
   - They are active over a wide range of pH, but is reduced in presence of organic matter.
   - E.g. '*Tego compounds*': Used as antiseptics in dental practice, but cause allergic reactions.

Cationic surfactants (Quaternary ammonium compounds):
- Acetyl trimethyl ammonium bromide (cetavlon or cetrimide)
- Alkyltrimethylammonium salts
- Benzalkonium chloride and Cetylpyridinium chloride
- Anionic surfactants, e.g. soaps
- The amphoteric surfactants, e.g.Tego compounds.

Dyes

Aniline and acridine dyes have been used extensively as skin and wound antiseptics.

1. **Aniline dyes:** E.g. crystal violet, gentian violet, brilliant green and malachite green:
   - They are more active against gram-positive bacteria than gram-negative and have no activity against *M. tuberculosis*.
   - They are non-toxic and non-irritant to the tissues.
   - Their activity is reduced in presence of organic material such as pus.
   - They interfere with the synthesis of peptidoglycan component of the cell wall.
   - These dyes are used in the laboratory as selective agents in culture media (e.g. malachite green in LJ medium)
2. **Acridine dyes:** These include acriflavine, euflavine, proflavine and aminacrine:
   - They are affected very little by the presence of organic material.
   - More active against gram-positive bacteria but are not as selective as the aniline dyes.
   - They interfere with the synthesis of nucleic acids and proteins in bacterial cells.

Gaseous Sterilization

Ethylene Oxide (ETO)

Ethylene oxide sterilizer is one of the widely used gaseous chemical sterilants in present days.

- *It has high penetration power*, has both microbicidal and sporicidal activity; acts by combining with cell proteins.
- However, it is highly inflammable, irritant, explosive and carcinogenic. Hence it is usually supplied in a 10 to 20% concentration mixed with inert gases.

Prions are the most resistant structure. Recommended methods are:
- Autoclaving at 134°C for 1–1.5 hour,
- Treatment with 1 N NaOH for 1 hour
- 0.5% sodium hypochlorite for 2 hours.

- *Sterilization condition*: 5 to 8 hours at 38°C or 3 to 4 hours at 54°C.
- Sterilization control: *Bacillus globigii*.
- Use: For sterilization of many heat sensitive items such as disposable plastic petri dishes and syringes, heart-lung machine, sutures, catheters, respirators and dental equipments.

The decreasing order of resistance of microorganisms to disinfectant or sterilizing agents is as follows:

Prions (highest resistance) > Cryptosporidium oocysts > Coccidian cyst > Bacterial spores > Mycobacteria > Other parasite cysts (Giardia) > Small non-enveloped viruses > Protozoan Trophozoites > Gram-negative bacteria > Fungi > Large non-enveloped viruses > Gram-positive bacteria > Enveloped viruses.

Sporicidal agents include:
- EFGH: Ethylene oxide, Formaldehyde, Glutaraldehyde, Hydrogen peroxide.
- 3P: Peracetic acid, O-Phthalic acid and Plasma sterilization.
- Autoclave and Hot air oven.

Testing of Disinfectants:
- Phenol coefficient (Rideal Walker) test
- Chick Martin test
- Capacity (Kelsey-Sykes) test
- In-use (Kelsey and Maurer) test.

TESTING OF DISINFECTANTS

1. **Phenol coefficient (Rideal Walker) test**:
   - Determined by the dilution of the disinfectant in question which sterilizes the suspension of *Salmonella* Typhi in a given time divided by the dilution of phenol which sterilizes the suspension in the same time.
   - Phenol coefficient of > 1 is taken as satisfactory.
   - Drawbacks:
     - Only the phenolic compounds can be assessed
     - It does not assess disinfectant ability to act in presence of organic matter.
2. **Chick Martin test:** Modified rideal and walker test in which the disinfectants act in the presence of organic matter (e.g. dried yeast, feces, etc.) to simulate the natural conditions.
3. **Capacity (Kelsey-Sykes) test**: It tests the capacity of a disinfectant to retain its activity when repeatedly used microbiologically.
4. **In-use (Kelsey and Maurer) test**: It determines whether the chosen disinfectant is effective in actual use in hospital practice.

Table 1.2.3: Biological sterilization indicator

Hot air oven	*Clostridium tetani nontoxigenic strain, B. subtilis* subsp.*niger*
Autoclave	*Geobacillus stearothermophilus*
Filtration	*Brevundimonas diminuta, Serratia*
Ionizing radiation	*Bacillus pumilus*
Ethylene oxide	*Bacillus globigi*
Plasma sterilization	*Geobacillus stearothermophilus, Bacillus subtilis* subsp.*niger*

Table 1.2.4: Methods of sterilization/disinfection used in different clinical situations

Material	Method of sterilization/disinfection
Clinical thermometer	Isopropyl alcohol
Paraffin, glass syringe, flask, slide, oil, grease, fat, glycerol	Hot air oven
OT, entryway, ward, laboratory fumigation, Preservation of anatomical specimen, woolen blanket	Formaldehyde gas > UV > BPL
Cystoscope, bronchoscope	Orthophthaldehyde > glutaraldehyde 2% (cidex)
Heart lung machine, respirator, dental equipments	Ethylene oxide

Contd...

Contd...

Material	Method of sterilization/disinfection
Vaccine, sera, antibiotic, sugar solution, antibiotic and body fluids	Filtration
Sharp instruments	Cresol
Milk	Pasteurization
Plastic syringe, catgut suture, swab, catheter, bone and tissue grafts, adhesive dressing	Ionizing radiation
Culture media, metal instruments, glassware and all suture materials except catgut	Autoclave
Metallic inoculation wire	Red hot by Bunsen burner
Infective material like soiled dressing, bedding, animal carcasses	Burning (incineration)
Metallic surgical instruments	Autoclave, infra-red radiation
Water	Chlorine as hypochlorite 0.2%
Skin	Tincture iodine, spirit (70% ethanol), savlon
Contact lenses	H_2O_2

Table 1.2.5: Common disinfectants and their spectrum of action

Germicide and their concentrations	Level of disinfectant	Bacteria and enveloped viruses	Fungi	Unenveloped viruses	M. tubercu-losis	Spore	Inactivated by organic matter
Glutaraldehyde (2%)	High/CS	+	+	+	+	+	–
Formaldehyde(3–8%)	High/CS	+	+	+	+	+	–
H_2O_2 (3–25%)	High/CS	+	+	+	+	+	+/–
Chlorine (100–1000 ppm)	High	+	+	+	+	+/–	+
Isopropyl alcohol (60–95%)	Intermediate	+	+	+/–	+	–	+/–
Phenol (0.4–5%)	Intermediate	+	+	+/–	+	–	–
Iodophor (30–50 ppm of free iodine)	Intermediate	+	+	+	+/–	–	+
Quaternary–ammonium (0.4–1.6%)	Low	+	+/–	–	–	–	+

Table 1.2.6: Spaulding's classification of medical devices according to the degree of risk for infection involved

Medical device	Definition	Examples	Recommended method
Critical device	Enter a normally sterile site	Surgical instruments, cardiac and urinary catheters, implants, eye and dental instruments	Heat based sterilization, Chemical sterilant or high level disinfectant
Semicritical device	Come in contact with mucous membranes or minor skin breaches	Respiratory therapy equipments, anesthesia equipments, endoscopes, laryngoscope, rectal/vaginal/ esophageal probes	High level disinfectant
Noncritical devices	Comes in contact with intact skin	BP cuff, ECG electrodes, bedpans, crutches, stethoscope, thermometer	Intermediate level or low level disinfectant
Noncritical surfaces	Less direct contact with patient	Surfaces of medical equipments, examination table, computers	Low level disinfectant

MULTIPLE CHOICE QUESTIONS

STERILIZATION

1. Sterilization accuracy is assessed by using: *(PGI Nov 2016)*
a. Clostridium perfringenes
b. Geobacillus stereothermophilus
c. Staphylococcus aureus
d. Clostridium botulinum
e. Bacillus subtilis subsp. Niger

2. Best method of sera sterilization is: *(APPG 2014)*
a. Filtration b. Autoclaving
c. Radiation d. Heating

3. Which of the following is used to test the efficiency of sterilization in an autoclave? *(AIIMS Nov 2011, AIIMS Nov 2010, MHPG 2014)*
a. Clostridium tetani
b. Bacillus stearothermophilus
c. Bacillus pumilus
d. Bacillus cereus

4. Spore of which bacteria is used as sterilization control of Plasma sterilization: *(AIIMS Nov 2010)*
a. B. subtilis b. B. pumilis
c. Cl. tetani d. B. stearothermophilus

5. Condition required for autoclave is? *(DNB June 2012)*
a. 121°C temperature for 20 min
b. 121°C temperature for 15 min
c. 100°C temperature for 60 min
d. 100°C temperature for 90 min

6. Which of the following is most resistant to sterilization? *(AI 2012, 2008)*
a. Cysts
b. Prions
c. Spores
d. Viruses

7. Choose the correct ones for the decreasing order of resistance to sterilization: *(PGI Dec 2007)*
a. Prions, Bacterial spores, Bacteria
b. Bacterial spores, Bacteria, Prions
c. Bacteria, Prions, Bacterial spores
d. Bacterial spores, Prions, Bacteria

DISINFECTION

8. Low Level disinfectant is: *(TNPG 2015)*
a. Benzalkonium chloride
b. Isopropyl alcohol
c. Glutaraldehyde
d. Hydrogen peroxide

9. Heart lung machine is best sterilized by: *(JIPMER, May2015)*
a. Cidex
b. Ethylene oxide
c. Isoprophyl alcohol
d. Formaldehyde

10. An upper GI endoscope was performed in a TB suspect. To reuse the instrument it should be sterilized by: *(Recent Question 2015)*
a. 2% Glutaraldehyde
b. Hot air oven
c. Autoclave

11. Best disinfectant for endoscopes is:
a. Hypochlorite *(JIPMER Nov 2014)*
b. Formaldehyde
c. Glutaraldehyde
d. Chlorhexidine

12. Prions are best killed by: *(NEET Pattern Based)*
a. Autoclaving at 121°C
b. 5% formalin
c. Sodium hydroxide for 1 hr
d. Sodium hypochloride for 10 min

13. Sterilization of fiberoptic is done by: *(DNB DEC 2012, AIIMS Nov 2003)*
a. Glutaraldehyde b. Chlorine
c. Autoclave d. Phenol

14. According to Spaulding classification, laparoscope and arthroscopic instruments are under:
a. Critical item *(Recent Question 2015)*
b. Semi critical item
c. Noncritical item

15. Which is false about spaulding classification? *(AIIMS Nov 2010)*
a. Noncritical items also included in classification
b. Semicritical items — contact with mucus membrane
c. Semicritical items — needs low disinfectant
d. Cardiac catheter, e.g. of critical items

16. Phenol coefficient indicates: *(DNB DEC 2012, JIPMER 2009)*
a. Efficacy of a disinfectant
b. Dilution of a disinfectant
c. Quantity of a disinfectant
d. Purity of a disinfectant

17. Sputum can be disinfected by all except: *(AIIMS May 2012, PGI Dec 2008)*
a. Autoclaving
b. Boiling
c. Cresol
d. Chlorhexidine

18. Surgical blade is sterilized by? *(DNB June 2009)*
a. Autoclave
b. Gamma radiation
c. Hot air oven
d. Steaming

19. Sporicidal agents are: *(PG June 2009, June 2006, JIPMER 2010)*
a. Glutaraldehyde
b. Ethylene oxide
c. Formaldehyde
d. Benzalkonium chloride
e. Chlorine

20. Savlon contains: *(AIIMS May 2010)*
a. Cetrimide + Chlorhexidine
b. Cetrimide + Butyl alcohol
c. Cetrimide + Chlorhexidine + Butyl alcohol
d. Cetrimide + Cetavlon

21. Boiling of milk is an example for: *(TNPG 2015)*
a. Concurrent disinfection
b. Precurrent disinfection
c. Terminal disinfection
d. Sterilization

22. Which of the following are used for sterilization of surgical instrument: *(PGI May 2013)*
a. Ethylene oxide
b. Gamma radiation
c. Autoclaving
d. Glutaraldehyde
e. Hot air oven

23. All of the following are the tests to check the efficiency of disinfectant Except: *(TNPG 2014, Kerala 2016)*
a. Chick Martin test
b. Riedel Walker test
c. Hugh Leifson test
d. Kelsey Sykes test

EXPLANATIONS

STERILIZATION

1. **Ans. (b,e) (Geobacillus.., B.subtilis subsp. Niger)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p40
   - *Geobacillus stereothermophilus* and *Bacillus subtilis subsp*. Niger are used as biological indicators in sterilization.
2. **Ans. (a) (Filtration)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p35, Ananthanarayan 9/e p33
   Filtration is the best method of sterilization of heat labile liquids such as sera and solutions of sugars or antibiotics, toxins and vaccines. It helps to remove bacteria.
3. **Ans. (b) (Bacillus stearothermophilus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p34, Ananthanarayan 9/e p32, 8/e p34
   Biological Sterilization Indicator: (Detail list — Refer text)
   - **Autoclave:** *B. stearothermophillus*
   - **Hot air oven:** *Clostridium tetani nontoxigenic strain*
4. **Ans. (d) (B. stearothermophilus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p34, Ananthanarayan 8/e p32, Mackie McCartney's practical microbiology 14/e p817
   Biological Sterilization Indicator of plasma sterilization: B. stearothermophillus, B. subtilis subspecies niger **(Detail list — Refer text)**
5. **Ans. (b) (121°C temperature....)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p34, Ananthanarayan 9/e p31-32, 8/e p32, Mackie McCartney's practical microbiology 14/e p817
   - Autoclave: Recommended condition- 121–124°C temperature for 15 min at 1.1 bar pressure
   - Alternate: 134–138°C temperature for 3 min at 2.2 bar pressure
   - Hot air oven: 160°C temperature for 120 min or 180°C temperature for 30 min
6. **Ans. (b) (Prions)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p31, Patrick Murray 9/e p69
   - *Prions are the highest resistance structure*
   - *Refer text to know detail*
7. **Ans. (a) (Prions, Bacterial spores, Bacteria)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p31, Patrick Murray 9/e p69
   Refer text to know detail

DISINFECTION

8. **Ans. (a) (Benzalkonium chloride)** Ref:Apurba Sastry's Essentials of Medical Microbiology 1/e p41
   - Isopropyl alcohol, Phenol: Intermediate level disinfectant
   - Quaternary ammonium compounds like Benzalkonium chloride: Low level disinfectant
   - Glutaraldehyde, formaldehyde, Hydrogen peroxide, Chlorine: High level disinfectant.
9. **Ans. (b) (Ethylene oxide)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p41
   - Heart lung machines, respirators and dental equipments are best sterilized by Ethylene oxide.
10. **Ans (a) (2% Glutaraldehyde)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p41
11. **Ans (c) (Glutaraldehyde)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p41
    2% Glutaraldehyde and Ophthaldehyde are recommended disinfectants for endoscopes.
12. **Ans. (c) (Sodium hydroxide for 1 hour)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p524, Ananthanarayan 9/e p34, 8/e p36, 37, Mackie McCartney's practical microbiology 14/e p827
    - **Prions are sterilized by:** 0.5% Hypochlorite for 2 hr or 1N NaOH for 1 hour or Autoclave for 134°C for 1–1.5 hour
13. **Ans. (a) (Glutaraldehyde)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p37, Ananthanarayan 9/e p34
    - A fiberoptic is a flexible, transparent fiber made of high quality extruded glass (silica) or plastic used for laryngoscopes.
    - Fiberoptic laryngoscopes are best sterilized by ***Glutaraldehyde*** or ortho-ophthaldehyde.

14. **Ans. (a) (Critical item)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p42
15. **Ans. (c) (Semicritical items — needs low disinfectant)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p42
    - Semicritical items are those that come in contact with *mucous membranes or minor skin* breaches and they needs high to intermediate level disinfectant for sterilization.
    - For the detail of Spaulding`s criteria of devices –Refer text
16. **Ans. (a) (Efficacy of a disinfectant)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p40
    - **Testing efficacy of disinfectants by *Phenol coefficient (Rideal Walker) test* -** Phenol coefficient is determined by the dilution of the disinfectant in question which sterilizes the suspension of *S. Typhi* in a given time *divided* by the dilution of phenol which sterilizes the suspension in the same time.
    - For detail of Testing efficacy of disinfectants — *Refer text*
17. **Ans. (d) (Chlorhexidine),** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p30
    - The commonest pathogen suspected to be present in sputum — Tubercle bacilli. So it has to be disinfected by an appropriate technique.
    - Chorhexidine is a skin antiseptic, used for burns or hand disinfection. It is not mycobactericidal.
    - Sputum can be disinfected by:
        - Autoclaving: Ideal method
        - Other methods include: ***Burning*** of the material, ***Cresol 5% or Phenol or Boiling***
18. **Ans. (a) (Autoclave)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p41
    - Steam is the preferred method for sterilizing critical medical and surgical instruments that are not damaged by heat, steam, pressure, or moisture..... CDC Guideline, 2008
    - Metal instruments and surgical blades are better sterilized by autoclave.
19. **Ans. (a), (b), (c) (Glutaraldehyde, Ethylene oxide, Formaldehyde)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p37, Ananthanarayan 9/e p34
    - List of sporicidal agent. Refer chapter review
    - Benzalkonium chloride is a surface active agent, widely used as wetting agents, detergents and emulsifiers.
20. **Ans. (a) (Cetrimide + Chlorhexidine)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p39, Park 22/e p119
    - *Savlon contains — Cetrimide 3% (Cetavlon) + Chlorhexidine 0.3% (Hibitane)*
21. **Ans. (b) Precurrent disinfection.** Ref: Park 23/e p127, 22/e p119

    Already explained.
22. **Ans. (c) (d) (e) (Autoclaving, Glutaraldehyde, Hot air oven)** Ref: Guideline for Disinfection and Sterilization in Healthcare Facilities, CDC, 2008
    - For the sterilization of surgical instruments: Most medical and surgical devices are made of materials that are heat stable and therefore can be sterilized best by autoclave (moist heat)
    - Heat labile instruments (e.g. plastics) can be sterilized by Ethylene oxide gas or other new, low-temperature sterilization systems (e.g. hydrogen peroxide gas plasma, peracetic acid immersion, ozone)
    - Dry-heat sterilizers such as hot air oven should be used only for materials that might be damaged by moist heat or that are impenetrable to moist heat (e.g., powders, petroleum products, sharp instruments)
    - Ionizing radiation: There are no FDA-approved ionizing radiation sterilization processes for use in healthcare facilities.
23. **Ans. (c) (Hugh Leifson test)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p39, Ananthanarayan 9/e p36
    - Hugh Leifson test is used to differentiate micrococci from staphylococci

Culture Media and Methods, Identification of Bacteria by Conventional, Automated and Molecular Methods

CHAPTER 1.3

CULTURE MEDIA

The basic constituents of culture media are:

- **Peptone:** Mixture of partially digested proteins
- **Agar**: It is used for solidifying the culture media. **It has no nutritional property.**
  - It is prepared from seaweeds (red algae of species *Gelidium* and *Gracilaria*).
  - *Agar is preferred over gelatine*, as it is bacteriologically inert, and it melts at 98°C and usually solidifies at 42°C
  - *Concentration of agar*:
    - For solid agar: 1–2% (Japanese agar 2% or New Zealand agar 1.2%)
    - For semisolid agar: 0.5%
    - For solid agar to inhibit *Proteus* swarming: 6%
- **Others:** Meat extract, Yeast extract, Blood and serum, Water and Electrolytes (NaCl).

Concentration of agar:
- For solid agar preparation 1–2% (Japanese agar 2% or New Zealand agar 1.2%)
- For semisolid agar 0.5%
- For solid agar to inhibit Proteus swarming 6%

Simple/Basal Media

They contain minimum ingredients that support the growth of non-fastidious bacteria. Examples include:

1. **Peptone water:** It contains peptone (1%) + NaCl (0.5%) + water
2. **Nutrient broth:** It is made-up of peptone water + meat extract (1%)
3. **Nutrient agar:** It is made-up of nutrient broth + 2% agar

The basal media are used for:

- Testing the non-fastidiousness of bacteria
- They serve as the base for the preparation of many other media
- Nutrient broth is used for studying the bacterial growth curve
- Nutrient agar is the preferred medium for:
  - Performing the biochemical tests such as oxidase, catalase and slide agglutination
  - To study the colony character and Pigment demonstration.

Simple/Basal Media:
- Peptone water: It contains peptone (1%) + NaCl (0.5%) + water
- Nutrient broth: It is made up of peptone water + meat extract (1%).
- Nutrient agar: It is made up of nutrient broth + 2% agar

Enriched Media

When a basal medium is added with additional nutrients such as blood, serum or egg, it is called enriched medium. They also support the growth of fastidious bacteria, e.g.:

1. **Blood agar:** Prepared by adding 5–10% of sheep blood to the molten nutrient agar at 45°C. It is used to test the hemolytic property of the bacteria
2. **Chocolate agar:** It is the heated blood agar, blood is added to the molten nutrient agar at **70°C**. It is more nutritious than blood agar, and even supports *Haemophilus influenzae*.
3. **Loeffler's serum slope** is used for isolation of *Corynebacterium diphtheriae.*
4. **Blood culture media:** Used for blood culture. They are of two types:
   - *Monophasic medium* is made-up of brain heart infusion (BHI) broth
   - *Biphasic medium* has a liquid phase (BHI broth) and a solid agar slope (BHI agar).

Differential Media:
- MacConkey aAgar
- CLED agar

Enrichment Broth

Liquid media that allow certain organism (pathogens) to grow and inhibit others (normal flora):

- Selenite F and Tetrathionate broth used for *Salmonella* and *Shigella*
- Alkaline peptone water (APW) used for *Vibrio cholerae*.

Selective Media

Solid media that allow certain organism (pathogens) to grow and inhibit others (normal flora):

1. Lowenstein Jensen (LJ) medium is used for isolation of *Mycobacterium tuberculosis*

2. Thiosulphate Citrate Bilesalt Sucrose (TCBS) agar used for isolation of *Vibrio* species
3. For the isolation of enteric *pathogens* such as *Salmonella* and *Shigella* from stool:
   - DCA (Deoxycholate Citrate Agar)
   - XLD (Xylose Lysine Deoxycholate) agar
4. Potassium tellurite agar (PTA) is used for isolation of *Corynebacterium diphtheriae*.
5. Wilson Blair bismuth sulphite medium: It is used for isolation of *Salmonella* Typhi.

Transport Media

They are used for the transport of specimens containing delicate organism or when the delay is expected. Bacteria do not multiply in the transport media, they only remain viable.

Table 1.3.1: Transport media used for common bacteria

Organism	Transport media
Streptococcus	Pike's medium
Neisseria	Amies medium, Stuart's medium
Vibrio cholerae	VR (Venkatraman-Ramakrishnan) medium, Cary Blair medium and Autoclaved sea water
Shigella, Salmonella	Buffered glycerol saline, and Cary Blair medium

Differential Media

These media differentiate between two groups of bacteria by using an indicator.

1. **MacConkey agar:** It differentiates organisms into LF or lactose fermenters (produce pink colonies, e.g. *E. coli and Klebsiella*) and NLF (produce colorless colonies, e.g. *Shigella* and *Salmonella*).
2. **CLED agar** (Cysteine lactose electrolyte-deficient agar): This is similar to MacConkey agar, differentiates between LF and NLF. It is used as an alternative to combination of blood agar and MacConkey agar, for the processing of urine specimens:
   - Advantages over MacConkey agar: It is less inhibitory than MacConkey agar, supports gram-positive bacteria (except β hemolytic *Streptococcus*) and *Candida*.
   - Advantage over blood agar: It can prevent the swarming of *Proteus*.

Anaerobic Culture Media

Anaerobic media contain reducing substances which take-up oxygen and create lower redox potential and thus permit the growth of obligate anaerobes such as *Clostridium*. Examples are:

1. **Robertson's cooked meat (RCM) broth:** It contains chopped meat particles (beef heart), which provide glutathione and unsaturated fatty acids.
2. **Other anaerobic media include:**
   - BHIS agar: Brain heart infusion agar with supplements (vitamin K and hemin)
   - Thioglycollate broth, Anaerobic blood agar
   - Neomycin blood agar, Egg yolk agar and Phenylethyl agar.

CULTURE METHODS

Various Aerobic Culture Methods

1. **Streak culture** by using loop with intermittent heating: It is the routinely used method.
2. **Lawn or carpet culture**: Useful for Carrying out antimicrobial susceptibility testing by disk diffusion method, Bacteriophage typing and producing large amount of bacterial growth for preparation of bacterial antigens and vaccines.
3. **Stroke culture** (zigzag fashion by straight wire): Used for citrate, urease and TSI (triple sugar iron test)

Aerobic culture methods:
- Streak culture
- Lawn or carpet culture
- Stroke culture
- Stab culture
- Liquid culture
- Pour-plate culture

4. **Stab culture:** Stabbing the semisolid agar butt by a straight wire. It is used for: (i) maintaining stock cultures, (ii) OF test (iii) Mannitol motility medium, (iv) Nutrient agar semisolid butts, (v) TSI (here both stroke and stab cultures are made).
5. **Liquid culture:**
   - Uses: Liquid cultures are useful for: (i) blood culture, (ii) for sterility testing.
   - Advantages: Used for: (i) When bacteria load is less, (ii) specimens (e.g. blood) containing antibiotics it is neutralized by dilution in the medium, (iii) when large yields of bacteria are required, (iv) for demonstration of bacterial growth curve
   - Disadvantages: Liquid cultures do not provide a pure culture from a mixed inoculum.
6. **Pour-plate culture:** Quantitative culture method, used to estimate viable bacterial count.

Incubatory Conditions

- Most of the pathogenic organisms grow best at 37°C.
- **Candle jar:** It provides capnophilic atmosphere (3–5% CO_2). This is useful for *Brucella abortus*, *Streptococcus*, pneumococcus and gonococcus.
- **Microaerophilic** bacteria such as *Campylobacter* and *Helicobacter* require 5% oxygen.

Anaerobic Culture Methods

Anaerobic Culture Methods:
- Production of vacuum
- By displacement and combustion of oxygen:
  - McIntosh and Filde's anaerobic jar
  - Anoxomat System
- Gas pak System
- Anaerobic Glove Box
- By reducing agents
- PRAS media

1. **Production of vacuum**
2. **By displacement and combustion of oxygen:** This principle is used in:
   - McIntosh and Filde's anaerobic jar (It involves evacuation of air and replacement with hydrogen gas manually)
   - Anoxomat System (principle is same, but done by automated instrument)
3. **Gas pak System (**Absorption of oxygen chemically, e.g. using alkaline pyrogallol). Indicators of anaerobiosis are:
   - Chemical indicator: Reduced methylene blue
   - Biological indicator: Pseudomonas
4. **Anaerobic Glove Box** (or anaerobic chamber)
5. **By reducing agents:** Such as glucose, thioglycollate, meat, cysteine and ascorbic acid.
6. **PRAS** media (Prereduced, Anaerobically Sterilized).

Preservation of Microorganisms

- **Short-term methods:** (i) Sub-culturing, (ii) Immersing the culture in glycerol, or sterile distilled water, (iii) Freezing at –20°C and (iv) Drying (for moulds and spores).
- **Long-term methods:** By Ultra temperature freezing and Lyophilization (freeze-drying).

IDENTIFICATION OF BACTERIA

Identification of bacteria can be done by: (i) conventional (culture and identification by biochemical reactions), (ii) automated culture techniques (iii) molecular methods.

Automated Culture Techniques

Conventional culture methods often yield poor results because of low bacterial load. Therefore, various automated blood culture techniques have been in use since last decade.

Advantages: The major advantages of automated blood culture techniques are:
- *Continuous automated monitoring* (once in every 15–20 min by the instrument).
- *Other advantages*: More sensitive,↑ yield, rapid, less labor intensive

Disadvantages: (i) high cost (ii) inability to observe the colony morphology as liquid medium is used, (iii) no separate detection in mixed cultures, (iv) radioactive hazards for BACTEC.

MOLECULAR METHODS

Nucleic acid amplification techniques (NAATs) include:
- Polymerase chain reaction (PCR) and its modification including Real time PCR
- Ligase chain reaction (LCR) and Transcription-mediated amplification (TMA)

Table 1.3.2: Automated culture systems in diagnostic bacteriology

For bacterial culture	Principle used
1. BACTEC	In was radiometry based, but later changed to fluorescent based detection technique
2. BacT/Alert	CO_2 liberated from bacteria, causes a pH change, detected by colorimetry
3. ESP culture system	CO_2 liberated from bacteria, causes a pressure change, detected by manometry
For bacterial identification	• Phoenix bacterial identification system • MALDI –TOF (Matrix-assisted laser desorption/ionization time-of-flight), e.g. VITEK MS • VITEK 2 bacterial identification and antimicrobial sensitivity system • Microscan Walkaway system
For *M. tuberculosis*	MGIT (Mycobacterial Growth Indicator Tube)

- Nucleic acid sequence based amplification (NASBA)
- Strand displacement amplification (SDA).

Polymerase Chain Reaction (PCR)

PCR is a technology in molecular biology used to amplify a single or few copies of a piece of DNA to generate millions of copies of DNA. It was developed by Kary B Mullis.

Principle: PCR involves three basic steps.

1. ***DNA extraction*** from the organism
2. ***Amplification of extracted DNA:*** The extracted DNA is subjected to repeated cycles (30–35 numbers) of amplification in a thermocycler which takes about 3–4 hours. Each amplification cycle has three steps:
   - *Denaturation at 95°C*: This involves separation the dsDNA into two separate ssDNA.
   - *Primer annealing (55°C):* Primer is a short oligonucleotide complementary to a small sequence of the target DNA. It anneals to the complementary site on ssDNA.
   - *Extension of the primer (72°C):* This step is catalyzed by Taq Polymerase enzyme.
3. ***Gel electrophoresis*** of amplified product: The amplified DNA is electrophoretically migrated according to their molecular size to form bands; seen under UV rays.

PCR involves three basic steps:
- DNA extraction from the organism
- Amplification of extracted DNA
- Gel electrophoresis of amplified product

Each amplification cycle has three steps:
- Denaturation at 95°C
- Primer annealing (55°C)
- Extension of the primer (72°C)

Advantages: PCR has the following advantages compared to the conventional culture:

- *More sensitive*: It can amplify very few copies of a specific DNA, so it is more sensitive.
- *More specific*: By use of primers targeting specific DNA sequence of the organism
- Detects the organism: (i) either from sample, (ii) to confirm culture isolate.
- Detect the organisms that are highly fastidiousor noncultivable by conventional culture methods.
- Detect genes coding drug resistance (e.g. *MecA* gene detection in *Staphylococcus aureus*)
- Detects genetic diseases such as sickle cell anemia, phenylketonuria, etc.

Disadvantages:

- Conventional PCR detects only the DNA, but not the RNA (detected by RT-PCR).
- *Qualitative, not quantitative* (Quantitation is done by real time PCR).
- *Viability*: PCR cannot differentiate between viable or nonviable organisms.
- *False positive* amplification may occur due to contamination with environmental DNA.
- *False negative*: by PCR inhibitors present in some specimens such as blood, feces, etc.

Modification of PCR

1. **Reverse transcriptase PCR** (RT-PCR): For amplifying RNA, RT-PCR is done.
   - After RNA extraction, the first step is addition of reverse transcriptase enzyme that coverts RNA into DNA. Then, the amplification of DNA and gel documentation steps are similar to that described for conventional PCR.
   - Useful for detection of RNA viruses or 16SrRNA genes of the organisms.
2. **Nested PCR**: The amplified products of the first round PCR is subjected to another round of amplification using a second primer targeting a different gene of same organism.

Modification of PCR:
- Reverse transcriptase PCR
- Nested PCR
- Multiplex PCR

Bacteriophage typing is done for:
- *Staphylococcus aureus*
- *SalmonellaTyphi*
- *Vibrio cholerae*
- *Brucella*
- *Corynebacterium diphtheriae*

- *More sensitive*: Double round of amplification yields high quantity of DNA.
- *More specific*: Use of two primers targeting same organisms makes more specific.
- *Disadvantage*: There is more chance of contamination of the PCR tubes, which may lead to false positive results.

4. **Multiplex PCR:** It uses more than one primer which can detect many DNA sequences of several organisms in one reaction.
   - *Syndromic approach*: To diagnose infectious syndrome caused by more than one organism.
   - Contamination chances of reaction tubes with environmental DNA.

Real-time PCR (rt-PCR)

Real time PCR, though expensive, but has many advantages over a conventional PCR:
- *Quantitative*, hence can be used for monitoring treatment response, e.g. in HIV or HBV.
- *Takes less time*: As the amplification can be visualized even when the amplification cycle is going on.
- *Contamination rate is extremely less.*
- *Sensitivity and specificity* of rt-PCR assays are extremely higher.

Bacteriocin typing is done for:
- *Shigella sonnei* (colicin typing)
- *Klebsiella* (klebocin typing),
- *E.coli* (colicin typing)
- *Proteus* (proticin typing),
- *Pseudomonas* (pyocin typing)

Detection of amplification products of real time PCR

- *Nonspecific methods* use *SYBR green dye* that stains any nucleic acid nonspecifically.
- *Specific methods* use fluorescent labelled DNA probe such as (i) TaqMan or hydrolysis probe, (ii) Molecular beacon and (iii) FRET (Fluorescence Resonance Energy Transfer) probe.

Biotyping is done for:
- *C. diphtheriae*
- *Vibrio cholerae*

MICROBIAL TYPING

Microbial typing refers to characterization of an organism beyond its species level. It is used to determine the relatedness between different microbial strains of the same species and thereby helps to (i) investigate outbreaks, (ii) determine the source and routes of infections.

Genotypic methods are more reliable and have better reproducibility and discriminative power than phenotypic methods, however they are expensive.

Table 1.3.3: Typing methods

Phenotypic methods	Genotyping methods
Bacteriophage typing: Based on their susceptibility to bacteriophages. It is done for • *Staphylococcus aureus* and *Salmonella*Typhi • *Vibrio cholerae, Brucella* and *Corynebacterium diphtheriae*	**Non Amplification based methods** 1. Plasmid profile analysis 2. Chromosomal DNA analysis 3. RFLP (Restricted fragment length polymorphism) 4. Ribotyping (RFLP analysis of ribosomal DNA) 5. Pulse field gel electrophoresis (PFGE)-Gold standard method **Amplification based methods** 1. PCR-RFLP 2. Amplified Fragment Length Polymorphism (AFLP) 3. Sequencing-based methods 4. Microarrays
Bacteriocin typing: Based on the ability of a strain to produce particular bacteriocin which inhibits the growth of a set of selected indicator strains. It is done for: • *Shigella sonnei* (colicin typing) • *Klebsiella* (klebocin typing), *E.coli (*colicin typing) • *Proteus* (proticin typing),*Pseudomonas* (pyocin typing)	
Biotyping: Based on different biochemical properties of the organism. It is done for • *C.diphtheriae* (gravis, intermedius and mitis • *Vibrio cholerae*O1(classical and El Tor) and *Yersinia pestis*	
Antibiogram typing: Based on their resistance pattern to different antimicrobials. It is the most commonly used typing method.	
Auxotyping: Based on nutritional requirement of the organism (e.g. Gonococcus)	
Morphotyping: Based on different type of colonies in culture (e.g. *Pseudomonas)*	
Serotyping: Based on the antigenic property of an organism. • *Streptococcus* (Lancefield grouping) • Based on capsular antigen, e.g. pneumococcus, meningococcus and *H. influenzae* • Based on somatic antigen- *E. coli, Shigella, Salmonella* and *Vibrio cholerae.*	

MULTIPLE CHOICE QUESTIONS

CULTURE MEDIA/METHODS

1. Antibiotic sensitivity testing is usually done on: *(Recent Question 2015)*
a. Blood agar
b. Chocolate agar
c. MacConkey agar
d. Mueller Hinton agar

2. CLED medium is used in preference to MacConkey agar in relation to culture of organism involved in urine infection. The reason is *(AIIMS MAY 2016)*
a. It prevents swarming of Proteus
b. Differentiates LF and NLF
c. Promotes *Staphylococcus*, *Streptococcus* and *Candida*
d. Identifies *Pseudomonas*

3. Which of following culture media combination is/are true except: *(PGI Nov 2014)*
a. Thayer-Martin media: Gonorrhea
b. Chocolate agar: Enriched media
c. Lowenstein-Jensen medium: Mycobacterium tuberculosis
d. Mueller-Hinton agar: Corynebacterium diphtheriae
e. MacConkey's agar: Nonlactose fermenters form colourless colonies

4. TSI is: *(Recent Questions 2014)*
a. Selective b. Enrichment
c. Enriched d. Composite

5. Selective media for Shigella: *(Recent Questions 2014)*
a. Wilson blair b. TCBS
c. DCA d. Blood agar

6. Recommended transport medium for stool specimen suspected to contain enteric pathogens is: *(TNPG 2015, NEET Pattern Based)*
a. Amie's medium
b. Buffered glycerol saline medium
c. MacConkey medium
d. Blood agar

7. Agar conc. In nutrient agar is: *(NEET Pattern Based)*
a. 2% b. 4%
c. 1% d. 3%

8. Robertson cooked meat broth is an example: *(NEET Pattern Based)*
a. Enriched media
b. Enrichment media
c. Nutrient media
d. Anaerobic media

9. Blood agar is an example: *(DNB June 2012)*
a. Enriched media
b. Enrichment media
c. Nutrient media
d. Special media

10. Which one of the following is true: *(AIIMS Nov 2006, AI 2007)*
a. Agar has nutrient properties
b. Chocolate medium is selective medium
c. Addition of selective substances in a solid medium is called enrichment media
d. Nutrient broth is basal medium

11. Smith Noguchi's media is used for: *(DNB 2007)*
a. Salmonella
b. Klebsiella
c. Spirochetes
d. Bacillus

12. To prevent swarming the % agar in Nutrient agar has to be increased at least to ___% . *(TNPG 2014)*
a. 2% b. 4%
c. 6% d. 8%

MOLECULAR AND AUTOMATED METHODS

13. PCR is used to: *(AIMS Nov 2013)*
a. Detect target plasmids
b. Amplify small amount of DNA
c. Seal the cut ends of DNA
d. Cleave the bacterial plasmid

14. Reverse transcriptase is: *(PGI June 2011)*
a. DNA dependent RNA polymerase
b. RNA dependent DNA polymerase
c. DNA dependent DNA polymerase
d. RNA dependent RNA polymerase
e. RNA polymerase

15. Enzyme(s) used in polymerase chain reaction is/are:
a. Restriction endonuclease *(PGI June 2011)*
b. DNA polymerase
c. Alkaline phosphate
d. RNA polymerase
e. Reverse transcriptase

16. All of the following are required in PCR except:
a. Deoxyribonucleotides *(AIIMS Nov 2011)*
b. Thermostable enzyme/DNA polymerase
c. Dideoxyribonucleotides
d. Magnesium/ssDNA/Template DNA

17. Chromosomal mutation can be identified by all except:
a. Single strand polymorphism *(AIIMS Nov 2011)*
b. Agarose gel electrophoresis
c. Denaturating Gradient gel electrophoresis
d. Dideoxynucleotide trail sequencing

18. Northern bloting is used for separation of:
a. DNA *(DNB June 2012)*
b. RNA
c. Proteins
d. None

19. For PCR, Thermus acquaticus plant is used to prepare:
a. DNA polymerase *(Recent Question 2013)*
b. RNA polymerase
c. Primers
d. Restriction endonuclease II

20. DNA hybridization is called: *(Recent Question 2013)*
a. Southern blot
b. Northern blot
c. Eastern blot
d. Western blot

EXPLANATIONS

CULTURE MEDIA/METHODS

1. **Ans. (d) (Mueller Hinton agar)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p83

2. **(c) (Promotes *Staphylococcus*, *Streptococcus* & *Candida*) Reference: Apurba Sastry's Essentials of Medical Microbiology/ p46**

   CLED agar (Cysteine lactose electrolyte-deficient agar): This is similar to MacConkey agar, differentiates between LF and NLF. It is used as an alternative to combination of blood agar and MacConkey agar, for the processing of urine specimens.
   - Advantages over MacConkey agar: It is less inhibitory than MacConkey agar, supports the growth of gram-positive bacteria (except β hemolytic *Streptococcus*) and *Candida*.
   - Advantage over blood agar: It can prevent the swarming of *Proteus*.

3. **Ans. (d) (Mueller-Hinton agar)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p44-46
   - Thayer-Martin media: Gonorrhea (Selective media)
   - Chocolate agar: Enriched media
   - Lowenstein-Jensen Medium: Mycobacterium tuberculosis (selective media)
   - Muller-Hinton agar: For antibiotic susceptibility test
   - Mac Conkey's agar: Differential media, differentiates LF (pink colony) and NLF (colourless colonies).

4. **Ans. (d) (Composite)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p57, Ananthanarayan 9/e p52
   - TSI is a popular composite medium, which indicated whether bacteria ferments glucose only, or lactose and sucrose also, with/without gas formation, besides indicating H_2S production as well.

5. **Ans. (c) (DCA)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p306, Ananthanarayan 9/e p285
   - Deoxycholate citrate agar (DCA) and Xylose lysine deoxycholate (XLD) are selective media for Shigella.

6. **Ans. (b) (Buffered...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p45 Ananthanarayan 9/e p42, 8/e p39

   Recommended transport medium for stool specimen is:
   - Buffered glycerol saline medium (when Salmonella or Shigella is suspected)
   - VR medium-(when Vibrio cholerae is suspected).

7. **Ans. (a) (2%)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p43, Ananthanarayan 9/e p39, 8/e p39
   - 2% of agar is routinely used for making solid media.

8. **Ans. (d) (Anaerobic...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p46, Ananthanarayan 9/e p43, 8/e p39
   - *Roberson cooked meat broth is used for culture of anaerobic organisms.*

9. **Ans. (a) (Enriched...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p45, Ananthanarayan 9/e p40, 8/e p39
   - **Enriched media** contains extra nutritional factors like blood, serum, egg so that it supports the fastidious organisms, e.g. Blood agar, Chocolate agar, Loeffler's serum slope.

10. **Ans. (d) (Nutr....)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p44 Ananthanarayan 9/e p40, 8/e p39
    - ***Nutrient broth***, Peptone water and Nutrient Agar are the ***basal media.***
    - Used to prepare solid media, but it has no nutritive value
    - Chocolate medium is enriched medium
    - Addition of selective substances in a solid medium is called selective medium

11. **Ans. (c) (Spirochetes)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p375, Ananthanarayan 9/e p42
    - ***Smith Noguchi's medium:*** Is used for ***Nonpathogenic Treponemes***
    - Pathogenic Treponemes cannot be grown in artificial culture medium.

12. **Ans. (c) (6%)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p43, Ananthanarayan 9/e p42

    Agar concentration routinely used: (i) In solid media 2%, (ii) Semisolid media 0.5-1%, (iii) To inhibit swarming 6%

MOLECULAR AND AUTOMATED METHODS

13. Ans. (b) (Amplify small...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 60, Ananthanarayan 9/e p65

- The polymerase chain reaction (PCR) is a biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA, generating thousands to millions of copies of a particular DNA sequence.

14. Ans. (b) (RNA dependent DNA polymerase) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p62

- *Reverse transcriptase has RNA dependent DNA polymerase activity and it uses the genome RNA as a template and 1st synthesizes single stranded DNA by reverse transcription of genomic RNA, then finally a double stranded DNA.*
- *The HIV and other retroviruses possess reverse transcriptase.*

15. Ans. (b) (DNA...) Apurba Sastry's Essentials of Medical Microbiology 1/e p62

- Taq Polymerase is a DNA Polymerase that amplifies the target gene into millions of copy without denaturation. It is one of the basic requirements of PCR along with other requirements like primers, Nucleotide and Mg^{++} ion.

About Other Options

- Restriction endonuclease: Require for RFLP (Restricted Fragment Length Polymorphism)
- Reverse transcriptase: Require for Reverse transcriptase PCR (RT-PCR)
- Alkaline phosphate: Required for ELISA.
- RNA polymerase: Not required.

16. Ans. (c) (Dideoxyribonucleotides) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p61, Ananthanarayan 9/e p65, 8/e p70-71, Bailey and Scott's Diagnostic Microbiology 12/e p127-131

- ***Dideoxyribonucleotides is not used in PCR.***

Requirement of PCR

- Taq Polymerase: Obtained from Thermus aquaticus plant which can withstand the high temperature.
- Primers-short oligonucleotide complementary to a small sequence of the target DNA
- Deoxynucleotide, Mg++ ion
- Target DNA present in the sample.

17. Ans. (b) (Agar..): Ref: Apurba Sastry's Ess of Med Microbiology 1/e p62, Prescott's Microbiology 6/e p251-54, Wikipedia

- ***Agarose Gel electrophoresis*** *is used to separate the DNA by charge or by size. It is usually performed to visualize the amplified DNA after PCR, but may be used as a preparative technique prior to use of other methods such as mass spectrometry, RFLP, PCR, cloning, DNA sequencing, or Southern blotting for further characterization.*
- ***Please remember,*** *it is just a method of visualizing the DNA after separating by size. So it is always used as a part of any molecular method to visualize the DNA. But alone it cannot be used to detect any mutations.*

18. Ans. (b) (RNA) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p77, Ananthanarayan 9/e p65, 8/e p69

- Northern blotting is used for detection of- RNA
- Southern blotting is used for detection of- DNA
- Western blotting is used for detection of- Protein

19. Ans. (a) (DNA pol...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p61, Mackie McCartney 14/e p227

The heat stable DNA polymerase enzyme used for PCR is derived from plant Thermus acquaticus.

20. Ans. (a) (Southern blot) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p77, Ananthanarayan 9/e p65

- DNA hybridization is called Southern blot
- RNA hybridization is called Northern blot.

CHAPTER

1.4

Bacterial Genetics and Antimicrobial Resistance

BACTERIAL GENETICS

Bacterial genetics deals with the study of heredity and variation seen in bacteria. All hereditary characteristics of the bacteria are encoded in their DNA which is present in chromsome as well in extrachromosomal genetic material as plasmid.

Plasmid

Plasmids are the extrachromosomal ds circular DNA molecules that exist in free state in the cytoplasm of bacteria and also found in some yeasts:

- Not essential for life: Bacteria may gain or lose plasmid during their life time.
- Numbers: They may be present singly or in multiple numbers up to > 40 plasmids per cell.
- Capable of replicating independently
- Episome: Plasmid may integrate with chromosomal DNA of bacteria and such plasmids are called episomes.
- Curing: The process of eliminating the plasmids from bacteria is known as curing.

Type of Plasmids based on function:
- Fertility or F-plasmids
- Resistance (R) plasmids
- Col plasmids
- Virulence plasmids
- Metabolic Plasmids

Classification of Plasmids

1. **Based on ability to perform conjugation:**
   - Conjugative plasmids or self-transmissible plasmids
   - Nonconjugative plasmids or nontransmissible plasmids. They cannot transfer themselves.
2. **Based on compatibility:** Compatible plasmids and Incompatible plasmids. Only compatible plasmids can stay together inside a cell.
3. **Based on function:** There are five main classes of plasmids:
   - Fertility or F-plasmids: Code for sex pili that forms the conjugation tube.
   - Resistance (R) plasmids, which contain genes that code resistance to various antibiotics.
   - Col plasmids: Contain genes that code for bacteriocins.
   - Virulence plasmids: Codes for virulence factors like toxins, adhesins.
   - Metabolic plasmids: They enable the host in various metabolic activities.
4. **Plasmid as vector:** By their ability to transfer DNA from one cell to another, plasmids have become important vectors in genetic engineering. Plasmids contain certain sites where genes can be inserted artificially by recombinant DNA technology. Such plasmids can be used for protein production, gene therapy, etc.

Horizontal Gene Transfer in Bacteria

Gene transfer in bacteria can be broadly divided into:

- Vertical gene transfer (transmission of genes from parents to offspring)
- Horizontal gene transfer (transmission of genes from one bacterium to another bacterium). This occurs by:

Transformation

Transformation is the process of random uptake of free or naked DNA fragment from the surrounding medium by a bacterial cell and incorporation of this molecule into its chromosome in a heritable form.

- It has been studied so far only in certain bacteria: *Streptococcus, Bacillus, Haemophilus, Neisseria, Acinetobacter and Pseudomonas*.
- The Griffith experiment (1928) on mice using pneumococci strains provided the direct evidence of transformation.

Transduction

Transduction is defined as transmission of a portion of DNA from one bacterium to another by a bacteriophage.

Types of transduction

1. **Generalized transduction:** It involves transfer of any part of the donor bacterial genome into the recipient bacteria.
2. **Restricted or specialized transduction:** Here, only a particular genetic segment of the bacterial chromosome that is present adjacent to the phage DNA is transduced.

Role of transduction

- In addition to chromosomal DNA, transduction is also a method of transfer of episomes and plasmids.
- Drug resistance, e.g. plasmid coded penicillin resistance in staphylococci.
- Treatment: As a method of genetic engineering in the treatment of some inborn metabolic defects.

Lysogenic Conversion

During the temperate or lysogenic life cycle, the phage DNA remains integrated with the bacterial chromosome as prophage.

- The prophage acts as an additional chromosomal element which encodes for new characters to the daughter cells.
- Imparts toxigenicity to the bacteria: Phage DNA may code for various toxins abbreviated as *ABCDE*:
  A and C of Streptococcus pyrogenic exotoxin (SPE), **B**otulinum toxin C and D, **C**holera toxin, **D**iphtheria toxin and **E**. coli (Verocytotoxin).

Conjugation

Conjugation refers to the transfer of genetic material from one bacterium (donor or male) to another bacterium (recipient or female) by mating with each other and forming the conjugation tube. It was discovered first by Lederberg and Tatum.

- **F+ × F– Mating:** When the F+ cell (containing a plasmid called F factor or fertility factor) comes close to the F– cell (lacking F factor), the F factor forms conjugation tube, through which the F factor is transmitted to F– cell ultimately making F– cell into F+ cell.
- **HFR Conjugation**: F factor being a plasmid, it may integrate with bacterial chromosome and behave as episome.
  - Such donor cells are able to transfer chromosomal DNA to recipient cells with high frequency in comparison to F+ cells, therefore, named as Hfr cells (high frequency of recombination).
  - During conjugation of Hfr cell with an F- cell, only few chromosomal genes along with only a part of the F factor get transferred. Hence, F- recipient cells do not become F+ cells.
- **F′ Conjugation:** The conversion of an F+ cell into an Hfr cell is reversible.
  - When the F factor reverts from the integrated to free-state, it may sometimes carry with it some chromosomal DNA from adjacent site of its attachment. They are named as F′ factor (F prime factor).
  - When F′ cell conjugates with a recipient (F-), it transfers, along with the F factor, the host DNA incorporated with it. The recipient becomes F′ cell. This process is called sexduction.
- Conjugation plays an important role in the transfer of plasmids coding for antibacterial drug resistance [resistance transfer factor (RTF)] and bacteriocin production [Colicinogenic (Col) factor].
- **R factor** (or the resistance factor) is a plasmid which has two components.
  - Resistance transfer factor (RTF) is the plasmid responsible for conjugational transfer (similar to F factor)
  - Resistance determinant (r): Codes for resistance to one drug. An R factor can have several r determinants.

Types of Conjugation:
- F+ × F- Mating
- HFR Conjugation
- F′ Conjugation

Section 1

ANTIMICROBIAL RESISTANCE

Antimicrobial resistance can be of two types; intrinsic and acquired.

- Intrinsic Resistance: It is the innate ability of a bacterium to resist a class of antibiotics
- Acquired Resistance: It is the emergence of resistance in bacteria, by acquiring the drug resistant genes either by – (i) mutational or by (ii) transferable drug resistance

Table 1.4.1: Intrinsic antimicrobial resistance

Organism	Intrinsic resistance against
Anaerobic bacteria	Aminoglycosides
Aerobic bacteria	Metronidazole
Gram-negative bacteria	Vancomycin
Klebsiella species	Ampicillin
Pseudomonas	Sulfonamides, trimethoprim, tetracycline, or chloramphenicol
Enterococci	Aminoglycosides and All cephalosporins

Table 1.4.2: Mutational vs transferable drug resistance

Mutational drug resistance	Transferable drug resistance
Resistance to one drug at a time	Multiple drug resistance at the same time
Low degree resistance	High degree resistance
Resistance can be overcome by combination of drugs	Cannot be overcome by drug combinations
Virulence of resistance mutants may be lowered	Virulence not decreased
Resistance is not transferable to other organisms; but spread to offsprings by vertical spread only	Resistance is transferable to other organisms- *Spread by*: horizontal spread (conjugation, or rarely by transduction/ transformation)

Mechanism of Antimicrobial Resistance

Bacteria develop antimicrobial resistance by several mechanisms.

1. **Decreased permeability across the cell wall:** Certain bacteria modify their cell membrane porin channels; thereby preventing the antimicrobials from entering into the cell. This strategy has been observed in many gram-negative bacteria such as *Pseudomonas, Enterobacter* and *Klebsiella* species against drugs such as imipenem, aminoglycosides and quinolones.
2. **Efflux pumps:** Certain bacteria possess efflux pumps which mediate expulsion of the drug(s) from the cell, soon after their entry; thereby decreasing the intracellular accumulation of drugs. This strategy has been observed in:
   - *Escherichia coli and* other Enterobacteriaceae against tetracyclines, chloramphenicol
   - Staphylococci against macrolides and streptogramins
   - *Staphylococcus aureus* and *Streptococcus pneumoniae* against fluoroquinolones.
3. **By modification of the antimicrobial target sites within the bacteria:** This strategy has been observed in:
   - MRSA (Methicillin resistant *Staphylococcus aureus*): (see chapter-3.1 for details).
   - VRE (Vancomycin resistant Enterococci) (See chapter-3.2 for details)
   - Streptomycin resistance in *Mycobacterium tuberculosis*: Due to modification of ribosomal proteins or 16SrRNA.
   - Rifampicin resistance in *Mycobacterium tuberculosis*: Due to mutations in RNA polymerase.
   - Quinolone resistance (seen in *S. aureus* and *S. pneumoniae*): Due to mutations in DNA gyrase enzyme.
4. **By enzymatic inactivation:** Certain bacteria can inactivate the antimicrobial agents by producing various enzymes such as:
   - Aminoglycoside modifying enzymes like (acetyltransferases, adenyltransferases, and phosphotransferases, produced by both gram-negative and gram-positive bacteria): They destroy the structure of aminoglycosides.
   - Chloramphenicol acetyl transferase: It is produced by members of Enterobacteriaceae
   - β lactamase enzyme production.

Mechanism of Antimicrobial Resistance:
- Decreased permeability across the cell wall
- Efflux pumps
- By modification of the antimicrobial target sites within the bacteria
- By enzymatic inactivation

Beta-Lactamase Enzymes

β-lactamase enzymes are capable of hydrolysing the β-lactam rings (the active site) of β-lactam antibiotics; thereby deactivating their antibacterial properties.

- It is observed in both gram-positive and gram-negative bacteria
- They are plasmid coded, and transferred from one bacterium to other mostly by conjugation, (except in *Staphylococcus aureus* where they are transferred by transduction). Beta lactamases can be classified in two ways:
  - Ambler's classification (structural or molecular classification)—See table
  - Bush Jacoby Medeiros classification or functional (phenotypic)—Advanced and complex classification

Table 1.4.3: Ambler classification of beta-lactamases

Class A-ESBL (Extended spectrum β-lactamases)
Organisms producing ESBL enzymes are resistant to all Penicillins and 1st, 2nd and 3rd generation cephalosporins and monobactams, however remain sensitive to carbapenems and cephamycins • Resistance can be overcome by β-lactam + β-lactamase inhibitor (e.g. sulbactum or clavulanic acid) • Detected by Combination disk test (Ceftazidime and cetftazidime + clavulanic acid), Three dimensional test (best method)
Class B-MBL (Metallo beta-lactamase)
These organisms are resistant to all those antibiotics to which AmpC beta-lactamase producers are resistant plus they are resistant to carbapenems. • Resistance cannot be overcome by β-lactam + β-lactamase inhibitor combination • Detected by EDTA disk synergy test, modified Hodge test
Class C- AmpC beta-lactamase
These organisms are resistant to all those antibiotics to which ESBL producers are resistant plus they are resistant to cephamycins (e.g. cefoxitin and cefotetan). But they are sensitive to carbapenems. • Resistance cannot be overcome by β-lactam + β-lactamase inhibitor combination • Detected by AmpC disk test using cefoxitin disk
Class D- oxacillinase
Resistance can be overcome by β-lactam + β-lactamase inhibitor combination

Antimicrobial Susceptibility Testing Methods:
- Disc diffusion methods
- Dilution tests
- Epsilometer or E-test
- Automated methods
- Molecular methods

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods include:

- **Disk diffusion methods:** Kirby-Bauer disk diffusion method and Stokes disk diffusion method:
  - Mueller-Hinton agar (MHA) is considered as the best medium
  - Lawn culture method is used to inoculate the organism onto MHA
  - Control strains: ATCC (American Type Culture Collection) strains are used
  - Reporting is done according to CLSI (Clinical and Laboratory Standards Institute) guidelines
- **Dilution tests:** Broth dilution method and Agar dilution method:
  - Here, the antimicrobial agent is serially diluted, each dilution is tested with the test organism for antimicrobial susceptibility test and the MIC is calculated.
  - MIC (minimum inhibitory concentration) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism.
- **Epsilometer or E-test:** This is a quantitative method detecting MIC by using the principles of both the dilution and diffusion of antibiotic into the medium.
- **Automated methods** such as VITEK 2, Phoenix System and MicroScan WalkAway system
- **Molecular methods** (PCR detecting drug resistant genes) e.g. *MecA* gene for MRSA.

MULTIPLE CHOICE QUESTIONS

BACTERIAL GENETICS

1. Bacteria used in Griffith experiment is: *(PGI Nov 2016)*
a. Streptococcus pyogenes
b. Capsulated Streptococcus pneumoniae
c. Staphylococcus aureus
d. MRSA
e. Non-capsulated Streptococcus pneumoniae

2. Pick the true statement regarding Plasmids:
a. Nonself-replicative *(JIPMER May 2015)*
b. Acts as Messenger RNA
c. Involved in Conjugational transfer between strains
d. Involved in transformation

3. Nontoxigenic C. diphtheriae changes to toxigenic C. diphtheriae by the help of bacteriophage... by which method this conversion occur? *(AIIMS Nov 2015)*
a. Transfection
b. Transduction
c. Conjugation
d. Recombinant Technology

4. Movement of DNA from one bacteria to another connection tube or pilus is called: *(JIPMER 2014, 2012)*
a. Transformation b. Transduction
c. Conjugation d. Lysogenic conversion

5. Mechanism of direct transfer of free DNA: *(NEET Pattern Based)*
a. Transformation b. Conjugation
c. Transduction d. None

6. Phage mediate transfer of cDNA into host is known as: *(DNB Dec 2012)*
a. Transduction b. Transformation
c. Transmission d. Conjugation

7. Horizontal transmission of 'R' factor is by: *(JIPMER 2009)*
a. Transduction b. Transformation
c. Conjugation d. Fusion

ANTIMICROBIAL RESISTANCE

8. Most common method of bacteria responsible for drug resistance: *(PGI Nov 2016)*
a. Conjugation b. Transduction
c. Transformation d. Enzyme inactivation
e. Mutation

9. Not true about bacterial drug resistance mechanism: *(AI 2012, AIIMS Nov 2011, AIIMS May 2012)*
a. Most common mechanism is production of neutralizing enzymes
b. If resistance is plasmid mediated, it is always transferred vertically
c. Alteration of target seen in pneumococcal resistance
d. Complete removal of target is cause of resistance to Vancomycin

10. Multiple drug resistance is spread by: *(TN 2008)*
a. Transformation b. Transduction
c. Mutation d. Conjugation

11. A patient is kept on ceftriaxone and amikacin, ESBL Klebsiella infection. What will you do next? *(AIIMS Nov 2010)*
a. Continue with same antibiotic but in higher dose
b. Change ceftriaxone and add ceftazidime
c. Start imipenem in place of ceftriaxone
d. Remove Amikacin

12. Drug against ESBL producing Pseudomonas: *(AIIMS Nov 2014, JIPMER Nov 2014)*
a. Ceftriaxone + Piperacillin
b. Ceftriaxone + Tazobactam
c. Piperacillin + Tazobactam
d. Ceftriaxone + Piperacillin + Tazobactam

13. MIC (minimum inhibitory concentration) can be calculated by all of the following antibiotic sensitivity methods except: *(Recent MCQ 2013)*
a. E test
b. Agar dilution method
c. Kirby Bauer's disk diffusion method
d. Broth dilution method

14. For antibiotic sensitivity test, the organism broth prepared should match with: *(Recent MCQ 2013)*
a. Mc Farland standard 0.5
b. Mc Farland standard 1
c. Mc Farland standard 2
d. Mc Farland standard 3

15. Beta lactamase is produced by: *(Recent MCQ 2013)*
a. E.coli b. Gonococcus
c. Staphylococcus aureus d. All of the above

16. Which of the following disease(s) is/are not toxin mediated: *(PGI Nov 2014)*
a. Diphtheria b. Tetanus
c. Pertussis d. Anthrax
e. Syphilis

17. A strain of E. coli isolated from urine is resistant to third generation cephalosporins. The mechanism of development of resistance is: *(JIPMER Nov 2014)*
a. Extended spectrum Beta-Lactamases
b. Decreased permeability
c. Active efflux of Beta-Lactam agents
d. Alteration of PBP

EXPLANATIONS

BACTERIAL GENETICS

1. **Ans. (b, e) (Capsulated S. pneumoniae, Non-capsulated S.pneumoniae)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p71

   Capsulated dead S.pneumoniae + non-capsulated live S.pneumoniae → Transformation of gene coding capsule from dead to live pneumococci → Results in capsulated live pneumococci

2. **Ans (c) (Involved in conjugational...)** Ref: ApurbaSastry's Essentials of Medical Microbiology 1/e p67
   Plasmids are self replicative extra-chromosomal elements frequently transferred by conjugation.

3. **Ans (b) Transduction** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p73
   - Transduction is the transfer of bacterial genes from one bacterial to other by bacteriophage
   - Lysogenic Conversion would have been a better answer here. It is the process by which the phage DNA is integrated to bacterial DNA and remains as lysogenic phage. In such case, certain phage gene (e.g. gene coding for diphtheria toxin) imparts toxigenicity to the bacteria.

4. **Ans. (c) Conjugation** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p73, Ananthanarayan 9/e p61

5. **Ans. (a) (Trans...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p70, Ananthanarayan 9/e p59, 8/e p63
   - ***Transformation is*** the process of the transfer of free DNA itself from one bacterium to another.

6. **Ans. (a) (Transduction)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p671, Ananthanarayan 9/e p59
   Refer text

7. **Ans. (c) (Conjugation)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p73, Ananthanarayan 9/e p60
   - Conjugation is the process where there is transfer of genetic elements from one bacterium (male) to another (female) along sex pilus or conjugation tube- Horizontal genetic transfer.

ANTIMICROBIAL RESISTANCE

8. **Ans (a,d) (Conjugation, Enzyme inactivation)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p82-83.

   Transferrable resistance by conjugation is the most common method of transfer of bacterial resistant genes. Enzyme inactivation is the most common mechanisms of bacteria drug resistance.

9. **Ans. (b) (If resistance is plasmid mediated, it is always transferred vertically)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p82, Ananthanarayan 9/e p63, 8/e p67, Harrison 18/e p1157
   - If resistance is chromosomally mediated, it is usually transferred vertically from parent to daughter bacteria.
   - If resistance is plasmid mediated, it is usually transferred by horizontal route mainly by conjugation.

   About Other Options
   - Clinically, enzymatic drug inactivation is the most common mechanism for acquired microbial resistance by bacteria
   - Most common mechanism of bacterial drug resistance:
   - ***Pneumococcal resistance:*** is mainly due to Alteration of target, i.e. Penicillin binding protein (PBP)
   - Resistance to Vancomycin is due to complete removal of target D alanyl-D alanine present the bacterial cell wall is the target site for Vancomycin, which binds there and inhibits it and thus inhibits the cell wall synthesis.

   The four Major Mechanisms of Antibacterial Resistance: Refer chapter review.

10. **Ans. (d) (Conjugation)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p73, Ananthanarayan 9/e p60-61
    - Resistance (R) factors are extrachromosomal plasmids responsible for spread of multiple drug resistance among bacteria.
    - They are circular double stranded DNA carry genes for variety of enzymes that can destroy antibiotics
    - R factor consists of 2 components: Resistance transfer factor (RTF) and resistant determinant (r).
    - The resistance transfer factor is responsible for conjugational transfer while each r determinant carries resistance for one of the several antibiotics.

11. Ans. (c) (Start imipenem in place of ceftriaxone) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p83

- ESBL **(Extended spectrum beta lactamases)** are resistant to all Penicillin and 1st/2nd/3rd cephalosporin and monobactam
- Which can be overcome by addition of β lactamase inhibitor like clavulinic acid
- Other alternate which can be given are:
  - Carbapenams like Imipenem and meropenem
  - Cephamycins (like cefoxitin and cefotetan)
  - Different class of antibiotics like aminoglycoside

12. Ans. (c) (Piperacillin + Taz...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p83

Extended Spectrum-β-Lactamases (ESBL) producing Pseudomonas can be treated with an antipseudomonial β lactam (e.g. piperacillin) plus β lactamase inhibitor such as tazobactam combination therapy.

13. Ans. (c) (Kirby Bauer's disk diffusion method) Ref: Ananthanarayan 8/e p619 & 9/e p635

- Kirby Bauer's disk diffusion method is used to know the zone of inhibition of the streaked organism surrounding the disk by which we can know whether the organism is sensitive or resistant to the antibiotic disk. However, we cannot know the MIC.
- MIC (Minimal inhibitory concentration) of the antibiotic is defined as the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism.
- MIC is calculated by: (i) Agar dilution method, (ii) Broth dilution method and (iii) Epsilometer (E test)

14. Ans. (a) (McFarland standard 0.5) Ref: Mackie McCartney 14/e p851-852

- In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range.
- A 0.5 McFarland standard is prepared by mixing 0.05 mL of barium chloride dihydrate with 9.95 mL of 1% sulfuric acid and its equivalent to 150 million no. of bacteria/mL in a broth.
- For antibiotic sensitivity test, the organism broth prepared should match with- 0.5 McFarland standard.

15. Ans. (d) (All of the above) Ref: Ananthanarayan 9/e p62, 233

Beta lactamase enzymes are plasmid coded, produced by both gram positive and gram negative organisms.

16. Ans. (e) (syphilis...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 93

Syphilis is caused by T. pallidum. Refer chapter review.

17. Ans. (a) (Extended spectrum Beta-Lactamases) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p83

Out of several mechanisms of beta lactam resistance in E.coli, Beta-Lactamase production is the MOST COMMON; particularly Extended spectrum Beta-Lactamases (ESBL).

NOTE

SECTION 2

Immunology

CHAPTER OUTLINE

CHAPTER

2.1 Immunity

INNATE IMMUNITY

The term 'immunity' is defined as the resistance offered by the host against microorganism(s) or any foreign substance(s). Immunity can be broadly classified into:

1. Innate immunity: present right from the birth
2. Acquired/Adaptive immunity: acquired during the course of the life.

Table 2.1.1: Differences between innate and acquired immunity

Innate immunity	Acquired/Adaptive immunity
Resistance to infection that an individual possesses from birth	Resistance to infection that an individual acquires during his lifetime
Immune response occurs in minutes	Immune response occurs in days
Prior exposure to the antigen is not required	Develops following the antigenic exposure
Diversity is limited, acts through a restricted set of reactions	More varied and specialized responses
Immunological memory responses are absent	Immunological memory responses are present
Respond to microbial antigens that are not specific to some microbe, rather shared by many microbes called microbes-associated molecular patterns (MAMP)	Respond to specific microbial antigens
Host cell receptors (pattern recognition receptors) are nonspecific, e.g. Toll-like receptor	Host cell receptors are specific, e.g. T cell receptors and B cell immunoglobulin receptors
Components of innate immunity • Anatomical barriers, such as skin and mucosa • Physiological barriers (e.g. body temperature) • Phagocytes (neutrophils, macrophages and monocytes) • Natural killer (NK) cells • Other Classes of lymphocytes -γδ T cells , NK-T cells, B-1 cells and marginal-zone B cells • Mast cells • Dendritic cells • Complement pathways: alternate and mannose binding pathways • Fever and inflammatory responses • Normal resident flora • Cytokines- TNF, certain interleukin (IL-1, IL-6, IL-8, IL-12, IL-16, IL-18), IFN-α, β and TGF- β • Acute phase reactant proteins (APRs)	**Components of acquired immunity:** • T cell • B cell • Classical complement pathway • Antigen presenting cells • Cytokines (IL-2, IL-4, IL-5, IFN-γ) **Types of acquired immunity:** It can be classified in two ways: • Active and passive immunity • Artificial and natural immunity

Factors Influencing Innate Immunity

- Depends on the Species, Race, Individual (genetic influence)
- Age, Hormonal influene and Nutrition.

Toll Like Receptors

They are so named because they are similar to Toll receptors present in the fruit fly- Drosophila, where it is the main receptor for induction of innate immunity by bind to particular MAMP molecules on microbial surfaces. They are of 13 types, out of which important ones are:

- TLR-2 binds to bacterial peptidoglycan
- TLR-3 binds to dsRNA of viruses

Toll like receptors:
They are the principle host cell receptors of innate immunity

- TLR-4 binds to LPS of Gram-negative bacteria
- TLR-5 binds to flagella of bacteria
- TLR-7 & 8 bind to ssRNA of viruses
- TLR-9 binds to bacterial DNA.

Acute Phase Reactant Proteins (APRs)

They are the proteins synthesized by liver at steady concentration, but their synthesis either increases or decreases exponentially during acute inflammatory conditions.

Though liver is the primary site, APRs can also be synthesized by various other cells such as endothelial cells, fibroblasts, monocytes and adipocytes.

Positive APRs are the proteins whose levels increase during acute inflammation. Examples:

- Serum Amyloid A
- C- Reactive protein
- Complement proteins: Complement factors (C1–C9), factor B, D, and properdin
- Coagulation protein, e.g. fibrinogen, von Willebrand factor
- Proteinase inhibitors, e.g. α1 antitrypsin
- α1 acid glycoprotein
- Mannose binding protein
- Haptoglobin
- Metal binding proteins, e.g. ceruloplasmin

Negative APRs: They are the proteins whose levels are decreased during acute inflammation thus creating a negative feedback that stimulates the liver to produce positive APRs. Examples of negative APRs include: albumin, transferrin, and antithrombin.

Role of APRs: They have a wide range of activities that contribute to the host defense:

- APRs have various antimicrobial and anti-inflammatory activities (e.g. complement)
- Metal binding proteins can chelate various metals such as iron, copper, etc. making them unavailable for the bacteria.

C-Reactive Protein (CRP)

CRP is an example of APR that rise in acute inflammatory conditions including bacterial infections. It belongs to beta globulin family.

- CRP is so named because it precipitates with C- carbohydrate (polysaccharide) antigen of Pneumococcus. However, it is not an antibody against the C-carbohydrate antigen of Pneumococcus; it is nonspecific, can be raised in any inflammatory conditions.
- Commonest markers of acute inflammation, used in most diagnostic laboratories.

CRP
- Normal level < 0.2 mg/dl.
- Detection limit of latex agglutination test– 0.6 mg/dl
  - Insignificant increase (< 1 mg/dl), e.g. in heavy exercise, common cold, and pregnancy
  - Moderate increase (1–10 mg/dl), e.g. bronchitis, cystitis, malignancies, pancreatitis, myocardial infarction.
  - Marked increase (> 10 mg/dl), e.g. acute bacterial infections, major trauma and systemic vasculitis.

CRP Level

The normal level of CRP is < 0.2 mg/dl. However, it increases by several folds in acute inflammatory conditions.

- *Insignificant increase of CRP* (< 1 mg/dl): Occurs in conditions such as heavy exercise, common cold, and pregnancy
- *Moderate increase* (1–10 mg/dl): Occurs in conditions such as bronchitis, cystitis, malignancies, pancreatitis, myocardial infarction.
- *Marked increase of CRP* (> 10 mg/dl): Occurs in conditions such as acute bacterial infections, major trauma and systemic vasculitis.

CRP can be detected by

- Precipitation method using C carbohydrate antigen (obsolete, not in use now)
- Latex (passive) agglutination test using latex particles coated with anti-CRP antibodies.
  - It is the most widely used method employed worldwide.
  - Detection limit of CRP by latex agglutination test 0.6 mg/dl.
- **Highly sensitive CRP** (hs-CRP) test: Minute quantities of CRP can be detected by various methods (e.g. nephelometry, enzyme immunoassays). This is useful in assessing the risk to cardiovascular diseases.

ACQUIRED OR ADAPTIVE IMMUNITY

Acquired immunity is defined as the resistance against the infecting foreign substance that an individual acquires or adapts during the course of his life.

- It is of two types: Active immunity and passive immunity.
- Active immunity can again be divided into:
  - Primary immune response (which develops after first microbial exposure)
  - Secondary immune response (which develops after subsequent microbial exposure).

Table 2.1.2: Differences between active and passive immunity

Active immunity	Passive immunity
Produced actively by host immune system	Immunoglobulins received passively
Induced by: • Infection (*natural*) • Vaccination (*artificial*)	Acquired by: • Mother to fetus IgG transfer (*natural*) • Readymade antibody transfer (*artificial*)
Long lasting	Lasts for short time
Lag period present	No Lag period
Memory present	No Memory
Booster doses are useful	Subsequent doses are less effective
Negative phase may occur	No Negative phase
In immunodeficiency individuals not useful	Useful in immunodeficient individuals

Table 2.1.3: Differences between primary and secondary immune response

Primary immune response	Secondary immune response
Immune response against primary antigenic challenge	Immune response against subsequent antigenic challenge
Slow, sluggish (appear late) and short lived	Prompt, powerful and prolonged (long lasting)
Lag period is longer (4–7 days)	Lag period is absent or short (1–3 days)
No negative phase	Negative phase may occur
Antibody produced in low titer and is of IgM type. Antibodies are more specific but less avid	Antibody produced in high titer and is of IgG type Antibodies are less specific but more avid
Antibody producing cells: Naive B cells	Antibody producing cells: Memory B cells
Both T dependent and T independent antigens are processed.	Only T dependent antigens are processed.

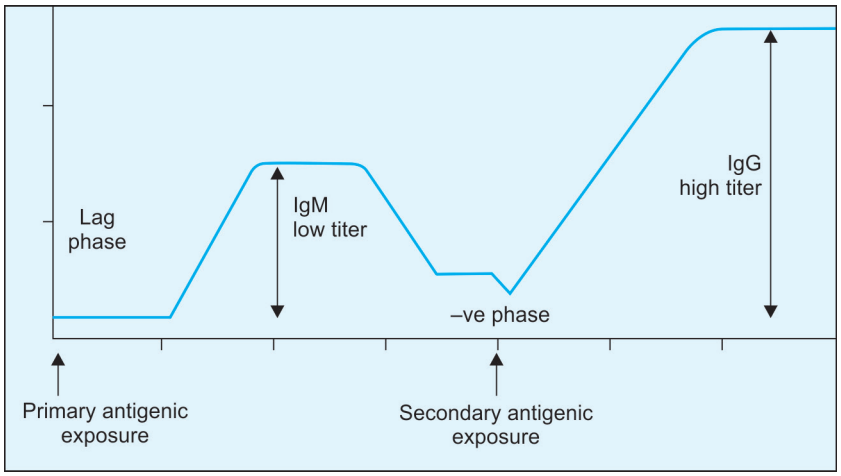


Fig. 2.1.1: Primary and secondary immune responses

Primary immune response
Immune response against primary antigenic challenge
- Slow, sluggish (appear late) and short lived
- Lag period is longer (4–7 days)
- No negative phase
- Antibody produced in low titer and is of IgM type.
- Antibodies are more specific but less avid
- Antibody producing cells- Naive B cells
- Both T depd and T indepd Ag are processed.

OTHER TYPES OF IMMUNITY

Local (or mucosal) Immunity

Local or mucosal immunity is the immune response that is active at the mucosal surfaces such as intestinal or respiratory or genitourinary mucosa:

- It is usually mediated by a type of IgA antibody called secretory IgA.
- Local immunity can only be induced by natural infection or by live vaccination, e.g. after OPV (but not by killed vaccines).

Local or mucosal immunity
Immune response that is active at the mucosal surfaces
- Mediated by secretory IgA.
- Induced by natural infection or by live vaccination

Herd Immunity

Herd immunity is defined as the overall immunity of a community (or herd) towards a pathogen:

- Herd immunity plays a vital role in preventing epidemic diseases. If the herd immunity is good, that means large population of the community are immune towards a pathogen. Hence, epidemics are less likely to occur and eradication of the disease may be possible.
- Elements that contribute to create a strong herd immunity are:
  - Occurrence of clinical and subclinical cases in the herd
  - Ongoing immunisation programme
  - Herd structure, i.e. type of population involved
  - Type of pathogen: Herd immunity may not be strong in a community against all the pathogens.
- Herd immunity develops following effective vaccination against some diseases like:
  - Diphtheria and Pertussis vaccine
  - Measles, Mumps and Rubella (MMR) vaccine
  - Polio (Oral polio vaccine)
  - Smallpox vaccine.

Herd immunity develops following vaccination against:
- Diphtheria and Pertussis vaccine
- Measles, Mumps and Rubella (MMR) vaccine
- Polio (Oral polio vaccine)
- Smallpox vaccine

Adoptive Immunity

It is the process of transfer of CMI from one individual to other.

- It occurs following injection of immunologically competent T-lymphocytes known as Transfer factor.
- It is useful for treatment when the CMI is low, e.g. in lepromatous leprosy.

MULTIPLE CHOICE QUESTIONS

1. **Toll like receptors: correct statement is:** *(Recent Question 2015)*
   a. Antigen specific
   b. Acts by cytokine release
   c. Part of adaptive immunity
2. **Not true about innate immunity:** *(NEET Pattern Based)*
   a. Not influenced by hormones
   b. Dependent on genetic constitution
   c. Identical twins have same degree of resistance
   d. Not influenced by exposure to antigen
3. **All are true about innate immunity except:**
   a. Acts as first line of defense *(NEET Pattern Based)*
   b. Complements are examples
   c. Nonspecific
   d. Not effected by genetic influences
4. **Transfer factor is an example of:** *(NEET Pattern Based)*
   a. Artificial active immunity
   b. Natural active immunity
   c. Adoptive immunity
   d. Artificial passive immunity
5. **Components of innate immunity:** *(PGI Nov 2010)*
   a. T lymphocyte
   b. B lymphocyte
   c. Complements
   d. NK cells
   e. Integrins
6. **Innate immunity is stimulated by which part of bacteria?** *(DNB Dec 2011)*
   a. Carbohydrate sequence in the cell wall
   b. Flagella
   c. Bacterial cell membrane
   d. Nucleus
7. **Innate immunity active against viral cells:** *(AI 2007)*
   a. NK cells
   b. Cytotoxic T cells
   c. B cells
   d. Memory B cell
8. **All of the following are a part of the innate immunity except:** *(AIIMS May 2005)*
   a. Complement
   b. NK cells
   c. Macrophages
   d. T cells
9. **Active immunity can be induced by:** *(PGI May 2013)*
   a. Toxoids
   b. Subclinical infection
   c. Antitoxin
   d. Immunoglobulins
   e. Antigen exposure
10. **True about passive immunity:** *(PGI May 2013)*
    a. Cannot be given with active immunity
    b. Last for 4–5 days only
    c. It can be given before disease occurrence
    d. Can be transferred by antibodies from another Host
    e. Takes longer time to develop
11. **Superantigen is produced by:**
    a. Staphylococcus aureus *(Recent Question 2013)*
    b. Streptococcus pneumoniae
    c. Pseudomonas
    d. Clostridium

ACUTE PHASE REACTANTS AND CRP

12. **Span of C reactive protein half-life:** *(TNPG 2014)*
    a. 18 hrs
    b. 2 hrs
    c. 12 hrs
    d. 15 hrs

EXPLANATIONS

1. **Ans. (b) (Acts by .....)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p98

   TLR is receptor of innate immunity, antigen non specific.

2. **Ans. (a) (Not influenced by hormones)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p97, Ananthanarayan 9/e p78, 8/e p84, Kuby's Immunology 6/e p53
   - Innate immunity is influenced by hormones: Endocrine disorders like diabetes are associated with enhanced susceptibility to infection due to altered innate immunity.
   - Innate immunity is dependent on genetic constitution of the individual. Homogenous identical twins exhibit similar degree of innate immunity.
   - Innate immunity is nonspecific, it is not influenced by exposure to antigen.

3. **Ans. (d) (Not effected by genetic influences)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p97, Ananthanarayan 9/e p78, 8/e p83, Kuby's Immunology 6/e p53
   - Innate immunity refers to the resistance to infection that an individual possess from birth by its genetic or constitutional make up.
   - Other options are correct- for explanation, refer text.

4. **Ans. (c) (Adoptive...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p103, Ananthanarayan 9/e p81, 8/e p89, 150

   Adoptive immunity
   - Acquired by injection of immunologically competent T-lymphocytes known as Transfer factor
   - Used for treatment when the CMI is low, e.g. Lepromatous leprosy.

5. **Ans. (c) (d) (e) (Complements, NK cells, Integrins)** Apurba Sastry's Essentials of Medical Microbiology 1/e p97, Ref: Ananthanarayan 9/e p79, 8/e p83, Kuby's Immunology 6/e p53, Harrison 18/e p2654
   - Complement pathways (alternate and mannose binding , NK cells, and Pattern recognition receptors like Integrins are components of Innate immunity
   - For the detail list of components of Innate immunity- Refer text.

6. **Ans. (a) (Carbohydrate sequence in cell wall)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p97, Harrison 18/e p2654
   - Pattern recognition receptors are important component of innate immunity
   - Toll-like receptors are, e.g. of PRR Protein Family which bind to carbohydrate antigens on bacterial and viral surfaces.

   Examples of microbial ligands that bind to Pattern recognition receptors on host cells include:
   - Bacterial and viral carbohydrates residues on cell wall
   - Terminal mannose and Carbohydrate on HLA molecules
   - Lipopolysaccharide (LPS)
   - Viral DNA Bacterial muramyl dipeptide

7. **Ans. (a) (NK cells)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p97, Ananthanarayan 9/e p137, 8/e p131
   - Natural killer cells are components of innate immunity Kuby's Immunology 6/e p53
   - Natural killer cells possess spontaneous cytotoxicity towards virus infected cells and malignant cells and their cytotoxicity is not antibody dependent nor MHC restricted Ananthanarayan 8/e p131
   - About Other options: Cytotoxic T cells, B cells and Memory B cell are components of adaptive/acquire immunity Kuby's Immunology 6/e p53
   - Components of Innate immunity- refer text.

8. **Ans. (d) (T cells)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p97, Kuby's Immunology 6/e p53
   - Alternate pathways of Complement , NK cells and Macrophages (as phagocytes) are the components of innate immunity
   - B cell, T cell, Classical complement and Antigen presenting cell are components of adaptive/acquire immunity.

9. **Ans. (a) (b) (e) (Toxoids, Subclinical infection, Antigen exposure)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p102, Ananthnarayan 9/e p81-82
   - Active immunity can be induced by any substance that actively stimulates the immune system to produce antibody. Vaccines, toxoids, infection or antigen exposure can induce active immunity
   - Antitoxin and immunoglobulins are, e.g. of Passive immunity.

10. **Ans. (c) (d) (It can be given before disease occurrence, can be transferred by antibodies from another Host)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p103, Ananthanarayan 9/e p82-83
   - Immunoglobulins can be given along with vaccine in postexposure prophylaxis, e.g. Rabies immunoglobulins
   - Passive immunity last for days to months
   - Immunoglobulins should be given immediately after the exposure but before the disease occurrence
   - Passive immunity can be transferred between individuals by Serum therapy (antibodies)
   - Passive immunity works immediately

11. **Ans. (a) (Staphylococcus aureus)** Ref: Stite's Medical Immunology 10/e p152

ACUTE PHASE REACTANTS AND CRP

12. **Ans. (a) (18 hrs) Journal:** Apurba Sastry's Essentials of Medical Microbiology 1/e p101, C-reactive protein: a critical update, J Clin Invest. 2003.

    The plasma half-life of CRP is about 19 hours and is constant under all conditions of health and disease, so that the sole determinant of circulating CRP concentration is the synthesis rate.

Antigen, Antibody, Antigen-Antibody Reaction, Complement

CHAPTER 2.2

Antigen has two distinct properties:
- Immunogenicity: Ability of Ag to induce immune response (CMI/AMI)
- Immunological reactivity: Ability of Ag to combine with the final products of the above two responses (i.e., antibodies and/or T-cell-surface receptors).

ANTIGEN

Antigen is defined as any substance that satisfies two distinct immunologic properties:

1. **Immunogenicity:** Ability of an antigen to induce immune response in the body (both humoral and/or cell mediated).
   - B-cells + antigen → effector B-cells (plasma cell) + memory B-cells
   - T-cells + antigen → effector T-cells (helper or cytotoxic T-cell) + memory T-cells
2. **Antigenicity (immunological reactivity):** Ability of an antigen to combine specifically with the final products of the above two responses (i.e., antibodies and/or T-cell-surface receptors).
   All molecules having immunogenicity property, also show antigenicity, but the reverse is not true (e.g. haptens– which are antigenic, but not immunogenic).

Epitope

Epitope or *antigenic determinant* is the smallest unit of antigenicity:
- It is defined as a small area present on the antigen that is capable of sensitizing T- and B-cells and reacting with specific site of T-cell receptor or an antibody.
- The specific site of an antibody that reacts with the corresponding epitope of an antigen is called as *paratope*.

Epitopes may be grouped into two types:

1. **Sequential** or **linear** epitope: Present as a single linear sequence of few amino acid.
2. **Conformational** or **nonsequential** epitopes are found on the flexible region of complex antigens having tertiary structures.

In general, T-cells recognize sequential epitopes, while B-cells bind to the conformational.

Haptens

Hapten-carrier conjugate produce antibodies against:
- Epitopes of hapten
- Unaltered epitopes on the carrier protein
- New epitopes formed by combined parts of both the hapten and carrier

Haptens are low molecular weight molecules that:
- *Lack immunogenicity* (cannot induce immune response), but:
- *Retain antigenicity* or immunological reactivity (i.e. can bind to their specific antibody or T-cell receptor).

How ever, Haptens can become immunogenic when combined with a larger protein molecule called 'carrier'.

It is observed that hapten-carrier conjugate induce antibodies specific for:
- Epitopes of hapten
- Unaltered epitopes on the carrier protein and
- New epitopes formed by combined parts of both the hapten and carrier

Haptens may be classified as complex or simple:
- **Complex haptens** contain two or more epitopes; they can react with specific antibodies and the hapten-antibody complex can be visualized by various methods such as precipitation reaction.
- **Simple haptens** usually contain only one epitope (univalent). Such haptens can bind to the antibodies but the hapten antibody complex cannot be not visualized as it is believed that precipitation to happen, it requires the antigen to have at least two or more epitopes.

Factors Influencing Immunogenicity

There are various factors that influence immunogenicity of an antigen:

1. **Size of the antigen:** Larger is the size (e.g. hemoglobin), more is the immunogenicity.

2. **Chemical nature:** Proteins are stronger immunogens than carbohydrates followed by lipid and nucleic acids.
3. **Susceptibility of antigen to tissue enzymes** – It increases immunogenicity by exposing more epitopes of the Ag.
4. **Structural complexity** of the antigen increases immunogenicity.
5. **Foreignness to the host:** More is the foreignness of Ag, more is the immunogenicity.
6. **Genetic factor**
7. **Optimal dose of antigen can only induce immune response.** A too little dose fails to elicit immune response and a too large dose causes *immunological paralysis.*
8. **Route of antigen administration**
9. **Repeated doses of antigens over a period of time**
10. **Multiple antigens:** One antigen may diminish (due to antigenic competition) or enhance (due to adjuvant like action) the immunogenicity of other antigen.
11. **Heterophile nature of the antigens** (explained below).
12. **Adjuvant** (explained below)

Chemical nature of antigen:
- Proteins are stronger immunogens than carbohydrates followed by lipid and nucleic acids.

Adjuvant

The term *adjuvant* refers to any substance that enhances the immunogenicity of an antigen. They are usually added to vaccines to increase the immunogenicity of the vaccine antigen. Examples of adjuvant include:

- *Alum* (aluminum hydroxide or phosphate)
- *Mineral oil* (liquid paraffin)
- *Freund's incomplete adjuvant*: It is a water-in-oil emulsion; with Ag in the aqueous phase.
- *Freund's complete adjuvant* is the mixture of Freund's incomplete adjuvant and killed tubercle bacilli in the oil phase.
- LPS of *Bordetella pertussis* acts as an excellent adjuvant for diphtheria and tetanus toxoids.
- Other bacteria or their products:
  - *Mycobacterium bovis*
  - Toxoid (DT and TT act as adjuvant for *Haemophilus influenzae*-b vaccine)
- Nonbacterial products: Silica particles, beryllium sulfate, squalene, and thimerosal.

Adjuvant examples:
- Alum
- Mineral oil (liquid paraffin)
- Freund's adjuvant
- LPS of Bordetella pertussis
- Other bacterial products
  - Mycobacterium bovis
  - Toxoid (DT and TT)
- Nonbacterial products: Silica particles, beryllium sulfate, squalene, and thimerosal.

Heterophile Antigens

Heterophile antigens share epitopes with each other. Antibody produced against antigen of one species can react with the other and vice versa:

- *Weil–Felix reaction* is done for diagnosis typhus fever. Antibodies against rickettsial antigens are detected by using cross reacting *Proteus* antigens.
- *Paul-Bunnell test* is done for infectious mononucleosis (caused by EBV). Here, sheep red blood cell (RBC) antigens are used to detect cross-reacting antibodies in patient's sera.
- *Cold agglutination test* and *Streptococcus MG* test are done for primary atypical pneumonia. Here, antibodies against *Mycoplasma pneumoniae* are detected by using human O blood group RBC and *Streptococcus* MG antigens respectively.
- *Forssmann antigen* is universal heterophile antigen, present in all animals, plants and bacteria, but absent in rabbits.

Heterophile antigens: Applications
- Weil-Felix reaction
- Paul-Bunnell test
- Cold agglutination test
- Streptococcus MG
- Forssmann antigen

Biological Classes of Antigens

Depending on the mechanisms of inducing antibody formation, antigens are classified as T-cell dependent (TD) and T-cell independent (TI) antigens.

T-dependent (TD) Antigens

Most normal antigens are T-cell dependent, they are processed and presented by antigen presenting cells (APCs) to T-cells. The activated T-cells secrete cytokines that in turn stimulate the B-cells to produce antibodies.

T-independent (TI) Antigens

There are a few antigens such as *bacterial capsule, flagella* and *LPS* that do not need the help of T-cells and APCs. They directly bind to Ig receptors present on B-cells and stimulate B-cells polyclonally leading to hypergammaglobulinemia.

T-independent antigen	T-dependent antigen
Structurally simple: LPS, capsular polysaccharide, flagella	Structurally complex: protein in nature
Dose dependent immunogenicity	Immunogenic over wide range of dose
No memory	*Memory present*
No antigen processing	Antigen processing step is needed
Slowly metabolized	Rapidly metabolized
Activate B-cells polyclonally	Activate B-cells monoclonally
Activate both mature and immature B-cells	Activate mature B-cells only
B-cells stimulated against T independent antigen do not undergo-*Affinity maturation* and *Class switch over*	B-cells stimulated against T dependent antigen undergo *Affinity maturation* and *Class switch over*
Antibody response is restricted to IgM and IgG3	Antibodies of all classes can be produced

Superantigens

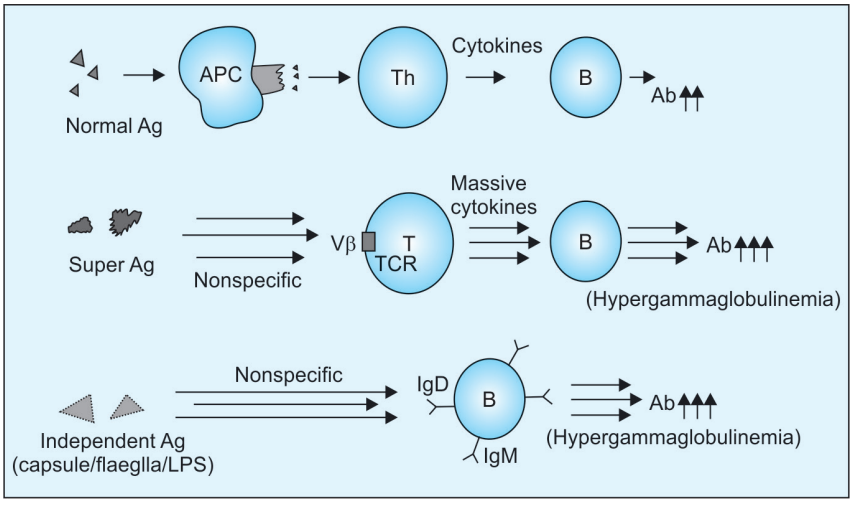
Superantigens are the third variety of biological class of antigens, recently described in the last decade. The unique feature of superantigens is, they can activate T-cells directly without being processed by antigen presenting cells (APCs).

- The variable β region of T-cell receptor (vβ of TCR) appears to be the receptor for superantigens.
- They directly bridge non-specifically between MHC-II of APCs and T-cells.

Superantigens:
- Acts directly on T cell, without APC involvement
- Receptor of super Ag-variable β region of T-cell receptor

Examples of Superantigens

- Bacterial superantigen:
  - Staphylococcal toxin: TSST, Exfoliative toxin, Enterotoxins
  - Streptococcal toxin: Streptococcal pyrogenic exotoxin (SPE)-A and C
  - Mycoplasma arthritidis mitogen-I
  - *Yersinia enterocolitica*
  - *Yersinia pseudotuberculosis*
- Viral: EBV, CMV, Rabies nucleocapsid, HIV encoded nef (negative regulatory factor)
- Fungal superantigen: *Malassezia furfur*



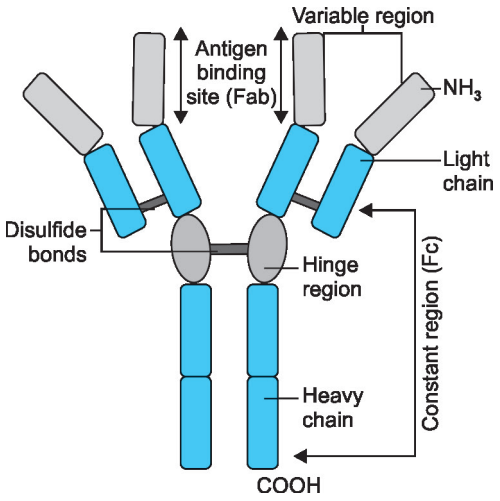


Fig. 2.2.1: Mode of action of various antigens

ANTIBODY

Structure of Immunoglobulins

Antibody or immunoglobulin is a 'Y- shaped' heterodimer;composed of four polypeptide chains: two light (L) chains and two heavy (H) chains:

- All four H and L chains are bound to each other by *disulfide bonds*, and by noncovalent interactions such as salt linkages, hydrogen bonds, and hydrophobic bonds.
- Chains have two ends: An aminoterminal end (NH_3) and a carboxylterminal end (COOH).
- There are five classes of H chains (γ, α,μ,δ and ε) and two classes of light chains (κ and λ).
- Any antibody contains only one type of light chain and one type of heavy chain.
- Based on the constant region of the heavy chains (γ, α,μ,δ and ε); Ig has been classified into five types; lgG, IgA, IgM, IgD and IgE respectively.
- Each H and L chain comprises of two regions: Variable region and constant region
- Within the variable region, there are some zones or hot spots called as hypervariable regions or complementarity determining regions that show higher variability. There are three hot spots in the L and four in the H chain.
- Paratope is the site on the hypervariable regions that make actual contact with the epitope of an antigen.
- **Hinge region:**
  - It is the junction formed between constant region of heavy chains of IgG, IgA and IgD. It is absent in IgE and IgM.
  - This region is rich in proline and cysteine. The hinge region is quite flexible, thus helps the antibody in reaching towards the antigen.
- **Enzymatic digestion:**
  - *Papain digestion*: Papain cleaves the Ig molecule at a point above the hinge region; resulting in three fragments: *Two Fab fragments* (Ag binding fragment) and one *Fc fragment* (crystallisable fragment)
  - *Pepsin digestion:* Pepsin cleaves the Ig molecule at a point below the hinge region; resulting in formation of-*One F(ab')2 fragment and Many smaller fragments*
  - *Mercaptoethanol digestion:* Generates four fragments (two H and 2 L chains) as it cleaves only disulphide bonds sparing the peptide bonds.
- **Immunoglobulin chains coded by different chromosomes**
  - Heavy chains: It is coded by chromosome -14
  - Light chain kappa: It is coded by chromosome -2
  - Light chain lambda: It is coded by chromosome -22.
  - After the synthesis, germ line recombination (rearrangement) of chains occurs.

Enzymatic digestion:
- Papain digestion: generates Two Fab and one Fc fragment
- Pepsin digestion: generates One F(ab')2 and Many smaller fragments
- Mercaptoethanol digestion: generates four fragments (two H and 2 L chains)

Functions of Immunoglobulins

- Antigen binding (by Fab region)
- Effector functions (by Fc region)
  - *Fixation of complement:* Antibody coating the target cell binds to complement through its Fc receptor which leads to complement mediated target cell lysis
  - *Binding to various cell types:* Phagocytic cells, lymphocytes, platelets, mast cells, NK cell, eosinophils and basophils bear Fc receptors (FcR) that bind to Fc region of immunoglobulins.

Immunoglobulin chains coded by different chromosomes:
- Heavy chains is coded by chromosome-14
- Light chain kappa is coded by chromosome-2
- Light chain lambda is coded by chromosome-22

Properties of Various Immunoglobulin

IgG Antibody:
- *IgG is highest for **DHS** (decreasing order for DHS is—GAMDE i.e. highest is IgG and lowest IgE):*
  - **D**aily production,
  - **H**alf life (23 days),
  - **S**erum concentration

- Four subtypes: IgG1-4 (Decreasing order for DHS is IgG 1 > IgG 2 > IgG 3 > IgG 4)
  - IgG is Responsible for:
  - Precipitation,
  - Neutralization,
  - NK cell binding (to perform ADCC)
  - Classical complement binding (IgM > IgG 3 > IgG 1 > IgG2) (IgG 4 does not fix complement)
  - Coagglutination by binding to *S.aureus* protein A (Except IgG 3)
  - Opsonization
- IgG appears late, so indicates past/chronic infection
- IgG avidity increases with time – So, detection of less avidity IgG indicates relatively recent infection
- Secreted in placenta (Maximum placental transfer IgG1, minimum IgG2)
- Secreted in breast milk
- Helps in phagocytosis by binding to FcR on phagocytes (Except IgG2)

IgG is Responsible for:
- Precipitation
- Neutralization
- NK cell binding (to perform ADCC)
- Classical complement binding
- Coagglutination by binding to *S.aureus* protein A (Except IgG3)
- Opsonization

Ig E Antibody:
- It is the only Heat labile antibody
- Lowest for *DHS*
- Responsible for- Type I hypersensitivity reaction
- Homocytotropic (Species specific) antibody
- Also called as Reagin antibody
- Raised in helminthic infections

IgA Antibody:

IgA is the second most abundant antibody (2nd highest for DHS). It is of two types:
- Serum IgA: Predominantly in monomeric form.
- Secretory IgA (SIgA): It is dimeric (valency four); both are joined by J chain. In addition, there is another joining segment called **secretory component** (synthesized by mucosal epithelium).
- Secretory IgA is responsible for Mucosal /local immunity.

IgA also exist in two subclasses/isotypes: IgA is mainly found in serum. IgA2 predominates in secretions.

Ig D Antibody:
- Surface immunoglobulin on the surface of B-cells
- Possess highest carbohydrate content

IgM Antibody:
- *IgM is highest for **MIS***:
  - **M**olecular weight (900,000),
  - **I**ntravascular distribution (blood Antibody) (80%),
  - **S**edimentation coefficient (19),
- Pentameric in nature with 10 valency
- IgM (and IgD) act as surface immunoglobulin on the surface of B-cells
- IgM is the first antibody to appear following infection, indicates **recent infection**
- IgM is the first antibodyto appear in intrauterine life also (20 weeks): Indicates congenital infection
- IgM is responsible for (or mediates):
  - Agglutination,
  - Haemolysis,
  - Opsonization,
  - Classical complement pathway binding

Example:
- Antibody in typhoid,
- Reagin Antibody (syphilis)
- Natural antibody of ABO, Rh system.

IgM is responsible for (or mediates):
- Agglutination
- Hemolysis
- Opsonization
- Classical complement pathway binding

Abnormal Immunoglobulin:
- Bence Jones proteins
- Waldenstrom's macroglobulinemia
- Heavy cthain disease
- Cryoglobulinemia

Abnormal Immunoglobulin

1. **Bence Jones Proteins:** They are produced in multiple myeloma (light chain disease).
   - The cancerous plasma cells produce excess of light chain (Bence Jones proteins) which are accumulated in patient's serum and excreted in urine.
   - Such proteins have a unique property of getting coagulated at 50°C and redissolving again at 70°C.

2. **Waldenstrom's Macroglobulinemia:** It is lymphoma affecting B-cells producing excess IgM. It has been seen in multiple myeloma. Somatic mutations in MYD 88 gene occur in over 90% of patients.
3. **Heavy Chain Disease:** It is characterized by an excessive production of heavy chains that are short and truncated. Four types of heavy chain disease have been recognized based on H chain involved—alpha (Seligmann's disease), gamma (Franklin's disease), mu and delta chain disease.
4. **Cryoglobulinemia:** It is a condition where the blood contains cryoglobulins; a type of Ig that becomes insoluble (precipitate) at low temperatures but redissolves again if the blood is heated:
   - Cryoglobulins usually consist of IgM directed against the Fc region of IgG.
   - They have been associated with multiple myeloma and hepatitis C infection.

Ig Specificity or Antigenic Determinants of Ig

Isotypes

The five classes of Igs (lgG, IgA, IgM, IgD and IgE) and their subclasses are called as isotypes, they vary from each other in the amino acid sequences of the constant region of their heavy chains. Such variation is called as isotypic variation.

Idiotypes

The unique amino acid sequence present in paratope region (in V_H and V_L regions) of one member of a species acts as antigenic determinant to other members of the same species:

- Such antigenic determinants are called as idiotopes. Immunoglobulins vary from each other in their idiotopes present in variable region, which is called as idiotypic variation.
- Idiotypes in an individual arise continuously from mutations (*somatic hypermutations*) in the genes of variable region. Hence, idiotypes may act as foreign to the host itself; however do not evoke *autoimmune response* because they are present in small numbers.

Allotypes

The antigenic determinants present in the isotype genes in the constant region of H and L chains, encoded by *multiple alleles* are called as allotypes:

- Although all members of a species inherit the same set of isotype genes, multiple alleles exist for some of the allele genes. Hence, allotypes are present in the constant region of Ig molecules of the same class, in some, but not all, members of a species.
- They have been characterized—kappa light chain, γ and α heavy chains.
- Anti allotype specific antibodies may also be developed following blood transfusion or by maternal passage of IgG into the fetus.

- Epitope: Antigenic determinant of Ag against which Ab is raised
- Paratope: Specific site of Ab that reacts with the corresponding epitope
- Idiotope: Antigenic determinant of Ab against which Ab is raised, vary in variable region of H & L chain
- Isotope: Classes and subclasses of Ab, which vary in constant region of H chain

Monoclonal Antibody

Monoclonal antibodies (mAb) are defined as the antibodies derived from a single clone of plasma cell; all having the same antigen specificity— i.e. produced against a single epitope of an antigen.

Hybridoma technique is used to produce mAb (Nobel prize Gwardee Kohler and Milstein):

- Clone of mouse splenic B-cell stimulated against a single epitope of antigen is fused with an immortal cell, e.g. myeloma cell (capable of multiplying indefinitely) to produce a hybridoma cell. This hybridoma cell has two unique properties:
  - Produces mAb of same antigen specificity (due to B-cell component)
  - Multiplies indefinitely producing clone of identical cells (due to myeloma cell)
- However, in the reaction chamber, along with hybridoma cells, there will be some unfused mouse B-cell and unfused myeloma cells which are removed by sub-culturing the fluid on a special medium called HAT media.
- **HAT medium** contains hypoxanthine, aminopterin and thymidine.
  - Purine synthesis in mammalian cell (e.g. splenic B-cell) occurs by either de novo or salvage pathways.

- Aminopterin blocks the de novo pathway so that the cell has to perform the salvage pathway to synthesize purines for its survival.
- Salvage pathway requires two important enzymes: HGPRT (hypoxanthine guanine phosphoribosyl transferase) and thymidine kinase.
- So any cell (e.g. myeloma cell) that lacks HGPRT cannot grow on HAT medium.

Fate of three type of cells on HAT media:
- *Unfused splenic B-cells:* Can grow but do not survive long as they are not immortal.
- *Unfused myeloma cells:* Cannot grow as they lack HGPRT enzyme to perform the salvage pathway.
- *Hybridoma cells:* Can grow and survive long.

- **Fate of three type of cells on HAT media:**
  - Unfused splenic B-cells: Can grow but donot survive long as they are not immortal.
  - Unfused myeloma cells: Cannot grow as they lack HGPRT enzyme to perform the salvage pathway.
  - Hybridoma cells: Can grow and survive long.
- **Selection of individual hybridoma cells**: By radioimmunoassay or ELISA using the specific antigen fragments

Types of Monoclonal Antibodies

Types of monoclonal antibodies:
- *Mouse mAb* contains 100% mouse derived proteins.
- *Chimeric mAb:* 34% mouse proteins (variable region) and 66% human proteins (constant region).
- *Humanized mAb:* Ag binding site is mouse derived (10%) and the remaining human derived.
- *Human mAb:* 100% of amino acids are human derived.

- **Mouse mAb** contains 100% mouse derived proteins. The mouse proteins being foreign; can induce immune response in humans producing human anti-mouse antibody (HAMA); that in turn eliminate the mAb faster from the body.
- **Chimeric mAb** is prepared by recombination of 34% mouse proteins (variable region) and 66% human proteins (constant region).
- **Humanized mAb**: Here, only the antigen binding site is mouse derived (10%) and the remaining part of mAb is human derived.
- **Human mAb**: 100% of amino acids are human derived. It is the best accepted mAb.

ANTIGEN ANTIBODY REACTIONS

Antigen (Ag): Antibody(Ab) reactions are characterized by the following general properties:

Noncovalent interactions of Ag-Ab Reaction:
- Hydrogen bonds
- Electrostatic interactions
- Hydrophobic interactions
- van der Waal forces

- **Specific:** Involves specific interaction of epitope of an antigen with the corresponding paratope of its homologous antibody.
- **Non-covalent interactions** exist between antigen and its antibody such as:
  - Hydrogen bonds
  - Electrostatic interactions
  - Hydrophobic interactions
  - van der Waal forces
- **Strength:** The strength or the firmness is influenced by the affinity and avidity
  - **Affinity:** It refers to sum total of non-covalent interactions between a single epitope of an antigen with its corresponding paratope present on antibody. It can be measured by: (i) by equilibrium dialysis and (ii) by surface plasmon resonance method
  - **Avidity:** It is a term used to describe the affinities of all the binding sites when multivalent antibody reacts with a complex antigen carrying multiple epitopes.

Marrack's Lattice Hypothesis

Marrack's Lattice Hypothesis:
- *Zone of equivalence:* Ag and Ab level equal
- *Prozone:* Excess Antibody
- *Prozone:* Excess Antigen

When the sera containing antibody is serially diluted (in normal saline), gradually the antibody level decreases. To such a set of test tubes containing serially diluted sera, when a fixed quantity of antigen is added, then:
- In the middle tubes, Ag-Ab reaction occurs at its best, because the amount of antigen and antibody are equivalent to each other (*zone of equivalence*).
- In the earlier test tubes, *antibodies are excess*, hence the Ag-Ab reaction does not occur: This is called as **prozone phenomenon.**
- In the later test tubes, *antigen is excess*, hence the Ag-Ab reaction fails to occur: This is called as **postzone phenomenon.**

This lattice hypothesis holds true for any Ag-Ab reactions.

TYPES OF ANTIGEN-ANTIBODY REACTIONS

- Conventional techniques: Precipitation, Agglutination reaction, Complement fixation test and Neutralization test
- Newer techniques: ELIS, IFA, RIA, CLIA, Immunohistochemistry, Rapid tests (Lateral flow assay or ICT and Flow through assay), Western blot and Immunoassays using electron microscope.

Precipitation Reaction:
- Uses soluble antigen
- Ab is mainly IgG type

Precipitation Reaction

When a *soluble antigen* reacts with its antibody in the presence of optimal temperature, pH and electrolytes (NaCl), it leads to formation of the antigen-antibody complex in the form of:

- Insoluble precipitate band when gel containing medium is used or
- Insoluble floccules when liquid medium is used.

A. Precipitation in Liquid Medium

- Ring test: Streptococcal grouping by Lancefield technique, and Ascoli's thermoprecipitation test done for anthrax.
- Slide flocculation test: VDRL and RPR tests used for diagnosis of syphilis.
- Tube flocculation test: Kahn test used previously for syphilis.

B. Precipitation in Gel (Immunodiffusion)

It has many advantages over liquid medium: (i) Clear visible bands are formed, which can be preserved for longer time, (ii) Individual antigens from a mixture can be differentiated, e.g.

- Single diffusion in one dimension (Oudin procedure)
- Double diffusions in one dimension (Oakley–Fulthorpe procedure)
- Single diffusion in two dimensions (Radial immunodiffusion)
- Double diffusions in two dimensions (Ouchterlony procedure), e.g. Elek's test (diphtheria toxin) and Eiken test (*E.coli* toxin).

C. Precipitation in Gel in Presence of Electric Current

The movement of Ag and Ab can be made faster if immunodiffusion in gel is carried out in presence of electric current. Examples include:

- Electroimmunodiffusion (EID)
- CIEP (Countercurrent immunoelectrophoresis)
- Rocket electrophoresis.

Agglutination Reaction:
- Uses particulate or insoluble antigen
- Ab is mainly IgM type
- More sensitive, easy to interpret

Agglutination Reaction

When a **particulate** or **insoluble** antigen is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated.

Agglutination is more sensitive than precipitation test and the clumps are better visualized and interpreted.

Diagnostic Applications of Agglutination Tests

Slide agglutination: To confirm the identification and serotyping of bacterial colonies grown in culture.

Tube agglutination is routinely used for:

- Typhoid fever (Widal test): Detects Ab against both H (flagellar) and O (somatic) Ag
- Acute brucellosis (Standard agglutination test)
- Blood grouping (ABO and Rh grouping)
- Coombs test or Antiglobulin test: Detects incomplete Rh antibodies:
  - Direct Coombs test: Detects bound Rh antibodies in fetus/baby's serum
  - Indirect Coombs test: Detects free Rh antibodies present in maternal serum.

Coombs test or Antiglobulin test:
Detects incomplete Rh Ab-
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- Indirect Coombs test: Detects free Rh antibodies present in maternal serum

- Heterophile agglutination tests:
  - Typhus fever (Weil–Felix reaction)
  - Infectious mononucleosis (Paul Bunnell test)
  - Mycoplasma pneumonia (Cold agglutination test).

Microscopic agglutination test (MAT) for leptospirosis

Indirect or passive agglutination test: Antigen is coated on carriers such as Latex or RBCs to detect Ab in serum. Examples:
- Indirect hemagglutination test (IHA)
- Latex agglutination test (LAT) for antibody detection' e.g. ASO.

Reverse passive agglutination test: Antibody is coated on carriers such as Latex or RBCs to detect Ag in serum. Examples:
- RPHA (Reverse passive hemagglutination assay), e.g. HBsAg detection
- Latex agglutination test for antigen detection, e.g. CRP, RA factor, capsular antigen in CSF and streptococcal grouping.
- Coagglutination test (here, *S. aureus* protein A is used as carrier).

Complement Fixation Test

CFT detects complement fixing antibodies in patient's serum. It is now almost obsolete:
- *Wasserman test* was the most popular CFT, used for the diagnosis of syphilis.
- CFT was also widely used for detection of antibodies in *Rickettsia, Chlamydia, Brucella, Mycoplasma* infections and some viral infections such as arboviruses, rabies, etc.
- *Indirect complement fixation test:* Detects certain avian (e.g. duck, parrot) and mammalian (e.g. horse, cat) serum antibodies which cannot fix guinea pig complement.
- *Conglutination* test: To perform CFT using nonhemolytic complements, e.g. horse complements.

Complements are also used for various serological tests, other than CFT such as:
- *Treponema pallidum* immobilization test (for detecting antibodies to *T. pallidum*)
- Sabin-Feldman dye test for detecting *Toxoplasma* antibodies.
- Vibriocidal antibody test for *V. cholerae.*

Neutralization tests,e.g.
- Viral neutralization test
- Plaque inhibition test-for bacteriophages
- Toxin-antitoxin neutralization test:
  - Schick test for *C. diphtheriae*.
  - Nagler's reaction for *C.perfringens*
  - ASLO detection
- Hemagglutination inhibition (HAI) test

Neutralization Test

Neutralization tests are also less commonly used in modern days. Examples include:
- Viral neutralization test: Detects viral neutralizing antibodies
- Plaque inhibition test: Done for bacteriophages.
- Toxin-antitoxin neutralization test:
  - Schick test for *Corynebacterium diphtheriae.*
  - Nagler's reaction: Due to α-toxin of *Clostridium perfringens*
  - ASLO detection in past (now it is done by latex agglutination)
- Hemagglutination inhibition (HAI) test.

NEWER TECHNIQUES OF ANTIGEN ANTIBODY REACTION

The newer techniques use a detector molecule to label antibody or antigen which in turn detects the corresponding antigen or the antibody in the sample by producing a visible effect. Most of the newer techniques use the same principle, but they differ from each other by the type of labeled molecule used and the type of visible effect produced.

Abbreviation	Immunoassay method	Molecules used for labeling	Type of visible effect
ELISA	Enzyme linked immunosorbent assay	Enzyme	Color change is detected by spectrophotometer
IFA	Immunofluorescence Assay	Fluorescent dye	Emits light, detected by fluorescence microscope

Contd...

Contd...

Abbreviation	Immunoassay method	Molecules used for labeling	Type of visible effect
RIA	Radioimmunoassay	Radioactive isotope	Emits β and γ radiations, detected by β and γ counters
CLIA	Chemiluminescence-linked immunoassay	Chemiluminescent compounds	Emits light, detected by luminometer
IHC	Immunohistochemistry	Enzyme or Fluorescent dye	Color change (naked eye) or Fluorescence microscope
WB	Western blot	Enzyme	Color band (naked eye)
Rapid test	Immunochromatographic test	Colloidal gold or silver	Color band, (naked eye)
	Flow through assay	Protein A conjugate	Color band, (naked eye)
IEM	Immunoferritin electron microscopy	Electron dense molecules (e.g ferritin)	Appears as black dot under electron microscope

ELISA (Enzyme Linked Immunosorbent Assay)

ELISA is so named because of two of its components:

- Immunosorbent: It is an absorbing material used (e.g. polystyrene, polyvinyl), that specifically absorbs the antigen or antibody present in serum.
- Enzyme is used to label one of the components of immunoassay (i.e. antigen or antibody).

ELISA is the method of choice in big laboratories as large number of samples can be tested together using the 96 well microtiter plate. But it is not preferred in small laboratories:

- It is economical, takes 2-3 hours (rapid tests take 10–20 min)
- ELISA is the ***most sensitive*** immunoassay, thus is the preferred screening test at blood banks and tertiary care sites.
- Its specificity used to be low. But now, with use of more purified recombinant and synthetic antigens, and monoclonal antibodies, ELISA has become more specific.
- It needs expensive equipments such as ELISA washer and reader.

ELISA type	Used for detection of	Enzyme is labeled with
Direct ELISA	Antigen	Primary antibody
Indirect ELISA	Antibody or antigen	Secondary antibody
Sandwich ELISA	Antigen	Primary antibody in sandwich direct ELISA Secondary antibody in sandwich indirect ELISA
Competitive ELISA	Antigen or antibody	Secondary antibody
ELISPOT	Cells producing antibody or cytokine	Primary antibody

Note: Primary antibody is directed against the antigen, Secondary antibody is an anti-human (or other species) Ig directed against Fc region of any human/other species Ig.

Immunofluorescence Assay

It is a technique similar to ELISA, but differs by some important features:

- Fluorescent dye is used instead of enzyme for labelling
- Detects cell surface antigens or antibodies bound to cell surface antigens, unlike ELISA which detects free Ag or Ab.

Types of Immunofluorescence Assays:

- Direct Immunofluorescence Assay: Detects cells carrying surface antigens
- Indirect Immunofluorescence Assay: This detects antibodies bound to cell surface Ag
- Flow cytometry: This is a laser-based fluorescence technology detects 4 properties:
  - *Cell Counting, e.g.* CD4 T-cell count
  - *Differentiating between two cells*, e.g. CD4 and CD8 T-cells

Flow cytometry detect four important properties:
- Cell Counting, e.g. CD4 T-cell count
- Differentiating between two cells, e.g. CD4 and CD8 T-cells
- Scattering of cells:
  - Forward scatter: Refers to size of the cell
  - Side scatter: Refers to intracellular complexity
- Sorting out of cells from a mixture

  - *Scattering of cells*:
    - Forward scatter is proportional to *size of the cell*; larger cells have greater forward scatter.
    - Side scatter is proportional to *intracellular complexity*; cells with more granules (e.g. neutrophils) have more side scatter than cells with a simple cytoplasm (lymphocytes).
  - *Sorting out of cells from a mixture.*

Western Blot

Western blot detects specific proteins (antibodies) in a sample containing mixture of antibodies each targeted against different antigens of same microbe:
- It is so named for its similarity to Southern blot (detects DNA fragments) and Northern blot (detects mRNAs).
- The **Eastern blot** is the latest addition to the list; it is a modification of Western blot, which detects the carbohydrate epitopes present on proteins or lipids.

Western blot comprises of three basic components:
1. Separation of complex protein antigen mixture into fragments by SDS PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis)
2. NCM blotting: The antigen fragments in the gel are transferred (blotted) to the Nitrocellulose membrane (NCM)
3. Enzyme immunoassay: Detects specific antibodies separately in patient's serum containing mixture of antibodies.

Western blot Three Steps:
- Separation of Ag mixture into fragments by SDS PAGE
- NCM blotting: Ag fragments in the gel are transferred to the NCM
- Enzyme immunoassay: Detects specific Ab separately in sample against Ag fragments blotted on NCM

Rapid Test

Rapid tests are revolutionary in the diagnosis of infectious diseases:
- They are very simple to perform (one step method), rapid (result obtained in 10–20 min), require minimal training, does not need any sophisticated instruments.
- These tests are also called **Point of care** (POC) tests, because unlike ELISA and other immunoassays, the Point of Care tests can be performed independent of laboratory equipment and deliver instant results.

Two principles of rapid tests are available:
- **Lateral flow assay** or Immunochromatographic test (ICT): (i) colloidal gold or silver is used for labelling, (ii) the sample flows *laterally* through the NCM. Example includes antigen detection in malaria
- **Flow through assay**: (i) Protein A is used for labelling, instead of gold conjugate and (ii) the sample flows *vertically* through the NCM as compared to lateral flow in ICT. Example includes HIV tridot test.

Rapid tests: Two types
- Lateral flow assay or Immunochromatographic test (ICT)
- Flow through assay

COMPLEMENT

Complement are a group of proteins normally found in serum in inactive form, but when activated they augment the immune responses:
- Constitute about 5% of normal serum proteins
- Antigen nonspecific: Their level does not increase following either infection or vaccination.
- Species nonspecific
- Heat labile: 56°C for 30 minutes
- Binds to Fc region of antibody: IgM (binds strongly) followed by IgG3 → 1 → 2
- Site of Synthesis of Complements: Mainly by liver, also by GIT, macrophage and spleen.

Complement Pathways

There are three pathways of complement activation *(Refer figure 2.2.2)*:
1. *Classical pathway*: This is an Ab dependent pathway, triggered by the Ag-Ab complex.
2. *Alternative pathway*: This is an Ab independent pathway, triggered by the antigen directly.
3. *Lectin pathway* resembles classical pathway, but it is Ab independent.

Stages of Complement Activation

There are four main stages in the activation of any of the complement pathways:

1. Initiation of the pathway
2. Formation of C3 convertase
3. Formation of C5 convertase
4. Formation of membrane attack complex (MAC)

All the three pathways (figure) differ from each other in their initiation till formation of C3 convertase. Then, the remaining stages are identical in all the pathways.

Biological Role of Complement

- Target cell lysis: MAC makes pores or channels in the target cell membrane
- Inflammatory response: Complement by-products such as C3a, C4a and C5a act as anaphylatoxins and chemotactic.
- Opsonization: C3b and C4b act as major opsonins
- Mediate hypersensitivity reaction II and III
- Removes the immune complexes from blood to spleen- by C3b
- Immune adherence: CR2 acts as EBV receptors
- Kinin like activity (↑vascular permeability): C2b
- Viral neutralization

Biological Role of Complement:
- Target cell lysis
- Inflammatory response- C3a, C4a and C5a
- Opsonization-C3b and C4b
- Mediate HSN II and III
- Removes the immune complexes from blood to spleen- byC3b
- Immune adherence- CR2 acts as EBV receptors
- Kinin like activity- C2b
- Viral neutralization

Table 2.2.1: Differences between three complement pathways

	Classical	Alternative pathway	Lectin pathway
Activator (initiator)	Antigen antibody complex	Endotoxin IgA, IgD, Cobra venom Nephritic factor	Carbohydrate residue of bacterial cell wall (mannose binding protein) that binds to host lectin antigen.
1st complement activated	C1	C3b	C4
C3 convertase	C14b2a	C3bBb	MBL/MASP-C4b2a
C5 convertase (C3 convertase + 3b)	C14b2a3b	C3bBb3b	MBL/MASP-C4b2a3b
Complement level in the serum	All C1-C9: Low	C1,C4,C2- Normal Others- Low	C1- Normal Others- Low
Immunity	Acquired	Innate	Innate

Table 2.2.2: Complement deficiency diseases

i. Complement protein deficiencies	**Pathway(s) involved**	**Disease/pathology**
C1, C2, C3, C4	C1, C2,C4-Classical pathway C3- Common deficiency	SLE, glomerulonephritis and pyogenic infections
Properdin, Factor D	Alternative pathway	*Neisseria* and pyogenic infection
Membrane attack complex (C5-C9)	Common deficiency	Disseminated *Neisseria* infection
ii. Complement regulatory protein deficiencies		**Diseases**
C1 esterase inhibitor	Overactive classical pathway	Hereditary angioneurotic edema
ii. Complement regulatory protein deficiencies		**Diseases**
DAF (Decay accelerating factor) and CD59	Deregulated C3 convertase Increased RBC lysis	PNH (Paroxysmal nocturnal hemoglobinurea)
Factor I	Deregulated classical pathway with over consumption of C3	Immune complex disease; recurrent pyogenic infections
Factor H	Deregulated alternative pathway with increased C3 convertase activity	Immune complex disease; pyogenic infection

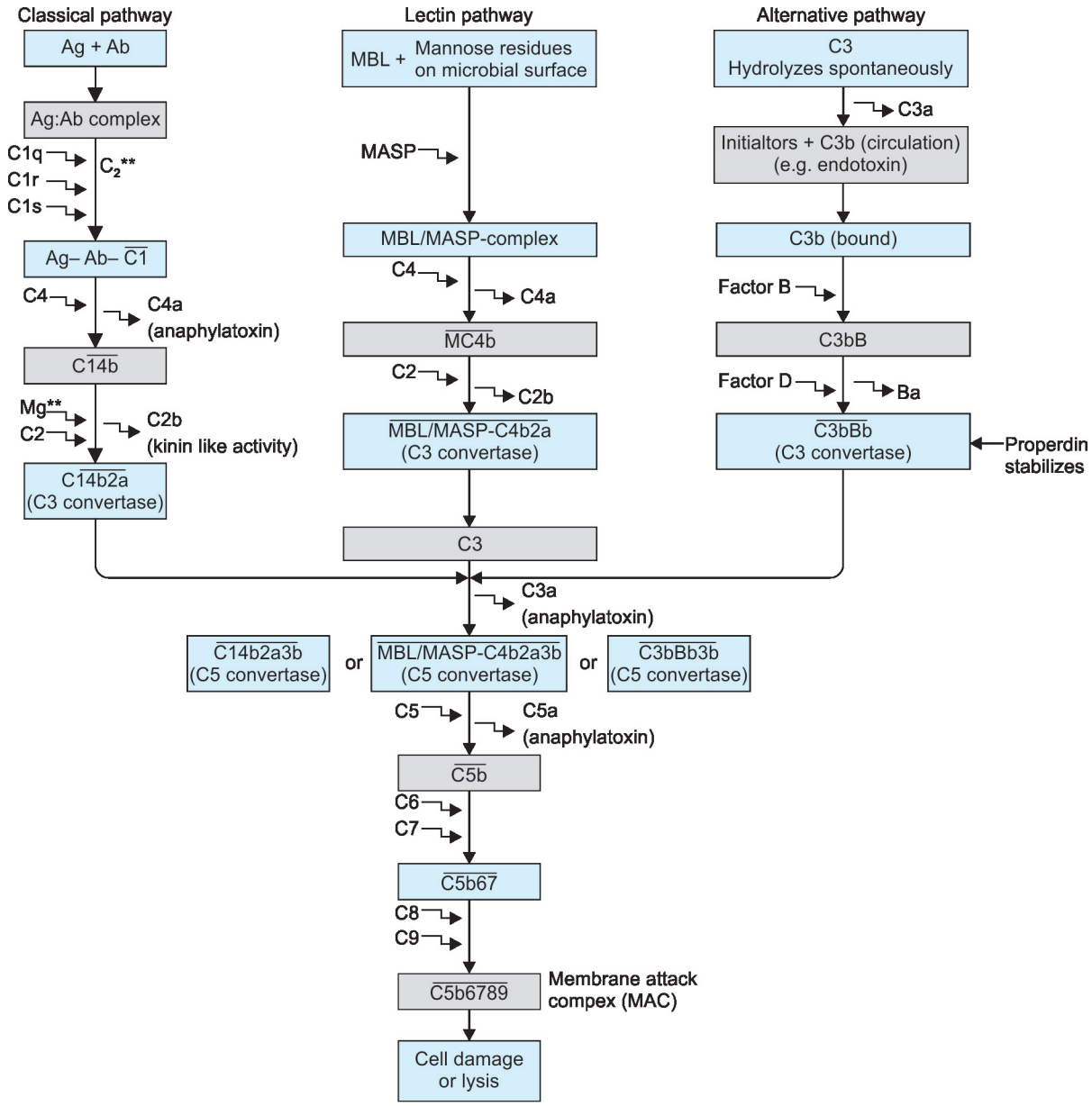


Fig. 2.2.2: Complement pathways

MULTIPLE CHOICE QUESTIONS

ANTIGEN

1. Smallest unit of antigenicity? *(JIPMER Nov 2015)*
a. Epitope
b. Paratope
c. Complex hapten
d. Simple hapten

2. Superantigens are produced by all the following pathogens, except: *(AI 2010, APPG 2014, similar MCQ in PGI June 2005, JIPMER 2014)*
a. Staphylococcus aureus
b. Enterococcus faecalis
c. Rabies virus
d. HIV

3. All of the following statements about carbohydrate antigen are true except: *(AIIMS Nov 2008)*
a. It has lower immunogenicity
b. Memory response is seen
c. Cause polyclonal B-cell stimulation
d. Does not require stimulation by T-cells

4. Heterophile antibody is found in:
a. Weil-Felix test *(NEET Pattern Based)*
b. Widal test
c. Standard agglutination test
d. All

5. The main aim of an adjuvant is to increase:
a. Distribution *(NEET Pattern Based)*
b. Absorption
c. Antigenicity
d. Metabolism

ANTIBODY

6. What is the percentage of immunoglobin in plasma protein? *(Recent Question 2015)*
a. 5 to 10% b. 10 to 15%
c. 15 to 20% d. 25 to 30%

7. Variable portion of an immunoglobulin:
a. Amino terminal *(NEET Pattern Based)*
b. Carboxy terminal
c. Acid terminal
d. Amoxy terminal

8. Vaccination is based on the principle of: *(AI 2012)*
a. Agglutination
b. Phagocytosis
c. Immunological memory
d. Clonal detection

9. Antibody diversity is due to: *(PGI Dec 2008)*
a. Gene rearrangement
b. Gene translocation
c. Antigenic variation
d. CD40 molecules
e. Mutation

10. All of the following statements about hybridoma technology are true except: *(AIIMS Nov 2008)*
a. Specific antibody producing cells are integrated with myeloma cells
b. Myeloma cells in salvage pathway grows well in HAT medium
c. Aminopterin, a folate antagonist, inhibits denovo pathway
d. HGPRT ase and thymidylate synthetase are required for salvage pathway

11. In which of the following(s), the two allele are inherited together: *(PGI Nov 2014)*
a. Idiotype b. Genotype
c. Phenotype d. Allotype
e. Isotype

12. When Papain cleaves IgG antibody, it produces: *(Recent Question 2013, DNB 2010)*
a. 2 Fab and 1 Fc b. 2 Fab and 2 Fc
c. 1 Fab and 1 Fc d. 1 Fab and 2 Fc

IgG

13. True about an immunoglobulin: *(NEET Pattern Based)*
a. IgG has max conc. in serum
b. IgM has max conc. in serum
c. IgA has max conc. in serum
d. IgE has max conc. in serum

14. Ig that helps in Opsonization: *(NEET Pattern Based)*
a. IgA b. IgG
c. IgD d. IgE

15. The serum concentration of which of the following human IgG subclass is maximum: *(AI 2005)*
a. IgG1 b. IgG2
c. IgG3 d. IgG4

16. Which IgG can cross placenta most efficiently? *(Recent Question 2015)*
a. IgG1 b. IgG2
c. IgG3 d. IgG4

17. Which of the following immunoglobulins can cross placenta? *(DNB DEC 2012, PGI June 2001)*
a. IgA b. IgM
c. IgG d. IgD

18. True about antibody: *(PGI MAY 2013, APPG 2012, Similar MCQ)*
a. IgM is produced in primary response
b. IgD protects mucosa
c. IgE is main antibody in secondary response
d. IgG is main antibody in secondary response
e. IgA protects body surface

19. Which antibody deficiency causes recurrent infection by organisms with polysaccharide capsule? *(Recent Question 2013)*
a. IgA b. IgG1
c. IgG2 d. IgM

20. Which of the following antibodies shows anamnestic response? *(Recent Questions 2014)*
a. IgA b. IgM
c. IgG d. IgD

21. Of all the IgG subclasses smallest is: *(Recent Question 2015)*
a. IgG 1 b. IgG 2
c. IgG 3 d. IgG 4

IgM

22. Which antibodies are found in Cryoglobulinemia? *(Recent Question 2015)*
a. IgG b. IgM
c. IgE d. IgA

23. Cold antibody is: *(Recent Question 2015)*
a. IgA b. IgG
c. IgE d. IgM

24. Activator of classical pathway of complement?
a. IgA b. IgG *(NEET Pattern Based)*
c. IgM d. IgE

25. Pentameric antibody with a J chain is? *(NEET Pattern Based, DNB Dec 2010, DNB June 2009)*
a. IgA b. IgG
c. IgM d. IgE

26. Which immunoglobulin acts as receptor on B-cell? *(DNB Dec 2010)*
a. IgG b. IgA
c. IgM d. IgE

27. Inutero infection leads to raise of which immunoglobulin first? *(AI 2003, DNB DEC 2012)*
a. IgG b. IgA
c. IgG d. IgM

28. Rheumatoid arthritis is best diagnosed by: *(AIIMS Nov 2011) (Modified repeat of PGI 1998)*
a. Anticitrulline antibody
b. IgG antibody
c. IgA antibody
d. IgM antibody

29. Which antibody is elevated in primary immune response? *(Recent Question 2013)*
a. IgA b. IgM
c. IgG d. IgE

30. Which is the first antibody is elevated in fetal life? *(Recent Question 2013)*
a. IgA b. IgM
c. IgG d. IgE

IgA

31. Synthesis of an immunoglobulin in membrane bound or secretory form is determined by: *(AIIMS May 2012)*
a. One turn to two turn joining rule
b. Class switching
c. Differential RNA processing
d. Allelic exclusion

32. Immunoglobulin in Peyer's patch is: *(DNB June 2011)*
a. IgM b. IgG
c. IgA d. IgD

33. Immunoglobulin present in local secretions is: *(DNB DEC 2012, DNB DEC 2009)*
a. IgG b. IgA
c. IgM d. IgD

34. The Ig which activates alternate complement pathway: *(JIPMER 2014, 2013)*
a. IgG b. IgE
c. IgA d. IgM

IgE

35. Immunoglobulin that is inactive by heating is? *(DNB DEC 2012)*
a. IgG b. IgA
c. IgM d. IgE

36. Immunoglobulin that is elevated in helminthic infection? *(WBPG 2016, MHPG 2015, NEET Pattern Based)*
a. IgG b. IgA
c. IgM d. IgE

37. IgE is secreted by: *(PGI 2005)*
a. Mast cell b. Basophil
c. Eosinophils d. Plasma cells
e. Neutrophils

ABNORMAL IMMUNOGLOBULIN

38. Which precipitates at 50 to 60°C but redissolve on heating: *(AI 2012; AI 2000)*
a. Bence Jones proteins
b. Heavy chain
c. Both light and heavy chains

Section 2

ANTIGEN ANTIBODY REACTION

39. Which one is the example of gel precipitation test? *(PGI Nov 2016)*
a. Immunoelectrophoresis b. VDRL
c. WIDAL d. Coomb's test
e. Elek's test

40. Overall strength of antigen with its antibody bond is referred to as? *(Recent Question 2015)*
a. Affinity b. Avidity
c. Antigenicity

41. Rose waller test is: *(Recent Question 2015)*
a. Passive agglutination test for Rheumatoid arthritis
b. Active agglutination for rheumatoid arthritis
c. Done for syphilis

42. Type of Ag-Ab reaction seen in VDRL? *(Recent Question 2015)*
a. Agglutination b. Passive agglutination
c. Flocculation d. Gel precipitation

43. Naegler reaction is example of: *(NEET Pattern Based)*
a. Precipitation b. CFT
c. Agglutination d. Neutralization

44. Wasserman test is: *(Recent Question 2015)*
a. Agglutination test b. Precipitation test
c. Neutralization test d. Complement fixation

45. All of the following interaction occurs between antigen antibody reaction except: *(Recent Question 2013)*
a. Ionic bond
b. Covalent bond
c. Hydrogen bond
d. van der Waals forces

46. Paul Bunnel test is example of: *(NEET Pattern Based)*
a. Agglutination b. Precipitation
c. Neutralization d. CFT

47. Which are example of agglutination test: *(NEET Pattern Based)*
a. Widal test b. VDRL test
c. Kahn test d. Ascoli's test

48. Which of the following acts as an opsonin? *(DNB June 2011)*
a. C3a b. C3b
c. C5a d. LTB4

49. The following methods of diagnosis utilize labeled antibodies except: *(AIIMS May 2005)*
a. ELISA (Enzyme Linked Immunosorbent Assay)
b. Hemagglutination inhibition test
c. Radioimmunoassay
d. Immunofluorescence

50. Heterophile agglutination is/are used in all test except: *(PGI MAY 2013)*
a. Widal test b. Weil-Felix reaction
c. Paulbunnel test d. ELISA
e. Cold agglutination test

51. Regarding to lattice formation which is true? *(Recent Question 2015)*
a. Associated with precipitation but not agglutination
b. Associated with agglutination but not precipitation
c. Associated with both
d. Neither associated with precipitation nor with agglutination

52. Antigen antibody reaction is seen maximum in?
a. Excess antibody *(AIIMS May 10)*
b. Excess antigen
c. Antigen and antibody are equal
d. Antigen and antibody are low

53. Which of the following is a neutralization test?
a. Kahn test *(Recent Question 2015)*
b. ASLO
c. Hemagglutination test

COMPLEMENT SYSTEM

54. Complement which lead to killing of organism and protects us? *(AIIMS MAY 2016)*
a. C2345 b. C56789
c. C34567 d. C3456

55. Powerful activator of classical complement pathway: *(NEET Pattern Based)*
a. IgA b. IgG
c. IgM d. IgD

56. Complement formed in liver: *(NEET Pattern Based)*
a. C2, C4 b. C3, C6, C9
c. C5, C8 d. C1

57. Which of the following acts as a chemoattractant? *(MHPG 2015, DNB DEC 2012)*
a. C3a b. C3b
c. C5b d. LTB4

58. Which of the following best denotes classical complement pathway activation in immuno inflammatory condition: *(AIIMS 2004)*
a. C2, C4, C3 decreased
b. C2 and C4 normal, C3 is decreased
c. C3 normal and C2 C4 decreased
d. C2, C4, C3 all are elevated

59. Which compliment binds with Fc portion of IgM in Classic pathway or which of the following complement components attaches to the crystallizable fragment of IgM? *(Recent Questions 2014)*
a. C1 b. C2
c. C3 d. C4

EXPLANATIONS

ANTIGEN

1. **Ans. (a) (Epitope)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p105
   Epitope or antigenic determinant is the smallest unit of an antigen that binds to paratope of an antibody.
2. **Ans. (b) (Enterococcus faecalis)** Ref: Apurba Sastry's Essentials of Medical Microbiology p108, Journal-Superantigen IJMM 2004 Vol.22 Iss.4
   Refer text
3. **Ans. (b) (Memory response is seen)** Ref: Apurba Sastry's Essentials of Medical Microbiology p109, Ananthanarayan 9/e p90
   - *Carbohydrate antigen like Lipopolysaccharide (LPS) is an example of T Independent Antigen*
   - ***T Independent Antigen:***
     - ***Directly stimulates B-cell for antibody production without participation of T-cell.***
     - Dose dependent immunogenic with Limited antibody response-IgM and IgG3
     - Lack memory response
     - LPS can cause polyclonal B-cell activation.
4. **Ans. (a) (Weil-Felix test)** Ref: Apurba Sastry's Essentials of Medical Microbiology p106, Ananthanarayan 9/e p90, 8/e p93
   - Weil-Felix test is an example of heterophile agglutination test. Ricketssial antibodies are detected by using Proteus Ox2, Ox19 and OxK antigens.
5. **Ans. (c) (Antigenicity)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 107, Ananthanarayan 9/e p150
   - Adjuvant is any substance that enhances immunogenicity of Antigen.

ANTIBODY STRUCTURE AND PROPERTIES OF ANTIBODY

6. **Ans. (c) (15 to 20%)** Ref: Internet Sources
   Immunoglubulins constitute 20% of total serum protein.
7. **Ans. (a) (Amino...)** Ref: Apurba Sastry's Essentials of Medical Microbiology p114, Ananthanarayan 9/e p94, 8/e p96
   - Variable region end—Amino terminal (NH_4)
   - Constant region end—carboxy terminal (CHO)
8. **Ans. (c) (Immunological memory)** Ref: Apurba Sastry's Essentials of Medical Microbiology p149, Ananthanarayan 9/e p83
   Vaccination leads to production of memory cells against the immunogens which play an important role in prevention of the infection by producing antibodies on subsequent exposure of the organism.
9. **Ans. (a), (e) (Gene rearrangement, mutation)** Ref: Apurba Sastry's Essentials of Medical Microbiology p118, Kuby's Immunology 6/e p 123
   Somatic hypermutation and Recombination of V-(D)-J segments joining (Gene rearrangement) are one of the important mechanisms involved for in Antibody diversity.

   Antibody Diversity

   There are 10^{10} antibodies that can be generated against various antigenic stimuli.
   This antibody diversity is possible due to:
   - Presence of Multiple germ-line gene segments
   - Recombination of V-(D)-J Segments joining
   - Junctional flexibility
   - P-region nucleotide addition (P-addition)
   - N-region nucleotide addition (N-addition)
   - Somatic hypermutation
   - Combinatorial association of light and heavy chains.

10. **Ans. (b) (Myeloma cells...)** Ref: Apurba Sastry's Essentials of Medical Microbiology p117
'Myeloma cells lacks HGPRT enzyme, hence they cannot grow well in HAT medium.'
For detail of Hybridoma technology, refer chapter review

11. **Ans. (d) (Allotypes)** Ref: Apurba Sastry's Essentials of Medical Microbiology p115, Ananthanarayan 9/e p96
ALLOTYPES, or allelic variants within the constant regions, are known to exist for some of these isotypes, and are inherited in a Mendelian co-dominant fashion in allelic manner ("allelic type").

12. **Ans. (a) (2 Fab and 1 Fc)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p, Ananthanarayan 9/e p95
- Papain cleaves antibody to: 2 Fab and 1 Fc
- Pepsin cleaves antibody to: 2 (Fab)'

IgG IMMUNOGLOBULIN

13. **Ans. (a) (IgG has...)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p113, Ananthanarayan 9/e p96, 8/e p98
- **Decreasing order of Serum level of various immunoglobulin is GAMDE: That is highest is IgG and lowest is IgE**
- ***Decreasing order of* Serum level of IgG subtypes: IgG1 > 2 > 3 > 4**

14. **Ans. (b) (IgG)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p113, Ananthanarayan 9/e p96
- **Most important opsonization- C3b and Fc (IgG)**
- **Examples of opsonin molecules include:**
  - Antibodies: IgG and IgM
  - Components of the complement system: C3b, C4b, and iC3b
  - Mannose-binding lectin (initiates the formation of C3b).

15. **Ans. (a) (IgG1)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p113, Ananthanarayan 9/e p97, 8/e p98
- Serum level of IgG subtypes: IgG1-65%, IgG2-23%, IgG3-8%, IgG4- 4%

16. **Ans. (a) (IgG1)** Ref: Journal:Vaccine. 2003 Jul 28;21(24):3365-9

17. **Ans. (c) (IgG)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p113, Ananthanarayan 9/e p96, 8/e p98
- IgG is secreted in placenta, breast
- Among the four subclasses: IgG2- poorly crosses placenta.

18. **Ans. (a) (d) (e) (IgM is produced in primary response, IgG is main antibody in secondary response, IgA protects body surface)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p113, Ananthanarayan 9/e /p96-98
Refer chapter review for detail.

19. **Ans. (c) (IgG2 deficiency)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p113, Jawetz 25/e p130, Robins Pathology 7/e p 144-147
IgG2 subclass is the predominant antibody raised against the polysaccharise capsular antigens and its deficiency is associated with recurrent pyogenic infection due pyogenic capsulated bacteria.

20. **Ans. (c) (IgG),** Ref: Journal: An evaluation of modified Widal test in the diagnosis of enteric fever, J Indian Med Assoc. 1989
Anamnestic response indicates secondary immune response which may be non-specific and mediated by IgG.

21. **Ans. (d) (IgG 4)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p112

IgM IMMUNOGLOBULIN

22. **Ans. (b) (IgM)** Ref: Internet Sources
Cryoglobulins usually consist of IgM directed against the Fc region of IgG.

23. **Ans. (d) (IgM)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p113

24. **Ans. (c) (IgM)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p114, Ananthanarayan 9/e p98, 8/e p99
- Classical complement is activated by both IgG and IgM. However, IgM is a powerful activator than IgG.

25. **Ans. (c) (IgM)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p114, Ananthanarayan 9/e p98, 8/e p99
- IgM is pentameric having a valency of 10, the five molecules of IgM are joined together by J-chain.

26. Ans. (c) (IgM) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p114, Ananthanarayan 9/e p98, 8/e p99
- Immunoglobulin acts as receptor on B-cell (i.e. surface immunoglobulin)- IgM and IgD

27. Ans. (d) (IgM) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p114, Ananthanarayan 9/e p98, 8/e p99
- IgM is phylogenetically oldest immunoglobulin and the earliest to be synthesized by the fetus, beginning by about 20 weeks.

28. Ans. (a) > (d) (Anticitrulline antibody, IgM antibody) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p185
- ***Anticitrulline antibodies***
  - ***Autoantibodies that are frequently detected in the blood of rheumatoid arthritis patients.***
  - Research suggests that in the joints of patients with rheumatoid arthritis, proteins may be changed to citrulline as part of the process that leads to inflammation of the rheumatoid joint.
  - ***When the citrulline antibody is found in a patient's blood, there is a 90-95% likelihood that the patient has rheumatoid arthritis.***
- ***Rheumatoid arthritis is also diagnosed by detecting RF (Rheumatoid factor)***
- *RF is a IgM antibody directed against Fc portion of IgG antibody.*
- ***RF is detected by passive agglutination tests like:***
  - Rose-Waaler test
  - *Latex agglutination test*

29. Ans. (b) (IgM) Ref: Apurba Sastry's Essentials of Medical Microbiology/p114, Ananthanarayan 9/e p146
- IgM: Elevated in primary immune response
- IgG: Elevated in secondary immune response

30. Ans. (b) (IgM) Ref: Apurba Sastry's Essentials of Medical Microbiology/p114, Ananthanarayan 9/e p98
- IgM: First antibody is elevated in fetal life
- IgG: Only antibody that crosses placenta.

IgA IMMUNOGLOBULIN

31. Ans. (c) (Differential...) Ref: Apurba Sastry's Essentials of Medical Microbiology/p114, Kuby's Immunology 6/e p130-131
- *'Differential RNA processing of a common primary transcript determines whether the secreted or membrane form of an immunoglobulin will be produced'…….Kuby 6/e p131*
  Membrane-bound or secreted form of immunoglobulin:
- *Mature naive B-cells* produce only membrane-bound antibody (IgD or IgM), whereas *differentiated plasma cells* produce secreted antibodies (IgA).
- Membrane-bound or secreted form of immunoglobulin is synthesized by ***alternate RNA splicing.***

32. Ans. (c) (IgA) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p114,
- Peyer's patches are composed of 30-40 lymphoid follicles present in intestinal submucosa
- They are required for intestinal secretory immunoglobulin A responses which provides local or intestinal immunity or mucosal immunity.
- Peyer's patches also contain Intra epithelial CD8 lymphocyte containing TCR ϒδ receptors

33. Ans. (b) (IgA) Ref: Apurba Sastry's Essentials of Medical Microbiology/p114, Ananthanarayan 9/e p97, 8/e p99-100
- Refer text

34. Ans. (c) (IgA) Ref: Apurba Sastry's Essentials of Medical Microbiology/p114 , Ananthanarayan 9/e p121-24

IgE IMMUNOGLOBULIN

35. Ans. (d), (IgE) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p114, Ananthanarayan 9/e p98, 8/e p100
- *Only heat labile immunoglobulin is – IgE*

36. Ans. (d), (IgE) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p114, Ananthanarayan 9/e p99, 8/e p100
- *Helminthic infection is characterized by an increase of IgE antibodies.*

37. **Ans. (d), (Plasma...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p114, Ananthanarayan 9/e p99, 8/e p100
    - IgE, like other immunoglobulins is secreted by plasma cells.
    - IgE after secreting from plasma cell, gets bound to mast cell by Fc portion. When antigen comes and binds to fab region of IgE, it in turn stimulates mast cell and mast cell degranulation occurs. (Type I hypersensitivity reaction)

ABNORMAL IMMUNOGLOBULIN

38. **Ans. (a) (Bence-Jones...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p115, Ananthanarayan 9/e p99
    Bence-Jones Protein:
    - Abnormal immunoglobulin found in urine
    - ***Coagulates at 50°C, redissolves at 70°C***
    - Elevated in Multiple myeloma

ANTIGEN ANTIBODY REACTION

39. **Ans. (a,e) (immunoelectrophoresis, Elek's test)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p124
    - **Refer text for detail**
    - **VDRL is a flocculation test and Widal and Coomb's tests are agglutination tests.**
40. **Ans. (b) (Avidity)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p121
    - Affinity is strength between single epitope of an antigen and its paratope of the antibody.
    - Avidity is the sum of total strength between all epitopes of a multivalent antigen and their paratopes.
41. **Ans. (a) (Passive...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p126
42. **Ans. (c) (Flocculation)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p123
43. **Ans. (d) (Neutralization)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p129, Ananthanarayan 9/e p112
    - Naegler reaction is an alfa toxin anti alfa toxin neutralization test
44. **Ans. (d) (Complement fixation)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p128
45. **Ans. (b) (Covalent bond)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p121, Ananthanarayan 9/e p103
    The antigen antibody reaction is reversible and the antigen and antibody molecules are bound together by weaker bonds such as ionic bond, van der Waal's forces and hydrogen bond rather than stronger bond like covalent bond
46. **Ans. (a) (Agglutination)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p126, Ananthanarayan 9/e p109, 8/e p111, 120,
    - Paul Bunnel test is an heterophile agglutination test done in infectious mononucleosis.
47. **Ans. (a) (Widal test)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p126, Ananthanarayan 9/e p109, 8/e p111, 120,
    - Widal test is an agglutination test
    - VDRL: Slide flocculation test (Precipitation test)
    - Kahn test: Tube flocculation test (Precipitation test)
    - Ascoli's thermo precipitation test: Ring precipitation test done for Anthrax antigen detection
48. **Ans. (b) (C3b)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p170, Ananthanarayan 9/e p112, 8/e p111,120
    - *Opsonins (like Fc of IgG and complement factors like C3b, C4b and ic3b) play a very important role in uptake of bacteria by binding to the specific molecules on the surface of bacteria thus facilitating the engulfment by the phagocyte (which bear the receptors for the opsonins).*
49. **Ans. (b) (Hemagglutination inhibition test)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p129, Ananthanarayan 9/e p112,
    - *Hemagglutination inhibition test is detected by inhibition of hemagglutination of anti RBC antibody present in patient's serum with respective RBC antigen.*

Methods of diagnosis utilizing labeled antibodies:

- Labeled antibodies are used for:
  - Antibody detection: Labeled anti human gamma globulin is used to detect human gamma globulin present in patient's serum.
  - Antigen detection: Labeled specific antibodies are used to detect corresponding antigens from the sample.

50. Ans. (a) (d) (Widal, ELISA) Ref: Apurba Sastry's Essentials of Medical Microbiology/p126, Ananthanarayan 9/e p90

Refer chapter review to know the list of heterophile agglutination tests

51. Ans. (c) (Ass. with both) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p122

52. Ans. (c) (Antigen and antibody are equal) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p122, Ananthanarayan 9/e p105, 8/e p104

- Antigen antibody reaction results when a large lattice is formed consist of alternate antigen and antibody molecules which occur in the *zone of equivalence.*

53. Ans. (b) (ASLO) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p129

COMPLEMENT SYSTEM

54. Ans (b) C56789 Ref: Apurba Sastry's Essentials of Medical Microbiology/p140

- **C56789 is known as membrane attack complex, causes microbial lysis.**

55. Ans. (c) (IgM) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p139, Ananthanarayan 9/e p122, 8/e p122

- Only IgM followed by IgG 3 > 1 > 2 can fix to classical pathway of complements.

56. Ans. (b) (C3...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p138, Ananthanarayan 9/e p122, 8/e p122

- Site of Complement synthesis: Liver: C3, C6, C9, GIT- C1, Macrophage- C2, C4, Spleen-C5, C8

57. Ans. (a) (C3a) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p142, Ananthanarayan 9/e p122, 8/e p122

Role of Byproducts of complements:

- Chemotaxis and Anaphylaxis- C5a and C3a
- Opsonization- C3b
- Kinin like activity (↑vascular permeability)- C2b

58. Ans. (a) (C2, C4, C3...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p142-3, Ananthanarayan 9/e p121

C2, C3, and C4 all are utilized in Classical pathway, hence their serum level will be low.

Classical pathway:

- Starts by Activation of C1 by antigen antibody complex
- Activated C1 in turn activates C4 to C4a (anaphylotoxin) and C4b
- C1 4b in turn activates C2 (in presence of ***Mg*** ion) to form C2a and C2b (kinin like, increases vascular permeability)
- C14b2a is known as C3 convertase which activates C3 to C3a (chemotactic and anaphylotoxin) and C3b
- C14b2a3b is known as C5 convertase which activates C5 to C5a (chemotactic and anaphylotoxin) and C5b
- Late components add to C5b – to form ***C56789*** (membrane attack complex) which forms pores on cell membrane causing cell lysis and also activates bystander cells to make them susceptible to lysis.

59. Ans. (a) (C1), Ref: Apurba Sastry's Essentials of Medical Microbiology/p113 , Ananthanarayan 9/e p123

Refer chapter review for detail

Structure of Immune System and Immune Response

CHAPTER 2.3

STRUCTURE OF IMMUNE SYSTEM

1. **Lymphoid Organs:** Consist of central and peripheral lymphoid organs
   - *Central* or *Primary* lymphoid organs, examples include thymus and bone marrow
   - *Peripheral* or *Secondary* lymphoid organs, examples include lymph node, spleen, and MALT
2. **Lymphoid Cells:** Consist of lymphocytes such as T-cells, B-cells and NK cells
3. **Other cells of immune system:** Include phagocytes, such as macrophage and microphages (neutrophil, eosinophil and basophil), dendritic cells, masT-cells and platelets.
4. **Cytokines:** Soluble products secreted from various cells of immune system

Structure of immune system:
- Lymphoid Organs
- Lymphoid Cells
- Other cells of immune system
- Cytokines

Central Lymphoid Organs

Bone Marrow

The progenitor T- and B-cells originate in bone marrow. Further development of B-cells occurs in bone marrow itself; whereas the progenitor T-cells proliferate in thymus.

Thymus

Thymus is developed in the embryonic life (third month) from *third/fourth pharyngeal pouch.* It is highly active at birth, reaches its peak size at puberty, and then it degenerates.

Structure: Thymus has two lobes, each is differentiated into an outer cortex and an inner medulla.

- **Cortex** is densely populated and contains: Numerous thymocytes (thymic lymphocytes), epithelial cells and *nurse cells* (specialized epithelial cells with long membrane extensions that surround many thymocytes)
- **Medulla** is sparsely populated and contains: Few thymocytes, epithelial cells, dendritic cells and *Hassall's corpuscles* (concentric layers of degenerating epithelial cells)
- Thymic hormones such as thymulin, thymopoietin and thymosin are produced which help in T-cell development.

Maturation of T-cells:

- **DN cell:** T-cell precursors after entering into the thymus transform into double negative (DN) T-cells ($CD4^-$ $CD8^-$)
- **DP cells:** Then the DN cells acquire CD4 and CD8 to become Double positive (DP) T-cells. The DP cells have the following fates.
  - Death by neglect: Majority (95%) do not specifically recognize their MHC molecules and are destroyed
  - Positive selection: The remaining 5% of DPT-cells undergo *Positive selection*
  - Negative selection: Out of 5% of DPT-cells that are positively selected, 2–5% are self-reacting and hence they are negatively selected, i.e. destroyed. (Central tolerance)
- **Mature T-cells:** The remaining DP T-cells (2–5%) loose either CD4 or CD8 to form Mature T-cells (e.g. $CD4^+$ helper T-cells and CD8+ cytotoxic T-cells). They acquire thymus specific antigens and then are released into the circulation and migrate to the peripheral lymphoid organs where they respond to the antigenic stimulus.

Fate of Double positive T-cells:
- Majority (95%): Undergo death by neglect
- The remaining 5%: Undergo Positive selection, out of which
  - 2–5%: Undergo Negative selection
  - 2–5%: Become Mature single positive T-cells.

Peripheral Lymphoid Organs

Lymph Node

Lymph nodes are small bean shaped organs; divided into three parts: cortex, medulla (both are B-cell areas) and paracortex (T-cell area). Cortex contains lymphoid follicles which are mainly of two types.

- *Primary lymphoid follicles:* They are present before the antigenic stimulus; they are smaller and contain resting B-cells.
- *Secondary lymphoid follicles*: They are formed following contact with an antigen, contain activated B-cells (i.e. plasma cells and memory B-cells). They are divided into central area (*germinal center)* and peripheral zone (*mantle area)*.

T and B cell area:
Lymph node:
- T-cell area: Paracortical area
- B-cell area: Cortex, medulla

Spleen:
- T-cell area: Periarteriolar lymphoid sheath
- B-cell area: Marginal zone

Spleen

Spleen is the largest secondary lymphoid organ. It is divided into two compartments; central *white pulp* and outer *red pulp*.

- *White pulp* has two parts: (i) Periarteriolar lymphoid sheath (T-cell area), (ii) *Marginal zone* (B-cell area)
- *Red pulp* contains the sinusoids, filled with RBCs. The older and defective RBCs are destroyed here.

Defect in spleen: As spleen is the site of destruction of most of the microbes, abnormalities of spleen or splenectomy, often leads to an increased incidence of bacterial sepsis caused primarily by capsulated bacteria.

Mucosa Associated Lymphoid Tissue (MALT)

MALT are present lining the mucosa of intestine, respiratory, and urogenital tract.

MALT in intestine are the best studied MALT, present in different layers of intestinal wall:

- Submucosa contains *Peyer's patches, composed of* lymphoid follicles
- Lamina propria contains loose clusters of lymphocytes and macrophages.
- Epithelial layer contains:
  - Few specialized lymphocytes called *intraepithelial lymphocytes* (γδ T-cells)
  - *M cells:* They are specialized flattened epithelial cells that do not have microvilli. They act as the portal of entry of a number of microbes such as *Salmonella, Shigella, Vibrio,* and Poliovirus.
  - They are lined by *Secretory IgA* which provide local or mucosal immunity.

M-cell
- Specialized flattened epithelial cells
- Do not have microvilli.
- Many microbes enter intestine via M-cells such as Salmonella, Shigella, Vibrio, and Poliovirus.

Lymphocytes

Lymphocytes can be of three types: T lymphocytes, B lymphocytes and NK (natural killer) cells.

T- and B-cells can also be classified into naive lymphocytes (prior antigenic contact) and lymphoblasts (following antigenic contact) which eventually differentiate into effector cells or memory cells.

Function of Lymphocytes
- Naive cell-Transforms to effector cell on primary exposure to antigen
- Effector cell-Eliminate antigen
- Memory cell-Transforms to effector cell on secondary exposure to antigen

Table 2.3.1: Differences between naive cell, effector cell and memory cell

	Naive cell	Effector cell	Memory cell
Location (present mostly in)	Secondary lymphoid organs	Inflamed tissues and mucosal surfaces	Both the locations of naive and effector cell
Cell cycle	Dormant (G0 phase)	Active	Dormant (G0 phase)
Morphology	Small lymphocyte	Large lymphocyte	Small lymphocyte
Life span	Short	Short	Long
Function	Transforms to effector cell on primary exposure to antigen; occurs slow due to lag period	Eliminate antigen	Transforms to effector cell on secondary exposure to antigen, occurs fast without lag period

Contd...

Contd...

Surface markers			
CD127 (IL-7R)	High	Low	High
CD45 isoform	CD45RA	CD45RO	CD45RO
CD25 (IL-2R α) on T-cells	No	Yes	Yes
CD27 on B-cells	No	Yes	Yes
B-cells producing Ig types and their affinity	IgM and IgD Low affinity	IgG, IgA, IgE High affinity	IgG, IgA, IgE High affinity

T Lymphocytes

There are two types of effector T-cells $CD4^+$ helper T-cells and $CD8^+$ cytotoxic T-cell. Rare subtypes are T_{REG} cells and γδ T-cells

Regulatory T-cells (T_{REG} cells, formerly known as suppressor T-cells):

- They provide tolerance to self-antigens (peripheral tolerance), and prevent the development of autoimmune disease.
- **Surface markers:** T_{REG} cells possess surface markers such as CD4, CD25 and Foxp3
- *Deficiency of Foxp3* receptors leads to a severe form of autoimmune disease known as **I**mmune dysregulation, **P**olyendocrinopathy, **E**nteropathy **X**-linked *(IPEX) syndrome.*

γδ T-cells:

They constitute 5% of total T-cells, express γ/δ chains of TCR chains; instead of α/β chains.

- They lack both CD4 and CD8 molecules.
- They do not require antigen processing and MHC presentation of peptides.
- They are part of innate immunity as the γδ receptors exhibit limited diversity for the antigen.
- They are usually found in the gut mucosa, as *intraepithelial lymphocytes* (IELs).
- The function of γδ T-cells is not known, they may encounter the lipid antigens that enter through the intestinal mucosa.

Table 2.3.2: Differences between T-cell and B-cell

Property	T-cell	B-cell
Origin	Bone marrow	Bone marrow
Maturation	Thymus	Bone marrow
Peripheral blood	70–80% of total lymphocytes	10–15% of total lymphocytes
Antigen recognition receptors	T-cell receptors complexed with CD3	B-cell receptor-Surface IgM or IgD complexed with Igα/Igβ
CD markers	CD 3,4,8	CD19, 21, 24
Thymus specific Ag	Present	Absent
Microvilli on the surface	Absent	Present

γδ T-cells:
- 5% of total T-cells
- Lack both CD4 and CD8
- Do not require APC
- Part of innate immunity
- Found in the gut mucosa, as intraepithelial lymphocytes (IELs)
- May encounter the lipid antigens in intestine.

B Lymphocytes

B-cells proliferate through various stages, first in bone marrow, then in peripheral lymphoid organs.

B-cell development in bone marrow is described below.

	Pro B-cells (progenitor B-cells)	Pre B-cells (precursor B-cells)	Immature B-cells	Mature B-cells
Events	Only express a heterodimer Igα/Igβ	μ heavy chain is formed Surrogate light chain is formed Pre BCR synthesis occurs Allelic exclusion occurs which allows the expression of only one out of the two alleles coding for an Ig chain	Light chain genes rearrangement occurs Membrane IgM expression Tolerance to self Ag is produced by: *Receptor editing* *Negative selection*	Express both membrane IgM and IgD

B-cell development stages:
Pro B-cells (progenitor B-cells) → Pre B-cells (precursor B-cells) → Immature B-cells → Mature B-cells

Development in peripheral lymphoid organs:

Immature B-cells migrate from bone marrow to peripheral lymphoid organs (lymph node and spleen) where they transform into mature or naive B-cells. Following antigenic stimulus, the mature B-cells transform into activated B-cells (lymphoblasts) which further differentiate into either effector B-cells, i.e. plasma cells (majority) or memory B-cells.

Natural Killer (NK) Cells

NK cells are large granular lymphocytes that constitute 10–15% of total lymphocytes, described later under CMI.

Other Cells of Immune System

Other cells of immune system include: Macrophages, dendritic cells, Granulocytes (e.g. neutrophils, eosinophil and basophils) and mast-cells.

Macrophages differ from monocytes by:
- 5–10 folds larger than monocytes
- Contain more lysozymes, organelles, enzymes and cytokines
- Possess greater phagocytic activity
- Have a longer life in tissues (months to years)

Macrophage

Monocytes/macrophages originate from bone marrow, from a separate granulocyte-monocyte progenitor cells. **Monocytes** are the largest blood cells present in blood. They do not divide and within 8 hours they migrate to tissues.

Macrophages differ from monocytes in the following:
- 5–10 folds larger than monocytes
- Contain more lysozymes, organelles, enzymes and cytokines
- Possess greater phagocytic activity and have a longer life in tissues (months to years)

Two major functions of macrophage are Phagocytosis and Antigen presentation.

Table 2.3.3: Types of macrophages

Body sites	Macrophage designation	Body sites	Macrophage designation
Peripheral blood	Monocytes	Inflammation site	Epithelioid cells Multinucleated cell (Langhans giant-cells)
Tissues	Macrophages		
Liver	Kupffer cells	Connective tissues	Histiocytes
Brain	Microglial cells	Placenta	Hofbauer cell
Kidney	Mesangial cells	Lymphoid follicle	Tingible body macrophage
Lungs	Alveolar macrophages		
Bone	Osteoclasts		

Table 2.3.4: Distribution and function of dendritic cells

Types of dendritic cells	Site	Function
Langerhans cells	Skin and mucosa	Antigen presentation Express high MHC-II and B7 molecules
Interstitial dendritic cells	Organs (lungs, liver, spleen, etc.)	
Interdigitating dendritic cells	Thymus	
Circulating dendritic cells	Blood and lymph	
Follicular dendritic cells	Lymph nodes	B-cells maturation, and differentiation; MHC-II and B7 molecules absent; Coated with Ag-Ab complex

Note: Langerhans cell is a dendritic cell; whereas Langhans giant cell is a macrophage.

Major Histocompatibility Complex

- MHC molecules or human leukocyte antigens (HLA) serve as a unique identification marker for every individual as the genetic sequence of MHC genes is different for every individual.
- They also determine the histocompatibility between the donor graft and the recipient.
- In humans, HLA complex coding for MHC proteins are located in *short arm of chromosome-6*.
- The genes are clustered in three regions named as MHC region-I, II and III
- MHC I and II help in antigen presentation to T-cells:
  - MHC I presents intracellular antigen on viral/tumor cells to cytotoxic T-cells
  - MHC II presents extracellular antigen on APCs to helper T-cells
- MHC III does not help in Ag presentation, but code for various proteins such as complement factors (C2, C4, C3 convertase, factor B and properdin), heat shock protein, TNF-α and β and steroid 21-hydroxylases.

MHC class I
- Present on all nucleated cells (except sperms) & platelets
- Presents the endogenous or intracellular Ag (viral/tumor) to CD8 T-cells

MHC class II
- Present on all APCs
- Presents the extracellular Ag to CD4 T-cells

Table 2.3.5: Differences between MHC class I and MHC class II molecules

	MHC class I	MHC class II
Present on	All nucleated cells (except sperms) and platelets	Antigen presenting cells (APCs)
Peptide antigen is	presented to CD8 T-cells	presented to CD4 T-cells
Nature of peptide antigen	Endogenous or intracellular (viral / tumor Ag)	Exogenous
Peptide antigen (size)	8–10 amino acid long	13–18 amino acid long
Antigen presentation	By Cytosolic pathway	By Endocytic pathway
Peptide-binding site	α1/α2 groove	α1/β1 groove
CD4 or CD8 binding site	α3 binds to CD8 molecules on T_C cells	β2 binds to CD4 on T_H cells

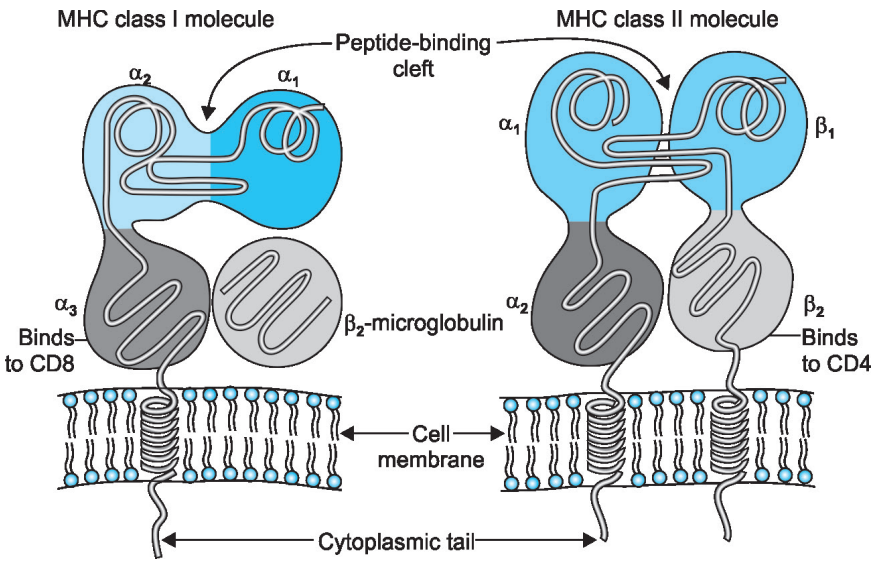


Fig. 2.3.1: Structure of MHC molecules

Table 2.3.6: Sources and functions of cytokines

Cytokine	Cytokine secreting cells	Targe T-cells and functions
Interleukins (IL)		
IL-1	Produced by all nucleated cells (Mainly by APCs such as Macrophages, monocytes dendritic cell, B-cells and endothelial cell)	1. T_H cells - IL1 produced by APCs stimulates T_H cells activation and proliferation: ○ Promotes IL2 secretion by T_H cells ○ Induces IL-2 receptor expression on T_H cells ○ Induces ↑MHC-II expression on APCs 2. B-cell: Promotes B-cell development and maturation 3. Liver: Induces synthesis of acute phase reactant proteins 4. Hypothalamus: induction of fever 5. Macrophage and neutrophil activation: ↑ expression of ICAM
IL-2	T_H 1 cells	Induces proliferation of activated T_H cells, T_C cells and some NK cells (Previously called T-cell growth factor)
IL-3	T_H cell, NK cell, Mast cell	1. Stimulates hematopoiesis (acts as multi-CSF) 2. Mast cell degranulation-↑ histamine secretion
IL-4	T_H 2 cells	1. T_H cells Promote T_H 2 cell activity and inhibit T_H 1 cell 2. B-cell: Promote B-cells activation and proliferation and induce B-cell class switch over to produce IgE, IgG4, IgG1; previously called B-cell growth factor 3. Macrophage and APCs: Induce ↑MHC-II expression
IL-5	T_H 2 cells	Promote eosinophil growth and differentiation
IL-6	T_H 2 cells, macrophages	IL-1 andTNF like effects (synergistic effect) Promotes B cell proliferation and antibody production
IL-7	Bone marrow/thymic stromal cells	Serves as a growth factor for T-cell and B-cell precursors.
IL-8	Macrophages, endothelial cells	Attracts neutrophils, NK cells, eosinophils and basophils.
IL-9	T_H cells	Hematopoietic and thymopoietic effects
IL-10	T_H 2 cells	Reduces cytokine production by T_H1 cell.
IL-11	Bone marrow stromal cells	Hematopoietic effect (B-cell and platelet development) Liver: Induce synthesis of acute phase reactant protein
IL-12	Macrophages	Promote T_H 1 cell induction and inhibit T_H 2 activity; promotes CMI responses, NK cell stimulatory factor
IL-13	T_H 2 cells	Mimic IL-4 function
IL-17	$CD4^+$ activated memory T_H cell	Initiates and maintains inflammation
Interferons (IFN)		
IFN-α	Leukocytes	Antiviral activity
IFN-β	Fibroblasts	Antiviral activity
IFN-γ	T_H and T_C cells, NK cells	1. Macrophage: Activates the resting macrophages into activated macrophage 2. B-cells: Activate B-cells to produce IgG 3. Promotes inflammation of delayed type of hypersensitivity (along with TNF-β) 4. T_H 2 cell: Inhibits T_H 2 cell proliferation
Tumor necrosis factors (TNF)		
TNF-α	Macrophage	1. IL-1 like effect 2. Tumor cells: Promote vascular thrombosis and tumor necrosis 3. Inflammatory cells: Induce cytokine secretion 4. Induces lipolysis, causes extensive weight loss associated with chronic inflammation
TNF- β	T_H 1 cell and T_C cell	Tumor cells: Similar effect like TNF-α Macrophage: Enhance phagocytic activity
Colony stimulating factor (CSF)		
GM-CSF	Fibroblasts, endothelium, T-cells, macrophages	Macrophage and granulocyte growth stimulation
G-CSF	Bone marrow stromal cells, macrophages	Granulocyte growth stimulation
M-CSF	Fibroblasts, endothelium	Macrophage growth stimulation
Others		
TGF- β	Macrophages, masT-cells T and B-cells, platelet	1. Inhibit T and B cell proliferation and hematopoiesis 2. Promote wound healing 3. Promotes class switching of B-cells to the IgA class

IMMUNE RESPONSES

Immune response refers to the highly coordinated reaction of the cells of immune system and their products. It has two arms Humoral or Antibody Mediated Immune response (AMI) and Cell-Mediated Immune response (CMI)

- Bothe CMI and AMI interdependent, regulated by the helper T (T_H) cells
- There is common pathway first before CMI/AMI; which involves antigen presentation to helper T-cells followed by activation of helper T-cells.
- Activated helper T-cells differentiate in to either T_H1 or T_H2 subsets. Induction of T_H1 cells secrete cytokines that stimulate CMI whereas if T_H2 derived cytokines induce the B-cells to produce antibodies.

Antigen-Presenting Cells (APCs)

APCs implies to cells that present the antigenic peptide along with MHC class II to T_H cells. They may be grouped into:

- Professional APCs: e.g. Macrophages, Dendritic cells and B-cells
- Nonprofessional APCs: e.g. Fibroblasts (skin), Thymic epithelial cells, Pancreatic beta cells, Vascular endothelial cells, Glial cells (brain) and Thyroid epithelial cells.

Antigen-presenting cells (APCs)
Professional APCs:
- Macrophages
- Dendritic cells
- B-cells

Non-professional APCs: e.g.,
- Fibroblasts (skin)
- Thymic epithelial cells
- Pancreatic beta cells
- Vascular endothelial cells
- Glial cells (brain)
- Thyroid epithelial cells

Table 2.3.7: Antigen Processing Pathways

Property	Cytosolic pathway	Endocytic pathway
Antigen processed	Endogenous	Exogenous
Antigen is complexed with	MHC I molecules	MHC II molecules
Antigen is presented to	TC cells	TH cells

Helper T-cells (Activation and Differentiation)

Activation of T_H cells requires generation of these specific signals:

1. *Antigen-specific signal*: Involves binding of antigenic peptide on APCs to TCR of T_H cells.
2. CD4 molecules of T_H cells also interact with β2 domain of MHC-II.
3. *Costimulatory signal* involves binding of CD28 molecule on T_H cells to B7 molecules on APCs.
4. *Cytokine signal*: IL-1 secreted from APCs acts on T_H cells.

Following signal transduction, naive T_H cells differentiates into effector and memory T-cells. Effector T_H cells further differentiate into either T_H1 or T_H2 subsets, regulated by IL-12 which promotes T_H1 subset proliferation.T_H1 and T_H2 derive cytokines mediates various functions.

T_H cells Activation-
Requires specific signals:
- *Antigen-specific signal:* Ag on APCs to TCR of T_H cells.
- CD4s of T_H cells with β2 domain of MHC-II.
- *Costimulatory signal:* CD28 on T_H cells to B7 on APCs.
- *Cytokine signal:* IL-1 acts on T_H cells

Table 2.3.8: TH_1 cytokines and their functions

T_H1 cytokines and their functions	
IL-2	Promotes activation of T_H and T_C cells and NK cell
IFN-γ	1. Activates the resting macrophages into activated macrophage 2. Activates B-cells to produce IgG 3. Promotes inflammation of delayed type of hypersensitivity (along with TNF-β) 4. Inhibits T_H2 cell proliferation
TNF-β	Enhances phagocytic activity of macrophage
T_H2 cytokines and their functions	
IL-4	1. Inhibits T_H1 cell differentiation 2. Stimulates B-cells to produce IgE and also IgG4 and IgG1
IL-5	1. Enhances proliferation of eosinophils 2. IL-4 and IL-5 together provide protection against helminthic infections and mediate allergic reaction
IL-6	Promotes B-cell proliferation and antibody production
IL-10	Inhibits T_H1 cell differentiation

Cell-Mediated Immune Response

Components of CMI:
- Ag specific: T_C Cell
- Ag Nonspecific:
  - NK cell
  - ADCC performing cells: NK cells, Neutrophils, Eosinophils and Macrophages

CMI provides immunity against: (i) microbes residing in intracellular milieu (both obligate and facultative) (ii) tumor cells, (iii) Mediate delayed/type IV hypersensitivity (iv) plays key role in transplantation immunity and graft-versus- host reaction.

Effector cells of CMI include:
- Antigen specific cells: (i) Cytotoxic T-cells (principal mediator of CMI)
- Antigen nonspecific cells: (ii) NK cells, (iii) Cells performing ADCC (NK cells, macrophages, neutrophil and eosinophils)

Cytotoxic T Lymphocytes

Tc cells Activation:
Requires specific signals:
- *Antigen-specific signal:* Ag on target cells to TCR of T_c cells.
- *CD4 of T_H cells:* with α3 domain of MHC-I.
- *Costimulatory signal:* CD28 on T_c cells to B7 on target cells.
- *Cytokine signal:* IL-2 acts on T_c cells

CD8TC cells are the principal effector cells of CMI. Activation of Naive TC cells requires these specific signals.

1. Antigen-specific signal: TCR of naive T_C cells binds to MHC I -peptide complex of target cells.
2. CD8 of T_C cells also interacts with α domain of MHC-I.
3. Costimulatory signal: CD28 of naive T_C cells interacts with B7 molecule on target cells.
4. Cytokine signal: IL-2 (secreted by T_H 1 cell) acts on T_C cells

The activated TC cells produce two types of lethal enzymes; called (i) perforins (for pores on the target cells) and (ii) granzymes (destroy the target cells).

Natural Killer Cells

NK cell mediated cytotoxicity occurs by:
- Receptor interaction followed by
- Target cell destruction

NK cells are large granular lymphocytes that constitute 10–15% of peripheral blood lymphocytes:
- They are derived from a separate lymphoid lineage. NK cells are cytotoxic but antigen nonspecific.
- They are part of innate immunity, act as first line of defense and do not require prior contact with the antigen.
- **Mechanism of NK cell mediated cytotoxicity:**
  - *Receptor interaction:* When activation receptors (e.g. NKR-P1, CD16) present on NK cells are engaged with ligands present on the target cells; NK cells become activated.
  - *Target cell destruction:* is similar to that of TC cells, i.e. via secreting perforins and granzymes. However, the NK cells enzymes are constitutively expressed (i.e. they are cytotoxic all the time, even without exposure to the antigen).

Table 2.3.9: Differences between NK cells and T_C cells

Property	NK cells	T_c cells
Surface markers	CD16, D56	CD3, CD8
MHC restriction	No	MHC-I restricted
Memory	No	Yes
Immunity	Part of Innate immunity, Ag nonspecific	Acquired immunity, Ag specific
Target cell	Virus infected cells and Tumor cells	Same as NK cells
Mechanism of destruction	Perforins and granzymes (Constitutive)	Same as NK cells, but inducible
Immune response	CMI	CMI

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

A number of nonspecific cytotoxic cells express receptors (FcR) on their surface that can bind to the Fc region of any Ig:
- These cells can bind to Fc portion of the antibody coated on the target cells, and subsequently cause lysis of the target cell by releasing various cytotoxic factors such as:
  - NK cells secrete perforins, and granzymes

- Neutrophils releases lytic enzymes
- Eosinophils can release lytic enzymes and perforins; protects against helminths
- Macrophages produce lytic enzymes and TNF

- Although these cytotoxic cells are nonspecific for the antigen, the specificity of the antibody directs them towards the specific target cells. This type of cytotoxicity is referred to as *antibody-dependent cell-mediated cytotoxicity* (ADCC).

Assessment/Detection of CMI

(i) Mixed-lymphocyte reaction (MLR), (ii) Cell-mediated lympholysis (CML), (iii) The graft-versus host reaction (GVH) in experimental animals.

Humoral/Antibody Mediated Immune Response (AMI)

AMI provides protection to the host by **secreting antibodies;** that prevent invasion of microbes present on the surface of the hosT-cells and in the extracellular environment but has no role against intracellular microbes.

AMI occurs through the following three sequential steps:

1. **Activation of B-cells** carrying microbial antigen (B-cells act as APCs) and presenting to T_H cells. This requires the following signals:
   - Signal-1 is induced by the cross linking of mIg on B cell membrane with the microbial antigen.
   - Signal-2 provided by binding of CD40 on B-cell with CD 40L (ligand) on activated T_H cells.
   - Signal-3 Cytokines produced by the activated T_H cells act on B-cells
2. **Differentiation of B-cells** into effector cells (plasma cells) and memory cells. This occurs in germinal centre of secondary lymphoid follicles.

B-cells Activation:
Requires three signals:
- mIg on B-cell with the microbial antigen
- CD40 on B-cell with CD40L (ligand) on activated T_H cells.
- Cytokines produced by the activated T_H cells act on B cells

Following Ag contact, the naive B-cells transforms to → **Centroblasts** → undergo somatic hypermutation and affinity maturation to form **Centrocytes** expressing high affinity surface Ig → High affinity centrocytes are selected by **follicular dendritic cells** coated with Ag Ab complexes→ Selected centrocytes interact with T_H cells (as described above) and undergo **class switch over** (by the cytokine stimulus, see table below) to produce Ig of classes other than IgM→Then, the selected centrocytes differentiate into plasma cells or memory B-cells.-

Table 2.3.10: Cytokines rsponsible for Ig class/subclass switch over

Cytokine(s)	Ig class produced
IFN-γ	IgG2a or IgG3
IL-5 + TGF-β	IgA or IgG2b
IL-4	IgE or IgG1 or IgG4
IL-2,4,5	IgM
IL-4,5,6 + IFN-γ	IgG

3. **Effector functions**: Production of secreted antibodies by plasma cells which in turn counter act with the microbes in many ways such as neutralization, opsonization, complement activation, ADCC, mucosal immunity, etc.

MULTIPLE CHOICE QUESTIONS

LYMPHOID ORGAN

1. **All are peripheral lymphoid organ except:** *(PGI May 10)*
   a. LN
   b. Spleen
   c. MALT
   d. Thymus
   e. Bone marrow
2. **T-cell dependent region in lymph node:** *(NEET Pattern Based)*
   a. Cortex
   b. Medulla
   c. Paracortical area
   d. Mantle layer

CELLS OF IMMUNE SYSTEM

3. **Which is not an example of antigen presenting cells (APC)?** *(AIIMS MAY 2016)*
   a. M cells
   b. Thymocytes
   c. Langerhan cells
   d. Macrophages
4. **True regarding thymus is:** *(JIPMER Nov 2014)*
   a. Mature thymocytes express CD 4 and CD 8
   b. Thymocytes whose T-cell receptor bind with high affinity to self-antigens and MHC Complexes are clonally deleted
   c. Mature thymocytes express surface IgM and IgD
   d. CD 4 and CD 8 double positive cells are completely eliminated by a process of negative selection
5. **Which of the following features is not shared between 'T-cells' and 'B-cells'?** *(AI 2012, AIIMS Nov 2012)*
   a. Positive selection during development
   b. Class I MHC expression
   c. Antigen specific receptors
   d. All of the above
6. **Cell mediated immunity is by virtue of:**
   a. Helper T-cell *(DNB June 2011)*
   b. Suppressor T-cells
   c. Cytotoxic T-cells
   d. All above
7. **Common between B- and T-cells:** *(PGI June 2007)*
   a. Origin from same cell lineage
   b. Site differentiation
   c. Antigenic marker
   d. Perform Both humoral and cellular immunity
   e. Further differentiation seen
8. **Most potent stimulator of Naive T-cells:**
   a. Mature dendritic cells *(AI 2011, AIIMS Nov 08)*
   b. Follicular dendritic cells
   c. Macrophages
   d. B-cells
9. **All of the following are functions of CD4 helper cells, except:** *(AI 2009)*
   a. Immunogenic memory
   b. Produce immunoglobulins
   c. Activate macrophages
   d. Activate cytotoxic cells
10. **True statement regarding NK cells are all except:** *(PGI May 2015)*
    a. Also called large granular lymphocyte
    b. Can kill virus infected cell
    c. Forms first line of defence
    d. Can kill tumour cell
    e. No role in cell mediated immunity
11. **Null cells that lack characteristics of B- or T-cells constitute what percentage of peripheral lymphocytes:** *(JIPMER 2014, 2013)*
    a. 0–1
    b. 2–3
    c. 5–10
    d. 15–20
12. **A New agent was developed to increase the recognition of foreign antigens by Antigen presenting cells. True regarding the physiological aspects of APCs:** *(JIPMER Nov 2014)*
    a. Antigen is presented via MHC–I complexes
    b. APCs are required before a response to viruses are generated
    c. Direct antibody stimulation still requires APC's
    d. Antigen processed by APC are recognized by CD 4+ T Cells
13. **Macrophages are major source of:** *(DNB June 2010)*
    a. IL-1
    b. IL-5
    c. IL-7
    d. IFN-Y

MHC COMPLEX

14. **The role played by MHC 1 and 2 is to:** *(AIIMS Nov 2013, AIIMS May 2014, AIIMS Nov 2014)*
    a. Stimulate interleukin production
    b. Immunoglobulin class switch over
    c. Transduce the signal to T-cell following antigen binding
    d. Presenting the antigen for recognition by T-cell antigen binding receptors

15. MHC molecules are coded by: *(NEET Pattern Based)*
a. Chromosome-6
b. Chromosome-7
c. Chromosome-8
d. Chromosome-9

16. Gene coding for MHC-I include: *(NEET Pattern Based)*
a. A, B, C
b. DR
c. DP
d. DQ

17. Peptide binding site on MHC-I for presenting processed antigen to CD8 T-cells is formed by: *(AI 2010)*
a. Junction of proximal domains made-up of Alpha subunit
b. Junction of distal domains made-up of Alpha subunit
c. Junction of proximal domains made-up of beta subunit
d. Junction of distal Proximal domains made-up of beta subunit

18. MHC II are presented on: *(PGI Nov 10)*
a. Macrophages
b. Dendritic cells
c. Lymphocytes
d. Eosinophils
e. Platelet

19. MHC III codes for: *(Recent MCQ 2013)*
a. Complements
b. Interleukin
c. Prostaglandins
d. Interferon

CYTOKINES

20. Which of the following is not pyrogenic in nature? *(AI 2012)*
a. IL 1
b. IL 18
c. TNF alpha
d. Interferon alpha

21. IL–2 produced by: *(Recent MCQ 2014, AI 2000)*
a. T-cells
b. B-cells
c. Monocytes
d. Neutrophils

22. Most important chemical mediators of gram negative septicemia: *(Recent MCQ 2013)*
a. IL-2B
b. TNF-α
c. TGB-β
d. IL-20

EXPLANATIONS

LYMPHOID ORGAN

1. **Ans. (d), (e) (Thymus, Bone marrow)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p145, Ananthanarayan 9/e p128, 8/e p123, 132, Kuby's Immunology 6/e p295

   Lymphoid Organ:
   - Central/Primary: Thymus and Bursa of fabricus (birds) / Bone Marrow (human)
   - Peripheral/Secondary: Spleen, Lymph Node, MALT (GIT and Respiratory mucosa).

2. **Ans. (c) (Paracortical...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p146-7, Kuby's Immunology 6/e p45-46

Lymph node	Spleen
T-cell area- Paracortical area B-cell area- Cortex and medulla	T-cell area- Periarteolar lymphoid sheath B-cell area- marginal zone

CELLS OF IMMUNE SYSTEM

3. **Ans : (b) (Thymocytes)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p162

   Thymocytes are the T cells present in thymus; and T cells NEVER act as APC; in fact APCs present the antigen to T cells.

4. **Ans. (b) (Thymocytes whose..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p150

   CD 4 and CD 8 double positive (DP) T-cells are not eliminated completely. They have three fates:
   - Positive selection: The 5% of DP T-cells, whose αβ receptors are capable of recognizing their MHC molecules are positively selected. This results in MHC restriction.
   - Death by neglect: Majority of DP cells (95%) fail positive selection because they do not specifically recognize their MHC molecules.
   - Negative selection: The survived cells that undergo positive selection (5%) are MHC restricted. However, some of these surviving cells (2–5%) react to the self antigens and therefore, they are selected to be killed by apoptosis and removed (negatively selection).
   - The remaining DP T-cells (2–5%) having αβ type TCR selectively loose either CD4 or CD8 and become single positive mature T-cells

   About Other options:
   - Mature thymocytes are single positive; i.e. they express either CD 4 or CD 8; but never both.
   - Mature B cells (not thymocytes) express surface IgM and IgD.

5. **Ans. (a) (Positive selection during development)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p150
   - *During embryonic life, for deletion of self reacting clones, T-cells undergo positive selection followed by negative selection where as B-cells undergo only negative selection.*

   About Other Options
   - Class I MHC molecules are expressed on all nucleated cells. Both T and B-cells being nucleated, so they express class I MHC. Whereas class II MHC are expressed on all APCs (so present on B-cells but not on T-cells).
   - Antigen specific receptor:
     - Antigen specific receptor on T-cell- TCR (T-cell receptor)
     - Antigen specific receptor in B-cell- BCR (B-cell receptor), i.e. surface immunoglobulins like IgM and IgD

6. **Ans. (d) (All of the above)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p162, Ananthanarayan 9/e p152
   - Cell mediated immunity refers to specific immune response mediated by effector T-cells (both T-helper, Cytotoxic T-cells and Suppressor T-cells) generated against an antigen.

7. **Ans. (a), (e) (Origin from same cell lineage, Further differentiation seen)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p145, Kuby's Immunology 6/e p 29, 34
   - Both T and B-cells originate from same lymphoid progenitor cell lineage in bone marrow.

- ***Site of differentiation*** **–**T cell- in Thymus ***and*** B cell- In bone marrow
- Antigenic marker are also different for T & B-cells (Refer text)
- **Immune response-** T cell mediates- cellular immunity and B cell mediates- humoral immunity
- **Further differentiation:**
  - T cell differentiated to TH (TH1 and TH2) and Tc cells
  - B cell differentiated to plasma cells

8. Ans. (a) (Mature dendritic cells) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p155

- Mature dendtritic cells possess higher level of MHC-II & costimulatory B-7 molecules, hence they are the most potent stimulator of T-cells.
- Detail-Refer text

9. Ans. (b) (Produce immunoglobulins) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p162-3

- When resting T_h cell binds to a MHC class II- antigen complex on APC, that initiates the activation of T_h cell.
- The activated T_h cell divides many times to produce effector T_h cell and ***memory T_h cell***.
- Effector T_h cell further divides to T_h 1 and 2 and perform the following actions:
  - Activation and proliferation of ***Tc cell***
  - Regulate monocyte ***macrophage*** system
  - Helps in activation of B-cell to produce plasma cell that secretes immunoglobulins, (but never produce immunoglobulins).

10. Ans (a), (b), (c), (d) (Also..., Can..., Forms..., Can...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p166

NK cell is an important component of CMI along with TC cell and ADCC.
Refer chapter review for detail of NK cell.

11. Ans. (c) (5-10) Ref: Apurba Sastry's Essentials of Medical Microbiology/p166, Ananthnarayan 9/e p137

12. Ans (d) (Antigen processed by APC..) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p162

Exogenous (foreign) antigens are first phagocytosed by APCs, then they are processed by APCs and presented on the surface of APCs along with MHC II to be recognized by CD 4+ T Cells.

13. Ans. (a) (IL-1) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p153-4, Kuby's Immunology 6/e p209

Macrophage produces various secretory molecules:

- Enzymes- Lysozyme, proteases, elastases, collagenases, plasminogen activator,
- Cytokines-IL-1, 8, 12, TNF-α, TGF-β, prostaglandins
- Platelet derived growth factor (PDGF), Platelet activated factor (PAF),
- CSF (colony stimulating factor), ACE (Angiotensin Converting Enzyme)
- Complements- C2, C4
- Reactive O_2 species- H_2O_2, NO, OH

MHC COMPLEX

14. Ans. (d) (Presenting the antigen for recognition by T-cell antigen binding receptors) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p156, Ananthanarayan 9/e p140

The major function of MHC I and II molecule is to present the peptide antigen to the T-cell receptors of T-cells.

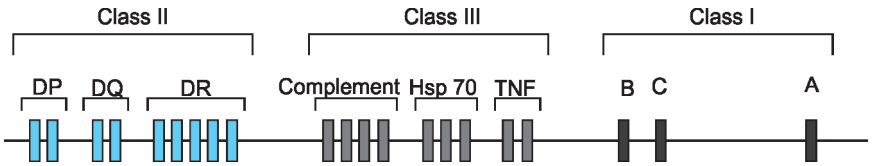
- MHC I molecule presents the peptide antigen to T_c cells.
- MHC II molecule presents the peptide antigen to T helper cells.

15. Ans. (a) (Chromosome-6) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p156-7, Ananthanarayan 9/e p139

- MHC genes are located on short arm of Chromosome-6

16. Ans. (a) (A, B, C) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p156-7, Ananthanarayan 9/e p139

- Class I MHC gene includes- A, B, C
- Class II MHC gene includes – DP, DQ, DR
- Class III MHC gene includes- genes for certain complement factors, Heat shock protein, TNF



17. Ans. (b) (Junctio...) Ref: Apurba Sastry's Essentials of Medical Microbiology1e/p156-7, Kuby's Immunology 6/e p194

Binding sites of MHC I & II molecules:

- ***The antigen binding site:***
  - For MHCI- groove between α1 and α2
  - For MHCII- groove between α1 and β1
  - Bothe these grooves lie in the distal part of MHC molecules
- ***The co-receptor binding site:***
  - For MHCI- α3 domain binds to CD8 of T_c cell
  - For MHCII- β2 domain binds to CD4 of T_H cell
  - Bothe these grooves lie in the proximal part of MHC molecules.

18. Ans. (a) (b) (Macrophages, Dendritic cells) Ref: Apurba Sastry's Essentials of Medical Microbiology/p156-7, Ananthanarayan 9/e, p142, 8/e p134

- *MHC I molecules are present on all nucleated cells (RBC and platelets are non nucleated, so MHC I molecules are not found)*
- MHC II molecules are more restricted in distribution, present on macrophages, dendritic cells and B-cells.

19. Ans. (a) (Complements) Ref: Apurba Sastry's Essentials of Medical Microbiology1/e, p156-7, Ananthanarayan 9/e p140

- MHC III codes for Complements C2, C4, C3 convertase, factor B, properdin, HSP and TNF.

CYTOKINES

20. Ans. (b) (IL18) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p160, Kuby's Immunology 6/e p279

Journal- Circulating Cytokines as Mediators of Fever, Mihai G. Netea et al Clin Infect Dis. (2000) 31 (Supplement 5): S178-S184.

- Cytokines ***IL-1, TNF-α and IL-6*** acts on the hypothalamus to induce a fever response ……. *Kuby's Immunology 6/e p279*
- **IFN-α is also a potent inducer of fever**
- *Bacterial LPS stimulates macrophages and endothelial cells to release TNF and IL1 which in turn activates IL-6. Finally Prostaglandin E2 (PGE2) is stimulated which is the main mediator of fever.*

21. Ans. (a) (T-cell) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p160, Kuby's Immunology 6/e p307

Action of cytokines: Refer chapter review

22. Ans. (b)(TNF-α) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p160, Stite'sMedical Immunology, 10/e p152

- Bacterial septic shock apparently develops because bacterial cell-wall endotoxins stimulate macrophages to overproduce IL-1 and TNF-α to higher levels that cause septic shock
- TNFα is the principle cytokines involved in gram-negative septic shock.
- TNFα is primarily responsible for Shwartzman reaction, in which repeated injection of LPS (obtained from gram-negative cell wall) into a solid tissue leads to hemorrhagic infarction.

CHAPTER

2.4 Hypersensitivity

Hypersensitivity or allergy refers to the injurious consequences in the sensitized host, following subsequent contact with specific antigens.

Table 2.4.1: Gell and Coombs classification of hypersensitivity reactions and their features

	Type–I	Type–II	Type–III	Type–IV
Immune response altered	Humoral	Humoral	Humoral	Cell mediated
Immediate or delayed	Immediate	Immediate	Immediate	Delayed
Antigen contact to symptoms interval	2 to 30 min	5–8 hrs	2–8 hrs	24 to 72 hrs
Antigen	Soluble	Cell surface bound	Soluble	Soluble or bound
Mediator	IgE	IgG	Ag-Ab complex	T_{DTH} cell
Effector mechanism	Mast cell degranulation	1. ADCC 2. Complement mediated cytolysis	Complement activation and inflammatory response	Macrophage activation leads to phagocytosis or cell cytotoxicity
Desensitization to the allergen	Easy, but short lasting	Easy, but short lasting	Easy, but short lasting	Difficult but sustained
Typical manifestations	1. Anaphylaxis 2. Asthma 3. Atopic dermatitis	1. Transfusion reactions 2. Rh incompatibility 3. Hemolytic anemia	1. Arthus reaction 2. Serum sickness 3. Glomerulone-phtiritis 4. Rheumatoid arthritis	1. Tuberculin test 2. Granuloma formation in tuberculosis and leprosy 3. Contact dermatitis

TYPE-I HYPERSENSITIVITY REACTION

Mechanism

Type-I Hypersensitivity reaction occurs through two phases:

Sensitization phase: Priming dose of antigen (allergen)→ processed by APC → antigenic peptide presented to T-cell → T_H2 activated → secretes IL4 → Acts on B-cell → IgE produced → Mast cells coated with IgE (Fc) at mucosal sites.
Effector phase: Shocking (subsequent) dose of allergen → IgE (Fab) binds to Antigen → Mast cell degranulation to release mediators which can be primary mediators (preformed, released immediately) and secondary mediators (released after synthesis). The mediators have various pharmacological actions.

Table 2.4.2: Mediators of Type-1 hypersensitivity reaction

Primary Mediators	**Action**
Histamine, Heparin and Serotonin	↑Vascular permeability, ↑Smooth-muscle contraction
Eosinophil chemotactic factor (ECF-A)	Eosinophil chemotaxis
Neutrophil chemotactic factor (NCF-A)	Neutrophil chemotaxis
Proteases	Bronchial mucus secretion; Degradation of blood-vessel basement membrane
Secondary Mediators	**Action**
Platelet-activating factor	Platelet aggregation and degranulation; Contraction of pulmonary smooth muscles

Contd...

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Secondary Mediators	Action
Leukotrienes (slow reactive substance of anaphylaxis, SRS-A)	↑ Vascular permeability; Contraction of pulmonary smooth muscles
Prostaglandins	↑Vasodilation; Contraction of pulmonary smooth muscles; Platelet aggregation
Bradykinin	↑Vascular permeability; Smooth-muscle contraction
Cytokines (IL-1 and TNF-α)	Systemic anaphylaxis; ↑ Expression of cell adhesion molecules (CAMs) on venular endothelial cells

Table 2.4.3: Common allergens associated with type I hypersensitivity reaction

Food	Nuts, egg, peas, sea food, beans, milk
Plants and pollens	Rye grass, rag weed, timothy grass
Proteins	Foreign serum vaccines
Drugs	Penicillin, sulfonamides, local anesthetics and salicylates
Insect bite products	Venom of bee, wasp, ant, cockroach calyx and dust mites
Others	Mold spores, animal hair and dander

Experiments to demonstrate type I HSN
- P-K reaction
- Schultz Dale phenomenon
- Theobald Smith phenomenon

Examples of Type I Hypersensitivity Reaction

- **Experiments to** demonstrate type I hypersensitivity reaction: P-K reaction, Schultz Dale phenomenon and Theobald smith phenomenon
- **Systemic anaphylaxis**
- **Localized anaphylaxis** (atopy) such as:
  - Allergic rhinitis (or hay fever)
  - Asthma
  - Food allergy
  - Atopic urticarial
  - Atopic dermatitis (allergic eczema)
  - Drug allergy
  - Wheal and flare reaction.
- **Parasitic diseases/tests:**
  - Casoni test (hydatid disease)
  - Tropical Pulmonary Eosinophilia (TPE)
  - Loeffler's pneumonia (Ascaris)
  - Ground itch (Hookworm)
  - Leakage of hydatid fluid
  - Cercarial dermatitis/swimmer's itch (Schistosoma).

Detection of type I HSN
- Skin prick test
- RIST: detects the total serum IgE
- RAST: Detects allergen specific serum IgE

Detection of Type I Hypersensitivity

- Skin prick test
- Radioimmunosorbent test (RIST): It quantitatively detects the total serum IgE
- Radioallergosorbent test (RAST): It quantifies the serum level of allergen specific IgE.

Hyposensitization to treat type I HSN
Repeated exposure to increased subcutaneous doses of allergens can reduce or eliminate the allergic response to the same allergen

Treatment of Type I Hypersensitivity Reaction

- Avoidance of contact with known allergens
- Hyposensitization: Repeated exposure to increased subcutaneous doses of allergens can reduce or eliminate the allergic response to the same allergen.
- Humanized Monoclonal anti-IgE-It can bind and block IgE.
- Drugs: Several drugs are useful in suppressing type-1 response such as antihistamines, adrenaline, cortisone, theophylline and cromolyn sodium.

TYPE-II HYPERSENSITIVITY REACTION

In type-II reactions, the host injury is mediated by antibodies (IgG or rarely IgM) which interact with various types of antigens such as:

- Host cell surface antigens (e.g. RBC membrane antigens like blood group and Rh antigens)
- Extracellular matrix antigens or
- Exogenous antigens absorbed on host cells (e.g. a drug coating on RBC membrane).

After Ag-Ab binding occurs, the Fc region of antibody initiates the type-II reactions by the following three broad mechanisms:

Antibody (Fc) Activating Complement System

By complement dependent cytolysis (due to MAC), inflammation (by C5a, C3a), opsonization (by C3b and C4b)

It is seen in following conditions:

- Transfusion reaction (ABO incompatibility)
- Erythroblastosis fetalis
- Autoimmune hemolytic anemia, agranulocytosis, or thrombocytopenia
- Drug induced hemolytic anemia
- Pemphigus vulgaris
- Hyper acute graft rejection.

Antibody dependent cellular dysfunction (ADCD). Examples:
- Grave's disease
- Myasthenia gravis
- Good pasture syndrome
- Pernicious anemia
- Rheumatic fever
- Myocarditis in Chagas disease

Antibody (FC Portion) Interacting with Fc Receptors on Target Cells

- Antibody dependent cellular cytotoxicity (ADCC)
- Opsonization.

Antibody Dependent Cellular Dysfunction or ADCD

Autoantibody Mediated:

- Activation of receptor, e.g. Grave's disease
- Inhibition of receptor, e.g. Myasthenia gravis
- Other examples of ADCD:
  - Good pasture syndrome (antibody produced against type IV collagen)
  - Pernicious anemia (antibody directed against intrinsic factor)
  - Rheumatic fever (antibody against streptococcal antigens cross reacting with heart)
  - Myocarditis in Chagas disease.

TYPE-III HYPERSENSITIVITY REACTION

Type-III hypersensitivity reactions are as a result of excess formation of immune complexes (Ag-Ab complexes) which initiate an inflammatory response through activation of complement system leading to tissue injury:

- Localized or Arthus reaction
  - In skin: (i) following insect bites or (ii) during allergic desensitization
  - In lungs (i) Farmer's lung (Saccharopolyspora species), (ii) Bird-Fancier's disease
- Generalized or Systemic type III Reactions, e.g. Serum sickness.

Parasitic example of type III HSN:
- Nephrotic syndrome in Plasmodium malariae
- Katayama fever in schistosomiasis
- African trypanosomiasis

Table 2.4.4: Diseases associated with generalized type III hypersensitivity reactions

Connective tissue disorders: Due to autoantibodies forming immunocomplexes with self-antigens • SLE (Systemic lupus erythematosus): Anti-DNA Ab • Rheumatoid arthritis: Ab against human immunoglobulin • PAN (Polyarteritis nodosa)
Parasitic diseases: Resulting from immunocomplex deposition • Nephrotic syndrome in *Plasmodium malariae* • Katayama fever in schistosomiasis • African trypanosomiasis

Contd...

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Bacterial diseases resulting from immunocomplex deposition
- *Streptococcus pyogenes*: Poststreptococcal glomerulonephritis
- *Mycobacterium leprae* (Lepra reaction type 2)

Viral diseases with immunocomplex deposition
- Hepatitis B (arthritis)
- Hepatitis C (arthritis)
- Infectious mononucleosis (Epstein Barr Virus)
- Dengue (arthritis)

Others:
- Subacute bacterial endocarditis
- Serum sickness

In Rheumatoid arthritis: There is complex of both type III and IV hypersensitivity Reaction. But Type III is a more appropriate answer than type IV.

TYPE-IV HYPERSENSITIVITY REACTION

Type-IV hypersensitivity reactions differ from other types in various ways:
- It is delayed type (occurs after 48–72 hours of antigen exposure)
- Cell mediated: Characteristic cells called TDTH cells are the principal mediators
- Tissue injury occurs predominantly due to activated macrophages.

Type IV HSN differ from other types by:
- Delayed type (occurs after 48–72 hours of Ag exposure)
- Cell mediated (TDTH cells)
- Tissue injury - due to activated macrophages.

Mechanism of Type-IV Reactions

- **Sensitization phase** (occurs 1–2 weeks following Ag exposure): APCs process and present the antigenic peptides to TH cells. T_H cells are differentiated to T_H1 cells which further differentiates to form T_{DTH} cells
- **Effector phase:** The T_{DTH} cells, on subsequent contact with the antigen, secrete variety of cytokines such as:
  - Interferon γ
  - IL-2
  - MCAF (*Monocyte* chemotactic and activating factor)
  - TNF-β
  - MIF (migration inhibitory factor)
  - IL-3
  - GM-CSF (granulocyte-monocyte colony stimulating factor).

Cytokines in turn perform various functions which may be either protective type or tissue damage type.

Lepra reactions
- Lepra reaction type I, e.g. of HSN IV
- Lepra reactions type II, e.g. of HSN III

Table 2.4.5: Infections/conditions associated with type IV hypersensitivity reactions

Intracellular bacteria: • *Mycobacterium leprae* • *M.tuberculosis* • *Listeria monocytogenes* • *Brucella abortus*	**Intracellular fungi:** • *Pneumocystis jirovecii* • *Candida albicans* • *Histoplasma capsulatum* • *Cryptococcus neoformans*	**Noninfectious conditions:** • Diabetes mellitus type 1 • Multiple sclerosis • Peripheral neuropathies • Hashimoto's thyroiditis • Crohn's disease • Chronic transplant rejection • Graft-versus-host disease
Intracellular viruses: • Herpes simplex virus • Variola (smallpox) • Measles virus	**Skin tests to demonstrate DTH:** • Tuberculin test (Mantoux test) • Lepromin test • Montenegro test (leishmaniasis) • Frie test – done in LGV	**Granuloma formation seen in:** Tuberculosis, sarcoidosis, schistosomiasis and other trematode infections **Other example:** Lepra reaction type I
Contact dermatitis: Following exposure to contact antigens: Nickel, poison ivy, poison oak, picryl chloride		

MULTIPLE CHOICE QUESTIONS

TYPE-I HYPERSENSITIVITY

1. **Asthma is due to which type of hypersensitivity reaction?** *(Recent Question 2015)*
   a. Type 1 b. Type 2
   c. Type 3 d. Type 4
2. **Schultz dale phenomenon, the following is true statement:** *(TNPG 2015)*
   a. Anaphylaxis in vitro
   b. Test to detect atopy
   c. Type V hypersensitivity reaction
   d. Test for allergic contact dermatitis
3. **Type I hypersensitivity is mediated by which of the following immunoglobulins?** *(NEET Pattern Based Kerala 2016)*
   a. IgA b. IgG
   c. IgM d. IgE
4. **Anaphylaxis is mediated by:** *(PGI May 2013)*
   a. 5-hydroxytryptamine
   b. Heparin
   c. Prostaglandin
   d. Anaphylotoxins from complement activation
   e. Platelet activating factor

5 **Allergic rhinitis is an example of:** *(Recent MCQ 2013)*
   a. Type I hypersensitivity reaction
   b. Type II hypersensitivity reaction
   c. Type III hypersensitivity reaction
   d. Type IV hypersensitivity reaction

TYPE-II HYPERSENSITIVITY

6. **Lysis of red cells in autoimmune anemias and hemolytic disease of the newborn are examples of:** *(MHPG 2015)*
   a. Type I hypersensitivity
   b. Type II hypersensitivity
   c. Type III hypersensitivity
   d. Type IV hypersensitivity
7. **Type 2 hypersensitivity reaction seen in:** *(Recent Question 2013)*
   a. Myasthenia gravis
   b. Good pasture syndrome
   c. Sarcoidosis
   d. Grave's disease
   e. Hyper acute graft rejection
8. **2 days after penicillin administration, antibodies developed that resulted in hemolysis. Which type of hypersensitivity reaction is this?** *(AIIMS Nov 2014)*
   a. Type-I b. Type-II
   c. Type-III d. Type-IV

TYPE-III HYPERSENSITIVITY

9. **Serum sickness is which type of reaction?** *(AIIMS MAY 2016)*
   a. Type 1 b. Type 2
   c. Type 3 d. Type 4
10. **Which of the following is an immune complex mediated disease?** *(PGI May 2016)*
    a. SLE
    b. Bronchial asthma
    c. Rheumatoid arthritis
    d. Good pasture
    e. Tuberculin skin test
11. **Type II lepra reaction is an example hypersensitivity type:** *(JIPMER 2013, 2014)*
    a. I b. II
    c. III d. IV
12. **The hypersensitivity reaction involved in the hyper acute rejection of renal transplant is:** *(AIIMS May 2005)*
    a. Type I b. Type II
    c. Type III d. Type IV

TYPE-IV HYPERSENSITIVITY

13. **Which of the following is type IV hypersensitivity reaction?** *(NEET Pattern Based; PGI June 2005)*
    a. Arthus reaction
    b. Serum sickness
    c. Schwartzman reaction
    d. Granulomatous disease
14. **Skin tests are used for which hypersensitivity reactions?** *(PGI June 2007)*
    a. I b. II
    c. II d. IV
    e. V

EXPLANATIONS

TYPE-I HYPERSENSITIVITY

1. **Ans. (a) (Type 1)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p174

2. **Ans. (a) (Anaphylaxis...)** Ref :Apurba Sastry's Essentials of Medical Microbiology 1/e p172, Ananthanarayan 9/e p164
   - Schultz dale phenomenon is an example of type I hypersensitivity (Anaphylaxis in vitro)

3. **Ans. (d) (IgE)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p174, Ananthanarayan 9/e p162, 8/e p163
   - *Type 1 hypersensitivity reaction is dependent on IgE antibody (also known as Reagin antibody or cytotropic antibody).*

4. **Ans. ALL (a) (b) (c) (d) (e)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p172-5, Ananthanarayan 9/e p164
   Refer chapter review to know the list of mediators of anaphylaxis (i.e. type-I hypersensitivity reaction)

5. **Ans. (a) (Type I hy...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p172-5, Ananthanarayan 9/e p165
   - Allergic rhinitis or Hay fever is, e.g. of Type I hypersensitivity reaction.

TYPE-II HYPERSENSITIVITY

6. **Ans. (b) (Type II hypersensitivity)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p177
   Refer chapter review.

7. **Ans. (a) (b) (d) (Myasthenia gravis, Good pasture syndrome, Grave's disease)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p176-7, Ananthanarayan 9/e p16
   Refer chapter review for explanation.

8. **Ans. (b) (Type-II)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p176-7Penicillin in high doses can induce immune mediated hemolysis via the hapten mechanism in which antibodies are targeted against the combination of penicillin in association with red blood cells. Complement is activated by the attached antibody leading to the removal of red blood cells by the spleen. This is an example of drug induced type-II hypersensitivity reaction.

TYPE-III HYPERSENSITIVITY

9. **Ans: (c) (type 3)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p179
   Refer text

10. **Ans (a,c) (SLE, RA)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p176-79
    - SLE and Rheumatoid arthritis – Type III (Immune complex mediated hypersensitivity)
    - Bronchial asthma (HSN type I), Good pasture (HSN type II) and Tuberculin test (HSN type IV)
    - Rheumatoid arthritis, there is complex of both type III and IV hypersensitivity Reaction. But Type III is a more appropriate answer than type IV. (Kuby and Roitt's immunology).

11. **Ans. (c) (III)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p179 , Ananthanarayan 9/e p165

12. **Ans. (c) (Type III)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p178, Ananthanarayan 9/e p166, 8/e p162
    - *Hyperacute graft rejection is an examples of Types III Hypersensitivity reaction whereas chronic graft rejection and Graft-versus-host disease is Types IV Hypersensitivity reaction.*

TYPE-IV HYPERSENSITIVITY

13. **Ans. (d) (Granu...)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p180, Ananthanarayan 9/e p166, 8/e p162
    - Granulomatous disease– type IV *Hypersensitivity reaction*
    - Arthus reaction, Serum sickness and Schwartzman reaction – type III *Hypersensitivity reaction.*

14. **Ans. (a), (d) (Type I, IV)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p180, Ananthanarayan 9/e p162
    - Type I skin test: Casoni skin test
    - Type IV skin test: Tuberculin, Lepromin and patch test.

Autoimmunity, Immunodeficiency, Transplantation, and Immunoprophylaxis

CHAPTER 2.5

AUTOIMMUNITY

Autoimmunity is a condition in which the body's own immunologically competenT-cells or antibodies act against its self-antigens resulting in structural or functional damage.

Immunological Tolerance

Immunological tolerance is a state in which an individual is incapable of developing an immune response against his own tissue antigens. It is mediated by two broad mechanisms—central tolerance and peripheral tolerance.

Central Tolerance

This refers to the deletion of self-reactive T and B lymphocytes during their maturation in central lymphoid organs

- In thymus: Removes the self reacting T-cells by negative selection
- In bone marrow for B-cells: Removes the self reacting B-cells by negative selection and receptor editing

Central tolerance (Mechanism):
- *In thymus:* Removes the self reacting T-cells by negative selection
- *In bone marrow:* Removes the self reacting B-cells by negative selection and receptor editing.

Peripheral Tolerance

This refers to several back-up mechanisms that occur in the *peripheral* tissues to counteract the self-reactive T-cells that escape central tolerance. It is provided by several mechanisms.

1. *Ignorance:* The self-reactive T-cells might never encounter the self-antigen which they recognize and therefore remain in a state of ignorance.
2. *Anergy:* By blocking co-stimulatory signal by binding of B7 molecules on APC to CTLA-4 molecules on T-cells
3. *Phenotypic skewing*: Self-reactive T-cells stimulated by self-antigens secrete nonpathogenic cytokines
4. *Apoptosis of Self-reactive T-cells by* activation-induced cell death (AICD)
5. *Regulatory T-cells* (T_{reg} *cells)* can down regulate the self-reactive T-cells
6. *Dendritic cells (DCs):* When certain dendritic cells, such as immature DCs and tolerogenic DCs capture the self-antigen for processing, they down regulate the expression of molecules of co-stimulatory ligands, such as CD40 and B7 molecules or act indirectly by induction of regulatory T-cells.
7. *Sequestration of self-antigen* in immunologically privileged sites, e.g. corneal proteins, testicular and brain antigens.

Peripheral tolerance (Mechanism):
- Ignorance
- Anergy (CTLA-4 mediated)
- Phenotypic skewing
- Apoptosis of self-reactive T-cells
- Regulatory T-cells mediated
- Dendritic cells (immature DCs and tolerogenic) mediated
- Sequestration of self-antigen.

Mechanisms of Autoimmunity

- Breakdown of CTLA-4 mediated T-Cell Anergy: Seen in multiple sclerosis, rheumatoid arthritis and psoriasis
- Failure of AICD (activation-induced cell death): Seen in SLE
- Loss of T_{reg} cells
- Providing T-cell help to stimulate self-reacting B-cells
- Release of Sequestered Antigens (spermatozoa and ocular antigens) due to injury to organs
- Molecular Mimicry, e.g. in post streptococcal acute rheumatic fever and glomerulo-nephritis
- Polyclonal Lymphocyte Activation: Mediated by Superantigens, EBV and HIV
- Exposure of cryptic self-epitopes
- Epitope spreading
- Bystander activation.

Table 2.5.1: Autoimmune diseases

Single Organ or Cell Type Autoimmune Diseases		
Disease	**Self-antigen present on**	**Type of immune response and important features**
Autoimmune anemias		
Autoimmune hemolytic anemia	RBC membrane proteins	Auto-antibodies to RBC antigens triggers complement mediated lysis or antibody-mediated and opsonization of the RBCs
Drug induced hemolytic anemia	Drugs alter the red cell membrane antigens	Drugs such as penicillin or methyldopa interact with RBCs so that the cells become antigenic
Pernicious anemia	Intrinsic factor (a membrane-bound protein on gastric parietal cells)	Auto-antibodies to intrinsic factor block the uptake of vitamin B_{12}; leads to **megaloblastic anemia**
Idiopathic Thrombocytopenic Purpura	Platelet membrane proteins (glycoproteins IIb-IIIa or Ib-IX)	Auto-antibodies against platelet membrane antigens leads to ↓platelet count
Goodpasture syndrome	Renal and lung basement membranes	Auto-antibodies bind to basement-membrane antigens on kidney glomeruli and the alveoli of the lungs, followed by complement mediated injury leads to progressive kidney damage and pulmonary hemorrhage
Myasthenia gravis	Acetylcholine receptors	**Blocking type of autoantibody** directed against Ach receptors present on motor nerve endings, leads to progressive weakening of the skeletal muscles
Graves' disease	Thyroid-stimulating hormone (TSH) receptor	Anti TSH- autoantibody (stimulates thyroid follicles, leads to hyperthyroid state)
Hashimoto's thyroiditis	Thyroid proteins and cells	Autoantibodies and T_{DTH} cells targeted against thyroid antigen leads to suppression of thyroid gland: • Seen in middle aged females • Hypothyroid state is produced (↓ production of thyroid hormones)
Post-streptococcal glomerulonephritis	Kidney	Streptococcal antigen: antibody complexes are deposited on glomerular basement membrane
Insulin-dependent diabetes mellitus	Beta cells present in islets of Langerhans of pancreas	T_{DTH} cells and auto-antibodies directed against pancreatic beta cells cause ↓ production of insulin
Spontaneous infertility	Sperm antigens released due to testicular injury	Autoantibodies directed against sperm antigens lead to infertility
Addison's disease	Adrenal cells	Autoimmune destruction of the adrenal cortex, caused by autoantibodies against the enzyme 21-hydroxylase leads to chronic primary adrenal insufficiency (↓ steroid hormones production)
Systemic Autoimmune Diseases		
Systemic lupus erythematosus	Autoantibodies are produced against various tissue antigens such as DNA, nuclear protein, RBC and platelet membranes.	• **Age and sex**: Women (20–40 years of age) are commonly affected; female to male ratio is 10:1. • **Immune complexes** (self Ag- auto Ab) are formed; which are deposited in various organs • **Major symptoms**: Fever, **butterfly rash** over the cheeks, arthritis, pleurisy, and kidney dysfunction
Rheumatoid arthritis	Here, a group of autoantibodies against the host IgG antibodies are produced called **RA factor**. It is an IgM antibody directed against the Fc region of IgG. **ACPA** (Anti citrullinated peptide antibodies) are also produced	• **Age and sex**: Women (40–60 years of age) affected • **Auto-antibodies** bind to circulating IgG, forming IgM-IgG complexes that are deposited in the joints and can activate the complement cascade. • ***Main feature***: Arthritis (chronic inflammation of the joints, begins at synovium; most common joints involved are Small joints of the hands, feet and cervical spine) • ***Other features***: Hematologic, cardiovascular, and respiratory systems are also frequently affected
Sjögren syndrome	Ribonucleoprotein (RNP) antigens SS-A (Ro) and SS-B (La) present on salivary gland, lacrimal gland, liver, kidney, thyroid	Auto-antibodies to the RNP antigens SS-A (Ro) and SS-B (La); leads to immune-mediated destruction of the lacrimal and salivary glands resulting in dry eyes (*keratoconjunctivitis sicca*) and dry mouth (xerostomia)

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Single Organ or Cell Type Autoimmune Diseases		
Disease	**Self-antigen present on**	**Type of immune response and important features**
Scleroderma (Systemic Sclerosis)	Nuclear antigens such as DNA topoisomerase and centromere present in heart, lungs, GIT, kidney, etc.	Helper T-cell (mainly) and autoantibody mediated. Excessive fibrosis of the skin, throughout the body **Two types:** 1. **Diffuse scleroderma**: Autoantibodies against DNA topoisomerase I (anti-Scl 70) is elevated 2. **Limited scleroderma** ↑Anticentromere antibody, characterized by **CREST syndrome:** Calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia
Seronegative Spondyloarthropathies	Sacroiliac joints and other vertebrae Several types: • Ankylosing spondylitis • Reiter syndrome • Psoriatic arthritis • Spondylitis with inflammatory Bowel disease • Reactive arthritis	Common characteristics: They present as rheumatoid arthritis like features, but differ from it by: • Association with HLA-B27 • Pathologic changes begin in the ligamentous attachments to the bone rather than in the synovium • Involvement of the sacroiliac joints, and/or arthritis in other peripheral joints • Absence of RFs (hence the name 'seronegative') • Auto-Ab and immune complex mediated
Multiple sclerosis	Brain (white matter)	Self-reactive T-cells produce characteristic inflammatory lesions in brain that destroys the **myelin** sheath of nerve fibers; leads to numerous neurologic dysfunctions

IMMUNODEFICIENCY DISORDERS

Immunodeficiency is a state where the defense mechanisms of the body are impaired, leading to enhanced susceptibility to microbial infections as well as to certain forms of cancer. It is broadly classified as primary or secondary.

- Primary immunodeficiency diseases result from inherited defects affecting immune system development.
- Secondary immunodeficiency diseases are secondary to some other disease process that interferes with the proper functioning of the immune system (e.g. infection, malnutrition, aging, immunosuppression, autoimmunity, or chemotherapy). They are more common than primary immunodeficiency diseases.

Table 2.5.2: Primary immunodeficiency diseases

Humoral immunodeficiency (B-cell defects)	
1	**Bruton disease (X-linked agammaglobulinemia)** It is characterized by failure of pre-B-cells to differentiate into immature B-cells in the bone marrow- due to absence of an enzyme called Bruton's tyrosine kinase leading to total absence of B-cells, plasma cells and all classes of Ig: • The B-cell maturation stops at pre B cell stage; after the synthesis of heavy-chain without forming the light chains. Hence the cytoplasm of pre B cell may have incomplete immunoglobulins. • X linked, seen primarily in males; rarely in females. Onset -after 6 months of life. • Secondary infection: Recurrent bacterial infections, viruses (enteroviruses) and parasites (*Giardia lamblia*) • Autoimmune diseases (such as SLE and dermatomyositis) also occur in up to 20% of cases.
2	**Common variable immunodeficiency** The clinical manifestations (secondary infection, autoimmune diseases) are superficially similar to those of Bruton diseases; but differ in the following aspects: • Both sexes are affected equally • Onset of symptoms is much later, in the second or third decade of life. • It is also B-cell development defect; B-cells may be present in circulation in normal numbers, but they appear defective in their ability to differentiate into plasma cells and secrete immunoglobulins. • The diagnosis is usually one of exclusion (after other causes of immunodeficiency are ruled out); the basis of the immunoglobulin deficiency is variable (hence the name). • The defect in the antibody production has been variably attributed to intrinsic B-cell defects, deficient T-cell help, or excessive T-cell suppressor activity.

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3	**Isolated IgA deficiency** It is the most common of all the primary immunodeficiency diseases, affects about 1 in 700 white individuals: • IgA deficiency leads to defective mucosal immunity; predispose patients to recurrent sinopulmonary infections and diarrhea. There is also a significant autoimmune diseases. • Pathogenesis is due to a block in the terminal differentiation of IgA-secreting B-cells to plasma cells, which in turn is due to altered T-cell production of cytokines that drive IgA responses (e.g. TGF-β and IL-5) or due to intrinsic B-cell defect. The levels of other immunoglobulins are usually normal or even excess.
4	**Hyper-IgM syndrome** Hyper-IgM syndrome is an X-linked disorder; results due to a defect in *isotype class switch over* of B-cells, due to mutations in either CD40L or CD40 genes; prevent the T- and B-cell interaction; needed for class switch over: • Hyper IgM levels with lack of synthesis of other classes of antibodies • Lack of other classes of Ig leads to secondary infection, autoimmune diseases
5	**Transient hypogammaglobulinemia of infancy** This occurs due to an abnormal delay in the initiation of synthesis IgG (or some time IgA or IgM): • IgG synthesis usually starts by 2 months of age. But in some infants, it is delayed leading to defect in opsonization or complement activation resulting in recurrent otitis media and respiratory infections. • However, spontaneous recovery occurs usually by 18–24 months of age. • Interestingly, these infants show a normal antibody response against vaccines.
Cellular immunodeficiencies (T–cell defects)	
1	**DiGeorge syndrome (Thymic hypoplasia)** It results from a congenital defect in thymic development leading to defect in T-cell maturation: • Infants are extremely vulnerable to viral, fungal, intracellular bacterial and protozoan infections. • **Pathogenesis:** In 90% of cases, there occurs a deletion affecting chromosome 22q11 → leads to malformation of third and fourth pharyngeal pouches in embryonic life • As a result, all the structures that develop from third and fourth pharyngeal pouches such as thymus, parathyroid glands, and portions of the face and aortic arch become defective. • Thus, in addition to the thymic defects, there may be associated: ○ Parathyroid gland hypoplasia resulting in neonatal tetany and hypocalcemia ○ Anomalies of the heart and the great vessels (Fallot'sTetralogy). ○ Characteristic facial appearance • B-cells and serum immunoglobulin levels are generally unaffected. • Treatment: Thymus transplantation
2	**Chronic mucocutaneous candidiasis** It represents an impaired CMI against *Candida albicans* leads to superficial infections of the skin, mucosa and nails: There is no↑ risk of infections but it is often associated with endocrinopathies and autoimmune disorders. Transfer factor therapy, along with amphotericin B has been reported to be effective.
3	**Purine nucleoside phosphorylase (PNP) deficiency** It is a rare autosomal recessive disorder (chromosome 14), characterized by deficiency of an PNP enzyme: • PNP is a key enzyme required for purine degradation; catalyzes the conversion of guanosine to hypoxanthine. • Its deficiency leads to elevated deoxy-GTP levels resulting in T-cell toxicity. However, B-cells are not affected. • T-cell depletion predisposes to increased susceptibility to infection and autoimmune disorders.
Combined immunodeficiencies (B and T-cell defects)	
1	**Severe combined immunodeficiencies** SCID represents groups of genetically distinct syndromes; all having in common defects in both humoral and CMI. • *Types of genetic defect in SCID include:* ○ Mutation in cytokine receptors (IL-7 mainly and others like IL-2, IL-4, IL-9, and IL-15)- MC type defect (50–60%), X-linked,seen in males ○ The remaining cases of SCID are inherited as autosomal recessive manner; include: ▪ *Adenosine deaminase (ADA)* deficiency ▪ Recombinase-activating genes (RAG) mutation ▪ Jak3 (intracellular kinase) mutation ▪ Class II MHC deficiency (bare lymphocyte syndrome) • *Infections:* Affected infants are susceptible to severe recurrent infections by a wide array of pathogens • *Treatment:* Bone marrow transplantation and gene therapy replacing the mutated genes.
2	**Wiskott-Aldrich syndrome** It is an X-linked recessive disease, characterized by immunodeficiency with thrombocytopenia, eczema: • The severity of WAS increases with age. • It first manifests itself by defective responses to bacterial polysaccharides and by lower IgM levels. IgG levels are usually normal. Paradoxically the levels of IgA and IgE are often elevated. • Other T and B cell responses are normal initially, but with increase of age, there are recurrent bacterial infections and a gradual loss of humoral and cellular responses.

Contd...

Contd...

	• Patients are also prone to develop nonhodgkin B-cell lymphomas. • Patients may present with bloody diarrhea secondary to thrombocytopenia. • ***Pathogenesis:*** Mutation in the gene coding WAS protein present in precursor lymphoid cells of bone marrow. It is a cytoskeletal glycoprotein (sialophorin or CD43), required for actin polymerization.
3	**Ataxia telangiectasia:** The syndrome is characterized by: • Difficulty in maintaining balance while walking (cerebellar ataxia) • Appearance of broken capillaries (telangiectasia) in the eyes and choreoathetoid movements (in infancy). • Deficiency of IgA and sometimes IgE • Profound sinopulmonary infections The primary defect appears to be in a kinase involved in regulation of the cell cycle. The relationship between the immune deficiency and the other defects in ataxia telangiectasia remains obscure.
4	**Nezelof syndrome** It is an autosomal recessive condition characterized by cellular immunodeficiency resulting from thymus hypoplasia: • In some patients, B-cells are normal, whereas in others a B-cell deficiency is secondary to the T-cell defect. • Affected individuals suffer from chronic diarrhea, viral and fungal infections, and a general failure to thrive.
Disorders of phagocytosis	
1	**Chronic granulomatous disease** **Pathogenesis** involves inherited defects in the gene encoding NADP oxidase of phagocyte which breaks down hydrogen peroxide → leads to decreased oxidative burst which predisposes to (i) recurrent catalase producing bacterial infections, (ii) ↑inflammatory reactions that result in gingivitis, swollen lymph nodes, etc. CGD is a genetic disease that runs in family in two forms: • In X-linked form (more common, 70%): membrane component of phagocyte oxidase is defective. • In autosomal recessive form: cytoplasmic component of phagocyte oxidase is defective. • Nitroblue tetrazolium reduction test (NBT) is positive
2	**Myeloperoxidase deficiency** Deficiency in either quantity or function, of myeloperoxidase, an enzyme produced by neutrophils. Patients present with immune deficiency and recurrent infections, especially with *Candida albicans*.
3	**Chediak-Higashi syndrome:** It is an autosomal recessive disease, characterized by: • Defective fusion of phagosomes and lysosomes in phagocytes → leads to ↑ susceptibility to pyogenic infections • Abnormalities in melanocytes leading to albinism (lack of skin and eye pigment) • Abnormalities in cells of the nervous system (associated with nerve defects) • Platelets abnormalities, causing bleeding disorders • Aggressive but nonmalignant infiltration of organs by lymphoid cells. **Pathogenesis:** Due to a *mutation in a protein called LYST* which is believed to regulate lysosomal trafficking: • The mutation impairs the targeting of proteins to secretory lysosomes, which makes them unable to lyse bacteria. • Phagocytes contain *giant granules* but do not have the ability to kill bacteria.
4	**Leukocyte adhesion deficiency** is rare autosomal recessive disorder, characterized by a defect in the adhesion of leukocytes which results in poor leukocyte chemotaxis particularly neutrophil, inability to form pus and neutrophilia. Thus it predisposes to various bacterial and fungal infections. LAD is of two types. • **LAD 1**: Mutations in β2 integrin subunit (CD18), of the leukocyte cell adhesion molecule (in chromosome 21). • **LAD 2**: Mutations in fucosyl transferase required for synthesis of sialylated oligosaccharide
5	**Lazy leukocyte syndrome** It is an idiopathic condition due to defect in neutrophil chemotaxis which results in increased pyogenic infections such as gingivitis, abscess formation, pneumonia and neutropenia.
6	**Job's syndrome or Hyper-IgE syndrome:** Rare, characterized by eczema, recurrent staphylococcal skin abscesses, recurrent lung infections (pneumatocele), eosinophilia and high serum levels of IgE. **Mechanism:** Due to a defect in neutrophil chemotaxis. Most cases are sporadic, but some familial cases have also been reported which may be: • Autosomal dominant cases: Linked to mutations in the STAT3 gene • Autosomal recessive cases: Due to mutations in DOCK8 gene.
7	**Tuftsin deficiency** Tuftsin is a tetrapeptide (Thr-Lys-Pro-Arg) produced primarily in the spleen, by the cleavage of the Fc-portion of the heavy chain of IgG. It stimulates phagocytosis. Tuftsin deficiency results in ↑ susceptibility to capsulated organisms.
8	**Shwachman's disease** It is a rare congenital disorder characterized by neutropenia, exocrine pancreatic insufficiency, bone marrow dysfunction, skeletal abnormalities, and short stature
Disorders of complement (Described in chapter 2.2)	
1	**Complement component deficiencies**
2	**Complement regulatory protein deficiencies**

Contd...

Table 2.5.3: Risk of secondary infections in various immunodeficiencies

Pathogen	T-cell defect	B-cell defect	Granulocyte defect	Complement defect
Bacteria	Bacterial sepsis	Streptococci, Staphylococci, *Haemophilus influenzae*	Staphylococci, *Pseudomonas Nocardia*	*Neisseria*, *Toxoplasma* Other pyogenic Infections
Viruses	CMV, EBV, Severe varicella, Chronic infections with respiratory and intestinal viruses	Enterovirus encephalitis	-	
Fungi	*Candida, Pneumocystis jirovecii*	-	*Candida, Aspergillus*	
Parasites	-	Giardiasis	-	
Special features	Aggressive disease with opportunistic pathogens, failure to clear infections	Recurrent sinopulmonary infections, Sepsis, chronic meningitis	-	

TRANSPLANT IMMUNOLOGY

Based on the genetic relationship between the donor and the recipient:

- Autograft is self-tissue transferred from one part of the body site to another in the same individual, e.g. skin grafts
- Isograft or syngeneic graft is tissue transferred between genetically identical individuals (e.g. monozygotic twins)
- Allograft is tissue transferred between genetically non-identical members of the same species (e.g. most transplants)
- Xenograft is tissue transferred between different species (e.g. the graft of a baboon heart into a man).

Table 2.5.4: Types of graft rejection

Graft rejection	Time taken for rejection	Immune mechanisms involved
Hyperacute	Minutes to hours	Preformed antibodies (Anti ABO and/or anti-HLA)
Acute	Weeks to months	Cytotoxic T-cell mediated
Chronic	Months to years	Chronic DTH mediated and Antibody mediated

Immune mechanisms of graft rejection:
- *Hyperacute:* Preformed Ab mediated
- *Acute:* Tc cell mediated
- *Chronic:* DTH mediated and Ab mediated.

Second set rejection of the graft is always faster than the first set rejection, i.e. if, in a recipient that has rejected a graft by the first set response, another graft from the same donor is transplanted, it will be rejected in an accelerated fashion.

Mechanism of Graft Rejection

The process of graft rejection can be divided into two stages:

1. **Sensitization phase:** Involves alloantigen (mainly graft MHC molecules) presentation to recipient's T-cells either by direct or indirect pathways:
   - Direct pathway: The MHC molecules on graft's APCs are directly presented to the recipient's helper T-cells. This pathway is responsible for most of the acute graft rejections mediated by cytotoxic T-cells.
   - Indirect pathway: This is similar to that for recognition of any foreign antigen by the host APCs. The graft alloantigens are processed and presented by recipient APCs to recipient's helper T-cells. This pathway is responsible for most of the chronic rejection mediated by helper T-cells via specialized form of chronic DTH reaction.
2. **Effector phase:** This involves a variety of effector mechanisms leading to immune destruction of the graft such as:
   - Cell-mediated reactions involving delayed-type hypersensitivity T-cells and cytotoxic T-cells.
   - Antibody mediated mechanisms: Complement mediated lysis and ADCC.

Laboratory Tests to Determine Histocompatibility

Prior to transplantation, various laboratory tests should be carried out to assess the histocompatibility:

- ABO blood group compatibility testing by blood grouping and cross matching.
- Immunosuppressive therapy
- HLA typing: In this test, donor's antigens expressed on the surface of leukocytes or their gene to that of recipient are matched. The HLA compatibility is determined by:
  - Phenotypic method: Microcytotoxicity (serology) and Mixed lymphocyte reaction (tissue typing)
  - Genotypic methods, such as:
    - PCR detecting HLA genes and PCR-RFLP
    - Variable number tandem repeat (or VNTR) typing and STR (Short tandem repeat) typing
    - DNA Sequence based typing and Karyosome analysis.

Graft-Versus-Host Reaction

GVH reaction is a condition, where graft mounts an immune response against the host (i.e. recipient) and rejects the host, in contrary to the usual situation where the recipient mounts an immune response against the graft antigens.

GVH reaction occurs when the following three conditions are present:

- The graft must contain immunocompetent T-cells (e.g. stem cells or bone marrow or thymus transplants)
- The recipient should possess transplantation antigens that are absent in the graft.
- The recipient may be immunologically suppressed and therefore cannot mount immune response against the graft.

GVH reaction occurs in two forms. Acute or fulminant (occurs < 100 days) and chronic GVH disease (occurs after 100 days)

- The acute GVH disease is characterized by selective damage to the liver (hepatomegaly), skin (rash), mucosa, and the intestine (diarrhea) mediated graft's immunocompetent T-cell.
- Chronic GVH disease also attacks the above organs, but in addition, it causes damage to the connective tissues and exocrine glands.
- Experimentally, GVH reaction can be produced in mice, called Runt disease.
- Treatment: Glucocorticoids.

GVH reaction occurs when:
- The graft must contain immunocompetent T-cells
- The recipient should possess transplantation antigens that are absent in the graft
- The recipient may be immunologically suppressed.

Tumor Antigens

Two types of tumor antigens have been identified on tumor cells:

1. **Tumor-specific transplantation antigen (TSTA):** They are present only on tumor cells and are absent in normal cells.

   TSTA are induced on tumor cells either by chemical or by physical carcinogens, and also by viral carcinogens.
   - In chemically/physically induced tumors, the TSTA is tumor specific. Different tumors possess different TSTA, even though induced by the same carcinogen.
   - In contrast, the TSTA of virus induced tumors is virus specific; all tumors produced by one virus would possess the same antigen.
2. **Tumor-associated antigens (TATAs):** In addition to tumor cells, they may also be expressed by normal cells but at a very low level, e.g. Oncofetal antigens, Carcinoembryonic antigen (CEA), Carbohydrate antigens (CA 125, CA 19-9), prostate specific antigen and β2 macroglobulin.

Tumor-associated antigens (TATAs):
- Oncofetal antigens
- Carcinoembryonic antigen (CEA)
- Carbohydrate antigens (CA 125, CA 19-9)
- Prostate specific antigen and β2 macroglobulin.

IMMUNOPROPHYLAXIS

Table 2.5.5: Characteristics of killed and live vaccines

Characteristic	Killed Vaccine	Live Vaccine
Number of doses	Multiple	Single*
Need for adjuvant	Yes	No
Duration of immunity	Shorter	Longer
Effectiveness of protection	Lower	Greater
Mimics natural infection	Less closely	More closely
Immunoglobulins produced	IgG	IgA and IgG
Mucosal immunity	Absent	Induced
Cell-mediated immunity	Poor	Induced
Reverse back to virulent form	No	Possible
Feco-oral spread	No	Possible
Interference by other microorganisms in host	No	Possible
Stability at room temperature	High	Low
Immunodeficiency and pregnancy	Safe	Unsafe

*Exception is OPV, which is given in multiple doses at spaced intervals to achieve effective immunity

Table 2.5.6: Examples of vaccines

Live Vaccines		Killed/Inactivated vaccine	
Bacterial	**Viral**	**Bacterial**	**Viral**
BCG vaccine	Oral polio vaccine (OPV, Sabin vaccine)	Typhoid vaccine	Injectable polio vaccine (IPV or Salk vaccine)
Typhoral vaccine	Live attenuated influenza	Cholera vaccine	Killed influenza vaccine
Epidemic typhus	Yellow fever 17D vaccine	Pertussis vaccine	Rabies vaccine
	Chickenpox vaccine	Plague vaccine	Hepatitis A
	Japanese B encephalitis (14-14-2 strain)		Japanese B encephalitis (Nakayama strain)
	Measles vaccine	**Combined vaccine**	
	Mumps vaccine	**Bacterial**	**Viral**
	Rubella vaccine	DPT vaccine	MMR vaccine (mumps, measles, rubella)
	Hepatitis A vaccine	Pentavalent vaccine (DPT + Hib + Hep B)	
	Rotavirus vaccine	**Toxoid vaccine**	**Subunit vaccine**
		DT (Diphtheria toxoid)	Hepatitis B
Cellular fraction	Meningococcal, Pneumococcal, *Haemophilus influenzae* type b (Hib)	TT (Tetanus toxoid)	HPV (Human papilloma virus) vaccine

Table 2.5.7: National Immunization Schedule (NIS) for infants, children and pregnant women (India)

Vaccine	When to give	Dose	Route	Site
For Pregnant Women				
TT-1	Early in pregnancy	0.5 ml	Intramuscular	Upper arm
TT-2	4 weeks after TT-1*	0.5 ml	Intramuscular	Upper arm
TT-Booster	If received 2 TTdoses in a pregnancy within the last 3 years*	0.5 ml	Intramuscular	Upper arm
For Infants				
BCG	At birth or as early as possible till one year of age	0.1 ml (0.05 ml until 1 month age)	Intradermal	Left upper arm
Hepatitis B	At birth or as early as possible within 24 hours	0.5 ml	Intramuscular	Anterolateral side of mid thigh
OPV-0	At birth or as early as possible within the first 15 days	2 drops	Oral	Oral
OPV 1, 2 and 3	At 6 weeks, 10 weeks and 14 weeks	2 drops	Oral	Oral
DPT1, 2 and 3	At 6 weeks, 10 weeks and 14 weeks	0.5 ml	Intramuscular	Anterolateral side of mid thigh
Hepatitis B 1, 2 and 3	At 6 weeks, 10 weeks and 14 weeks	0.5 ml	Intramuscular	Anterolateral side of mid thigh
Measles	9 completed months-12 months (give up to 5 years if not received at 9-12 months age)	0.5 ml	Subcutaneous	Right upper arm
Vitamin A (1st dose)	At 9 months with measles	1 ml (1 lakh IU)	Oral	Oral
For Children				
DPT booster	16–24 months	0.5 ml	Intramuscular	Anterolateral side of mid thigh
OPV Booster	16–24 months	2 drops	Oral	Oral
Measles (2nd dose)	16–24 months	0.5 ml	Subcutaneous	Right upper arm
Japanese Encephalitis**	16–24 months with DPT/OPV booster	0.5 ml	Subcutaneous	Left upper arm
VitaminA*** (2nd to 9th dose)	16 months with DPT/OPV booster. Then one dose every 6 months up to the age of 5 years	2 ml (2 lakh IU)	Oral	Oral
DPT Booster	5–6 years	0.5 ml	Intramuscular	Upper arm
TT	10 years and 16 years	0.5 ml	Intramuscular	Upper arm

* Give TT-2 or Booster doses before 36 weeks of pregnancy. However, give these even if more than 36 weeks have passed. Give TT to a woman in labor, if she has not previously received TT.
** SA 14-14-2 Vaccine, in selected endemic districts of Uttarpradesh, Karnataka, West Bengal and Assam
*** The 2nd to 9th doses of Vitamin A can be administered to children 1–5 years old.

Table 2.5.8: Passive immunoprophylaxis

Immunoglobulin preparations	Source	Indications
Diphtheria antitoxin	Equine	Treatment of respiratory diphtheria
Tetanus immune globulin (TIG)	Equine, Human	Treatment of tetanus as PEP, for people not adequately immunized with tetanus toxoid
Botulinum antitoxin	Equine, Human	Treatment of botulism
Varicella-zoster immune globulin (VZIG)	Human	PEP for immunosuppressed contacts of acute cases or new born contacts
CMV-IG	Human	PEP in hematopoietic stem cell and kidney transplant recipients
Rabies immune globulin (RIG)	Equine, Human	Treatment of rabies and PEP in people not previously immunized with rabies vaccine

Contd...

Contd...

Immunoglobulin preparations	Source	Indications
Hepatitis B immune globulin (HBIG)	Human	PEP for-Percutaneous or mucosal or sexual exposure and Newborn of mother with HBsAg +ve
Hepatitis A immune globulin (HAIG)	Human	Post exposure prophylaxis for Family contacts and Travelers
Rubella	Human	Women exposed during early pregnancy
Measles	Human	Infants or immunosuppressed contacts of acute cases exposed < 6 days previously
Rh iso immunization (RhIG)	Human	**Treatment –** Rh-ve mother on delivery of a Rh+ve baby

MULTIPLE CHOICE QUESTIONS

NATIONAL IMMUNISATION SCHEDULE

1. Which vaccine can be given to pregnant women: *(Recent Question 2013, PGI Dec 2006)*
a. Hepatitis B
b. Meningococcus
c. Rabies
d. Measles
e. BCG

2. According to national immunisation schedule, which of the following is recommended for a child of 5-year of age? *(AIMS Nov 2013)*
a. Pentavalent vaccine and vitamin A
b. DT booster
c. DT, OPV and vitamin A
d. DPT booster and vitamin A

3. Live attenuated vaccine: *(Recent Question 2015)*
a. Mumps
b. Hepatitis B
c. Salk vaccine
d. Typhoral

AUTOIMMUNITY

4. Which of the following is a immunologically privileged site? *(AIIMS Noc 2015)*
a. Seminiferous tubule
b. Area postrema
c. Optic nerve
d. Loop of henle

5. Which is not an autoimmune disorder? *(TNPG 2015)*
a. Myasthenia gravis
b. Sickle cell anemia
c. Graves' disease
d. SLE

6. Autoimmunity can be caused due to all of the following except: *(AIIMS May 2005)*
a. The pressure of forbidden clones
b. Expression of cryptic antigens
c. Negative selection of T-cells in the thymus
d. Inappropriate expression of the MHC proteins

IMMUNODEFICIENCY

7. All are true about severe combined immunodeficiency except: *(PGI May 2015)*
a. B & T cell deficiency
b. Adenosine deaminase deficiency may occur
c. Affected child can survives beyond adolescence without treatment
d. Can transmit either as X-linked or autosomal recessive defect
e. Person susceptible to recurrent and severe infections

8. Chronic granulomatous disorder is due to defect in: *(NEET Pattern Based)*
a. B-cell
b. NADPH oxidase
c. IgA
d. T-cell

9. Purine Nucleoside phosphorylase deficiency: *(NEET Pattern Based)*
a. Humoral immunity deficiency
b. Acquired immunity deficiency
c. SCIDs
d. Cell mediated immunity deficiency

10. Decreased IgM, bleeding tendency with eczema is seen in: *(AI 2012)*
a. Wiskott-Aldrich syndrome
b. Chronic granulomatous disease
c. Job's syndrome
d. Chediak Higashi syndrome

11. In a 5-year-old boy who has history of pyogenic infections by bacteria with polysaccharide-rich capsules, which of the following investigations should be done? *(AI 2012, AIIMS May 2012)*
a. IgA deficiency
b. IgG1 deficiency
c. IgG2 deficiency
d. IgA and IgG2 deficiency

12. Adenosine deaminase deficiency is seen in the following: *(NEET Pattern Based) (AI 2005, 2001)*
a. Common variable immunodeficiency
b. Severe combined immunodeficiency
c. Chronic granulomatous disease
d. Nezel of syndrome

13. The commonest primary immunodeficiency is: *(PGI June 2005)*
a. Common variable immunodeficiency
b. Isolated IgA immunodeficiency
c. Wiskott – Aldrich syndrome
d. AIDS

14. Which is found in DiGeorge's syndrome *(PGI 2001)*
a. Tetany
b. Eczema
c. Mucocutaneous Candidiasis
d. Absent B-and T-cells
e. Total absence of T-cells

15. Disorders of phagocytosis are all except:
a. Job's syndrome *(PGI May 2013)*
b. Chediak-Hegashi syndrome
c. Myeloperoxidase deficiency
d. Wiskott-Aldrich Syndrome
e. Tuftsin deficiency

TRANSPLANTATION IMMUNOLOGY

16. A woman with infertility receives an ovary transplant from her sister who is an identical twin. What type of graft is? *(AI 2005)*

a. Xenograft
b. Autograft
c. Allograft
d. Isograft

17. Hyperacute graft rejection is seen in: *(Recent MCQ 2013)*

a. Bone marrow
b. Kidney
c. Liver
d. Heart

18. Allograft rejection is an example of: *(JIPMER 2014)*

a. GVHD
b. Delayed (cell mediated) hypersensitivity
c. Immediate hypersensitivity
d. Acute rejection

19. Skin transplant was done from sister to brother. After few years, brother to sister skin transplant was done, but rejection occurred. What is this phenomena called? *(Recent MCQ 2013)*

a. Schwartzman reaction
b. Theobald Smith phenomena
c. Eichwald Silmser effect
d. Schultz Dale phenomena

EXPLANATIONS

NATIONAL IMMUNISATION SCHEDULE

1. **Ans. (a), (b), (c) (Hepatitis B, Meningococcus, rabies)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p205 Park 22 /e p98/, 21/e p98

   All live vaccine such as Measles and BCG are contraindicated in pregnancy

2. **Ans. (d) (DPT booster and vitamin A)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p205, Park 22/e p115

   According to national immunization schedule in India:
   - DPT schedule: 1st/2nd/3rd dose at 6/10/14 weeks and two boosters at 16–24 months and 5–6 yr
   - Vitamin A: 1st dose at 9 month (along with measles), 2nd dose at 16–24 months (along with DPT booster) and the 3rd to 9th dose given every 6 month till 5 yrs.
   - OPV schedule: Zero dose at birth, then 1st/2nd/3rd dose at 6/10/14 weeks and booster at 16–24 months.

3. **Ans. (d) (Typhoral)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p203

AUTOIMMUNITY

4. **Ans. (a) (Seminiferous tubule)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p184

   Seminiferous tubule, spermatozoa, lens protein, placenta and the fetus are the immunologically privileged site. Brain is no longer considered immunologically privileged.

5. **Ans. (b) (Sickel cell anemia)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p185

   Refer chapter review of chapter 2.5.

6. **Ans. (c) (Negative selection of T-cells in thymus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p150, Harrison 17/e p2071-2073

   Negative selection of T-cell is a process by which self reactive T-cells are killed in thymus–this is one of the mechanism of preventing development of Autoimmunity.

   The other options are the mechanisms of preventing Autoimmunity:
   - Forbidden clones with self reactivity arise as a result of failure of negative selection of autoreactive T-cell.
   - Cryptic antigens are the antigenic epitopes, which are normally not exposed, gets exposed to the immune system as a result of injury or infection and leads to development of immune response.
   - Inappropriate expression of MHC proteins also leads to development of self-destructive immune response.

IMMUNODEFICIENCY

7. **Ans. (a,b,d,e) (B & T cell.., Adenosine deaminase..,Can transmit.., Person susceptible..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p190

   In Severe Combined Immunodeficiencies (SCID), both CMI are AMI are affected. It runs as:
   - X linked: Mutation in cytokine receptor
   - Autosomal recessive: It occurs as: (i) Adenosine deaminase (ADA) deficiency, (ii) Jak3 mutation, (iii) RAG mutation and (iv) Class II MHC deficiency
   - The affected infants are susceptible to severe recurrent infections by a wide array of pathogens, including Candida, Pneumocystis, cytomegalovirus and Pseudomonas.
   - Prognosis of SCID is poor. Bone marrow transplantation is the mainstay of treatment.

8. **Ans. (b) (NADPH oxidase)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p191, Ananthanarayan 9/e p172

   Chronic granulomatous disease:
   - Due to defect in phagocytosis (results from absence of NADPH oxidase)
   - ↑Recurrent catalase +ve pyogenic infection (catalase –ve organisms are handled normally)
   - *Screening test used- Nitroblue tetrazolium (NBT) reduction test is negative.*

9. **Ans. (d) (Cell mediated immunity deficiency)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p190

- Purine Nucleoside phosphorylase deficiency is an autosomal recessive inherited trait, leads to low CMI & ↑Recurrent infection (Candidiasis), hypoplastic anemia & ↓uric acid.

10. Ans. (a) (Wiskott-Aldrich syndrome) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p190, Ananthanarayan 9/e p172, 8/e p158, Harrison 17/e p2060

Wiskott-Aldrich syndrome (Refer Chapter review for detail)

11. Ans. (c) (IgG2 deficiency) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p188, Jawetz 25/e p130.

- IgG2 subclass is the predominant antibody raised against the polysaccharide capsular antigens and deficiency of IgG2 subclass is commonly associated with recurrent pyogenic infection due to bacteria with polysaccharide capsule like Str. pneumoniae or H.influenzae.

According to the memory recall of some other students….

The question asked as- '*Boy with history of recurrent sinopulmonary infections by bacteria with polysaccharide-rich capsules*'.

- For this question… the answer could be. '***Both IgG2 & IgA deficiency***'
- IgA deficiency: Due to *history of recurrent sinopulmonary infections*
  - IgA is the major immunoglobulin in mucosal secretions and weakened mucosal defenses predispose patients to recurrent sinopulmonary infections and diarrhea… Robins 7/e p144-147
- *IgG2* deficiency: Due to *infections by bacteria with polysaccharide-rich capsules.*

12. Ans. (b) (Severe combined immunodeficiency) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p190, Ananthanarayan 9/e p174, 8/e p158.

- Severe combined immunodeficiency results from various mechanisms: MC mechanism is X-linked (mutation in IL7 receptor). Other mechanism include Adenosine deaminase (ADA) deficiency.

13. Ans. (b) (Isolated IgA immunodeficiency) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p189, Ananthanarayan 9/e p173, 8/e p 158 and Harrison 17/e p2058

- Among immunodeficiency diseases, primary immunodeficiency is relatively uncommon, when compared to secondary immunodeficiency (common).
- ***The most common is isolated IgA deficiency, occurs in approximately 1 in 600 individuals (in Europe and North America) and it is reported in about 0.2% of normal populations.***
- Next most common disorder: Common variable immunodeficiency, characterized by pan hypogammaglobulinemia.
- Both of these immunodeficiency states often become clinically evident in young adults.
- The more severe forms of primary immunodeficiency are relatively rare, have their onset early in life, and frequently result in death during childhood.

14. Ans. (a), (c) (Tetany and Mucocutaneous Candidiasis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p189

Refer chapter review

15. Ans. (d) (Wiskott-Aldrich Syndrome) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p190

Refer chapter review for the detail list of disorders of phagocytosis.

TRANSPLANTATION IMMUNOLOGY

16. Ans. (d) (Isograft) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p193, Ananthanarayan 9/e p183, 8/e p178

Refer chapter review for detail.

17. Ans. (b) (Kidney) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p194, Ananthanarayan 9/e p184, 166

- Hyperacute graft rejection is seen in kidney and skin grafting and is preformed antibody mediated (Type III Hypersensitivity reaction)

18. Ans. (b) (Delayed) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p180, Ananthanarayan 9/e p184

19. Ans. (c) (Eichwald Silms....) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p194, Ananthanarayan 9/e p185

- While transplants between members of a highly inbred strain of animals are successful, an exception is seen when the donor is a male and the recipient a female.
- Such grafts are rejected as the grafted male tissue (XY) will have antigens determined by the Y chromosome which will be absent in the female (XX) recipient.
- Grafts from the female to the male will succeed.
- This unilateral sex linked histoincompatibility is known as the Eichwald-Silmser effect.

SECTION 3

Systemic Bacteriology

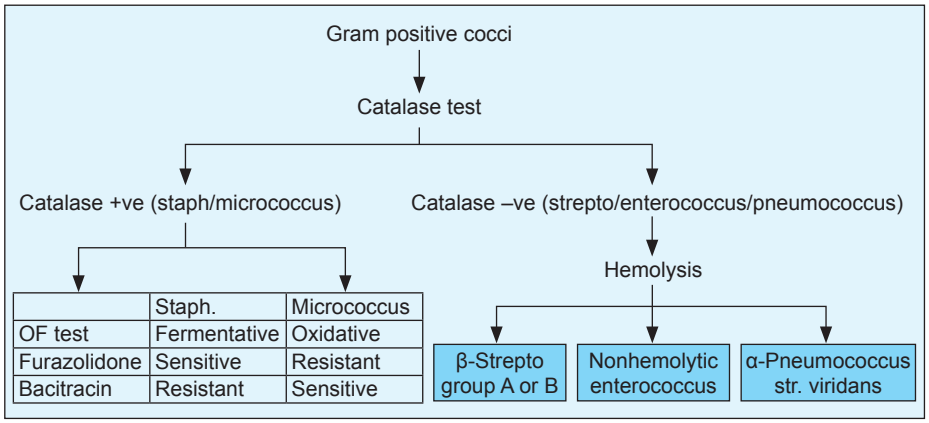
CHAPTER OUTLINE

CHAPTER

3.1 Staphylococcus

Gram positive cocci can be classified into: Micrococcaceae and Streptococcaceae Family

Family	Micrococcaceae family	Streptococcaceae family
Examples	*Staphylococcus*, *Micrococcus*	*Streptococcus*, *Pneumococcus*, *Enterococcus*
Catalase	Catalase positive	Catalase negative
Arrangement in Gram Stain	GPC in cluster (*Staphylococcus*) GPC in tetrad (*Micrococcus*) (as cell division takes place in multiple planes)	GPC in chain (*Streptococcus*) GPC in pair (Pneumococci, Enterococci) (as cell division takes place in single plane)



Catalase test differentiates
- *Staphylococcus* (positive)
- *Streptococcus* (negative)

STAPHYLOCOCCUS AUREUS

Staphylococcus aureus is catalase positive, coagulase positive, facultative anaerobe, non-motile, non-sporing and occasionally capsulated.

- In Greek, *Staphyle* means bunch of grapes.
- *Staphylococcus* was discovered by Sir Alexander Ogston and *S.aureus* was named by Rosenbach.

S. aureus cell wall proteins
- Protein A: Responsible for Coagglutination reaction
- Clumping factor/Bound coagulase: Responsible for slide coagulase reaction

Virulence Factors of S. aureus

Cell wall factors	Activity
Peptidoglycan	More thicker, Confers cell rigidity and induces inflammatory response
Teichoic acid	Helps in adhesion to mucosal surfaces and prevent opsonisation
Clumping factor/Bound coagulase	Responsible for slide coagulase reaction
Protein A	Antiphagocytic, anticomplementary, chemotactic Binds to Fc region of IgG leaving Fab region free to bind to an Antigen - ***Basis of Coagglutination reaction***
Toxins	**Activity**
Membrane active toxins	
A. Hemolysins	
α Hemolysin	Inactivated at 70°C; reactivated **paradoxically** at 100°C (due to denaturation of a heat labile inactivator at 100°C) Leucocidal, Cytotoxic, dermonecrotic, lethal

Contd...

Contd...

Toxins		Activity
	β Hemolysin	Sphingomyelinase ***Lyses sheep RBC***, but not human or rabbit RBC Exhibits **hot-cold** phenomenon
	γ Hemolysin	Bicomponent protein, Lyses rabbit sheep and human RBCs
	δ Hemolysin	Detergent like, Lyses rabbit, sheep and human RBCs
B. Leucocidins/ Panton valentine (PV) toxins		Two components F and S Damage PMN and macrophages Associated with Community acquired MRSA ***Synergohymenotropic toxins***: Bicomponent toxins such as γ and PV toxin act synergistically and are called as Synergohymenotropic toxins
Other toxins		
Epidermolytic toxin (Exfoliative toxin)		Mainly belong to phage group II Scalded skin syndrome (**Nikolsky's sign**-epidermal layer separated) Severe- **Ritter** disease (newborn), toxic epidermal necrolysis **(TEN)** (adult) Milder- Pemphigus neonatorum, bullous impetigo
Enterotoxins		Produced by **50%** of clinical isolates Cause food poisoning Incubatory Period: **1-6 hr** due to **preformed toxin** Site of action: The toxin stimulates the **vagus nerve** and **vomiting center** of the brain. It also stimulates the intestinal peristaltic activity. Heat stable (not destroyed after heating food) Most common food items involved are milk products, bakery food, custards, potato salad, or processed meats. Multiple antigenic type (A–E, G–I, R-T and V) **(MC- type A)**
Toxic shock syndrome toxin		Most strains belong to phage group I Enterotoxins F (pyrogenic exotoxin C) is the most common TSST, followed by Enterotoxin B,C **Risk factor:** *Use of vaginal tampon by menstruating females* (however, males and nonmenstruating females also get effected rarely) Anti TSST1 Antibody is protective **Manifestations**: Rash, fever, hypotension and Multi organ failure **Diagnosis:** Detection of TSST by latex agglutination test and enzyme immunoassay. Detection of TSST genes 1 and 2 by PCR **Treatment**: ***Clindamycin*** (reduces toxin synthesis)
Extracellular enzymes: • Specific to *S.aureus*: Coagulase, heat stable thermonuclease, DNase, phosphatase • Present in most staphylococci: Protease, lipase, staphylokinase (fibrinolysin), hyaluronidase		

Pathogenesis

***Staphylococcus aureus* is the MC agent of the following conditions:**

- Skin and soft tissue infections
- Botryomycosis (mycetoma-like condition)
- Tropical pyomyositis – *S. aureus*, (acute bacterial myositis – Group A *Streptococcus*) (Overall - *S.aureus*)
- Osteomyelitis and septic arthritis (MC site- knee)
- Postoperative parotitis
- Paronychia
- Pyomyositis (skeletal muscle infection): In tropics and HIV infected people (Overall MC agent - *S.aureus*, except in acute bacterial myositis – Group A *Streptococcus* is the MC agent)
- Pneumatocele-Shaggy, thin-walled cavities in lungs) in neonates
- Abscess: Psoas abscess and epidural abscess
- Surgical wound infection
- Folliculitis, furuncle, carbuncle and Hidradenitis suppurativa
- Mastitis and breast abscess (in nursing mothers)
- Toxin-mediated diseases: Toxic shock syndrome, food poisoning, scalded-skin syndrome

Contd...

Contd...

Infections associated with Community: Associated Methicillin Resistant S. aureus (CA-MRSA)
While skin and soft tissues are the most common sites for CA-MRSA strains; 5–10% of strains are invasive and can cause various invasive infections, such as:
- Necrotizing pneumonia
- Sepsis with Waterhouse: Friderichsen syndrome or purpura fulminans (*S. aureus* is rare cause; most commonly caused by meningococci).
- Necrotizing fasciitis (*S. aureus* is a rare cause, *Streptococcus pyogenes* is the most common cause)

Endocarditis:
- ***MC cause of Native valve endocarditis***: *Overall or Hospital acquired-S.aureus, Community acquired- S.viridans*
- ***MC cause of Prosthetic valve endocarditis***
  - Early prosthetic valve endocarditis (<12 months): *Staphylococcus epidermidis*
  - Late prosthetic valve endocarditis (>12 months): *Viridans streptococci*
  - Overall MC cause of prosthetic valve endocarditis. *Staphylococcus epidermidis*
- ***MC cause of Endocarditis in IV drug users:***
  - Rt sided – *Staphylococcus aureus*,
  - Lt sided – *Enterococcus >Staphylococcus aureus*
  - Over all – *Staphylococcus aureus*
- ***MC cause of Subacute endocarditis*** – Viridans streptococci

Laboratory Diagnosis

- **Direct smear microscopy**: Pus cells with gram positive cocci in cluster
- **Culture:**
  - Nutrient agar: Golden yellow pigmented colonies (pigments made up of beta carotene)
  - Blood agar: Colonies with narrow zone of β-hemolysis
  - Selective media:
    - Mannitol salt agar (yellow colonies due to mannitol fermentation)
    - Salt milk agar
    - Ludlam's medium
- **Culture smear microscopy:** Gram-positive cocci in clusters
- **Biochemical identification**
  - Catalase test-positive
  - Tests differentiating staphylococci from micrococci- Of test (shows fermentative pattern)
  - Tests differentiating *S. aureus* (positive) from CoNs (negative)
    - Coagulase test (slide and tube)-positive
    - Heat stable thermo nuclease test-positive
    - DNase test-positive
    - Phosphatase test-positive
    - Mannitol sugar is fermented
    - Black colored colonies on potassium tellurite agar
    - Gelatin liquefaction-positive
    - Protein A detection
- **Typing of *S.aureus*:**
  - MC method for typing of *S.aureus* – ***Phage typing*** (pattern method)
  - National reference centre for phage typing—in Maulana Azad Medical College, Delhi.
  - Epidemic strain of *S. aureus* is **Phage type 80/81**. It causes outbreaks in hospitals.

Enzymes specific to *S. aureus* (absent in CoNS):
- Coagulase
- Heat stable thermonuclease
- DNase test
- Phosphatase test

Tube coagulase	Slide coagulase
Due to coagulase enzyme	Due to clumping factor
Requires CRF in plasma	Does not requires CRF in plasma
Done in tube	Done on slide
Test +ve: coagulum formed/plasma clotted	Test +ve: clumps formed
S. lugdunensis –ve	*S.lugdunensis* +ve
S. schleiferi +ve	*S.schleiferi* –ve
Both tube and slide coagulase positive: *S. aureus*, *S. hyicus* and *S. intermedius*	

Coagulase test:
- Both Tube and Slide coagulase positive: *S. aureus, S. hyicus and S. intermedius*
- Only Slide coagulase positive: *S. lugdunensis*
- Only Tube coagulase positive: *S. schleiferi*

Treatment of Staphylococcus aureus Infections

Since *S. aureus* rapidly develops drug resistance, antibiotics should be cautiously chosen

Parenteral therapy for serious infections:	
Sensitive to penicillin	DOC: Penicillin G
Sensitive to methicillin	DOC: Nafcillin or oxacillin
Resistant to methicillin (MRSA)	**DOC: Vancomycin** (15–20 mg/kg bd) Alternate drugs: See text below
Empirical therapy (if MRSA status not yet known):	Vancomycin with or without an aminoglycoside Vancomycin is indicated only if MRSA risk is high or condition is serious, e.g. cardiac implant
Oral therapy for skin and soft tissue infections	
Sensitive to methicillin	Dicloxacillin, cephalexin
Resistant to methicillin (MRSA)	Clindamycin Alternate drugs: Cotrimoxazole, doxycycline, linezolid

Drug Resistance in S. aureus

Resistance in S. aureus to β lactam antibiotics

S. aureus shows resistance to β lactam antibiotics in various ways:

1. **Production of β lactamase enzyme:** β lactamase or penicillinse enzymes cleave the β lactam ring:
   - This resistance is plasmid coded, can be transferred between *S.aureus* strains by **transduction.**
   - It is produced by > 90% of strains of *S.aureus.*
   - This resistance can be overcome by addition of β lactamase inhibitors such as clavulanic acid or sulbactam.
2. **By alterations of PBP:** It is shown by MRSA strains (see below)

Community associated MRSA (CA-MRSA):
- Mediated by mecA gene subtype IV, V, VI.
- More virulent and express PV toxin.
- Cause invasive skin and soft tissue infections, such as necrotizing fasciitis

Methicillin Resistant *Staphylococcus aureus* (MRSA)

MRSA is mediated by ***mecA gene;*** which is a chromosomally coded. It alters penicillin binding protein (PBP) present on *S.aureus* cell membrane to PBP-2a:

- PBP is an essential protein needed for cell wall synthesis of bacteria. β lactam drugs bind and inhibit this protein, there by inhibiting cell wall synthesis.
- The altered PBP2a of MRSA strains has less affinity for β lactam antibiotics; hence MRSA strains are resistant to all β lactam antibiotics.
- **BORSA** strains (Borderline Oxacillin resistant *S.aureus*): Occasionally a non-*mecA* gene mediated low level resistance to oxacillin is observed in some strains of *S.aureus,* which is due to hyper production of β lactamase.
- There is an increasing trend of MRSA rate over last few decades. Though it varies from place to place, overall about 30–40% strains of *S. aureus* are MRSA.
- MRSA rate in India is 30-40%. It is lowest in Scandinavian countries

Types of MRSA: MRSA are either community or hospital associated.

Community associated MRSA (CA-MRSA)	**Hospital associated MRSA (HA-MRSA)**
It is mediated by *mecA* gene subtype IV, V, VI	It is mediated by *mecA* gene subtype I,II,III
They are usually more virulent and express several toxins such as PV toxin.	They are multidrug resistant (but their virulence is low)
They cause invasive skin and soft tissue infections such as necrotizing fasciitis	They cause perioperative wound infections in hospitals and nosocomial outbreaks (*hospital staff are the major carries*)

Note: CA-MRSA and HA-MRSA terminologies are becoming artificial nowadays; as many CA-MRSA strains have been isolated in hospitals and vice versa.

Detection of MRSA

- Antimicrobial susceptibility test: Disk diffusion test can be done by using cefoxitin or oxacillin disks.
  - Cefoxitin is the recommended disk to be used.
  - If oxacillin disk is used, then certain conditions to be maintained such as—using media containing 2–4% NaCl, incubation at 30 °C for 24 hours.
- Oxacillin screen agar: Adding oxacillin 6 µg/ml and NaCl (2–4%) to the medium.
- PCR detecting *mecA* gene
- Latex agglutination test detecting PBP-2a

Treatment of MRSA

- Vancomycin is the drug of choice for MRSA.
- Alternate drugs include:
  - Teicoplanin, linezolid, quinupristin-dalfopristin, tigecycline, oritavancin
  - Daptomycin (for endocarditis and complicated skin infections),
  - Mupirocin 2% ointment (for nasal carriers of MRSA)
- However, even simple orally effective drugs such as tetracycline, erythromycin or cotrimoxazole may also be effective. These can be indicated in non-serious conditions, caused by CA-MRSA strains if found to be susceptible based on antimicrobial susceptibility report.
- All β lactam drugs should be avoided. However, 5th generation cephalosporins, such as **Ceftobiprole, ceftaroline, ceftolozane** have shown some activity against MRSA.

Resistance to Vancomycin (VRSA and VISA)

Erroneous and overuse of vancomycin may lead to emergence of resistance to vancomycin, which may be of two types:

- VRSA (Vancomycin Resistant *S. aureus*): High grade resistance with MIC ≥ 16 µg/ml
- VISA (Vancomycin Intermediate *S. aureus*): Low grade resistance with MIC 4-8 µg/ml
- **Epidemiology:** VRSA is very rare. In India, it is reported from few places such as Hyderabad, Kolkata and Lucknow. However, VISA is more frequently reported.
  Mechanisms:
  - VRSA is mediated by *van* gene. *A* The *van A* gene is believed to be acquired from a vancomycin-resistant strain of *Enterococcus fecalis* by horizontal conjugal transfer.
  - VISA is due to increase in cell wall thickness of *S. aureus*.
- **Treatment** of VRSA/VISA: Linezolid, telavancin, daptomycin and quinupristin/dalfopristin are the effective drugs. Vancomycin and Teicoplanin are not effective.

Resistance to vancomycin:
- VRSA: MIC ≥ 16 µg/ml, Due to Van gene
- VISA: MIC 4-8 µg/ml, Due to cell wall thickeing

S. aureus carriers

- About 25–50% of healthy population are carriers of *S.aureus.*
- MC site of colonization: Anterior nares and ***Skin,*** (perineum, axilla, groins)
- MC way of spread of infection in hospital- through the ***hands of hospital staff***
- Most effective way to prevent the hospital infection – ***handwashing***
- DOC for nasal carriers of MRSA: Mupirocin 2% ointment.

COAGULASE NEGATIVE STAPHYLOCOCCUS (CoNS)

They are mostly the normal flora of skin.

Staphylococcus Epidermidis

- MC CoNS—Accounts for 60–70% of CoNS
- Produces polysaccharide glycocalyx (slime) (Biofilm production)
- Adhere to any implanted foreign bodies like valvular shunts, prosthetic devices
- Infections:
  - Endocarditis with insertion of valvular **prosthesis**
  - Ventricular shunt infections and Stitch abscess.

S. epidermidis Infections:
- Endocarditis with insertion of valvular prosthesis
- Ventricular shunt infections
- Stitch abscess

Staphylococcus Saprophyticus

- It causes UTI in young sexually active females.
- It is resistant to novobiocin.

MULTIPLE CHOICE QUESTIONS

1. How to differentiate micrococci and staphylococci? *(Recent Question 2015)*
a. Catalase
b. Oxidase
c. Gram staining

2. Pneumatoceles in chest X-ray are characteristically seen in pneumonia due to: *(APPG 2015)*
a. Streptococcus pneumoniae
b. Staphylococcus aureus
c. Streptococcus pyogenes
d. Hemophilus influenzae

3. Toxic epidermal necrosis and Scalded Skin Syndrome are associated with which toxin? *(West Bengal 2016)*
a. Exfoliative toxin of S.aureus
b. TSST of S.aureus
c. Enterotoxin of S.aureus
d. SPE of S.pyogenes

4. Surrogate marker for MRSA detection is? *(Recent Questions 2014)*
a. Cefotaxime b. Ceftazidime
c. Cephazolin d. Cefoxitin

5. All the following are true about Staphylococcus aureus except: *(JIPMER 2014, 2013)*
a. Coagulase positive
b. Catalase negative
c. DNAse positive
d. Indole negative

6. Cephalosporin with anti MRSA activity: *(AIIMS Nov 2014)*
a. Ceftriaxone b. Aztreonam
c. Cefazolin d. Ceftobiprole

7. Drug(s) used in MRSA is/are: *(PGI Nov 2014)*
a. Linezolid b. Cephalothin
c. Vancomycin d. Meropenam
e. Piperacillin + Tazobactum

8. Staph aureus causes: *(NEET Pattern Based)*
a. Erythrasma
b. Acne vulgaris
c. Chancroid
d. Bullous impetigo

9. What is the best way to control the outbreak of MRSA infection in a hospital ward? *(JIPMER May 2015, Nov 2014, AIIMS 2012)*
a. Vancomycin is given empirically to all the patients
b. Frequent fumigation of wards
c. Wearing mask before invasive procedure
d. Washing of hands before and after treating the patients

10. Protein A of Staphylococcus aureus is part of bacterial? *(Recent Questions 2014)*
a. Genome
b. Cell wall
c. Limiting membrane
d. Plasmid

11. Synergohymenotropic toxins of Staphylococal consists of: *(PGI June 2011)*
a. a toxin b. β toxin
c. γ toxin d. d toxin
e. Panton – Valentine toxin

12. A person had infection due to gram positive organism treated with methicillin and then culture sensitivity shows resistance to it. Hence all can be given in MRSA except: *(AIIMS Nov 2011) (AI 2012)*
a. Cotrimoxazole
b. Cefaclor
c. Vancomycin
d. Ciprofloxacin

13. Most common cause of pyomyositis is: *(DNB Dec 2011)*
a. Streptococcus pyogenes
b. Pseudomonas
c. Staphylococcs aureus
d. E. Coli

14. Preformed toxin produces diarrhea in which organism? *(DNB June 2010)*
a. Staphylococcus aureus
b. Vibrio cholera
c. Salmonella
d. Escherichia coli

15. In a postoperative ward, 4 out of 10 patients developed wound discharge, which on culture was found to be positive for coagulase positive gram positive cocci. Antimicrobial susceptibility showed that the strain was resistant to Methicillin. On surveillance cultures, a health care personnel attending to the patients was found to be a nasal carrier for the same agent. The following are true regarding the agent responsible for the outbreak: *(AI 2011)*
a. The major route of spread causing the outbreak is air borne
b. The resistance to methicillin is plasmid mediated
c. The organism has an alteration in its penicillin binding proteins.
d. It will be sensitive to treatment with antibiotics containing amoxicillin + clavulinic acid combination.

16. Toxic shock syndrome is caused by: *(JIPMER 2011 and 2010, PGI Dec 2007)*
a. Streptococcus pyogenes
b. Staphylococcus aureus
c. Strept. albicans
d. Enterococcus durans

17. False statement about Staphylococcus epidermidis? *(NIMHANS 2016)*
a. It is sensitive to penicillin
b. It is Gram+ve CONS
c. It secrets slime layer around it
d. It causes infection through catheters and cardiac implants.

18. All are true about PBP except: *(AI 2010)*
a. PBP is localized in outer cell wall
b. PBP is essential for cell wall synthesis
c. PBP acts as carboxypeptidase and transpeptidase
d. Alteration in PBP – basis of MRSA

19. All true about S.aureus except: *(AI 2010)*
a. MC source of infection – cross infection from infected persons in hospital
b. 30% of general population is healthy carriers
c. TSS and epidermolysin are Superantigens
d. MRSA- chromosomally mediated

20. A patient has prosthetic valve replacement and he develops endocarditis 8 months later. Organism responsible is: *(AIIMS Nov 2010)*
a. Staphylococcus aureus
b. Streptococcus Viridans
c. Staphylococcus epidermidis
d. HACEK

21. A 25-year-old man with 3 weeks fever presented with tricuspid valve vegetation. Patient is intravenous drug abuser. Most common cause of endocarditis in this patient is: *(AIIMS Nov 2009)*
a. Staph. aureus
b. Candida albicans
c. Pseudomonas
d. Strep. Viridian

22. The following are characteristic features of staphylococcus food poisoning except: *(AIIMS 2004)*
a. Optimum temperature for toxin production is 37 °C
b. Intradietic toxin is responsible for intestinal symptoms
c. Toxin can be destroyed by boiling for 30 minutes
d. Incubation period is 1-6 hours

23. Staph. aureus causes vomiting in 6–8 hours. The mechanism of action is: *(AIIMS May 2002)*
a. Stimulation of cAMP
b. Vagal stimulation
c. Stimulation of cGMP
d. Acts through ganglioside GM receptor

24. Which of the following organism is implicated in the causation of botryomycosis? *(PGI 2001)*
a. Staphylococcus aureus
b. Staphylococcus albus
c. Pseudomonas aeruginosa
d. Streptococcus pneumoniae
e. Streptococcus pyogenes

25. MC phage type of Staphylococcus aureus:
a. 79/80 *(Recent Question of 2013)*
b. 3A/3B
c. 80/81

26. A 25-year-old female presented to the hospital on the third day of menstruation with complaints of high fever, vomiting and a rash on her trunk and extremities. On investigation she had leucocytosis and a negative blood culture. She is diagnosed as:
a. Staphylococcal food poisoning *(MHPG 2014)*
b. Scalded skin syndrome
c. Toxic shock syndrome
d. Varicella zoster infection

EXPLANATIONS

1. **Ans. (b) (Oxidase)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p211

   Micrococci are positive for modified oxidase test, and staphylococci are oxidase negative.

2. **Ans (b) (S. aureus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p215
   Pneumatocele is shaggy, thin-walled cavities in lungs of neonates (commonly) and S. aureus is the MC cause.

3. **Ans (a) (Exfoliative toxin of S.aureus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p213
   Toxic epidermal necrosis and SSSS (Staphylococcal Scalded Skin Syndrome) are associated with epidermolytic or exfoliative toxin of S.aureus.

4. **Ans. (d) (Cefoxitin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p219, CLSI Guideline 2014
   Surrogate marker for MRSA detection: Cefoxitin (Best), Oxacillin and Methicillin

5. **Ans. (b) (Catalase negative)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p212, Ananthanarayan 9/e p205

6. **Ans. (d) (Ceftobiprole)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p218 Clin Dermatol. 2008;9(4):245-54.
   - Fifth-generation cephalosporins such as ceftobiprole and ceftaroline are effective against MRSA.
   - Ceftobiprole has additional activity against penicillin-resistant Streptococcus pneumoniae, Pseudomonas aeruginosa, and Vancomycin resistant enterococci (VRE).

7. **Ans. (a) (c) (Linezolid), (Vancomycin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p218
   - MRSA strains are resistant to all β lactam drugs.

8. **Ans. (d) (Bullous impetigo)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p215 Ananthanarayan 9/e p202
   - Bullous Impetigo is a cutaneous condition that characteristically occurs in the newborn, presenting with bullae.
   - Bullous impetigo is caused by Staphylococcus aureus, which produces exfoliative toxins, whereas non-bullous impetigo is caused by either Staphylococcus aureus, or Streptococcus pyogenes.

9. **Ans. (d) (Washing of...)** Ref: Apurba Sastry's Essentials of Medical Microbiology p609, Harrison 18/e p1166, 19/e p959
   - Handwashing is the most effective way to prevent hospital acquired infections.
   - Prevention of the spread of S. aureus infections in the hospital setting involves handwashing and careful attention to appropriate isolation procedures……. Harrison 18/e p1166.

10. **Ans. (b) (Cell wall)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p212, Ananthanarayan 9/e p201
    Refer chapter review.

11. **Ans. (c) (e) (γ toxin, Panton Valentine toxin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p212
    - Gamma hemolysin and Panton Valentine toxin are membrane active toxins of Staph. aureus that are composed of two components (bicomponent toxins) and together called as Synergohymenotroic toxins.

12. **Ans. (b) (Cefaclor)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p218
    - MRSA strains are resistant to all β lactam antibiotics as it results from alternation of penicillin binding protein. (For detail- refer chapter review).

13. **Ans. (c) (Staphyloco...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p215, Harrison 19/e p957, 18/e p1164
    - *Pyomyositis* is an unusual infection of skeletal muscles that is seen primarily in tropical climates but also occurs in immunocompromised and HIV-infected patients. Pyomyositis presents as fever, swelling, and pain overlying the involved muscle.
    - Tropical pyomyositis – MC cause is S. aureus, (acute bacterial myositis: MC cause is Group A Streptococcus) (Overall: MC cause of pyomyositis is S.aureus).

14. **Ans. (a) (Staphy...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p213, Harrison 19/e p959, 18/e p1165
    - Example of Preformed toxin i.e. toxin secreted in food: S.aureus enterotoxin, Bacillus cereus emetic type of enterotoxin and botulinum toxin.

15. **Ans. (c) (The orga...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p219, Ananthanarayan 9/e p205, 8/e p197
    - Coagulase positive gram positive cocci: Indicates Staphylococcus aureus
    - Resistant to Methicillin: Indicates MRSA (Methicillin resistant *Staphylococcus aureus)*
    - Health care personnel of the hospital found to be nasal carrier for the same agent: Indicates that the infection has spread to the patient from health care personnel of the hospital
    - ***"The MC way of spread of infection in hospital: Through the hospital staff (not from other patient from the hospital)"***
    - *Mechanism of Resistance of MRSA*: Due to chromosomally mediated Mec A gene which codes for altered PBP 2a (Penicillin Binding protein or receptor 2a) which has less affinity for β lactam drugs.

    About Other Options
    - The major route of spread causing the outbreak is through the hands of hospital staff
    - The resistance to Methicillin is chromosomally mediated
    - Since MRSA coded altered PBP 2a **(Penicillin Binding protein 2a) has less affinity for β lactam drugs, so** MRSA strains are resistant to all β lactam drugs. Even combination of β lactam and β lactamase inhibitor will not work.

16. **Ans. (a), (b) (Streptococcus pyogenes, Staphyloco...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p213
    - Staph TSST1 (enterotoxin F/pyrogenic Exotoxin C): causes vaginal tampon associated TSS
    - Staph enterotoxin B,C Nonmenstrual cases of TSS
    - Streptococcus pyrogenic exotoxin A,B,C: Can also cause TSS.

    Also know:
    - Streptococcal TSS: Bacteraemic, ↑soft tissue necrosis (necrotizing fasciitis), but rash less common
    - Staphylococcal TSS: Rash more common, bacteraemia less common, less tissue necrosis

17. **Ans. (a). It is sensitive to Penicillin)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p220

    Most staphylococci are resistant to Penicillin due to beta lactamase production.

18. **Ans. (a) (PBP is localized in outer cell wall)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p219
    - PBP (Penicillin Binding protein or receptor) is localized in outer cell membrane (not in the cell wall).
    - PBP acts as transpeptidase or carboxypeptidase enzyme (essential for cell wall peptidoglycan synthesis).
    - Penicillin binds to PBP and inhibits transpeptidation thus inhibiting peptidoglycan synthesis.
    - In MRSA – Mec A gene alters PBP to PBP 2a which has less affinity for β lactam drugs.

19. **Ans. (a) (MC source of infection: Cross infection from infected persons in hospital)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p219, 609, Harrison 19/e p959, 18/e p1160
    - MC source of infection: Cross infection from ***hands of hospital staffs***
    - Most effective way to prevent the hospital infection: ***handwashing***
    - 25–30% of general population is healthy carriers of S.aureus
    - MC site of colonization: anterior nares, Skin (perineum, axilla, groins)
    - Example of Staphylococcal super antigen: TSS toxin and Epidermolytic toxin and enterotoxin
    - MRSA: Chromosomally mediated while Penicillinase production is plasmid mediated.

20. **Ans. (c) (Staphylococ...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p220, Harrison 19/e p960, 18/e p1053
    Refer text (chapter review) for explanation.

21. **Ans. (a) (S.aureus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p215, Harrison 19/e p958, 18/e p1053,
    Refer text (chapter review) for explanation.

22. **Ans. (c) (Toxin can be ...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p212-13
    Staphylococcal enterotoxin is heat stable. So cooking the contaminated food and leaving at room temp for some time leads to toxin accumulation.

    About Other Options

    Option a: Optimum temperature for toxin production is same as Optimum temperature for S.aureus growth i.e. at 37°C. So it seems to be a correct statement.
    Remember: Optimum temperature for pigment production is **22°C** and for MRSA expression is **37°C**
    Option b: St.aureus enterotoxin is intradietic in nature i.e. it is preformed, secreted in the diet
    Option d: IP of S.aureus food poisoning is 1–6 hour due to preformed toxin

23. Ans. (b) (Vagal stimulation) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p213, Jawetz 25/e p188

Jawetz 25/e p188: '*The emetic effect of enterotoxin is probably the result of central nervous system stimulation (vomiting center) after the toxin acts on neural receptors in the gut.*'

Harrison 18/e p1165**:** '*The toxin stimulates the vagus nerve and the vomiting center of the brain. It also appears to stimulate intestinal peristaltic activity.*'

24. Ans. (a), (c), (e) (S.aureus, Pseudomonas, Strept. Pyogenes) Ref: Jagdish Chander's Mycology, 3/e p158/table 11.6

Agent of Botryomycosis:

- S.aureus (MC agent)
- Others: Streptococcus, Pseudomonas, E.coli, Proteus, CoNS, Peptostreptococcus, Streptococcus spp

25. Ans. (c) (80/81) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p218, Ananthanarayan 8/e p203

Staphylococcus aureus phage type 80/81 is the most common type associated with hospital infections.

26. Ans. (c) (Toxic...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p213, Ananthanarayan 9/e /p202

Refer chapter review.

CHAPTER

3.2

Streptococcus, Enterococcus and Pneumococcus

Family Streptococcaceae are catalase negative gram-positive cocci, arranged in pairs or chains (due to single plane of division).

- *Streptococcus, Enterococcus* and *Pneumococcus* are the important members of this family.
- However, according to the molecular structure, *Enterococcus* is now reclassified under separate family Enterococcaceae.

Typing of Streptococcus:

- Serogrouping (Lancefield's): Based on carbohydrate antigen, 20 groups
- Serotyping (Griffith typing): Based on M protein (>100 M types)
- emm genotyping: Based on gene coding for M protein, >124 emm

CLASSIFICATION

On the basis of hemolysis, streptococci can be divided into 3 groups

1. α Hemolytic: (Partial or green hemolysis), e.g. *Streptococcus Viridans, Streptococcus pneumoniae*
2. β Hemolytic: (Complete or yellowish hemolysis), e.g. β haemolytic *Streptococcus*
3. γ Hemolytic: (no hemolysis is seen), e.g. Enterococci

Lancefield's grouping (for β haemolytic streptococci)

Based on carbohydrate antigen in cell wall, the β haemolytic streptococci are further divided into 20 serogroups: Group A to V except I and J.

Carbohydrate antigen extracted by HCl (Lancefield's method), Formamide (Fuller's method), Enzymatic (Maxted's) or autoclaving.

Streptococcus group A (S. pyogenes) is further subdivided based on

- **Griffith typing:** Based on M protein (> 100 M serotypes) or
- ***emm* typing:** Based on gene coding for M protein, > 124 *emm* genotypes identified.

STREPTOCOCCUS PYOGENES (GROUP A)

Virulence Factors

Cell wall antigens	• Inner thick peptidoglycan layer (confers cell wall rigidity, induces inflammatory response and has thrombolytic activity)
	• C-carbohydrate antigen: Present as middle layer and is group specific
	• Outer layer of protein (M, T, R) and lipoteichoic acid (helps in adhesion)
	• **M protein:**
	  ○ Mediates adherence to epithelial cells, inhibits phagocytosis
	  ○ Binds to fibrinogen and neutrophils leadings to release of inflammatory mediators that induce vascular leakage (streptococcal toxic shock).
	  ○ M protein is further divided into Class I and Class II. Antibodies to class IM protein are responsible for pathogenesis of rheumatic fever.

Capsule	• Expressed by mucoid strains, made-up of hyaluronic acid. • Capsule is anti-phagocytic, helps in adhesion; but it is not antigenic.

Contd...

SPE is associated with the pathogenesis of:
- Scarlet fever
- Necrotizing fasciitis
- Streptococcal TSS.

Contd...

SPE (Streptococcal pyrogenic exotoxin) or Erythrogenic toxin	• 3 Types (SPE A, B and C): Type A and C are, e.g. of Superantigens • Type **A and C** bacteriophage coded, **B** toxin chromosomal mediated • **Pathogenic role**: Associated with the pathogenesis of scarlet fever, necrotizing fasciitis and streptococcal toxic shock syndrome. • **Dick test:** Intradermal injection of SPE produces erythema only in those children who are susceptible to develop scarlet fever. • **Schultz Charlton reaction** (blanching of rash after injection of anti SPE antibodies): Used for diagnosing scarlet fever in past
Hemolysins	Streptolysin O and Streptolysin S (see table below)
Streptokinase	Fibrinolysin (activates plasminogen) Rapid spread: By preventing the formation of fibrin barrier. Therapeutically used in treatment of coronary thrombosis.
DNase	Also called Deoxyribonuclease or Streptodornanse (4 types: A, B,C,D) • **Diagnostic use: Anti-DNase B** > 300–350 U is useful for the retrospective diagnosis of skin infections (pyoderma) and acute glomerulonephritis where ASO titer is low • **Therapeutic use:** Preparation containing streptodornase and streptokinase can be used to liquefy the thick exudates in empyema cases.
Other enzymes	• **Hyaluronidase (spreading factor):** Expressed by noncapsulated strains, such as M type 4 and 22. It breaks down the hyaluronic acid of the tissues, thus helps in the spread of infection along the intercellular space • Serum opacity factor: Lipoproteinase enzyme in nature • NADase, C5a peptidase and SpyCEP (inactivates IL- 8)

Antistreptolysin-O antibodies (ASO):
- Raised in most of the streptococcal infections
- Used as a standard marker for retrospective diagnosis of streptococcal infections
- Except in glomerulonephritis and pyoderma; where ASO titer is low.

Streptolysin-O (SL-O)	Streptolysin-S (SL-S)
Oxygen labile (hence named streptolysin-O) Heat labile	Oxygen stable Serum soluble (hence named streptolysin-S)
Hemolysis is seen only in deep colonies (pour plate) as it is inactivated in the presence of oxygen	Causes hemolysis on the surface of blood agar plate
It is cytotoxic for neutrophils, platelets and cardiac tissue	It has leucocidal activity
Strongly antigenic	Not antigenic
Antistreptolysin-O antibodies (ASO) are raised in most of the streptococcal infections and are used as a standard marker for retrospective diagnosis of streptococcal infections (except in glomerulonephritis and pyoderma; where ASO titer is low)	Not useful for serological diagnosis of streptococcal infections
Streptolysin-O is structurally and functionally similar to: • Tetanolysin of *Clostridium tetani* • Pneumolysin of *S. pneumoniae* • Theta toxin of *Clostridium perfringens* • Listeriolysin O of *Listeria* • Cereolysin of *Bacillus cereus*	

Manifestations

Streptococcus pyogenes causes both suppurative and nonsuppurative manifestations.

Scarlet fever:
Caused by S. pyogenes, Now rare, characterized by:
- Pharyngitis and Sandpaper rashes, strawberry tongue
- Pastia's lines: prominent rashes in skin folds
- Pathogenesis is due to SPE toxin (Dick test +ve).

Suppurative Manifestations
Respiratory infections: • Pharyngitis/sore throat (MC cause, 20–40% of all cases) • Pneumonia and empyema
Scarlet fever (MC cause): Now rare, characterized by: • Pharyngitis and Sandpaper rashes, strawberry tongue • Pastia's lines- prominent rashes in skin folds • Pathogenesis is due to SPE toxin (Dick test +ve)

Contd...

Section 3

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Suppurative Manifestations
Skin and soft tissue infections: • **Impetigo (pyoderma): (MC cause)** ○ Seen in children, poor hygiene, warm climate ○ Characterized by pustular lesions that become honeycomb like crusts, no fever, painless. ○ Associated with higher M types, and nephritogenic strains. • **Cellulitis and erysipelas (MC cause):** ○ Tender, bright red, swollen and indurated peaud'orange texture of skin (due to involvement of the superficial lymphatics) along with fever and chills. ○ MC site- malar area of the face, seen in older people.
Deep soft tissue infections: • Necrotizing fasciitis or streptococcal hemolytic gangrene- *S. pyogenes* is MC cause (60%), it is rapidly spreading, hence *S. pyogenes* is also called flesh eating bacteria • Toxic shock syndrome (staphylococcal TSS is MC, but bacteremia is MC in streptococcal TSS) • Streptococcal myositis (*S. aureus* is MC cause of myositis)
Complications: • Puerperal sepsis (Group B *Streptococcus* is MC cause), • Others: Otitis media, Quinsy, Ludwig's angina, pneumonia (post viral), osteomyelitis, meningitis

Nonsuppurative Complications

Streptococcal antigens show molecular mimicry with human antigens. Due to antigenic cross reactivity, antibodies produced against previous streptococcal infections cross react with human tissues to produce lesions. This accounts for a number of nonsuppurative complications such as:

- Acute rheumatic fever
- Poststreptococcal glomerulonephritis (PSGN)
- Guttate psoriasis
- Reactive arthritis
- Pediatric Autoimmune Neuropsychiatric Disorders Associated with *Streptococcus pyogenes* (PANDAS)

Nonsuppurative complications of *S. pyogenes*:
- Acute rheumatic fever
- Post streptococcal glomerulonephritis (PSGN)
- Guttate psoriasis
- Reactive arthritis
- PANDAS

Antigenic cross reactivity between streptococcal antigens and the corresponding human antigens

Streptococcal Ag	Mammalian Ag
Cell wall **M** protein (of serotypes M1, M5, M6, and M19)	Myocardium (tropomyosin and myosin)
Cell wall **C** carbohydrate	Cardiac valves
Cytoplasmic membrane	Glomerular vascular intima
Peptidoglycan	Skin antigens
Hyaluronic acid	Synovial fluid

Differences between acute rheumatic fever and poststreptococcal glomerulonephritis

Feature	Acute rheumatic fever (ARF)	Poststreptococcal glomerulonephritis (PSGN)
Prior history of infection with	Pharyngitis strains	Mainly pyodermal strains, or rarely pharyngitis strains
Serotype	Most of the strains of *S. pyogenes*	Pyodermal strains: 49, 53–55, 59–61 and Pharyngitis strains: 1, 12
Immune response	Marked	Moderate
Complement level	Unaltered	Low (due to deposition in glomeruli)
Genetic susceptibility	Present	Absent
Repeated attacks	Common	Uncommon
Penicillin prophylaxis	Indicated	Not indicated

Contd...

Serology:
- ASO Ab: Titer > 200 Todd unit/ml in most streptococcal infections except in pyoderma and PSGN
- Anti-DNase-B Ab – Titer > 300–350 units/ml is diagnostic of PSGN and pyoderma.

Contd...

Feature	Acute rheumatic fever (ARF)	Poststreptococcal glomerulonephritis (PSGN)
Course	Progressive	Spontaneous resolution
Prognosis	Variable	Good
Hypersensitivity reaction	Type II	Type III

Laboratory Diagnosis of Streptococcus Pyogenes

- **Transport medium**: Pike's medium
- **Direct smear microscopy**: Pus cells with gram-positive cocci in short chains
- **Culture:**
  - Blood agar: Pinpoint colony with a wide zone of β-hemolysis
  - Selective media: Crystal violet blood agar and PNF (polymyxin B, neomycin, fusidic acid) media
  - Liquid media: Granular turbidity with powdery deposit
- **Biochemical identification**: Catalase negative, Bacitracin sensitive and Pyrrolidonyl Arylamidase (PYR) test is positive
- **Typing:**
  - Lancefield grouping: Shows group A *Streptococcus*
  - Typing of group A *Streptococcus*: Griffith typing and emm typing
- **Serology:** ASO antibodies and Anti-DNase B antibodies
  - ASO antibodies titer is elevated > 200 Todd unit/ml in most streptococcal infections except in pyoderma and PSGN.
  - Anti-DNase-B Ab – Titer > 300–350 units/ml is diagnostic of PSGN and pyoderma.
  - Other antibodies elevated are Antihyaluronidase and antistreptokinase antibodies.

Treatment of Necrotizing fasciitis:
- Surgical debridement (most crucial) plus
- Penicillin G plus
- Clindamycin

Treatment of streptococcal infection

Penicillin is the drug of choice for all type of streptococcal infections

Conditions	Treatment recommended
Pharyngitis	Benzathine penicillin G, IM single dose or oral penicillin V for 10 days
Erysipelas/Cellulitis	Mild- Procaine penicillin Severe- Penicillin G
Necrotizing fasciitis	Surgical debridement (most crucial) + Penicillin G + Clindamycin
Pneumonia and empyema	Penicillin G + drainage of empyema
Streptococcal TSS	Penicillin G + Clindamycin + immunoglobulin (to SPE)
Rheumatic fever	Benzathine penicillin G, IM single dose; or oral Penicillin V for 10 days
	Long-term maintenance therapy with penicillin G monthly: • For 5 yrs or until 21 yrs of age, (without carditis) • For 10 yrs (with carditis) • up to 40 yrs of age/lifelong (with residual heart disease)
PSGN	Benzathine penicillin G, IM single dose; or oral penicillin V for 10 days
Treatment of asymptomatic carriers	
Pharyngeal carrier	Penicillin V + rifampicin
Rectal carriers	Vancomycin + rifampicin

Rheumatic fever:
- **Treatment:** Benzathine penicillin G, IM single dose; or oral Penicillin V for 10 days
- **Long-term maintenance** therapy, with penicillin G monthly
  - For 5 years or until 21 years of age, (without carditis)
  - For 10 years (with carditis)
  - Up to 40 years of age/ lifelong (with residual heart disease)

Prophylaxis

Long-term maintenance therapy with penicillin (alternative-sulfadiazine or erythromycin in penicillin allergy) is required for children who develop early signs of rheumatic fever. This prevents streptococcal reinfection and further damage to heart.

GROUP B STREPTOCOCCUS (S. AGALACTIAE)

Pathogenesis: Approximately 30% of women are vaginal or rectal carriers of group B *Streptococcus*. Hence, the infection is common in neonates and in pregnancy. It is a major cause of:

- Neonatal sepsis and meningitis: Neonatal sepsis can be of two types-early onset and late onset type
- Puerperal sepsis and peripartum fever
- Infections in elderly people with underlying illness, such as diabetes mellitus or malignancy: Cellulitis and soft tissue infections, UTI, pneumonia, and endocarditis.

Laboratory Diagnosis: It can be differentiated from Group A *Streptococcus* by following biochemical tests (see table)

- CAMP positive, Hippurate hydrolysis test positive, Bacitracin resistant and PYR test is negative
- Orange pigment production- enhanced in Islam's medium.
- β hemolytic colonies, which are mucoid and slightly larger (2 mm).
- It has a capsular polysaccharide, which can be typed into nine serotypes.

Group B Streptococcus Causes:
- Neonatal sepsis and meningitis
- Puerperal sepsis and peripartum fever
- Infections in elderly people with underlying illness

Early and late onset Group B *Streptococcus* disease in neonates

Characteristics	Early-onset disease	Late-onset disease
Age of onset	0–6 days of birth	7–90 days of birth
Increased risk following obstetric complications	Prematurity and prolonged labor	Not associated
Mode of transmission to the baby	During or before birth from the colonized maternal genital tract	Contact with a colonized mother and nursing personnel
Common clinical manifestations	Pneumonia and/or respiratory distress syndrome followed by meningitis	Bacteremia and meningitis (most common)
Common serotypes	Ia, III, V, II, Ib	III predominates
Case fatality rate	4.7%	2.8%

Characters	*S. pyogenes*	*S. agalactiae*
Lancefield group	Group A	Group B
Bacitracin sensitivity test	Sensitive	Resistant
PYR test	Positive	Negative
Hippurate hydrolysis test	Negative	Positive
CAMP test	Negative	Positive
β hemolytic colonies	0.5–1 mm, pin point	Mucoid, larger (2 mm)

ENTEROCOCCUS

The enterococci were initially grouped under group-D *Streptococcus*, but later, it has been reclassified as a separate genus *Enterococcus* under family Enterococcaceae.

- Enterococci are the part of normal flora of human GIT. At the same time, they are also increasingly important agents of human disease especially in hospitals mainly because of their resistance to antibiotics.
- *E. faecalis* is the most common species found in clinical specimens; whereas *E. faecium* is more drug resistant than *E. faecalis*.

Various Clinical Manifestations Include

- Urinary tract infections (cystitis, urethritis, pyelonephritis and prostatitis)
- Bacteremia and mitral valve endocarditis (in IV drug abusers)
- Intra-abdominal, pelvic, and soft tissue Infection

- Late-onset neonatal sepsis and meningitis
- Infection on burn surface.

All cocci are Nonmotile except:
- *Enterococcus gallinarum*
- *Enterococcus casseliflavus*

Laboratory Diagnosis

Enterococci show the following characteristics that help in the identification:
- They are gram-positive oval cocci arranged in pairs (spectacle eyed appearance)
- Nonmotile cocci (except *E. gallinarum* and *E. casseliflavus*)
- Blood agar: It produces nonhemolytic, translucent colonies (rarely produces α or β hemolysis)
- MacConkey agar: It produces minute magenta pink colonies.
- Bile aesculin hydrolysis test is positive
- PYR test is positive
- Growth occurs in presence of:
  - 6.5% NaCl, 40% bile and pH 9.6
  - Heat tolerance test: They are relatively heat resistant, can survive 60°C for 30 minutes.

Treatment

Most strains of enterococci are resistant to penicillins, aminoglycosides and sulfonamides. They show intrinsic resistance to cephalosporins and cotrimoxazole.
- Resistance is overcome by combination therapy with penicillin and aminoglycoside (due to synergistic effect) and is the standard therapy for life-threatening enterococcal infections; however in UTI, monotherapy with ampicillin or nitrofurantoin is sufficient. Resistance to this combination therapy may also develop.
- Vancomycin is usually indicated in resistant cases but resistance to vancomycin has also been reported.

Vancomycin Resistant Enterococci (VRE)

Vancomycin resistance in enterococci has been increasingly reported nowadays.
- **VRE is mediated by *Van* gene**, which codes for altered target site for vancomycin in the cell wall (i.e. D-alanyl-D-alanine side chain of peptidoglycan layer is altered to D-alanyl-D-serine or D-alanyl-D-lactate) and this altered side chains have less affinity for binding to vancomycin.
- ***Van* gene has 9 genotypes. Important ones are**: *VanA* to *VanE*.
  - Strains with *VanA* gene show high level resistance to both glycopeptides- vancomycin and teicoplanin.
  - Strains with *VanB* gene show low level resistance to vancomycin, but sensitive to teicoplanin.
  - *E. gallinarum* and *E. casseliflavus* possess *VanC* genes which is chromosomal coded (other genotypes transposon coded), and they show intrinsic resistance to both glycopeptides.
- **Screening of patients for VRE** is carried out by: Rectal swab culturing on Bile esculin azide agar with 6 µg/ml vancomycin

Pathogenic Viridans streptococci:
- *S.mutans:* Causes dental caries and plaques
- *S.sanguis:* Causes subacute bacterial endocarditis
- *S.milleri* group: Produces suppurative infections

VIRIDANS STREPTOCOCCI

Viridans streptococci are α hemolytic, commensal of mouth:
- *S. mutans:* Causes dental caries and plaques
- *S. sanguis*: Causes subacute bacterial endocarditis
- *S. milleri* group: Produces suppurative infections, differ in hemolytic pattern (may be α, β or γ hemolytic).

Treatment: Usually sensitive to penicillin (except neutropenic patients with bacteremia; where vancomycin is given).

STREPTOCOCCUS PNEUMONIAE OR PNEUMOCOCCUS

Virulence Factors and Pathogenesis

S. pneumoniae possesses a number of virulence factors such as:

- **Capsular polysaccharide:** It protects the cocci from phagocytosis:
  - It is type specific (> 90 capsular serotypes are recognized), serotypes are detected by Quellung reaction.
  - **Quellung reaction**: Capsular swelling occurs when colonies are added with type-specific antiserum and methylene blue dye.
  - It diffuses (soluble) into culture media, tissue and exudates, hence also called *soluble specific substance*.
- **C-carbohydrate antigen** (C-polysaccharide or C-substance): It is species specific. CRP (*C-reactive protein)* present in sera of patients with acute inflammation. It is so named because; it precipitates with pneumococcal C-antigen.
- **Pneumolysin:** It is a membrane damaging toxin, inhibits neutrophil chemotaxis.
- **Autolysin:** It is an amidase enzyme that cleaves peptidoglycan leading to autolysis of cells. This property is responsible for bile solubility and draughtsman appearance of pneumococcal colonies.

Virulence Factors of S. pneumoniae:
- Capsule (detected by Quellung reaction)
- C carbohydrate antigen: CRP is named after this
- Pneumolysin
- Autolysin: Responsible for bile solubility and draughtsman colony

Clinical Manifestation

S. pneumoniae is the most common cause of:

- Lobar pneumonia
- Pyogenic meningitis in all ages (except in neonates)
- Noninvasive manifestations such as otitis media and sinusitis.

Other invasive manifestations: *S. pneumoniae* can cause osteomyelitis, septic arthritis, endocarditis, pericarditis, primary peritonitis, rarely, brain abscess and hemolytic-uremic syndrome. Empyema and parapneumonic effusion may occur as complications of pneumonia.

S.pneumoniae is the most common cause of:
- Lobar pneumonia
- Pyogenic meningitis in all ages (except in neonates)
- Noninvasive manifestations, such as otitis media and sinusitis.

Epidemiology

- *Source of infection* in humans is upper respiratory tract of carriers (less often patients).
- *Carrier rate* > 90% of children of 6 months to 5 yrs of age harbor *S. pneumoniae* in the nasopharynx.
- *Mode of transmission* is by inhalation of contaminated droplet nuclei.

Risk factors:

- Children (< 2yrs)
- Splenectomy, sickle cell disease and other hemoglobinopathies: As spleen is the site of destruction of capsulated bacteria, the conditions where the opsonization and clearance of circulating bacteria by the spleen is hampered, there is increase risk of pneumococcal infection.
- Underlying comorbid diseases, such as chronic lung, heart, kidney and liver disease, cochlear implants, diabetes mellitus and immunosuppression (e.g. HIV).
- Underlying viral upper respiratory tract infections (e.g. Influenza).
- Nature of infecting serotypes:
  - In children: Serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F are common
  - In adults: Serotypes 1–8 are common
  - Virulent serotypes: Serotype 3 followed by 7 are more virulent and they produce mucoid colonies.

Nature of infecting serotypes of Pneumococcus:
- In children: Serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F are common
- In adults: Serotypes 1–8 are common
- Virulent serotypes: Serotype 3 followed by 7 are more virulent and they produce mucoid colonies.

Laboratory Diagnosis

S. pneumoniae can be differentiated from Viridans streptococci by various features:

Properties	Pneumococcus	Viridans streptococci
Morphology	Lanceolate or flame shaped	Round/oval
Arrangement	Gram-positive cocci in pairs	Gram-positive cocci in long chains
Capsule	Present	Absent
On blood agar	Draughtsman or carom coin colony	Convex shaped colony
Liquid medium	Uniform turbidity	Granular turbidity
Bile solubility	Soluble in bile	Insoluble in bile
Inulin fermentation	Fermenter	Nonfermenter
Optochin	Sensitive	Resistant
Mice Pathogenicity	Pathogenic	Nonpathogenic

Treatment

Penicillin G remains the drug of choice; cephalosporins, such as ceftriaxone can be given alternatively.

Oral amoxicillin is recommended for children with acute otitis media.

Treatment of Pneumococcal Infections:
- Penicillin G remains the DOC
- Ceftriaxone can be given alternatively.
- Oral amoxicillin is recommended for children with acute otitis media.

Prevention (Capsular Polysaccharide Vaccines)

Two types of pneumococcal vaccines are available.

23-Valent Pneumococcal Polysaccharide Vaccine (PPV23)

PPV23 includes capsular polysaccharide of 23 serotypes of pneumococci. It gives protection for about 5 years.

Indication: It is recommended for people with

• Asplenia or splenic dysfunction	• Diabetes mellitus
• Sickle cell disease or celiac disease	• Cochlear implants
• Chronic lung, heart, kidney and liver disease	• Cerebrospinal fluid leaks
• Immunocompromised patients (HIV)	• Age above 65 years

Contraindication to PPV-23 include:

Malignancies, pregnancy and children of < 3years: As capsular antigens are examples of T-independent antigen, they are poorly immunogenic to children of < 3 years. Hence, PPV-23 is not useful among children of < 3 years.

Contraindications to PPV-23 include:
- Malignancies
- Pregnancy
- Children of < 3 years

7-Valent Polysaccharide Conjugate Vaccine (PCV-7)

It consists of capsular polysaccharide of 7 serotypes added to a protein conjugate. It mainly includes the childhood serotypes (such as 6B, 9V, 14, 19F, 23F, and 18C):

- When a protein conjugate is added, it increases the immunogenicity of capsular antigen (act as adjuvant), hence can be given to children of < 3years.
- Schedule: 4 doses administered at 2, 4, 6, and 12–15 months of age.
- As, resistance to antibiotics is most often noted in pneumococcal serotypes 6, 9, 14, 19, and 23; hence use of PCV-7 has shown to decrease pneumococcal resistance.

MULTIPLE CHOICE QUESTIONS

STREPTOCOCCUS AND PNEUMOCOCCUS

1. **Boy presented with skin ulcer on leg. Culture reveals beta hemolytic Streptococci. Culture from school children with sore throat some days back also revealed beta hemolytic Streptococci. What is the characteristic which can tell both the strains are same or different?** *(AIIMS Nov 2010)*
   a. C-carbohydrate Ag
   b. M protein
   c. Emu protein
   d. Mec A gene
2. **Lancefield grouping of streptococci is done by using:** *(Recent Question 2013, AIIMS Nov 2007, June 1998)*
   a. M Protein
   b. Group C peptidoglycan cell wall
   c. Group C carbohydrate antigen
   d. Staining properties
3. **Streptokinase is taken from which source:** *(Recent Question 2015)*
   a. Streptococcus equisimilis
   b. Streptococcus bovis
   c. Streptococcus canis

GROUP A STREPTOCOCCUS

4. **Streptococcus toxin which is responsible for connective tissue breakdown?** *(Recent Questions 2014)*
   a. Hyaluronidase
   b. Streptolysin O
   c. Streptolysin S
   d. Streptococcus pyogenic exotoxin
5. **False regarding Streptococcus pyogenes:** *(Recent questions 2014, NEET Pattern Based)*
   a. Causes necrotizing fascitis
   b. Beta hemolytic
   c. M protein is virulence factor
   d. Resistant to bacitracin
6. **Which streptococcal antigen cross reacts with synovial fluid?** *(AI 2008)*
   a. Carbohydrate (group A)
   b. Cell wall protein
   c. Capsular hyaluronic acid
   d. Peptidoglycan
7. **A child presents with infective skin lesion of the leg. Culture showed hemolytic colonies which were gram + ve cocci in chains. The test to confirm the organism is:** *(Recent questions 2014, AI 2012, AIIMS Nov 2006; AIIMS Nov 2011, AIIMS May 2007, AI 2007)*
   a. Bile solubility
   b. Optochin sensitivity
   c. Bacitracin sensitivity
   d. Catalase positive
8. **Treatment for streptococcal necrotizing fasciitis:** *(PGI June 2008)*
   a. Surgical debridement
   b. Penicillin
   c. Clindamycin
   d. Metronidazole
   e. Vancomycin
9. **Group A hemolytic pharyngitis is due to:** *(DNB June 2010)*
   a. Local infection
   b. Systemic toxicity
   c. Attachment to mucosa
   d. Local toxins

GROUP B STREPTOCOCCUS

10. **A child presents with sepsis. Bacteria isolated showed β hemolysis on blood agar, resistant to bacitracin and a positive CAMP test. The most probable organism is:** *(Recent Question 2013, AI 2001, 2010)*
    a. S. pyogenes
    b. S. agalactiae
    c. Enterococcus
    d. Pneumococcus
11. **Neonatal meningitis acquired during passage through birth canal is due to:** *(MHPG 2015, TN 2002)*
    a. Streptococcus agalactiae
    b. S. equisimilus
    c. S. pyogenes
    d. Pnemococci

ENTEROCOCCUS

12. **Vancomycin resistance in enterococcus is due to?** *(Recent Question 2015)*
    a. Thickening of cell wall
    b. Altered target site
13. **Which group of streptococcus grow at 60°C:** *(NEET Pattern Based)*
    a. A
    b. B
    c. C
    d. D
14. **A patient admitted to an ICU is on central venous line for the last one week. He is on ceftazidime and amikacin. After 7 days of antibiotics, he develops a spike of fever and his blood culture is positive for gram positive cocci in chains, which are catalase negative. Following this, vancomycin was started but the culture remained positive for the same organism even after 2 weeks of therapy. The most likely organism causing infection is:** *(AIIMS Nov 2011, May 2006, Nov 2006, AI 2007)*
    a. Staphylococcus aureus
    b. Viridans streptococci
    c. Enterococcus faecalis
    d. Coagulase negative Staphylococcus

15. **Which of the following organisms, when isolated in the blood, requires the synergistic activity of penicillin plus an aminoglycoside for appropriate therapy:** *(AIIMS Nov 2004)*
    a. Enterococcus faecalis
    b. Staphylococcus aureus
    c. Staphylococcus pneumoniae
    d. Bacteroides fragilis

STREPTOCOCCUS VIRIDANS

16. **A patient of RHD developed infective endocarditis after dental extraction. Most likely organism causing this is:** *(AIIMS Nov 01)*
    a. Streptococcus viridans
    b. Streptococcus pneumoniae
    c. Streptococcus pyogenes
    d. Staphylococcus aureus

PNEUMOCOCCUS

17. **Patient is presented with cough with rusty sputum. On examination, lower lobe consolidation and bronchial breath sounds were heard. It gives a positive Quellung reaction. What is the probable Gram staining appearance?** *(JIPMER May 2016)*
    a. Gram -ve bacilli
    b. Gram +ve cocci
    c. Gram+ve bacilli
    d. Gram - ve diplococci

18. **Most common organism causing acute otitis media in a 4-8 year child?** *(NIMHANS 2016)*
    a. Streptococcus pneumoniae
    b. Moraxella catarrhalis
    c. Staphylococcus aureus
    d. Shigella

19. **Most common cause of meningitis in alcoholics:** *(Recent Question 2015)*
    a. Pneumococcus
    b. H. influenzae

20. **Mechanism of development of resistance to penicillin in Streptococcus pneumoniae is:** *(JIPMER Nov 2014)*
    a. Production of Beta lactamase
    b. Mutations in the proteins on the bacterial surface
    c. Changes in the membrane permeability to penicillin
    d. Production of an alternative penicillin binding protein

21. **A person presents with pneumonia. His sputum was sent for culture. The bacterium obtained was gram positive cocci in chains and alpha haemolytic colonies on sheep agar. Which of the following will help in confirming the diagnosis:** *(AIIMS May 2012)*
    a. Novobiocin
    b. Optochin
    c. Bacitracin
    d. Oxacillin

22. **The following statements are true regarding Streptococcus pneumoniae except:** *(AI 2011)*
    a. It is bile-sensitive
    b. The capsule of S. pneumoniae allows establishment of infection
    c. It is an etiological agent of pneumonia and otitis media
    d. Pneumococcal meningitis is the least virulent of the major bacterial meningitides

23. **65 years old patient presented to the emergency with high grade temperature and increased respiratory rate. He complained of pain in the chest and had developed cough with expectoration. His sputum was sent to the laboratory for gram straining which showed the presence of pus cells and gram-positive cocci in pair. The culture on the blood agar medium was also positive. Which of the following laboratory tests will help to differentiate the specific pathogen from the other commensal gram-positive cocci?**
    a. Bacitracin sensitivity *(AIIMS Nov 2009)*
    b. Catalase test
    c. Bile solubility
    d. Coagulase test

24. **'C' in C reactive protein stands for:** *(AI 2011)*
    a. Capsular polysaccharide in Pneumococcus
    b. Concanavalin-a
    c. Calretinin
    d. C-carbohydrate antigen

25. **In a splenectomized patient there is increase of infection by all the organisms except :** *(NEET Pattern Based, PGI 2000, SGPGI 2005)*
    a. Pneumococci
    b. Klebsiella
    c. H. influenzae
    d. Staph. aureus

26. **Most common causative organism for lobar pneumonia is:** *(AIIMS 2004)*
    a. Staphylococcus aureus
    b. Streptococcus pyogenes
    c. Streptococcus pneumoniae
    d. Haemophilus influenzae

27. **The most common organism causing acute otitis media is:** *(MHPG 2014)*
    a. H. influenza
    b. S. pneumoniae
    c. M. catarhalis
    d. S. aureus

EXPLANATIONS

STREPTOCOCCUS AND PNEUMOCOCCUS

1. **Ans. (a) (C-carbohydrate Ag)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/r p227, Ananthanarayan 9/e p209
   - β hemolytic Streptococci was obtained from two cases-Boy with skin ulcer and children with sore throat.
   - To know the relatedness between Beta hemolytic Streptococci, Lancefield grouping is done
   - Lancefield grouping is based on C-carbohydrate Ag.

   Classification of Streptococcus- Refer text (chapter review) for explanation.
2. **Ans. (c) (Group C carbohydrate antigen)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p221, Ananthanarayan 9/e p209
   - β hemolytic Streptococcus is classified to group A-V based on C carbohydrate antigen (Lancefield classification)
   - M protein is used to further classify group A Streptococcus (Griffith typing).
3. **Ans. (a) (Streptococcus equisimilis)** Ref: Internet Sources
   - Streptococcus equisimilis is used a source for preparation of streptokinase.

GROUP A STREPTOCOCCUS

4. **Ans. (a) (Hyaluronidase)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p223, Ananthanarayan 9/e p213

   Refer chapter review.
5. **Ans. (d) (Resistant to bacitracin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p227, Ananthanarayan 9/e p213
   - Streptococcus pyogenes (Group A) is sensitive to bacitracin whereas Str. agalactiae (Group B) is resistant to bacitracin.
6. **Ans. (c) (Capsul...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p225, Ananthanarayan 9/e p212
   - Capsular hyaluronic acid cross reacts with synovial fluid
   - Structural components of Strepto pyogenes cross reacts with human tissues-Refer text (chapter review).
7. **Ans. (c) (Bacitracin...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p227, Ananthanarayan 9/e p215
   - Hemolytic colony with gram-positive cocci in chain is suggestive of β hemolytic Streptococcus.
   - Option c: Bacitracin sensitivity is used to differentiate between β hemolytic Streptococci Group A- Bacitracin sensitive and Group b: Bacitracin resistant
   - Option a and b: Bile solubility and Optochin sensitivity are used to differentiate Pneumococcus and S. Viridans
   - Option d: Catalase test is used to differentiate Streptococcus and Staphylococcus.
8. **Ans. (a), (b), (c) (Surgical debridement, Penicillin, Clindamycin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p225, Harrison 19 e/p966 18/e p1176,
   - Treatment of Necrotizing fasciitis: Surgical debridement (most crucial) + Penicillin G + Clindamycin
   - If single option to be selected: Then Answer should be Surgical debridement
   - Harrison 17/e p 886 states: 'Drainage and debridement are central to the management of necrotizing fasciitis; antibiotic treatment is a useful adjunct, but surgery is life-saving'.
9. **Ans. (c) (mucosal attachment)** Ref: Harrison 19/e p964, 18/e p1172

   The capsular polysaccharide may also play a role in GAS colonization of the pharynx by binding to CD44, a hyaluronic acid–binding protein expressed on human pharyngeal epithelial cells.

GROUP B STREPTOCOCCUS

10. **Ans. (b) (S. agalactiae)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p229, Ananthanarayan 9/e p216, 8/e p207

    Already explained

- Points favoring to S. agalactiae:
  - Septicemia in a child
  - β hemolysis on blood agar
  - Resistant to bacitracin and a positive CAMP test
- S. pyogenes: sensitive to bacitracin and CAMP test is negative.

11. Ans. (a) (Streptococcus agalactiae) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p229, Ananthanarayan 9/e p216

Refer chapter review.

ENTEROCOCCUS

12. Ans. (b) (Altered target site) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p231

13. Ans. (d) (D) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p230, Ananthanarayan 8/e p206

Group D Streptococci like Enterococci can grow > 60°C.

14. Ans. (c) (Enterococcus faecalis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 231

This is case of VRE (Vancomycin resistant Enterococci)

Points in favor:

- Gram+ve cocci in chain and Catalase –ve points towards Streptococcaceae family
- Resistant to aminoglycoside, cephalosporins and vancomycin
- S, aureus and CONS are catalase +ve, hence they are ruled out.

15. Ans. (a) (Enterococcus faecalis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 231,

Resistance in Enterococci – Refer chapter review

STREPTOCOCCUS VIRIDANS

16. Ans. (a) (Streptococcus...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p231, Harrison, 18/e p1052-53

- MC cause of Native valve endocarditis: S. aureus
- MC cause of subacute endocarditis: MC agent Streptococcus Viridans
- MC Streptococcus Viridans causing endocarditis: S. sanguis
- Following tooth extraction, transient bacteremia occurs and Streptococcus Viridans gets lodged into pre damage valve.
- So, prophylactic antibiotic is implemented before tooth extraction.

PNEUMOCOCCUS

17. Ans (b) (Gram + ve cocci) Ref: Apurba Sastry's Essentials of Medical Microbiology/p234

- History of lobar consolidation –indicates lobar pneumonia
- Quellung test positive- indicates pneumococcal pneumonia
- Pneumococci are Gram positive diplococci, lanceolate shaped

18. Ans (a). (Streptococcus pneumoniae) Ref: Apurba Sastry's Essentials of Medical Microbiology/p232

- Streptococcus pneumoniae is the MC cause of acute otitis media in children.

19. Ans. (a) (Pneumococcus) Ref: Community-Acquired Bacterial Meningitis in Alcoholic Patients, http://www.ncbi.nlm.nih. gov/pmc/articles/PMC2817003/

20. Ans (d) (Production of an alternative penicillin binding protein) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p234.

Alterations in the target enzymes for β-lactam antibiotics, the penicillin-binding proteins (PBPs), have been recognized as a major resistance mechanism in Streptococcus pneumoniae.

21. Ans. (b) (Opt...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p234 , Ananthanarayan 9/e p222, 8/e p220

- α-hemolytic colonies and gram-positive cocci: Isolated from a sputum of a patient with fever and respiratory distress - Suggestive of:
  - Pneumococcal Pneumonia or
  - Pneumonia due to Str. viridans
- To differentiate Pneumococcus from Str. viridans—Optochin sensitivity test is done.

22. Ans. (d) (Pneumococcal meningitis is the least virulent of the major bacterial meningitis)

Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p234, Ananthanarayan 9/e p223, 8/p219-222

Among the major bacterial meningitides, Pneumococcus is one of the most virulent organism.

About Other Options

Pneumococcus is:

- Capsulated principle virulence factor
- Bile soluble
- MC cause of lobar pneumonia and otitis media.

23. Ans. (c) (Bile solubility) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p234, Ananthanarayan 9/e p222

Points in favor:

- Fever and ↑respiratory rate, chest pain and cough with expectoration: Suggestive of Pneumonia
- Gram positive cocci in pair was isolated from Sputum: Suggestive of Pneumococcus
- Test to differentiate Pneumococcus from commensal like Strept Viridans: Bile solubility
- Refer text for differentiating properties between Pneumococcus and S.viridans.

It can be differentiated from other commensal in sputum, i.e. Strept Viridans:

- Optochin sensitivity (Ethyl hydrocuprein)
- Bile solubility

Out of this, Bile solubility is a better Option because:

- Few strains of Strept Viridans also can be sensitive to Optochin while few strains of Pneumococcus can be resistant.
- Bile solubility is a constant property of Pneumococcus, hence is of diagnostic importance. .. Ananthanarayan 8/e p219

24. Ans. (d) (C-carbohydrate antigen) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p234, Ananthanarayan 9/e p222

C-reactive protein: Refer chapter review

25. Ans. (d) (Staph. aureus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p235, 170, Ananthanarayan 9/e p18, 200

- Capsulated organism can resist killing by preventing phagocytosis.
- Spleen has an important role in removing these capsulated organisms where opsonization takes place.
- Certain complements (opsonins) can attach to these organisms and once delivered to spleen, their uptake is facilitated by complement receptors like CR1, 2. etc. present on splenic phagocyte surface (Process known as Opsonization).
- Hence, in a splenectomized patient there is increase risk of infection by capsulated organisms.
- Among the options, all are capsulated except S. aureus.

26. Ans. (c) (Streptococcus pneumoniae) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p233 , Ananthanarayan 9/e p222

- Streptococcus pneumoniae is the Most common causative organism for lobar pneumonia
- Adult: MC serotype associated: Type 1-8
- Children: Type 6, 14, 19, 23 are frequently associated serotypes
- Most Virulent: Type 3.

27. Ans. (b) (S. pneumoniae) Ref: Ear Infections/CDC Website

- Streptococcus pneumoniae followed by Haemophilus influenzae and Moraxella catarrhalis are the most common bacterial isolates from the middle ear fluid of children with acute otitis media.

CHAPTER

3.3

Neisseria and Moraxella

- *N.meningitidis*: Ferments glucose and maltose
- *N.gonorrheae*: Ferments only glucose

Gram negative cocci include *Neisseria, Moraxella catarrhalis* and *Veillonella*

Neisseriae are catalase and oxidase positive, non-motile, aerobic gram-negative diplococci:

- Two species are pathogenic to humans: *N.meningitidis* and *N.gonorrhoeae*
- Others are commensals of intestine, genital tract or oral cavity.

N. meningitidis	N. gonorrhoeae
Capsulated	Noncapsulated
Lens shaped/half-moon shaped (diplococci with adjacent sides flattened)	Kidney shaped (diplococci with adjacent sides concave)
Ferments glucose and maltose	Ferments only glucose
Rarely have plasmids	Usually possess plasmids, coding for drug resistance genes
Exist in both intra and extracelular forms	Predominantly, exist in intracelular form
Colony—circular	Colony—vary in size with irregular margin
Habitat—Nasopharynx	Habitat—genital tract (urethra, cérvix), rarely pharynx.

NEISSERIA MENINGITIDIS (MENINGOCOCCUS)

Meningococcal Disease pattern:

- Epidemic (Africa): Due to A and also by W135
- Outbreaks: Due to serogroup C
- Hyperendemic disease: Due to serogroup B
- Sporadic cases: Due to all, i.e. A, B, C, Y, and W135

Virulence Factors

- **Capsular polysaccharide:** It prevents the bacteria from phagocytosis, can be typed into 13 serogroups:
  - Only 5 serogroups account for the majority of cases: **A, B, C, Y,** and **W135**;
  - Other capsular serotypes and noncapsulated strains (16% of isolates are not capsulated) colonize the nasopharynx of asymptomatic carriers.
- Outermembrane proteins: OMP is used to classify serogroups to serotypes.
- *Lipopolysaccharide and endotoxin*: Causes endothelial injury leads to:
  - Increased vascular permeability leading to loss of fluid and shock
  - Intravascular thrombosis leading to disseminated intravascular coagulation (DIC)
  - Waterhouse-Friderichsen syndrome
  - Myocardial dysfunction
- IgA proteases—Cleave mucosal IgA
- Transferrin binding protein.
- Adhesins: Mediated by OPA protein and pili.

Epidemiology

Worldwide, nearly 5 lakh cases of meningococcal disease occur each year, and 10% of those die.

- **Disease pattern**: There are several patterns of the disease noted:
  - Epidemics occurs mainly in sub-Saharan Africa—Due to group A (mainly) and W135.
  - Outbreaks—mainly due to serogroup C (in semi-closed communities such as schools, military camps , etc.).
  - Hyperendemic disease (> 10 cases per 100,000 population) Due to serogroup B.
  - Sporadic cases (0.3–5 cases per 100,000 population) can occur due to all, i.e. A, B, C, Y, and W135.
- **High prevalence area** is sub-Saharan belt of Africa (from Ethiopia to Senegal)
- **Seasonal variation** is seen commonly in winter and spring (cold and dry climate)
- **Age:** Meningitis is common in early childhood (3 months to 5 years).
- **Risk factors that promote colonization include**:
  - Overcrowding and semi-closed communities such as schools, military and refugee
  - Travellers (Hajj pilgrims) and smoking
  - Viral and *Mycoplasma* infection of the respiratory tract

- **Risk factors that promotes disease include:**
  - Deficiency of terminal complement components (C5–C9)
  - Hypogammaglobulinemia
  - Hyposplenism.

Risk factors that promote Meningococcal Infections disease:
- Deficiency of terminal complement components (C5–C9)
- Hypogammaglobulinemia
- Hyposplenism

Pathogenesis

- *Source:* MC source of infection is human nasopharyngeal carriers (mainly children).
- Carrier rate may vary from 5–10% (during inter epidemic period) up to 70–80% (during epidemic).
- *Mode of transmission* is by droplet inhalation
- *Spread of infection*: From nasopharynx, meningococci reach the meninges either by:
  - Hematogenous route (most common) or
  - By direct olfactory nerve spread through cribriform plate or
  - Rarely through conjunctiva.
- *Case fatality ratio* is 80% (falls to 10% if early treatment is started).

Clinical Manifestations

Asymptomatic colonization is the most common presentation. Various manifestations include

- *Rashes*: A nonblanching rash (petechial or purpuric) develops in > 80% of cases.
- *Septicemia*: It is attributed to endotoxin induced endothelial injury
- *Waterhouse-Friderichsen syndrome:* It is a severe form of fulminant meningococcemia, characterized by large purpuric rashes (purpura fulminans), shock, DIC, bilateral adrenal hemorrhage and multi-organ failure.
- *Pyogenic meningitis*: Commonly affects young children (3–5 years of age).
- *Chronic meningococcemia*: Occurs rarely and characterized by petechial rash, fever, arthritis, and splenomegaly.
- *Postmeningococcal reactive disease*: Immune complexes develop 4–10 days later, lead to manifestations like arthritis, rash, iritis, pericarditis, polyserositis and fever.

Meningococcal Infections:
- Rashes
- Septicemia
- Waterhouse Friderichsen syndrome
- Pyogenic meningitis
- Chronic meningococcemia
- Post meningococcal reactive disease

Laboratory Diagnosis

- Specimen:
  - For cases: Blood and CSF
  - For carriers: Nasopharyngeal swab
- CSF examination:
  - First portion is centrifuged and used for:
    - Capsular antigen detection
    - Biochemical analysis: ↑CSF pressure, ↑protein and ↓glucose in CSF
    - Gram staining: Pus cells with gram-negative diplococci, lens-shaped
  - Second portion: For culture on blood agar, chocolate agar
  - Third portion is enriched in BHI broth and incubated for 7 days
- Nasopharyngeal swab culture: On Thayer Martin medium
- Biochemical tests:
  - Oxidase and catalase positive
  - Ferment glucose and maltose but not sucrose
- Serogrouping: by latex agglutination test:
- Serology: Antibodies to capsular Ag (ELISA), Useful in retrospective diagnosis of disease
- Molecular diagnosis: By multiplex PCR.

Direct examination of CSF:
- Capsular Ag detection
- Biochemical analysis: ↑CSF pressure, ↑protein and ↓glucose in CSF
- Gram staining: Pus cells with gram-negative diplococci, lens-shaped

Treatment

- DOC for treatment → Ceftriaxone and cefotaxime
- DOC for carriers and prophylaxis→ Ceftriaxone (DOC), others: Rifampicin and ciprofloxacin.

Vaccine

Polyvalent vaccine containing → Four groups A,C,Y, W135

- No vaccine for Group B:
  - As Group B capsule is made up Sialic acid residue which is encephalitogenic and poorly immunogenic
  - However, Outer membrane vesicles (OMVs) based vaccines trails are going on.

- Dose 50 µg single dose, immunity starts in 10 days, lasts for 3 years
- Vaccine is indicated to travellers like Hajj pilgrimage
- Contraindications pregnancy and below 3 year (capsule being T independent, is poorly immunogenic < 3 year).

Treatment of Meningococcal Infections:
- DOC for treatment → Ceftriaxone and cefotaxime
- DOC for carriers and prophylaxis→ Ceftriaxone (DOC), others: Rifampicin and ciprofloxacin

No vaccine for Group B Meningococcus:
- As Group B capsule is made up Sialic acid residue which is encephalitogenic and poorly immunogenic

NEISSERIA GONORRHOEAE (GONOCOCCUS)

N.gonorrhoeae is noncapsulated, Gram negative kidney shaped diplococci.

Virulence Factors

- **Pili or fimbriae:** Principal virulence factor, helps in adhesion and inhibit phagocytosis.
- **Outer membrane proteins:**
  - ***Porin (protein I):*** They form membrane channels (pores)
    - There are two major serotypes: Por B.1A and PorB.1B serotypes.
    - PorB.1A strains associated with local and disseminated gonococcal infections (DGI)
    - PorB.1B strains usually cause local genital infections.
  - ***Opacity-associated protein (Protein II):*** It helps in adhesion, and invasion
- Transferrin-binding and lactoferrin binding protein: It is required for uptake of iron
- IgA1 protease: It degrades mucosal IgA antibody
- Lipooligosaccharide (LOS): It differs from LPS of Enterobacteriaceae by lacking the repetitive O side chain.

Typing of Gonococci

- ***Serotyping*** is based on protein-I (porin).
- ***Auxotyping***: Typing is based on nutritional requirements of the strains, e.g. AHU auxotype needs arginine, hypoxanthine and uracil as growth factors.

Clinical Manifestations

Gonorrhea is a venereal disease reported since ancient time.

1. **In males:**
   - Acute urethritis is the most common manifestation. Purulent urethral discharge (the word 'gonorrhea' is derived from flow of seed resembling semen)
   - The usual incubation period is 2–7 days.
   - Complications: Epididymitis, prostatitis, edema of the penis, and balanitis.
   - Infection may spread to periurethral tissues causing abscess with sinus formation (known as **water-can perineum**).
2. **In females:** ***Gonococcal infection is less severe in females with more asymptomatic carriage:***
   - Mucopurulent cervicitis is the most common presentation.
   - Vulvovaginiti seen in prepubertal girls and postmenopausal women, but not in adult females as the adult vagina is resistant to gonococcal infection (due to its low pH and thick stratified squamous epithelium).
   - Salpingitis and pelvic inflammatory disease may lead to sterility.
   - *Fitz-Hugh-Curtis syndrome:* It is a rare, characterised by peritonitis and associated perihepatitis.
3. **In both the sexes:**
   - Anorectal gonorrhea (as acute proctitis): Rectal isolates are usually drug resistant.
   - Pharyngeal gonorrhea (spread by orogenital sex)
   - Ocular gonorrhea.
4. **In neonates (Ophthalmia neonatorum):**
   - Characterized by purulent eye discharge, occurs within 2–5 days of birth.
   - Transmission occurs during birth from colonized maternal genital flora.
   - Treatment: Silver nitrate solution into the eyes of newborn (Crede's method).
5. **Disseminated gonococcal infection (DGI):** ***Occurs in 0.5–3% of untreated persons:***
   - DGI is characterized by polyarthritis and rarely dermatitis and endocarditis.
   - It is most commonly associated with PorB.1A serotypes and AHU auxotypes.
   - Menstruation and complement (C5–C9) deficiency are risk factors for DGI
6. **In HIV-infected persons:** Enhances the transmission of HIV.

Gonococcal infection:
- In males: Acute urethritis is MC
- In females: Less severe with more carriage

Laboratory Diagnosis

Sample

- Males: Urethral discharge
- Females: Endocervical swab (high vaginal swab not recommended).
- For DGI: Blood culture and synovial fluid culture.

Transport media

- Charcoal impregnated swabs/medium (Stuart/Amies media)
- For longer holding period: CO_2 generating system (JEMBEC system)

Gram staining

- For males: Gram staining of urethral discharge is more sensitive (90%): Based on which treatment can be started
- For females: Gram staining is less sensitive (50–60%) due to presence of commensal *Neisseria* spp. in genital tract. So, Endocervical culture is recommended.
  Endocervical culture: Gonococci are difficult to grow than meningococci:
- Culture media in acute gonorrhoeae: Chocolate agar and Mueller-Hinton agar
- Selective media are useful in chronic cases:
  - Thayer: Martin media
  - Modified New York city medium
  - Martin: Lewis Media

Treatment

- DOC: Single-dose regimen of Ceftriaxone and cefotaxime
- Treat both the partners, regimen should also include azithromycin for *Chlamydia*
- Most strains are resistant to penicillin due to penicillinase production. Such strains are called as PPNG strains (Penicillinase producing strains of *N. gonorrhoeae*)

Properties	Gonococcal urethritis (GU)	Nongonococcal urethritis (NGU)
Agent	*N. gonorrhoeae*	Bacterial: • *Chlamydia trachomatis*- MC cause of NGU • *Ureaplasma urealyticum* • *Mycoplasma hominis* • Some cases may be due to gonococcal infection, the cocci persisting as L forms and hence undetectable by routine tests • Viruses: Herpesvirus and cytomegalovirus • Fungi: *Candida albicans* • Parasites: *Trichomonas vaginalis*
Onset	48 hours	Longer (> 1 week)
Urethral discharge	Purulent (flow like seed)	Mucous to mucopurulent
Complication	DGI Water-can perineum	Reiter's syndrome: Characterized by conjunctivitis, urethritis, arthritis and mucosal lesions
Other complications are common to both GU and NGU such as: • Males: Epididymitis, prostatitis, seminal vesiculitis and balanitis • Females: Salpingitis and pelvic inflammatory disease and Fitz-Hugh-Curtis syndrome		
Diagnosis	• Gram stain, • Culture on Thayer-Martin media	For *Chlamydia*: Culture on McCoy and HeLa cell lines *Trichomonas*: Detection of trophozoite *Candida*: Detection of budding yeast cells in discharge PCR: Can be done for HSV or *Chlamydia*
Treatment	Ceftriaxone	For *Chlamydia*: Doxycycline For *Trichomonas*: Metronidazole For *Candida*: Clotrimazole (as vaginal cream or tablet)

Disseminated gonococcal infection (DGI):
- Occurs in 0.5–3% of untreated persons
- Characterized by polyarthritis and rarely dermatitis and endocarditis
- Associated with PorB.1A serotypes and AHU auxotypes
- Risk factors: Menstruation and complement (C5-C9) deficiency.

Gram staining of urethral discharge:
- For males: More sensitive (90%), based on which treatment can be started
- For females: Less sensitive (50–60%) due to presence of commensal Neisseria species.

Selective media are useful in chronic gonococcal cases:
- Thayer Martin media
- Modified New York city medium
- Martin Lewis Media.

MULTIPLE CHOICE QUESTIONS

MENINGOCOCCUS

1. **Vaccine available against which of the serotype of meningococcus?** *(Recent Question 2015)*
   a. Serotype b only
   b. Serotype a and c only
   c. Serotype b and c only
   d. Serotype a, b and c only
2. **Patient gives h/o low grade fever, skin rash and increased WBC counts. What will you do next?**
   a. Blood culture *(JIPMER Nov 2015)*
   b. CT scan for meningococci
   c. Serology
3. **A 16-year-old male patient presents with headache, fever and neck stiffness for the past 24 hours. Similar history was present one year back. CSF analysis shows WBC count–400/ml, with 90% neutrophils. Gram staining shows gram negative diplococci. The immune system affected in this condition is:** *(JIPMER Nov 2014)*
   a. B lymphocytes
   b. T lymphocytes
   c. Immunoglobulins
   d. Complement system
4. **Waterhouse-Friedrichsen syndrome is a complication seen in infection with:** *(NEET Pattern Based, TNPG 2014)*
   a. Neisseria gonorrhoeae
   b. Neisseria meningitidis
   c. Escherichia coli
   d. Mycobacterium tuberculosis
5. **Which among the following differentiates Neisseria meningitidis from Neisseria gonorrhoeae?** *(NEET Pattern Based, DNB Dec 2010)*
   a. It is oxidase positive
   b. It ferments glucose
   c. It ferments maltose
   d. It reduces nitrates
6. **Virulence factors for Meningococci:** *(PGI Dec 2007)*
   a. Capsule
   b. Pili
   c. Endotoxin
   d. Coagulase
   e. M. protein
7. **Xavier and Yogender stay in the same hostel of same university. Xavier develops infection due to group B Meningococcus. After few days Yogender develops infection due to Group C Meningococcus. All of the following are true statements except:** *(AI 2002)*
   a. Educate students about meningococcal transmission and take preventive measures
   b. Chemoprophylaxis to all against both group B and group C
   c. Vaccine prophylaxis of contacts of xavier
   d. Vaccine prophylaxis of contacts of yogendra
8. **Conjugate vaccines are available for the prevention of invasive disease caused by all of the following except:** *(AIIMS 2004)*
   a. H influenzae
   b. Strep pneumoniae
   c. N.meningitidis (group C)
   d. N.meningitidis (group B)

GONOCOCCUS

9. **Protein degrading enzyme secreted by:** *(JIPMER, May 2015)*
   a. Pneumococci
   b. Staphylococcus aureus
   c. Gonococci
   d. Enterococci
10. **19 years old male patient comes to the emergency department with complaints of urethral discharge, 1 week after having unprotected sex. Gram staining reveals numerous neutrophils, some gram negative intracellular diplococci. He was treated with ceftriaxone 250 mg I.M; 5 days later he returns with the same complaints. What is the diagnosis?**
    a. Chlamydia trachomatis *(JIPMER Nov 2014)*
    b. Penicillin resistant Neisseria gonorrhoeae
    c. Reinfection with Neisseria gonorrhoeae
    d. Ureaplasma urealyticum
11. True about Neisseria gonorrhoeae?
    a. Kidney shaped *(Recent Questions 2014)*
    b. Isolated in PIKES medium
    c. It is not transmitted through sexual contact
    d. Protein II is useful for typing
12. **Gonococci can be identified by?** *(DNB Dec 2011)*
    a. Growth on Macconkey medium
    b. Growth at 22 °C
    c. By the fermentation of glucose
    d. Growth in 45 to 60% bile
13. **The virulence factor of Neisseria gonorrhoeae includes all of the following except:** *(AIIMS May 2003)*
    a. Outer membrane proteins
    b. IgA protease
    c. M proteins
    d. Pili

14. Which is the true statement regarding gonococcal urethritis? *(PGI Dec 2001)*

a. Symptoms are more severe in females than in males
b. Rectum and prostate are resistant to gonococci
c. Most patients present with symptoms of dysuria
d. Single dose of ciprofloxacin is effective in treatment
e. Commonly leads to arthritis

15. A patient has history of sexual intercourse with a commercial sex worker 3 days back, has developed genital discharge resembling 'flow of seed'. What medium should be used for culture of the discharge material? *(Recent Question 2013, PGI June 2005))*

a. Mannitol salt agar
b. Thayer-Martin media
c. Potassium tellurite agar
d. TCBS

MORAXELLA

16. Cause of angular conjunctivitis and all options was of moraxella: *(Recent Question 2015)*

a. Moraxella catarrhalis
b. Moraxella lacunata

EXPLANATIONS

MENINGOCOCCUS

1. **Ans. (b) (Serotype a and c)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p238

2. **Ans (a) (Blood culture)** Ref: ApurbaSastry's Essentials of Medical Microbiology 1/e p 237
   Definitive diagnosis of meningococcal meningitis is done by CSF or blood culture. Serology (antibody detection) only helps in retrospective diagnosis.

3. **Ans (d) (Complement system)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p144
   - This is most probably a case of pyogenic meningitis due to Meningococcus.
   - Late complement (C5-C9) deficiency is an important risk factor for meningococcal infection

4. **Ans. (b) (N. meningitidis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p237, Ananthanarayan 9/e p229
   Refer chapter review for detail.

5. **Ans. (c) (It ferments...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p237, Ananthanarayan 9/e p228
   - Meningococcus can ferment glucose and maltose where as Gonococcus can ferment only glucose.

6. **Ans. (a), (b), (c) (Capsule, Pili, Endotoxin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p236
   Virulence factor of Meningococcus:
   - Refer text (chapter review) for explanation:
   - Option d: Coagulase is a Virulence factor for Staphylococcus
   - Option e: M protein is a Virulence factor for Streptococcus.

7. **Ans. (c) (Vaccine prophylaxis of contacts of Xavier)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p239, Ananthanarayan 9/e p230, Harrison 19/e p1001, 18/e p1219
   - Meningococcal Polyvalent vaccine contains à A,C,Y, W135, immunity lasts for 3 years
   - No vaccine available for Group B:
   - As its capsule is poorly immunogenic- -α-2, 8-N-acetylneuraminic acid is expressed on the surface of neural cells in the fetus such that the B polysaccharide is perceived as 'self' and therefore is not immunogenic in humans.
   - Also remember, vaccine is not effective in children below 3 years of age because capsular polysaccharide being T independent Antigen, is poorly immunogenic < 3 years.

8. **Ans. (d) (N. meningitidis...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p239, Ananthanarayan 9/e p230
   Already explained:

GONOCOCCUS

9. **Ans (c) (Gonococci)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p239
   - Gonococci and Meningococci produced IgA1 protease that splits and inactivates IgA.

10. **Ans (b) (Penicillin resistant...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p241, Harrison 19/e p1005-07
    It's a case of drug resistance of infected strain, not a case of reinfection.
    - H/o urethral discharge with ↑neutrophils, and gram negative intracellular diplococci- Suggestive of Gonococcus.
    - DOC for Neisseria gonorrhoeae infection is -ceftriaxone
    - However some strains fail to respond to beta lactams due to – i) production for beta lactamase; they are called as PPNG (Penicillinase producing Neisseria gonorrhoeae) or ii) may be due to altered penicillin-binding protein 2. (Resistance to ceftriaxone is due to altered PBP2)
    - Reinfection may be seen only with infected women with antibody to gonococcal OMP called Rmp (reduction modifiable protein); because Rmp antibodies block the effect of bactericidal antibodies to porin and LOS. Reinfection is NOT seen following treatment.

11. Ans. (a) (Kidney...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p239, Ananthanarayan 9/e p230-31.
Refer chapter review.

12. Ans. (c) (By the...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p239, Ananthanarayan 9/e p234

- Gonococcus cannot grow on MacConkey medium
- Gonococcus can grow at 37 °C but not at 22 °C
- Gonococcus can ferment glucose
- Gonococcus cannot grow in presence of 40% bile (It is a property of Enterococcus)

13. Ans. (c) (M pro...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p239, Ananthanarayan 9/e p231
Virulence factor for Gonococcus – Refer text for (chapter review) explanation.

14. Ans. (c) (Most patients present with symptoms of dysuria) Ref: Harrison 18/e p1222-23, 19 e/p1005
'Acute urethritis is the most common clinical manifestation of gonorrhea in males and major symptoms are Urethral discharge and dysuria, usually without urinary frequency or urgency' .....Harrison 18/e p1222-23

About Other Options

- Fluoroquinolones offered the advantage of antichlamydial activity when administered for 7 days
- Third-generation cephalosporins have remained highly effective as single-dose therapy for gonorrhea
- Adult vagina is resistant to Gonococcus, so infection is less severe in female
- In males, it can involve rectum and prostate and epididymitis. (never involve **testes**).

About detail of DGI (gonococcal arthritis)- Refer chapter review

15. Ans. (b) (Thayer-Martin...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p240, Ananthanarayan 9/e p233

- Genital discharge resembling 'flow of seed'.... Suggestive of Gonococcal urethritis
- Selective media for culture of Gonococcus: Thayer-Martin medium.

MORAXELLA

16. Ans. (b) (M. lacunata) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p242

CHAPTER

3.4

Corynebacterium and Bacillus

CORYNEBACTERIUM

Corynebacterium are club-shaped gram-positive, noncapsulated, non-sporing, non-motile rods.

Corynebacterium diphtheriae (or Klebs-Loeffler bacillus) shows two additional features:

- **Chinese letter or cuneiform arrangement** in smear (V or L shaped)- due to bacterial cells divide and daughter cells tend to lie at acute angles to each other. This type of cell division is called snapping type of division.
- **Metachromatic granules** present at ends or poles of bacilli (also called polar bodies or Babes Ernst bodies or volutin granules):
  - They are storage granules composed of polymetaphosphates.
  - They are better stained with special stains, such as Albert's, Neisser's and Ponder's stain.
  - Granules are well developed on enriched media, such as blood agar or Loeffler's serum slope.
  - Volutin granules can also be possessed by:
    - *Corynebacterium xerosis*
    - *Gardnerella vaginalis*
    - *Spirillium*
    - Few *Mycobacterium* species
    - Enterobacter aerogene
    - Few yeasts.

Volutin granules can also be possessed by:
- *Corynebacterium xerosis*
- *Gardnerella vaginalis*
- *Spirillium*
- Few *Mycobacterium* species
- Enterobacter aerogene
- Few yeasts

Virulence Factor (Diphtheria Toxin)

Mechanism of Action

DT is the primary virulence factor responsible for the diphtheria:

- It has two fragments: Fragment A (active unit) and B (binding unit)
- Fragment B binds to the host cell receptors (such as epidermal growth factor) and helps in entry of fragment A.
- Fragment A is internalized into the host cells and then causes→ ADP ribosylation of elongation factor 2(EF2)→ inhibition of EF2→ irreversible inhibition of translation step of protein synthesis→ cell death.
- Mechanism of DT is similar to exotoxin A of *Pseudomonas*.

Diphtheria Toxin (Mechanism):
- Fragment B binds to the host cell receptors
- Fragment A causes→ ADP ribosylation of elongation factor 2(EF2) → ↓ of EF2→ ↓ protein synthesis→ cell death.
- Mechanism: Similar to exotoxin A of *Pseudomonas*.

Toxin Production is Dependent on

- Phage coded: DT is coded by β corynephage carrying tox gene.
- Iron concentration: Toxin production depends on optimum iron concentration (0.1 mg per liter). Higher levels of iron inhibit toxin synthesis by up regulating DT repressor gene in the bacterial chromosome.
- DT repressor gene (DtxR) is an iron dependent negative regulator of DT production and iron uptake in *C.diphtheriae*.
- Biotypes: Among the three biotypes of *C.diphtheriae*, all strains of gravis, 95–99% strains of intermedius and 80–85% of mitis strains are toxigenic. However, toxins produced by different biotypes are antigenically similar.
- Other species: DT is also produced by *C.ulcerans* and *C.pseudotuberculosis.*

Diphtheria Toxin production is dependent on:
- Phage coded
- Iron concentration
- DT repressor gene
- Biotypes
- Other species: DT is also produced by *C.ulcerans* and *C.pseudotuberculosis*

Toxoid is used for Vaccination

- Diphtheria toxin is antigenic and antitoxins are protective in nature. However, as it is virulent, cannot be given directly for vaccination.
- Toxin can be converted to toxoid which is used for vaccination. Toxoid is a form of toxin, where the virulence is lost, retaining its antigenicity.
- Toxoid formation is promoted by formalin, acidic pH and prolonged storage.
- Park William 8 strain of *C.diphtheriae* is used as a source of toxin for preparation of vaccine.
- Lf unit: DT is expressed as Loeffler's flocculating unit. 1 Lf unit is the amount of toxin which flocculates most rapidly with one unit of antitoxin.

Park William 8 strain of *C.diphtheriae*
Used as a source of toxin for preparation of vaccine

Pathogenicity and Clinical Manifestations

Diphtheria is toxemia but never a bacteremia:

- Bacilli are noninvasive, present only at local site (pharynx), secrete the toxins which spread by bloodstream to various organs.
- It is the toxin which is responsible for all types of manifestations including local (respiratory) and systemic complications (except the skin lesions which may be caused due to the organism).

Respiratory Diphtheria

This the most common form of diphtheria. Tonsil and pharynx (faucial diphtheria) are the most common sites followed by nose and larynx and rarely non-respiratory mucosa, such as conjunctiva or vagina. Incubation period is about 3–4 days:

- Faucial diphtheria: Toxin elicits an inflammatory response that leads to necrosis of the epithelium and exudate formation leading to formation of **pseudomembrane** (tough leathery greyish white coat composed of an inner band of fibrin surrounded by neutrophils, RBCs and bacteria).
- Pseudomembrane is so named, as it is adherent to the mucosal base and bleeds on removal in contrast to the true membrane which can be easily separated (e.g. as in *Candida*).
- Extension of pseudomembrane: In severe cases, it may extend into **larynx and medium-sized bronchial airways** which may result in fatal airway obstruction leading to asphyxia, which mandates immediate tracheostomy.
- **Bull-neck appearance**: Characterized by massive tonsillar swelling and neck edema.

Pseudomembrane:
- Tough leathery greyish white coat
- Composed of an inner band of fibrin surrounded by neutrophils, RBCs and bacteria
- So named, as it is adherent and bleeds on removal

Cutaneous Diphtheria

- Characterized by punched-out ulcerative lesions with necrosis, or rarely pseudomembrane.
- Cutaneous diphtheria is due to the organism itself and is not toxin mediated.
- Skin lesions can also be caused by nontoxigenic strains.
- There is increasing trend of cutaneous diphtheria nowadays, especially in vaccinated children, because antitoxins present in vaccinated people cannot prevent the disease.

Systemic Complications

Neurologic manifestations (cranial nerve involvement, Peripheral neuropathy, ciliary paralysis) and myocarditis are late toxic manifestations, occurring after weeks of infection.

Laboratory Diagnosis

The diagnosis of diphtheria is based on clinical signs and symptoms plus laboratory confirmation.

- Because of risk of respiratory obstruction, specific treatment should be instituted immediately on clinical suspicion of diphtheria without waiting for laboratory reports.

Laboratory diagnosis of diphtheria is necessary only for:
- Confirmation of clinical diagnosis
- Initiating the control measures
- Epidemiological purposes (Not to start treatment)

- Laboratory diagnosis is necessary only for:
  - Confirmation of clinical diagnosis
  - Initiating the control measures
  - Epidemiological purposes
- Laboratory diagnosis consists of isolation of the bacilli and toxin demonstration.

Isolation of the Diphtheria Bacillus

Culture media:
- *Enriched medium:* Such as Loeffler's serum slope
  - Detects growth early (6–8 hrs)
  - Best medium for metachromatic granules production
- *Selective medium:* Such as PTA and Tinsdale medium
  - Best media for isolation
  - Black colonies appear only after 48 hours

- **Specimen**: Throat swab (one or two) containing fibrinous exudates and a portion of membrane
- **Direct smear microscopy**:
  - Gram stain: *C.diphtheriae* appears as irregularly stained club shaped gram-positive bacilli arranged in Chinese letter or cuneiform arrangement (V or L shaped). It is difficult to differentiate them from other commensal coryneforms found in the respiratory tract.
  - Albert's stain is more specific for *C.diphtheriae*, where they appear as green bacilli with bluish black metachromatic granules.
- **Culture media**:
  - ***Enriched medium: such as Loeffler's serum slope:***
    - Advantages: (i) detects growth early (6–8 hrs), (ii) best medium for metachromatic granules production.
    - Disadvantage: As it is an enriched medium, if incubated beyond 6–8 hrs, it supports growth of other throat commensals also.
  - ***Selective medium: such as Potassium tellurite agar (PTA) and Tinsdale medium:***
    - Advantage: Throat commensals are inhibited, hence they are best media for isolation of *C.diphtheriae* from cases as well as carriers; as the normal flora will be inhibited.
    - Disadvantage: The black colonies appear only after 48 hours of incubation.
- **Biochemical identification:**
  - Hiss's serum sugar media: *C.diphtheriae* ferments glucose and maltose (by all biotypes) and starch (by only gravis)
  - Pyrazinamidase test: Negative for *C.diphtheriae*, *C.ulcerans* and *C.pseudotuberculosis*
  - Urease test: Negative for *C.diphtheriae*; but *C.ulcerans* and *C.pseudotuberculosis* are urease positive.
  - *Corynebacterium* is catalase positive but oxidase negative and nonmotile.

Toxin detection:
In vivo tests (Guinea pigs inoculation)
In vitro tests:
- Elek's gel precipitation test
- Detection of tox gene- by PCR
- Detection of diphtheria toxin by ELISA
- Cytotoxicity produced on cell lines

Toxin Demonstration

As the pathogenesis is due to diphtheria toxin, mere isolation of bacilli does not complete the diagnosis. Toxin demonstration should be done following isolation, which can be of two types; *in vivo* and *in vitro*:

- *In vivo* tests (Guinea pigs inoculation) by (i) subcutaneous test, (ii) Intracutaneous inoculation
- *In vitro* tests:
  - Elek's gel precipitation test
  - Detection of *tox* gene by PCR
  - Detection of diphtheria toxin by ELISA or immunochromatographic test (ICT)
  - Cytotoxicity produced on cell lines.

Typing of C. diphtheriae

- Biotyping (McLeod's classification): *C.diphtheriae* can be typed into four biotypes, such as gravis, intermedius, mitis and belfanti based on various properties. Biotype belfanti is a nitrate negative variant of mitis biotype.
- Other methods: Serotyping, Bacteriophage typing, Bacteriocin typing and Molecular typing methods, such as PFGE.

	C.diphtheriae biotypes		
Properties	**Gravis**	**Intermedius**	**Mitis**
Morphology	Short, no granules	Long barred, poor granules	Long curved, prominent granules
Colonies on PTA	**Daisy head**	**Frog's egg** colony	**Poached egg** colony
Starch fermentation	+ve	–ve	–ve
Toxigenic strains	100%	95–99%	80–85%
Virulence	Severe	Moderate	Mild
Occurrence	Epidemic	Epidemic	Endemic
Complications	Paralytic and hemorrhagic	Hemorrhagic	Obstructive
Hemolysis	Variable	Nonhemolytic	Hemolytic

Epidemiology

Diphtheria is an endemic disease known from ancient time. However, there is declining trend of diphtheria cases in most developed as well as a few developing countries including India due to widespread vaccination coverage.

- Source of infection: Carriers (95%) are more common source of infection than cases (5%).
- Carriers may be temporary (persists for a month) or chronic (persists for a year). Nasal carriers are more dangerous due to frequent shedding than throat carriers. Incidence of carrier rate varies from 0.1 to 5%.
- Transmission is via the aerosol route, or rarely by contact with infected skin lesions.
- Reservoir: Humans are the only reservoir
- Age: Diphtheria is common in children aged 1–5 years. With wide spread immunization, a shift in age has been observed from preschool to school age. Newborns are usually protected due to maternal antibodies.

Treatment

Treatment should be started immediately on clinical suspicion of diphtheria:

- **Antidiphtheritic horse serum or ADS (antitoxin):** It is the treatment of choice as it neutralizes the toxin.
- **Antibiotics:** Penicillin or erythromycin is the drug of choice. Antibiotic plays a minor role as it is of no use once the toxin is secreted. However, antibiotics are useful:
  - If given early (< 6 hrs of infection), before the toxin release
  - Prevent further release of toxin by killing the bacilli
  - Treatment for cutaneous diphtheria.
- Treatment of carriers: Drug of choice is erythromycin

Antibiotics are useful in diphtheria only:
- If given early (< 6 hrs of infection), before the toxin release
- To prevent further release of toxin by killing the bacilli
- To treatment for cutaneous diphtheria

	Vaccine or ADS	Antibiotic
Carrier	Not effective	Effective
Clinical Diphtheria	ADS is treatment of choice Vaccine is given for prevention	Not effective (Except early stage)
Cutaneous Diphtheria	Not effective	Effective

Prophylaxis (Vaccination)

- Active immunization is done by diphtheria toxoid that induces antitoxin production in the body.
- Protective titer of antitoxin is > 0.01 Unit/ml.
- Herd immunity of > 70% is required to prevent epidemic spread of diphtheria.
- However, vaccine is not effective for:
  - Prevention of cutaneous diphtheria
  - Elimination of carrier stage

- Types of vaccine:
  - Single vaccine: Diphtheria toxoid (alum or formal precipitated)
  - Combined vaccine:
    - DPT: Contains DT (diphtheria toxoid), Pertussis (whole cell) and TT (tetanus toxoid)
    - DaPT: Contains DT, TT and acellular pertussis (aP)
    - DT: Contains DT and TT
    - dT: Contains adult dose diphtheria toxoid (d) and TT.

dT
- Contains TT and adult dose (2Lf) of diphtheria toxoid .
- Given to adults > 12 years

DPT Vaccine

- DPT is the preparation of choice for vaccinating infants, because:
  - Infants can be immunized simultaneously against three important childhood diseases: diphtheria, tetanus and pertussis by single injection.
  - Pertussis component acts as adjuvant and increases immunogenicity of DT and TT.
- There are two types of DPT:
  - Plain formol toxoid (or fluid toxoid): Toxoid is prepared by incubating toxin with formalin
  - Adsorbed (alum adsorbed): Alum acts as adjuvant and increases the immunogenicity of toxoid.
- **Administration of DPT:**
  - ***Schedule:*** Under national immunisation schedule of India, total five doses are given; three doses at 6, 10 and 14 weeks of birth followed by two booster doses at 16–24 months and 5 years.
  - ***Site:*** DPT is given deep intramuscularly (IM) at anterolateral aspect of thigh, (gluteal region is not preferred as fat may inhibit DPT absorption)
  - ***Thiomersal*** (0.01%) is used as preservative
  - ***Storage:*** DPT should be kept at 2–8°C, if accidentally frozen then it has to be discarded.
  - ***Dose***: 1 dose (0.5 ml) contains:
    - Glaxo: 25 Lf (DT), 5 Lf (TT), 20,000 million (Pertussis killed bacilli)
    - Kasauli: 30 Lf (DT), 10 Lf (TT), 32,000 million (pertussis killed bacilli)
  - **dT**: It contains TT and adult dose (2Lf) of diphtheria toxoid. dT is given to adults > 12 years (3 doses at 0, 1 month, and 1 year).
- **Reactions following DPT administration:**
  - Mild: Fever and local reaction (swelling and indurations) are observed commonly.
  - Severe: Whole cell killed Bordetella pertussis is encephalitogenic. It is associated with neurological complications. Hence, DPT is not recommended after 6 yrs of age.
  - Absolute contraindication to DPT:
    - Hypersensitivity to previous dose
    - Progressive neurological disorder

Acelluar pertussis component (aP) is devoid of neurological complication and is given safely to older children.

Schick test: It is a toxin-antitoxin neutralization test, obsolete nowadays:

- It was in use long back when the vaccine was introduced initially.
- The test was being done on the people before starting immunization to identify susceptible individuals.
- Susceptible individuals used to develop erythema and induration on test arm following intradermal inoculation of toxin. Vaccine was administered only to those susceptible people.
- Since now safer toxoid preparations are available, Schick test is not performed.

Nondiphtheriae Corynebacterium species
- *C. ulcerans:* Mimics respiratory diphtheria
- *C. pseudotuberculosis* (Preisz Nocard bacilli): Affect sheep and horse
- *C. minutissimum:* Causes erythrasma, which gives coral red color under wood lamp.
- *C. jeikeium:* Multidrug resistant species, causes opportunistic infection in human.
- *C.parvum:* Example of immunomodulator

Nondiphtheria Corynebacterium

- *Clinical diphtheriae: C.ulcerans* and *C.pseudotuberculosis* can also produce DT and produce clinical diphtheriae. However, they are urease positive and usually affect animals.
  - *C.ulcerans:* Mimics respiratory diphtheria, transmitted by cow milk
  - *C.pseudotuberculosis* (Preisz Nocard bacilli): Affect sheep and horse
- *C.minutissimum*: Causes cutaneous lesion called **erythrasma**, which gives **coral red color** under wood lamp.
- *C.jeikeium:* Multidrug resistant species, causes opportunistic infection in human.
- *C.parvum*, e.g. of immunomodulator.

BACILLUS

Spore forming bacilli belong to two genera:

- *Bacillus:* They are obligate aerobes; having non bulging spores
- *Clostridium:* They are obligate anaerobes with bulging spores.

Bacillus Anthracis

B.anthracis is the causative agent of an important zoonotic disease called anthrax. It also gained importance recently because of its ability to be used as biological weapon.

Virulence Factors and Pathogenesis

Pathogenesis of anthrax is due to two important virulence factors; anthrax toxin and capsule.

Anthrax toxin

- It is a tripartite toxin, consisting of three fragments:
  - Edema factor: It is the active fragment; acts as adenylcyclase → increases host cell cyclic AMP.
  - Protective factor: It is the binding fragment that binds to the host cell receptor.
  - Lethal factor: It causes cell death, inhibits mitogen activated protein kinase pathway.
- These fragments are not toxic individually, but in combination, they produce local edema and generalized shock.
- Toxin synthesis is controlled by a plasmid (pX01). Loss of plasmid makes the strain avirulent. This was probably the basis of original anthrax vaccine prepared by Pasteur.
- Live attenuated spore vaccine (Sterne, Mazucchi): prepared by deleting the capsule genes.

Anthrax Toxin: Tripartite toxin, consisting of:
- Edema factor: ↑ host cell cyclic AMP.
- Protective factor: Helps in binding
- Lethal factor: It causes cell death

Anthrax capsule

B.anthracis has a polypeptide capsule made-up of polyglutamate (in contrast to the polysaccharide capsule present in other capsulated bacteria).

- Capsule is plasmid (pX02) coded.
- It inhibits complement mediated phagocytosis.

Anthrax Transmission:
- Cutaneous (MC mode)
- By inhalation of spores
- Ingestion of carcasses
- Indirectly through fomites

Pathogenesis and Clinical Manifestations

Anthrax is primarily a zoonosis. Herbivorous animals such as cattle, sheep and less often horses and pigs are affected more commonly than the carnivorous animals.

Human Transmission: Human beings acquire infection by:

- Cutaneous mode: By spores entering through the abraded skin; seen in people with occupational exposure to animals (most common mode)
- By inhalation of spores
- Ingestion of carcasses of animals dying of anthrax containing spores (manifested as bloody diarrhea)

Types of human anthrax: Mainly three types:

i. Cutaneous,
ii. Pulmonary and
iii. Intestinal anthrax

	Cutaneous anthrax	Pulmonary anthrax
Also called	**Hide porter's disease** (as it commonly occurs in dock workers carrying loads of hides and skins on their bare backs)	**Wool sorter's disease** (as it is seen in workers of wool factory, due to inhalation of dust from infected wool)
Transmission	Cutaneous exposure to spores (enter through abraded skin)	Inhalation of spores
Characterized by	**Malignant pustule:** • Painless coal-black, necrotic eschar surrounded by non-pitting indurated edema. • The name anthrax, which means coal, comes from the black color of the eschar. • However, it is a nonmalignant condition.	**Hemorrhagic pneumonia**: Bacilli spread by lymphatics or blood, leading to: • Bacteremia • Hemorrhagic mediastinitis • Hemorrhagic meningitis
Occupations	Dock worker, butcher, abattoir and farmer	Wool factory
Occurrence	Most Common (95%)	Rare
Prognosis	Self-limiting, rarely becomes fatal if untreated.	Fatal
Bioterrorism	Rarely associated with bioterrorism	Most common form to be associated with bioterrorism

Cutaneous Anthrax:
- Called as Hide porter's disease
- Feature: Malignant pustule

Pulmonary Anthrax:
- Called Wool sorter's disease
- Feature: Hemorrhagic pneumonia

B. anthracis as an Agent of Bioterrorism

- *B. anthracis* has been a major agent of bioterrorism and biologic warfare such as outbreaks in Sverdlovsk in 1979 and in United States in 2001
- Pulmonary anthrax is the most common form seen in bioterrorism outbreaks.
- It occurs via inhalation of anthrax spores from contaminated animal products.

Laboratory Diagnosis (Anthrax vs Anthracoid Bacilli)

B. anthracis can be differentiated from other *Bacillus* species (Anthracoid Bacilli) by the presence of following properties:

- *B. anthracis* is Nonmotile and Capsulated
- MC Fadyean reaction: Polypeptide capsule is seen as amorphous purple material surrounding blue bacilli when stained with polychrome methylene blue.
- Gram staining: chain of bacilli arranged in **bamboo stick** appearance.
- On Agar plate: **Medusa head** appearance colony (under low power microscope)
- Gelatin stab: Appear as **inverted fir tree** appearance
- Solid media with penicillin: **String of pearl** appearance in culture smear
- Blood agar: nonhemolytic colonies
- Selective medium: PLET media
- DFA (Direct fluorescent antibody test): Detects capsular antigen. It is used for confirmation of diagnosis during bioterrorism outbreaks.
- Ascoli's thermo precipitin test: It is a ring precipitation test, detects anthrax antigens.
- Spores can be demonstrated by phase contrast microscope or use of special stains such as hot malachite green (Ashby's method) or 0.25% sulfuric acid (spores are acid fast).
- Lipid granules can be demonstrated by Sudan black B (Burdon's method).

Lab Diagnosis of Anthrax:
- *Gram staining:* Bamboo stick appearance
- *Medusa head* appearance colony
- *Gelatin stab:* Appear as inverted fir tree colony
- *Solid media* with penicillin: String of pearl appearance colonies
- *Blood agar:* Nonhemolytic colonies

Guidelines for Diagnosis of Anthrax during Bioterrorism Attacks (CDC, 2001)

Following 2001 USA attack, CDC has prepared guidelines for identification of *B. anthracis* during bioterrorism.

- For presumptive identification of anthrax: Any large gram-positive bacillus with morphology and cultural properties similar to anthrax bacillus
- For initial confirmation, the tests done are Lysis by gamma phage and DFA
- Further confirmation is done by PCR.

Treatment

Anthrax can be successfully treated if the disease is promptly recognized and appropriate therapy is initiated early.

- *Antibiotics for treatment:* Consists of ciprofloxacin or doxycycline, plus clindamycin, and/or rifampin, for 60 days.
- *Antibiotics for post-exposure prophylaxis:*
  - Ciprofloxacin for 60 days and
  - Doxycycline, for 60 days orAmoxicillin, for 60 days
- *Raxibacumab:* It is a monoclonal antibody that neutralizes anthrax toxin (protective antigen). It is intended for the prophylaxis and treatment of inhaled anthrax.

Prevention

- *Live attenuated, noncapsulated spore vaccine* (Stern vaccine): It is extensively used in animals, but not for human use.
- *Alum precipitated toxoid vaccine:* It is prepared from the protective antigen. It is safe and effective for human use.

Other Bacillus Species

Bacillus Cereus

It is a normal habitant of soil, also widely isolated from food items, such as vegetables, milk, cereals, spices, meat and poultry.

Manifestations:

- Food poisoning: It produces two types of toxins; diarrheal toxin and emetic toxin
- Ocular disease: Causes severe keratitis and panophthalmitis following trauma to the eye.
- Other conditions: It rarely causes systemic infections, including endocarditis, meningitis, osteomyelitis, and pneumonia; the presence of a medical device or intravenous drug use.

Laboratory diagnosis: *B. cereus* is motile, noncapsulated and not susceptible to gamma phage. It can be isolated from feces by using selective media such as;

- MYPA (Mannitol, egg yolk, phenol red, polymyxin and agar)
- PEMBA(polymyxin B, egg yolk, mannitol, bromothymol blue and agar)

Treatment: *B. cereus* is susceptible to clindamycin, erythromycin, vancomycin. It is resistant to penicillin (by producing β-lactamase) and trimethoprim.

***B. cereus* (Emetic type toxin):**
- Preformed toxin
- IP: 1–5 hours
- Heat stable
- MC food: Rice (Chinese fried rice)
- MC feature: Vomiting, abdominal cramps
- MC serotype: 1,3,5

B. cereus	Diarrheal type	Emetic type
Incubation period	8–16 hours	1–5 hours
Toxin	Secreted in intestine (Similar to *Clostridium perfringens* enterotoxin)	Preformed toxin (formed in diet, similar to *S.aureus* enterotoxin)
	Heat labile	Heat stable
Food items contaminated	Meat, vegetables, dried beans, cereals	Rice (Chinese fried rice)
Clinical feature	Diarrhea, fever, rarely nausea	Vomiting, abdominal cramps
Serotype involved	2,6,8,9,10,12	1,3,5

Bacillus Thuringiensis

It may occasionally produce food poisoning. It is also used as larvicidal agent for mosquito control.

Bacillus used as Sterilization Control

- *B. stearothermophilus* and *B. subtilis:* Both are used as biological controls for autoclave and plasma sterilization.
- *B. pumilus* is used as biological control for ionizing radiation.
- *B. globigii* is used as biological control for ethylene oxide.

MULTIPLE CHOICE QUESTIONS

CORYNEBACTERIUM DIPHTHERIAE

1. A child with bull neck and membrane over tonsil. What is mechanism of the toxin responsible for this condition? *(JIPMER May 2016)*
a. Increased cAMP
b. Inhibits GABA neurons
c. Inhibits Elongation Factor-2

2. Which Corynebacterium carries toxin similar to that causing diphtheria toxin? *(Recent Question 2015)*
a. C. auris
b. C. jivelai
c. C. ulcerans

3. True about Corynebacterium diphtheriae: *(AIIMS May 2007/Nov 2007. DNB Dec 2010, AI 2000/2011, NEET Pattern Based)*
a. All types produce toxin
b. Toxin production is dependent upon critical concentration of iron
c. Coded by plasmid
d. Inhibit cAMP

4. Protein synthesis is inhibited by the toxin(s) of:
a. Pertussis *(AIIMS 2000, PGI May 2012)*
b. Cholera
c. Pseudomonas
d. Diphtheria
e. Shiga toxin

5. Metachromatic granules are seen in: *(DNB Dec 2011)*
a. Corynebacterium
b. E. coli
c. Yersinia
d. Pseudomonas

6. The type of diphtheria with highest mortality is:
a. Pharyngeal *(JIPMER 2009)*
b. Nasal
c. Laryngeal
d. Conjunctival

7. True about diphtheria is: *(SGPGI 2008)*
a. Cause cranial nerve palsies in 2nd and 3rd weeks
b. Treatment with erythromycin
c. It is gram-negative organism
d. Passive immunization is harmful and should not be tried

8. The special stain used to identify C. diphtheriae in a throat swab is: *(MHPG 2015)*
a. Albert's stain
b. Giemsa stain
c. Gram's stain
d. India ink

9. In a completely and adequately immunized child against Diphtheria, the throat swab was collected. It showed the presence of Corynebacterium diphtheria like organisms on Albert staining. These organisms can have one of the following properties on further laboratory processing: *(AIIMS Nov 2004)*
a. It can grow on Potassium tellurite medium
b. It would show a positive Elek's gel precipitation test
c. It can be pathogenic to experimental guinea pigs
d. It can produce cytotoxicity in tissue cultures

10. A 12-year-old child presents with fever and cervical lymphadenopathy. Oral examination shows a grey membrane on the right tonsil extending to the anterior pillar. Which of the following medium will be ideal for the culture of the throat swab for a rapid identification of the pathogen? *(NEET Pattern Based, AIIMS Nov 2002)*
a. Nutrient agar
b. Blood agar
c. Loffler's serum slope
d. Lowenstein-Jensen medium

11. Metachromatic granules seen in: *(PGI 2000)*
a. C.diphtheriae
b. Mycoplasma
c. Gardnerella
d. Chlamydia
e. Staphylococcus

12. Positive Schick's test indicates that person is: *(NEET Pattern Based, AI 2002, AIIMS 2000, AIIMS Nov 2007, MP 2007, Kolkata 2003)*
a. Immune to diphtheria
b. Hypersensitive to diphtheria
c. Susceptible to diphtheria
d. Carrier of diphtheria

13. Herd immunity over…….% is required to prevent epidemic spread of diphtheria: *(DNB 2000)*
a. 50
b. 55
c. 60
d. 70

14. DOC for diphtheria carrier: *(DNB 03)*
a. Erythromycin
b. Tetracycline
c. Penicillin
d. DPT

15. Diphtheria carriers are diagnosed by: *(MP 2000)*
a. Throat culture
b. Gram's staining
c. Albert's stain
d. Schick's test

BACILLUS ANTHRACIS

16. Wool sorter disease is: *(Recent Question 2015)*
a. Cutaneous anthrax
b. Pulmonary anthrax
c. Intestinal anthrax

17. Eschar is formed by which of the following organism? *(Recent Questions 2014)*
a. B. henselae
b. B. anthracis
c. Staph aureus
d. E. coli

18. An abattoir worker presented with a malignant pustule on his hand that progressed to form an ulcer. Smear was taken from the ulcer and sent to laboratory for investigation. The diagnosis is: *(AIIMS Nov 2012)*
a. Cutaneous anthrax
b. Carbuncle
c. Ulcerating melanoma
d. Infected rodent ulcer

19. Bacillus used to test efficacy of sterilization by autoclave is: *(DNB Dec 2010)*
a. Bacillus subtilis
b. Bacillus pumilis
c. Bacillus stearothermopilus
d. Coxiella burnetti

20. True about anthrax: *(PGI June 2009)*
a. Cause by Gram-positive bacilli
b. Soil reservoir
c. Spore formation takes place inside the body
d. More common in carnivorous than herbivores
e. Penicillin is drug of choice

21. Inverted fir tree appearance is characteristic:
a. Bacillus anthrax *(JIPMER 2004)*
b. Haemophilus influenzae
c. Yersinia pestis
d. Brucella

22. Which of the following is true regarding anthrax: *(PGI Dec 2001)*
a. McFadyean reaction shows capsule
b. Humans are usually resistant to infection
c. Less than 100 spores can cause pulmonary infection
d. Gram stain shows organism with bulging spores
e. Sputum microscopy helps in diagnosis

23. Anthrax bacilli differs from Anthracoid bacilli by being: *(PGMEE 2006)*
a. Noncapsulated
b. Strict aerobe
c. Nonmotile
d. Haemolytic colonies on blood agar

24. All are true about cutaneous anthrax except: *(JIPMER 2009)*
a. Extremely painful
b. The whole area is congested and edematous
c. Central crustation with black eschar
d. Satellite nodule around inguinal region

25. A person working in an abattoir presented with a pustule on his hand that progressed to form an ulcer. Smear is taken from the ulcer and sent to laboratory for investigation. Which of the following is the best stain to determine the causative agent of the ulcer? *(AIIMS Nov 2012, AI 2007, AIIMS Nov 2006)*
a. Trichrome methylene blue
b. Carbol fuschin
c. Acid fast stain
d. Calcoflour white

26. Bacillus anthracis, false statement is:
a. Gram-negative *(Recent Question 2013)*
b. Bacilli in long chain
c. Nonhemolytic colony in blood agar
d. Medusa head appearance on nutrient agar

27. Medusa head colony is seen in: *(Recent Questions 2014)*
a. Bordetella pertussis
b. Bacillus anthracis
c. Bartonella henselae
d. Bacteroides species

BACILLUS CEREUS

28. Bacillus cereus food poisoning is associated with which food? *(West Bengal 2016)*
a. Fired rice
b. Baked potato
c. Dairy products

29. Characteristic of Bacillus cereus food poisoning is:
a. Presence of Fever *(AIIMS 2010)*
b. Presence of Pain abdomen
c. Absence of Vomiting
d. Absence of Diarrhea

30. Incubation period for B. cereus: *(PGI June 2008)*
a. 1–6 hrs
b. 8–16 hrs
c. 24 hrs
d. > 24 hrs
e. Weeks

31. Chinese restaurant syndrome after eating fried rice and vanilla sauce is due to: *(APPG 2014, MHPG 2014)*
a. Clostridium perfringens
b. Bacillus cereus
c. Staphylococcus aureus
d. Clostridium botulinum

32. Coral red color under wood lamp examination. What is the diagnosis? *(JIPMER Nov 2015)*
a. Ecthyma
b. Erythema
c. Erythrasma

EXPLANATIONS

CORYNEBACTERIUM DIPHTHERIAE

1. **Ans (c) (Inhibits Elongation Factor-2)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p244
   - A child with bull neck and membrane over tonsil….suggestive of diphtheria
   - Diphtheria toxin inhibits Elongation factor-2, which leads to inhibition of translation step of protein synthesis.
2. **Ans. (c) (C. ulcerans)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p244
3. **Ans. (b) (Toxin production is dependent upon critical concentration of iron)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p244, Ananthanarayan 8/e p 238-39
   - Optimum conc. of iron (0.1 mg/L) is required for expression of diphtheria toxin.
   - Among the biotypes of C.diphtheriae, all most all strains of gravis, 95–99% strains of intermedius and 80–85% strains of mitis are toxigenic.
4. **Ans. (c) (d) (e) (Pseudomonas, Diphtheria, Shiga)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p239, 244

   Mechanism of action of exotoxin:
   - Toxin that increases cAMP: Pertussis toxin, Cholera toxin, heat labile toxin of E. coli, Anthrax toxin (edema factor)
   - Toxin that increases cGMP: Heat stable toxin of E. coli
   - Toxin that inhibits protein synthesis:
     - By inhibiting ribosome: Shiga toxin and Verocytotoxin of E. coli
     - By inhibiting elongation factor 2-Diphtheria toxin and Pseudomonas toxin
   - Neurotoxin:
     - Blocks the release of glycine and GABA: Tetanospamsin
     - Blocks the release of acetylcholine: botulinum toxin
5. **Ans. (a) (Corynebacterium)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p243, Ananthanarayan 9/e p236
   - Metachromatic Granule is produced By C.diphtheriae, C.xerosis (Refer chapter review for the detail)
6. **Ans. (c) (Laryngeal)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p244, Harrison 19/e p979, 18/e p 1189

   Formation of extensive pseudomembrane over larynx carries the risk of airways obstruction.
7. **Ans. (a) (Cause…)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p245, Harrison 19 e/p979, 18/e p1190
   - Manifestations may appear during the first or second week of illness, typically beginning with dysphagia and nasal dysarthria and progressing to other signs of cranial nerve involvement.

   About other options

   Option b: Prompt administration of diphtheria antitoxin is critical in the management of respiratory diphtheria.
   - Antibiotics have no role once the toxin is formed and are mainly used to prevent transmission to other susceptible contacts.
   - Procaine penicillin G given for treatment and erythromycin given for carriers.

   Option c: C.diphtheriae is a gram +ve bacilli

   Option d: Passive immunization is critical in the management of respiratory diphtheria.

LAB DIAGNOSIS OF DIPHTHERIA

8. **Ans (A) (Albert's stain)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p243
9. **Ans. (a) (It can grow on Potassium tellurite medium)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p246
   - This child is a carrier for C.diphtheriae.
   - Adequate vaccination can prevent the disease but not the colonization.

   'Mass immunization might result in disappearance of toxigenic strains from many populations but has no effect on carriage of nontoxigenic strains.'

- Since the child is completely vaccinated, antitoxin level in serum can always neutralize the toxin but it can not prevent the colonization of nontoxigenic strain.
- So, the organism can be grown on Potassium tellurite medium but since it might be a nontoxigenic strain, so any test to detect the toxigenicity will be negative.

10. **Ans. (c) (Loffler's…)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p246, Ananthanarayan 9/e p237
    - Rapid identification of C. diphtheria: Loffler's serum slope (6–8 hour)
    - Best media for isolation in carriers/contacts/convalescent: PTA (48 hour)
    - Metachromatic granules best develops on: Loffler's serum slope
11. **Ans. (a), (c) (C. diphtheriae, Gardnerella)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p243, Ananthanarayan 9/e p 237
    - For detail: Refer chapter review
12. **Ans. (c) (Susceptible…)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p248, Ananthanarayan 9/e p 241
    - Positive test means that person is susceptible to diphtheria: So immunization is required.
    - **Shick test**: For detail, Refer chapter review
13. **Ans. (d) (70%)** Ref:, Park 23/e p161, 22/e p152
    - Herd immunity over 70% is required to prevent epidemic spread of diphtheria.
14. **Ans. (a) (Erythromycin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p248, Park 23/e p161, 22/e p153
    - DOC or carriers of diphtheria: Erythromycin
15. **Ans. (a) (Throat culture)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p246, Park 23/e p161, 22/e p152
    - Carriers harbor the diphtheria bacilli in throat, hence can be effectively diagnosed by throat culture.

BACILLUS ANTHRACIS

16. **Ans. (b) (Pulmonary anthrax)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p251
17. **Ans. (b) (B. anthracis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p252, Ananthanarayan 9/e p245
18. **Ans. (a) (Cutaneous anthrax)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p251, Ananthanarayan 9/e p247
    - Malignant pustule is the characteristic feature of cutaneous anthrax
19. **Ans. (c) (Bacillus…)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p255, Ananthanarayan 9/e p32
    Industrial Application of Bacillus species: Refer text
20. **Ans. (a), (b) (Cause by Gram-positive bacilli, Soil reservoir)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p251-53, Ananthanarayan 8/e p243-248
    - ***Option a:*** Bacillus anthracis is a Gram-positive bacilli
    - ***Option b:*** Reservoir is animal and soil. Animal gets infection by inhalation of spores in soil
    - ***Option c:*** Spore formation never takes place inside the body
    - Please remember 'spore formation takes place in unfavorable conditions and human body is a favorable environment for the organism like Bacillus anthracis causing anthrax and Clostridium perfringens causing gas gangrene.'
    - ***Option d:*** Anthrax is more common in herbivores like sheep and cattle than carnivorous
    - ***Option e:*** Jawetz 24/e p205 and 25/e p167 says- 'Ciprofloxacin is recommended for treatment; penicillin G, along with gentamicin or streptomycin, has previously been used to treat anthrax.'
21. **Ans. (a) (Bacillus anthracis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p253, Ananthanarayan 9/e p245
    - Inverted fir tree appearance in gelatin stab culture seen by Bacillus anthracis
    - **Bacillus anthracis laboratory findings** Refer text.
22. **Ans. (a), (b), (c), (e) (McFadyean reaction shows capsule, Humans are usually resistant to infection, Less than 100 spores can cause pulmonary infection, Sputum microscopy helps in diagnosis)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p250-53, Ananthanarayan 9/e p247-48, 8/e p243
    - ***Option b:*** Greenwood 16/e p226 says 'Humans are relatively resistant to infection with B anthracis'

- ***Option c:*** Harrison 17/e p1344 and 18/e p1769 says 'While an LD50 of 10,000 spores is a generally accepted number for anthrax it has also been suggested that as few as one to three spores may be adequate to cause disease in some settings.'
- ***Option d:*** Gram stain shows organism with non bulging spores.
- Bulging spores are seen for Clostridium
- ***Option e:*** Microscopy of the sample like lesion, blood, sputum reveals gram-positive bacilli in chain.

23. Ans. (c) (Nonmotile) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p254, Ananthanarayan 9/e p249

- Anthrax bacillus is capsulated, nonmotile and produce nonhemolytic colony on blood agar.
- Both Anthrax bacilli and Anthracoid bacilli are strict aerobe.
- Also Refer the table on the text.

24. Ans. (a) (Extremely painful) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p251, Ananthanarayan 9/e p247
Cutaneous lesions in Anthrax are painless.

25. Ans. (a) (Tric...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p251-52, Ananthanarayan 9/e p248, 8/e p246

- This is a case of Cutaneous anthrax (Points in favor abattoir occupation, pustule and ulcer on hand)
- Provisional diagnosis of Anthrax: demonstration of Capsule by polychrome methylene blue (M' fadyean reaction)
- Confirmed diagnosis by immunofluorescence microscopy
- Cutaneous anthrax (Hide porter's disease): MC (95%), but usually self limiting
- Occupation associated: Dock worker, butcher, abattoir, farmer.

26. Ans. (a) (Gram-negative) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p253, Ananthanarayan 9/e p244
Bacillus anthracis is gram-positive bacilli.

27. Ans. (b) (Bacillus anthracis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p253, Ananthanarayan 9/e p245
Refer chapter review.

BACILLUS CEREUS

28. Ans (a) (Fired rice) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p255

29. Ans. (b) (Presence…) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p255, Ananthanarayan 9/e p249

- Fever is not a consistent feature of B. cereus food poisoning.
- Abdominal cramps may be seen in both emetic and diarrheal type of B. cereus food poisoning.
- For detailed explanation refer text (chapter review).

30. Ans. (a), (b) (1–6, 8–16) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p255, Ananthanarayan 9/e p249
Emetic type: IP 1–5 hour
Diarrheal type: IP 8–16 hour

31. Ans. (b) (Bacillus cereus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p255, Ananthanarayan 9/p249

- Chinese restaurant syndrome means: Food poisoning within 1–6 hr of consumption of Chinese fried rice …… Most probable agent is Bacillus cereus.

32. Ans (c) (Erythrasma) Ref: ApurbaSastry's Essentials of Medical Microbiology 1/e p249
Erythrasma is a skin lesion caused by *C. minutissimum* and under wood lamp examination, it gives coral red color.

CHAPTER

3.5

Anaerobes: Clostridium and Non-Sporing Anaerobes

Anaerobic bacteria do not have cytochrome system for oxygen metabolism and hence are unable to neutralize toxic oxygen metabolites. They can be classified as:

- **Obligate anaerobes**: They cannot grow in presence of oxygen as they completely lack superoxide dismutase and catalase and are susceptible to the lethal effects of oxygen. For example, *Clostridium* and non-sporing anaerobes
- **Aerotolerant anaerobes**: They do not utilize oxygen for growth, but tolerate its presence. This is because they possess small amounts of superoxide dismutase and peroxidase (but lack catalase) which may neutralize the toxic oxygen radicals. Examples include *Clostridium histolyticum* and *Bacteroides.*

Anaerobic bacteria:
- Obligate anaerobes: Cannot grow in presence of oxygen
- Aerotolerant anaerobes: Do not utilize oxygen for growth, but tolerate its presence.

CLOSTRIDIUM

Clostridia are obligate anaerobic gram-positive bacilli, having bulging spores:

- Clostridia are saprophytes found in soil, organic matter, and also in intestine of animals including humans
- Only few infect humans, such as *C. perfringens*, *C. tetani, C. botulinum* and *C. difficile*
- Industrial importance: Some clostridia, such as *C. acetobutylicum* and *C. butyricum* are used to prepare chemicals, such as acetone and butanol.
- They are motile (exhibit stately motility) except *C. perfringens*, *C. ramosum* and *C. tetani* VI.
- They are noncapsulated except *C. perfringens* and *C. butyricum.*
- Most of the clostridia bear a sub-terminal spores except:
  - *C. tetani*: Produces spherical and terminal spore (drum stick appearance)
  - *C. tertium:* Produces oval and terminal spore (tennis racket appearance)
  - *C. bifermentans*: Produces central and oval spore.

CLOSTRIDIUM PERFRINGENS

C. perfringens (previously, *C. welchii*) is a saprophyte and commensal in the large intestine of human beings and animals:

- It is capsulated, nonmotile, gram-positive bacillus
- It bears sub-terminal bulging spores; but the gas gangrene strains do not produce spores.

Virulence Factors

C. perfringens is invasive as well as toxigeniC. The toxins and the other virulence factors can be grouped into:

- Four major toxins: Alpha (α), beta (β), epsilon (ε) and iota (ι).
  Alpha toxin (or lecithinase or phospholipase C) is the principle virulence factor for gas gangrene and food poisoning.
- Eight minor toxins: Gamma (γ), delta (δ) lambda (λ), kappa (κ), theta (θ), eta (η), mu (μ) and nu (υ)
- They also produce heat labile enterotoxin.
- Soluble substances produced by *C. perfringens* are neuraminidase, histamine, bursting factor (produce muscle lesions) and circulating factor (inhibit phagocytosis).

Clinical Manifestations

C. perfringens infections are mostly polymicrobial involving other clostridia species. Various manifestations include:

Enteritis necroticans (gas gangrene of the bowel):
- Ischemic necrosis of the jejunum and gas in tissue plane
- Also known as pigbel in Papua New Guinea and darmbrand in Germany.
- Caused by *C.perfringens* type C producing β toxin.

1. Clostridial Wound Infection

MacLennan has classified them as: (i) *Simple wound contamination, (ii) Anaerobic cellulitis, (iii) Anaerobic myositis (gas gangrene)*

2. Clostridial Enteric Infection

- Food poisoning: Caused by *C. perfringens* type A enterotoxin (coded by gene *cpe*)
- Enteritis necroticans (gas gangrene of the bowel):
  - Ischemic necrosis of the jejunum and gas in tissue plane
  - Also known as pigbel in Papua New Guinea and darmbrand in Germany.
  - Caused by *C. perfringens* type C producing β toxin.
- Necrotizing enterocolitis: Resembles enteritis necroticans but associated with *C. perfringens* type A, has been found in previously healthy adults.
- Gangrenous appendicitis

3. Other Clostridial Infections

- Bacteremia: *C. perfringens* followed by *C. tertium* and *C. septicum*.
- Skin and soft-tissue infections: *C. perfringens*, *C. histolyticum*, *C. septicum*, *C. novyi*, and *C. sordellii* cause necrotizing infections of the skin and soft tissues.
- Infection of the endometrium leading to toxic shock syndrome (*C. sordellii).*
- Meningitis and brain abscess
- Panophthalmitis (due to *C. sordellii* or *C. perfringens*).

Gas Gangrene

Gas gangrene (by (Oakley) is defined as a rapidly spreading, edematous myonecrosis, occurring in association with severely crushed wounds contaminated with pathogenic clostridia, particularly with *C. perfringens*. It is always polymicrobial:

- Established agents: *C. perfringens* (MC, 60%) and *C. novyi* and C. *septicum* (20–40%).
- Probable agents: *C. histolyticum, C. sporogenes, C. fallax, C. bifermentans, C. sordellii, C. aerofoetidum* and *C. tertium.*

Pathogenesis of Gas Gangrene

The development of gas gangrene requires:

- *Anaerobic environment*: Crushing injuries of muscles (road traffic accidents) or foreign bodies (bullet injuries)
- *Contamination of wound* with clostridial spores present in the soil (during war or road traffic accident) or clothes.
- Rarely, spontaneous *non-traumatic gas gangrene* occurs via invasion of bowel clostridia; occurs in people with gastrointestinal pathologies (e.g. colonic malignancy).

Incubation period for gas gangrene:
- 10-48 hrs for *C.perfringens*
- 2-3 days for *C.septicum*
- 5-6 days for *C.novyi*

Incubation Period of Gas Gangrene

The incubation period is variable, depending upon the nature of injury, infective dose and clostridial species involved:

- 10–48 hrs for *C. perfringens*, 2–3 days for *C. septicum* and 5–6 days for *C. novyi*

Gas Gangrene is Clinically Characterized By

- Sudden onset of excruciating pain at the affected site
- Rapid development of a foul-smelling thin serosanguineous discharge
- Gas bubbles (crepitus) in the muscle planes and brawny edema and induration
- Shock and organ failure develop later
- Associated with higher mortality rate (50%).

Laboratory Diagnosis

- Specimen: Necrotic tissues, muscle fragments and exudates from deeper wound.
- Direct microscopy:

- Thick, stubby, boxcar-shaped gram-positive bacilli without spore – suggestive of *C. perfringens*
- Gram-positive bacilli with spore- suggestive of other *Clostridium* species

- Culture Media: Robertson cooked meat broth (RCM), egg yolk agar, etC.
- **Identification of *C. perfringens:***
  - Target hemolysis (double zone hemolysis)
  - Nagler's reaction: Opalescence surrounding the streak line on egg yolk agar, inhibited by adding anti-alpha toxin.
  - Reverse CAMP test: Positive.

Identification of *C. perfringens*:
- Thick, stubby, boxcar-shaped gram-positive bacilli without spore
- Target hemolysis (double zone hemolysis)
- Nagler's reaction Positive
- Reverse CAMP test: Positive

Treatment

- Early surgical debridement is the most crucial step in the management of gas gangrene.
- Antibiotics: Penicillin + clindamycin are recommended for 10–14 days.
- Hyperbaric oxygen: It may kill the obligate anaerobic clostridia, such as *C. perfringens*
- Passive immunization with anti α toxin antiserum.

Treatment of gas gangrene:
- Early surgical debridement (main stay)
- Antibiotics: Penicillin + clindamycin
- Hyperbaric oxygen
- Anti α toxin antiserum

CLOSTRIDIUM TETANI

C. tetani is obligate anaerobic, gram-positive bacillus with terminal round spores (drum stick appearance):

- It is the causative agent of 'tetanus' manifested by skeletal muscle spasm and autonomic nervous system disturbance.
- *C. tetani* is widely distributed in soil, hospital and intestine of man and animals.

Virulence Factors

C. tetani produces two exotoxins: Tetanolysin and tetanospasmin:

- **Tetanolysin** is a heat labile, oxygen labile hemolysin. It plays no role in the pathogenesis.
- **Tetanospasmin or tetanus toxin (TT)** is a neurotoxin responsible for the pathogenesis of tetanus:
  - It is oxygen stable but heat labile; coded by plasmid.
  - Mechanism of action: Toxin acts pre-synaptically at the inhibitory neuron terminals and prevents release of inhibitory neurotransmitter GABA and glycine → leads to spastic muscle contraction.
  - Strychnine poisoning has a similar mechanism except that it acts post-synaptically.

Tetanospasmin or tetanus toxin (TT):
- Acts presynaptically at the inhibitory neuron terminals and prevents release of inhibitory neurotransmitter GABA and glycine → leads to spastic muscle contraction
- Strychnine poisoning similar mechanism but acts postsynaptically

Mode of Transmission

Tetanus bacilli enter through:

- Injury like road traffic accidents, unsterile surgery/abortion/delivery, otitis media (otogenic tetanus)
- It is noninfectious: There is *no person to person spread*

Clinical Manifestations

- **Incubation period** is about 6–10 days. Shorter the IP, graver is the prognosis.
- Muscles of the face and jaw are often affected first (due to shorter distances for the toxin to reach the nerve terminals).
- **1st symptom**: Increase in the masseter tone leading to **trismus** or **lock jaw**, followed by muscle pain and stiffness, back pain, and difficulty in swallowing.
- In neonates, difficulty in feeding is the usual presentation
- As the disease progresses, painful muscle spasm develops which may be:
  - Localized: involves the affected limb
  - Generalized painful muscle spasm: leads to descending *spastic paralysis*.
- Hands, feet are spared and mentation is unimpaired
- Deep tendon reflexes are exaggerated

Complications of tetanus:
- Risus sardonicus
- Opisthotonos position

- **Autonomic disturbance** is maximal during the second week of severe tetanus-characterized by low or high blood pressure, tachycardia, intestinal stasis, sweating, increased tracheal secretions and acute renal failure.

Complications

- **Risus sardonicus:** Characteristic, abnormal, sustained spasm of the facial muscles that appears to produce grinning.
- **Opisthotonos position** of the body occurs due to generalized spastic contraction of extensor muscles.
- Respiratory muscles spasm-may cause airway obstruction.

Warm climate, rural area with fertile soil is associated with increased risk.

Laboratory Diagnosis

Treatment should be started immediately based on clinical diagnosis. Laboratory diagnosis helps only in confirmation.

- **Specimen:** Excised tissue bits from the necrotic depths of wounds.
- **Gram staining:**
  - Reveal gram-positive bacilli with terminal and round spores (drumstick appearance)
  - However microscopy alone is unreliable as it cannot distinguish *C. tetani* from morphologically similar clostridia like *C. tetanomorphum* and *C. sphenoides*.
- **Culture:** Culture is more reliable than microscopy:
  - In RCM broth: *C. tetani*, being proteolytic turns the meat black and produces foul odor.
  - Blood agar: *C. tetani* produces characteristic swarming growth.
- **Toxigenicity Test**: For demonstration of toxin production
  - *In vitro* hemolysis inhibition test: detects tetanolysin
  - *In vivo* mouse inoculation test: detects tetanospasmin

Treatment

- **Passive immunization** (tetanus immunoglobulin): It is the treatment of choice:
  - Two preparations are available:
    - HTIG (Human tetanus immunoglobulin): 250 IU (protects for 30 days)
    - ATS (anti tetanus serum, equine derived): 1500 IU (protects for 7–10 days)
  - HTIG is preferred as ATS is associated with side effects, such as serum sickness and anaphylactoid reactions
- **Combined immunization** (Both active and passive immunization): Indicated **i**n non-vaccinated person
- **Antibiotics:** Antibiotics has minor role as they cannot neutralize the toxin once released:
  - However, they are useful (i) In early infection before expression of the toxin (before 6hrs), (ii) To prevent further release of toxin
  - Metronidazole is the drug of choice. Penicillin can be given alternatively.

Prevention by Active Immunization (Vaccine)

Tetanus toxoid (TT) is used for active immunization. It is available either as (i) Monovalent vaccine as TT and (ii) Combined vaccine as DPT (details are discussed in chapter 3.4)

- **Primary immunization of children:** Under national immunization schedule of India, total ***seven doses*** are given; three doses of DPT at 6, 10 and 14 weeks of birth followed by two booster doses of DPT at 16–24 weeks and 5 years followed by two additional doses of TT at 10 yrs and 16 yrs.
- **Adult immunization**:
  - It is indicated if primary immunization is not administered in childhood.
  - **Four doses** of TT is given; 2 doses of TT at 1 month interval followed by 2 booster doses at 1 yr and 6 yrs.
- **Site:** TT is given deep IM at anterolateral aspect of thigh (children) and in deltoid (adults).
- **Protective titer** of tetanus antitoxin is **≥ 0.01 unit/ml.**

Tetanus Toxoid Immunization schedule:
- Primary immunization of children: total seven doses
- Adult immunization: Four doses of TT is given

Prevention of Tetanus After Injury

All types of wounds need surgical toilet followed by immunization which depends on the wound type and immunization status of the individual.

Recommendation for prevention of tetanus after injury

Immunity Category	Simple wound Wound < 6 hrs, clean, non-penetrating, no/negligible tissue damage	Complicated wounds (Other wounds)
Category A	Nothing required	Nothing required
Category B	Toxoid 1 dose	Toxoid 1 dose
Category C	Toxoid 1 dose	Toxoid 1 dose + HTIG
Category D	Toxoid complete dose	Toxoid complete dose + HTIG

- Category A: Taken complete course of TT/booster within the past 5 years
- Category B: Taken complete course of TT/booster within the past > 5 years < 10 years
- Category C: Taken complete course of TT/booster within the past >10 years
- Category D: Not Taken complete course of TT/booster or immunity status is unknown.

Prevention of Neonatal Tetanus

Neonatal tetanus is defined by WHO as 'child loses ability to suck and cry between day 3 and 28 of life and becomes rigid and has spasms'. It is also known as **'8th day disease'** as the symptoms usually starts after 1 week:

- **Most common reason:** Unhygienic practices during deliveries, such as infected umbilical stumps due to application of cow dung, rarely by circumcision or by ear piercing.
- **Seasonal:** More common in July, August and September months.
- **Neonatal tetanus can be prevented by:**
  - Discouraging home deliveries and promoting hospital or attended deliveries.
  - Following aseptic clean practices are followed during deliveries: clean hand, clean surface, clean blade for cutting cord, clean cord tie, clean cord stump, clean towel and clean water.
  - TT (2 doses) are given to all pregnant women during 2nd trimester at 1 month gap.
- **Neonatal tetanus elimination is based on:**
  - Neonatal tetanus rate < 1/1000 live births
  - TT coverage to pregnant women > 90%
  - Attended deliveries > 75%.

Neonatal tetanus elimination is based on:
- Neonatal tetanus rate < 1/1000 live births
- TT coverage to pregnant women: > 90%
- Attended deliveries: > 75%

CLOSTRIDIUM BOTULINUM

Clostridium botulinum produces botulinum toxin and causes ***botulism***.

Pathogenesis (Botulinum Toxin)

C. botulinum is noninvasive and the pathogenesis is due to production of powerful neurotoxin 'botulinum toxin'(BT), probably the most toxic substance known to be lethal to mankind:

- **Serotype:** Based on light chain, there are eight serotypes-A, B, C1, C2, D, E, F and G:
  - Serotypes A, B, E commonly cause human disease; most severe being serotype A.
  - All serotypes produce neurotoxin; except C2 which produces an enterotoxin
  - Botulinum toxin Type C and D are bacteriophage coded
- BT is produced intracellularly, not secreted and appears outside only after autolysis of bacterial cell.
- BT is synthesized as protoxin, converted into active form by proteolytic enzymes.
- **Mechanism:** BT blocks the release of acetylcholine in neuromuscular junction, which leads to flaccid paralysis.
- **Therapeutic uses:** As BT produces flaccid paralysis it can be used therapeutically for the treatment of spasmodic conditions, such as strabismus, blepharospasm and myoclonus.

Mechanism of Botulinum toxin: BT blocks the release of acetylcholine in neuromuscular junction, which leads to flaccid

- **BT is also produced by** other clostridia, such as *C. butyricum,C. baratti* and *C. argentinense.*
- **Recovery:** Blocking of acetylcholine release is permanent, but the action is short lasting as the recovery occurs *in 2–4 months,* once the new terminal axons sprout.

Clinical Manifestations

The manifestations of botulism are due to decreased acetylcholine in cranial nerve and parasympathetic nerve terminals. Common symptoms include:

- 5Ds-Diplopia, dysphasia, dysarthria, descending symmetric *flaccid paralysis* and ↓deep tendon reflexes
- Constipation
- Respiratory muscle paralysis may lead to death
- There is no sensory or cognitive deficits.

Botulism (Symptoms)
- 5Ds-Diplopia, dysphasia, dysarthria, descending symmetric flaccid paralysis and ↓deep tendon reflexes
- Constipation

Types of Botulism

1. **Food borne botulism:** Results from consumption of foods contaminated with preformed botulinum toxin; commonly due to consumption of homemade canned food.
2. **Wound botulism** results from contamination of wounds with *C. botulinum* spores. It presents like food borne botulism except for absence of gastrointestinal features.
3. **Infant botulism:** By ingestion of contaminated food (usually *honey*) with spores of *C. botulinum* in children ≤ 1 year of age. Spores germinate in intestine releasing the toxin:
   - Manifestations include inability to suck and swallow, weakened voice, ptosis, and floppy neck, extreme weakness (called **floppy child syndrome**)
   - It is usually self-limiting, rarely, progress to generalized flaccidity.
4. **Iatrogenic botulism:** Results from injection of overdose of the toxin.

CLOSTRIDIUM DIFFICILE

Clostridium difficile is the agent of '**pseudomembranous colitis'**which occurs almost exclusively in association with prolonged antimicrobial use. It was so named due to unusual difficulties involved in the isolation of *C. difficile*.

Risk factor for *C. difficile* infection:
- Prolonged hospital stay
- Prolonged antimicrobial
- Cephalosporins and other antibiotics
- Toxin production

Pathogenesis

Clostridium difficile infection is associated with the following risk factors:

- **Prolonged hospital stay:** Spores from hospital environment gets colonized in colon of patients.
- **Prolonged antimicrobial use** can result in disruption of the normal colonic flora:
  - Cephalosporins (e.g. ceftriaxone) are frequently responsible for this condition.
  - Other antibiotics, such as clindamycin, ampicillin and fluoroquinolones (ciprofloxacin)
  - However, all antibiotics, including vancomycin and metronidazole (which are the DOC in *C. difficile* infection) have been found to carry a risk of infection, if given for prolonged duration.
- **Toxin production:** Pathogenesis is toxin mediated. *C. difficile* (nontoxigenic strains) may be a part of normal intestinal flora, however only the toxigenic strains can cause pseudomembranous colitis:
  - It produces two toxins: Toxin A (enterotoxin) and Toxin B (cytotoxin). Both are important for pathogenesis.
  - Infants do not develop symptoms because they lack suitable mucosal toxin receptors.
- **Other risk factors** include older age, underlying illness, intestinal surgery, use of electronic rectal thermometers and antacid treatment.

Clinical Manifestations

- **Diarrhea** is the most common manifestation caused by *C. difficile.* Other manifestations include fever, abdominal pain and leukocytosis. Blood in stool is uncommon.
- **Pseudomembrane** formation over colonic mucosa with a relapse seen in 15–30% of cases.

Laboratory Diagnosis

Laboratory diagnosis of *C. difficile* infection depends on isolation of the bacilli followed by toxigenicity testing:

- **Stool culture** on selective media, such as **CCFA** (*cefoxitin cycloserine fructose agar*) or **CCYA** (*cefoxitin cycloserine egg yolk agar*). Stool culture is highly sensitive but not specific. Toxin demonstration is more meaningful.
- **Toxin demonstration:** Toxins can be detected by various methods:
  - Cell culture cytotoxin test: It is highly specific but not as sensitive as stool culture; it is time consuming.
  - Enzyme immunoassay for toxin A and/toxins B in stool is rapid, but not sensitive.
  - PCR for *C. difficile toxin B* gene in stool it is highly specific and sensitive.
- **Colonoscopy** is highly specific if pseudomembranes are seen, but sensitivity is low.
- **Histopathology** of colonic pseudomembrane is also highly specifiC.

Treatment of *C. difficile* infection:
- *Initial episode, mild to moderate cases:* Oral metronidazole is DOC
- *Recurrent episodes or severe cases:* Vancomycin is DOC
- *Severe complicated or fulminant infection:* The combination of vancomycin plus IV metronidazole

Treatment

- *Initial episode, mild to moderate cases:* Oral metronidazole is the drug of choice
- *Recurrent episodes or severe cases:* Vancomycin is the drug of choice
- *Severe complicated or fulminant infection:* Vancomycin plus IV metronidazole

NON-SPORING ANAEROBES

Classification of Non-sporing anaerobes

Gram-positive cocci	*Peptostreptococcus, Peptococcus*
Gram-negative cocci	*Veillonella*
Gram-positive bacilli	*Bifidobacterium, Propionibacterium, Eubacterium Lactobacillus, Mobiluncus, Actinomyces*
Gram-negative bacilli	*Bacteroides, Prevotella, Porphyromonas Fusobacterium, Leptotrichia*
Spirochete	*Treponema, Borrelia*

Anaerobes as a part of normal flora

Anatomical site	Common anaerobic normal flora
Mouth (saliva)	Anaerobic cocci, *Actinomyces*, *Bifidobacterium, P.melaninogenica,* Spirochetes
Stomach	*Lactobacillus*
Jejunum/ileum and Colon	Anaerobic cocci, *Bacteroides fragilis*, *Fusobacterium, Bifidobacterium P. melaninogenica*
Vagina	Anaerobic cocci, *Lactobacillus*, *P. melaninogenica, Bifidobacterium*
Skin	*Propionibacterium*

Common disease

Mobilincus: Bacterial vaginosis (also caused by *Gardnerella*)
Leptotrichia* or *Fusobacterium fusiformis: Agent of Vincent's angina (also caused by *Borrelia vincentii*)
Anaerobic cocci: Puerperal sepsis and other female genital tract infection (also by *B. fragilis* and *Prevotella*) Skin and soft tissue infections.
***Bacteroides fragilis*:** • Non-sporing anaerobe, capsulated gram-negative bacilli • MC commensal in human intestine • MC anaerobe to cause infection, causes abdominal infection • Endotoxin is less toxic than that of aerobic gram-negative bacilli
Fusobacterium necrophorum: Agent of Lemierre's syndrome (is a form of thrombophlebitis)

MULTIPLE CHOICE QUESTIONS

GENERAL

1. **True about obligate anaerobes:** *(JIPMER May 2016)*
   a. In absence of oxygen it grows well
   b. In absence of oxygen, it does not grow
   c. In presence of oxygen, it grows but does not utilize the oxygen
2. **Gas gangrene and Tetanus caused by which group of bacteria:** *(JIPMER, May 2015)*
   a. Campylobacter
   b. Clostridium
   c. Citrobacter
   d. Cardiobacterium
3. **Nonmotile clostridia is:** *(JIPMER 2010, AI 2009)*
   a. Cl. perfringens
   b. Cl. novyi
   c. Cl. botulinum
   d. Cl. difficile
4. **Oval bulging terminal spores seen in:** *(AI 2008, PGI Dec 08)*
   a. Cl. tertium
   b. Cl. welchii
   c. Cl. perfringens
   d. Cl. histolyticum

CLOSTRIDIUM PERFRINGENS

5. **A male is presented with left mid thigh crushed injury. Which is the most essential step to prevent gas gangrene in this patient?** *(JIPMER May 2016)*
   a. Wound debridement
   b. Anti gas gangrene serum
   c. Anti tetanus
   d. Hyperbaric oxygen
6. **Which of the following most likely cause necrotizing enteritis?** *(Recent Question 2015)*
   a. Clostridium botulinum
   b. Clostridium tetani
   c. Clostridium perfringens A
   d. Clostridium perfringens C
7. **True about gas gangrene:** *(PGI May 2015)*
   a. α-toxin is main cause of the toxaemia associated with gas gangrene
   b. Caused by mainly Clostridium intestinale
   c. Low oxygen tension in tissue is important precondition
   d. Devitalized tissue predisposes to gas gangrene
   e. Not occur if dead tissue is not present
8. **Best treatment for contaminated wound with necrotic material is:** *(Recent Question 2013, AIIMS Nov 2013)*
   a. Anti-gas gangrene serum
   b. Debridement
   c. Antibiotics
   d. Tetanus toxoid
9. **Regarding Clostridium perfringens gas gangrene, false is:** *(PGI June 2008, AIIMS May 2009)*
   a. Common cause of gas gangrene
   b. Nagler reaction positive
   c. Most common toxin is Hyaluronidase
   d. Food poisoning strain of Cl. Perfringens produces heat resistant spores
10. **Gastrointestinal enteritis necroticans is caused by:**
    a. Clostridium difficile *(PGI Dec 2000)*
    b. Clostridium perfringens
    c. Botulinum
    d. Campylobacter jejuni
    e. Pseudomonas
11. **True about gas gangrene:** *(PGI May 2013)*
    a. Underlying skin and muscles are normal
    b. Caused by tetanospasmin toxin
    c. Muscle rigidity and spasms are characteristic
    d. Most common organism implicated is C. perfringens
    e. Passive immunization does not help

CLOSTRIDIUM TETANI

12. **Which is the principle virulence factor in Clostridium tetani?** *(Recent Question 2015)*
    a. Tetanolysin
    b. Tetanospasmin
13. **A patient is presented with trismus with opisthotonus position. The probable causative agent is:**
    a. Clostridium tetani *(Recent Question 2015)*
    b. Clostridium perfringens
    c. Clostridium difficile
14. **Mechanism of action of tetanospasmin:**
    a. Inhibition of GABA release *(NEET Pattern Based)*
    b. Inhibition cAMP
    c. Inactivation of Ach receptors
    d. Inhibition of cGMP
15. **Which of the following is false for tetanus?** *(AI 2011)*
    a. Produces heat resistant spores
    b. Person to person transmission does not occur
    c. Incubation period is 6–10 days
    d. Three doses of primary vaccination is protective

16. Site of action of tetanus toxin: *(JIPMER 2007)*
a. Presynaptic terminal of spinal cord
b. Postsynaptic terminal of spinal cord
c. Neuromuscular junction
d. Muscle fibers

17. True about tetanus: *(PGI Dec 2004, Kerala 2016)*
a. Gram –ve spore forming organism
b. Produces Telanolysin and Tetanospasmin
c. Trismus and neck stiffness are early sign
d. Generalized tonic-clonic seizure occurs on hyper stimulation
e. Wound debridement is necessary

18. A 25-year-boy is presented with deep injury and abrasions on the left shoulder, thigh and leg with immunization status unknown. What is to be given now? *(JIPMER Nov 2015)*
a. DTaP only
b. DTaP + Ig
c. dTonly
d. dT + Ig

19. A 10-year-old boy following a road traffic accident presents to the casualty with contaminated wound over the left leg. He has received his complete primary immunization before preschool age and received a booster of DT at school entry age. All of the following can be done except: *(AIIMS 2001)*
a. Injection of TT
b. Injection of human antiserum
c. Broad spectrum antibiotics
d. Wound debridement and cleaning

20. A person has received complete immunization against tetanus 10 years ago. Now he presents with a clean wound without any lacerations from an injury sustained 2.5 hours ago. He should now be given: *(AI 2001, Karnataka 2011)*
a. Full course of tetanus toxoid
b. Single dose of tetanus toxoid
c. Human Tet globulin
d. Human Tet globulin and single dose of toxoid

21. The causative organism can be isolated in which among the following conditions: *(PGI Dec 2001)*
a. Serum in toxic shock syndrome
b. Meningococcal rash
c. Rheumatic valvulitis
d. CSF in tetanus
e. Diphtheritic myocarditis

22. All are done to prevent neonatal tetanus except:
a. Two TT doses to all pregnant *(AI 2007)*
b. TT to all females in the reproductive age
c. TT to all new borne
d. Penicillin injection to all new borne

23. Three doses of TT provide immunity for: *(DPG 2006)*
a. 1 yr
b. 5 yr
c. 10 yr
d. 15 yr

24. Wrong about Cl.tetani: *(AIIMS Nov 2010)*
a. Main reservoir- Soil, human and animal intestine
b. MC mode of transmission- Trauma and contaminated wound
c. Herd immunity- not useful
d. MC season –winter and dry

25. For neontatal tetanus elimination, the incidence should be….: *(DNB 08)*
a. Less than 0.5/1000 live birth
b. Less than 1/1000 live birth
c. Less than 0.1/1000 live birth
d. Less than 10/1000 live birth

26. Period of communicability for tetanus: *(UP 07, RJ 01)*
a. 7 days
b. 14 days
c. 21 days
d. None

27. A person has got a non penetrating wound 11 hour back. He had taken complete course of TT 1 year back. What treatment is recommended next? *(MP 09)*
a. Full course of tetanus toxoid
b. Single dose of tetanus toxoid
c. Nothing required
d. Human Tet globulin and single dose of toxoid

28. An adult with no immunization history presents with a clean non penetrating wound 2 hrs back. What measure has to be taken? *(Karnataka 2011)*
a. Full course of tetanus toxoid
b. Single dose of tetanus toxoid
c. Nothing required
d. Human Tet globulin and single dose of toxoid

CLOSTRIDIUM BOTULINUM

29. True about food botulism: *(Recent Question 2015)*
a. Symptoms resembles strychnine poisoning
b. Most common botulinum toxin serotype is type C and D
c. The bacteria invades the intestine
d. Botulinum toxin acts by blocking A.ch release

30. True about Botulinum toxin: *(PGI May 2015)*
a. Interfere with adrenergic transmission
b. Interfere with Cholinergic transmission
c. Increase release of synaptic vesicles
d. Inhibit release from synaptic vesicles
e. Act also on CNS

31. Botulism is most commonly due to: *(NEET Pattern Based)*
a. Egg
b. Milk
c. Meat
d. Canned vegetables

32. Botulisim causes: *(NEET Pattern Based)*
a. Descending flaccid paralysis
b. Descending spastic paralysis
c. Ascending paralysis
d. Ascending spastic paralysis

33. Botulinum affects all except: *(AI 2007, NEET Pattern Based)*
a. Neuromuscular junction
b. Preganglionic junction
c. Postganglionic nerves
d. CNS

34. An 18-year-old male presented with acute onset descending paralysis of 3 days duration. There is also history of blurring of vision for the same duration. On examination, the patient has quadriparesis with areflexia. Both the pupils are nonreactive. The most probably diagnosis is: *(AIIMS 2006)*
a. Poliomyelitis
b. Botulism
c. Diphtheria
d. Porphyria

35. The following statements are true regarding botulism except: *(AIIMS May 2003)*
a. Infant botulism is caused by ingestion of preformed toxin
b. Clostridium botulinum A, B, C and F cause human disease
c. The gene for botulinum toxin is encoded by a bacteriophage
d. Clostridium baratti may cause botulism

36. True about Clostridium tertium: *(PGI June 2008)*
a. Gram variable
b. Terminal spore
c. Produces exotoxin
d. Causes septicemic arthritis

37. Among the toxin produced by Clostridium botulinum, the non neurotoxic one is: *(JIPMER 2000)*
a. A
b. B
c. C1
d. C2
e. D

CLOSTRIDIUM DIFFICILE

38. Cause of clostridium difficile associated diarrhea: *(NEET Pattern Based)*
a. Trauma
b. Dairy products
c. Fried rice
d. Antibiotic use

39. Pseudomembranous colitis is caused by: *(DNB June 2012, Kerala 2016)*
a. Clostridium perfringens
b. Clostridium difficile
c. Clostridium tetani
d. Clostridium botulinum

40. Clostridium difficile infection occurs after:
a. After prolong antibiotic therapy *(PGI June 2008)*
b. Pantoprazole increases the risk
c. Associated with use of rectal thermometer
d. Increased with proportion of hospital stay

41. Pseudomembranous colitis, all are true except: *(AIIMS May 2007, AI 2000)*
a. Toxin A is responsible for clinical manifestation
b. Toxin B is responsible for clinical manifestation
c. Blood in stools is a common feature
d. Summit lesions is an early histopathological finding

42. A patient of Acute lymphocytic leukemia with fever and neutropenia develops diarrhea after administration of amoxycillin therapy, which of the following organism is most likely to be the causative agent: *(AIIMS Nov 2005)*
a. Salmonella Typhi
b. Clostridium difficile
c. Clostridium perfringens
d. Shigella flexneri

43. DOC for pseudomembranous enterocolitis:
a. Oral Vancomycin *(AIIMS Nov 2014)*
b. Penicillin
c. Oral Ampicillin
d. Clindamycin

NONSPORING ANAEROBES

44. With reference to Bacteroides fragilis the following statements are true, except: *(AIIMS Nov 2012, 11, 06, May 2006, 03, AI 2007)*
a. B. fragilis is the most frequent anaerobe isolated from clinical samples
b. B. fragilis is not uniformly sensitive to metronidazole
c. The lipopolysaccharide formed by B. fragilis is structurally and functionally different from the conventional endotoxin
d. Shock and disseminated intravascular coagulation are common in Bacteroides bacteremia

45. A patient presents with frontal abscess. Foul smelling pus is aspirated. Pus shows red fluorescence on ultraviolet examination. The most likely organism causing the frontal abscess is: *(AIIMS May 2002)*
a. Bacteroides
b. Peptostreptococcus
c. Pseudomonas
d. Acanthamoeba

EXPLANATIONS

GENERAL

1. **Ans. (a) (In absence of oxygen it grows well)** Ref:Apurba Sastry's Essentials of Medical Microbiology/p28
   - Obligate anaerobes grow only in absence of oxygen as oxygen is lethal to them.
   - Aerotolerant anaerobes tolerate oxygen for some time and grow in presence of oxygen, but do not utilize.
2. **Ans. (b) (Clostridium)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p256, Ananthanarayan9/e p251
   - Gasgangrene is caused by Clostridium perfringens and Tetanus is caused by Clostridium tetani.
3. **Ans. (a) (Cl. perfringens)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p256, Ananthanarayan 9/e p252
   - All Clostridia are motile except Cl. perfringens and Cl. tetani type VI.
   - Clostridia show ***stately*** type of motility.
   - All Clostridia are noncapsulated except Cl. perfringens and Cl. butyricum.
4. **Ans. (a) (Cl. tertium)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p256, Ananthanarayan 9/e p252
   All clostridia produce bulging spore (spore are wider than the bacilli) except Cl. bifermentans and Cl. sordelli
   - C. tertium produces Oval and terminal spore (tennis racket shaped)
   - Type of spore produced by Clostridium species: Refer Chapter review:

CLOSTRIDIUM PERFRINGENS

5. **Ans (a) (Wound debridement)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p260
   Early surgical debridement is the most crucial step in the management of gas gangrene to remove all devitalised tissues so as to remove conditions that produce anaerobiosis.
6. **Ans. (d) (C. perfringens C)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p258
   Necrotizing enteritis is caused by β-toxin of Type C Clostridium perfringens.
7. **Ans (a, c, d, e) (a-toxin..., low ..., devitalized ..., not occur...)** Ref: Harrison 19th/p990-02, Apurba Sastry's Essentials of Medical Microbiology 1/e p258
   - α-toxin is the principle virulence factor of gas gangrene
   - Gas gangrene is mainly caused Clostridium perfringens
   - The development of gas gangrene requires an anaerobic environment and contamination of a wound with C. perfringens. Devitalized tissue, foreign bodies, and ischemia reduce locally available oxygen levels and favor outgrowth of C. perfringens. ….Harrison 19/e p992
   - In the absence of devitalized tissue, the presence of clostridia does not necessarily lead to infection.
8. **Ans. (b) (Debridement)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p260, Harrison 19 /e p990, 18/e p1205
   - Contaminated wound with necrotic material is suggestive of higher risk of clostridial infection and gas gangrene.
   - Treatment of gas gangrene: Refer Chapter review
9. **Ans. (c) (Most...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p258, 59, Ananthanarayan 9/e p254
   - Most common toxin is '**α toxin'** not 'Hyaluronidase'
   - Cl. perfringens produces 4 Major (Lethal) Toxins – alfa, beta, iota, epsilon
   - *Commonest cause of gas gangrene is Clostridium perfringens type A (60%)*

   About Other Options
   Option b: Nagler reaction positive for Cl. perfringens and Cl. bifermentans
   For detail of Naegler reaction: Refer Chapter review
   Option d: Food poisoning strain of Cl. perfringens (mostly Type A) characterized by their marked heat resistant spores but *feeble production of α and theta toxin*.
   Instead they produce heat labile enterotoxin (similar to LT of E. coli)

Also remember

'Gas gangrene producing Cl. perfringens strains do not produce Spores in tissue/media.

10. **Ans. (b) (Cl. perfringens)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p258, Ananthanarayan 9/e p255, 56

Enteritis necroticans (Pigbel in New Guinea):

- Caused by Cl. Perfringens type-C strains with ***heat resistant spores***
- *Spores germinates in intestine producing* **beta toxin**
- *Usually following a pig meat diet along with a trypsin inhibitors like* ***sweet potato***

11. **Ans. (d) (Most common...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p258, Ananthanarayan 9 /e p254

- Most common organism implicated in Gas gangrene is Clostridium perfringens followed by C. novyi and C. septicum
- Underlying skin and muscles are gangrenous
- Gas gangrene is caused by α-toxin
- Pain and crepitus in muscle are characteristic of Gas gangrene
- Passive immunization – Anti α- toxin (anti gas gangrene serum) is of great value for the treatment of Gas gangrene.

CLOSTRIDIUM TETANI

12. **Ans. (b) (Tetanospasmin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p261

13. **Ans. (a) (Clostridium tetani)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p261

14. **Ans. (a) (Inhibition...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p261, Ananthanarayan 9/e p262

- Mechanism of action of tetanospasmin is: Acts presynaptically and inhibits of glycine and GABA release that leads to spastic contraction of muscles.

15. **Ans. (d) (Three doses of primary...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p263

According to National immunisation schedule:

- Primary course of immunisation includes:
- '3 doses of DPT at 4–8 week apart followed by booster of PDT at 16–24 month followed by booster of DPT at 5–6 year followed by booster of TT at 10 and 16 year.' Park 23/e p312, 22/e p115, 21/e p113

About Other options:

- C. tetani produces heat resistant spores
- Person to person transmission does not occur in tetanus
- Incubation period of tetanus is 6–10 days.

16. **Ans. (a) (Presynaptic...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p261, Ananthanarayan 9/e p261

- Tetanospasmin: Blocks release of inhibitory transmitters glycine and GABA at CNS and Pre synaptic terminal of spinal cord → leads to spastic paralysis
- The manifestations of Tetanus and strychnine poisoning are similar except Tetanospasmin acts at Presynaptic terminal whereas strychnine acts at postsynaptic terminal.

17. **Ans. (b), (c), (d), (e)** Ref:Apurba Sastry's Essentials of Medical Microbiology 1/e p261, Ananthanarayan 9/e p261

- Option a: Cl. tetani is Gram +ve spore forming organism
- Option b: Tetanolysin and Tetanospasmin are the principle toxin produced by Cl. tetani
- Option c: 1st symptom-↑masseter tone (trismus/lock jaw) then → descending tetanus
- Option d: 'Tetanus patient should be isolated to protect them from noise and light which may provoke convulsion'
- Option e- Prophylactic measures include- Wound toilet, antibiotic and most importantly immunization.

18. **Ans (b) (DTaP + Ig)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 263

DTaP (complete immunization) + Human Tetanus Ig is recommend for this condition.

- Human Tetanus Ig- because it's a deep injury
- DTaP (complete immunization)- because no h/o immunization. Acellular pertussis (aP) is given instead of killed pertussis vaccine after 5 year of age.

19. **Ans. (b) (Injection...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p262, Park 23/e p312, 22/e p286-87

- Treatment of tetanus consists of (Wound debridement + Antibiotic + immunization)
- *For treatment of wound injury, we have to know the type of wound and the immune category that the patient belongs to.*
- This history is suggestive of:
  - 10-year-old child Taken complete immunization with booster of DT at school entry age—indicates taken complete immunization > 5 to < 10 year back So belongs to Immunity category B
  - Type of wound- contaminated (other wound category)
  - Treatment required for Category B with contaminated (other wound) Toxoid 1 dose
- Antibiotic has role to eradicate the source of toxin (No role after 6 hour when the toxin is already formed)
- HT Ig has role in category C and D persons, i.e. when the complete immunization taken > 10 year or the immunization is not complete/unknown.

Treatment: All type of wounds need surgical toilet followed by: Immunization. (Refer chapter review)

20. Ans. (b) (Toxoid 1 dose) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p263, Park 23/e p314, 22/e p286

- **Type of wound:** Clean wound without any lacerations from an injury sustained 2.5 hours ago
- Patient belongs to Immunity category C complete immunization taken 10 years ago
- So, Treatment required for Category C with simple wound is: Toxoid 1 dose

21. Ans. (a), (b) (Serum in Toxic..., Meningococcal rash) Ref: Topley 10/e p813, Harrison 19/e p992, 18/e p1117

- Option a: *Lab test for diagnosis of Toxic Shock Syndrome includes isolation of S.aureus from mucosal or normally body site (Topley 10/e p813) and isolation of Str. pyogenes from mucosal or normally body site like blood* (Harrison 19/e p992)
- Option b: '*Meningococci may sometime be demonstrated in petechial lesions by culture.*' Ananthanarayan 8/e p226
- Rheumatic fever is due to cross reacting antigen between Strept M protein and human myocardium.
- Diphtheria and tetanus both can cause toxemia but never bacteremia.

22. Ans. (d) (Penicillin injection to all new borne) Ref: Park 23/e p312, 22/e p286

- ATS is recommended to all neonates borne to unimmunized mother. (Detail- refer chapter review)

23. Ans (c) (10 years) Ref: Park 23/e p312, 22/e p286

- Individuals sustaining tetanus-prone wounds should be immunized if their vaccination status is incomplete or unknown or if their last booster was given >10 years earlier
- Two doses of immunization provides immunity for several years Park 22/e p285

24. Ans. (d) (MC season –winter and dry) Ref: Park 23/e p312, 22/e p284-85

- Tetanus shoes seasonal variation, in India, > 50% of cases occur in- July to September
- Main reservoir: Soil, human and animal intestine
- MC mode of transmission: Trauma and contaminated wound
- Herd immunity not useful for tetanus

25. Ans (c) (Less...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p263, Park 23/e p312, 22/e p286, 21 e/p287

26. Ans (d) None Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p263, Park 23/e p312, 22/e p284

- Tetanus is NOT infectious from man to man.

27. Ans (b) (Single dose of tetanus toxoid) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p263, Park 22/e p286

- It is a case of Simple wound (Non penetrating wound 1hr back) and category C (h/o complete course of TT 11 yr).
- The recommendation for simple wound and category C is Single dose of tetanus toxoid

28. Ans (a) (Full course...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p263, Park 23/e p312, 22/e p286

- It's a case of simple wound (clean and non penetrating) and category-D (unimmunized)
- The recommendation for simple wound and category D is Full dose of tetanus toxoid

CLOSTRIDIUM BOTULINUM

29. Ans. (d) (Botulinum toxin…) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p264

- **MC botulinum toxin is serotype A, B, and E. Most severe is type A**
- **Tetanus resembles strychnine poisoning**
- **C. botulinum is non invasive. Disease is toxin mediated.**

30. **Ans (b, d) (Interfere…, inhibit...)** Ref: Harrison 19th/p987-88, Apurba Sastry's Essentials of Medical Microbiology 1/e p264
Botulinum toxin blocks the release of Acetyl choline at synapses of NM junction (MC site), peripheral ganglia and parasympathetic nerve ending; however it has no action on CNS.

31. **Ans. (d) (Canned vegetables)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p264, Park 22/e p217
- The most common food associated with botulism are home preserved foods such as home canned vegetables, smoked or pickled fish, home made cheese and similar low acid food.

32. **Ans. (a) (Descending flaccid paralysis))** Apurba Sastry's Essentials of Medical Microbiology 1/e p264
- Botulism blocks acetylcholine release, hence causes Descending flaccid paralysis

33. **Ans. (d) (Central...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p264, Harrison 19/e p987, 18/e p1200
- 'Botulinum neurotoxin, acts on peripheral cholinergic nerve terminals, but not on CNS:

34. **Ans. (b) (Botulism)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p264, Harrison 19/e p987, 18/e p1201

Points in favor:

Descending paralysis, blurring of vision, quadriparesis (flaccid paralysis), areflexia and nonreactive pupils

Also know:
- Causes of Descending paralysis: Tetanus, botulism, polio, diphtheria
- Tetanus causes spastic paralysis whereas botulism causes flaccid paralysis

35. **Ans. (a) (Infant...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 264; Harrison 19/e p988, 18/e p1200
- *'Infant botulism occurs* due to ingestion of spores, *toxin released inside*

Whereas food borne botulism occurs due to preformed toxin mixed with canned food'

36. **Ans. (a), (b), (d) (Gram..., terminal..., causes septic arthritis)** Apurba Sastry's Essentials of Medical Microbiology 1/e p264

Cl. tertium:
- Nonexotoxin-producing, Aerotolerant, ***Gram variable***
- Oval and terminal spore – ***tennis racket shaped***
- Resembles lactobacilli
- It is a rare human pathogen
- Causes: Necrotizing Fasciitis and Gangrene, septicemia, ***septic arthritis***

37. **Ans. (d), (C2)** Ref: Ananthanarayan 9/e p264, 8/e p262, Apurba Sastry's Essentials of Medical Microbiology 1/e p264
All subtypes (A–G) of Botulinum toxin are pharmacologically similar (neurotoxin), except C2 (enterotoxin)

CLOSTRIDIUM DIFFICILE

38. **Ans. (d)(Antibiotic use)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p265, Ananthanarayan 9/e p265
- Prolonged broad spectrum antibiotic therapy is the most important risk factor for Clostridium difficile associated diarrhea.

39. **Ans. (b) (Clostridium...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p265, Ananthanarayan 9/e p265
- *Pseudomembranous colitis is caused by Clostridium difficile*

40. **Ans. (a), (b), (c), (d) (After prolong antibiotic therapy, Pantoprazole increases the risk, Associated with use of rectal thermometer, Increased with proportion of hospital stay)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p265, Harrison 19/e p857, 18/e p1091
Risk factors for Pseudo membranous colitis: Refer chapter review.

41. **Ans. (c) (Blood...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p265, Harrison 19/e p858, 18/e p1092
Says: St*ools are almost never Grossly bloody and range from unformed to watery or mucoid in consistency, with a characteristic odor.*

About Other option:
- Cl. difficile Toxins: A → Enterotoxin and B → Cytotoxin
- Both are required for manifestation.
- In the earliest stage, Summit lesion, i.e. tiny superficial intercryptal erosions may be found. Topley 10/e p1120

42. Ans. (b) (Clostridium difficile) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p265, Harrison 19/e p858 18/e p 1091, Ananthanarayan 9/e p265, 8/e p263

- Points favoring diarrhea after administration of amoxicillin therapy

43. Ans. (a) (Oral...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p266, Harrison 19/e p860 18/e p1093-94

Etiological agent for pseudomembranous enterocolitis is Clostridium difficile.
Treatment of Clostridium difficile infection: Refer chapter review

NONSPORING ANAEROBE

44. Ans. (d) (Shock...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p268; Harrison 18/e p1332, 1338

'B. fragilis LPS are 100–1000 times less biologically potent than endotoxin associated with aerobic gram-negative bacteria. This accounts for the lower frequency of DIC and purpura in Bacteroides bacteremia than in facultative aerobic gram-negative bacillary bacteremia.'

About Other options:

Option a: '*Bacteroides are the most common anaerobes isolated in clinical specimen.*' Ananthanarayan 9/e p269, 8/e p267
Option b: '*Metronidazole is active against gram-negative anaerobes, including the B. fragilis group; resistance is rare but has been reported*' Harrison 18/e p1332, 1338, 17/e p810

45. Ans. (a) (Bacteroides) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p268, Ananthanarayan 9/e p269

- Frontal abscess with foul smelling pus: Points towards anaerobic infection
- Fluorescence on ultraviolet examination: Indicates Prevotella melaninogenica which is a type of Bacteroides infection.

Also know, the Anaerobic/Aerobic Ratio in human GIT flora- (Harrison 18/e p1332, 1338, 17/e p 903)
1:1 (stomach, ileum, jejunum, saliva)
10^3: 1 (Terminal ileum and colon and Gingival crevices)
10:1 (Female genital tract)

CHAPTER

3.6 Mycobacteria

Mycobacteria are obligate aerobic weakly gram-positive, straight or slightly curved bacilli, sometimes show branching filamentous forms resembling fungal mycelium. They have the following minimum properties:

1. **Acid fastness**: This is due to:
   - Presence of mycolic acids in the cell wall and
   - Integrity of the cell wall.
2. ***Guanine plus cytosine*** (G + C) content of DNA is 61–71 mol %

   Mycobacteria are classified into:
   - *M.tuberculosis* complex
   - *M.leprae*
   - Nontuberculous mycobacteria (NTM).

Acid fastness is due to:
- Presence of mycolic acids in the cell wall
- Integrity of the cell wall

MYCOBACTERIUM TUBERCULOSIS COMPLEX

M. tuberculosis complex consists of genetically related species that cause tuberculosis in man:

- *M.tuberculosis*: It is the most common cause of tuberculosis in man
- Others: *M.bovis*, *M.caprae*, *M.africanum, M.microti, M. pinnipedii* and *M.canetti*

Pathogenesis

About one third of world population is infected with *M.tuberculosis*, of which 5–10% develop clinical disease. India accounts for *one fourth (25%)* of global TB cases with prevalence and incidence rates of 256 and 185 cases per one lakh.

Mode of Transmission

- By inhalation of droplet nuclei (< 5–10 µm size) directly from cases of pulmonary tuberculosis
- Inoculation (rare): Direct skin contact with an infected person
- Ingestion (rare): Swallowing of sputum (in infants) or unpasteurized milk.

Risk Factors Favoring the Transmission and Disease Progression Include

At least 10^4 bacilli/ml MTB load in sputum is needed for:
- Effective transmission
- Patient to become smear positive

- Sputum positive patients transmit more efficiently
- Bacillary load: At least **10^4 bacilli/ml** in sputum is required for an effective transmission
- Adult patients with cavitary lesions in lung have more bacillary load
- Overcrowding in poorly ventilated rooms
- Low cell mediated immunity as occurs in HIV infected people
- Other co-morbid conditions such as post silicosis, post-transplantation (renal, cardiac), jejuno-ileal bypass, gastrectomy, chronic renal failure/hemodialysis, diabetes, IV drug abuse, smoking, etc
- Age: Late adolescence and early adulthood periods are more prone.
- Sex: Risk is higher in women at 25–34 years, while at older ages, men have greater risk.

The Sequence of Pathogenic Events that Take Place is as Follows:

Intracellular Survival of MTB is due to:
- Impairs phagosome-lysosome fusion to bacterial cell wall lipoarabinomannan

1. *Droplet nuclei* containing tubercle bacilli from infectious patients are inhaled.
2. Majority are trapped in the upper airways and expelled out by the ciliary action of the mucosal cells; only a fraction (usually < 10%) of small droplets reach the alveoli.
3. *Adhesion to macrophages*: Mycobacterial surface lipoarabinomannan (LAM) binds to complement receptors and mannose receptors present on the surface of macrophages.

4. *Phagocytosis by macrophages* is enhanced by complement (C3b) mediated opsonization.
5. *Survival inside the macrophages:* This is due to bacterial cell-wall lipoarabinomannan which **impairs phagosome-lysosome fusion** by inhibiting increase in intracellular Ca^{2+} and phosphatidylinositol 3-phosphate (PI3P).

Clinical Manifestations

Tuberculosis (TB) is classified as pulmonary (80%) and extrapulmonary forms (20%).

Pulmonary Tuberculosis

It is classified into Primary PTB and Post-primary/secondary PTB

Features	Primary PTB	Post-primary/secondary PTB
Results due to	Initial exogenous infection with tubercle bacilli	• Exogenous reinfection • Endogenous reactivation of latent primary lesion
Age group affected	Children	Adults
Parts of the lungs commonly affected	Sub pleural lesion (upper part of lower lobe and lower part of upper lobe)	Apical and posterior segments of the upper lobes (areas of high oxygen tension)
Lesions formed at the initial sites	Fibrotic nodular lesions are formed (Ghon focus)	Calcified nodules are formed (Assmann focus) Early hematogenous seedling in the apex of lungs called as Simon's focus
Lymph node	Ghon focus with hilar lymphadenopathy (called as primary complex)	Lymph node involvement is unusual
Clinical feature	It may be asymptomatic or may present with fever, cough, hemoptysis, chest pain, night sweating, weight loss	Lesions undergoing necrosis and tissue destruction, leading to cavity formation. Symptoms are similar, but more pronounced.
Fate	In the majority of cases: • Lesions heal spontaneously. • Primary complex becomes calcified (*Ranke complex*) Rarely, develops to **progressive primary TB** which spreads by local invasion and by lymphatics	In majority of cases: The necrotic material breaks into the airways, leading to: • Bronchogenic spread to the same or opposite lung • Expectoration of bacteria laden sputum • Hematogenous spread leading to seedling of bacilli in various parts of the body • Spontaneous healing is rare

Extrapulmonary Tuberculosis (EPTB)

EPTB results from hematogenous dissemination of tubercle bacilli to various organs.

Though EPTB constitutes about 15 to 20% of all cases of TB, in HIV-positive patients the frequency is much higher accounting for 20–50% of all cases of tuberculosis.

The sites commonly involved (in order of frequency) are:

1. **Tuberculous lymphadenitis** is the MC form (35% of all EPTB cases). The most common sites are posterior cervical and supraclavicular lymph nodes.
2. **Pleural tuberculosis** accounts for 20% of all EPTB cases. It presents as pleural effusion.
3. **Tuberculosis of the upper airways** involving larynx, pharynx, and epiglottis
4. **Genitourinary tuberculosis:**
   - Renal tuberculosis
   - Genital tuberculosis: In female patients, fallopian tubes and the endometrium are commonly involved causing infertility. In male, epididymis is the MC site.
5. **Skeletal tuberculosis:** Weight-bearing joints such as spine (Pott's disease or tuberculous spondylitis is most common), hips and knees are commonly affected.
6. **Tuberculosis of CNS** occurs commonly in children. Tuberculous meningitis and tuberculoma are the common forms
7. **Gastrointestinal tuberculosis**: Terminal ileum and cecum are the MC sites involved. Transmission is due to swallowing of sputum, hematogenous spread, or ingestion of cow's milk contaminated with *M.bovis*

Tuberculous lymphadenitis
- MC form (35%) of all EPTB cases
- MC sites are posterior cervical and supraclavicular lymph nodes

8. **Tuberculous pericarditis**
9. **Tuberculous skin lesions:**
   - *Scrofuloderma* is a skin condition caused by tuberculous involvement of the skin by direct extension, usually from underlying tuberculous lymphadenitis
   - *Lupus vulgaris:* Apple jelly nodules are formed over the face in females.
10. **Miliary or disseminated tuberculosis:** Hematogenous spread of tubercle bacilli results in the formation of granulomatous lesions resembling *millet seeds* in various organs. It is more common in HIV infected people.
11. **HIV-associated tuberculosis:** TB is the MC opportunistic diseases among HIV-infected persons. Worldwide, TB affects 70–80% of HIV infected individuals, EPTB being more common than PTB.

Laboratory Diagnosis of Tuberculosis

Diagnosis of Active Tuberculosis

- **Specimen collection:**
  - In pulmonary TB- sputum (2 specimens—spot and early morning), gastric aspirate (in children)
  - In EPTB—specimens vary depending on the site involved.
- **Digestion, decontamination and concentration of specimen:**
  - Petroff's method (4% NaOH)
  - NALC (N-acetyl-L-cysteine) + 2% NaOH.
- **Acid-fast staining by Ziehl Neelsen (ZN) technique:**
  - *Procedure:*
    - Smear is covered with primary stain, strong carbol fuchsin for 5 minutes with intermittent heating
    - Decolorization with 25% sulfuric acid for 3 minutes
    - Counter staining with methylene blue for 1 min.
  - *Advantages:* Smear microscopy is rapid, easy to perform at peripheral laboratories and is cheaper.
  - *Disadvantages:*
    - Low sensitivity: Detection limit is 10,000 bacilli/ml of sputum.
    - Viability of bacilli cannot be determined
  - *Reporting*: *M.tuberculosis* appears as ***long slender, beaded***, less uniformly stained red color acid fast bacilli.
  - *Acid alcohol* can be used as decolorizer in renal tuberculosis to differentiate *M.tuberculosis* (acid and alcohol fast) from *M.smegmatis* (acid fast but not alcohol fast) in urine sample.
  - *Sputum microscopy is the method of choice case finding tool for tuberculosis under RNTCP*
  - *RNTCP grading:* This grading is useful for:
    - Monitoring the treatment response of the patients
    - Assessing the severity of disease
    - Assessing the infectiousness of the patient: Higher the grade more is the infectiousness. Smear negative patients are less infectious.
- **Others staining methods:**
  - *Kinyoun's cold acid fast staining*
  - *Auramine phenol technique:* It is a fluorescent staining technique.
- **Conventional culture media**:
  - *Advantage:* Culture is more sensitive with detection limit of 10-100 viable bacilli.
  - *Disadvantage:* It is time consuming. Minimum 8 weeks of incubation is needed.

  Examples of media:
  - Solid media, e.g.
    - *Egg based:* Lowenstein Jensen (LJ), Dorset egg media, Petragnani media

      Lowenstein Jensen (LJ): *M.tuberculosis* ***produces rough, tough and buff colonies, recommended by RNTCP***

RNTCP grading of sputum smear is useful for:
- Monitoring the treatment response of the patients
- Assessing the severity of disease
- Assessing the infectiousness of the patient

Section 3

    - *Agar based:* Middlebrook7H11 and 7H10. They are preferred for isoniazid resistant strains of *M.tuberculosis*
  - Liquid media: Middlebrook 7H9, Dubos, Proskauer, Sula, and Sauton media.
- **Automated culture methods:** These systems monitor the growth continuously and detect growth faster (2-3 weeks); however, they are expensive:
  - Example include BACTEC, MGIT and BACT/Alert
  - **BACTEC MGIT** (Mycobacteria Growth indicator Tube)—Uses an oxygen sensitive fluorescent compound to detect:
    - Mycobacterial growth and
    - Resistance to first line anti-tubercular drug
- **Biochemical identification:** Positive for Niacin test, Nitrate reduction test, Pyrazinamidase test and Resistant to TCH.
- **Serology:**
  - Antigen detection—LAM and antigen-5 detection by ELISA
  - Antibody detection—not useful in endemic area.
- **Molecular methods:**
  - PCR detecting *IS6110* gene and other genes such as 65 KDa and 38 KDa genes
  - **Line probe assay:** Detects drug resistance from samples (takes one day), but only from smear positive cases. It is less sensitive for smear negative cases because it is based on hybridization techniques.
  - **Gene Expert:** It detects growth and resistance to rifampicin. It takes very less time (2 hours). It is a cartridge based nucleic acid amplification technique (based on real time PCR). Pleural biopsy is better specimen than pleural fluid for Gene Expert.
- **Animal pathogenicity**: Using Guinea pig and rabbit

BACTEC MGIT (Mycobacteria growth indicator tube)
Uses an oxygen sensitive fluorescent compound to detect
- Mycobacterial growth
- Resistance to first line anti-tubercular drug

Line probe assay
- Detects drug resistance from samples (takes one day), but only from smear positive cases
- Less sensitive for smear negative cases

Gene Expert:
- It detects growth and resistance to rifampicin
- It takes very less time (2 hours)
- It is a cartridge based real time PCR technique

Property	*M. tuberculosis*	*M. bovis*
Acid fast stain	Curved, long, beaded, less uniformly stained	Straight, short, stout, uniformly stained
LJ media	Rough, tough, buff colony	White, smooth, moist colony
Growth	Eugonic	Dysgonic
Glycerol	Helps in growth	No effect on growth
TCH	Resistant to TCH	Sensitive to TCH
Niacin test	Positive	Negative
Nitrate test	Positive	Negative
Rabbit pathogenicity	Not pathogenic	Pathogenic
Oxygen	Obligate aerobe	Microaerophilic
Both are pathogenic to guinea pig and human beings		

Diagnosis of EPTB: EPTB differs from PTB in many aspects:

As such EPTB specimens are paucibacillary, hence smear microscopy is less sensitive.

- Molecular methods are more useful.
- Pleural fluid reveals elevated ADA (adenosine deaminase) and (IFN)-γ levels.
- Renal tuberculosis: Urinary excretion of bacilli is intermittent; hence 3–6 consecutive early morning urine samples are collected, centrifuged and the sediment is used for processing. Acid alcohol is used as decolorizer.
- CSF examination shows *cobweb coagulum* on standing, ↑CSF pressure, protein and chloride; whereas ↓ glucose levels.

Diagnosis of Latent Tuberculosis

It is diagnosed by demonstration of delayed or type IV hypersensitivity reaction against the tubercle bacilli antigens.

A. Tuberculin test

It is discovered by Von Pirquet in 1907.

- **Antigens used**:
  - *Old tuberculin (OT):* Crude preparation of tubercle bacilli, was used before.
  - *Purified protein derivative (PPD):* Purified preparation of the active tuberculo protein, prepared by Seibert
  - Dosage: It is expressed in tuberculin unit (TU). One TU is equal to 0.01 ml of OT or 0.00002 mg of PPD.
- **Procedure:**
  - *Mantoux test* is the MC method used. 0.1 ml of PPD containing 1TU is injected intradermally into flexor surface of forearm.
  - *Heaf* and *Tine* multiple puncture tests: Both the techniques are not in use.
- **Reading:** Induration surrounded by an erythema is produced at 48–72 hrs. If the width of the induration is:
  - ≥ 10 mm: Positive (tuberculin reactors)
  - 6–9 mm: Equivocal/doubtful reaction
  - < 5 mm: Negative reaction
- **Interpretation of result**:
  - Adults: Positive test in adults only indicates present or past infection with tubercle bacilli but does not confirm presence of active stage of the disease. It is only used as epidemiological marker:
    - Prevalence is calculated by counting all tuberculin reactors in a community
    - Incidence–By counting new converters to tuberculin test in a community.
  - Children: Positive test indicates active infection and used as *diagnostic marker*.
- **False positive:** The test becomes false positive after:
  - BCG vaccination (after 8–14 weeks),
  - NTM infection
- **False negative:** The test may become false negative in conditions such as:
  - Early or advanced TB,
  - Miliary TB and post measles
  - Low immunity, HIV infected people.
- **Two step testing:** In adults, tuberculin reactivity slowly wanes with time and it may become negative after some years. Repeat test 1–2 weeks after the first test exerts a booster effect and gives a strong positive reaction (> 20 mm).

Mantoux test Interpretation:
- Adults: Positive test indicates present or past infection with tubercle bacilli
  - But does not confirm active disease
  - Used as epidemiological marker.
- Children: Positive test indicates active infection and used as diagnostic marker.

B. Interferon Gamma Release Assay (IGRA)

- **Procedure:** Sensitized T lymphocytes of suspected individuals, are exposed to highly specific *M.tuberculosis* antigen such as CFP10 (culture filtrate protein) and ESAT6 (early secreted antigenic target-6), which leads to release of high level of IFNγ.
- It is an in vitro test. ELISA formats are available (called as QuantiFERON-TB Gold assay)
- Advantage**:** It is highly specific; there are no false positive conditions.

Treatment

Anti-tubercular drugs can be classified into:

- **First line drugs:** Isoniazid (H), Rifampin (R), Pyrazinamide (Z), Ethambutol (E), Streptomycin (S)
- **Second line drugs:**
  - Ethionamide, Cycloserine, Macrolides (Clarithromycin)
  - Quinolones (Ofloxacin, ciprofloxacin)
  - Aminoglycosides: Kanamycin, capreomycin and amikacin

The following strategies are followed:

1. Multidrug therapy for Short course lasting for 6 months (or 8 months in previously treated cases)

2. Two phase chemotherapy**:** *Intensive phase* (2–3 months) followed by *Continuation phase* (4–5 months)
3. DOTS strategy (Directly Observed Treatment, Short course) is recommend by RNTCP and WHO
4. Treatment regimens**:** Category I and II regimens are followed as per RNTCP.

Drug Susceptibility Testing (DST)

Several DST methods are available for tubercle bacilli.

Phenotypic methods: Commonly used methods are:

- **Proportion method** is the gold standard method
- Automated systems such as **BACTEC MGIT** are widely used these days.
- Others:
  - Absolute concentration method
  - Resistance ratio method.

Molecular methods: To detect drug resistant genes by methods such as PCR based assays, DNA Probe based (Line probe assay), DNA microarray and Gene Expert

Drug resistance genes present in *M.tuberculosis (acquired by mutation)*

Drugs	Drug-resistant genes
Isoniazid	Enoyl ACP reductase (inhA), Catalase-peroxidase (katG) Alkyl hydroperoxide reductase (AhpC)
Rifampicin	RNA polymerase subunit B (rpoB)
Pyrazinamide	Pyrazinamidase (pncA)
Ethambutol	Ribosomal protein subunit 12 (rpsL)
Streptomycin	Ribosomal protein subunit 12 (rpsL), 16s ribosomal RNA (rrs) Aminoglycoside phosphotransferase gene (strA)
Fluoroquinolones	DNA gyrase (gyr A and B)

MDR-TB
Resistance to isoniazid and rifampicin with or without resistance to other first line drugs.

Multidrug Resistant Tuberculosis (MDR-TB)

MDR-TB is defined as resistance to isoniazid and rifampicin with or without resistance to other first line drugs.

Epidemiology:

- As per WHO, 60% of total MDR-TB cases resides in BRICS countries: Brazil, Russia, India, China, South Africa, with India accounting for the maximum cases .
- In India, MDR-TB accounts for 2.8% of all new TB cases and 12–17% of re-treatment cases.

Treatment of MDR-TB is done under **DOTS-Plus** programme by using complex regimen of 2nd line drugs : intensive phase (6 drugs) for 6–9 months followed by continuation phase (4 drugs) for 18 months.

Extensively Drug Resistant Tuberculosis (XDR-TB)

1. **Definition:** These are MDR-TB cases that are also resistant to:
   - Fluoroquinolones (ofloxacin/levofloxacin) and
   - At least one injectable aminoglycosides (kanamycin, amikacin or capreomycin).
2. **Epidemiology:** In USA, 3% of MDR-TB cases have been found to be XDR. The exact incidence of XDR-TB in India is not known. The MDR-TB treatment failure cases (2–6%) may be presumed to be XDR-TB cases.
3. **Treatment** of XDR-TB is extremely difficult. XDR-TB has a very rapidly progressing clinical course with high mortality.

Extensively Drug-Resistant Tuberculosis (XDR-TB):
These are MDR-TB cases that are also resistant to:
- Fluoroquinolones (ofloxacin/levofloxacin)
- At least one injectable aminoglycosides (kanamycin, amikacin or capreomycin)

Prophylaxis by BCG Vaccine (Bacillus Calmette Guerin)

BCG vaccine was developed by Calmette and Guerin (1921). They attenuated the strain by serial sub culturing in glycerol bile potato medium for 230 times over a period of 13 years.

Administration of BCG:
- Dose and strength: 0.1 ml containing 0.1 mgTU
- Alcohol should not used to wipe the skin
- Site: It is given above insertion of left deltoid
- Route: By intradermal route

Protection of BCG:
- Variable efficacy of 0–80%.
- Duration of immunity lasts for 15–20 years

Indications of BCG:
- Direct BCG: BCG is given to newborn soon after birth directly, followed in developing countries.
- Indirect BCG: BCG is given after performing tuberculin test, followed in less prevalence country

Other uses of BCG:
- Stimulates the immune system, providing some protection against leprosy and leukemia
- In malignancies such as bladder carcinoma (Onco TICE strain of BCG)
- BCG may be superior to PPD for tuberculin test

- **BCG Strain:** Live attenuated *M.bovis w*as the strain originally used:
  - Though the same strain is used currently, due to different methods of maintenance in various vaccine laboratories, many substrains have evolved in past few decades.
  - In India, WHO recommended ***Danish 1331*** strain of BCG is used. It is prepared in Central BCG laboratory, Guindy, Chennai.
- **Reconstitution:** BCG is available in lyophilized form which should to be reconstituted in normal saline before administration. Distilled water is never used as it is irritant. Once reconstituted; it is administered within 1 hour.
- **Administration of BCG:**
  - *Dose and strength*: 0.1 ml containing 0.1 mgTU
  - Alcohol should not used to wipe the skin
  - *Site:* It is given above insertion of left deltoid
  - *Route:* By intradermal route by using a 26 gauge tuberculin syringe.
- **Phenomena after BCG:** If properly injected intradermally, then at inoculation site:
  - 6–12 weeks: Permanent tiny round scar (4–8 mm diameter) is formed
  - 8–14 weeks: Mantoux test becomes positive.

  If overdose is given: The lesion or scar becomes larger and irregular size.
- **Protection:**
  - *Efficacy:* Many trials have shown that BCG has a variable efficacy of 0–80%.
  - *Duration* of immunity lasts for 15–20 years
  - Though BCG may not protect from the risk of tuberculosis infection, but it surely gives protection to infants and young children against the more serious types of the disease, such as meningitis and disseminated tuberculosis.
- **Complications following BCG:**
  - *MC complications* include ulceration at the vaccination site and regional lymphadenitis
  - Rarely, keloid or lupus lesion, and osteomyelitis may develop
  - Very rarely, non-fatal meningitis and progressive tuberculosis, disseminated BCG infection ('BCGitis') are reported in people with low immunity.
- **Indication of BCG:**
  - *Direct BCG:* BCG is given to newborn soon after birth directly. This strategy is followed by most of the developing countries including India. If not given at birth it can be given later, maximum up to 2 years.
  - *Indirect BCG:* BCG is given after performing tuberculin test.
- **Contraindications to BCG:** HIV positive child, Child borne to AFB positive mother, Child with low immunity, Generalized eczema and Pregnancy
- **Other uses of BCG:**
  - BCG provides some protection against leprosy and leukemia
  - BCG has been tried as adjunctive therapy in malignancies such as bladder carcinoma (Onco TICE strain of BCG)
  - BCG may be superior to PPD for tuberculin test, as reported by some workers.

NONTUBERCULOUS MYCOBACTERIA

Non Tuberculous Mycobacteria (NTM) or atypical mycobacteria or Mycobacteria other than tubercle bacilli (MOTT) are isolated from birds, animals, soil and water. They are opportunistic pathogens in humans.

Table 3.6.1: Runyon's classification of non-Tuberculous mycobacteria (NTM)

Runyon group	Property	Species
I. Photochromogens	Produce pigments only in light	***MASK-*** *M. **m**arinum, M. **a**siaticum, M. **s**imiae, M. **k**ansasii*
II. Scotochromogens	Produce pigments even in dark, but intensity of color may increase on exposure to light	*M. scrofulaceum, M. szulgai, M. gordonae, M. celatum, M. flavescens*

Contd...

Contd...

Runyon group	Property	Species
III. Non-photochromogens	No Pigmentation	*M. avium-intracellulare complex (MAC), M. ulcerans, M. xenopi, M. paratuberculosis, M. malmoense*
IV. Rapid growers	Grow within one week	*M. chelonae, M. fortuitum, M. smegmatis, M. abscessus*

NTM differentiated from MTB complex by:

- Resistance to paranitrobenzoic acid (PNB), but sensitive to thiophen-2-carboxylic acid hydrazide (TCH)
- Aryl sulfatase test positive
- Strong Catalase positive
- Nonpathogenic for guinea pig; but pathogenic for mouse
- Resistant to antitubercular drugs.

Common NTM infections:
- *M.marinum:* Swimming pool granuloma or fish tank granuloma
- *M.ulcerans:* Buruli ulcer
- *M.fortuitum* and *M.chelonae* cause post-trauma injection abscess
- MAC: Causes opportunistic infection in HIV

Diseases Caused by Nontuberculous Mycobacterium

Photochromogens (Produce Pigments Only in Light)

- *M.marinum:* It causes the following infections
  - *Swimming pool granuloma* or *fish tank granuloma.*
  - Tendonitis and tender nodules – spread in a sporotrichoid pattern.
- *M.asiaticum* is rarely associated with pulmonary disease and bursitis.
- *M.simiae:* It gives a positive niacin test. It produces pulmonary lesions.
- *M.kansasii*: Causes chronic pulmonary disease resembling tuberculosis.

Scotochromogens (Produce Pigments in Light and Dark)

- *M.scrofulaceum* causes scrofula (cervical lymphadenitis) in children.
- *M.gordonae* is often found as commensal in tap water.
- *M. szulgai:* It may occasionally cause pulmonary disease and bursitis.
- *M.celatum* is a rare cause of pulmonary infection.

Nonphotochromogens (Do not Produce Pigments)

- *M. avium-intracellulare* complex (MAC):
  - They comprise of two related organisms: *M.avium* (Battey bacillus) and *M.intracellulare.*
  - They are opportunistic pathogens in HIV with low CD4 T cell count (< 50/µl).
  - Manifestations-lymphadenitis, respiratory infection and disseminated disease.
- *M.xenopi* has been isolated from hospital water supplies, and nosocomial outbreaks.
- *M.ulcerans* is a waterborne skin pathogen, found mainly in the **tropics** of Africa, America and Southeast Asia:
  - It is the agent of **Buruli ulcer**.
  - It can also cause osteomyelitis and limb deformities.
  - Exotoxin: It produces mycolactone toxin.
- *M.malmoense* can cause pulmonary disease and rarely lymphadenitis.
- *M.paratuberculosis* (Johne's bacillus) is associated with the pathogenesis of Crohn's disease, but this link has not been proved yet.

Rapid Growers (Grow within 1 Week of Incubation)

- *M.fortuitum* and *M.chelonae* cause **post-trauma injection abscess** and catheter infections
- *M.abscessus* can cause pulmonary infection
- Test for rapid growers: Arylsulfatase test—Positive for rapid growers.

Skin Lesions of Lepromatous Leprosy:
- Many, symmetrical
- Margin is irregular
- Appear as: Multiple nodules (lepromata), Plaques and Xanthoma-like papules
- Leonine facies and eyebrow alopecia

MYCOBACTERIUM LEPRAE

Mycobacterium leprae or Hansen's bacillus is the causative agent of leprosy, an ancient disease that remained as a social stigma over many years due to the superstitious beliefs and characteristic deformities produced in the patients.

Clinical Manifestations and Classification

Leprosy primarily involves cooler parts but is capable of affecting any tissue or organs causing bony deformities and disfigurements in untreated cases.

Incubation period: Leprosy has a long incubation period, an average of 3–5 years.

- This can be explained due to the longer generation time of lepra bacilli (12–13 days)
- Lepromatous leprosy (LL) cases have longer incubation period than tuberculoid (TT).

Table 3.6.2: Clinical classification of leprosy

Ridley Jopling classification	Madrid classification	Indian classification
Lepromatous leprosy (LL)	Lepromatous type	Lepromatous type
Borderline Lepromatous leprosy (BL)	Borderline	Borderline
Borderline leprosy (BB)	Indeterminate type	Indeterminate type
Borderline Tuberculoid leprosy (BT)	Tuberculoid type	Pure neurotic type
Tuberculoid leprosy (TT)	-	Tuberculoid type

Slik Lesions of Tuberculoid Leprosy:
- One or few, asymmetrical
- Margin is sharp
- Appear as: Hypopigmented, annular macules with elevated borders
- Tendency towards central clearing

Leprosy is a bipolar disease. Under any classification scheme, lepromatous and tuberculoid cases are the two extreme poles of the disease (described in table). Other varieties are:

- **Borderline type:** It is seen in patients possessing characteristics in between tuberculoid and lepromatous types. They may shift to either type depending on chemotherapy or alterations in the host resistance.
- **The indeterminate type:** This denotes those early unstable cases with one or two hypopigmented macules and definite sensory impairment. Bacteriologically negative.
- **Pure neuritic type:** Shows neural involvement without any skin lesion. Cases are bacteriologically negative.

Characters	Lepromatous leprosy (LL)	Tuberculoid leprosy (TT)
Bacillary load	Multibacillary	Paucibacillary
Bacteriological index	4–6+	0–1+
Skin lesions	Many, symmetrical, Margin is irregular, Lesions appear as Multiple nodules (lepromata), Plaques and Xanthoma-like papules, Leonine facies and eyebrow alopecia	One or few, asymmetrical, Margin is sharp, Lesions appear as, Hypopigmented, annular macules with elevated borders, Tendency towards central clearing
Nerve lesion	Appear late Hypoesthesia is a late sign	Early anesthetic skin lesion, Enlarged thickened nerves (MC nerves involved are ulnar nerve followed by post auricular nerve), Nerve abscess seen (common in BT)
Cell mediated immunity	CMI low	CMI normal
Lepromin test	Negative	Positive
Lymphocyte transformation test	Negative	Positive
CD4/CD8 T cell ratio	1:2	2:1 (normal)
Humoral immunity	Exaggerated	Normal
Auto Antibodies	Elevated	Not seen
VDRL test	Biological false positive	VDRL test negative
Antibodies to PGL-1	Elevated in 95% of cases	Elevated in 60% of cases
Macrophages	Foamy type (lipid laden)	Epithelioid type
Langhans giant cells	Not seen	Found

Deformities

About 25% of untreated cases develop deformities in due course which may arise due to:

- Nerve injury leading to muscle weakness or paralysis or
- Disease process (facial deformities or loss of eye brow) or
- Infection or injury (ulcers).

Common deformities include:

- Face: Leonine facies, sagging face, loss of eye brow/eye lashes and corneal ulcers.
- Hands: Claw hand and wrist drop
- Feet: Foot drop, clawing of toes, inversion of foot, and plantar ulcers.

Lepra Reaction Type I:
- If occurs before treatment: Progresses towards LL (down grading reaction)
- If occurs after treatment: Progress towards TT (reversal reaction)

Lepra Reactions

Though leprosy runs as a chronic disease, several allergic type acute exacerbations occur throughout its course, called as lepra reactions which are of two types: I and II.

Characters	Lepra Reaction Type I	Lepra Reaction Type II
Hyper sensitivity reaction	Type IV (delayed hyper sensitivity)	Type III (immune complex mediated)
Seen with	Borderline leprosy	Lepromatous variety (BL,LL)
Manifests as	Inflammation of previous lesions, new skin lesions and neuritis	Crops of painful erythematous papules which become nodular
Progresses as	If occurs before treatment—Progresses towards LL (*down grading reaction)* If occurs after treatment—Progress towards TT (*reversal reaction*)	It usually occurs following the start of chemotherapy.
T helper response	T_H1 predominates	T_H2 predominates
Other organs	Usually not affected	Eyes, testes and kidney are affected
Treatment	Glucocorticoid	Glucocorticoid, thalidomide, clofazimine

Epidemiology

- **Source of infection:** Multibacillary (LL and BL) cases are the most important source.
- **Mode of transmission:** Nasal droplet infection (MC) > Contact transmission > breast milk, vertical mode, tattooing
- **Communicability:** Leprosy is not highly communicable. Intimate and prolonged contact is necessary.

Leprosy elimination level:
- Leprosy elimination level: < 1/10,000 population
- Prevalence in India: 0.68 cases per 10,000 population
- Chhattisgarh and Dadra and Nagar Haveli are above the level of leprosy elimination

Geographical Distribution

Currently leprosy is almost exclusively confined to the developing nations of Asia, Africa, Latin America, and Pacific.

India accounts for maximum cases globally. However, the disease burden is declining:

- The prevalence rate in India is about **0.68 cases per 10,000 population** in 2012.
- About 32 states/Union Territory (UT) have already achieved the level of leprosy elimination i.e. prevalence rate of less than 1 case per 10,000 population.
- Chhattisgarh and Dadra and Nagar Haveli are the only state/UT in which it remains above the level of leprosy elimination. Bihar lies at the borderline.

Laboratory Diagnosis

Smear Microscopy

Smear microscopy is done to demonstrate acid fast bacilli in the lesions:

- Total six samples are collected; four from skin (forehead, cheek, chin and buttock), ear lobe and nasal mucosa
- The **edge of the lesion** is the preferred site.
- **Slit skin smear** is the technique followed to collect the skin and ear lobe specimens.
- Nasal specimens are collected by nasal blow, ii) nasal scraping
- **Biopsy** from the thickened nerves and nodular lesions may be necessary in some cases.
- **Acid fast staining is done** by Ziehl-Neelsen technique by using 5% sulfuric acid.
- **Appearance:** Under oil immersion objective, red acid fast bacilli are seen, arranged singly or in groups, bound together by lipid-like substance, the *glia* to form **globi** (called

Smear microscopy:
- Site: Edge of the lesion
- Method: Slit skin smear
- Appearance: Cigar bundles of AFB arranged in globi, which are present in foamy macrophages called Virchow's 'lepra cells'

as **cigar bundle** appearance). The globi are present inside the foamy macrophages called as **Virchow's 'lepra cells' or 'foamy cells':**
  - Live bacilli will be uniformly stained with parallel sides and round ends and length is five times the width.
  - Dead bacilli are less uniformly stained and have *fragmented and granular* appearance.
- **Grading of the smear** is done by:
  - *Bacteriological index (BI)* is based on the total number of bacilli (live and dead) seen per oil immersion field.
  - *Morphological index (MI):* Percentage of uniformly stained bacilli out of the total number of bacilli counted. MI is a better marker to monitor the treatment response.
  - *SFG percentage* (solid, fragmented granular rod percentage).

Mouse Foot Pad Cultivation

M.leprae is not cultivable either in artificial culture media or in tissue culture. The only certain way to cultivate *M.leprae* is by inoculating the specimens into:
- Nine banded armadillo and
- Foot pad of mice.

Antibody Detection in Leprosy:
- FLA-ABS
- ELISA detecting Ab to PGL-1

Antibody Detection

- **FLA-ABS** (Fluorescent Leprosy Antibody Absorption test)
- **ELISA** detecting IgM antibodies to PGL-1 (phenolic glycolipid-1) antigen of *M.leprae*

Test for Detecting CMI (Lepromin Test)

Lepromin test demonstrates the delayed hypersensitivity reaction and an intact CMI against the lepra antigen.

Uses of Lepromin Test:
- Classifying lesions of leprosy
- Assessing prognosis
- Assessing resistance to leprosy in individuals

- **Procedure:** 0.1 ml of lepromin antigen is given intradermally to forearm and reading is taken twice; at 48 hrs and 21 days.
- **Early reading or Fernandez reaction** at 48 hrs: Induration and erythema (red area) of > 10 mm diameter indicates delayed hypersensitivity reaction and hence past exposure to lepra bacilli. However, it does not indicate active infection.
- **Late reading** or **Mitsuda reaction** at 21 days: Nodule of > 5 mm size formed at the site of inoculation which ulcerates later on. It indicates that the patient's CMI is intact.
- **Uses of Lepromin test:** The late reaction measures the CMI; hence can be used for:
  - *Classifying lesions of leprosy:* In TT patients with intact CMI, the test is strongly positive. In LL patients; the test is negative indicating a low CMI.
  - *Assessing prognosis:* Intact CMI (as in TT patients) indicates good prognosis.
  - *Assessing resistance to leprosy in individuals:* Lepromin negative persons are at higher risk of developing multibacillary leprosy than lepromin positive persons.

Treatment

Because of risk of developing resistance, WHO recommends multidrug therapy (MDT) for treatment of leprosy:
- Recommended drugs: Dapsone, rifampicin and clofazimine
- Alternate drugs: Ethionamide, quinolones (ofloxacin), minocycline and clarithromycin
- WHO treatment regimens are administered based on the clinical type of leprosy.

Table 3.6.3: Clinical classification of leprosy and WHO treatment regimens

Criteria	Paucibacillary leprosy	Multibacillary leprosy
Skin lesions	1–5	6 or more
Nerve involvement	1	2 or more
Microscopy	Smear negative	Smear positive

Contd...

Contd...

Criteria	Paucibacillary leprosy	Multibacillary leprosy
Leprosy type	TT, BT and Intermediate	BB,BL, LL
Treatment regimen	1. Dapsone (100 mg) given daily, self-administered 2. Rifampicin (600 mg) given once a month under supervision	1. Dapsone (100 mg) given daily 2. Rifampicin (600 mg) given once a month 3. Clofazimine–300 mg once a month under supervision, followed by 50 mg daily, self-administered
Duration of treatment	Up to 6 months	Upto 1 yr or till smear negative
Follow up	Annually till 2 yrs	Annually till 5 yrs

Prevention of Leprosy

- Active case finding and effective treatment of cases is the most important measure.
- **BCG Vaccine:** Trials using BCG had shown efficacy of 28–60%.
- **Chemoprophylaxis:** Dapsone can be given to contacts of TT patients (but not LL patients)
- Hospitalized patients need not be isolated as transmission requires prolonged contact.

Leprosy Elimination

National Leprosy Eradication programme in India aims at two major components:

- Elimination of leprosy (already achieved in India except in Chattishgarh, Dadra and Nagar Haveli)
- Decentralize the services through general health care system.

Though India has achieved the elimination level (< 1 case per 10,000 population), still India accounts for the highest number of leprosy cases worldwide. Hence, the long-term aim is to eradicate leprosy.

However eradication of leprosy is difficult because of:

- Long and variable Incubation period
- Disputed mode of transmission
- More subclinical cases
- Low immunity in patients with LL
- Absence of effective vaccine
- Bacterial resistance
- Complicated spectrum of disease
- Poor patient compliance because of longer duration of treatment
- Social issues

The national institutes of leprosy are:

- National **JALMA** Institute of Leprosy and other Mycobacterial Diseases, Agra.
- **CLTRI** (Central Leprosy Training and Research Institute), Chengalpattu, Tamil Nadu

National Institutes of Leprosy:
- National JALMA Institute of Leprosy and other Mycobacterial Diseases, Agra.
- CLTRI (Central Leprosy Training and Research Institute), Chengalpattu, Tamil Nadu

MULTIPLE CHOICE QUESTIONS

MYCOBACTERIUM TUBERCULOSIS

1. Two sputum samples of TB suspect given one at spot and other in morning are labelled as: *(AIIMS MAY 2016)*
a. A B
b. 1 2
c. Alpha beta
d. Y Z

2. Pleural fluid from a suspected patient of TB is sent to lab. This cannot be used for measuring which of the following parameter? *(AIIMS MAY 2016)*
a. Gene Xpert
b. LDH
c. Albumin
d. ADA

3. Which of the following stains is/are used for detection of M. tuberculosis? *(PGI May 2016)*
a. Auramine
b. ZN
c. Gram stain
d. Giemsa stain
e. Albert's stain

4. Agent used in interferon gamma release assay for diagnosing latent tuberculosis is: *(JIPMER Nov 2014)*
a. MPT 64
b. Mycolic acid
c. Lipoarabinomannan
d. ESAT-6

5. Xpert MTB/RIF test is/are used for: *(PGI May 2013)*
a. For assessing resistance to isoniazid
b. For assessing multi drug resistant TB
c. For assessing rifampicin resistance
d. Monitoring drug response in MDR TB
e. Diagnosis of TB

6. RNTCP diagnosis is based on: *(AIIMS Nov 2014)*
a. Sputum microscopy
b. Chest X-ray
c. LJ culture

7. Mycobacteria can be stained by: *(PGI Nov 2014)*
a. Ziehlneelsen stain
b. Kinyoun stain
c. Auramine rhodamine
d. GMS (gomori methanamine stain)
e. Albert stain

8. XDRTB is defined as: *(PGI Nov 2014)*
a. Resistant to Amikacin + Ofloxacin
b. Resistant to INH + Rifampicin
c. Resistant to INH + Rifampicin + Amikacin
d. Resistant to Rifampicin + Amikacin + Ofloxacin
e. Resistant to INH + Rifampicin + Amikacin + Ofloxacin

9. Which is used in digestion and decontamination of sputum in smear preparation? *(PGI May 2013)*
a. NaOH
b. KOH
c. NaCl
d. KCl
e. N-acetyl-L-cysteine

10. Most appropriate method to assess the incidence of tuberculosis in community: *(AIIMS Nov 05, AI 07, AI 04, DNB 07)*
a. Identify all positives to Tuberculin test
b. Sputum examination of symptomatic patients
c. Identify new converter to tuberculin test
d. Screen all under 5r - for tuberculin test

11. National Institute of Tuberculosis located in:
a. Chennai *(AIIMS Nov 03, DNB 03)*
b. Bangalore
c. Delhi
d. Chengalpettu

12. Prevalence of tuberculosis in India: *(Orissa 2011, Kolkata 04)*
a. 25% b. 30%
c. 40% d. 50%

13. Best criteria for tuberculosis diagnosis by WHO:
a. Sputum examination *(Bihar 05, DNB 01)*
b. Chest pain
c. X-ray finding
d. Mantoux positive

14. True about tuberculosis: *(MP 2000, MH 2002)*
a. Smear positive- requires > 10,000 bacilli/ml
b. Mantoux test can differentiate recent and past infection
c. Can grow on ordinary media
d. Drug sensitivity test- by disk diffusion

15. In India, a tubercular mother is advised for: *(RJ 2003)*
a. BCG to baby
b. ATT to mother
c. With hold breastfeeding
d. Separated from mother

16. Multidrug therapy is given for tuberculosis because.
a. To delay development of resistance *(MP 2002)*
b. To reduce toxicity
c. To broaden antimicrobial spectrum
d. To prevent toxin release from the organism

17. Which of the following statements is true about BCG vaccination? *(AIIMS May 2011)*
a. Distilled water or normal saline is used as diluents for BCG vaccine
b. The site for injection should be cleaned thoroughly with spirit
c. Tuberculin test is positive after 6 weeks of vaccination
d. WHO recommends Danish 1331 strain for vaccine production

18. Analysis of the IFN-gamma responses of whole blood cells from BCG-vaccinated or non-BCG-vaccinated donors or patients with tuberculosis, stimulated with PPD, ESAT-6 or CFP-10 antigens, and evaluation of the specificity and sensitivity of the test INF-gamma assay in TB is done. Which of the following is not true? *(AI 2012)*
a. 1st generation uses ESAT
b. 2nd generation uses ESAT and CFP-10
c. ESAT and CFP-10 cannot differentiate between mycobacteria and other atypical mycobacteria
d. Replacing the PPD test for screening of tuberculosis.

19. Acid fast organism(s) is/are: *(SG PGI 2003, APPG 2011, PGI May 2012)*
a. Atypical mycobacteria
b. Rickttesia
c. Nocardia
d. Chlamydia

20. Method of testing resistance to drugs in TB are all expect: *(AIIMS May 2004, DNB Dec 2010)*
a. Radiometric broth method
b. Molecular method
c. Disk diffusion method
d. PCR

21. Sterile pyuria is present in: *(AI 2011)*
a. Renal tuberculosis
b. Chronic hydronephrosis
c. Wilm's tumor
d. Neuroblastoma

22. The best diagnostic procedure of M. tuberculosis:
a. PCR *(SG PGI 2008)*
b. Auramin rhodamine stain
c. Sputum culture
d. ESR

23. True about Mantoux test: *(PGI June 2003)*
a. < 5 cm always +ve
b. Usually –ve after treatment
c. Positive reaction in children < 2 yrs is not important than adult
d. Usually read after 48–72 hours
e. False +ve in post measles state

24. True regarding Mycobacterium tuberculosis is:
a. Produces visible colonies in 1 weeks time on Lowenstein–Jensen media *(PGI June 2002)*
b. Decolorized by 20% sulfuric acid
c. Facultative aerobe
d. Niacin positive

25. Commonest mycobacterial infection in patient residing in tropical countries: *(PGI 2002)*
a. M. leprae
b. M. avium intracellulare
c. M.tuberculosis
d. M. kansasii

26. Basanti, 29-year-aged female from Bihar present with active TB. She delivers baby. All of the following are indicated except: *(AI 2001)*
a. Administer INH to baby
b. Withhold breastfeeding
c. Give ATT to mother for 2 years
d. Ask mother to ensure proper disposal of sputum

27. Collection of urine sample of a patient of TB/kidney:
a. 24 hrs urine *(AIIMS June 2000)*
b. 12 hrs urine
c. In early morning
d. Any time

28. Tuberculin test is done for: *(AI 2000)*
a. Previous or present sensitization to tuberculous protein
b. Patient is resistant to TB
c. Patient is susceptible to TB
d. Individual is suffering from TB

29. Not easily cultivable but well viable and used in epidemiology are/is: *(PGI Dec 2000)*
a. Staph
b. Mycobacterium tuberculosis
c. E. coli
d. Salmonella

30. The tuberculosis bacilli was discovered by: *(TN 2004)*
a. Robert Koch
b. Edward Jenner
c. Louis Pasteur
d. Jonas Salk

NONTUBERCULOUS MYCOBACTERIUM

31. Pigment producing atypical mycobacteria:
a. M. fortuitum and M. chelonae
b. M. gordonae and M. szulgai *(NEET Pattern Based)*
c. M. xenopi and MAC
d. M. ulcerans

32. Fish tank granuloma is caused by: *(MHPG 2015, NEET Pattern Based, PGI June 2011)*
a. M. kansasi
b. M.marinum
c. M.paratuberculosis
d. M.gordonae
e. M.scrofulaceum

33. Rapidly growing Atypical organism involved in lung infection: *(AIIMS May 2013)*
a. M. chelonae
b. M. fortuitum
c. M. abscessus
d. M. kansasi

34. Which is not a mycobacteria tuberculosis complex organism? *(NEET Pattern Based, DNB June 2010)*
a. M. africanum
b. M. tuberculosis
c. M. bovis
d. M. kansasii

35. Which one of the following statement is true regarding pathogenicity of Mycobacteria species? *(AI 2006)*
a. M. tuberculosis is more pathogenic than M. bovis to the humans
b. M. kansasii can cause a disease indistinguishable from tuberculosis
c. M. africanum infection is acquired from the environmental source
d. M. marinum is responsible for tubercular lymphadenopathy

36. Which of the following is known as Battey bacillus? *(Recent Questions 2014)*
a. Mycobacterium intracellulare
b. Mycobacterium tuberculosis
c. Mycobacterium leprae
d. Mycobacterium kansaai

M. LEPRAE

37. At the end of paucibacillary multidrug therapy for 6 months, if the skin lesions (persistence erythema and induration in the plaque) persists, then what is the next step according to WHO guidelines?
a. Stop antileprosy treatment *(AIIMS May 01)*
b. Continue same treatment till erythema subsides
c. Biopsy of the lesion to document activity
d. Continue dapsone alone for 6 month more

38. False about leprosy: *(AI 04)*
a. Multi bacillary leprosy: > 5 lesions
b. New case detection rate: Indicator of incidence
c. Target elimination of leprosy: Prevalence < 1/10,000 population
d. Defaulter: Not taken treatment for > 6 months

39. ENL occurs in: *(NEET Pattern Based, Karnataka 07)*
a. Due to lepromin test
b. Due to multi drug therapy
c. In LL patients
d. In TT patients

40. True about leprosy in India: *(PGI Dec 08)*
a. Lepra bacilli: Cannot survive outside humans
b. Bacteria load: High in TT
c. Insect can transmit
d. Relapse rate: Indicator of drug efficacy
e. If prevalence is high in childhood: It means disease is under control

41. False about lepromin test: *(AIIMS 2006, AIIMS 2007, AIIMS May 2010)*
a. Negative in children < 6 month age
b. It is a diagnostic test
c. Used to classify leprosy
d. BCG vaccine may convert a negative lepromin test to positive

42. Mitsuda reaction is read at: *(DNB 04, Orissa 08)*
a. 3rd day
b. 10th day
c. 21st day
d. 45th day

43. In multibacillary leprosy, after the treatment, follow up is done yearly for: *(DNB 08)*
a. 3 years
b. 5 years
c. 10 years
d. 2 years

44. Treatment of leprosy according to WHO is done by all except: *(Bihar 05)*
a. Dapsone
b. Ciprofloxacin
c. Clofazemine
d. Rifampicin

45. In paucibacillary leprosy, dapsone is continued for: *(UP 08, RJ 07)*
a. 6 months
b. 9 months
c. 12 months
d. 3 months

46. Lepromin test is valuable for: *(MP 09, AIIMS 2006, AI 2002, MP 01, MP 05)*
a. Diagnosis
b. Response to treatment
c. Epidemiological reason
d. To test humoral immunity

47. Bacteriological index of 1+ indicates: *(UP 2004, MP 03)*
a. < 100 bacilli/Oil immersion field
b. 1–10 bacilli/100 oil immersion field
c. No Bacilli in all fields
d. Bacilli in all fields

48. Which is NOT included in Madrid classification but included in indian classification? *(MH 03)*
a. Inderminate leprosy
b. Borderline leprosy
c. Tuberculoid leprosy
d. Pure neuritic type leprosy

49. Multibacillary leprosy, the bacteriological index is more than: *(RJ 05)*
a. 1
b. 2
c. 3
d. 4

50. Erythema nodosum leprosum (ENL) occur in:
a. Borderline leprosy *(PGI June 2011)*
b. Lepromatous leprosy
c. Indeterminate type
d. Histoid leprosy
e. Tuberculoid type

51. Leprosy affects all except: *(AI 2007)*
a. Testes
b. Ovary
c. Eye
d. Nerve

52. The following drug is used for the treatment of type II lepra reaction, except: *(AIIMS 06)*
a. Chloroquine
b. Thalidomide
c. Cyclosporine
d. Corticosteroid

53. Which of the following is true regarding globi in a patient with Lepromatous leprosy? *(AI 2002)*
a. Consists of lipid laden macrophages
b. Consists of macrophages filled with AFB
c. Consists of neutrophils filled with bacteria
d. Consists of activated lymphocytes

54. Exacerbation of lesions in patients of borderline leprosy is seen in: *(PGI June 2001)*
a. ENL (erythema nodosum leprosum)
b. Lepra reaction type I
c. Jarisch–Herxheimer reaction
d. Resolving leprosy
e. Rifampicin and minocycline for 6 months

55. Lupus vulgaris is caused by: *(Recent Question 2013)*
a. M tuberculosis
b. M lepare
c. M ulcerans
d. M marinum

56. Criteria for diagnosing multibacillary leprosy include(s): *(PGI May 2013)*
a. 2-6 skin lesion
b. Skin smear 1+
c. Skin smear 2+
d. Deformity ±
e. Eye lesion +

57. All are true statement regarding leprosy except: *(PGI Nov 2012)*
a. Multibacillary leprosy means person having 6 or more skin lesions
b. Regular MDT means patients received 2/3rd of months of treatment schedule
c. In paucibacillary leprosy > 2 nerves are involved
d. Loss of sensation may present
e. Lepra reaction if not treated can leads to permanent deformities

58. Leprae bacilli doubling time: *(TNPG 2014)*
a. 20 hours
b. 20 minutes
c. 12 days

59. Satellite papules are characteristic of following type of leprosy: *(MHPG 2014)*
a. Borderline Tuberculoid (BT)
b. Polar Tuberculoid (TT)
c. Borderline Lepromatous (BL)
d. Polar Lepromatous (LL)

EXPLANATIONS

MYCOBACTERIUM TUBERCULOSIS

1. **Ans: (a) (A, B)** Ref: RNTCP manual for laboratory technicians
   - **The two sputum specimens (early morning and spot) are labelled as A and B**

2. **Ans (a) (Gene Xpert)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p278 https://www.ncbi.nlm.nih.gov/books/NBK254320/

   For Gene Xpert; pleural biopsy is the preferred specimen. Pleural fluid is suboptimal specimen.

3. **Ans (a, b) (Auramine, ZN)** (Ref: Apurba Sastry's Essentials of Medical Microbiology/p276
   - Auramine and ZN stains are used for detection of M. tuberculosis.
   - Though M. tuberculosis is gram-positive, it is not usually done for diagnosis.

4. **Ans (d) (ESAT-6)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p279
   - **Interferon Gamma Release Assay (IGRA) uses highly specific M. tuberculosis antigens such as CFP10 (culture filtrate protein) and ESAT6 (early secreted antigenic target-6).**

5. **Ans. (c), (e) (For assessing rifampicin resistance, Diagnosis of TB)** Ref: CDC website

   The Xpert MTB/RIF is a cartridge-based, fully automated diagnostic test that can:
   - Identify Mycobacterium tuberculosis DNA and resistance to rifampicin (RIF) simultaneously
   - It works on the principle of PCR i.e. Only detects the DNA but cannot quantify the DNA, hence NOT suitable for monitoring disease progression. Real time PCR is the best for this purpose.
   - Provides accurate results in less than two hours so that early treatment can be started
   - Has minimal bio-safety requirements, training, and can be done in any laboratories.

6. **Ans. (a) (Sputum...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p276, Park 23/e p182, 22/e p170

   Sputum smear microscopy is the recommended method for the diagnosis of tuberculosis under RNTCP.

7. **Ans. (a), (b), (c) (Ziehl Neelsen, Kinyoun Stain, Auramine rhodamine)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p276, Ananthanarayan 9/e p353

   Mycobacterium tuberculosis being acid fast can be stained by acid fast staining:
   - Ziehl-Neelsen staining (hot method) or its modifications such as:
     - Kinyoun's stain (cold acid fast staining) or
     - Gabbett's staining method
   - Fluorescent staining such as Auramine rhodamine staining method.

8. **Ans. (e) (Resistant to INH...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p281, Ananthnarayan 9/e p357

   XDRTB is defined as at least MDR TB (i.e. rifampicin + isoniazid) + Resistant to one fluoroquinolone (ofloxacin) + one injectable second line aminoglycosides (Amikacin or kanamycin or capreomycin).

9. **Ans. (a) (e) (NaOH, N-acetyl-L-cysteine)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p275, Ananthnarayan 9/e p352

   Sputum and specimens from non-sterile sites need prior treatment for digestion (to liquefy the thick pus cells and homogenization) and decontamination (to inhibit the normal flora) and concentration (to increase the yield).
   - Petroff's method (4% NaOH): Most commonly followed
   - NALC (N-acetyl-cysteine) + 2% NaOH: This is superior to Petroff's method for isolation. NALC liquefies the sputum and NaOH kills the normal flora. This method is better compatible with automated culture systems.
   - If for only smear microscopy, then formalin or hypochlorite can also be used as mucolytic and for killing the bacilli. However, they are not useful for culture or animal pathogenicity.

10. **Ans. (c) (Identify new converter to tuberculin test)** Ref: Park 22/e p168, , 23/e p185
    - Identify all positives to Tuberculin test: Marker of prevalence of tuberculosis
    - Sputum examination of symptomatic patients by Zn stain: Method of choice case finding tool for tuberculosis under RNTCP.
    - Identify new converter to tuberculin test: Marker of incidence of tuberculosis.

11. Ans. (b) (Bangalore) Ref: Internet source

Important National centers for Tuberculosis in India:

- Tuberculosis research centre: Chennai
- National institute of tuberculosis: Bangalore
- L R S Institute of Tuberculosis and Respiratory Diseases: Delhi
- JALMA Institute for Leprosy and Other Mycobacterial diseases: Agra.

12. Ans. (c) (40%) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p275, Park 22/e p167

- Prevalence of tuberculosis infection in India: 40% (i.e. 2 out of five Indians are Mantoux positive)
- In June 2011, the revised estimate of tuberculosis in India:
  - Prevalence tuberculosis cases in India – 256 cases/100,000 population
  - Incidence tuberculosis cases in India - 185 cases /100,000 population.

13. Ans. (a) (Sputum...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p276, Park 23/e p182, 22/e p170

- Sputum examination of symptomatic patients by Zn stain: It is case finding tool for tuberculosis under RNTCP.

14. Ans. (a) (Smear positive...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p276, Park 23/e p182, 22/e p170

- For smear to be acid fast stain positive: It requires > 10,000 bacilli/ml
- Mantoux test cannot differentiate recent and past infection
- M.tuberculosis is fastidious, it cannot grow on ordinary media
- Disk diffusion test is NOT useful for drug sensitivity testing of M.tuberculosis.

15. Ans. (b) (ATT to mother) Ref: Park 22/e p172, 21/e p168

- After delivery: If mother chest X-ray and sputum AFB +ve then

3 Dos	3 Do nots
Mother to be given ATT(HRE)	Do not separate the baby from mother
Baby (INH for 9-12 months)	Do not with hold of breastfeeding
Screening of household contacts	Do not give to the baby BCG

16. Ans. (a) (To delay...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p281, Park 23/e p192, 22/e p171-72

- Resistance to anti-tubercular drug is mainly due to mutational drug resistance which can be overcome by combination of drugs.

17. Ans. (d) (WHO recommends Danish 1331 strain for vaccine production) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p281, Park's 23/e p197, 22/e p178-79

- WHO recommends Danish 1331 strain for vaccine production (M.bovis). In India, it is prepared in Guindy, Chennai
- Normal saline is recommended as diluents for BCG vaccine as distilled water is irritant
- The site for injection should be cleaned thoroughly with soap but disinfectant or antiseptic should not be used. If alcohol is used then it should be evaporated before the vaccination is given
- Tuberculin test is positive after 8 weeks of BCG vaccination but in some it might require 14 weeks.

18. Ans. (c) (ESAT and CFP-10...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p279, Harrison 18/e p1351

- *'IFN-γ Release Assays (IGRAs) are more specific than the Tuberculin Skin Testing (TST) as a result of less cross-reactivity due to BCG vaccination and sensitization by nontuberculous mycobacteria.'*

For details of diagnosis of latent tuberculosis- refer chapter review.

19. Ans. (a) (c) (Atypical mycobacteria, Nocardia) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p16

Refer chapter review- for the list of acid fast organisms.

20. Ans. (c) (Disk...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p280, Ananthanarayan 9/e p354

- Disk diffusion method is used for drug susceptibility testing for most of the bacteria; however, it is not used for M.tuberculosis.
- Various methods used for drug susceptibility testing for M.tuberculosis: Refer chapter review for detail.

21. Ans. (a) (Renal tuberculosis) Ref: Harrison 18/e p1347, 17/e p1011

'The documentation of culture-negative pyuria in acidic urine raises the suspicion of tuberculosis'

22. Ans. (c) (Sputum culture) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p277-78

- Culture is the gold standard for diagnosis of tuberculosis with detection thresh hold of 10–100 bacilli/ml.
- Also, it detects the viability of the organism.
- It is more sensitive than acid fast smear and approaches 100% specificity.

About Other Options:

- PCR though can detect even 1 bacilli/ml, but it is not the gold standard because:
  - False –ve PCR might occur by presence of PCR inhibitors in sample or
  - False +ve PCR might occur due to contamination during the procedure
  - More so it can not detect the viability of the organism.
- However PCR is useful in:
  - Extrapulmonary tuberculosis (where Culture is less sensitive)
  - For rapid diagnosis along with rapid drug sensitivity detection.
- Staining (ZN and Auramin rhodamine stain) though it is rapid, but sensitivity is low (detection limit of 10^4 bacilli/ml.
- ESR is non specific, can be elevated in no. of condition.

23. Ans. (d) (Usually read after 48–72 hours) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p279, Ananthanarayan 9/e p354-55, Park 23/e p185, 22/e p172-73

- ***Option a:*** Mantoux test: Induration < 5 mm- always –ve
- ***Option b:*** Does not always turn –ve after treatment
- ***Option c:*** Mantoux test is the indicator of Active infection in infants
- ***Option e:*** Mantoux test becomes false -ve in post measles state
  'A positive tuberculin test may occasionally revert –ve after INH' Park 22/e p172, 20/e p164
- Diagnosis of latent tuberculosis- refer chapter review for explanation.

24. Ans. (d) (Niacin positive) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p277-78, Ananthanarayan 9/e p347

- Human strains of M.tuberculosis is Niacin +ve where as no other Mycobacterium are +ve. (Except M. simiae, few strains of M. chelonae.)

About Other Options:

- ***Option a:*** Produces visible colonies in 6–8 weeks time on Lowenstein–Jensen media
- ***Option b:*** Resists Decolorization by 20% sulfuric acid (Acid fast)
- ***Option c:*** M. tuberculosis is strict aerobe while M. bovis is Microaerophilic.

25. Ans. (c) (M.tuberculosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p275, Park 23/e p176, 22/e p166

- In tropical country, the most common Mycobacterial infection is due to M.tuberculosis.
- India ranks first globally accounting for 20% of total tuberculosis cases of the world.

26. Ans. (b) (Withhold breastfeeding) Ref: Nelsen 17/e p971

- If mother chest X-ray and sputum AFB +ve then, ***'mother to be given ATT(HRE) + baby (INH for 9–12 m) + screening of household contacts'***
- *'Separation from mother or with hold of breastfeeding is not recommended'.*

27. Ans. (c) (In early morning) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p278

'The 1st urine passed on the day i.e. Early morning sample is recommended for tuberculosis of urinary tract.'

- *Most commonly collected Sample for UTI: Midstream urine*
- *Best Sample for UTI: supra pubic aspiration*
- *Sample for UTI for infant: supra pubic aspiration*
- *Best Sample when urethritis or prostatitis is suspected: Initial flow of urine*
- *If delay is expected to process, then: Store by refrigeration at 4°c or add boric acid 1.8%.*

28. Ans. (a) (Previous...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p279, Park 23/e p185, 22/e p172

Positive tuberculin test indicates:

- Active infection in infants (suffering from TB)
- Prevalence of infection
- Past exposure to TB bacilli in adult (Previous or present sensitization to tuberculous protein)

Option b and c: *'Tuberculin test does not indicate susceptibility or resistance to TB where as Lepromin test is used to assess individual resistance to Leprosy'.*

29. Ans. (b) (M.tuberculosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p276, Ananthanarayan 9/e p346, 353
M.tuberculosis is slow growing takes 4–8 weeks of time to grow in culture.
Other options: Staph, E.coli and Salmonella can be grown easily with in 24 hour.
Also know: Generation time of '*E.coli is 20 min, M.tuberculosis is 20 hour and M. leprae is 20 days.*'

30. Ans. (a) (Robert...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p270, Ananthanarayan 9/e p345, 8/e p3477
'Robert Koch in 1882 isolated mammalian tubercle bacillus and proved its causative role in tuberculosis by satisfying Koch's postulate where as: Lepra bacillus was 1st described by Hansen.'

NONTUBERCULOUS MYCOBACTERIUM

31. Ans. (b) (M. gordona...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p283, Ananthanarayan 9/e p360
Pigment producing atypical mycobacteria:
- **Photochromogen:** *M marinum, M simiae, M. asciaticum, M kansasii,*
- **Scotochromogen:** *M. scrofulaceum, M szulgai, S.gordonae.*

32. Ans. (b) (M. mar...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p283, Ananthanarayan 9/e p360, 8/e p359
- *M. marinum (e.g. of Photochromogen) is originally isolated from fish is the causative agent of swimming pool or fish tank granuloma.*
- *This condition is associated with development of superficial granulomatous lesions in the skin.*

33. Ans. (c), (M.abscessus), Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p283, Harrison 18/e p1369
- *Any of the rapidly growing Mycobacteria such as M chelonae, M fortuitum and M. abscesses can cause pulmonary infection but infection with M. Abscessus may be particularly dangerous*
- *M. kansasii* ..... Belongs to photochromogen:
  - Can cause a clinical syndrome that strongly resembles tuberculosis, consisting of hemoptysis, chest pain, and cavitary lung disease.
- MAC: Most Common Causes of pulmonary nontuberculous mycobacterial infection.
- The rapidly growing NTM, *M chelonae, M fortuitum and M. abscessus*, acquired via skin contamination from surgical instruments (especially in cosmetic surgery), injections, and other procedures. These infections are typically accompanied by painful, erythematous, draining subcutaneous nodules, usually without associated fever or systemic symptoms.

34. Ans. (d) (M. Kansasii) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p283, Ananthanarayan 9/e p354, 359
M. tuberculosis complex include following species:
- M. tuberculosis
- M. bovis (bovine tubercle bacillus)
- M. africanum
- M. microti (vole tubercle bacillus)

35. Ans. (b) (Mycobacterium...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p283, Harrison 18/e p1369
- "*Mycobacterium kansasii produce human disease indistinguishable from tuberculosis*"
- *M. kansasii i* is the most pathogenic non tuberculous mycobacterial species affecting the lung, and the clinical features of *M. kansasii* disease resemble those of tuberculosis. Harrison 17/e p1030
- NTM causing human infection- Refer chapter review.

About Other Options:
Option a: M.tuberculosis is equally pathogenic than M.bovis to the humans and Guinea pig. However, M.bovis but not M.tuberculosis is pathogenic to rabbit.
Option c: M. africanum belongs to the members of Mycobacterium tuberculosis complex. It is commonly found in West African countries, causing up to a quarter of cases of tuberculosis in countries such as the Gambia. '*It is an infection of humans only and is spread by an airborne route from individuals with open cases of disease.*' Source- Wikipedia
Option d: '*M. marinum causes swimming pool granuloma/fish trank granuloma/ fish fancier's finger. Secondary lesions appear along with dermal lymphatics.*' (Sporotrichoid spread) Greenwood 16/e p217

36. Ans. (a) (Mycobacterium...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p283, Ananthanarayan 9/e p360
Refer chapter review.

M. LEPRAE

37. Ans. (a) (Stop anti-leprosy treatment) Ref: Park 22/e p298, 23/e p323
- Refer chapter review

38. Ans. (d) (Defaulter: Not taken treatment for > 6 months) Ref: Park 23/e p323, 22/e p287-302
- Multibacillary leprosy: Bacteriological index ≥ 2, skin lesion: > 5, nerve involvement: ≥ 1
- New case detection rate: Indicator of incidence
- Target for elimination of leprosy: Prevalence < 1/10,000 population
- Defaulter: Not taken treatment for ≥ 2 months.

39. Ans. (c) (In LL patients) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p288, Park 23/e p324, 22/e p297
- ENL occurs in type II lepra reaction in LL and BL patients.

40. Ans. (c) (Insect...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p289, Park 23/e p317, 22/e p287-302
- Lepra bacilli can survive outside humans in animals like foot pad of mice and armadillo.
- Bacteria load – high in LL
- Insect can occasionally transmit lepra bacilli
- If prevalence is low in childhood: It means disease is under control.

41. Ans. (b) (It's a...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p290, Park 23/e p320, 22/e p294

For details on Lepromin test– Refer chapter review

42. Ans. (c) (21st day) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p290, Park 23/e p320, 22/e p293-94
- Early reading (Fernandez) is taken at 3 days; late reading (Mitsuda reaction) is taken at 3 weeks i.e. on 21st day.

43. Ans. (b) (5 year) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p291, Ref: Park 23/e p323, 22/e p299
- In multibacillary leprosy, after the treatment, follow up is done yearly for 5 years
- In paucibacillary leprosy, after the treatment, follow up is done yearly for 2 years.

44. Ans. (b) (Ciprofloxacin) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p291, Ref: Park 23/e p323, 22/e p296
- Common drugs given in treatment of leprosy that included in WHO protocol- Dapsone, rifampicin, clofazemine
- Other drugs which can be given in treatment of leprosy- Ethionamide, quinolones, clarithromycin, minocycline.

45. Ans. (a) (6 months) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p291, Park 23/e p323, 22/e p297
- In paucibacillary leprosy, dapsone is continued for- 6 months
- In multibacillary leprosy, dapsone is continued for- 12 months

46. Ans. (b) (Response...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p290, Park 23/e p323, 22/e p294
- Uses of Lepromin test:
  - Classify lesions of leprosy
  - Assess prognosis
  - Assess resistance to leprosy in individuals.

47. Ans. (b) (1–10 bacilli/100...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p290, Park 22/e p293
- Bacteriological index:
  - 0 indicates No Bacilli in all fields
  - 1+ indicates - 1–10 bacilli/100 oil immersion field
  - 2+ indicates - 1–10 bacilli/10 oil immersion field
  - 3+ indicates – 1–10 bacilli/ each oil immersion field
  - 4+ indicates – 10–100 bacilli/ each oil immersion field
  - 5+ indicates – 100–1000 bacilli/ each oil immersion field
  - 6+ indicates – >1000 bacilli/ each oil immersion field
- Morphological index: No. of live bacilli detected by: Solid uniformly stained, parallel sides, rounded ends, length 5 times than width.
- Solid fragmented granular (SFG) percentage of solid fragmented and granular bacilli. It is the most sensitive indicator for monitoring response to treatment.

48. Ans. (d) (Pure neuritic type leprosy) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p286, Park 22/e p291

Classification of leprosy:

- Ridley-Jopling classification – TT, BT, BB, BL, LL
- Madrid classification – LL, TT, borderline/dimorphous, indeterminate (early unstable type)
- Indian classification – Madrid + pure neuritic type

49. Ans. (b) (2) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p291, Park 23/e p323, 22/e p291-92

- *Paucibacillary leprosy:* Given for-I ,TT, BT and if bacteriological index < 2, skin lesion: 1–5, one nerve involvement
- *Multibacillary leprosy:* Given for BB,BL, LL if bacteriological index ≥ 2, skin lesion: > 5, nerve involvement: ≥ 1

50. Ans. (a) (b) (Borderline Leprosy, Lepromatous leprosy) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p287, Harrison 18/e p1363

- *Erythema nodosum leprosum (ENL) occurs exclusively in patients near the lepromatous end of the leprosy spectrum (BL-LL), affecting nearly 50% of this group* Harrison 18/e p1363

51. Ans. (b) (Ovary) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p288, Harrison 17/e p1020-1024, Internet source

- Grows well in cooler part of body skin, **testes, peripheral nerve, anterior eye**
- It can involve any organ – except CNS and lungs, **ovary** and also warm area of skin (axilla, groin, scalp)
- *LL leprosy, the anterior chamber of the eye is invaded by bacilli, and ENL may result in uveitis, with consequent cataracts and glaucoma. Thus leprosy is a major cause of blindness in the developing world.*
- *M. leprae invades the testes, and ENL may cause orchitis.*
- *Tuberculoid leprosy usually affects Peripheral Nerves*

52. Ans. (c) (Cyclosporine) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p291, Harrison 18/e p1366

Treatment of lepra reactions: Refer chapter review for detail

53. Ans. (b) (Consists...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p289, Ananthanarayan 9/e p364

*'Intracellularly, M.leprae are arranged as – parallel **cigar** bundles of bacilli bound with lipid like glia (**globi**) present inside foamy macrophage (**Virchow's leprae cell**)'*

54. Ans. (b) (Lepra reaction type I) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p288, Harrison 18/e p1363

- For details about Lepra reaction type I– Refer chapter review.

55. Ans. (a) (M.tuberculosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p274, Harrison 18/e p420

- Lupus vulgaris (also known as Tuberculosis luposa) are painful cutaneous tuberculosis skin lesions with nodular appearance, most often on the face around the nose, eyelids, lips, cheeks, ears and neck. It is the most common M. tuberculosis skin infection.

56. Ans. (b), (c) (Skin smear 1+, skin smear 2+) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p291, Park 22/e p295

Refer chapter review for detail

57. Ans.(c) (In paucibacillary…) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p291, Park 22/e p292-295

- Refer chapter review for detail.
- Regular multidrug therapy of leprosy means patients should receive atleast 2/3rd of the total duration of treatment schedule (i.e. at least 8 full months out of 12 months).

58. Ans. (c) (12 days) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p285, Ananthanarayan 9/e p22-23

- Refer chapter review.

59. Ans. (a) Borderline Tuberculoid (BT) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p287

- Satellite papules are characteristic of Borderline Tuberculoid (BT) type of leprosy.

Enterobacteriaceae (E. Coli, Klebsiella, Proteus, Shigella, Salmonella, Yersinia)

CHAPTER 3.7

Enterobacteriaceae
Classification:
- LF: *Escherichia, Klebsiella and Citrobacter*
- NLF: *Salmonella, Shigella, Proteus, Morganella, Providencia and Yersinia*
- Late LF: *Shigella sonnei*

FAMILY CHARACTERS (GENERAL PROPERTIES)

Members of the family Enterobacteriaceae should have the following properties:

1. They are gram-negative, aerobes and facultative anaerobic bacilli
2. Nonfastidious, can grow in ordinary media like nutrient agar.
3. Ferment glucose, and reduce nitrate to nitrite
4. Oxidase test negative and catalase positive (except *Shigella dysenteriae* type-1)
5. They are generally motile, except *Shigella* and *Klebsiella.*
6. Natural habitat: Most of them are commensals in human intestine, called *coliform bacilli.*

Table 3.7.1: Classification of family Enterobacteriaceae based on lactose fermentation

Groups	Lactose fermentation	Colonies on MacConkey agar	Examples
Lactose fermenters (LF)-all are coliform bacilli	Ferment lactose producing acid	Produce pink colored colonies	*Escherichia, Klebsiella Citrobacter*
Nonlactose fermenters (NLF)	Do not ferment lactose	Produce pale or colorless colonies	*Salmonella, Shigella Proteus, Morganella, Providencia* and *Yersinia*
Late lactose fermenters (LLF or previously called as paracolon bacilli)	Ferment lactose after 2–8 days of incubation	At 24 hrs incubation- produce pale or colorless colonies, After 2 days produce pink color colonies	*Shigella sonnei*

ESCHERICHIA COLI

E.coli is the most important species encountered as human pathogen.

- It is also the commonest aerobe to be harbored in the gut of humans and animals.
- After excreted in feces, it remains viable only for some days in the environment. Hence detection of fecal *E.coli* (*thermotolerant E.coli* that survives at 44°C) is taken as an *indicator of recent contamination of drinking water* with human or animal feces.

Clinical Manifestations of *E.coli* Infection

1. **Urinary tract infection** (UTI): Caused by uropathogenic *E.coli* (UPEC) (described later)
2. **Diarrhoea**: Caused by six types diarrheagenic *E.coli* (described later)
3. **Other syndromes:**
   - *Abdominal infections*: *E.coli* is the most common cause of both primary bacterial peritonitis (occurs spontaneously) and secondary bacterial peritonitis (occurs secondary to intestinal perforation. It also causes visceral abscesses, such as hepatic abscess.
   - *Pneumonia* (especially in hospitalized patients: ventilator associated pneumonia)
   - *Meningitis* (especially **neonatal meningitis**)
   - *Wound and soft tissue infection.*
   - Osteomyelitis
   - Endovascular infection and bacteremia.

LT (heat labile toxin)	ST (heat stable toxin)	Verocytotoxin or Shiga-like toxin
Produced by: Enterotoxigenic *E.coli*	**Produced by:** Enterotoxigenic *E.coli*	**Produced by:** Enterohemorrhagic *E.coli*

Contd...

Contd...

LT (heat labile toxin)	ST (heat stable toxin)	Verocytotoxin or Shiga-like toxin
It resembles cholera toxin in its function **Mechanism of action:** It has two peptide fragments: A and B **Fragment A-** active fragment, causes ADP ribosylation of G protein → upregulates adenylate cyclase → ↑**cyclic AMP** → ↑water and electrolytes secretion→ diarrhea **Fragment B:** Binds to GM1ganglioside receptors on intestinal epithelium	**Mechanism of action:** Binds to guanylate cyclase→ ↑ **cyclic GMP**→ diarrhea	It is so named because it is cytotoxic to Vero cell lines. Also called Shiga-like toxin **Mechanism of action:** It has two fragments: A and B **Fragment B:** Binds to a globotriosyl ceramide (Gb3) receptor on intestinal epithelium **Fragment A** is the active fragment. It inhibits protein synthesis by inhibiting ribosome.
Plasmid coded	Plasmid coded	Bacteriophage coded
Detection of LT: *In vivo* tests: • Ligated **rabbit ileal loop** test +ve at 18 hrs • Adult rabbit skin test is positive *In vitro* Tissue culture tests: Steroid production in mouse adrenal cell Elongation in Chinese hamster ovary cells	**Detection of ST:** *In vivo* tests: • Ligated rabbit ileal loop test – positive at 6 hrs • Infant mouse intra gastric test is positive at 4 hrs. *In vitro* tests are negative	**Detection of VT:** Serologically, e.g. ELISA Molecular methods Cytotoxicity on Vero and HeLa cell lines

Urinary Tract Infection (UTI)

E.coli (uropathogenic *E.coli* or UPEC) is the single most common pathogen, accounting for 85–95% of all cases of UTI. **UPEC** serotypes O1, O2, O4, O6, O7 and O75 are responsible for most UTIs.

E.coli exotoxins:
- LT (heat labile toxin): ↑cyclic AMP
- ST (heat stable toxin): ↑ cyclic GMP
- Verocytotoxin or Shiga-like toxin: ↓ Protein synthesis by ↓ ribosome

Route of Spread

- **Ascending route:** After colonizing the periurethral area, *E.coli* ascends the urinary tract to reach bladder and later to kidney.
- **Descending route:** Hematogenous seeding of *E.coli* into kidneys result in pyelonephritis.

Types of UTI

Depending on the site involved, there are two types of UTI: Lower and upper UTI

	Lower UTI	Upper UTI
Syndromes	Cystitis and urethritis	Pyelonephritis
Symptoms	Local manifestations: dysuria, urgency, frequency,	Systemic manifestations: Fever, vomiting, abdominal pain
Route of spread	Ascending route	Both ascending (common) and descending route
Occurrence	More common	Less common
Virulence factors	Fimbriae (e.g. P fimbriae)	Capsular K antigen
Common virulence factors responsible are: • Cytotoxins (CNF 1-cytotoxic necrotizing factor 1 and SAT-Secreted auto transporter toxin) • Hemolysins		

Detection of LT:
- In vivo tests:
  - Ligated rabbit ileal loop test +ve at 18 hrs
  - Adult rabbit skin test is positive
- In vitro Tissue culture tests:
  - Steroid production in mouse adrenal cell
  - Elongation in Chinese hamster ovary cells

Predisposing Factors that Promote UTI

- Females: Due to short urethra and close proximity to anus.
- Pregnancy: Physiological obstruction in urinary tract due to growing fetus may lead to prolonged stasis of urine and asymtpomatic bacteriuria.
- Others: Presence of urinary catheters, urinary stones or prostate enlargement.

Upper UTI due to E.coli:
- Pyelonephritis
- Manifestations: Fever, vomiting, abdominal pain
- Both ascending (common) and descending route
- Less common than lower UTI
- Express Capsular K antigen

Laboratory Diagnosis of UPEC

- **Specimen collection:**
  - Clean voided midstream urine: It is the most common specimen for UTI.
  - Suprapubic aspiration is the most ideal specimen (for coma or infants).
  - In catheterized patients: Collected from the catheter tube and not from the bag.

Kass concept of 'significant bacteriuria:
- ≥10^5 CFU/ml of urine: considered as significant
- ≤ 10^4 CFU/ml: considered as commensal bacteria

Culture Media–Urine is inoculated onto:
- MacConkey agar and blood agar combination
- CLED (cysteine lactose electrolyte deficient) agar

Quantitative culture:
- Semi quantitative method such as standardized loop technique
- Quantitative method: Such as pour plate method

- **Transport:** Stored in *refrigerator* or stored by adding *boric acid*
- **Direct examination:** Screening tests done are as follows:
  - *Wet mount examination* is done to demonstrate the pus cells in urine. Pyuria of > 8 pus cells/mm^3 or 4 lakh pus cells excreted in urine/hour is taken as significant.
  - *Others:* Leukocyte esterase test, Nitrate reduction test (Griess test), Catalase test
  - *Gram staining* of urine is not a reliable indicator as (i) low bacterial count in urine, (ii) pus cells rapidly deteriorate and may not be seen well. It may be limited to pyelonephritis and invasive UTI cases and a count of ≥ 1 bacteria/oil immersion filed is taken as significant.
- **Culture:**
  - ***Culture Media*:** Urine is inoculated onto:
    - MacConkey agar and blood agar combination or
    - CLED (cysteine lactose electrolyte deficient) agar.
  - ***Kass concept of significant bacteriuria:***
    - A count of ≥ *10^5 colony forming units (CFU)/ml* of urine is considered as significant – indicates infection (referred as 'significant bacteriuria' developed by Kass)
    - *Low count of ≤ 10^4 CFU/ml* is due to commensal bacteria (due to contamination during voiding) and is of no significance. However, low counts may be significant in following conditions:
      a. Patient on antibiotic treatment or on diuretic drugs
      b. Infection with some gram-positive bacteria like *S.aureus*, and *Candida*.
      c. Pyelonephritis and acute urethral syndrome
      d. Sample taken by suprapubic aspiration
  - ***Quantitative culture*** is done to count the number of colonies. This is done by:
    - Semi quantitative method, such as standardized loop technique
    - Quantitative method, such as pour plate method.
- **Antibody coated bacteria test** is used to differentiate upper and lower UTI.

Diarrhea (Diarrheagenic *E. coli*)

1. Enteropathogenic E. coli (EPEC)

EPEC frequently cause *infantile diarrhea* (outbreaks) and rarely sporadic diarrhea in adults.

Diarrheagenic E.coli:
- EPEC frequently cause infantile diarrhea
- ETEC is the most common cause of traveler's diarrhea
- EIEC: Causes ulceration of bowel, dysentery

- It is nontoxigenic and noninvasive
- **Mechanism of diarrhea:**
  - *Adhesion* to intestinal mucosa, mediated by plasmid coded *bundle-forming pili*
  - *A/E lesions* (attaching and effacing lesions) on the intestinal epithelium.

2. Enterotoxigenic E. coli (ETEC)

ETEC is the most common cause of **traveler's diarrhea** causing 25–75% of cases:
- It causes acute watery diarrhea in infants and adults.
- Common serotypes associated are: O6, O8, O15, O25, O27, O153, O159, etc.
- It is toxigenic but not invasive
- **Pathogenesis:**
  - *Attachment* to intestinal mucosa is mediated by CFA (Colonization Factor Ag)
  - *Toxins:* (i) heat labile toxin or LT (↑cAMP), (ii) heat stable toxin or ST (↑cGMP)
  - Diagnosis is done by detection of toxins by *in vitro* and *in vivo* methods.

3. Enteroinvasive E. coli (EIEC)

Common serotypes associated with EIEC are O28, O112, O114, O124, O136, O152, etc.
- **Pathogenesis**: EIEC is not toxigenic but invasive. The epithelial cell invasion is mediated by a plasmid coded antigen called virulence marker antigen (VMA).
- EIEC is biochemically, genetically and pathogenically closely related to *Shigella*.

- **Manifestations** include ulceration of bowel, dysentery (resembling shigellosis).
- **Diagnosis:**
  - Detection of VMA by ELISA
  - HeLa cell invasion assay
  - Sereny test (inoculation into guinea pig eyes produces conjunctivitis)
  - EIEC are biochemically atypical being nonmotile and lactose nonfermenters.

4. Enterohemorrhagic E. coli (EHEC)

- **Serotypes associated with EHEC are:**
  - O157: H7 (most common serotype)
  - Other serotypes are rare (O26: H11, O6, O55, O91, O103, O111 and O113).
- EHEC is usually transmitted by contaminated food, i.e. lettuce, spinach, sprouts and undercooked ground beef.
- Prevalent in industrialized countries (other Diarrheagenic *E.coli* are common in developing regions)
- Low infective dose (< 10^2 CFU) of EHEC can also initiate the infection.
- **Pathogenesis:** EHEC secretes a toxin called verocytotoxin or Shiga-like toxin (refer table for its mechanism)
- **Manifestations:** VT has predilection for endothelial cells causing capillary microangiopathy which leads to:
  - *HC (hemorrhagic colitis):* Manifests as gross bloody diarrhea, abdominal pain and fecal leukocytosis but no fever.
  - *Hemorrhagic uremic syndrome (HUS):* Injury to small vessels of the kidney and brain can lead to bloody diarrhea, thrombocytopenia, renal failure and encephalopathy but without fever. It is more common in children.
- **Diagnosis:**
  - Sorbitol MacConkey agar: EHEC in contrast to other *E.coli*, does not ferment sorbitol
  - Toxin detection:
    - Cytotoxicity in Vero cell lines (gold standard method)
    - Fecal toxin antigen detection by ELISA or rapid tests
  - PCR can be used to detect gene coding for VT.

EHEC:
- MC Serotypes: O157: H7
- MC food: Lettuce, spinach, sprouts and undercooked ground beef
- Prevalent in industrialized countries
- Complications:
  - Hemorrhagic colitis
  - Hemorrhagic uremic syndrome (HUS)

E. coli O104:H4
- EAEC strain that has caused major outbreaks in Germany in 2011.
- Peculiarity is, it produces Shiga toxin and can cause HUS

5. Enteroaggregative E. coli (EAEC)

- It is so named because it adheres to HEp-2 cells in a stacked-brick fashion
- Most strains are 'O' untypeable but 'H' typeable
- **Pathogenesis:**
  - Colonization mediated by aggregative adhesion fimbriae I (regulated by *aggR* gene)
  - Produce *EAST 1 toxin* (enteroaggregative heat stable enterotoxin 1)
- **Manifestations:** Persistent and acute diarrhea are common; in developing countries
- **E. coli O104: H4** is an enteroaggregative strain that has caused major outbreaks in Germany in 2011. Peculiarity is, it produces Shiga toxin and can cause HUS.

6. Diffusely Adherent E. coli (DAEC)

- It is characterized by Ability to adhere to HEp-2 cells in a diffuse pattern
- Expresses diffuse adherence fimbriae which contribute to the pathogenesis.

KLEBSIELLA

- Nonmotile, lactose fermenter
- Capsulated, produce mucoid colonies
- *K. pneumoniae*: Urease positive. It causes pneumonia, UTI, abdominal, wound and surgical site infection
- *K.ozaenae:* It causes ozaenae (foul smelling nasal discharge)/atrophic rhinitis
- *K.rhinoscleromatis*: causes rhinoscleroma.

Organisms that swarm:
- *Proteus*
- *Clostridium tetani*
- *V. parahaemolyticus*
- *Serratia*

PROTEUS

- Motile, Nonlactose fermenter
- Tribe character: PPA test +ve (phenylalanine deaminase test +ve)
- Both *P.mirabilis* and *P.vulgaris* are H_2S and Urease positive
- Indole test: Positive for *P.vulgaris*, negative for *P.mirabilis*
- Pleomorphism: Gram-negative, appear in various forms—coccobacilli, bacillary and in filamentous forms.
- Odor: Produce characteristic putrid 'fishy' or 'seminal' odor in cultures.
- **Swarming**-*Proteus* has an ability to swarm (or spread) on solid media.
  Organism which swarm: *Proteus*, *Clostridium tetani*, *V. parahemolyticus*, and *Serratia.*
- Diene's phenomenon: It is done to know the relatedness between different strains.
- *Proteus* forms struvite stone in bladder in alkaline urine.
- Nonmotile strains of *Proteus* (Basis of Weil felix test): OX 19, OX 2 (*P.vulgaris*), OX K (*P.mirabalis*) forms the basis of Weil Felix test.

Most common Shigella species:
- In world: *S. sonnei*
- In India: *S. flexneri*

SHIGELLA

Shigella species are the agent of bacillary dysentery.

- Four species has been recognized: *S. dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei*.
- Most hardier: *S. sonnei*
- Most common species: In world: *S. sonnei*, In India: *S. flexneri*

Infective dose
- *Shigella:* 10 to 100 bacilli.
- *Salmonella Typhi:* 10^3–10^6 bacilli
- *Vibrio cholerae:* 10^6–10^8 bacilli

Pathogenicity

- **Transmission:** (i) ingestion through contaminated fingers (MC), food, and water or flies and (ii) rarely by homosexuals
- **Infective dose:**
  - *Shigella* has a low Infective dose (10 to 100 bacilli). Others with low infective dose include EHEC, *Entamoeba histolytic*a and *Giardia*.
  - *Salmonella* Typhi: 10^3–10^6 bacilli
  - *Vibrio cholerae*: 10^6–10^8 bacilli
- Bacilli enter the mucosa via M cells.
- Invasion: Mediated by a large virulence plasmid
- Direct cell to cell spread: This occurs by inducing actin polymerization of host cells.
- Exotoxins:
  - Shigella enterotoxin (ShET 1 and 2)- found essentially in *S. flexneri*
  - Shiga toxin is a cytotoxin, produced by *S.dysenteriae* type1.
- Endotoxin induces intestinal inflammation and ulcerations.

Shigella Exotoxins
- *Shigella enterotoxin* (ShET 1 and 2): found essentially in *S.flexneri*
- Shiga toxin is a cytotoxin, produced by *S. dysenteriae* type1.

Complications:

- *Intestinal* complications, such as toxic megacolon, perforations and rectal prolapse.
- *Metabolic* compilations, such as hypoglycemia, hyponatremia, and dehydration.
- *Ekiri syndrome* or toxic encephalopathy is a metabolic complication of shigellosis.
- *Postinfectious phase*: Patients expressing HLA-B27 develop autoimmune reactions, such as reactive arthritis, ocular inflammation, and urethritis months after *S. flexneri* infection (3% of cases).
- HUS and HC is produced by *S. dysenteriae* Type 1 (due to producing Shiga toxin, hence called Shiga bacillus)
- Rarely, it causes bacteremia, meningitis, pneumonia, vaginitis and keratoconjunctivitis.

Ekiri syndrome
It is a metabolic complication of shigellosis, characterized by toxic encephalopathy

Laboratory Diagnosis:

- **Specimen**: mucus flakes of stool
- **Culture Media**: (Common media for both *Shigella* and *Salmonella*):
  - Transport media: Sach's buffered glycerol saline

- Selective media: DCA (deoxycholate citrate agar), XLD, SS Agar
- Enrichment Broth: Gram-Negative broth, selenite F broth, tetrathionate broth.

- **Important biochemical properties:**
  - Nonmotile, Nonlactose fermenter except: *S. sonnei* (Late lactose fermenter)
  - Catalase +ve except: *S. dysenteriae* type 1
  - Mannitol fermenting except: *S. dysenteriae*
- **Typing of *Shigella*:**
  - Serotyping *S. dysenteriae*: 15, *S. flexneri* 6 , *S. boydii* 19 serotypes, *S. sonnei* 1
  - Colicin typing (Bacteriocin typing) done for *S. sonnei* (has 26 colicin types).

Typing of Shigella
- Serotyping is done for *S. dysenteriae, S. flexneri, S. boydii*
- Colicin typing done for *S. sonnei* (has 26 colicin types)

Treatment:

Because of the prompt transmissibility, every case of shigellosis should be treated with antibiotics, in addition to ORS.

- Ciprofloxacin is the drug of choice.
- Alternative drugs: Ceftriaxone, azithromycin, pivmecillinam, and some fifth-generation quinolones.
- Duration of treatment is about 3 days except for:
  - *S. dysenteriae* type 1 infection 5 days
  - Infections in immunocompromised patients 7–10 days.

Shigella (imp biochemical properties)
- NonMotile
- NLF except: *S. sonnei* (Late LF)
- Catalase +ve except: *S. dysenteriae* type 1
- Mannitol fermenting except: *S. dysenteriae*

SERRATIA

- *S. marcescens* produces characteristic red nondiffusible pigment called **prodigiosin.**
- It is a saprophyte found in water, soil and food.
- It may grow in sputum after collection and makes the sputum red (due to pigment production). This condition is known as **'pseudohemoptysis'.**
- It is increasingly reported in various **nosocomial infections**. The hospital strains are often nonpigmented and multidrug resistant (produce AmpC β-lactamases).

Serratia marcescens
- Prodigiosin pigment (red) producing strain – sputum commensal, causes 'pseudohemoptysis'.
- Nonpigmented strains: Cause nosocomial infections

YERSINIA PESTIS

Yersinia pestis is the agent of plague, a zoonosis, transmitted from rodents by rat flea.

Epidemiology of Plague

Plague is one of the greatest killer known to mankind. Africa accounts for maximum cases (97%).

Plague Pandemics: There were three pandemics reported, each with a different biotype of *Y.pestis*.

- First pandemic (in AD541) associated with biotype Medievalis.
- Second pandemic (in 14th century) was called **black death:** with biotype Antiqua
- Third pandemic (1884): It mainly affected India and China: with biotype Orientalis.

Recent outbreaks of plague in India

- 1994 (Surat epidemic), 2002 (Shimla outbreak) and 2004 (bubonic plague in Uttaranchal)
- Four potential endemic foci are there in India at present which include: (i) region near Kolar, (ii) Beed-Latur belt in Maharashtra (iii) Rohru in Himachal Pradesh (iv) Dangud village, Uttaranchal.

Epidemiological Factors

- **Reservoir:** Wild rodents, such as gerbils (*Tatera indica*), field mice, and forest bandicoot
- **Source of infection** are infected wild rodents, rat fleas and cases of pneumonic plague.
- **Vector: Rat flea** is the commonest vector of plague.
  - *Xenopsylla cheopis* – the most efficient vector (found in north India)
  - *Xenopsylla astia* (less efficient, found in south India).
  - Human flea (*Pulex irritans*) may rarely serve as vector.

Plague in India (Recent outbreaks)
- 1994 (Surat epidemic)
- 2002 (Shimla outbreak)
- 2004 (bubonic plague in Uttaranchal)

Human plague (Transmission):
- Bite of an infected rat flea (most common)
- Direct contact with tissues of infected animal
- Droplet inhalation (man to man) from cases of pneumonic plague
- Bite of an infected human flea *(Pulex irritans)*

Cheopis index
- Average number of *X. cheopis* per rat
- > 1 Indicates Plague outbreak is likely to occur

Pneumonic plague:
- Results from inhalation of bacilli in droplets
- Incubation period is short, about 1–3 days
- Respiratory symptoms
- Rare (< 1%), but highly infectious and highly fatal.
- Can be used in bioterrorism

Yersinia pestis–Identifying feature:
- Wayson stain (or methylene blue staining): Bipolar/safety pin appearance
- Culture: Shows Stalactite growth on nutrient broth

Treatment of plague:
- Streptomycin was given in the past
- Now Gentamicin is for treatment
- Levofloxacin followed by doxycycline for Chemoprophylaxis

- **Plague cycles:** Plague exists in two natural cycles-
  - ***Domestic cycle:*** Occurs between humans, rat fleas and rodents.
  - ***Wild or sylvatic cycle*** occurs in nature between wild rodents independent of human.
- **Mode of transmission:** Human plague is frequently contracted from:
  - Bite of an infected rat flea (most common)
  - Direct contact with tissues of infected animal
  - Droplet inhalation (man to man) from cases of pneumonic plague
  - Bite of an infected human flea (*Pulex irritans*).
- **Blocked flea:**
  - The bacilli multiply and block the proventriculus of flea. The flea regurgitates the blood mixed bacteria into the bite, thus transmitting the infection.
  - A partially blocked flea is more dangerous than a completely blocked flea as it survives longer inside burrows, may be up to 4 years in certain species.
- **Extrinsic incubation period** is the interval between the flea acquiring infection through blood meal and becoming a blocked flea; which is usually about **two weeks**.
- **Cheopis index** (No. of *X.cheopis* per rat) of > 1- Indicates Plague outbreak is likely to occur
- **Seasonality:** Plague is seasonal in north India (September to May) and throughout the year in South India.

Clinical Types of Human Plague

Bubonic plague: It is the most common type, transmitted by the bite of an infected rat flea.
- Incubation period is about 2–7 days.
- **Buboes**: Enlarged regional lymph nodes are called buboes (MC site inguinal LN)
- It cannot spread from person to person as the bacilli are locked up in buboes.

Pneumonic plague: Results from inhalation of bacilli in droplets expelled from patients/animals with pneumonic plague.
- Incubation period is short, about 1–3 days
- Clinical feature: Respiratory symptoms (cough or hemoptysis, dyspnea, and chest pain)
- Though rare (< 1%), it is highly infectious and highly fatal.
- Agent of bioterrorism-Aerosolized *Y.pestis* is a possible source of bioterrorism attack.

Septicemic plague: Occurs secondary to spread of bubonic or pneumonic plague.
- Incubation period is about 2–7 days.
- Massive involvement of blood vessels results in hemorrhages in the skin and mucosa, hence the name ***black death.***

Laboratory Diagnosis

- Specimen: Pus aspirated from bubo, Sputum and blood.
- **Direct Microscopy**:
  - Gram staining: Reveals pus cells and gram-negative oval coccobacilli with rounded ends surrounded by capsule.
  - Wayson stain (or methylene blue staining): Demonstrates bipolar appearance.
- **Culture:**
  - *Y. pestis* grows best at 27°C but the capsule develops best at 37°C.
  - Blood agar: Colonies are nonhemolytic and ***dark brown pigmented***
  - MacConkey agar: NLF colonies
  - ***Stalactite growth*** seen on nutrient broth with oil or ghee floated on top.
- F1 Antigen detection by direct immunofluorescence test, ELISA
- Antibodies to F-1 antigen may be detected by CFT, ELISA
- PCR can be done targeting gene coding F1 antigen, pesticin gene.

Treatment

- Streptomycin was given in the past, now Gentamicin is recommended for treatment.
- β-lactams and macrolides are generally not recommended as the response is poor.

- Chemoprophylaxis should be given to all contacts of pneumonic plague. Levofloxacin followed by doxycycline is DOC

Vaccine

WHO recommends using vaccine only for prevention of outbreak and not for general use.

- **Formalin killed vaccine (Sokhey's modification of original Haffkine vaccine):**
  - It is prepared in Haffkine institute, Mumbai.
  - It is given subcutaneously, two doses 4 weeks apart and a booster after 6 months.
  - Protection is short lasting (< 6 months).
  - It is not protective against pneumonic plague and has considerable side effects, C/I in < 6 month of age.
- **Live attenuated vaccine:** Based on strain EV76, used in Soviet Union; causes side effects.

Vaccine in Plague:
- Formalin killed vaccine (Sokhey's modification of original Haffkine vaccine)
- Live attenuated vaccine

Yersiniosis

Yersiniosis refers to *Yesinia* infection other than *Y. pestis* i.e. *Y. pseudotuberculosis* and *Y.enterocolitica*

Yersiniosis:
- Yesinia infection other than *Y. pestis*, i.e. *Y. pseudotuberculosis* and *Y. enterocolitica*

Clinical Manifestations

Overall, *Y.enterocolitica* is more frequently reported clinically than *Y.pseudotuberculosis*.

- **Self limited gastroenteritis** (diarrhea with or without blood) occurs in younger children.
- **Intestinal complications** occur in older children, characterized by:
  - Terminal ileitis (mostly in *Y.enterocolitica*)
  - Mesenteric adenitis (mostly in *Y. pseudotuberculosis*)
  - Pseudoappendicitis (by both species, appendectomy is not indicated)
- **Postinfective phenomena** (in adults): Occurs commonly with *Y.enterocolitica* as a result of autoimmune activity. Manifestations include:
  - Reactive arthritis (people with in HLA-B 27)
  - Erythema nodosum and Graves' disease (independently of HLA-B 27)
- **Super antigen:** Some strains of *Y.pseudotuberculosis* express a super antigen ***mitogen*** which has caused scarlet-like fever in Russia and Japan (called **Izumi-fever**) and also linked to the pathogenesis of Kawasaki's disease.

Intestinal complications of Yersiniosis:
Occur in older children, characterized by:
- Terminal ileitis (mostly in *Y. enterocolitica*)
- Mesenteric adenitis (mostly in *Y. pseudotuberculosis*)
- Pseudoappendicitis (by both species, appendectomy is not indicated)

Laboratory Diagnosis

***Y. enterocolitica* and *Y. pseudotuberculosis* can be differentiated from *Y. pestis*:**

- Differential motility: They are motile at 22°C (but not at 37°C)
- Cold enrichment: Growth improves on refrigeration (4°C)
- Urease positive

Tests to differentiate *Y. enterocolitica* from *Y. pseudotuberculosis*:

- Sugar fermentation
- Ornithine decarboxylase and VP test positive only for *Y. enterocolitica.*

SALMONELLA

Classification and Nomenclature

Salmonella is antigenically complex. There are several classifications proposed so far.

1. **Clinical classification of *Salmonella*** into Typhoidal and Nontyphoidal groups:
   - Typhoidal *Salmonella,* e.g. *S.* Typhi and *S.* Paratyphi—They are restricted to human hosts, cause enteric fever
   - Nontyphoidal *Salmonella* or NTSI: The remaining serotypes can colonize the intestine of a broad range of animals. They cause food-borne gastroenteritis and septicemia in man.
2. **Antigenic classification (Kauffmann-White scheme):** It is based on the somatic (O) and flagellar (H) antigens which can be detected by agglutination with antisera.

Postinfective phenomena (in adults):
- Occurs commonly with *Y. enterocolitica*
- Reactive arthritis (people within HLA-B 27)
- Erythema nodosum and Graves' disease (independently of HLA-B 27)

- **Serogroups:** Based on O antigen, salmonelleae are classified into 67 serogroups:
  - Serogroup 2 (*S*.Paratyphi A): Contains O antigen type 2
  - Serogroup 4 (*S*.ParatyphiB): Contains O antigen type 4
  - Serogroup 9 (*S*.Typhi): Contains O antigen type 9
- **Serotypes**: Each serogroup is further typed into > 2500 serotypes based on flagellar Ag.

3. **Molecular classification**- According to molecular classification:
   - Genus *Salmonella* consists of two species: *Salmonella enterica* and *S.bongori.*
   - Most of the pathogenic serotypes are placed under subspecies enterica.
   - Nomenclature of *S*. Typhi is *Salmonella* species *enterica*, subspecies enterica, serotype Typhi.

Antigenic Structure

Salmonellae possess three important antigens:

- Somatic antigen (O) and Flagellar antigen (H): Present in all serotypes/species
- Surface envelope antigen (Vi): Found in some serotypes/species

O antigen	H antigen
Somatic antigen It is a part of cell wall lipopolysaccharide (LPS)	**Flagellar antigen** Made-up of proteins: flagellin, confers motility
Heat stable, Alcohol stable, but formaldehyde labile	Formaldehyde stable but Heat labile, alcohol labile
In Widal test: O antigen of *S*.Typhi is used	In Widal test: H Ag of *S*.Typhi, *S*.Paratyphi A and B are used
O Ag is less immunogenic	H Ag is more immunogenic
O antibody appears early, disappears early – indicates recent infection	H antibody appears late, disappears late- Indicates convalescent stage
When O antigen reacts with O antibody: forms compact, granular, chalky clumps Agglutination takes place slowly. Optimum temperature for agglutination is 55°C	When H antigen reacts with H antibody- forms large, loose, fluffy clumps. Agglutination takes place rapidly. Optimum temperature for agglutination is 37°C
Serogrouping is based on the O antigen.	Serogroups are differentiated into serotypes based on H Ag

Vi Antigen

Vi Antigen is present in:
- S. Typhi
- S. Paratyphi C
- S. Dublin
- *Citrobacter freundii*

Vi antigen is a surface polysaccharide envelope or capsular antigen covering the O antigen. The naming is due to the belief that Vi antigen is related to virulence.

- It is expressed in only few serotypes, such as *S*.Typhi, *S*.Paratyphi C, *S*.Dublin and some stains of *Citrobacter freundii*
- When Vi antigen is present, it renders the bacilli inagglutinable with the O antiserum.
- As Vi antigen is *poorly immunogenic* and antibody titers are low, not helpful in the diagnosis of cases. Hence the Vi antigen is not employed in the Widal test.
- However, the complete absence of the Vi antibody in a proven case of typhoid fever indicates **poor prognosis**.
- Vi antibody usually disappears early in convalescence, but if persists, indicates the development of the **carrier state**.
- **Phage typing** of *S*. Typhi can be done by using Vi specific bacteriophages.
- Vi antigens can also be used for **vaccination**.

Risk factors for promoting transmission:
- Stomach acidity (< 1 year age)
- Antacid ingestion
- Achlorhydria
- Prior *H. pylori* infection
- Intestinal integrity (infl. bowel disease, prior GIT surgery or on antibiotics)

Pathogenesis

- **Mode of transmission** by oral route, MC through contaminated food or water.
- **Infective dose** of *Salmonella*: Minimum 10^3–10^6 bacilli are needed to initiate the infection.
- **Risk factors** that promote transmission include the conditions that decrease:
  - Stomach acidity (an age of < 1 year age, antacid ingestion, or achlorhydria
  - Intestinal integrity (inflammatory bowel disease, prior GIT surgery or on antibiotics)

- **Entry:** *Salmonella* enter through epithelial cells (M cells) lining the intestinal mucosa.
- The an uptake is called **bacteria-mediated endocytosis**, favored by bacterial type III secretion system
- Following entry, the bacilli remain inside vacuoles in the cytoplasm.
- **Entry into macrophages:** Salmonellae containing vacuoles cross the epithelial layer to reach submucosa, where they are carried by macrophage to bloodstream.
- **Primary bacteremia** occurs and then **Spread** organs, such as liver, spleen, lymph nodes and bone marrow
- **Massive secondary bacteremia** occurs from the seeded organs, which leads to onset of clinical disease.

Clinical Manifestations (Enteric Fever)

Incubation period is about 10–14 days. Enteric fever is a misnomer as the manifestations are more extraintestinal than intestinal. Various manifestations include:

- Fever (*step ladder pattern* type of prolonged continuous fever)
- Other symptoms: Headache, chills, cough, sweating, myalgia and arthralgia
- Rashes (called *rose spots*) - seen in 30% of patients at the end of the first week.
- Early intestinal manifestations such as abdominal pain, nausea, vomiting and anorexia.
- Late intestinal manifestations: GI bleeding and intestinal perforation (3-4 weeks).
- Important signs include hepatosplenomegaly, epistaxis and relative bradycardia.
- Neurologic manifestations occur rarely which include meningitis, cerebellar ataxia and neuropsychiatric symptoms (described as 'muttering delirium' or 'coma vigil') such as paranoid psychosis and delirium.

Epidemiology

- Host: Humans are the only natural hosts for typhoidal Salmonellae.
- Incidence is highest (> 100 cases per 100,000 population per year) in south central and southeast Asia.
- Locality and age-Enteric fever is:
  - More common in urban than rural areas
  - More common among young children and adolescents than in adults.
- Most important risk factor: Poor sanitation and lack of access to clean drinking water.
- *S*.Typhi to *S*.Paratyphi A ratio is 4:1. However, *S*.Paratyphi A appears to be increasing, especially in India; may be due to increased vaccination for *S*.Typhi.
- **Carriage:** Up to 10% of untreated patients become carriers.
  - *Carriers of are of two types:*
    - Fecal carriers (more common): Multiply in the gallbladder and are excreted in feces.
    - Urinary carriers: Multiplication takes place in kidneys and bacilli are excreted in urine.
  - *Duration of shedding*: Carriers continue to shed bacilli in feces and urine for:
    - Convalescent carriers: Three weeks to three months (after clinical cure)
    - Temporary carriers: Three months to one year
    - Chronic carriers: for more than 1 year
  - *Chronic carriers* occur in about 1–4% of infected people. It is more common in:
    - Women, infants and old age
    - Biliary tract abnormalities leads to increased fecal excretion
    - Abnormalities of urinary tract and associated *Schistosoma haematobium* infection of bladder- leads to increased urinary excretion.
- **Reference centers** for *Salmonella* in India:
  - National *Salmonella* phage typing centre: Lady Hardinge medical college, Delhi.
  - National *Salmonella* Reference Centre: Central Research Institute, Kasauli
  - National *Salmonella* Reference Centre for animal origin: Izatnagar

Locality and age-Enteric fever is:
- More common in urban than rural areas
- More common among young children and adolescents than in adults.
- S. Typhi to S. Paratyphi A ratio is 4:1.

Chronic carriers:
- Occur in about 1–4%, More common in:
- Women, infants and old age
- Biliary tract abnormalities
- Urinary tract abnormalities
- *Schistosoma haematobium* infection of bladder

Laboratory Diagnosis of Enteric Fever

Duration	Specimen used and test done
First week	**Culture of Blood, Bone marrow aspirate, Duodenal aspirate:** • Blood culture is the ideal method of diagnosis in the 1st week of fever (90% positivity) • Clot culture has shown a higher isolation rate than blood culture • If blood culture is negative then →Bone marrow aspirate culture → if negative then Duodenal aspirate culture is done (best is combination of all three specimens) • If patients is on antibiotics →Bone marrow aspirate culture is preferred over blood c/s • Medium: There are two types of media used in blood culture bottles: ○ Monophasic medium: contains brain heart infusion (BHI) broth. ○ Castaneda's biphasic medium: consists of BHI agar slope and BHI broth • SPS (Sodium polyanethol sulfonate) is added to the medium as anticoagulant.
Second and third week	Antibody detection by Widal test is the investigation of choice **Stool culture** is preferred if patients is on antibiotics • Enrichment broth, such as Selenite F broth, tetrathionate broth and gram-negative broth • Selective media such as: ○ Low selective media MacConkey agar ○ Highly selective medium: DCA, XLD agar, SS agar, Wilson Blair's Brilliant green Bismuth sulfite medium (jet black colonies with a metallic sheen)
Fourth week	Stool and urine culture
Carriers	Stool and urine culture Serum for detection of antibodies to Vi antigen Sewage culture: indirect way

Week wise Investigation of choice in enteric fever:
- First week: Culture of Blood, Bone marrow aspirate, Duodenal aspirate
- Second and third week: Widal test
- Fourth week: Stool and urine culture
- Carrier
  - Stool and urine culture
  - Antibodies to Vi antigen
  - Sewage culture: indirect way

Four antigens used in Widal tests:
- O antigens of S. Typhi (TO)
- H antigens of S. Typhi (TH), S. Paratyphi A (AH) and S. Paratyphi B (BH)

O antibody vs H antibody:
- O antibody appears early, disappears early: Indicates recent infection
- H antibody appears late, disappears late: Indicates convalescent stage

Widal Test

Widal test is the investigation of choice second week and third week. As antibodies appear only after the end of the first week, it is not preferred in first week of illness.

- **Principle:** It is an agglutination test where H and O antibodies are detected in the patient's sera by using H and O Ag.
- **Antigens used:** Four antigens are used:
  - O antigens of *S.*Typhi (TO), H antigens of *S.*Typhi (TH), *S.*Paratyphi A (AH) and *S.*Paratyphi B (BH)

  (Paratyphoid O Ag are not used as they cross-react with the *S.*Typhi O Ag due to sharing of factor 12)
- **Strains used** for Ag preparation: *S.*Typhi 901 'O' and 'H' strains and lab strains for *S.*Paratyphi A, and B.
- **Results:**
  - O agglutination appears as compact granular chalky clumps (disc-like pattern), with clear supernatant fluid.
  - H agglutination appears as large loose fluffy cotton-woolly clumps, with clear supernatant fluid.
  - If agglutination does not occur, *button* formation occurs due to deposition of antigens and the supernatant fluid remains hazy.
  - *Antibody Titer:* The highest dilution of sera, at which agglutination occurs.
- **Interpretation:**
  - *Significant titer* in most of the places in India is taken as: H agglutinin titer > 200 and O agglutinin titer > 100
  - Low titers should be ignored and considered as baseline titers in endemic areas (due to prior exposure, people will always have some base line antibodies)
  - O Ab appear early and disappear early and indicate recent infection. H Ab appear late and disappear late.
  - O Ab are nonspecific. They are raised in all, i.e. *S.*Typhi, *S.*Paratyphi A and B
  - H Ab are specific. TH, AH and BH antibodies are raised in *S.*Typhi, *S.*Paratyphi A and B infections respectively.
- **False positive** Widal test may occur due to:
  - Anamnestic response: It refers to a transient rise of titer due to unrelated infections (malaria, dengue) in persons who have had prior infection or immunization.

- If bacterial antigen suspensions are not free from fimbriae
- Persons with inapparent infection or prior immunization (with TAB vaccine)

- **Four-fold rise** in antibody titer in paired sera at 1week interval is more meaningful than a single high titer:
  - After 1 week if titer rises-indicates true infection
  - After 1 week if titer falls- indicates anamnestic responses
- **False negative** Widal test may occur in:
  - Early stage (1st week of illness), Late stage (after fourth week) and in carriers
  - Patients on antibiotics
  - Due to prozone phenomena (antibody excess): This can be obviated by serial dilution of sera.

Widal test result	Suggestive of
Rise of TO and TH antibody	Enteric fever due to *S.*Typhi
Rise of TO and AH antibody	Enteric fever due to *S.*Paratyphi A
Rise of TO and BH antibody	Enteric fever due to *S.*Paratyphi B
Rise of only TO antibody	Recent infection: Due to any serotype *S.*Typhi or *S.*Paratyphi A or B
Rise of only TH antibody	Convalescent stage/Anamnestic response
Rise of all three TH, AH, BH antibodies	Post TAB vaccination

When H antigen reacts with O antibody:
- Forms compact, granular, chalky clumps
- Agglutination takes place slowly.
- Temperature for agglutination is 55°C

When O antigen reacts with H antibody:
- Forms large, loose, fluffy clumps.
- Agglutination takes place rapidly.
- Temperature for agglutination is 37°C

Treatment of Enteric Fever

	Drug of choice	Alternate drug
Empirical t/t	This is the treatment given before antimicrobial susceptibility report is available	
	Ceftriaxone	Azithromycin
Fully Susceptible	If susceptible to all the drugs given for enteric fever	
	Ciprofloxacin	Amoxicillin, Chloramphenicol, Cotrimoxazole
MDR strains (Multidrug-resistant)	Defined as resistant to chloramphenicol, ampicillin, and cotrimoxazole-antibiotics used to treat enteric fever long back.	
	Ciprofloxacin	Ceftriaxone, Azithromycin
NAR strains (Nalidixic acid resistant)	Defined as strains resistant to nalidixic acid with reduced susceptibility to ciprofloxacin	
	Ceftriaxone	Azithromycin, Ciprofloxacin (higher dose and longer course)
Carriers	Ampicillin or Amoxicillin plus probenecid for 6 weeks	Cotrimoxazole or Ciprofloxacin

Interpretation of Widal test:
- Significant titer: H agglutinin titer > 200 and O agglutinin titer > 100
- Low titers: Considered as baseline titers in endemic areas

MDR S. Typhi:
- Defined as resistant to chloramphenicol, ampicillin, and cotrimoxazole
- DOC: Ciprofloxacin

NARST strain:
- R/nalidixic acid, Sn/ciprofloxacin
- DOC- Ceftriaxone > Ciprofloxacin higher course

Vaccines for Typhoid Fever

Parenteral TAB Vaccine

It is heat-killed whole cell typhoid/paratyphoid A and B; no longer in use due to its side effects.

Parenteral Vi Polysaccharide Vaccine

It is composed of Vi capsular polysaccharide antigen derived from *S.*Typhi strain Ty2.

- Dosage: Single dose containing 25 µg of Vi antigen is given IM or subcutaneously.

Typhoral:
- Live attenuated vaccine
- Strain used: S. Typhi Ty21a
- Indicated only after 6 years of age
- Given as Enteric coated capsules.
- Boosters: Every 3 years

- Vaccine confers protection for 2 years.
- VI antigen elicits T independent IgG antibody response, booster given @ 2 years.
- Age: It is given only after 2 years of age.

Typhoral (Oral Live Attenuated S. Typhi Ty21a Vaccine)

- Typhoral is a stable live attenuated mutant of *S.*Typhi strain Ty21a, which lacks the enzyme UDP-galactose-4-epimerase (Gal E mutant).
- On ingestion, it multiplies for sometime, initiates the immune response but self destructs (dies of its own after 4–5 cell divisions, due to lack of enzyme) and therefore cannot induce any pathogenesis.
- It is indicated only after 6 years of age.
- Lyophilized form of the vaccine is available as *enteric coated capsules*.
- It is given orally before food, on alternate days- 1, 3, 5 and/or 7 (total of three or four doses). No antibiotics should be given during this period.
- *Protective immunity* starts after 7 days of the last dose and lasts for 4 years.
- *Boosters* are recommended every 3 years in endemic areas and every year for travelers.

Characteristics	NTS (Non-typhoidal salmonellae)	Typhoidal salmonellae
Host	Zoonotic	Strictly human pathogens
Transmission	Animal food products	Mainly water-borne
Resistance	Relatively resistant to many environmental factors such as drying, salting, smoking and freezing	Less resistant
Prevalence	Both developed as well as developing countries	Mainly in developing countries
Hospitals outbreaks	Common	Rare
Pathogenesis	Similar to that of enteric fever except neutrophil infiltration occurs into intestinal mucosa	Mononuclear cells infiltration in intestinal mucosa
Clinical Manifestations	• Gastroenteritis • Bacteremia: Common with *S.*Choleraesuis and *S.*Dublin • Endovascular infections such as endocarditis • Metastatic localized infections: ○ Intra-abdominal infections, such as hepatic or splenic abscesses or cholecystitis ○ *Salmonella* osteomyelitis is commonly associated with sickle cell disease ○ *Reactive arthritis* (Reiter's syndrome): seen in persons with HLA-B27 histocompatibility antigen.	Enteric fever
Treatment	Conservative with fluid replacement, Antibiotics limited only to invasive NTS infection or severe gastroenteritis	Antibiotics started early
Drug resistant	NTS are more drug resistant	Less drug resistant

MULTIPLE CHOICE QUESTIONS

ESCHERICHIA COLI

1. **Which is not an important cause of neonatal sepsis?** *(AIIMS Nov 2013)*
   a. E. coli
   b. Group B Streptococci
   c. Acinetobacter
   d. Staph. auerus
2. **Enterobacteriaceae are:** *(PGI Dec 2006)*
   a. Pseudomonas
   b. Klebsiella
   c. V.cholerae
   d. Proteus
   e. E. coli

GENERAL PROPERTIES

3. **Which is not member of enterobacteriaceae family?** *(PGI Nov 2016)*
   a. E. coli
   b. Salmonella
   c. Citrobacter
   d. Vibrio
   e. Pseudomonas

DIARRHEAGENIC E. COLI

4. **EHEC strain associated with HUS is:** *(JIPMER May 2015)*
   a. O157 : H7
   b. O7 : H157
   c. O37 : H14
   d. O157 : H9
5. **MC cause of diarrhea in children of developing country is:** *(NEET Pattern Based)*
   a. EHEC
   b. ETEC
   c. EPEC
   d. EIEC
6. **Traveller's diarrhea is caused by:**
   a. ETEC *(NEET Pattern Based, AIIMS Nov 2010)*
   b. EHEC
   c. EPEC
   d. EIEC
7. **Which of the following is not true about HUS?**
   a. May present with hemorrhagic colitis *(AI 2012)*
   b. Shiga like toxin has no role in HUS
   c. Usually self-limited
   d. Fever is typically absent
8. **Culture media used for diagnosis of EHEC O157: H7 is:** *(AI 2009)*
   a. O7 culture
   b. Sorbitol MacConkey media
   c. XLD agar
   d. Deoxycholate media
9. **A 20-year-old man presented with hemorrhagic colitis. The stool sample grew Escherichia coli in pure culture. The following serotype of E.coli is likely to be causative agent:** *(AI 2004, AIIMS May 2003))*
   a. O157:H7
   b. O159:H9
   c. O107:H7
   d. O55:H7
10. **MC cause of HUS in developing country?**
    a. EHEC *(MHPG 2015, Recent Question 2013)*
    b. Shigella dysentriae type 1
    c. ETEC
    d. Pneumococcus
11. **Sereny test is employed for laboratory diagnosis of:**
    a. Enterotoxigenic E. coli *(APPG 2014)*
    b. Enteropathogenic E. coil
    c. Enterohaemorrhagic E. coil
    d. Enteroinvasive E. coli

UROPATHOGENIC E. COLI

12. **Most common cause of pyelonephritis in pregnant women?** *(Recent Questions 2014)*
    a. E. coli
    b. Klebsiella
    c. N. gonorrhea
    d. S. aureus
13. **Regular drinking of which of the following can help prevent UTI?** *(AIIMS Nov 2011)*
    a. Grape juice
    b. Raspberry juice
    c. Orange juice
    d. Cranberry juice
14. **A young lady presents with fever, dysuria and pain abdomen. Uncomplicated acute cystitis was diagnosed. Which among the following is not true?** *(AI 2011)*
    a. E.coli count was < 10^3/ml
    b. 1 pus cell per 7 fields
    c. 1 bacilli per field
    d. Nitrate test positive

PROTEUS

15. **Dienes' phenomena is seen with:** *(PGI 2002)*
    a. Proteus mirabilis
    b. Klebsiella
    c. Proteus vulgaris
    d. Providentia
    e. Morganella

16. All of the following are true except: *(AI 2001)*
a. E.coli is an aerobe and facultative anaerobe
b. Proteus forms uric acid stones
c. E.coli is motile by peritrichous flagella
d. Proteus causes domination of phenylalanine to phenylpyruvic acid

SHIGELLA

17. Hemolytic uremic syndrome mc causative organism in children: *(Recent Question 2015)*
a. E. coli (ETEC)
b. Shigella dysenteriae
c. Salmonella

18. All are catalase positive except:
a. Shigella flexneri *(Recent Question 2015)*
b. Shigella boydii
c. Shigella dysenteriae type 1
d. Shigella sonnei

19. In Tropical countries (India) bacillary dysentery is by: *(TNPG 2015, MHPG 2015)*
a. Shigella dysentriae b. Shigella flexneri
c. Shigella sonnei d. Shigella boydii

20. Which of the following toxins acts by inhibiting protein synthesis: *(AI 2004)*
a. Cholera toxin
b. Shiga toxin
c. Pertussis toxin
d. LT of Enterotoxigenic E.coli

SALMONELLA

21. Salmonella Typhi is the causative agent of typhoid fever. The infective dose of S. Typhi is: *(TNPG 2015, AI 2012, AIIMS Nov 2006)*
a. One bacillus b. 10^8–10^{10} bacilli
c. 10^2–10^5 bacilli d. 1–10 bacilli

22. 15-year-old girl had splenomegaly, leucopenia, fever and died in a few days. Longitudinal ulcers were found in intestine. What should be the probable diagnosis?
a. Typhoid *(AI 2012)*
b. Tuberculosis
c. Amebiasis

23. All are correct regarding widal test, except: *(AIIMS Nov 2009)*
a. Baseline titer differs depending on the endemicity of the disease
b. High titer value is a single widal test is not confirmative
c. O antibody last longer and hence is not indicative of recent infection
d. O antibody cannot differentiate between types

24. All of the following salmonellae are motile except:
a. S. Typhi *(SGPGI 2009)*
b. S. Enteridis
c. S. Gallinarum pullorum
d. S. Chester

25. Drug commonly used against enteric fever are all except: *(AI 2008)*
a. Amikacin
b. Ciprofloxacin
c. Ceftriaxone
d. Azithromycin

26. Incubation period of salmonella Typhi: *(PGI June 2005, AIIMS May 94)*
a. 2–5 days b. 3–21 days
c. 14–25 days d. > 60 days

27. Vi antigen found in: *(PGI June 2005)*
a. Salmonella paratyphi 'A'
b. Salmonella paratyphi 'C'
c. Salmonella dublin
d. Klebsiella pneumoniae
e. Citrobacter freundii

28. There has been an outbreak of food born salmonella gastroenteritis in the community and the stool samples have been received in the laboratory. Which is the enrichment medium of choice: *(AIIMS May 2003)*
a. Cary Blair medium
b. VR medium
c. Selenite 'F' medium
d. Thioglycollate medium

29. A 24-year-old cook in a hostel mess suffered from enteric fever 2 years back. The chronic carrier state in this patient can be diagnosed by: *(AIIMS Nov 2002)*
a. Vi agglutination test
b. Blood culture in Brain Heart infusion broth
c. Widal test
d. Bone marrow culture

30. In a patient with typhoid, diagnosis after 15 days of onset of fever is best done by: *(AI 2001, 2002)*
a. Blood culture
b. Widal
c. Stool culture
d. Urine culture

31. Pea – soup stool is characteristically seen in:
a. Cholera *(JIPMER 2000)*
b. Typhoid
c. Botulism
d. Polio

32. Typhoid carrier is diagnosed by: *(AIIMS 08)*
a. Detection of Core antigen
b. Detection of Vi antigen
c. Detection of Vi antibody
d. Typhoid bacilli in stool

33. DOC for typhoid carrier: *(DNB 01,02)*
a. Ampicillin b. Chloramphenicol
c. Cotrimoxazole d. Clindamycin

34. True about typhoid: *(DNB 08)*
a. Female carriers- less common
b. Male carriers are less common but more dangerous
c. Gallbladder not involved in acrriers
d. Tetracycline –DOC

35. Typhoid revaccination is recommended every ..... years in endemic area: *(Recent Question 2015)*
a. 1 b. 3
c. 5 d. 10

36. Typhoral schedule: *(UP 08)*
a. 1,3,5 days b. 1,2,3,days
c. 1,2,4, days d. 1,7,14 days

37. H_2S forming Salmonella: *(PGI Nov 2012)*
a. S. Typhimurium b. S. Typhi
c. S. Paratyphi A d. S. Paratyphi B
e. S. Cholerasuis

38. Red pigment producing bacteria is?
a. E coli *(Recent Questions 2014)*
b. Bordetella parapertussis
c. Pseudomonas aeruginosa
d. Serratia marcescens

YERSINIA

39. In pneumonic plague, transmission occurs from human to human by? *(Recent Question 2015)*
a. Rate flea bite b. Rat bite
c. Respiratory droplet

40. True about plague: *(PGI May 2015)*
a. Seasonal spread
b. No vaccine is available
c. Tetracycline is used both for chemoprophylaxis and treatment
d. Caused by gram negative motile bacteria

41. Children with Thalassemia and iron overload are at an increased risk for infection with: *(MHPG 2015)*
a. Yersinia enterocolitica b. Campylobacter jejuni
c. Escherichia coli d. Vibrio cholerae

42. What is not true about yersiniosis:
a. Zoonosis *(NEET Pattern Based)*
b. Caused by Y.pestis
c. Caused by Yersinia enterocolitica
d. Caused by Yersinia pseudotuberculosis

43. Farmer presents with the features of high fever, painful inguinal lymphadenopathy, vomiting and diarrhea and hypotension. Which stain will help in the diagnosis? *(AI 2011, AIIMS Nov 2012)*
a. Neisser stain
b. Wayson's stain
c. Albert's stain
d. McFadyean's stain

44. A girl from Shimla presented to OPD with fever, hypotension, malaise and axillary and inguinal lymphadenopathy. Culture in glucose broth shows stalactite growth. Most likely causative organism is:
a. Yersinia pestis *(AIIMS Nov 2008)*
b. Francisella tularensis
c. Brucella abortus
d. Coxiella burnetii

45. A young boy had a flea bite while working in a wheat grain goes down. After 5 days he developed fever and had axillary lymphadenopathy. A smear was sent to the laboratory to perform a specific staining. Which one of the following staining method would help in the identification of the suspected pathogen: *(AI 2006)*
a. Albert staining
b. Ziehl – Neelsen staining
c. McFadyean's staining
d. Wayson staining

46. True statements about Y. Pestis are: *(PGI June 2004)*
a. Gram-positive
b. Nonmotile
c. Benzyl penicillin is given in prophylaxis
d. Patients are kept isolated till 48 hours of treatments
e. Repeated blood culture is diagnostic

47. The drug of choice for chemoprophylaxis in contacts of a patient of pneumonic plague is: *(AIIMS Nov 2002)*
a. Penicillin b. Rifampicin
c. Erythromycin d. Tetracycline

48. Plague epidemic in Surat had occurred after a silence period of: *(DPG 05)*
a. 18 yrs b. 20 yrs
c. 28 yrs d. 30 yrs

49. Maximum explosiveness of plague is determined by:
a. Total flea index *(AP 03, DPG 06)*
b. Cheopis index
c. Burrow index
d. Specific percentage of flea

50. Highly infectious plague: *(TN 03)*
a. Bubonic plague b. Pneumonic plague
c. Septicemic plague d. Any of the above

51. Which of the following is NOT a source of infection in plague? *(MHPG 2014)*
a. Case of bubonic plague
b. Case of pneumonic plague
c. Infected rodents
d. Infected rat fleas

52. Appendicitis like syndrome is caused by: *(Recent Question 2015)*
a. Y. pestis b. Y. pseudotuberculosis
c. Pasturella septica d. Brucella abortis

EXPLANATIONS

ESCHERICHIA COLI

1. **Ans. (c) (Acinetobacter)** Ref: Nelson Textbook of Pediatrics, 18/e Chapter 176

Among the options, Acinetobacter is the best answer.

The infectious agents associated with sepsis in pediatric patients:

- ***In the neonatal age group, group B streptococcus, Escherichia coli, Listeria monocytogenes, enteroviruses, and herpes simplex virus are the pathogens most commonly associated with sepsis.***
- In older children *Streptococcus pneumoniae, Neisseria meningitidis,* and *Staphylococcus aureus* are more common.
- Toxic shock syndrome from group A streptococcus or *S. aureus* can also be seen in older children.
- Infections with gram-negative bacteria (e.g. *Escherichia coli, Pseudomonas, Acinetobacter, Klebsiella, Enterobacter, Serratia*) and fungi (e.g. *Candida, Aspergillus*) most often occur in immunocompromised and hospitalized patients colonized with these organisms.
- **Pseudobacteremia** may be associated with contaminated heparin flush solutions, intravenous solutions, albumin, cryoprecipitate, and infusion equipment. Contaminants include waterborne organisms, such as *Burkholderia cepacia, Pseudomonas aeruginosa,* and *Serratia.*

2. **Ans. (b), (d), (e) (Klebsiella, Proteus, E.coli)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p299
   - **Classification of Enterobacteriaceae – Refer chapter review.**

GENERAL PROPERTIES

3. **Ans (d,e) (Vibrio, Pseudomonas)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p298
   - Vibrio and Pseudomonas are not members of enterobacteriaceae family

DIARRHEAGENIC E. COLI

4. **Ans. (a) (O157:H7)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p304, Ananthanarayan9/e p 279
   - **HUS is caused by EHEC strain O157:H7.**

5. **Ans. (c) (EPEC)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p304, Ananthanarayan 9/e p 279, 8/e p277
   - Next to Rotavirus, EPEC is the MC cause of diarrhea in children of developing country.

6. **Ans. (a) (ETEC)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p304, Ananthanarayan 9/e p 279, 8 /e p277
   - ETEC is the MC cause of traveler's diarrhea.

7. **Ans. (b) (Shiga like toxin has no role in HUS)** Apurba Sastry's Essentials of Medical Microbiology 1/e p304' Harrison 18/e chapter 286/Vascular Injury to the Kidney, Chapter-149/p1251

	Hemolytic Uremic Syndrome	Thrombotic Thrombocytopenic Purpura
Age	Children	Adult (40 yr)
Pathogenicity	Hemorrhagic diarrhea due to- Shiga toxin mediated (S.dys-1) Verocytotoxin mediated (EHEC)- 157/H7	Absence of metalloprotease ADAMTS13 specific for vWF
Neurologic symptoms	Less common	More pronounced
Fever	Typically absent	Present
Treatment	Usually self-limited Plasma exchange: Not Required	Plasma exchange: Required for treatment

8. **Ans. (b) (Sorbitol MacConkey media)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p304
   - EHEC can be differentiated from other E.coli as it is sorbitol non fermenter, hence it produces colorless colony on Sorbitol MacConkey media whereas others can ferment sorbitol producing pink colony.

9. **Ans. (a) (O157:H7)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p304, Ananthanarayan 9/e p279, 8/e p277
   - Enterohemorrhagic E.coli dysentery can lead to complications like hemorrhagic uremic syndrome and hemorrhagic colitis.
   - Serotypes' associated with EHEC are O157:H7 and O26:H1
   - Similar illness also produced by Shigella dysenteriae type1.

10. **Ans. (b) (S.dysentriae type 1)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p304, Ananthanarayan 9/e p287
    MC cause of HUS in developing country is Shigella dysentriae type 1; whereas in developed country, it is EHEC O157:H7.

11. **Ans. (d) (Enteroinvasive E. coli)** Apurba Sastry's Essentials of Medical Microbiology 1/e p304, Ananthanarayan 8/e p277
    Sereny's test is an animal pathogenicity test employed for laboratory diagnosis of Enteroinvasive E. coli and Shigella. Instillation of organism into the eyes of guinea pigs leads to conjunctivitis and severe keratitis.

UROPATHOGENIC E.COLI

12. **Ans. (a) (E. coli)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p303, Harrison's 18/e chapter 288
    E.coli is the most common cause of UTI-Both upper UTI (pyelonephritis) and Lower UTI (Cystitis).

13. **Ans. (d) (Cranberry juice)**
    Ref: Journal- Inhibitory activity of cranberry extract on the bacterial adhesiveness in the urine of women: An ex-vivo study'. Int. journal of immunopathology and pharmacology, *Tempera et al* 2010-23 (2): 611–8.
    - ***Cranberry juice may help prevent and relieve the symptoms of urinary tract infections***
    - ***Mainly by 3 mechanisms:***
      - Directly by altering the molecular structure of the fimbriae on the pathogenic strains.
      - Proanthocyanidins in cranberries prevents the bacteria adherence to the bladder and urinary tract.
      - Indirectly on the bacteria by reducing the intravesical pH.

14. **Ans. (a) (E.coli count was < 10^3/ml)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p303
    - *UTI is diagnosed when the bacterial count exceeds 10^5/ml of urine (significant Bacteriuria).*

    About other options

    Option (b): *'Under appropriate condition, finding of 1 leukocyte per 7 high power field correlates with 10^4 leucocytes/ml and which implies significant'.* …..Mackie and McCartney Practical Microb. 14/e p86

    Option (c): *Gram staining is not a reliable method to estimate the significance of UTI.*
    Using >1/Oil immersion field, the sensitivity is 91% which correlates with significant Bacteriuria.
    ……. Baily and Scott Diagnostic Microbiology 12/e p848

    Option (d): The most common organisms causing UTI are E.coli (70%), Klebsiella, Proteus.
    All these belong to family Enterobacteriaceae and *all can reduce nitrate to nitrite.*

PROTEUS

15. **Ans. (a) and (c) (P. mirabilis, P. vulgaris)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p310
    Diene's phenomenon – to know the relatedness between different strains of Proteus
    - *When two Proteus strains are streaked at two ends of a blood agar, they start swarming and join to each other.*
    - *If both the strains are related, there will not be any line of separation and if both the strains are not related, they will be separated by a line of demarcation.*

16. **Ans. (b) (Proteus forms uric acid stones)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p310
    Proteus forms struvite stone in bladder in alkaline urine.

SHIGELLA

17. **Ans. (b) (S. dysenteriae)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p306

18. **Ans. (c) (S. dysenteriae type 1)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p306

19. **Ans. (b) (S.flexneri)** (Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p306, Ananthanarayan 9th /p 288)
Bacillary dysentery is caused by Shigella species:
- In India and other tropical countries- MC Shigella species is S. flexneri
- In developed countries- MC Shigella species is S. sonnei

20. **Ans. (b) (Shiga...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p306, Ananthanarayan 9/e p286, 8/e p284
Toxin which acts by inhibiting protein synthesis:
- Diphtheria toxin and Pseudomonas Exotoxin A: Inhibiting elongation factor 2
- Shiga toxin and Verotoxin (Shiga like toxin) of EHEC: Inhibiting 60s ribosome.

SALMONELLA

21. **Ans. (c) (10^2 –10^5 bacilli)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p319, Harrison 19/e p1049-53
- *All Salmonella infections begin with ingestion of organisms, most commonly in contaminated food or water. The infectious dose is 10^3–10^6 colony-forming units.*

22. **Ans. (a) (Typhoid)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p310, 19/e p1049-53, Harrison 18/e p1276
- ***Splenomegaly, leucopenia, fever and Longitudinal ulcers found in intestine are characteristic features of enteric fever.***

23. **Ans. (c) (O antibody...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p321, Ananthanarayan 9/e p298
- O antibody appears early and goes early and rise of O Antibody without rise of H Antibody indicates recent infection.
- Details about Widal test- refer chapter review.

24. **Ans. (c) (S. gallinarum...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p319, Ananthanarayan 9/e p 299
Salmonelle are motile except S. gallinarum pullorum which are nonmotile (aphasic)

25. **Ans. (a) (Amikacin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p326, Harrison 19/e p1049-53, 18/e p1277,
- Aminoglycosides are not given for enteric fever
- Treatment for Enteric fever- Refer Chapter Review

26. **Ans. (b) (3-21 days)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p320, Harrison 19e/1049-53 , 18/e p1275, 17/e p957
- *'The incubation period for S. Typhi averages 10–14 days but ranges **from 3 to 21 days**, with the duration likely reflecting the inoculum size and the host's health and immune status.'*

27. **Ans. (b), (c), (e) (S. Paratyphi C, S. Dublin, Citrobacter)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p318
- Vi Antigen is possessed by ***S Typhi and S. Paratyphi C, S. Dublin, Citrobacter (Ballerup-Bethesda group).***
- For detailed explanation refer chapter review.

28. **Ans. (c) (Selenite 'F' medium)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p321
Outbreak of food born salmonella gastroenteritis
For the stool culture, 1st it has to be processed in an enrichment medium followed by streaking in a selective media.
Enrichment medium for Salmonella: Tetrathionate broth, Selenite F broth, Gram-negative broth.

29. **Ans. (a) (Vi aggluti...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p325, Ananthanarayan 9/e p299
- Vi agglutinins of titer of > 1:10 is taken significant for diagnosis of Typhoidal carriers.

30. **Ans. (b) (Widal)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p324, Ananthanarayan 9/e p298, 8/e p297
- *'Agglutinins in Widal test start appearing at the end of 1st week and increases steadily till 3rd/4th week then fall.'*
- Week wise diagnosis of choice of Enteric fever- Refer chapter review.

31. **Ans. (b) (Typhoid)** Ref: Farlex's Free Online Medical dictionary
- 'Pea soup stool is used for the khaki-green, slimy stools typically occurs in the 3rd week of typhoid fever, at which point the Patients are in a 'toxic state' and at greatest risk for intestinal perforation and hemorrhage; Similar stools occur in enteropathic E coli infections of infants'.

32. **Ans. (d) > (c) (Typhoid... > Detection...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p350
Typhoid carrier is diagnosed by:
- Detection of Vi antibody
- Culture in stool and urine (Best method)

33. **Ans. (a) (Ampicillin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p326, Park 23/e p237, 22/e p216
DOC for typhoid carrier: Ampicillin or Amoxycilin plus probenecid for 6 weeks.

34. **Ans. (b) (Male...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p326, Park 23/e p236 , 22/e p213-16
- Male carriers are less common but more dangerous
- MC site of for typhoid carrier: Gallbladder and billiary tract and also urinary tract
- DOC for typhoid carrier: Ampicillin or Amoxycilin plus probenecid for 6 weeks.

35. **Ans. (b) (3 years)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p326

36. **Ans. (a) (1,3,5)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p326, Ananthanarayan 9/e p295-96
- Typhoral schedule: 3 doses on alt day (1/3/5) in capsules, Effective after 7 days, Booster needed @5 years.

37. **Ans. (a),(b),(d),(e) (S.Typhimurium, S.Typhi, S.Paratyphi B , S.Cholerasuis)** Apurba Sastry's Essentials of Medical Microbiology 1/e p321
H_2S forming Salmonella:
- Salmonella Paratyphi -A : Absent H_2S
- Salmonella Paratyphi –B : Big H_2S (Produce large quantity of H_2S)
- Salmonella Typhi and S.Cholerasuis- Tiny H_2S (i.e. small amount of H_2S)

38. **Ans. (d) (Serratia marcescens)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p309, Ananthnarayan 9/e p281
Serratia marcescens produces red pigment called prodigiosin.

YERSINSIA

39. **Ans. (c) (Respiratory...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p313

40. **Ans. (a, c) (Seasonal spread)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p312-14, Park 23rd/p293
- **Plague outbreaks are usually seasonal in North India (from September until May) which depends on field rodent factors. No seasonality is found in South India.**
- **Tetracycline is DOC for chemoprophylaxis and it can also be given for treatment (DOC for treatment is Gentamicin)**
- **Y.pestis is gram negative but non motile.**

41. **Ans (a) (Yersinia enterocolitica)** Ref: Harrison 19th/p1076, Apurba Sastry's Essentials of Medical Microbiology 1/e p 315
Any condition involving iron overload (including thalassemia and hemochromatosis) is a risk factor for infection with Yersinia enterocolitica.

42. **Ans. (b) (Caused by Y.pestis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p311, Ananthanarayan 9/e p324
- The term Yersiniosis denotes infection with Yersiniae other than Y.pestis. These include zoonotic infections by Y.pseudotuberculosis and Y.enterocolitica.

43. **Ans. (b) (Wayson stain)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p313, Ananthanarayan 9/e p323
This is a case of bubonic plague

Points in favor:
- Farmer: High-risk of exposure to rodents
- Presented with high fever, painful inguinal lymphadenopathy,
- So, now we have to use a stain that will stain the causative agent Y. pestis.
- *'When stained with a polychromatic stain (e.g. Wayson or Giemsa), Y. pestis isolated from clinical specimens exhibits a characteristic bipolar appearance, resembling closed safety pins'.*

Other stains given in the question:
- Neisser stain and Albert's stain: Used to demonstrate metachromatic granules
- McFadyean's stain: Used to demonstrate capsule of B.anthracis.

44. **Ans. (a) (Yersinia pestis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p312-13, Ananthanarayan 9/321-22
Points in favor:
Girl from Endemic area of plague (Shimla) presenting with lymphadenopathy.
Culture showing ***stalactite*** growth.

45. Ans. (d) (Wayson staining) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p313, Ananthanarayan 9/e p323
Refer previous explanation.

46. Ans. (b), (d), (e) (Nonmotile, Patients are kept isolated... Repeated blood...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p312-13, Ananthanarayan 9/e p323-24, Harrison 19e/1071-75, 18/e p1305-09

- ***Option a, b:*** Y.pestis is Nonmotile Gram-negative Coccobacilli
- ***Option c:*** Treatment Streptomycin (DOC) ……Harrison 17/e p984
- *Others*- Gentamicin, Tetracycline, Doxycycline and Chloramphenicol
- '***Option d:*** *Patients in whom respiratory plague is suspected should be managed* ***under isolation****, until pneumonia has been ruled out or* ***until 48 hour*** *of antimicrobial therapy* ……Harrison 17/e p984
- ***Option e:***

'Cultures of three blood samples taken over a 45-min period before treatment usually results in isolation of the bacterium. Y. pestis' ……Harrison 17/e p 983

47. Ans. (d) (Tetracycline) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p314 Harrison 19/e p1075, 10, 18/e p1309

DOC for **chemoprophylaxis of plague**: Tetracycline (Others: Doxy, Cotrimoxazole, Ciprofloxacin)
DOC for treatment of plague: Streptomycin was given in the past, now Gentamicin is is recommended for treatment.

48. Ans. (c) (28 years) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p311, Park 23/e p292-97, 22/270

- Plague epidemic in Surat in 1994 had occurred after 28 years of last outbreak that had occurred in Karnataka in 1966.

49. Ans. (b) (Cheopis index) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p312, Park 23/e p292-97, 22/269

- Cheopis index: Average no. of X.cheopis per rat
- It is the most efficient indicator to measure the explosiveness of plague.

50. Ans. (b) (Pneumonic plague) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p313, Park 23/e p292-97, 22/269

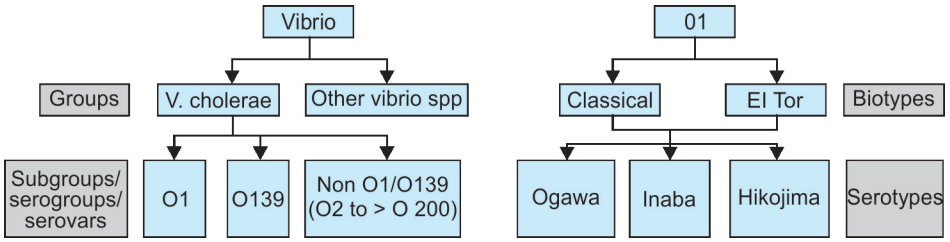
- MC form of plague: Bubonic plague
- Highly infectious and highly fatal: Pneumonic plague.

51. Ans. (a) (Case...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p311-13, Ananthnarayan 9/e p320-22
MC source for bubonic plague is infected rat fleas followed by Infected rodents
Source for pneumonic plague is case of pneumonic plague.

52. Ans. (b) (Y. pseudotuberculosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p315
Y. enterocolitica commonly causes terminal ileitis and Y. pseudotuberculosis commonly causes mesenteric adenitis, leading to pseudoappendicitis.

CHAPTER

3.8 Vibrio



Classification of Vibrio:
- Nonhalophilic: *V. cholerae* and *V. mimicus*
- Halophilic: *V. parahaemolyticus* *V. alginolyticus and* *V. vulnificus*

CLASSIFICATION (GARDNER AND VENKATRAMAN)

Based on Salt Requirement

- Nonhalophilic vibrios: They cannot grow at higher salt concentrations. Examples, *V. cholerae* and *V. mimicus*
- Halophilic vibrios: Salt is their absolute requirement. They cannot grow in the absence of salt. They can tolerate and grow at higher salt concentration of up to 7–10, e.g. *V. parahaemolyticus, V. alginolyticus* and *V. vulnificus*.

V. cholerae is Further Classified

- **Serogrouping:** Based on somatic O antigen side chain of LPS (lipopolysaccharide), *V. cholerae* can be grouped into more than 200 serogroups or serovars:
  - O1 serogroup was responsible for all pandemics and most of the epidemics of cholera.
  - Nonagglutinable (NAG vibrios) or non-cholera vibrios (NCV)- refers to non-O1 serogroups.
  - O139 serogroup was identified in 1992 and causes cholera outbreaks India and Bangladesh.
  - Non O1/O139 serogroups have occasionally caused sporadic outbreaks of diarrhea and extraintestinal manifestations, but have never caused epidemic cholera so far.
- **Serotyping:** O1 serogroup can be further divided into three serotypes: Inaba, Ogawa, and Hikojima; based on minor antigenic differences of O antigen:
  - Ogawa is the most common serotype isolated from clinical samples followed by Inaba.
  - However, during epidemics, shifting between Ogawa to Inaba shift can takes place.
  - Hikojima represents an unstable transitional state where both Inaba and Ogawa antigens are expressed.
- **Biotyping:** Serogroup O1 has two biotypes classical and El Tor; differentiated by various biochemical reactions:
  - *Classical biotype* was responsible for the first six pandemics of cholera.
  - *Currently,* most of cholera cases are due to El Tor, although occasional classical isolates are still seen.
  - However, some isolates do not fit in to both the biotypes and are called as *El Tor variants.*
  - ***El Tor variants:*** Few variants of El Tor biotype have been described recently in Bangladesh and in few other places of Asia and Africa which show properties overlapping with that of the classical biotype, e.g. include:

Typing:
- Serogrouping: Based on major differences of O Ag, > 200 serogroups
- Serotyping: Based on minor differences of O Ag, 3 serotypes (Inaba, Ogawa, and Hikojima)
- Biotyping: Based on biochemical property, 3 types (Classical and El Tor and El Tor variants)

El Tor variants:
Some isolates have mixed property of Classical and El Tor biotypes:
- Matlab variants (El Tor hybrid)
- Mozambique variant (2004–2005)

- *Matlab variants (El Tor hybrid):* These strains could not be biotyped because they have a mixture of both classical and El Tor properties, were described first in Bangladesh in 2002.
- *Mozambique variant (2004–2005):* It has a typical phenotypic properties and genome of El Tor, except that the cholera toxin and its gene (CTX) are of classical type.

Biotypes of *V. cholerae* O1	Classical biotype	El Tor biotype
β hemolysis on sheep blood agar	Negative	Positive
Chick erythrocyte agglutination	Negative	Positive
Polymyxin B (50 IU)	Sensitive	Resistant
Group IV phage susceptibility	Susceptible	Resistant
El Tor Phage V susceptibility	Resistant	Susceptible
VP (Voges Proskauer) test	Negative	Positive
CAMP test	Negative	Positive
Cholera toxin gene	CTX-1	CTX-2

Cholera toxin (CT) Mechanism:
- Fragment B: Binds to GM1 ganglioside receptors on GI epithelium
- Fragment A causes ADP ribosylation of G protein → ↑adenylate cyclase → ↑ cAMP.

PATHOGENESIS OF CHOLERA

Pathogenesis of cholera is toxin-mediated. Both *V. cholerae* O1 and O139 are capable of producing cholera toxin, thus resulting in cholera:

- **Transmission:** Ingestion of contaminated water, or food.
- **Infective dose:** Being acid-labile; a high infective dose of 10^8 bacilli is required to bypass the gastric barrier.
- **Factors** promoting transmission include conditions reducing gastric acidity, such as hypochlorhydria, use of antacids.
- **Crossing of the protective layer of mucus**: In the small intestine, vibrios penetrate the mucus layer which may be achieved by—Its highly active motility, producing mucinase and hemagglutinin protease (cholera lectin)
- **Adhesion** to the intestinal epithelium is facilitated by fimbria called *toxin co-regulated pilus* (TCP).
- **Cholera toxin (CT):** Consists of two peptide fragments: A & B
  - *Fragment B* is the binding fragment and t binds to GM1ganglioside receptors present on the intestinal epithelium
  - *Fragment A* is the active fragment (27kDa), causes ADP ribosylation of G protein → upregulates the activity of adenylate cyclase → result is the intracellular accumulation of cyclic AMP.
- **Increase in cyclic AMP leads to:**
  - Accumulation of sodium chloride in the intestinal lumen.
  - Water moves passively into lumens leads to accumulation of isotonic fluid resulting in watery diarrhea.
  - Loss of fluid and electrolytes leads to shock (due to profound dehydration) and acidosis (due to loss of bicarbonate).
- **Gene for cholera toxin** (CTX) is phage coded.
- ***ToxR* gene** regulates the expression of CT, TCP and other virulence factors
- **Chromosomes:** *V. cholerae* has two circular chromosomes (in contrast to most other bacteria having one chromosome)
- **Other virulence factors:**
  - Zonaoccludens toxin: Disrupts the tight junctions between mucosal cells
  - Siderophore: Required for iron acquisition
  - Bacterial endotoxin (LPS): Unlike other gram-negative bacilli, the LPS of *V. cholerae* does not contribute to the pathogenesis of cholera. However, it is immunogenic, and is included as a component in killed vaccines.

CLINICAL MANIFESTATIONS (CHOLERA)

V. cholerae O1 or O139 infection produces a ranging from: (i) asymptomatic (75%), (ii) mild diarrhea or cholera (20%), (iii) explosive diarrhea (*cholera gravis*, in 5% of cases)

- **Incubation period**: Varies from 24 to 48 hours.
- **Watery diarrhea:** Sudden onset of *painless* watery diarrhea
- **Rice water stool:** The stool is typically non-bilious, slightly cloudy, gray, and watery with mucus flakes but without blood or pus cells, with a fishy, inoffensive odor.
- Vomiting and muscle cramps may be present but fever is usually absent.
- **Complications** are directly proportional to the fluid loss resulting in loss of body weight.

Loss of body weight by	Symptoms
< 5%	Increased thirst
At 5–10%	Postural hypotension, weakness, tachycardia Decreased skin turgor
At > 10%	Renal failure (due to acute tubular necrosis) and fluid loss result in: • Oliguria and weak or absent pulses • Sunken eyes and fontanelles in infants • Wrinkled 'washer woman' skin • Somnolence and coma

Watery diarrhea (Rice water stool):
- Sudden onset of painless watery diarrhea
- Nonbilious, slightly cloudy, gray, and watery with mucus flakes
- Without blood or pus cells (noninvasive)
- Fishy, inoffensive odor

EPIDEMIOLOGY

History of Pandemics

Cholera can occur in many forms: sporadic, limited outbreaks, endemic, epidemic or pandemic.

- **Home land** of cholera was the *delta region* of the Ganges and Brahmaputra in West Bengal (India) and Bangladesh
- **First six pandemics** (1817 to 1923): All were caused by the classical biotype of *V. cholerae* which had spread from Bengal to involve most of the world which resulted in several thousands of deaths.
- **Seventh pandemic** started in 1961 and it differed from the first six pandemics by:
  - It was the only pandemic that originated outside India, i.e. from Indonesia (Sulawesi, formerly Celebes Island) in 1961. India was affected in 1964 and the whole world was encircled by 1991.
  - It was the only pandemic to be caused by El Tor biotype
  - El Tor produced a much milder cholera; with more carrier rate than the classical. This is due to the fact that El Tor is much hardier but less virulent than the classical vibrios.
- **O139 (Bengal strain)** was isolated first from Chennai in 1992. Since it was not agglutinated by any of the antisera available at that time (O1 to O138), it was designated as a new serogroup O139 or the *Bengal strain*.
  - O139 appears to be a derivative of O1 El Tor, but differs from the latter in having a distinct LPS and being **capsulated**. Thus, it is invasive and can cause bacteremia and extraintestinal manifestations also.
  - There is no cross protection between O1 and O139.
  - Currently, O139 still causes a minority of cases in India and Bangladesh.

Seventh pandemic:
- Only pandemic that originated outside India
- Only pandemic to be caused by El Tor biotype
- Much milder cholera; with more carrier

Classical vs El Tor:
El Tor has more carrier rate.
Classical is more virulent.
The case–carrier ratio is
- 1: 50 for the Classical biotype
- 1:90 for the El Tor biotype

Epidemiological Determinants Include

- **Reservoir:** Humans are the only reservoir. There is no animal reservoir.
- **Source** of infection may be either asymptomatic cases or carriers.
- **Carriers** are apparently healthy people who shed the bacilli in feces. Carriers may be:
  - *Incubatory carriers:* They are less common as cholera has a short incubation period of 1–2 days.

Persistence of V. cholerae:
- During epidemics: Maintained by carriers
- In inter epidemic period: Maintained in sea water, crustaceans and planktons.

Resistance of V. cholerae:
- Acid-labile but stable to alkali.
- Heat-labile but stable to refrigeration

Selective media:
- Alkaline bile salt agar (BSA)
- Monsur's GTTTA medium
- TCBS agar

  - *Convalescent carriers* are the recovered patients who shed the bacilli for 2–3 weeks.
  - *Contact* or *healthy carriers* result from a subclinical infection. They shed the bacilli for less than 10 days.
  - *Chronic carriers*: Minority of convalescent carriers become chronic carriers.

  In general, biotype El Tor has more carrier rate than classical. The case–carrier ratio is 1: 50 for the Classical biotype and 1:90 for the El Tor biotype.
- **Cholera season:** Maximum transmission occurs in, heavy rainfall and flooding
- **Factors determining severity** of the disease include:
  - Lack of pre-existing immunity
  - Persons with *'O' blood group* are at greater risk of severe disease if infected, while those with type AB blood group are at least risk.
- **Age:** During inter epidemic period, all the age groups are affected equally, however during epidemics it affects more commonly children.
- **Habitat:** *V. cholerae* is a natural inhabitant of coastal sea salt water and brackish estuaries.
- **Persistence** of *V. cholerae:*
  - During epidemics, it is maintained by carriers and subclinical cases.
  - In inter epidemic period, it is maintained in sea water, crustaceans and planktons.
- **Resistance:**
  - *V. cholerae* is acid- labile but stable to alkali.
  - It is heat: Labile but stable to refrigeration and can remain in ice for 4–6 weeks.

LABORATORY DIAGNOSIS

Specimens

- Freshly collected watery stool before starting the antibiotics.
- Rectal swab is preferred specimen for convalescent patients or carriers.

Transport/Holding Media

- Venkatraman-Ramakrishnan (VR) medium
- Alkaline salt transport medium
- Cary-Blair medium
- Autoclaved sea water.

Direct Microscopy

- **Gram staining** of fecal smear reveals short curved **comma-shaped** gram-negative rods, arranged in parallel rows, *(fish in stream appearance)*
- **Darting motility or shooting star motility (**actively motile frequently changing their direction, also seen in *Campylobacter* and *Aeromonas*).

Culture

Cultural conditions: *V. cholerae* ***is:***
- Nonfastidious and strongly aerobic
- Hemodigestion on blood agar
- Growth is better in alkaline medium. The optimum pH is 8.2
- NaCl (0.5–1%) stimulates the growth, however, high concentrations of NaCl (> 6%) are inhibitory.

Culture medium:
- **Enrichment broth:**
  - Alkaline peptone water (APW)
  - Monsur's taurocholate tellurite peptone water (pH 9.0).
- **Selective media:**
  - Alkaline bile salt agar (BSA)
  - Monsur's gelatin taurocholate trypticase tellurite agar (GTTTA) medium

- TCBS agar (thiosulfate, citrate, bile salts, sucrose and pH of 8.6)-*V. cholerae* produces yellow colonies due to sucrose fermentation. Sucrose nonfermenters (*V. mimicus* and *V. parahemolyticus*) produce green colonies.

Biochemical Reactions

Important biochemical properties of *V. cholerae* include:

- Catalase and Oxidase positive
- Indole and nitrate test positive (together called **Cholera red reaction)**
- String test positive
- **Susceptible to O/129** (vibriostatic agent)

Biochemical reactions
- Oxidase positive
- Cholera red reaction
- String test positive

TREATMENT OF CHOLERA

- DOC in adult: Macrolide (Azithromycin and erythromycin) followed by Doxycycline
- DOC in children: Cotrimoxazole
- DOC in pregnancy: Furazolidone
- DOC for chemoprophylaxis: Tetracycline.

Treatment of Cholera
- DOC in adult: Azithromycin
- DOC in children: cotrimoxazole
- DOC in pregnancy: Furazolidone
- DOC for chemoprophylaxis: Tetracycline

VACCINE

Injectable killed vaccines: They are no longer in use. As they provide little protection, produces adverse effects.

Oral cholera vaccines (OCV) are currently in practice. Two types of oral vaccines.

1. ***Killed Whole-Cell Vaccine***
   - Two types: Whole cell (WC) vaccine and Whole cell recombinant B subunit cholera vaccine (WC/rBS) (Dukoral)
   - *Schedule*: Two oral doses are given at 7 days gap. C/I to children < 2 years.
   - *Protection* is short lived. (At 6 months, 58% for WC vaccine and 85% for WC/rBS vaccine)
   - Children are better protected than adults.
   - WHO recommends for using vaccine during epidemics in the community but not during inter epidemic period.
2. ***Oral live attenuated vaccines:***
   - CVD 103-HgR, Peru-15 and *V. cholerae* 638 for classical and/or El Tor biotypes of *V. cholerae* O1.
   - CVD-112 and Bengal-15 vaccine trials are ongoing for *V. cholerae* O139.

HALOPHILIC VIBRIOS

Halophilic vibrios can withstand higher salt concentration (> 6%).

V. parahaemolyticus

- **Manifestations** include:
  - Food-borne gastroenteritis is the MC presentation, following raw or uncooked sea food (e.g. Oyster) intake. It presents as watery diarrhea (MC) or rarely dysentery with abdominal cramps.
  - Extraintestinal manifestations are rare, such as wound infection, otitis and sepsis.
- **Laboratory diagnosis**: The distinct properties are:
  - Morphology: It is capsulated, shows bipolar staining in fresh isolates and pleomorphism in older cultures
  - Motile by peritrichous flagella (but does not show darting motility)
  - On TCBS agar it produces green colonies (sucrose nonfermenter)
  - **Kanagawa phenomenon**: It causes β hemolysis on Wagatsuma agar

V. parahaemolyticus
- Food-borne gastroenteritis following raw or uncooked sea food
- Kanagawa phenomenon: β hemolysis on Wagatsuma agar

- Swarming on blood agar
- Urease test is positive in few strains
- Salt tolerance test: It can resist maximum of 8% NaCl.

V. vulnificus

Also called (L+) *Vibrio* as it is the only vibrio that ferments lactose. It produces the most severe infection of all vibrios.

- ***Primary sepsis*** usually occurs in patients with underlying liver disease and iron overload or rarely in renal insufficiency and immunosuppression.
- ***Primary wound infection***
- Occasionally, it may cause gastroenteritis.

V. alginolyticus

- *V. alginolyticus* can occasionally cause eye, ear, and wound infections.
- Few cases of otitis externa, otitis media, and conjunctivitis have been reported.
- It rarely causes bacteremia in immunocompromised hosts.
- It is the most salt-tolerant *Vibrio* and can grow at salt concentrations of > 10%.

MULTIPLE CHOICE QUESTIONS

VIBRIO

1. Vibrio cholerae differs from E.coli by? *(JIPMER May 2016)*
a. Oxidase test
b. Catalase test
c. Optochin sensitive
d. Lactose fermenter

2. Vibrio cholerae toxin acts by disrupting which of the following structures? *(AIIMS May 2015)*
a. Hemidesmosome
b. Gap junctions
c. Zona occludens
d. Zona adherens

3. Channel activated by cholera toxin: *(JIPMER Nov 2014)*
a. Adenylate cyclase
b. Guanyl cyclase
c. ABC transport channel
d. Iron transport channel

4. Which of the following statement(s) is/are wrong about the 8th pandemic of cholera? *(PGI Nov 2014)*
a. Caused by Vibrio cholerae O139
b. Started in 1992
c. Started in Bangladesh
d. Mostly confined to Bangladesh & India
e. Capsulated

5. True about mechanism of bacterial toxins: *(PGI May 2013)*
a. Cholera toxin acts by inhibition of guanylcyclase
b. Botulinum toxin inhibits Ach release
c. Shiga toxin of Shigelladysenteriae act by inhibiting protein synthesis
d. Diphtheria toxin act by inhibiting protein synthesis

6. Invasive infections caused by all except: *(NEET Pattern Based)*
a. Shigella
b. Salmonella
c. V. cholerae
d. Yersinia

7. The endotoxin of the following gram-negative bacteria does not play any part in the pathogenesis of the natural disease: *(AI 2012, AIIMS 2006, AIIMS Nov 2012)*
a. E. coli
b. Klebsiella
c. Vibrio cholerae
d. Pseudomonas

8. Tetracycline is used in prophylaxis of: *(AI 2011, AI 2005)*
a. Shigellosis
b. Cholera
c. Brucellosis
d. Leptospirosis

9. Not true about EIT or Vibrio 01: *(AI 2010)*
a. Animals are the only reservoir
b. Epidemiologically indistinguishable from V. cholerae 0139
c. Human acts as vehicle for spread
d. The efficacy of vaccine against E1tor Vibrio is great

10. El Tor vs classic Vibrio- True is: *(Recent Question 2015)*
a. ElTor is more common
b. ElTor is more severe
c. Classical is associated with more carriers

11. Cholera is caused by: *(DNB June 2009, PGI June 2009)*
a. Vibrio cholerae – 01
b. Vibrio cholerae – 0139
c. Vibrio parahemolyticus
d. NAG Vibrio

12. True about Vibrio cholerae is: *(SGPGI 2009)*
a. Disease more common in woman
b. Classical Vibrio protect against development of O-139
c. ElTor is milder than classical
d. Erythromycin is used in treatment

13. Darting motility which occur in V.cholerae, also found in: *(PGI Dec 2008)*
a. Shigella
b. Campylobacter jejuni
c. Pneumococcus
d. Bacillus anthrax
e. Aeromonas

14. Selective media for Vibrio: *(AIIMS May 2008, Nov 2007)*
a. TCBS
b. Stuart
c. Skirrow's
d. MYPA

15. Which of the following bacteria act by increasing c-AMP: *(AI 07, 2012, AIIMS Nov 2006)*
a. Vibrio cholerae
b. Staphylococcus aureus
c. E. coli, heat stable toxin
d. Salmonella

16. True about V. cholera 0139 all except: *(AI 2007, 2008)*
a. Clinical manifestations are similar 01 ElTor strain
b. First discovered in Chennai
c. Epidemiologically indistinguishable from 01 ElTor strain
d. Produces 01 Lipopolysaccharide

17. True about Cholera is: *(PGI Dec 2006)*
a. Can be transmitted by food
b. Vaccination gives 90% efficiency
c. Healthy carrier
d. Chlorination is not effective

18. V. cholerae is able to stay in GIT because of: *(PGI Dec 2006)*
a. Acid resistance
b. Bile resistance
c. Motility
d. Binds to specific
e. Anaerobic potential receptors

19. Cholera toxin: *(AI 2005, AIIMS May 2006)*
a. Increases the levels of intracellular cGMP
b. Acts through the receptor the opiates
c. Causes continued activation of Adenylate cyclase
d. Inhibits the enzyme phosphodiesterase

20. Which of the following is the drug of choice of chemoprophylaxis of cholera: *(AI 2011, TNPG 2014) (AIIMS Nov 2005, UP 2004)*
a. Tetracycline b. Doxycycline
c. Furazolidone d. Cotrimoxazole

21. For cholera control: all recommended except: *(TNPG 2014, AI 2011, AIIMS May 05, Nov 05, UP 04)*
a. Mass chemoprophylaxis
b. Early case detection
c. Chlorination of water
d. Proper disposal of excreta

22. Which of the following is true of cholera: *(PGI 2002)*
a. Recent epidemic was due to classical type
b. Causes secretory diarrhea
c. Caused by endotoxin
d. Vibriocidal antibodies correspond to susceptibility

23. True about V. cholerae is: *(AIIMS May 2002)*
a. One attack of V. cholerae gives live-long immunity
b. Affects adults and children with equal propensity in nonepidemic regions
c. In between epidemics, carrier states maintain the organism
d. Pathogenicity of 0-139 Vibrio is due to O antigen

24. In a patient presenting with diarrhea due to Vibrio cholerae, which of the following will be present:
a. Abdominal pain *(PGI Dec 2001)*
b. Presence of leukocytes in stool
c. Fever
d. Neutrophilia

25. Red leg disease is caused by: *(PGI 2001)*
a. Pseudomonas b. Moldy sugarcane fiber
c. Conidiosporium d. Aeromonas

26. A stool examination was carried out which showed organism with darting motility. Which of the following organism may be present in stool: *(PGI May 2013)*
a. V.cholerae b. Shigella
c. Salmonella d. Campylobacter jejuni
e. E. coli

27. Type of diarrhea in Vibrio cholerae: *(Recent Question 2013)*
a. Osmotic b. Secretory
c. Colloidal d. Bloody

28. The best way to classify organism associated with epidemics of watery diarrhea in Southeast Asia? *(Recent Question 2013)*
a. Phage typing
b. Biotyping
c. Colicin typing
d. Antibiogram typing

HALOPHILIC VIBRIO

29. A Gram-negative marine bacterium that causes ear infection: *(AI 2012)*
a. Vibrio cholerae
b. Vibrio parahemolyticus
c. Vibrio alginolyticus
d. Vibrio mimicus

30. Vibrio parahemolyticus is seen in undercooked: *(DNB Dec 2009)*
a. Crab b. Shellfish
c. Prawn d. Fish

31. All of the following Vibrio sp. are halophilic, except: *(AI 2005)*
a. V. cholerae
b. V. parahaemolyticus
c. V. alginolyticus
d. V. fluvialis

32. 32-year-old male, Kallu, who recently visited a sea coast presented with ulcer over left leg. The probable cause is: *(AIIMS May 2001)*
a. Pasteurella multocida
b. Micrococcus halophillus
c. Vibrio vulnificus
d. Neisseria gonorrheae

33. Vibrio parahemolyticus true is: *(Recent Questions 2014)*
a. Cannot tolerate maximum 1% salt
b. Cannot tolerate maximum 3% salt
c. Cannot tolerate maximum 7% salt
d. Cannot tolerate maximum 10% salt

EXPLANATIONS

VIBRIO

1. **Ans. (a) (oxidase test)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p334
   - Vibrio cholerae is oxidase positive; E.coli is oxidase negative
   - Vibrio cholerae can ferment lactose late, hence called as late lactose fermenter.

2. **Ans. (c) (Zona Occludens)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p331
   Vibrio cholerae elaborates Zona occludens toxin (Zot), a protein that increases the permeability of small intestinal mucosa by disrupting the intercellular tight junctions between mucosal cells.

3. **Ans (a) (Adenylate cyclase)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p330
   - Cholera toxin causes ADP ribosylation of G protein → upregulates the activity of adenylate cyclase → result is ↑cAMP.

4. **Ans. (c) (Started in Bangladesh)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 332, Ananthanarayan 9th/p308
   O139 (The Bengal strain) was isolated first from Chennai in 1992 and spread rapidly along the costal region of way of Bengal up to West Bengal, then to the adjacent areas of Bangladesh (Detail-Refer chapter review).

5. **Ans. (b) (c) (d) (Botulinum…, Shiga toxin…., Diphtheria toxin …..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 330, Ananthanarayan 9th /p307,286,264,237
   - Cholera toxin acts by activating cAMP
   - Botulinum toxin inhibits Acetylcholine release at NM junction → flaccid paralysis
   - Shiga toxin of Shigella dysenteriae acts by inhibiting protein synthesis by inhibiting ribosome
   - Diphtheria toxin act by inhibiting protein synthesis by inhibiting elongation factor-2

6. **Ans. (c) (Vibrio cholerae)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 330, Ananthanarayan 9/e p308
   - V. cholerae is noninvasive. The pathogenesis of V. cholerae is due to exotoxin.

7. **Ans. (c) (Vibrio...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 331, Harrison 18/e p1291, 19e/p1061-65
   - Cholera Vibrios also possess lipopolysaccharide O antigen (LPS, endotoxin) as in other gram-negative intestinal bacilli. But unlike other gram-negative bacilli like E.coli, Klebsiella and Pseudomonas, etc. (where endotoxin is the principle virulence factor), LPS apparently plays no role in pathogenesis of cholera but is responsible for the immunity induced by killed vaccine.
   - For Vibrio cholerae the principle virulence factor is the exotoxin, i.e. cholera toxin.

8. **Ans. (b) (Cholera)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 336, Harrison 18/e p1293, 19e/p1061-65
   - DOC for chemoprophylaxis for cholera- Tetracycline

9. **Ans. (a) > (d) (Animals are the only reservoir, the efficacy of vaccine against El tor Vibrio is great)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 332, Harrison 18/e p1289-93, 19e/p1061-65
   - ***Option a:*** 'The natural habitat of V. cholerae is coastal salt water and brackish estuaries, where the organism lives in close relation to plankton. There is no known animal reservoir' ….Harrison 17/e p968
   - ***Option d:*** Classical and ElTor are biotypes, i.e. they are biochemically different but antigencically same. Hence, the efficacy of both Classical and ElTor should be same.
   - ***Option b:*** 'The clinical manifestations and epidemiologic features of the disease caused by V. cholerae O139 Bengal are indistinguishable from those of O1 cholera'. ….Harrison 17/e p969
   - ***Option c:*** 'Humans become infected incidentally but, once infected, can act as vehicles for spread.' Harrison 17/e p968

10. **Ans. (a) (ElTor is MC)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p329

11. **Ans. (a), (b), (d) (Vibrio cholerae – 01,Vibrio cholerae – 0139, NAG Vibrio)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 329, Jawetz 24/p271 & 25/p235, Harrison 18/e p1289-93

- Epidemic and pandemic Cholera is caused by both Vibrio cholerae O1 and O139 strains.
- However, Cholera like illness can also be caused by NAG (Non agglutinable Vibrio), i.e. V cholerae serogroups non-O1/non-O139.

Organism	Human Disease
V .cholerae serogroups O1 and O139	Epidemic and pandemic cholera
V .cholerae serogroups non-O1/non-O139	Cholera-like diarrhea; mild diarrhea; rarely, extra intestinal infection

12. Ans. (c) (ElTor...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 329, Park 22/p209-10, 23e/p228-233

- 'ElTor is much hardier and resistant than classical, But ElTor produces less severe cholera
- Hence, ElTor infection leads to more carrier than cases

About Other Options:

- Classical Vibrio belongs to serovar O1, which is antigenically different from Serovar O139, it cannot protect against it.
- But Classical Vibrio protect against development of ElTor.
- Cholera Vibrio affects all ages and sexes but in epidemic area it affects more children. Park 22/e p209

13. Ans. (b) (Campylobacter...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 362, Ananthanarayan 9/e p399

- Campylobacter jejuni possess single polar flagella and, they exhibit darting motility appreciated under dark field or phase contrast microscopy.

14. Ans. (a) (TCBS) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 334, Ananthanarayan 9/e p304, 8/e p303

- TCBS (Thiosulfate Citrate Bile salts Sucrose Agar) is a Selective media for Vibrio.
- V. Cholerae can ferment sucrose and it produces Yellow colonies on TCBS.
- Whereas V.parahemolyticus produces green colonies on TCBS

Culture medias for Vibrio: Refer text (chapter review).

15. Ans. (a) (Vibrio...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 330, Ananthanarayan 9/e p308, 8/e p307

- Mechanism of action of cholera toxin – refer text (chapter review).
- Toxin acts by cAMP are: LT of E.coli, Cholera toxin, Pertussis toxin, Anthrax toxin (edema factor).

16. Ans. (d) (Produces 01 ...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 332, Ananthanarayan 9/e p308

- V. Cholerae O139 possesses LPS serogroup O139, not O1.
- ***Detail of O139 Vibrio – Refer text (chapter review)***

17. Ans. (a), (c) (Can..., Healthy...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 332, Park 23e/p228-233, 22/p208-09, 205

- Cholera is transmitted by contaminated water & food, direct contact through finger from carriers
- For details about carriers of cholera refer chapter review.

About Other Options:

- ***Option b:*** Vaccination gives protection rate of 50–60% ..... Ananthanarayan 9/e p311
- ***Option d:*** Chlorination is effective. 'In rural area, water can be made safe from cholera bacilli by boiling or chlorination' Park 22/e p209.

18. Ans. (b), (c), (d) (Bile resistance, Motility, Binds to specific receptors) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 332, Ananthanarayan 9/e p305, 8/e p304

- V. cholerae is acid labile that is why high infective dose of 10^6 bacilli is required to cross the gastric juice barrier. (and achlorohydria facilitates transmission)
- V. cholerae is bile resistant, hence can survive in the intestine. (bile resistant property helps to grow on TCBS
- V. cholerae is strongly aerobic but still grows slowly and scanty anaerobically.

Other reasons include:

- Factors that help to cross the protective mucus layer in small intestine are motility, chemotaxis, mucinase, and other proteolytic enzymes.
- Other factors: Siderophore, neuraminidase, accessory colonizing factor
- Adhere to epithelium by TCP (Toxin Co-regulatory Pilus)
- Cholera Toxin: Principle virulence factor binds to specific GM1 receptor on intestinal epithelium.

19. **Ans. (c) (Causes ....)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 330, Ananthanarayan 8/e p307
Cholera Toxin (CT): ***causes ADP ribosylation of G protein → Adenyl cyclase → cAMP***

20. **Ans. (a) (Tetracycline)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 336, Harrison 17/e p970 & 18/e p1293
- DOC for chemoprophylaxis- Tetracycline

21. **Ans. (a) Mass chemoprophylaxis** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 336, Park 22/e p211
- Mass chemoprophylaxis is not recommended for the control of cholera
- Measures for cholera control includes:
  - Early case detection
  - Water control by Chlorination of water
  - Proper disposal of excreta.

22. **Ans. (b) (Causes...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 330, Harrison 18/e p1289-93
- Vibrio causes secretory diarrhea.

About Other options:
- Recent epidemics are due to O1 ElTor and also O139 serovar.
(Classical still exists and few cases were reported from Bangladesh)
- Vibriocidal antibodies in serum (titer 1:20) have been associated with protection against colonization and disease. …… Jawetz 25/e p237
- Endotoxin (LPS) does not play any role in pathogenicity of cholera but is responsible for immunity developed by killed vaccine.

23. **Ans. (b) (Affects ...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 332, Harrison 18/e p1290
- 'Cholera is predominantly a pediatric disease in endemic areas, but it affects adults and children equally when newly introduced into a population.' ……. Harrison 17/e p968

About Other options:
- Vaccination gives protection rate of 50–60% for 3–6 months …… Ananthanarayan 9/e p311
- In between epidemics, it is maintained in sea water. During the epidemics, carrier states maintain the organism
- Pathogenicity of 0-139 Vibrio is due to cholera toxin and capsule.
- LPS has no role in pathogenesis of O139, however since it is different from LPS of O1, so it is not neutralized by antisera to O1. ……..Harrison 18/e p1290

24. **Ans. (d) (Neutrophilia)** Ref: Harrison 18/e p1291, 17/e p970
Clinical feature of cholera (detail- refer chapter review):
- Sudden onset of painless voluminous watery diarrhea
- Fever is usually absent.
- Mild neutrophilic Leucocytosis
- Non invasive diarrhea (So no pus cells in stool)

25. **Ans. (d) (Aeromonas)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 338, Wikipedia
- Red Leg Disease is a severe, acute, bacterial infection of amphibians.
- It is termed «Red Leg Disease» because it causes hemorrhages of the leg (often the inner thigh) as a result of septicemia.

26. **Ans. (a) (d) (V. cholerae, Campylobacter jejuni)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 333,362
Darting or shooting star motility is exhibited by V. cholerae and Camplyobacter jejuni.

27. **Ans. (b) (Secretory)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 330, Ananthanarayan 9/e p307
- Cholera stool is painless voluminous watery secretory diarrhea with non bilious, gray, slightly cloudy fluid with flecks of mucus, no blood, and sweet, inoffensive, non invasive diarrhea (So no pus cells in stool).

28. **Ans. (b) (Biotyping)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 329, Ananthanarayan 9/e p306
- Organism associated with epidemics of watery diarrhea in Southeast Asia- suggestive of V. cholerae.
- V. cholerae is commonly typed by serotype (O1 to O139) and O1 is further biotypes to classical and Eltor.

HALOPHILIC VIBRIO

29. Ans. (c) (Vibrio alginolyticus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 337, Harrison 18/e p1295

- V. alginolyticus occasionally causes eye, ear, and wound infections. …….. Harrison 18/e p1295
- V. alginolyticus is associated with infection of ear, eye and wounds in human beings exposed to sea water.

30. Ans. (b) (Shellfish) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 337, Harrison 18/e p1295

- Refer chapter review

31. Ans. (a) (V. cholerae) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 337, Ananthanarayan 9/e p311

Examples of Halophilic Vibrio –V. alginolyticus, V. parahemolyticus, V. vulnificus & V. fluvialis
Examples of Non Halophilic Vibrio – V. cholerae and V. mimicus

32. Ans. (c) (V. vulnificus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 337, Ananthanarayan 9/e p312

Points in Favor: Person belongs to Sea coast presented with ulcer over the left leg
'Halophilic Vibrio usually cause infection in sea coastal region following ingestion of seafood like Oysters, etc.'

33. Ans. (d) (Cannot tolerate...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p337, Ananthanarayan 9/e p311

V. parahemolyticus can tolerate up to 8% of salt concentration, but not 10%.

Pseudomonas and Other Nonfermenters and Haemophilus, Bordetella, Brucella (HBB)

CHAPTER 3.9

NONFERMENTERS

Nonfermenters utilize the sugars oxidatively. Important members are *Pseudomonas, Burkholderia and Acinetobacter*.

Pseudomonas

Virulence Factors and Pathogenesis

1. **Colonization:** To colonize the host surface by pili or fimbria (the organ of attachment)
2. **Toxin mediated immune evasion and Tissue Injury:**
   - *Nondiffusible toxins* (e.g. exotoxins S, U, T, and Y)
   - *Diffusible toxins* (e.g. exotoxin A, proteases, phospholipases, hemolysins, elastases, pyocyanin, etc.)

   Exotoxin A is the most important virulence factor. It inhibits protein synthesis by inhibiting EF-2 (mechanism of action is similar to diphtheria toxin).
3. **Host's inflammatory response:** Against lipid A of LPS and flagellin
4. **Pigment production**:
   - Pyocyanin (a blue green pigment, produced only by *P.aeruginosa*)
   - Fluorescein (or pyoverdin): Gives greenish yellow color, produced by all species
   - Pyorubin (imparts red color)
   - Pyomelanin (imparts brown black color)
5. **Alginate coat:** Mucoid strains of *Pseudomonas* have a slime layer or alginate layer which facilitates biofilm formation, thus helps in adhesion to purulent mucus. Such strains can cause infections in patients with cystic fibrosis.
6. Capsular polysaccharide-prevents the bacteria from phagocytosis.
7. **Multi-drug resistance and Multi-disinfectant resistance.**
8. **Wide temperature range** (5–45 °C).

Exotoxin A of Pseudomonas:
- Most important virulence factor
- Inhibits protein synthesis by inhibiting EF-2 (similar to diphtheria toxin)

Mucoid Strains of Pseudomonas:
- Possess slime layer or alginate layer which facilitates biofilm formation
- Thus helps in adhesion to purulent mucus.
- Such strains can cause infections in patients with cystic fibrosis.

Clinical Manifestations

Most of the infections are encountered in hospitalized patients.

- Pneumonia: (VAP or Ventilator-Associated Pneumonia).
- **Chronic respiratory tract infections:** Occurs in patients with cystic fibrosis (in Caucasian populations), bronchiectasis or chronic panbronchiolitis (in Japan):
  - The mucoid strains (possessing alginate layer) of *Pseudomonas* commonly cause such infections.
  - Structural abnormalities of the airways result in mucus stasis.
- **Ear infections:** ***Swimmer's ear*** (among children) and ***malignant otitis externa*** (in elderly diabetic patients).
- **Eye infections** such as corneal ulcers (in contact lens wearers) and endophthalmitis
- **Shanghai fever:** A mild febrile illness resembling typhoid fever.
- **Skin and soft tissue infections:**
  - *Burns patients*: *Pseudomonas* is the most common organism to infect the burn wounds.
  - *Ecthyma gangrenosum* is an acute necrotizing condition results from bacteremia), occurs more commonly in patients with febrile neutropenia and AIDS.
  - *Pseudomonas dermatitis:* Cause outbreaks in spas, and swimming pools
  - *Toe-web infections* (in the tropics)
  - *Green nail syndrome*: It is a 'paronychia' results from prolonged submersion of the hands in water.

Ear Infections due to Pseudomonas:
- *Swimmer's ear* (among children)
- *Malignant otitis externa* (in elderly diabetic patients)

- **Other infections:**
  - Cellulitis (characterized by *blue green pus*)
  - Bone and joint infections such as osteomyelitis and septic arthritis
  - Meningitis (in postoperative or post-traumatic patients)
  - UTI (urinary tract infection) in catheterized patients.

Laboratory Diagnosis

Culture Media of Pseudomonas:
- Selective media-cetrimide agar
- Pigment enhanced in- King's media

Pseudomonas is nonfastidious, obligate aerobe and is motile with single polar flagellum:
- It produces large, opaque, irregular colonies with a metallic sheen (***iridescence***)
- Diffusible pigments: Blue green (pyocyanin) or yellow green (pyoverdin) pigmentation
- Pigment production can be enhanced in special media such as **King's media**
- Most colonies have a characteristic sweet ether or alcohol-like fruity odor
- Blood agar: It produces β hemolytic colonies on blood agar
- MacConkey agar: Produce pale nonlactose fermenting colonies
- Selective media-*cetrimide agar*
- Oxidase and catalase positive
- Nonfermenter: It does not ferment any sugars, but utilizes sugars oxidatively.
- **OF test** (Hugh and Leifson oxidative fermentative test) shows oxidative pattern.

Treatment

Antipseudomonial Cephalosporins:
- Ceftazidime
- Cefoperazone
- Cefepime

Pseudomonas species are inherently resistant to most of the antibiotics. Only limited anti-pseudomonials are available:
- Penicillins: Piperacillin, mezlocillin, ticarcillin
- Cephalosporins: Ceftazidime, cefoperazone, and cefepime
- Carbapenems: Imipenem, meropenem
- Monobactam: Aztreonam
- Aminoglycoside: Tobramycin, gentamicin, amikacin
- Quinolones: Ciprofloxacin, levofloxacin
- Polymyxins: Polymyxin B, colistin.

Drug Resistance

Pseudomonas possesses a number of drug resistant plasmids which confer multiple drug resistance. Many strains are producers of β lactmases such as ESBL (extended spectrum β lactamases), carbapenemases, and AmpC β lactamases. Many strains are resistant to aminoglycosides and quinolones.

Burkholderia

Burkholderia species differ from Pseudomonas in being:
- Bipolar stained (safety pin appearance)
- Resistant to polymyxin B

Burkholderia species are also oxidase positive nonfermenters; however they differ from *Pseudomonas* in being:
- Bipolar stained (safety pin appearance)
- Resistant to polymyxin B

Burkholderia Pseudomallei (Melioidosis)

Burkholderia infections:
- *B. pseudomallei:* Causes Melioidosis or Vietnam time-bomb disease
- *Burkholderia mallei:* Causes glanders and farcy in horses
- *B. cepacia:* Infections in hospitals

B.pseudomallei is the causative agent of melioidosis.
- Clinical feature: Melioidosis is characterized by:
  - Pulmonary infection: Ranges from abscesses, pneumonia or severe necrotizing lung disease to chronic pulmonary tuberculosis.
  - Skin ulceration
  - Lymphadenopathy
- Long latent period: This disease is also called 'Vietnam time-bomb'.
- Bioterrorism: *B.pseudomallei* can be used as a potential agent of biological warfare.
- Ashdown medium is used as a selective medium.

- Treatment of melioidosis consists of:
  - Intensive phase (2 weeks): Ceftazidime or a carbapenem is given
  - Maintenance phase for 12 weeks: Oral cotrimoxazoleis given to prevent relapse.

Burkholderia Mallei

B.mallei is a pathogen of horses; causes glanders (nasal discharge and ulcers in the nasal septum) and farcy (skin lesions and lymph node involvement).

- Human infection is characterized by:
  - Local skin nodules and lymphadenitis (if transmitted by inoculation)
  - Pneumonia, ulceration of the trachea and sepsis (if transmitted by inhalation)
- *B.mallei* differs from *B.pseudomallei* in being:
  - Nonmotile and Oxidase negative
  - Inability to grow on MacConkey agar
  - Inoculation into Guinea pigs can cause testicular swelling (Strauss reaction).

Burkholderia Cepacia

B.cepacia is currently the most commonly encountered *Burkholderia* species:

- *B.cepacia* inhabits moist environments, detergents and IV fluids.
- LPS of *B.cepacia* is among the most potent of all gram-negative bacteria.
- Cepacia syndrome characterized by a rapidly fatal respiratory infection and septicemia in cystic fibrosis patients.
- Nosocomial pathogen in ICU patients because as it is resistant to multiple antibiotics.

Acinetobacter

Acinetobacter are saprophytic bacilli. However, it is recognized as a nosocomial pathogen:

- It can cause ventilator associated pneumonia, Central line associated bloodstream infection, Catheter associated UTI.
- Wound and soft tissue infections and infections in burn patients.
- *A.baumannii* is nonfermenter, but differs from *Pseudomonas* being Oxidase negative and Nonmotile.

HAEMOPHILUS

Haemophilus species are oxidase positive, capsulated pleomorphic gram-negative bacilli. It (Pfeiffer's bacillus) is blood loving organism; requires two accessory growth factors present in blood.

- Factor X- hemin present freely in blood
- Factor V is a NAD (present in side RBC)

Virulence Factors and Typing

- Capsule-Based on Capsular polysaccharide, *H.influenzae* is typed into six serotypes *(a to f):*
  - *H.influenzae* serotype b (*Hib*) is the most virulent and accounts for most of the invasive infections.
  - Hib capsule has unique chemical structure, made up of polyribosylribitol phosphate (PRP) antigen. It is strongly immunogenic, hence used for vaccination.
- Next to Hib, nontypeable strains are commonly isolated clinically. Other capsular serotypes are very rarely isolated.
- *H.influenzae* was the first free-living organism whose entire genome was sequenced.

Serotypes *H.influenzae*:
- *H.influenzae* serotype b (Hib) is the most virulent and accounts for most of the invasive infections
- Next to Hib, non-typeable strains are commonly isolated clinically

Clinical Manifestations

H.influenzae type b (Hib) is the most common and most invasive serotype.

- Central nervous system infections:
  - Pyogenic meningitis in < 2 years of age
  - Subdural effusion, MC CNS complication

Biotype IV of *H.influenzae*:
- Causes Puerperal sepsis and neonatal bacteremia
- Colonizes the female genital tract

- Epiglottitis: Seen in older children (2-7 years), absence among Navajo Indians and Alaskan Eskimos.
- Lobar Pneumonia in infants
- Less common invasive conditions seen in children include:
  - Cellulitis of neck and head region
  - Osteomyelitis, septic arthritis
  - Orbital cellulitis, endophthalmitis

Next to Hib, non-typeable strains are the commonest group clinically. They are noninvasive, spread by contagious spread and usually affect adults. Their clinical manifestations include:

- Childhood otitis media
- Exacerbations of COPD: They are the MC bacterial cause for this condition.
- Pneumonia in adults among patients with COPD or AIDS
- Puerperal sepsis and neonatal bacteremia- by strains of *biotype IV*.
- Sinusitis in adults and children.

As *H.influenzae* is highly sensitive to low temperature, the specimens should never be refrigerated

Laboratory Diagnosis

- **Specimen collection and transport:**
  - CSF, blood, sputum, pus, aspirates from joints, middle ears or sinuses.
  - As it is highly sensitive to low temperature, the specimens *should never be refrigerated.*
- **Gram staining** of CSF and other specimen shows pleomorphic gram-negative coccobacilli
- **Capsule detection:** By Quellung reaction or Latex aggl. test
- **Culture:** *H.influenzae* is largely aerobic, growth is enhanced by 5–10% CO_2.
  - Blood agar with *S.aureus* streak line: Colonies of *H.influenzae* grow adjacent to *S.aureus* streak line (this property is called as **satellitism).** This is due to release of V factor by lysis of RBCs mediated by *S.aureus*.
  - Chocolate agar: It grows well on chocolate agar but sparsely on blood agar.
  - Fildes agar and Levinthal's agar
- **Disk test for X and V requirement**:
  - Require only X factor: *H.ducreyi* and *H.aphrophilus*
  - Require only V factor: *H.parainfluenzae, V.parahaemolyticus* and *H.paraphrophilus*
  - Require both X and V factor: *H.influenzae, H.aegyptius* and *H.haemolyticus*
- **Biotyping**: It is done by IOU tests (indole, urease test and ornithine decarboxylase test).
- **Slide agglutination test:** Serotyping is carried out using type-specific antisera.

X and V requirement:
- Only require X factor: *H.ducreyi* and *H.aphrophilus*
- Only require V factor: *H.parainfluenzae*, *V.parahaemolyticus* and *H.paraphrophilus*
- Require both X and V factor: *H.influenzae*, *H.aegyptius* and *H.haemolyticus*

Treatment

- **Invasive infection** due to Hib: Cephalosporins are the drugs of choice.
- **Nontypeable** strains of *H.influenzae* are often resistant to β lactams [due to β-lactamase production (20–35% of strains) or rarely altered penicillin binding protein-3]. DOC is quinolones (levofloxacin) or macrolides (azithromycin).
- **Chemoprophylaxis**: Oral rifampin is indicated to household contacts or healthcare staff (if two or more cases occur within 60 days).

Treatment of H.influenzae infections:
- Invasive infection due to Hib: DOC is Cephalosporins
- Nontypeable strains: DOC is quinolones (levofloxacin)

Hib Conjugate Vaccine

The PRP capsular antigen of *H.influenzae* type b is used as vaccine.

- As capsular antigens are poorly immunogenic to children, they are conjugated with adjuvants such as diphtheria toxoid, tetanus toxoid.
- It also reduces the rates of pharyngeal colonization with Hib.
- Conjugate vaccines has dramatically reduced the incidence of Hib disease.

H. aegyptius:
- Koch's – Week's bacillus
- Pink eye syndrome (Egyptian ophthalmia)
- Brazilian purpuric fever

H. aegyptius

- Koch's –Week's bacillus
- Pink eye syndrome (Egyptian ophthalmia)
- Brazilian purpuric fever

H. Ducreyi

- Causes **Chancroid/soft sore**: Characterized by painful lymph node, tender non-indurated and bleeding genital ulcer
- Chancroid increases both transmission and the degree of susceptibility to HIV infection
- **In direct smear**: Pleomorphic gram-negative coccobacilli that:
  - Show bipolar staining
  - Occurs in parallel chains called in '*School of fish*' or '*rail road track*' appearance
- Antigenically homogenous
- **Culture Medium used:**
  - Rabbit blood agar or Chocolate agar with 1% isovitalex, Vancomycin
  - Chorioallantoic membrane (CAM)
- **Drug of choice:** Azithromycin (1 g oral; single dose), treatment of all sexual partners.

Chancroid/soft sore:
- Caused by *H. ducreyi*
- Characterized by painful lymph node, tender non-indurated genital ulcer

Haemophilus Aegyptius

It is also called as Koch-Weeks bacillus; closely resembles *H.influenzae* biotype III. It causes:

- **Brazilian purpuric fever:** A fulminant condition, characterized by fever, purpura, hypotension and shock
- Purulent contagious conjunctivitis (**Egyptian ophthalmia**).

Eikenella corrodens:
- Produces twitching or jerky motility
- Pitting or corroded colonies on blood agar

HACEK Group

HACEK organisms are a group of highly fastidious, gram-negative bacteria, normally residing in the oral cavity as commensal, but occasionally have been associated with local infections in the mouth and systemic infections, such as bacterial endocarditis:

- ***H**aemophilus* species: *H.aphrophilus, H.paraphrophilus* and *H.parainfluenzae*
- ***A**ggregatibacter* (formerly ***A**ctinobacillus*) *actinomycetemcomitans:* Most common member
- ***C**ardiobacterium hominis*
- ***E**ikenella corrodens:* Produces **twitching or jerky** motility and **pitting or corroded colonies** on blood agar
- ***K**ingella kingae*

Treatment: Ceftriaxone (2 g/day) is the DOC except for *Eikenella corrodens* where ampicillin is indicated.

BORDETELLA

Bordetella is described first by Bordet and Gengou, causes a violent paroxysmal productive cough in children called as *whooping cough* or *100 days fever*.

Pertussis toxin (PT):
- Expressed only by *B.pertussis*
- Similar to cholera toxin in its structure and function (↑ cAMP)

Virulence Factors

Toxins:

- Pertussis toxin (PT) expressed only by *B.pertussis*, similar to cholera toxin in its structure and function (↑cAMP)
- Other toxins: Tracheal cytotoxin, adenylate cyclase toxin, dermonecrotic toxin and Endotoxin
- Adhesins: They play a role in bacterial attachment:
  - Filamentous hemagglutinin (FHA)
  - Pertactin, an outer-membrane protein
  - Fimbriae or pili or agglutinogens.

Pertussis-Clinical stages:
- *Catarrhal* phase: Nonspecific symptoms but Infectious stage and smear and cultures are positive
- *Paroxysmal* phase: Specific symptoms (whooping cough), but noninfectious and smear and culture are negative.

Clinical Manifestations

Whooping cough (or pertussis) passes through three stages following an IP of 7–10 days.

1. **Catarrhal phase:** It lasts for 1–2 weeks, is characterized by common cold like nonspecific symptoms. It is highly infectious stage and smear and cultures are likely to be positive.

2. **Paroxysmal phase:** It is characterized by specific symptoms such as:
   - Whooping cough, Post tussive vomiting
   - In this stage, patient is less infectious; smear and culture become negative.
3. **Convalescent stage**: Severity decreases. Antibodies appear in serum.

Differential diagnosis: Whooping cough like symptoms may be seen with:

- *Mycoplasma pneumoniae*
- *Chlamydophila pneumoniae*
- Adenovirus
- Influenza and other respiratory viruses
- Use of angiotensin-converting enzyme (ACE) inhibitors
- Reactive airway disease
- Gastro-esophageal reflux disease

Recent outbreak of whooping cough Washington epidemic in 2012

Epidemiology

Whooping cough is exclusively human disease. There is no animal reservoir:

- **Source:** Early cases (catarrhal stage) are the main source. There is no carrier state.
- **MC age affected** < 5 years.
- **Mode of transmission** is via inhalation of droplets or rarely through direct contact.
- **Recent outbreak:** Washington epidemic in 2012
- There is no cross protection to *B.parapertussis* infection.

B.pertussis- Identifying feature:
- Specimen: Nasopharyngeal aspirate (best)
- Media: Regan and Lowe medium, Bordet-Gengou glycerine-potato-blood agar
- Colonies: Mercury drops or bisected pearls appearance
- Culture smear: Coccobacilli arranged in thumb print appearance

Laboratory Diagnosis

- Best Specimen: *Nasopharyngeal secretions*, obtained by nasopharyngeal aspiration (best method) or pernasal swab
- *Type of swabs used:* Alginate swabs are the best followed by dacron swabs for culture. However, for PCR, only dacron or rayon swabs are recommended.
- If delay is expected, then suitable charcoal based transport medium (Amies or Stuart's) can be used.
- Cough plate method and postnasal swabs used before are no longer recommended.
- **Antigen detection:** Direct fluorescent antibody tests of nasopharyngeal secretions
- **Culture:** Nasopharyngeal aspirate culture is the Gold standard method
  - Media: Regan and Lowe medium, Bordet-Gengou glycerine-potato-blood agar
  - Colonies: ***Mercury drops or bisected pearls*** appearance.
- **Culture smear:** Reveals small, ovoid coccobacilli arranged in ***thumb print*** appearance. Capsules and bipolar metachromatic granules may be seen occasionally.
- **PCR:** Most sensitive, gives quicker results, but yet to be standardized properly. The most common targeted genes are IS481 and the PT promoter region genes.
- **Antibody detection:** Enzyme immunoassays detecting IgA and IgG to pertussis toxin, filamentous hemagglutinin.

Treatment

Antibiotics eliminates the bacteria from nasopharynx, but less useful for treatment as pertussis is toxin mediated.

- Macrolides are the drugs of choice (e.g. erythromycin for 7–14 days)
- Cotrimoxazole is recommended as an alternative in macrolide resistance.
- Chemoprophylaxis: Erythromycin is DOC.

Vaccine

Whole-Cell Pertussis Vaccines

It is prepared by heating followed by chemical inactivation and purification of whole *B.pertussis* bacilli.

WC Pertussis vaccine is contraindicated in:
- Children > 5–6 years age
- Progressive neurological conditions
- Family history of epilepsy
- Hypersensitivity to previous dose

Section 3

- It is given along with DPT to children < 5 years age
- Efficacy is good, average being 85%.
- **Adverse effects**
  - Common: Fever, injection-site pain, erythema, swelling, and irritability.
  - Rare: Neurological complications and *hypotonic hyporesponsive syndrome*
- **WC vaccine is contraindicated in:**
  - Children > 5–6 years age
  - Associated progressive neurological conditions or family history of epilepsy
  - Hypersensitivity to previous dose.

Acellular Pertussis Vaccine

- It is composed of pertussis toxoid and ≥ 2 other bacterial components such as FHA, pertactin or fimbriae.
- Though the efficacy is same as WC vaccine, it is associated with fewer side effects and safely given after 5–6 years.

Nomen species classification is based on:
- Preference of animal host
- CO_2 requirement
- H_2S production
- Genetic composition
- Bacteriophage susceptibility
- Tolerance to bacteriostatic dyes
- Agglutination with monospecific antisera

BRUCELLA

Brucellosisis primarily a zoonotic disease acquired from animals such as sheep, goat, or cattle.

Nomen System of Classification

DNA hybridization reveals that *Brucella* are very closely related and probably represent variants of a single species.

However for the sake of convenience, these have been classified into **nomen species.**

Nomen species: Six nomen species identified so far, further classified into several biovars

- *B.melitensis:* Infects sheep, goat and camel (it has 3 biovars)
- *B.abortus:* Infects cattle and buffalo (it has 9 biovars)
- *B.suis:* Infect pigs. (it has 5 biovars)
- *B.canis:* Causes abortion in dogs
- *B.ovis:* Causes reproductive disease in sheep
- *B.neotomae:* Infects desert rodents.

Preference of animal host:
- *B. melitensis:* Sheep, goat and camel
- *B.abortus:* Cattle and buffalo
- *B. suis:* Pigs
- *B. canis:* Dogs
- *B. ovis:* Sheep
- *B. neotomae:* Desert rodents

Pathogenesis

B.melitensis is most pathogenic followed by *B.abortus* and *B.suis*. Human infection with other species is extremely rare.

- **Transmission**—is usually from infected animals to man. There is no evidence of man to man transmission.
  Direct contact (MC mode) ***with the infected animal tissue > Ingestion of raw milk or dairy products >*** *Air borne*
- **Organs affected:** *Brucellae* are facultative intracellular pathogens, primarily infecting organs of *reticuloendothelial system*.
- **Incubation period** varies from 1 week to several months and the onset is often insidious.

Transmission of Brucella:
- Direct contact (MC mode) with the infected animal tissue > Ingestion of raw milk or dairy products > Air borne
- No man to man transmission.

Clinical Manifestations

- **Classic triad:** Fever with night sweats; arthralgia/arthritis and hepatosplenomegaly
- **Typhoid-like** illness: Overall, brucellosis resembles typhoid like illness except that it is less acute, less severe with **undulating** pattern of fever (or Malta fever or Mediterranean fever) and more musculoskeletal symptoms.

Typhoid-like illness:
Overall, brucellosis resembles typhoid like illness except that it is less acute, less severe with undulating pattern of fever and more musculoskeletal symptoms

Laboratory Diagnosis

Culture and Identification

- Sample: Blood, bone marrow, CSF, joint fluid or other tissues.

False negative SAT may occur due to:
- Prozone phenomenon (due to excess of antibodies in patient's sera)
- Presence of blocking or non-agglutinating IgG or IgA antibodies

Diagnosis of brucellosis in animals:
- **Mil**k ring test
- Rose Bengal card test
- Whey agglutination test

Treatment of Brucellosis
- Gold standard regimen in adults - Streptomycin plus doxycycline
- WHO regimen in adults- Rifampin plus doxycycline

- Cultural media: Biphasic blood culture bottles media (Castaneda's) made up of Brain heart infusion (BHI) broth/agar
- Erythritol: Improves growth
- Automated techniques such as BACTEC and BacT/Alert systems.

Antibody Detection by Standard Agglutination Test (SAT)

It remains the gold standard test serological test:
- It is a tube agglutination test detecting antibodies in serum by using standard strain of *B.abortus:*
- SAT detects IgM antibodies against antigens of smooth LPS: Hence useful for acute brucellosis
- False negative SAT may occur due to:
  - Prozone phenomenon (due to excess of antibodies in patient's sera)
  - Presence of blocking' or non-agglutinating IgG or IgA antibodies
- False positive SAT may occur due to-antigenic cross reacting gram-negative bacteria such as *E.coli.*
- **CFT and ELISA**: Are preferred in chronic brucellosis for Ab detection.

Other Tests

- PCR using primers for *rrs-rrl* gene, *Omp2* gene
- Brucellin skin test
- Guinea pig inoculation
- Tbilisi phage typing is done
- **Diagnosis of Brucellosis in Animals: Antibody detection in milk by (i) Milk ring test, (ii) Rose Bengal card test and (iii) Whey agglutination test**

Treatment

- Gold standard regimen in adults: Streptomycin plus doxycycline
- WHO regimen in adults: Rifampin plus doxycycline
- Relapse or treatment failure occurs in 5–10% of cases.
- For CNS involvement: Ceftriaxone is added to the regimen and treatment is prolonged for 3–6 months.

MULTIPLE CHOICE QUESTIONS

PSEUDOMONAS

1. Most common Gram-negative organism in cystic fibrosis: *(Recent Questions 2014)*
a. Pseudomonas
b. E. coli
c. Klebsiella
d. Legionella

2. Significance of mucoid strain Pseudomonas aeruginosa: *(AIIMS Nov 2014)*
a. Exhibit more drug resistant
b. Produces biofilms
c. Secrete more toxins
d. Produce more bacteriocin

3. Ecthyma gangrenosum is caused by: *(NEET Pattern Based)*
a. Pseudomonas
b. Streptococcus
c. Staphylcoccus
d. H. influenzae

4. Drugs used in pseudomonas treatment: *(PGI June 2011)*
a. Cefixime
b. Ceftazidime
c. Ceftriaxone
d. Colistin
e. Ampicillin

5. Which one of the following drugs is an Anti pseudomonal penicillin? *(AI 2006)*
a. Cephalexin
b. Cloxacillin
c. Piperacillin
d. Dicloxacillin

BURKHOLDERIA

6. Lysine positive non fermenter bacteria:
a. *Burkholderia pseudomallei* *(PGI May 2016)*
b. *Burkholderia cepacia*
c. *Acinetobacter baumannii*
d. *Pseudomonas*

7. Whitemore bacillus: *(Recent Question 2015)*
a. H. influenzae
b. Burkholderia mallei
c. Burkholderia pseudomallei

8. Burkholderia cepacia infection is/are typically associated with: *(PGI May 2015)*
a. Cystic fibrosis
b. Chronic bronchitis
c. Chronic granulomatous disease
d. Multiple myeloma
e. Myeloperoxidase deficiency

9. Burkholderia is infrequently seen in: *(DNB June 2011)*
a. Pools
b. Plants
c. Soil
d. Air

10. Cause of melioidosis is: *(TNPG 2015; DNB Dec 2011)*
a. Burkholderia mallei
b. Burkholderia pseudomallei
c. Burkholderia cepacia
d. None

11. Cause of chest infection in a child with cystic fibrosis: *(AIIMS May 2011, Recent Question 2015)*
a. Burkholderia
b. Pseudomonas (nonmucoid strain)
c. Klebsiella
d. Streptococcus pneumoniae

12. The following statements are true regarding melioidosis except: *(AI 2005)*
a. It is caused by Burkholderia mallei
b. The agent is a gram-negative aerobic bacteria
c. Bipolar staining of the etiological agent is seen with methylene blue stain
d. The most common form of melidiosis is pulmonary infection

13. A 50-year-old chronic alcoholic male agricultural worker presented with high grade fever of one week duration with spells of chills and rigor. Examination of the respiratory system revealed bilateral crepitation with scattered rhonchi. Multiple subcutaneous nodules were found on the extensor surface of the left forearm, arm and left leg. Direct microscopy of the pus aspirated from the skin nodule revealed plenty of Gram-negative bacilli with bipolar staining. Culture revealed distinct rough corrugated grey-white colonies on blood agar. The organisms were motile and oxidase positive. The most likely diagnosis is: *(AIIMS Nov 2003)*
a. Plague
b. Melioidosis
c. Bartonellosis
d. Actinomycosis

14. True about Burkholderia mallei: *(PGI Nov 2012)*
a. Causes glanders disease
b. Affects horses
c. Nonmotile
d. Cause melioidosis

STENOTROPHOMONAS MALTOPHILIA

15. Treatment of choice for *S.maltophilia*: *(PGI May 2016)*
a. Azithromycin
b. Aminoglycosides
c. Co-trimoxazole
d. Levofloxacin
e. Ticarcillin/clavulanate

HAEMOPHILUS

16. In a child admitted with Haemophilus Influenzae meningitis, Cefotaxime was started instead of ampicillin. Which of these is the likely reason for this?
a. H.influenzae stains are known to produce Betalactamase *(AIIMS May 2015)*
b. H.influenzae stains are known to have altered penicillin binding proteins
c. Cefotaxime is easier to administer than ampicillin
d. Drug of choice for this condition is sulphamethoxazole and trimethoprim but cannot be given.

17. All are the feature(s) of chancroid:
a. Ulcer bleed easily *(PGI May 2015)*
b. Painful
c. Bubo formation
d. Typically indurated
e. Caused by H. ducreyi

18. Which of these need both V and X factors?
a. Haemophilus influenzae *(PGI June 2011)*
b. H.ducreyi
c. H.paraphrophilus
d. H.aegyptius
e. H.haemolyticus

19. Capsular polysaccharide constitutes an important virulence factor responsible for protective antibody response in infections caused by all the following bacteria except: *(AI 2011)*
a. Haemophilus influenzae
b. Streptococcus pneumoniae
c. Neisseria meningitides
d. Bordetella pertussis

20. Acute epiglottitis is caused most commonly by: *(Recent Question 2015)*
a. Haemophilus influenzae
b. Bordetella
c. Streptococcus pyogenes

21. Disease caused by Haemophillus: *(PGI June 2009)*
a. Chancroid
b. Influenza
c. Acute epiglottitis
d. Brain abscess
e. Brazilian purpuric fever

22. HACEK group includes all except:
a. Haemophilus aprophillus
b. Acinetobacter baumanii
c. Eikenella corrodens *(AIIMS Nov 2007, May 2008)*
d. Cardiobacterium hominis

23. Chancroid is caused by: *(NEET Pattern Based)*
a. H. ducreyi b. T. pallidum
c. Gonococcus d. HSV

24. A 20-year-old male patient present to the STD clinic with a painful genital ulcer. The gram stain of the smear from ulcer shows gram-negative coccobacilli. The most appropriate media for culture would be:
a. Thayer-Martin Medium *(AI 2004)*
b. Blood agar with X and V factors
c. Chocolate agar with isovitalex X
d. Tellurite blood agar

25. Haemophilus parainfluenzae requires which factors: *(Recent Question 2015)*
a. Only factor X
b. Only factor V
c. Both factor X and V
d. Nothing required

26. All of the following are true regarding Haemophilus influenzae except: *(AIIMS Nov 2003)*
a. It can be a part of the normal flora in some persons
b. The serotyping is based on the bacterial outer membrane proteins
c. It requires Haemin and NAD for growth in culture medium
d. Type b is responsible for invasive disease

27. A 2 years old child is brought to the emergency with history of fever and vomiting. On examination he has neck rigidity. The CSF examination shows polymorphs more that 2000/ml protein 100 mg/dl and glucose 10 mg/dl. The gram stain shows the presence of Gram-negative coccobacilli. The culture shows growth of bacteria only on chocolate agar and not on blood agar. The causative agent is: *(AIIMS Nov 2002)*
a. Neisseria meningitides
b. Haemophilus influenzae
c. Branhamella cartarrhalis
d. Legionella pneumophila

28. Nontypable H.influenzae can cause all except:
a. Meningitis *(AIIMS Nov 2014)*
b. Otitis media
c. Sinusitis in adults
d. Puerperal infection

29. True about H.influenzae: *(PGI May 2013)*
a. Also called as Pfeiffer's bacilli
b. In acute infections capsulated strains are often isolated
c. Gram-negative motile bacilli
d. Easily cultured
e. VP Test positive

30. A patient presented to a STD clinic with painful genital ulcer and painful, soft nonindurated enlarged inguinal lymph node. Identify the pathogen? *(Recent Question 2013)*
a. H.ducreyi b. T.pallidum
c. Calymmatobacter d. Herpes

BORDETELLA

31. Drug of choice for whooping cough is: *(Recent Question 2015)*
a. Tetracycline b. Ceftriaxone
c. Erythromycin

32. Which does not has a known animal reservoir: *(NEET Pattern Based)*
a. Bordetella pertussis b. Francisella tularensis
c. Brucella melitensis d. Pasturella multocida

33. Components of acellular pertussis vaccine:
a. PT + FHA + fimbriae *(AIIMS May 2011)*
b. PT + cytotoxin + fimbriae
c. Endotoxin + fimbriae + pili
d. Pertactin + FHA + outer membrane protein + fimbriae

34. A-7-month old infant with the history of incomplete childhood vaccination presents with bouts of spasmodic cough with cyanosis and a typical inspiratory whoop. Which is most appropriate clinical specimen to be collected for the isolation of pathogen? *(AI 2011)*
a. 'Cough plate culture' b. Per oral swab
c. Nasopharyngeal swab d. Endotracheal aspirate

35. Pertussis toxin acts by all of the following mechanisms except? *(AIIMS Nov 2008)*
a. ADP ribosylation of proteins associated with receptors
b. Increase cyclic AMP
c. Increased calcium release from sarcoplasmic reticulum
d. Acts through G alpha subunit

36. The usual incubation period of pertussis is: *(AIIMS Nov 1996, 2005, AIIMS June 2000)*
a. 7–14 days b. 3–5 days
c. 21–25 days d. Less than 3 days

37. Treatment for pertussis contacts of children:
a. Prophylactic antibiotic for 10 days *(UP 2000)*
b. Prophylactic antibiotic for 14 days
c. Prophylactic antibiotic for 20 days
d. Prophylactic antibiotic for 12 days

38. A child with pertussis should be isolated for: *(Kolkatta 2003)*
a. 1–2 weeks b. 2–4 weeks
c. 3–4 weeks d. 4–6 weeks

BRUCELLA

39. Milk ring test is seen in: *(NEET Pattern Based)*
a. Brucellosis b. Tuberculosis
c. Bacteroides d. Salmonellosis

40. Brucella melitensis is commonly found in (animal):
a. Pig *(PGI June 2011)*
b. Camel
c. Sheep
d. Goat
e. Reindeer

41. Malta fever is caused by: *(PGI June 2002)*
a. Treponema pallidum
b. Borrelia burgdorferi
c. Brucella melitensis
d. Pseudomonas aeruginosa

42. Treatment of Brucellosis: *(PGI June 2008, JIPMER 2010)*
a. Doxycycline
b. Streptomycin
c. Erythromycin
d. Penicillin
e. Rifampicin

43. Brucellosis can be transmitted by all of the following, except: *(AIIMS May 2007, PGI June 2002) (AIIMS Nov 2006, AI 2007)*
a. Contact with infected placenta
b. Ingestion of raw vegetables from infected forms
c. Person to person transmission
d. Inhalation of infected dust or aerosol

44. A farmer presenting with fever off and on for the past 4 years was diagnosed to be suffering from chronic brucellosis. All of the following serological tests would be helpful in the diagnosis at this state except:
a. Standard Agglutination test *(AI 2004)*
b. 2 Mercaptoethanol test
c. Complement fixation test
d. Coomb's test

45. Castaneda method of blood culture is usually used for diagnosis of: *(MHPG 2015)*
a. Lobar pneumonia
b. Toxic shock syndrome
c. Relapsing fever
d. Brucellosis

EXPLANATIONS

PSEUDOMONAS

1. **Ans. (a) (Pseudomonas)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p340
   Mucoid (alginate capsule producing) strains of Pseudomonas are the most common organism associated with cystic fibrosis.
2. **Ans. (b) (Produces biofilms)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p340, Harrison 18/e p1267-73,19/e p1042-47
   Mucoid strain of Pseudomonas aeruginosa are able to produce biofilm (or alginate layer), hence can cause infection in patient with cystic fibrosis.
3. **Ans. (a) (Pseudomonas)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p340, Ananthanarayan 9/e p315
   Ecthyma gangrenosum is caused by Pseudomonas aeruginosa
4. **Ans. (b) (d) (Ceftazidime, Colistin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p341; Harrison 19/e p1042-47
   Antipseudomonal drugs: Refer text (chapter review).
5. **Ans. (c) (Piper...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p341, Harrison 19/e p1042-4718/e p1269
   Antipseudomonal drugs: Refer text (chapter review).

BURKHOLDERIA

6. **Ans (b) (*Burkholderia cepacia*)** Ref: Indian J Med Microbiol. 2009 Apr-Jun;27(2):128-33. doi: 10.4103/0255-0857.49425
   - *Burkholderia cepacia* complex (BCC) and *Stenotrophomonas maltophilia* are lysine positive nonfermenters.
7. **Ans. (c) (B. pseudomallei)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p342
8. **Ans. (a), (b), (c) (Cystic fibrosis, Chronic bronchitis, Chronic granulomatous)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p343, Harrison 19/e p1048
   B.cepacia causes of a rapidly fatal syndrome of respiratory distress (**chronic bronchitis** and bronchiectasis) and septicemia in **Cystic fibrosis** patients called the Cepacia syndrome.
   - It is as an antibiotic-resistant nosocomial pathogen in ICU patients.
   - Patients with **chronic granulomatous disease** are predisposed to B. cepacia lung disease.
   - B. cepacia is an environmental organism that inhabits moist environments
   - B. cepacia appears as an airway colonizer and is a cause of VAP, catheter-associated infection and wound infections.

   Myeloperoxidase deficiency is associated with *S.aureus, E.coli and Candida* infections.
9. **Ans. (b) (plants)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p342, Harrison 18/e p1273, 19/e p1042-47
   - *B. pseudomallei* is found in ***soil and water (pools)***. Humans and animals are infected by inoculation, ***inhalation*** (***from air***), or ingestion; rarely from person to person
10. **Ans. (b) (Burkholderia...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p342, Ananthanarayan 9/e p317, 18
    - B. pseudomallei is the causative agent of meliodiosis (Vietnam time bomb disease)
11. **Ans. (a) (Burkholderia)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p342-43, Harrison 18/e p2147, 48
    'Mucoid strain of pseudomonas is often found in sputum of cystic fibrosis patients. Burkholderia is also recovered from CF sputum and is pathogenic.'
    Cystic fibrosis patients exhibit characteristic sputum microbiology profile:
    - *Haemophilus influenzae* and *S. aureus:* First organisms recovered in newly diagnosed patients with CF.
    - *Mucoid P. aeruginosa* (biofilm producing, antibiotic-resistant): MC organism recovered from CF.
    - *Burkholderia*: 2nd most common (next to mucoid pseudomonas)
    - Other gram-negative rods recovered from CF sputum include:
      - *Alcaligenes xylosoxidans*, occasionally mucoid forms of *Proteus*, *Escherichia coli*, and *Klebsiella*.

- *Mycobacterium tuberculosis* is rare in patients with CF.
- However, 10–20% of adult patients have nontuberculous Mycobacteria
- MC fungus isolated: *Aspergillus fumigatus* (allergic bronchopulmonary Aspergillosis)

12. **Ans. (a) (It is caused by Burkholderia mallei)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p343
    - B.mallei causes glanders and farcy
    - B. pseudomallei: Causative agent of melidiosis (Glanders like disease)
        - **Motile** and Zoonotic - rodents
        - Humans: Produces pulmonary infections like TB (MC), Multiple abscesses, LN↑
13. **Ans. (b) (Mel...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p342, Ananthanarayan 9/e p317, 8/e p318
    Explained earlier
14. **Ans.(a), (b),(c) (Causes glanders disease, Affects horses, Nonmotile)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p342-43, Ananthanarayan 9/e p317
    Refer chapter review for detail.

STENOTROPHOMONAS MALTOPHILIA

15. **Ans (c, d, e) (Co-trimoxazole, Levofloxacin, Ticarcillin/clavulanate)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p344

    S. maltophilia is intrinsically resistant to most antibiotics. Recommended antibiotics are co-trimoxazole, ticarcillin/clavulanate and levofloxacin etc.

HAEMOPHILUS

16. **Ans. (a) (H.influenzae...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p348
    - DOC for Invasive infection due to H.influenzae type b is Cephalosporins such as ceftriaxone, cefotaxime. This is the best answer that's why cefotaxime was started. But this not there in option.
    - Hence the next best answer is β-lactamase production.
    - Nontypeable strains of H. influenzae are often resistant to β lactams [mostly due to β-lactamase production (20–35% of strains) or rarely by expressing altered penicillin binding protein-3].
17. **Ans. (a), (b), (c), (e) (Ulcer bleeds easily, Painful, Bubo formation, Caused by H.ducreyi)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p348
    Chancroid is caused by H. ducreyi and characterized by painful ulcer and painful soft non indurated lymph nodes (bubo).
18. **Ans. (a) (d) (e) (*H.influenzae, H.aegypticus and H.haemo...*)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p345
    - ***Haemophilus that need both X and V factors: H.influenzae, H.aegypticus and H.haemolyticus***
19. **Ans. (d) (B.pert...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p351, Ananthanarayan 9/e p332, 8/e p332
    H.influenzae, N.meningitidis and Pneumococcus are capsulated and capsular polysaccharide vaccine is available against these organism.
20. **Ans. (a) (H. influenzae)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p347
21. **Ans. (a), (c), (d), (e) (Chancroid, Acute epiglottitis, Brain abscess, Brazilian purpuric fever)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p348, Ananthanarayan 9/e p329, 331, 8/e p 329, 331
    - Influenza is caused by influenza virus.
    - Pfeiffer observed Haemophilus in sputum of Influenza pandemic patients and wrongly named as H.influenzae
    - Brazilian Purpuric fever: H.aegypticus
    - Chancroid (Soft Chancre): H.ducreyi
    - Laryngoepiglottitis (Croup), Meningitis Brain abscess: H.influenzae
22. **Ans. (b) (Acinetobacter...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p349 Ananthanarayan9/e p331
    HACEK group of organisms: Refer text (chapter review).
23. **Ans. (a) (H. d...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p348, Ananthanarayan 9/e p330, 8/e p333
    - Chancroid is caused by – H. ducreyi

24. Ans. (c) (Chocolate...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p348, Ananthanarayan 9/e p330

- Causes of Genital ulcer includes:
  - Chancroid or Herpes: Painful ulcer
  - Donovanosis or primary syphilis or LGV: Painless ulcer
- Painful Genital ulcer showing gram-negative coccobacilli: Indicates Chancroid
- **Chocolate agar with isovitate X and vancomycin is used as a medium for H.ducreyi, the causative agent of Chancroid.**

About Other Options

- Thayer-Martin Medium: For Gonococcus
- Blood agar with X and V factors: For H.influenzae
- Tellurite blood agar: For C.diphtheriae

25. Ans. (b) (Only factor V) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p345

26. Ans. (b) (The serotyping ...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p346, Ananthanarayan 9/e p328

- Serotyping of H.influenzae is based on the capsular polysaccharide

About Other Options:

- H.influenzae can be typed to Six serotypes (a-f), out of which type b is responsible for most of the invasive infection.
- Type b and nontypable strains are the most relevant strains clinically, responsible for invasive disease
- Haemin (X factor) and NAD (V factor) are essential for growth in culture medium
- Can be a part of the normal flora in some persons
- *Nontypable strains colonize the upper respiratory tract of up to three-fourths of healthy adults*
- *Further details refer chapter review.*

27. Ans. (b) (H. influenzae) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p343-47, Ananthanarayan 9/e p328, 30

Points in favor: Case of meningitis, gram stain shows the presence of Gram-negative coccobacilli. The culture shows growth of bacteria only on chocolate agar and not on blood agar.

- H. influenzae requires X and V factor for its growth.
- In blood agar, X factor is available but V factor is not free as it is inside RBC. Where as chocolate agar is a heated blood agar, So RBC gets lysed to release V factor in medium. Hence, H.influenzae can grow in chocolate agar but not in blood agar.
- However, if S.aureus is streaked on blood agar, it provides V factor, hence H.influenzae can grow in blood agar adjacent to the S.aureus steak line (called as ***Staellitism***).

Media for H. influenzae:

- Levinthal's agar
- Field's agar,
- Chocolate agar
- Blood agar with S.aureus streak line

28. Ans. (a) (Meningitis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p347, Harrison 18/e p1229-33

Nontypable H.influenzae

- Next to H.influenzae-b (Hib), Nontypable strains are the commonest group of Haemophilus influenzae clinically. They are noninvasive, spread by contagious spread. They cause the following infections.
- Childhood otitis media
- Exacerbations of COPD (chronic obstructive pulmonary disease): They are the most common bacterial cause.
- Pneumonia in adults among patients with COPD or AIDS
- Puerperal sepsis and neonatal bacteraemia: Especially by nontypable strains of **biotype IV** that colonizes the female genital tract.
- Sinusitis in adults and children
- Rarely causes invasive infections such as empyema, adult epiglottitis, pericarditis, cellulitis, septic arthritis, osteomyelitis in countries where Hib vaccines are used widely.

29. Ans. (a) (b) (Also called as Pfeiffer's bacilli, In acute infections capsulated strains are often isolated) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p345, Ananthanarayan 9/e p327

H.influenzae or the Pfeiffer's bacillus is nonmotile, Gram-negative pleomorphic bacilli. VP is negative.
Capsulated strains are isolated in invasive acute infections whereas the nontypable strains are often responsible for non-invasive conditions such as otitis media.

30. **Ans. (a) (H. ducreyi)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p348, Ananthanarayan 9/e p331
    - Painful genital ulcer and painful, soft nonindurated enlarged inguinal lymph node. Suggestive of soft sore/chancroid which is caused by Haemophilus ducreyi.

BORDETELLA

31. **Ans. (c) (Erythromycin)** Ref: Harrison 19/e p1024
    Macrolide antibiotics are the drugs of choice for treatment of pertussis.

32. **Ans. (a) (B. pertussis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p352, Park 22/e p155, 23/e p163-65
    - Bordetella pertussis does not have a known animal reservoir.

33. **Ans. (a) (PT + FH...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p354, Harrison, 18/e p1245, 19/e p1021-23
    - ***'Although a wide variety of acellular pertussis vaccines were developed, only a few are still widely marketed; all contain pertussis toxoid and filamentous hemagglutinin.'***
      Acellular pertussis contains: Pertussis toxoid
      - Filamentous hemagglutinin
      - Agglutinogen 1,2,3
      - Pertactin (in some vaccine preparations)

34. **Ans. (c) (Nasopharyngeal swab)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p353, Ananthanarayan 9/e p336, 8/e p 938, Harrison 18/e p1244, 19/e p1021-23
    - History of Incomplete childhood vaccination with bouts of spasmodic cough with cyanosis and a typical inspiratory whoop is suggestive of Whooping cough due to Bordetella pertussis.
    - ***'The best specimen is collected by nasopharyngeal aspiration,*** *by fine flexible plastic catheter.*
    - *Is attached to a 10-ml syringe is passed into the nasopharynx and withdrawn while gentle suction is applied.*
    - *Since B. pertussis is highly sensitive to drying, secretions for culture should be inoculated without delay onto appropriate medium'*

    About Other Options
    - Cough plate method though offers advantage like direct bedside plating without delay, but chance of contamination is more.
    - Per oral swab is used to collect secretions from posterior pharyngeal wall.
    - Endotracheal swab is not useful as the secretion will be predominantly in nasopharynx.

35. **Ans. (c) (Increased...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p351, Jawetz 24/e p283, 25/e p248
    - Increased calcium release from sarcoplasmic reticulum is not a mechanism of Pertussis toxin
    - Pertussis toxin
    - Promotes lymphocytosis
    - Sensitization to histamine
    - Enhances insulin secretion
    - ***ADP-ribosylating activity, with an A/B structure and mechanism of action similar to that of cholera toxin.***
    - ADP ribosylation of GTP binding protein → activation of Adenyl cyclase → activation of cAMP

36. **Ans. (a) (7–14 days)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p352, Harrison 18/e p1243, Park 22/e p155, 23/e p163-65
    Incubation period varies averaging 7–10 days (Harrison), 7–14 days (Park)

37. **Ans. (a) (Prophylactic...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p354, Park 22/e p156
    - Treatment for pertussis contacts of children, Prophylactic antibiotic like erythromycin for 10 days

38. **Ans. (c) (3–4 weeks)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p352, Park 22/e p156
    - A child with pertussis should be isolated till he is considered non infectious, probably for 3–4 weeks

BRUCELLA

39. **Ans. (a) (Bruce...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p360, Ananthanarayan 9/e p339, 8/e p343
    - Milk ring test is done for diagnosis of Brucellosis in animals.

40. Ans. (b) (c) (d) (Camel, Sheep, Goat) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p355, Ananthanarayan 9/e p339, 8/e p343, Harrison 18/e p1296 , 19/e p1066(eChapter 194e)

- *The nomen system recognizes B. melitensis, which is the most common cause of symptomatic disease in humans and for which the main sources are sheep, goats, and camels …Harrison 18/e p1296*

41. Ans. (c) (Brucella...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p355, Ananthanarayan 9/e p339, 8

'Brucellosis is also known as Malta or Undulant fever or Mediterranean fever'.

42. Ans. (a), (b), (e) (Doxycycline, Streptomycin, Rifampicin) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p360, Harrison 18/e p1296

Treatment of brucellosis: Refer chapter rivew

43. Ans. (c) (Person to person transmission) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p357; Ananthanarayan 9/e p342, 8/e p343, Harrison 18/e p1296

'Person-to-person transmission is extremely rare'

Mode of Transmitted by:

- Intake of Raw milk (MC), or milk products/meat contaminated with infected animal feces/urine
- Contact with animal feces/urine
- Inhalation of dried material of animal origin

44. Ans. (a) (Standard...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p359, Ananthanarayan 9/e p343, 8/e p345

- Standard Agglutination test identifies mainly IgM antibody, hence they are useful in acute but not in chronic brucellosis.
- In acute brucellosis- Following 7-10 days of infection, both IgM and IgG appear, however the IgM antibodies gradually fall and only IgG antibodies persist in chronic brucellosis

45. Ans. (d) (Brucellosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p359

CHAPTER

3.10

Spirochetes

Spirochetes are thin, flexible, elongated spirally coiled helical bacilli (*speira*, meaning coil; and *chaite*, meaning hair).

Most spirochetes are saprophytes. Only three are human pathogens: *Treponema*, *Borrelia* and *Leptospira*.

Pathogenic Spirochetes	Disease	Transmission
Treponema		
T.pallidum	Syphilis	Sexual
T.pertenue	Yaws	Direct contact (Non-veneral *Treponema*)
T.endemicum	Endemic syphilis	
T.carateum	Pinta	
Borrelia		
B.recurrentis	Relapsing fever (epidemic)	Louse borne
B.duttonii, B.hermsii	Relapsing fever (endemic)	Tick borne
B.burgdorferi	Lyme disease	Tick borne
B.vincentii	Vincent's angina	Direct contact
Leptospira		
L.interrogans	Leptospirosis (Weil's disease)	Contact with rodent urine

SYPHILIS

Syphilis is one of the ancient sexually transmitted disease. Name was derived from a famous poem in the year 1530 which described a legend of a shepherd boy named Syphilus, who had suffered from the disease.

- **Transmission:** It is acquired by sexual contact. However, it can also be transmitted by nonvenereal modes such as direct contact, blood transfusion or transplacental transmission.
- **Incubation period** is variable (9–90 days) and is inversely proportional to the number of organisms inoculated.

Clinical Manifestations

- Approximately, 30% of persons who have sexual exposure with an infected partner develop syphilis; which can pass through various stages: primary, secondary, latent and tertiary (or late) stages.

Primary Syphilis

- *Primary (or hard) chancre*: Single painless indurated ulcer. MC sites are penis (in males), cervix or labia (in females).
- *Regional (usually inguinal) lymphadenopathy*: **Painless** firm, nonsuppurative, and often bilateral.
- If syphilis acquired by nonvenereal mode:
  - If transmitted by direct contact → the primary chancre is extragenital, usually on the fingers.
  - If transmitted by blood transfusion → the primary chancre does not occur.

If syphilis acquired by non-venereal mode:
- If transmitted by direct contact→ the primary chancre is extragenital, usually on the fingers.
- If transmitted by blood transfusion→ the primary chancre does not occur

Secondary Syphilis

It usually develops 4–8 weeks after the healing of primary lesion and is characterized by:

- Skin rashes (palms and soles)
- Condylomata lata (mucocutaneous lesion)
- Mucous patches (superficial mucosal erosions)
- Generalized lymphadenopathy

Late or tertiary syphilis:
- Gumma
- Cardiovascular syphilis: Aneurysm of ascending aorta and aortic regurgitation
- Neurosyphilis
  - General paresis of insane
  - Tabes dorsalis
  - Meningeal syphilis (meningitis)
  - Meningovascular syphilis

Latent Syphilis

It is characterized by absence of clinical manifestations of syphilis with positive serological tests for syphilis and normal CSF findings in patients with history of primary and secondary syphilis in the past.

- Early latent syphilis (occurs within first year after infection) and late latent syphilis (occurs after the first year).
- Patients are still infectious transmitting the infection either by bloodstream or in utero.
- Latent syphilis may have one of the following fates:
  - Persistent lifelong infection (common)
  - Development of late syphilis (rare)
  - Spontaneous cure with the serological tests becoming negative (rare).

Late or Tertiary Syphilis

One-third of untreated patients develop tertiary syphilis after several decades.

- Gumma (late benign syphilis): Occurs in 15% people
- Cardiovascular syphilis (10% people): Characterized by, aneurysm of ascending aorta and aortic regurgitation.
- Neurosyphilis (Occurs in 10% people): Invasion of CNS occurs in first few weeks of infection, but manifestations develop only in late stage. CNS and CVS lesions are called as *quaternary syphilis:*
  - General paresis of insane
  - Tabes dorsalis: (Demyelination of the posterior columns)
  - Meningeal syphilis (meningitis)
  - Meningovascular syphilis (vasculitis of arteries leading to embolic stroke)

Cultivation:
- Pathogenic treponemes cannot be grown in artificial culture media but are maintained rabbit testes.
- However, nonpathogenic treponemes (e.g. Reiter treponemes) can be cultured in Smith Noguchi media

Congenital Syphilis

Placental transmission may occur at any stage of pregnancy, but fetal damage occurs only after fourth month of gestation. **Manifestations of congenital syphilis include:**

- ***Earliest manifestations*** occur within 2 yrs of age. Affected children are infectious and they suffer froy rhinitis (or snuffles), smucocutaneous lesions, bone changes, hepatosplenomegaly and lymphadenopathy.
- ***Late congenital syphilis*** occurs after 2 years and is noninfectious. It is characterized by interstitial keratitis, eighth-nerve deafness bilateral knee effusions (Clutton's joints)
- **Residual stigmata** may remain for long time such as:
  - Hutchinson's teeth notched central incisors)
  - Mulberry molar
  - Saddle nose and saber shins.

Laboratory Diagnosis of Syphilis

1. **Microscopy**
   - Dark ground microscopy:
     - *T.pallidum* appears as slender, flexible, spirally coiled bacilli with tapering ends, and three types of motility: (i) *flexion extension type,* (ii) *corkscrew motility*, (iii) rotational with *soft bending* at midpoint.
     - The sensitivity is 80% with a detection limit of 10^4 bacilli/ml.
   - Direct fluorescent antibody staining for *T. pallidum* (DFA-TP-Sensitivity is 100%)
   - Silver impregnation method: (i) Levaditi stain (for tissue section), (ii) Fontana stain (smear)

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2. Culture: ○ Pathogenic treponemes cannot be grown in artificial culture media but are maintained rabbit testes. ○ However, nonpathogenic treponemes (e.g. Reiter treponemes) can be cultured in Smith Noguchi media		
3. Serology (antibody detection)		
A. Nontreponemal or nonspecific tests or STS (standard tests for syphilis): Reagin antibodies are detected by using nonspecific cardiolipin antigen derived from bovine heart		
	i. Wassermann test	CFT (complement fixation test)
	ii. Kahn Test	Tube flocculation
	iii. VDRL (Venereal disease research laboratory) test	Slide flocculation
	iv. RPR (Rapid plasma reagin)	Slide flocculation
	v. USR (Unheated serum reagin test)	Slide flocculation
	vi. TRUST (toluidine red unheated serum test)	Slide flocculation
B. Specific/Treponemal tests: Specific antibodies are detected by using *T.pallidum* antigens		
	i. TPI (*Treponema pallidum* immobilization test)	Uses live *T.pallidum*
	ii. FTA-ABS (Fluorescent treponemal antibody absorption test)	Uses killed *T.pallidum*
	iii. TPA (*T.pallidum* agglutination test)	
	iv. TPIA (*T.pallidum* immune adherence test)	
	v. TPHA (*T.pallidum* hemagglutination test)	Uses antigenic extract of *T.pallidum*
	vi. TPPA (*T.pallidum* particle agglutination test)	
	vii. Western blot	
	viii. Enzyme immunoassay (EIA)	
C. Group specific: RP CFT (Reiter protein Complement fixation test): Uses Reiter strain		
4. Polymerase chain reaction (PCR)		

VDRL (Venereal Disease Research Laboratory)

VDRL test is a slide flocculation test (type of precipitation reaction)

- **Antigen preparation:** VDRL antigen is a cardiolipin antigen derived from bovine heart to which cholesterol and lecithin are added. Antigen has to be reconstituted with buffer and should be used within 24 hrs.
- **Patient's serum preparation**: Inactivating serum by heating at 56°C for 30 min to remove the non-specific inhibitors.
- **VDRL slide** containing 12 concave rings are used.
- **Qualitative test:** Drop of inactivated serum is mixed with a drop of VDRL antigen and the slide is rotated for 4 minutes:
  - Non reactive: Uniformly distributed fusiform crystals represent the presence of VDRL antigen only
  - Reactive: Medium to large clumps signifies antigen antibody complexes
- **VDRL-CSF**: VDRL test can also be performed to detect CSF antibodies. However, no pre heating of CSF is needed.

VDRL test:
- Example of Slide flocculation test (type of precipitation reaction)
- Reagin antibodies are detected by using non-specific cardiolipin antigen derived from bovine heart

VDRL (Venereal Disease Research Laboratory) test	RPR (Rapid Plasma Reagin) Test
Results read microscopically (low power) as clumps are smaller in size.	Results read macroscopically (carbon particle coated cardiolipin antigens are used, forms larger clumps).
Antigen, once reconstituted, should be used within 24 hours	**EDTA** is used as stabilizer; hence RPR antigen can be stored longer (up to 6 months at 4-10°C)
Preheating of serum is required to remove nonspecific inhibitors	Preheating of serum is not required as **choline chloride** is used to remove inhibitors
Blood, plasma, serum, and CSF can be tested	Blood, plasma and serum can be tested ***but not CSF***
Rotation of slide is done for 4 mins	Rotation of card is done for 8 mins
Sensitivity in primary syphilis is 78%	Sensitivity in primary syphilis is 86%
It is cheaper; one vial of VDRL antigen can be used for 250 tests. It is preferred for field studies and for antenatal screening	RPR is expensive than VDRL. It is preferred when sample load is less.

Advantages of non-treponemal tests
- Monitoring the response to treatment
- Useful in Neurosyphilis (VDRL-CSF)
- Reagin antibody becomes detectable in 7–10 days

Disadvantages of non-treponemal tests
- Biological false positive (BFP) reactions
- Prozone phenomena
- Sensitivity low in late stage of syphilis
- Can be used as only Screening tests

Most Sensitive Serological test:
- Primary syphilis—Western blot and EIA > TPPA > RPR > FTA-ABS
- Secondary syphilis—All tests equally sensitive (100%)
- Latent syphilis—All treponemal test equal
- Late syphilis—FTA-ABS > TPHA

Overall most specific:
TPHA, EIA,USR,TRUST (All 99%) > TPPA

Serial IgG in baby (by VDRL test Screening):
- If maternal transfer→ titer falls in 3 months,
- If congenital infection → titer rises in 3 months

Advantages of nontreponemal tests
- Monitoring the response to treatment: Reagin tests usually become negative 6–18 months after the effective treatment for syphilis, depending on the stage at which treatment is given.
- Neurosyphilis: VDRL can also be used to detect CSF antibodies.
- Reagin antibody becomes detectable 7–10 days after the appearance of primary chancre (or 3–5 weeks after infection).
- Sensitivity of non treponemal tests varies from 78–85% in primary, 100% in secondary and 95–98% in latent stage.

Disadvantages of nontreponemal tests:
- ***Biological false positive (BFP) reactions:*** Defined as positive nontreponemal tests, with negative treponemal tests and not caused by technical faults and no clinical feature. It occurs in 1% of cases:
  - BFP antibody is usually of IgM type, while reagin antibody in syphilis is mainly IgG.
  - *Types of BFP:* Conditions in which BFP reactions occur can be classified into:
    - *Acute BFP reactions* persist for < 6 months, e.g. acute infections, injuries or inflammatory conditions
    - *Chronic BFP reactions* last for > 6 months, e.g. SLE and other collagen diseases such as rheumatoid arthritis.
    - *Other conditions* include lepromatous leprosy, relapsing fever, malaria, tropical pulmonary eosinophilia, viral hepatitis, infectious mononucleosis, HIV, pregnancy and IV drug abusers.
- ***Prozone phenomena:*** False negative if antibody titer in patient's sera is high
- ***Sensitivity*** of nontreponemal tests is low in late stage of syphilis.
- ***Screening tests:*** Nontreponemal tests are used as screening tests which should be confirmed by treponemal tests.
  - If treponemal tests are found positive: It confirms syphilis
  - If treponemal tests are found negative: It indicates false positive nontreponemal tests.

One-Liners for Serological Tests in Syphilis
- For monitoring treatment: Ideal test is VDRL > RPR
- Primary syphilis: Most Sensitive: Western blot and EIA > TPPA > RPR > FTA-ABS
- Secondary syphilis: All tests equally sensitive (100%)
- Latent syphilis: Most Sensitive: All treponemal test
- Late syphilis: Most Sensitive: FTA-ABS > TPHA
- Overall most specific: TPHA, EIA, USR, TRUST (All 99%) > TPPA
- First test to be positive: FTA-ABS
- Rapid and mass screening: VDRL.

Diagnosis of Congenital Syphilis
Definitive diagnosis: Demonstration of *T.pallidum* by DGM of umbilical cord, placenta, nasal discharge, or skin lesion

Presumptive diagnosis:
1. Infant born to a mother who had syphilis at the time of delivery regardless of findings in the infant and
2. Reactive treponemal test in infant and
3. One of the following additional criteria:
   - Clinical signs/symptoms of congenital syphilis
   - Abnormal CSF findings without other cause
   - Reactive VDRL-CSF test
   - Reactive IgM antibody test specific for syphilis (IgM FTA ABS or IgM ELISA): As IgM does not cross the placenta, its presence in neonatal serum confirms the diagnosis of congenital syphilis.

Screening of Congenital syphilis based on VDRL test (IgG):

Serial IgG in baby (VDRL):

- If maternal transfer → titer falls in 3 months,
- If Congenital infection → titer rises in 3 months

Simultaneous VDRL test of mother and baby:

- If mother's titer > baby's titer → Indicates maternal transfer
- If baby's titer > mother's titer → Indicates congenital infection
- Sample : Baby serum (Most appropriate) > cord blood > Maternal serum

Treatment of Syphilis Penicillin is the DOC in all stages in penicillin allergy:

- Primary, secondary, latent, CVS or benign tertiary syphilis: Tetracycline is recommended
- Neurosyphilis or pregnancy or associated HIV Desensitization to penicillin

Treatment of Syphilis

- **Penicillin** is the drug of choice for all the stages of syphilis:
  - Primary, secondary, or early latent syphilis: Single dose of Penicillin G
  - Late latent CVS or benign tertiary stage: Penicillin G, single dose weekly for 3 weeks.
  - Neurosyphilis or associated HIV: Aqueous crystalline or procaine penicillin G for 10–14 days.
- **Alternate drug is used** in patients with penicillin allergy:
  - Primary, secondary, latent, CVS or benign tertiary syphilis: Tetracycline is recommended.
  - Neurosyphilis or pregnancy or associated HIV: Desensitization to penicillin has to be done following which penicillin is administered.

Nonveneral Treponema:

- *T. pertenue*: Causes Yaws
- *T. endemicum*: Causes Endemic syphilis
- *T. carateum:* Causes Pinta

NONVENEREAL TREPONEMATOSES

Endemic treponematoses can traditionally be distinguished from venereal syphilis by (Table given below):

- Mode of transmission (direct contact, not sexual)
- Age of acquisition (childhood)
- Geographic distribution (rural areas of developing nations of tropics, travellers in developed nations)
- Associated with poor hygiene
- Clinical features (described in the text)

Feature	Venereal Syphilis	Yaws	Endemic Syphilis	Pinta
Agent	*T. pallidum*	*T.pertenue*	*T.endemicum*	*T.carateum*
Mode of transmission	Sexual, Transplacental Blood	Skin-to-skin	Household contacts: kissing, sharing utensils or insect	Skin-to-Skin
Age	Adulthood	Early childhood	Early childhood	Late childhood
Primary lesion	Chancre- painless nonindurated Lymphadenopathy	Papilloma, often ulcerative Lymphadenopathy	Rarely seen	Nonulcerating pruritic papule
Site of lesion	Genital, oral, anal	Extremities	Oral	Extremities, face
Secondary lesions	Skin rashes Mucosal patches, Condylomata lata	Skin lesions: Macular or papular, Periostitis	Oral mucous patches Periostitis, lymphadenopathy	**Pintides**, Pigmented and pruritic
Relapses	25%	Common	Unknown	None
Late complications	Gummas, CVS and CNS lesion	Destructive gummas of skin, bone, cartilage Destruction of the nose, maxilla, palate, and pharynx is termed as *gangosa,* No CNS or CVS lesion		Nondestructive, *dyschromic macule* No CNS or CVS lesion

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	Syphilis	Chancroid	LGV	Donovanosis	Herpes
Ulcer	Single, Painless, indurated	Painful, non indurated irregular	Painless	Painless	Multiple, painfull, vesicular, bilateral
LN	Painless	Painful	Painful	Not enlarged	Not enlarged
IP	9–90 days	< 1 week	3 days to 6 weeks	1–4 week	< 1 week (2–3 days)

BORRELIA

Relapsing Fever

- Relapsing fever is so named because: Alternate periods of febrile and afebrile episodes
- Reason for relapse is antigenic variation, shown by its causative agent *B. recurrentis.*

Borrelia (Disease and their Transmission):
- *B. recurrentis:* Causes Relapsing fever (epidemic), Louse borne
- *B. duttonii, B. hermsii:* Causes Relapsing fever (endemic), Tick borne
- *B. burgdorferi:* Causes Lyme disease, Tick borne
- *B. vincentii:* Causes Vincent's angina, Direct contact

Lab diagnosis

- Microscopic examination of Blood:
  - Wet film under dark ground or phase contrast microscope
  - Giemsa and Leishman staining or QBC
- PCR: More sensitive and speciation possible
- Serology (Antibody detection)
  - ELISA, IFA: Not reliable, gives false positive
  - GlpQ assay: It is the most reliable serological method. It is an immunoblot assay detecting antibody against the recombinant GlpQ antigen (Glycerophosphodiester phosphodiesterase).
- Animal inoculation into mice: Causes intraperitoneal infection
- Molecular methods: Multiplex Real-time PCR has been developed targeting 16SrRNA and glpQ genes

Characters	Epidemic relapsing fever	Endemic relapsing fever
Agent	*B.recurrentis*	*B.duttoni, B.hermsii*
Natural Host	Humans	Rodents
Transmitted by	Louse - by crushing or rubbing	Tick bite
Distribution	East Africa (Sudan & Ethiopia)	North America, Central Asia, and throughout Africa
Hemorrhage, CNS features	More common	Less common
Treatment	Doxycycline: Single-dose	Doxycycline for 1 week

Lyme Disease

- Agent: *B. burgdorferi*
- Transmission: Tick borne (*Ixodes ricinus*)
- **Clinical Manifestation:** Consists of triad of:
  - Stage 1: Erythema migrans (annular lesions)
  - Stage 2: Disseminated infection: skin lesions, meningitis, neuritis, nodal block, or joint pain.
    (Neurological abnormalities: Occur in 15% of cases which include meningitis, encephalitis and a typical lymphocytic meningoradiculitis seen in cases from Europe and Asia; called as ***Bannwarth's syndrome***)
  - Stage 3: Persistent infection: arthritis, encephalopathy or acrodermatitis.
- **Lab Diagnosis:** Diagnosis mainly on clinical ground.
  - Isolation from skin lesions or blood- modified Kelley's medium
  - Microscopic detection – Dark ground, phase contrasts, Immunfluroscence, silver staining
  - Antigen in urine

- Antibody detection:
  - ELISA and western blot: First month-both IgM and IgG; later IgM disappears
  - Limitations: Antibodies persist for long time and false positive in unrelated infections
  - Fourfold rise of antibody is more significant
  - Two test approach: CDC recommends to perform ELISA first → confirmed by western blot.
  - C6 peptide IgG ELISA
- PMNs in joint fluid but lymphocytosis in CSF

- **Treatment:**
  - DOC for all cases (except CNS or AV block): Oral doxycycline > amoxicillin,
  - DOC for CNS or AV block- IV Ceftriaxone > Cefotaxime

Borrelia Vincentii

- It is a commensal of mouth
- Vincent's angina: Ulcerative gingivostomatitis (predisposing conditions such as malnutrition, viral infection).
- Often associated with fusiform bacillus (*Leptotrichia* which is formerly called *Fusobacterium fusiformis*).

LEPTOSPIRA

Classification

Species: *Leptospira*, comprises of two species:

- *L.interrogans* (pathogenic for humans): Causes leptospirosis or Weil's disease involving liver and kidney.
- *L.biflexa* (saprophyte)

Serovars and serogroups:

- *L.interrogans* comprises of 25 serogroups which further consist of over 250 serovars.
- Although all serogroups and serovars are morphologically identical, produce similar clinical picture but they differ in their geographical distribution and in severity of infection.

Leptospira transmission:
- Indirect contact with water, moist soil and wet surfaces contaminated with animal urine
- Direct contact with urine and products of parturition, placenta of infected animals
- Direct human-to-human transmission does not occur.

Epidemiology

- **Mode of transmission:** It is zoonotic. Direct human-to-human transmission does not occur. It is transmitted by:
  - Indirect contact with water, moist soil and wet surfaces contaminated with animal urine or
  - Direct contact with urine and products of parturition, placenta of infected animals
- **Source:** Although > 100 animals can be infected; but important sources of infection are rats, dogs, cattle, and pigs.
- **3R's**: Important epidemiological determinants for leptospirosis include exposure to **r**odents, **r**ainfall and **r**ice field.
- **Global distribution:** Leptospirosis is worldwide in distribution. Highest burden is seen in Brazil, India and Thailand.
- **In India:** Leptospirosis is endemic in Tamil Nadu, Kerala and Andaman (called as Andaman hemorrhagic fever)
- **Regional Medical Research Centre** is the national leptospirosis reference centre of India, located in Portblair.
- **Classic serovar: Animal associations** include:
  - Icterohemorrhagiae and Copenhageni in domestic rats (*Rattus norvegicus, R.rattus*): MC serovar in India

3R's
Important epidemiological determinants for leptospirosis include exposure to rodents, rainfall and rice field

Weil's disease:
- High grade fever
- Jaundice and raised liver enzymes
- Hemorrhages: Pulmonary (MC)
- Kidney: Raised serum urea and creatinine

- Grippotyphosa in opossums and raccoons (emerging in USA)
- Canicola in dogs
- Hardjo in cattle and buffalo
- Pomona in pigs

Clinical Manifestations

The incubation period is around 5–14 days. In general, the manifestations can be divided into two distinct clinical syndromes.

- **Mild anicteric febrile illness:** Occurs in 90% of patients. It is biphasic; septicemic phase followed by immune phase
- **Weil's disease** (Hepato-renal-hemorrhagic syndrome): It is a severe form of icteric illness, occurs in 10% patients. *Typical biphasic course may not be present*. It is more severe and fulminant

	Mild anicteric febrile illness		**Weil's disease**	
	First stage 3–10 days (septicemic)	Second stage 10–30 days (immune)	First stage 3–10 days (septicemic)	Second stage 10–30 days (immune)
Clinical Findings	1. Fever, 2. Myalgia, 3. Headache, 4. Conjunctival suffusion 5. Abdominal pain, 6. Pharyngeal erythema without exudates 7. Vomiting	1. Meningitis, 2. Uveitis, optic neuritis, chorioretinitis 3. Rash, 4. Fever 5 Peripheral neuropathy	1. High grade fever 2. Jaundice and raised liver enzymes 3. Hemorrhages: ○ Pulmonary hemorrhage, ○ Petechiae and purpura ○ Conjunctival hemorrhage, ○ GI hemorrhage 4. Kidney: Raised serum urea and creatinine and Renal failure	
Isolation	From blood and CSF	From Urine	Blood and CSF	Urine
Serum IgM	Absent	Present	Absent	Present
Antibiotics	Susceptible to antibiotics	Refractory to treatment	Susceptible to antibiotics	Refractory to treatment

Leptospira (Dark Ground Microscopy):
Tightly and regularly coiled, with hooked ends like umbrella handle.

Culture Media for Leptospira:
- EMJH medium
- Korthof's media with rabbit blood
- Fletcher's semisolid

Microscopic Agglutination Test (MAT):
- Detects antibodies against specific serovars of *L. interrogans*.
- It is the gold standard method and reference test for the diagnosis of leptospirosis

Laboratory Diagnosis

- **Specimens:** CSF and blood (in first 10 days of infection) and urine (between 10–30 days of infection)
- **Microscopy:** *Leptospira* are extremely thin, hence seen under dark ground microscope
  - They are *tightly* and regularly coiled, with characteristic *hooked ends like umbrella handle.*
  - They are highly motile; exhibit *spinning* and *translational* movements.
- **Isolation:**
  - Culture condition: *Leptospira* is obligate aerobe and slow growing. Incubated at 30°C for 4–6 weeks
  - **Culture media:** As *Leptospira* is highly fastidious, requires enriched media such as:
    - **EMJH medium** (Ellinghausen, McCullough, Johnson, Harris) -most commonly media
    - **Korthof's** media with rabbit blood and **Fletcher's semisoli**d media
  - **Advantages:**
    - Isolation confirms the diagnosis.
    - Useful to maintain the stock culture of the *Leptospira* in the laboratories.
  - **Disadvantages:**
    - Culture technique is laborious, technically demanding and time consuming.
    - *False positive results:* It may occur due to contamination of culture media
    - *False negative results:* It may occur due to prior antibiotics, or incubating in improper temperature and pH
- **Animal Inoculation:** Hamsters (4–6 weeks old) and young guinea pigs

- **Serology for antibody detection:** Antibody detection tests can be broadly classified into:
  - ***Genus specific tests*** uses broadly reactive genus specific antigen prepared from nonpathogenic *L.biflexa* Patoc 1 strain. They cannot detect the infecting serovar. Various tests available are:
    - Macroscopic slide agglutination test
    - Microcapsule agglutination test (MCAT)
    - Latex agglutination test, ELISA and ICT ***Serovar specific test*****: Microscopic agglutination test (MAT)** detects antibodies against specific serovars of *L.interrogans*. It is the *gold standard* method and *reference test* for the diagnosis of leptospirosis.
- **Molecular methods:**
  - Various genes such as 16S or 23SrRNA or IS1533 insertion sequence are targeted.
  - PCR detects early before seroconversion occurs. However, PCR is not serovar specific.
  - PCR-RFLP or PFGE are the methods to determine the genomospecies of *Leptospira*.
- **Faine'scriteria** is a WHO approved guideline used for the diagnosis of leptospirosis. It is based on clinical, epidemiological and laboratory findings.
- **Nonspecific findings** such as: Altered renal function and liver function tests.

Faine's Diagnostic criteria:
It is a WHO approved guideline used for the diagnosis of leptospirosis. It is based on clinical, epidemiological and laboratory findings

Leptospirosis Treatment:
- Mild: Oral doxycycline is DOC
- Severe: Penicillin is DOC

Treatment

- **Mild leptospirosis** should be treated with oral doxycycline. Amoxicillin can be given alternatively.
- **Severe leptospirosis:** Penicillin is the drug of choice, alternatives being ceftriaxone or cefotaxime.

MULTIPLE CHOICE QUESTIONS

SYPHILIS

1. Centripetal rash is seen in all except: *(AIIMS Nov 2016)*
a. Measles
b. Typhoid
c. Epidemic typhus
d. Secondary syphilis

2. Which stage of syphilis is known as great imitator?
a. Primary syphilis *(Recent Question 2015)*
b. Secondary syphilis
c. Tertiary syphilis

3. T.pallidum can be grown in: *(Recent Question 2015)*
a. Mice b. Armadillo
c. Rodent d. Cannot be grown

4. Non-treponemal test includes: *(PGI Nov 2014)*
a. RPR b. VDRL
c. FTA-ABS d. TPHA
e. TPI

5. Which of the following is/are NOT spirochete(s): *(PGI Nov 2014)*
a. Borrelia b. Fusobacterium
c. Lactobacillus d. Leptospira
e. Leptotrichia

6. Syphilis was first identified by: *(NEET Pattern Based)*
a. Fraenkel b. Nicolaicu
c. Schaudinn & Hoffman d. Ogston

7. In a syphilis patient, site which does not help in isolation of organism: *(DNB DEC 2010, JIPMER 2002, NEET Pattern Based)*
a. Gumma b. Primary chancre
c. Mucosal patch d. Maculopapular rash

8. Stain for treponema: *(NEET Pattern Based)*
a. Fontana's b. Acid-fast
c. Methenamine-silver d. PAS

9. In a pregnant lady of 8 weeks, allergic to penicillin, VDRL was done which found to be positive. The drug of choice for treatment is: *(AI 2012)*
a. Erythromycin b. Penicillin
c. Tetracycline d. desensitization

10. False +ve VDRL test is/are seen in: *(PGI DEC 2008, JIPMER 2011)*
a. Leprosy
b. Malaria
c. Relapsing fever
d. IV drug user
e. HIV infection

11. Correct combination of incubation period is:
a. Syphilis: 9–90 days *(PGI June 2011)*
b. Herpes genitalis: 4–5 week
c. LGV: 3 d –6 week
d. Donovanosis: 1–4 week
e. Chancroid: 2–3 week

12. A newborn premature baby presented with bullous lesions on skin and a shin on knee. X-ray shows periostitis. Best investigation for diagnosis is:
a. VDRL from mother and baby *(AIIMS Nov 2011)*
b. PCR for tuberculosis
c. HBsAg detection
d. ELISA for HIV

13. In India, syndromic management is applicable for:
a. Chancroid and chancre *(AIIMS Nov 2011)*
b. Chancroid and herpes genitalis
c. Chancroid, chancre, herpes genitalis
d. Chancre and herpes genitalis

14. VDRL is most sensitive in the diagnosis of which stage of syphilis? *(JIPMER 2011)*
a. Primary
b. Secondary
c. Tertiary
d. Reactivation

15. 25-year-old laborer 3 years back presented with penile ulcer not treated. Later he presented with neurological symptoms for which he got treated. Test to monitor response to treatment is: *(MHPG 2015; AIIMS Nov 2009)*
a. VDRL
b. FTA-ABS
c. TPI
d. RPR

16. True about primary chancre: *(PGI Dec 2008, 2004)*
a. Painless ulcer
b. Painless lymphadenopathy
c. Covered with exudates
d. Indurated lesion
e. Organism can be cultured from exudative fluid

17. A VDRL reactive mother gave birth to an infant. All of the following would help in determining the risk of transmission to the infant except: *(AI 2006)*
a. TPHA test on the serum sample of the mother
b. TPHA test on the serum sample of the infant
c. VDRL on the paired serum sample of the infant and mother
d. Time interval between the treatment of the mother and her delivery

18. 23-year-old guy with painless penile ulcer and painless lymphadenopathy. What is the diagnosis? *(Recent Question 2015)*
a. Chancroid b. Donovanosis
c. Syphilis d. Herpes

19. A 23-year-old male had unprotected sexual intercourse with a commercial sex worker. Two weeks later, he developed a painless, indurated ulcer on the glans that exuded clear serum on pressure. Inguinal lymph nodes in both groins were enlarged and not tender. The most appropriate diagnostic test is: *(AIIMS May 2004)*
a. Gram's stain of ulcer discharge
b. Dark field microscopy of ulcer discharge
c. Giemsa stain of lymph node aspirate
d. ELISA for HIV infection

20. Investigation of choice for detection of syphilis in a patient after 2 course of complete therapy: *(AI 2002)*
a. FTA ABS
b. VDRL
c. TPI
d. Dark ground microscopy

21. True about VDRL test: *(AIIMS 2001)*
a. Non –specific
b. Slide flocculation test
c. Best followed for drug therapy
d. All

22. Congenital syphilis can be best diagnosed by: *(AI 2001)*
a. IgM FTA – ABS b. IgG FTA – ABS
c. VDRL d. TPI

23. All are true about FTA – ABS in Syphilis, except: *(AI 2000)*
a. FTA –ABS becomes negative after treatment
b. Present in secondary syphilis
c. It is a specific test
d. May be positive in Lyme's disease

24. Hutchinson's triad a feature of: *(TNPG 2014)*
a. Primary Syphilis
b. Secondary Syphilis
c. Tertiary Syphilis
d. Late Congenital Syphilis

25. T.pallidum can survive in blood under refrigeration for maximum...? *(COMED-K 2016)*
a. Few hours b. 1 day
c. 3 days d. Several weeks

NONVENEREAL TREPONEMA

26. Bejel is caused by: *(NEET Pattern Based)*
a. Trepnema pertenue
b. Treponema caratenum
c. Treponema pallidum
d. Treponema endemicum

27. Causative agent of Yaws is:
a. Trepnema pertenue *(NEET Pattern Based)*
b. Treponema caratenum *(DNB June 2011)*
c. Treponema pallidum
d. Treponema endemicum

28. Nonvenereal treponemes are: *(PGI June 2004)*
a. T. pertenue b. T. Carateum
c. T. pallidum d. T. cuniculi

29. True about Yaws: *(PGI May 2013)*
a. Sexually transmitted disease
b. Transmitted by fomites
c. Mother-child transmission
d. Periostitis occurs
e. Caused by T. pallidum subspecies endemicum

BORRELIA

30. True about B. recurrentis: *(NEET Pattern Based)*
a. Causes leptospirosis
b. Water borne disease
c. Transmitted by louse
d. Transmitted by flea

31. Lyme's disease is caused by:
a. Borrelia parkeri *(DNB Dec 2009, PGI June 2001)*
b. Borellia burgdorferi
c. Borrelia recurrentis
d. Borrelia hermsii

32. Which one of the following microorganisms uses antigenic variation as a major means of evading host:
a. Streptococcus pneumoniae *(AIIMS Nov 2004)*
b. Borrelia recurrentis
c. Mycobacterium tuberculosis
d. Listeria monocytogenes

33. The following are true regarding Lyme's Disease, except: *(AI 2003)*
a. It is transmitted by ixodid tick
b. Erythema chronicum migrans may be a clinical feature
c. Borrelia recurrentis is the etiological agent
d. Rodents act as natural hosts

LEPTOSPIRA

34. Most common Mode of acquisition of leptospirosis is:
a. Skin and mucous membrane *(TNPG 2015)*
b. Rat bite
c. Ingestion
d. Inhalation

35. Which serogroup of Leptospira interrogans is commonly responsible for causing Weil's disease? *(MHPG 2015)*
a. Icterohaemorrhagica b. Hebdomadis
c. Australis d. Canicola

36. Leptospirosis is transmitted by: *(NEET Pattern Based, DNB June 2009)*
a. Rat b. Cat
c. Dog d. Fish

37. Weil's disease is caused by? *(DNB June 2011)*
a. Leptospira b. Plague
c. Yersinia d. Rickettsial Fever

38. A sewer worker comes with high grade fever, neck rigidity and signs of meningismus. Lab findings suggestive of renal failure and elevated liver enzymes. Most appropriate drug? *(AI 2011)*
a. Benzylpenicillin b. Ciprofloxacin
c. Doxycycline d. Cotrimoxazole

39. Which one of these is true regarding Leptospirosis?
a. Rat is the principal animal reservoir. *(AI 2011)*
b. Transmission is orofecal.
c. Renal and hepatic involvement is seen in half of the affected children.
d. Fluoroquinolones are the drugs of choice.

40. Which of the following is not used to diagnose Leptospirosis? *(AIIMS May 2010)*
a. Microscopic agglutination test
b. Dark field microscopy
c. Macroscopic agglutination test
d. Weil-Felix reaction

41. Culture media of Leptospira: *(AIIMS May 2009)*
a. Korthoff
b. Perkin
c. Tinsdale
d. Baker's

42. Which human infection spreads through urine: *(AI 2002, PGI Dec 2006)*
a. Leptospira
b. Legionella
c. Plague
d. Diphtheria

43. A bacterial disease that has been associated with the 3 "R" i.e rats, rice fields, and rainfall is: *(AI 2005)*
a. Leptospirosis
b. Plague
c. Melioidosis
d. Rodent – bite fever

44. A 25-year-old farmer presented with history of high grade fever for 7 days and altered sensorium for 2 days. On examination, he was comatosed and had conjunctival hemorrhage. Urgent investigations showed hemoglobin of 11 gm/dl, serum bilirubin 8 mg/dl and urea 78 mg/dl. Peripheral blood smear was negative for malarial parasite. What is the most likely diagnosis: *(AIIMS Nov 2005)*
a. Brucellosis
b. Weil's disease
c. Acute viral hepatitis
d. Q-fever

45. A fourteen year old body is admitted with history of fever, icterus, conjunctival suffusion and hematuria for twenty days. Which of the following serological test can be of diagnostic utility: *(AIIMS Nov 2004)*
a. Widal test
b. Microscopic Agglutination Test
c. Paul Bunuel test
d. Weil-Felix reaction

46. Which of the following is not a features of Weil's disease: *(APPG 2014)*
a. Fever and jaundice
b. Hepatic encephalitis
c. Renal failure
d. Conjunctival hyperaemia and Skin purpura

47. Which of the following infection is transmitted by rat's urine: *(TNPG 2014)*
a. Leptospirosis b. Brucella
c. Anthrax d. Plague

EXPLANATIONS

SYPHILIS

1. **Ans (d) (Secondary syphilis)** Ref: Internet Sources

   Refer the chapter review

2. **Ans. (c) (Tertiary)** Ref: Internet source/Wikipedia

   Syphilis was referred to as "the great imitator" by Sir William Osler due to its varied presentations (Gummas, neurological and cardiac) seen in its tertiary stage.

3. **Ans. (d) (Cannot be grown)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p376

4. **Ans: (a, b) (RPR, VDRL)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 375

   Refer chapter review.

5. **Ans. (b),(c),(e) (Fusobacterium, Lactobacillus, Leptotrichia)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p371, Ananthanarayan 9/e p370
   - Spirochetes include Treponema, Borrelia and Leptospira

6. **Ans. (c) (Schaudinn...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p5, Ananthanarayan 8/e p5
   - Schaudinn and Hoffman were the first to identify Treponema pallidum.

7. **Ans. (a) (Gumma)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p374, Ananthanarayan 9/e p372, 8/e p377
   - Demonstration of T.pallidum in gumma is very rare.

8. **Ans. (a) (Font...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p376, Ananthanarayan 9/e p372, 8/e p377
   - Sliver impregnation stains like Fontana and Levaditi stains are used for T.pallidum.

9. **Ans. (d) (desensitization)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p379, Harrison 19/e p1038
   - DOC of syphilis in pregnancy: Penicillin
   - DOC of syphilis in pregnancy allergic to Penicillin: Desensitization followed by penicillin
   - For detail: Refer chapter review.

10. **Ans. (a), (b), (c), (d), (e) (Leprosy, Malaria, Relapsing fever, IV drug user and HIV infection)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p377, Ananthanarayan 9/e p374
    - ***Biological false positive (BPF)*** is defined as positive reaction obtained in nontreponemal test with negative treponemal test in absence of past or present treponemal infection.
    - For detail- refer chapter review

11. **Ans. (a) (c) (d) (Syphilis: 9 – 90 days, LGV : 3 d – 6 week, Donovanosis: 1– 4 week)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p373, Ananthanarayan 9/e p372, 421, 331, 420 468, 470, Harrison 18/e p1108

Feature	Syphilis	Herpes	Chancroid	LGV	Donovanosis
Incubation period	9–90 days	2–7 days	1–14 days	3 days–6 weeks	1–4 weeks (up to 6 months)

12. **Ans. (a) (VDRL from mother and baby)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p379, Clin. Microbiol. Rev, Larsen et al. 1995, V8/no1, p/14
    - ***Clinical clue:*** Newborn with bullous lesions on skin and a shin on knee with periostits on X-ray is pointing towards congenital syphilis.
    - Among the options: ***Only option a*** is lab method used for of syphilis. (Other option can easily be ruled out).
    - However, even 'VDRL from mother and baby' is not a satisfactory answer. Because: VDRL detects IgG antibodies, hence cannot differentiate between congenital syphilis and maternal transfer. However, there are certain guideline to for screening of congenital syphilis based on VDRL test.

13. **Ans. (c) (Chancroid, chancre, herpes genitalis)** Ref: Harrison 18/e p1108-09, 19/e pp869-75
    - ***Immediate syndrome-based treatment is recommended for the*** **usual causes** of ***acute genital ulcerations like syphilis, chancroid and herpes.***

14. **Ans. (b) (Secondary)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p377, Ananthanarayan 9/e p375
    - VDRL is 100% sensitive in Secondary syphilis.
15. **Ans. (a) (VDRL)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p376-77, Ananthanarayan 9/e p374-75
    - **History of 'present** neurological symptoms with penile ulcer 3 years back' is suggestive of tertiary Syphilis.
    - Test to monitor response to treatment for Syphilis – VDRL followed by RPR.
16. **Ans. (a), (b), (c), (d) (Painless ulcer, Painless lymphadenopathy, Covered with exudates, Indurated lesion)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p373, Ananthanarayan 8/e p373, Jawetz 24/e p333, 25/e p302
    - **Clinical features of Syphilis- Refer chapter review.**
17. **Ans. (a) (TPHA test on the serum sample of the mother)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p378, Review article- Laboratory Diagnosis and Interpretation of Tests for Syphilis, CMR 1995/V8/no 1, Harrison 18/e p1384

    TPHA test on the serum sample of the mother:
    - *Indicates mother has syphilis. But it doesn't say about the congenital syphilis. Moreover, TPHA remains elevated even after treatment. So, an adequately treated mother before 4th month of gestation has a very less risk of transmission but can still have an elevated TPHA.*

    About Other Options:
    - Since all the nontreponemal tests like VDRL mainly detects IgG, so they cannot be used to detect congenital syphilis as maternally transferred IgG will be there till 12-18 months of birth. *However, VDRL is used for screening in India, and the interpretation should be done as follows…*
    - Simultaneous VDRL test of mother and baby:
        - If mother's titer > baby's titer → Indicates maternal transfer
        - If baby's titer > mother's titer → Indicates congenital infection
    - TPHA test on the serum sample of the infant—suggestive of established congenital syphilis
    - Adequate treatment of the mother before the 16th week of pregnancy should prevent fetal damage.
18. **Ans. (c) (Syphilis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p373
19. **Ans. (b) (Dark field ...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p375, Ananthanarayan 9/e p373
    - History of unprotected sexual intercourse with a commercial sex worker followed by:
        - Painless, indurated ulcer on the glans
        - Enlarged and not tender Inguinal lymph nodes
    - Suggestive of primary Syphilis.
    - Diagnosis of primary Syphilis can be done by Dark field microscopy of ulcer discharge.
    - For further details about laboratory diagnosis of syphilis refer chapter review.
20. **Ans. (b) (VDRL)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p379, Ananthanarayan 9/e p375, 8/e p377
    - ***For monitoring treatment- VDRL is best followed by RPR***
    - *VDRL Sero- reversal occurs in primary syphilis in 4 months, in secondary and early latent syphilis in 12-18 months. In late syphilis it might take 5 years.*
21. **Ans. (d) (All)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p376-77, Ananthanarayan 9/e p374, 8/e p375

    Refer Chapter review
22. **Ans. (a)(IgM FTA – ABS)** Ref:Apurba Sastry's Essentials of Medical Microbiology 1/e p379
    - Explained earlier
    - Nontreponemal test (VDRL) detects mainly IgG, So cannot differentiate from passive maternal transfer
      Method to diagnose congenital syphilis is: A reactive IgM test specific for syphilis
    - 19s IgM FTA-ABS, Captia syphilis M test (IgM ELISA) and Western blot
23. **Ans. (a) (FTA –ABS becomes negative after treatment)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p379
    - Specific Treponemal test for syphilis remain elevated even after treatment, so cannot be used to monitor response to treatment.
24. **Ans. (d) (Late Congenital Syphilis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p374

    Hutchinson's triad, which consists of Hutchinson's teeth (notched incisors), keratitis and deafness and occurs in 63% of cases of late congenital syphilis.
25. **Ans (c) (3 days)** Ref:http://cdn.intechopen.com/pdfs-wm/23790.pdf

NONVENEREAL TREPONEMA

26. Ans. (d) (T. endemicum) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p380, Ananthanarayan 9/e p377
- Bejel or endemic syphilis is caused by Treponema endemicum

27. Ans. (a) (T. pertenue) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p380, Ananthanarayan 9/e p377
- Trepnema pertenue is the causative agent of yaws.

28. Ans. (a), (b), (T. Pertenue ,T. Carateum) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p380
T.cuniculi is pathogenic to rabbit but mostly nonpathogenic to human.

29. Ans. (b) (d) (Transmitted by fomites, Periostitis occurs) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p380
Yaws is caused by T.pertenue, transmitted by nonsexual mode such as direct or indirect contact through fomites/files feeding on to lesions.

BORRELIA

30. Ans. (c) (Transmitted...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p381, Ananthanarayan 9/e p378
- Epidemic relapsing fever is transmitted by louse, endemic relapsing fever is transmitted by tick.

31. Ans. (b) (B. burgdorferi) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p382, Ananthanarayan 9/e p378
- *Lyme's disease is caused by Borellia burgdorferi*

32. Ans. (b) (B. recurrentis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p381, Ananthanarayan 9/e p378
- Borrelia recurrentis shows antigenic variation due to DNA rearrangement in the linear plasmid of Borrelia.
- This explains the reason for relapse of fever in relapsing fever.

33. Ans. (c) (Borrel...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p381, Ananthanarayan 9/e p378, 8/e p381
- Borrelia burgdorferi is the causative agent of Lyme's disease
- Lyme's disease is transmitted by ixodid tick
- Erythema migrans (annular skin lesions) occurs in 1st stage
- Rodents act as natural hosts.

LEPTOSPIRA

34. Ans. (a) (Skin...) Ref :Apurba Sastry's Essentials of Medical Microbiology 1/e p384, Ananthanarayan 9/e p 381
Leptospira enters body via skin mucous membrane through direct contact with rodents.

35. Ans (a) (Icterohaemorrhagica) Ref: Harrison 19/e p1142, ApurbaSastry's Essentials of Medical Microbiology 1/e p384
L. interrogans serovar Icterohaemorrhagiae and Copenhageni are mostly commonly associated with Weil's disease.

36. Ans. (a) (Rat) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p384, Ananthanarayan 9/e p381, 8/e p384, 85
- Leptospira is transmitted by direct contact with the rodents

37. Ans. (a) (Leptospira) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p385, Ananthanarayan 9/e p381
- **Weil's disease is caused by-** Leptospira

38. Ans. (a) (Benzyl...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p387, Ananthanarayan 9/e p 384
The history is suggestive of Weil's disease
Points in favor:
- Sewer worker
- High grade fever, neck rigidity and signs of meningismus.
- Lab findings suggestive of renal failure and elevated liver enzymes.
- ***DOC for Weil's disease - IV Penicillin***

39. Ans. (a) (Rat is the principal animal reservoir) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p384
- Transmission is – contact with animal
- Renal and hepatic involvement is seen in 10% of the affected cases
- Penicillin: DOC in severe cases, doxycycline is DOC in milder cases.

40. Ans. (d) (Weil...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p386, Harrison 18/e p1395, 19/e pp1040-45

- Weil-Felix test is used for diagnosis of rickettsiosis.

Diagnosis of Leptospirosis- Refer chapter review.

41. Ans. (a) (Kort...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p386, Harrison 18/e p1395. 19/e p1040-45

Culture media for Leptospira- EMJH (Ellinghausen-McCullough-Johnson-Harris), Korthoff and Fletcher media

42. Ans. (a) (Leptospira) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p384, Harrison 18/e p1395

- Transmission of leptospira to humans may follow direct contact with urine, blood, or tissue from an infected animal or exposure to a contaminated environment;
- Human-to-human transmission is rare.
- *Legionella antigen detection test is done in urine sample but it spreads through inhalational route*

43. Ans. (a) (Leptospirosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p384, Harrison 18/e p1394

Leptospirosis

- More common in tropics
- During the summer (Western) and during the ***rainy season*** in the tropics
- ***Rodents***, especially rats, are the most important reservoir
- High-risk occupational exposures include: Veterinarians, ***agricultural workers***, sewage workers, slaughterhouse employees, and workers in the fishing industry

44. Ans. (b) (Weil's...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p385, Harrison 17/e p1049, 50, 18/e p1394, 94

- Farmer with high grade fever, altered sensorium and conjunctival hemorrhage with hepatic (raised bilirubin) and renal (raised creatinine) involvement is suggestive of Leptospirosis with hepatorenal syndrome (Weil's disease)
- **Clinical Stage of Leptospirosis-** Refer chapter review.

45. Ans. (b) (Microscopic...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p386, Harrison 18/e p1395

- History of fever, icterus, conjunctival suffusion and hematuria is suggestive of Leptospirosis with hepatorenal syndrome (Weil's disease)
- The Gold Standard test for Leptospirosis—Microscopic Agglutination Test

46. Ans. (b) (Hepatic encephalitis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p385, Harrison 18/e p1393-96

- Weil's disease is characterized by jaundice, acute kidney injury, hypotension, and haemorrhage (MC site lungs).
- Other syndromes include aseptic meningitis with ↑PMNs, uveitis, cholecystitis, acute abdomen, pancreatitis (with hypo- or hyperglycemia), hypotension, hepatosplenomegaly and electrolyte abnormalities (Hypokalemia, hypomagnesemia and hypermagnesemia).

47. Ans. (a) (Leptospirosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p384, Ananthanarayan 9/e p383

Refer chapter review for detail.

CHAPTER

3.11

Rickettsia, Chlamydia and Mycoplasma

RICKETTSIA

General Properties

Members of Rickettsiae possess the following common characteristics:

- They are obligate intracellular organisms
- Cannot grow on artificial media, but can grow on cell line/egg/mice inoculation (except *Bartonella*)
- Transmitted by arthropod vector (except *Coxiella*, transmitted by inhalational mode)

The order Rickettsiales comprises of genera, such as *Rickettsia, Orientia* and *Ehrlichia*

Former members, such as *Coxiella* and *Bartonella* are now excluded from the family.

Group	Species	Disease	Vector	Distribution	Rash	Eschar	LN	Weil Felix
Typhus Group	*R. prowazekii*	**E**pidemic Typhus	**L**ouse (Rubbing) Code-(**LET**)	Worldwide (Africa and South America)	80% (All over except palm and sole)	-	-	OX 19 ++++ OX 2 +/-
		Brill Zinsser						-ve or weakly +ve
	R. typhi	**En**demic Typhus	**F**lea Code- **(FEN)**	Worldwide	80% (trunk)	-	-	OX 19 ++++ OX 2 +/-
Spotted Fever Group	*R. rickettsii*	**R**MS (Rocky mountain spotted fever)	Tick Code-(**TRIA**)	America	90% (extremities and hemorrhagic)	< 1%	+	OX 19 ++ OX 2++
	R.conori	**I**ndian tick typhus (ITT)	**T**ick	Europe, Asia	97%	50%	+	
	R.africae	**A**frican Tick bite fever	Tick	Sub-Saharan Africa	50% (Vesicular)	90%	++++	
	R.akari	Rickettsial **P**ox	**M**ite(gamasid) Code- **(PSM)**	USA, Ukraine	100% (Vesicular)	90%	+++	All negative
Scrub Typhus	*Orientia tsutsuga-mushi*	**S**crub typhus	**M**ite (trombiculid)	Asia, Australia	50%	35%	+++	OX K +++

Clinical Manifestation

- Most severe form with systemic involvement: Rocky mountain spotted fever
- Mildest form: R.pox
- Eschars are seen: Rickettsial Pox, African Tick bite fever, Indian tick typhus (ITT) and Scrub typhus
- Distribution of Rash:
  - Epidemic typhus (all over, except palm and sole)
  - Endemic typhus (trunk followed by extremities)
  - RMS, Indian tick typhus (ITT) (palm and sole)
  - Vesicular/vericelliform rash: Rickettsial pox, African Tick bite fever
  - No rash: Q fever
- Q fever:
  - Acute Q fever: Fever, pulmonary feature, hepatitis
  - Chronic Q fever: Endocarditis
- CNS involvement like mental confusion and coma: Epidemic and endemic typhus
- Recrudescent illness (Brill-Zinsser disease)-*R.prowazekii*

Vectors for Rickettsiae:
- Epidemic Typhus: Louse (Rubbing)
- Endemic Typhus: Flea
- RMS and ITT: Tick
- Rickettsial Pox: Mite (gamasid)
- Scrub typhus: Mite (trombiculid)

Distribution of Rash:
- Epidemic typhus (all over, except palm and sole)
- Endemic typhus (trunk followed by extremities)
- RMS: Palm and sole
- Vesicular/vericelliform rash: Rickettsial pox, African Tick bite fever
- No rash: Q fever

Clinical Triad of Scrub typhus:
- Eschar (at the site of bite)
- Regional lymphadenopathy
- Maculopapular rash

Disease caused by Ehrlichia:
- *E.chaffeensis:* Human Monocytic Ehrlichiosis (HME)
- *E.ewingii* and *A.phagocytophilum:* Human Granulocytic Ehrlichiosis
- *N. sennetsu:* Human Lymphocytic Ehrlichiosis

- Tick and mite are transmitted by (i) bite, (ii) transovarial transmission
- Louse and flea borne rickettsiae are transmitted by:
  - Autoinoculation following rubbing the insect
  - Aerosol (by inhaling dried louse or flea feces in the laboratory or as part of bioterrorism).

Scrub Typhus

- **Agent:** *Orientia tsutsugamushi.* It differs from *Rickettsia* by both genetically as well as lacking LPS in cell wall.
- **Vector:** Trombiculid mites of genus *Leptotrombidium (L.akamushi* in Japan and *L.deliensis* in India). The larval (called **chiggers**) stage of mite are the only stage that feed on humans. Hence, scrub typhus is also called *chiggerosis*.
- **Clinical manifestations:** Classic presentation consists of *triad* of an eschar (at the site of bite), regional lymphadenopathy and maculopapular rash. However, the classical triad is seen only in 40–50% of cases.
- **Antigenic diversity:** Three major antigenic types have been identified: *Karp, Gilliam* and *Kato*. Because of this remarkable antigenic diversity exhibited by the organism, immunity wanes over 1–3 years.
- **Zoonotic tetrad:** Four elements are essential to maintain *O. tsutsugamushi* in nature:
  - Trombiculid mites
  - Small mammals (e.g. field mice, rats, shrews)
  - Secondary scrub vegetations or forests (hence named as scrub typhus)
  - Wet season (when mites lay eggs).
- **Global Scenario:** Scrub typhus is prevalent in Japan, China, Philippines, and South East Asia including India, Pakistan, Afghanistan, tropical Australia, New Guinea, and Pacific Islands.
- **Indian scenario:** Scrub typhus is the moist common rickettsial disease found in India.
- **Diagnosis** by Weil Felix test (↑OXK titer).

Ehrlichiosis

Family Anaplasmataceae comprises of four obligatory intracellular organisms named *Ehrlichia, Wolbachia, Anaplasma,* and *Neorickettsia*

	Ehrlichia chaffeensis	***Ehrlichia ewingii***	***Anaplasma phagocytophilum***	***Neorickettsia sennetsu***
Causes	Human Monocytic Ehrlichiosis (HME)	Human Granulocytic Ehrlichiosis	Human Granulocytic Anaplasmosis	Human Lymphocytic Ehrlichiosis
Feature	Leucopenia Thrombocytopenia Elevated liver enzymes *Risk factor-* ↓Immunity	Features similar to HME but less severe *Risk factor-* ↓Immunity	Leucopenia Thrombocytopenia	Mononucleosis like illness Atypical lymphocytosis Lymphadenopathy
Transmitted by	Tick (*Amblyomma americanum*)	Tick (*Amblyomma americanum*)	Tick (*Ixodes scapularis*)	Ingestion of fish carrying infected flukes
Reservoir	White-tailed deer (rarely dogs)	White tailed deer and dogs	Mice, squirrels, and white-tailed deer	? (not known)
Distribution	USA	USA	USA	Japan and Malaysia

Section 3

Common to all three Species:

- Obligate intracellular parasite, Cannot be cultivated in artificial media
- Grow in cluster inside the phagosome as mulberry like inclusions called as MORULA
- DOC: Doxycycline.

Weil Felix Results:
- In epidemic and endemic typhus: ↑OX 19 Ab
- In tickborne spotted fever: ↑OX 19 and OX 2 Ab
- In scrub typhus: ↑OX K Ab
- Negative in rickettsial pox, Q fever, ehrlichiosis and bartonellosis

Laboratory Diagnosis of Rickettsiosis

Weil Felix Test

It is heterophile agglutination test; where rickettsial antibodies are detected by using certain *Proteus* strains (OX 19, OX 2 and OX K strains) due to sharing cross reactive alkali stable LPS antigen.

- **Procedure:** It is a tube agglutination test; serial dilutions of patient's serum are treated with nonmotile strains of *P.vulgaris* OX 19, and OX 2 and *P.mirabilis* OX K.
- **Results:**
  - In epidemic and endemic typhus- Sera agglutinate mainly with OX 19 and sometimes with OX 2.
  - In tickborne spotted fever-Antibodies to both OX 19 and OX 2 are elevated.
  - In scrub typhus- Antibodies to OX K are raised.
  - The test is negative in rickettsial pox, Q fever, ehrlichiosis and bartonellosis.
- **False positive titer** may be seen in presence of underlying *Proteus* infection. Hence fourfold rise of antibody titer in paired sera is more meaningful than a single high titer.
- **False negative result** may occur due to excess antibodies in patient's sera (*prozone* phenomena). This can be obviated by testing with serial dilutions of patient's sera.
- Weil Felix test being a nonspecific test should always be confirmed by specific tests.

Specific Antibody Detection Tests

- Indirect immunofluorescence assay: It is the most common serologic test used for confirmation of diagnosis
- CFT (specific, but less sensitive)
- IgM capture ELISA: It is useful in early diagnosis (< 1 week) with excellent sensitivity and specificity
- Latex agglutination test.

Neil Mooser reaction:
Positive tunica reaction (testicular inflammation) seen in
- *R. conori*
- *R. akari*
- *R. typhi*

Other Methods of Diagnosis Include

- **Histological examination** of a cutaneous biopsy sample from a rash lesion can be done even during acute illness.
- **Isolation:** Rickettsiae cannot be cultivated in cell free media. Isolation can be done by cell lines (Vero, primary chick embryo, WI-38, HeLa), egg (yolk sac inoculation), or animal inoculation (guinea pig).
- **Neil Mooser reaction:** Specimens are inoculated intraperitoneally into male guinea pigs. The changes observed in the animal (over 3–4 weeks), varies among various rickettsial species.
  - *R.rickettsii*: Produces scrotal necrosis
  - *R.prowazekii*: Produces only fever without any testicular inflammation
  - *R.**c**onori, R.**a**kari* and *R.**t**yphi, (code-CAT)*: Produce fever and *positive tunica reaction* (testicular inflammation).
- PCR is available targeting *16SrRNA* gene or *OMP* genes.

Treatment of Rickettsiosis

Doxycycline is the drug of choice for treatment of most rickettsial illnesses. Chloramphenicol is used as alternative.

Bartonellosis

Bartonella species are fastidious, intracellular, gram-negative bacteria that have ability to invade RBCs. They differ from other rickettsiae being capable of growing on blood agar.

Bartonella	Diseases	Reservoir	Transmission
B.henselae	**Cat-scratch disease:** • Typical from (90%): LN↑ (MC site- axillary/ epitrochlear) and painless pustule • Atypical from (10%): Involving various organs • Warthin-Starry sliver nitrate staining **Bacillary angiomatosis:** Neovascular lesions in skin and other organs, seen in HIV infected people **Others**: Bacillary peliosis, bacteremia, endocarditis	Cats, Other felines	Exposure to cat – by scratch or bite Cat fleas associated with cat-to-cat transmission, but not cat-to-human transmission
B.quintana	Trench fever: (quintanor 5 days fever) Chronic bacteremia, endocarditis Bacillary angiomatosis	Humans	Louse (*Pediculus humanus corporis*)
B.bacilliformis	Carrion's disease or Oroya fever: systemic illness Verruga peruana-cutaneous vascular lesions	Humans	Sand fly (*Lutzomyia verrucarum*)

Bartonella Infections:
- *B.henselae:* Cat-scratch disease
- *B.quintana:*
  - Trench fever: (quintan or 5 days fever)
  - Chronic bacteremia, endocarditis
  - Bacillary angiomatosis
- *B.bacilliformis*
  - Carrion's disease or Oroya fever
  - Verruga peruana

Treatment of Bartonella Infections

- Typical Cat scratch diseases: Azithromycin
- Atypical Cat scratch diseases: Doxycycline
- Trench fever: Gentamicin + Doxycycline
- Bacillary angiomatosis peliosis: Erythromycin
- Oroya fever: Chloramphenicol or Ciprofloxacin
- Verruga peruana: Rifampin or Streptomycin.

CHLAMYDIA

General Properties

- Obligate intracellular gram-negative bacteria.
- Cannot grow on artificial media, but can grow on cell line/egg/mice inoculation
- They are filterable and produce inclusion bodies like viruses.
- But they differ from viruses being possessing both RNA and DNA.
- Possess modified peptidoglycans.
- Cannot produce their own ATP: They are called Energy parasite as they depend on host cell ATP.
- Shows tropism for squamous epithelium and LN.
- Life cycle: They exist in two distinct morphological forms: Elementary body (EB) and Reticulate body.

Elementary body	Reticulate body
Extracellular form	Intracellular form
Infectious form	Replicating form
Metabolically inactive	Metabolically active
Rigid cell wall	Fragile cell wall
Small size (0.20–0.30 µm)	Large size (1–1.5 µm)
Nucleoid is electron dense	Nucleoid is diffuse
DNA and RNA contents are same	RNA content is more than DNA

Species	Character	Biovar	Serotype	Disease
Chlamydia trachomatis	Forms compact inclusions mixed with glycogen matrix, Sensitive to sulphonamide, Natural human pathogen, Leave the host cell with a scar	TRIC	A, B, Ba, C	Trachoma
			D-K	Genital chlamydiasis Inclusion conjunctivitis Infant pneumonia
		LGV	L1,L2,L3	Lymphogranuloma venereum
Chlamydophila psittaci	Forms diffuse vacuolated inclusions without glycogen, Resistant to sulphonamide, Natural pathogen of birds, Leave the host cell by lysis	Nil	Many serotypes	Psittacosis (Atypical pneumonia) Transmission-Inhalational route - pet birds (parrots) and poultry (turkeys and ducks) No man to man transmission
Chlamydophila pneumoniae TWAR agent	Exclusive human pathogen, Forms inclusions without glycogen matrix, Resistant to sulfonamide	Nil	Only 1 serotype	Community acquired atypical pneumonia Associated with Atherosclerosis, and asthma

Chlamydia Trachomatis

C. trachomatisis the MC cause of:

- STD, nongonococcal urethritis (NGU) and post-gonococcal urethritis (PGU)
- Pelvic Inflammatory Disease (PID) and acute epididymitis
- Inclusion conjunctivitis: Swimming pool conjunctivitis (adult) and ophthalmic neonatorum or inclusion blennorrhea (neonate).

Complications:

- Reiter syndrome
  - Characterized by: CUP (conjunctivitis + urethritis + polyarthritis) and mucocutaneous lesions
  - Associated with people with HLAB27.
  - MC cause of peripheral inflammatory arthritis in young men, MC site: Large joints of the legs
- Fitz Hugh Curtis syndrome: Perihepatitis in, sexually active women
- Urethral Syndrome in Women: Dysuria and frequency, urethritis, pyuria, and no bacteriuria.

Reiter syndrome:
- Characterized by: CUP (conjunctivitis + urethritis + polyarthritis) and mucocutaneous lesions
- Associated with HLAB27

Trachoma

- Chronic conjunctivitis: (follicular hypertrophy + papillary hyperplasia + pannus + cicatrization)
- Stages: Trachoma dubium, protrachoma, established trachoma I –IV
- Inclusion body (HP- Halberstaedter - Prowazek) seen only in, established trachoma stages I–IV.

LGV (Lymphogranuloma Venereum)

LGV is an invasive STD, caused by *C.trachomatis* Serovar L1, L2, and L3, characterized by:

- MC Serotype by L2 > L1, L3
- LGV serovars are more invasive than others
- Incidence is falling, Male: female: 3.4:1,
- Clinical feature:
  - Pain less ulcer + painful lymph nodes ↑(enlarged inguinal LN called **bubo**),
  - Esthiomone (elephantiasis of vulva) rectal stricture, proctitis
- Skin test positive → Frie test.

LGV (Clinical feature:
- Pain less ulcer + painful lymph nodes or bubo,
- Esthiomone (elephantiasis of vulva) rectal stricture, proctitis

Laboratory Diagnosis of Chlamydial Infection

- **Microscopy:**
  - Gram staining: Often reveals sterile pyuria (↑ neutrophils, no organisms) as they are poorly gram-negative.

Chlamydial Inclusion bodies:
- LCL body (Levinthal-Cole-Lillie) body: Psittacosis
- Miyagawa corpuscle: LGV
- HP (Halberstaedter: Prowazek) body- trachoma

  - Other stains, such as Castaneda, Machiavello or Gimenez stains are better methods to detect chlamydiae from samples. The inclusion bodies can also be detected in cytoplasm; which are known by different names:
    - LCL body (Levinthal-Cole-Lillie) body: Psittacosis
    - Miyagawa corpuscle: LGV
    - HP (Halberstaedter – Prowazek) body: trachoma
  - Lugol's I2- used only for *C. trachomatis* (stains the glycogen inclusion body)
- **Direct immunofluorescence test (DIF)** for direct detection of inclusion bodies in clinical material.
- **Antigen detection:** Enzyme Immunoassays detects chlamydial group specific antigens (LPS).
- **Culture:**
  - Mice inoculation (infective by only *C.psittaci* and LGV biovars)
  - Yolk sac inoculation
  - Cell culture inoculation
    - McCoy Cells and HeLa
    - HEp2 for *C.pneumoniae*
- **Serology:**
  - Micro-IF test: It is the serological test of choice, detects serovar specific antibody by using outer membrane protein (OMP) antigen
  - CFT (genus specific) detects Genus specific antibody by using LPS antigen
  - High antibody titer seen in LGV, infant pneumonia, salpingitis
- **Nucleic acid amplification tests** (NAATs), i.e. PCR: Diagnosis of choice, most sensitive and specific assay, almost replacing, the so called gold standard culture.

Chlamydia Cell line Culture:
- McCoy Cells and HeLa-C.trachomatis
- HEp2 for C.pneumoniae

Chlamydial Serology:
- Micro-IF test: Detects serovar specific antibody by using OMP antigen
- CFT (genus specific):detects genus specific antibody by using LPS antigen

Treatment of Chlamydial Infections

- *C. trachomatis*: Azithromycin (1 gram single dose) is the overall DOC except for:
  - For complicated genital infection: Doxycycline or erythromycin are DOC.
  - For neonatal infections (Ophthalmianeonatorum and infant pneumonia): Erythromycin is DOC.
- *C. psittaci*: Tetracycline > Erythromycin for 7–14 days.
- *C. pneumoniae*: Tetracycline or erythromycin for 14 days.

Non Gonococcal Urethritis (NGU)- Refer chapter 3.3

DOC of Chlamydial Infections:
- *C. trachomatis:* Azithromycin
- *C. psittaci:* Tetracycline
- *C. pneumoniae:* Tetracycline

MYCOPLASMA

Mycoplasma are the smallest free living organism known.

General Properties

- Filterable (Hence known as **Eaton's agent**)
- Formerly called PPLO- Pleuro pneumonia like organism.
- Lack rigid cell wall. Peptidoglycan layer is absent; replaced by cholesterol.
- Hence they are resistance to cell wall active antibiotics like beta lactams.

Mycoplasma is also called:
- Eaton's agent
- PPLO: Pleuro pneumonia like organism

Clinical Feature

- Incubation period is 2–4 weeks , person to person spread by respiratory droplets.
- MC manifestation-upper respiratory illness
- MC cause of community acquired atypical pneumonia in adults.
- Pneumonia is called **Primary atypical pneumonia** (PAP) or 'walking' pneumonia or **Eaton agent pneumonia**
- Extrapulmonary Manifestations-neurologic, dermatologic, cardiac, rheumatologic, and hematologic.

Note: *Ureoplasma urealyticum* cause NGU, epididymitis, vaginitis and cervicitis.

Laboratory Diagnosis

- Poorly gram-negative, shows pleomorphism, resemble like L forms
- Staining with Dienes stain: Block of agar containing *Mycoplasma* colony added to methylene blue is observed under microscope.
- Show gliding mobility (however, they lack flagella, pili)
- Culture medium: PPLO broth and PPLO agar
- Produces **Fried egg** colonies.
- Antigen detection by Direct Immunofluorescence test
- PCR: More sensitive while culture is more specific.
- ***Detection of Antibody***:
  - Heterophile antibody:
    - Cold agglutination test: Detects *Mycoplasma* antibody by using human 'O' RBC antigen.
    - *Streptococcus* MG test: Detects *Mycoplasma* antibody by using *Streptococcus* MG antigen.
  - Specific antibody:
    - CFT (complement fixation test)
    - ELISA for IgM, IgG and IgA detection
- The combination of PCR for respiratory tract secretions and serologic testing constitutes the most sensitive and rapid approach to the diagnosis of *M. pneumoniae* infection.

M.pneumoniae causes Primary atypical pneumonia, also called walking pneumonia or Eaton agent pneumonia

Heterophile antibody detection in Mycoplasma Infections:
- Cold agglutination test: detects antibody by using human 'O' RBC antigen.
- *Streptococcus MG* test: detects antibody by using *Streptococcus MG* antigen

Treatment

- *Mycoplasma pneumoniae* and *Ureoplasma urealyticum*: DOC—Azithromycin
- *M. hominis:* DOC—Doxycycline

Treatment of Mycoplasma Infections:
- *M. pneumoniae* and *Ureoplasma:* DOC: Azithromycin
- *M. hominis* – DOC: Doxycycline

Characters	Chlamydia	Rickettsia	Mycoplasma
Obligate Intracellular	Yes	Yes	No, free living
Make ATP	No ATP	Limited ATP	Normal ATP
Peptidoglycan in cell wall	Modified	Normal	Absent
Growth on artificial media	No	No	Yes

MULTIPLE CHOICE QUESTIONS

RICKETTSIA

1. Which are/is serologically diagnosed? *(PGI May 2016)*
a. Typhoid
b. Q fever
c. Gonorrhea
d. Actinomyces
e. Scrub typhus

2. Which of the following statement is True: *(Recent Question 2015)*
a. Scrub typhus-Weil Felix test negative
b. Neil Mooser reaction: RMSF shows scrotal necrosis
c. Only Proteus vulgaris antigens are used in Weil Felix test

3. A patient is presented with rashes all over body sparing palm and soles. He does not have h/o of animal exposure. This condition may be associated with which of the following rickettsial infection? *(JIPMER Nov 2015)*
a. Epidemic typhus
b. Q fever
c. RMSF
d. Rickettsial pox

4. Which one is rickettsial disease? *(Recent Question 2015)*
a. Weil's disease
b. Rocky mountain fever
c. Relapsing fever

5. Brill Zinsser disease- True statement is: *(Recent Question 2015)*
a. Latent period < 1 month
b. Severe form of recrudescence
c. Mild illness, for several months

6. Which is transmitted by louse: *(PGI Nov 2012, PGI June 2011)*
a. Endemic typhus
b. Scrub typhus
c. Trench fever
d. Q fever
e. Relapsing fever

7. Well felix reaction for Scrub typhus shows positivity for: *(NEET Pattern Based)*
a. OXK
b. OXK & OXI9
c. OX-2
d. OX-19

8. All are true about scrub typhus except? *(AI 2010)*
a. Mite is vector
b. Adult mite feeds on vertebral host
c. Caused by Orientia tsutsugamushi
d. Tetracycline is DOC

9. Which of the following gives positive test with both Weil Felix) OX 2 and OX 19? *(DNB Dec 2011)*
a. Spotted fever
b. Scrub typhus
c. Epidemic typhus
d. None of the above

10. Neil Mosser reaction or tunica reaction is useful to differentiate between: *(DNB Dec 2010)*
a. R. prowazekii and R. typhi
b. R. typhi and R. rickettsii
c. R. prowazekii and R. rickettssii
d. R. rickettsia and C. burnetti

11. Q fever is caused by: *(MHPG 2015, DNB June 2010)*
a. Rickettsia typhi
b. Coxiella burnettii
c. Salmonella
d. Escherichia coli

12. Mite is a vector for: *(PGI June 2008)*
a. R. typhi
b. R.prowazeki
c. R. rickettsii
d. R. tsutsugamushi
e. R.conori

13. Scrub typhus transmitted by: *(AIIMS Nov 2007), DNB 2006, RJ 2000)*
a. Reduviid bug
b. Trombiculid mite
c. Enteric pathogens
d. Cyclops

14. Which of the following species belong(s) to the genus Rickettsia? *(PGI Dec 2007)*
a. Rickettsia tsutsugamushi
b. Rochalimaea quintana
c. Rickettsia prowazekii
d. Rickettsia typhi

15. An army jawan posted in remote forest area following a tick bite had fever and headache. His fever was 104°F and pulse was 70 per min. He had an erythematous lesion of about 1 cm on the leg surrounded by small vesicles, along with generalized lymphadenopathy at the time of presentation to the referral hospital. His blood sample was collected to perform serology for the diagnosis of Rickettsial disease. Which one of the following results in Weil-felix reaction will be diagnostic in this clinical setting: *(AI 2006, AI 2005)*
a. High OX -2
b. High OX -19
c. High OX – K
d. High OX – 19 and OX – 2

Section 3

16. The following is the etiological agent of Rocky mountain spotted fever: *(AIIMS May 2005)*
a. Rickettsia rickettsii
b. Rochalimaea quintana
c. Rickettsia tsutsugamushi
d. Coxiella burnetii

17. All of the following statement are true regarding Q fever except: *(AI 2003, AIIMS May 2003, SGPGI 2005)*
a. It is zoonotic disease
b. Human disease is characterized by an interstitial pneumonia
c. No rash is seen
d. Weil–Felix reaction is very useful for diagnosis

18. A man with very high fever shows strongly positive agglutination test with OXK antigen. The most likely diagnosis is: *(AI 2000)*
a. Tsutsugamushi fever b. Trench fever
c. Undulant fever d. Relapsing fever

19. Not true about scrub typhus: *(PGI Nov 2012)*
a. Chigger-borne
b. Vector is mite
c. Caused by Orientiatsut sugamushi
d. Chlamydial disease
e. Has 3 serotypes

20. Which is obligate intracellular? *(AIIMS MAY 2016, AIIMS Nov 2016)*
a. Coxiella burnetti
b. Ehrlichia chaffeensis
c. Bartonella henselae
d. Tropheryma whippelii

21. Morula in RBC seen in: *(Recent Question 2015)*
a. Babesia
b. Ehrlichia
c. Spirochete

BARTONELLOSIS

22. Drug of choice for Bacillary angiomatosis?
a. Macrolides *(Recent Questions 2014)*
b. Aminogylcosides
c. Cephalosporins
d. Carbapenems

23. Bartonella henselae causes all except:
a. Oroya fever *(NEET Pattern Based)*
b. Cat scratch disease
c. Bacillary angiomatosis
d. SABE

24. All are true about B. quintana except: *(NEET Pattern Based)*
a. Causes trench fever
b. Not detected by Weil felix reaction
c. Recurrence is common
d. Tick is the vector

25. Bacillary Angiomatosis occur due to: *(PGI May 2012)*
a. Mycoplasma
b. Gardenella
c. Bartonella bacilliformis
d. Hemophilus influenzae
e. No relation with any above mentioned

26. Cat scratch disease is: *(PGI 2000)*
a. Associated with positive Frie skin test
b. Caused by a DNA virus
c. Associated with a pathognomonic histological picture
d. Associated with regional lymphadenopathy
e. Associated with a positive Weil felix test

27. A patient has presented with a macule in hand followed by axillary lymphadenopathy. What will you ask in the history to the patient?
a. History of exposure to rat *(Recent Question 2013)*
b. History of exposure to cat
c. History of similar complain in family members

28. Ehrlichia chaffeensis is causative agent of: *(NEET Pattern Based)*
a. HME
b. HGE
c. Glandular fever
d. None

29. Verruga peruana caused by: *(PGI Nov 2014)*
a. Bartonellabacilliformis
b. Bartonellaquintana
c. Bartonellahensale

30 Oroya fever is caused by: *(PGI Nov 2014)*
a. Bartonellahenselae
b. Bartonellabaciliformis
c. Bartonellaquintana

CHLAMYDIA

31. Which one of the following is implicated in the etiology of Fitz-Hugh-Curtis syndrome? *(APPG 2015)*
a. Chlamydial infection
b. Gastric perforation
c. Perforation of dermoid cyst
d. Liver metastases in ovarian cancer

32. True about chlamydia are all except: *(NEET Pattern Based)*
a. Obligate intracellular organism
b. Gram-positive
c. Reticulate body is metabolically active
d. Replicated by biniary fission

33. Inclusion body present in psittacosis is called: *(Recent Question 2015)*
a. HP body
b. Miyagawa corpuscles
c. Levinthal cole Lillie

34. In a patient with urethral syndrome, urine microscopy shows full of polymorph, but no bacteria. The most appropriate culture medium is? *(AIIMS May 2012, Nov 2011, 2006)*
a. McCoy cell
b. Thayer martin *(AI 2012)*
c. Cooked meat
d. PPLO broth

35. Chlamydia escape killing by: *(DNB June 2011)*
a. Causes cell membrane perforation
b. Produces factors that Camouflage it
c. Molecular mimicry
d. Inhibit phagolysosome fusion

36. Which of the following is true about Chlamydia trachomatis? *(AI 2011)*
a. It is usually symptomatic
b. It is routinely treated with penicillin
c. It is diagnosed by culture of cervical purulent discharge
d. Women using oral pills are more prone for infection

37. Chlaymydia in asymptomatic carriers, the most sensitive test is: *(AIIMS Nov 2009, AI 2004)*
a. Tissue culture
b. Nucleic acid amplification test
c. Serology
d. Serum electrophoresis

38. Chlamydia psittacosis all are true except: *(AI 2007)*
a. Acquired from bird's droppings
b. Causes urethritis
c. Causes pneumonia
d. Treatment is tetracycline

39. Triad of Reiter's syndrome: *(PGI Dec 2007)*
a. Conjunctivitis
b. Uveitis
c. Mucosal lesions
d. Glaucoma

40. Chlamydia trachomatis false is: *(AIIMS Nov 2006, AI 2007)*
a. Elementary body is metabolically active
b. It is biphasic
c. Reticulate body divides by binary fission
d. Inside the cell it evades phagolysozome fusion

41. Which one of the following statements is true regarding Chlamydia pneumoniae: *(AI 2005, AIIMS May 2005)*
a. Fifteen serovars have been identified as human pathogens
b. Mode of transmission is by the airborne bird excreta
c. The Cytoplasmic inclusions present in the sputum specimen are rich in glycogen
d. The group specific antigen is responsible for the production of complement fixing antibodies

42. Chlamydia trachomatis is associated with the following except: *(AI 2005)*
a. Endemic trachoma
b. Inclusion conjunctivitis
c. Lymphogranuloma venereum
d. Community acquired pneumonia

43. The following is not a method of isolation of Chlamydia from clinical specimens: *(AIIMS Nov 2005)*
a. Yolk sac inoculation
b. Enzyme immunoassay
c. Tissue culture using irradiated McCoy cells
d. Tissue culture using irradiated BHK cells

44. Chlamydia grows in which of the following cell lines:
a. HeLa *(PGI Dec 2001)*
b. HeP2
c. McCoy
d. Human diploid fibroblast series
e. Vero cells

45. Frie test is used for: *(Recent Questions 2014, JIPMER 2014, 2012)*
a. LGV
b. Syphilis
c. Gonorrhea
d. Chancroid

46. A 32-year-female is presented with vaginal discharge resembling chlamydial urethritis. Most sensitive test for diagnosing this condition is: *(AIIMS Nov 2014)*
a. Nucleic acid amplification test
b. Gram stain
c. Culture on McCoy cell line
d. Serum antibody detection by MIF

47. DOC of Chlamydia in pregnancy?
a. Tetracycline *(Recent Question 2013)*
b. Doxycycline
c. Amoxycillin
d. Metronidazole

48. Which of the Chlamydia species is having one serotype? *(Recent Question 2013)*
a. Chlamydia psittaccii
b. Chlamydia trachomatis
c. Chlamydia pneumophilla
d. All of the above

MYCOPLASMA

49. Diene's method is used for: *(NEET Pattern Based)*
a. Mycoplasma
b. Chlamydiae
c. Plague
d. Diphtheria

50. Ureaplasma is naturally resistant to: *(NEET Pattern Based)*
a. Erythromycin
b. Chloramphenicol
c. Cephalosporins
d. Tetracyclines

51. Which is Eaton agent? *(JIPMER 2011)*
a. Mycoplasma
b. H.influenzae
c. Klebsiella
d. Chlamydia pneumoniae

52. In reference to the Mycoplasma, the following are true except: *(AIIMS May 2005)*
a. They are inhibited by penicillin
b. They can reproduce in cell free media
c. They have an affinity for mammalian cell membranes
d. They can pass through filters of 450 nm pore size

53. DOC for Mycoplasma pneumoniae: *(West Bengal 2016)*
a. Azithromycin
b. Amoxyclav
c. Doxycycline

EXPLANATIONS

RICKETTSIA

1. **Ans. (a,b,e) (Typhoid, Q fever, Scrub typhus)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p321,393,394
   Serological diagnosis of antibody detection is useful both for Typhoid, Q fever and also scrub typhus by Weil felix and ELISA. Serology is not useful for gonorrhea and Actinomyces.

2. **Ans. (b) (Neil Mooser...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p393

3. **Ans (a) (Epidemic typhus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p389
   Distribution of rashes in rickettsial infection: Refer Chapter review

4. **Ans. (b) (Rocky mountain..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p389

5. **Ans. (c) (Mild illness..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p389
   It is a mild recrudescent illness occurring years after acute epidemic typhus.

6. **Ans: (c), (e) (trench fever, Relapsing fever)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p315
   Louse borne diseases are: Epidemic typhus, Epidemic Relapsing fever, Trench fever and Pediculosis.

7. **Ans. (a) (OXK)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 389, Ananthanarayan 9/e p408
   - In Scrub typhus, Well Felix reaction shows a raise in OXK titer.

8. **Ans. (b) (Adult...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 392, Ananthanarayan 9/e p408
   - Mite is vector for scrub **typhus**
   - Larval form of mite, e.g. **chiggers feed on man, other forms of mite including adult form do not feed man.**
   - Scrub typhus Caused by **Orientia tsutsugamushi**
   - Tetracycline is DOC.

9. **Ans. (a) (Spotted.)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 389, Ananthanarayan 9/e p410, 8/e p410
   Weil Felix reaction:
   - Antibodies to OX19: Epidemic and endemic typhus
   - Antibodies to OX 2 and OX19: Rocky mountain spotted fever
   - Antibodies to OX K: Scrub typhus.

10. **Ans. (a) (R. pr...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 393, Ananthanarayan 9/e p409, 8/e p409
    - **Neilmooser reaction is done to differentiate epidemic typhus** R. prowazekii which gives a negative tunica reaction and **endemic typhus** (R. typhi) which gives a positive tunica reaction.
    - ***For detail refer chapter review***

11. **Ans. (b) (Coxiella burnetii)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 394, Ananthanarayan 9/e p411, 8/e p410
    - Q fever is caused by Coxiella burnetii.

12. **Ans. (d) (R. tsu...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 392, Harrison 19/e p1155, 18/e p1408
    - Mite transmits: Rickettsial Pox and Scrub Typhus
    - Agent for Rickettsial Pox: R.akari
    - Agent for Scrub Typhus: Orientia tsutsugamushi (old name- R. tsutsugamushi)

13. **Ans. (b) (Trom...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 392, Harrison 19/e p1155 , 18/e p1408
    - Rickettsial Pox: Vector is gamasid mite
    - Scrub Typhus: Vector is Trombiculid mite

14. **Ans. (a), (c), (d) (Rickettsia tsutsugamushi, Rickettsia prowazekii, Rickettsia typhi)**
    Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 389, Harrison 119/e p1155-59, 8/e p1407, 08
    - Rickettsia are characterized by:
      - Obligate intracellular parasite

- Cannot be cultivated in artificial cell free media
- Transmitted by arthropods

- **Bartonella quintana (R.quintana)** (can be cultured in blood agar) and **Coxiella** (No vector, but transmitted by droplet) are recently excluded in Rickettsia …….. Ananthanarayan 9/e p405,8/e p405
- R. Quintana is the old name of Bartonella Quintana

15. **Ans. (d) (High...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 389, Harrison 19/e p1155-59
    - History states: Tick bite followed by development of fever, headache, erythematous lesion surrounded by small vesicles and lymphadenopathy…… Its cases of African tick Typhus
    - Vesicular rash seen in Rickettsial Pox and African tick Typhus
    - Rickettsial Pox Weil Felix reaction is –ve
    - In African tick Typhus Weil Felix reaction shows High OX-19 and OX2
    - ***Weil Felix is negative for Q*** fever, R.pox, Ehrlichia, Bartonella.
16. **Ans. (a) (Rickettsia rickettsii)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 389, Harrison 19/e p1155-59, 18/e p1408, 12
    Already explained.
17. **Ans. (d) (Weil–Felix reaction is very useful for diagnosis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 393, Harrison 18/e p1408, 12
    Weil-Felix reaction is not useful for Q fever, R.pox, Ehrlichia, Bartonella.
18. **Ans. (a) (Tsutsugamushi fever)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 392, Harrison 18/e p1413
    - For details refer chapter review.
19. **Ans. (d), (Chlamydial...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 392, Ananthnarayan 9/e p408
    - Scrub typhus is rickettsial disease, caused by Orientiatsutsugamushi which has three major antigenic types: Karp, Gilliam and Kato.
    - Scrub typhus is transmitted by larval form of trombiculid mite called chiggers.
20. **Ans: (b) (Ehrlichia chaffeensis)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p392, Harrison 19th/p 1078,1154-9
    - Ehrlichioses are obligately intracellular organisms comprised by four genera: Ehrlichia, Anaplasma, Wolbachia, and Neorickettsia.'
    - The location of bacterial rRNA in tissues from patients with Whipple's disease provides evidence that bacteria are growing outside cells and suggests that T. whippelii is not an obligate intracellular pathogen. http://www.ncbi.nlm.nih.gov/pubmed/11262205
    - Bartonella species are fastidious, facultative intracellular, slow-growing, gram-negative bacteria.
    - Though rickettsiae are obligately intracellular gram-negative coccobacilli, Coxiella burnetii Rickettsia prowazekii and R. typhi have the well- documented ability to survive for an extended period outside the reservoir or vector--------- Harrison 19th/p1154
21. **Ans. (b) (Ehrlichia)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p393

BARTONELLOSIS

22. **Ans. (a) (Macrolides)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p396 , Harrison's 18/e table 160-1
    DOC for Bacillary angiomatosis is erythromycin or doxycycline
23. **Ans. (a) (Oroy...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 395, Harrison 19/e p1078-79 , 18/e p1314
    - *B. henselae* is associated with Cat-scratch disease, bacillary angiomatosis, bacillary peliosis, bacteremia, endocarditis
24. **Ans. (d) (Tick is...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 396, Harrison 19/e p1078-79, 18/e p1314
    - Vector for B.quintana- Louse
    - Trench Fever is often periodic and recurrent, caused by B.quintana
25. **Ans. (e), (No...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 396, Harrison , 18/e p1318
    - *Bacillary angiomatosis is a disease of severely immunocompromised patients, is caused by B. henselae or B. quintana.*
    - *Characterized by neovascular proliferative lesions of skin and other organs.*

26. Ans. (c), (d) (Associated with a pathognomonic histological picture, Associated with regional lymphadenopathy)
Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 396, Harrison 18/e p1314, 15, 17/e p 987
Frie skin test is positive for LGV
Warthin-Starry stains of biopsy/aspirated LN tissue reveal typical clusters of pleomorphic gram-negative organisms within areas of necrosis, blood vessel walls, or erythrocytes.

27. Ans. (b) (History of...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 396, Ananthanarayan 9/e p413
- Patient with a macule in hand followed by axillary lymphadenopathy. More suggestive of Cat scratch disease, hence we should ask first about the history of exposure to cat.

28. Ans. (a) (HME) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 392, Ananthanarayan 9/e p409, 8/e p409
Refer chapter review

29. Ans. (a) (B. bacilliformis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 395, Ananthnarayan 9th/p412
VerrugaPeruana or Peruvian Wart is caused by Bartonella baciliformis, characterized by cutaneous rashes produced by a proliferation of endothelial cells.

30. Ans. (b) (B. bacilliformis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 395, Ananthanarayan 9th/p412
- Bartonella baciliformis causes Oroya fever or Carrion's disease.

CHLAMYDIA

31. Ans (a) (Chlamydial infection) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p400
Fitz–Hugh–Curtis syndrome (perihepatitis) is a complication seen in Chlamydial and gonococcal infections.

32. Ans. (b) (Gram-positive) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 397, Ananthanarayan 9/e p418
- Chlamydia is poorly gram-negative.

33. Ans. (c) (Levinthal cole Lillie) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p402.

34. Ans. (a) (McCoy cell) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 402, Bailey and Scott's Diagnostic Microbiology 12/e p846, Ananthanarayan 9/e p418, 8/e p418
- *Chlamydia trachomatis is by far the most common cause of Urethral syndrome. So 1st attempt should be made for the isolation of Chlamydia trachomatis.*
- *McCoy and HeLa cell lines are used for the isolation of Chlamydia trachomatis.*

Among the options:
- McCoy cell: Cell line used for Chlamydia…… *answer*
- Thayer martin: Culture media used for Gonococcus
- Cooked meat: Culture media used for anaerobic culture
- PPLO broth: Culture media used for Mycoplasma

35. Ans. (d) (Inhibit...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 398, Harrison 19/e p1165-73, 18/e p1422
- The Elementary Body is the infective form which enters the cell by receptor-mediated endocytosis and resides in an inclusion, where the entire growth cycle is completed. The chlamydiae prevent phagosome-lysosome fusion by which it can survive in the intracellular environment.

36. Ans. (d) (Women using oral pills are more prone for infection) Ref: Harrison 19/e p1165-73 , 18/e p1422
'Use of oral contraceptive pills and the presence of cervical ectopy also confer an increased risk of chlamydial infection.'
About Other Options:
- Chlamydial infections are usually asymptomatic.
- 'The proportion of infections that are asymptomatic appears to be higher for C. Trachomatis than for N. gonorrhoeae, and symptomatic C. Trachomatis infections are clinically less severe'.
- It is routinely treated with Single dose Azithromycin
- It is diagnosed by isolation in McCoy and HeLa cell line. But they vary in their infectivity to cell lines.

37. Ans. (b) (Nucleic acid amplification test) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 403, Harrison 19/e p1165-73 , 18/e p1426, Table 169.1
- Nucleic acid amplification test (NAAT) is the Confirmatory Test of Choice for all type of C.trachomatis infection
- Diagnosis of C. Trachomatis infection- Refer chapter review.

38. **Ans. (b) (Causes...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 399, Harrison 19/e p1165-73, 18/e p1429
    - Urethritis does not occur in psittacosis

    Clinical feature of Psittacosis: Refer chapter review

39. **Ans. (a), (c) (Conjunctivitis, Mucosal lesions)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 399, Harrison 19/e p1165-73, 18/e p1423
    - Reiter's syndrome: *Consists of conjunctivitis, urethritis (or, in female, cervicitis), arthritis, and characteristic Mucocutaneous lesions in* HLA-B27 *phenotype patients*
    - *C. trachomatis:* MC case, Others *Salmonella, Shigella,* or *Campylobacter*

40. **Ans. (a) (Elemen...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 397, Harrison 19/e p1165-73, 18/e p1421

    Refer chapter review
    - Chlamydia biphasic elementary and reticulate body
    - Elementary body is extracelluar, infectious form
    - Reticulate body is the replicating form, divides by binary fission and it is metabolically active
    - Chlamydia evades phagolysozome fusion, hence can survive intracellularly.

41. **Ans. (d) (The group..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 398, Harrison 18/e p1426

    Refer chapter review

42. **Ans. (d) (Community...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 399, Ananthanarayan 9/e p421

    Refer text for explanation.

43. **Ans. (b) (Enzyme...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 402, Ananthanarayan 9/e p417, 18

    Enzyme immunoassay detects either antigen or antibody (Does not help in isolation)

    Cultivation of Chlamydia:
    - Not cultivable in artificial media
    - Mice inoculation (infective by only C.Psittaci and LGV)
    - Yolk sac, cell culture (McCoy Cells and HeLa) useful for LGV and C.Psittaci
    - Pretreatment with irradiation, cycloheximide, DEAE dextran, centrifugation – enhance detection
    - Hep2 for *C.Pneumoniae*

44. **Ans. (a), (b), (c) (HeLa, HeP2 , McCoy)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 402, Ananthanarayan 9/e p418

    Refer Text.

45. **Ans. (a) (LGV)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p400 , Ananthanarayan 9/e p421

    Frei's test (an intradermal test), using crude chlamydial antigen from bubo pus was previously used for detection of LGV.

46. **Ans. (a) (Nuc...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p403, Harrison 18/e p1427-29, 19/e p1165-73

    Nucleic Acid Amplification Test (NAAT) has revolutionized the diagnosis of chlamydial infections.
    - Advantage: It is highly sensitive and specific, takes less time, and detects even few copies of DNA from the sample. It can also differentiate the species and serovars.
    - NAATs are currently the diagnostic assays of choice for chlamydial infection as recommended by the CDC, replacing the so called gold standard culture methods.

47. **Ans. (c) (Amox..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p403, Harrison 19/e p1165-73, 18/e p1426
    - Treatment of genital Chlamydiasis: Single dose azithromycin regimen is DOC.
    - Treatment of Chlamydia in pregnancy:
        - Although not approved by the FDA for use in pregnancy, single dose regimen of azithromycin regimen appears to be safe and effective for this purpose.
        - However, amoxycillin (500 mg three times daily for 7 days) can also be given to pregnant women.
        - The fluoroquinolones are contraindicated in pregnancy.

48. **Ans. (c) (Chlamydia..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p401, Ananthanarayan 9/e p416
    - Chlamydia pneumoniae has one serotype, C.trachomatis has 15 serotype and C.psittaccii has many serotypes.

MYCOPLASMA

49. Ans. (a) (Myco...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 407, Ananthanarayan 9/e p387, 8/e p388
Dienes method is used to stain mycoplasma.

50. Ans. (c) (Cephalosporins) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 405, Ananthanarayan 9/e p389
- As there is lack of peptidoglycan layer, hence Ureoplasma is naturally resistant to all beta lactams.

51. Ans. (a) (Mycoplasma...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 405, Ananthanarayan 9/e p386
- Eaton had isolated Mycoplasma pneumoniae from hamsters and cotton rats. As it was filterable, so it was 1st considered as virus as **'Eaton agent'.**

52. Ans. (a) (They are inhibited by penicillin) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 405, Harrison , 19/e p1163-64, 18/e p1417, Ananthanarayan 9/e p386, 87

About Other Options
- Mycoplasma is completely resistant to penicillin because they lack the cell wall structures at which penicillin acts, but they are inhibited by tetracycline or erythromycin.
- Mycoplasma have an affinity for mammalian cell membranes
- Filterable, can pass through filters of 450 nm pore size
- Mycoplasma can reproduce in cell-free media; on agar, the center of the whole colony is characteristically embedded beneath the surface.

53. Ans (a) (Azithromycin) Ref:Apurba Sastry's Essentials of Medical Microbiology 1/e p408
Macrolides are drug of choice (oral azithromycin) for Mycoplasma infections.

CHAPTER

3.12 Miscellaneous Bacteria

MISCELLANEOUS GRAM-POSITIVE BACTERIA

LISTERIA MONOCYTOGENES

Listeria monocytogenes is a food-borne zoonotic pathogen that can cause serious human infections, particularly in neonates, pregnant women and elderly people.

Listeria Transmission:
- Contaminated food (raw milk, MC)
- Vertical transmission

Pathogenicity

- It can grow in refrigerated food and can tolerate preserving agents.
- Mode of transmission: Contaminated food (raw milk, MC) followed by vertical transmission (mother to fetus).
- Intracellular survival: It is facultative intracellular organism. Survival inside the host cells is due to inhibition and lysis of phagosome by forming pores (mediated by listeriolysin O).
- Direct cell-to-cell spread is due to host cell actin polymerization (mediated by listeriopods).

Clinical Manifestations

- Infection in pregnancy: Before 20 weeks is rare, may lead to abortion, still birth.
- Neonatal disease: Two types: (1) early onset and (2) late onset
- Adult:
  - Associated with steroid therapy, HIV, DM, malignancy (fludarabine treated)
  - Bacteremia > meningitis
  - MC cause of meningitis in kidney transplanted patient after 1 month
  - Also causes Gastroenteritis: Following contaminated milk, meat and salad.

Intracellular survival of Listeria:
- It is facultative intracellular organism.
- Survival inside the host cells is due to inhibition and lysis of phagosome by forming pores (mediated by listeriolysin O).

Early onset neonatal disease	Late onset neonatal disease
Occurs < 5 days of birth	Occurs > 5 days of birth
Acquired from maternal *genital flora*	Acquired from environment
Associated with Obstetrical complications	Not associated
Most common form is neonatal sepsis	Most common form is neonatal meningitis
Granulomatosis infantiseptica occurs rarely	Not seen
Mortality rate is > 30%	Mortality rate is < 10%
Does not cause nosocomial outbreaks	Nosocomial outbreaks are seen

Lab Diagnosis

- Gram-positive coccobacilli, catalase positive
- Shows tumbling type of motility
- Motile at 25°C but nonmotile at 37°C (Differential motility)
- Growth improves if cultured in thioglycollate broth at 4°C (cold enrichment)
- Media: Blood agar, chocolate agar, PALCAM agar (selective medium)
- Anton test: Instillation to rabbit eye causes conjunctivitis.

Listeria–Motility:
- Shows tumbling type of motility.
- Motile at 25 °C but non motile at 37 °C (Differential motility)

Actinomycetes:
- *Actinomyces:* Non Acid fast and anaerobe, Infections occur endogenously
- *Nocardia:* Partially acid fast and Obligate aerobe, Infections occur from soil

Treatment

- DOC: Ampicillin (also penicillin)
- Alternate: Cotrimoxazole (if allergic to penicillin)
- Cephalosporin not affective

ACTINOMYCETES

Actinomycetes are Gram-positive branching filamentous bacteria

Human pathogenic actinomycetes: (1) Actinomyces, (2) Streptomyces, (3) Nocardia, (4) Actinomadura

- Actinomyces is non-acid fast and anaerobic
- *Nocardia*: Aerobe and acid fast (1% sulfuric acid)
- Streptomyces and Actinomadura are aerobes and non-acid fast – Can cause actinomycetoma

	Actinomyces	*Nocardia*
Acid-fastness	Non-Acid fast	Partially acid fast
O_2 requirement	Anaerobe	Obligate aerobe
Sugar	Fermenter	Utilizes sugar oxidatively
Habitat	Found as oral flora Infections occur endogenously	Usual habitat is soil Infections occur exogenously
Risk factors	Disease occurs in immunocompetent host	Usually affects people with low immunity
Clinical forms	Cervicofacial, abdominal and other forms	Pulmonary, CNS, Actinomycetoma
Granules	*Sulfur granules* are hard and not emulsifiable, consist of branching filamentous bacilli and surrounded by clubs (*sunray appearance*)	Granules are soft and lobulated. Commonly found in mycetoma, rare in other conditions
Culture	*Spidery molar* teeth colony in solid media *Fluffy ball* at bottom of the liquid medium	Colonies are creamy, wrinkled and orange to pink. Recovery done by: • Selective media • Paraffin bait technique • LJ medium
Drug of choice	Penicillin	Sulfonamide or Cotrimoxazole

Whipple's disease:
- Characterized by fever, abdominal pain, diarrhea, weight loss and migratory polyarthralgia.
- Agent: *Tropheryma whipplei*

TROPHERYMA WHIPPLEI

Tropheryma whipplei is a gram-positive actinomycete not closely related to any known genus. It is the agent of **Whipple's disease** affecting the small intestine.

- Whipple's disease is characterized by fever, abdominal pain, diarrhea, weight loss and migratory polyarthralgia.
- Mesenteric lymph nodes of the small intestine are primarily involved.

Laboratory diagnosis:

- Histopathological staining of intestinal biopsy shows vacuoles within the macrophage containing PAS stain positive bacilli.
- Culture of *T.whipplei* has been unsuccessful.
- PCR targeting 16S rRNA can be done to identify the bacilli.

Treatment of Whipple's disease include:

- Penicillin, ampicillin, tetracycline, or cotrimoxazole for 1–2 years or doxycycline ***plus***
- Hydroxychloroquine for 12 to 18 months.

ERYSIPELOTHRIX RHUSIOPATHIAE

- Gram-positive bacilli
- Catalase negative, H_2S positive
- Causes erysipeloid skin lesion violaceous swelling with severe pain, but no pus.
- Most common site is finger (called 'seal finger' and 'whale finger').
- Treatment: DOC is penicillin G, however it is intrinsically resistant to vancomycin.

MISCELLANEOUS GRAM-NEGATIVE BACTERIA

CAMPYLOBACTER

Campylobacter species are microaerophilic curved gram-negative bacilli. They are zoonotic, cause both diarrheal (*C.jejuni*) and systemic diseases (*C.fetus*).

Campylobacter triggers:
- Guillain-Barre syndrome (mainly by *C.jejuni serotype* O19) and α chain disease

Epidemiology

- Mode of transmission: (i) by ingestion of raw or undercooked food-poultry (most common), raw (unpasteurized) milk or water, (ii) through direct contact with animals, (iii) oral-anal sexual contact
- MC Age affected: *C.jejuni* (children) and *C.fetus* (extremes of age)
- Developing versus developed countries:
  - In developing countries: It is hyperendemic, mostly asymptomatic except < 2 years (symptomatic).
  - In developed countries, *Campylobacter* is the leading bacterial cause of diarrheal disease.
- Seasonality: Incidence peaks during summer and early autumn.

Pathogenesis

- Motility of the strain (possesses single polar flagellum and exhibits darting motility)
- Capacity to adhere to host tissues
- Toxins play a minor role:
  - Enterotoxin (Heat-labile, similar to cholera toxin)
  - Cytotoxins (cytolethal distending toxin, or CDT)
- Proteinaceous capsule-like structure (S-layer) expressed by *C.fetus.*

Clinical Manifestations

- Intestinal infection: Characterized by inflammatory diarrhea, abdominal pain and fever, rarely bloody stools.
- Extraintestinal infection is mainly due to *C.fetus* developing mostly in immunocompromised hosts and at the extremes of age.
- In persons with the HLA-B27: Reactive arthritis and other rheumatologic manifestations.
- Campylobacter triggers: Guillain-Barre syndrome (mainly by *C.jejuni* serotype O19) and α chain disease.

Campylobacter triggers:
- Curved gram-negative bacilli
- Gull wing shaped
- Darting motility
- Microaerophilic

Laboratory Diagnosis

- Gram staining of smear of feces may show curved gram-negative bacilli appearing comma (resembling *Vibrio*), S-shaped or spiral (gull wing shaped).
- Dark ground microscopy demonstrates the darting motility of the bacilli.
- Culture media are:
  - Transport medium: Cary-Blair medium can be used.
  - Selective media: (i) Skirrow's, (ii) Butzler's and (iii) Campy BAP selective media.
- Culture conditions: Microaerophilic (5% O_2) and Thermophilic Grow at 42°C except *C.fetus* (nonthermophilic).

Treatment

Fluid and electrolyte replacement is the mainstay of treatment. Antibiotics can be given,

- Diarrheal disease: Oral macrolides are the drug of choice (erythromycin or azithromycin).
- Systemic infection: Parenteral gentamicin (or imipenem or chloramphenicol)

HELICOBACTER

Helicobacter pylori is curved gram-negative rod that colonizes stomach and is associated with peptic ulcer disease and gastric carcinoma.

Colonization of H. pylori:
H. pylori colonizes the stomachs of:
- 50% of the world's human population
- Vary from 30% in developed countries
- 80% in developing countries

Pathogenesis

- **Colonization:** *H.pylori* colonizes the stomach of 50% of the world's human population (30% in developed countries to nearly 80% in developing countries).
- **Adhesins:** Few (~2%) strains bind to mucosal epithelium by expressing:
  - Blood group antigen-binding adhesion- binds to Lewis blood group antigen
  - Adherence-associated lipoprotein
- **Induces of pathological changes by producing toxins**
  - Vacuolating Cytotoxin (VacA): Induces the formation of vacuoles in the cytoplasm of epithelial cells.
  - Cytotoxin: Associated Gene A (cagA)
- **Molecular mimicry:** LPS of *H.pylori* cross reacts with Lewis blood group antigen, which contributes to pathogenesis of chronic active gastritis.
- **Environmental risk factors**:
  - Smoking increases the risks of ulcers and cancer in *H. pylori* colonized individuals.
  - Diets high in salt and preserved foods increase cancer risk, whereas antioxidants and vitamin C are protective.

Peptic ulcer disease:
80% of duodenal ulcers and 60% of gastric ulcers are due to *H. pylori*

Clinical Manifestations

- **Acute gastritis** (Antrum is the most common site involved, cardiac end is not involved),
- Antral gastritis: Predisposes to duodenal ulcers
- Pan gastritis: Predisposes to adenocarcinoma of stomach
- **Peptic ulcer disease:** 80% of duodenal ulcers and 60% of gastric ulcers are due to *H.pylori.*
- Chronic atrophic gastritis
- Autoimmune gastritis
- Promotes pernicious anemia
- Adenocarcinoma of stomach Non-Hodgkin's gastric lymphoma

Protective role for H. pylori: Colonization of *H.pylori* (especially with cagA+ strains) has an inverse relation with the occurrence of Gastro esophageal reflux disease (GERD), Barrett's esophagus, Adenocarcinoma of esophagus and asthma.

Laboratory Diagnosis

Invasive Test

Endoscopy guided multiple biopsies can be taken from gastric mucosa (antrum and corpus) and are subjected to:

- **Histopathology** with Warthin starry silver staining
- **Microbiological methods:**
  - Gram staining: Curved gram-negative bacilli with seagull shaped morphology
  - Culture media for *H.pylori*: Culture is the most specific test, however, it is not sensitive.
    - Media for *Campylobacter* can be used, such as Skirrow's media
    - Chocolate agar can be used
    - Plates are incubated at 37°C under microaerophilic condition

Protective role for H. pylori:
- Colonization of *H. pylori* (cagA+ strains) protects us from:
- Gastroesophageal reflux disease (GERD)
- Barrett's esophagus
- Adenocarcinoma of esophageus and asthma.

- Biochemical tests: Oxidase, catalase and urease tests are positive.

- **Biopsy urease test** (rapid urease test): Detects urease activity in gastric biopsies. It is rapid, sensitive, and cheap.

Noninvasive Test

- **Urea breath test:** It is very popular now a days as it is noninvasive and is:
  - Most consistent and accurate test
  - Most sensitive, quick and simple
  - Used for monitoring of treatment (becomes negative after improvement)
- **Stool antigen (coproantigen) assay**: Used for (i) Monitoring of treatment, (ii) Screening of children.
- **Antibody (IgG) detection** by ELISA: Used for (i) Screening before endoscopy, (ii) Sero-epidemiological study

Urea breath test is very popular nowadays as it is noninvasive and is:
- Most consistent and accurate test
- Most sensitive, quick and simple
- Used for monitoring of treatment

Treatment Regimen for *H.pylori* Infection

Treatment in *H.pylori* infections is indicated for (i) duodenal or gastric ulceration, (ii) low-grade gastric B cell lymphoma

However, treatment is not recommended for asymptomatic colonizers or primary prophylaxis for gastric cancer because of risk of adverse side effects and development of antibiotic resistance

1st line triple drug therapy (OCM or OCA regimen)-
Omeprazole + Clarithromycin + Metronidazole or Amoxicillin
given for 7–14 days.
↓
Urea breath test is done after 1 month gap
↓
If 1st line regimen fails (Urea Breath test is +ve)
↓
2nd line Quadruple drug therapy (OBMT regimen)-Omeprazole + Bismuth subsalicylate + Metronidazole + Tetracycline given for 14 days
↓
If 2nd line Quadruple drug therapy fails then –
Culture of Endoscopic guided biopsy is done and treatment is given based on antimicrobial susceptibility test

LEGIONELLA

Legionellae are fastidious gram-negative, short rods, associated with two respiratory syndromes: Pontiac fever and Legionnaires' disease

- Most cases are associated with ***serogroup 1*** followed by 4, and 6.
- Other species rarely cause human infection, such as *L.micdadei* (Pittsburgh pneumonia agent, acid fast) and *L.longbeachae*.

Epidemiology

- **Reservoir**: *Legionella* inhabits on aquatic bodies: (i) Natural water sources, such as rivers or even inside amebae, (ii) Artificial aquatic sources, such as air conditioners.
- **Transmission:**
  - Aspiration (MC mode) via oropharyngeal colonization or directly via drinking of contaminated water.
  - Aerosols from contaminated air conditioners, nebulizers, and humidifiers
  - Direct instillation into the lungs during respiratory tract manipulations
  - There is no man to man transmission, there is no animal reservoir and no carrier.

Reservoir of Legionella:
Inhabits on aquatic bodies
- Natural water sources such as rivers or even inside amoebae
- Artificial aquatic sources such as air conditioners, water coolers

MC mode of Transmission of Legionella:
Aspiration via oropharyngeal colonization or directly via the drinking of contaminated water.

Pathogenesis

After Legionellae enter the lungs through aspiration or direct inhalation:

- Attachment and invasion to respiratory mucosa
- ***Coiling phagocytosis***: Macrophages phagocytose legionellae by a coiling mechanism.
- *Legionella* evades intracellular killing by inhibiting phagosome-lysosome fusion.

Clinical Manifestations

- **Pontiac Fever:** It is an acute, flu-like illness
- **Legionnaires' Disease (Pneumonia):** This is an interstitial atypical pneumonia:
  - It is the fourth common cause of community-acquired pneumonia, accounting for 2–9% of cases).
  - It is responsible for 10–50% of cases of nosocomial pneumonia. Serogroup 6 is more commonly involved in hospital outbreaks.
- **Extrapulmonary Legionellosis:**
  - The most common extrapulmonary site is heart; (myocarditis, pericarditis and prosthetic-valve endocarditis)
  - Other manifestations include: Sinusitis, peritonitis, pyelonephritis, skin and soft tissue infection.

Pathogenesis of *Legionella* is due to Coiling phagocytosis

Laboratory Diagnosis

- **Gram stain** reveals numerous neutrophils but no organisms (as weakly stained, often missed)
- **Direct fluorescent antibody (DFA)**
- **Culture:** Buffered charcoal, yeast extract **(BCYE)** agar is the recommended media.
- **Antibody Detection:** used for epidemiologic purpose.
- **Urinary Antigen:** (i) It is rapid, cheaper, easy to perform, (ii) Remains positive even after antibiotics. (iii) Next to culture, it is highly sensitive, and specific
- **PCR** is useful for isolation from environmental water.

Legionnaires' Disease:
- MC form: Community-acquired pneumonia
- MC extrapulmonary site is heart

Treatment

Macrolides (azithromycin) and the respiratory quinolones are antibiotics of choice.

CALYMMATOBACTERIUM GRANULOMATIS

It is the agent of granuloma inguinale (also called Granuloma venereum or Donovanosis)

- Present name is *Klebsiella granulomatis*
- **Clinical feature:**
  - Painless ulcer: Ulcerogranulomatous ***beefy red ulcer*** that bleeds readily
  - No lymphadenopathy, however *pseudobubos* seen due to subcutaneous abscess.
- **Donovan bodies** (stained by Wrights, Giemsa): Large cyst like macrophages filled with deeply stained capsulated bacilli having a safety-pin (bipolar) appearance.
- **Culture:** Embryonated hens egg and on HEp-2 cell lines.
- **Treatment:** DOC is Azithromycin, Alternate: Cotrimoxazole, Erythromycin and Tetracycline.

Donovanosis:
- Painless beefy red ulcer
- No lymphadenopathy, however pseudobubos seen

RAT BITE FEVER

Agents: *Streptobacillus moniliformis* and *Spirillum minus*

	Streptobacillary rat bite fever	Spirillary rat bite fever
Agent	*Streptobacillus moniliformis*	*Spirillum minus*
Disease also called	Haverhill fever (USA), also called Erythema arthriticum epidemicum	Sodoku (Japan)

Contd...

Agents of Rat Bite Fever:
Inhabits on aquatic bodies
- *Streptobacillus moniliformis*
- *Spirillum minus*

Contd...

	Streptobacillary rat bite fever	Spirillary rat bite fever
Incubation period	7–10 days	1–3 weeks
Clinical feature	Septic fever, rashes, painful polyarthritis with frequent relapses	Similar features *plus* lymph node enlargement
Laboratory diagnosis	• Gram-negative, pleomorphic bacilli in chain with beaded or with fusiform swellings • Nonmotile • Readily developing into L forms • Culture: It grows on media containing serum protein, egg yolk, or starch.	Gram-negative, spirally coiled bacilli Motile (amphitrichous flagella) Not cultured on artificial media Can be isolated from guinea pigs or mice
Artificial media	Cultivable	Noncultivable
Drug of choice	Penicillin	Penicillin

Amsel's criteria: Bacterial vaginosis:
- Profuse thin vaginal discharge
- pH > 4.5
- Fishy odor
- Clue cells

GARDNERELLA VAGINALIS

It causes bacterial vaginosis.

- It is a gram variable (mostly gram-negative coccobacilli), possess metachromatic granules
- Other organisms implicated in BV- *Mycoplasma hominis*, *Mobilincus*, *Prevotella*, *Ureaplasma* and *Peptostreptococcus*
- **Amsel's criteria**: Bacterial vaginosis is diagnosed if any 3 of the following 4 findings are present:
  - Profuse thin (low viscous), white homogeneous vaginal discharge
  - pH of vaginal discharge > 4.5 (due to Decrease in the concentrations of Lactobacilli)
  - Fishy odor accentuated by vaginal secretions mixed with 10% solution of KOH (Whiff test).
  - Clue cells: Vaginal epithelial cells coated with coccobacilli.
- **Nugent's score** is followed to count the no. of *Gardnerella vaginalis*, *Mobiluncus* and Lactobacilli in the Gram stained smear of vaginal discharge. A score of ≥ 7 is diagnostic.
- **Treatment**: DOC Metronidazole.

Nugent's score for bacterial vaginosis:
- Followed to count the no. of *Gardnerella vaginalis*, *Mobiluncus* and *Lactobacilli* in smear
- A score of ≥ 7 is diagnostic.

PASTEURELLA MULTOCIDA

It is probably the most common organism in human wounds inflicted by the bites of cats and dogs.

- **Clinical findings:** Causes wound infection, regional lymphadenopathy and respiratory infection.
- **Laboratory diagnosis:**
  - Nonmotile gram-negative coccobacilli with a bipolar staining.
  - They resemble yersiniae, however differ from the latter in being:
    - Oxidase-positive and indole positive
    - Failure to grow on MacConkey agar
- **Treatment:** Penicillin G is considered as the drug of choice for *P.multocida* infections.

Pasteurella Multocida:
- MC organism in human wounds inflicted by the bites of cats and dogs.

FRANCISELLA TULARENSIS (TULAREMIA)

- **Transmission:** Direct contact with rodents > Tick bite > Inhalation and Ingestion
- **Subspecies:** *F.tularensis* has four subspecies: *tularensis, holarctica, novicida*, and *mediasiatica*.
  - The first three are found in North America; whereas subspecies *mediasiatica* is found in central Asia
  - Subspecies *tularensis* is the most common and the most virulent among all.

Francisella Tularensis:
- Transmission is by direct contact with rodents> Tick bite>Inhalation and Ingestion

- **Clinical Manifestations:** Tularemia is characterized by various clinical syndromes
  - Ulceroglandular tularemia (MC form): Ulcerative lesion at the site of inoculation, with regional lymphadenopathy
  - Pulmonary tularemia
  - Oropharyngeal tularemia and Lemming fever in Norway
  - Oculoglandular tularemia
  - Typhoid-like illness
  - Agent of bioterrorism (under class A).
- **Laboratory diagnosis:**
  - Small gram-negative coccobacillus with bipolar appearance, nonmotile and capsulated and filamentous
  - Filterable, fastidious and obligate aerobe.
  - Media: Francis blood dextrose cysteine agar
  - Antibody detection is the mainstay of diagnosis.
- **Treatment:** Gentamicin is considered as the drug of choice.

CHROMOBACTERIUM VIOLACEUM

It is a saprophyte of water and soil in tropics.

- It occasionally causes skin lesions, sepsis, and liver abscesses
- It is a motile, gram-negative, facultative anaerobe, non-sporing, coccobacillus.
- It produces characteristic violet color non diffusible pigment (called **violacein**).

Capnocytophaga:
Occasionally cause periodontal diseases, and sepsis in immunocompromised hosts

CAPNOCYTOPHAGA SPECIES

Several species, such as *C.ochracea, C.gingivalis* and *C.sputigena* have been a part of human mouth flora.

- They occasionally cause periodontal diseases, and sepsis in immunocompromised hosts.
- Species, such as *C.canimorsus* and *C.cynodegmi* are commensals in mouth of dogs and are transmitted by dog bites.
- **Laboratory diagnosis:**
  - They are fusiform or filamentous gram-negative coccobacilli
  - Highly fastidious, require carbon dioxide for optimal growth
  - They produce yellow orange pigment
  - They lack flagella but exhibit *gliding motility* on agar surface.
- **Treatment:** Due to their ability to produce β lactamases, ampicillin + sulbactam is the drug of choice.

MULTIPLE CHOICE QUESTIONS

LISTERIA

1. **True about Listeria monocytogenes :** *(PGI May 2016)*
   a. Pregnant women are at high risk
   b. Infants at high risk
   c. Elderly at high risk
   d. Infection transmitted by inhalation
   e. Ampicillin is drug of choice

2. **Gram-positive short rods are seen in the CSF sample of a new borne child suffering from lethargy, fever and seizure. Organism responsible is:**
   a. Group B Streptococcus *(AIIMS Nov 2014)*
   b. Listeria
   c. Clostridium tetani
   d. Bacillus anthracis

3. **Tumbling motility is shown by:**
   a. Listeria monocytogenes *(NEET Pattern Based)*
   b. Proteus vulgaris
   c. Borrelia
   d. Clostridia

4. **Drug of choice for Listeria monocytogenes is:**
   a. Ampicillin *(DNB Dec 2009)*
   b. Amoxycillin
   c. Vancomycin
   d. Amikacin

5. **A 39-year-old female comes with high grade fever and signs suggesting meningitis. On CSF examination a gram-positive bacilli was isolated. The organism involved is:** *(AI 2011)*
   a. Streptococcus pneumoniae
   b. Listeria monocytogenes
   c. H. influenzae
   d. Staph aureus

6. **Which is not true about bacterial transmission:**
   a. Legionella – through water aerosol *(AI 2009)*
   b. Listeria – Refrigerated food
   c. Leptospira – Urine
   d. Tetanus – droplet/dust

7. **A 3-week-old child presented to the pediatrician with meningitis. A presumptive diagnosis of late onset of a perinatal infection was made. The CSF culture was positive for gram-positive bacilli. Which of the following characteristic of this bacteria would be helpful in differentiating it from other bacteria agents:**
   a. Ability to grow on blood agar *(AIIMS May 2005)*
   b. Ability to produce catalase
   c. Fermentative attack on sugars
   d. Motility at 25°C

8. **A 30-year-old woman with a bad obstetric history presents with fever. The blood culture from the patient grows gram-positive small to medium coccobacilli that are pleomorphic, occurring in short chains. Direct wet from the culture shows tumbling motility. The most likely organism is:** *(AI 2004)*
   a. Listeria monocytogenes
   b. Corynebacterium sp.
   c. Enterococcus spp
   d. Erysipelothrix rhusiopathiae

9. **Seal finger and whale finger are associated with:**
   a. Listeria *(NIMHANS 2006)*
   b. Erysipelothrix
   c. Corynebacterium
   d. Treponema

10. **In patient with Listeria meningitis who is allergic to penicillin the treatment of choice is:** *(AIIMS 2004)*
    a. Vancomycin
    b. Gentamycin
    c. Trimethoprim – sulphamethoxazole
    d. Ceftriaxone

ACTINOMYCES

11. **Microbiological organism can be recovered from:**
    a. Sulphur granules of actinomycetes *(PGI May 2015)*
    b. Streptococci from valve leaflet lesion in rheumatic valvulitis
    c. Petechial purpura for Meningococci
    d. Corynebacterium in pseudomembrane in throat

12. **Patient has history of brain abscess. On examination, branching weak acid fast and Gram positive bacilli were seen.** *(JIPMER Nov 2015)*
    a. Nocardia
    b. Actinomyces
    c. Pseudomonas
    d. Acinetobacter

13. **Actinomyces israelii has following feature** *(MHPG 2015)*
    a. Exogenous infection
    b. Branching filaments
    c. Aerobic
    d. Black granules

14. **Actinomycetes, most common site is:**
    a. Cervicofacial *(NEET Pattern Based)*
    b. Thoracic
    c. Abdomen
    d. Brain

15. Color of granule of Actinomycetes: *(NEET Pattern Based)*
a. Black
b. Yellow
c. Red
d. Brown

NOCARDIA

16. Nocardia and Actinomyces can be differentiated by which stain? *(Recent Question 2015)*
a. Gram
b. PAS stain
c. Silver stain
d. Acid fast stain

17. Nocardia infection is most commonly associated with: *(AI 2012)*
a. Liver abscesses
b. Kidney abscesses
c. Brain abscesses
d. Subcutaneous

18. True about Nocardia are all EXCEPT: *(DNB June 2009)*
a. Penicillin is drug of choice
b. Most common disease caused is pneumonia
c. Aerobic
d. Acid fast

19. A patient comes with history of unresponsive fever and cough. On examination of sputum it is negative for mycobacterium. Chest X-ray shows pneumonia. BAL shows gram-positive branching filaments. Organism was partially AFB positive. Causative organism is: *(AI 2011)*
a. Actinomycosis
b. Nocardiosis
c. Aspergillus
d. Penicillium

20. A clinical specimen was obtained from the wound of a patient diagnosed as Nocardiosis. For the selective isolation of Nocardia sp. which one of the following would be the best method: *(AIIMS May 2004)*
a. Paraffin bait technique
b. Castaneda's culture method
c. Craige's tube method
d. Hair bait technique

21. Nocardia is differentiated from Actinomyces by:
a. Gram stain *(PGI June 2002)*
b. ZN stain
c. Nocardia causes mycetoma, actinomyces does not
d. Nocardia is facultative anaerobe

CAMPYLOBACTER

22. The following statements are true for Campylobacter except: *(AI 2011, AI Nov 09)*
a. Campylobactereriosis in humans is induced mainly by campylobacter jejuni.
b. Poultry is a major source of infection
c. Humans are the reservoir of infection
d. Gullian-Barre syndrome is a sequelae of campylobacter infection

23. Campylobacter culture media are: *(PGI Dec 2008)*
a. Schaedler's agar
b. CVA medium
c. Regan-Lowe medium
d. Skirrow medium
e. Campylobacter blood agar

24. A 35-year-old patient complains of abdominal cramps along with profuse diarrhea. The treating physician wants to process the stool specimen for isolation of Campylobacter jejuni. Which of the following is the method of choice for the culture of stool: *(AIIMS Nov 2004, AI 2005)*
a. Culture on TCBS medium incubated at 37°C under aerobic conditions
b. Culture on Skirrow's medium incubated at 42°C under micro-aerophilic conditions.
c. Culture on MacConkey medium incubated at 42°C under anaerobic conditions
d. Culture on Wilson and Blair's medium incubated at 37°C under micro-aerophilic conditions

HELICOBACTER

25. 45 year old female patient presents with duodenal ulcer. The most sensitive test for the detection of H. pylori is: *(JIPMER Nov 2014, JIPMER May 2015, MHPG 2015)*
a. Urea breath test
b. Serology
c. Biopsy and culture
d. Stool antigen test

26. H.pylori causes all except *(West Bengal 2016)*
a. Gastric lymphoma
b. Gastric ulcer
c. Duodenal ulcer
d. Fundal gastritis

27. Incidence of H.pylori in Gastric ulcer is:
a. 5% *(JIPMER 2011, PGI Dec 2000)*
b. 20%
c. 60%
d. 80%

28. Noninvasive test for H.pylori: *(PGI June 2008)*
a. Rapid urease test
b. Urease breath test
c. Stool antigen assay
d. Stomach aspiration culture
e. Biopsy

29. True about H.pylori: *(PGI June 2004, 2002 AIIMS Nov 2003)*
a. It is flagellated
b. Involved in causation of peptic ulcer disease
c. Hypergastrinomia caused by it
d. Eradication leads to improved lifestyle
e. It is a gram –ve organism

30. Maximum urease +ve is produced by: *(PGI 2000)*
a. H.pylori
b. P.mirabilis
c. K.rhinoscleromatis
d. Ureoplasma

31. Which of the following is false regarding H.pylori:
a. With chronic infection urease breath test become negative
b. H.pylori infection remains lifelong if untreated
c. Endoscopy is diagnostic *(AIIMS 2000)*
d. Toxigenic strains usually cause ulcer

32. True about H.pylori is a all except: *(PGI June 2000)*
a. It splits urea and produces ammonia to survive
b. Produces gastric carcinoma
c. Gram –ve curved rod
d. Cag –A gene is not associated with risk of duodenal ulcer

33. Endoscopic biopsy is positive for urease test and organism grows on Skirrows media. Identify the organism. *(Recent Question 2013)*
a. Campylobacter
b. Helicobacter pylori
c. Proteus

34. True about H. pylori: *(PGI Nov 2014)*
a. Spiral
b. Microaerophilic
c. Gram-negative bacillus
d. Survives in acidic environment by producing urease

LEGIONELLA

35. Coccobacillus grown in BCYE medium:
a. Legionella *(Recent Question 2015)*
b. Streptobacillus
c. Gardnerella

36. Sensitivity of urinary Antigen test of Legionella is:
a. 80% *(Recent Questions 2014)*
b. 70%
c. 95%
d. 99%

37. All of the following are true regarding Legionella except: *(PGI Nov 2014)*
a. Cause Pontiac fever
b. Aerobic gram negative bacilli
c. Can grow on simple medium
d. Grow on BCYE agar
e. Communicable from infected patients to others

38. Legionella pneumophila spreads by:
a. Person to person *(AIIMS Nov 2012, May 2013)*
b. A.C Aerosol
c. Infected meat
d. Contaminated air

39. An elderly man presented with fever and cough. Sputum examination revealed gram-negative organisms that were grown on Buffered charcoal yeast extract agar. The organism involved is: *(AIIMS Nov 2011, AI 2007, AIIMS Nov 2006)*
a. H. influenzae
b. Legionella pneumophila
c. Burkholderia cepacia
d. Brucella

40. Legionella pathogenicity is due to: *(DNB June 2010)*
a. Capsule
b. Toxin
c. Bacteriophage
d. Failure of oxidative burst of neutrophils

41. Pontiac fever is caused by: *(Recent Question 2015)*
a. Legionella micdadei
b. Legionella pneumophila serogroup 1
c. Legionella adelaidensis
d. Legionella anisa

42. Pontiac fever is caused by: *(PGI Dec 2007)*
a. Legionella
b. Listeria
c. Scrub typhus
d. Leptospira
e. Rickettsia

43. True about Legionella: *(PGI Dec 2006)*
a. Epidemics seen
b. Splenomegaly
c. Easily seen on sputum
d. Scanty neutrophils with fed organisms
e. Purulent sputum common

44. A 50-Year-old man is diagnosed to be suffering from Legionnaires' disease after the returns home from attending a convention. He could have acquired it: *(AI 2003)*
a. From a person suffering from the infection while travelling in the aeroplane
b. From a chronic carrier in the convention center
c. From inhalation of the aerosol in the air conditioned room at convention center
d. By sharing an infected towel with a fellow delegate at the convention

45. Which of the following is a good media to use for diagnosis of Legionnaires disease? *(Recent Questions 2014, AIIMS Nov 2001, PGI 1999)*
a. Thayer Martin Media
b. BCYE agar
c. Bordet Gengou media
d. Chocolate agar

46. Devi, a-28-year female, has diarrhea, confusion high grade fever with bilateral pneumonitis. The diagnosis is: *(AI 2000)*
a. Legionella
b. Neisseria meningitidis
c. Streptococcus pneumoniae
d. H. influenzae

MISCELLANEOUS

47. Rhinoscleroma is caused by *(APPG 2015)*
a. Candida guilliermondii
b. Rhinosporidium seeberi
c. Aspergillus fumigatus
d. Klebsiella rhinoscleromatis

48. Which one of the following is a Gram negative fusiform gliding bacillus? *(APPG 2015)*
a. Eikinella
b. Capnocytophaga
c. Kingella
d. Moraxella

49. Donovan bodies seen in: *(Recent Question 2015)*
a. Leishmania donovani
b. Klebsiella granulomatis

50. In donovanosis: *(NEET Pattern Based)*
a. Pseudolymphadenopathy
b. Penicillin is used for treatment
c. Painful ulcer
d. Suppurative lymphadenopathy

51. Clue cell is seen in: *(AI 2011, JIPMER 2011, 2010, APPG 2012)*
a. Trichomonas vaginalis
b. Bacterial vaginosis
c. Candidiasis
d. Herpes

52. Beefy red bleeding ulcer: *(JIPMER 2010)*
a. Chancroid
b. Syphilis
c. Donovanosis
d. LGV

53. Which of the following is a common feature of Spirillary rat bite fever? *(Recent Question 2015)*
a. Lymph node swelling
b. Lymphadenopathy
c. Endocarditis
d. Hepatospleenomegaly

54. Drug of choice for rat bite fever: *(AIIMS Nov 2014)*
a. Penicillin G
b. Cephalosporin
c. Amikacin
d. Tetracycline

55. Pasteurella multocida mainly is transmitted by:
a. Animal bite *(Recent Question 2014)*
b. Insect bite
c. Droplets
d. Sexual contact

56. Bacterial vaginosis is identified by? *(JIPMER May 2016)*
a. pH <4.5, green coloured vaginal discharge
b. pH >4.5, clue cells present, fishy odour with KOH
c. pH >4.5, white creamy discharge
d. pH >4.5, foul smelling discharge

57. Which of the following is/are true about Pasteurella multocida: *(PGI Nov 2014)*
a. May cause meningitis
b. Transmitted by unpasteurized milk
c. Cause disease exclusively in human
d. Gram-negative coccobacillus

58. Bacteriorhodopsin is associated with *(PGI Nov 2016)*
a. Helicobacter
b. Halobacteria
c. Hafnia
d. Antibacterial protein
e. Act as proton pump

EXPLANATIONS

LISTERIA

1. **Ans (a, b,c,e) (Pregnant women …, Infants at …., Elderly at …, Ampicillin is drug..)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p297
   - Listeriosis transmitted by contaminated food/water followed vertical route
2. **Ans. (b) (Listeria)** Apurba Sastry's Essentials of Medical Microbiology 1/e p296, Harrison 19/e p982-83, 18/e p1194-97
   Common cause of meningitis in new borne – Group B Streptococcus (Gram-positive cocci), Listeria (Gram-positive short bacilli) and E.coli (Gram-negative bacilli).
3. **Ans. (a) (Listeria...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p296, Ananthanarayan 9/e p396
   - Listeria shows characteristic tumbling motility at 25 °C however, it is non motile at 37 °C.
4. **Ans. (a) (Ampicillin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p296, Harrison 19/e p982-83, 18/e p1196

   Treatment for Listeriosis:
   - Ampicillin is the drug of choice, high doses (2 g IV every 4 h)
   - Cephalosporins are *not* effective and should not be used.
5. **Ans. (b) (Listeria...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p296, Harrison 19/e p982-83, 18/e p1194, 96
   Points in favor:
   - Meningitis in newborn
   - Gram-positive bacilli isolated from CSF.
   - Among the options, the only gram positive bacilli - L.monocytogenes.
6. **Ans. (d) (Tetanus-droplet/dust)** Apurba Sastry's Essentials of Medical Microbiology 1/e p296; Ananthanarayan 9/e p61, 40 18/e p259, 400, Harrison 19/e p982-83, 18/e p1194, 1393
   'Tetanus is transmitted through injury (preferably punctured), surgery done aseptically, following otitis media (otogenic tetanus), by unhygienic practice like circumcision, ear boring, cow dung on umbilical stump.' ……..Ananthanarayan 8/e p259
   I have not got any reference for Droplet spread of tetanus.
7. **Ans. (d) (Motility at 25°C)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p296, Harrison 19/e p982-83
   - Agents of Perinatal meningitis – E.coli, Strept. agalactiae, Listeria monocytogenes
   - Perinatal meningitis showing gram-positive bacilli in CSF – Suggestive of Listeria

   Next step is:
   The characteristic feature of Listeria which differentiate from other organism causing neonatal meningitis is - Differential Tumbling motility at 25 °C but nonmotile at 37 °C. (Gillespie Bacteriology 2/e p129)
   Listeria has > 6 species and all show Differential Tumbling motility.
   However Listeria monocytogenes is the main pathogen whereas others rarely cause human infection.
   Test to differentiate L.monocytogenes from other Listeria spp:
   - Beta hemolysis
   - Fermentative pattern of sugars
   - I think, the intention of the question is how to differentiate Listeria from other organism causing neonatal meningitis. So I suggest you to go with ***Option'd'.***
8. **Ans. (a) (Listeria monocytogenes)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p296, Harrison 19/e p982-83, 18/e p1194, 95
   - Listeria in pregnancy can lead to abortion.
   - Febrile woman with a bad obstetric history presents with gram-positive coccobacilli and tumbling motility is suggestive of Listeria monocytogenes.
9. **Ans. (b) (Erysipelothrix)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p296, Jawetz 25/e p181, 24/e p219
   *'The most common E rhusiopathiae infection in humans is called **erysipeloid** it usually occurs on the fingers by direct inoculation at the site of a cut or abrasion and has been called **seal finger and whale finger**'.*

10. **Ans. (c) (Trimethoprim – sulphamethoxazole)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p296, Harrison 19/e p982-83, 18/e p1196
    - Treatment for Listeria- Explanation of Refer Q.No.1

ACTINOMYCES

11. **Ans (a), (c), (d) (Sulphur…, Petechial…,Corynebacterium…)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p293, 225, 238, 246
    - Sulphur granules are composed of organisms.
    - Rheumatic heart disease is a post streptococcal sequel due to autoimmune cause. Hence, organism cannot be isolated. High ASO titre is diagnostic.
    - Meningococci can be isolated from cultures of scrapings or aspirates from skin lesions but less value than CSF/blood culture… Harrison 19/e p1000
    - Pseudomembrane is the specimen of choice to isolate C.diphtheriae.
12. **Ans. (a) (Nocardia)** Ref: ApurbaSastry's Essentials of Medical Microbiology 1/e p294
    Nocardia is branching weak acid fast and Gram positive bacillus and is associated with brain abscess.
13. **Ans. (b) (Branching filaments)** Ref: ApurbaSastry's Essentials of Medical Microbiology 1/e p292
    Actinomyces are branching Gram positive filamentous bacilli
14. **Ans. (a) (Cervicofacial)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p292-93, Ananthanarayan 9/e p391
    - Actinomycosis occurs in four clinical forms- Cervicofacial (most common form), thoracic, abdominal and pelvic.
15. **Ans. (b) (yellow)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p292-93, Ananthanarayan 9/e p391
    - Sulfur granules in Actinomyces exhibit white to yellow color.

NOCARDIA

16. **Ans. (d) (Acid fast stain)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p292
17. **Ans. (c) (Brain abscesses)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p294-95, Harrison 18/e p162-1323
    - MC form of nocardiosis: Pulmonary nocardiosis.
    - The most common site of extrapulmonary dissemination is the brain.
    - Other common sites include the skin and supporting structures, kidneys, bone, and muscle, but almost any organ can be involved.
    - The typical manifestation of extrapulmonary dissemination is a subacute brain abscess.
    - Brain abscesses are usually supratentorial, are often multiloculated, and may be single or multiple.
    - Brain abscesses tend to burrow into the ventricles or extend out into the subarachnoid space.
18. **Ans. (a) (Penicillin...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p294-95, Harrison 19/e p1084-85
    - MC form of nocardiosis: Pulmonary nocardiosis
    - Nocardia is Obligate Aerobe and partially Acid fast
    - DOC: Sulfonamides are the drugs of choice. Cotrimoxazole may work even better but pose toxicity. Duration 6–12 months depending on site of involvement.
19. **Ans. (b) (Nocardiosis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p294-95, Ananthanarayan 9/e p393
    - ***Gram-positive branching filaments*** – Actinomyces or Nocardia
    - ***Partially AFB positive***- Suggestive of Nocardia
20. **Ans. (a) (Paraffin...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p294-95, Harrison 19/e p1084-87
    'Since Nocardia are among the few aerobic microorganisms that use paraffin as a carbon source, paraffin baiting can be used to isolate the organisms from mixed cultures'.
21. **Ans. (b) (ZN stain)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p294-95, Ananthanarayan 9/e p393
    - *Nocardia are partially acid fast while Actionomyces are non acid fast.*

    About other options
    - *The most common causes of Actinomycetoma are Nocardia asteroides, Nocardia brasiliensis, Streptomyces somaliensis, and Actinomadura madurae.* Jawetz 24/e p220

- *Actinomyces israelii and A.bovis also can cause Mycetoma* Ananthanarayan 9/e p393
- Both are gram +ve filamentous bacteria
- Nocardia are obligate aerobe.

CAMPYLOBACTER

22. Ans. (c) (Humans are the reservoir of infection) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p361-62, Harrison 19/e p1058-59, 18/e p1286

- Humans are not the only reservoir of infection. *Campylobacter are found in the gastrointestinal tract of many animals used for food* (including poultry, cattle, sheep, and swine) and many household pets.

About Other Options

- *'The principal diarrheal pathogen is C. jejuni, which accounts for 80–90% of all cases of recognized illness due to campylobacters and related genera'*
- *'Ingestion of contaminated In cooked poultry is the most common mode of acquisition (30–70% of cases). Other modes include ingestion of raw (unpasteurized) milk or untreated water, contact with infected household pets, travel to developing countries'*
- *'Campylobacter infections, because of their high incidence, may trigger 20–40% of all cases of Guillain-Barré syndrome'.*

23. Ans. (b), (d), (e) (CVA medium, Skirrow medium, Campylobacter blood agar) Ref:Apurba Sastry's Essentials of Medical Microbiology 1/e p361-62

- Culture media for Campylobacter-Refer chapter review

About Other Options

- *Schaedler* Agar with Vitamin K- used for anaerobic microorganisms.
- *Regan-Lowe* medium –used for Bordetella

24. Ans. (b) (Culture...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p361-62, Ananthanarayan 9/e p399

Explained already

HELICOBACTER

25. Ans. (a) (Urea breath test) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p364

- The best established test (and a very accurate one) is the urea breath test.
- Most consistent and accurate test
- Most sensitive, quick and simple
- Used for monitoring of treatment (becomes negative after improvement)
- Microbiologic culture is most specific but may be insensitive because of difficulty with *H. pylori* isolation

… Harrison 19th/p1040.

26. Ans (d) (Fundal gastritis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p363

H.pylori causes

- Acute gastritis (Antrum is the most common site involved, cardiac end is not involved).
- Peptic ulcer disease: 80% of duodenal ulcers and 60% of gastric ulcers are due to H. pylori.
- Non-Hodgkin's gastric lymphoma

27. Ans. (c) (60%) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p363-64, Harrison 19/e p1058-59, 18/e p1262

- 'Worldwide, > 80% of duodenal ulcers and > 60% of gastric ulcers are related to H. pylori colonization'

28. Ans. (b), (c) (Urease breath test, Stool antigen assay) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p363-64, Ananthanarayan 9/e p401, 8/e p400

Diagnosis of H.pylori infection: Refer text for explanation.

29. Ans. (a), (b), (c), (e) (Flagellated, Associated peptic ulcer disease, Hypergastrinomia caused..., It is a gram –ve organism) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p363-64, Ananthanarayan 9/e p400, 01, Harrison 19/e p1058-59, 18/e p1261, 63

- Improved lifestyle leads to Eradication of H.pylori, i.e. why the colonization of H. pylori is low (30%) in developed country compared to 80% in developing country. But Eradication of H.pylori cannot cause Improved lifestyle Harrison 17/e p949 also states that H.pylori eradication is found to increase the risk of Emerging diseases such as asthma, obesity, and type 2 diabetes mellitus reflecting aspects of the current Western lifestyle. Also increase risk to Diarrheal diseases and GERD and its complications, including esophageal Adenocarcinoma.

About Other Options

- H.pylori is a gram –ve spirally coiled curved organism
- H.pylori has Unipolar tuft of lophotrichous flagella
- Definite Association exists with peptic ulcer disease
- *H. pylori–induced gastritis diminishes the number of somatostatin-producing D cells. Since somatostatin inhibits gastrin release, gastrin levels become higher.*

30. Ans. (a) *(H.pylori)* Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p363-64

Urease production:

- *Maximum- H.pylori followed by Brucella canis, Proteus*
- *Moderate- Klebsiella.*

Urease producing Fungus:

- *Cryptococcus neoformans*
- *Trichophyton mentagrophytes*
- *Trichosporon beigelii*
- *Malasseiza furfur.*

31. Ans. (a) (With...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p363-64, Jawetz 25/e p241, 24/e p276

After treatment, urease breath test becomes negative (not chronic infection)

About Other Options

- Once colonized, the *H pylori* infection persists for years and even for a lifetime
- Endoscopic guided biopsy usually done for the diagnosis.
- H.pylori infection has been associated with two important virulent gene-Cytotoxin associated gene (Cag) and Vacuolating Cytotoxin gene (Vac)
- Toxins and lipopolysaccharide may damage the mucosal cells, and the ammonia produced by the urease activity may directly damage the cells.

32. Ans. (d) (Cag –A gene...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p363-64, Harrison 19/e p1058-59, 18/e p1261, 62

- ***H. pylori colonization increases the lifetime risk of:***
  - Peptic ulcer disease
  - Noncardia gastric cancer
  - B cell non-Hodgkin's gastric lymphoma
- *Cytotoxin associated gene (Cag), Vacuolating Cytotoxin gene (Vac) and urease enzyme are the principle virulence factors.*
- ***However, CagA+ H.pylori strains protect against:***
  - Adenocarcinoma of the esophagus
  - Adenocarcinoma of gastric cardia
  - Premalignant lesions such as Barrett's esophagus.

33. Ans. (b) (Heli..) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p363-64, Ananthanarayan 9/e p400, 401

- Gastric biopsy showing urease positive and growth in Skirrow's media- Suggestive of Helicobacter pylori.

34. Ans. (b) (c) (d) (Microaerophilic, Gram negative bacilli, Survives in acidic environment by producing urease) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p363-64, Ananthanarayan 9/e p400

LEGIONELLA

35. Ans. (a) (Legionella) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p366

36. Ans. (b) (70%) Ref: Apurba Sastry's Essentials of Medical Microbiology/p366, Harrison 18/Chapter 147, table 147.2

Urinary antigen testing for Legionella has sensitivity for 70% and specificity of 100%

37. Ans. (c), (e) (Can grow.., Communicable…) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p366-67

Legionella can grow on complex media like BCYE media and it cannot be transmitted from man to man.

38. Ans: (b), (A.C Aerosol), Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p365-66, Harrison 19/e p1014-20

- Aspiration is now known to be the predominant mode of transmission
- Aerosolization of *Legionella* by devices filled with tap water, including whirlpools, nebulizers, and humidifiers, cooling tower, air-conditioners, has been implicated.

39. **Ans. (b) (Legionella)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p365-66, Harrison 19/e p1014-20
Fever, chest pain and dry cough is suggestive of atypical pneumonia.
Among the agents of atypical pneumonia, Legionella grows on charcoal yeast medium.

40. **Ans. (d) (Failure...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p365-66, Harrison 19/e p1014-20
- *The role of neutrophils in immunity appears to be minimal: neutropenic patients are not predisposed to Legionnaires' disease.*
- *Although L. pneumophila is susceptible to oxygen-dependent microbiologic systems in vitro, it resists killing by neutrophils*

41. **Ans. (b) (L. pneumophila)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p365

42. **Ans. (a) (Legionella)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p365-66, Harrison 18/e p1237
- Pontiac fever is an acute, self-limiting, flu-like illness with an incubation period of 24–48 hour.
- It is the milder form of Leginella infection.
- Severe for is known as Legionnaire's disease.

43. **Ans. (a) (Epidemics seen)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p365-66, Harrison 19/e p1014-20,
- *Pontiac fever (milder form of Legionella infection) occurs in epidemics.*
- In Sputum numerous neutrophils but no organisms revealed by Gram's staining of respiratory secretions
- Legionella has been identified in lymph nodes, spleen, liver, or kidneys in autopsied cases but splenomegaly is uncommon. (*Textbook of pediatric infectious diseases, V-1,Ralph D. Feigin*)
- The clinical picture includes a relatively nonproductive cough and a low incidence of grossly purulent sputum. ……..

44. **Ans. (c) (From...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p365-66, Ananthanarayan 9/e p401
- Human infection occurs by inhalation of aerosol produced by cooling tower, AC, shower heads which acts as disseminators.
- Natural source: Legionella is widely distributed in natural water (stagnant water, mud and hot spring) where the growth requirement is provided by algae and protozoa like ameba.
- ***No carrier state, No animal reservoir and No man to man transmission***

45. **Ans. (b) (BCYE agar)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p365-66, Ananthanarayan 9/e p401
BCYE media: Buffered charcoal yeast extract with cysteine and antibiotics

46. **Ans. (a) (Legionella)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p365-66, Harrison 19/e p1014-20
Atypical Pneumonia– 4th MC cause of community-acquired pneumonia (CAP)
1st three cause of CAP are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Chlamydia pneumonia*

MISCELLANEOUS

47. **Ans. (d) (Klebsiella rhinoscleromatis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p308
Rhinoscleroma, or simply Scleroma, is a chronic granulomatous bacterial disease of the nose, caused by Klebsiella rhinoscleromatis

48. **Ans. (b) (Capnocytophaga)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p370
Capnocytophaga are fusiform or filamentous gram-negative coccobacilli. They are part of human oral flora, occasionally cause periodontal diseases, and sepsis in immunocompromised hosts.

49. **Ans. (b) (K. granulomatis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p367

50. **Ans. (a) (Pseudolym...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p367, Harrison 18/e p1320
- Donovaniasis is characterized by pseudo bubo, i.e subcutaneous swelling without lymphadenopathy.

51. **Ans. (b) (Bacterial..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p367, Ananthanarayan 9/e p403
Bacterial vaginosis: Refer text for explanation.

52. **Ans. (c) (don...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p367, Harrison 19/e p1014-20, 18/e p1320
Donovanosis presents as: Beefy lesions, which bleed readily on contact.
Donovanosis: Refer text for explanation.

53. **Ans. (a) (LN swelling)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p368

54. **Ans. (a) (Penicillin G)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p368, Ananthnarayan 9/e p398
- Penicillin is considered to be the treatment of choice for both forms of rat bite fever, i.e. Streptobacillus moniliformis and Spirillum minus

- Alternative drug: Streptomycin, tetracycline, doxycycline, cephalosporin
- Penicillin resistance, though rare, but has been reported for S. moniliformis.

55. Ans. (a) (Animal bite) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p370, Ananthanarayan 9/e p 325.

56. Ans (b) (pH >4.5, clue cells present, fishy odour with KOH) *Ref:Apurba Sastry's Essentials of Medical Microbiology/p367*
Refer text for Amsel's criteria for diagnosis of Bacterial vaginosis

57. Ans. (a), (d) (May cause... Gram-negative...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p369
Pasteurella is zoonotic, causes wound infection and rarely meningitis especially following head injury. It is transmitted by bites of cats and dogs (not through milk).

58. Ans (b,e) (Halobacteria, Acts as proton pump) Ref: Internet Sources

- Bacteriorhodopsin is a protein used by Archaea, most notably by ***Halobacteria***.
- It acts as a ***proton pump***; that is, it captures light energy and uses it to move protons across the membrane. The resulting proton gradient is subsequently converted into chemical energy.

NOTE

NOTE

SECTION 4

Virology

CHAPTER OUTLINE

CHAPTER

4.1

General Properties of Viruses

Viruses are the smallest unicellular organisms that are obligate intracellular. They differ from bacteria and other prokaryotes, as:

- They are obligate intracellular
- They possess either DNA or RNA, but never both.
- Filterable: They are smaller than bacteria, can be passed through the bacterial filters.
- They cannot be grown on artificial cell free media (However, grow in animals, eggs or tissue culture).
- They multiply by a complex method, but not by binary fission as seen in bacteria.
- Viruses do not have a proper cellular organization.
- They do not have cell wall or cell membrane or cellular organelles including ribosomes.
- They lack the enzymes necessary for protein and nucleic acid synthesis.
- They are not susceptible to antibacterial antibiotics.

CLASSIFICATION AND MORPHOLOGY OF VIRUSES

DNA Viruses: Examples include: 3P + 2H + AB

DNA Virus Family	DNA Viruses	DNA Virus Family	DNA Viruses
Poxviridae (largest virus in size)	Variola virus, Vaccinia virus, Molluscum contageosum virus	**H**erpesviridae	Herpes simplex virus 1 and 2, Varicella zoster virus, CMV, EBV, Human herpesvirus-6, 7 and 8
Papovaviridae	Human Papilloma virus, Polyoma virus, BK and JC virus	**H**epadnaviridae	Hepatitis B virus
		Adenoviridae	Adenovirus
Parvoviridae (smallest virus in size)	Parvovirus (only DNA virus to have ss DNA)	**B**acteriophage	

RNA Viruses

Family	RNA Viruses	Family	RNA Viruses
Picornaviridae	Poliovirus, Coxsackievirus Echovirus, Enterovirus Rhinovirus, Hepatitis A virus	Paramyxoviridae	Parainfluenza virus , Mumps virus Measles virus, Respiratory syncytial virus Newcastle disease virus, Metapneumovirus
Caliciviridae	Hepatitis E virus, Norwalk agent	Orthomyxoviridae	Influenza viruses: A, B, and C
Togaviridae	Rubella virus Eastern equine encephalitis virus Western equine encephalitis virus	Bunyaviridae	Hantavirus California encephalitis virus Sandfly fever virus
Flaviviridae	Yellow fever virus, Dengue virus St. Louis encephalitis virus West Nile virus, Hepatitis C virus	Arenaviridae	Lymphocytic choriomeningitis virus Lassa fever virus South American hemorrhagic fever virus
Coronaviridae	Coronaviruses	Reoviridae	Rotavirus, Reovirus, Colorado tick fever (only RNA virus family to have ds DNA)
Rhabdoviridae	Rabies virus Vesicular stomatitis virus	Retroviridae	HTLV (Human T Lymphotropic virus) HIV (Human immunodeficiency virus)
Filoviridae	Marburg virus and Ebola virus		

Size of Viruses

- Size of the viruses is determined by
  - Ultrafiltration: Passage through membrane filters membrane of graded porosity
  - Ultracentrifugation
  - Electron microscopy
- Largest virus: Pox virus (300 nm)
- Smallest virus: Parvovirus (20 nm)

- **Largest:Poxviridae**
- **Smallest:Parvoviridae**

Shape of Viruses

Most of the viruses are roughly spherical except:

Rabies: Bullet shaped	Tobacco mosaic virus: Rod shaped	Rotavirus: Wheel shaped
Pox virus: Brick shaped	Adenovirus: Space vehicle shaped	Astrovirus: Star shaped peplomers
Ebola virus: Filamentous shaped	Corona virus: Petal shaped peplomers	

Genome size
- Largest genome-Retroviridae (7–115 kb)
- Smallest genome-Hepatitis D (1.6 kb) < Hepadnaviridae (3.2 kb) < Parvoviridae (5.6 kb)

Viral Structure

Viruses consist of nucleocapsid (nucleic acid and capsid), which is further surrounded by envelope (in some viruses). Capsid is a protein layer; is made up capsomer units.

Nucleic Acid: Viruses possess either DNA or RNA, but never both

- Genomic size:
  - Largest is retroviruses (7–11.5 kbp),
  - Smallest Hepatitis D (1.7 kb) followed by Hepatitis B (3.2 kbp)
- All the DNA viruses are double stranded, except Parvoviruses (the only SS DNA virus)
- All the RNA viruses have one copy of single stranded unsegmented RNA except:
  - Reo viruses (the only double stranded RNA virus)
  - Retroviruses including HIV (possess 2 copies of SS RNA)
- ***Segmented RNA Viruses (code-BIRA)***
  - Bunya virus (3 segments)
  - Influenza virus (8 segments),
  - Rotavirus (11 segments),
  - Arenavirus (2 segments) e.g. LCM i.e. lymphocytic choriomeningitis virus
- **Most RNA viruses possess positive sense RNA** except: Myxoviruses, Rabies, Filoviruses, Bunyaviruses and Arenaviruses (bear negative sense SS RNA).

Segmented RNA Viruses (code-BIRA)
- Bunya virus
- Influenza virus
- Rotavirus
- Arenavirus

Symmetry

- Icosahedron symmetry: E.g. All DNA viruses (except Pox) and most of the RNA viruses
- Helical symmetry: Few RNA viruses (Myxo, Rhabdo, Filoviridae, Bunya) (MRF-Bat)
- Pox: Complex symmetry.

Envelope

Certain viruses possess an envelope surrounding the nucleocapsid. Envelope is lipoprotein in nature.

- Made up lipoprotein subunits called peplomere
  - Lipid part is host cell membrane-derived and protein part is virus-derived,
  - Envelop provides chemical, physical and biological properties to cell.
- Enveloped Viruses are ether sensitive, heat labile, pleomorphic.
- Example – All, other than nonenveloped viruses are enveloped virus (See below).

Nonenveloped Virus

- Ether resistant, heat stable and nonpleomorphic
- DNA: Parvovirus, Adenovirus, Papovavirus (PAP)
- RNA: Picorna, Astrovirus, Reovirus, Calicivirus, and Hepatitis A and E.

VIRAL REPLICATION

Viruses do not undergo binary fission (seen in bacteria), but undergo a complex way of cell division. Replication of viruses passes through six sequential steps:

1. **Adsorption/attachment** is the first and the most specific step of viral replication. It involves receptor interactions between virus and host.
2. **Penetration:** After attachment, the virus particles penetrate into the host cells either by
   - *Phagocytosis (or viropexis)*-Through receptor mediated endocytosis
   - *Membrane fusion:* seen in HIV
   - *Injection of nucleic acid:* seen in bacteriophages
3. **Uncoating**: Capsid is lysed (due to host lysozymes) and the nucleic acid is released. This step is absent for bacteriophages.
4. **Biosynthesis** of various viral components: i) nucleic acid, ii) capsid protein, iii) enzymes iv) other regulatory proteins

   Site of Nucleic acid replication
   - In DNA viruses, the DNA replication occurs in the nucleus; except in poxviruses (cytoplasm).
   - In RNA viruses: The RNA replication occurs in cytoplasm; except in retroviruses and orthomyxoviruses (nucleus).
5. **Assembly:** Viral nucleic acid and proteins are packaged together to form progeny viruses (nucleocapsids).
   - DNA viruses are assembled in the nucleus except hepadnaviruses and poxviruses (in cytoplasm)
   - RNA viruses are assembled in the cytoplasm.
6. **Maturation**: Take place either in the nucleus or cytoplasm or membranes
7. **Release** of daughter virions occur either by:
   - *Lysis of the host cells* as shown by nonenveloped viruses and bacteriophages.
   - *Budding through host cell membrane* as shown by enveloped viruses.

Eclipse phase: It is the interval between entry of the virus into host cell till appearance of first infectious virus particle.

- During this period, the virus cannot be demonstrated inside the host cell.
- The duration of eclipse phase is about 15 to 30 minutes for bacteriophages and 15-30 hours for most of the animal viruses.

- All DNA viruses replicates in nucleus; except in poxviruses (cytoplasm).
- All RNA viruses replicates in cytoplasm; except in retroviruses and orthomyxoviruses (nucleus).

- **Virion:** contains capsid protein and nucleic acid
- **Prion:** consist of abnormal infectious protein molecules without nucleic acid.
- **Viroids:** comprise of naked, cyclical, small ssRNA without a capsid.

Viruses can be classified to:

- ***Virion:*** True viral particles (contain both capsid protein and nucleic acid)
- ***Prion:*** consist of abnormal infectious protein molecules without nucleic acid.
- ***Viroids*** comprise of naked, cyclical, small ssRNA without a capsid. They are mostly restricted to plants. They depend on host enzymes for replication.

VIRAL CULTIVATION

Viruses cannot be grown on artificial cell free media (However, grow in animals, eggs or tissue culture)

Animal Inoculation

Suckling Mice is used for cultivation of certain viruses such as Coxsackie and arboviruses.

- Coxsackie A-produces flaccid paralysis in mice
- Coxsackie B-produces spastic paralysis in mice

Embryonated Egg Inoculation

Embryonated egg has four sites for cultivation of virus

1. Chorioallantoic membrane (CAM): Few viruses produce lesions called pocks, e.g. Vaccinia, Variola, HSV 1 and 2

2. Yolk sac: Arboviruses (e.g. JEV, Saint Louis and West Nile virus), *Rickettsia, Chlamydia* and *Hemophilus ducreyi*.
3. Amniotic membrane: Influenza culture (for diagnosis)
4. Allantoic cavity: Used for vaccine preparation for Influenza, Yellow fever (17D), Rabies (Flury).

Tissue Culture

- Organ culture: Whole organ is used, tracheal ring used for coronaviruses
- Explant culture: Minced organ is used, e.g. adenoid explant used for Adenovirus
- Cell Line: Tissues are completely digested and the individual cells are mixed with viral growth medium and dispensed in tissue culture flask.

Types of Cell Lines
Primary cell line
- Rhesus Kidney cell line
- Human amniotic cell line
- Chick embryo fibroblast

Secondary cell line
- Human fibroblast cell line
- MRC-5 and WI-38 cell line

Continuous cell line
- HeLa cell line
- HEp-2 cell line
- KB cell line
- McCoy cell line
- Vero cell line
- BHK cell line
- Detroit 6 cell line
- Chang C/I/L/K cell line

Types of Cell Lines
Primary cell line Undergo limited divisions (5–10), possess diploid karyosome. Examples include: • Rhesus Kidney cell line-used for myxoviruses, enteroviruses and adenoviruses • Human amniotic cell line, Chick embryo fibroblast
Secondary cell line Undergo moderate cell divisions (10–50), possess diploid karyosome. Examples include: • Human fibroblast cell line used for CMV • MRC-5 and WI-38 (human embryonic lung cell strain) • Used for preparation of various viral vaccine, e.g. for rabies, Chickenpox, Hepatitis-A and MMR vaccines • They also support the growth of HSV, VZV, CMV, adenoviruses, and picornaviruses.
Continuous cell line Derived from cancerous cells, hence have indefinite divisions and possess haploid karyosome. They are easy to maintain in the laboratories by serial sub culturing, hence widely used. • HeLa cell line (Human carcinoma of cervix cell line) • HEp-2 cell line (Human epithelioma of larynx cell line): used for RSV, adenoviruses and HSV • KB cell line (Human carcinoma of nasopharynx cell line) • McCoy cell line (Human synovial carcinoma cell line): useful for isolation of viruses as well as Chlamydia • Vero cell line (Vervet monkey kidney cell line): used for rabies vaccine production. • BHK cell line (Baby hamster kidney cell line) • Detroit 6 cell line (Sternal marrow cell line) • Chang C/I/L/K cell line: Human conjunctiva (C), Intestine (I), liver (L), and kidney (K) cell line

Detection of Viral Growth in Cell Line

- **Cytopathic effect (CPE)**
  - It is defined as the morphological change produced by the virus in the cell line detected by light microscope.
- **Viral Interference:** The growth of a non-CPE virus in cell culture can be detected by the subsequent challenge to the cell line with a known CPE virus. The growth of the first virus inhibit infection by the second virus. For example, rubella is a non-CPE virus but prevents the replication of enteroviruses which are known to produce CPE.
- **To detect viral antigens** in infected cell line- i) Direct IF assay, ii) Immunoperoxidase staining, iii) Hemadsorption.
- **Electron microscopy:** to detect viral particles in infected cell lines
- **Viral genes detection:** by using PCR or nucleic acid probes.

Type of Cytopathic Effect (CPE)	Virus
Rapid crenation and degeneration of the entire cell sheet	Enteroviruses
Syncytium or multinucleated giant cell formation	Measles, RSV, HSV
Diffuse roundening and ballooning of the cell line	HSV
Cytoplasmic vacuolations	SV 40 (Simian vacuolating virus-40)
Large granular clumps resembling bunches of grapes	Adenovirus

Inclusion body

They are the aggregates of virions or viral proteins and other products of viral replication by which they can be demonstrated in virus infected cells under the light microscope.

Intracytoplasmic inclusion bodies	Intranuclear inclusion bodies
Negri bodies–seen in Rabies virus Paschen body–seen in Variola virus Guarnieri bodies–seen in Vaccinia virus Bollinger bodies–seen in Fowl pox virus Molluscum bodies–seen in Molluscum contagiosum virus Perinuclear cytoplasmic body- seen in Reovirus	A) Cowdry type A inclusions • Torres body–seen in Yellow fever • Lipschultz body–seen in Herpes simplex B) Cowdry type B inclusions–seen in Poliovirus (non specific, may be illusory type)
Intracytoplasmic and intranuclear inclusion bodies: Seen in Measles and Cytomegalovirus (Owl's eye appearance)	

Intracytoplasmic inclusion bodies
- Negri bodies–seen in Rabies virus
- Paschen body–seen in Variola virus
- Guarnieri bodies–seen in Vaccinia virus
- Bollinger bodies–seen in Fowl pox virus
- Molluscum bodies–seen in Molluscum contagiosum virus
- Perinuclear cytoplasmic body-seen in Reovirus

Assay of Infectivity of Viruses

Physical Methods: These methods estimate the total virus count (or viral antigen or gene count) and cannot distinguish between infectious and noninfectious virus particles.

- Real time PCR
- Antigen detection assay
- Electron microscopy

Biological Methods: Detect the infectious virions. Example include:

- Qualitative assay (end point biological assays)
- Quantitative assays (plaque assay and pock assay)

Persistent Viral Infection

Some viruses undergo a period of latency, which may be of various types:

- *Latent infection with periodic exacerbations*: Seen with Herpesviridiae family
- *Cell Transformation*: Oncogenic viruses
- *Latency seen in HIV infection:* Viral genome integrates with host cell chromosome and leads to clinical latency
- *Latency seen in slow virus infection*: They have unsual long incubation period (years)
- *Persistent tolerant infection*: The classical example is lymphocytic choriomeningitis virus infecting mice. Here, the host is immunologically tolerant to the virus, doesnot show any immune response, but the virus is readily demonstrable in the tissues.

Transplacental Transfer of Viruses

- Teratogenic Virus (viruses causing fetal malformation): CMV, Rubella, Herpes, Varicella and Parvovirus B19
- Viruses Transfer through placenta but does not cause fetal malformation: Hepatitis B and C, HIV, Coxsackie B, Measles and Mumps.

Interferons

Interferons (IFNs) are the cytokines, produced by host cells on induction by viral or nonviral inducers.

- **Classification**: Interferons are classified into three groups, designated as IFN-α, β and γ.
- **Mechanism of action:** IFN has *no direct action* on viruses and it does not protect the virus-infected cell that produces it. However, it induces the other host cells to produce certain proteins called translation inhibition proteins (TIPs), that inhibit viral protein synthesis by selectively inhibiting the translation of viral mRNA, without affecting cellular mRNA.
- **IFNs are host specific but not virus specific**

- **Inducers**: both viral and nonviral agents can induce IFN synthesis. In general, RNA viruses and avirulent viruses are strong inducers. Examples of potent inducers are:
  - Viruses: Togaviruses, vesicular stomatitis virus, Sendai virus and NDV (New Castle Disease virus)
  - Nucleic acids (double-standard RNA)
  - Synthetic polymers (e.g. Poly I:C)
  - Bacterial endotoxin
- **IFN induction is much quicker** than the antibody response: IFN synthesis begins within about an hour of induction and reaches high levels in 6–12 hours
- **Resistance**: IFNs are proteins, hence inactivated by proteases, but not by nucleases or lipases. They are heat stable and also stable to wide ranges of pH (except IFN-γ).
- **Interferon assay**: is based on their biological activity. Being poorly antigenic, they cannot be detected serologically.

Application of IFN

- IFN-α is used in:
  - Topically: in rhinovirus infection, genital warts and herpetic keratitis.
  - Systemically: in chronic hepatitis B, C and D infections, hairy cell leukemia and Kaposi's sarcoma
- IFN-β: It is used in multiple sclerosis
- IFN-γ: It is used in chronic granulomatous disease and osteopetrosis.

Property	IFN-α	IFN-β	IFN-γ
Formerly called as	Leukocyte IFN	Fibroblast IFN	Immune IFN
Type of designation	Type I	Type I	Type II
Produced by host cell	Most cell types (Mainly macrophages)	Most cell types-(mainly fibroblasts)	Lymphocytes (mainly T_H1 cells, rarely CD8 T cells, NK cell)
Inducing agent	Viruses; dsRNA	Viruses; dsRNA	Mitogens
Action	Antiviral action ↑MHC-I expression Activates NK cells Antiproliferative function	Antiviral action ↑MHC-I expression Activates NK cells Antiproliferative	1) Immunoregulatory function- Stimulates macrophages ↑MHC-I & II expression 2) Anti-proliferative function
Stability at pH 2.0	Stable	Stable	Labile
Chromosomal location of genes	9	9	12
IFN receptor	IFN -α/β receptor	IFN -α/β receptor	IFN-γ receptor
IFN receptor genes located on chromosome number	21	21	6

Viral vaccines and their preparation source			
Inactivated vaccine		**Examples**	**Derived from**
Rabies			
	Rabies Neural Vaccine	Semple vaccine	Sheep brain derived, inactivated with phenol
		BPL vaccine	Sheep brain derived, beta propiolactone inactivated
		Infant mouse brain vaccine	Neural tissue of newborn mice
	Rabies Non-neural Vaccine	PCEV (purified chick-embryo cell) vaccine	Chicken fibroblast cell line
		HDC (human diploid cell) vaccine	Human fetal lung fibroblast cell line(WI-38 & MRC-5)
		Purified Vero cell (PVC) vaccine	Vero cell line
Kyasanur Forest Disease (KFD)		Killed KFD Vaccine	Formalin-inactivated chick embryo vaccine
		Subunit vaccine	
Hepatitis B		HBsAg (Hepatitis B surface antigen)	Yeast (recombinant DNA technology)
Papilloma		L1 protein	Yeast (recombinant DNA technology)

Contd...

Contd...

Both live and inactivated vaccines	Examples	Derived from
Poliovirus	Live Oral Polio Vaccine (OPV)	Poliovirus types 1,2,3
	Poliovirus types 1,2,3	Killed Injectable Polio Vaccine (IPV)
Japanese B encephalitis	Nakayama strain (killed)	Formalin inactivated mouse brain derived
	Formalin inactivated mouse brain derived	Beijing strain (killed) SA 14-14-2 strain (live)
	Primary hamster kidney cell line	
Influenza	Killed vaccine	Embryonated chicken egg
	Embryonated chicken egg	Live attenuated (intranasal)
Yellow fever	17D live attenuated	Embryonated chicken egg
	Mouse brain derived	Dakar strain (killed)
Hepatitis A	Inactivated	Human fetallung fibroblast cell line (WI-38 & MRC-5)
	Live attenuated	Human diploid cell line (H2 & L-A-1)
Live vaccines	**Examples**	**Derived from**
Mumps	Mumps-Jeryl-Lynn strain	Embryonated chicken eggs and chicken embryo fibroblast cell line
Measles	Edmonston-Zagreb Strain	Chicken embryo fibroblast cell line
Rubella	RA 27/3 Strain	Human fetallung fibroblast cell line (WI-38 & MRC-5)
Chickenpox	Oka-strain of varicella zoster (live attenuated)	Human fetallung fibroblast cell line (WI-38 & MRC-5)
Smallpox	Live vaccinia virus	Calf lymph
Rotavirus	Live attenuated	Vero cell line
Adenovirus	Live attenuated	Human fetal lung fibroblast cell line (WI-38 & MRC-5)

MULTIPLE CHOICE QUESTIONS

1. **Which one of the following vaccines is contraindicated in children with egg allergy?** *(APPG 2015)*
   a. MMR b. BCG
   c. DPT d. Yellow Fever
2. **Which of the following is an example of RNA oncogenic virus?** *(Recent MCQ 2013)*
   a. Retroviruses b. Reoviruses
   c. Coronaviruses d. Roboviruses
3. **Virus quantification is done by:** *(NEET Pattern Based)*
   a. Egg inoculation b. Hemadsorption
   c. Plaque assay d. Electron microscopy
4. **Brick-shaped virus:** *(NEET Pattern Based)*
   a. Chicken pox b. Small pox
   c. CMV d. EBV
5. **Suckling mice is used for isolation of:** *(NEET Pattern Based)*
   a. Coxsackie virus b. Herpes
   c. Pox d. Adenovirus
6. **Human fibroblast cell line is used for cultivation of:** *(NEET Pattern Based)*
   a. Adenovirus b. Poliovirus
   c. CMV d. Measles
7. **Both intranuclear and cytoplasmic inclusion is seen in:** *(NEET Pattern Based)*
   a. Poxvirus b. Herpesvirus
   c. Measles virus d. Mumps virus
8. **True about interferon is:** *(NEET Pattern Based)*
   a. Host protein
   b. Viral protein
   c. Inactivated by nucleases
   d. Virus specific
9. **Which of the following is primary cell line?** *(NEET Pattern Based)*
   a. Chick embryo fibroblast
   b. HeLa cells
   c. Vero cells
   d. WI-38
10. **Continuous cell line for viruses not present for:** *(DNB Dec 2011)*
    a. Vero b. Hep2
    c. WI-38 d. Hela
11. **Viral inclusion bodies are all except:** *(DNB June 2010)*
    a. Psamomma bodies b. Molluscum
    c. Negri d. Bollinger
12. **Viral infection that is least transmitted transplacentally is:** *(AI 2011)*
    a. Hepatitis B b. Rubella
    c. Herpes simplex d. HIV
13. **Latency seen in viral infections:** *(PGI Dec 2008, June 2005)*
    a. HSV – II b. CMV
    c. Rotavirus d. HIV
    e. EBV
14. **Which of the following is not an RNA virus?** *(AIIMS Nov, 08)*
    a. Ebola
    b. Simian 40
    c. Rabies
    d. Vesicular stomatitis virus
15. **Both DNA and RNA are found in:** *(AIIMS May 2014)*
    a. All Bacteria b. Prion
    c. Viroid d. Plasmid
16. **All the following vaccines are developed from embryonated eggs except:** *(PGI May 2013)*
    a. Influenza
    b. Hepatitis-A
    c. Yellow fever
    d. Rabies
    e. CMV
17. **The following is not a live vaccine:** *(JIPMER 2014, 2013)*
    a. Measles b. BCG
    c. Yellow fever d. Salk's vaccine
18. **True about interferon:** *(NEET Pattern Based, PGI June 2015)*
    a. Viral protein
    b. Virus specific
    c. Host protein
    d. Inactivated by nucleases
19. **True about interferon:** *(PGI June 2005)*
    a. It is virus specific
    b. It is bacteria specific
    c. Produced from bacteria
    d. Effective against viral infection
    e. It is species specific
20. **Interferon gamma secreted by:** *(DNB Dec 2009)*
    a. Activated T-cell
    b. CD 8 cells
    c. RBC
    d. Neutrophils

EXPLANATIONS

1. **Ans. (d) (Yellow Fever)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p427
   Vaccines prepared in embryonated egg (e.g. yellow fever-17D, Rabies-Flury and Influenza vaccines) are contraindicated in persons having egg allergy.
2. **Ans. (a) (Retroviruses)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p413, Ananthanarayan 9/e p565
   - Oncogenic RNA viruses-HTLV1 (Retroviruses) and HCV.
3. **Ans. (c) (Plaque...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p429, Ananthanarayan 9/e p437, 8/e p436
   Assay of infectivity of viruses:
   - **Quantal assay**: End point biologic assays
   - **Quantitative infectivity assay**: Plaque assay and Pock assay
4. **Ans. (b) (Small...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p412, Ananthanarayan 9/e p429, 8/e p427
   - Most of the viruses are roughly spherical except
     - **Rabies:** Bullet shaped, Pox virus- Brick shaped, Tobaco mosaic virus– Rod shaped
     - **Adenovirus**: Space vehicle shaped, Ebola virus- Filamentous shaped
5. **Ans. (a) (Coxsackie...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p426, Ananthanarayan 9/e p452, 8/e p451
   - Suckling mice is used for isolation of Coxsackie and Arbovirus
6. **Ans. (c) (CMV)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p428, Ananthanarayan 9/e p434, 8/e p433
   - Human fibroblast cell line is used for cultivation of CMV
7. **Ans. (c) (Measles virus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p422, Ananthanarayan 9/e p452
   - Both intranuclear and cytoplasmic inclusion are seen in:- Measles and CMV
8. **Ans. (a) (Host...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p423, Ananthanarayan 9/e p452, 8/e p451
   - Interferons are Host specific protein, not virus specific.
   - Interferons are inactivated by proteolytic enzymes but resistant to nucleases and lipases.
9. **Ans. (a) (Chick embryo...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p428, Ananthanarayan 9/e p436
   - Examples of primary cell lines: Rhesus Kidney cell line, Human amniotic cell line, Chick embryo fibroblast
10. **Ans. (c) (WI-38)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p428, Ananthanarayan 9/e p436
    - *WI-38: Human embryonic lung cell strain is an example of Secondary cell line.*
11. **Ans. (a) (Psamomma bodies)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p422,
    - Psammoma bodies (PBs) are concentric lamellated calcified structures, observed most commonly in papillary thyroid carcinoma (PTC), meningioma, and papillary serous cystadenocarcinoma of ovary.
    - Other options are example of viral inclusion bodies seen in:
      - Molluscum body (Molluscum contagiosum virus)
      - Negri body (Rabies)
      - Bollinger body (Fowlpox)
12. **Ans. (a) (Hepatitis B)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p604
    - Rubella and HSV are teratogenic virus, so their transplacental transmission is expected to be high
    - HIV has an overall transplacental transmission of 30%
    - HBV can also transmit transplacentally, but among the options, HBV has least chance of transplacental transmission.
      - List of teratogenic viruses- refer chapter review.
      - List of viruses that transfer through Placenta – Refer chapter review
13. **Ans. (a), (b), (d), (e) (HSVII, CMV, HIV, EBV)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p421,
    Persistent Infections are characterized as those in which the virus is not cleared but remains in specific cells of infected individuals.

Responsible for 20% of human cancers. It is of 3 overlapping varieties.

1. *Latent infection:* Defined as a state of infection in which the virus is not replicating i.e. no demonstrable infectious virus between episodes of recurrent disease. For example, Refer chapter review.
2. *Chronic infection:* Characterized by the continued presence of infectious virus following the primary infection. For example, HBV, HCV
3. *Slow infection:* Characterized by a prolonged incubation period followed by progressive disease. Without any acute period of viral multiplication. For example, Slow viruses.

14. Ans. (b) (Simian 40) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p413, Ananthanarayan 9/e p552

- Simian Vacuolating virus (SV40) is an example for Papilloma virus (DNA virus) affecting monkey …..Ananthanarayan 9/e p552, 8/e p549
- Vesicular stomatitis virus and Rabies are example of Rhabdovirus (RNA virus)
- *Ebola virus belongs to Filoviridae (RNA Virus)*

15. Ans. (a) (All Bacteria) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p411, Ananthnarayan 9/e p428, 442

Refer chapter review.

16. Ans. (b) (e) (Hepatitis-A, CMV) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p413, Ananthnarayan 9/e p434

The vaccines prepared in embryonated eggs are- Influenza vaccine, Rabies (Flury vaccine) and Yellow fever 17 D vaccine. Site of inoculation in egg for all the above vaccine production- Allanotic sac.

17. Ans. (d) Salk's vaccine Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p431, Ananthnarayan 9/e p83-84

18. Ans. (c) (Host protein) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p160, 423, Ananthanarayan 9/e p449

Detail about interferon-refer chapter review

19. Ans. (d) (e) (Effective against viral infection, It is species specific) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p160, 423, Ananthanarayan 9/e p449, 8/e p447

Detail about interferon-refer chapter review

20. Ans. (a) (Activated T- cell) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p160, 423, Ananthanarayan 9/e p449

Detail about interferon-refer chapter review

CHAPTER

4.2

Herpesviruses and Other DNA Viruses

HERPESVIRUSES

The members of Herpesviridae possess the following properties:

- Large (150–200 nm size), spherical in shape with icosahedral symmetry.
- Tegument: It is an amorphous, asymmetric structure present between the capsid and envelope
- Establish **latent or persistent infections** in their hosts and undergo periodic reactivation
- Possess dsDNA and replicates by *rolling circle* mechanism.

Subfamily	Duration of replication (Cytopathology)	Site of latency	Species
Alpha	Short (12-18 hours) Cytolytic	Neurons	Herpes simplex virus type 1 and 2 Varicella-zoster virus
Beta	Long (> 24 hours), Cytomegalic	Glands, Kidneys	Cytomegalovirus
	Long (> 24 hours) Lymphoproliferative	T-cells	Human herpesvirus 6 and 7
Gamma	Variable, Lymphoproliferative	B-Cells	Epstein-Barr virus and Human herpesvirus 8

Nucleic acid:
Herpesviruses have linear dsDNA which replicates by rolling circle mechanism.

Transmission:
- HSV-1: Direct contact with mucosa or abraded skin
- HSV-2: Sexual mode or vertical mode.

Latency:
- HSV-1: Trigeminal ganglia
- HSV-2: Sacral ganglia.

HERPES SIMPLEX VIRUS

Properties	Herpes simplex virus-1	Herpes simplex virus-2
Transmission	Direct contact with mucosa or abraded skin	Sexual mode or vertical mode
Latency	Trigeminal ganglia	Sacral ganglia
Age affected	Young children	Young adults
Antibody distribution in adults	Present in 70–90% of people	Present in 20% of people
Common manifestations	• Oralfacial mucosal lesions • Encephalitis, meningitis and ocular lesions • Skin lesions: Above the waist	• Genital lesions • Skin lesions: Below the waist • Neonatal Herpes
Egg (CAM*)	Forms smaller pocks	Forms larger pocks
Chick embryo fibroblast	Does not grow well	Replicates well
Neurovirulence	Less	More
Drug resistance	Less	More
Antigenic homology	HSV 1 and 2 show > 80% antigenic homology	
Genomic sequence homology	HSV 1 and 2 show > 50% homology in the genomic sequence	

Clinical Manifestations

The incubation period ranges from 1 to 26 days (median, 6–8 days).

Orofacialmucosal lesions: They are the most common manifestations of HSV.

- Most common affected site is buccal mucosa
- Most frequent primary lesions are gingivo stomatitis and pharyngitis.
- Most frequent recurrent lesion is herpes labialis (painful vesicles near lips).

CNS Infections of HSV:
- HSV is the MC cause of acute sporadic viral encephalitis (MC site temporal lobe)
- Mollaret's meningitis.

Nervous system

- *Encephalitis:* HSV is the MC cause of acute sporadic viral encephalitis (MC site temporal lobe), HSV-1 causes > 95% of cases.
  - Children get primary infection: HSV is acquired exogenously and invades CNS via the olfactory bulb
  - Adults get recurrent infections due to reactivation of HSV in trigeminal nerve.
- *Meningitis:* HSV can cause recurrent lymphocytic meningitis called as *Mollaret's meningitis*
- *Other manifestations:*
  - Autonomous nervous system involvement (sacral region)
  - Transverse myelitis
  - Guillain-Barré syndrome
  - Peripheral nervous system involvement (e.g. Bell's palsy)

Cutaneous lesions: HSV usually infects through abraded skin and causes various lesions.

- *Herpetic whitlow*: Lesions present on the fingers of dentists and hospital personnel.
- *Febrile blisters*:
- *Herpes gladiatorum:* Mucocutaneous lesions present on the body of wrestlers.
- *Eczema herpeticum:* Caused by HSV-1 in patients with chronic eczema. Similar lesions are also produced by vaccinia virus infection; both conditions together are designated as *Kaposi's varicelliform eruptions*.
- *Erythema multiforme:* HSV is the most common to be associated.

Cutaneous lesions due to HSV:
- Herpetic whitlow
- Febrile blisters
- Herpes gladiatorum
- Eczema herpeticum
- Erythema multiforme

Ocular lesions: HSV-1 is more common than HSV-2 to infect eyes.

- Severe conjunctivitis is the most common manifestations
- Recurrent lesions develop in to *dendritic ulcers* of cornea or vesicles on the eyelids.
- Corneal blindness:

Genital lesions: HSV-2 is more common than HSV-1 to cause primary as well as recurrent genital lesions.

- Genital lesions are described as bilateral, painful multiple, tiny vesicular ulcers
- This may be associated with fever and inguinal lymphadenopathy.

Genital lesions due to HSV:
- Described as bilateral, painful multiple, tiny vesicular ulcers
- HSV-2 is more common

Visceral and disseminated herpes

- *Risk factors*: Immunocompromised patients, underlying malnutrition or AIDS, pregnancy.
- *Common manifestations* include: Pneumonitis, tracheobronchitis and hepatitis.

Neonatal Herpes: HSV is one of the common causes of congenital infections, along with the other TORCH agents.

- *Transmission:* Can also occur in utero or after birth; but MC being during birth.
- *Risk* of developing neonatal herpes is maximum (10 times more) if the mother recently acquires the virus (primary infection).
- *HSV-2* is more common to cause neonatal herpes (75% of total cases) than HSV-1.
- Have a higher tendency to develop visceral infections.

Laboratory Diagnosis

- **Cytopathology**: **Tzanck smear** preparation by Wright's or Giemsa stain:
  - Detects inclusion bodies (Lipschultz body) and formation of multinucleated giant cells, ballooning of infected cells
  - It cannot differentiate between HSV-1, HSV-2, and VZV
  - Sensitivity of staining is low (< 30% for mucosal swabs).
- **Virus isolation**: Remains the most definitive tool for HSV diagnosis
  - Conventional cell lines: Detects diffuse rounding and ballooning of cell lines.
- **Viral antigen detection:** in specimen by direct IF. It is sensitive, specific and can differentiate HSV-1 from HSV-2.
- **HSV DNA detection by PCR** the most sensitive test and can differentiate HSV-1 and HSV-2.
- **Antibody detection** by ELISA or other formats
  - Most available tests usually detect IgG or total antibodies, hence cannot differentiate recent and past infections.
  - Serologic assays based on the type-specific antigens can differentiate between HSV-1 and HSV-2.

Tzanck smear of Herpes lesions shows:
- Inclusion bodies (Lipschultz body)
- Multinucleated giant cells
- Ballooning of infected cells

Treatment

- Acyclovir is the drug of choice. It acts by inhibiting viral DNA polymerase.
- Foscarnet is the DOC for acyclovir resistance cases.

VARICELLA ZOSTER VIRUS

VZV produces vesicular rashes on the skin and mucous membranes in two forms:

- **Chickenpox:** Diffuse bilateral vesicular rashes, occur following primary infection, usually affecting children.
- **Zoster or Shingles:** Occur following reactivation of latent VZV in the ophthalmic branch of trigeminal ganglia (which is also the site of latency of VZV). It occurs mainly in adult life. Vesicular rashes are unilateral and segmental.

VZV produces two types of infections:
- Chicken pox: Diffuse bilateral vesicular rashes, occur following primary infection in children.
- Zoster or Shingles: Occur in adults following reactivation of latent VZV. Vesicular rashes are unilateral and segmental.

Chickenpox

Clinical Manifestations

- Incubation period is about 10–21 days (2–3 weeks).
- Typical description of chickenpox rashes
  - Vesicular, bilateral, diffuse and centripetal (start on the face and trunk, spread rapidly to involve flexor surfaces).
  - Rashes appear in multiple crops, fever appears with each crop of rashes
- Chickenpox is a disease of childhood.
- When occurs in adults, it is more severe with bullous and hemorrhagic rash.

Complications

Complications are more common in adults and in immunocompromised individuals.

- MC infectious complication is: Secondary bacterial infection of the skin.
- **MC** extracutaneous complication: CNS involvement (cerebellar ataxia, encephalitis and aseptic meningitis): Usually occurs in children.
- **Most serious** complication is: Varicella pneumonia, develops commonly in pregnant women.
- **Reye's syndrome**: Fatty degeneration of liver following salicylate (aspirin) intake.

Chickenpox in pregnancy affects both mother and the fetus.

- **Mothers** are at high-risk of developing varicella **pneumonia**
- **Fetal or congenital varicella syndrome**: VZV is highly teratogenic.
  - Risk is maximum when mother acquires a primary infection during pregnancy.
  - Late first trimester/early second trimester: Congenital malformation in fetusis more frequent, characterized by *cicatricial skin lesions, limb hypoplasia* and *microcephaly*.
- **Infection near delivery**
  - If mother gets infection > 5 days before delivery: Then baby is mostly asymptomatic due to protective maternal Ab
  - If mother gets infection **before 5 days to after 2 days** of delivery: Maternal Ab would not have produced in such a short time. This leads to dissemination of virus in the baby to cause **neonatal varicella** (a severe form of chickenpox).

Complications of chickenpox:
- MC infectious complication: Secondary bacterial infection
- MC extracutaneous complication: CNS involvement
- Most serious complication is: Varicella pneumonia
- Following salicylate intake: Reye's syndrome.

Chickenpox in pregnancy:
- Late first trimester/early second trimester is associated worst outcome
- Infected at 6–12 weeks- max interruption with limb development
- Infected at 16–20 weeks- eye and brain involvement.

Epidemiology

Chickenpox is a highly contagious disease.

- Period of infectivity: 2 days before the onset of rash to 5 days after thereafter, until the vesicles are crusted.
- One attack gives lifelong immunity
- Reservoir: Humans are the only known reservoir hosts.
- Source of infection: Patients are the only source, there are no carriers.
- Secondary attack rate is about 70–90%.

Common features of MMR and 2 Pox (Mumps, Measles, Rubella, Chickenpox and Smallpox):
- No carrier
- Source of infection: Case
- No animal reservoir
- Humans are: only host
- High secondary attack rate
- All have live vaccines
- Once infected/vaccinated: probably get life long immunity.

Zoster or Shingles

Zoster usually occurs due to reactivation of latent VZV in old age (> 60 year age), in ↓immunity or rarely in healthy adults.

- **Rashes**: There are unilateral and segmental, confined to the area of skin supplied by the affected nerves.
- Complications
  - Post-herpetic neuralgia (pain at local site): MC complication in elderly patients.
  - Zoster ophthalmicus: Unilateral painful crops of skin rashes surrounding the eye.
  - Ramsay Hunt syndrome develops when geniculate ganglion of facial nerve is involved. It is characterized by tetrad of facial nerve palsy plus vesicle on tympanic membrane, external auditory meatus and the tongue.

Zoster ophthalmicus:
Unilateral painful crops of skin rashes surrounding the eye.

Vaccine

- Live attenuated vaccine using *Oka strain* of VZV is available.
- It is given to children after 1 year of age; 2 doses, first dose is given at 12–15 months and second dose at 4–6 yrs.

Treatment

- **Acyclovir** is the drug of choice. It can prevent the complications of chickenpox and can also halt the progression of zoster in adults, but cannot prevent post-herpetic neuralgia.
- **VZIG (Varicella zoster immunoglobulin)**
  - It is recommended for postexposure prophylaxis. It is given within 96 hrs (preferably within 72 hrs) of exposure.
  - It is also indicated for neonates born to mothers suffering from chickenpox if the onset of chickenpox in the mother is *between < 5 days before delivery till 48 hrs after delivery*. VZIG not indicated if the mother has zoster.

VZIG (Varicella zoster immunoglobulin):
- Given within 96 hrs of exposure
- Given to Neonates born to chickenpox mother: If the onset of chickenpox in the mother is between < 5 days before delivery till 48 hrs after delivery.

CYTOMEGALOVIRUS (CMV)

CMV is the largest virus in Herpesviridae family. It is so named because it causes massive enlargement of infected host cells.

- **Host specificity:** CMV are strictly species-specific.
- **Cell type specificity**: CMV infects kidney and salivary glands.
- **Cell–to-cell spread**: CMV is almost always closely associated with the cells and spread primarily cell-to-cell, so that very little virus may be cell-free.

Clinical Manifestations

Congenital CMV Infection

- CMV is probably the MC intrauterine infection associated with congenital defects, affecting near 1% of infants born.
- Cytomegalic inclusion disease develops in about 5% of the infected fetus. The remaining are although asymptomatic at birth, 5–25% of them may develop significant psychomotor, hearing, ocular, or dental defects within 2 years.
- **Congenital defects** include:
  - MC defects are petechiae, hepatosplenomegaly, and jaundice.
  - Less common: Microcephaly, cerebral calcifications, IUGR, and prematurity.
- **Risk is maximum** if the infection occurs in early pregnancy and if the mother is primarily infected during pregnancy.

Congenital CMV Infection:
- CMV is probably the MC intrauterine infection
- Affects near 1% of infants born
- 5% of the infected fetus develops disease.

Perinatal CMV Infection

- Transmission to newborn occurs during: (i) Delivery, (ii) Postnatal—through infected breast milk/secretion of mother.

- Mostly asymptomatic, but shed virus in urine up to several years.
- Few infants, especially premature babies develop interstitial pneumonitis.

Immunocompetent Adults

- **Mononucleosis like syndrome** develops in healthy adults following blood transfusion.

	Infectious mononucleosis	Mononucleosis like syndrome
Agent	Epstein Barr Virus (EBV)	CMV (20-50%) HHV-6, Toxoplasma, Ehrlichia, HIV
Atypical lymphocytosis	Seen	Seen
Clinical symptoms	Fever, myalgia, rashes Hepatosplenomegaly Exudative pharyngitis, Cervical lymphadenopathy	Similar presentation, except that exudative pharyngitis, cervical lymphadenopathy are absent
Heterophile antibodies	Elevated (Paul-Bunnell test)	Negative
Specific antibodies	Antibodies to specific EBV antigens are elevated	Antibodies to CMV or other agents may be elevated.

Mononucleosis like syndrome:
- MC cause is CMV (20–50%)
- Atypical lymphocytosis seen
- Heterophile antibodies (Paul Bunnell test) is Negative.

In the Immunocompromised Host

CMV produces severe infection in immunosuppressed individuals; due to reactivation of latent CMV viruses.

- **In untreated AIDS patients** with CD4 T-cell count < 50/µl—CMV may cause Chorioretinitis (MC presentation), gastroenteritis and dementia.
- **Organ transplant recipients**: CMV is probably the MC viral infection in transplant recipients.
  - CMV infection occurs usually between *1 to 4 months* following transplantation.
  - Bilateral interstitial pneumonia in bone marrow transplant recipients.

CMV is the MC viral infection in transplant recipients:
- Bilateral interstitial pneumonia in BM transplants
- Febrile leukopenia in solid organ transplants
- Obliterative bronchiolitis in lung transplants
- Graft atherosclerosis in heart transplants
- Rejection of renal allografts.

Epidemiology

- **Transmission:** In contrast to HSV, CMV transmission requires **c**lose contact
  - Oral and respiratory contact is the predominant mode
  - Others-Transplacental, blood transfusion (risk is 0.1–10%) and sexual
- **Reservoir**: Humans are the only known host for CMV.
- **Prevalence:** In under developed nations, 90% of people being seropositive in contrast to 40–70% in developed nations.

Laboratory Diagnosis

- **Detection of inclusion bodies** in urine: CMV produces both perinuclear cytoplasmic and intranuclear inclusions (describe as Owl's eye appearance)
- **Virus Isolation**: CMV can be isolated from throat washings and urine.
  - *Human fibroblasts* are the most ideal cell lines, growth occurs in 2–3 weeks.
  - Shell vial technique: Used to detect growth in 4–5 days.
- **Antibody detection**:
  - IgM antibodies are indicative of active infection.
  - Four fold rise of IgG indicates recurrent infection.
  - Antibodies are often undetectable in immunocompromised patients.
- **Antigen detection**: CMV-specific **pp65** antigens. It is highly specific and reliable method
- **PCR** detects specific CMV DNA in blood or body fluids such as CSF.

CMV Isolation:
- Specimen: Washings and urine.
- Cell line: Human fibroblasts
- CPE: Owl's eye appearance in 2–3 weeks.

Treatment

CMV does not respond to acyclovir.

- **Ganciclovir** is the DOC for cytomegalic inclusion disease or retinitis or transplant infections.
- **Others:** Valganciclovir (given orally), foscarnet (DOC in ganciclovir-resistant cases), cidofovir and CMV Ig.

EPSTEIN-BARR VIRUS

Pathogenesis

EBV is transmitted by oropharyngeal contact through infected salivary secretions

- **Binds to EBV receptors**: Complement receptors (CD21 or CR2) on B-cell and pharyngeal epithelial cells.
- **Primary infection** occurs in the oropharynx. EBV replicates in epithelial cells or B-lymphocytes of the pharynx and salivary glands.
- **Inside** B-cells, EBV directly enters into latent phase.
- **Pathogenesis in children,** developing infectious mononucleosis:
  - Infected B-cells become *immortalized* by the virus and synthesize large number of variety of immunoglobulins (polyclonal), many of those are autoantibodies
  - In response to this, the bystander CD8 T-lymphocytes are stimulated and appear atypical.
- **Pathogenesis in people developing EBV induced cancers (*Mechanism of oncogenesis):*** EBV can induce malignant transformation of infected B-cells and epithelial cells by expressing latent EBV antigens such as LMP and EBNA.
  - **LMP-1** (latent membrane protein-1) is the most important viral oncogene.
  - **Viral EBNA-2** (EBV nuclear antigen-2) activates host cell *cyclin-D*, thus promotes cell proliferation.

Oncogenesis in EBV is due to:
- LMP-1 (latent membrane protein-1)
- Viral EBNA-2.

Clinical Manifestations

Infectious Mononucleosis

It is also called as kissing disease (salivary contact) or glandular disease

- Age-Young adults of developed countries are usually affected.
- It is characterized by:
  - Headache, fever, malaise and Exudative pharyngitis
  - Cervical lymphadenopathy and Hepatosplenomegaly
  - Rashes following ampicillin therapy
  - Atypical lymphocytosis (CD8 T-cells)
  - Autoantibodies reactive to sheep RBC antigens (detected by Paul-Bunnell test).

EBV Associated Malignancies

EBV is associated with several malignancies:

- **Burkitt's lymphoma** (tumor of the jaw seen in children and young adults): EBV is associated with 90% of African and 20% of non-African cases of Burkitt's lymphoma.
  - Most of the cases have pre-existing mutation [t(8;14)] that in turn activates the growth promoting MYC oncogene
  - Falciparum malaria may impair host CMI and stimulates the EBV-infected B-cells
- **Nasopharyngeal Carcinoma**: Seen among *Chinese* having h/o intake of *salted fish* (nitrosamine) and herbal snuff (phorbol ester)
- **Hodgkin's lymphoma** (especially the mixed-cellularity type).
- **NHL (Non-Hodgkin's lymphoma):** All central nervous system non-Hodgkin's lymphomas and 50% of systemic non-Hodgkin's lymphomas are EBV positive.

EBV associated malignancies:
- Burkitt's lymphoma
- Nasopharyngeal Carcinoma
- Hodgkin's lymphoma
- NHL (Non-Hodgkin's lymphoma).

Other Conditions Associated with EBV

- Lymphoproliferative disorder such as *Duncan syndrome,* an X-linked recessive disease affecting young boys.
- Hairy cell leukoplakia: Wart-like growth of epithelial cells of the tongue developed in some HIV-infected patients and transplant recipients
- Chronic fatigue syndrome

Laboratory Diagnosis

Heterophile Antibody Detection by Paul-Bunnell Test

- It is a heterophile agglutination test that uses sheep RBCs to detect heterophile antibodies in patient's serum
- Differential absorption test and Monospot test are done for confirmation.

EBV Specific Antibody Detection

- **Antibody to viral capsid antigen** (VCA):
  - IgM type: It indicates current infection.
  - IgG type: It is a marker of past infection and indicates immunity.
- **Antibodies to early antigen** also indicate current viral infection.
  - EA-D antibody (antibody to early antigen that occurs in diffuse pattern in nucleus and cytoplasm of the infected cells): It is elevated in *acute infection and Burkitt's lymphoma*
  - EA-R antibody, antibody to early antigen restricted to the cytoplasm: It is elevated in *nasopharyngeal carcinoma*
- **Antibodies to EBNA** (EBV nuclear antigen) reveal past infection, but four fold rise of titer suggest current infection.

EBV Specific antibody detection:
- Antibody to viral capsid antigen (VCA)
- Antibodies to early antigen
- Antibodies to EBNA (EBV nuclear antigen)

Treatment

- **Acyclovir** is useful in the treatment of oral hairy leukoplakia, but not effective for infectious mononucleosis and other malignancies.
- **Antibody to CD20** (rituximab) has been effective in some cases.

HUMAN HERPESVIRUS-6

HHV-6 infects the T-cells by binding to CD46 receptor. It has two variants 6A and 6B.

- **Sixth disease**: In children, HHV-6 (usually the 6B variant) causes sixth disease, also called as exanthema subitum or roseola infantum.
- In older age groups, HHV-6 has been associated with mononucleosis-like syndrome.

HUMAN HERPESVIRUS-8

HHV-8 is also called as Kaposi's sarcoma-associated herpesvirus (KSHV)

- **Epidemiology**
  - In high prevalence area: HHV-8 is endemic in Africa where it is transmitted by oral secretion.
  - In low prevalence areas such as North America, Asia, northern Europe, it affects homosexual
  - Less common mode: Organ transplantation, IV drug abuse, and blood transfusion
- **Manifestations:** In immunocompromised individuals (e.g. HIV), HHV-8 causes:
  - Kaposi sarcoma
  - Primary effusion lymphoma (body cavity based lymphomas)
  - Castleman's disease (lymphoproliferative disorder of B-cells)
- However, in immunocompetent host, HHV-8 produces fever and rash
- **Treatment:** HHV infections respond well to foscarnet, ganciclovir, and cidofovir.

HHV-8 is associated with:
- Kaposi sarcoma
- Primary effusion lymphoma (body cavity based lymphomas)
- Castleman's disease (lymphoproliferative disorder of B cells)

OTHER DNA VIRUSES

PARVOVIRUSES

- Parvoviruses are the smallest viruses (18–26 nm size).
- Nonenveloped with icosahedral symmetry
- Possess linear single-stranded DNA, (the only DNA virus to have ssDNA).

Parvoviruses:
- Smallest viruses
- The only DNA virus to have ssDNA.

- **Transmission:** Parvovirus B19 exclusively infects humans, most commonly by the respiratory route, followed by blood transfusion and transplacental transmission (occurs in 30% of cases with maximum risk is in the second trimester).
- **Tropism for RBCs:** It infects RBC precursors by using P blood group Ag as receptor.
- **Genotyping:** Parvovirus B19 has three genotypes but single serotype. Genotype 1 is the MC in world and genotype 3 is MC in western Africa.

Syndrome	Host or condition	Clinical feature
Erythema infectiosum	Children (fifth disease)	Slapped cheek rashes
	Adults	Polyarthropathy
Transient aplastic crisis	Underlying hemolytic anemia	Severe acute anemia
Pure red cell aplasia	Underlying immunosuppression	Chronic anemia
Hydrops fetalis	Fetus	Fatal anemia
Papular purpuric gloves and socks syndrome	Young adult	Painful redness and swelling of the feet and hands

Polyomaviruses of human importance:
- JC virus: Causes Progressive Multifocal Leukoencephalopathy
- BK virus: Causes nephropathy in transplant recipients
- Merkel cell virus: Causes Merkel cell carcinoma of skin.

PAPILLOMAVIRIDAE AND POLYOMAVIRIDAE

The formerly known Papovaviridae family are currently separated into two different families

- Family Papillomaviridae has 16 genera, out of which *Human papillomavirus* infects man.
- Family Polyomaviridae has several genera infecting animals. Human infections are associated with:
  - **JC virus** causes Progressive Multifocal Leukoencephalopathy (PML)
  - **BK virus** causes nephropathy in transplant recipients. It differs from JC virus, by its ability to grow in a wide range of cell lines and is less oncogenic.
  - **Merkel** cell virus causes Merkel cell carcinoma of skin
  - **SV40 virus** (Simian vacuolating 40 virus) is nonpathogen to man.

Human Papillomavirus (HPV)

HPV has selective tropism for epithelium of skin and mucous membranes and produces an array of infections.

Products of early genes E6 and E7 have oncogenic potential by following ways:

- E6 protein facilitates the degradation of the p53 tumor-suppressor protein
- E7 protein binds to the retinoblastoma gene product and related proteins.

Oncogenecity in HPV is due to:
- E6 protein facilitates the degradation of the p53 tumor-suppressor protein
- E7 protein binds to the retinoblastoma gene product and related proteins.

Pathogenesis

HPV has tropism for skin (squamous epithelium) and mucous membranes and produce various benign and malignant lesions

- **Benign warts**
  - Common warts (*verruca vulgaris*) and flat warts (verruca plana) in children.
  - Plantar warts (*verruca plantaris*) in young adults
  - Anogenital warts (*condyloma acuminatum*) seen among adults.
- **Epidermodysplasia verruciformis**
- **Cervix lesions**
  - CIN (Cervical Intraepithelial Neoplasia) is a benign condition, associated with low-risk HPV types 6 and 11
  - Carcinoma cervix (squamous cell) is associated with high-risk HPV types such as 16, 18, 31, 33, and 45.
  - High-risk serotypes are associated with squamous cell carcinoma involving other genital regions such as penis, anus, vagina, and vulva.

Epidermodysplasia verruciformis:
- Rare autosomal recessive benign condition
- Rarely progresses to squamous cell malignancy (seen with HPV serotypes 5 and 8)
- Affects the sun-exposed areas.

- **Head and neck lesions**
  - Benign: Recurrent laryngeal papillomas in children are associated with low-risk serotypes 6 and 11.
  - Malignant: Laryngeal and esophageal carcinomas (associated with high-risk serotypes 16 and 18).
- **Pityriasis versicolor**–like lesions

HPV Vaccine

HPV vaccine is recommended to adolescent and young adult females.

Subunit vaccine consists of virus-like particles composed of HPV L1 proteins produced in yeast by DNA recombinant technology. Both quadrivalent and bivalent vaccines are licensed.

- *Quadrivalent vaccine:* Includes serotype 6, 11, 16 and 18 (Gardasil, Merck)
- *Bivalent vaccine* includes only the high-risk serotype 16 and 18 (Cervarix, GlaxoSmithKline)

Barrier methods of contraception can block sexual transmission thus prevent anogenital HPV infections.

POXVIRIDAE

Poxviruses are the largest (400 nm) viruses- large enough to be seen under light microscope.

- Most complex symmetry.
- Brick-shaped or ellipsoid
- Nucleocapsid consists of: Biconcave dumbbell shaped DNA core.
- It is the only DNA virus that replicates in the cytoplasm

Important members include: Variola, Vaccinia, Molluscum contageosum.

Smallpox (Variola)

Smallpox, a highly contagious severe exanthematus (rashes) disease, was the first disease to be eradicated from the world.

Smallpox time line:
- Last natural case of variola major: Bangladeshi women in Assam in 1975
- Last natural case of variola minor: Somalia, 1977
- Eradication was declared: on 8th May 1980.

Timeline

1. Last natural case of variola major was seen in a Bangladeshi women in Assam in May 1975
2. Last natural case of variola minor was seen in Merca, Somalia, 26th October 1977
3. **Eradication** was declared by WHO nearly after three years, i.e. on 8th May 1980
4. **Agent of bioterrorism**: As vaccination was stopped following eradication people borne after 1980 are not immunized. Hence smallpox virus can be a potential agent of bioterrorism.

Reasons that Made Eradication Successful

- Variola was an exclusively human pathogen, no animal reservoir
- Source: Patients were the only source, there was no carrier state
- Case detection was easy due to characteristic appearance of rashes
- Subclinical disease were not transmitting the disease
- Global smallpox eradication programme
- Highly affective live vaccinia vaccine:
  - Freeze dried form was used (↑stability)
  - Multiple puncture technique was followed to administer the vaccine by using abifurcated needle, which was simple, effective and economical.

Smallpox	Chickenpox
Incubation perio: 12 days (7–17 days)	Incubation period:15 days (10–21 days)
Rash: Palm and sole and extensor surface	Rash: Axilla and flexor surface
Rash: Deep seated and appear in single stage, evolution is slow, centrifugal distribution	Rash: Superficial and pleomorphic (appear in crops) evolution is rapid, dew drop rashes, centripetal distribution
Fever subsides with appearance of rash	Fever rises with each crop of rash

Ceiling temperature:
- Highest temperature beyond which the pock formation is inhibited on CAM.
- It is higher for vaccinia virus (41 °C) than variola virus (38 °C).

Vaccination

- **Live vaccinia vaccine** was highly effective.
  - It was given as single dose between 1-2 years of age.
  - As un-attenuated live virus was used, adverse reactions were common.
- **Cowpox vaccine** discovered by Edward Jenner (the father of vaccination)
- **Variolation** (Healthy people were inoculated with the skin scraping of a smallpox patient).

Vaccinia

Vaccinia differs from variola by:

- It is nonpathogenic to humans or produces milder skin lesions.
- Produces an inclusion body called as Guarnieri body (variola produces Paschen body)
- On CAM vaccinia virus produces larger and hemorrhagic and necrotic pock lesions.
- **Ceiling temperature** is the highest temperature beyond which the pock formation is inhibited on CAM. It is higher for vaccinia virus (41 °C) than variola virus (38 °C).
- Vaccinia but not variola can produce plaques on chick embryo tissue cultures.

Molluscum contagiosum:
- Produces pink pearly wart-like lesions with a characteristic dimple at the center (umbilicated).
- Lack of associated inflammation and necrosis
- Found anywhere except palms and soles.

Molluscum Contagiosum Virus

- **Lesions:** It produces pink pearly wart-like lesions with a characteristic dimple at the center (umbilicated).
  - Lack of associated inflammation and necrosis
  - Lesion are found anywhere on the body except on the palms and soles. Genital lesions are seen in adults.
- Transmission:
  - Children (MC): Spread by direct and indirect contact.
  - Rarely sexual transmission has been reported in young adults.
- Laboratory diagnosis:
  - Molluscum bodies are the intracytoplasmic eosinophilic inclusions seen in skin scrapings (histopathological stains)
  - Not cultivable: It cannot be propagated in tissue culture, egg or in animals.
- **Treatment:** Surgical removal of the lesions by ablation.
- **Prognosis:** Self-limiting, except in HIV.

Swimming associated diseases:
- Swimming pool conjunctivitis:
  - Adenovirus 3.7 and 14
  - C. trachomatis
- Swimming pool granuloma-Mycobacterium marinum
- Swimmer's itch: Schistosoma mansoni
- Swimmer's ear: Pseudomonas
- Swimming is also a risk factor for Naegleria.

ADENOVIRIDAE

- It is nonenveloped DNA virus, ***space vehicle*** shaped

Disease produced by adenovirus	Adenovirus serotype associated
Hemorrhagic cystitis	Adenovirus type 11 and 21 (boys)
Infant diarrhea	Adenovirus serotype 40, 41
Ocular Infections	
Epidemic keratoconjunctivitis	Adenovirus type 8,19, 37 (Shepard eye, industrial worker)
Pharyngoconjunctival fever or Swimming pool conjunctivitis (follicular)	Adenovirus type 3,7,14 (tends to occur in outbreaks, at children's summer camps)
Respiratory Diseases	
Upper respiratory tract infection	Serotypes 1, 2, 3 and 5
Pneumonia	Serotypes 3, 7, and 21
Acute respiratory disease syndrome	In military recruits, associated with type 4 and 7
Transplant recipients	Serotypes 34 and 35. ↑ risk to develop pneumonia, hepatitis, nephritis, colitis, encephalitis, and hemorrhagic cystitis

BACTERIOPHAGES

Bacteriophages are viruses that attack bacteria

- Bacteriophages are typically tadpole-shaped possessing a hexagonal head and a tail attached with tail fibers.
- Hexagonal head contains tightly coiled dsDNA, enclosed by capsid (protein coat)

Altered morphology may be seen in some phages:

- Shape: Spherical or filamentous instead of hexagonal.
- Nucleic acid: May contain ssDNA or RNA instead of dsDNA.

Life Cycle of Bacteriophage

Based on two types of life cycle bacteriophages are classified into: Lytic and lysogenic or temperate phage

- **Lytic phase:** Phage replicates in the cytoplasm and lyses the host bacterium to come out. It resembles the replication of other DNA viruses; except that:
  - In penetration step: Phages are attached to bacterial cell wall as 'ghosts'.
  - There is no uncoating step as seen with other viruses.
  - Release of the daughter phages occur by lysis of the host bacterium.
  - Duration of Eclipse phase is about 15 to 30 minutes; in contrast to 15-30 hours for most of the animal viruses.
- **Lysogenic or Temperate phage:** Phage DNA is incorporated within host DNA and remains dormant as prophage.

Lysogenic to lytic interconversion: When the temperate phages want to come out, they get excised from bacterial chromosome, then transform to lytic phages, multiply in the cytoplasm and are released by lysis.

Uses of Bacteriophage

- **Phage typing:** Phage typing is employed for typing the following bacteria:
  - Staphylococcus aureus
  - Vi antigen typing of *Salmonella* Typhi,
  - *Vibrio cholerae* (Basu Mukherjee phage typing)
  - *Brucella* (Tbilisi phage typing)
  - *Corynebacterium diphtheriae*
- **Phage assay**: To estimate the no. of viable phages in preparations.
- **Used in treatment (Phage therapy):** Lytic phages can kill the bacteria, hence may be used for treatment of bacterial infection, such as post-burn and wound infections.
- **Used in diagnosis**: Mycobacteriophages are used for the identification of *M.tuberculosis*.
- **Used as a cloning vector**.
- **Transduction**: In *S.aureus,* the plasmids coding for β-lactamases are transferred between the strains by transduction.
- Codes for toxins: The phage genomes code for the following bacterial toxins
  - **A**, C of Streptococcal pyrogenic toxin
  - **B**otulinum toxin C, D
  - **C**holera toxin
  - **D**iphtheria toxin
  - **E**HEC (Enterohemorrhagic *E.coli*) (Verocytotoin)
- **Alter antigenic property of bacteria:** e.g. in Salmonella.

Uses of Bacteriophage:
- Phage typing
- Phage assay
- Used in treatment (Phage therapy)
- Used in diagnosis: Mycobacteriophages
- Used as a cloning vector
- Mediates transduction
- Codes for toxins
- Alter antigenic property of bacteria.

MULTIPLE CHOICE QUESTIONS

HERPES SIMPLEX VIRUS

1. **A 25 year female came with multiple painful tiny vesicular ulcers over vulva and vaginal walls. On examination there is painful enlarged lymph nodes. The causative organism might be?** *(JIPMER May 2016)*
   a. C.granulomatis b. Chlamydia trachomatis
   c. H.ducreyi d. HSV-2
2. **The most common cause of sporadic viral encephalitis is:** *(AIIMS 2004)*
   a. Japanese B encephalitis
   b. Herpes simplex encephalitis
   c. HIV encephalitis
   d. Rubella encephalitis
3. **True about Herpes virus:** *(PGI Dec 2003)*
   a. HSV encephalopathy is treated with acyclovir
   b. Oropharyngeal involvement is common in HSV:1
   c. Recurrent genital involvement is seen in HSV:2
   d. Recurrence is rare in HSV:1
4. **Regarding HSV: 2 infection:** *(PGI June 2002)*
   a. Primary infection is usually wide spread
   b. Recurrent attacks are due to reactivation of latent infection
   c. Encephalitis: HSV:2 is a common cause
   d. Newborn may acquire infection via the birth canal at the time of labor
   e. Treatment is with acyclovir

VARICELLA ZOSTER VIRUS

5. **Newborn is presented with chorioretinitis, hypoplasia of limbs and scaring of hands. Most probable diagnosis is?**
   a. Herpes
   b. Rubella
   c. CMV
   d. Fetal varicella syndrome
6. **A patient presented with a vesicle on skin. Microscopy of Tzank smear showed giant cells. Causative agent is:** *(AIIMS May 2015)*
   a. Vaccinia virus
   b. Varicella zoster
   c. Mycobacterium
   d. Molluscum contagiosum
7. **Indication for varicella immunoglobulin is:** *(JIPMER Nov 2014)*
   a. A pregnant woman nonimmune to Varicella Zoster, exposed to a child with chickenpox 12 days ago
   b. A pregnant woman nonimmune to Varicella Zoster, exposed to mother with shingles 2 days ago
   c. A pregnant woman with no history of chickenpox develops shingles
   d. Pregnant woman previously treated with varicella immunoglobulin 10 days ago, but re-exposed to a case of chickenpox.
8. **Shingles are seen in:** *(NEET Pattern Based)*
   a. IMN
   b. Herpes zoster
   c. Chickenpox
   d. Smallpox
9. **Congenital varicella infection causes all except:** *(NEET Pattern Based)*
   a. Microcephaly
   b. Limb hypoplasia
   c. Cortical atrophy
   d. Cataract
10. **A baby is delivered with scarring of the skin and deformed limbs. Which of the following intrauterine infection can be held responsible?** *(AIIMS May 2011)*
    a. CMV
    b. Treponema pallidum
    c. Varicella
    d. Rubella
11. **A mother delivers a baby three days after developing chickenpox. Which of the following is true?** *(AIIMS May 2011)*
    a. No risk to both mother and baby
    b. Baby has a risk of congenital Varicella infection
    c. Give antiviral treatment to mother
    d. Give no treatment to mother and give antiviral treatment to baby
12. **Treatment of varicella in immunocompetent host is:**
    a. Acyclovir *(DNB June 2010)*
    b. Acyclovir and vaccination
    c. Prevention of complications
    d. Immunoglobulin
13. **Varicella zoster remains latent in:** *(AI 2010)*
    a. Lymphocytes b. Monocytes
    c. Trigeminal ganglion d. Plasma cells
14. **Infectivity of chickenpox last for:** *(AI 2002, UP 2002, MP 2001)*
    a. Till the last scab falls off
    b. 6 days after onset of rash
    c. 3 days after onset of rash
    d. Till the fever subsides

15. Which of the viral disease is associated with reactivation? *(Recent MCQ 2013)*
a. Pleurodynia
b. Shingles
c. Infectious mononucleosis
d. Viral arthritis

16. A patient is suffering from painful vesicular eruption at T-4 dermatome. The cause is: *(AIIMS May 2014)*
a. EBV infection
b. Herpes zoster
c. CMV infection
d. Herpes simplex

CYTOMEGALOVIRUS

17. What is the most common infection of the retina for a person living with HIV... *(Recent Question 2015)*
a. CMV retinitis
b. VZV causing progressive outer retinal necrosis
c. Syphilitic retinitis

18. Owl's eye appearance inclusions are seen in:
a. Herpes simplex virus infections *(APPG 2015)*
b. Cytomegalovirus infections
c. Epstein-Barr virus infection
d. Adenovirus infection

19. CMV infection immediate diagnosis by:
a. Direct DNA estimation *(AIIMS May 2013)*
b. Antigen detection
c. Isolation of the virus
d. ELISA for serum antibody

20. A 40-year-old man underwent kidney transplantation. Two months after transplantation, he developed fever and feature suggestive of bilateral diffuse interstitial pneumonitis. Which of the following is most likely etiologic agent: *(AIIMS 2003, 2002)*
a. Herpes simplex virus
b. Cytomegalovirus
c. Epstein-Barr virus
d. Varicella Zoster virus

21. A person with renal transplant developed infection of graft within 2 months. Most probable cause of infection is? *(AIIMS May 2014)*
a. Polyoma BK virus
b. CMV
c. Hepatits C
d. Herpes simples virus

22. A neonate has hepatosplenomegaly. His urine was stained with Giemsa stain which revealed owl's eye appearance inclusions. Which will be the most probable cause? (two pictures given in exam: *(AIIMS May 2014)*
a. CMV
b. HIV
c. Rubella

EPSTEIN–BARR VIRUS

23. Which of the following is a receptor for EBV? *(Recent Question 2015)*
a. CR1
b. CR2
c. CR3
d. CR4

24. EBV receptors is: *(TNPG 2015)*
a. CD 21
b. CD 9
c. CD 10
d. CD8

25. Lymphocytosis with atypical lymphocytes are seen in infection with: *(NEET Pattern Based)*
a. HSV
b. HBV
c. EBV
d. RSV

26. How Epstein-Barr Virus causes autoimmunity?
a. Molecular mimicry *(AI 2012)*
b. Exposure of sequestered antigens
c. Antigenic cross reactivity
d. Polyclonal B-cell activation

27. EBV causes all EXCEPT: *(DNB Dec 2009, PGI June 2003, AI 2002)*
a. Nasopharyngeal carcinoma
b. Burkitt's lymphoma
c. Verrucous lymphoma
d. Hodgkin's lymphoma

28. Paul-Bunnel test is done for?
a. HBV *(NEET Pattern Based, DNB Dec 2010)*
b. EBV
c. CMV
d. HIV

29. Oral hairy leukoplakia is associated with:
a. Cytomegalovirus *(DNB June 2010)*
b. Human immunodeficiency virus
c. EBV
d. HPV

30. EBV causes all except: *(JIPMER 2010, AI 2006, PGI Dec 2006, PGI June 2002, AI 2004, AIIMS Nov 2004)*
a. Kaposi sarcoma
b. Infectious monocytosis
c. Burkit's lymphoma
d. Duncan's disease

31. True about infectious mononucleosis: *(PGI June 2008)*
a. Associated with heterophile antibodies
b. Monocytosis
c. Associated with cold agglutinin
d. Associated with CMV infection
e. Self limited disease

32. Kaposi sarcoma is related to which virus? *(DNB June 2010, Latest MCQ 2014)*
a. HPV-16
b. HHV-8
c. EBV
d. CMV

33. HHV-8 causes: *(NEET Pattern Based)*
a. Burkitt's lymphoma
b. Nasopharyngeal carcinoma
c. Kaposi sarcoma
d. Hepatic carcinoma

POX VIRUSES

34. Which is not oncogenic virus? *(Recent Question 2015)*
a. Molluscum contagiosum
b. HPV
c. HBV
d. EBV

35. Bollinger bodies are seen in: *(NEET Pattern Based)*
a. Chickenpox
b. Cowpox
c. Fowl pox
d. Smallpox

36. Following virus is of pox family: *(NEET Pattern Based)*
a. Variola
b. Coxsackie
c. ECHO
d. HSV

37. Which pox virus does not grow in egg, animal cells?
a. Cowpox *(NEET Pattern Based)*
b. Vaccinia
c. Variola
d. Molluscum

38. Smallpox eradication was successful due to all except: *(AI 2011, AIIMS Nov 2010)*
a. Subclinical cases did not transmit disease
b. Highly effective vaccine
c. Infection provides life long immunity
d. Cross infection occurs from animal pox viruses

39. Which of the following is not a pox virus? *(AIIMS 2002)*
a. Cowpox
b. Molluscum contagiosum
c. Smallpox
d. Chickenpox

ADENOVIRUSES

40. Adenovirus causes all except: *(NEET Pattern Based)*
a. Hemorrhagic cystitis
b. Diarrhea
c. Respiratory tract infection
d. IMN

41. Pharyngoconjunctival fever is caused by: *(NEET Pattern Based, DNB June 2009)*
a. Adenovirus
b. Parainfluenza
c. Coxsackie viruses
d. All of the above

42. Space vehicle virus is: *(Recent MCQ 2013)*
a. Reovirus
b. Rhabdovirus
c. Retrovirus
d. Adenovirus

PARVOVIRUS

43. Slapped cheek appearance with fever is seen in? *(PGI May 2016)*
a. Rubella
b. Rubeola
c. Parvovirus B19
d. HSV-6

44. Slapped cheek sign is seen in: *(NEET Pattern Based)*
a. Parvovirus B19
b. JC virus
c. Rotavirus
d. Mumps

45. Parvovirus infection is associated with: *(PGI June 2008, AIIMS May 2008, PGI Dec 2007)*
a. Hydrops fetalis
b. Aplastic anemia
c. Abortion
d. Sixth disease
e. Hemophagocytic syndrome

46. In parvovirus infection what is common in adult:
a. Bone marrow aplasia *(PGI June 2007)*
b. PRCA
c. Erythema infectiosum
d. Arthropathy

HUMAN PAPILLOMAVIRUSES

47. Which HPV causes Condyloma acuminate. Most common cause? *(AIIMS Nov 2016)*
a. HPV 1-4
b. HPV 6 &11
c. HPV 16 & 18
d. HPV 31 & 33

48. Human papilloma virus (HPV) causes cancer of all except: *(PGI Nov 2016)*
a. Bladder cancer
b. Oropharyngeal carcinoma
c. Squamous cell carcinoma
d. Cervical cancer
e. Testicular cancer

49. Human Papilloma Virus is associated with all of the following cancers except: *(AIIMS May 2015)*
a. Carcinoma base of tongue
b. Tonsillar carcinoma
c. Nasopharyngeal carcinoma
d. Recurrent respiratory papilloma

50. The following is low risk type human papilloma virus: *(MHPG 2015)*
a. HPV – 6
b. HPV – 18
c. HPV – 16
d. HPV – 31

51. HPV belongs to: *(NEET Pattern Based)*
a. Papovavirus
b. Parvovirus
c. Herpes virus
d. Poxvirus

52. Progressive multifocal leukoencephalopathy is caused by: *(NEET Pattern Based)*
a. CMV
b. EBV
c. JC virus
d. RSV

53. Condyloma acuminatum is caused by:
a. HSV *(TNPG 2015, NEET Pattern Based)*
b. HPV
c. HIV
d. VZV

54. HPV vaccine is: *(AIIMS Nov 2009)*
a. Monovalent
b. Bivalent
c. Quadrivalent
d. Both bivalent and quadrivalent

55. MC serotype of HPV causing CaCx: *(Recent MCQ 2013)*
a. HPV-6
b. HPV-11
c. HPV-16
d. HPV-33

56. Quadrivalent vaccine for HPV contains all except:
a. Type 7 *(PGI May 2013, APPG 2011)*
b. Type11
c. Type16
d. Type18
e. Type26

57. Oropharyngeal cancer/Head neck cancer is associated with? *(Recent Questions 2014)*
a. EBV
b. HPV
c. HSV
d. HBV

BACTERIOPHAGE

58. True about bacteriophage is: *(AIIMS May 2011)*
a. It is a bacteria infecting bacteria
b. Helps in gain of new toxigenic property
c. Transfers only chromosomal genes
d. Helps in transformation of bacteria

59. All of the following statements are true about bacteriophage except: *(AIIMS, May 2011, AIIMS Nov 2008)*
a. It is a virus that infect bacteria
b. It helps in transduction of bacteria
c. It imparts toxigencity to bacteria
d. It transfers only chromosomal gene

60. Most of the Bacteriophage capsid exhibits which symmetry? *(Recent Questions 2014)*
a. Helical
b. Icosahedral
c. Spherical
d. Filamentous

EXPLANATIONS

HERPES SIMPLEX VIRUS

1. **Ans. (d) (HSV-2)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p604
   - Painful multiple tiny vesicular ulcers on genitalia- suggestive of Herpes simplex virus-2 infection.

2. **Ans. (b) (Her...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p435, Harrison 19/e p1178, 18/e p3481
   'HSV encephalitis though rare, is most common sporadic acute viral encephalitis in most parts of the world'.

3. **Ans. (a) (b) (c) (HSV encephalopathy..., Oropharyngea..., Recurrent genital...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p435, Ananthanarayan 9/e p 468-69, 8/e p 468-71
   - **Mostly, HSV-1 produces above waist (orofacial**) and HSV-2 produces below waist (genital) lesions.
   - Treatment of choice for HSV encephalopathy is intravenous acyclovir.
   - Recurrences are common in both HSV 1 and 2 infections.
   - **Genital recurrences are common in HSV-2 infections**
   - Orofacial recurrences are common in HSV-1 infections

4. **Ans. (a) (b) (d) (e) Primary infection is usually wide spread, Recurrent attacks are due to reactivation of latent infection, Newborn may acquire infection via the birth canal at the time of labor and Treatment is with acyclovir** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p435, and Harrison 19/e p1178, 18/e p1457
   - All the above options about HSV-2 infections are true except option c-

   Encephalitis can be caused by HSV-2, but very rare. Majority of cases of HSV encephalitis are caused by HSV-1.

VARICELLA ZOSTER VIRUS

5. **Ans (d) (Fetal varicella syndrome)** Ref:Apurba Sastry's Essentials of Medical Microbiology/p438
   - Hypoplasia of limbs and scaring of hands- Suggestive of Fetal varicella syndrome.

6. **Ans. (b) (Varicella zoster)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p439
   - Giemsa staining of the scrapings from the Varicella-zoster ulcer base (Tzanck smear) reveals cytopathological changes similar to that of HSV infection, such as formation of multinucleated giant cells.

7. **Ans (b) (A pregnant woman ...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p439, Harrison 19th/p1186, CDC (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6228a4.htm)

   Recommendations for VZIG Administration
   - The following should receive VZIG as soon as possible but not >96 h after exposure:
     - In susceptible pregnant women exposed to person with chickenpox or zoster
     - Immunocompromised susceptible children exposed to person with chickenpox or zoster
     - Newborn (>28 weeks) whose mother had onset of chickenpox within -5 to +2 of delivery (VZIG not indicated if the mother has zoster)
     - Hospitalized premature infant (<28 weeks /<1000 gm weight), regardless of maternal h/o exposure
   - Patients receiving high-dose VZIG are likely to be protected and probably do not require VZIG on next exposure occurring within 3 weeks … CDC,2013
   - Pregnant woman developing chickenpox or zoster- should be treated with acyclovir or valacyclovir. VZIG is not recommended here.

8. **Ans. (b) (Herpes zoster)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p437, Ananthanarayan 9/e p472
   - Varicella zoster can cause chickenpox in primary exposure and zoster or shingles on reactivation of the primary lesion.

9. **Ans. (d) (Cat...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p438, Ananthanarayan 9/e p472, 8/e p472

   Fetal Varicella syndrome is characterized by
   - Cicatrization skin lesion, Chorioretinitis, CNS defects and Limb hypoplasia.

10. **Ans. (c) (Vari...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p438, Ananthanarayan 9/e p472, 8/e p472
Refer the previous explanation

11. **Ans. (b) (Baby has a risk of Congenital...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p438, Ananthanarayan 9/e p472, 8/e p472, Harrison 19/e p1184, 18/e p1466
Infection of Varicella during pregnancy:
- If mother gets chickenpox > 5 days before delivery: Then baby is mostly asymptomatic due to maternal antibody
- If mother gets chickenpox within 5 days of delivery: Mother would not have produced protective antibodies, hence: disseminated infection in baby occurs with high-risk of pneumonia and encephalitis.
- In such cases, treatment with Varicella zoster immunoglobulin (VZIG) is indicated within 96 hr.

12. **Ans. (c) (Pre...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p439, Harrison 19/e p1185, 18/e p1465
- ***Medical management of chickenpox in the immunologically normal host is directed toward the prevention of avoidable complications***.
- Good hygiene includes daily bathing and soaps. Secondary bacterial infection of the skin can be avoided by meticulous skin care, particularly with close cropping of fingernails. Pruritus can be decreased with topical dressings or the administration of antipruritic drugs. Tepid water baths and wet compresses are better for the relief of itching.

13. **Ans. (c) (Trigeminal ganglion)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p438, Ananthanarayan 9/e p472, 8/e p473, Harrison 19/e p1184, 18/e p1462
- Following an attack of chickenpox, Varicella zoster virus remains latent in the *sensory ganglion of trigeminal nerve*
- Reactivation is triggered by some precipitating factors
  - Immunocompromised state: HIV
  - Old age
  - Lymphoreticular malignancy
- Reactivation of this virus causes ***Herpes zoster/Shingles***

14. **Ans. (b) (6 days...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p438, Ananthanarayan 9/e p471
'Chickenpox patient is considered to be infectious during 2 days before and 5 days after the onset of rashes'
- *Hence option b is most appropriate answer.*

15. **Ans. (b) (Shingles)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p439, Ananthanarayan 9/e p472
- Varicella zoster can undergo reactivation to cause shingles or zoster.

16. **Ans. (b) (Herpes zoster)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p439, Ananthanarayan 9/e p471
Herpes zoster (shingles) is a sporadic, incapacitating disease of adults or immunocompromised individuals that is characterized by painful vesicular eruption limited in distribution to the skin innervated by a single sensory ganglion (e.g. T-4 dermatome). Zoster is the response of the partially immune host to reactivation of varicella virus present in latent form in neurons in sensory ganglia.

CYTOMEGALOVIRUS

17. **Ans. (a) (CMV retinitis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508

18. **Ans (b) (CMV infections)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p439
- Owl's eye appearance inclusions are seen in cells infected with CMV.

19. **Ans. (a) (Direct...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p441, Harrison 19/e p1191 18/e p1474
- *The most sensitive way to detect CMV in blood or other fluids may be by amplifying CMV DNA by PCR.*
- PCR detection of CMV DNA in blood may predict the risk for disease progression, particularly in immunocompromised hosts
- PCR detection of CMV DNA in cerebrospinal fluid is useful in the diagnosis of CMV encephalitis or polyradiculopathy.
- Detection of CMV antigens (pp65) in peripheral-blood leukocytes or of CMV DNA in blood or tissues may hasten diagnosis. Such assays may yield a positive result several days earlier than culture methods.

20. **Ans. (b) (CMV)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p339, Harrison 19/e p1191, 18/e p1472
- Persons at greatest risk for cytomegalovirus disease are
  - Those receiving organ transplants (***Kidney*** or solid organs),

- Those with malignant tumors who are receiving chemotherapy, and
- Those with AIDS.

- Viral excretion is increased and prolonged, and the infection is more apt to become disseminated.
- *Diffuse Interstitial Pneumonia is the most common complication.*

21. Ans. (b) (CMV) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p440, Ananthanarayan 9/e p437

Cytomegalovirus is the most common cause of infection following renal transplantation. It usually occurs after 1 month of transplantation and presented as bilateral interstitial pneumonia.

22. Ans. (a) (CMV) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p439, Ananthanarayan 9/e p473

Neonate with hepatosplenomegaly, Giemsa stain of urine reveals owl's eye appearance inclusions…..is suggestive of neonatal CMV infection.

EPSTEIN–BARR VIRUS

23. Ans. (b) (CR2) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e pp442

Receptor for EBV is CD 21 or CR2.

24. Ans. (a) (CD21) Ref : Apurba Sastry's Essentials of Medical Microbiology 1/e p442, Ananthanarayan 9/e p 475.

CD21 or CR2 are the receptors for EBV present on B cell and pharyngeal epithelial cells.

25. Ans. (c) (EBV) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p442, Ananthanarayan 9/e p475, 8/e p475

- Lymphocytosis with atypical lymphocytes are seen in infection with EBV

26. Ans. (d) (Poly...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p442-43, Kuby's Immunology 6/e p419

- A number of viruses and bacteria can induce nonspecific polyclonal B-cell activation.
- *Gram-negative bacteria, cytomegalovirus, and Epstein-Barr virus (EBV) are all known to be such polyclonal activators, inducing the proliferation of numerous clones of B-cells that express IgM in the absence of TH cells.*
- If B cells reactive to self-antigens are activated by this mechanism, auto-antibodies can appear.
- For instance, during infectious mononucleosis, which is caused by EBV, a variety of auto-antibodies are produced, including autoantibodies reactive to T- and B-cells, rheumatoid factors, and antinuclear antibodies.

27. Ans. (c) (Verrucous...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p442-43, Ananthanarayan 9/e p475,

- Verrucous lymphoma is NOT caused by EBV
- EBV can cause: Nasopharyngeal carcinoma, Burkitt's lymphoma and Hodgkin's lymphoma
- Detail: Refer chapter review.

28. Ans. (b) (EBV) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p442-43, Ananthanarayan 8/e p476

- Paul-Bunnel test is a heterophile agglutination test done for infectious mononucleosis (caused by *Epstein-Barr virus).*
- Sheep RBC antigen is used to detect EBV antibodies.

29. Ans. (c) (EBV) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p442-43, Ananthanarayan 9/e p475

- *Hairy cell leukoplakia is caused by Epstein-Barr virus.*

30. Ans. (a) (Ka...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p442-43, Harrison 19/e p1193, 18/e p1467-69

- **HHV8 is called as Kaposi's sarcoma associated herpesvirus.**
- **For detailed explanation refer chapter review.**

31. Ans. (a), (c), (e) (Associated with heterophile antibodies, Associated with cold agglutinin, Self limited disease) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p442-43, Harrison 19/e p1186, 18/e p1467-69

- *EBV is the causative agent of infectious mononucleosis*
- *EBV infection causes **lymphocytosis** and not monocytosis*
- *Produces **self-limited disease**, infectious mononucleosis/ glandular fever*
- *There is production of **heterophile antibodies**, which can be detected by Paul-Bunnel test*
- *EBV causes autoimmune hemolytic anemia due to production of **cold agglutinins***
- *CMV can cause infectious mononucleosis like syndrome following blood transfusion in adults*

32. **Ans. (b) (HHV-8)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p444, Ananthanarayan 9/e p477
    - *Human Herpes virus-8 has been identified from the tissue of patients with Kaposi sarcoma and often called as Kaposi sarcoma associated virus commonly occurs in HIV infected people, higher in men who have sex with men.*

33. **Ans. (c) (Kaposi...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p444, Ananthanarayan 9/e p477
    - *HHV-8 causes Kaposi sarcoma*

POX VIRUSES

34. **Ans. (a) (Molluscum contagiosum)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p542

35. **Ans. (c) (Fo...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p452-53, Ananthanarayan 9/e p461, 8/e p461
    - Bollinger bodies are seen in Fowlpox

36. **Ans. (a) (Va...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p452-53, Ananthanarayan 9/e p461, 8/e p461
    - Variola belongs to pox family.

37. **Ans. (d) (Molluscum)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p452-53, Ananthanarayan 9/e p465
    - Molluscum contagiosum virus does not grow in animal, egg or tissue culture.

38. **Ans. (d) (Cross...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p452-53, Park 22/e p135
    Smallpox eradication was successful due to: (Detail refer chapter review)
    - Subclinical cases did not transmit disease
    - Highly effective vaccine
    - *Once infected – leads to* lifelong immunity

39. **Ans. (d) (Chi...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p452-53, Ananthanarayan 9/e p471, 8/e p471
    - Chickenpox virus or Varicella zoster virus is Herpes Virus.

ADENOVIRUSES

40. **Ans. (d) (IMN)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p450, Ananthanarayan 9/e p482
    - Infectious mononucleosis is caused by EBV.

41. **Ans. (a) (Adenovirus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p450, Ananthanarayan 9/e p482
    - Pharyngoconjunctival fever: This syndrome consists of febrile pharyngitis and conjunctivitis seen in civilian population.....spelling is usually associated with Adenovirus serotype 3, 7 and 14.

42. **Ans. (d) (Adenovirus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p450, Ananthanarayan 9/e p480
    - Adenovirus virion is Space vehicle shaped.

PARVOVIRUS

43. **Ans (c) (Parvovirus B19)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p447
    - Refer text

44. **Ans. (a) (Par...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p44-47, Harrison 19/e p1115-16, 18/e p1478-79
    - The main manifestation of symptomatic Parvovirus B19 infection is **erythema infectiosum,** also known **as *fifth disease* or *slapped-cheek disease.***

45. **Ans. (a), (b), (c), (e) (Hydrops fetalis, Aplastic anemia, Abortion and Hemophagocytic syndrome)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 44-47, Harrison 18/e p1478-79
    Already explained

46. **Ans. (d) (Art...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p44-47, Harrison 19/e p1195, 18/e p1478-79
    - Arthropathy occurs in ~50% of adults and is more common among women than among men.
    - The distribution of the affected joints is often symmetrical, with arthralgia affecting the small joints of the hands and occasionally the ankles, knees, and wrists.
    - Resolution usually occurs within a few weeks, but recurring symptoms can continue for months. Arthropathy is uncommon among children

HUMAN PAPILLOMAVIRUS

47. Ans. (b) (HPV 6 &11) Ref:Apurba Sastry's Essentials of Medical Microbiology/p448 , Jawetz 26th/chapter 43

Anogenital condylomas; laryngeal papillomas; dysplasias and intraepithelial neoplasias (mucosal sites are associated with HPV types 6, 11, 40, 42–44, 54, 61, 70, 72, 81. They have a low suspected oncogenic potential.

48. Ans: (a,e) (bladder, testicular cancer) Ref: Apurba Sastry's Essentials of Medical Microbiology/p448, Harrison 19th/p1198

- HPV-associated cancers include cervical and anal cancer, other vulvar and vaginal cancer, penile cancer and oropharyngeal squamous cell carcinoma (OPSCC) involving larynx and esophagus.

49. Ans. (c) (Nas...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p448

Nasopharyngeal carcinoma is caused by Epstein-Barr virus.
Refer chapter review, for the list of malignancies caused by HPV.

50. Ans. (a) (HPV – 6) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p448

51. Ans. (a) (Pap...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p448-49, Harrison 19/e p1197, 18/e p1483-84

- HPV belongs to Papova family.

52. Ans. (c) (JC virus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p448-49, Harrison 18/e p1483-84

- Progressive multifocal leukoencephalopathy is caused by – JC virus

53. Ans. (b) (HPV) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p448-49, Harrison 18/e p1483-84

Condyloma acuminatum is caused by–HPV

54. Ans. (d) (Both...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p448-49, Harrison 19/e p1119, 18/e p1483-84

HPV vaccines under trial

- Recently developed HPV vaccines dramatically reduce rates of infection and disease produced by the HPV types in the vaccines.
- These products are directed against virus types that cause anogenital tract disease and are derived from expression of the major capsid protein (L1) gene in tissue culture
- Currently, one ***quadrivalent*** product containing ***HPV types 6, 11, 16, and 18*** has been licensed in the US and recommended by the Centers for Disease Control and Prevention for administration to girls and young women 9–26 years of age.
- Another product contains HPV types 16 and 18 ***(bivalent)***
- HPV types 6 and 11 cause 90% of anogenital warts
- HPV types 16 and 18 are responsible for 70% of cervical cancers.

55. Ans. (c) (HPV16) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p448-49, Ananthanarayan 9/e p565

MC serotype of HPV causing Coax- HPV 16 and 18.

56. Ans. (a), (e) (Type 7 and Type 26) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p448-49, Harrison 19/e p1199 18/e p1483-84

- Quadrivalent vaccine (Gardasil) for HPV contains serotypes 6, 11, 16 and 18
- Bivalent vaccine (Cervarix) for HPV contains serotypes 6 and 11

57. Ans. (b) (HPV) Ref: Harrison 18/e chapter 185, Jawetz 24/e Table 43-1

HPV infection may play a role in squamous cell carcinomas of the head and neck

Clinical Lesions due to HPV	Oncogenic Potential	HPV Types
Carcinomas of cervix and other genital mucosa/larynx/esophagus	High	16, 18, 30, 31, 33 and 45
Squamous cell carcinomas of the head and neck		

BACTERIOPHAGE

58. Ans. (b) (Helps in.....) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p454-56

Bacteriophage coded Toxin makes the bacterium toxigenic. (refer text for the list)

About Other Options

- Bacteriophage is a virus that infects a bacterium
- Bacteriophage transfers genes from one bacterium to another known as ***Transduction***
- Bacteriophage can transfer chromosomal as well as plasmid coded genes. For example, plasmid mediated beta lactamase resistance

59. Ans. (d) (It...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p454-56

- Bacteriophages act as carriers of genes from one bacterium to another: Transduction
- Bacteriophages can transfer chromosomal as well as plasmid coded genes.
- Plasmid mediated drug resistance in Staphylococcus is an example of a medically important property that is transmitted by transduction.

60. Ans. (b) (Icosahedral) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p454, Ananthanarayan 9/e p459

Bacteriophages have Icosahedral symmetry

CHAPTER

4.3

Myxoviruses and Rubella

Properties	Orthomyxoviridae	Paramyxoviridae
Size	80–120 nm	100–300 nm
Shape	Spherical; Rarely filamentous	Pleomorphic
Nucleic acid	Negative sense ssRNA, Segmented; eight pieces	Negative sense ssRNA Unsegmented; single piece
Genetic recombination	Seen	Not seen
Antigenic variation	Seen	Not seen
Site for RNA Replication	Nucleus	Cytoplasm
Important human pathogens	Influenza virus	Parainfluenza virus, Mumps virus Respiratory syncytial virus Measles virus, Metapneumovirus
Antigens: Hemagglutinin (HA) and Neuraminidase (NA)	Both HA and NA spikes present, So hemagglutination is reversible (Elution seen)	**HA spike present in:** Parainfluenza, Mumps, Measles, **NA spike present in:** Parainfluenza, Mumps **Inclusion body:** Intracytoplasmic (only for measles, it is both intracytoplasmic and intranuclear)

ORTHOMYXOVIRUSES

INFLUENZA VIRUS

General Properties

- Influenza virus is spherical and possesses helical symmetry
- Viral RNA comprises of eight segments of negative sense single stranded RNA
- Site of RNA replication: In the nucleus (in contrast to cytoplasm by most other RNA viruses)
- Viral proteins: Influenza virus contains eight structural proteins (PB1, PB2, PA, NP, HA, NA, M1 and M2) and two nonstructural proteins (NS1 and NS2).

Antigens and Typing

Influenza possess two glycoprotein antigens inserted into the lipid envelope: HA (Hemagglutinin) and NA (Neuraminidase).

- **Hemagglutinin (HA):** It binds to mucin or sialic acid receptors on RBCs, resulting in clumping of RBCs to cause **hemagglutination.** It also binds to the same receptors on host cells, thus facilitating viral entry. Antibody to HA is protective in nature.
- **Neuraminidase (NA):** It is present in fewer number. It is a sialidase enzyme that degrades the sialic acid receptors on RBCs; thus helps in:
  - It displaces HA from RBCs resulting in reversal of hemagglutination called **elution.**
  - It facilitates release of virus particles from infected cell surfaces.
  - NA helps the virus to pass through the mucin layer in the respiratory tract.

Antigens of Influenza:
- Hemagglutinin (HA): Cause hemagglutination
- Neuraminidase (NA): causes reversal of hemagglutination called Elution.

Typing

- **Three Genera:** Based on Nucleoprotein (NP) and Matrix (M) proteins:
  - Influenza: A (MC cause of outbreak/epidemic, only cause of pandemic)
  - Influenza: B (rarely seen) and Influenza C (not circulating now)
- **Subtypes**
  - Based on HA and NA antigens, Influenza A has distinct 16 H subtypes (H1 to H16) and 9 N subtypes (N1–N9).
  - Influenza B and C viruses though have subtypes; but are not designated.

Influenza Typing:
- Influenza: A (MC cause of outbreak/epidemic, only cause of pandemic)
- Influenza: B (rarely seen)
- Influenza: C (not circulating now).

Antigenic Variation

Influenza virus type A and to less extent type B undergo antigenic variation, which is of two types: antigenic shift and drift. Type C influenza virus is stable.

Antigenic Shift	Antigenic Drift
Occur in Type A	Occur in type A, to less extent type B
Results in Pandemic and major epidemic	Results in periodic Epidemic and sporadic cases
Due to genetic reassortment	Due to point mutation
Occurs every 10–20 years	Occurs every 2–3 years

Currently circulating strains:

- Type A/H1N1 (2009 Pandemic flu strain)
- Type A/H3N2 (Seasonal flu strain)
- Type A/H5N1 (Avian flu strains)
- Type B

Currently circulating Influenza strains:
- Type A/H1N1 (2009 Pandemic flu strain)
- Type A/H3N2 (Seasonal flu strain)
- Type A/H5N1 (Avian flu strains)
- Type B.

Clinical Features

- Incubation period: 18–72 hours
- Reservoir: Animals, birds and humans
- MC manifestation: Mild flue like illness (URTI)
- MC complication:
  - Bacterial pneumonia (*S. aureus* > pneumococci and *H. influenzae*) is more common than viral pneumonia
  - Reye's syndrome: Common with Type B (fatty liver following aspirin intake).

Laboratory Diagnosis

- **Specimen collection:** Nasopharyngeal swab (polyester or Dacron swabs).
- **Transported** in viral transport media, kept at 40°C up to 4 days, thereafter at 700°C.
- **Isolation of virus**: Embryonated eggs and primary monkey kidney cell lines.
- **Egg inoculation**
  - Amniotic cavity inoculation: It supports growth of Influenza A, B, C
  - Allantoic cavity inoculation: Supports growth of only Influenza A
  - Growth is detected by: Hemagglutination with Fowl and Guinea pig RBC
  - Type A: Agglutinates with Guinea pig RBC, Type C: Agglutinates with fowl RBC at 4°C; and Type B: Agglutinates with both.
- **Antigen detection** from nasopharyngeal cells by direct IF test.
- **Antibody detection:** Fourfold rise in the antibody titer is more significant:
  - Hemagglutination inhibition test (HAI)
  - Neutralization test: It is the most specific and the best predictor of susceptibility to infection, but is time-consuming and difficult to perform.
  - ELISA is more sensitive than other assays.
- **Molecular methods:**
  - Reverse transcriptase PCR (RT-PCR) is the most sensitive, 2 type specific.
  - Real time RT: PCR can be used to quantitate the viral load in the clinical sample.

Influenza Serology:
- Neutralization test: It is the most specific and the best predictor of susceptibility to infection, but is time-consuming and difficult to perform
- ELISA: Most sensitive test.

Vaccine

Killed Vaccine

Inactivated Influenza vaccines:
- Whole virus (WV) vaccine
- Subvirion (SV) vaccine
- Surface antigen vaccines.

- Prepared in allantoic cavity of egg, contains HA antigen (15 µg/dose).
- 2 doses, given by IM route, efficacy 70–90%, lasts for 6–12 months.
- Single dose is administered by IM or SC route. Protectivity is about 50–80%, Immunity lasts for 6–12 months.
- Indication: Annual inactivated influenza vaccination is recommended for high-risk groups such as children, older age, underlying chronic lung, cardiac, renal, hepatic, and CNS conditions, low immunity (HIV) and pregnancy.
- Contraindication: People who have allergy to eggs or have history of hypersensitivity to previous dose.
- Inactivated vaccines are of three types. All are efficacious.
  - Whole Virus (WV) vaccine: Contains intact, inactivated viruses
  - Subvirion (SV) vaccine: Contains purified virus disrupted with detergents.
  - Surface antigen vaccines contain purified HA and NA glycoproteins.

Live Nasal Spray (Trivalent)

Live nasal spray (Trivalent) contains:
- Influenza A (H1N1) virus
- Influenza A (H3N2) virus
- Influenza B virus.

- It is trivalent: Contains influenza A (H1N1) virus, influenza A (H3N2) virus and influenza B virus.
- Stimulates both local + systemic immunity, not given if immunity is low.
- Cold-adapted strain is used.
- Indication: Recommended to all healthy persons of 2–49 years age, but is not given to high risk groups.

Treatment

Matrix M2 inhibitor (amantadine) and neuraminidase inhibitor (oseltamivir).

Sialic Acid Receptors Determines Pathogenicity

Influenza virus entry into the host cells is dependent on type of sialic acid receptors present on the host cell surfaces; which are specific for HA antigens of influenza.

Sialic acid receptors determines pathogenicity of Influenza:
- α 2–6 receptors: Specific for human influenza
- α 2–3 receptors: Specific for avian influenza
- Both α 2–3 and α 2–6 receptors: Found in pigs, hence pigs are the MC mixing vessels.

- α **2–6 sialic acid receptors** are specific for human influenza strains and are found abundantly on human upper respiratory tract epithelium but not on lower upper respiratory tract. This explains why most human flu strains cause mild upper respiratory tract infections but not pneumonia.
- α **2–3 sialic acid receptors** are specific for avian influenza strains and are found abundantly on bird's intestine.
  - In humans, they are present in very few numbers on lower upper respiratory tract.
  - This explains why avian flu strains cannot easily infect humans and need close contact. However, once infected, they can infect lower respiratory tract and cause pneumonia.

Why pigs are the most common mixing vessels?
- Both α 2–3 and α 2–6 sialic acid receptors are found on the respiratory epithelium of pigs and swine flu strains have specificity for both the receptor types.
- Hence pigs can be infected simultaneously by human, swine and avian strains, thus serving as a mixing vessel where reassortment takes place.

Avian Flu Infection in Humans

Avian flu strains infecting humans:
- A/H5N1 (MC type)
- A/H7N7 (Netherlands)
- A/H9N2 (Hong Kong)
- A/H7N9 (caused an outbreak in China, 2013).

Birds are the primary reservoir for influenza viruses.

It is believed that, to date, all human pandemic strains have originated by reassortment between avian and human influenza viruses and the mixing has occurred in pigs.

- ***A/H5N1*** is the MC avian flu strain that has been endemic in the world.
  - *Origin:* It was first reported from Hong Kong in 1997 and has spread to various countries including India.

- *Transmission* to man occurs only from birds, and requires close respiratory contact.
- *Less morbidity*: As there is no human-to-human transmission, morbidity is less.
- *More mortality*: Avian flu strains are highly virulent (due to PB1F2 protein) and mortality rate is > 60%.
- *Clinical feature*: H5N1 avian flu strains are associated with higher rates of pneumonia (> 50%) and extrapulmonary manifestations such as diarrhea and CNS involvement.

- ***Other avian flu strains*** that can cause human infections are:
  A/H7N7 (Netherlands), A/H9N2 (Hong Kong) and A/H7N9 (caused an outbreak in China, 2013)
- **Laboratory diagnosis**: By real time RT- PCR detecting specific HA and NA genes.
- **Treatment**: Drug of choice is oseltamivir (Tamiflu).

A/H1N1 2009 Flu

It has caused the most recent pandemic of influenza, emerged in California in March 2009 and rapidly spread to the entire world including India, over the next few months. WHO declared the pandemic in 11th June 2009.

Epidemiology

- ***Origin:*** H1N1 2009 flu originated by genetic reassortment of four strains (1 Human strain + 2 swine strains + 1 avian strain) and the mixing has occurred in pigs.
- Though people commonly use the word 'swine flu' to describe H1N1 2009 flu, but this is not the correct terminology as it is a *reassortant of four strains*.
- ***Transmission:*** It can be transmitted from human to human, which has accounted for its rapid spread.
- However, it is less virulent (as it lacks the *PB1F2* protein). Therefore in contrast to H5N1, the H1N1 2009 flu has caused more morbidity but less mortality.
- ***Situation in World:*** Currently, World is in the post pandemic period except in India and New Zealand where still local intense transmission is ongoing.
- ***Situation in India:***
  - Since 2009 about 53,943 cases and 3,315 deaths due to H1N1 were reported from India, out of which in 2013 alone nearly 708 cases with 132 deaths have occurred.
  - However, a threatening outbreak of H1N1 started again in 2015 affecting 33,761 people with 2035 deaths (up to March 30th 2015). The worst hit states are Rajasthan, Gujarat, Maharashtra and Madhya Pradesh.

H1N1 situation in India:
- Since 2009 about 53,943 cases and 3,315 deaths due to H1N1were reported from India, out of which in 2013 alone nearly 708 cases with132 deaths have occurred.
- However, a threatening outbreak of H1N1 started again in 2015 affecting 33,761 people with 2035 deaths (up to March 30th 2015) .The worst hit states are Rajasthan, Gujarat, Maharashtra and Madhya Pradesh.

Clinical Feature

- Uncomplicated influenza: Most of the cases are present with mild URTI and diarrhea.
- Complicated/severe influenza can occur very rarely in high risk groups, characterized by features such as secondary bacterial pneumonia, dehydration, CNS involvement, and multiorgan failure.

H1N1 Origin:
- Originated by genetic reassortment of four strains (1 Human strain + 2 swine strains + 1 avian strain) and the mixing has occurred in pigs.

Laboratory Diagnosis

Real time RT-PCR can detect and quantify the specific HA and NA genes.

Treatment

H1N1 flu is resistant to amantadine. Drug of choice is neuraminidase inhibitors:
- Oseltamivir (Tamiflu) tablet: 75 mg twice a day for 5 days
- Zanamivir (10 mg, inhalational form)

Prophylaxis

- *Vaccine:* Both killed injectable and live nasal spray vaccines are available for A/H1N12009 flu.
- *Chemoprophylaxis:* Oseltamivir-75 mg once a day, duration depends on the clinical setting.

PARAMYXOVIRUSES

PARAINFLUENZA VIRUSES

Human parainfluenza viruses are one of the major causes of lower respiratory tract disease.

- Common cold syndrome such as rhinitis and pharyngitis is the MC presentation
- Croup (laryngotracheobronchitis): Seen with type 1 and 2 and involves older children
- Pneumonia or bronchiolitis: Occurs very rarely to 6 months, especially with type 3
- Otitis media is the MC complication of parainfluenza virus infection.
- Reinfections are common. There is no cross protection between the serotypes.

Human parainfluenza viruses:
- MC agent of Croup (laryngotracheobronchitis).

MUMPS VIRUS

Mumps virus is the most common cause of parotid gland enlargement in children. Transmission is through the respiratory route via droplets, saliva, and fomites. Incubation period is about 19 days (range, 7–23 days).

Incubation period of MMR:
- Measles: 10 days
- Mumps: 19 days
- Rubella: 14 days.

Clinical Manifestation

- **Inapparent infection:** (Most common).
- **Bilateral parotitis:** It is the MC manifestation (70–90%). Rarely, other salivary glands may also be involved.
- **Epididymo-Orchitis** is the next MC manifestation of mumps. Orchitis is unilateral in most of the cases hence, infertility is rare.
- **Aseptic meningitis** occurs in <10% of cases, with a male predominance. It is self-limiting condition except the deafness (due to cranial nerve palsy) which may be permanent.
- **Oophoritis** occurs in about 5% of women.
- **Pancreatitis** occurs in 4% of infections and may lead to diabetes.
- **Atypical mumps:** Parotitis may be absent in 10% of cases and patients are directly presented with aseptic meningitis.

Mumps virus:
- Bilateral parotitis: It is the MC manifestation
- Epididymo: Orchitis is the next MC Complication
- Aseptic meningitis: MC Complication in female.

Epidemiology

Mumps is endemic worldwide, sporadic cases occurring throughout the year, with a peak in cases typically in winter and spring. Epidemics occur every 3–5 years; typically associated with unvaccinated people living in overcrowded areas.

- **Period of communicability:** Patients are infectious from 1 week before to 1 week after the onset of symptoms.
- **Source:** Cases are the source of infection. There is no carrier state.
- **Reservoir:** Humans are the only reservoir of infection.
- **Age:** Children of 5–9 years age are MC affected. Disease tends to be more severe in adults.
- **Immunity:** One attack (either by vaccine or infection) gives lifelong immunity.
- Secondary attack rate is high (86%).

Prevention (Live Attenuated Vaccine)

- Jeryl Lynn strain is the recommended strain used worldwide. Other strains are RIT 4385, Urabe strain and L-Zagreb.
- It is prepared in chick embryo cell line.
- **Mumps vaccine is available as:**
  - Trivalent MMR vaccine (live attenuated measles-mumps-rubella vaccine) or
  - Quadrivalent MMR-V vaccine (contains additional live attenuated varicella vaccine)
  - Monovalent mumps vaccine (not commonly used)
- **Schedule:** Two doses of MMR is given by IM route at 1 year and 4–6 year (before starting of school)

- **Efficacy** is about 90% after the second dose. Neutralizing antibodies appear in 95% of the recipients.

MEASLES (RUBEOLA) VIRUS

Measles is an acute, highly contagious childhood disease characterized by fever and respiratory symptoms, and rash.

Transmission occurs predominantly via the *respiratory route*.

Clinical Manifestations

Incubation period is about 10 days which may be shorter in infants and longer (up to 3 weeks) in adults

- ***Fever*** is the first manifestation, occurs on day-1 (i.e. on 10th day of infection)
- ***Koplik's spots*** are pathognomonic of measles, appear after two days following fever and is characterized by:
  - White to blush spot (1 mm size) surrounded by an erythema
  - Appear first on buccal mucosa near second lower molars, rapidly spread to entire buccal mucosa
- ***Rash:*** Maculopapular dusky red rashes appear after four days of fever (i.e. at 14th day of infection).
  - Rashes appear first ***behind the ears*** → then spread to face, arm, trunk → then fade in the same order.
  - Rashes are typically absent in HIV infected people.

Incubation period (10 days) → Fever (10th day)→ Koplik's spot (12th day) → rash (14th day)

Measles—Sequence of manifestation:
Incubation period (10 days) → Fever (10th day) → Koplik's spot (12th day) → rash(14th day).

Complications

- **Secondary bacterial infections**: Following measles, there is profound CMI suppression which in turn predisposes to various secondary bacterial infections.
  - Otitis media and bronchopneumonia are the most common
  - Worsening of underlying tuberculosis with a false negative Mantoux test
- **Complications due to measles virus itself**
  - Giant-cell pneumonitis in immunocompromised children, and HIV infected people
  - Acute laryngotracheobronchitis (croup)
  - Diarrhea, leads to malnutrition including vitamin A deficiency
- **CNS complications** are rare but most severe.
  - *Postmeasles encephalomyelitis*
  - *Measles inclusion body encephalitis*
  - *Subacute sclerosing panencephalitis (SSPE)*-is a slowly progressive disease characterized by seizures and progressive deterioration of cognitive and motor functions.
    - SSPE belongs to group C slow virus infection, caused by a defective measles virus.
    - Occurrence is 1 in 300,000 measles cases
    - SSPE typically occurs in persons infected with measles virus at < 2 years of age.
    - SSPE usually develops after 7–10 years of initial infection.
    - It is fatal within 1–3 years of onset with mortality rate of 10–20%.
    - High titer antibody in CSF is diagnostic.

Complications of Measles:
- Otitis media and bronchopneumonia are the most common
- Subacute sclerosing panencephalitis (SSPE) is the rarest but severe most.

Laboratory Diagnosis

- Cell lines: Monkey or human kidney cells or a lymphoblastoid cell line (B95-a) are optimal cell lines used for isolation of measles. Vero/hSLAM cell line is the CDC recommended cell line.
- Cytopathic effect: Multinucleated giant cells (**Warthin-Finkeldey cells**) containing both intranuclear and intracytoplasmic inclusion bodies.

Cytopathic effect of Measles:
Multinucleated giant cells (Warthin-Finkeldey cells) containing both intranuclear and intracytoplasmic inclusion bodies.

Live Attenuated Measles Vaccine

- *Strains:* All are derived from the original Edmonston strain isolated in 1954, which includes:
  - Schwartz strain (currently serves as the standard in much of the world)
  - Edmonston-Zagrebstrain
  - Moraten strain
- Vaccine is prepared in chick embryo cell line
- *Reconstitution*: Vaccine is available in lyophilized form and it has to be reconstituted with distilled water and then should be used within 4 hours.
- Vaccine is thermolabile, hence it must be stored at 20°C.
- One dose (0.5 ml) containing > 1000 infective viral units is administered subcutaneously.
- *Indication:* It is given at 9 months (because maternal antibody disappears by this time) along with vitamin-A supplements.
- However, it can be given at 6 months during measles outbreak in that case a second dose should be given at 9 month.
- **Side effects include:**
  - Mild measles like illness develops (15–20%). There is no spread of the vaccine virus in the community.
  - Toxic shock syndrome (due to contamination of vaccine vial with *S. aureus* toxins).

Measures taken following exposure

- Measles vaccine is given within 3 days of exposure. This is because incubation period of measles induced by the vaccine strain is about 7 days, compared to 10 days for the naturally occurring measles.
- Measles immunoglobulin can also be given within 3 days, at a WHO recommended dose of 0.25 mg/kg of body weight.
- However, both should not be given together. At least 8–12 weeks of gap must be maintained.

Measures taken following exposure to measles patient:
- Measles vaccine or Ig can be given within 3 days
- However, both should not be given together
- At least 8–12 weeks of gap must be maintained.

Epidemiology

Measles is endemic throughout with epidemics recur regularly every 2–3 years, typically in late winter and early spring.

- **Source:** Cases are the only source of infection. There is no carrier stage.
- **Reservoir:** Humans are the only reservoir of infection. There is no animal reservoir.
- **Period of communicability:** Patients are infectious from four days before to four days after the onset of rash.
- **Secondary attack rate** is very high (> 90%)
- **Age:** Measles is a childhood disease
  - Children (6 months to 3 years) in developing countries.
  - Older children (> 5 years) in developed countries or in vaccinated population.
- **Immunity:** No age is immune if there is no previous immunity.
  - There is single serotype, hence one attack (vaccine or infection) gives lifelong immunity.
  - Infants are protected up to 6 months due to pre-existing maternal antibodies.
- **Measles genotypes:** There are 8 clades of measles which are further grouped into 23 genotypes (WHO). Globally, genotype B3 is the most common, where as in India, D8 is common.
- **Epidemic** of measles occurs if proportion of susceptible children exceeds 40%.
- **Recent outbreaks:** In 2014, outbreak of measles had occurred in Philippines and Vietnam.

Measles genotypes:
- Globally, genotype B3 is the most common
- Where as in India, D8 is common.

Measles Eradication

With the efficient and widespread immunization programme, it is possible to eradicate measles from the world.

WHO measles elimination strategy: '*Catch up, Keep up and Follow up*' the immunization programme.

- **Catch-up campaign** is a one-time effort to vaccinate all children between 9 months up to 10 years irrespective of their prior immunization status. The aim is to rapidly reduce the susceptible population in the community.
- **Follow-up campaigns** are done every 2–4 years following catch-up campaigns to vaccinate all children of > 9 months age who have born after the last catch-up campaign.

WHO measles elimination strategy:
- Catch up, Keep up and Follow up the immunization programme.

RESPIRATORY SYNCYTIAL VIRUS

- **Clinical Manifestations**
  - Infants: RSV is the most common cause of lower respiratory tract infection below 1 year of age, causing bronchiolitis, pneumonia, and tracheobronchitis.
  - Adults: RSV produces influenza-like upper respiratory symptoms.
  - RSV can cause exacerbation and worsening of asthma or COPD.
  - Recurrent infection is common, but is much milder (common cold).
- **Laboratory Diagnosis**
  - Virus isolation: HeLa and HEp-2 are the most sensitive cell lines for virus isolation.
  - A characteristic cytopathic effect, **syncytium formation (multinucleated giant cell)** appear after 10 days, hence it is named as syncytial virus.
- **Epidemiology**
  - Seasonality: Rain fall, in winter and spring.
  - Age: Infants between ages of 6 weeks to 6 months of age.
  - Subgroups: RSV can be typed into two subgroups; Subgroup A infections appear to cause more severe illness.
- **Treatment**
  - **Ribavirin** is the drug of choice. It is indicated for severe infections in infants. However its beneficial effect to older children and adult is doubtful. It is administered as aerosol for 3–6 days.

RSV:
- MC cause of LRTI in infants, causing bronchiolitis, pneumonia, and tracheobronchitis.

RUBELLA

Rubella is not a myxovirus, but discussed here because of its clinical overlapping with measles.

Rubella is also known as German measles. It belongs to family Togaviridae.

Epidemiology

- Source: Only cases, No carriers
- Once infected: Provides lifelong immunity
- In India, still 40% females of reproductive age group are susceptible to rubella infection.
- Period of communicability: 1 week to +1 week of rash
- IP-2–3 weeks (14 days)
- Transmission: Droplet, contact, sexual, in-utero.

Clinical Manifestations in Adult

- Subclinical infections: 50%
- Rash on day 1 (face): lasts for 3 days
- Lymphadenopathy (occipital and postauricular)
- Forschheimer spots: Pin-head sized petechiae seen on the soft palate and uvula. They appear with onset of rash.

Congenital Rubella Syndrome

- Risk of transmission and severity is maximum in 1st trimester of pregnancy, after 5th month: Risk negligible

Congenital Rubella Syndrome (Classical Triad):
- Ear defect: Nerve deafness (MC defect)
- Ocular defects: Salt-and-pepper retinopathy is the MC ocular defect followed by cataract
- Cardiac defect: PDA is MC > pulmonary artery stenosis > VSD.

- **Permanent congenital defects**
  - Classical Triad:
    - Ear defect: Nerve deafness (MC defect of congenital rubella syndrome)
    - Ocular defects: Salt-and-pepper retinopathy is the MC ocular defect followed by cataract
    - Cardiac defect: Patent ductus arteriosus (PDA) is the MC cardiac defect followed by pulmonary artery stenosis and ventricular septal defect.
  - CNS defects such as microcephaly and mental retardation, and motor delay and autism
- **Transient congenital changes** such as hepatosplenomegaly, bone lesion, intrauterine growth retardation (IUGR) and thrombocytopenia with petechiae (*Blueberry muffin syndrome*) may be seen.
- **Diagnosis:** IgM at birth or persistent IgG that doesn't fall 2 fold dilution/month, virus isolation (within 6m), RT-PCR (for viral RNA detection).

Laboratory Diagnosis

- Most widely used methods: Hemagglutination inhibition test (HAI) and ELISA
- **Culture:**
  - Ideal specimen: Nasopharyngeal or throat swabs
  - Ideal cell line: Monkey or rabbit origin cell lines may be used.
  - Identified by interference with Echovirus
- Interpretation of serology in congenital rubella infection
  - IgM antibodies do not cross placenta; their presence in a neonate is diagnostic of congenital rubella infection.
  - IgG antibodies cannot differentiate between maternal transfer and a true congenital infection. However, IgG persisting in baby's serum beyond the expected time of disappearance of maternal IgG can be used for diagnosis.

Rubella Vaccination (RA 27/3 Live Attenuated)

Rubella Vaccination (RA 27/3 live attenuated):
- Prepared from human diploid cell line
- As it is teratogenic, pregnancy should be avoided at least for 4 weeks (28 days) following vaccination.

- Prepared from Human diploid cell line, Single dose (0.5 ml) of vaccine is administered subcutaneously.
- Vaccine is contraindicated in pregnancy.
- As it is teratogenic, pregnancy should be avoided at least for 4 weeks (28 days) following vaccination.
- Infants below 1 year should not be vaccinated due to possible interference from persisting maternal antibody.
- Priority groups for rubella vaccine in India: indicated in all women of reproductive age (first priority group) followed by all children (1–14 years).

Daywise Appearance of Rashes

1st day–Rubella	3rd day–Smallpox	5th day–Parvovirus B19–Exanthem infectiosum
2nd day–Chickenpox	4th day–Measles	6th day–HHV6–Exanthem subitum or Roseola infantum

Vaccine Storage

- Deep Freezer: Polio, Measles (–20°C)
- Vaccine is stored at cold part (4°C) and never allowed to freeze: DPT, Typhoid, TT, DT, BCG diluents
- Vitamin A: Outside, at room temperature
- Most of the vaccine can be stored up to 5 weeks in refrigerator: between 4–80°C

Open multidose vials should be discarded:
- Within 1 hr (if no preservative is added, e.g. most live vaccines)
- Within 3 hr or till end of session (if preservative is added).

MULTIPLE CHOICE QUESTIONS

INFLUENZA

1. True about antigenic drift? *(AIIMS MAY 2016)*
a. Caused only by influenza A
b. Leads to seasonal epidemics
c. Leads to pandemic
d. Arises due to frame shift mutations

2. About Killed Influenza vaccine dosage, all are true except: *(Recent Question 2015)*
a. It can be given to pregnant patient
b. Adult dose is 0.5 ml
c. At age of 6–36 months , dose is 0.25 ml
d. Immunity lasts for 3 years

3. Trivalent Influenza vaccine contains all except:
a. H1N1 *(AIIMS Nov 2015)*
b. H2N1
c. Influenza B
d. H3N2

4. True about Swine flu: *(PGI May 2015)*
a. Older bird influenza vaccine is equally effective in swine flu
b. Oseltamivir is effective in prevention
c. Zanamivir can be used for treatment
d. Influenza vaccine provides immunity just after vaccination

5. Avian influenza is due to: *(Latest MCQ 2013)*
a. H1N1 b. H3N1
c. H5N1 d. H7N1

6. 70-year-old women refused to take influenza vaccine, developed flu. Death happened 1 week after pneumonia. Most common cause of Post influenza pneumonia is: *(AIIMS Nov 2014)*
a. Staphylococcus aureus
b. Measles
c. Legionella
d. CMV

7. Reason for H5N1 influenza not becoming a pandemic: *(AIIMS Nov 2014)*
a. Man to man transmission is rare
b. No human to human transmission occurs
c. Less virulent
d. Bird to bird transmission is not efficient

8. Outbreak of avian Influenza epidemic in china In 2013 is caused due to stain: *(PGI May 2013)*
a. H1N1
b. H3N2
c. H5N1
d. H7N7
e. H7N9

9. True about influenza: *(PGI June 05, 06)*
a. Asymptomatic cases rare
b. IP-10–12 days
c. Pandemic – rare
d. Extrahuman reservoir not seen
e. All age and sex equally affected

10. Swine flu in 2009 is caused by: *(DNB Dec 2012)*
a. H1N1
b. H5N1
c. H3N1
d. H3N3

11. Antigenic variation is seen in all except: *(DNB Dec 2011)*
a. Influenza type A b. Influenza type B
c. Influenza type C d. None of the above

12. Myxoviruses include: *(PGI Dec 2008)*
a. Orthomyxovirus
b. Influenza
c. Measles
d. Polio
e. HSV

13. H5N1 is a strain of:
a. Avian flu *(AI 2008, DNB 2014, Latest MCQ 2014)*
b. New vaccine against AIDS
c. Agent for Japanese encephalitis
d. Causes Chikungunya fever

14. Antigenic variation seen in which of the following?
a. Influenza virus *(PGI Dec 2004)*
b. Hepatitis virus
c. Yellow fever virus
d. Leptospira

15. Which of the following statement is/are true of all paramyxoviruses: *(PGI 2003)*
a. They contain a single stranded RNA genome of negative polarity
b. Envelope is derived from the host cells plasma membrane
c. They have a cytoplasmic site of replication
d. They enter the body by the respiratory route

16. Modality not employed in the diagnosis of respiratory viruses in laboratory: *(JIPMER Nov 2014)*
a. ELISA
b. Immunofluorescence
c. Single Radial Hemolysis
d. Hemagglutination

MEASLES

17. A child is presented with fever, conjunctivitis and bluish white spot on buccal mucosa. Four days later, she developed rashes. What is the characteristic feature of the virus that is responsible for this condition? *(JIPMER May 2016)*
a. ss- naked RNA virus
b. ds- naked RNA virus
c. ss- enveloped RNA virus
d. ds- enveloped RNA virus

18. Warthin Finkeldey cells are observed in: *(West Bengal 2016, TNPG 2015)*
a. Measles
b. Rubella
c. Varicella
d. Small pox

19. According to WHO's measles elimination strategy, vaccination campaigns are done in which phage?
a. Mop up *(AIMS Nov 2013)*
b. Follow up
c. Keep up
d. Catch up

20. Which of the following is not true about measles?
a. High secondary attack rate *(AI 2008)*
b. Only one serotype
c. Not infectious in prodromal stage
d. Infection confers lifelong immunity

21. Least common complication in measles?
a. Diarrhea *(AIIMS May 06, May 2007)*
b. Pneumonia
c. Otitis media
d. SSPE

22. Reservoir of measles: *(DPG 2007)*
a. Man
b. Soil
c. Monkey
d. Fomites

23. True about measles: *(PGI June 2004)*
a. Koplik spot appears in prodromal stage
b. Fever stops after onset of rash
c. Vaccine – at 9 month
d. IP- 6 days
e. Not diagnosed when coryza and rhinitis are absent

24. A baby was given measles vaccine at 6 month due to outbreak in the community. Which is correct statement regarding the subsequent dose?
a. Given at 9 month *(MH 2007, DNB 2003)*
b. Given 1 dose as soon as possible
c. No dose required
d. Given at 14–16 month age with booster dose

25. To eradicate measles, the % of infant to be vaccinated:
a. 70% *(DNB 2001, 2003)*
b. 80%
c. 85%
d. 95%

26. Most fatal complication of measles: *(Recent Question 2015)*
a. Pneumonia
b. Otitis media
c. SSPE

27. In measles, the patient is infectious: *(UP 2008)*
a. 3 days before to 4 days after the onset of rash
b. 4 days before to 3 days after the onset of rash
c. 4 days before to 5 days after the onset of rash
d. 5 days before to 4 days after the onset of rash

28. Giant cell (Hecht's) pneumonia is due to:
a. CMV *(PGI Dec 2000)*
b. Measles
c. Malaria
d. P. carinii

29. Which of the following is wrong about isolation of the patient: *(AIIMS May 2014)*
a. Chickenpox: 2 days before to 5 days after rash
b. Measles: up to 3 days of rash
c. Mumps: Until swelling subsides
d. Rubella: up to 7 days after of rash

30. Immune thrombocytopenic purpura is a complication following which of the following vaccine?
a. DPT *(AIIMS May 2014)*
b. OPV
c. MMR
d. Typhoid
e. Influenza

31. Chemoprophylaxis is not done for? *(AIIMS May 2014)*
a. Measles
b. TB
c. Diphtheria
d. Conjunctivitis
e. Cholera

32. Patient presented with fever, coughing, headache. He developed rash on 4th day of onset of fever, what is probable diagnosis? *Recent Questions 2014*
a. Measles
b. Mumps
c. Smallpox
d. Chickenpox

MUMPS

33. Parotitis and orchitis are common manifestations of:
a. Measles *(APPG 2015)*
b. Mumps
c. Rubella
d. Diphtheria

34. With reference to mumps which of the following is true: *(AI 2006)*
a. Meningoencephalitis can precede parotitis
b. Salivary gland involvement is limited to the parotid
c. The patient is not infectious prior to clinical parotid enlargement.
d. Mumps orchitis frequently leads to infertility

35. Commonest complication of mumps is:
a. Orchitis and oophritis *(AI 2000, RJ 2004)*
b. Encephalitis
c. Pneumonia
d. Myocarditis

RSV

36. RSV causes all except: *(DNB June 2009)*
a. Coryza in kids
b. ARDS
c. Bronchitis
d. Common cold

37. Regarding respiratory viruses all are true except: *(AIIMS Nov 2007)*
a. RSV is the most common cause of bronchiolitis in infants
b. Mumps causes septic meningitis in adult
c. Measles causes SSPE
d. EBV causes pleuritis

38. Which pathogens adhere to respiratory epithelium?
a. RSV *(PGI Dec 2006)*
b. Influenza
c. Parainfluenza
d. HBV
e. Picornavirus

39. Which of the following pair is correct? *(PGI Dec 2005)*
a. RSV: Bronchiolitis
b. Orf: Viral infection is transmitted from sheep
c. Parvovirus B 19: Exanthema subitum
d. HHV6: Kaposi Sarcoma

RUBELLA

40. A female became pregnant after 1month of taking MMR vaccine; though was advised to avoid pregnancy. What advice the doctor should give to the patient? *(JIPMER May 2016)*
a. Termination of pregnancy is mandatory
b. High risk of anomalies, serious consideration for termination
c. Low risk, no action needed
d. Wait and watch.

41. If lady has taken live vaccine recently, she can plan for pregnancy at least after: *(Recent Question 2015)*
a. 1 month
b. 3 months
c. 6 months
d. 1 year

42. All are true about congenital rubella except: *(AIIMS May 2011, AI 2005)*
a. IgG persists for more than 6 months
b. IgM antibody is present at birth
c. Most common anomalies are hearing and heart defects
d. Increased congenital malformation if infection after 16 weeks

43. Age group most prone to Rubella is: *(DNB June 2009)*
a. Children 3–10 years
b. Adolescent girls
c. Pregnant females
d. Women of child bearing age

44. Recommended vaccination strategy for rubella is to given first and foremost for: *(AI 2007, RJ 2008)*
a. Women 15–49 year
b. Infants
c. Children 1–14 year
d. Adolescent girls

45. Rubella: All are seen except: *(AP 2003)*
a. Tender LN in neck
b. Congenital infection: Cataract
c. IP < 10 days
d. RNA virus

46. Which of the following is a cause of acute laryngotracheal bronchitis? *(DNB June 2009)*
a. H influenzae
b. Parainfluenza virus
c. Influenza
d. Coxsakie virus

47. A new borne presents with PDA and cataract. Infection with which group of virus is likely to be the cause?
a. Rubella *(TNPG 2014)*
b. Togavirus
c. Measles
d. Chicken pox

48. Forschheimer spots seen in: *(TNPG 2014)*
a. Rubella
b. Measles
c. Mumps
d. Chickenpox

EXPLANATIONS

INFLUENZA

1. **Ans. b (Leads to seasonal epidemics)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p459
   - Antigenic drift is seen in both Influenza A and B; leads to seasonal periodic epidemics and minor outbreaks; Arises due to point mutation.
   - Antigenic shift is seen in only Influenza A; leads to endemics and major epidemics; Arises due to genetic recombination.

2. **Ans. (d) (Immunity lasts for 3 years)** Ref: Park 23 e/p155

   Immunity lasts for 6—12 months; hence, on an annual basis revaccination is recommended.

3. **Ans. (b) (H2N1)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p461
   - Trivalent influenza vaccine include H1N1 , H3N2, Influenza B

4. **Ans. (b, c) (Oseltamivir.., Zanamivir ..)** Ref: Park 23/e p157-8, Apurba Sastry's Essentials of Medical Microbiology 1/e p 463
   - Oseltamivir is DOC for chemoprophylaxis. For treatment, both Oseltamivir (DOC) and Zanamivir can be given.
   - Avian influenza (H5N1) vaccine is not effective for swine flu.
   - There is a separate pandemic influenza vaccine is available for swine flu, composed of H1N1 (both live and inactive forms). It is effective only after 14 days of vaccination.

5. **Ans. (c) (H5N1)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p462, Ananthanarayan 9/e p502

   Avian influenza strains are: H5N1 (most common), H7N9, H7N3, H7N7, and H9N2

6. **Ans. (a) (Staphylococcus...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p459, Harrison 19/e p1214, 18/e p1494-96

   The most common cause of postinfluenza secondary pneumonia are bacterial pathogens such as Streptococcus pneumoniae, Staphylococcus aureus, and Haemophilus influenzae.

7. **Ans. (b) (No human ..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p462, Harrison 19/e p1205, 18/e p1493-99

   Avian flu strain (H5N1)
   - Transmission to man occurs only from birds and require close respiratory contact.
   - There is no human to human transmission documented so far. Hence the morbidity is less. Only 500 cases were reported between 1977 to 2010 from Asia and Middle East.
   - However, the avian flu strains are highly virulent (due to presence of PB1F2 protein) and mortality rate is > 60%.

8. **Ans. (e) (H7N9)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p462, CDC website

   Recent outbreak of Avian Influenza reported from China in April 2013 was due to type A/H7N9, > 130 human infections were reported, 43 died. It was controlled later, due to containment measures taken by China Govt.

9. **Ans. (c) (Pandemic...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p460, Park 23/e p153, 22/e p145
   - Asymptomatic cases are more common with influenza infection.
   - Incubation period of influenza has ranged from 18 to 72 hr.
   - Pandemic – rare, occurs every 10–15 yrs
   - Major reservoir of influenza persists in animals and birds.
   - Influenza attack all the ages of both the sexes. But the attack rate is low in adults and mortality rate is high in children, older age, patient with diabetes, chronic heart/renal/respiratory disease.

10. **Ans. (a) (H1N1)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p463, Park 23/e p154, 22/e p147

    Swine flu in 2009 is due to H1N1

11. **Ans. (c) (Influenza type C)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p459, Park 23/e p153, 22/e p145

    Antigenic variation is commonly seen in type A and to less extent type B. Type C influenza is antigenically stable.

12. **Ans. (a), (b), (c) (Orthomyxovirus, Influenza, Measles)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p457
    - Myxoviruses are enveloped RNA viruses

They are divided as

- Orthomyxovirus: Influenza A,B,C
- Paramyxovirus: Parainfluenza, Measles, Mumps, RSV, Metapneumovirus

13. **Ans. (a) (Avian flu)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p462, Harrison 18/e p1494
    - In 1997, human cases of influenza caused by avian influenza viruses (A/H5N1) were detected in Hong Kong during an extensive outbreak of influenza in poultry.
    - Mortality rates have been high (60%)
    - Only bird to human transmission seen, but no human-human transmission seen
    - Highly virulent due to PB1F2 which targets host mitochondria, induces apoptosis

14. **Ans. (a) (Influenza virus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p458, Ananthanarayan 9/e p499
    - Antigenic variation is a unique feature of influenza virus.
    - The surface antigens hemagglutinin and neuraminidase are primarily responsible for antigenic variations exhibited by influenza viruses.

15. **Ans. (a), (b), (c), (d) (They contain a single..., Envelope is derived from..., They have a cytoplasmic... and They enter the...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p457,464, Ananthanarayan 9/e p507, 8/e p4504
    - Paramyxoviruses are *negative sense single stranded* Enveloped RNA viruses
    - Site of riboncleoprotein synthesis is *cytoplasm* and envelop is derived from *host cell plasma membrane*
    - They are important pathogens of infants and children and responsible for major part of acute respiratory infections and Infection is acquired by *respiratory route*.

16. **Ans. (c) (Single radial hemolysis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p460, internet sources
    - Single Radial Hemolysis is a very old and obsolete test, usually carried out for rubella and some time for other viral infections like influenza.
    - Other options ELISA, direct IF test and Hemagglutination- all are done for influenza virus and for some other respiratory viruses as well.

MEASLES

17. **Ans (c) (ss- enveloped RNA virus)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p468
    - The history of fever, conjunctivitis and Koplik spot's on buccal mucosa and rashes- suggestive of measles.

18. **Ans. (a) (Measles)** Ref:Apurba Sastry's Essentials of Medical Microbiology 1/e p468; Ananthnarayan 9/e p512
    Warthin Finkeldey cells are intranuclear and cytoplasmic multinucleated giant cells found in Measles.

19. **Ans. (d) (Catch up)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p470, Park 21/e p137
    WHO measles Elimination strategy: 'Catch up, Keep up and Follow up'
    - Catch-up is defined as onetime national wide vaccination campaigns targeting children of 9 months-14 years regardless of measles disease or vaccination status.
    - Keep-up is defined as routine services aimed at vaccinating >95% of each successive birth cohort.
    - Follow-up is defined as subsequent national wide vaccination campaigns conducted every 2–4 yrs targeting usually all children born after the catch-up campaign.

20. **Ans. (c) (Not…)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p467-470, Park 23/e p146, 22/e p139, Harrison 18/e p1600
    - Measles: infectious during prodromal stage
    - High secondary attack rate of 90%
    - Once infected provides lifelong immunity
    - Only one serotype (antigenically homogenous)

21. **Ans. (d) (SSPE)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p468, Park 23/e p147, 22/e p139, 21, Harrison 19/e p1298, 18/e p1603
    - SSPE is rare but severe most complication following measles with occurrence rate of 1:300,000
    - Most complications of measles result from secondary bacterial infections of the respiratory tract Otitis media and bronchopneumonia are most common and may be caused by S. pneumoniae, H. influenzae type b, or staphylococci.

22. **Ans. (a) (Man)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p467-470, Harrison 19/e p1298, 18/e p1600
    - There are no latent or persistent measles virus infections that result in prolonged contagiousness, nor are there animal reservoirs for the virus.
    - Thus, measles virus can be maintained in human populations only by an unbroken chain of acute infections, which requires a continuous supply of susceptible individuals

23. **Ans. (a) (c) (Koplik.., Vaccine ..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p467-470, Park 23/e p147, 22/e p139
    - Koplik spot appears in prodromal stage: 2 days after fever appears
    - Both fever and rash will gradually disappear in 3–4 days of onset of rash
    - Vaccine: indicated at 9 month
    - IP of measles: 10 days
    - Clinical diagnosis: Based on typical rashes and Koplik's spot. The diagnosis would be incorrect if red eye and cough are absent.

24. **Ans. (a) (Given at 9 month)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p467-470, Park 22/e p140
    - Age: Given at 9 months (because maternal antibody disappears by this time)
    - However, can be given at 6 months if measles outbreak seen.
    - In this case, the 2nd dose to be given at 9 month (provided at least 1 month gap should have elapsed from the 1st dose)

25. **Ans. (d) (95%)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p467-470, PSM Park 23/e p147, 22/e p141
    - To eradicate measles, 96% of infant to be vaccinated.
    - Epidemic of measles occurs if proportion of susceptible children > 40%.
    - If measles is introduced in a virgin community, it infects > 90% of children.

26. **Ans. (c) (SSPE)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p468

27. **Ans. (c) (4 days before...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p467-470, Park 22/e p139
    Refer chapter review

28. **Ans. (b) (Measles)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p467-470, Ananthanarayan 9/e p513, 8/e p510
    In children with immunodeficiency or severe malnutrition measles virus can cause fatal giant cell pneumonia.

29. **Ans. (d) (Rubella ..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p467-470, Park 22/e p112, 141
    Rubella is much less communicable than mumps and measles. Patient is infectious from 1 week before to 1 week after rash, however isolation is not needed except that woman is in third trimester or sexually active, nonimmune woman should not be exposed.

	Patient is infectious from	Duration of isolation recommended
Chickenpox	2 days before to 5 days after rash	Until all lesions are crusted, usually about 6 days
Mumps	1 week before to 2 weeks after parotitis	Until swelling subsides
Measles	4 days before to 5 days after rash	From the onset of catarrhal stage till 3rd day of rash
Rubella	1 week before to 1 week after rash	None, except that woman is in third trimester or sexually active, nonimmune woman should not be exposed

30. **Ans. (c) (MMR)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p467-470
    A causal association between MMR vaccine and ITP was confirmed in this study. The absolute risk of ITP within six weeks of immunisation was 1 in 22 300 doses, with two of every three cases occurring in the six week postimmunisation period being caused by MMR.

31. **Ans. (a) (Measles)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p467-470, Park 22nd/p117
    There is no antiviral drugs available for measles. Hence, vaccine prophylaxis is the only option for prevention of measles.
    Chemoprophylaxis is indicated for:
    - Cholera: Tetracycline
    - Conjunctivitis (bacterial): Erythromycin ointment
    - Diphtheria: Erythromycin and 1st dose of vaccine
    - Meningococcal meningitis: Sulfadiazine or Rifampicin or ciprofloxacin and vaccine against type A and C
    - Plague: Tetracycline (for contacts of pneumonic plague)
    - Influenza A: Amantadine and vaccine

32. Ans. (a) (Measles) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p467 , Park 22/e p139
Refer chapter review.

MUMPS

33. Ans (b) (Mumps) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p466
In Mumps: The common manifestation is Parotitis, and the common complication is orchitis

34. Ans. (a) (Meningoencephalitis can precede parotitis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p466, Harrison 18/e p1609
States - 'Aseptic meningitis, which may develop before, during, after, or in the absence of parotitis, is common in both children and adults……Atypical Mumps'.
- Parotid gland is commonly involved, occasionally sublingual, submandibular glands also may be involved
- Period of communicability: 4 days before to 5 days after appearance of rash
- Mumps orchitis is usually unilateral, Rarely can be bilateral and then it leads to low sperm count and sterility. Since orchitis is bilateral in < 15% of cases, sterility after mumps is rare.

35. Ans. (a) (Orchitis and Oophoritis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p466
- Edididymo orchitis and oophoritis are common complications seen in about a third of postpubertal patients
- The testis is painful, tender, and enlarged to several times its normal size; accompanying fever is common.
- Later, testicular atrophy develops in half of the affected men.
- Since orchitis is bilateral in < 15% of cases, sterility after mumps is rare.
- Less common complications: arthritis, nephritis, pancreatitis, thyroiditis and myocarditis.

RSV

36. Ans. (b) (ARDS) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p470-71, Harrison 18/e p1488-89, 19/e p1202-08
- RSV: In infants, 25–40% of infections result in lower respiratory tract involvement, including pneumonia, bronchiolitis, and tracheobronchitis.
- RSV: In adults, the most common symptoms common cold, with rhinorrhea, sore throat, and cough.
- RSV: Also called as Chimpanzee Coryza Agent

37. Ans. (d) (EBV causes pleuitis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p470-71
Options a, b and c are correct. (Already explained)
EBV causes glandular fever and it is oncogenic virus

38. Ans. (a), (b), (c), (e) (RSV, Influenza, Parainfluenza, Picornavirus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p470-71, Ananthanarayan 9/e p500, 509 and 8/e p497, 507
- Influenza and parainfluenza viruses attaches to the ciliated cells of the respiratory tract
- RSV is the pathogen of lower respiratory tract
- Rhinovirus, which belong to Picornavirus family attaches to ciliated cells of respiratory tract.

39. Ans. (a), (b) (RSV-Bronchiolitis, orf-viral infection is transmitted from sheep)
Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p470-71, Ananthanarayan 9/e p464, p510, 8/e p465, p508
- Orf/Contagious pustular dermatitis is a disease of sheep and goats transmitted to humans by contact
- RSV is responsible for half of the cases of bronchiolitis occurring in the first few months of life
- Exanthem subitum caused by HHV-6
- Kaposi sarcoma by HHV-8.

RUBELLA

40. Ans (d) (wait and watch) Ref: Apurba Sastry's Essentials of Medical Microbiology/p473

Receipt of Rubella vaccine during pregnancy is not ordinarily a reason to consider termination of the pregnancy. Harrison 19th/p1299 (230e), Nelson's paediatrics 20th/p1511

Vaccine should not be administered during pregnancy. If pregnancy occurs within 28 days of immunization, the patient should be counselled on the theoretical risks to the foetus. Studies of over 200 women who had been inadvertently immunized with rubella vaccine during pregnancy showed that none of their offspring developed congenital rubella syndrome. Therefore, interruption of pregnancy is probably not warranted …… Nelson's paediatrics 20th/p1511

41. **Ans. (a) (1 month)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p473

42. **Ans. (d) (Increased congenital...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p472-73, and Harrison 19/e p1299, 18/e p1606
Congenital malformations are commonest during the first trimester and if infection occurs very early in pregnancy fetus may die.

43. **Ans. (a) (Children...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p472-73, Park 22/e p142, 23e/151
Rubella is mainly a disease of childhood particularly 3–10 yrs.

44. **Ans. (a) (Women...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p472-73, Park 22/e p142, 23/e p151
Priority groups for rubella vaccine in India: Females (reproductive age) > All children (1–14 yr) > after 1 yr.

45. **Ans. (c) (IP < 10 days)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p472-73, Park 22/e p142, 23/e p151
- Tender Lymphadenopathy, particularly occipital and postauricular, may be noted during the second week after exposure of Rubella
- Cataract is one of the important Congenital manifestation.
- IP of Rubella: 14 days (2–3 weeks)
- Rubella is a RNA virus, belongs to togaviridae family.

46. **Ans. (b) (Parainfluenza...)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p472-73, Ananthanarayan 9/e p509
Parainfluenza virus type 2 (sometimes type 1) is the causative agent of croup (acute laryngotracheal bronchitis)

47. **Ans. (a) (Rubella)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p472-73, Ananthanarayan 9/e p555
Refer chapter review.

48. **Ans. (a) (Rubella)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p472-73, Ananthanarayan 9/e p555
Refer chapter review.

CHAPTER

4.4

Arboviruses, Picornaviruses and Rabies Virus

ARBOVIRUSES

Arboviruses (arthropod-borne viruses) are diverse group of RNA viruses that are transmitted by bloodsucking arthropods (insect vectors) from one vertebrate host to another.

- **Transmission cycle:** Arboviruses are zoonotic, maintained in the nature between animals and their insect vectors.
- **Humans are the accidental hosts** and do not play any role in the maintenance or transmission cycle of the virus, except for urban yellow fever and dengue.
- **Arboviruses found in India:** Over 40 arboviruses have been detected in India:
  - Common: Dengue, chikungunya, and Japanese B encephalitis viruses
  - Rare: Kyasanur forest disease, West Nile, Sindbis, Crimean Congo hemorrhagic fever, Ganjam, Vellore, Chandipura, Bhanja, Umbre, Sathuperi, Chittoor, Minnal, Venkatapuram, Dhori, Kaisodi and sandfly fever viruses.

Virus	Manifestation	Distribution	Vector	Reservoir
Family—Togaviridae				
Chikungunya virus	Fever and arthritis	Asia, Africa	*Aedes aegypti*	Monkeys (?)
O'nyong-nyong virus	Fever and arthritis	Africa	*Anopheles*	?
Mayaro virus	Fever and arthritis	South America	*Aedes aegypti*	Monkeys
Ross River virus	Epidemic polyarthritis	Australia	*Aedes*	Small animals
Sindbis virus	Arthralgia, and rash	Africa, Europe, Australia	*Culex*	Birds, mammals
Semliki Forest virus	Fever and arthralgia	Africa	*Aedes*	Birds, rodents
Eastern equine encephalitis virus	Encephalitis	Eastern part of North America	*Aedes, Culex*	Birds
Western equine encephalitis virus	Encephalitis	Western part of North America	*Culex tarsalis, Aedes*	Birds
Venezuelan equine encephalitis virus	Encephalitis	South and central America	*Aedes, Culex*	Horses
Family—Flaviviridae				
Japanese B encephalitis virus	Encephalitis	South East Asia	*Culex tritaeniorhynchus*	Pigs, Birds
St. Louis encephalitis virus	Encephalitis	United States	*Culex*	Wild birds
West Nile encephalitis virus	Encephalitis	East Africa (Uganda), Algeria, Romania	*Culex, Aedes, Anopheles*	Birds
Murray Valley encephalitis virus	Encephalitis	America	*Culex annulirostris*	Birds
Rocio virus	Encephalitis	São Paulo, Brazil	*Culex*	?
Russian spring-summer encephalitis virus	Encephalitis	Central Europe, Russia	Tick	Rodents, other mammals, birds
Powassan virus	Encephalitis	America	Tick	Rodents
Louping-ill	Encephalitis	Europe	Tick	Sheep
Dengue virus	Hemorrhagic fever	India	*Aedes aegypti*	?
Yellow fever virus	Hemorrhagic fever	West Africa, Central South America	*Aedes aegypti*	Monkeys
Kyasanur Forest Disease virus	Hemorrhagic fever	India (Karnataka)	Tick	Monkeys and rats

Contd...

Contd...

Virus	Manifestation	Distribution	Vector	Reservoir
Omsk Hemorrhagic fever virus	Hemorrhagic fever	Russia	Tick	Small mammals
Family—Bunyaviridae				
California encephalitis virus	Encephalitis	USA	*Aedes triseriatus*	Rodents
Oropouche virus	Rash and aseptic meningitis	Central and South America	*Culicoides paraensis*	Not known
Sandfly fever	fever and myalgia	Southern Europe, North Africa, India	Sandfly	Small mammals
Rift Valley fever virus	fever and myalgia	Africa	*Aedes*	Sheep, cattle
Crimean Congo hemorrhagic fever virus	Hemorrhagic fever	Africa	Tick	Small mammals
Ganjam virus	?	India	Tick	Small mammals
Family—Reoviridae				
Colorado tick fever virus	Fever, rarely encephalitis	America (mountains)	Tick	Rodents
Orungo virus	Fever	Sub-Saharan Africa	*Aedes*	?
Kemerovo virus	Fever, meningism	Russia	Tick	?
Family—Rhabdoviridae				
Vesicular stomatitis virus	Oral mucosal vesicles	Indiana	Sandfly	?
Chandipura virus	Encephalitis	India	Sandfly	?

?- Not yet identified

Reasons for Chikungunya re-emergence:
- New mutation (E1-A226V)
- New vector (Aedes albopictus).

CHIKUNGUNYA

Chikungunya fever is a re-emerging disease characterized by fever with arthralgia.

- **Human Transmission:** *Aedes* mosquito, primarily *Aedes aegypti* which bites during day time, rarely by vertical transmission from mother to fetus, blood transfusion
- **Clinical Manifestations:**
  - Incubation period is about 5 days (3–7 days)
  - Most common symptoms are fever and severe joint pain (due to arthritis)
  - **Arthritis** is polyarticular (migratory), affecting the small joints.

Features	Chikungunya	Dengue
Fever	Common	Common
Polyarthritis and tenosynovitis	Common	None
Rashes	Day1–4	Day 3–7
Myalgia	Possible	Common
Leukopenia and thrombocytopenia	None	Common
Retro-orbital pain, hypotension, minor bleeding	Rare	Common

- **Epidemiology:**
  - **India:** Reported during 1963–1973; e.g. Kolkata in 1963 and South India in 1964. Since then, it was **clinically quiescent in world** between 1973–2005.
  - **Re-emergence** (Reunion Outbreak): In 2005, Chikungunya re-emerged in Reunion Island of Indian Ocean and then spread to India and other countries.
  - **India (at present):** Chikungunya is endemic in several states.
    - States: Karnataka, Tamil Nadu, Andhra Pradesh and West Bengal have reported higher number of cases.
    - Karnataka accounted for the maximum cases in the year 2013 and 2014.
- **Genotypes:** It has three genotypes: West African, East African and Asian genotypes.
  - Most Indian cases before 1973 were due to Asian genotypes.

- However, Reunion outbreak was caused due to a mutated strain and is responsible for most of the current outbreaks in India as well as in other parts of the world.

- **Reasons for re-emergence:**
  - **New mutation** (E1-A226V): Chikungunya virus underwent an important mutation. Alanine in the 226 position of E1 glycoprotein gene is replaced by valine.
  - **New vector** (*Aedes albopictus*): Mutated virus was found to be 100 times more infective to *A.albopictus* than to *A. aegypti.*
- **Laboratory diagnosis**
  - **Viral isolation** (in mosquito cell lines) and real time **RT- PCR** are best for early diagnosis.
  - **Serum antibody detection:** ***MAC ELISA*** is the best serology test.
  - **Biological markers** like IL-1β, IL-6 and are increased and RANTES levels are decreased in chikungunya infection.

Chikungunya Genotypes:
- Three genotypes: West African, East African and Asian genotypes
- Most Indian cases before 1973 were due to Asian genotypes.
- Reunion outbreak: Due to a mutated strain.

Japanese B Encephalitis (JE)

Japanese B encephalitis is the leading cause of viral encephalitis in Asia, including India. It was first seen in Japan (1871); however, it is now uncommon in Japan.

- ***Vector:*** *Culex* mosquito:
  - *C. tritaeniorhynchus* is the major vector worldwide including India.
  - *C. vishnui* is the next common vector found in India.
- ***Transmission cycle:*** JE virus infects several animals and birds. Two transmission cycles are predominant.
- Pigs → *Culex* → Pigs
- Ardeid birds → *Culex* → Ardeid birds
- ***Animal hosts:***
  - Pigs are considered as the amplifier host. JE virus multiplies exponentially in pigs without causing any manifestation.
  - Cattle and buffaloes may act as ***mosquito attractants.***
  - Horses are probably the only animal to be symptomatic and show encephalitis.
  - Humans are considered as dead end; there is no man → mosquito → man cycle (unlike in dengue)
- ***Bird hosts:*** Ardeid (wading) birds such as herons, cattle egrets, and ducks are the important reservoir.

Vector from JE:
Culex mosquito
- C. tritaeniorhynchus: Worldwide including India.
- C. vishnui is the next common vector found in India.

Animal hosts for JE:
- Pigs are the amplifier host.
- Cattle and buffaloes may act as mosquito attractants.
- Horses: The only animal to be symptomatic
- Humans are considered as dead end
- There is no man→ mosquito →man cycle (unlike in dengue).

Epidemiology

- **Geographical distribution:** Currently, JE is endemic in Southeast Asian region.
  - It is common India, Nepal, Pakistan, Thailand, Vietnam and Malaysia.
  - Because of immunization, its incidence has been declining from Japan and Korea.
  - ***In India*:** JE has been reported since 1955. JE is endemic in 15 states; Uttar Pradesh (***Gorakhpur*** district) accounting for the largest burden followed by Assam, West Bengal, Bihar, Tamil Nadu and Karnataka.
- **Age:** 85% of cases occur in children below 15 years (but infants are not affected).
- **Seasonal Variation:** Common in rainy season with (maximum mosquito activity).

JE in India:
- JE is endemic in 15 states
- Uttar Pradesh (Gorakhpur district) accounting for the largest burden
- Followed by Assam, West Bengal, Bihar, Tamil Nadu and Karnataka.

Clinical Manifestations

JE is the most common cause of epidemic encephalitis:

- **Incubation period**: Varies from 5–15 days.
- **Subclinical infection is common:** JE typically shows *iceberg phenomena*. Cases are much less compared to subclinical/inapparent infection with a ratio of 1:300-1000.
- Even during an epidemic the number of cases are just 1–2 per village.
- **Clinical course** of the disease can be divided into three stages: Prodromal stage, Acute encephalitis stage and Late stage with sequelae of neurological deficits permanently.

Vaccine for JE:
- Live attenuated SA 14-14-2 vaccine
- Inactivated vaccine (Nakayama strain and Beijing strain)
- Inactivated vaccine (Beijing P3 strain): It is a cell line derived vaccine.

Vaccine Prophylaxis

1. **Live attenuated SA 14-14-2 vaccine:**
   - It is prepared from SA 14-14-2 strain
   - It is cell line derived; primary hamster kidney cells are commonly used.
   - Single dose is given subcutaneously, followed by booster dose after 1 year.
   - It is manufactured in China, but now licensed in India.
   - Under Universal Immunization Programme, it is given to children (1–15 years) targeting 83 endemic districts of four states–UP, Karnataka, West Bengal and Assam.
2. **Inactivated vaccine (Nakayama strain and Beijing strain):**
   - It is a mouse brain derived formalin inactivated vaccine.
   - It is prepared in Central Research Institute, Kasauli (India).
3. **Inactivated vaccine (Beijing P3 strain):** It is a cell line derived vaccine.

Vector (Aedes aegypti) for Dengue:
- They bite during the day time.
- Patient is infectious to Aedes from –1 to + 5 days later
- Extrinsic IP of 8–10 days
- Once infected, it remains infective for life.
- Transovarial transmission seen in Aedes.

Dengue Viruses

Dengue virus is the most common arbovirus found in India. It has four serotypes (DEN-1, to DEN-4). Recently, the fifth serotype (DEN-5) was discovered in 2013 from Bangkok.

Vector

Aedes aegypti is the principal vector (most efficient vector) followed by *Aedes albopictus*. They bite during the day time.

- *Aedes* acquires infection by feeding on viremic patients (from a day before to 5 days later, i.e. the end of the febrile period).
- Extrinsic incubation period of 8–10 days is needed before *Aedes* becomes infective.
- Once infected, it remains infective for life.
- *Aedes* can pass the dengue virus to its offsprings by transovarial transmission.
- Transmission cycle: Man and *Aedes* are the principal reservoirs. Transmission cycle does not involve other animals.

Antibody dependent enhancement (ADE):
- The severity of secondary dengue infection is due to ADE
- Here, the non-neutralizing antibody produced against the first serotype will combine, cover and protect the second serotype from host immune response.

Pathogenesis

- *Primary dengue infection* occurs when a person is infected with dengue virus for the first time with any one serotype.
- Months to years later, a more severe form of dengue illness may appear (called *secondary dengue infection*) due to infection with another second serotype which is different from the first serotype causing primary infection.
- The severity of *secondary dengue infection* occurs due to a unique immunological phenomena called **antibody dependent enhancement** (ADE), i.e. the non-neutralizing antibody produced against the first serotype will combine, cover and protect the second serotype from host immune response.
- **ADE is remarkably observed** when serotype 1 infection is followed by serotype 2, which also claims to be the most severe form and prone to develop into DHF and DSS.
- Serotype 2 is apparently more dangerous than other serotypes.

Criteria for DHF:
- High continuous fever
- Hepatomegaly
- Thrombocytopenia (platelet count < 1 Lakh/mm^3)
- Raised hematocrit (packed cell volume) by 20%
- Hemorrhages:
  - Positive tourniquet test (> 20 petechial spots per square inch area)
  - Spontaneous bleeding from skin, nose, mouth and gums.

Clinical Classifications

The traditional (1997) WHO classification, divides dengue into three clinical stages:

- ***Dengue Fever (DF):*** It is characterized by:
  - High fever (called as biphasic fever, break bone fever or saddle back fever)
  - Maculopapular rashes over the chest and upper limbs
  - Others: Frontal headache, Muscle and joint pains, lymphadenopathy, loss of appetite, nausea and vomiting
- ***Dengue Hemorrhagic Fever* (DHF)** is characterized by:
  - High continuous fever
  - Hepatomegaly
  - Thrombocytopenia (platelet count < 1 Lakh/mm^3)

- Raised hematocrit (packed cell volume) by 20%
- Evidence of hemorrhages which can be detected by:
  - Positive tourniquet test (> 20 petechial spots per square inch area in cubital fossa
  - Spontaneous bleeding from skin, nose, mouth and gums

- ***Dengue Shock Syndrome* (*DSS*):** All the above criteria of DHF are present, *plus* manifestations of shock.

The 2009 WHO classification grades dengue into two stages of severity of infection:

- Dengue with or without warning signs
- Severe dengue.

Geographical Distribution

- **Global Scenario:** Tropical countries of Southeast Asia and Western Pacific are at highest risk.
- **Situation in India:**
  - Disease is prevalent in most of the **urban cities/towns** affecting almost 31 states/ Union territories.
  - Maximum cases have been reported from Kerala, Tamil Nadu, Karnataka, Orissa, Delhi, Maharashtra and Gujarat.
  - Maharashtra followed by Orissa accounted for maximum cases in 2014.
  - All four dengue serotypes have been isolated from India. (DEN-1 and 2 are widespread).

Laboratory Diagnosis

- **NS1 antigen detection:** ELISA and ICT are available for detecting NS1 antigen in serum.
  - Early detection: NS1 antigen becomes detectable from day-1 of fever and remains positive up to 18 days.
  - Highly specific: It differentiates between flaviviruses. It can also be specific to different dengue serotypes.
- **Antibody detection:**
  - ***In primary infection***: IgM appears first after 5 days of fever and disappears within 90 days, followed by IgG (14–21 days of illness).
  - ***In secondary infection***: Four fold rise of IgG antibody titers occurs.
  - ***MAC: ELISA*** is the most recommended test with excellent sensitivity and specificity. It can detect IgM and IgG separately.
  - ***Other antibody detection assays*** used previously are:
    - HAI (Hemagglutination inhibition test)
    - CFT (Complement fixation test)
    - Neutralization tests such as plaque reduction test, neutralization and microneutralization tests
- **Virus detection:** Dengue virus can be detected in blood from 1 day before the onset of symptoms and 5 days thereafter. It is done by:
  - Virus isolation can be done by inoculation into mosquito cell line or in mouse
  - Detection of specific genes of viral RNA by real time RT- PCR.

Vaccine

Live-attenuated tetravalent vaccine based on chimeric yellow fever-dengue virus **(CYD-TDV)** has been developed by Sanofi Pasteur Company. It was found to be safe and effective in Phase III clinical trial done in Latin America. It is recently approved for human use in Mexico.

Yellow Fever Virus

Yellow fever is endemic in West Africa and Central South America. It is not found in the rest of the World including India.

- ***Typing*:** At least *seven genotypes* of yellow fever virus have been identified based on genomic sequence, five in Africa and two in South America. There is only *one serotype*.
- ***Vector*:** Humans get the infection by the bite of *Aedes aegypti* or the tiger mosquito.

NS1 antigen detection for dengue:
- ELISA and ICT formats
- Becomes detectable from day 1 of fever, then up to 18 days.
- Highly specific to different dengue serotypes.

MAC-ELISA:
- It is the most recommended Antibody detection test for dengue.
- It can detect IgM and IgG separately.

Live-attenuated tetravalent vaccine:
- Based on chimeric yellow fever-dengue virus (CYD-TDV)
- Developed by Sanofi Pasteur Company.
- It is recently approved for human use in Mexico.

Absence of yellow fever in India is due to:
- Airport Measures:
  - Unvaccinated travelers from endemic zone quarantine for 6 days
  - Breteau index < 1; surrounding 400 mt of airport
- Cross reacting dengue antibody.

- ***Transmission cycle*:** Two major cycles of transmission have been recognized:
  - Jungle cycle: Occurs between monkeys and forest mosquitoes.
  - Urban cycle: Occurs between humans and urban mosquitoes (*Aedes aegypti*)
- ***India:*** Yellow fever has not invaded India yet. Various reasons have been hypothesized to explain the absence of yellow fever in India:
  - **Measures** for the travelers taken at the international airports in India:
    - Unvaccinated travelers coming from endemic zone to India will be kept in quarantine for 6 days
    - Breteau index or the *Aedes aegypti* index should be less than one; surrounding 400 mt of airport.
  - **Cross reacting dengue antibody** provides protection against yellow fever. However, yellow fever immunization does not protect from dengue.
- **Clinical manifestations:** Incubation period is about***: 3–6 days***. Common features include:
  - Jaundice (hence the name yellow fever)
  - Mid-zonal necrosis and presence of councilman bodies
  - Intranuclear inclusions may be seen inside the hepatocytes called as ***Torres*** bodies.

Yellow Fever 17D Vaccine

It is a live attenuated vaccine, which is prepared from allantoic cavity of chick embryo:

- There is no risk of encephalitis (unlike the previously used Dakar vaccine).
- In India: It is prepared in Central Research Institute (CRI), Kasauli
- Strict cold chain has to be maintained –30°C to + 5°C.
- It is available in lyophilized form and has to be reconstituted with diluents such as *physiological saline* before use. Once reconstituted, it should be used within ½ hr.
- Dosage: Single dose, given subcutaneously
- Vaccine is effective within 7 days of administration, which lasts for 35 years.
- Validity of yellow fever vaccine certificate: Certificate is issued after 10 days of vaccination and renewed (i.e. reimmunization) every 10 years.
- Cholera and yellow fever vaccine interact with each other, hence shouldnot be given together (3 weeks gap to be maintained).
- Contraindication of yellow fever vaccine include: Children < 9 months, (< 6 months - during epidemic), pregnancy (except during outbreak), HIV, people with allergy to egg.

Immunity and validity of YF vaccine:
- Effective within 7 days of administration, which lasts for 35 years
- Vaccine certificate is issued after 10 days of vaccination and renewed (i.e. reimmunization) every 10 years.

Kyasanur Forest Disease Virus (KFD)

KFD virus was identified in 1957 from monkeys from the Kyasanur Forest in Shimoga district of Karnataka, India.

- **Vector:** Hard ticks (Haemaphysalis spinigera)
- **Hosts:**
  - Reservoirs are the rats and squirrels
  - Amplifier hosts are the monkeys (KFD is known as Monkey's disease).
  - Man is an incidental host and considered as dead end.
- **Clinical Manifestation in humans:** Incubation period varies from 3–8 days. First stage (hemorrhagic fever) occurs followed by second phase of meningoencephalitis.
- **Seasonality:** KFD is increasingly reported in dry months (January-June) which coincides with human activity in the forest.
- **Situation in India:**
  - **Endemic in 5 Districts** of Karnataka, Shimoga, North Kannada, South Kannada, Chikkamagaluru and Udupi
  - **Largest outbreak** had occurred in 1983–84. There is a *declining trend* of incidence after the initiation of vaccine in 1999. Currently only focal cases occur.
- **Killed KFD vaccine:** It is recommended in endemic areas of Karnataka (all villages within 5 km of endemic foci).

KFD Situation in India:
- Endemic in 5 Districts of Karnataka: Shimoga, North Kannada, South Kannada, Chikkamagaluru and Udupi
- Largest outbreak had occurred in 1983–84.

ZIKA VIRUS OUTBREAK

Microbiology

- Zika virus belongs to family Flaviviridae and the genus Flavivirus.
- It is ssRNA virus, related to other viruses of same family such as dengue, yellow fever, Japanese encephalitis, and West Nile viruses.

History

- It is named after the Zika Forest, Uganda in 1947.
- Though it was wide spread among human population, was never a threat. Only 14 cases since discovery (1947) till 2007.
- The first outbreak was reported in 2007 in Yap Islands. *Aedes hensilli* was the predominant mosquito. 49 confirmed and 59 probable cases were reported.
- Monkeys are the reservoirs.

Epidemiology

Transmission

- **Mosquito borne**—Mainly spread by the *Aedes aegypti* but also by *Aedes albopictus* and other Aedes species.
- Mother-to-child transmission through placenta (common in first trimester), (during delivery rare, but possible)
- Sexual transmission is also possible (17 cases as of 26 August 2016)- Transmission is possible from:
  - Asymptomatic males to their female partners
  - Symptomatic female to her male partner
  - Longer shedding of Zika virus in semen.

Current Outbreak (2015-2016)

- Zikavirus current outbreak began in April 2015 in Brazil.
- Subsequently it spread to other countries in South America, Central America, and the Caribbean.
- Imported cases have also been reported from Europe and the United States and Australia.
- **As of 30th January 2017**
  - 174,665 suspected cases, 528,157 confirmed cases and 18 deaths have been reported so far; out of which Brazil alone witnessed nearly 109,596 suspected and 200,465 confirmed cases.
  - Next to Brazil, other countries reported maximum cases are Puerto Rico, Colombia and Mexico.
- In February 2016, the WHO declared the Zika virus outbreak a public health emergency of international concern.

Situation in India

No cases have been reported so far, but there is evidence of seroprevalence (i.e. Indian patients have in the past tested positive for Zika virus antibodies). As the vector is prevalent, so India may be affected in near future.

Clinical Manifestations

- Incubation period: unknown, few days to 1 week
- Majority Asymptomatic-The asymptomatic: symptomatic ratio is 5:1
- Zika fever- Minor illness known such as fever and a rash, conjunctivitis
- Congenital transmission leads to newborn microcephaly
- In very few cases, Guillain-Barré syndrome have been reported from French Polynesia.

Lab Diagnosis

- IgM ELISA is available. But it cross reacts with Dengue antibodies
- Plaque-reduction neutralization test -may be more specific.
- RT-PCR is done in acutely ill patients

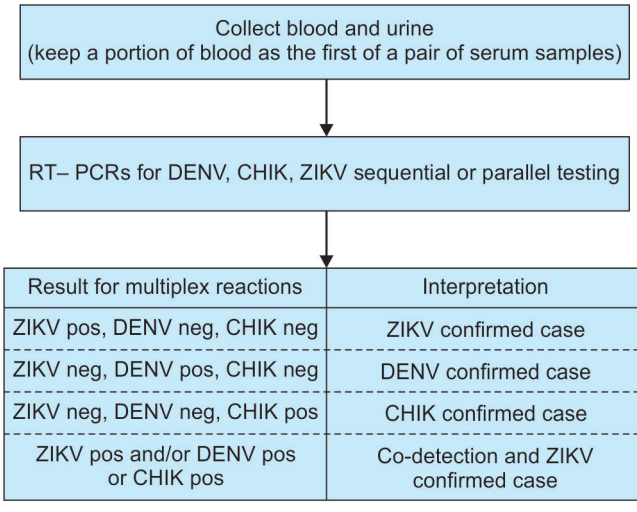
Treatment and Vaccine

- No effective treatment and vaccine is available so far. Intense research is ongoing for vaccine development by many companies including India's Bharat Biotech.
- An investigational Zika vaccine developed by NIAID and the NIH enters phase 1 clinical trials. It contains a genetically engineered plasmid—a small, circular piece of DNA—that encodes Zika virus protein.
- Only symptomatic treatment available such as fluid replacement and analgesic such as acetaminophen

Prevention

- Affected countries including Brazil, Colombia, Ecuador, El Salvador and Jamaica advise women to postpone getting pregnant until more was known about the risks.
- Travel of pregnant women from other countries (including India) to Zika affected countries have been restricted.
- Mosquito control measures- Same as that is done for Dengue prevention
- Infected patients should prevent mosquito bites for the first week of illness.
- CDC recommendation on Sex or pregnancy restriction:
    - Men - consider using condoms or not having sex for at least 6 months after travel (if don't have symptoms) or for at least 6 months from the start of symptoms (or Zika diagnosis)
    - Women- consider using condoms or not having sex for at least 8 weeks after travel (if don't have symptoms) or for at least 8 weeks from the start of symptoms (or Zika diagnosis)
    - Use condoms from start to finish, every time for vaginal, anal, or oral sex or do not have sex for the entire pregnancy.

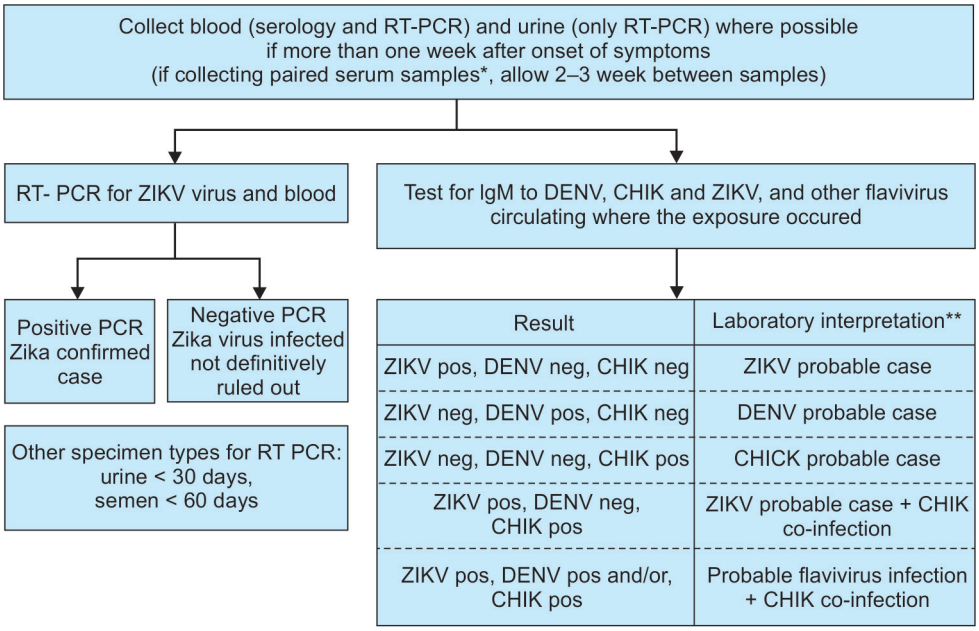
WHO Guideline: Algorithm for Diagnosis of Zika Virus Infection



Result for multiplex reactions	Interpretation
ZIKV pos, DENV neg, CHIK neg	ZIKV confirmed case
ZIKV neg, DENV pos, CHIK neg	DENV confirmed case
ZIKV neg, DENV neg, CHIK pos	CHIK confirmed case
ZIKV pos and/or DENV pos or CHIK pos	Co-detection and ZIKV confirmed case

A negative result for any PCR test does not conclusively rule out the infection

Proposed testing algorithm for suspected cases of arbovirus infection identified within seven days of onset of symptoms



*For period serum sample, a four-fold rise in Igm in the absence of a rise in antibody litre to other flavivirus is further evidence of recent Zika virus infection

**Final interpretation of result should be done in conjuction with clinical presentation

Proposed testing algorithm for suspected cases of arbovirus infection more than one week after onset of symptoms

PICORNAVIRUSES

Picornaviruses are very small (28–30 nm size) and nonenveloped viruses; divided into two major groups:

- **Enteroviruses:** Transmitted by fecal-oral route. However, they do not cause intestinal symptoms, but are associated with systemic manifestations. Examples:
  - Polio (3 serotypes)
  - Coxsackie A (1–24)
  - Coxsackie B (1–6)
  - Echovirus (1–33)
  - Parechovirus (1–3)
  - Enteroviruses (68–71)
  - Enterovirus 72 is reclassified as Hepatitis A virus.
- **Rhinoviruses:** Transmitted by respiratory mode. It is the MC cause of common cold.

Polio serotypes:

Type 1
- MC serotype to cause epidemics.
- Only serotype that is endemic currently in the world.

Type 2
- Most antigenic and hence easiest serotype to be eradicated.
- No natural case since 1999.
- MC serotype found among the VDPV strains.

Type 3
- No natural case since 2013.
- MC serotype to cause VAPP.

Polioviruses

Serotypes

- **Type 1** (Brunhilde and Mahoney strains): Most common serotype to cause epidemics. Only serotype that is endemic currently in the world.
- **Type 2** (Lansing and MEF-1 strain): It is the most antigenic and hence easiest serotype to be eradicated. No natural case has been reported since 1999. It is the MC serotype found among the VDPV (vaccine derived poliovirus) strains.
- **Type 3** (Leon and Saukett strain): No natural case caused by serotype-3 has been reported since 2013. However, It is considered as the most common serotype to cause VAPP (vaccine associated paralytic poliomyelitis).

Polio pathogenesis:
- MC Transmission: Feco-oral route
- Host cell Receptor: Via *CD155* receptors
- Site of action: Motor nerve ending, i.e. anterior horn cells of the spinal cord that leads to flaccid paralysis.
- Spread- Hematogenous (MC) > Direct Neural spread.

Pathogenesis

- **Transmission:** MC is feco-oral route, or rarely by inhalation or conjunctival contact.
- **Receptor**: Viral entry into the host cells is mediated by binding to *CD155* receptors present on the host cell surface.
- **Spread to CNS/spinal cord:** *Hematogenous spread* (MC)**>***Direct Neural spread* (occurs following tonsillectomy, where the virus may spread via glossopharyngeal nerve present in the tonsillar fossa).
- **Site of action:** Motor nerve ending, i.e. *anterior horn cells of the spinal cord* that leads to flaccid paralysis.
- **Neuron degeneration:** Earliest change in neuron is the degeneration of ***Nissl body*** (aggregated ribosomes, normally found in the cytoplasm of neurons).
- Pathological changes are always more extensive than distribution of paralysis.

Clinical Manifestations

The incubation period is usually 7–14 days. It manifests in four forms:

- **Inapparent infection**: Following infection, the majority (91–96%) of cases are asymptomatic.
- **Abortive infection:** 5% of patients develop minor illness (fever and malaise).
- **Nonparalytic Poliomyelitis:** Seen in 1% of patients, presented as aseptic meningitis.
- **Paralytic Poliomyelitis** is the least common form (< 1%):
  - Characterized by: Descending **asymmetric acute flaccid paralysis (AFP)**
  - Proximal muscles are affected earlier than the distal muscles; paralysis starts at hip → proceeds towards extremities—that leads to the characteristic **tripod sign** (child sits with flexed hip, both arms are extended towards the back).
  - **Site of involvement** can be spinal, bulbospinal and bulbar.
  - Cranial nerve involvement seen, but there is no sensory loss.

Risk factors: Paralytic disease is more common among:

- Older children and adults, pregnant women and heavy muscular exercise
- Tonsillectomy predisposes to bulbar poliomyelitis
- IM injections increase the risk of paralysis in the involved limb.

Laboratory Diagnosis

- **Specimen:** Blood (3–5 days), throat swab (up to 1 week), CSF, feces (up to 6–8 week)
- **Virus isolation:** Primary monkey kidney cell line is used.
  - Virus growth can be identified by various methods.
    - *CPE:* Described as crenation and degeneration of the entire cell sheet.
    - *Antigen* can be detected and serotyped by neutralization with specific antiserum.
    - *Specificgene detection* by PCR
  - Stool culture: Recommended for AFP surveillance and laboratory confirmation
  - Cultures of CSF, serum or throat swab are positive less frequently, but indicative of disease.
- **Antibody detection:** Neutralizing Antibody and CFT Antibody

Stool culture:
Recommended for AFP surveillance and laboratory confirmation.

Polio Vaccines

Two types of polio vaccines are in use (OPV and IPV). See table given below.

Vaccine Vial Monitor

It is a tool to monitor the stability/potency of OPV (and efficiency of cold chain)

- It is heat sensitive label lining the OPV vial: Contains a ring of two circles—outer circle is blue and inner square is white
- WHO grading: Outer circle remain blue always, but inner square may be white (grade-I) or changes to light blue (grade II), blue (grade III), black/purple (grade IV)
- OPV is usable up to grade I and II and should be discarded for grade III and IV.

Polio Vaccine	Salk (Injectable)	Sabin (Oral)
Preparation	Formalin killed preparation in MKC (Monkey kidney cell line) Total 80 units of D-antigen of: • Type 1 (40 units), • Type 2 (8 units), • Type 3 (32 units)	Each dose contains TCID50 of: Type 1: 3 lakh, Type 2: 1 lakh, Type 3: 3 lakh, *Markers of attenuation: Vaccine virus:* • Phenotypic markers: Vaccine virus will not grow in presence of low levels bicarbonate, at 40°C, MKC and is inactivated by specific antisera • Genotypic markers: Detects specific genes of attenuated virus
Dose	Four doses: 1st three doses: at 1-2 months gap, 4th booster: after 6-12 months gap	Total five doses: Zero dose: Given at birth 1st/2nd /3rd: at 6/10/14 weeks of age, Booster: 16-24 months
Safety	Relatively safer than OPV	Safe except in: Immunocompromised patients, pregnancy, old age
Efficacy	80-90% by full course of IPV Immune response is slower than OPV	90-100% efficacy is achieved even by 1 or 2 doses of OPV Efficacy decreases by: • Interference by other enteroviruses • Diarrheal diseases • Breastfeeding
Economy	Relatively expensive	Economical
Duration of Protection	Short, need booster doses periodically	Long lasting
In epidemics	Can precipitate paralysis	Can be used safely
Herd immunity	Not provided	Provided due to feco-oral spread of vaccine virus
Local immunity	Weakly stimulated	Strongly stimulated (due to IgA antibody)
Can prevent	Only paralysis	Paralysis and intestinal reinfection
Storage condition	Relatively stable Does not require stringent condition	Should be stored at (–20°C) Stabilized in $MgCl_2$, $pH<7$
VAPP and VDPV	Zero chance	Relatively more chance

Vaccine-Induced Cases (VAPP and VDPV)

Vaccine-Associated Paralytic Poliomyelitis

VAPP cases occur following OPV administration; due to some OPV strains undergo mutation.

- VAPP strains are ***OPV-like isolates***, which differs from OPV by < 1% gene.
- VAPP cases are ubiquitous in places where OPV is used extensively.
- VAPP can occur among OPV recipients as well as to their close contacts.
- However, VAPP strains are not capable of circulating in the community and do not cause outbreaks. This is largely because the spread of OPV-related virus is largely limited by high population immunity.
- **VAPP rate:** VAPP occurs at a rate of one case per 2.5 million doses of OPV.
- **VAPP occurs more frequently:**
  - Following the first dose of OPV than the subsequent doses
  - Among people with primary immunodeficiency disorder (↑ risk by 3000 fold)
- **MC serotype** associated with VAPP-Sabin type 3 (60%), followed by type 2.

Vaccine-Derived Polioviruses (VDPVs)

VDPV isolates exhibit a higher level of genetic divergence from their parental OPV strains at VP1 sequence, which helps in their prolonged replication, and transmission.

- The genetic divergence of VDPVs from parental OPV strains is about
  - > 1% for Sabin types 1 and 3
  - > 0.6% for Sabin type 2
- Isolates showing genetic divergence lower than this cutoff are considered as ***OPV-like isolates.***
- VDPV isolates are indistinguishable from wild polioviruses both clinically (due to regain of neurovirulence and phenotypically (by reversal of markers of attenuation)
- Most VDPV isolates belong to **Sabin type** 2 (90%) followed by type 1. This is because wild type 2 strains are already eradicated and not circulating in the community since 1999.

VAPP and VDPV:
- VAPP strains are OPV-like isolates, which differ from OPV by < 1%
- Vaccine-derived polioviruses (VDPVs)
- VDPV differ from OPV by > 1% for Serotypes 1 and 3 and > 0.6% for Sabin type 2.

VDPVs can be categorized as:
- Circulating VDPVs (cVDPVs)
- Immunodeficiency-associated VDPVs (iVDPVs)
- Ambiguous VDPVs (aVDPVs).

VDPVs can be categorized as:

1. **Circulating VDPVs (cVDPVs):** These strains are capable of person-to-person transmission in the community and can cause outbreaks in areas with low OPV coverage:
   - They pose the same threat to the community as that of wild polioviruses
   - Since 2000, cVDPV outbreaks have occurred in 18 countries including India, with majority (87.1%) of reported cases are associated with type 2.
   - In 2015, over **27 cases** due to VDPVs have been isolated worldwide. Madagascar reported maximum.
2. **Immunodeficiency-associated VDPVs (iVDPVs)** are isolated from persons with primary immunodeficiency disorder:
   - They do not develop disease, but excrete the iVDPVs for many years.
   - iVDPVs exhibit greater genetic diversity than cVDPVs some strains may be diverse by **> 10%**
   - The extent of sequence divergence is proportional to the duration of the infection.
   - Unlike cVDPV, infections due to iVDPV cannot be prevented by high OPV coverage.
3. **Ambiguous VDPVs (aVDPVs)** are heterogeneous; They are either cVDPVs for which only 1 case isolate had yet been detected, or they may be sewage isolates obtained from developed countries with unknown source (probably iVDPV).

Clinical: subclinical ratio in Polio:
For every clinical case, there may be 1000 children and 75 adults of subclinical cases.

Epidemiology

- **Reservoir:** Man is the only known reservoir. Most cases are sub clinical.
- **Clinical: subclinical ratio:** For every clinical case, there may be 1000 children and 75 adults of subclinical cases.
- There are **no chronic carriers**. However, immunodeficient individuals may excrete the virus for longer periods.
- **Source:** Infective material such as stool and oropharyngeal secretions are the sources of infection.
- **Age:** Younger children and infants are more susceptible than adults. However in developed countries, there is shift of age; affecting older children.
- **Period of communicability:** Patients are infectious shedding the virus in the feces from 7–10 days before the onset of symptoms up to 2–3 weeks thereafter, sometimes as long as 3–4 months.

Polio cases reported in the World:
- In 2016, only 35 wPV cases (71 cases in 2015) and 4 cVDPV cases (27 cases in 2015) have been reported.
- Pakistan accounted for maximum no. of natural cases.
- Lao People's Democratic Republic is the only non endemic country to report cVDPV cases (3 no.).

Polio Eradication

Poliomyelitis is now at the verge of eradication. This is attributed to the extensive immunization programme being conducted globally.

Pulse Polio Immunization (PPI):

- Two rounds of PPI (6 weeks apart) are scheduled every year during the **winter season**, where all children under the age of **five years** are vaccinated with OPV **irrespective of** their OPV vaccination status.
- PPI doses of OPV are considered as **extra doses** and they do not replace the OPV doses received under the routine national immunization schedule.

Polio Situation in the World

- **Endemic countries:** Currently polio is endemic only in three countries: Pakistan, Afghanistan and Nigeria (Nigeria was declared polio free in 2015; but again became endemic in 2016).
- **Countries** no longer infected by wild poliovirus, but which remain vulnerable to international spread include: Cameroon Equatorial, Guinea, Ethiopia, Iraq, Israel, Nigeria, Somalia, Syrian, Arab Republic.
- **No. of cases reported in the World (till January 2017):**
  - In 2016, only 35 wPV cases (71 cases in 2015) and 4 cVDPV cases (27 cases in 2015) have been reported. Pakistan accounted for maximum no. of natural cases. Lao People's Democratic Republic is the only non endemic country to report cVDPV cases (3 no.).

IPV and bOPV in India:
- *IPV introduced in India:* from November 2015
- *Bivalent OPV will be introduced in India:* from April 2016

Section 4

- Currently, all natural cases are due to type-1. Type-2 and 3 have not been reported since 1999 and 2013.
- **India** has been declared polio-free since 2014, the last natural case was detected three years back (Jan 2011).

- **The Global Polio Eradication Initiative (GPEI)** had launched *'Eradication and Endgame Strategic Plan'* (2013–2018) aiming to wipe out polio from the entire world by 2018.

Endgame Strategic Plan (2013–2018)

GPEI had initiated an end game strategic plan for polio eradication, which has four objectives:

1. *Interruption of polio virus transmission*
2. *Strengthening immunization* systems by step wise withdrawal of OPV along with switching over to IPV
   - Introduction of one dose of IPV by the end of 2015: Third dose of OPV will be withdrawn and replaced by IPV.
   - Withdrawal of Serotype-2: Trivalent OPV will be replaced by Bivalent OPV (serotype 1 and 3) six months after starting IPV (i.e. mid 2016)
   - IPV only: Complete withdrawal of OPV and replacement with IPV only immunization schedule by 2019.
3. *Implementing containment of polioviruses* and to certify the world as polio-free by end of 2018.
4. *Legacy planning:* The infrastructure, fund, man-power, knowledge and experience that have been created through the global polio programme will be utilized to support other health programmes following postpolio eradication.

IPV in India:
- Govt. of India introduced IPV under universal immunization programme
- From November 2015.
- One dose of IPV
- This IPV dose is extra dose; over and above the Trivalent OPV
- In a phased manner
- In the first phase, IPV has been introduced in six high risk states: Assam, Gujarat, Punjab, Bihar, Madhya Pradesh, and Uttar Pradesh.

Indian Govt. Action plan: Switch over of tOPV to bOPV and IPV
- *From November 2015 to March 2016:* Three doses of trivalent OPV plus one dose of IPV along with the third dose of OPV at 14 weeks.
- *From April 2016:* Bivalent OPV three dose plus one dose of IPV along with the third dose of bOPV at 14 weeks.

Coxsackieviruses	
Group A Coxsackieviruses	**Group B Coxsackieviruses**
Suckling mouse intracerebral inoculation:	
• Flaccid paralysis in mice • Generalized myositis	• Spastic paralysis in mice • Focal myositis and Necrosis of brown fat
Manifestations	
1. Aseptic meningitis (A7, A9) 2. Herpangina (vesicular Pharyngitis) 3. Hand foot and mouth disease (also by Enterovirus:71) 4. Acute hemorrhagic conjunctivitis- Caused by Coxsackie-A24 and Enterovirus 70	**More organ involvement:** 1. Aseptic meningitis (B1-6) 2. Pleurodynia (Epidemic myalgia or Bornholm disease) 3. Myocarditis, pericarditis 4. Hepatitis and Pneumonia 5. Pancreatitis leading to Juvenile Diabetes mellitus – Coxsackie B4 6. Generalized disease of infants

Endgame Strategic Plan:
- Interruption of polio virus transmission
- Strengthening immunization
- Implementing containment of polioviruses
- Legacy planning.

RABIES VIRUS

Morphology

- Bullet-shaped, enveloped virus. Envelope is embedded with glycoprotein antigen spikes.
- Nucleocapsid (made up of nucleoprotein) has a helical symmetry and comprises of negative sense ssRNA
- Antigens: Rabies has two major antigens; Glycoprotein G and Nucleoprotein.

Speed of Rabies virus progress in axon:
- 250 mm/day.

Glycoprotein G	Nucleoprotein
Peplomers or spikes embedded in envelope	Capsid proteins associated with viral RNA
Species-specific	Group-specific and cross-reacts with rabies related viruses.
Role in pathogenesis: It binds to acetyl choline receptors in neural tissues (attachment): 1st step in pathogenesis	Does not have any role in pathogenesis
Diagnostic role: It induces hemagglutination inhibiting antibodies which can be detected in patient's serum by hemagglutination inhibition test	1. CFT-It induces complement fixing antibodies 2. Antiserum prepared against the purified nucleocapsid is used in direct- IF test for Ag detection
Role in immunity: It induces neutralizing antibodies which are protective in nature; hence used for vaccination	Antibodies are not protective, hence it is not used for vaccination

Types of Rabies:
- Furious Rabies (Encephalitic Rabies)
  - Accounts for 80% of all cases
- Dumb/ Paralytic Rabies
  - Accounts for 20% of all cases
  - Associated with partial vaccine course.

Types of Rabies virus:
Street rabies:
- Causes disease
- Produce Negri body
- Affects salivary gland
- IP -3months

Fixed rabies:
- Avirulent
- Produced by repeated passage
- Used as vaccine strain
- Doesn't produce Negri body
- Doesn't affect salivary gland
- IP-4-5days

Pathogenesis

- **Transmission:**
  - Bite: Rabies virus is usually transmitted to humans by the bite of an infected animal.
    - Dog bite is the most common mode
    - Other animal bites: monkey, sheep, goat, etc. (except rat bite and human bite)
    - Human-to-human transmission is theoretically possible but is extremely rare.
  - Non-bite exposures are rare such as:
    - Lick on abrasion or mucosa
    - Inhalation of from infected bats aerosols.
    - Corneal transplantation
- **Route of spread:** Viral replication in muscle → bind to nicotinic A ch receptors at NM junctions → spreads centripetally along peripheral motor nerves → dorsal route ganglia of spinal cord → infect brain neurons (brainstem and mental system) → centrifugal spread via sensory and autonomic nerves to cornea, salivary gland, skin and other organs
- Speed of Rabiesvirus progress in axon – **250 mm/day**.

Clinical Manifestations

- Incubation period: 1–3 month (20–90 days)
- IP is shorter in children and upper limb bite and for short heighted people (than leg bite and taller people)
- Earliest symptom: Neuritic pain at bite site
- The clinical spectrum can be divided into three phases:
  - Short prodromal phase
  - Acute neurologic phase: It presents in two forms: Encephalitic or dumb rabies
  - Coma and death: Occurs within 14 days of encephalitic rabies and 30 days of dumb rabies.

Furious Rabies (Encephalitic Rabies)	Dumb/Paralytic Rabies
Accounts for 80% of all cases	Accounts for 20% of all cases
Encephalitis: Hydrophobia, aerophobia and hyper excitability Hydrophobia is due to dysfunction of infected brainstem neurons. The act of swallowing precipitates an involuntary, painful spasm of the respiratory muscles. ***ANS dysfunction:*** Hypersalivation (foaming at the mouth), gooseflesh, arrhythmia and priapism Lucid interval gradually decreases	Cardinal features of encephalitic rabies absent Quadriparesis and facial weakness present
Seen in unvaccinated individuals	Associated with partial vaccine course

Direct IF test for rabies:
- The best specimen is hair follicle of nape of neck (most sensitive).

Laboratory Diagnosis

1. Rabies antigen detection

- Direct IF test detecting rabies nucleoprotein antigens in specimens.
- The best specimen is hair follicle of nape of neck (most sensitive).
- Corneal impression smear: Positive in late stage with a sensitivity of 30%.

Section 4

2. **Viral Isolation**
   - **Mouse inoculation:** Intracerebral inoculation into suckling mice
   - **Cell lines:** Mouse neuroblastoma cell lines and baby hamster kidney (BHK) cell lines are the preferred.
3. **Antibody detection:** Detection of CSF antibodies is more significant than serum antibodies as serum antibodies appear late and can also be present after vaccination. Various antibody detection tests include:
   - Mouse neutralization test (MNT)
   - Rapid fluorescent focus inhibition test (RFFIT)
   - Fluorescent antibody virus neutralization (FAVN)
   - Indirect fluorescence assay (IFA)
   - Hemagglutination inhibition test (HAI)
   - Complement fixation test (CFT)
4. **Viral RNA detection**
   - Reverse transcription-polymerase chain reaction (RT-PCR)
   - Most sensitive and specific assay available at present for the diagnosis of rabies.
5. **Negri body detection**
   - Negri body detection is pathognomonic for post mortem diagnosis of rabies. However, it may not be detected in 20% of cases. Therefore, the absence of Negri bodies does not rule out the diagnosis of rabies.
   - They are intracytoplasmic eosinophilic inclusions with characteristic basophilic inner granules.
   - Sharply demarcated, spherical to oval, and about 2–10 µm in size.
   - **MC sites** are neurons of cerebellum and hippocampus; however, less frequently in cortical and brainstem neurons.
   - **Commonly used stains are:** Histological stains such as H and E and Sellers stains (basic fuchsin and methylene blue).

Negri body detection:
- Detected in 20% of cases
- Intracytoplasmic eosinophilic inclusions
- MC sites: Cerebellum and hippocampus
- MC stain used: Sellers stains.

Prevention of Human Rabies

Postexposure prophylaxis (PEP) includes local wound care and both active and passive immunization.

Non-neural Vaccines for Rabies:
- Purified chick embryo cell (PCEC)
- Purified Vero cell (PVC)
- Human diploid cell (HDC).

Local Treatment

- Prompt cleaning of the wound, scrub with soap and water and apply antiseptics.
- Bite wounds are not sutured immediately.
- Confirmation whether or not the animal is rabid (for 10 days): Indicated in category II and III bites.

Passive Immunization

- Human rabies immune globulin (HRIG): 20 IU/kg, maximum injected locally, rest IM in gluteal region. It is Indicated in only in category III bites.
- Equine rabies immunoglobulin (ERIG)- It is given at dose of 40 IU/kg . Being heterologous in origin (horse), it is associated with serum sickness; hence not in use.

Active Immunization (Rabies Vaccine)

Neural Vaccines: They are poorly immunogenic and encephalitogenic; hence not used.
- Semple vaccine: Derived from infected sheep brain
- BPL: Beta propiolactone derived vaccine (Prepared in Coonoor)
- Infant mouse brain derived vaccine

Non-neural Vaccines: Cell line derived, they are the recommended vaccine currently:
- Purified chick embryo cell (PCEC)
- Purified Vero cell (PVC)
- Human diploid cell (HDC).

Category of risk	Type of exposure	Recommended prophylaxis (WHO)
Category I (No risk)	Touching, or feeding of animal Licks on intact skin	No treatment needed if history is reliable
Category II (Minor risk)	Minor scratches or abrasions without bleeding or nibbling of uncovered skin	Wound management Rabies vaccine (6 doses) Observe the dog for 10 days
Category III (Major risk)	Single or multiple transdermal bites with oozing of blood, Licks on broken skin (fresh wounds) or mucous membrane Bite by wild animals /bat	Same as for Category II plus Rabies immunoglobulin

National Guideline on Rabies Prophylaxis

(Adapted from National Center for Disease Control, India)

Regimens for Rabbies vaccine:
- Postexposure prophylaxis: Five doses on days 0, 3, 7, 14 and 28 and booster at 90 days
- Preexposure prophylaxis: Three doses on day 0, 7, 28 days and booster every 2 years
- Postexposure prophylaxis to previously vaccinated people
  - Severe bite or titer unknown: 3 doses of vaccine- 0,3,7 days
  - Less severe bite or titer > 0.5 IU/ml: 2 doses of vaccine 0,3 days.

Regimen for Post-Exposure Prophylaxis

- **IM regimen or** Essen regimen (1-1-1-1-1): Five doses (0.5 or 1 ml per dose) each given on days 0, 3, 7, 14 and 28 followed by booster at 90 days. Day 0 indicates the date of first dose of vaccine; not the date of exposure.
- **ID Regimen** (or Thai Red Cross Schedule) (2-2-2-0-2): This involves injection of 0.1 ml of reconstituted vaccine on two sites per visit on days 0, 3, 7 and 28.
- Potency: Single intramuscular dose should have a minimum potency of 2.5 IU.
- **Site of injection:**
  - Deltoid region is ideal site. Gluteal region is not recommended because fat retards the absorption of antigen.
  - Infants and young children: Anterolateral part of the thigh is the preferred site.

Regimen for Pre-Exposure prophylaxis

- Recommended for high risk groups like veterinarians, animal handlers and travellers of rabies free areas.
- Three doses of vaccine: 0, 7, 28 days and booster @ 2 years
- Antibody titer should be checked every 6 months for 2 years and thereafter every 2 yearly.
- Booster dose is given if antibody titer is less than 0.5 IU/ml.

Regimen for Post-exposure Prophylaxis to Previously Vaccinated People

- Severe bite or titer unknown: 3 doses of vaccine: 0, 3, 7 days
- Less severe bite or titer > 0.5 IU/ml: 2 doses of vaccine 0, 3 days.

Epidemiology

Rabies is enzootic and epizootic disease of both wild and domestic animals worldwide.

Rabies-free area:
- World: Australia, Antarctica, Britain, Iceland, Ireland, China (Taiwan), Cyprus, Japan, Malta, New Zealand
- India: Andaman & Nicobar, Lakshadweep.

- **Worldwide:** Rabies is endemic in > 150 countries. About 55,000 deaths occur due to human rabies each year. India accounts for maximum cases (20,000 deaths/year).
- **Source:** Infected dog virus is present in saliva from 3-4 days before the onset of symptoms till death of the dog.
- **Rabies free area:** Defined as countries/areas where no cases have been reported in last two years. Examples include:
  - World: Australia, Antarctica, Britain, Iceland, Ireland, China (Taiwan), Cyprus, Japan, Malta, New Zealand
  - India: Andaman and Nicobar, Lakshadweep
- **Control of Urban Rabies:** Elimination of stray dogs and mass immunization to at least 80% dogs in an area.

MULTIPLE CHOICE QUESTIONS

ARBOVIRUSES

1. Which of the following is a true arbovirus? *(Recent MCQ 2013)*
a. HSV
b. JE
c. ECHO
d. Hanta

2. Viralhemorrhagic fever found in India are: *(PGI Nov 2014)*
a. KFD
b. Dengue
c. Yellow fever
d. Crimean Congo fever

3. Diseases not transmitted by Aedes aegypti is/are: *(PGI Nov 2012)*
a. Dengue fever
b. Chikungunya fever
c. Yellow fever
d. Rift valley fever
e. Japanese encephalitis

4. All are mosquito borne viral fevers except: *(APPG 2014)*
a. Dengue
b. Kysanur Forest disease
c. Yellow fever
d. Japanese encephalitis

5. Drug of choice to treat H1N1 influenza is: *(MHPG 2014)*
a. Acyclovir
b. Cidofovir
c. Oseltamivir
d. Tenofovir

6. Which of the following is/are Arboviral diseases? *(PGI June 2009, PGI June 2003)*
a. Japanese encephalitis
b. Dengue
c. Yellow fever
d. Hand-foot-mouth disease
e. Rocky mountain spotted fever

7. Group B (flaviviruses) Arboviruses is /are: *(PGI Dec 2006)*
a. Dengue fever
b. Rift valley fever
c. Chikungunya fever
d. JE
e. Yellow fever

JAPANESE ENCEPHALITIS

8. The upper age limit administration of JE vaccine is: *(Recent Question 2015)*
a. 3 years
b. 5 years
c. 10 years
d. 15 years

9. Japanese encephalitis in India all except: *(AI 2011, NEET PG Based)*
a. In an Epidemic are 2–3 cases in a village
b. Mosquito bite is always associated with disease
c. Apparent and nonapparent ratio 1:100
d. Incubation period varies 5–15 days

10. In Japanese Encephalitis, pigs acts as: *(PGI 2000)*
a. Amplifier
b. Definitive host
c. Intermediate host
d. Any of the above

11. Human to human transmission not seen in: *(PGI June 2005, Bihar 06)*
a. SARS
b. Japanese B encephalitis
c. Bird's flu
d. Poliomyelitis
e. HIV

12. Culex tritaeniorhynchus is vector of …… disease: *(CMC Vellore 2016)*
a. JE
b. Dengue
c. KFD
d. Yellow fever

13. Not a cause of epidemic encephalitis: *(PGI May 2013)*
a. Herpes simplex virus
b. Rabies
c. West Nile virus
d. Nipah virus
e. Japanese encephalitis

DENGUE

14. In a suspected patient of dengue, all of these are acceptable investigations at day 3 of presentation except: *(AIIMS Nov 2016)*
a. NS1 antigen detection
b. Viral culture and isolation in Aedes albopticus C6/36 cell line
c. RT-PCR
d. ELISA for antibody against Dengue virus

15. Torniquet test is used for diagnosis of: *(AIIMS May 2016)*
a. Dengue
b. Zika virus
c. Chikungunya virus
d. Yellow fever virus

16. Antibody dependant enhancement (ADE) is a feature of which of the following? *(AIIMS Nov 2015)*
a. Dengue hemorrhagic fever
b. SSPE
c. SSSS

17. Most virulent dengue fever strain is: *(NEET Pattern Based)*
a. 1
b. 2
c. 3
d. 4

18. True statements regarding dengue fever: *(PGI June 2011)*
a. It can cause both endemic and epidemic
b. MC arboviral disease
c. Unaffected by ambient temperature
d. Decreased incidence in last 30-year in India
e. Self-limiting

19. Dengue hemorrhagic fever is caused by: *(AI 2009)*
a. Type I dengue virus
b. Reinfection with same serotype of dengue virus
c. Reinfection with a different serotype of dengue virus
d. Infection in an immuno compromised host

20. Most sensitive diagnostic test for dengue is:
a. IgM ELISA *(AIIMS Nov 2008)*
b. Complement fixation test
c. Neutralization test
d. Electron microscopy

21. True about dengue hemorrhagic fever: *(PGI Dec 06)*
a. Increase in hematocrit
b. Decrease in platelet count
c. +ve tourniquet test
d. Pleural effusion present
e. Aedes aegypti bites in day time

22. Dengue virus appears to have a direct man-mosquito-man cycle in india. The mechanism of dengue virus survival in inter epidemic period is: *(DPG 2011)*
a. Non human reservoir
b. Dormant or latent phase
c. Transovarian transmission in Aedes
d. Poor housekeeping in public

23. Vector of dengue virus: *(Karnataka 2011)*
a. Aedes aegypti
b. Aedes albopictus
c. Aedes polynesiensis
d. Aedes scutellaris

24. MOST important factor for prognosis of patient in DHF? *(AIIMS MAY 2016)*
a. Platelet count
b. Hematocrit
c. Hemoglobin

25. All are true about dengue hemorrhagic fever except:
a. Malnutrition is protective *(AIIMS Nov 2014)*
b. Lamivudine is drug of choice
c. Causative agent belongs to flaviviridae group
d. Aedes aegypti acts as vector

26. True about dengue fever: *(PGI May 2013)*
a. Caused by 4 serotypes
b. Effective vaccine is available
c. Presents with fever and joint pain
d. Virus belongs to flavivirus genus
e. Contain segmented RNA

YELLOW FEVER VIRUS

27. Egg allergy, which vaccine is contraindicated:
a. MMR vaccine *(Recent Question 2014)*
b. Hepatitis B
c. DPT
d. Yellow fever vaccine

28. Which of the following arbovirus disease is not found in India? *(AI 2011)*
a. Sandfly fever
b. Rift valley
c. Westnile
d. Yellow fever

29. Incubation period of yellow fever: *(AIIMS May 04)*
a. 3–6 days
b. 3–4 weeks
c. 1–2 weeks
d. 8–10 weeks

30. According to international health regulation, yellow fever transmission risk is minimal if Aedes aegypti index is less than… : *(AI 2004)*
a. 1%
b. 5%
c. 8%
d. 10%

31. All are true about yellow fever except: *(AI 03)*
a. Belongs to Flavivirus
b. Vaccination is valid after 10 days and lasts for 10 yrs
c. IP-16–46 days
d. Case fatality ratio 80%

KYASANUR FOREST DISEASE

32. Which of the following statements is true regarding Arbo viruses? *(AI 2004)*
a. Yellow fever is endemic in India
b. Dengue virus has only one serotype
c. Kyasanur forest disease (KFD) is transmitted by ticks
d. Mosquito of culex visnoi-complex is the vector of Dengue fever

33. Which is not useful for prevention of KFD?
a. Vaccination *(AIIMS May 2001)*
b. Deforestation
c. Prevention of roaming cattle
d. Personal protection

ZIKA VIRUS

34. True about Zika virus: *(PGI May 2016)*
a. Can be transmitted by sexual route
b. NAAT from amniotic fluid can be used for prenatal diagnosis
c. 50% infected patients develop symptoms
d. Aedes mosquito transmission
e. Antiviral therapy is treatment of choice for ZIKV

POLIOVIRUS

35. Vaccine associated polio occurs how many days after vaccination? *(JIPMER Nov 2015)*
a. 4–30 days
b. 20–60 days
c. 60–90 days
d. 90–120 days

36. Which of the following is not a Vaccine-Derived Poliovirus? *(AIIMS Nov 2014)*
a. aVDPV
b. cVDPV
c. mVDPV
d. iVDPVans - mVDPV

37. Polio is still transmitting in: *(PGI Nov 2014)*
a. India
b. Pakistan
c. Nigeria
d. Afghanistan
e. Srilanka

38. True about polio eradication initiative in India is: *(AIMS Nov 2013)*
a. Last natural case due to wild virus was reported in 13 January 2011
b. No case of VAPP was reported from 2012
c. India is the only country that has not achieved the goals of polio eradication
d. Currently the vaccine used for polio eradication in India is IPV

39. Diagnosis of polio: *(NEET Pattern Based)*
a. Detection of polio virus in stool
b. Serology
c. Limb wasting
d. AFP

40. True about polio: *(AIIMS Nov 2007, May 2008)*
a. Paralytic polio is most common
b. Spastic paralysis
c. IM injections and increased muscular activity lead to increased paralysis
d. Polio drops given only in < 3 year

41. All of the following statements are true regarding poliovirus except: *(AI 2004)*
a. It is transmitted by feco-oral route
b. Asymptomatic infections are common in children
c. There is a single serotype causing infection
d. Live attenuated vaccine produces herd immunity

42. All are true about poliovirus, except:
a. Type I is responsible for most epidemics
b. Very difficult to eliminate type I *(AIIMS May 2002)*
c. Type I responsible for vaccine paralytic polio myelitis
d. Type I most commonly associated with paralysis

43. Which sample is recommended for polio isolation? *(AIIMS Nov 04)*
a. Stool
b. Blood
c. Throat
d. CSF

44. True about side effect of OPV: *(PGI June 03)*
a. VAPP in recipient of OPV
b. VAPP in contacts or recipient of OPV
c. Guillein Barré syndrome
d. Vomiting and fever

45. Zero dose of OPV is given at: *(DNB 06)*
a. Before birth
b. At birth
c. When child is having diarrhea
d. When child is having polio

OTHER PICORNA VIRUSES

46. Common causative agent of hand-foot-and-mouth disease is: *(PGI May 2016)*
a. Enterovirus
b. Coxsackie B4
c. Coxsackievirus A16
d. HPV
e. HAV

47. Which of the following is Picornaviridae? *(PGI May 2015)*
a. Polio virus
b. Coxsackievirus
c. Rhinovirus
d. Corona virus
e. Reovirus

48. Coxsackie virus is: *(NEET Pattern Based)*
a. Herpes virus
b. Pox virus
c. Enterovirus
d. Myxovirus

49. Herpangina is caused by: *(NEET Pattern Based)*
a. Enterovirus
b. Rhinoviruses
c. Myxovirus
d. Rabies virus

50. Enterovirus 72 is: *(NEET Pattern Based)*
a. Hepatitis A
b. Hepatitis E
c. Hepatitis B
d. Hepatitis C

51. The commonest viruses that can cause meningoencephalitis in children are: *(AI 2011)*
a. Arboviruses
b. Enteroviruses
c. Herpes viruses
d. Mumps viruses

52. Enterovirus causes all except: *(AI 2009, AIIMS Nov 2001)*
a. Hemorrhagic fever
b. Pleurodynia
c. Herpangia
d. Aseptic Meningitis

53. Herpangina is due to: *(Recent MCQ 2013)*
a. HSV
b. HIV
c. Coxsackie A
d. Coxsackie B

54. True statement about Enteroviruses: *(PGI May 2013)*
a. Composed of segmented RNA genome
b. Stable at pH 4
c. Cause pleurodynia
d. Cause encephalitis
e. Cause meningitis

55. Infantile pericarditis and myocarditis is caused by: *(TNPG 2014)*
a. Coxsackie B
b. Coxsackie A
c. Polio
d. Adenovirus

56. Enterovirus associated with acute hemorrhagic conjunctivitis is: *(MHPG 2014)*
a. Serotype 68
b. Serotype 69
c. Serotype 70
d. Serotype 71

RHABDOVIRUS (RABIES)

57. A patient presented to the hospital with severe hydrophobia. Corneal scrapings from the patient is obtained. What test should be done on this specimen for a diagnosis of Rabies? *(AIIMS Nov 2016)*
a. RT-PCR for Rabies virus
b. Negri bodies (Sellar stain)
c. Antibodies to rabies virus by Indirect Immunofluorescence
d. Tzanck smear

58. Negri body- MC site is: *(West Bengal PG 2016)*
a. Limbic system
b. Purkinje cells of cerebellum
c. Thalamus

59. Negri bodies are abundant in the following cells of CNS except: *(JIPMER 2010, 2014)*
a. Subcortical white matter
b. Purkinje cells of cerebellum
c. Hippocampus
d. Basal ganglia

60. Rabies is identified by: *(AI 2007, NEET Pattern)*
a. Guarneri bodies
b. Negri bodies
c. Cowdry A bodies
d. Cowdry B bodies

61. A 15-year-old girl was admitted to the infectious disease hospital with a provisional diagnosis of rabies. The most suitable clinical sample that can confirm the ante mortem diagnosis is: *(AIIMS Nov 2004)*
a. Serum for antirabies IgG antibody
b. Corneal impression smear for immunofluorescence stain
c. CSF sample for viral culture
d. Giemsa stain on smear prepared from salivary secretions

62. Rabies free zone in India: *(DPG 06, RJ 08)*
a. Lakshadweep island
b. Rajasthan
c. Sikkim
d. Nagaland

63. Rabies free country: *(DNB 2000, 2001, 05)*
a. China
b. Russia
c. France
d. Australia

64. Bitten dog should be observed for at least: *(RJ 2007)*
a. 5 days
b. 10 days
c. 15 days
d. 21 days

PROPHYLAXIS OF RABIES

65. For the prevention of human rabies, immediate flushing and washing the wound(s) in animal bite cases, with plenty of soap and water, under running tap should be carried out for how much time? *(MHPG 2015)*
a. 2 minutes
b. 1 minute
c. 15 minutes
d. 5 minutes

66. Antirabies vaccine is prepared by: *(DNB June 2012)*
a. Street virus
b. Fixed virus
c. Live virus
d. Wild virus

67. For the treatment of case of class III dog bite, all of the following are correct except: *(AI 2005)*
a. Give Ig for passive immunity
b. Give ARV
c. Immediately stitch wound under antibiotic coverage
d. Immediately wash wound with soap and water

68. Class II exposure in animal bites includes the following: *(AI 2003)*
a. Scratches without oozing of blood
b. Nibbling of uncovered area
c. Scratch with oozing of blood on palm
d. Bites from wild animals

69. Neurological complications following Rabies vaccine is common with: *(AI 2002)*
a. HDCS Vaccine
b. Chick embryo Vaccine
c. Semple Vaccine
d. Duck Egg Vaccine

70. All of the following rabies vaccines are commercially available except: *(AI 2001)*
a. Purified chick embryo cell vaccine (PCEC)
b. Human diploid cell vaccine
c. Vero continuous cell vaccine
d. Recombinant glycoprotein

71. A boy got unprovoked bite from a neighbor's dog. The animal control authority caught the dog and it was found to be healthy. What will be the next step?
a. Test antibody level of dog *(AIIMS May 2014)*
b. Withhold immunization and observe dog for 10 days for signs of rabies
c. Start postexposure prophylaxis and watch the dog for 10 days
d. Kill the dog and watch for negri body in brain

EXPLANATIONS

ARBOVIRUSES

1. **Ans. (b) (JE)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p484, Ananthanarayan 9/e p520
   - Japanese encephalitis belongs to flavivirus group of arboviruses.
   - Hantaviruses, though a part of arboviruses, but are not arthropod borne.
2. **Ans. (a) (b) (d) (KFD, Dengue, Crimerian Congo fever)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p484
   Recently, few cases of Crimean Congo hemorrhagic fever have been reported from Gujarat.
3. **Ans (e) (Japanese...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p484, Ananthanarayan 9/e p518
   Refer chapter review to know the detail list of vectors of various abroviral diseases.
4. **Ans. (b)(Kysanur...)** Ref: Apurba Sastry's Essentials of Medical Parasitology,p/315, Ananthanarayan 9/e p518
   Kysanur Forest disease virus is transmitted by tick.
5. **Ans. (c) (Ose...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p484, Harrison 19/e p1213, 18/e chapter-187
   Neuraminidase inhibitors like Oseltamivir or Zanamivir are the DOC of H1N1.
6. **Ans. (a), (b), (c) (Japanese ..., Dengue, Yellow fever)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p484
   - ***Options a, b*** and **c** already explained in chapter review
   - ***Option d-*** Hand-foot-mouth disease: Exanthematous fever affecting mainly young children, caused by Coxsackie A16, 9 and B 1-3 and Enterovirus-71- Ananthanarayan 9/e p517, 8/e p489
   - ***Option e -*** Rocky mountain spotted fever – Spotted fever caused by *Rickettsia rickettsii.*
7. **Ans. (a), (d), (e) (Dengue fever virus, JE and Yellow fever)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p484
   Refer chapter review

JAPANESE ENCEPHALITIS

8. **Ans. (d) (15 years)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p489
9. **Ans. (c) (Apparent and Nonapparent ratio 1:100)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p488-89
   - ***Option a-*** Correct
   - As the cases of encephalitis represent only the tip of the iceberg. PSM Park 23e/284-86, 22/e p260-61 states
     'Encephalitis cases are scattered in distribution.
     It was observed in North India, the number of cases recorded per village were no more than 1-2'.
   - ***Option b -*** correct: Culicine mosquitoes ***(C. tritaeniorhynchus, C. vishnui)*** and some Anopheline mosquitoes are the vectors
   - ***Option c -*** Incorrect: The ratio of overt disease to inapparent infection varies from 1:300 to 1:1000
   - ***Option d -*** Correct: Incubation period varies 5–15 days.
10. **Ans. (a) (Amplifier)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p488-89, Ananthanarayan 9/e p 520
    Japanese encephalitis:
    - *Reservoir: Ardeid (wading) birds: Herons, cattle ergets, ducks*
    - *Amplifying hosts: Pigs (asymptomatic), bats*
    - *Mosquito attractant: Cattle and Buffalo (infected but not natural host of JE)*
    - *Incidental hosts: Horses (only animal to be symptomatic), humans(dead end), others.*
11. **Ans. (b) (Japanese...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p488-89, Ananthanarayan 9/e p521
    - **Japanese encephalitis*: Human infection is tangential dead end and it occurs when the infected mosquitoes reach high density***
    - SARS and Bird's flu will be transmitted from man to man by direct contact with infected respiratory secretions
    - HIV-transmitted from human to human by parenteral route, vertical (infected mother) and sexual route
    - Poliomyelitis transmitted through: Feco-oral route (ingestion), Inhalation or through conjunctiva.

12. **Ans. (a) (Japanese B Encephalitis)** Ref: Apurba Sastry's Medical Microbiology 1/e p488-89

13. **Ans. (a) (b) (Herpes simplex virus, Rabies)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p488-89, Ananthanarayan 9/e p469,518
    - Herpes simplex encephalitis though RARE, is the MOST COMMON cause of acute sporadic viral encephalitis in most part of the world.
    - Rabies encephalitis is directly related dog bite, it is never epidemic.
    - Nipah virus (belongs to paramyxovirus) had caused an outbreak of neurological and respiratory disease in pig farms in Malaysia(1999), resulting in 257 human infections.

DENGUE

14. **Ans. (d) (ELISA for antibody….)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p491
    In dengue infection, IgM antibody appears after 5th day, where as NS1 antigen appears in blood from day-1 itself.

15. **Ans (a) (Dengue)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p491
    - Tourniquet test of >20 petechial spots is one of the criteria used to diagnose DHF.

16. **Ans. (a) (Dengue hemorrhagic fever)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p490.
    Antibody dependant enhancement (ADE) is seen in DHF. Refer chapter review for detail.

17. **Ans. (b) (2)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p489-91, Park 23/e p246-54,, 22/e p224-25
    - Serotype 2 is the most virulent serotype of dengue virus.
    - Mixed serotype infection is more severe than single serotype infection.
    - Among all, serotype 1 followed by 2 is the most dangerous combination.

18. **Ans. (a) (b) (e) (It can cause both endemic and epidemic, MC arboviral disease, Self-limiting)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p489-91, Park 23/e p246-54, 22/e p224-25
    - *Dengue fever can occur epidemically or endemically.*
    - *Over past 10–15 years, Dengue has become the leading cause of hospitalization and death next to diarrheal and respiratory infection among children in South East Asia region (SEAR) including India.*
    - *Temperature also plays an important role in the transmission of Dengue by the Mosquito. Aedes aegypti kept at 26°C fails to transmit DEN-2 serotype.*
    - *Dengue fever is a self limiting condition and represents the majority of Dengue cases where as both Dengue hemorrhagic fever and Dengue Shock Syndrome are fatal.*
    - *Of all arthropod borne viral diseases, Dengue fever is the most common in India.*

19. **Ans. (c) (Reinfection...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p489-91, and Harrison 18/e p2546.
    - *Dengue hemorrhagic fever or dengue shock syndrome occurs in most patients who have had prior infection with one or more dengue serotypes.*
    - *Possible reasons for Dengue hemorrhagic fever:* **Due to antibody-dependent enhancement**
    - *Antibody raised against the 1st serotype persists and binds to the second serotype and protect it from the host immune system.*

20. **Ans. (c) (Neut..)** Ref: Journal – Apurba Sastry's Essentials of Medical Microbiology 1/e p489-91, CMR Vol.11,1998
    'Neutralization Test- ***Most specific and sensitive serologic test for dengue viruses'***
    Serologic Diagnosis of Dengue for antibody detection:
    - *Hemagglutination-inhibition (HI)*
    - *Complement fixation (CF)*
    - *Neutralization test (NT)*
    - *IgM capture ELISA (MAC-ELISA)*
    - *Indirect IgG ELISA.*

21. **Ans. (a) (b) (c)(d)(e) (↑Hematocrit, ↓ Platelet, +ve tourniquet test, pleural effusion , Aedes aegypti bites in day time)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p489-91, Park, 22/e p225-27, 23/e p246-54
    - *Respiratory distress due to pleural effusion and ascites may occur in any time*

    Criteria for Dengue Hemorrhagic Fever (DHF):
    - Fever: Acute, high, continuous for 2–7 days
    - Positive Tourniquest test: *Petechial spot > 20 per square inch in cubital fossa*

- Enlargement of liver
- Thrombocytopenia (< 100,000/mm^3)
- Hematocrit raised by > 20%).

22. Ans. (c) (Transovarian transmission in Aedes) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p489-91, Park 22/e p225, 23/e p246-54

- Aedes aegypti: Once infected, remains infective for life
- Transovarian transmission seen by which infection can be spread to offsprings.

23. Ans. (a) (Aedes aegypti) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p489-91, Park, 22/e p225, 23/e p246-54

- Vector for dengue: Aedes aegypti mosquito (MC), but also by (Aedes albopictus, A. polynesiensis and A. scutellaris)

24. Ans. (a) (Platelet count) Ref: Apurba Sastry's Essentials of Medical Microbiology/p491

Platelet count is the most important prognostic factor in Dengue management.

25. Ans. (b) (Lamivudine is drug of choice) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p489-91

There is no anti-viral drug available for dengue hemorrhagic fever.

26. Ans. (a) (c) (d) (Caused by 4 serotypes, Presents with fever and joint pain, Virus belongs to flavivirus genus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p489-91

- Dengue virus has 4 serotypes (DEN-1 to DEN-4). However, the fifth serotype was discovered in 2013 from Bangkok
- No effective vaccine is available for Dengue yet, however, recently a vaccine trial has cleared the Phase III clinical trial.
  - Live-attenuated tetravalent vaccine based on chimeric yellow fever-dengue virus (CYD-TDV) has been developed by Sanofi Pasteur Company. It was found to be safe and effective in Phase III clinical trial done in Latin America and is expected to be marketed by 2015.
- Fever and joint pain are the cardinal manifestation of dengue fever.
- Dengue virus belongs to Family -Flaviviridae, Genus- Flavivirus.
- Segmented RNA is present in- Bunyavirus, Influenza, Rotavirus and Arena virus (Code-BIRA).

YELLOW FEVER VIRUS

27. Ans. (d) (Yellow fever vaccine) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p493

Yellow fever vaccine and influenza vaccine are contraindicated in persons who develop allergy to egg

28. Ans. (b), (d) (Rift valley, Yellow fever) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p492-93

- ***Yellow fever*** NOT found in India:
- Rift Valley fever: It is a mosquito borne viral disease causing influenza like illness and caused hemorrhagic fever outbreaks in Kenya, Yemen and Soudi Arabia (not found in India)
- Other options- explained in chapter review.

29. Ans. (a) (3–6 days) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p492-93, Park, 22/e p257

- **Incubation period of yellow fever 3–**6 days *(6 days for International Health Regulations)*

30. Ans. (a) (1%) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p492-93, Park 22/e p257, 23/e p283

- *According to international health regulation, yellow fever transmission risk is minimal if Breteau index (Aedes aegypti index)* ***is < 1,*** *surrounding* ***400 mt*** *of airport*

31. Ans. (c) (IP- 16–46 days) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p492-93, Park 22/e p257

- Yellow fever virus:
  - Belongs to Flavivirus
  - Vaccination is valid after 10 days and lasts for 10 yrs
  - Case fatality ratio 80%
  - Vaccine is effective within 7 days of inoculation, lasts for 35 years
  - However, validity of yellow fever certificate (by WHO): Certificate is issued after 10 days and Reimmunization required, i.e. certificate is renewed every 10 years for international travel.
  - IP-3–6 days.

KYASANUR FOREST DISEASE

32. Ans. (c) (Kyasanur...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p493

- ***Option a*** and **b**: Incorrect- already explained
- ***Option c:*** Correct-KFD is a hemorrhagic fever disease and infection is transmitted by bite of ticks **(*Hemaphysalis spinigera*)**
- ***Option d:*** Incorrect- Vectors for transmission of Dengue fever- *Aedes aegypti*, *A. albopictus*, *A. polynesiensis* mosquitoes.

33. Ans. (b) (Deforestation) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p493, Park, 22/e p263, 23/e p288

KFD control measures:

- Vector (tick) control
- Killed KFD vaccine
- Personal protection: By mosquito repellents like dimethylphthalate
- Since heavy tick population in the forest is attributed to free roaming of cattle, the restriction of cattle movement is thought to cause reduction of vector population.

ZIKAVIRUS

34. Ans. (a,b,d) (Can be transmitted by sexual route, NAAT from amniotic fluid…, Aedes…) Ref: CDC guideline on Zikavirus

- Zika virus is transmitted by bite of Aedes, sexual or by vertical route.
- No antiviral drug is available
- Symptomatic to asymptomatic ratio 1:5.
- NAAT can be used for prenatal diagnosis.

POLIOVIRUS

35. Ans. (a) (4-30 days) Ref: Polio Vaccines/WHO, ApurbaSastry's Essentials of Medical Microbiology 1/e p479

The onset of symptoms with VAPP usually occurs 4–30 days following receipt of oral polio vaccine (OPV) or within 4–75 days after contact with a recipient of OPV…….....Polio Vaccines/WHO.

36. Ans. (c) (mVDPV) Ref: Journal: Apurba Sastry's Essentials of Medical Microbiology 1/e p479, Vaccine-Derived Polioviruses. JID 2014:210 (Suppl 1)S283

Vaccine-derived polioviruses (VDPVs)

VDPVs isolates exhibit a higher level of genetic divergence from their parental OPV strains at VP1 sequence, which helps in their prolonged replication, and transmission. Refer chapter review for detail

37. Ans. (b), (c) and (d) (Pakistan, Nigeria, Afghanistan) Refer: Apurba Sastry's Essentials of Medical Microbiology 1/e p480, www.cdc.gov/polio

Polio is still endemic in Pakistan and Afghanistan. Nigeria is declared polio free in 2015, but again became endemic in 2016. To know detail about polio eradication update, Refer annexure at the beginning of the book.

38. Ans. (a) (Last natural...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p480, Park 22/e p184, 23/e p203

Polio Situation in India (till 2013):

- ***Last natural case was reported on 13th Jan 2011 (Howrah, Kolkata, due to WPV-1)***
- 1 case of VAPP by type-2 strain was reported from West Bengal in 2012
- Type-2 is not reported since 1999
- India was removed from the list of polio endemic countries as of 25th Feb 2012. It has to remain polio free till Feb 2014 to achieve the goal of polio eradication and to be certified as polio free country.
- Still now, OPV is recommended in national immunization schedule in India.
- Countries which are endemic for polio are - Pakistan, Afghanistan, Nigeria.

39. Ans. (a) (Detection...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p477, Park 23/e p203-9, 22/e p188

Polio virus is best indentified by isolation of the virus from stool culture.

40. Ans. (c) (IM injections and increased muscular activity lead to increased paralysis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p477

- IM injections and increased muscular activity: risk factor for increased paralysis in poliomyelitis
- Paralytic polio occurs in < 0.1% of all cases of poliomyelitis
- Poliomyelitis: results in Acute ***flaccid*** paralysis (AFP), not spastic paralysis
- Oral Polio drops given to all children of < 5 year.

41. Ans. (c) (There is single serotype causing infection) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p476

- There are three serotypes of Poliovirus: 1, 2, 3 and any of them can cause infection
- Polio is transmitted most commonly by feco-oral route followed by inhalation and conjunctival route
- Asymptomatic infections are common in children. The most vulnerable group for Polio is children of 6 months to 3 years.
- Herd immunity is produced by live attenuated vaccine: Because live vaccine progeny viruses can be excreted in feces and spread to other house hold contacts.

42. Ans. (c) (Type I responsible...) Apurba Sastry's Essentials of Medical Microbiology 1/e p 479

- ***Polio virus type 3,*** undergoes mutation in the course of their multiplication in vaccinated children, and causes ***vaccine associated paralytic polio***
- The developing countries in the tropics, more than half of the vaccinees fail to show serological response to 2/3 doses of polio vaccine, especially with polio type 1
- Type 1 is responsible for most of cases of paralysis and it frequently causes epidemics
- So remember:
  - ***Type 1 – MC cause of wild virus vaccine associated paralytic polio***
  - ***Type 3 – MC cause of vaccine associated paralytic polio.***

43. Ans. (a) (Stool) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p477, Park 22/e p188, 23/e p203-9

- Stool culture: Recommended for AFP surveillance and lab confirmation
- Isolation in stool: Does not correlate with disease as shedding occurs in subclinical cases also.
- Cultures of CSF, serum or throat swab are positive less frequently, but indicative of disease.

44. Ans. (a) (b) (VAPP in recipient of OPV, VAPP in contacts or recipient of OPV) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p479, Park 23/e p203-9, 22/e p188

- VAPP occurs to recipients of OPV as well as to their contacts.

45. Ans. (b) (At birth) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p478, Park, 22/e p188, 23/e p203-9

- OPV Schedule:
  - Total five doses: Zero dose at birth
  - 1st/2nd /3rd: 6/10/14 wks, booster: 16–24 m.

OTHER PICORNA VIRUSES

46. Ans (a, c) (Enterovirus, Coxsackievirus A16) Ref: Apurba Sastry's Essentials of Medical Microbiology/p481

- Coxsackievirus A5,10 and 16, Enterovirus 71 are the common causes of hand, foot, and mouth disease.

47. Ans. (a, b, c) (Polio, Coxasackie, Rhinovirus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 475

- **Picornaviridae include Enteroviruses (Polio, Coxasackie, Echovirus, Parechovirus, Enterovirus 68-72) and Rhinovirus**

48. Ans. (c) (Enterovirus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p480-81, Ananthanarayan 9/e p491

- Coxsackie virus belongs to Picornaviridae family.

49. Ans. (a) (Enterovirus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p480-81, Ananthanarayan 9/e p491

- Herpangina or vesicular pharyngitis is caused by Coxsackie virus-A.

50. Ans. (a) (Hepatitis A) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p480-81, Ananthanarayan 9/e p491

- **Hepatitis A virus belongs to** Picornaviridae family and it is Enterovirus-72.

51. Ans. (b) (Enterovirus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p480-81

- *Most common cause of meningoencephalitis in children is Enterovirus*
- *Detail list of agents of aseptic meningitis, Refer chapter review of chapter-7.1*

52. Ans. (a) (Hemorrhagic fever) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p480-81

- *Enteroviruses do not cause hemorrhagic fever.*

53. **Ans. (c) (Coxsackie A)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p480-81, Ananthanarayan 9/e p491
    - Herpangina (vesicular pharyngtitis) is due to Coxsackie A virus serotype 1–6, 8, 10.

54. **Ans. (b) (c) (d) (e)(Stable at pH 4, Cause pleurodynia, Cause encephalitis, Cause meningitis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p479-81, Ananthanarayan 9/e p485
    - Enteroviruses (e.g. Polio and Coxsackie virus and Enterovirus type 1-33) are acid stable, hence can survive in GIT.
    - Many enteroviruses can cause aseptic meningitis and encephalitis
    - Pleurodynia is caused by Coxsackie virus B
    - Segmented RNA is present in- Bunyavirus, Influenza, Rotavirus and Arena virus (Code-BIRA).

55. **Ans. (a) (Coxsackie B)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p480-81, Ananthanarayan 9th/491
    Refer chapter review.

56. **Ans. (c) (Serotype 70)** Apurba Sastry's Essentials of Medical Microbiology 1/e p480-81, Ananthanarayan 9/e p491-2
    Acute hemorrhagic conjunctivitis is caused by Enterovirus 70 and Coxsackie A-24.

RHABDOVIRUS (RABIES)

57. **Ans. (a) (RT-PCR for rabies virus)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p499
    Antigen detection by direct-IF or RT-PCR is the preferred methods done on corneal smear of Rabies suspects.

58. **Ans (b) (Purkinje cells of cerebellum)** Ref: Infections of the Nervous System

59. **Ans. (a) (Subcortical...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p498 , Ananthanarayan 9/e p537

60. **Ans. (b) (Negri bodies)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p498, Ananthanarayan 9/e p533, 8/e p529
    Negri bodies
    - Diagnosis of Rabies may be made postmortem by demonstration of Negri bodies in the brain, but they may be ***absent in 20% of cases.***
    - Negri bodies are most abundant in ***hippocampus and cerebellum***
    - Impression smears made from brain tissue are stained by ***Seller's technique.***
    - Negri bodies are seen as intracytoplasmic, round/oval, purplish pink structures with characteristic basophilic inner granules.

61. **Ans. (b) (Corneal...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p499, Ananthanarayan 9/e p533, 8/e p529
    - *For the antemortem diagnosis of rabies, viral antigens can be demonstrated in the corneal smear, skin biopsy from the face or neck or saliva.*

62. **Ans. (a) (Lakshadweep island)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p501-2, Park, 22/e p251
    - Rabies free zone in India: Andaman and Nicobar Island and Lakshadweep Island.

63. **Ans. (d) (Australia)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p501-2, Park, 22/e p251
    Rabies free area- Refer text for detail.

64. **Ans. (b) (10 days)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p502, Park 23/e p276-81, 22/e p255
    - Bitten dog and cat have to be observed for sign and symptoms for 10 days. If they are healthy, vaccine is discontinued, if they develop symptoms, then they are humanly killed and tissue is examined.
    - For other animal bite, this period of observation is not required. They are immediately humanly killed and examined for the presence of rabies antigen in brain sections.

PROPHYLAXIS OF RABIES

65. **Ans. (c) (15 minutes)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p500, Park 23/e p278
    Immediate flushing and washing the animal bite wound, with plenty of soap and water, under running tap should be carried out at least for 15 minutes.

66. **Ans. (b) (Fixed virus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p500-501, Ananthanarayan 9/e p535
    - Vaccine is prepared from fixed virus (i.e. viruses grown serial subcultures in cell line). They differ from wild viruses in many ways…… (Refer chapter review for detail)

67. Ans. (c) (Immediately stitch wound under antibiotic coverage)

Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p500-501, PSM Park 22/e 23/e p276-81

- ***Bite wounds are not sutured immediately to prevent additional trauma which may help spread of virus deeper in to tissues.***
- *Detail- Refer chapter review*

68. Ans. (b) (Nibbling of uncovered area) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p500-501

Category II bites include

- Minor scratches or abrasions without bleeding or
- Nibbling of uncovered skin

69. Ans. (c) (Semple vaccine) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p500-501

Antirabies Vaccines: Refer chapter review

70. Ans. (d) (Recombinant glycoprotein) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p500-501

- Surface glycoprotein cloned and recombinant vaccine (subunit vaccine) still in the experimental stage.
- In India following cell culture vaccines are available.

71. Ans. (c) (Start...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p500-501, Park, 22/e p/254, 23/e p276-81

For category II and III type of exposure to Dog, the guideline is that post exposure prophylaxis (vaccine) should be started immediately and the dog should be observed for 10 days.

- Immunoglobulin is given in addition for category III exposure.
- If the dog is found to have abnormal behavior, it is humanly killed and brain tissue is examined for presence of negri bodies.

CHAPTER

4.5 Hepatitis Viruses

Hepatitis viruses are heterogeneous group of viruses that are taxonomically diverse (belong to different families) but all are hepatotropic; cause acute inflammation of the liver producing identical histopathologic lesions and similar clinical illness such as fever, nausea, vomiting, and jaundice.

- Hepatitis viruses are classified into six types: (HAV, HBV, HCV, HDV, HEV and HGV).
- Other viruses that can cause sporadic hepatitis, are yellow fever virus, CMV, EBV, HSV, rubella virus, and enteroviruses.

Table 4.5.1: Features of hepatitis viruses

Properties	HAV	HBV	HCV	HDV	HEV
Common name	Infectious hepatitis	Serum hepatitis	Non A non B or post-transfusion hepatitis	Delta agent	Non A non B enteric transmitted hepatitis
Family	Enterovirus-72 (Picornaviridae)	Hepadnaviridae	Flaviviridae	Unclassified viroid-like	Unclassified Caliciviridae-like
Virion	27 nm, icosahedral	42 nm, spherical	60 nm, spherical	35 nm, spherical	30–32 nm, icosahedral
Envelope	No	Yes (HBsAg)	Yes	Yes (HBsAg)	No
Genome	ssRNA	dsDNA	ssRNA	ssRNA	ssRNA
Stability	Heat and acid-stable	Acid-sensitive	Ether-sensitive, acid-sensitive	Acid-sensitive	Heat-stable
Onset	Abrupt	Insidious	Insidious	Insidious	Abrupt
Age	Children, Young adults	Young adults babies, toddlers	Any age, but more common in adults	Any age (similar to HBV)	Young adults (20–40 years)
Route	Feco-oral	Blood (MC) Sexual, Vertical	Blood (MC) Sexual (+/-) Vertical (+/-)	Blood (MC) Sexual(++) Vertical(+)	Feco-oral
Incubation period	15–45 days (Average 30)	30–180 days (Average 60–90)	15–160 days (Average 50)	30–180 days (Average 60–90)	14–60 days (Average 40)
Fulminant disease	Rare (0.1%)	Rare (0.1–1%)	Rare (0.1%)	Frequent (5–20%)	Usually rare (1–2%) Pregnancy- 20–40%
Carrier	None	Yes (0.1–30%)	Yes (1.5–3.2%)	Variable	None
Chronicity	None	Occasional (1–10%)	Common (85%)	Common	None
Oncogenic	No	Yes (neonate)	Yes	+/-	No
Prevalence	High	High	Moderate	Low, regional	Regional
Associated other features	Secondary attack rate 10–20%	HCC, cirrhosis, Autoimmune disorder like AGN, arthritis, PAN	HCC, cirrhosis, Autoimmune- AGN, arthritis, cryoglobu-linemia	HCC, cirrhosis, fulminant hepatitis	Secondary attack rate (1–2%) Rarely seen in western countries
Prognosis	Excellent	Worse with age	Moderate	Acute-good Chronic- poor	Good
Prophylaxis	Immunoglobulin, Inactivated vaccine	HBIG, Recombinant vaccine	None	HBV vaccine (no vaccine for HBV carriers)	Vaccine (HEV239) (only in China)
Therapy	None	Pegylated interferon Lamivudine	Pegylated interferon plus ribavirin	Interferon ±	None

HEPATITIS A VIRUS

Hepatitis A virus belongs to family Picornaviridae (called enterovirus 72 or genus *Hepatovirus*) HAV is 27 to 32 nm in size, with icosahedral symmetry, containing a linear ssRNA.

Hepatitis A (Age affected):
- Developing countries: Children and adolescents (mostly subclinical)
- Developed countries with improved hygiene: Trend of shift of towards the older age (more *icteric*).

Epidemiology

- **Mode of transmission**
  - HAV transmission is by the **feco-oral route**
  - Rarely, by sexual (oro-genital contact) and parenteral routes.
- **Hosts:** Humans are the only host for HAV.
- **Age:**
  - Children and adolescents (5–14 years of age) are MC affected, majority remain *sub-clinical* (80–95%)
  - However, in *developed countries* with improved hygiene, the incidence is decreasing and there is trend to shift of infection towards the older age.
  - Adults are more *icteric* (75–90%), with higher mortality rate than children.
- **Anicteric to icteric cases:** ratio is about in children – 12:1 and in adults – 1:3.
- **Risk factors:** Poor personal hygiene and overcrowding.
- **Seasonality**: Widespread throughout the year (peak in late rainfall and in early winter).
- **Virus excretion**: Viral excretion in feces may be 2 weeks before to 2 weeks after the appearance of jaundice.

MC cause of viral hepatitis in India:
- Acute sporadic and epidemic hepatitis- HEV (30%–70%)
- Acute hepatitis in children- HAV
- 15–30% of acute hepatitis is due to HBV
- Chronic hepatitis- HBV >HCV
- Cirrhosis- HBV >HCV
- Primary liver cell cancer- HBV >HCV
- Post-transfusion hepatitis- HBV (1:220,000)>HCV (1:1800,000)

Laboratory Diagnosis

- **Anti HAV Antibody detection**
  - IgM antibodies appear during the acute phase, peak about 2 weeks after the elevation of liver enzymes, and disappear within 3–6 months.
  - IgG appears a week after the appearance of IgM, persists for decades and indicates past infection or recovery.
- **Detection of HAV particles** by immune electron microscopy: HAV appears in stool from -2 to +2 weeks of jaundice
- **Detection of HAV antigens** in stool by ELISA from -2 to +2 weeks of jaundice
- **Isolation**: HAV is the only hepatitis virus where isolation has been attempted though difficult (primate cell lines)
- **Non-specific:** Elevated liver enzymes and serum bilirubin level.

Vaccines

- Formaldehyde inactivated vaccine: It is prepared from human fetal lung fibroblast cell lines, given by IM
- Live attenuated vaccine: It uses H2 and L-A-1 strains, prepared in human diploid cell line (China)
- Both vaccines are highly immunogenic, produce long lasting immunity.

HAV-Ig

It is useful for post-exposure prophylaxis of intimate contacts (household, day care centers) of persons with hepatitis A or to the travellers. It should be administered within 2 weeks of exposure and gives protection for 1–2 months.

HEPATITIS B VIRUS

Hepatitis B virus is the most widespread hepatitis viruses.

HBV is the only DNA virus among hepatitis viruses; discovered by Blumberg in 1963 and belongs to the family Hepadnaviridae.

Hepatitis B: Morphologic forms:
Electron microscopy reveals 3 forms:
- Spherical forms (MC)
- Tubular or filamentous forms
- Complete form or Dane particles.

Morphology

Electron microscopy of serum of the patients infected with HBV reveals 3 morphologic forms:

1. **Spherical forms**: Most numerous, (22 nm size), exclusively made up of HBsAg.
2. **Tubular or filamentous forms**: 200 nm long, also exclusively made up of HBsAg.
3. **Complete form or Dane particles:** They are less frequently observed, 42-nm size spherical virions; made up of-
   - Outer surface envelope: HBsAg (Hepatitis B surface antigen or envelope antigen or Australian antigen).
   - Inner 27 nm size nucleocapsid: Consists of core antigen (HBcAg) and pre-core antigen (HBeAg) and DNA polymerase.

Hepatitis B genome:
- S gene: Code for HBsAg
- C gene: Code for two nucleocapsid proteins
  - Pre-C region codes for HBeAg
  - C-region codes for HBcAg
- X gene: Codes for HBxAg
- P gene: Codes for polymerase (P) protein.

Viral Genome

- **S gene** has three regions: S, pre-S1 and pre-S2. They code for surface antigen (HBsAg).
- **C gene:** Consists of pre-C and C-regions, which code for two nucleocapsid proteins
  - Pre-C region codes for HBeAg
  - C-region codes for HBcAg
- **X gene**-codes for HBxAg.
  - It may contribute to carcinogenesis by binding to p53.
  - HBxAg and its antibody are elevated in patients with severe chronic hepatitis and hepatocellular carcinoma.
- **P gene** is the largest and codes for polymerase (P) protein, having 3 enzymatic activities: (i) DNA polymerase activity, (ii) Reverse transcriptase activity and (iii) RNase H activity.

Serotypes of HBV:
- ADW: Seen in in Europe, Australia and America
- In India:
  - ADR: In South and East India
  - AYW: In Western and Northern India.

Typing of HBV

Serotypes: HBV is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope protein HBsAg.
- adw is the predominant subtype in Europe, Australia and America
- In India adr is the prevalent subtype in South and East India whereas ayw is prevalent in Western and Northern India.

Genotypes: Has eight genotypes (A–H). Genotypes A and D are prevalent in India.

Hepatitis B Virus Mutants

Pre-core Mutants

- They have defect in pre-core region of C gene which leads to their inability to synthesize HBeAg.
- Geographical distribution: Identified in Mediterranean countries and in Europe. Such patients may be diagnosed late and they tend to have *severe chronic hepatitis* that progresses to cirrhosis.
- Markers: They lack HBeAg. Other viral markers are present as such.

Escape Mutants

They have mutations in the S gene, leads to alteration of HBsAg (usually in *a* antigen). They may pose problems in hepatitis B vaccination strategies as well as in the diagnosis of the disease. These mutations are observed in:
- Infants born to HBeAg positive mothers
- Liver transplant recipients.
- A small proportion of recipients of active and passive immunization.

YMDD mutation:
HBV infected patients on lamivudine therapy may develop resistance due to mutation in the YMDD locus, present on reverse transcriptase region of P gene of HBV.

YMDD Mutation

HBV infected patients on lamivudine therapy may develop resistance to the drug due to mutation in the YMDD locus of the HBV reverse transcriptase region of polymerase gene.

Transmission

HBV transmission occurs via multiple routes.

- **Parenteral route**: In developing countries, the most common mode of transmission is via blood and blood products transfusion and needle prick injuries.
- Transmission risk of HBV following needle prick injury is nearly 30% as compared to 3% and 0.3% with HCV and HIV respectively. As little as 0.00001 ml of blood can be infectious for HBV.
- **Sexual transmission** is commonest route in developed countries. (homosexual males)
- **Vertical (perinatal) transmission:** Particularly in China and SE Asia.
  - Transmission occurs at any stage; in utero, ***during delivery*** (maximum risk) and during breast feeding
  - Risk is maximum if the mother is HBeAg positive.
- **Direct skin contact** with infected open skin lesions, e.g. impetigo (especially in children).

Transmission of HBV:
- Parenteral route: MC mode in developing countries
- Sexual transmission: MC mode in most developed countries
- Vertical (perinatal) transmission
- Direct skin contact.

Epidemiology

Hepatitis B virus infection occurs throughout the world.

- **Reservoir of infection:** Humans are the only reservoir of infection who can be either cases or carriers. Carries may be temporary (harbor the virus for weeks to months) or persistent/chronic (harbor the virus for > 6 months).
- **Carriers** can also be grouped into:
  - *Simple carriers:* Low infectivity, transmit the virus at a lower rate. They possess low level of HBsAg and no HBeAg.
  - *Super carriers:* They are highly infectious and transmit the virus efficiently. They possess higher levels of HBsAg and also have HBeAg, DNA polymerase, and HBV DNA.
- **Prevalence:** Based on HBsAg carrier rates, three epidemiological patterns are observed:
  - *Type 1 pattern* (low endemicity, < 2% carrier rate): It is observed in Sri Lanka and Nepal
  - *Type 2 pattern* (intermediate endemicity, 2–8% carrier rate): Observed in India, Bhutan, Indonesia and Maldives
  - *Type 3 pattern* (high endemicity, > 8% carrier rate): It is observed in Bangladesh and DPR Korea.
- **Situation in India:** Overall, India accounts for the second largest burden of HBV infection, next to China.
  - India is considered to have an intermediate level of HBV endemicity. South Indians have higher carrier rates.
  - HBV is the second most common cause of acute viral hepatitis after HEV in India.
- **Period of infectivity:** Infectious as long as the HBsAg is present in blood, i.e. during incubation period (a month before jaundice) up to several months thereafter (occasionally years for chronic carriers).
  - Become non-infectious once HBsAg disappears and is replaced by anti-HBs antibody.
  - Maximum infectivity is observed when HBeAg is elevated in serum.
- **HBV and HIV Co-infection:**
  - It is estimated that 10% of the total HIV infected people worldwide are co-infected with HBV.
  - Although HBV does not alter the progression of HIV, the presence of HIV greatly enhances the risk of developing HBV associated cirrhosis and liver cancer.
- **Age:** The outcome of HBV infection depends on the age. Following HBV infection:
  - Chance of developing acute hepatitis is directly related to the age
  - Chance of developing chronic hepatitis or carrier state is inversely related to age.

HBV Carriers:
- Simple carriers: Low infectivity. Possess low level of HBsAg and no HBeAg.
- Super carriers: Highly infectious. Possess higher levels of HBsAg and also have HBeAg, DNA polymerase, and HBV DNA.

Situation in India:
- Overall, India accounts for the second largest burden of HBV infection, next to China.
- India is considered to have an intermediate level of HBV endemicity. South Indians have higher carrier rates.
- HBV is the second most common cause of acute viral hepatitis after HEV in India.

Age	Chance of developing acute hepatitis	Chance of developing chronicity or carrier
Perinatal	1%	80–90%
Early childhood (1–5 years)	10%	30%
Late childhood (> 5 years)	30%	5%

Laboratory Diagnosis of HBV

Definitive diagnosis of HBV depends on the serological demonstration of the viral markers. It does not grow in any conventional culture system.

HBsAg:
- First marker to be elevated following infection
- Indicates onset of infectivity
- It remains elevated in the entire duration of acute hepatitis.

HBeAg and HBV DNA:
They are the markers of:
- Active viral replication
- High viral infectivity (i.e. they are highly infectious to others).

Hepatitis B Surface Antigen (HBsAg)

HBsAg is the first marker to be elevated following infection; appears within 1–12 week:

- It appears during incubation period; 2–6 weeks before the biochemical and clinical evidence of hepatitis
- Presence of HBsAg indicates onset of infectivity
- It remains elevated in the entire duration of acute hepatitis;
  - Becomes undetectable 1–2 months after the onset of jaundice and replaced by HBsAb- indicate recovery
  - Or, rarely persists beyond 6 months during chronic hepatitis and carrier state
- HBsAg is used as an epidemiological marker of hepatitis B infection (i.e. to calculate prevalence of infection).

Hepatitis B Pre-core Antigen (HBeAg) and HBV DNA

They appear concurrently with or shortly after appearance of HBsAg in serum

- They are the markers of
  - Active viral replication
  - High viral infectivity (i.e. they are highly infectious to others)
- However, they are present in either acute, chronic and carrier state and it cannot differentiate between these stages. Their presence just indicates that the virus is actively multiplying, which could be either-
  - Acute active hepatitis
  - Chronic active hepatitis
  - Or a carrier in whom HBV is actively multiplying and highly infectious (such carriers are called as super carriers).

Hepatitis B Core Antigen (HBcAg)

- HBcAg is a hidden antigen due to surrounding HBsAg coat. It is also non-secretory in nature; hence it cannot be detected in blood.
- However, HBcAg may be detected in hepatocytes by immunofluorescence microscopy.

Anti-HBc IgM (Hepatitis B Core Antibody)

- Anti-HBc IgM is the first antibody to elevate following infection
- It appears within the first 1–2 weeks after the appearance of HBsAg and lasts for 3–6 months
- Its presence indicates acute hepatitis B infection
- It is probably the only marker (sometimes anti-HBc IgG) present during the period between appearance of anti-HBs antibody and disappearance of HBsAg.

Anti-HBc IgG (Hepatitis B Core Antibody)

- Anti-HBc IgG appears in late acute stage and remains positive indefinitely whether the patient proceeds to:
  - Chronic stage (with persistence of HBsAg, symptomatic and elevated liver enzymes)
  - Carrier state (with persistence of HBsAg but asymptomatic) or
  - Recovery (appearance of Anti-HBs antibody).
- It can also be used as epidemiological marker of HBV infection.

Anti-HBe (Hepatitis B Precore Antibody)

- Anti-HBe antibodies appear after the clearance of HBeAg
- Its presence signifies diminished viral replication and decreased infectivity.

Anti-HBs (Hepatitis B Surface Antibody)

- Appears after the clearance of HBsAg and remains elevated indefinitely
- Its presence indicates recovery, immunity and non-infectivity (i.e. stoppage of transmission)
- It is also the only marker of vaccination.

Anti-HBs (Hepatitis B surface antibody):
Its presence indicates:
- Recovery and immunity
- Non-infectivity (i.e. stoppage of transmission)
- It is also the only marker of vaccination.

Table 4.5.2: Interpretation of HBV sero-markers

HBsAg	Anti-HBs	Anti-HBc	HBeAg	Anti-HBe	Interpretation
+	-	-	-	-	Early acute hepatitis (Incubating)
+	–	IgM	+	–	Acute hepatitis B, high infectivity
+	–	IgG	+	–	1.Chronic hepatitis B, high infectivity 2.Super carrier (If asymptomatic, normal liver enzymes)
+	–	IgG	–	+	1.Chronic hepatitis B, low infectivity 2.Simple carrier (If asymptomatic, normal liver enzymes)
+	–	IgG	–	-	Precore–mutant hepatitis B
-	+	+	-	+/–	Recovery
-	-	IgG	-	-	1.Remote infection (Common) 2.False positive immunoassay (rare)
+	+	IgG	+/–	+/–	1.HBsAg of one subtype and heterotypic anti-HBs (common, seen in 10–20%) 2.Process of seroconversion from HBsAg to anti-HBs (rare)
-	+	-	-	-	Postvaccination
–	–	IgM	+/–	+/–	Time period between appearance of anti-HBs antibody and disappearance of HBsAg

Treatment

In acute hepatitis B infection among previously healthy adults, recovery occurs in 99%; therefore, antiviral therapy is unnecessary. Specific antiviral drugs are indicated in stages of fulminant hepatitis or severe chronic hepatitis.

- Pegylated interferon
- Nucleoside/nucleotide analogues: Lamivudine, adefovir, entecavir, telbivudine and tenofovir.

HBV vaccine:
Booster doses are needed after 5 years especially to high-risk group if the antibody titer falls below 10 IU/ml.

Prophylaxis

Active Immunization (Hepatitis B Vaccine)

Hepatitis B vaccine is a recombinant subunit vaccine.

- The surface antigen (HBsAg) is used as vaccine candidate which is prepared in Baker's yeast by *DNA recombinant technology* by cloning the S gene into the yeast chromosome.
- **Route of administration:** By IM route over deltoid (in infant-anterolateral thigh)
- **Dosage:** 10–20 µg/dose (half of the dose is given to children below 10 years)
- **Schedule:**
  - Recommended schedule for adults: Three doses are given at 0, 1 and 6 months.
  - Under National immunization schedule: It is given at 6, 10, 14 weeks (along with DPT vaccine). Additional dose at birth may be given in areas with prevalence of HBV > 8%
  - Minimum interval between the doses- 4 weeks.

- **Marker of protection**: Recipients are said to be protected if the anti-HBsAg antibody titer is > 10 IU/ml.
- However, *non responders* (do not show seroconversion) and *low responders* (seroconversion occurs slowly) may be seen in 5–10% of vaccinated individuals.
- Protection may last for about 15 yrs or even longer
- Booster doses are needed after 5 years especially to high-risk group if the antibody titer falls below 10 IU/ml.

Neonates born to HBV infected mother:
- HBIG + vaccine
- Given within 12 hours of birth.

Passive Immunization (Hepatitis B Immunoglobulin or HBIG)

- **Indications:** Where an **immediate protection** is warranted.
  - Acutely exposed to HBsAg positive blood, e.g. surgeons, nurses, laboratory workers
  - Sexual contact of acute hepatitis B patients
  - Neonates borne to hepatitis B carrier mothers
  - Postliver transplant patients who need protection against HBV infection.
- Following accidental exposure, HBIG should be started immediately (ideally within 6 hrs, but not later than 48 hrs).
- **Recommended dose** is *0.05–0.07ml/kg* body weight, two doses of HBIG should be given 30 days apart.
- HBIG gives short term passive protection which lasts for about 100 days.

Combined Immunization

Combined immunization with **HBIG + vaccine** is recommended for **neonates** born to HBV infected mother, where a single injection of 0.5 ml of HBIG is given to the neonate immediately after the birth, followed by full course of vaccine given at a different site (the first dose being given within 12 hours of birth).

The guideline for post-exposure prophylaxis is as follows:

- If the exposed person is vaccinated and the antibody titer is protective (i.e. >10 IU/ml) No further treatment is needed
- If the exposed person is vaccinated and the titer is not protective (i.e. < 10 IU/ml):
  - HBIG: Should be started immediately
  - Vaccine: Single dose should be given within 7 days of exposure.
- If the exposed person is not vaccinated: HBIG and full course of vaccine (3 doses) are needed.

HEPATITIS C VIRUS

Hepatitis C virus (HCV) is the common cause of post-transfusion hepatitis in developing countries.

- HCV is classified under family Flaviviridae, genus *Hepacivirus*
- It is spherical, 60 nm size and enveloped virus, contains positive sense ssRNA.

HCV genotypes distribution:
- Genotype 1 is the MC type, distributed worldwide
- In India, genotypes 1 and 3 of HCV are more prevalent
- Patients with genotype-1b respond poorly to therapy than other genotypes.

Genetic Diversity of HCV

HCV displays diversity in the RNA genome due to high rates of mutations in the virus.

- **Genotypes:** HCV is divided into 6 genotypes or clades, which differ from each other by 25–35% in RNA sequence.
- **Subtypes:** Genotypes are further divided into 100 subtypes, which differ from each other by 15–25% in their RNA nucleotide sequence. In any given patient, the subtypes of HCV circulate as complex closely related viral population known as ***quasispecies.***
- **E2 envelope protein** is the most variable region of HCV genome and hence more prone to undergo mutations, which results in: (i) Chronic infection, (ii) Failure of vaccine
- HCV genotypes do not vary in clinical severity but they vary in their epidemiological distribution:
  - Genotype 1 is the most common type, distributed worldwide.
  - In India, genotypes 1 and 3 of HCV are more prevalent.
  - Genotypes also vary in their susceptibility to antiviral drugs. Patients with genotype-1b respond poorly to therapy than other genotypes.

Transmission

- HCV is transmitted by **Parenteral** (most common), **Vertical-**less risk (6%) than that of HBV (20%) and **Sexual**
- HCV ***does not spread*** through breast milk, food or casual contacts (hugging or kissing).

Clinical Manifestations

Incubation period is about 15–160 days (average 50 days). Following an infection with HCV:

- About 20% of people develop acute hepatitis
- About 75–80% directly develop chronic disease; out of which:
  - 60–70% develop chronic hepatitis
  - 5–20% develop cirrhosis
  - 1–5% develop hepatocellular carcinoma. (HCV accounts for 25% of total liver cancer patients).
- **Extrahepatic** manifestations: Due to deposition of circulating immune complexes in various sites leading to manifestations such as: (i) Mixed cryoglobulinemia (ii) Glomerulonephritis (iii) Arthritis and joint pain.

Laboratory Diagnosis

- **Serum antibody detection:** Anti-HCV antibodies appear in about 8–9 weeks after exposure. It is detectable in >95% of chronic cases; however; in acute hepatitis antibodies are variably present. **Third-generation ELISAs** are the most popular assays currently available, which employ the antigens from the core, NS3, NS4, and NS5 regions, to detect anti-HCV antibodies:
  - Acute diagnosis: Anti-HCV (C33c, C22-3, NS5) are detected
  - Chronic diagnosis: Anti-HCV (C100-3, C33c, C22-3, NS5) are detected.
- **HCV RNA detection** is the most sensitive and gold standard test:
  - It is detected even before rise of liver enzymes and HCV antibodies.
  - Useful in predicting response to therapy, but not a reliable marker of severity.

Anti-HCV antibodies:
- Acute diagnosis: Anti-HCV (C33c, C22-3, NS5) are detected
- Chronic diagnosis: Anti-HCV (C100-3, C33c, C22-3, NS5) are detected.

Treatment

The most recommended regimen is combined therapy with pegylated interferon + ribavirin, should be started within 2–3 months after the onset of disease and continued for 24-weeks.

Predictors of Treatment Response

- Genotypes: People with HCV genotype 1b show the worst prognosis among all genotypes
- Viral RNA load: Higher the viral load (> 800,000 IU/mL), worse is the prognosis
- Interleukin 28B is a strong inducer of interferon-α.
  - The presence of a certain variation of IL28B called ***CC genotype*** produces a stronger IFN-α release.
  - Caucasians and African Americans lack *CC genotype*, hence show a poor treatment response than that of Asians.
- Metabolic disorders such as insulin resistance, obesity decrease the chance of responding to HCV therapy.

Worst prognosis to treatment response:
- HCV genotype 1b show the worst prognosis
- Higher the viral load, worse is the prognosis
- Lack of IL28B subtype ***CC genotype*** worse is the prognosis
- Metabolic disorders such as insulin resistance, obesity.

HEPATITIS D VIRUS

Hepatitis D is a defective virus; cannot replicate by itself; depends on Hepatitis B for its survival. The association of HDV with HBV is of two types: Co-infection and Super-infection.

- **Morphology:** Hepatitis D is taxonomically unclassified though resembles viroids. It is 35 nm in size, consisting of:
  - Circular, negative-sense ssRNA
  - Protein coat made up of single protein called as hepatitis D antigen (HDAg)
  - Surrounded by envelope protein derived from HBsAg from hepatitis B; hence it is called as defective virus.

- **Transmission:** Parenteral route is the most common mode; followed by sexual and vertical routes.

Features	Co-infection	Super-infection
Definition	HBV and HDV infection occurs simultaneously	HDV infection occurs to a carrier of HBV
Patient status	Healthy	HBV carrier
Complications	Less risk for fulminant hepatitis, chronicity, cirrhosis and HCC	Much greater risk for fulminant hepatitis, chronicity, cirrhosis and HCC
Mortality	Rare	>20%
Diagnosis	HBsAg, Anti-HBc (IgM), Anti-HDV (IgM), HDV RNA	HBsAg , HBeAg, and Anti-HBc (IgG) Anti-HDV-IgM (in acute), IgG and IgM (in chronic) and HDV RNA

HEPATITIS E VIRUS

Hepatitis E virus (HEV) causes an enterically transmitted hepatitis primarily occurring in young adults which occurs as epidemics in developing countries.

- Although HEV resembles caliciviruses, taxonomically it belongs to genus, *Hepevirus*, under family Hepeviridae.
- **Genotypes**: HEV has single serotype; however, five genotypes exist in nature
  - Only four genotypes have been detected in humans
  - Genotypes 1 and 2 appear to be more virulent and found exclusively in humans
  - Genotypes 3 and 4 are found both in humans and other mammals. They are more attenuated and account for subclinical infections.
- Fulminant hepatitis may occur rarely in 1–2% of cases; except for the *pregnant women* who are particularly at higher risk **(20%)** of developing fulminant hepatitis.

HDV diagnosis:
- Co-infection: Anti-HBc (IgM)
- Super-infection: Anti-HBc (IgG).

Epidemiology

- **Transmission**: It is feco-orally transmitted via sewage contamination of drinking water or food.
- There is no chronic infection or carrier state.
- **Epidemics** have been reported primarily from India, Asia, Africa, and Central America; in those geographic areas, HEV is the most common cause of acute hepatitis in this zone.
- HEV is unique among the known hepatitis viruses, in which it has an animal reservoir. HEV genotypes 3 and 4, animal to human transmissions occur.
- **In India,** HEV infection accounts for maximum (30–60%) cases.
- Though it resembles to HAV, features that differentiate HEV from that of HAV are:
  - Secondary attack rate (spread from patients to their contacts) is rare (1–2%) in HEV, compared to 10–20% in HAV.
  - Age: Young adults (20–40 years age) are commonly affected in HEV, compared to children in HAV.

HEV Genotype distribution:
- Genotypes 1 and 2: More virulent and found exclusively in humans
- Genotypes 3 and 4:
  - Found in humans and other mammals
  - They are more attenuated and account for subclinical infections.

Laboratory Diagnosis

- **HEV RNA** (by reverse transcriptase PCR) and **HEV virions** (by electron microscopy) can be detected in stool and serum even before the onset of clinical illness
- **Serum antibody** detection by ELISA.

HEPATITIS G VIRUS

Hepatitis G virus (HGV, also referred to as GB virus C) was discovered in 1995.

- It is related to Hepatitis C virus, belongs to family Flaviviridae, under the genus *Pegivirus*
- HGV is transmitted by contaminated blood or blood products, or via sexual contact
- It replicates in the bone marrow and spleen; however, it is not associated with any known human disease so far.
- HIV co-infection: HGV commonly co-infects people infected with HIV (prevalence 35%); but surprisingly this dual infection is *protective against HIV* and patients survive longer.

MULTIPLE CHOICE QUESTIONS

HEPATITIS A VIRUS

1. **Hepatitis A is:** *(Recent Question 2014)*
   a. Enveloped RNA virus
   b. Nonenveloped RNA virus
   c. Enveloped DNA virus
   d. Nonenveloped DNA virus

2. **The correct statement about Hepatitis A is all except:**
   a. It is difficult to culture *(Recent MCQ 2013)*
   b. It belongs to enterovirus
   c. Vaccines are available
   d. Chronicity is the hallmark of the disease

3. **True about HAV:** *(PGI June 06)*
   a. Causes milder illness in children
   b. 3% incidence of carrier
   c. Sexual route—common
   d. 10% chance of Ca liver
   e. Vertical transmission—never seen

4. **Hepatitis A virus can be inactivated by:**
   a. Boiling over at 120° C over 1 min *(DNB Dec 2009)*
   b. Ether
   c. Chloroform
   d. Iodine

5. **Cultivable (in vitro) hepatitis virus is:**
   a. Hepatitis A *(AIIMS May 2007)*
   b. Hepatitis B
   c. Hepatitis C
   d. Hepatitis D

6. **HAV shedding in feces:** *(UP 04)*
   a. 1 week before the symptoms appear
   b. 2 weeks before the symptoms appear
   c. 2 weeks before the symptoms appear to 2 weeks later
   d. 1 week before the symptoms appear to 1 week later

HEPATITIS B VIRUS

7. **A patient is positive for both HbsAg and HbeAg. It indicates which statement?** *(NIMHANS 2016)*
   a. He is infected in the past and a chronic carrier
   b. He is previously vaccinated
   c. He is infectious and is in active phase
   d. He has HEV infection

8. **Reverse transcriptase is a RNA dependent DNA polymerase. Which of these viruses has it?**
   a. Hepatitis A virus *(AIIMS May 2015)*
   b. Hepatitis B virus
   c. Hepatitis E virus
   d. Hepatitis C virus

9. **Long history was given. Then the question was which has maximum risk of transmission by needle prick?**
   a. HIV *(JIPMER Nov 2015)*
   b. Hepatitis A
   c. Hepatitis B
   d. Hepatitis C

10. **DNA of HBV is:** *Recent Question 2014*
    a. Single stranded
    b. Double stranded
    c. Partially single stranded
    d. Partially double stranded

11. **Which of the following hepatitis viruses is a DNA virus?** *(PGI Dec 2004, AIIMS May 2003)*
    a. Hepatitis C virus
    b. Hepatitis B virus
    c. Delta agent
    d. Hepatitis E virus

12. **Which of the following hepatitis virus have significant perinatal transmission?** *(AI 2003)*
    a. HEV b. HCV
    c. HBV d. HAV

13. **HBV present in India is:** *(AI 2002)*
    a. Adw b. Ayw
    c. Adr d. Ayr

14. **Reverse transcriptase of HBV is coded on following:** *(AI 2000)*
    a. C gene
    b. S gene
    c. P gene
    d. X gene

LABORATORY DIAGNOSIS OF HBV

15. **In acute hepatitis B, all are raised except:** *(Recent Question 2015)*
    a. HBsAg
    b. IgM anti-HBc
    c. Total anti-HBc
    d. Anti-HBsAb

16. **The serological test of a patient shows HBsAg negative, IgM anti HBc antibody negative, HBV DNA not detectable, anti-Hbc IgG antibody positive and anti HBs antibody positive. What is the most probable diagnosis?** *(AIIMS Nov 2015)*
    a. Acute hepatitis window period
    b. Chronic hepatitis in active stage
    c. Chronic hepatitis with precore mutant
    d. Recovery from previous infection of hepatitis B

17. Nurse is presented with HBsAg and HBeAg. What does it indicate? *(AIIMS Nov 2014, MHPG 2014)*
a. Acute infectious hepatitis
b. Infection with HBV and HEV
c. Acute noninfectious hepatitis
d. Chronic hepatitis B infection

18. Marker for diagnosing acute hepatitis B in 2–5 weeks: *(AIIMS Nov 2014)*
a. IgM Anti HBc
b. HBsAg
c. Anti HCV
d. HBeAg

19. Serological testing of patient shows HBsAg, IgM anti-HBc and HBeAg positive. The patient has: *(NEET Pattern Based, AIIMS Nov 01)*
a. Chronic hepatitis B with low infectivity
b. Acute hepatitis B with high infectivity
c. Chronic hepatitis with high infectivity
d. Acute on chronic hepatitis

20. First antibody to appear in hepatitis: *(NEET Pattern Based)*
a. IgM anti-HBe
b. IgG-anti-HBe
c. IgM anti-HBc
d. IgM anti-HBs

21. Infection of HBsAg is best/commonly diagnosed by: *(NEET Pattern Based)*
a. HBeAg
b. HBV DNA
c. HbsAg
d. Anti HBsAg

22. Finding of acute hepatitis B are: *(PGI June 2011, AIIMS Nov 2014, AIIMS Nov 2001)*
a. HbsAg
b. Anti-Hbe
c. HBV DNA
d. IgM anti-HBc
e. IgG anti-HBc

23. A 30-year-old patient presented with history of jaundice for 10 days. His liver function tests showed bilirubin of 10 mg/dl, SGOT/SGPT – 1100/1450, serum alkaline phosphatase – 240 IU. He was positive for Hbs Ag. What should be the confirmatory test to establish acute hepatitis B infection? *(AIIMS 2006, AIIMS Nov 2001)*
a. IgM anti-HBc antibody
b. HbeAg
c. HBV DNA by PCR
d. IgG Anti-HBc antibody

24. Hepatitis B vaccination is given to a patient. His serum will reveal: *(AIIMS May 2002)*
a. HBsAg
b. Anti-HBsAg
c. IgM Anti-HBcAg and HBsAg
d. IgM and IgG Anti-HbcAg

25. Epidemiological marker in HBV infection: *(UP 08, Karnataka 2009)*
a. HBsAg
b. HBeAg
c. Anti HBe
d. Anti HBs

26. The precore mutants in Hepatitis B are characterized by notable absence of: *(MHPG 2014)*
a. HBV DNA
b. HBeAg
c. HBcAg
d. Anti HBeAg

PROPHYLAXIS AND TREATMENT

27. What should be given to the newborn borne to a HBsAg +ve mother to prevent neonatal infection? *(AI 2012, AIIMS Nov 2005)*
a. Hepatitis B vaccine and Hepatitis B immunoglobulins within 12 hrs of delivery
b. Immunoglobulins
c. Hepatitis B vaccine only

28. Best describing hepatitis B vaccine: *(MHPG 2015, PGI June 2011)*
a. Recombinant DNA vaccine
b. Edible vaccine
c. Nuclear transformation
d. RNA vaccine

29. A blood donor is not considered for safe transfusion, if he has: *(AI 2000)*
a. Anti HBsAg +ve
b. Anti HBsAg and HBc Ag (+) ve
c. HBsAg +ve and IgM anti HBc +ve
d. Anti HBc +ve

HEPATITIS C VIRUS

30. Which hepatitis is shows maximum chronicity? *(West Bengal 2016, Recent Question 2014)*
a. Hepatitis A
b. Hepatitis B
c. Hepatitis D
d. Hepatitis G

31. Which of the following statements about Hepatitis 'C' is true? *(AI 2009)*
a. DNA virus
b. Most common indication for liver transplant
c. Does not cause liver cancer
d. Does not cause coinfection with hepatitis B

32. HCV is: *(PGI June 2008, Dec 2005)*
a. Enveloped RNA
b. Nonenveloped RNA
c. Nonenveloped positive strand RNA
d. Enveloped negative strand RNA

33. Maximum Hepatitis C virus transmission to fetus in pregnancy depends on: *(PGI 2000)*
a. Duratation of illness
b. Time of infection
c. Route of delivery
d. HIV infection
e. High level of HCV RNA

34. Chronic liver disease is caused by: *(AI 2000)*
a. Hepatitis B
b. Hepatitis A
c. Hepatitis C
d. Hepatitis E

35. HCV is associated with: *(AI 2000)*
a. Anti LKM-1 antibody
b. Scleroderma
c. SLE
d. Polyarteritis nodosa

36. According to drug and cosmetic act which is not mandatory for screening of donated blood:
a. HIV *(PGI May 2013)*
b. Hepatitis B
c. Hepatitis C
d. West Nile virus
e. CMV

HEPATITIS D VIRUS

37. Viruses that is dependent on other virus for survival:
a. EBV *(Recent Question 2014, AI 2001)*
b. Hepatitis C
c. Hepatitis D
d. Herpes

38. HBV and HDV false is: *(NEET Pattern Based)*
a. Both can infect simultaneously
b. HDV causes more serious infection d/t superinfection
c. HDV cannot infect in absence of HBV
d. HDV is a DNA virus

39. The Hepatitis D virus is a defective virus that requires its helper to provide: *(MHPG 2014)*
a. An envelope protein
b. Transcriptase to transcribe mRNA
c. Replicase for its RNA
d. Reverse transcriptase

HEPATITIS E VIRUS

40. Which is not transmitted by oral route? *(Kerala 2016)*
a. HAV
b. HEV
c. HAV and HEV
d. HDV

41. With which of the following of viral hepatitis infection in pregnancy, the maternal mortality is highest: *(AIIMS 2006, AI-2004, 2001, 2000, MHPG 2014)*
a. Hepatitis A
b. Hepatitis B
c. Hepatitis C
d. Hepatitis E

42. Which of the following is Calcivirus?
a. Hepatitis E *(MHPG 2015, AIIMS Nov 2001)*
b. Hepatitis B
c. Hepatitis C
d. Hepatitis A

EXPLANATIONS

HEPATITIS A VIRUS

1. **Ans. (b) (Nonenveloped...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 528, Ananthanarayan 9/e p541-2
   Hepatitis A is nonenveloped and RNA virus, belong to Family Picornaviridae, Enterovirus 72.
2. **Ans. (d) (Chronicity...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p528-29
   - HAV belongs to enterovirus-72, presents only in acute stage (there is no chronic stage), culture though difficult but can be performed.
3. **Ans. (a) (Causes...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p528-29, Park 22/e p191-92, 23/e p210-20
   HAV:
   - Age: Children are more affected, excrete virus in feces for longer time but subclinical. Adults are more icteric than children with higher mortality rate
   - Mode of transmission: Feco-oral (MC), Rare- sexual and parenteral routes
   - No carrier and No oncogenicity potential (Zero chance of Ca liver).
4. **Ans. (a) (Boiling...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p528-29, Park 22/e p191-92, 23/e p210-20
   - Hepatitis A virus can be inactivated by: ultra-violet rays, boiling for 5 min, autoclaving and formalin
   - It can resist chlorine, heating 60 °C for 1 hr.
5. **Ans. (a) (Hepatitis A)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p528-29
   - Hepatitis A virus can be grown in some human and simian cell cultures
   - It is the only human hepatitis virus which can be cultivated in vitro.
6. **Ans. (c) (2 week...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p528-29, Park 22/e p191, 23/e p210-20
   - *Period of infectivity of HAV: 2 weeks before to 1 week after appearance of jaundice (however, viral excretion in feces may be -2 to +2 weeks of jaundice).*

HEPATITIS B VIRUS

7. **Ans (c) (He is infectious and is in active phase)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p534
   HBeAg is marker of high infectivity and active infection.
8. **Ans. (b) (Hepatitis B virus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p531
   The polymerase gene of Hepatitis B virus has three enzymatic activities: (i) DNA polymerase, (ii) Reverse transcriptase activity, (iii) RNAse
9. **Ans. (c) (Hepatitis B)** Ref: ApurbaSastry's Essentials of Medical Microbiology 1/e p 532
   Following needle prick injury the risk of transmission of Hepatitis B is 30%, Hepatitis C is 3% and HIV is 0.3%.
10. **Ans. (d) (Partially...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 530, Ananthanarayan 9/e p543
    Hepatitis B has a partially/incomplete double stranded DNA.
11. **Ans. (b) (Hepatitis B virus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p527, Ananthanarayan 9/e p543
    - Among Hepatitis viruses only Hepatitis B virus is a DNA virus.
12. **Ans. (c) (HBV)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p532, Harrison 18/e p2547, 19/e p2004-2022
    - Perinatal transmission occurs primarily in infants born to HBsAg carrier mothers or mothers with acute hepatitis B during the third trimester of pregnancy or during the early postpartum period.
    - 10% of infections may be acquired in utero, at the time of delivery.
    - ***90% of HBeAg-positive mothers transmit HBV infection to their offspring***
    - Acute infection in the neonate is clinically asymptomatic, but the child is very likely to become an HBsAg carrier
    - ***The chances of sexual and perinatal transmission of Hepatitis C virus ~ 5%.***

13. **Ans. (c) (Adr)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p531, Ananthanarayan 9/e p543
    - The HBsAg glycoprotein contains a *group specific antigen* termed ***a*** and *type specific antigens* termed ***d* or *y* and *w* or *r***
    - Combination of these antigens results in 4 major subtypes of HBV (*adw*, *adr*, *ayw* and *ayr*)
    - These subtypes show a distinct geographic distribution
    - Subtype ayw is common in ***northern India***
    - Subtype *adr* is common in ***south and east India***
    - Prevalence of HBV is higher in South India than North India
    - ***Hence overall adr is commonest in India.***

14. **Ans. (c) (P gene)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p531
    - Gene P-codes for DNA polymerase that has reverse transcriptase activity
    - RT activity of polymerase can repair the gap and makes HBV genome to completely double stranded.

LABORATORY DIAGNOSIS OF HBV

15. **Ans. (d) (Anti-HBsAb)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p532

16. **Ans. (d) (Recovery…)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p534
    Only Anti-Hbc IgG antibody and Anti HBs antibody are positive, rest all markers are negative. Hence this is a probably a case of recovery.

17. **Ans. (a) (Acute infectious hepatitis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p534-35
    Refer chapter review for explanation.

18. **Ans. (a) (IgM Anti HBc)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p534-35
    Refer chapter review for explanation.

19. **Ans. (b) (Acute hepatitis B with high infectivity)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p534-35
    - Markers elevated in Acute hepatitis B with high infectivity- HBsAg, IgM anti-HBc and HBeAg

20. **Ans. (c) (IgM anti-HBc)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p534-35, Ananthanarayan 9/e p548
    - IgM anti-HBc is the first antibody to appear in hepatitis B infection and indicates acute stage.

21. **Ans. (c) (HbsAg)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p534-35, Ananthanarayan 9/e p548
    - HBsAg is the first marker to appear in hepatitis B infection and indicates infection with HBV.

22. **Ans. (a) (c) (d) (HbsAg, HBV DNA, IgM anti-HBc)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p534-35, Ananthanarayan 9/e p548, Park 23/e p210-20, 22/e p194-95
    - ***HBsAg* :** Appears in blood during incubation period, acute, chronic and carrier state
    - ***HBeAg and HBV DNA:*** They can be present in acute or chronic stage or in carriers. The presence of HBeAg/HBV DNA indicates a high infectivity and transmissibility and activeness of disease
    - ***HBcAb* :** *Demonstration of antibodies (HBcAb) against hepatitis B core antigen can differentiate acute and chronic infection.*

23. **Ans. (a) (IgM anti–HBc antibody)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p534-35, Ananthanarayan 9/e p548, 8/e p544
    Refer text for detail

24. **Ans. (b) (Anti-HBs Ag)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p534-35, Ananthanarayan 9/e p548
    - As HBV vaccine contains HBsAg, following vaccination only titers of anti-HBsAg will be raised
    - All other serological markers will be negative.

25. **Ans. (a) (HBsAg)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p534-35, Park 22/e p194, 23/e p210-20
    - **Epidemiological marker in HBV infection: HbsAg and Anti- HBc IgG**

26. **Ans. (b) (HBeAg)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p534-35, Ananthanarayan 9/e p546-8
    - The precore mutants in Hepatitis B: Unable to form HBeAg
    - Escape mutants in Hepatitis B: Unable to form HBsAg.

PROPHYLAXIS AND TREATMENT

27. Ans. (a) (Hepatitis B vaccine + immunoglobulin within 12 hours of delivery)
Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p535-36, Ananthanarayan 9/e p548, 8/e p544

- **For babies born to HBV carrier mothers**
- *A single injection of 0.5 ml of HBIG given IM immediately after birth (passive immunization) followed by the full course of vaccine (active immunization) at a different anatomical site, the first dose being given within 12 hours of birth.*

28. Ans. (a) (Recombinant DNA vaccine) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p535-36

- *Hepatitis B vaccine is a recombinant DNA vaccine composed of Hepatitis B surface antigen (HBsAg) prepared in Baker's yeast.*

29. Ans. (c) (HBsAg +ve and IgM anti HBc +ve) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p535-36

- ***Option a:*** Blood donor with Anti-HBs Ag indicates recovery/resistance to HBV infection, hence their blood is safe for donation
- ***Option b:*** Wrong statement as HBcAg is never found in blood, it can be demonstrated only in infected hepatocyte by immunofluorescence
- ***Option c: Presence of HBsAg +ve and IgM anti HBc +ve indicates patient is suffering from acute hepatitis, hence not suitable for blood donation***
- ***Option d:*** Anti HBc +ve indicates remote infection/resistance to infection and infectivity is nil.

HEPATITIS C VIRUS

30. Ans. (b) (Hepatitis B) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p533
Chronicity is seen maximum (50–80%) with HCV infection, followed by HBV infection. As HCV is not there in option, HBV is correct option.

31. Ans. (b) (Most common...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p536-38, Harrison 18/e p2545
'Hepatitis C accounts for 40% of chronic liver disease, is the most frequent indication for liver transplantation'
About other options:

- HCV is RNA virus, it causes liver cancer
- It may cause co-infection with HBV.

32. Ans. (a) (Enveloped...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p536-38, Ananthanarayan 9/e p549

- ***Belongs to Flaviviridae***
- ***Single stranded RNA, enveloped virus***

33. Ans. (e) (High level of HCV RNA)
Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p536-38, CMR review article -Volume 61, Number 10

- *The maternal viral titer appears to be an important determinant of vertical HCV transmission.*
- *In general, the* ***higher the concentration of serum HCV RNA,*** *the more likely the chance of vertical transmission.*
- ***Other risk factors are***
  - HCV and HIV co-infection
  - Prolonged rupture of the membranes
  - Female infants are twice as likely to be infected as compared with males.
- The rates of vertical transmission were similar for vaginal delivery and cesarean section.

34. Ans. (c) (Hepatitis C) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p536-38

- ***50–80% of patient infected with HCV develop: Chronic infection***
- 1–10% of patient infected with HBV develop: Chronic infection
- Hence among hepatitis viruses, chronicity is highest with HCV infection.

35. Ans. (a) (Anti LKM-1 antibody) Ref: Hematology 2005 - The American Society of Hematology

- *Several studies have observed a relationship between hepatitis C virus infection and anti-liver/kidney microsome-1 (anti-LKM-1) positive chronic hepatitis.*
- *It has been suggested that hepatitis C may induce an autoimmune phenomenon that leads to the development of a specific type (type II anti-LKM-1 positive) autoimmune chronic hepatitis*- Journal- J Viral Hepat. 1995; 2(4):175-9.

36. Ans. (d) (e) (West Nile virus, CMV) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p536-38, Drugs and Cosmetics Act & Rule, 1945

- According to the Drugs and Cosmetics Act, & Rule (1945), the blood banks who intend to supply blood units/components shall perform the following mandatory tests for screening of donated blood:
  - Blood grouping
  - Antibody testing
  - Hemoglobin content
  - Hepatitis B (HBsAg detection)
  - Hepatitis C (Anti HCV antibody detection)
  - Syphilis serology (VDRL)
  - HIV I and II antibody detection
  - Malaria parasite detection.

HEPATITIS D VIRUS

37. Ans. (c) (Hepatitis D) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p538, Ananthanarayan 9/e p549
Hepatitis D virus is a defective virus; it is dependent on the helper function of Hepatitis B virus.

38. Ans. (d) (HDV...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p538-39, Ananthanarayan 9/e p549

- HDV contains SS-RNA
- Both HBV and HVD can infect simultaneously to hepatocyte: Called as coinfection
- Superinfection is most severe form of HDV infection.
- HDV always depends on HBV and it cannot infect in absence of HBV.

39. Ans. (a) (An envelope...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p538-39, Ananthanarayan 9/e p549
The envelop protein part of Hepatitis D virus is derived from HBsAg of Hepatitis B Virus.

HEPATITIS E VIRUS

40. Ans. (b) (HEV) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p540, Park 22/e p198, 23/e p210-20

- HEV is transmitted by only feco-oral route
- HAV, though - mainly transmitted by only feco-oral route, but can rarely transmitted by vertical and sexual routes.

41. Ans. (d) (Hepatitis E) Ref: : Apurba Sastry's Essentials of Medical Microbiology 1/e p540, Ananthanarayan 9/e p550

- ***HEV causes a serious infection in pregnant women***
- It causes fulminant disease in pregnant women especially in last trimester of pregnancy and ***has a high fatality rate of 15–20%.***
- ***Hepatic encephalopathy*** and disseminated intravascular coagulation are the important causes of death.
- The rate of fulminant hepatic failure in infected pregnant women is very high.

42. Ans. (a) (Hepatitis E) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p540, Ananthanarayan 9/e p550

- HEV is currently classified in the family Calciviridae
- It resembles calciviruses such as Norwalk virus.

CHAPTER

4.6

HIV and Other Retroviruses

Retroviruses possess a unique enzyme '*reverse transcriptase*' that directs the synthesis of DNA from the viral RNA after infection into a host cell. Only two genera are pathogenic to humans; HIV and HTLV.

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Morphology

Envelope: HIV and other lentiviruses are spherical and 80–110 nm in size, possess an **envelope**; made-up of a lipid layer in which two proteins are embedded:

- Glycoprotein 120 (gp 120) are projected as knob like spikes on the surface
- Glycoprotein 41 (gp 41): They form anchoring transmembrane pedicles.

Nucleocapsid: Capsid is icosahedral in symmetry, made-up of core protein. Inside, there is an inner core which encloses:

- RNA: Two identical copies of single-stranded positive sense linear RNA
- Viral enzymes, such as reverse transcriptase, integrase and proteases that are closely associated with HIV RNA.

HIV Genes and Antigens

HIV contains 3 structural genes: *gag, pol,* and *env* and 6 nonstructural or regulatory genes.

Structural genes of HIV:
- *gag gene* (p55)
  - p18 (codes for matrix or shell antigen)
  - p24 and p15- both constitute the core antigens
- *pol gene:* codes for viral enzymes such as RT, protease and integrase
- *env gene*: Codes for the envelope glycoprotein (gp120 and gp41)

Structural Genes

Structural genes code for various components of the virus.

- ***Gag*** gene: It is expressed as a precursor protein, p55 which is cleaved into 3 proteins: p18 (constitutes the matrix or shell antigen); p24 and p15 both constitute the core antigens
- ***Pol*** gene codes for viral enzymes, such as reverse transcriptase, protease and integrase. It is expressed as a precursor protein, which is cleaved into proteins p31, p51 and p66.
- ***Env* gene** codes for the envelope glycoprotein (gp 160), which is cleaved into gp 120 and gp41.

Non-Structural Genes

Non-structural genes regulate viral replication and are important in disease pathogenesis *in vivo*.

- ***Tat*** is a transcriptional transactivator gene
- ***Nef*** (negative factor gene), ***Rev*** (regulator of virus gene), ***Vif*** (viral infectivity factor gene)
- ***Vpu*** gene, ***Vpr*** gene, ***Vpx*** gene and **LTR** (long terminal repeat) sequences.

Antigenic Variation and Diversity of HIV

HIV shows extensive antigenic diversity because of undergoing high rates of mutation especially *env* gene and due to the error prone nature of reverse transcriptase enzyme.

HIV Serotyping

Based on sequence differences in *env* gene, HIV comprises of two serotypes HIV-1 and 2.

HIV–1 is divided into three distinct groups (M, N and O). Recently, *group P* has been identified.

- 'M'is the dominant group worldwide. It comprises of ten subtypes or 'clades' (A-J).

- There are also 'circulating recombinant forms' or CRFs derived from recombination between different subtypes. For example, CRF01_AE is a recombination between subtypes A and E.
- The same infected host may have a group of closely related viral subtypes and/or CRF at a given time which are collectively called *quasispecies*.
- HIV-1 subtypes or clades do not vary in pathogenesis or biology; but they differ in geographical distribution and transmission.
- **Geographical distribution:**
  - Subtype A is common in West Africa
  - Subtype B is predominant in Europe, America, Japan, and Australia
  - Subtype C is the MC form worldwide (47%). It is also the dominant form in South East Africa, India, and China
  - In Cameroon (West Africa), all known HIV virus groups and subtypes are found. It is the place of origin of the virus.
- **Transmission:** Asian and African subtypes (C and E) are more readily transmitted heterosexually; whereas American strains (subtype B) preferentially spread through blood and homosexual contact.

HIV-2 comprises of eight groups (A–H); they are confined to Africa and some time in other places including India. Group A is the MC form.

Genotype distribution:
- *Subtype A* is common in West Africa
- *Subtype B* – in Europe, America, Japan, and Australia
- *Subtype C* is the MC form worldwide (47%). It is also the dominant form in South East Africa, India, and China
- *In Cameroon* (West Africa), all HIV virus groups and subtypes are found.

Pathogenesis

Mode of Transmission

- **Most common mode of transmission in World:** Sexual (75%) (vaginal, 60% > anal, 15%) > Parent to child (10%) > Injection drug abuse (10%) > Blood transfusion (5%) > Needle-stick exposure (0.1%)
- **Most common mode of transmission in India:** Heterosexual (87.4%) > Parent to child (5.4%) > Injection drug abuse (1.6%) > Homosexual (1.5%) > Blood transfusion and Needlestick exposure (together 1%).
- **Risk of transmission:** Blood transfusion (90-95%) > Parent to child (20–40%) > Injection drug abuse (0.5–1.0%) > Needlestick exposure (0.3%) > Sexual intercourse (Anal 0.065–0.5% > vaginal 0.05–0.1% > oral 0.005–0.1%).

Most common mode of transmission in World:
Sexual (75%) (vaginal, 60% > anal,15%) > Parent to child (10%) > Injection drug abuse(10%) > Blood transfusion (5%) > Needle stick exposure (0.1%)

Points to be noted:

- Transmission may occur at anytime during pregnancy and breastfeeding but the risk is maximum during delivery.
- Risk is maximum if mother is recently infected or has already developed AIDS
- There is no evidence of HIV transmission by casual contact or kissing or insect bite.
- **Viral load** is maximum in blood, genital secretions, and CSF; variable in breast milk and saliva; zero to minimal in other body fluids or urine.
- Saliva may contain inhibitory substances like fibronectin & glycoproteins, which prevent transmission of the virus.

Most common mode of transmission in India:
Heterosexual (87.4%) > Parent to child (5.4%) > Injection drug abuse (1.6%) >Homosexual (1.5%) > Blood transfusion and Needle stick exposure (together 1%).

Receptor Attachment and Fusion

- **Main receptor:** gp120 of HIV binds to the CD4 receptor on host cell surface. CD4 molecules are mainly expressed on helper T-cells; but also on the surface of various other cells like monocytes, macrophages, langerhans cells, astrocytes, keratinocytes and glial cells.
- **A second coreceptor** is necessary for fusion of HIV by binding to gp120 and to gain entry into the host cell, e.g.
  - CXCR4 molecules present on T-lymphocytes
  - CCR5 molecules present on cells of macrophage lineage
- **DC-SIGN**, a dendritic cell-specific lectin receptor can also bind to HIV-1 but does not mediate cell entry. Rather, it may facilitate transport of HIV by dendritic cells to lymphoid organs where HIV replicates further in T-cells.

Risk of transmission:
Blood transfusion (90–95%) > Parent to child(20–40%) > Injection drug abuse (0.5–1.0%) > Needle stick exposure (0.3%) > Sexual intercourse (Anal 0.065–0.5% > vaginal 0.05–0.1% > oral 0.005–0.1%)

Receptor interaction:
- *Main receptor*-gp120 of HIV binds to the CD4 receptor on host cell surface
- *Second co-receptor:*
  - CXCR4 molecules present on T lymphocytes
  - CCR5 molecules present on cells of macrophage lineage

Mutation in CCR5 (delta 32 mutation) results in blockade of HIV entry into the cells. It is observed among some lucky Europeans who are either:
- *Completely resistant to HIV infection:* If they are homozygous for delta 32 mutation genes (seen in 1% of Northern Europeans, particularly Swedes) or
- *Susceptible but progress of AIDS is delayed*: If they are heterozygous, seen in 10–15% of Europeans).

Replication

- After fusion, **HIV undergoes penetration & uncoating** → HIV RNA is converted to HIV DNA by RT enzyme→ **Preintegration complex** is formed, comprises of linear dsDNA, gag matrix protein, accessory Vpr protein and viral integrase which is transported into the host cell nucleus.
- **Integration:** The viral dsDNA gets integrated into the host cell chromosome; mediated by viral integrase. The integrated virus is called *provirus*.
- **Latency:** In the integrated state, HIV establishes a latent infection for variable period. However, HIV is different from other latent viruses as it is able to replicate even in latent state and is infectious to the neighboring cells.

Mutation in CCR5 (delta 32 mutation):
Results in blockade of HIV entry into the cells.
Observed among some lucky Europeans who are:
- Completely resistant to HIV infection (If homozygous) or
- Show delayed Susceptible to progress to AIDS (If heterozygous)

Immunopathogenesis

The natural course of the disease passes through the following stages:
- **Acute HIV Disease or Acute Retroviral Syndrome**
- **Asymptomatic Stage**
- **Persistent Generalized Lymphadenopathy (PGL)**
- **Symptomatic HIV Infection (or AIDS related complex, ARC)**
- **AIDS**

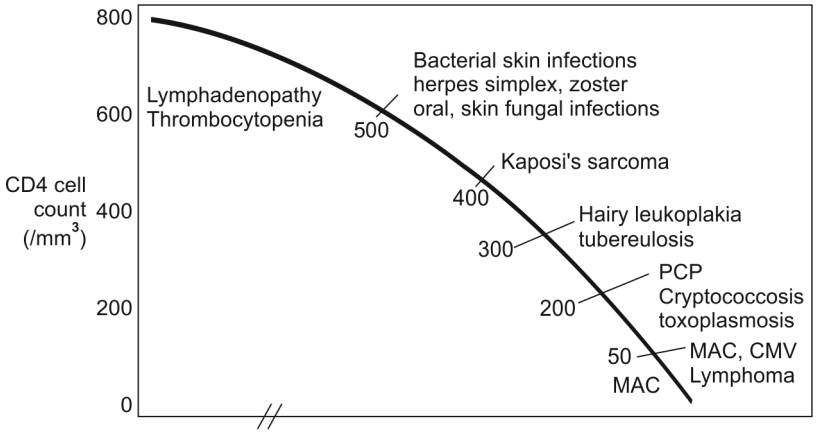


Fig. 4.6.1: Opportunistic infections associated with HIV infection and correlation with CD4 T-cell counts

Persistent Generalized lymphadenopathy (PGL)
It is defined as enlarged lymph nodes of > 1 cm size in two or more non-contiguous sites that persist for at least 3 months. It is seen in 25–30% of infected people.

Clinical Diagnosis of HIV/AIDS

Classification systems are useful for tracking and monitoring the HIV epidemic and for providing clinicians and patients with important information about HIV disease stage and clinical management. Two such systems are currently in use:

1. **CDC classification system** (revised 1993): This system classifies HIV infection into nine stages based on associated clinical conditions and CD4 T-cell count of the patient.
2. **WHO clinical staging of HIV/AIDS for adults** (revised 2007) is based only on the clinical conditions. It is useful for resource poor countries like India, where facilities for the CD4 T-cell count are not available widely.

Clinical Diagnosis of HIV/AIDS:
- CDC classification system: Nine stages based on clinical conditions and CD4 T count
- WHO clinical staging: Four sagens, only based on clinical conditions (Useful in India)

Table 4.6.1: WHO clinical staging of HIV/AIDS for adults (Revised, 2007)

Clinical Stage 1	Clinical Stage 4
Asymptomatic HIV infection	• **HIV Wasting syndrome** (*Slim disease*): Characterized by profound weight loss (> 10%), chronic diarrhea (> 1 month), prolonged unexplained fever (1 month) • **Bacterial opportunistic infections**: ○ Recurrent severe bacterial infections ○ Extrapulmonary tuberculosis ○ Disseminated nontubercular mycobacterial infection ○ Recurrent septicemia (including nontyphoidal salmonellosis) • **Viral opportunistic infections:** ○ Chronic HSV infection ○ Progressive multifocal leukoencephalopathy ○ CMV (retinitis, or other organ infection excluding liver, spleen , and lymph node) • **Fungal opportunistic infections**: ○ *Pneumocystis jirovecii* pneumonia ○ Esophageal candidiasis ○ Extrapulmonary cryptococcosis (meningitis) ○ Disseminated mycoses (histoplasmosis and coccidioidomycoses) • **Parasitic opportunistic infections:** ○ *Toxoplasma* encephalitis ○ Chronic intestinal isosporiasis (> 1 month) ○ Atypical disseminated leishmaniasis ○ Chronic intestinal cryptosporidiosis (> 1 month) • **Neoplasia:** ○ Kaposi's sarcoma ○ Invasive cervical cancer, ○ Lymphoma (cerebral, B-cell and non-Hodgkin) • **Other conditions (direct HIV induced):** ○ HIV encephalopathy ○ Symptomatic HIV associated nephropathy or cardiomyopathy
Persistent generalized lymphadenopathy	
Clinical Stage 2	
Unexplained moderate weight loss (< 10%)	
Recurrent respiratory tract infection (sinusitis, tonsillitis, otitis media, pharyngitis)	
Herpes zoster	
Angular cheilitis	
Recurrent oral ulcers	
Papular pruritic eruptions	
Seborrheic dermatitis	
Fungal nail infection	
Clinical Stage 3	
Unexplained severe weight loss (> 10%)	
Unexplained chronic diarrhea > 1 month	
Unexplained persistent fever 1 month	
Oral candidiasis	
Oral hairy leukoplakia	
Pulmonary tuberculosis	
Severe bacterial infection	
Acute necrotizing ulcerative stomatitis, gingivitis, and periodontitis	
Unexplained anemia	

Epidemiology of HIV/AIDS

Global Situation of HIV/AIDS

- **Prevalence:** By 2013, about 35 million people were living with HIV with a *global prevalence of 0.8%* in adults.
- **Sub-Saharan Africa** remains most severely affected, with nearly one in every 20 adults living with HIV and accounting for nearly 71% of the people living with HIV worldwide.

HIV/AIDS Situation in India

- By the end of 2015, the adult *HIV prevalence in India* was reported as ***0.26%.***
- Number of PLHA (people living with HIV/AIDS) were over 21.1 lakh.
- *Andhra Pradesh* (undivided) was the worst affected state followed by Maharashtra and Karnataka in terms of PLHA.
- However, as far as prevalence (number of cases per 100 population) is concerned, Northeast states, such as Nagaland, Mizoram and Manipur are worst affected.

Laboratory Diagnosis

Table 4.6.2: Tests for detecting HIV infection

Specific tests for HIV infection	Nonspecific/Immunological methods
Screening tests (ERS) (IgG antibody detection, high sensitivity): • ***E***LISA (takes 2–3 hours) • ***R***apid/ ***S***imple test (takes < 30 minutes)	Low CD4 T-cell count

Contd...

Neoplasia in HIV:
- Kaposi's sarcoma
- Invasive cervical cancer,
- Lymphoma (cerebral, B cell and non-Hodgkin)

HIV prevalence:
- *Global prevalence* of 0.8% in adults
- *Prevalence in India:* **0.26%**
- *Number of PLHA in India:* 21.17 lakh

Worst affected State in India:
- *PLHA and to total absolute no. of HIV cases:* Andhra Pradesh followed by Maharashtra and Karnataka
- *Prevalence highest in:* Northeast states (Nagaland, oram and Manipur)

Contd...

Supplemental tests (antibody detection, high specificity): • Western blot assay • Immunofluorescence assay • Radioimmuno-precipitation assay (RIPA) • Line immunoassay (LIA)	Hypergammaglobulinemia due to polyclonal B cell activation leading to formation of abnormal Ig, such as: Neopterin β2-macroglobulin
Confirmatory tests	Altered CD4 : CD8 T-cell ratio
• p24 antigen detection	
• Viral culture by Cocultivation technique	
• HIV RNA ('gold standard' for confirmation of HIV diagnosis) ○ Reverse transcriptase PCR (RT-PCR) ○ Branched DNA assay ○ NASBA (Nucleic acid sequence based amplification) ○ Real time RT-PCR for estimating viral load • HIV DNA detection: Useful for diagnosis of pediatric HIV	

Confirmatory tests:
- p24 antigen detection
- Viral culture: by Co-cultivation technique
- Reverse transcriptase PCR (RT-PCR): for HIV RNA detection (gold standard)
- Real time RT-PCR: for estimating viral load
- HIV DNA detection: Useful for diagnosis of paediatric HIV

NACO Strategy for HIV Diagnosis

For the resource poor countries, it is impracticable to confirm the result of HIV screening tests by PCR or western blot as these assays are expensive and available only at limited centers.

NACO (National AIDS Control Organisation, India) has formulated a strategic plan for HIV diagnosis.

- Depending on the situation/condition, for which the test is done, the positive result of the first screening test should be either considered as such or confirmed by another one or two screening tests.
- The first screening test should be highly sensitive, whereas the second and third screening tests should have high specificity.
- The three screening tests should use different principles or different antigens. The same kit should not be used again.
- Supplemental or confirmatory tests should be used only when the screening test(s) result equivocal/intermediate.

There are four NACO strategic plans/algorithms

1. **Strategy I:** t is done for blood donors in blood banks. Only one test is done.
2. **Strategy IIa:** It is done for seroprevalence or epidemiological purpose. Two tests are done.
3. **Strategy IIb:** Purpose: It is followed for the diagnosis of HIV/AIDS in symptomatic patients. Two tests are done.
4. **Strategy III:** It is done for the diagnosis of asymptomatic HIV patients, antenatal screening and screening of patients awaiting surgeries. Three tests are done.

NACO strategy:
- *Strategy I:* Done for blood transfusion and transplantation
- *Strategy IIa:* Done for seroprevalence or epidemiological purpose
- *Strategy IIb:* Done in symptomatic HIV patients
- *Strategy III:* Done for asymptomatic HIV patients

Prognosis/Monitoring of HIV

Various tools available for monitoring the response to antiretroviral therapy include:
- CD4 T-cell count: Most commonly used
- HIV RNA load: Most consistent and best tool at present
- p24 antigen detection
- Neopterin and β2 macroglobulin level

Note: Antibody levels are inconsistent during late stage due to immune collapse; hence not reliable for prognosis.

Diagnosis of Pediatric HIV

The routine screening methods (ELISA or rapid/simple tests) detect IgG antibodies.

- They cannot differentiate between baby's IgG or maternally transferred IgG, hence cannot be used for the diagnosis of pediatric HIV.
- As all maternal antibodies would disappear by 18 months; IgG assays can only be performed after 18 months of birth.

The recommended methods for diagnosis of pediatric HIV include:

- HIV DNA detection: Most recommended
- HIV RNA detection
- p24 antigen detection
- IgG ELISA: Only after 18 months of age.

Diagnosis of HIV in Window Period

Definition: Window period refers to the initial time interval between the exposure and appearance of detectable levels of antibodies in the serum.

- The antibodies appear in blood within 2–8 weeks after infection but usually become detectable after 3 to 12 weeks with the assays available presently. It can be as low as 22 days; when third generation antibody detection kits with high sensitivity are used.
- p24 antigen detection (30% sensitive): detects by 1–2 weeks (average 16th day)
- HIV RNA detection (PCR) is the best method, detects earliest by 12th day.

Prognosis/monitoring of HIV:
- *CD4 T cell count:* Most commonly used
- *HIV RNA load:* Most consistent and best
- *p24* antigen detection
- *Neopterin* and β2 *macroglobulin* level

Treatment: Antiretroviral Therapy (ART)

Indication to Start ART

NACO guidelines recommend initiation of ART based on CD4 T-cell count and WHO clinical staging.

- Clinical Stage I and II: Start ART if CD4 T-cell count < 500 cells/mm^3
- Clinical Stage III and IV: Start ART irrespective of CD4 T-cell count
- For HIV and TB coinfected patients: Start ART irrespective of CD4 T-cell count and type of tuberculosis (Start antitubercular drugs first, initiate ART after -2 months when TB drugs are well tolerated, if CD4 below 50, ART and ATT may be started together).
- HIV and HBV/HCV coinfection with evidence of severe chronic liver disease: Start ART irrespective of CD4 count
- HIV infected pregnant women - start ART irrespective of CD4 count
- Patients with HIV nephropathy
- Patients with HIV-Visceral Leishmaniasis co-infected --Start ART irrespective of CD4 count

Diagnosis of Pediatric HIV:
- HIV DNA detection-most recommended
- HIV RNA detection
- p24 antigen detection
- IgG ELISA-only after 18 months of age

Table 4.6.3: Antiretroviral drugs

NRTI (Nucleoside reverse transcriptase inhibitors)	NNRTI (Non-nucleoside reverse transcriptase inhibitors)	PI (Protease inhibitors)	Fusion inhibitors
Zidovudine Stavudine Lamivudine Didanosine Zalcitabine Abacavir	Nevirapine Efavirenz Delavirdine	Saquinavir Ritonavir Nelfinavir Amprenavir Indinavir Lopinavir Ritonavir Fosamprenavir Atazanavir Darunavir Tipranavir	Enfuvirtide Integrase inhibitors Raltegravir Dolutegravir Elvitegravir CCR5 receptor inhibitor Maraviroc
NtRTI (Nucleotide reverse transcriptase inhibitor)			
Tenofovir			

Diagnosis of HIV in window period:
It is about 3 weeks to 12 weeks
- *p24 Ag detection*: detects by 1–2 weeks (average 16th day)
- *HIV RNA detection* (best method): detects earliest by 12th day

Principles for Selecting the First-line Regimen

Highly active ART (HAART) is referred to as the combination of ***at least three*** antiretroviral (ARV) drugs to maximally suppress the HIV virus and stop the progression of HIV disease. Monotherapy with single drug is contraindicated.

Problems Pertaining to Use of ART

- Adverse side effects, High cost of ARTs, Limited therapeutic options
- Risk of development of drug resistance and dissemination of resistant virus
- **IRIS**: Immune reconstitution inflammatory syndrome (IRIS) occurs in some cases of AIDS during the recovery phase following the start of ART. As the viral load decreases, the immune system begins to recover, but then responds to a previously acquired opportunistic infection with an overwhelming inflammatory response that paradoxically makes the symptoms of infection worse.

Immune reconstitution inflammatory syndrome (IRIS):
- Occurs during the recovery phase following the start of ART and restoration of immune
- Bouncing back of previously acquired opportunistic infection with an overwhelming inflammatory response.

NACO Guidelines for Postexposure Prophylaxis (PEP)

TLE Regimen

- Single tablet containing Tenofovir (TDF) 300 mg plus Lamivudine (3TC) 300 mg plus Efavirenz (EFV) 600 mg once daily for 4 weeks.
- Ideally, therapy should be started within 2 hours and definitely within 72 hours of exposure.
- Indicated to HCW exposed to
  - Source positive for HIV (low risk/asymptomatic or high risk/symptomatic) and
  - Any kind of exposure (mild, moderate or severe exposure).
- Source unknown

HUMAN T-CELL LYMPHOTROPIC VIRUS (HTLV)

Two important members are HTLV-I & II. HTLV-I is only pathogenic, described below.

- **Transmission** by: (i) from mother to child via breast milk (most common); (ii) homosexual; (iii) infected blood
- **TargeT-cells:** It infects CD4 T-cells; but occasionally also infect CD8 T-cells, dendritic cells and B-cells.
- **Target receptor:** Viral gp binds to hosT-cell receptor ***GLUT1*** (Human glucose transporter protein-1)
- **Tax gene** of HTLV-I acts as a transactivator, and responsible for its *oncogenecity*
- **Distribution**-HTLV-I is endemic in certain parts of Japan (10% prevalence) and the Caribbean basin of Africa
- **Genotypes:** It has 7 genotypes; type-A is most common, others are found only in central Africa
- **Clinical manifestations:** HTLV-I is a potential human oncogenic virus:
  - Adult T-cell leukemia/lymphoma
  - Cutaneous T-cell lymphoma
  - Tropical spastic paraparesis
  - Autoimmune features, such as Inflammatory disease, uveitis and arthropathies.

Clinical manifestations of HTLV-I:
- Adult T cell leukemia/ lymphoma
- Cutaneous T-cell lymphoma
- Tropical spastic paraparesis
- Auto immune: Inflammatory disease, uveitis and arthropathies

MULTIPLE CHOICE QUESTIONS

TRANSMISSION AND PATHOGENICITY

1. Tropical spastic paraparesis is caused by: *(AIIMS May 2015)*
a. Epstein Barr Virus
b. Human T-cell Lymphotropic Virus (HTLV)
c. Human Immunodeficiency Virus
d. Hepatitis B virus

2. Which of the following is/are true about HIV-2: *(PGI May 2015)*
a. HIV-2 first detected in West Africa in 1986
b. Donated blood is only screened for HIV-1, not HIV 2
c. More virulent than HIV 1
d. More closely related to simian immunodeficiency virus (SIV) than HIV 1
e. Mode of transmission is like HIV 1

3. HIV infects which of the following cells? *(JIPMER Nov 2014, West Bengal 2016)*
a. NK cells
b. T-helper cells
c. T suppressor cells
d. Plasma cells

4. Resistance to HIV infection occurs due to mutation of: *(PGI Nov 2012)*
a. CCR 5
b. CXCR4
c. gp41
d. CD4
e. gp120

5. Causative agent of AIDS was discovered in: *(AIIMS May 2014)*
a. 1983
b. 1976
c. 1994
d. 1967

6. WHO, AIDS defining illness is/are: *(PGI Nov 2012)*
a. Herpes zoster
b. Oropharyngeal candidiasis
c. CMV retinitis
d. Cryptococcal meningitis
e. Primary brain lymphoma

7. Which is a nonstructural gene of HIV? *(APPG 2014)*
a. Gag
b. Env
c. Tat
d. Pol

8. In the absence of any intervention, the risk of HIV transmission from mother to baby in pregnancy, labor, delivery and breastfeeding in nonbreastfeeding population is: *(AIMS Nov 2013)*
a. 5–10%
b. 10–15%
c. 15–30%
d. 40–50%

9. Nef gene in HIV is for use: *(NEET Pattern Based)*
a. Enhancing the expression of genes
b. Enhancing viral replication
c. Decreasing viral replication
d. Maturation

10. What is p24? *(NEET Pattern Based)*
a. Envelop antigen in HIV
b. Genome of HIV
c. Core antigen in HIV
d. Shell antigen

11. HTLV or HIV contains an extra gene that is not there in standard retrovirus: *(NEET Pattern Based)*
a. Gag
b. Pol
c. Env
d. Tex

12. HIV infects most commonly: *(AI 2009, June 2000,DNB June 2012, DNB 2010, 2014)*
a. CD4 + helper cells
b. CD8 + cells
c. Macrophage
d. Neutrophils

13. HIV can: *(PGI June 2008)*
a. Cross blood brain barrier
b. RNA virus
c. Inhibited by 0.3 % H_2O_2
d. Thermostable

14. Latent phase of HIV: *(PGI Dec 2006)*
a. Viral replication
b. Sequestered in lymphoid tissue
c. Infective
d. Not infective
e. Rapid decline of CD4 count is common

15. What is the sequence which a retrovirus follows on entering a host cell? *(AI 2000)*
a. RNA-DNA-RNA
b. RNA-DNA
c. DNA-RNA
d. DNA-RNA-DNA

16. True about HIV: *(PGI 2000)*
a. Not transmitted through semen
b. More chances of transmission during LSCS than normal labor
c. More infectious than hepatitis B
d. Male to female transmitted > female to male

17. Which of the following is HIV structure? *(PGI 2002)*
a. Gag
b. Tat
c. P2500
d. Kinase
e. P24

18. State with maximum no. of AIDS cases: *(AIIMS Nov 2004)*
a. Delhi
b. Kerala
c. Bihar
d. AP

19. True about HIV: *(PGI June 2002)*
a. MTC 25%
b. Semen is more infectious than vaginal discharge
c. Infectious in window period
d. Southern Africa has 72% of total global cases
e. Children infection is rare

OPPORTUNISTIC INFECTION

20. The most common fungal eye infection in HIV infected patient: *(AIIMS Nov 2015)*
a. Cryptococcosis
b. Aspergillosis
c. Coccidioidomycosis
d. Candidiasis

21. Features of stage III HIV infection is/are:
a. Fever > 38.5 °C *(PGI May 2015)*
b. Oral hairy leukoplakia
c. Candidiasis
d. Diarrhoea of > 20 day duration
e. > 26% CD4 count in adults

22. A 25 years old male presents with 2 months history of loose stools and weight loss. Laboratory diagnostic tests are positive for HIV. Presence of which of the following disease is most likely in diagnosing HIV/AIDS: *(JIPMER Nov 2014)*
a. Lyme disease
b. Glandular fever like syndrome
c. Oropharyngeal candidiasis
d. Pulmonary MTB

23. Parotid enlargement in a HIV infected child is characterized in which stage of AIDS, according to WHO? *(JIPMER Nov 2014)*
a. Stage 1 b. Stage 2
c. Stage 3 d. Stage 4

24. Common CNS lesions in HIV is caused by: *(PGI June 2009, PGI Dec 2003)*
a. Cryptococcus b. Toxoplasma
c. Neurocysticercosis d. Mucormycosis
e. Lymphoma

25. Fungal infection in AIDS: *(PGI Dec 2006)*
a. Mucormycosis
b. Aspergillosis
c. Disseminated candidiasis
d. Mucocutaneous candidiasis
e. Pneumocytis jiroveci

26. The most common organism amongst the following that causes acute meningitis in an AIDS patients is: *(AI 2005)*
a. Streptococcus pneumoniae
b. Streptococcus agalactiae
c. Cryptococcus neoformans
d. Listeria monocytogenes

27. In a patient having HIV infections, oral ulcer is most commonly due to: *(JIPMER 2005)*
a. Candida b. Cryptococcosis
c. Histoplasma d. Trichophyton

28. The tissue of origin of the Kaposi's sarcoma is: *(AIIMS May 2005)*
a. Lymphoid b. Vascular
c. Neural d. Muscular

29. An HIV patient complains of visual disturbances. Fundal examination shows bilateral retinal exudates and perivascular hemorrhages. Which of the following viruses are most likely to be responsible for this retinitis: *(AI 2004)*
a. Herpes simplex b. Varicella zoster
c. Cytomegalovirus d. EBV

30. Which of the following lesions is associated with HIV infection? *(AIIMS May 2004)*
a. Hairy leukoplakia b. Erythroplakia
c. Oral lichen planus d. Bullous pemphigoid

31. HIV infection is associated with: *(PGI June 2002)*
a. A glandular fever like illness
b. Generalized lymphadenopathy
c. Gonococcal septicemia
d. Presenile dementia

32. CMV retinitis in HIV occurs when the CD4 counts falls below: *(AI 2002)*
a. 50 b. 100
c. 200 d. 150

33. Important features of AIDS are: *(PGI June 2001)*
a. Follicular tonsillitis
b. Lichen planus
c. Oral candidiasis
d. Hairy leukoplakia
e. Mitotic lesions of oral cavity

34. Persistent diarrhea in AIDS is caused by A/E: *(PGI 2001)*
a. Microspora b. Cryptosporidia
c. Cryptococcus d. Isospora belli
e. Giardia lamblia

35. Multifocal tumor of vascular origin in a patient of AIDS: *(AI 2000, MHPG 2014)*
a. Kaposi sarcoma
b. Astrocytoma
c. Gastric carcinoma
d. Primary CNS lymphoma

36. In India most common cause of TB in HIV: *(PGI 2000)*
a. Myco. tuberculosis
b. Myco. avium intracellulare
c. M. bovis
d. M. scrofulaceum

37. WHO stage IV includes all except? *(AIIMS May 2009)*
a. Toxoplasma
b. Pneumocystis
c. HIV wasting syndrome
d. Oral thrush

38. Major signs of HIV/AIDS case definition according to WHO: *(PGI 07, PGI Dec 2001)*
a. Generalized lymphadenopathy
b. Prolonged Fever > 1 month
c. Chronic cough > 1 month
d. Prolonged diarrhea > 1 month
e. Weight loss > 10%

39. MC opportunistic infection in HIV positive patient in India is: *(Recent MCQ 2013)*
a. M. tuberculosis
b. Cryptococcus
c. Candida
d. Cryptosporidiosis

LAB DIAGNOSIS OF HIV INFECTION

40. Most sensitive test for HIV infection: *(NEET Pattern Based)*
a. Western blot
b. ELISA
c. Agglutination test
d. CFT

41. In HIV window period indicates: *(AIIMS Nov 2007)*
a. Time period between infection and onset of first symptoms
b. Time period between infection and detection of antibodies against HIV
c. Time period between infection and minimum multiplication of the organism
d. Time period between infection and maximum multiplication of the organism

42. HIV can be detected and confirmed by: *(AI 2005)*
a. Polymerase Chain Reaction (PCR)
b. Reverse transcriptase PCR
c. Real time PCR
d. Mimic PCR

43. Antenatal maternal HIV diagnosis is of importance in:
a. To prevent vertical transmission *(JIPMER 2004)*
b. To terminate
c. To discharge
d. To isolate the patient

44. All of the following methods are used for the diagnosis of HIV infection in a 2 month old child except:
a. DNA-PCR *(Recent Question 2014)*
b. Viral culture
c. HIV ELISA
d. p24 antigen assay

45. A patient comes to hospital with a history of sore throat, diarrhea and sexual contact 2 weeks before. The best investigation to rule out HIV is: *(AI 2000)*
a. P24 antigen assay
b. ELISA
c. Western blot
d. Lymph node biopsy

PROPHYLAXIS

46. A resident doctor sustained a needlestick injury while sampling blood of a patient who is HIV positive. A decision is taken to offer him post-exposure prophylaxis. Which one of the following would be the best recommendation? *(AIIMS Nov 2004, 2003)*
a. Zidovudine + Lamivudine for 4 weeks
b. Zidovudine + Lamivudine + Nevirapine for 4 weeks
c. Zidovudine + Lamivudine + indinavir for 4 weeks
d. Zidovudine + Lamivudine + Nevirapine for 4 weeks

47. Which of the following is used to prevent HIV transmission from an HIV positive pregnant mother to child? *(AIIMS Nov 2011)*
a. Lamivudine
b. Stavudine
c. Nevirapine
d. Didanosine

48. Which of the following is an opportunistic organism in AIDS? *(TNPG 2014)*
a. Ascaris
b. Strongyloidosis
c. Hookworm
d. Enterobius

EXPLANATIONS

TRANSMISSION AND PATHOGENICITY

1. **Ans. (b) (HTLV)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p516
   - HTLV-I is associated with: (i)Tropical spastic paraparesis, (ii) Adult T cell leukemia/lymphoma, (iii) Cutaneous T-cell lymphoma, (iv) Auto immune manifestations such as uveitis and arthropathies.

2. **Ans. (a, d, e) (HIV-2 first.., More closely related, Mode of..)** Ref: CDC guidelines, Wikipedia, Apurba Sastry's Essentials of Medical Microbiology 1/e p503-5

   Characteristic Features of HIV-2
   - HIV-2 was **first reported in 1986** in West Africa and then in 1987 in USA… CDC report, 1988
   - Donated blood should be screened **for both** HIV-1 and HIV 2
   - **Less virulent:** Compared to HIV-1 infection, HIV-2 infection is characterized by a longer asymptomatic stage, lower plasma HIV-2 RNA levels, and lower mortality; however, progression to AIDS does occur.
   - HIV-2 was more similar to SIV strains than to HIV-1. HIV-1 is closely related to SIV found in chimpanzees, and HIV-2 to SIV found in sooty mangabeys
   - HIV-2 has the same routes of transmission like HIV-1 but less infectious than HIV-1.
   - HIV-2 is far rarer than HIV-1, and geographically restricted to Africa.
   - HIV-2 is intrinsically resistant to NNRTI and to Enfuvirtide.

3. **Ans. (b) (T-helper cells)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p506

   HIV infects CD4 positive cells, i.e. mainly T-helper cells and also on the surface of various other cells like monocytes, macrophages, Langerhans cells, astrocytes, keratinocytes and glial cells.

4. **Ans (a) (CCR5)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p506

   CCR5-delta 32 mutation:
   - CCR-5 molecules are the coreceptors for HIV virus, present on human macrophages, dendritic cells and other immune cells.
   - A genetic mutation of CCR-5 is known as CCR5-delta 32 results in blockade of HIV entry into the cells so that those lucky people having this mutation are resistant to HIV infection.
   - It is observed among some Europian people:
     - Homozygous: 1% of people from Northern Europeans, particularly Swedes are homozygous for CCR5-delta 32 genes & are absolutely immune to HIV infection
     - Hetrozygous: Another 10–15% of European people inherit one copy of the gene. It does not prevent against infection, however reduce carrier's chances of infection and delays the progress of AIDS.
   - This mutation has not been found in Africans, East Asians, or Amerindians.

5. **Ans. (a) (1983)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p503, Ananthanarayan 9/e p570

   In 1983, Luc Montagnier and colleagues from the Pasteur Institute, Paris, isolated HIV from a West African patient with persistent generalized lymphadenopathy.

6. **Ans. All options (a), (b),(c), (d) (e)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p507, Harrison 18/e p1507

   Refer chapter review to know the detail about WHO classification of AIDS

7. **Ans. (c) (Tat)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p504, Ananthanarayan 9/e p572
   - Tat is a nonstructural gene of HIV.

8. **Ans. (c) (15–30%)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p505, Park 23/e p343-54, 22/e p320
   - In the absence of prophylactic antiretroviral therapy to the mother during pregnancy, labor, and delivery, and to the fetus following birth, the probability of transmission of HIV from mother to infant/fetus ranges from ***15 to 25% in industrialized countries and from 25 to 35% in developing countries***. ..... Harrison 18/e p1515
   - In the absence of any intervention, the rate of transmission of HIV from mother to infant/fetus ranges from 20 to 25%

9. **Ans. (c) (Decreasing viral replication)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p504
   - Nef gene: Down regulates viral replication

10. **Ans. (c) (Core antigen in HIV)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p504
    - **P24 is the** Core antigen in HIV, coded by gag gene.

11. **Ans. (d) (Tex)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p504, Ananthanarayan 9/e p567, 8/e p566
    - All retrovirus carry three genes for viral replication: Gag, pol and env.
    - Some retroviruses (transregulatroy viruses) HTLV or HIV contains an extra gene that is not there in standard retrovirus – tex or tat

12. **Ans. (a) (CD4+ helper cells)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p507
    - Receptor for the HIV is CD4 antigen and therefore virus may infect any cell ***bearing CD4 antigen on the surface.***
    - ***This is primarily/most commonly - CD4+ helper T-lymphocytes such as:***
    - Other cells bearing CD4 antigens are:
        - B-lymphocytes: 5–10%
        - Monocytes and Macrophages: 10–20%
        - Glial cells and Microglia in CNS
    - Follicular dendritic cells are susceptible to HIV without involvement of CD4 antigen.

13. **Ans. (a) (b) (c) (Cross blood brain barrier, RNA virus, Inhibited by 0.3% H_2O_2)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p503-9, Ananthanarayan 9/e p571-76
    - ***Option a: HIV crosses blood-brain barrier*** and cause encephalopathy leading to loss of higher functions, progressing to dementia.
    - ***Option b:*** HIV is spherical, enveloped virus and its genome is composed of 2 identical ***single stranded positive sense- RNA.***
    - ***Option c:*** HIV is inactivated by treatment with 50% ethanol, 35% isopropanol, 0.5% lysol, 0.5% formaldehyde, ***0.3% hydrogen peroxide***, and 10% bleaching powder in 10 minutes. A 2% solution of glutaraldehyde is effective for disinfection of medical instruments.
    - ***Option d:*** Incorrect:
        - HIV is a ***thermolabile virus.*** It is readily inactivated at 60 °C in 10 minutes and at 100 °C in seconds.

14. **Ans. (a) (b) (c) (Viral replication, Sequestered in lymphoid tissue, Infective)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p506
    - All persons infected with HIV pass through a phase of symptomless infection (clinical latency) lasting for several years.
    - During latent phase, patient show positive HIV antibody tests and ***they are infectious.***
    - There will be ***varying degrees of viral multiplication.***
    - ***CD4+ T-cell count declines steadily*** and not rapidly.
    - Lymphoid organs play a central role in HIV infection and in the lymphoid organs specific immune responses are generated.
    - Throughout the course of untreated infection—even during the stage of clinical latency—***HIV is actively replicating/ sequestered in lymphoid tissues.*** The microenvironment of the lymph node is ideal for the establishment and spread of HIV infection. Cytokines are released, activating a large pool of CD4 T-cells that are highly susceptible to HIV infection- ……. Jawetz 25/e p614-15, 24/e p611

15. **Ans. (a) (RNA-DNA-RNA)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p506, Ananthanarayan 9/e p574

    When the HIV infects a cell following sequence of events take place:
    - ***Viral RNA*** is transcribed by the enzyme, reverse transcriptase into single stranded **DNA**
    - Then single stranded DNA into ***Double stranded DNA*** (provirus), which is integrated into the host cell genome.
    - In response to viral promoters, the provirus initiates viral replication by directing synthesis of ***viral RNA*** and other components.

16. **Ans. (d) (Male to female transmitted > female to male)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p505, Harrison 19/e p1215-17, 18/e p1512-13
    - *'It is clear that the virus can be transmitted to either partner through vaginal intercourse.*
    - *Studies have found that* ***male-to-female HIV transmission is usually more efficient than female-to-male transmission.***
    - *The differences in reported transmission rates between men and women may be due in part to the prolonged exposure to infected seminal fluid of the vaginal and cervical mucosa, as well as the endometrium (when semen enters through the cervical os).*
    - *By comparison, the penis and urethral orifice are exposed relatively briefly to infected vaginal fluid'.*

17. **Ans. (a), (b), (e) (Gag, Tat and p24)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p504, Ananthanarayan 9/e p571
Refer chapter review

18. **Ans. (d) (AP)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Park 23/e p343, 22/e p317-18
Andhra Pradesh is worst affected state, followed by Maharashtra, Karnataka and Tamilnadu.

19. **Ans. (ALL ARE CORRECT)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p505, Park 22/e p318-20
- Mother to child transmission (MTC) – around 20–25%
- Semen is more infectious than vaginal discharge
- HIV is infectious even during the latent phase and window period
- Africa accounts for maximum no. of total global cases
- Children infection is rare (3%).

OPPORTUNISTIC INFECTION

20. **Ans. (d) (Candidiasis)** Ref: Harrison 19/e p1267
Opportunistic Ocular Infection in HIV infected patient:
- Cytomegalovirus Retinitis: MC ocular infection in HIV
- HSV and VZV induced acute retinal necrosis, or progressive outer retinal necrosis (PORN)
- Toxoplasma Retinochoroiditis
- Candida Endophthalmitis: MC fungal ocular infection in HIV
- Bacterial Retinitis
- Cryptococcus Chorioretinitis
- Pneumocystis Choroiditis
- Acute Retinal Necrosis
- Syphilis uveitis.

21. **Ans. (a, b, c, d) (Fever, Oral hairy.., Candidiasis, Diarrhoea..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, WHO manual/HIV staging
Refer chapter review for detail of conditions enlisted in WHO HIV stage III.

22. **Ans. (b) (Glandular fever...)** Ref: Harrison 19/e p1249,1257, Apurba Sastry's Essentials of Medical Microbiology 1/e p507
- The Acute HIV Syndrome presents as acute infectious mononucleosis (glandular fever) like syndrome and characterized by:
  - General: Fever, pharyngitis, lymphadenopathy, headache, arthralgia/myalgia, weight loss and vomiting/diarrhea
  - Neurologic: Meningitis, encephalitis, peripheral neuropathy and myelopathy
  - Dermatologic: Rash and mucocutaneous ulceration
- Lyme disease is not associated with HIV
- Diarrhea in HIV patient is associated with fungal infection such as Histoplasmosis, coccidioidomycosis, and penicilliosis but not Oropharyngeal candidiasis
- Diarrhea in HIV patient is associated with MAC infection; but not Pulmonary MTB
- Also remember, Causes of Diarrhea in HIV infected patients-
- Bacterial: Salmonella, Shigella, and Campylobacter, Clostridium difficile and MAC
- Parasitic: Cryptosporidium, microsporidia, and Isospora belli
- Fungal: Histoplasmosis, coccidioidomycosis, and penicilliosis
- Viral: CMV colitis
- AIDS enteropathy (chronic diarrheal syndrome for which no etiologic agent)

23. **Ans. (b) (Stage 2)** Ref: http://www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf, Apurba Sastry's Essentials of Medical Microbiology 1/e p507
Unexplained persistent parotid enlargement is seen in WHO Clinical Staging 2 for children with confirmed HIV infection.

24. **Ans. (a), (b) and (e) (Cryptococcosis, Toxoplasma and Lymphoma)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Harrison 18/e p1558, 17/e p1182
Refer table given below
- Neurologic Diseases in Patients with HIV Infection. Refer chapter review.

25. **Ans. (c), (d), (e) (Disseminated Candidiasis, Mucocutaneous Candidiasis, Pneumocystis jiroveci)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Ananthanarayan 8/e p576, Harrison 18/e p1507
Refer chapter review for detail

26. **Ans. (c) (Cryptococcus...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Harrison 18/e p1507
- *C. neoformans* is the leading infectious cause of meningitis in patients with AIDS.
- It is the initial AIDS-defining illness in ~2% of patients and generally occurs in patients with CD4+ T-cell counts < 100/µl.
- **But bacterial pathogens predominate in pediatric AIDS as the most common cause of meningitis.**

..... Jawetz 25/e p616, 24/e p612

27. **Ans. (a) (Candida)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Harrison 18/e p1507
- Oral lesions in HIV infected patients oral thrush, hairy leukoplakia, and aphthous ulcers
- Oral thrush/oral ulcers occur commonly due to ***Candida infection,*** and oral hairy leukoplakia is due to EBV generally occur in patients with CD4+ T-cell counts of < 300/µl.
- Sometimes, palatal, glossal, or gingival ulcers may result from Cryptococcal disease or histoplasmosis.

28. **Ans. (b) (Vascular)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Jawetz 25/e p617, 24/e p613
- Kaposi's sarcoma is a ***vascular tumor*** of endothelial origin
- It appears in skin, mucous membranes, lymph nodes and visceral organs.
- Other tumors commonly seen in AIDS patients:
  - Hodgkin's and non-Hodgkin's lymphomas
  - Cervical cancer
  - Anogenital cancer
  - Burkitt's lymphoma.

29. **Ans. (c) (Cytomegalovirus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Harrison 18/e p1563
The history provided gives clearly clue that patient is suffering from CMV retinitis.
Refer explanation given below
- ***CMV* retinitis**
  - The majority of cases of CMV retinitis occur in patients with a CD4+ T cell-count < 50/µl.
  - CMV reactivation syndrome seen in 25–30% of patients with AIDS.
  - CMV retinitis presents as a ***bilateral painless, progressive loss of vision, blurring of vision, 'floaters,' and scintillations***.
  - There is characteristic ***perivascular hemorrhage and exudates of retina.***
  - Therapy for CMV retinitis consists of oral valganciclovir, IV ganciclovir, or IV foscarnet, with cidofovir as an alternative.
- ***HSV and varicella zoster virus*es**
  - Cause a rapidly progressing, bilateral necrotizing retinitis referred to as the acute retinal necrosis syndrome, or progressive outer retinal necrosis (PORN).
  - This syndrome, in contrast to CMV retinitis, is associated with pain, keratitis, and iritis.
  - It is often associated with orolabial HSV or trigeminal zoster.
  - Ophthalmologic examination reveals widespread pale gray peripheral lesions.
  - This condition is often complicated by retinal detachment.

30. **Ans. (a) (Hairy leukoplakia)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Ananthanarayan 9/e p576
- Oral hairy leukoplakia is one of the oral manifestations in AIDS patients.

31. **Ans. (a)(b)(d) (A glandular fever like illness, Generalized lymphadenopathy, and Presenile dementia)** Ref: Ananthanarayan 9/e p576-77, 8/e p575, Harrison 18/e p1507, 17/e p1169-1170
- ***Presenile dementia*** may occur in AIDS patients by direct cytopathogenic damage by HIV in CNS leading to progressive loss of higher functions
- ***For other options refer chapter review.***

32. **Ans. (a) (50)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Harrison 18/e p1563
- The majority of cases of CMV retinitis occur in patients with a CD4+ T-cell count < 50/µl.
- CD4+ T-cell count < 50/µl is more prone for infections with MAC, T.gondii & CMV

- CD4+ T-cell count < 200/µl is more prone for infections with Pneumocystis jirovecii.
- Patients with HIV infection should have CD4+ T-cell measurements performed at the time of diagnosis and every 3–6 months thereafter.
- CD4 T-cell count < 350/µl is an indication for consideration of initiating ARV therapy.
- Decline in CD4+ T-cell count of > 25% is an indication for considering a change in therapy.
- Once the CD4+ T-cell count is < 200/µl, patients should be placed on a regimen for *P. jiroveci* prophylaxis,
- Once the count is < 50/µl, primary prophylaxis for MAC infection is indicated.

33. Ans. (c), (d) (Oral candidiasis, Hairy leukoplakia) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Harrison 18/e p1550-51

GIT involvement in AIDS patients

- Diseases of oral cavity: **Oral thrush (oral Candidiasis), Oral Hairy leukoplakia,** aphthous ulcers very common
- Eosophagitis due to Candida (commonly), HSV and CMV
- HIV encephalopathy
- Diarrhea commonly by protozoan parasites like *Cryptosporidia, Microsporidia and Isospora belli*

34. Ans. (c), (e) (Cryptococcus and Giardia lamblia) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Harrison 18/e p1506

Refer chapter review for detail

35. Ans. (a) (Kaposi sarcoma) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Jawetz 25/e p617, 24/e p613

- Kaposi's sarcoma is a ***multifocal vascular tumor*** of endothelial origin
- It appears in skin, mucous membranes, lymph nodes and visceral organs.

36. Ans. (a) (M.tuberculosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Ananthanarayan 9/e p576

- ***In developing countries like India, the most important pathogen is M.tuberculosis, with many strains being multi drug resistant.***

37. Ans. (d) (Oral thrush) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p508, Park 22/e p323

- Oral thrush belongs to WHO clinical stage 3 (Refer chapter review for detail)

38. Ans. (b)(d)(e) (Prolonged fever > 1 month, prolonged diarrhea > 1 month, Weight loss > 10%) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p507-08, Park 22/e p322

WHO case definition for AIDS surveillance (> 12 yrs) - At least 2 major + 1 minor sign

- Major sign: Weight loss > 10%, chronic diarrhea > 1 month, fever > 1 month
- Minor sign: Cough > 1 month, generalized pruritic dermatitis, herpes zoster, chronic progressive disseminated Herpes simplex infection, Oral candidiasis, Persistent generalized lymphadenopathy.

39. Ans. (a) (M. tuberculosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p506, Ananthanarayan 9/e p576

- MC opportunistic infection in HIV positive patient in India is Tuberculosis
- MC opportunistic infection in HIV positive patient in world is Tuberculosis (Harrison 19th/p1233)

LAB DIAGNOSIS OF HIV INFECTION

40. Ans. (b) (ELISA) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p510-13, Ananthanarayan 9/e p579, 8/e p578

- ELISA is considered as the most sensitive test for the diagnosis of HIV.

41. Ans. (b) (Time period between infection and detection of antibodies against HIV) Ref: Apurba Sastry's Essentials of Medical Microbiology/ p510-13

- It takes 2–8 weeks to months for antibodies to appear HIV infection—this period is called window period or sero negative infective stage.
- During this period, individual may be highly infectious.
- HIV infection can be detected during this period by p24 antigen assay and PCR.

42. Ans. (b) (Reverse transcriptase PCR) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p510-13

- RT-PCR is done to detect HIV RNA.
- Real time RT-PCR is done to quantify HIV RNA load.

43. Ans. (a) (To prevent vertical transmission) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p510-13, Harrison 18/e p1515, NACO guidelines for HIV

- HIV infection can be transmitted from an infected mother to her fetus during pregnancy, ***during delivery,*** or by ***breast-feeding.***
- The relative proportions of mother-to-child transmissions were 23–30% before birth, 50–65% during birth, and 12–20% via breastfeeding.
- Treatment of an HIV-infected mother with nevirapine during pregnancy and the infant during the first weeks following birth has proved ***very effective in dramatically decreasing mother-to-child transmission of HIV***

44. Ans. (c) (HIV ELISA) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p510-13, NACO Guidelines for HIV testing

- Anti-HIV antibody detection by ELISA or any other method is done only after 18 months of birth when all maternal antibodies would have disappeared.
- HIV diagnosis <18 months: (i) HIV DNA detection (best), (ii) p-24 antigen, (iii) HIV RNA detection, (iv) virus isolation

45. Ans. (a) (P24 antigen assay) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p510-13

- It takes 2–8 weeks to months for antibodies to appear HIV infection—this period is called ***window period or sero negative infective stage.***
- During window period: HIV infection can be detected by ***p24 antigen assay and PCR***

PROPHYLAXIS

46. Ans. (c) (Zidovudine + Lamivudine + Indinavir for 4 weeks)
Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p513-14, NACO guidelines for HIV and Harrison 18/e p1582

- Post exposure prophylaxis: Refer chapter review
- Current NACO recommendation is TLE regimen- tenofovir, lamivudine and efavirenz - Single daily dose, for 4 weeks for any exposure
- Earleir recommendation was- Zidovudine + Lamivudine + Indinavir for 4 weeks for severe expsoure

47. Ans. (c) (Nevirapine) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p513-14, Harrison 18/e p1515, NACO Guideline for HIV Testing 2007

NACO Guidelines to prevent neonatal HIV:
Single dose Nevirapine (NVP) to mother during labor and to the baby within 72 hours after birth.

48. Ans. (b) (Stron....) Ref: Harrison 18/e Chapter 189, table-189.1, Apurba Sastry's Essentials of Medical Parasitology /e p240.

CHAPTER

4.7

Miscellaneous Viruses

RODENT-BORNE VIRAL INFECTIONS

Rodent-borne viruses or roboviruses are transmitted from rodents to man by contact with body fluids or excretions without participation of arthropod vectors. Hence, they are not arboviruses. They are mainly in two groups.

Hantaviruses

Hantaviruses cause:
- Hemorrhagic fever with renal syndrome (interstitial nephritis) -by Hantaan virus, Dobrava virus, Puumalavirus (nephropathia epidemica) and Seoul virus.
- Hantavirus pulmonary syndrome –by Sin Nombre virus

Hantaviruses are spherical, enveloped viruses; contain triple-segmented, negative-sense ssRNA, belong to Bunyaviridae.

- Rodents are the reservoir of infection
- Transmission to humans occurs by inhaling aerosols of rodent excreta.
- They cause two fatal human diseases
  - Hemorrhagic fever with renal syndrome (interstitial nephritis) is caused by several species of hantaviruses such as Hantaan virus, Dobrava virus, Puumalavirus (nephropathia epidemica) and Seoul virus.
  - Hantavirus pulmonary syndrome is caused by the species—Sin Nombre virus

Arenaviruses

Arenaviruses
- New world viruses: Cause South American hemorrhagic fever
- Old world viruses: Lassa fever viruses and LCM viruses

Arenaviruses are pleomorphic, 50–300 nm in size, enveloped with large, club-shaped peplomers and contain a segmented ssRNA (two segments).

- New world viruses: Junin, Machupo, Guanarito & Sabia viruses. They cause South American hemorrhagic fever.
- Old world viruses: Lassa fever viruses (in Africa, ribavirin is DOC) and Lymphocytic choriomeningitis (LCM) viruses.

FILOVIRUSES

Include two genera: *Ebolavirus* and *Marburgvirus* both cause African hemorrhagic fever.

- **Morphology:** They are pleomorphic, mostly appear as long *filamentous*, ranging from 80–1000 nm, the average size being 665 nm (Marburg) to 805 nm (Ebola).
- **Highly fatal**: All the viral hemorrhagic fevers, with highest mortality rates (25–90%).

Ebola virus

Ebola virus (largest outbreak)
- Occurred in 2014
- Three West African countries affected: Guinea, Liberia and Sierra Leone.
- As of Jan 2016, about 28,637 suspected cases and 11,315 deaths
- Max cases from Sierra Leone, but max deaths from Liberia

Ebola virus has become a global threat, because of its recent outbreak in 2014; which was declared by WHO, as a public health emergency of international concern.

- **History:** In humans, it appeared first in 1976 in African near the *Ebola River*, from which the disease takes its name.
- **Species:** Ebola virus has five stable subtypes or species ((*Zaire, Sudan, Taï Forest, Reston and Bundibugyo)*.Species are of epidemiological importance. The virus causing the 2014 West African outbreak belongs to the *Zaire* species.
- **Geographical distribution**: Since its discovery, Ebola virus has caused several outbreaks in various African countries affecting > 17,000 documented cases with nearly 7,000 deaths.
- **The largest outbreak** occurred in 2014; affecting three West African countries—Guinea, Liberia and Sierra Leone.

- As of January 2016, about 28,637 suspected cases and 11,315 deaths were reported.
- Maximum cases were reported from Sierra Leone, but Liberia accounted for maximum deaths.
- Guinea is the only countries at present with widespread transmission whereas control measures have been established in Sierra Leone and Liberia.

- **Reservoir:** Though unknown, but are suspected to be infected animals, such as a *fruit bat or primates* (apes and monkeys).
- **Transmission:**
  - Through *direct close contact* with the blood, secretions, organs or other body fluids of infected animals/humans, infected surfaces and materials (e.g. bedding, clothing, syringes etc.)
  - Health care workers and close contacts/family members are at greater risk of contracting the infection.
  - Ebola can stay in semen for up to 3 months, although sexual transmission has not been reported yet.
- **Clinical manifestations:**
  - Incubation period is about 2–12 days (average being 8–10 days)
  - Common symptoms include: Fever, headache, muscle pain and sore throat, followed by abdominal pain, vomiting, diarrhea and rash, with hemorrhages (bleeding or bruise), often leading to shock and death.
- **Laboratory diagnosis:**
  - **Antibody** detection: ELISA detects both IgM and IgG separately by using recombinant nucleoprotein (NP) and glycoprotein (GP) antigens
  - **Serum antigen** detection by capture ELISA: The target proteins are NP, VP40, and GP.
  - **Molecular methods** such as reverse transcriptase PCR (RT-PCR) assay and real time RT-PCR assay to detect the viral RNA
  - **Electron microscopy** of the specimen shows typical filamentous viruses
  - **Virus isolation** in Vero cell line: Processing the specimen should be carried out in biosafety level-4 cabinets as there is a great risk of laboratory spread of the virus.
- **Treatment:** Supportive care such as rehydration is required. There is no treatment or vaccine available.
- **Measures taken in India**: There is no confirmed case documented yet in India.
  - However, because of risk of contracting infection from travellers, strict vigilance is going on in the airports of India.
  - Any person presenting with an acute onset of fever who has been in Guinea, Liberia, Sierra Leone or Mali i*n past 21 days* are kept in quarantine in the airports until tested negative for Ebola virus infection.

Transmission of Ebola
Through *direct close contact* with the blood, secretions, organs or other body fluids of infected animals/humans, infected surfaces and materials (e.g. bedding, clothing, syringes etc.)

Marburg Virus

Marburg virus disease was first reported in Germany and Yugoslavia (1967) among laboratory workers exposed to tissues of African green monkeys imported from Africa.

- Since then, over 450 cases have been reported in various African countries such as Kenya, South Africa, Democratic Republic of Congo, Uganda and Angola.
- The most recent outbreak was in Angola (2005), affecting 252 people with 227 deaths (mortality rate of 90%).

CORONAVIRUSES

Coronaviruses are enveloped; carrying petal or club-shaped peplomer spikes giving appearance of solar corona. Most of them infect animals and birds. Human infection is extremely rare.

- Most human coronaviruses are widespread affecting people of most part of the world and produce mild upper respiratory tract infection and occasional diarrhea.

- Two exceptions are SARS-CoV and MERS-CoV which are geographically restricted, transmitted from man to man and have produced outbreaks of severe respiratory disease with higher mortality.
- **Transmission:** Human coronaviruses spread by coughing and sneezing, and close personal contact, such as touching mouth, nose, or eyes or shaking hands. SARS-CoV can also spread via droplets and rarely spread through the air (airborne spread).

SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus)

- **History:** SARS was first recognized in China in 2003 by WHO physician Dr. Carlo Urbani. He diagnosed it in a businessman who had traveled from the China, through Hong Kong, to Hanoi, Vietnam. The businessman and the doctor who first diagnosed SARS both died from the illness.
- **Epidemiology**: During 2003 outbreak, the SARS virus, spread from Asia to various regions of the world causing nearly 8098 cases in 29 countries, with over 774 deaths in 2003. However, India remained free from the infection. Since 2004, no case has been reported from anywhere in the world.
- **Source**: SARS-CoV infection in humans is believed to be contracted from animals, including monkeys, Himalayan palm civets, raccoon dogs, cats, dogs, and rodents.
- **Clinical manifestation** includes severe lower respiratory tract infection, characterized by muscle pain, headache, sore throat and fever, followed in 2–10 days by the onset of respiratory symptoms mainly cough, dyspnea, and pneumonia.

MERS-CoV
- Middle East respiratory syndrome Coronavirus
- Causes lower respiratory illness with a mortality of 30%.
- Reported first in Saudi Arabia in 2012
- Now, confined to middle least

MERS-CoV (Middle East Respiratory Syndrome Coronavirus)

MERS-CoV has recently caused a severe form of lower respiratory illness with a mortality of 30%.

Epidemiology: It was first reported in Saudi Arabia in 2012.

- Since then, several hundreds of cases have been reported from various countries located in and around the Arabian Peninsula such as Saudi Arabia, UAE, Qatar, Oman, Jordan, Kuwait, Yemen, Lebanon and Iran.
- It is not reported from India yet.

Source though unknown, it is believed to have been acquired from camels and bats.

People at increased risk for MERS-CoV infection include:

- Recent history of travel from the Arabian Peninsula within 14 days
- Close contacts of a confirmed case of MERS
- Healthcare personnel not using recommended infection control precautions
- People with exposure to infected camels

Clinical manifestation:

- Incubation period is about 2–14 days.
- Severe acute respiratory symptoms like fever, cough and shortness of breath may appear.
- Some people develop gastrointestinal symptoms including diarrhea and nausea/vomiting.
- Complications occur such as pneumonia and kidney failure especially in people with underlying comorbid conditions.

Slow virus diseases
- Long IP
- Predilection for CNS
- Strong genetic predisposition
- Lack in antigenicity -leads to
  - Lack of immune response
  - Lack of associated inflammation
- Does not produce cytopathologic effect *in vitro*

SLOW VIRUSES AND PRIONS

Slow virus diseases including prion diseases, are a group of neurodegenerative conditions affecting both humans and animals, characterized by:

- *Long incubation period*, ranging from months to years
- *Predilection for CNS*: Slow viruses usually affect the CNS
  - Cause vacuolation of neurons (spongiform changes), with deposition of amyloid like plaques and gliosis

- Symptoms include loss of muscle control shivering, tremors and dementia
- Invariably fatal

- Strong *genetic* predisposition
- Slow viruses and prions *lack in antigenicity;* hence there is:
  - Lack of immune response and interferon production against the viral proteins
  - Lack of associated inflammation
- Does not produce cytopathologic effect in vitro

Slow virus diseases are either caused by: (i) Conventional viruses or (ii) Unconventional viruses termed as 'prions'

Table 4.7.1: Slow viral diseases

Due to conventional viruses	Agent	Hosts	Nature of disease
Subacute sclerosing panencephalitis	Measles virus variant	Humans	Chronic sclerosing panencephalitis
Progressive multifocal leukoencephalopathy (PML)	Polyomavirus JC Virus	Humans	CNS demyelination
Visna virus	Retrovirus	Sheep	CNS demyelination
Maedi virus	Retrovirus	Sheep	Progressive pneumonia
Due to Unconventional viruses: Prions	Agent	Hosts	Nature of Disease
Kuru	Prion	Humans, monkeys chimpanzees	Spongiform encephalopathy
Creutzfeldt-Jakob disease	Prion	Humans, monkeys chimpanzees	
Gerstmann-Sträussler-Scheinker disease	Prion	Humans	
Fatal familial insomnia	Prion	Humans	
Scrapie	Prion	Sheep, goats, mice	
Bovine spongiform encephalopathy	Prion	Cattle	
Transmissible mink encephalopathy	Prion	Mink	
Chronic wasting disease	Prion	Mule deer, elk	

Prion Diseases

Prions are infectious protein particles that lack any nucleic acid. They are filterable like viruses; but are resistant to wide range of chemical and physical agents of sterilization. There are several prion diseases of humans and animals.

Prion Proteins have Two Isoforms

- PrP^{Sc} is the prion protein that causes disease. It is so named because, it was first identified in scrapie.
- PrP^{C} is the normal cellular isoform of the prion protein present on the cell membrane of mammals. It is encoded in chromosome 20. It is the precursor of PrP^{Sc}, they differ from each other in many respects.

	PrP^{C}	PrP^{Sc}
Full form	Prion protein cellular	Prion protein scrapie
	Normal isoform of prion protein present in man/animals	Prion protein that causes prion disease in man/animals
Structure	Elongated polypeptide, rich in α-helix and has little β-structure	Globular polypeptide, Contains less α-helix and a more of β-structure
Location	Anchored to the cell membrane	Cytoplasmic vesicles

Contd...

Prion proteins have two isoforms:
- PrP^{Sc} is the prion protein that causes disease
- PrP^{C}: Normal prion protein, found in human cells (chromosome 20)

Contd...

	PrP^C	PrP^{Sc}
Protease	Sensitive	Resistant
Turnover	Hours	Days

Mechanism of Prion Diseases

Theory proposed by *Stanley B. Prusiner* (Nobel prize winner, 1997) had clearly explained the detailed mechanism.

- Once infected, the prion proteins (PrP^{Sc}) are carried to the neurons. They bind to the normal PrP^C on the cell surface.
- This causes the release of PrP^C from cell surface followed by their conversion into the disease-causing isoform (PrP^{Sc}). This is a *post translational modification* by which the elongated polypeptide PrP^C become globular polypeptide PrPSc.
- The cell synthesizes new PrP^C and the cycle is repeated; as a result, large amount of PrP^{Sc} is formed.
- PrP^{Sc} are aggregated as *amyloid-like plaques* in the brain. As these plaques consist of host proteins, there is lack of an immune response or inflammation.
- PrP^{Sc} are internalized by neurons and get accumulated inside the cytoplasmic vacuoles giving the cell a spongiform appearance.

Laboratory diagnosis of Prion diseases
- Measurement of PrP^{Sc} by *conformation dependent immunoassay*
- Brain MRI
- Neuropathological diagnosis in brain biopsies
- Sequencing the PRNP gene
- Stress protein 14-3-3
- Abnormal EEG

Laboratory Diagnosis

- Measurement of PrP^{Sc} by *conformation dependent immunoassay* (definitive diagnostic tool).
- Brain MRI: > 90% of patients show increased intensity in the basal ganglia and cortical ribboning.
- Neuropathological diagnosis in brain biopsies: The pathologic hallmarks of prion diseases seen under light microscopy, are spongiform degeneration and astrocytic gliosis with lack of inflammatory response.
- Sequencing the PRNP gene to identify the mutation: This is important in familial forms of Prion diseases.
- Stress protein 14-3-3 is elevated in the CSF.
- Abnormal EEG: In late stage of the disease, high-voltage, triphasic sharp discharges are observed.

Decontamination of Prions
- Autoclaving at 134 °C for 1–1.5 hour,
- Treatment with 1 N NaOH for 1 hour and
- 0.5% sodium hypochlorite for 2 hours

Treatment

There is no known effective therapy for preventing or treating prion diseases. Several trials using drugs such as quinacrine and anti-PrP antibodies have shown to eliminate PrPSc from the cultured cells, but they failed to do so in vivo.

Decontamination

Prions are extremely resistant to most of the common sterilization procedures. Recommended methods for sterilization of material contaminated with prion proteins are:

- Autoclaving at 134 °C for 1–1.5 hour
- Treatment with 1 N NaOH for 1 hour
- 0.5% sodium hypochlorite for 2 hours.

ROTAVIRUS

Rotaviruses belong to the family Reoviridae; the only RNA virus family to have dsRNA.

- Surrounded by a triple layered capsid
- Possess segmented dsRNA (11 segments)
- Proteins: There are 6-structural viral proteins (VPs) and 6-nonstructural proteins (NSPs).

Classification of Rotaviruses

1. **Traditional Classification**: Based on group specific VP6 antigen, there are seven groups (A-G) of rotaviruses. Most human diarrhea is caused by group A and, to a much lesser extent, by groups B and C.
2. **Binary system of typing**: **VP7 (**a glycoprotein or G-type antigen) and **VP4 (**a protease sensitive protein or P-type antigen) are used to type rotaviruses.

- Serotyping (by neutralization test) and genotyping (by sequencing) methods are available.
- Currently, 19G and 28[P] types are known. The most common type seen in the world as well as in India is **G1P [8]**type, which accounts for nearly 70% of total isolates.

Clinical Manifestation

- Rotaviruses are the most common cause of diarrheal illness in children.
- IP is about 1–3 days.
- It has an abrupt onset, characterized by vomiting followed by watery diarrhea, fever, and abdominal pain.

Laboratory Diagnosis

- **Direct detection of virus**: Rotaviruses can be demonstrated in stool by:
  - *Immunoelectron microscopy* (IEM): Rotaviruses have sharp edged triple shelled capsids; look like spokes grouped around the hub of a wheel.
  - *Isolation* of rotavirus is difficult. Rolling of tissue cultures may be attempted to enhance replication.
- **Detection of viral antigen** in stool- by ELISA and latex agglutination based methods.
- **RT-PCR** is the most sensitive detection method for detection of rotavirus from stool.
- **Serologic tests** (ELISA) can be used to detect the rise of antibody titer. This may be useful for seroprevalence purpose.

Treatment and Control

Treatment is mainly supportive, to correct the loss of water and electrolytes such as oral or parenteral fluid

Vaccine: Human live attenuated rotavirus vaccine (*Rotarix*) is available.

- It consists of live attenuated human strains of rotavirus serotypes G1, G2, G3, G4 and G9.
- *Schedule*: It is administered by oral route, two doses at 2 and 4 months after birth.
- Most serious complication following rotavirus vaccine is intussusceptions.

Rotavirus types
- Currently, 19G and 28 [P] types are known.
- MC type in world as well as in India: **G1P [8]** type.
- Areas with poor hygiene reported other diverse types.

Rotavirus Vaccine (Rotarix)
- Live attenuated serotypes G1, G2, G3, G4 and G9.
- *Schedule*- By oral route, two doses at 2 and 4 months after birth.
- Most serious complication - intussusceptions

Norovirus
- MC cause diarrheal illness in older children and adults
- Occurs following shellfish intake

Viral agents of Gastroenteritis and their features	
Rotavirus	Group A: Most common cause of severe endemic diarrheal illness in children worldwide Group B: Causes outbreaks of diarrhea in adults in China.
Caliciviruses	Possess cup-like depressions on the capsid surface
Norovirus (or Norwalk)	Most common cause diarrheal illness in older children and adults (associated with vomiting and fever), affect all age groups
Sapovirus	Causes sporadic cases and occasional outbreaks of diarrheal illness in infants and children
Astrovirus	
Adenovirus 40 and 41	Common viral agent of endemic diarrheal illness of infants and young children worldwide
Respiratory viruses	Diarrhea has also been reported as a part of manifestations of certain respiratory viruses- SARS CoV, Influenza A/H5N1and A/H1N1 virus
Others	Toroviruses, Picobirnaviruses, Pestiviruses, and Parvovirus B Some enteroviruses like Coxsackie A (type 18, 20–22, 24) and many Echovirus serotypes

MULTIPLE CHOICE QUESTIONS

HANTAVIRUSES

1. **Which of the following is true?** *(AIIMS May 2008)*
   a. Hantavirus pulmonary syndrome is caused by inhalation of rodent urine and feces
   b. KFD is caused by bite of wild animal
   c. Lyssa virus is transmitted by tick
   d. Yellow fever is endemic in India
2. **True about Hantavirus:** *(PGI 2002)*
   a. Hantavirus pulmonary syndrome
   b. Transmitted by arthropod
   c. Transmitted by rodents
   d. Hemorrhagic fever with renal failure

EBOLA VIRUS

3. **True about Ebola virus:** *(PGI Nov 2014)*
   a. Belongs to family Filoviridae
   b. Transmission may occur from dead bodies
   c. Transmitted by sexual contact
   d. Incubation period is less than 48 hr
   e. Contains single-stranded RNA
4. **Ebola virus: True statement(s) is/are:** *(PGI Nov 2014)*
   a. Incubation less than 48 hr
   b. Transmission is by oral route
   c. Specific treatment available
   d. Cases are restricted to Guinea, Liberia and Sierra Leone

ROTAVIRUS

5. **The most common organism causing diarrhea in adults associated with shell fish ingestion:**
   a. Calicivirus *(AIIMS Nov 2015)*
   b. Enterovirus type 40, 41
   c. Norovirus
   d. Rota virus
6. **The viruses causing gastroenteritis are:**
   a. Rotavirus *(PGI Dec 2008)*
   b. Norwalk virus
   c. Adenovirus
   d. Hepadnavirus
   e. Enterovirus
7. **Electron microscopy is helpful in diagnosis of:**
   a. Rotavirus *(PGI June 2003)*
   b. RSV
   c. Herpes virus
   d. Prion
8. **Rotavirus is detected by:** *(NEET Pattern Based, AIIMS May 2002, AI 2000)*
   a. Antigen in stool
   b. Antibody in serum
   c. Demonstration of virus
   d. Stool culture
9. **Rotavirus vaccine is given by which route:**
   a. Intramuscular *(TNPG 2014)*
   b. Intravenous
   c. Oral
   d. Subcutaneous

SARS

10. **SARS is a type of:** *(Recent Questions 2014)*
    a. Rhinovirus
    b. Flavivirus
    c. Corona virus
    d. Enterovirus
11. **All are true about SARS EXCEPT:**
    a. Epidemic in India *(DNB Dec 2012, JIPMER 2003)*
    b. Spreads by droplet
    c. Diagnosed by PCR
    d. Caused by SARS CoV
12. **SARS virus is a:** *(NEET Pattern Based, DNB June 2009, PGI June 2006, DNB 2014)*
    a. Coronavirus
    b. Lentivirus
    c. Calciviridae
    d. Hepadnaviridae

SLOW VIRAL DISEASES

13. **Which of the following is correct about prions?**
    a. Long incubation period *(AIIMS Nov 2012)*
    b. Destroyed by autoclaving at 121 °C
    c. Nucleic acid present
    d. Immunogenic
14. **Prion is a:** *(DNB June 2009, AI 2008, AIIMS Nov 2007)*
    a. DNA
    b. RNA
    c. Protein
    d. Polysacchride
15. **Regarding prion protein which of the following statement is true:** *(AIIMS Nov 2008)*
    a. It is protein product coded in viral DNA
    b. It catalyses abnormal folding of other proteins
    c. It protect disulfide bonds from oxidation
    d. It cleaves normal proteins

16. All of the following statements are true regarding CNS infection, except: *(AIIMS 2004)*

a. Measles virus is the causative agent for subacute sclerosing panencephalitis
b. Cytomegalovirus causes bilateral temporal lobe hemorrhagic infarction
c. Prions infection causes spongiform encephalopathy
d. JC virus is the causative agent for progressive multifocal leucoencephalopathy

17. True about prion protein diseases is all, except: *(AIIMS Nov 2001)*

a. Myoclonus is seen in 10% of the patients
b. Caused by infectious protein
c. Brain biopsy is diagnostic
d. Commonly manifests as dementia

18. PML is caused by: *(PGI 2000)*

a. CMV
b. Papovavirus
c. HIV
d. Poliovirus

EXPLANATIONS

HANTAVIRUSES

1. **Ans. (a) (Hanta...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p517, Jawetz 25/e p531, 24/e p526 and Ananthanarayan 9/e p527, 8/e p522
Hantaviruses are natural pathogens of rodents, viremia is present in infected rodents and virus is shed in urine, feces and saliva in high titres.
Transmission from rodent to humans is primarily respiratory; by inhalation of the virus contained in the dried excreta.
About other options
   - Option b: Incorrect –Kyasanur forest disease (KFD) is transmitted by bite of infected ticks
   - Option c: Incorrect –Lyssa virus (Rabies virus) transmitted by bite of infected rabid dogs/ mammals.
   - Option d: Incorrect – already explained.
2. **Ans. (a), (c), (d) (Hantavirus pulmonary syndrome, Transmitted by rodents and Hemorrhagic fever...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p517, Jawetz 25/e p531, 24/e p526 and Ananthanarayan 9/e p527
Two important syndromes associated with hanta viruses-
   - Hantavirus Pulmonary Syndrome: Severe respiratory illness, hantavirus pulmonary syndrome (HPS) caused by hantavirus (belongs to Arboviruses).
   - Hemorrhagic fever with renal syndrome (HFRS) is an acute viral infection that causes an interstitial nephritis, acute renal insufficiency and renal failure

EBOLA VIRUS

3. **Ans. (a, b, c, e) (Belongs to…, Transmission may…, Transmitted by…, Contains single stranded RNA)**
Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 520, Ebola as a STI. Curr Opin Infect Dis. 2015 Feb;28(1):83-5
Refer chapter review.
There is theoretical plausibility for sexual transmission of Ebola virus but there has been no evidence of this occurring.
Patients are advised for sexual abstain or to use condoms for 3 months.
4. **Ans. (d) (Cases are..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p519, www.cdc.gov/vhf/ebola

Ebola virus:
   - Incubation period is about 2–12 days (average being 8–10 days)
   - Ebola then spreads among people via DIRECT CLOSE CONTACT (through broken skin or mucous membranes of eyes, nose, or mouth)
   - There is no specific drug available, only symptomatic treatment can be given.
   - The largest outbreak occurred in 2014; mainly in three West African countries: Guinea, Liberia and Sierra Leone.

ROTAVIRUS

5. **Ans. (c) (Norovirus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p526, Harrison 19/e p1286
   - Noroviruses may be the most common infectious agents of mild gastroenteritis in the community and affect all age groups
   - Noroviruses account for the majority of adult outbreaks of gastroenteritis
   - Shellfish and salad ingredients are the foods most often implicated in norovirus outbreaks.
6. **Ans. (a), (b), (c), (e) (Rotavirus, Norwalk virus, Adenovirus and Enterovirus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p524-26
Refer chapter review for explanation.
7. **Ans. (a) (Rotavirus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p524-26, Ananthanarayan 9/e p561
   - Rotavirus culture is difficult
   - So, electron microscopy is useful in diagnosis of Rotavirus infection
   - They characteristically appear as little wheels with short spokes radiating from a wide hub to a clearly defined outer rim.
   - The name Rota is derived from Latin word 'rota' means wheel.
8. **Ans. (a) > (c) (Antigen in stool > Demonstration of virus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p524-26, Jawetz 25/e p511, 24/e p505

States 'Laboratory diagnosis rests on demonstration of virus in stool collected early in the illness and on a rise in antibody titer. Virus in stool is demonstrated by IEM, latex agglutination tests, or ELISA. Genotyping of rotavirus nucleic acid from stool specimens by the polymerase chain reaction is the most sensitive detection method'

- Immunoelectron microscopy (IEM) can detect virus particle, if their concentration is more than 10^6/ml of stool.
- Use of ELISA in demonstration of virus in stool indirectly indicates detection of viral antigens as ELISA is useful in detection of either antigen or antibody.
- 'Antigen in stool' is a better answer than 'Demonstration of virus'

9. **Ans. (c) (Oral)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p524-26, www.cdc.gov/rotavirus.html
   - Rotarix (human rotavirus, live, attenuated, oral vaccine), is indicated for active immunization against rotavirus gastroenteritis in infants caused by the G1, G2, G3, G4 and G9 serotypes.
   - Rotavirus vaccine is administered by oral route, 2 doses at 2 and 4 months after birth.
   - Most serious complication following rotavirus vaccine: Intussusception.

SARS

10. **Ans. (c) (Coronavirus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p521, Ananthanarayan 9/e p560
    Severe acute respiratory syndrome (SARS) was caused by a corona virus.

11. **Ans. (a) (Epidemic in India)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p521
    - In November 2002, Guangdong province in South China experienced outbreak of SARS and it affected over 30 countries, with many thousands of cases and over 800 deaths.
    - India ***escaped the SARS epidemic;*** however, a few suspect cases were detected and quarantined.
    - For further detail, refer chapter review

12. **Ans. (a) (Coronavirus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p521, Harrison 18/e p1487-88
    Already explained.

SLOW VIRAL DISEASES

13. **Ans. (a) (Long incubation period)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 522-24
    Slow virus diseases are characterized by a ***very long incubation period*** and slow relentless course, terminating fatally.

14. **Ans (c) (Protein)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p522-24, Ananthanarayan 9/e p556,
    Prions are small infectious proteinaceous particles without any detectable nucleic acid

15. **Ans. (b) (It catalyzes...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p522-24, Harrison 18/e p3441-42
    - Prions are infectious proteins that cause degeneration of the central nervous system (CNS). Prion diseases are disorders of protein conformation,
    - Prions reproduce by binding to the normal, cellular isoform of the prion protein (PrP^C) and stimulating conversion of PrP^C into the disease-causing isoform (PrP^{Sc}).
    - PrP^C is rich in α-helix and has little β structure, while Pr P^{Sc} has less α -helix and a high amount of β-structure.
    - This α-to β-structural transition in the prion protein (PrP) is the fundamental event underlying prion diseases
    - PrP^{Sc} is a misfolded PrP and they can cause other normally folded prions to misfloded state.

16. **Ans. (b) (Cytomegaloviru...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p522-24
    CNS manifestations of CMV infections
    - CMV rarely causes meningoencephalitis in healthy individuals.
    - Causes ventriculoencephalitis characterized by cranial-nerve deficits, nystagmus, disorientation, lethargy, and ventriculomegaly.
    - In immunocompromised patients, CMV can also cause subacute progressive polyradiculopathy, which is often reversible if recognized and treated promptly.
    - JC virus, which is a polyomavirus causes progressive multifocal leucoencephalopathy
    - Prions infection causes Bovine spongiform encephalopathy
    - Measles virus really causes late complications as: Subacute sclerosing panencephalitis (SSPE)

17. **Ans. (a) (Myoclo...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p522-24, Harrison 18/e p3441-43
    - Most patients (~90%) with Cruetzfeldt-Jacob Disease (CJD) exhibit myoclonus that appears at various times throughout the illness.
    - Unlike other involuntary movements, myoclonus persists during sleep. Startle myoclonus elicited by loud sounds or bright lights is frequent.

18. **Ans. (b) (Papo..)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p522-24, Ananthanarayan 9/e p554, 8/e p550
    JC virus, which is a polyomavirus belonging to Papovaviridae family, causes progressive multifocal leucoencephalopathy (PML).

NOTE

SECTION 5

Mycology

CHAPTER OUTLINE

CHAPTER

5

Mycology

CLASSIFICATION OF FUNGI

Morphological Classification

Based on the morphological appearance, there are four main groups of fungi.

1. **Yeast:** They grow as round to oval cells that reproduce by *budding*, e.g. *Cryptococcus neoformans* and *Saccharomyces*
2. **Yeast like:** They exist as yeasts with pseudohyphae, e.g. *Candida*
3. **Molds:** They grow as long branching filaments of 2–10 µm wide called **hyphae.** Examples include, Dermatophytes, *Aspergillus*, *Penicillium*, *Rhizopus and Mucor*, etc.
4. **Dimorphic fungi**: They exist as molds (hyphal form) in the environment at ambient temperature (25°C) and as yeasts in human tissues at body temperature (37°C). Examples include:
   - *Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides, Paracoccidioides*
   - *Penicillium marneffei* and *Sporothrix schenckii:*

Morphological Classification:
- Yeast: *Cryptococcus neoformans* and *Saccharomyces*
- Yeast like: *Candida*
- Molds: Dermatophytes, *Aspergillus*, *Zygomycetes*
- Dimorphic fungi.

Dimorphic fungi:
- Hyphal form at 25°C and yeasts at body temperature (37°C)

Examples include:
- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*
- *Coccidioides, Paracoccidioides*
- *Penicillium marneffei*
- *Sporothrix schenckii.*

Taxonomical Classification

Based on the production of sexual spores, the Kingdom Fungi has been divided into four medically important phyla.

1. **Zygomycota:** They are lower fungi, produce sexual spores known as zygospores and possess aseptate hyphae, e.g. *Rhizopus, Mucor* and *Absidia*
2. **Ascomycota:** They produce sexual spores known as ascospores and possess septate hyphae, e.g. Aspergillus
3. **Basidiomycota:** They produce sexual spores known as basidiospore, e.g. Cryptococcus
4. **Deuteromycota (Fungi Imperfecti):** Sexual state is either absent or unidentified yet, e.g. most medically important fungi.

Deuteromycota (Fungi Imperfecti):
- Sexual state is either absent or unidentified yet
- E.g. Most medically important fungi.

Table 5.1: Types of fungal spores

Sexual spore	**Observed in**
Zygospores	Zygomycetes
Ascospores	*Aspergillus*
Basidiospores	*Cryptococcus*
Asexual spore	**Observed in**
Vegetative asexual spore	
Arthrospore	*Coccidioides* and *Trichosporon*
Blastospore	*Candida*
Chlamydospore	*Candida albicans*
Aerial asexual spore	
Conidiospore or conidia	*Aspergillus*
Sporangiospore	Zygomycetes
Microconidia and Macroconidia	Dermatophytes, Fusarium

Classification of Fungal Diseases

1. **Superficial mycoses:** Tinea versicolor, Tinea nigra, Piedra and Dermatophytosis
2. **Subcutaneous mycoses:** Mycetoma, Sporotrichosis, Chromoblastomycosis and Rhinosporidiosis
3. **Systemic mycoses:** Histoplasmosis, Blastomycosis, Coccidioidomycosis and Paracoccidioidomycosis
4. **Opportunistic mycoses:** Candidiasis, Cryptococcosis, Zygomycosis, Aspergillosis, Penicilliosis, Pneumocystis and Fusariosis
5. **Mycotoxicoses.**

SUPERFICIAL MYCOSES

Tinea Versicolor or Pityriasis Versicolor

Tinea Versicolor:
- Agent: *Malassezia furfur*
- Microscopy: *Spaghetti and meat balls* appearance
- SDA culture: *Fried egg* colonies appear
- Urease test is positive
- Wood's lamp: *Golden yellow* fluorescence.

It is a chronic recurrent condition involving stratum corneum of skin, caused by a lipophilic fungus- *Malassezia furfur.*

Clinical manifestation: Characterized by scaly patches of hypo to hyper pigmentation of skin of moist humid areas.

- Lesions are noninflammatory and nonpruritic (rarely pruritic)
- Areas rich in sebaceous glands are commonly involved (neck, chest, or upper arms).

Other manifestations include:

- Seborrheic dermatitis: Also called dandruff in adults and cradle cap in babies.
- Atopic dermatitis, folliculitis (hair follicle infection) and disseminated infection.

Laboratory diagnosis: Diagnosis is largely made clinically.

- 10% KOH mount: Mixture of budding yeasts and short septate hyphae (described as *Spaghetti and meat balls* appearance)
- SDA culture with olive oil overlay: *Fried egg* colonies appear
- Urease test is positive
- Wood's lamp examination: Scaly lesions show *golden yellow* fluorescence.

Treatment: Topical lotions like selenium sulfide shampoo, ketoconazole shampoo or cream, terbinafine cream should be used for 2 weeks.

Tinea Nigra

It is characterized by painless, black, non scaly patches present on palm and sole; more commonly in females**.** It is caused by a black colored yeast like fungus called *Hortaea werneckii.*

Piedra

Piedra is characterized by nodule formation on hair shaft (either black or white in color).

1. **White Piedra:** White nodules are formed on the hair shaft, which are less firmly attached.
   - Agent: *Trichosporon beigelii*
   - Identifying feature**:** *T. beigelii* is a urease positive produces creamy white colonies, containing hyaline septate hyphae intervening with rectangular *arthrospores.*
2. **Black Piedra:** Black nodule are formed which firmly attach to the hair shaft
   - Agent: *Piedraia hortae*
   - Identifying feature**:** It is a phaeoid fungus; produces reddish brown colonies; containing dark brown thick septate hyphae with ascus containing ascospores.

Dermatophytoses

Dermatophytoses (or tinea or ringworm) is the commonest superficial mycoses infecting keratinized tissues:

1. *Trichophyton* species: Infect skin, hair and nail
2. *Microsporum* species: Infect skin and hair
3. *Epidermophyton* species: Infect skin and nail

Dermatophytoses:
- *Trichophyton:* Infect skin, hair and nail
- *Microsporum:* Infect skin and hair
- *Epidermophyton:* Infect skin and nail.

Depending on the usual habitat dermatophytes are classified as follows:

- Anthropophilic species (infect only humans): Most common type of dermatophyte infection in man, produce mild and chronic lesions but respond poorly to treatment.
- Geophilic (soil species) and zoophilic species (infects animals): Produce more acute inflammatory response and severe infections; but they tend to resolve more quickly.

Table 5.2: Classification of dermatophytes based on their usual habitat

	Trichophyton	***Microsporum***	***Epidermophyton***
Anthropophilic	*T. rubrum, T. mentagrophytes* *T. schoenleinii, T. tonsurans* and *T. violaceum*	*M. audouinii*	*E. floccosum*
Zoophilic	*T. equinum T. verrucosum*	*M. canis, M. equinum*	
Geophilic (soil)	*T. ajelloi*	*M. gypseum*	*E. stockdaleae*

Table 5.3: Clinical types of dermatophytoses

Clinical types		**Area involved**
Tinea capitis (Scalp hair)		Scaly patches are produced on scalp, in which hair shafts are broken off right above the skin. It is of various types
1	Kerion	Painful inflammatory reaction producing boggy lesions on scalp (Agent: *T. verrucosum)*
2	Favus	Cup like crust (scutula) forms around the infected hair follicle with minimal hair shaft involvement. Agent: *T. schoenleinii*
3	Ectothrix	Arthrospore formation occurs on the surface of hair shaft Agent: *M. audouinii*, *M. canis* and *T. mentagrophytes.*
4	Endothrix	Arthrospore formation occurs within the hair completely filling hair shaft, alopecia can result. Agent: *T. tonsurans* and *T. violaceum*
Tinea corporis		Infection of the nonhairy skin of the body (trunk and limbs).
Tinea pedis		Infect first the webs between the toes, then spread to the sole in a '*moccasin'* pattern. (Athlete foot)
Tinea cruris		Infection of the groin area (also called jock itch)
Tinea barbae		Infection of the beard and moustache area of face.
Tinea faciei		Infection of the nonbearded area of face
Tinea imbricata		Concentric lesions of the skin, caused by *Trichophyton concentricum*
Tinea unguium		Infection of nail beds, commonly caused by *T. mentagrophytes and E. floccosum*
Tinea manuum		Infection of the palmar aspect of hands

Dermatophytid or ID reaction

Occasionally, hypersensitivity to dermatophyte antigens may occur which leads to appearance of secondary eruption in sensitized patients because of circulation of allergenic products. However, these lesions are distinct from the primary ringworm lesions as they occur distal to primary site and fungal culture often turns negative.

Laboratory Diagnosis

Woods lamp examination: Certain dermatophytes fluoresce when the infected lesions are viewed under Woods lamp.

- This is due to presence of pteridine pigment in cell wall.
- It is positive for various *Microsporum* species and *Trichophyton schoenleinii*. Negative for other dermatophytes.

Specimen collection: Skin scrapings, hair plucks (broken or scaly ones) and nail clippings are obtained from the active margin of the lesion and are kept in folded black paper.

Direct examination (10% KOH mount): Reveals thin septate hyaline hyphae with arthroconidia.

Culture on SDA followed by LPCB mount of the colonies: Reveals two types of spores (macroconidia and microconidia), based on which speciation is done.

Table 5.4: Distribution of conidia of dermatophytes

Dermatophytes	Macroconidia	Microconidia
Trichophyton	Rare, thin walled, smooth, pencil shaped	Abundant
Microsporum	Numerous, thick walled, rough, spindle shaped	Rare
Epidermophyton	Numerous, smooth walled, club shaped	Absent

Other methods of diagnosis

Apart from culture, there are several other methods for identification of dermatophytes:

- Hair perforation test is positive for *Trichophyton mentagrophytes* and *Microsporum canis*.
- Urease test: Positive for *Trichophyton mentagrophytes*
- Dermatophyte test medium and dermatophyte identification medium
- PCR can be used to detect species specific genes (e.g. chitin synthase gene)
- Skin test for detecting hypersensitivity to dermatophyte antigen (trichophytin).

Treatment of Dermatophytoses:
- DOC-Oral terbinafine or itraconazole
- Duration of treatment:
  - 1–2 weeks for skin lesions
  - 6 weeks for hair infection
  - 3 months for onychomycosis

Treatment

- Oral terbinafine or itraconazole are the drugs of choice for treatment. Duration of treatment is 1–2 weeks for skin lesions, 6 weeks for hair infection, 3 months for onychomycosis.
- Alternate: Oral griseofulvin and ketoconazole may be given
- Topical lotion, such as whitfield ointment or tolnaftate can be applied.

SUBCUTANEOUS MYCOSES

Mycetoma

Mycetoma is a chronic, slowly progressive granulomatous infection of the skin and subcutaneous tissues.

Clinical triad of Mycetoma:
- Subcutaneous Swelling
- Discharging sinuses
- Presence of granules in the discharge

- Clinically, it is manifested as triad of swelling, discharging sinuses and presence of granules in the discharge.
- Mycetoma (also known as Maduramycosis or Madura foot) is of two types: eumycetoma and actinomycetoma.

Table 5.5: Agents and clinical manifestations of mycetoma

Agents of mycetoma		Clinical manifestations of mycetoma		
Eumycetoma	**Actinomycetoma**		**Eumycotoma**	**Actinomycotoma**
Black granules: *Madurella mycetomatis* *Madurella grisea* *Exophiala jeanselmei* *Curvularia* species **White granules:** *Pseudallescheria boydii* *Aspergillus nidulans* *Acremonium* species *Fusarium* species	**White to yellow granules:** *Nocardia* species-(Most common agent) *Streptomyces somaliensis* *Actinomadura madurae* **Pink to red granules:** *Actinomadura pelletieri*	Tumor	Single, well defined margins	Multiple tumor masses with ill defined margins
		Sinuses	Appear late, few in number	Appear early, numerous with raised inflamed opening
		Discharge	Serous	Purulent
		Grains	Black/white	White/red
		Bone	Osteosclerotic lesions	Osteolytic lesions
		Grains contain	fungal hyphae (> 2 µm)	Filamentous bacteria(< 2 µm)

Epidemiology

Mycetoma is endemic in Africa, India and the Central and South Americas.

- Overall, actinomycetoma is more common (60%) than eumycetoma (40%) globally.
- Eumycetoma is more common in Africa.
- In India, Rajasthan reports maximum cases of mycetoma per year followed by Tamil Nadu and West Bengal. Actinomycetoma predominates in India (65%) except in Rajasthan where eumycetoma is more common.

Laboratory Diagnosis

Direct Examination

Granules are thoroughly washed in sterile saline; crushed between the slides and examined

1. *Macroscopic appearance of granules,* such as color, size, shape, texture
2. *If eumycetoma is suspected*: Grains are subjected to KOH mount which reveals hyphae of 2–6 µm
3. *If actinomycetoma is suspected*: Gram staining which reveals filamentous Gram-positive bacilli (0.5–1 µm wide). Modified acid fast stain is performed if *Nocardia* is suspected as it is partially acid fast.
4. *Histopathological staining* of the granules:
   - Eumycetoma: Reveals granulomatous reaction with palisade arrangement of hyphae in the cement substance
   - Actinomycetoma: Shows granulomatous reaction with filamentous bacteria at the margin.

Culture

Granules obtained from deep biopsies are the best specimen for culture. Both fungal (e.g. SDA) and Lowenstein Jensen media (for *Nocardia*) should be used.

Treatment

Treatment of mycetoma consists of surgical removal of the lesion followed by use of:

- Antifungal agents for eumycetoma (itraconazole or amphotericin B for 8–24 months)
- Antibiotics for actinomycetoma, such as Welsh regimen (amikacin plus cotrimoxazole).

Mycetoma occurs in 2 forms:

- In World: Actinomycetoma (60%) is MC except in Africa (Eumycetoma)
- In India: Actinomycetoma is MC except in Rajasthan (Eumycetoma)
- Max cases India reported: Rajasthan followed by Tamil Nadu and West Bengal

Sporotrichosis

Sporotrichosis or Rose Gardner's disease is chronic subcutaneous pyogranulomatous disease; caused by a thermally dimorphic fungus *Sporothrix schenckii*. Various clinical manifestations have been observed.

- Nodulo-ulcerative lesions (painless) spreading along the lymphatics
- Lymph nodes become enlarged, suppurative and indurated
- Other rare clinical types are osteoarticular type, pulmonary type, disseminated sporotrichosis.

Epidemiology

- Prevalent in tropical countries with high humidity (South Africa and India).
- *In India,* sporotrichosis is prevalent in sub Himalayan hilly areas (from Himachal Pradesh to Assam).
- *Risk factors* include people walking bare foot (such as farmers and gardeners).

Laboratory Diagnosis

- **Direct Microscopy** by H and E staining of tissue sections reveals **cigar shaped asteroid bodies**. It is described as central basophilic yeast cell surrounded by eosinophilic mass, composed of antigen-antibody complexes. Such eosinophilic halo is described as *Splendore-Hoeppli* phenomenon.

- **Culture:** Specimens are inoculated on SDA and incubated at 25°C and 37°C
  - At 25°C: It produces mycelial form, consisting of slender hyphae with conidia arranged in ***flower like pattern***
  - At 37°C: It produces yeast form, characterized by moist creamy white colonies
- **Serology:** Latex agglutination test detects serum antibodies in patients with extracutaneous form
- **Skin test** may demonstrate delayed type of hypersensitivity reaction against sporotrichin antigen.

Treatment

Itraconazole is the DOC, except for disseminated infection (amphotericin B is DOC).

Chromoblastomycosis

Sporothrix schenckii:
- At 37°C: Reveals **cigar shaped asteroid bodies**
- At 25°C: Produces hyphae with conidia arranged in ***flower like pattern***

Chromoblastomycosis refers to slow growing chronic subcutaneous lesions caused by group of dematiaceous or phaeoid fungi (i.e. darkly pigmented fungi) that produce a characteristic morphology called ***sclerotic body.***

- **Agents:** *Fonsecaea pedrosoi*, *Phialophora verrucosa*, *Cladosporium carrionii* and *Rhinocladiella aquaspersa*
- **Lesions:** Verrucose type (most common), crusted, ulcerative and nodular.
- **Sclerotic bodies:** Thick walled round cells (5–12 µm size) with multiple internal transverse septa. They are also called Medlar bodies or muriform cells.
- **Treatment** consists of surgical removal (cryosurgery or laser therapy) of the lesion followed by antifungals (itraconazole).

Phaeohyphomycosis

Phaeohyphomycosis refers to chronic subcutaneous lesions caused by dematiaceous or phaeoid fungi other than that are described in chromoblastomycosis (i.e. they do not produce sclerotic bodies). They exist in hyphal form. Agents include:

- *Alternaria*, *Bipolaris*, *Curvularia*, *Exophiala jeanselmei*
- *Cladophialophora bantiana* (it is neurotrophic, produces brain abscess, affecting frontal lobe).

Rhinosporidiosis

Chromoblastomycosis:
- Dematiaceous or phaeoid fungi (i.e. darkly pigmented fungi)
- Produce ***sclerotic body***
- **Agents:**
  - *Fonsecaea pedrosoi*
  - *Phialophora verrucosa*
  - *Cladosporium carrionii*
  - *Rhinocladiella aquaspersa.*

Rhinosporidiosis is a chronic granulomatous disease, characterized by **large friable polyps** in the nose (MC site), conjunctiva and occasionally in ears, larynx, bronchus and genitalia.

- **Agent:** It is caused by *Rhinosporidium seeberi*, recently re-classfied as an Aquatic Protistan Parasite(Mesomycetozoa); previously classified as lower acquatic fungi.
- **Source:** Stagnant water is the main source of infection. Fungal spores are inhaled while taking bath in ponds and rivers.
- **Distribution:** Tropical countries especially in Sri Lanka and India (Tamilnadu, Kerala, Orissa and Andhra Pradesh)
- **Diagnosis:**
  - Histopathology of polyp reveals **spherules** (large sporangia containing numerous endospores).
  - *R.seeberi* has not been cultivated yet.
  - It is stained better with mucicarmine stain.
- **Treatment:** Radical surgery with cauterization is the mainstay of treatment. Dapsone has been found to be affective. Recurrence is common.

SYSTEMIC MYCOSES

All the four fungi causing systemic mycoses are dimorphic. Transmission is by inhalation of spores, which then transform into the yeast phase in lungs. Pulmonary manifestation is the MC form.

Histoplasmosis

Histoplasmosis or Darling's disease is caused by dimorphic fungus-*Histoplasma capsulatum*. It has three biovars.

- *H. capsulatum* var. *capsulatum:* Causes classical histoplasmosis (most common type)
- *H. capsulatum* var. *duboisii:* Causes African histoplasmosis with frequent skin and bone involvement.
- *H. capsulatum* var. *farciminosum*: Causes epizootic histoplasmosis (in horses and mules).

Rhinosporidium seeberi:
- Lower aquatic fungus or hydrophilic protist
- Produces large friable polyps in the nose
- **Source:** Stagnant water
- **Distribution:** Tropical countries
- Histopathology of polyp reveals **spherules**
- Not been cultivated yet
- It is stained better with mucicarmine stain.

Epidemiology

- Histoplasmosis is endemic in USA (Ohio River valley and the lower Mississippi River).
- In India, it is reported frequently from the region of West Bengal along the Ganga River.
- Reservoir: Humid and acidic soil containing bird or bat droppings
- Transmission: By inhalation of spores (i.e. microconidia) circulating in the air being contaminated with soil.

Clinical Manifestations

- Pulmonary histoplasmosis: It is the most common form.
  - Acute form starts as mild flu like illness
  - Chronic cavitary histoplasmosis
- Mucocutaneous oral lesion (particularly seen in Indian patients)
- Disseminated histoplasmosis develops if CMI is very low (HIV).

H. capsulatum biovars:
- *var. capsulatum:* Causes classical histoplasmosis (MC type)
- *var. duboisii:* Causes African histoplasmosis with frequent skin and bone involvement.
- *var. farciminosum*: Causes epizootic histoplasmosis.

Laboratory Diagnosis

- **Histopathological staining** of specimens reveals **tiny oval yeast cells (2–4 µm size)** with **narrow based budding** within the macrophages and underlying granulomatous response
- **Culture** is the gold standard method of diagnosis: *Histoplasma* is a dimorphic fungus, hence:
  - At 25°C: Produces white mycelial colonies that consist of two types of conidia: Thick **tuberculate macroconidia** (characteristic) and thin microconidia
  - At 37°C: It gets converted into yeast form (creamy white colonies) in special Kelley's media.
- **Antibodies** in serum can be detected by CFT and immunodiffusion test.
- **Skin test** may be done to demonstrate delayed type hypersensitivity.

Histoplasma:
- At 25°C: Produces thick tuberculate macroconidia and thin microconidia
- At 37°C: Produces tiny oval yeast cells (2–4 µm size) with narrow based budding

Treatment

Liposomal amphotericin B is the antifungal of choice in acute pulmonary and disseminated histoplasmosis. Itraconazole is recommended for chronic cavitary pulmonary histoplasmosis.

Blastomycosis

Blastomycosis (also known as North American blastomycosis or Gilchrist's disease or Chicago disease) is a fungal infection of humans and other animals, notably dogs and cats, caused by the dimorphic fungus *Blastomyces dermatitidis*.

- **Clinical manifestations:** Acute pulmonary (MC form). Others include: Skin lesions, osteomyelitis and CNS involvement in AIDS patients (brain abscess)
- **Epidemiology: E**ndemic in North America particularly in states bordering the Ohio River and Mississippi River.
- **Laboratory diagnosis:**
  - *Histopathological staining* of the tissue biopsy specimens reveals thick-walled round yeast cells of 8–15 µm size with single broad-based budding ***(figure of 8 appearance)***
  - *Antibody detection* by immunodiffusion test against yeast phase antigens, such as antigen-A, BAD-1 and ASWS antigen (alkali soluble water soluble)
  - *Antigen detection* assay to detect *Blastomyces* antigen in urine (more sensitive) and in serum.
- **Treatment:** Liposomal amphotericin B is the drug of choice. Itraconazole can be given in immuncompetent patients with mild blastomycosis.

Blastomycosis:
Histopathology reveals thick-walled round yeast cells of 8–15 µm size with single broad-based budding ***(figure of 8 appearance).***

Coccidioidomycosis

Coccidioidomycosis (also called desert rheumatism or Valley fever or California fever), is a systemic fungal disease caused by a dimorphic soil dwelling fungus- *Coccidioides* which has two species, *C.immitis and C.posadasii.*

- **Clinical manifestations:**
  - Pulmonary coccidioidomycosis (MC form). Other forms: Skin lesions, erythema nodosum and arthritis in women.
  - *Disseminated* infection in males and persons with low CMI (HIV with CD4 count < 250/µl) are at higher risks. Common sites for dissemination include skin, bone, joints, soft tissues, and meninges.
- **Epidemiology:** Endemic in certain parts of Arizona, California, New Mexico, Texas, and Northern Mexico.
- **Laboratory diagnosis**
  - *Histopathological staining* of sputum or tissue biopsy specimens demonstrates **Spherules** (large sac like structures filled with endospores)
  - Cultures on SDA produces mycelial growth described as-fragmented hyphae consisting of **barrel shaped arthrospores** with alternate cells distorted (*empty cells*):
    - *Coccidioides* differs from other dimorphic fungi as it grows as mold at both 25°C and 37°C in usual culture media. It forms spherules at 37°C in certain special culture media only.
    - Cultures are highly infectious; require biosafety level-3 precautions
  - Serology: Antibodies are detected by immunodiffusion test and CFT.
  - Skin test with fungal extracts (coccidioidin or spherulin).
- **Treatment:** Itraconazole is the DOC (Amphotericin-B is given in diffuse pneumonia).

Coccidioidomycosis:
- *Yeast form:* **Spherules** seen
- Cultures on SDA produces mycelial growth (**barrel shaped arthrospores**).

Paracoccidioidomycosis

Paracoccidioidomycosis (also known as South American blastomycosis, Lutz-Splendore-de Almeida disease) is a systemic disease caused by the dimorphic fungus- *Paracoccidioides brasiliensis*.

- **Clinical manifestations:** It occurs as two major forms.
  - *Acute form (or juvenile type):* It affects young adults under 30 years age. It is less common variety, but more severe form, manifests as disseminated infection involving multiple viscera and is refractory to treatment.
  - *Chronic form (or adult form):* Common (90%) variety affecting older men, but less severe, manifested as progressive pulmonary disease, skin lesions and cervical lymphadenopathy.
- **Epidemiology**: Endemic in Brazil and other South American countries.
- **Laboratory diagnosis:**
  - *Histopathological staining* of pus, tissue biopsies or sputum reveals round thick-walled yeasts, with multiple narrow-necked buds attached circumferentially giving rise to ***Mickey Mouse* or *pilot wheel appearance*.**
  - *Culture* on SDA yields mycelial form at 25°C which converts into yeast phase at 37°C
  - *Serology:* Antibodies are detected by immunodiffusion, and most recently by ELISA (using gp43 antigen).
  - *Skin test* demonstrates delayed type hypersensitivity response against paracoccidioidin antigen.
- **Treatment:** Itraconazole is the treatment of choice except for the seriously ill patients where Amphotericin B is recommended.

Paracoccidioidomycosis:
Histopathology reveals:
- Mickey Mouse appearance
- Pilot wheel appearance

OPPORTUNISTIC MYCOSES

Opportunistic mycoses are caused by a group of fungi, which are normally a part of human anatomical flora (e.g. *Candida*) or found in nature and frequently isolated as laboratory contaminants (e.g. *Aspergillus, Rhizopus* and *Penicillium*).

Candidiasis

Candidiasis accounts for the most common fungal infection in humans both in HIV and non-HIV infected people; caused by *Candida*, a yeast like fungus that produces pseudohyphae. Various species of *Candida* include:

- *Candida albicans:* The most common and most pathogenic species
- Other rare species are *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. dubliniensis*, *C. kefyr*, and *C. viswanathii.*

Blastomycosis Misnomers:
- North American Blastomycosis: Due to *Blastomyces dermatiditis*
- South American Blastomycosis: Due to *Paracoccidioides immtis*
- European Blastomycosis: Due to *Cryptococcus*

Pathogenesis

Predisposing factors that are associated with increased risk of infection with *Candida* include-

- Physiological state: Extremes of age (infancy, old age), pregnancy
- Low immunity: Patients on steroid or immunosuppressive drugs, post transplantation, malignancy, HIV
- Patients on broad spectrum antibiotics suppresses the normal flora
- Diabetes mellitus, febrile neutropenia and zinc or iron deficiency.

Clinical Manifestations

Candida species are a part of normal flora of the skin and mucosa including gut flora. In presence of opportunistic conditions, they can cause various infections.

Mucosal candidiasis:
- Oropharyngeal candidiasis (oral thrush)
- Candidal vulvovaginitis-thin whitish ***curd like*** discharge
- Balanitis and balanoposthitis (in uncircumcised males)
- Esophageal candidiasis
- Angular stomatitis and denture stomatitis
- *Chronic mucocutaneous candidiasis.*

1. **Mucosal candidiasis:**
   - Oropharyngeal candidiasis (oral thrush) presents as white, adherent, painless patches in the mouth
   - Candidal vulvovaginitis thin whitish ***curd like*** vaginal discharge
   - Balanitis and balanoposthitis (occurring in uncircumcised males)
   - Esophageal candidiasis
   - Angular stomatitis and denture stomatitis
   - *Chronic mucocutaneous candidiasis:* Seen in infants with deficient CMI, resistant to treatment.
2. **Cutaneous candidiasis:**
   - *Intertrigo*, *Paronychia* and onychomycosis (fungal infection of nail)
   - *Diaper candidiasis* in infants, *Perianal candidiasis*
   - *Erosio interdigitalis blastomycetica*, an infection between the digits of the hands or toes
   - *Generalized* disseminated cutaneous candidiasis, seen in infants.
3. **Invasive candidiasis:** Results from hematogenous or local spread of the fungi. Various forms are:
   - UTI, Pulmonary candidiasis, meningitis, osteomyelitis and Hepatosplenic and disseminated candidiasis
   - Septicemia (*C.albicans* and *C.glabrata*).
   - Ocular: Keratoconjunctivitis and endophthalmitis
   - Nosocomial candidiasis (mainly by *C. glabrata*)
4. **Allergic candidiasis Include:**
   - *Candidid*: Vesicular lesions in the web space of hands, similar to that of dermatophytid reaction (both conditions are together called *'ID'* reaction)
   - *Other allergic reactions* include: Gastritis, irritable bowel syndrome and eczema.

Tests for species identification of *C.albicans:*
- *Germ tube test*
- *Dalmau plate culture-* produces chlamydospores
- *CHROM* agar
- *Growth at 45°C*
- *Sugar fermentation test and sugar assimilation test*

Laboratory Diagnosis

- **Direct microscopy:** Gram-positive oval budding yeast cells (4–6 µm size) with pseudohyphae.
- **Culture on SDA:** Colonies are described as creamy white, smooth, and pasty with typical yeasty odor.
- **Tests for species identification:**
  - *Germ tube test*: It is also called Reynolds Braude phenomenon, specific test for *C. albicans*.

        - It is differentiated from pseudohyphae as there is no constriction at the origin
        - Though the test is specific for *C. albicans*, it may also be positive for *C. dubliniensis*.
    - *Dalmau plate culture* on Cornmeal agar *C. albicans* produces thick walled chlamydospores
    - *CHROM* agar: Different *Candida* species produce different colored colonies on CHROM agar.
    - *Growth at 45°C*: It differentiates *C. albicans* (grows well) from *C.dubliniensis* (does not grow at 45°C).
    - *Sugar fermentation test and sugar assimilation test.*
- **Immunodiagnosis:**
    - Antibody detection: Against cell wall mannan antigen.
    - Antigen detection: Cell wall mannan and cytoplasmic antigens can be detected by ELISA
    - Enzyme detection: Specific for *Candida* such as enolase, aspartate proteinase, etc.
    - Test for metabolites specific for *Candida* such as mannitol, arabinitol can be detected.
    - G test is done for detection of α1-3 glucan.

	Pseudohyphae	True hyphae
Septa	Constricted	No constriction
Origin of branches	Constricted and Septate	No constriction, No septum present
Grows by	Budding	Apical elongation

Treatment

The antifungal drugs recommended depends upon the type of candidiasis:

- Cutaneous candidiasis or oral thrush–drug of choice is topical azole
- Esophageal and vulvovaginal candidiasis–drug of choice is oral fluconazole
- Disseminated candidiasis- drug of choice is Amphotericin B
- *C.glabrata* and *C.krusei* exhibit intrinsic resistance to azoles and are refractory to treatment with azoles.

Cryptococcosis

Cryptococcus ***has two species,*** *C. neoformans* ***and*** *C.gattii* ***and four serotypes A,B,C and D.***

- *C.neoformans* occurs in two varieties - *Cryptococcus neoformans* var. *grubii* and *Cryptococcus neoformans* var. *neoformans*; which correlate with serotypes A and D, respectively.
- *C.gattii* is antigenically diverse, corresponds to the serotypes B and C.
- Most laboratories do not routinely distinguish between the types, and report all isolates simply as *C.neoformans*.
- *CNS spread*: The unique feature of *Cryptococcus* is its ability to cross blood-brain barrier which occurs by yeast cells either migrate directly across the endothelium or carried inside the macrophages as 'Trojan horse'.
- **Virulence factors** of *Cryptococcus* that favor invasion and spread of infection include:
    - Polysaccharide capsule
    - Ability to make melanin by producing phenyl oxidase enzyme.
    - Production of other enzymes, such as phospholipase and urease
- **Risk factors:** Individuals at high-risk for cryptococcosis include:
    - Patients with advanced HIV infection with CD4 T-cell counts < 200/µl is the most important risk factor for *C.neoformans*. However, *C.gattii* is not associated with HIV. It usually causes infection in immunocompetent individuals.
    - Patients with hematologic malignancies
    - Transplant recipients
    - Patients on immunosuppressive or steroid therapy.

Clinical manifestations of cryptococcosis:

- Pulmonary cryptococcosis (MC from)
- Chronic meningitis
- Skin lesions-seen with var. *neoformans* (serotype D)
- Osteolytic bone lesions.

Clinical Manifestation

Various clinical manifestations of cryptococcosis include:

- Pulmonary cryptococcosis is the first and the most common presentation
- Chronic meningitis
- Skin lesions are commonly seen with *C.neoformans* var. *neoformans* (serotype D)
- Osteolytic bone lesions.

Geographical distribution of cryptococcosis:
- var. *grubii* (serotype A) strains are found worldwide
- var. *neoformans* (serotype D) - Europe
- *C.gattii* is confined to tropics.

Epidemiology

- **Geographical distribution:** *C. neoformans* var. *grubii* (serotype A) strains are found worldwide. *(var. neoformans in Europe).*
- **Habitat:** *C. neoformans* is frequently found in soils contaminated with avian excreta and pigeon droppings. In contrast, *C.gattii* inhabits in eucalyptus tree.

Habitat of Cryptococcus:
- *C.neoformans*: In soil contaminated with avian excreta and pigeon droppings.
- In contrast, *C.gattii* inhabits in eucalyptus tree.

Laboratory Diagnosis

Direct detection methods:

- **Negative staining** by *modified India ink stain* and nigrosin stain to demonstrate the capsule which appears as refractile delineated clear space surrounding the round budding yeast cells against black ground. Sensitivity is 60–70%.
- **Gram staining** may show Gram-positive round budding yeast cells
- **Other stains:** Mucicarmine stain Masson-Fontana stain, Alcian blue stain
- **Capsular antigen detection** from CSF or serum by latex agglutination test is a rapid and sensitive (95%).

Confirmation of Cryptococcus species is made by:

- Culture on SDA: Colonies appear as mucoid creamy white yeast like colonies
- Niger seed agar and bird seed agar is used to demonstrate melanin production
- Growth at 37°C, urease test positive
- Assimilation of inositol and nitrate and mouse pathogenicity test postive.

Treatment

Treatment depends upon the type of cryptococcosis.

- Cryptococcosis without CNS involvement: Fluconazole is the drug of choice.
- HIV infected patients with CNS involvement Induction phase for two weeks (Amphotericin-B ± flucytosine) followed by lifelong maintenance therapy with fluconazole.

Zygomycosis

Zygomycosis or mucormycosis represents group of life-threatening infections caused by aseptate fungi belonging to the phylum zygomycota. Examples include: *Rhizopus*, *Mucor and Absidia*

Predisposing factors: Agents of mucormycosis require iron as growth factor. Hence conditions with increased iron load are at higher risk of developing invasive mucormycosis, such as:

- Diabetic ketoacidosis (DKA) is the most important risk factor
- End stage renal disease
- Patients taking iron therapy or deferoxamine (iron chelator)
- Defects in phagocytic functions (e.g. neutropenia or steroid therapy).

Predisposing factors of mucormycosis:
- Diabetic ketoacidosis (DKA): Most important
- End stage renal disease
- Patients taking iron therapy
- Defects in phagocytic functions (e.g. neutropenia or steroid therapy).

Clinical Manifestations

Agents of mucormycosis are angioinvasive in nature. There are six types of clinical presentations:

1. *Rhinocerebral* mucormycosis: MC form; presents as orbital cellulitis, proptosis and vision loss

Rhinocerebral mucormycosis:
- MC form
- Presents as orbital cellulitis, proptosis and vision loss

2. Pulmonary mucormycosis is the second MC form, occurs in patients with leukemia.
3. Cutaneous mucormycosis
4. Gastrointestinal mucormycosis, such as necrotizing enterocolitis; seen commonly in premature neonates
5. Disseminated mucormycosis: Brain is the most common site of dissemination
6. Miscellaneous forms may involve any body site, including bones, trachea and kidneys, etc.

Zygomycetes Species can be differentiated based on rhizoid:
- *Rhizopus* bears nodal rhizoid
- *Absidia* bears internodal rhizoid
- *Mucor*- rhizoid is absent.

Laboratory Diagnosis

- **Histopathological staining** of tissue biopsies shows broad aseptate hyaline hyphae with wide angle branching
- **Culture on SDA** at 25°C reveals cottony woolly colonies which are initially white, later become brown black due to sporulation giving rise to ***salt* and *pepper*** appearance
- **Microscopic appearance:** LPCB mount of the colonies reveals broad aseptate hyaline hyphae, from which sporangiophore arise which ends at sporangium containing numerous sporangiospores
- **Rhizoid:** Some species bear a unique root like growth called rhizoid which provides initial clue for identification of the fungus. Species can be differentiated depending on the position of the rhizoid with respect to sporangiophore:
  - *Rhizopus* bears nodal rhizoid
  - *Absidia* bears internodal rhizoid
  - *Mucor*: rhizoid is absent.

Treatment

Amphotericin B deoxycholate remains the drug of choice for all forms of mucormycosis except the mild localized skin lesions in immunocompetent patients, which can be removed surgically.

Risk Factors for invasive aspergillosis:
- Glucocorticoid use (most important)
- Profound neutropenia or Neutrophil dysfunction
- Underlying pneumonia or COPD, tuberculosis or sarcoidosis
- Anti-tumor necrosis factor therapy.

Aspergillosis

Aspergillus species are widely distributed on decaying plants, producing chains of conidia.

Risk Factors for invasive aspergillosis are:
- Glucocorticoid use (the most important risk factor)
- Profound neutropenia or Neutrophil dysfunction
- Underlying pneumonia or COPD, tuberculosis or sarcoidosis
- Antitumor necrosis factor therapy.

Clinical Manifestations

Clinical manifestations of aspergillosis depend on the site of involvement. The incubation period varies from 2 to 90 days.

1. **Pulmonary aspergillosis** (MC form): Various forms include Allergic bronchopulmonary aspergillosis (ABPA), Asthma, Extrinsic allergic alveolitis, Aspergilloma (fungal ball) and Chronic cavitary pulmonary aspergillosis
2. **Invasive sinusitis:** Invasive sinusitis, chronic granulomatous sinusitis, maxillary fungal ball and allergic fungal sinusitis
3. **Ocular** aspergillosis: Keratitis and endophthalmitis
4. **Ear** infection: Otitis externa
5. **Others:** Endocarditis, brain abscess, skin lesions and onychomycosis.

Clinical manifestations also depend on the species involved:
- *A. fumigatus* accounts for most of the cases of acute pulmonary and allergic aspergillosis.
- *A. flavus* is more common in hospitals and causes more sinus, skin and ocular infections than *A. fumigatus*.
- *A. niger* can cause invasive infection but more commonly colonizes the respiratory tract and causes otitis externa.

	A.fumigatus	*A.flavus*	*A.niger*
Macroscopic appearance of colony	Smoky green, velvety to powdery, reverse is white	Yellow green, velvety, reverse is white	Black, cottony type, reverse is white
Microscopic appearance of colony (LPCB mount)	Vesicle is conical-shaped. Phialides are arranged in single row Conidia arise from upper third of vesicle Conidia are hyaline	Vesicle is globular shaped Phialides in one or two rows Conidia arise from entire vesicle Conidia are hyaline	Vesicle is globular shaped Phialides in two rows Conidia arise from entire vesicle Conidia are black

Laboratory Diagnosis

- *Direct examination*: Reveal narrow septate hyaline hyphae with acute angle branching
- *Culture on SDA followed by colonies subjected to LPCB mount (see table)*
- *Antigen detection:* ELISA detecting galactomannan antigen
- *Antibody detection:* Useful for chronic invasive aspergillosis and aspergilloma, where the culture is usually negative.
- In allergic syndromes, such as ABPA and severe asthma, specific serum IgE levels are elevated.
- *Detection of metabolites,* such as α1-3 glucan (by G test) or mannitol

Treatment

Following are the first line treatment recommended in different forms of aspergillosis.

- For invasive aspergillosis: Voriconazole is the drug of choice.
- For ABPA: Itraconazole is the drug of choice
- For single aspergilloma: Surgery is indicated.
- For chronic pulmonary aspergillosis: Itraconazole or voriconazole is the drug of choice
- For prophylaxis, posaconazole is indicated.

Penicilliosis

Penicillium species are usually found in environment and as laboratory contaminants. Rarely infects humans.

- *Common manifestations*: endophthalmitis, otomycosis, onychomycosis and allergic pneumonitis
- *Microscopy*: Reveal hyaline thin septate hyphae, vesicles are absent, and conidia arranged as ***brush border appearance.***

Penicillium Marneffei

- *Penicillium marneffei* is a thermally dimorphic fungus that causes opportunistic infection in HIV infected patients.
  - Systemic infection mimicking that of diss°eminated histoplasmosis
  - Skin lesions: Warty lesions mimicking that of Molluscum contagiosum are seen.
- It is endemic in SE Asian countries including Thailand, Vietnam and India (Manipur).
- *P. marneffei* is found mostly in rural areas and bamboo rats are the reservoirs of infection
- **Lab Diagnosis:**
  - Direct Microscopy: Shows ***oval or elliptical yeast cells with central septation,*** which indicates that these cells divide by transverse fission rather than budding
  - Culture: *P. marneffei* being dimorphic; produces yeast like colonies at 37°C and mold form at 25°C. The mold form has a characteristic ***brick red pigment.***
- **Treatment:** AIDS patients with severe penicilliosis are treated with amphotericin B till the condition improves followed by maintenance therapy with itraconazole for 12 weeks. In mild penicilliosis, itraconazole is recommended for 12 weeks.

Penicillium marneffei:
- At 37°C: Shows ***oval or elliptical yeast cells with central septation***
- At 25°C: The mold form has a characteristic ***brick red pigment***

Pneumocystis Pneumonia (PcP)

Pneumocystis is classified under fungus based on nucleic acid sequence studies:

- **Exist in two forms:** In environment cysts are found, whereas in human tissues both cysts and trophozoites are found.
- Cysts are inhaled, carried to the lungs, transformed into trophozoite stage which induces an inflammatory response that leads to recruitment of plasma cells resulting in formation of ***frothy exudate*** filling the alveoli. Hence, PcP is also called plasma cell pneumonia.

Pneumocystis pneumonia:
- Plasma cells pneumonia
- Formation of ***frothy exudate.***

Pneumocystis Exist in two forms:
- In environment: Cysts are found
- In human tissues: Both cysts and trophozoites are found.
- Cysts are inhaled, carried to the lungs, transformed into trophozoite stage.

Gomori's methenamine silver (GMS) staining:
- Method of choice to demonstrate the cysts of *P. jirovecii*
- The cysts resemble black colored ***crushed ping-pong balls***, against the green back ground

- **Laboratory Diagnosis:**
  - Gomori's methenamine silver (GMS) staining is the method of choice to demonstrate the cysts of *P. jirovecii*. The cysts resemble black colored ***crushed ping-pong balls***, against the green back ground.
  - *Pneumocystis* is not cultivable and there is no serological test available.
- **Treatment:** Cotrimoxazole is the DOC for *Pneumocystis* pneumonia. It is given for 14 days in non-HIV patients and 21 days in patients with HIV. It is also the recommended drug for primary and secondary prophylaxis in patients with HIV.

Fusariosis

Fusarium is a saprophytes; rarely cause human infections.
- In immunocompetent individuals, they cause: Keratitis in contact lens wearers and Onychomycosis
- In immunocompromised patients: They are angioinvasive and cause pulmonary and sinus infection.
- In neutropenic patients and with hematologic malignancies: Disseminated fusariosis occurs with frequent skin lesions.

Lab Diagnosis: LPCB mount of the colony reveals hyaline septate hyphae bearing round microconidia, sickle shaped large macroconidia and chlamydospores.

Treatment: Liposomal amphotericin B, voriconazole or posaconazole are recommended.

MYCOTOXICOSES

Mycotic poisoning can be classified into two varieties:
1. Mycotoxicosis: Occurs following consumption of food contaminated by toxins liberated by certain fungi
2. Mycetism: Refers to the toxic effects produced by eating poisonous fleshy fungi (e.g. mushrooms).

Table 5.6: Features of common mycotoxins

Mycotoxin	Produced by fungal species	Source	Clinical condition
Aflatoxin	*Aspergillus flavus* *Aspergillus parasiticus, A.nomius* *Penicillium puberulum*	Nuts, Maize	*Hepatoma*, Hepatitis Indian childhood cirrhosis Reye's syndrome
Fumonisins	*Fusarium moniliforme*	Maize	Equine leukoencephalomalacia Porcine pulmonary edema Carcinoma esophagus
Trichothecenes	*Fusarium graminearum*	Maize, wheat, sorghum	Alimentary toxic aleukia Biological warfare (yellow rain)
Ochratoxin	*Aspergillus ochraceus, A.niger* *Penicillium verrucosum*	Cereals, bread	Nephropathies (Balkan endemic nephropathy)
Cyclopiazonic acid	*Aspergillus flavus, A.versicolor, A.oryzae* *Penicillium cyclopium*	Groundnut, corn	Kodua poisoning Co-contaminant with aflatoxin
Zearalenones	*Fusarium graminearum*	Wheat, maize	Genital disorder in pigs

Table 5.7: Features of common mycetism

Mushroom poisoning	Produced by fungal species	Source	Clinical condition
Ergot alkaloid	*Claviceps purpurea*	Rye flour	St. Anthony's fire
Coprine poisoning	*Coprine atrementarius*	Butter	Antabuse like reaction
Muscarine	*Inocybe fastigiata*	Food	Cholinergic effect
Ibotenic acid, muscimol	*Amanita pantherina*	Edible mushroom	Abdominal pain, vomiting, diarrhea
Cyclopeptide	*Amanita phalloides*	Toadstools	Hepatocellular failure, Green death cap
Zearalenones	*Fusarium graminearum*	Wheat, maize	Genital disorder in pigs

MULTIPLE CHOICE QUESTIONS

GENERAL MYCOLOGY

1. C. albicans is: *(West Bengal 2016)*
a. Yeast b. Yeast like
c. Dimorphic d. Mould

2. Commonly used stain(s) for identifying fungus include(s): *(PGI Nov 2014)*
a. Periodic acid-Schiff (PAS) stain
b. von Kossa stain
c. Mucicarmine stain
d. Gomori's methenamine silver
e. Giemsa stain

3. Lower fungi which have nonseptate hyphae and which form sporangiospores are called: *(APPG 2015)*
a. Ascomycetes b. Phycomycetes
c. Deuteromycetes d. Basidiomycetes

4. Dimorphic fungi grow as yeasts at: *(MHPG 2015)*
a. 25°C b. 37°C
c. 42°C d. Room Temperature

5. Fungi of medical importance belongs to: *(Recent MCQ 2013, ,TNPG 2008)*
a. Basidiomycetes b. Ascomycetes
c. Phycomycetes d. Deuteromycetes

6. The only pathogenic true yeast is: *(APPG 2014)*
a. Candida b. Saccharomyces
c. Cryptococcus d. Trichophyton

7. Special stain fungus: *(AIIMS May 2013)*
a. Masson trichrome
b. Silver methanamine stain
c. Alizarin red
d. Congo red

8. Stains used for degenerated fungi in tissue:
a. PAS *(DNB Dec 2012)*
b. Gomori methamine silver
c. H and E
d. Muciramine

9. All are dimorphic fungi except: *(NEET Pattern Based, DNB Dec 2011, 2010, AIIMS Nov 2003, May 2009)*
a. Histoplasma b. Paracoccidiodes
c. Cryptococcus d. Blastomyces

10. Fungus which cannot be grown on artificial media/ not cultivable fungus is? *(NEET Pattern Based, DNB June 2010, PGI June 2007)*
a. Rhinosporidium seeberi
b. Penicillium marneffei
c. Aspergillus flavus
d. Sporothrix schenckii

11. Fungal spores formed by condensation of hyphal elements: *(JIPMER 2010)*
a. Arthrospores b. Conidiospore
c. Basidiospores d. Ascospores

12. Which dye is most suitable for fungus demonstration in biopsy? *(AIIMS Nov 2006)*
a. Alizarin red b. Veirhoff dye
c. Manson's trichome d. PAS

13. Correctly matched stain: *(PGI June 2006)*
a. Mucicarmine – Cryptococcus
b. Giemsa – Candida
c. Methanamine silver -Histoplasma
d. Gram's – Pneumocystis carinii

14. A sporangium contains: *(AIIMS 2002)*
a. Spherules b. Sporangiospores
c. Chlamydospores d. Conidia

SUPERFICIAL MYCOSES

15. Seborrheic dermatitis - causative agent is *(Recent Question 2015)*
a. Candida b. Dermatophytes
c. Cryptococcus d. Malassezia furfur

16. Feature(s) of Taenia capitis is/are all except:
a. May presents as a boggy swelling *(PGI Nov 2014)*
b. Most commonly occurs in elderly
c. May present as black dot
d. Caused by Trichophyton and Microsporum but not by Epidermophyton
e. Scutula formation

17. Trichophyton species which is zoophilic: *(NEET Pattern Based)*
a. T. tonsurans b. T. violaceum
c. T. schoenleinii d. T. mentagrophytes

18. Wood's Lamp used in: *(PGI June 2006)*
a. Tinea pedis b. Pityriasis versicolor
c. Sporotrichosis d. Vitiligo

19. Which of the following does not produces dermatophytosis in India? *(PGI Dec 2004)*
a. Trichophyton rubrum
b. Trichophyton mentagrophytes
c. Microsporum distortum
d. Epidermophyton floccosum

20. Which of the following fungi is/are difficult to isolate culture? *(PGI Dec 2003)*
a. Candida b. Dermatophytes
c. Cryptococcus d. Malassezia furfur
e. Coccoidiodomycosis

21. A patient made a self-diagnosis of athlete's foot (tinea pedis) and began using a product advertised on television. The condition improved but did not clear and then the patient showed himself to a Dermatologist. A skin scraping was sent to the laboratory for culture, including culture for fungi. The fungal culture yielded a slow growing colony, which produced a few small microconidia. This is consistent with isolation of a dermatophyte of the genera: *(AIIMS Nov 2003)*

a. Trichophyton b. Microsporum
c. Epidermophyton d. Trichosporon

22. T. Capitis (endothrix) is caused by: *(PGI Dec 2000)*

a. Epidermophyton b. T. tonsurans
c. T. violaceum d. Microsporum
e. T. rubrum

23. Ectothrix is due to: *(Recent MCQ 2013)*

a. Trichophyton tonsurans
b. Micosporum audounii
c. Trichophyton schoenleinii
d. Trichophyton violaceum

SUBCUTANEOUS MYCOSES

Mycetoma

24. All statements are true about mycetoma except:

a. Eumycetoma is caused by bacteria *(PGI Nov 2014)*
b. Surgery is important component of treatment
c. Usually painless
d. Diagnosis can be made by examination of lesion
e. Can affect lower and upper extremities

25. Yellow black granules are seen in which fungal infection: *(DNB Dec 2012)*

a. Mucormycosis b. Mycetoma
c. Aspergillosis d. Rhinosporidiosis

26. Color of granules produced by actinomycetes is: *(DNB Dec 2010)*

a. Black b. Yellow
c. Blue d. White

27. True about mycetoma: *(PGI Dec 2008, 2004)*

a. Commonly occurs in hands
b. Commonly erodes bone
c. Drains through lymphatics
d. Antibiotics has no role

28. Actinomycetoma is caused by: *(PGI Dec 2005)*

a. Actinomyces b. Nocardia
c. Streptomyces d. Madura mycosis

29. The granules discharged in mycetoma contains: *(AIIMS May 2002)*

a. Bone specules b. Fungal colonies
c. Pus cells d. Inflammatory cells

30. A farmer presents with multiple discharging sinuses in the leg not responding to antibiotics: Most likely diagnosis is: *(AIIMS May 2002)*

a. Madurella b. Actinomycetoma
c. Nocardia d. Sporothrix

31. Which of the following organisms is implicated in the causation of botryomycosis? *(PGI Dec 2001)*

a. Staphylococcus aureus
b. Staphylococcus albus
c. Pseudomonas aeruginosa
d. Streptococcus pneumonia
e. Streptococcus pyogenes

OTHER SUBCUTANEOUS MYCOSIS

32. Asteroid bodies is seen in: *(TNPG 2015)*

a. Sporotrichosis b. Blastomycosis
c. Coccidioidomycosis d. Cryptococcosis

33. The causative agent of recurrent ulcer in sub-himalayan region is:

a. Cladosporium
b. Sporothrix *(AI 2012, AIIMS Nov 2012, AIIMS 2003)*
c. Chromoblastomycosis
d. Mucor

34. Cigar body is seen in: *(NEET Pattern Based)*

a. Cryptococcosis b. Sporotrichosis
c. Histoplasmosis d. Aspergillosis

35. Sclerotic bodies are found in: *(NEET Pattern Based, DNB Dec 2010)*

a. Rhinosporidiosis b. Histoplasmosis
c. Coccidiomycosis d. Chromoblastomycosis

36. A gardener has multiple vesicles on hand and multiple eruptions along the lymphatics. Most common fungus responsible is: *(AIIMS Nov 2008)*

a. Sporothrix schenckii
b. Cladosporium
c. Histoplama
d. Candida

37. Which of the following medium is used to grow Rhinosporidium seeberi? *(Recent Question 2015)*

a. SDA b. HeLa cell line
c. Not cultivable

38. Rhinosporidium seeberi is a: *(Recent Question 2015)*

a. Fungi
b. Bacteria
c. Parasite
d. Virus

39. True about Rhinosporidium Seebri: *(PGI June 2006)*

a. Fungi
b. Bacteria
c. Ketoconazole –treatment
d. Present in coastal India

SYSTEMIC MYCOSES

40. A HIV +ve patient came with pain in the hip region. Aspiration stained with Giemsa staining shows multiple monocytes and macrophages with yeast like organism of 4-6 µm size in the cytoplasm of cells. The organism might be: *(NIMHANS 2016)*

a. Candida
b. Blastomyces
c. Histoplasma
d. Cryptococcus

41. Darling disease is caused by: *(NEET Pattern Based 2013)*

a. Histoplasma
b. Candida
c. Cryptococcus
d. Rhizopus

42. Most Common deep Mycosis in India is:

a. Histoplasmosis *(DNB June 2011)*
b. Blastomycosis
c. Coccidiomycosis
d. Cryptococcus

43. What is true about Histoplasmosis?

a. In early stage it is indistinguishable from TB
b. Culture is not diagnostic *(AIIMS May 2008)*
c. Mycelial forms are infectious form
d. Person to person spread occurs by droplet infection

44. Endemic fungal infection: *(PGI Dec 2005)*

a. Coccidioides immitis
b. Cryptococcus
c. Histoplasmosis
d. Aspergillus
e. Blastomyces

OPPORTUNISTIC MYCOSES

Candida

45. Which fungus gives germ tube test positive? *(PGI Nov 2016)*

a. *Cryptococcus*
b. *Aspergillus*
c. *Candida albicans*
d. *Candida dubliniensis*
e. *All Candida species*

46. Raynaud Braude phenomenon is seen in: *(NEET Pattern Based)*

a. Candida albicans
b. Candida pscitasi
c. Histoplasma
d. Cryptococcus

47. Most common fungal infection in non HIV infected (immunocompetent) persons is: *(AI 2011)*

a. Candida
b. Cryptococcus
c. Mucor
d. Aspergillus

48. A vitreous aspirate from a case of metastatic endophthalmitis on culture yields Gram-positive round to oval cells, 12-14 µm in size. The aspirate on Gram staining shows the presence of pseudohyphae. Which of the following is the most likely etiological agent? *(AI 2006)*

a. Aspergillus
b. Rhizopus
c. Candida
d. Fusarium

49. A HIV positive female presents with an indurated ulcer over the tongue. Laboratory findings show growth in cornmeal agar at 20 degree celcius, microscopy showing hyphae and growth in human serum at 37 degree celcius show budding yeasts. The probable cause is: *(AIIMS May 2001)*

a. Candida albicans
b. Histoplasmosis
c. Blastomycosis
d. Coccidioidomycosis

50. MC fungal infection in febrile neutropenia is: *(AI 2001)*

a. Aspergillus niger
b. Candida
c. Mucormycosis
d. Aspergillus fumigatus

51. Which fungal infection spreads in neonates through care giver's hands? *(AIIMS May 2014)*

a. Candida albicans
b. Candida parapsilosis
c. Candida tropicalis
d. Candida glabrata

CRYPTOCOCCUS

52. A HIV+ve patient with CD4 Count 20 cells/mm^3 came with complaints of headache, mild neck stiffness and weakness suspected Meningitis, lumbar puncture revealed yeast with no pseudohyphae it might be: *(NIMHANS 2016)*

a. Candida
b. Cryptococcus
c. Aspergillus
d. Mucor

53. The most sensitive and rapid test for diagnosis of cryptococcal meningitis is: *(MHPG 2015)*

a. Culture on SDA
b. Culture on Bird Seed Agar
c. India Ink
d. Latex Agglutination

54. Budding is seen with: *(NEET Pattern Based)*

a. Cryptococcus and Candida
b. Candida and Rhizopus
c. Rhizopus and Mucor
d. Candida and Aspergillus

55. The capsule of Cryptococcus neoformans in a CSF sample is best seen by: *(AI 2005)*

a. Gram stain
b. India ink preparation
c. Giemsa stain
d. Methanamine –silver stain

56. The most common organism amongst the following that causes acute meningitis in an AIDS patient is: *(AI 2005)*

a. Streptococcus pneumoniae
b. Streptococcus agalactiae
c. Cryptococcus neoformans
d. Listeria monocytogenes

57. Cryptococcus can be readily demonstrated by: *(PGI June 2002)*
a. Albert's stain
b. India ink stain
c. Giemsa stain
d. Gram's stain
e. Z-N stain

58. Neurotropic fungus is /are: *(PGI Dec 2002)*
a. Cryptococcus neoformans
b. Histoplasmosis
c. Trichophyton
d. Candida
e. Aspergillosis

59. Latex agglutination study of the antigen in CSF helps in the diagnosis of: *(AI 2000)*
a. Cryptococcus
b. Candidiasis
c. Aspergillosis
d. Histoplasmosis

60. Cryptococcal meningitis is common in: *(PGI 2000)*
a. Renal transplant recipient
b. Agammaglobulinemia
c. Neutropenia
d. IgA deficiency

ASPERGILLUS

61. The following are main diagnostic criteria for allergic bronchopulmonary aspergillosis except: *(APPG 2015)*
a. Pulmonary infiltrates
b. Distal bronchiectasis
c. Peripheral eosinophilia
d. Bronchial asthma

62. Drugs used in treatment of Aspergillosis: *(PGI June 2011)*
a. Itraconazole
b. Voriconazole
c. Clotrimazole
d. Amphotericin B
e. Ketoconazole

63. Aflatoxins is produced by: *(AI 2011, DNB Dec 2009)*
a. Aspergilus flavus
b. Aspergilus niger
c. Candidia
d. Cryptococcus/Nocardia

64. A 25 years old female complains of recurrent rhinitis, nasal discharge and bilateral nasal blockage since 2 years. She has history of asthma and allergy. On examination multiple ethmoidal polyps are noted with mucosal thickening and impacted secretions in both the nasal cavities. Biopsy is taken and the material is cultured which shown the growth of many hyphae and pseudhohyphae with dichotomous branching typically at 45. Which of the following is the most likely responsible organism? *(AI 2010, AIIMS Nov 2003)*
a. Aspergillus fumigatus
b. Rhizopus
c. Mucor
d. Candida

65. In HIV infected individual Gram stain of lung aspirate shows yeast like morphology. All of the following are the most likely diagnosis except: *(AIIMS Nov 2005)*
a. Candida tropicalis
b. Cryptococcus neoformans
c. Pencillium marneffi
d. Aspergillus fumigatus

66. In a patient, corneal scraping reveals narrow angled septate hyphae, which of the following is likely etiologic agent: *(Recent Question 2014)*
a. Mucor
b. Aspergillus
c. Histoplasma
d. Candida

67. Most common Aspergillus infection in humans: *(TNPG 2014)*
a. Aspergillus niger
b. Aspergillus flavus
c. Aspergillus fumigatus
d. Aspergillus nidulans

MUCORMYCOSIS

68. Zygomycosis is caused by: *(TNPG 2015)*
a. Aspergillus
b. Candida
c. Yeast
d. Rhizopus

69. A Diabetic patient suffers from a soft tissue infection and microbiological examination reveal the infection been caused by with fungi with aseptate and broad hyphae. Which of the following fungus is responsible for this infection? *(AI 2012)*
a. Candida
b. Aspergillus
c. Pencillium
d. Apophysomyces

70. Which of the following is aseptate fungus?
a. Aspergillus *(DNB Dec 2012, NEET Pattern Based)*
b. Candida
c. Nocardia
d. Rhizopus

71. True about mucormycosis is: *(DNB June 2011)*
a. Nose is a common site
b. Diabetics is common predisposing factor
c. Common in India
d. All of the above

72. Long history and then on examination broad based aseptate hyphae was found. What is the most probable fungus responsible for this condition? *(Recent Question 2016)*
a. Mucor
b. Aspergillus
c. Penicillium

PNEUMOCYSTIS JIROVECII

73. Morphological appearance of Pneumocystis jirovecii infection of lung is best characterized by: *(JIPMER Nov 2014)*

a. Hemorrhagic and necrotizing pneumonia
b. ARDS with wide spread hyaline membrane formation
c. Interstitial pneumonitis with foamy intra-alveolar exudates
d. Bronchopulmonary abscess formation

74. Select the false statement about P.jirovecii: *(AI 2008)*

a. It is seen in only immunocompromised individuals
b. Frequently associated with CMV
c. May be associated with pneumatocele
d. Diagnosed with sputum microscopy

75. Which of the following is a fungus?

a. Klebsiella *(PGI June 2006, 07)*
b. Clostridia
c. Pneumocystis jirovecii
d. Listeria

76. A young man aged 30 years, presents with difficulty in vision in the left eye for the last 10 days. He is immunocompetent, a farmer by occupation, comes from a rural community and gives history of trauma to his left eye with vegetative matter 10–15 days back. On examinations, there is an ulcerative lesion in the cornea, whose base has raised soft creamy infiltrate. Ulcer margin is feathery and hyphae. Branching aseptate hyphae were noted. There are a few satellite lesions also. Corneal scrapping shows sickle shaped macro conidia. What should be the probable diagnosis? *(AI 2012)*

a. Fusarium
b. Aspergillus
c. Mucormycosis
d. Dermatophytes

77. Fungal toxin causing liver cancer: *(Recent MCQ 2013)*

a. Aflatoxin
b. Trichothecenes
c. Ochratoxin
d. Fumonisins

78. DOC of Pneumocystis jirovecii: *(TNPG 2014)*

a. Cotrimoxazole
b. Penicillin
c. Amphotericin B
d. Clotrimazole

EXPLANATIONS

GENERAL MYCOLOGY

1. **Ans (b) (Yeast like)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p568
   - **C.albicans is yeast like fungus showing pseudohyphae.**
2. **Ans. (a, c, d, e) ((PAS, Mucicar..,Gomori's .., Giemsa…)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 551
   - von Kossa stain is used to quantify mineralization in cell culture and tissue section.
   - Refer Chapter review
3. **Ans (b) (Phycomycetes)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p550
   Lower fungi which have nonseptate hyphae and which form sporangiospores belong to zygomycetes or phycomycetes.
4. **Ans (b) (37°C)** Ref: ApurbaSastry's Essentials of Medical Microbiology 1/e p449
5. **Ans. (d) (Deuteromycetes)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p549, Ananthanarayan 9/e p590
   - Most of the fungi of medical importance belongs to: Deuteromycetes or fungi imperfectii (sexual spore is not found yet).
6. **Ans. (c) (Cryptococcus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p549, Ananthanarayan 9/p590
   Cryptococcus is the only true yeast which is pathogenic.
7. **Ans. (b) (Silver methenamine stain)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p551, Harrison 18/p1639
   - The stains most commonly used to identify fungi are periodic acid–Schiff and Gomori methenamine silver. Harrison 18/e p1637
   - Masson Trichrome stain used for stool sample to demonstrate the parasites.
8. **Ans. (b) (Gomori...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p551, Jagdish Chander 3/e p519
   - Gomori methamine silver is a better fungal stain than others because.
   - It stains both live and dead (degenerated) fungus as compared to PAS which stains only live fungi.
   - It also stains Actinomycetes and cyst of Pneumocystis jerovecii and algae (Prototheca).
9. **Ans. (c) (Cryptococcus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p550, Jagdish Chander 3/e p29
   - Dimorphic Fungi (Thermal Dimorphism)- Mycelial forms at 25°C and as yeast forms at 37°C, e.g. Coccidioides immitis, Paracoccidioides brasiliensis, Histoplasma capsulatum, Blastomyces dermatitidis, Penicillium marneffi and Sporothrix schenckii.
10. **Ans. (a) (Rhinosporidium...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p550, Ananthanarayan 9/e p603
    - Not cultivable Fungi-Rhinosproridium, Pneumocystis jerovecii and Locazia
    - R.seeberi cannot be grown on artificial culture media; however it grows in vivo in epithelial carcinoma cell culture lines.
11. **Ans. (a) (Arthrospores)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p550, Ananthanarayan 9/e p590-91
    - Arthrospores are asexual spores that are formed along the mycelium/hyphae by segmentation and condensation of hyphae.
    - Detail- Refer text
12. **Ans. (d) (PAS...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p551, Jagadish Chander 3/e p518
    - PAS: Very useful for demonstrating fungi in tissues, as they are stained darker than surrounding tissues.
    - The nuclei stained blue and fungi magenta color.
    - It stains only the live fungi.
13. **Ans. (a) and (c) (Mucicarmine – Cryptococcus and Methanamine silver -Histoplasma)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p551, Jagadish Chander 3/e p518, 519

Fungus	Stains used
Cryptococcus	For Capsule: India ink/Nigrosin stain, and Alcian blue For melanin: Masson Fontana For cell wall: Mayer's mucicarmine For biopsy: H and E, Methanamine silver, PAS, Calcofluor white
Candida	Gram stain, Calcofluor white H and E (tissue biopsy specimens)
Histoplasma	Geimsa, PAS and Methanamine silver
Pneumocystitis	Methanamine silver

14. Ans. (b) (Sporangiospores) Apurba Sastry's Essentials of Medical Microbiology 1/e p550, Ref: Jagdish Chander 3/e p25

- **Option b:** Asexual spores of Zygomycetes (lower fungi), called Sporangiospores. They are found with in swollen sac like structures called sporangium
- **Option a:** Spherules are tissue form of dimorphic fungi, Coccidioides immitis.
- **Option c** and d - Asexual fungal spores.

SUPERFICIAL MYCOSES

15. Ans. d. (Malassezia furfur) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p554

16. Ans: (b) (Most commonly occurs..) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p555

- Tinea capitis occurs most commonly in children 3–7 years old.
- Tinea capitis exist as various forms – Favus (as Cup like crust or scutula), Keroin (as boggy swelling), ectothrix and endothrix (as black dot due to hair break and alopecia).

17. Ans. (d) (T. mentagrophyte) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p555, Jagdish Chander 3/e p135

- Zoophilic species: Trichophyton mentagrophyte and Microsporum canis.

18. Ans. (a) (b) (d) (Tinea pedis, Pityriasis versicolor, Vitiligo) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p557, Jagdish Chander 3/e p135

- Wood's lamp is a device that detects fluorescence structures which gives different color.
- In fair skin individuals Wood's Lamp detects Vitiligo (and other depigmentations) even when it is not visible to the eye under normal light conditions.
- List of Wood's lamp positive fungi- Refer Chapter Review

19. Ans. (c) (M.disto...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p555, Jagdish Chander 3/e p124

- T. rubrum, T. mentagrophytes and E. floccosum-Worldwide distribution
- **Option c-** M. distortum-America and Europe, not present in India

20. Ans. (d) (Malassezia...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p553, Jagdish Chander 3/e p99-100

- It is cumbersome to grow Malassezia furfur in routine SDA, as it is a lipophilic fungi-hence lipids like oleic acids are incorporated into SDA.
- Selective culture media used: Dixon agar and modified Dixon medium.
- Other options: Candida, Dermatophytes, Cryptococcus and Coccidiodes grow on routine SDA.

21. Ans. (a) (Trichophyton) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p557, Jagdish Chander 3/e p125

- Tinea pedis is Dermatophytic infection of plantar aspect of foot
- Caused by T. rubrum, E. floccosum and T. mentagrophytes.
- It is frequently seen among individual wearing shoes for long hours.
- Warmth and moisture produced by shoes are key factors in maintaining infection.
- Trichophyton spp. generally produces abundant Microconidia with few Macroconidia and Microsporum produces few Microconidia.
- Even though in this question it is mentioned that the isolate produced few Microconidia, I feel the most appropriate answer is Trichophyton, as Microsporum does not cause T. pedis.

22. Ans. (b) and (c) (T.tonsurans and T.violaceum) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p557-8, Jagdish Chander 3/e p135

- Ectothrix infection:
  - Arthrospores appear as mosaic sheath around the hair shaft as chains, e.g. M. canis, M. gypseum M. audounii, T. mentagrophytes, T. verrucosum, T. rubrum
- Endothrix infection:
  - Arthrospores found within hair shaft, e.g. T. schoenleinii, T. tonsurans, T. violaceum T. soudanense.

23. Ans. (b) (Micosporum audounii) Ref: Ananthanarayan 9/e p597

Ectothrix hair infection	Microsporum spp, T. rubrum
Endothrix hair infection	*T. schoenleinii*, *T. violaceum*, *T. tonsurans*

SUBCUTANEOUS MYCOSES

Mycetoma

24. Ans. (a) (Eumycetoma is…) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p559-60

- Eumycetoma is caused by fungi
- Treatment of mycetoma consists of surgical removal of the lesion followed by antifungals.
- Refer chapter review for remaining options.

25. Ans. (b) (Mycetoma) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p559

- All the agents of actinomycetoma produce whitish yellow granules where as that of eumycetoma produces white or black granules.

26. Ans. (d) (White) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p559, Jagdish Chander 3/e p150

- All the agents of actinomycetoma produce white to yellow granules except Actinomadura pelletieri (red)

27. Ans. (a), (b) (Commonly occurs in hands and Commonly erodes bone) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p559, Harrison 18/e p258, 1329, 17/e p1242 and p1266

- **Mycetoma**: Slowly progressive, chronic granulomatous infection of skin and subcutaneous tissues with involvement of underlying fasciae and bone
- Affects extremities like hand and foot, which are more prone for accidental trauma.
- Occupational disease: Common in farmers and who carry goods on head and back.

Other options

- **Option c:** Incorrect, it rarely spreads through lymphatics
- **Option d :** Incorrect, Mycetoma can be caused by both Fungus (Eumycotic) and Bacteria (Actinomycotic). Actinomycotic variety can be treated by prolonged combination therapy of antibiotics like Streptomycin and Cotrimoxazole or Streptomycin and Dapsone.

28. Ans. (b) and (c) (Nocardia and Streptomyces) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p559

29. Ans. (b) (Fungal...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p559

- Discharged granules in mycetoma are micro colonies of etiological agents.
- Color of the granules differ according to the etiological agent

30. Ans. (a) (Madurella) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p559

- Multiple discharging sinuses, not responding to antibiotics suggests the diagnosis of Eumycetoma (ture fungi) and common etiological agent is Madurella mycetomatis.

31. Ans. (a) and (c) (Staphylococcus aureus and Pseudomonas aeruginosa) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p215,558

- **Botryomycosis:**
  - Chronic, purulent, granule-producing, granulomatous infection of the skin, subcutaneous tissues and visceral organs by several bacterial spp.
  - Neutrophilic reaction is present, (in Mycetoma cement substance present between the agents).
  - Clinically and histopathologically, it resembles Mycetoma
  - Treatment- susceptible to variety of antibiotics.

Causative agents

• Staphylococcus aureus	• Non-hemolytic Streptococcus
• Escherichia coli	• Bacteroides
• Pseudomonas aeruginosa	• Rodococcus
• Proteus	• Actinobacillus lignieresii

Other Subcutaneous Mycosis

32. Answer (a) Sporotrichosis Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p560, Ananthanarayan 9/e p602

33. Ans. (b) (Sporothrix) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p561, Jagdish Chander 3/e p163

- Multiple recurrent ulcers and history of sub-Himalaya region is suggestive of Sporotrichosis.

34. Ans. (b)(Sporotrichosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p561, Jagdish Chander 3/e p163

- Cigar shaped asteroid body is seen in Sporotrix schenkii infection.

35. Ans. (d)(Chro...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p561, Jagdish Chander 3/e p178

- Skin scrapings from a patient with chromoblastomycosis shows characteristic brown walled, globose bodies 5–13 μ in size, called sclerotic bodies or muriform cells/Medlar body.

36. Ans. (a) (Sporothrix...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p561, Jagdish Chander 3/e p163

- Multiple vesicles on hand and multiple eruptions along the lymphatics in a gardener suggest typical clinical manifestation of Sporotrichosis.
- Sporotrichosis caused by thermally dimorphic fungi, Sporothrix schenckii
- Infection is introduced in skin by trauma (thorn prick injury), hence Sporotrichosis also called as 'Rose Gardener's disease'. Draining lymphatic system involvement is characteristic.

37. Ans. (c) (Not cultivable) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p562

38. Ans. (b) (Parasite) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p562

Rhinosporidium seeberiis recently reclassfied as an Aquatic Protistan Parasite (Mesomycetozoa)

39. Ans. (d) (Present in coastal India) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p562

- Rhinosporidium seeberi was thought to be a fungus but based on molecular studies it is now considered to be a hydrophilic protistian parasite, hence called as pseudofungal organism.
- R.seeberi cannot be cultured in cell-free artificial media.
- Infection is acquired by coming in contact with fresh or stagnant water and hence it is believed as hydrophilic organism.
- Common in India (Tamil Nadu, Kerala and Andhra Pradesh) and Sri Lanka.

SYSTEMIC MYCOSES

40. Ans. (c) (Histoplasma) Ref: Apurba Sastry's Essentials of Medical Microbiology/p563

Case of osteomyelitis in HIV infected patient, with intracellular yeast of 4-6 μm size - most likely diagnosis is histoplasmosis.

41. Ans. (a) (Histoplasma) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p563, Jagdish Chander 3/e p212-25

- Darling's disease is the other name of histoplasmosis. Named after the scientist Samuel Taylor Darling.

42. Ans. (d) (Cryptococcus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p563, Jagdish Chander 3/e p209

- Both systemic and opportunistic fungal infections are together called as deep mycoses
- Candidiasis is the most common deep mycotic infection happens to occur in India
- Among the options, cryptococcal infection would be the most appropriate answer

43. Ans. (c) (Mycelial...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p563, Harrison 18/e p1641, 17/e p1246

- Chronic cavitary histoplasmosis is seen in smokers who have structural lung disease (e.g. bullous emphysema) is characterized upper-lobe infiltrates, cavitation, and pleural thickening- mimics cavitary tuberculosis.
- Isolation of fungi by culture and demonstration of dimorphism is diagnostic.

- Reservoir of infection: Soil
- Microconidia (spores) are the infective form and mode of infection is inhalation.
- Person to person or animal to man transmission in not known so far for Histoplasmosis.

44. Ans. (a), (c) and (e) (Coccidioides immitis, Histoplasmosis, Blastomycosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p550, Jawetz 25/e p638, 24/e p634

- **Endemic Mycosis-** Fungal infection which is confined to particular geographical area. All these fungi show thermal dimorphism and cause systemic mycosis.

Mycosis	Endemic Area
Histoplasmosis	Central and Southeastern U.S.
Blastomycosis	North America
Coccidioidomycosis	Central and South America
Paracoccidioidomycosis	Latin America (Brazil)

OPPORTUNISTIC MYCOSES

Candida

45. Ans. (c, d) (*C. albicans, C. dubliniensis*) Ref: Apurba Sastry's Essentials of Medical Microbiology/p568

- **Germ tube test is positive for both *C. albicans, C. dubliniensis***

46. Ans. (a) (Candida albicans) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p568, Jagdish Chander 3/e p270

- Raynaud Braude phenomenon is the other name of positive germ tube test
- Germ tube test is usually positive by Candida albicans, rarely by Candida dubliniensis

47. Ans. (a) (Candida) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p568, Ananthanarayan 9/e p611, 8/e p607

- Candida species in immunocompetent host can cause infection of any warm and moist parts of the body exposed to environment. It causes infection of the nail, rectum, and other skin folds.
- Among the given options Candida is most appropriate, as other options Cryptococcus, Mucor and Aspergillus are mainly isolated from opportunistic infections.

48. Ans. (c) (Candida) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p567-8, Jagdish Chander 3/e p273

- The history provided suggests that it is a case of disseminated fungal endophthalmitis
- The fungal agent has morphology of yeast cells with pseudohyphae suggests diagnosis of Candida spp
- Other options provided- Aspergillus, Rhizopus and Fusarium are molds/filamentous fungi.

49. Ans. (a) (Candida...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p567-8, Harrison 18/e p1652, 17/e p1254

- The history of oral ulcer/oral thrush in an immunocompromised (HIV) patient suggests one of the opportunistic pathogen.
- The pathogen was able to grow in cornmeal agar at 20°C and produced hyphae and produced budding yeast in human serum at 37°C which suggests the cultural characteristics of Candida albicans.
- Other options provided- Histoplasmosis, Blastomycosis and Coccidioidomycosis are all Dimorphic fungi which cause systemic mycosis. They neither produce oral ulcers in HIV patients nor hyphae on cornmeal agar.

50. Ans. (b) (Candida) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p567-8, Jagdish Chander 3/e p268 and p273

- Febrile neutropenia- Fever more than 38.5°C and absolute neutrophil count less than 500/µl.
- Neutrophils/CMI play crucial role in controlling Candida infection, hence deep seated and disseminated Candida infections are common in Neutropenic patients.

51. Ans. (b) (C. parapsilosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p567-8, EID Journal Volume 10, 2004:Candida parapsilosis Characterization in an Outbreak Setting

C. parapsilosis is increasingly responsible for hospital outbreaks, and the hands of healthcare workers may be the predominant environmental source.

CRYPTOCOCCUS

52. Ans. (b). (*Cryptococcus*) Ref: Apurba Sastry's Essentials of Medical Microbiology/p570.

Case of meningitis in HIV infected patient- most likely diagnosis is cryptococcosis.

53. Ans (d) (Latex agglutination) Ref:Apurba Sastry's Essentials of Medical Microbiology 1/e p569

Latex Agglutination test detecting capsular antigen in CSF is the most sensitive test (95%) for diagnosis of Cryptococcal meningitis

54. Ans. (a) (Cryptococcus...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p569, Ananthanarayan 9/e p590

- Cryptococcus is yeast and reproduce by budding (blastoconidium)
- Candida is yeast-like fungi; reproduce by budding and binary fission.

55. Ans. (b) (India...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p569, Ananthanarayan 9/e p613, 8/e p610

- Cryptococcus is the capsulated yeast.
- Among the given staining techniques, India ink preparation is the best staining technique used for demonstration of capsule (negative staining) - sensitivity of the technique: 60–75%.
- Other capsular staining techniques are:
  - 10% Nigrosin staining
  - Modified India ink preparation with 2% chromium mercury
  - Alcian blue staining
- **Methanamine silver and Periodic acid- Schiff** – used for tissue sample.
- **Sensitivity of various diagnostic tests- Harrison 18/e p1652**
  - Cryptococcal antigen detection in CSF—90%
  - Blood culture: 10—30% in non-HIV patients and 60% in HIV patients
  - Sputum culture: 10%
  - Sputum antigen detection: 30%

56. Ans. (c) (Cryptoco...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p569, Harrison 18/e p1650, 17/e p1282

- Cryptococcosis is the predominant cause of fatal fungal infection and causes acute... meningitis in patients with AIDS.
- It may occur most common when CD4+ count falls below 200 cells/mm^3.
- The extra pulmonary cryptococcosis is one of the AIDS defining diseases.

57. Ans. (b) (India...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p569, Ananthanarayan 9/e p613, 8/e p610

- Refer earlier explanation.

58. Ans. (a), (b), (d) and (e) (Cryptococcus neoformans, Histoplasmosis, Candida and Aspergillosis) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p569, Jagdish Chander 3/e p299, p215, 272 and 347

- **Option a** Correct: Cryptococcus neoformans- causes fatal meningitis in immunocompromised patients
- **Option b** Correct: CNS Histoplasmosis manifesting as intramedullary spinal cord abscess is very rare
- **Option c** Incorrect: Trichophyton is Dermatophyte infects only skin, hair and nail
- **Option d** Correct: Candida meningitis occurs in low-birth weight neonates with candidemia
- **Option e** Correct: Aspergillosis- serious fungal infection occurs by hematogenous dissemination from pulmonary foci of infection

59. Ans. (a) (Cryptococcus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p569, Jagdish Chander 3/e p306

- Cryptococcal antigens can be detected in CSF, serum and urine by latex agglutination test.
- Antigen titer in CSF stays for longer duration and is directly proportional to mortality of the patients.

60. Ans. (a) (Renal transplant...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p569, Harrison 18/e p1650-51 Harrison 17/e p1251 states:

- **Individuals at high risk for cryptococcosis include:**
  - Patients with hematologic malignancies
  - Recipients of solid organ transplants who require ongoing immunosuppressive therapy
  - Persons whose medical conditions necessitate glucocorticoid therapy
  - Patients with advanced HIV infection and CD4+ T lymphocyte counts of < 200/L.

ASPERGILLUS

61. Ans (b) (Distal bronchiectasis) Ref: Harrison 19 /e p1348

ABPA represents a hypersensitivity reaction to A. fumigatus; occurs in ~1% of patients with asthma and in up to 15% of adults with cystic fibrosis.

- Presents with coughing fits, pneumonia, pulmonary infiltrates, consolidation, and breathlessness
- Eosinophilia and elevated serum level of total IgE
- Central bronchiectasis is characteristic (but not distal).

62. Ans. (a) (b) (d) (Itraconazole,Voriconazole, Amphotericin B)) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p573, Harrison 18/e p1659, 17/e p1260, Katzung 10/e p830

- Antifungal drugs active against Aspergillus include voriconazole, itraconazole, posaconazole, caspofungin, micafungin, and amphotericin B.
- Voriconazole is the preferred agent for invasive aspergillosis.
- For detail- refer test.

63. Ans. (a) (Aspergillus flavus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p573, Ananthanarayan 8/e p 614

- Mycotoxin is produced by A. flavus
- It is frequently present in mouldy foods, particularly in groundnuts, corn and peas.

64. Ans. (a) (Aspergillu...) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p573, Harrison 18/e p1656, 17/e p1257

- The history provided in the question, suggests involvement of paranasal sinuses with presence of multiple ethmoidal polyps in an asthmatic patient.
- Septate hyphae with dichotomous branching typically at 45° is observed in morphology of Filamentous fungi-Aspergillus.
- All of the above points suggest patient is suffering from fungal sinusitis due to Aspergillus fumigatus.

Clinical spectrum of diseases produced by Aspergillus: Refer chapter review

65. Ans. (d) (Aspergillus fumigatus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p573, Jagdish Chander 3/e p20, p21, p347, Ananthanarayan 8/e p607, p613, 610

- **Option a:** Correct- Candida tropicalis causes infection in immunocompromised patients and has a gram-positive budding yeast-like morphology
- **Option b:** Correct- Cryptococcus neoformans- causes infection in immunocompromised patients and has a gram-positive budding yeast morphology
- **Option c:** Correct- Pencillium marneffi - causes infection in immunocompromised patients and is a Dimorphic fungus with yeast morphology in body tissues/at 37°C
- **Option d:** Incorrect- Aspergillus fumigatus- Even though can cause infection in immunocompromised patients, it is a filamentous fungi showing hyphae with dichotomous branching typically at 45°C.

66. Ans. (b) (Aspergillus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p573, Jagdish Chander 3/e p401, Harrison 18/e p1656,17/e p1257

- **Option a:** Incorrect- Mucor has aseptate hyphae and not septate
- **Option b:** Correct-Aspergillus causes fungal keratitis and has narrow angled septate branching hyphae
- **Option c:** Incorrect- Histoplasma does not cause fungal keratitis
- **Option d:** Incorrect- Candida characteristically shows budding yeast-like cell morphology and also does not cause fungal keratitis.

67. Ans. (c) (Aspergillus fumigatus) Journal: Aspergillus fumigatus and Aspergillosis, CMR/1999

A. fumigatus is the most common species, being responsible for approximately 90% of human infections due to aspergillosis

MUCORMYCOSIS

68. Ans. (d) (Rhizopus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p570, Ananthanarayan 9/e p610

69. **Ans. (d) (Apophy...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p570-71, Ananthanarayan 9/e p590, 8
    - Broad aseptate hyphe: Belongs to the family zygomycetes or Phycomycetes, e.g. Rhizopus, Mucor, Absidia, Apophysomyces
    - Narrow septate hyphae, e.g. Aspergillus (belongs to Ascomycetes), Penicillium, Fusarium

70. **Ans. (d) (Rhizopus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p570-71, Jagdish Chander 3/e p362, Ananthanarayan 9/e p610, 8/e p601-02
    Refer chapter review

71. **Ans. (d)(All of the above)** Apurba Sastry's Essentials of Medical Microbiology 1/e p570-71
    - Rhinocerebral zygomycosis is the most common and fulminant type of zygomycosis, affecting nose, paranasal sinuses, orbit and palate.
    - Risk factor- MC is Diabetes followed by patient on dialysis, iron overload and Hematopoietic stem cell transplantation
    - Mucormycosis is increasingly recognized in recently developed countries, such as India, mainly in patients with uncontrolled diabetes or trauma. …… Journal-Clin Infect Dis. 2012 Feb; 54 Suppl 1:S23-34

72. **Ans (a) (Mucor)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p571

PNEUMOCYSTIS JIROVECII

73. **Ans (c) (Interstitial...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p576, Harrison 19/e p1359
    - Pneumocystis pneumonia (Pathology): Alveoli are filled with a typical foamy, vacuolated exudate.
    - Severe disease may include interstitial edema, fibrosis, hyaline membrane formation (but not ARDS) and malnourished infants develop plasma cell infiltrate hence called as interstitial plasma cell pneumonia.

74. **Ans. (b) (Frequently associated with CMV)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p576
    - P. jirovecii infection and CMV (Cytomegalo virus) infection is common in patient with renal transplant recipients, but association with CMV infection is not clearly documented. Ref: Clinical Infectious diseases by David Schlossberg p.616

    About other options:
    - **Option a:** Correct- It is an opportunistic pathogen
    - **Option c:** Correct- The common radiographic finding in P. jirovecii pneumonia is the presence ground-glass opacity in both lungs and atypical radiographic manifestations include cystic spaces (pneumatocele) and bullae, adenopathy, pleural effusions and pneumothorax. BMC Infectious Diseases 2009, 9:171)
    - **Option d:** Correct- Lab diagnosis is established based visualisation of P. jirovecii in expectorated sputum or BAL (Ideal sample).

75. **Ans. (c) (Pneumocystis jirovecii)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p576, Harrison 18/e 1673
    Pneumocystis jerovecii is considered to be fungus based on following points:
    - It takes fungal stains like Gomori's methanamine silver stain
    - It possesses chitin at all stages of development (component of fungal cell wall)
    - Based on molecular study of 5S –rRNA.

76. **Ans. (a) (Fusarium)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p576
    - Fusarium is a hyaline filamentous fungi, may cause a range of opportunistic infections in humans.
    - In humans with normal immune systems, fusarial infections may occur in the nails (onychomycosis) and in the cornea (keratomycosis or mycotic keratitis)
    - Diagnosed by presence of sickle shaped macroconidia.

77. **Ans. (a) (Aflatoxin)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p575, Ananthanarayan 9/e p615
    - Refer chapter review.

78. **Ans. (a) (Cotrimoxazole)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 576
    - Refer chapter review.

NOTE

SECTION 6

Parasitology

CHAPTER OUTLINE

CHAPTER

6.1 General Parasitology

Parasite is a living organism, which lives in or upon another organism (host) and derives nutrients directly from it, without giving any benefit to the host.

Parasites may be Classified as

- *Ectoparasite:* Inhabits the surface of the body of the host without penetrating into the tissues (e.g. fleas or ticks). Invasion by the ectoparasite called as *infestation*.
- *Endoparasite:* Lives within the body of the host. Invasion by the endoparasite is called as *infection*.
- Obligate parasite: Cannot exist without a parasitic life in the host (e.g. *Plasmodium* spp.)
- Facultative parasite: Can live a parasitic life or free-living life. (e.g. *Acanthamoeba*).
- Accidental parasite: Infects an unusual host (e.g. *Echinococcus granulosus* infects humans accidentally).
- Aberrant parasite or Wandering parasite: It cannot develop further when infects an unusual host (larva migrans such as *Toxocara* in humans).

HOST: Harbors the parasite and provides the nourishment and shelter.

- Definitive host: Sexual cycle takes place.
- Intermediate host: Asexual cycle takes place.
- Reservoir host: Harbors and serves as an important source of infection to other susceptible hosts.
- Paratenic host: It functions as a transport or carrier host. Parasite lives but it cannot develop further and the host is not essential for its life cycle (e.g. fresh water prawn for *Angiostrongylus cantonensis,* big suitable fish for plerocercoid larva of *Diphyllobothrium latum* and Freshwater fishes for *Gnathostoma spinigerum*).

Paratenic host
functions as a transport or carrier host. Parasite lives
but it cannot develop further and the host is not essential for its life cycle

Direct/Simple life cycle: Parasites that need only one host (man)

Protozoa	Helminths
• Entamoeba histolytica • Free living ameba • Giardia lamblia • Trichomonas vaginalis • Balantidium coli • Cryptosporidium parvum • Cyclospora cayetanensis • Isospora belli • Microsporidia	Cestodes: *Hymenolepis nana* Nematodes: • *Ascaris lumbricoides* • Hookworm • *Enterobius vermicularis* • *Trichuris trichiura* • *Strongyloides* spp.

Indirect/Complex life cycle: Parasites requiring one definitive host and one intermediate host

Man acts as definitive host		
Parasites	**Definitive host (man)**	**Intermediate host**
Leishmania spp.	Man	Sandfly
Trypanosoma cruzi	Man	Reduviid bugs
Trypanosoma brucei	Man	Tsetse fly
Taenia solium (intestinal teniasis)	Man	Pig
Taenia saginata	Man	Cattle

Contd...

Opportunistic infection in HIV
- Toxoplasma gondii
- Cryptosporidium
- Cyclospora
- Isospora

Coinfection in HIV
- Leishmania donovani
- Strongyloides stercoralis

Parasites according to their Size
- **Largest human intestinal Nematode:** Ascaris lumbricoides
- **Smallest Nematode:** Trichinella spiralis
- **Largest Trematode:** Fasciolapsis buski
- **Largest protozoan parasite:** Balantidium coli
- **Largest cestodes found in human's intestine:** Diphyllobothrium latum
- **Smallest tape worm:** Hymenolepis nana

Parasites transmitted by blood transfusion: Plasmodium Babesia, Toxoplasma, Leishmania Trypanosoma

Acid Fast Parasites
- Egg and scolex of Taenia saginata
- Oocyst of Isospora belli
- Oocyst of Cyclospora
- Oocyst of Cryptosporidium parvum
- Hooklet of hydatid cyst

Contd...

Man acts as definitive host		
Parasites	**Definitive host (man)**	**Intermediate host**
Hymenolepis diminuta	Man	Rat flea
Schistosoma spp.	Man	Snail
Trichinella spiralis	Man	Pig
Filarial worms	Man	Mosquito (*Culex, Aedes, Anopheles*) and flies (blackflies and deerflies)
Dracunculus medinensis	Man	Cyclops
Plasmodium spp.	Female *Anopheles* mosquito	Man
Babesia spp.	Tick	Man
Sarcocystis lindemanni	Cats, dogs	Man
Toxoplasma gondii	Cat	Man
Echinococcus granulosus	Dog	Man
Taenia solium(Cysticercosis)	Man	Man

Indirect/Complex life cycle: Parasites requiring one definitive host and two intermediate hosts

Parasites	Definitive host	First intermediate host	Second intermediate host
Diphyllobothrium spp.	Man	Cyclops	Fish
Fasciola hepatica	Man	Snail	Aquatic plants
Fasciolopsis buski	Man	Snail	Aquatic plants
Paragonimus spp.	Man	Snail	Crab and fish
Clonorchis spp.	Man	Snail	Fish
Opisthorchis spp.	Man	Snail	Fish
Gnathostoma spinigerum	Cat, dog and man	Cyclops	Fish

Unusual/Rare Mode of Transmission

- Parasites transmitted by sexual route: *Trichomonas, Entamoeba, Giardia, Enterobius*
- Parasites transmitted by Transplacental/Perinatal route: *Plasmodium, Toxoplasma, Trypanosoma*
- Parasites transmitted by blood transfusion: *Plasmodium, Babesia, Toxoplasma, Leishmania & Trypanosoma*
- Parasites entering through conjunctiva: *Acanthamoeba spp.*

Tropical Parasitic Diseases

Food and waterborne	Soil-transmitted	Vector-borne
Entamoeba histolytica	*Ascaris lumbricoides*	*Plasmodium* spp.
Giardia lamblia	*Ancylostoma duodenale*	*Leishmania donovani*
Cryptosporidium parvum	*A braziliense*	*Wuchereria bancrofti*
Isospora belli	*A caninum*	*Brugia malayi*
Cyclospora cayetanensis	*Trichuris trichiura*	*Onchocerca volvulus*
	Strongyloides stercoralis	*Trypanosome brucei*
		Trypanosoma cruzi

MULTIPLE CHOICE QUESTIONS

1. Which of the following parasite can enter through intact skin? *(DNB Dec 2011)*
a. Giardia
b. Whip worm
c. Strongyloides
d. Trichinella

2. Which among the following does not enter human body via skin? *(DNB Dec 2010)*
a. Ancyclostoma
b. Strongyloides
c. Trichinella
d. Necator

3. Which worm is longest: *(SGPGI 2009)*
a. T. solium
b. T. saginata
c. Hookworm
d. A. lumbricoides

4. Simple life cycle is seen in: *(PGI Dec 2006)*
a. Ascaris
b. T. solium
c. Toxoplasma
d. Giardia
e. Schistosoma

5. Aedes aegypticus transmits: *(PGI June 2004)*
a. JE
b. KFD
c. Yellow fever
d. Filaria
e. Dengue

6. Fish acts as intermediate host in: *(PGI June 2004)*
a. D. latum
b. Clonorchis sinensis
c. H. diminuta
d. H. nana

7. Two hosts are required in: *(PGI Dec 2002)*
a. T. solium
b. E. histolytica
c. T. saginata
d. Giardia
e. Toxoplasma

8. Culex mosquito is associated with the transmission of: *(PGI Dec 2001)*
a. Malaria
b. Filariasis
c. Dengue
d. Japanese encephalitis
e. Typhus

9. Pigs are reservoir for: *(AI 2000)*
a. T. solium
b. T. saginata
c. Trichinella spiralis
d. Ancyclostoma

10. Definitive host is one: *(PGI 2000)*
a. In which sexual multiplication takes place
b. In which asexual multiplication takes place
c. Harbors adult form
d. Harbors larval form

EXPLANATIONS

1. **Ans (c) (Strong...)** Ref: Apurba Sastry's Essentials of Medical Parasitology, 1/e p5
   Mode of transmission:
   - Giardia: Feco-oral route (infective form: cyst)
   - Whip worm: Feco-oral route (infective form: embryonated eggs)
   - Strongyloides: Skin penetration route (infective form: L3 filariform larva)
   - Trichinella: By ingestion of raw or uncooked pork containing First stage (L1) larva

2. **Ans (c) (Trichinella)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p5
   - Mode of transmission of Hookworm (Ancyclostoma and Necator): Skin penetration route (infective form- L3 filariform larva)
   - Other options: Refer previous explanation

3. **Ans. (b) (T.saginata)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p167
   - *T. solium*- adult worm measures 2–3 meters in length
   - *T. saginata*- adult worm measures **5–10 meters** in length
   - *Hookworm*- adult worm measures 12 cm
   - *A. lumbricoides* adult worm 20–30 cm

4. **Ans. (a), (d) (Ascaris, Giardia)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p6-7
   - Direct/Simple life cycle: Parasites that need only one host (man)- Refer table from chapter review

5. **Ans. (c), (d) and (e) (Yellow fever, Filaria and Dengue)** Ref: Park 22/e p712, Apurba Sastry's Essentials of Medical Parasitology 1/e p315
   - *Option a:* JE caused by Culex mosquito
   - *Option b:* KFD-Tick borne
   - *Option c, d,* and *e:* Caused by bite of Aedes aegyptii

6. **Ans. (a) and (b) (D. latum and Clonorchis sinensis)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p163, 207
   - ***Fish acts as intermediate host:*** *Diphyllobothrium latum, Clonorchis sinensis, Paragonimus westermani* (Crab fish), *Metagonimus spp., Heterophyes heterophyes*
   - In case of H. nana Human, rat and mouse acts as both definitive and intermediate host, no intermediate host.
   - In case of *H. diminuta*: Flea acts as intermediate host.

7. **Ans. (a), (c) and (e) (T. solium, T. saginata and Tox...)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p6-7
   - In case of T. solium: Definitive host: Man and intermediate—Pig and man
   - In Case of Entamoeba and Giardia infection: Only single host (man) required to complete life cycle.
   - In case of T. saginata: Definitive host: Man and intermediate—Cattle
   - In case of Toxoplasma: Definitive host: Cat and intermediate—Man and other mammals

8. **Ans. (b) and (d) (Filariasis and Japanese encephalitis)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p315
   - ***Option a:*** Malaria transmitted by – Anopheline mosquito
   - ***Option b*** *and* ***d:*** Filariasis and Japanese encephalitis transmitted by – Culex mosquito
   - ***Option c:*** *Dengue* transmitted by – Aedes aegypti.
   - ***Option e:*** Typhus transmitted by - Louse and Flea – Apurba Sastry's Essentials of Medical Microbiology 1/e p488

9. **Ans. (a) and (c) (T. solium and Trichinella spiralis)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p6-7
   - Both ***T. solium*** and ***Trichinella spiralis*** infection is acquired by ingestion of contaminated pork, (Pigs are reservoir hosts).

10. **Ans. (a) and (c) (In which sexual... and Harbors...)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p6

CHAPTER

6.2

Intestinal Amebae, Free-Living Ameba and Balantidium Coli

INTESTINAL AMEBAE : ENTAMOEBA HISTOLYTICA

Habitat: Large intestine of man

Morphology-3 stages: Trophozoites, Pre-cyst and cyst.

Life cycle

- Host: Humans are the only host
- Infective form: Mature quadrinucleate cyst
- Mode of transmission: Faeco-oral route and rarely sexual transmission (20–30% in homosexuals)
- Excystation occurs in small intestine → eight small metacystic trophozoites are released→ carried to large intestine → trophozoites colonize the GIT mucosa → depending on nutritional status and host immunity, there may have different courses (i) Asymptomatic cyst passers, (ii) Amebic dysentery, (iii) Amebic liver abscess.
- Encystation: Occurs in large intestine → first precyst → immature cysts and→ later mature quadrinucleated cysts are released in the feces (**diagnostic form**).

E.histolytica:
Infective form and diagnostic form – Mature quadrinucleate cyst

Virulence Factors

- Amebic Lectin antigen: Helps in adhesion
- Cysteine proteinase Amebapore, Hydrolytic enzymes, Neuraminidase and metallocollagenase

Intestinal Amebiasis

- Males and females are affected equally with a ratio of 1:1
- Amebic ulcer:
  - Flask-shaped (broad base with a narrow neck)
  - Most common site: Ileocecal region
- Complications of intestinal amebiasis:
  - Fulminant amebic colitis
  - Amebic appendicitis
  - Intestinal perforation and amebic peritonitis
  - Toxic megacolon and Intussusception
  - Perianal skin ulcers
  - Ameboma (Amebic granuloma): Diffuse pseudotumor like mass found in recto-sigmoid region, seen in chronic amebiasis.

Intestinal Amebiasis
Amoebic ulcer:
- Flask shaped (broad base with a narrow neck)
- Most common site: Ileo-cecal region

Amebic Liver Abscess

- MC site involved: Posterior superior surface of right lobe of liver
- Sex: Male:female ratio is 9:1
- ***Anchovy sauce pus:*** Liver abscess pus is thick chocolate brown color
- Other organs affected**:** Lung, brain, skin.

Amoebic Liver Abcsess
- MC site involved: Posterior – superior surface of right lobe of liver.
- Anchovy sauce pus: Liver abscess pus is thick chocolate brown color

Laboratory Diagnosis

- Minimum of three stool samples on consecutive days: As amebae are shed intermittently.
- **Stool Microscopy-**done to demonstrate:
  - Trophozoites: Indicates active infection
  - Quadrinucleated cysts: Indicates carrier state
- Cyst and trophozoites of E.histolytica should be differentiated from E.coli which is a commensal in stool.
- **Stool culture**

Laboratory Diagnosis:
- Stool Microscopy: Done to demonstrate
- Trophozoites: Indicates active infection
- Quadrinucleated cysts: Indicates carrier state

- **Polyxenic culture:**
  - Contains bacterial supplement providing nourishment to ameba.
  - Used in chronic and asymptomatic carriers passing less number of cysts.
  - 50–70% sensitivity and 100% specificity (gold standard).
  - Various culture media used are:
    - National Institute of Health (NIH) media
    - Boeck and Drbohlav egg serum medium containing Locke's solution
    - Balamuth's medium, Nelson's medium and Robinson's medium.
- **Axenic culture:** It lacks bacterial supplement, e.g. Diamond's medium. Axenic culture is useful when the bacterial flora interferes with the test results such as:
  - Studying pathogenicity of ameba
  - Testing antiamebic drug susceptibility
  - Preparation of amebic antigen in mass for serological tests
  - For harvesting the parasite to determine the zymodeme pattern.
- **Stool antigen detection (copro-antigen):** By ELISA or ICT detecting lectin antigen
- ***Amebic antigen*** in serum indicates recent and active infection. *ELISA* is done for lectin antigen
- ***Amebic antibody:*** Serum antibodies appear only in the later stages of intestinal amebiasis, e.g. ELISA, IFA, indirect hemagglutination test (IHA).
- Zymodeme Analysis: Detecting isoenzyme markers like malic enzyme, hexokinase, isomerase.
- Nested multiplex PCR
- Charcot Leyden crystal in stool and moderate leucocytosis in blood.

Laboratory Diagnosis of Amebic Liver Abscess

- Microscopy and Histopathology of liver pus shows trophozoites but never cyst. This is specific but of low sensitivity (< 25%)
- Stool microscopy and culture is not useful
- Lectin antigen is usually absent in stool but can be demonstrated in serum, liver pus and saliva
- Antibody detection: Antibodies are often elevated and can be detected by ELISA and IHA. However, antibodies persist even after the cure, so it cannot differentiate recent and old infection.
- PCR done on amebic liver pus
- USG of liver shows the site of the abscess and its extension.

Characteristics of trophozoites and cysts of common intestinal *Entamoeba* species

Trophozoite	**Entamoeba Histolytica**	**Entamoeba Coli**
Size	15–20 µm	20–25 µm
Motility	Very active, unidirectional purposeful motility. Pseudopodia with finger like projection	Sluggish, nonpurposeful, aimless motility Blunt pseudopodia
Cytoplasm	Differentiated to ectoplasm and endoplasm	Not clearly differentiated
Cytoplasm has-	RBC, leucocytes, tissue debris and bacteria	Same except it does not contain RBC
Nucleus	Karyosome is small and central Nuclear membrane is thin and lined by fine chromatin granules.	Karyosome is large and eccentric Nuclear membrane is thick and lined by coarse chromatin granules.
Cyst	**Entamoeba Histolytica**	**Entamoeba Coli**
Size	12–15 µm, round	15–25 µm, round
Nucleus	Same as trophozoite, 1–4 in no.	Same as trophozoite, 1–8 in no.
Chromatoid body	Present in immature cysts Thick bars with rounded ends	Present in immature cysts Filamentous and thread like ends.
Glycogen mass	Present in immature cysts	Present in immature cysts

E. Histolytica Comprises of Three Subspecies

- *E. histolytica* subspecies *histolytica, E. h. dispar* and *E. h. moshkovskii*
- Cysts and trophozoites of all three subspecies are morphologically identical.
- All colonize the large intestine, however, only E. h. histolytica causes invasive disease
- Differentiated by:
  - Lectin antigen (found only in E. h. histolytica)
  - RBC inside trophozoites (sign of invasion, found only in E. h. histolytica)
  - PCR
  - Distinct isoenzyme markers by zymodeme study.

Treatment

- Amebic dysentery or Amebic Liver Abscess: Tinidazole and Metronidazole
- Luminal Infection: Paromomycin and Iodoquinol

E. Histolytica has three sub-species
E.h. histolytica and E.h. dispar and E. h. moshkovskii.

Cysts and trophozoites of all: Morphologically identical.

FREE-LIVING AMEBAE

Character	Naegleria Fowleri	Acanthamoeba
Disease	Primary amebic meningoencephalitis	Granulomatous amebic encephalitis Ulcerative keratitis
Risk factor	Swimming in contaminated water	Immunodeficiency
Infective form	Trophozoites	Trophozoites and cysts
Transmission	Respiratory mode	Respiratory mode or rarely skin
Clinical course	Acute	Subacute to Chronic
Pathology	Diffuse suppurative changes	Focal granulomatous inflammation
Trophozoites	Two forms, ameboid and flagellated form Blunt pseudopodium (lobopodia) 8–15 µm size	One form, no flagellated form Thorn like pseudopodium (acanthopodia) 15–25 µm size
Cyst	Not present in tissue or CSF Small (7–15 µm), thick smooth double wall	Can be found in tissue or CSF Larger (12–20 µm), thin wrinkled double wall
Spread	Direct neural spread	Hematogenous spread
CSF Leukocytes	Neutrophils	Lymphocytes
Culture	Require bacterial supplement Do not grow with > 0.4% NaCl	Do not require bacterial supplement Not affected by NaCl
CT scan	Unremarkable, no specific feature	Space occupying lesion seen
Diagnostic form in CSF	Trophozoites	Trophozoites and cyst
Climate	Temperate	Tropics

Naegleria Fowleri
Primary amebic meningoencephalitis
Acanthamoeba
Granulomatous amebic encephalitis
Ulcerative keratitis

Other Free Living Ameba

- *Balamuthia mandrillaris:* Also causes granulomatous amebic encephalitis (GAE) and skin lesion
- *Sappinia diploidea*.

BALANTIDIUM COLI

- Only ciliate parasite of humans: Trophozoite is ciliated
- Largest protozoa invading human intestine
- Site involved: Large intestine
- Infective form: Cysts in contaminated food or drink
- Both trophozoite and cyst are ***bi-nucleated***: Macronucleus and Micronucleus
- Trophozoites divide by both binary fission and conjugation
- Dysentery: Ulcers mimic amebic ulcers but never invade muscular layer
- Diagnosis: Detection cyst, rarely trophozoites
- Treatment: Tetracycline – DOC.

Balantidium Coli
Only ciliate parasite of humans: Trophozoite is ciliated.

Both trophozoite and cyst are binucleated: Macronucleus and Micronucleus.

MULTIPLE CHOICE QUESTIONS

1. All are true about Entamoeba histolytica Except: *(AIIMS Nov 2014)*
a. Stool trophozoites are essential for diagnosis
b. Mostly (85%) are asymptomatic
c. Cause disease in brain
d. Cause disease in liver

2. Which of the following statement about amoebiasis is False: *(Recent Question 2015)*
a. Amoebic dysentery: Flask shaped ulcer
b. Entamoeba coli can be pathogenic
c. Liver is the MC extra intestinal site

3. The virulence factor that is responsible for adherence of Entamoeba histolytica to intestinal mucosa is:
a. Ionophore-like protein *(APPG 2014)*
b. Amebiclectin
c. Phosphatase
d. Proteinase

4. The most common site for Amebiasis is: *(MHPG 2014)*
a. Sigmoid colon
b. Transverse colon
c. Cecum
d. Hepatic flexure

5. Investigation of choice for amebiasis is:
a. ELISA *(DNB Dec 2012)*
b. Colonoscopy
c. Microscopy
d. Microscopy + ELISA

6. Number of nucleus present in mature cyst of E. histolytica is: *(DNB Dec 2011)*
a. 1 b. 2
c. 4 d. 8

7. Most common cause of dysentery in adults is:
a. Crypto parvum *(DNB Dec 2011)*
b. Giardia
c. Strongoloides
d. Entamoeba histolytica

8. Treatment given to Entamoeba cyst carriers is:
a. Metronidazole *(DNB June 2009)*
b. Diloxanidefuroate
c. Paromomycin
d. Nitzoxzanide

9. Largest protozoan is: *(MHPG 2015, DNB Dec 2009)*
a. E. Histolytica
b. Balantidium coli
c. E. Coli
d. Plasmodium

10. A patient presents with lower gastrointestinal bleed. Sigmoidoscopy shows ulcer in the sigmoid. Biopsy from this area shows flask–shaped ulcers. Which of the following is the most appropriate treatment:
a. Intravenous ceftriaxone *(AIIMS 2005)*
b. Intravenous metronidazole
c. Intravenous steroids and sulfasalazine
d. Hydrocortisone enemas

11. Which one of the following statements are false:
a. The presence of ingested erythrocytes is seen only in Entamoeba histolytica *(AIIMS Nov 2003)*
b. Young adult male of low socioeconomic status are most commonly affected by invasive amebiasis
c. A low iron content in the diet predisposes to invasive amebiasis
d. The pathogenic and nonpathogenic strains of E. histolytica can be differentiated by the electrophoretic study of zymodemes

12. True about amebic colitis is: *(PGI June 2002)*
a. Caused by E.histolytica
b. Cyst contains eight nuclei
c. Flask-shaped ulcers are present
d. Cecum is most commonly affected
e. Is premalignant

13. Invasive amebiasis can be best diagnosed by:
a. ELISA *(AIIMS Nov 2001)*
b. Countercurrent immunoelectrophoresis
c. Indirect hemaglutination test
d. Complement fixation test

14. Which is not causing neurodegenaration?
a. Balamuthia *(Recent Question 2015)*
b. Iodamoeba
c. Naegleria
d. Entamoeba

FREE-LIVING AMEBA

15. Most common organism causing keratitis infection in soft contact lens users is: *(JIPMER Nov 2015)*
a. Naegleria
b. Acanthamoeba
c. Gonococcus
d. Staphylococcus aureus

16. Acute meningoencephalitis is caused by:
a. Acantamoeba *(DNB Dec 2011)*
b. Naegleria
c. Meningococcus
d. Balmuthia

Section 6

17. A patient following use of contact lens, developed corneal ulcers and symptoms of conjunctivitis. Saline mount preparation of corneal scrapping shows polygonal cyst. What should be the probable diagnosis? *(AI 2012)*

a. Acanthamoeba
b. Naegleria
c. Entamoeba
d. Giardia

18. Acute Primary Amebic meningoencephalitis true is: *(AIIMS May 2008)*

a. Meningitis caused by Acanthamoeba species is acute in nature
b. Diagnosis is by demonstration of trophozoite in CSF
c. Caused by feco-oral transmission
d. More common in tropical culture

19. A 30-year-old patient presented with features of acute meningoencephalitis in the casualty. His CSF on wet mount microscopy revealed motile unicellular microorganisms. The most likely organism is: *(AIIMS May 2005)*

a. Naegleria fowleri
b. Acanthamoeba castellani
c. Entamoeba histolytica
d. Trypanosoma cruzi

20. Parasitic Encephalitis is caused by: *(PGI Dec 2005)*

a. Naegleria
b. Acanthamoeba
c. Balamuthia
d. Gnathostoma

21. Girl visits her friend's village, develops nasal discharge, meningitis and dies in 5 days. Organism responsible is: *(AIIMS Nov 2014)*

a. Naegleria fowleri
b. Acanthamoeba
c. Toxoplasma
d. P. falciparum

EXPLANATIONS

1. **Ans. (a) (Stool trophozoites are essential for diagnosis)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p29-34
   - Stool trophozoites are usually found in acute stage of amebic dysentery and indicate active infection but they soon disappear and are not found in later stage of dysentery or carriers or in invasive amebiasis.
   - Hence stool trophozoites are NOT essential for diagnosis; however their presence confirms active stage of amebic dysentery.
   - Confirmation of the diagnosis can also be done by amebic PCR or detection of lectin antigen in stool.
   - Other options:
     - Entamoeba histolytica infection is mostly asymptomatic
     - Invasive amebiasis: Liver is the most common site, but can also infect other sites such as lungs and brain.
2. **Ans. (b) (Entamoeba coli...)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p37
3. **Ans. (b) (Amebic lectin)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p29
   - Amebic lectin antigen is the principle virulence factor of Entamoeba histolytica that is responsible for adherence to large intestinal mucosa.
4. **Ans. (c) (Cecum)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p28

   The most common site for Amebiasis is ileocecal region followed by Sigmoid colon or may be generalized involving whole of large intestine.
5. **Ans. (d) (Microscopy + ELISA)** Ref: Harrison 18/e p1685, Apurba Sastry's Essentials of Medical Parasitology 1/e p34
   - *'Microscopy, often combined with serologic testing, remains the standard diagnostic approach in many hospitals and clinics worldwide for the diagnosis of amebiasis.'*
   - **Stool Microscopy —** done to demonstrate:
     - Trophozoites: Indicates active infection
     - Quadrinucleated cysts: Indicates carrier state
   - Microscopy is poorly sensitive (25–60% with single sample) but the sensitivity increases to 85–95% when three stool samples are examined. It cannot differentiate between *E. histolytica* and other *Entamoeba histolytica* subspecies such as *E. dispar* and *E. moshkovskii*.
   - *ELISA* detecting 170 kDa of lectin antigen in stool shows > 95% sensitivity and specificity. It can also differentiate pathogenic *E. histolytica* (lectin antigen positive) and nonpathogenic *E.dispar* ((lectin antigen negative).
   - Polyxenic culture: 50–70% sensitivity and 100% specificity (gold standard). Serves as a research tool but not available for clinical use.
   - PCR assay for DNA in stool samples is currently the most sensitive and specific method for identifying *E. histolytica* infection and has become a valuable epidemiologic and research tool.
6. **Ans. (c) (4)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p26
   - Mature cyst of E. histolytica: Contains four nuclei
   - Mature cyst of E. coli: Contains eight nuclei
7. **Ans. (d) (Entamoeba...)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p31
   - Entamoeba histolytica is one of the commonest cause of dysentery in man
   - Other Agents dysentery include: Bacterial dysentery (e.g. Shigella and Campylobacter and Vibrio parahemolyticus), Parasitic causes (schistosomiasis, Trichuris and Balantidium coli)
   - In Cryptosporidium parvum, Giardia and Strongoloides infection: Diarrhea is the chief manifestation (not dysentery).
8. **Ans. (c) (P*aromomycin)*** Ref: Harrison 18/e p1686, Apurba Sastry's Essentials of Medical Parasitology 1/e p35-36
   - *Luminal agent (paromomycin or iodoquinol) are given to asymptomatic cyst passers to ensure eradication of the infection Paromomycin is the preferred agent.*
   - *Asymptomatic individuals infected with E. histolytica should be treated because of the risks of developing amebic colitis or amebic liver abscess and of transmitting the infection to other*
   - Amebic dysentery or Amebic Liver Abscess: DOC is Tinidazole and Metronidazole.

9. **Ans. (b) (Balantidium coli)** Ref: Harrison 18/e p1733, Apurba Sastry's Essentials of Medical Parasitology 1/e p146
   - *Balantidium coli,* the largest protozoan and the only ciliated parasite of humans.
   - *Balantidium coli* is a large ciliated protozoal parasite that can produce a spectrum of large-intestinal disease analogous to amebiasis.
   - Trophozoite of B.coli: Oval shaped 30–300 µm in length and 30–100 µm in breadth.

10. **Ans. (b) (Intraveno...)** Ref: Harrison 18/e p1686, 17/e p1278, Apurba Sastry's Essentials of Medical Parasitology 1/e p28, 35
    - The given history of lower GIT bleed with '*classical flask shaped ulcers in Sigmoid colon*' suggests intestinal Amebiasis caused by *E. histolytica.*
    - The localized amebic ulcers are commonly present in the Ileocaecal region *and* less commonly in sigmoido-rectal region.
    - Treatment of intestinal Amebiasis: Tinidazole, 2 g/d PO with food for 3 days Metronidazole (750 mg tid PO or IV), for 5–10 days
    - Tinidazole appears to be better tolerated and slightly more effective than metronidazole for amebic colitis and amebic liver abscess.

11. **Ans. (c) (A low...)** Ref: Harrison 18/e p1684-85, 17/e p1275, Apurba Sastry's Essentials of Medical Parasitology 1/e p32
    Refer explanation given below:
    - ***Option a-*** Correct: RBCs, occasionally leucocytes and tissue debris found inside the endoplasm of Entamoeba histolytica trophozoite. Ref: KD Chatterjee 13/e p19
    - ***Option b-*** Correct: Amebiasis occurs *most* frequently in *adult males*, **children**, people with *low socioeconomic status*. This may be due to poor living conditions, over crowding and unhygienic practices which leads to fecal contamination of food and drinks which help in transmission of ameba. ***Ref:*** Journal of Teachers Association June 2008; Vol21 N1
    - ***Option d-*** Correct: The subspecies of E. histolytica have distinct surface antigens and isoenzyme markers, which can be differentiated by the electrophoretic study of zymodemes. Harrison 18/e p1684-85, 17/e p1275
    - ***Option c-*** Incorrect: Relation between low iron content and invasive amebiasis is not documented any where, probably is the wrong statement.

12. **Ans. (a), (c) and (d) (Caused by E. histolytica, Flask-shaped ulcers are present *and* Cecum is most commonly affected)** Ref: Harrison 18/e p1683, 17/e p1278, KD Chatterjee 13/e p22, Apurba Sastry's Essentials of Medical Parasitology 1/e p28-31
    - ***Option* a, c** and ***d-*** correct: Already explained
    - ***Option b-*** Incorrect: Each mature cyst contains **4 nuclei** (tetranucleate ameba)
    - ***Option e-*** Amebic colitis leading to carcinoma of colon not documented.

13. **Ans. (a) (ELISA)** Ref: Harrison 18/e p1685, Apurba Sastry's Essentials of Medical Parasitology 1/e p35
    - The diagnosis of invasive amebiasis like amebic liver abscess is based on the detection (generally by ultrasound or CT) of one or more space-occupying lesions in the liver and a positive serologic test for antibodies to E. histolytica antigens.
    - ELISA is now replacing IHA and has reported sensitivity of 90% and specificity of 85%.

14. **Ans. (b) (Iodamoeba)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p39

FREE LIVING AMEBAE

15. **Ans. (b) (Acanthamoeba)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p44
    Acanthamoeba causes keratitis in soft contact lens users.

16. **Ans. (b) (Naegleria)** Ref: Harrison 18/e p1686-87, Apurba Sastry's Essentials of Medical Parasitology 1/e p44
    - *Naegleria fowleri: Agent of Primary Amebic meningoencephalitis*
    - *Acanthamoeba: Agent of granulomatous amebic encephalitis.*

17. **Ans. (a) (Acanthamoeba)** Ref: Harrison 18/e p1687, Parija's Parasitology 3/e p53, Apurba Sastry's Essentials of Medical Parasitology 1/e p44
    - Acanthamoeba keratitis is associated with corneal injuries complicated by exposure to water or soil and with the wearing of contact lenses.
    - ***Acanthamoeba* keratitis:** Risk factors:
      - Extended wear of lens
      - Breaches in hygiene and disinfection procedures
      - Swimming with contact lenses in place
      - Use of homemade saline solutions contaminated with *Acanthamoeba*.

18. Ans. (b) (Diagnosis...) Ref: K.D.Chatterjee 13/e p45, Apurba Sastry's Essentials of Medical Parasitology 1/e p41-42

Primary Amebic meningoencephalitis

- Caused by *Naegleria fowleri*, which is acute and suppurative in nature (*Granulomatous amebic encephalitis* caused by Acanthamoeba species is chronic in nature)
- Diagnosis is by demonstration of trophozoite in CSF (GAE is diagnosed by demonstration of both Acanthamoeba trophozoite and cyst in CSF)
- Transmitted by: Respiratory mode (Acanthamoeba by Respiratory or rarely through skin penetration)
- More common in temperate climate (Acanthamoeba is common in tropics).

19. Ans. (a) (Naegleria fowleri) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p41-42

- Provided history of **acute meningoencephalitis** with demonstration of motile trophozoites in CSF wet mount suggests diagnosis of *N.fowleri.* (Already explained)
- Eventhough Acanthamoeba also causes meningoencephalitis, it is not acute in nature and it occurs mainly in immunocompromised host.

20. Ans. (a), (b), (c) and (d) (Naegleria, Acanthamoeba, Balamuthia and Gnathostoma) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p325

Parasites causing CNS infections

Protozoan Parasites	Helminths
• ***Acanthamoeba***	• ***Angiostrongylus cantonensis***
• ***Entamoeba histolytica***	• ***Gnathostoma spinigera***
• ***Naegleria fowleri***	• *Echinococcus granulosus (hydatid cyst)*
• ***Balamuthia***	• *Taenia multiceps and T. solium*
• *Plasmodium falciparum*	• *Schistosoma japonicum*
• *Trypanosoma brucei and* ***T. Cruzi***	• *Paragonimus westermani*
• ***Toxoplasma gondii***	• *Toxocara spp.*

21. Ans. (a) (Naegleria fowleri) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p42

History of sudden onset of nasal discharge, meningitis and death in 5 days: Indicates primary amebic meningoencephalitis (PAM). The agent of PAM is Naegleria fowleri.

CHAPTER

6.3 Flagellates

GIARDIA LAMBLIA (INTESTINAL FLAGELLATES)

Habitat: Mucosa of duodenum and upper ileum of man.

Morphology: 2 forms

- **Trophozoites**
  - **Front view**: Tear-drop shaped/racket shaped/piriform shaped, **Lateral view:** sickle/spoon shaped
  - Shows falling leaf like motility, 15—20 µm size
  - It has 2 nuclei, 4 pairs of flagella, 2 Axostyles, 2 parabasal body and two Ventral sucking disk
- **Cyst**: Mature cyst is oval, 10–14 µm size, consists of 4 nuclei and an axostyle. Cysts are passed in feces.

Life Cycle

- Infective stage: Cyst, Infective dose: As few as 10 to 25 cysts
- Route of infection: Feco-oral route
- Cyst transforms to trophozoites which multiply in duodenum, attach to GIT mucosa by adhesive disk and later on, shed in the lumen, transforms to cysts which are passed in feces. They are the diagnostic form of the parasite.
  Susceptibility to infection: Children, HIV and Individuals with Achlorhydria and Hypochlorhydria.

Pathogenesis
Malabsorption of fat (steatorrhea) - leads to foul smelling profuse frothy diarrhea.

Pathogenesis

- It causes abnormalities of villous structure and causes malabsorption (lipids and lipid soluble vitamins)
- *Malabsorption:* There could be various types which include:
  - Malabsorption of fat (steatorrhea): Leads to foul smelling profuse frothy diarrhea.
  - Disaccharidase deficiencies (lactate, xylose): Leading to lactose intolerance.
  - Malabsorption of vitamin B_{12} and folic acid and protein loosing enteropathy.

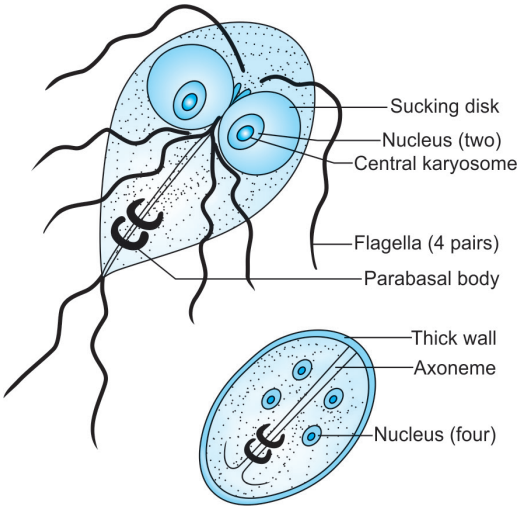


Fig. 6.3.1: Giardia trophozoite and cyst (front view)

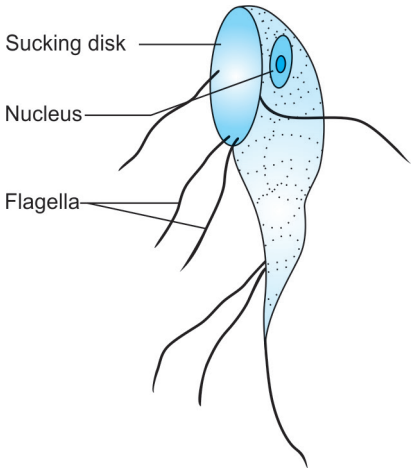


Fig. 6.3.2: Giardia trophozoite (lateral view)

- Antigenic variation in 'variant surface protein' of Giardia: results in chronic and persistent infection.

Lab Diagnosis

- Samples collected: Stool sample and duodenal contents
- Microscopy: Demonstrates either trophozoites (active infection) or cyst (carrier)
- **String test/Entero test**: Bile stained mucus is collected for examination of Trophozoites
- Antigen detection in stool (coproantigen) by ELISA or ICT: indicates active infection
- Serum Antibody detection by ELISA, IFA: indicates past infection

Treatment: Metranidazole/Tinidazole, Nitazoxanide or Furazolidone.

String test/Entero test- Bile stained mucus is collected for examination of Trophozoites.

TRICHOMONAS VAGINALIS (GENITAL FLAGELLATES)

MC parasitic cause of STD and NG (nongonococcal urethritis)

Habitat: Urethra, vagina and prostate.

Trichomonas- Trophozoite is the only form. No cystic stage.

Morphology

- Trophozoite is the only form. No cystic stage.
- Trophozoite: Shows twitching or jerky motility, pear-shaped consists of 5 flagella (4 anterior + 1 recurrent flagella) supported by undulating membrane and a fibrillary structure called as costa.

Life Cycle

- Mode of transmission: Sexual route
- Reservoir of infection: Woman
- Infective stage and diagnostic stage: Trophozoites
- Trophozoites divide by longitudinal binary fission.

Trichomoniasis- Strawberry appearance of vaginal mucosa (Colpitis macularis)- seen in 2% of cases

Clinical Disease

- **Incubation period: 4–28 days**
- In men: Asymptomatic or Urethritis, Prostatitis and Cystitis
- In women: Asymptomatic or vulvo-vaginitis—Characterized by:
  - Profuse vaginal discharge with offensive smell, high pH (> 4.5)
  - Smell gets accentuated by adding 10% KOH (whiff test)
  - Strawberry appearance of vaginal mucosa (***Colpitis macularis***)—seen in 2% of cases.

Lab Diagnosis

- Sample collected: Vaginal, and urethral discharges, Prostatic secretions or Urine sediment
- Microscopy of Vaginal, and urethral discharges: Actively motile **(Jerky motility)** trophozoites
- Staining: Giemsa and papanicolaou staining or Direct fluorescent antibody (DAF) test
- Culture: More sensitive, gold standard e.g. Lash's cysteine hydrolysate serum media
- Antigen detection in vaginal smear by ICT or ELISA: More sensitive than microscopy and indicates recent infection.
- Serology by ELISA: Antibodies persist for long, so indicates past infection
- PCR.

Treatment

Drug of choice: Metronidazole or tinidazole (2 grams, single dose) to both the sexual partners. Resistance is rare but has been reported.

HEMOFLAGELLATES: (LEISHMANIA AND TRYPANOSOMA)

- Exist in four morphological forms.

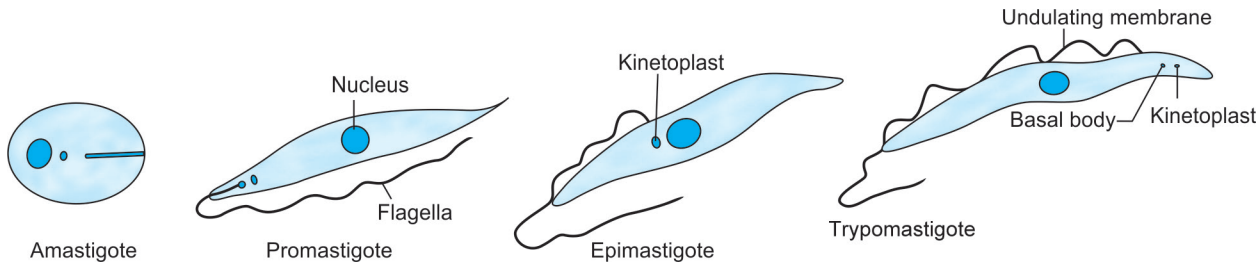


Fig. 6.3.3: Various morphological forms of hemoflagellates

LEISHMANIA

- *Infective form: Promastigote*
- *Mode of transmission- By bite of female sandfly* during the late evening or night time.
- Minimum 10–1000 promastigotes per infective bite are required to initiate the infection.
- Promastiogotes enter into skin macrophages, transform into amastigotes which are carried out in the circulation to various organs like liver, spleen, and bone marrow
- *Diagnostic from in humans Amastigote form inside the macrophages- k/a LD body.*

Leishmania
- Infective form Promastigote
- Mode of transmission: By bite of female sandfly
- Diagnostic from in humans: Amastigote form inside the macrophages- k/a LD body

Parasite	Epidemiology	Mode (Sandfly)	Syndromes		Leishmaniasis
L. donovani	Asia, Africa	Phlebotomus argentipes - India P. orientalis, P. martini- Africa	VL, PKDL	Anthroponotic	Old World
L. tropica	Mediterranean, Asia, Rajasthan	P. sergenti	CL/Oriental sore, LR	Anthroponotic	Old World
L. infantum	Middle East	P. perniciosus	Infantile VL, CL	Zoonotic	Old World
L. aethiopica	Ethiopia	P. longipes	CL, DCL	Zoonotic	Old World
L. major	Middle East India	P. papatasi	CL	Zoonotic	Old World
L. brasiliensis	Central and South America	Lutzomyia	MCL-Espundia, CL	Zoonotic	New World
L. mexicana	Central and South America	Lutzomyia	CL-Chiclero ulcer (pinna), DCL	Zoonotic	New World
L. chagasi	Central and South America	Lutzomyia	VL, CL	Zoonotic	New World

- VL: Visceral Leishmaniasis, CL: Cutaneous Leishmaniasis, LR: Leishmaniasis Recidivans, MCL: Mucocutaneous Leishmaniasis, DCL: Diffuse Cutaneous Leishmaniasis, PKDL: Post Kala-azar Dermal Leishmaniasis.

Kala-Azar

- Bone marrow involvement leads to:
  - Anemia, leucopenia, thrombocytopenia
  - Hypergammaglobulinemia
- Spleen ↑ , Liver ↑
- Fever and Hyperpigmentation (Indian cases)
- LN↑ (African but not in Indian cases)
- Kala-azar with HIV:
  - Absence of hepatosplenomegaly
  - GIT and resp. symptoms
  - Mainly it is reported from Southern Europe (France, Italy, Spain and Portugal)
  - In India, it is reported from Bihar and sub-Himalayan region and other North Indian states
  - Diagnosis: Amastigote detected from BAL and buffy coat region of blood. Antibodies are negative.

Kala-azar
- Fever and Hyper pigmentation (Indian cases)
- LN↑ (Seen in African but not in Indian cases)

PKDL (Post Kala-azar Dermal Leishmaniasis)

- Seen in 2–10 year after treatment of VL (< 6 months in Sudan)
- Incidence among patients with VL: 50%(Sudan), 2–20% (Indian)
- Nonulcerative hypopigmented macular lesions, becomes nodular later
- Persists for 20 years (few months for Sudan cases)
- Treatment: Longer courses of antimonials, resolve slowly.

Virulence Factors

- Glycoprotein (gp-63), Lipophosphoglycan (LPG) and Glycosylphosphatidylinositol.

Host Immune Response

- T helper 1 (Th1) Response:
  - Protective response
  - Leishmanin skin test +ve
- T helper 2 (Th2) Response
- Indicates: Disease progression
- Leishmanin skin test -ve:

Laboratory Diagnosis
Sample:
- Spleen (most Sensitive)/Bone marrow aspiration (Commonly preferred sample)
- Lymphnode aspirate (Not useful in Indian cases)
- Blood: Buffy coat region and BAL (for HIV infected)

Laboratory Diagnosis

- Sample:
  - Spleen (most Sensitive)/Bone marrow aspiration (Commonly preferred sample)
  - Lymphnode aspirate (Not useful in Indian cases)
  - Blood: Buffy coat region and BAL (for HIV infected)
- Microscopy: Stained peripheral blood smear examination demonstrates LD bodies (amastigotes)
- Culture: NNN medium (McNeal, Novy and Nicolle) and Schneider's Liquid medium (Amastigotes transform to promastigotes)
- Serological tests:
  - Hypergammaglobulinemia: Detected by Napier's aldehyde and Chopra's antimony test
  - CFT: Using tubercle bacilli antigen like WKK antigen
  - Immunochromatographic test (ICT): Antibodies to rK39 antigen
  - Direct agglutination test (DAT), ELISA, IFA
- Leishmanin (Montenegro) skin test (indicates delayed hypersensitivity reaction):
  - It is positive in people with good cell mediated immunity, Observed in Patients with CL, LR, Recovered from VL
  - However, this test is negative in (when CMI is low): Patients with active VL and DCL
- PCR: To detect Kinetoplast DNA
- Animal inoculation: Chinese and golden Hamsters.

- NNN medium: (McNeal, Novy and Nicolle) and Schneider's Liquid medium (Amastigotes transform to promastigotes)
- Immunochromatographic test (ICT): Antibodies to rK39 antigen

Treatment

- Pentavalent antimonial: DOC in most endemic regions of the world, except in Bihar
  - Sodium stibogluconate
  - Meglumine antimoniate
- Amphotericin B: DOC in Bihar
- Paromomycin - DIffuse CL (L.ethiopia)
- Miltefosine.
- Pentamidine - DOC in CL due to L.guyanensis

Treatment of Leishmaniasis
- **In VL:** Pentavalent antimonials are the DOC except Bihar (Amphoterecin B is DOC)
- **In CL:** Pentavalent antimonials are the DOC except for L.guyanensis (pentamidine is DOC) and L.ethiopia (paromomycin is DOC)

Prevention: Insect control. Sleeping at top floors also can prevent transmission.

TRYPANOSOMA CRUZI

Life Cycle

- Mode: Rubbing of reduviid bugs (*Triatomine)*
- Infective form: Metacyclic Trypomastigotes
- Invades macrophages, transforms to amastigotes which multiply to form pseudocyst
- Amastigotes again transform to trypomastigotes:
  - Slender highly motile forms: Invades various organs
  - Broader less motile forms: Infective form to reduviid bugs.

Trypanosoma cruzi:
- Mode of transmission: Rubbing of reduviid bugs (Triatomine)
- Infective form: Metacyclic Trypomastigotes

Section 6

Acute Chagas' Disease

- **Chagoma (**subcutaneous nodule)
- **Romana's sign: Periorbital edema**
- Lymphadenopathy and hepatosplenomegaly.

Chronic Chagas' Disease

- Autoimmune hypothesis: Occurs due to molecular mimicry
- Multiplication of the parasites in the muscles (skeletal, cardiac and GIT) and CNS leads to:
  - Cardiac form: Myocarditis and dilated cardiomyopathy
  - Gastrointestinal form: Megaesophagus and megacolon
  - Meningoencephalitis (↑in HIV infected people).

Diagnosis and Treatment

- Diagnostic form: C-shaped Trypomastigotes in blood smear. Often confused with *T. rangeli (non pathogen)*
- Culture-Blood is inoculated in NNN media
- Radioimmune precipitation assay (Chagas' RIPA): Highly sensitive & specific
- PCR detecting kinetoplast DNA: Useful in chronic disease
- Xenodiagnosis
- Animal inoculation in mice.

Treatment: Benznidazole (DOC) and Nifurtimox.

TRYPANOSOMA BRUCEI

Life Cycle

- Infective form: Metacyclic trypomastigotes
- Mode of transmission: By bite of Tsetse fly
- Transform to: long slender trypomastigotes followed by short stumpy forms (infective form to the tsetse fly)
- Undergo periodic antigenic variation of surface glycoprotein (VSG).

Clinical feature

- Trypanosomal chancre
- ***Stage I disease:*** Systemic febrile illness, lymphadenopathy
- ***Stage II disease:*** Causes ***African sleeping sickness*** – Progressive daytime somnolence restlessness and insomnia at night.

Diagnosis

- ***Diagnostic form:*** Trypomastigotes seen in blood smear
- CSF shows-Mott cells (abnormal plasma cells containing IgM)
- **Antigens from serum and CSF**: By ELISA. Useful for clinical staging of disease and for monitoring
- Antibody detection: Card agglutination test for try panosomes (CATT), ELISA and IFA.

Trypanosoma brucei:
- Infective form: Metacyclic trypomastigotes
- Mode of transmission: By bite of Tsetse fly

	T. brucei gambiense	T. brucei rhodesiense
Disease	West African sleeping sickness	East African sleeping sickness
Vectors	Tsetse flies (*Glossina palpalis*)	Tsetse flies (*Glossina morsitans*)
Primary reservoir	Humans	Animals (Antelope and cattle)
Human illness	Chronic CNS disease	Acute CNS disease: up to 9 months
Duration of illness	Months to years	< 9 months (before that the death occurs)
Lymphadenopathy	Frequent, Cervical Lymphadenopathy (Winter bottom sign)	Minimal (Axially and Inguinal)
Parasitemia	Low	High
Virulence	Less	More
Rodent inoculation	Not useful	Diagnostic
Epidemiology	Rural populations	Workers in wild areas, rural populations, tourists in game parks
Respond to drugs	Less resistant	More resistant
Treatment	DOC-Pentamidine Abnormal CSF-DOC-Eflornithine	Suramin Abnormal CSF-Melarsoprol

MULTIPLE CHOICE QUESTIONS

GIARDIA LAMBLIA

1. Giardia, true is: *(PGI May 2016)*
a. Mostly invade into small intestine
b. Present with bloody diarrhoea
c. Mostly in hyperglobulinemia patient
d. Nitroimidazole is effective
e. Passed in stool but non infectious

2. Which of the following is true with Giardia lamblia?
a. Malabsorption commonly seen *(PGI Dec 2005)*
b. Trophozoite form is binucleate
c. Diarrhea is seen
d. Jejunal wash fluid is diagnostic

3. Which of the following infestation leads to malabsorption? *(TNPG 2014)*
a. Giardia lamblia
b. Ascaris lumbricoides
c. Necater americana
d. Ancylostoma duodenale

4. An anxious mother brought her 4-year-old daughter to the pediatrician. The girl was passing loose bulky stools for the past 20 days. This was often associated with pain in abdomen. The pediatrician ordered the stool examination, which showed the following organisms. Identify the organism: *(AI 2003)*
a. Entamoeba histolytica
b. Giardia lamblia
c. Cryptosporidium
d. E. coli

TRYPANOSOMA CRUZI

5. All are true about Chagas' disease except: *(JIPMER Nov 2015)*
a. Romana's sign is a diagnostic feature of acute Chagas' disease
b. Tsetse fly is the vector (Glossina palpalis)
c. Cultured in NNN medium
d. Has amastigote and trypomastigote forms

6. Reduviid bug is a vector for the transmission of: *(MHPG 2015, AIIMS 2005, DNB Dec 2011)*
a. Relapsing fever
b. Lyme's disease
c. Scrub typhus
d. Chaga's disease

7. Strawberry cervix is seen in: *(Recent MCQ 2013)*
a. Gardenerella vaginalis
b. Trichomonas vaginalis
c. Mycoplasma hominis
d. Neisseria gonorrheae

8. NNN Media used for isolation of: *(PGI Nov 2016)*
a. *Leishmania donovani*
b. *Giardia*
c. *E. histolytica*
d. *Trichomonas vaginalis*
e. *Trypanosoma cruzi*

T. BRUCEI

9. Sleeping sickness is transmitted by: *(PGI 2001)*
a. Tsetse fly
b. House fly
c. Sand fly
d. Simulium fly

LEISHMANIA

10. Mucocutaneous Leishmaniasis is caused by: *(Recent Question 2015)*
a. Leishmania donovani
b. Leishmania braziliensis
c. Leishmania mexicana
d. Leishmania tropica

11. Espundia is a condition seen in: *(JIPMER Nov 2014)*
a. Endemic syphilis
b. Malaria
c. Lympho Granuloma Venereum
d. Mucocutaneous leishmaniasis

12. Vector for Kala-azar: *(AIIMS Nov 2007)*
a. Flea
b. Tsetse fly
c. Sand fly
d. Tick

13. Amastigote forms are seen in: *(PGI June 2001)*
a. Leishmania donovani
b. Toxoplasma gondi
c. Leishmania major
d. Entamoeba

14. Drug not used in visceral leishmaniasis: *(AIIMS Nov 2009)*

a. Sitamoqine
b. Paromycin
c. Miltefosine
d. Hydroxychloroquine

15. A 20-years-old female from Africa has hepatosplenomegaly, pallor and generalized lymphadenopathy. Lab test useful for diagnosis is /are: *(PGI June 2009)*

a. ESR
b. Electrophoresis
c. Parasite detection in aspirate
d. ELISA
e. Routine hemogram

16. The following tests help in laboratory diagnosis of Kala-azar except: *(AI 2002)*

a. Bone marrow examination
b. Immobilization test
c. Blood smear examination
d. Aldehyde test

17. Leishmania is cultured in ……..media:

a. Chocolate agar
b. NNN *(PGI 2002)*
c. Tellurite
d. Sabourauds

EXPLANATIONS

GIARDIA LAMBLIA

1. **Ans (d) (Nitroimidazole is effective)** Ref: Apurba Sastry's Essentials of Medical Parasitology/p53-55
   - Metronidazole, tinidazole, nitazoxanide and furazolidone are the drugs effective in giardiasis.
   - Giardiasis is associated with hypogammaglobulinemia, seen in Common variable immunodeficiency syndrome.
   - Giardia never invades GI mucosa.
   - Tetra nucleated cysts passed in stool are infectious.
   - Trophozoites passed in stool are non- infectious as they disintegrate.

2. **Ans. (b) and (c) (Trophozoite form is binucleate and Diarrhea is seen)** Ref: Harrison 18/e p1730, Apurba Sastry's Essentials of Medical Parasitology 1/e p50-52

   Option a: Incorrect: Disease manifestations of Giardiasis range from **asymptomatic carriage** (most common form) to fulminant diarrhea and malabsorption (rarely seen).
   Option b: Correct: Trophozoite of Giardia has 2 axostyles, **2 nuclei** and 4 pairs of flagella.
   Option c: Correct: Common symptoms—**diarrhea**, abdominal pain, bloating, belching, flatus and vomiting.
   Option d: Incorrect: For diagnosis of Giardiasis—**Duodenal aspirate/wash fluid** is diagnostic and not jejunal.

3. **Ans. (a) (Giardia lamblia)** Ref: Harrison 18/e p1739-30, Apurba Sastry's Essentials of Medical Parasitology 1/e p52

   Refer earlier explanation.

4. **Ans. (b) (Giardia lamblia)** Ref: Harrison18/e p1739-30, 17/e p1312, KD Chatterjee 13/e p47-48; Apurba Sastry's Essentials of Medical Parasitology 1/e p52

   The clues provided in the given history:
   - Affected patient: child–4 years
   - History Diarrhea: loose bulky stools for the past 20 days and associated with pain in abdomen
   - Suggest that probably child is suffering from Giardiasis.

TRYPANOSOMA CRUZI

5. **Ans. (b) (Tsetse fly is vector)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p80-83
   - Reduviid bug is the vector for Trypanosoma cruzi (the causative agent of Chagas' disease)
   - Romana's sign (periorbital edema) is a diagnostic feature of acute Chagas disease.
   - Trypanosoma cruzi can be cultured in NNN medium.
   - Trypanosoma cruzi has both amastigote (seen only in insect vector) and trypomastigote forms (seen in both humans and insect vector).

6. **Ans. (d) (Chaga's disease)** Ref: KD Chatterjee 13/e p61; Apurba Sastry's Essentials of Medical Parasitology 1/e p80
   - *Reduvid bug/triatomid bug is the vector for Trypanosoma cruzi (agent of Chagas'disease)*

7. **Ans. (b) (Trichomonas...)** Ref: Apurba Sastry's Essentials of Medical Parasitology p57
   - Strawberry appearance of vaginal mucosa is the characteristic feature of Trichomonas vaginalis infection.
   - Also k/a Colpitis macularis
   - Seen in 2% of cases.

8. **Ans (a, e) (*Leishmania donovani*, *Trypanosoma cruzi*)** Ref: Apurba Sastry's Essentials of Medical Parasitology/p305
   - NNN Media is used for culture of *Leishmania* and *Trypanosoma*

T. BRUCEI

9. Ans. (a) (Tsetse fly) Ref: KD Chatterjee 13/e p55; Apurba Sastry's Essentials of Medical Parasitology 1/e p85

Trypanosoma brucei, the causative agent of Sleeping sickness is transmitted by tsetse fly

- *Trypanosoma brucei gambiense* (West Africa): Bite of Glossina (tsetse fly)
- *Trypanosoma brucei rhodesiense* (Eastern and Central Africa): Bite of Glossina (tsetse fly)

LEISHMANIA

10. Ans. (b) (L. braziliensis) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p77

11. Ans. (d) (Mucocutaneous leishmaniasis) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p77

- Mucocutaneous leishmaniasis caused by L. Brasiliensis is presented as Espundia (infects mucous membrane of the nose, oral cavity, pharynx or larynx)

12. Ans. (c) (Sandfly) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p66

- **Sandflies** of genera ***Phlebotomus*** act as intermediate host for Leishmaniasis (Kala-azar).
- Human and other vertebrates act as definitive host.

13. Ans. (a) and (c) (Leishmania donovani and Leishman...) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p66

- Leishmania parasite exists in 2 stages.
- **Amastigote stage (Formerly called Leishmanial form) occurs in man**
- Promastigote stage (Formerly called Leptomonad form) occurs in gut of Sandfly/in artificial culture medias.

14. Ans. (d) (Hydroxy...) Ref: Harrison 18/e p1712, 17/e p1299; Apurba Sastry's Essentials of Medical Parasitology 1/e p76

Treatment regimen of Leishmaniasis:

- Pentavalent antimonial - DOC in most endemic regions of the world, except in Bihar
  - Sodium stibogluconate
  - Meglumine antimoniate
- Amphotericin B: DOC in Bihar
- Paromomycin
- Miltefosine

***Option* d: Sitamaquine (WR6026)** is an 8-aminoquinoline in development for the **oral** treatment of visceral leishmaniasis (VL). Ref: Journal- Am J Trop Med Hyg. 2005 Nov; 73 (5):871-6).

15. Ans. (a), (c) and (e) (ESR, Parasite detection in aspirate and Routine hemogram) Ref: Park 22/e p278-80, Apurba Sastry's Essentials of Medical Parasitology 1/e p71

- The history provided suggests probably patient is suffering from Kala-azar (visceral Leishmaniasis)
  Park 22/e p 279-80 states that:
  '*The classical features of VL* ***splenomegaly and hepatomegaly*** *accompanied by* ***anemia*** *and weight loss. Darkening of skin of face, hands, feet and abdomen are common (kala-azar – black sickness)* ***Atypical features such as generalized lymphadenopathy*** *may also occur in African leishmaniasis'.*

16. Ans. (b) (Immobilization test) Ref: KD Chatterjee 13/e p76; Apurba Sastry's Essentials of Medical Parasitology 1/e p71

- Immobilization test is done for Treponema pallidum

17. Ans. (b) (NNN) Ref: KD Chatterjee 13/e p67; Apurba Sastry's Essentials of Medical Parasitology 1/e p72

- Leishmania can be cultured in medium: ***NNN medium—Novy, MacNeal and Nicolle medium.***
- Material for culture is inoculated in water of condensation of the medium and incubated at 22°C.

CHAPTER

6.4

Plasmodium Species and Babesia

When transmitted by blood transfusion:
- There is no liver stage,
- Hypnozoites are not produced
- Hence, there is no relapse
- So no need of primaquine

Infective form:
- Infective form to man: Sporozoites present in the salivary gland of mosquito
- Infective form to man when transmitted by blood transfusion or vertical mode: trophozoite
- Infective form to mosquito: Gametocyte

To infect mosquitoes the gametocytes should be mature, viable, count > 12 per cubic mm of blood.

PLASMODIUM

Plasmodium spp. causing malaria as follows

Species	Disease	Periodicity (Hours)
Plasmodium vivax	Beningn tertian	48
Plasmodium falciparum	Malignant tertian	48
Plasmodium ovale	Ovale tertian	48
Plasmodium malariae	Quartan	72
Plasmodium knowlesi	Quotidian	24

Life Cycle

- Definitive host: **Female Anopheles** mosquitoes
- Intermediate host: Man.
- Infective form to man: **Sporozoites** presesnt in the salivary gland of mosquito
- Infective form to man when transmitted by blood transfusion or vertical mode- ***trophozoite***
- When transmitted by blood transfusion or vertical mode the difference in the life cycle is:
  - There is no liver stage, and infective form is trophozoite> merozoite
  - Hypnozoites are not produced
  - Hence, there is no relapse
  - So no need of primaquine
- Infective form to mosquito- **Gametocyte**
- To infect mosquitoes- the gametocytes should be mature, viable, count > 12 per cubic mm of blood.
- Asexual cycle in man:
  - After the mosquito bite: Sporozoites are discharged to blood, carried to liver
  - **Liver cycle (**pre or exoerythrocytic cycle): Sporozoites transform to trophozoites → pre-erythrocytic schizont undergoes schizogony to produce → PE merozoites
  - **RBC cycle:** PE merozoites infect RBCs → transform to early trophozoites (ring form) → late trophozoites → erythrocytic schizont → Undergoes schizogony to produce merozoites
  - Release of merozoites leads to appearance of clinical manifestations
  - Merozoites either attack RBCs to repeat the cycle or transform to gametocytes which infect mosquito
- Sexual cycle (Sporogony): Begins when gametocytes infect the female anopheles mosquito and then transform to gametes → zygote → ookinete → oocyst → sporozoites

Recrudescence

- Seen in *P. falciparum* and *P. malariae* infections
  - Falciparum malaria: Recrudescence is due to persistence of drug resistant parasites and fever reappears after 2–3 weeks of completion of treatment.
  - In *P. malariae infection,* long term recrudescences are seen for as long as for 60 years. This is due to long term survival of erythrocytic stages at a low undetectable level in blood.

Relapse

- Due to hypnozoites (resting stage) which may reactivate after 2-3 years of malaria
- Seen in *P. vivax* and *P. ovale*
- Treatment of relapse: Primaquine

Differences between the four malaria parasites

Properties	*P. vivax*	*P. falciparum*	*P. malariae*	*P. ovale*
Relapse (Hypnozoites)	Seen	Not seen	Not seen	Seen
Recrudescence	Not seen	Seen	Seen (60 yrs)	Not seen
Erythrocytic cycle	48 hours	36–48 hours	72 hours	48 hours
Prepatent period	8 days	5 days	13 days	9 days
Incubation period	14 days	12 days	28 days	17 days
R-G interval[a]	4–5 days	10–12 days	11–14 days	5–6 days
Extrinsic IP[b]	8–10 days	9–10 days	25–28 days	14–16 days

[a]R-G interval: Interval between appearance of gametocyte and ring form, [b]IP: Incubation period

Pathogenesis of P. falciparum

- Sequestration, i.e. holding back of the parasite in the blood vessels of deep visceral organs like brain, kidney, etc. leads to vascular occlusion.
  - Cytoadherence (binding of RBC to endothelium) – Pf EMP
  - Rossetting- unparasitized RBC clump together with parasitized RBC
- Cytokines released: d/t GPI-glucosylphosphatidyl Inositol
- Antigenic diversity of Pf EMP

Immunity

- Nature of Hemoglobin:
  - Sickle cell anemia trait: Protective from P. falciparum
  - Thalassemia trait: Protective from P. falciparum
  - Fetal Hb: Protective from P. falciparum
- Nature of RBC:
  - P. falciparum: Infect All RBC,
  - P. malariae: Infect old RBC,
  - P. ovale/vivax: Infect Young RBC
- Nature of Enzyme- G6PD deficiency: Protective from P. falciparum
- Ovalocytosis: RBC is rigid, so protected from P. falciparum
- Duffy negative RBC: Protected from P. vivax
- *HLA-BW53* and haplotypes bearing DRW13.02 antigen and R111 gene
- Nutritional status: Has a paradoxical effect
- **Predisposing factors:**
  - Density of the vector
  - Number of human bites per day per mosquito
  - Time of mosquito bite (more after the dusk)
  - Optimum temperature (20–30 °C)
  - Optimum humidity (60%)
  - Rainfall (July to November)
  - Altitude below 2000 meters
  - Mosquito longevity (as sporogony lasts for 7–30 days, thus, to transmit malaria, the mosquito must live > 7 day.

Nature of RBC:
- P. falciparum: Infects All RBC,
- P. malariae: Infects old RBC,
- P. ovale/vivax: Infects Young RBC

Duffy negative RBC: Protected from P. vivax

Epidemiology

Situation in World

- Most affected area Sub-Saharan Africa (85%) followed by South East Asia Region (SEAR) (10%) and Mediterranean (4%)
- *P.vivax* is the predominant species
- Age: Children

Situation in India:
P. falciparum - (51%) and *P. vivax* (49%). Few cases of mixed infections reported.

Situation in India (According to the WHO Malaria Report 2012)

- *P. falciparum* - (51%) and *P.vivax* (49%). Few cases of mixed infections reported.
  - 22% of people reside in high transmission area (≥ 1 case per 1000),
  - 67% in low transmission area (0–1 case per 1000)
  - 11% in malaria free area.
- Odisha: Affected the most (24%) where 92% of cases are due to *P.falciparum*
- *P.malariae*: (< 1%) in India. Mainly confined to Tumkur and Hassan districts of Karnataka.
- *P.ovale: Confined to* tropical Africa. Only few cases are reported from India (from Odisha, Delhi, Assam, Gujrat and Kolkata).
- Roll back malaria: Launched in 2000. Aims at reducing the malaria cases by 50% by the end of 2010 and 75% by the end of 2015.
- **World malaria day: Every year, 25th April.**

Antigen detection kits-
- pLDH and Aldolase: Common to all Plasmodium species
- HRP-2 Ag detection: Specific for *P. falciparum*

Clinical Features of Malaria

- Fever, Sweating
- Anemia
- Splenomegaly (enlarged spleen)
- Irritability
- Coma, Retinal Hemorrhages
- Algid Malaria (a shock like syndrome)
- Respiratory distress syndrome.

Complications

- Cerebral malaria
- Black water fever
- Tropical splenomegaly syndrome
- Malarial hyperpyrexia
- Gastrointestinal disorders.
- Algid malaria
- Nephrotic syndrome: ***P. malariae*** infection
- Promotes Burkitt's Lymphoma.

Plasmodium Knowlesi
- Parasite of monkey but can also affect humans: Recently reported from South east Asia.
- Blood smear examination
  Early trophozoite: Resembles to *P. falciparum*
  Late trophozoite: Resemble to *P. malariae*

Plasmodium Knowlesi

- It is a malaria parasite of monkey but can also affect humans, recently reported from Asia.
- Not reported from India yet, though the vector Anopheles leucosphyrus is present in south west costal region.
- Large focus *P. knowlesi* in humans: Recently identified in Malaysia, Singapore and Thailand.
- Blood smear examination: Early trophozoite- resembles to *P. falciparum* and Late trophozoite- Resemble to *P. malariae*
- Produces an acute illness and relatively high parasitaemia compared to that of *P. malariae.*
- Paroxysms of fever occur daily (quotidian malaria) because of short RBC cycle.

Blood smear examination
- Thick smear: More sensitive (40 times), used for quantification and malaria pigment detection
- Thin smears: Used for speciation

Laboratory Diagnosis

Microscopic Tests

- Examination of peripheral blood smears (thin and thick blood films): Gold standard method:
  - Thick smear: More sensitive (40 times), used for quantification and malaria pigment detection
  - Thin smears: Used for speciation
  - Feathery tail end of the smear should be examined
  - At least 200–300 oil immersion fields should be examined before considered as negative

- Stains used:
  - JSB (Jaswant Singh and Bhattacharya) stain – Used in malaria control programme in India
  - Leishman's, Giemsa, and Field's, Wright's
- Fluorescence microscopy (Kawamoto technique)- by Acridine orange stain
- Quantitative Buffy coat examination (QBC) Rapid method for detection of parasites:
  - Blood is collecetd in a capillary tube coated with acridine orange + centrifuge the capillary tube + Examine the buffy coat region (junction of RBC & WBC) under UV.

Antigen detection kits:
- pLDH and Aldolase: Common to all Plasmodium species
- HRP-2 Ag detection: Specific for *P. falciparum*

Non-microscopic Tests

- Antigen detection tests: Rapid diagnostic tests (RDTs) or Immunochromatographic tests (ICTs)
  - Rapid and simple but less sensitive, costly and may give false +ve in RA factor +ve cases
  - pLDH and Aldolase: Common to all Plasmodium species
  - HRP-2 Ag detection: Specific for *P. falciparum*
- Antibody detection methods:
  - Epidemiological survey in malaria.
  - Screening of blood bank: To identify the infected donors
- Culture of malarial parasites: Tragger and Jensen method using RPMI 1640 medium is used for research purpose
- Molecular methods- PCR using PBRK1 primer
  - It is 100 times more sensitive than that of thick blood smear.
  - Speciation can be done.
  - Drug resistance genes can be detected.

Parasitic changes	*P. vivax*	*P. falciparum*	*P. malariae*	*P. ovale*
Forms seen in peripheral blood smear examination	Trophozoites (early and late), Gametocytes, Schizonts	Ring forms (early trophozoites), Gametocytes	Similar to that of *P. vivax*	Similar to that of *P. vivax*
Ring forms (Early trophozoites)	Ring occupies 1/3rd of size of RBC, Late trophozoites: Large, amoeboid, prominent vacuole	Rings 1.5 µm size, smaller than in *P.vivax* occupying 1/6th of RBC *Variants of ring forms:* Multiple rings, Accole (appliqué) forms Double dot ring forms	Band forms	Similar to that of *P. vivax*, more compact
Merozoites/schizont	12–24 no.	18–24 no.	6–12 no.	8–12 no.
Gametocyte	Spherical, almost occupies the RBC	Banana shaped, larger than RBC size.	Similar to that of *P. vivax*	Similar to that of *P. vivax*
RBC infected	Young RBC	RBC of all age	Old RBC	Young RBC
RBC size	Enlarged, Round (frequently bizarre form)	Normal in size	Normal in size	Enlarged, oval, fimbriated margin
Stippling	Schuffner's dots (small red dots)	Maurer's cleft (large red spots)	Ziemann's dots (small red dots)	James's dots (small red dots)
Malarial Pigments	Yellowish brown	Dark brown	Dark brown	Dark brown

Comparison of Peripheral Smear, QBC and Rapid Diagnostic Tests

	Peripheral smear	QBC	Rapid diagnostic tests
Method	Cumbersome	Easy	Easy
Time	Longer, 60–120 minutes	Faster, 15–30 minutes	Faster, 15–30 minutes
Sensitivity	Detection limit: 5 parasites/µl in thick film 200/µl in thin film	Claimed to be more sensitive, at least as good as a thick film	> 100 parasites/µl Sensitivity > 90% < 100 parasites/µl Sensitivity falls
Specificity	Gold standard	False positives: Artifacts may be reported as positive by non trained technicians	False positive in RA factor (rheumatoid arthritis) positive cases

Contd...

Contd...

	Peripheral smear	QBC	Rapid diagnostic tests
Speciation	Accurate, gold standard	Difficult	Detect *P. falciparum* But cannot differentiate non *falciparum* species.
Cost	Inexpensive	Costly equipment and consumables	Kits are costly but no extra equipment required. Good for field study.
Experienced Microscopist	Required	Not Required, minimal training is sufficient	Not required, minimal training is sufficient.

Treatment

Uncomplicated Benign Malaria in India

- Chloroquine is still the drug of choice for uncomplicated benign malaria in India. It is given 25 mg/kg divided over three days.
- Relapse rate of vivax malaria is around 30% in India. Primaquine is given as 0.25 mg/kg daily for 14 days to prevent relapse.

Complicated or Falciparum Malaria in India

- Treatment of falciparum malaria in India is based on area resistant or sensitive to chloroquine.
- Artemisinin combination therapy (ACT) is recommended in chloroquine resistant areas where as chloroquine can be given in sensitive areas.
- Artemisinin combination therapy (ACT) consists of a combination of artemisinin derivative (Artemisinin or artemether or arte-ether) and long acting antimalarial drugs like sulfadoxine-pyrimethamine, mefloquine or lumefantrine.
- For treatment failure of *P. falciparum* cases: Quinine with combination of doxycycline or tetracycline is recommended.
- Chloroquine resistant areas where Artemisinin combination therapy (ACT) is recommended: Odisha, Jharkhand, Madhya Pradesh, Chhattisgarh and Andhra Pradesh.
- In pregnancy: Quinine is recommended in first trimester whereas Artemisinin combination therapy (ACT) is given in second and third trimester.
- Treatment of unconfirmed cases or mixed infection with *P. sfalciparum* should be treated as falciparum malaria plus primaquine is given for radical cure.

Babesia
– Not found in India
– Vector: Tick borne
– Zoonotic

BABESIA

- Intraerythrocytic protozoa
- Produces Malaria like illness in animal and Zoonotic causes opportunistic infection to human
- Not found in India
- Vector: Tick borne
- Treatment: Clindamycin with oral quinine and atovaquone and azithromycin.
- Differ from Plasmodium:
  - Hemozoin absent
  - Gametocyte not distinguished from asexual forms
  - Maltese cross form seen: Ring forms are arranged in tetrad

Diagnostic form of Babesia
– Maltese cross form seen: Ring forms are arranged in tetrad

Species	B. microti	B. bovis/divergens
Distribution	North America	Europe
Host	Rodent	Cattle
Immunity of host	Spleen is usually normal	Seen in spleenectomised and immunocompromised patient
Clinical feature	Asymptomatic/mild fever	Severe, but no cerebral involvement
Clinical Course	Self-limiting	Severe, fulminant

MULTIPLE CHOICE QUESTIONS

1. Characteristic morphological form associated with P. falciparum is/are: *(PGI May 2016)*
a. RBC enlarged
b. Ring-shaped gametocytes
c. Crescent shape gametocytes
d. Multiple rings trophozoite
e. All developmental stages are not seen in peripheral smears.

2. Duffy antigen associated with: *(Recent Question 2015)*
a. Plasmodium falciparum
b. Plasmodium ovale
c. Plasmodium malariae
d. Plasmodium vivax

3. Why are schizont and late trophozoite stages of Plasmodium falciparum are not seen in peripheral blood smear? *(AIIMS Nov 2016)*
a. They are sequestered in the spleen
b. Due to adherence to the capillary endothelium, they are not seen in peripheral blood
c. Due to antigen-antibody reaction and removal, antibody dependent cytotoxic killing
d. They are seen in mosquito blood

4. Transfusion associated malaria, the infective form is:
a. Trophozoite *(AIIMS May 2016)*
b. Sporozoites
c. Female Gametocyte
d. Male Gametocyte

5. In patient with Malaria, if fever has periodicity of 72 hours, which one of the following is likely to be causative agent? *(MHPG 2014, Recent MCQ 2013)*
a. *P. falciparum* b. *P. vivax*
c. *P. ovale* d. *P. malariae*

6. A patient is suffering from high grade fever with chill and rigor. Which of the following complication he may develop? *(Recent MCQ 2013)*
a. Renal failure
b. Black water fever
c. Meningitis

7. Malaria recrudescence is due to: *(AIIMS May 2014)*
a. Resistant to treatment
b. Relapse of infection
c. Relapse in vivax and ovale
d. Reappearance of sexual stage parasitemia after treatment

8. People with HLA type _____are protected from *Plasmodium falciparum:* *(PGI Nov 2014)*
a. HLA-Bw53
b. HLA B27
c. HLA B1

9. All are strategies of roll back malaria except:
a. Strengthening the health system *(AIIMS Nov 2013)*
b. Development of more effective insecticide
c. Use of insecticide treated mosquito nets
d. Training the health care workers

10. Life cycle of Malarial parasite was discovered in female Anopheles mosquito by:
a. Ronald Ross *(NEET Pattern Based)*
b. Paul Muller
c. Laveran
d. Pampania

11. Which type of malaria is associated with nephrotic syndrome and renal failure? *(NEET Pattern Based)*
a. Falciparum
b. Vivax
c. Malariae
d. Ovale

12. Features of Severe malaria are: *(PGI June 2011)*
a. Cerebral feature
b. ↑ LDH
c. Severe anemia
d. ↓ Platelet count
e. Metabolic acidosis

13. Case of plasmodium ovale in India is /are reported in:
a. Odisha *(PGI June 2011)*
b. Gujrat
c. Maharastra
d. Delhi
e. Karnataka

14. Which of the following acts as intermediate host of malaria parasite? *(NEET Pattern Based, DNB Dec 2011)*
a. Culex b. Female anopheles
c. Thromboculid mite d. Human

15. Schizonts are not seen in peripheral smear in which type of malaria? *(DNB Dec 2011)*
a. *P. Vivax* b. *P. Falciparum*
c. *P. Ovale* d. *P. Malariae*

16. Cerebral malaria is caused by: *(DNB June 2010)*
a. *Plasmodium falciparum*
b. *Plasmodium ovale*
c. *Plasmodium vivax*
d. *Plasmodium malariae*

17. Urban malaria is due to? *(PGI Dec 2000, 07)*
a. Anopheles stephensi
b. Anopheles culicifacies
c. Aedes
d. Cules vishnui

18. True about malaria epidemiology? *(PGI Dec 2006)*
a. Extrinsic IP -0–14 days
b. In India: common from Jan to June
c. Man- definitive host
d. Rare in urban
e. Mosquito-definitive host

19. IP of P.vivax: *(DNB 04)*
a. 5-7 days b. 7-10 days
c. 10-14 days d. 15-30 days

20. Which of the following is detected in peripheral blood smear? *(PGI Dec 2004)*
a. Malaria b. Toxoplasma
c. Babesia d. Brucella
e. Filaria

21. All are factors for Resurgence of malaria except: *(AI 2011, AIIMS Nov 2014, APPG 2011)*
a. Vector resistance
b. Host resistance
c. Use of bed nets
d. Mutation in parasite

22. Infective form of mosquito in plasmodium falciparum is: *(AIIMS Nov 2009)*
a. Merozoites b. Sporozoites
c. Gametocytes d. Trophozoites

23. Malarial parasite – which statement is false regarding communicability: *(AIIMS Nov 2006)*
a. The gametocytes appear in blood 4–6 days after a sexual phase in *P. vivax*
b. The gametocytes appear in blood 10–12 days after a sexual phase in *P. falciparum*
c. The number of gametocytes increases in blood with time
d. The number of gametocytes increases by 1000 times

24. Which one of the following is detected by the antigen detection test used for the diagnosis of *P. falciparum* malaria:? *(AIIMS Nov 2004)*
a. Circum –sporozoite protein
b. Merozoite surface antigen
c. Histidine –Rich-Protein I (HRP – I)
d. Histidine – Rich –Protein II (HRP – II)

25. P. falciparum causes: *(PGI June 2003)*
a. Thrombocytopenia
b. DIC
c. Hemolysis
d. Haematemesis

26. Chronic complication of malaria: *(PGI Dec 2002)*
a. Splenomegally
b. Nephrotic syndrome
c. Pneumonia
d. Hodgkin's disease

27. RBC's are enlarged in infection with: *(AIIMS 2002)*
a. P. vivax b. P. malariae
c. P. ovale d. P. falciparum
e. Hodgkin's disease

28. Senescent RBC's are mainly attacked in: *(PGI 2002)*
a. Vivax malaria b. Ovale malaria
c. Falciparum malaria d. Quartan malaria

29. Patient diagnosed to have malaria; smear shows all stages of schizonts 14-20 merozoites, yellowish brown pigment. The type of malaria is: *(AIIMS 2001)*
a. *Pl. falciparum* b. *Pl. malariae*
c. *Pl. vivax* d. *Pl. ovale*

BABESIA

30. Gold standard test for Babesia *(PGI May 2016)*
a. Giemsa stain
b. Culture
c. PCR
d. Antibody detection

31. Maltese cross is characteristic feature of: *(AI 2009)*
a. Cryptococcus neoformans
b. Babesia microti
c. Blastomycosis
d. Penicillium marfeni

32. True about Babesiosis: *(PGI June 2003)*
a. Caused by Babesia microti
b. Resides in RBC
c. Resides in WBC
d. Chloroquine is the treatment of choice

EXPLANATIONS

1. **Ans (c, d, e) (crescent shaped.., multiple rings., all developmental..)** (Ref: Apurba Sastry's Ess. of Medical Parasitology/p98
   - *P. falciparum*—RBC normal size, 3 types of ring forms are seen such as multiple ring forms, accolle form and double dot ring forms. Gametocytes are crescent shaped.
   - Most part of RBC cycle of *P. falciparum* occurs in deep vessels.
2. **Ans. (d) (P. vivax)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p102
3. **Ans. (b) (Due to adherence to the…)** Ref: Apurba Sastry's Essentials of Medical Parasitology/p94

   P.falciparum gets sequestrated by adhering to the endothelium of deep vessels. After the ring forms are formed, the remaining part of RBC cycle of P.falciparum occurs in deep vessels (not in peripheral blood). This is the reason why schizont and late trophozoite stages are not seen in peripheral blood smear.
4. **Ans. (a) (Trophozoite)** Ref: Apurba Sastry's Essentials of Medical Parasitology/p101
5. **Ans. (d) (P. malariae)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p96
   - In, P. malariae the febrile paroxysm occurs every 72 hrs, for others it occurs every 48 hrs
6. **Ans. (b) (Black water...)** Ref: Paniker's parasitology 7/e p77, Apurba Sastry's Essentials of Medical Parasitology 1/e p100
   - Black water fever is characterized by sudden intravascular hemolysis followed by fever, hemoglobinuria and dark urine. It occurs following quinine treatment to subjects previously infected with P. falciparum
7. **Ans. (a) (Resistant to treatment)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p93

   Recrudescence is seen in *P. falciparum* and *P. malariae* infections.
   - In falciparum malaria, it is due to persistence of drug resistant parasites following the treatment course.
   - In P. malariae infection, long-term recrudescences are seen for as long as for 60 years, this is due to long-term survival of erythrocytic stages at a low undetectable level in blood, but the cause is NOT identified.
8. **Ans. (a) (HLA-Bw53)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p102

   HLA-Bw53 and haplotypes bearing DRW13.02 antigen and R111 gene are protected from cerebral malaria.
9. **Ans. (b) (Development...)** Ref: Park 22/e p244, 385; Apurba Sastry's Essentials of Medical Parasitology 1/e p104
   - Roll back malaria (RBM): Launched in 2000. Aims at reducing the malaria cases by 50% by the end of 2010 and 75% by the end of 2015.
   - In sept 2008, RBM launched 'Global Malaria Action Plan' that defines the steps to achieve the target.

   Malaria control strategies
   - Surveillance and case management
   - Integrated vector management: Such as indoor residual spray, insecticide treated bed nets, antilarval measures.
   - Epidemic preparedness and early response
   - Supportive interventions
   - Capacity building
   - Behavior change communication
10. **Ans. (a) (Ronald ross)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p91
    - French army surgeon Alphonse Laveran (1880) was the first to discover the causative agent *Plasmodium*, in the RBC of a patient in Algeria.
    - Golgi had described the asexual cycle of the parasite in RBC.
    - Sir Ronald Ross, in 1897 had described the sexual cycle of the parasite in female *Anopheles* mosquito in Secunderabad, India.
11. **Ans. (c) (P.malariae)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p100
    - P.malariae is associated with nephrotic syndrome and renal failure
12. **Ans. (a) (c) (d) (e) (Cerebral feature, Severe anemia, Low Platelet count, Metabolic acidosis)** Ref: Harrison 18/e p1693-94 (Table 210-2 and 3); Apurba Sastry's Essentials of Medical Parasitology 1/e p100

Major manifestations of severe falciparum malaria *Harrison 18/e p1693 (Table 210-2)*	
Unarousable coma/cerebral malaria	Pulmonary edema/adult respiratory distress syndrome
Metabolic Acidemia/acidosis- low bicarbonate	Hypoglycemia
Severe normochromic, normocytic anemia	Hypotension/shock
Renal failure	Bleeding/disseminated intravascular coagulation
Hemoglobinuria	Convulsions
Other manifestations	
Impaired consciousness/arousable	Hyperparasitemia: Parasitemia level of > 5% in nonimmune patients (> 20% in any patient)
Extreme weakness	Jaundice

Features Indicating a Poor Prognosis in Severe Falciparum Malaria **Harrison 18/e p 1694 (Table 210-3)**	
Clinical: Marked agitation, Hyperventilation (respiratory distress), Hypothermia (< 36.5 °C), Bleeding, Deep coma, Repeated convulsions, Anuria, Shock ***Hematology:*** Leukocytosis, Severe anemia (PCV < 15%), Coagulopathy, low ***platelet count (< 50,000/µL),*** Prolonged prothrombin time, Prolonged partial thromboplastin time, Decreased fibrinogen	***Hyperparasitemia:*** Increased mortality at > 100,000/µL, High mortality at > 500,000/µL, > 20% of parasites identified as pigment-containing trophozoites and schizonts and > 5% of neutrophils with visible pigment ***Biochemistry:*** Hypoglycemia, Hyperlactatemia, Acidosis, Elevated serum creatinine, total bilirubin, liver enzymes, muscle enzymes, serum urate

13. Ans. (a) (b) (d) (Odisha, Gujarat, Delhi) Ref: Journals; Apurba Sastry's Essentials of Medical Parasitology 1/e p103

- Detection of *Plasmodium ovale* in ***Koraput district, Orissa*** state. *Indian J Med Res* 1989; *89:* 115–6.
- A case of Plasmodium ovale infection. Bull ***Calcutta*** Sch Trop Med 1966;14: 88–9.
- P. ovale malaria in ***Delhi.*** Mishra B et al., Indian J Pediatr 1999; 66: 143–4.
- P. ovale From ***Gujarat***, India, Marathe et al., J Vect Borne Dis 43, Dec 2006, p. 206–208
- P. ovale: First case report from ***Assam***. Mahanta et al., Ind J/of current science, vol. 84, no. 9, 10 may 2003

• Case reports of P.ovale reported from India -from Odisha, Kolkata, Delhi, Gujarat and Assam
• The sporadic presence of P. malariae (few thousands cases so far) has been reported from time to time in Orissa, West Bengal, Madhya Pradesh, Karnataka, Tamil Nadu, Kerala and Assam

14. Ans. (d) (Human) Ref: KD Chatterjee 13/e p91; Apurba Sastry's Essentials of Medical Parasitology 1/e p91

- Definite host (sexual cycle) of Plasmodium- Female anopheles mosquito,
- Intermediate host (asexual cycle) of Plasmodium - man

15. Ans. (b) (P. falciparum) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p94

- After the ring form is formed in the peripheral blood, the remaining of RBC cycle of P.falciparum occurs in the capillaries of brain and internal organs. Hence, only the ring forms are found in the peripheral blood by microscopic examination but not late trophozoites and schizonts.
- However, for other Plasmodium species, all erythrocytic stages occur in peripheral blood vessels.

16. Ans (a) (Plasmodium...) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p100

- ***Cerebral malaria:*** Occurs due to plugging of brain capillaries by sequestration of parasitized RBCs leading to vascular occlusion and cerebral anoxia.
- ***Sequestration:*** i.e. holding back of the parasite in the blood vessels of deep visceral organs like brain, kidney etc. leads to vascular occlusion.
- Cytoadherence *is mediated by a protein PfEmp* (P. falciparum erythrocytic membrane protein) which is only expressed by P.falciparum not by any other speices.

17. Ans. (a) (Anopheles stephensi) Ref: Park 22/e p233, Apurba Sastry's Essentials of Medical Parasitology 1/e p91

- Anopheles stephensi: Vector for Urban malaria
- Anopheles culicifacies: Vector for Rural malaria
- Anopheles fluviatilis: Highly anthrophilic, breeds in moving water
- Anopheles sundaicus: Breeds in brackish water

18. **Ans. (e) (Mosquito- definitive host)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p91, 102, 103
For details on epidemiology of malaria refer chapter review.

19. **Ans. (c) (10–14 days)** Ref: PSM Park 22/e p237, Apurba Sastry's Essentials of Medical Parasitology 1/e p96
Incubation period: The time interval between entry of the parasite to the body and appearance of first clinical feature is known as incubation period. It varies between the species.
- *P. vivax:* 14 days (ranges 8–17 days)
- *P. falciparum:* 12 days (ranges 9–14 days)
- *P. malariae:* 28 days (ranges 18–40 days)
- *P. ovale*: 17 days (ranges 16–18 days)

20. **Ans. (a), (b), (c) and (e) (Malaria, Toxoplasma, Babesia and Filaria)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p502
Parasites detectable in blood smear
- Plasmodium spp in RBCs
- Toxoplasma- Tachyzoites
- Filarial microfilaria
- Trypanosoma spp.: Trypomastigotes
- Leishmania amastigotes in macrophages
- Babesia- in RBC

21. **Ans. (c) (Use of bed nets)** Ref: Parija's Parasitology 3/e p139; Apurba Sastry's Essentials of Medical Parasitology 1/e p113
Multiple factors responsible for resurgence of malaria:
- Development of resistance of vector to insecticides
- Development of drug resistance to malarial parasites due to mutation
- Multiple water-logging areas, leading to increased mosquito breeding

Personal protective measures such as protective clothing, **usage of mosquito bed nets** or mosquito repellents are factors involved in **control of malaria out breaks** and not reasons for resurgence.

22. **Ans. (c) (gametocytes)** Ref: KD Chatterjee 13/e p92-94; Apurba Sastry's Essentials of Medical Parasitology 1/e p91
- Infective form of the parasites to humans- Sporozoites.
- Mature gametocytes are infective form to the female Anopheline mosquitoes.

23. **Ans. (c) (The...)** Ref: PSM Park 22/e p235-36, Apurba Sastry's Essentials of Medical Parasitology 1/e p95
- *'Malaria is communicable as long as mature, viable gametocytes exist in the circulating blood in sufficient density to infect vector mosquitoes.'*
- *RG interval (time interval between appearance of ring form & gametocyte):*
  - In vivax infections, - 4-5 days, In falciparum infections -10-12 days
  - In P.falciparum, sine the RBC cycle takes place in the vessels of internal organs, hence gametocyte takes time to come to the peripheral blood.
- *Gametocytes are numerous during early stages (may exceed 1,000 per cubic mm of blood")* and *do not increase in blood with time.*

24. **Ans. (d) (Histidine...)** Ref: Harrison18/e p1698, 17/e p1287; Apurba Sastry's Essentials of Medical Parasitology 1/e p108
Antigen detection tests [Rapid diagnostic tests (RDTs) or Immunochromatographic tests (ICTs)
- Rapid and simple but less sensitive, costly and may give false +ve in RA factor +ve cases
- pLDH and Aldolase: Common to all Plasmodium species
- HRP-2 Ag detection: Specific for P.falciparum

25. **Ans. (a), (b), (c) and (d) (Thrombocytopenia, DIC, Hemolysis and Hematemesis)** Ref: Harrison 18/e p1693, Apurba Sastry's Essentials of Medical Parasitology 1/e p100
- Refer chapter review for detail

26. **Ans. (a) and (b) (Splenomegaly and Nephrotic syndrome)** Ref: Harrison 18/e p1695, 17/e p1285-6; Apurba Sastry's 5Essentials of Medical Parasitology 1/e p100-101
For details on chronic complications of malaria, refer chapter review.

27. Ans. (a) and (c) (P.vivax and P.ovale) Ref: Harrison18/e p1689, 17/e p1280, K.D.Chatterjee 13/e p103; Apurba Sastry's Essentials of Medical Parasitology 1/e p98

- RBC size: Enlarged in –P. vivax and P. ovlae, others- normal size
- RBC shape: Oval in P. ovale, others- round

28. Ans. (d) (Quartan) Ref: Harrison 18/e p1689, 17/e p1280, KD Chatterjee 13/e p103; Apurba Sastry's Essentials of Medical Parasitology 1/e p98

- *P. malariae* which attacks mainly senescent (old) RBC's: Agent of Quartan malaria
- *P. ovale* and *P. vivax*: attack younger RBCs and reticulocytes: Agent of benign tertian malaria
- *P. falciparum:* attacks RBC of all ages: Agent of malignant tertian malaria.

29. Ans. (c) (*Pl. vivax*) Ref: K.D.Chatterjee 13/e p103; Apurba Sastry's Essentials of Medical Parasitology 1/e p98

This patient is suffering from malaria and his peripheral smear shows all stages of schizonts, 14–20 merozoites and yellowish-brown malarial pigment probably suggests vivax infection.

Species	Colour of pigment	No. of merozoites/Mature schizont
P. vivax	**Yellowish-brown**	**12–24**
P. falciparum	Dark brown	18–24
P. malariae	Dark brown	8 (6–12)
P. ovale	Dark Yellowish brown	8 (8–12)

BABESIA

30. Ans (a) (Giemsa stain) Ref: Apurba Sastry's Essentials of Medical Parasitology/p116

Gold standard test for most of all haemoparasites is stained peripheral smear. By this method direct parasitological diagnosis can be done as well as parasite density and response to treatment can be monitored. Demonstration of 2 or 4 rings inside the RBCs (called as maltese cross forms) in the Geimsa stained thick and thin blood smear is the diagnostic feature.

31. Ans. (b) (Babesia microti) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p115

- *Ring forms are arranged in tetrads – k/a Maltese cross appearance, Pathognomonic for babesiosis*
- Sporozoites of Babesia enter into RBCs where they transform into trophozoites and then multiply asexually by budding giving rise to two or four daughter pear shaped trophozoites (ring forms in tetrad called as ***Maltese cross form***) arranged inside RBCs.

32. Ans. (a) and (b) (caused by... and Resides...) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p114-115

- Intraerythrocytic protozoa
- Tick borne malaria like illness in animal
- Zoonotic: Opportunistic to human
- Species: *B. microti*, *B. bovis*, *B. diversus*
- Treatment of choice for Babesiosis: Clindamycin with oral quinine and atovaquone and azithromycin.

CHAPTER

6.5 Coccidian Parasites

COCCIDIAN PARASITES

- *Toxoplasma gondii*
- *Cryptosporidium parvum*
- *Isospora belli*
- *Sarcocystis lindermanii*
- *Cyclospora cayetanensis*

TOXOPLASMA GONDII

Life Cycle

- Worldwide distribution, infects a wide range of animals.
- Three morphological forms: Sporulated oocyst, crescentic tachyzoites, tissue cyst containing bradyzoites
- Infective form: All three morphological forms are infectious
- Definitive hosts are cat and other felines
- Intermediate hosts are man and other mammals (goat, sheep)
- Transmission:
  - MC mode: Ingestion of sporulated oocysts from contaminated soil, food, or water
  - Ingestion of tissue cyst containing bradyzoites from undercooked meat
  - By blood transfusion or vertical transmission tachyzoites are the infective form.
- Sporulated oocyst transforms into tachyzoites which multiply actively in blood, then finally transforms into tissue cyst containing the bradyzoites (resting stage) which get deposited in various organs.

Toxoplasma Gondii:
- MC mode: Ingestion of sporulated oocysts from contaminated soil, food, or water

Clinical Features

- Congenital toxoplasmosis:
  - 1st Trimester: More severe infection
  - 3rd Trimester: More chance of transmission
  - If Mother is previously infected: Fetus is asymptomatic.
  - Incidence: Approximately 1 per 1000 live births.
  - Featured by: 3C + 2M (chorioretinitis, cerebral calcification, convulsion, microcephaly and mental retardation)
  - Most common manifestation: Chorioretinitis
  - Diagnosis:
    - IgM detection in fetal blood, IgA can also be used (experimental but better sensitivity)
    - Toxoplasma Ag in amniotic fluid, PCR to detect Toxoplasma genes.
- Adult: Mostly asymptomatic, most common manifestation – Cervical LN↑
- In HIV pt:
  - Association rate 15–40%.
  - MC manifestation encephalitis
  - MC site involved: Brainstem
  - Occurs when CD4 < 100/µl
  - Other manifestations: Pulmonary infections and chorioretinitis.

Congenital toxoplasmosis:
- MC manifestation: Chorioretinitis
- More severe: 1st trimester
- More chance of infection: 3rd trimester.

Diagnosis

- Microscopy:
  - Blood smear: Comma shaped Tachyzoites (indicates active lesion)
  - Smear from biopsy from organs: Tissue cyst with bradyzoites (indicates chronic or past infection)

Microscopy for Toxoplasma:
- Blood smear: shows Comma shaped Tachyzoites (indicates active lesion)
- Smear from biopsy from organs- shows Tissue cyst with bradyzoites (indicates chronic or past infection)

- Antibody detection:
  - Sabin Feldman test:
    - Gold standard method, highly sensitive & specific but cannot differentiate recent and past infection
    - Pt serum + live tachyzoites + complement + methylene blue- Incubated
    - Ab in pt's serum binds to tachyzoites along with complement that leads to tachyzoites becomes distorted and colorless.
  - Other- ELISA, IFA
- PCR
- Animal Inoculation.

Treatment

- Immunocompetent adults: No t/t required
- Toxoplasmosis during pregnancy: Spiramycin is DOC
- HIV +ve patients Cotrimoxazole: DOC
  - Primary Prophylaxis:
    - Started in AIDS patients with Toxoplasma CD4 < 100/µl
    - Discontinued if responds to ART and CD4 > 200/µl for 3 months
  - Secondary Prophylaxis (Long-Term Maintenance Therapy):
    - Started if the CD4 < 200/µl.
    - Discontinued if pt asymptomatic, CD4 + T > 200/µl for 6 months.

COMPARISON OF COCCIDIAN PARASITES CAUSING DIARRHEA IN IMMUNOCOMPROMISED HOST

Property	Cryptosporidium	Cyclospora	Isospora
Infective form	Sporulated oocyst • Thick walled oocyst (80%) By contaminated food & water • Thin walled (20%) Autoinfection	Sporulated oocyst (Contaminated food & water)	Sporulated oocyst (Contaminated food & water)
Sporulated oocyst	4–6 µm, Round Contains four sporozoites	8–12 µm ,Round Contains 2 sporocyst, each having two sporozoites	23–36 µm Contains 2 sporocyst, each having four sporozoites
Acid fastness Detection limit > 50,000 oocyst/ml stool	Uniformly acid fast	Variable acid fast	Uniformly acid fast
Autofluorescence	No, but can be stained with fluorescent dye	Autofluorescence ++	Autofluorescence +/-
Sporulation of the oocyst	Occurs inside the host cells (enterocytes)	Occurs in soil (environment)	Occurs in soil (environment)
Diagnostic form	Sporulated oocyst	Unsporulated oocyst	Unsporulated oocyst
Autoinfection	Seen	Not seen	Not seen
Treatment	Nitazoxanide	Cotrimoxazole	Cotrimoxazole
Outbreaks	Common	Common	Less

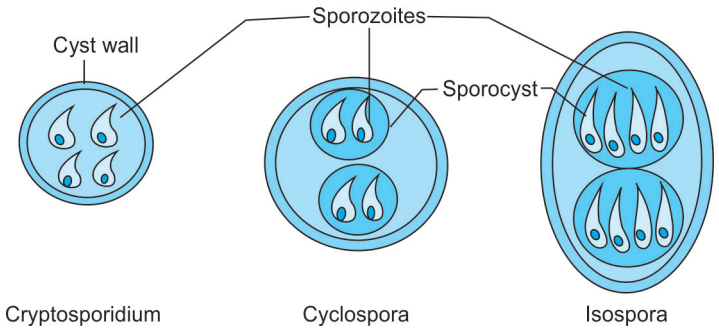


Fig. 6.5.1: Sporulated oocysts of various coccidian parasites

MULTIPLE CHOICE QUESTIONS

TOXOPLASMA

1 Triad of congenital toxoplasmosis is all except:
a. Intracranial calcification *(Recent Question 2015)*
b. Hydrocephalus
c. Chorioretinitis
d. Convulsion

2. All are true about Toxoplasma infection except:
a. May occur due to ingestion of oocyst from cat's feces *(PGI Nov 2014)*
b. May spread by organ transplantation
c. Toxoplasmosis is usually symptomatic in immunocompetent person
d. Infection is severe and progressive in immunocompromised host
e. Human infection is dead end for parasite

3. Sabin Feldman test is done for:
a. Toxoplasma *(NEET Pattern Based 2013)*
b. Ascaris
c. Filaria
d. Histoplasma

4. A pregnant lady presented with cervical lymphadenopathy. She was prescribed Spiramycin but was noncompliant. The baby has intracerebral calcification. What is the most probable diagnosis:
a. Toxoplasma *(AIIMS May 2011)*
b. Cytomegalovirus
c. Cryptococcus
d. Rubella

5. True about Toxoplasma gondii: *(PGI June 2011)*
a. Direct spread by blood/urine is main mode of transmission
b. Cerebellum is MC site of brain involvement
c. Isolation of parasite from blood is very easy
d. Laboratory test are useful for making diagnosis
e. Infection is severe and progressive in immunocompromised host

6. Cat acts as reservoir is which of the following?
a. Toxoplasma gondii *(DNB Dec 2009)*
b. Rabies
c. Streptocerca infection
d. Plague

7. Toxoplasmosis in the fetus can be best confirmed by: *(AIIMS 2002, AIIMS Nov 2001)*
a. IgM antibodies against Toxoplasma in the mother
b. IgM antibodies against Toxoplasma in the fetus
c. IgG antibodies against Toxoplasma in the mother
d. IgG antibodies against Toxoplasma in the fetus

8. Congenital toxoplasmosis false is: *(AIIMS May 2010)*
a. IgA is better than IgM in detection
b. Diagnosed by detection of IgM in cord blood
c. Dye test is gold standard and it detects IgG
d. Avidity test must be done to differentiate IgA and IgM

OTHER COCCIDIAN PARASITES

9. About Cryptosporidium. All are true except:
a. Shape round *(COMDEK 2016)*
b. Size (4–6 µm)
c. Causes cholangitis
d. Having two sporozoites

10. A patient was given a drug to inhibit oxidase-reductase system? What infestation was he having?
a. Beef tapeworm *(AI 2012)*
b. Whipworm
c. Cryptosporidium
d. Roundworm

11. True about cryptosporidium are all except:
a. Oocyst chorine resistant *(DNB Dec 2012, PGI 2003)*
b. Acid fast oocyst
c. Oocyst > 100 micro meters
d. Enzyme immune assay done

12. Which of the following is not a coccidian?
a. Isospora *(DNB June 2010)*
b. Cyclospora
c. Crytosporidia
d. Enterocytozoon

13. Cryptosporidium cyst identified by which stain in stool sample? *(DNB June 2012)*
a. PAS
b. H and E
c. Giemsa
d. Acid fast stain

14. 25-years-old male presented with diarrhea for 6 months. On examination the causative agent was found to be acid fast with 12 micro meter diameter. The most likely agent is: *(AIIMS May 2009)*
a. Cryptosporidium
b. Isospora
c. Cyclospora
d. Giardia

15. Which of the following is true with microsporidia?
a. It is fungus *(PGI Dec 2005)*
b. It is a protozoa
c. It is a bacteria
d. It is associated with diarrhea in HIV

EXPLANATIONS

TOXOPLASMA

1. **Ans. (d) (Convulsion)** Ref: Nelson's Paediatrics 18th/chapter 287
   - The characteristic triad of manifestations seen in congenital toxoplasmosis include chorioretinitis, hydrocephalus, and cerebral calcifications.
2. **Ans. (c) (Toxop...)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p120-22; Harrison 18/e p1722-26
   Refer chapter review.
3. **Ans. (a) (Toxoplasma)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p124
   For details about Sabin-Feldman dye test, refer chapter review.
4. **Ans. (a) (Toxoplasma)** Ref: Harrison 18/e p1728; Apurba Sastry's Essentials of Medical Parasitology 1/e p121-122
   - *Lymphadenopathy is the classical clinical sign of Toxoplasmosis and deep cervical lymph node are most commonly affected.*
   - *When pregnant lady was noncompliant, i.e. did not take spiramycin properly (which is the DOC of toxoplasmosis in pregnancy), the baby has developed congenital toxoplasmosis with intracerebral calcification.*
5. **Ans. (d) (e) (Laboratory test are useful for making diagnosis, infection is severe and progressive in immunocompromised host)** Ref: Harrison 18/e p1723-28; Apurba Sastry's Essentials of Medical Parasitology 1/e p121-124
   - ***Option a:*** The principal route of human toxoplasma infection is ***by the oral route*** by ingestion of either ***sporulated oocysts*** from contaminated soil, food, or water or ***bradyzoites*** from undercooked meat. Other Route-Transmission Via Blood or Organs and Transplacental Transmission……Harrison 18/e p1723
   - ***Option b:*** Although lesions can occur anywhere in the CNS, the areas most often involved appear to be the brainstem, basal ganglia, pituitary gland, and corticomedullary junction. ……Harrison 18/e p1725
   - ***Option c:*** Although difficult and available only at specialized laboratories, the isolation of *T. gondii* from blood or other body fluids can be accomplished ……. Harrison 18/e p1726
   - ***Option d:*** Serological procedures have great diagnostic value and has become the routine method of diagnosis.
   - ***Option e:*** Immunocompromised patient are at greatest risk for acute toxoplasmosis …Harrison 18/e p1728
6. **Ans. (a) (Toxoplasma gondii)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p120
   The life cycle of Toxoplasma gondii involves two hosts:
   - Definitive hosts are cat and other felines; where the sexual cycle takes place.
   - Intermediate hosts are man and other mammals (goat, sheep, pig, cattle and certain birds); where the asexual cycle takes place.
7. **Ans. (b) (IgM antibodies against Toxoplasma in the fetus)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p125
   - Refer earlier explanation.
8. **Ans. (d) (Avidity test must be done to differentiate IgA and IgM)** Ref: Harrison 18/e p1725-27
   - *Avidity test is done for IgG to differentiate recent and past infection.* Avidity low- indicates recent infection, Avidity strong- indicates past infection

   Test to differentiate recent and past infection:
   - IgG Avidity test: Avidity low- indicates recent infection, Avidity strong- indicates past infection
   - IgM detection by ELISA or IFA: Indicates recent infection
   - Detection of Toxoplasma antigen or genes.

OTHER COCCIDIAN PARASITES

9. **Ans (d) (Having two sporozoites)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p130
   - Cryptosporidium parvum is round, 4–6 µm in size, having four sporozoites inside.
   - Reversible sclerosing cholangitis secondary to cryptosporidiosis in a renal transplant patient.

10. **Ans. (c) (Cryptosporidium)** Ref: Harrison 18/e p1732, Goodman Gillman 11/e p40; Apurba Sastry's Essentials of Medical Parasitology 1/e p13
    - **Drug to inhibit oxidase-reductase system:** Suggestive of Nitazoxanide and this drug is usually indicated in Cryptosporidium parvum infections.
11. **Ans. (c) (Oocyst > 100 micro meters)** Ref: Apurba Sastry's Essentials of Medical Parasitology, p/126-130
    - Oocyst - round, 4–6 µm size, contains four sporozoites
    - Oocyst of Cryptosporidium are resistant to may disinfectants including chlorine
    - Oocyst are acid fast to 1% sulfuric acid or acid alcohol
    - ELISA has been developed to detect C. parvum specific coproantigen from stool, shows a sensitivity ranging from 66% to 100% with excellent specificity.
12. **Ans. (d) (Enterocytozoon)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p118
    - Coccidian parasites include: Toxoplasma, Cryptosporidium, Cyclospora and Isospora
    - Enterocytozoon belongs to the phylum Microspora.
13. **Ans. (d) (Acid fast stain)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p130
    - *Acid fast staining*: The oocysts of *C.parvum* are acid fast to 1% sulfuric acid or acid alcohol and appear as round, 4–6 µm red color oocyst against blue back ground. The sensitivity of acid fast staining is low and it requires a minimum concentration of >50,000 oocysts/ml of stool.
    - *Direct Fluorescent Antibody staining* is done to detect *C.parvum* oocyst by using fluorescent labeled monoclonal antibody directed against cyst wall antigens. This is more sensitive (10 times) and specific than acid fast staining. It is also useful to detect oocyst from water and other environmental samples. Currently, this method is considered as the gold standard test for cryptosporidiosis.
14. **Ans. (c) (Cyclospora)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p132
    - All the parasitic agents provided in options above can cause chronic diarrhea
    - Among which Cysts of Giardia: Non acid fast
    - Acid fast oocyst with 12 µm in size: Suggestive of Cyclospora (**8–12 µm)**
    - Isospora (23–36 µm) or Cyclospora (4–6 µm) are also acid fast but of different size.
15. **Ans. (a) and (d) (It is a fungus and it is associated with diarrhea in HIV)** Ref: Jawetz 24/e p684; Apurba Sastry's Essentials of Medical Parasitology 1/e p141
    - Microsporidium is a ***recently classfied under Fungi***.
    - It mostly causes infection in *severely immunocompromised patients with AIDS* (diarrhea).
    - Microsporidium is characterized by a unicellular spore containing a coiled spring-like tubular polar filament through which the sporoplasm is forcibly discharged into a host cell.

CHAPTER

6.6

Cestodes and Trematodes

Properties	Cestodes	Trematodes	Nematodes
Shape	Tape like, segmented	Leaf like, unsegmented	Elongated, cylindrical, unsegmented
Head end	Suckers present, some have attached hooklets	Suckers present No hooklets	No sucker, no hooklets. Some have well developed buccal capsule
Alimentary canal	Absent	Present but incomplete, no anus	Complete from mouth to anus
Body cavity	Absent	Absent	Present
Sexes	Monoecious	Monoecious (except: *Schistosomes*)	Diecious
Life cycle	Requires two hosts (except: *Hymenolepis and Diphyllobothrium*)	Requires three hosts (except: *Schistosoma*)	Requires one host (except: filarial worms and *Dracunculus*)
Larva form	Cysticercus, Hydatid cyst, Coenurus, Cystecercoid, Coracidium, Plerocercoid, Procercoid	Cercaria Metacercaria Redia Miracidium Sporocyst	Rhabditiform larva Filariform larva Microfilaria

CESTODES

- Cestodes or tapeworms are segmented worms.

Intestinal Cestodes — Humans are Definitive Host	Tissue Cestodes —Humans are Intermediate Host
• *Taenia saginata* and *T. solium* • *Diphyllobothrium* • *Hymenolepis nana* • *Dipylidium caninum*	• Echinococcus: Causing hydatid disease (liver) • *Taenia solium*: Causing cysticercosis (CNS) • Taenia multiceps: Causing coenurosis (CNS) • Spirometra: Sparganosis (muscle)

Cestodes Eggs

- All cestodes eggs have an egg shell and three pair of hooklets except D. latum eggs (operculated)

Cestodes Exist in three Morphological Forms

- Adult (tapeworm): Divided to head (scolex), neck and segments called as proglottids or strobila
- Some adults bear hooklets in scolex and called as armed tapeworm, e.g. T. solium, Echinococcus, H. nana
- Eggs: All cestodes eggs have an egg shell and three pair of hooklets except D. latum eggs (operculated)
- Larva: Eggs develop to larva which are called as:
  - Cysticercus: Larval stage of *Taenia* [T. saginata-Cysticercus bovis, T. solium-C. cellulosae]
  - Sparganum: Larval stage of *Spirometra*
  - Hydatid cyst: Larval stage of *Echinococcus*
  - Coenurus: Larval stage of *Multiceps*
  - Cysticercoid: Larval stage of *Hymenolepis, Dipyllidium*
  - *Diphyllobothrium*: has 3 larva stages, L1-Coracidium, L2-Procercoid, L3-Plerocercoid larva

Taenia

Taenia Species	Definitive Host	Intermediate Host	Organ Affected	Disease
T. saginata	Man	Cattle	Intestine	Intestinal taeniasis
T. solium	Man	Pig	Intestine	Intestinal taeniasis
T. solium	Man	Man	Muscle, CNS, eye	Cysticercosis
T. asiatica	Man	Pig (Liver)	Intestine	Intestinal taeniasis
T. multiceps	Dog	Sheep, rarely man	CNS	Coenurosis

Table 6.6.1: Differences between *Taenia saginata* and *Taenia solium*

Features	Taenia saginata	Taenia solium
Adult worm		
Length	4–6 meters or more	2–4 meters
Head/scolex	• Large and quadrangular • Four suckers present which may be plgmented • No rostellum, No hooklets	• Small and globular • Four suckers present—not pigmented • Bears rostellum with two rows of hooklets • Hence called as armed tapeworm
Neck	Longer	Shorter
Proglottids		
No. of Proglottids	1,000–2,000	800–1,000
Uterus	Bears in 15–20 lateral branches	Bears in 7–13 lateral branches
Lobes of Ovary	Two, No accessory lobe	Three-two lobes with an accessory lobe
Testes	300–400 follicles	150–200 follicles
Vaginal sphin	Present	Absent
Measurement	Gravid segment–20 mm × 5 mm	Gravid segment–12 mm × 6 mm
Expulsion of segments	Expelled singly in the faces	Expelled in chain of 5–6 segments
Eggs per segment	80,000 eggs per gravid segment	40,000 eggs per gravid segment
Larva		
	Cycticercus bovis present in cattle's muscle, but not in man	Cysticercus cellulosae present in pig's muscle and also in man (muscle, eye and brain)
Egg		
	Acid fast	Non acid fast
Disease	Causes intestinal taeniasis	Causes intestinal taeniasis and cysticercosis
Egg		
Host	Definitive host: Man Intermediate host: Cattle	For intestinal taeniasis: • Definitive host: Man • Intermediate host: Pig For Cysticercosis: • Both definitive and intermediate host: Man
Infective form	Larva (cysticercus bovis)	• For intestinal taeniasis–Larva (cysticercus cellulosae) • For cysticercosis–egg
Diagnostic form	Egg	• For intestinal taeniasis-Eggs • For cysticercosis–larva (cysticercus cellulosae deposited in tissue)
Mode of transmission	Ingestion of contaminated beef	For intetinal taeniasis–ingestion of contaminated pork For cysticercosis: • Contaminated food and water • Autoinfection

Cysticercosis

- Potentially dangerous systemic disease.
- Definitive host: Man, Intermediate host: Man
- Transmission: (i) Ingestion of food/water contaminated with eggs, (ii) autoinfection
- Eggs develop to larva (Cysticercus cellulosae) in human intestine
- Larvae penetrate the intestine and get deposited in-MC sites- CNS (60-90%) followed by Eye and muscle.

Neurocysticercosis (NCC)

- NCC: MC parasitic CNS infection of man and MC cause of adult onset epilepsy in world.
- MC site: Sub-arachnoid space followed by parenchyma.
- Seizure: MC manifestation (70% of cases). NCC accounts for 50% cases of late onset epilepsy.
- Hydrocephalus, intracranial hypertension and psychiatric disturbances.
- Four morphological stages: Vesicular, necrotic, nodular, calcified stages.
- Clinical feature depends on: (i) No. of cyst, (ii) Location-parenchymal or extra-parenchymal, (iii) Size (small cyst-C. cellulosae, big cyst-C. racemosus), (iv) Morphological stage and (v) Host immune response.
- Lab diagnosis:
  - Antibody detection by ELISA or Western blot (specific)
  - Detection of the cyst by CT/MRI
  - CT scan is superior to detect calcified cysts (appears as hyperdense dots).
  - MRI is superior to CT scan to detect the Extra parenchymal cysts in ventricle and cisterns, inflammatory changes, vesicular, necrotic lesions and noncystic lesions.
  - Del Brutto's criteria used for neurocysticercosis.
- Treatment: Albendazole, Praziquantel, Surgery (for ocular and spinal and ventricular lesions).

Neurocysticercosis (NCC)
- NCC: MC parasitic CNS infection of man and MC cause of adult onset epilepsy in world.
- MC site: sub-arachnoid space followed by parenchyma
- Four morphological stages: Vesicular, necrotic, nodular, calcified stages

Taenia Multiceps

- Definitive host: Dog, fox and wolf
- Intermediate host: herbivorous animals like sheep (or man)
- Transmission: Ingestion of food/water contaminated with eggs
- Eggs develop to larva (Coenurus) which is a unilocular cyst with multiple scolices.
- Larva penetrate the intestine and get deposited in CNS (Coenurosis)
- Man space occupying lesions in CNS (headache, vomiting, paralysis, seizure, etc.)
- In animals, it causes gid (CNS lesion)
- *Epidemiology:* African countries (like Uganda, Kenya).

Eggs–Infective stage of the Echinococcus

Echinococcus Granulosus

Life Cycle

- Definitive host: Dog and wild carnivores.
- Intermediate hosts: Man and other herbivorous animals.
- Man is an accidental host (dead end).
- Eggs: Infective stage of the parasite.
- Eggs transform to larva (hydatid cyst) that penetrate GIT and migrates to various organs like liver.

Clinical Features

- Hydatid disease: Hepatomegaly (60—70% of cases), then lungs
- *E. multilocularis*:
  - Causes Alveolar Hydatid disease because cyst has multiple locules but has no fluid/free brood capsule
  - 90% liver involvement, rapidly metastasizes (mimic malignant tumor)
- E. oligarthrus and E. vogeli: Causes Polycystic Hydatid disease.

Diagnosis

- Hydatid fluid microscopy:
  - Wet mount examination to demonstrates protoscolices and brood capsule
  - Acid fast staining of centrifuged deposit
  - Histological examination
- Casoni's skin test: Example of immediate hypersensitivity reaction
- Antibody: Indicates past infection, used for seroepidemiology:
  - Screening: IHA, CIEP, ELISA
  - Confirm: Western Blot (against antigen B fragment)
- Detection of antigen: Indicates Recent infection
- Imaging methods like USG, MRI and X-ray: Demonstrates size, exact location and extension of the cysts
- Water lily sign in USG: Due to collapsed cyst (floating membrane) floating in the abdomen
- Tests to monitor the response to treatment: Imaging methods and Antigen detection methods.

Casoni's test— Example of immediate hypersensitivity reaction, done for hydatid disease

Water lily sign in USG
Due to Collapsed cyst (floating membrane) floating in the abdomen

Treatment

- Treatment of choice: Surgery
- DOC: Albendazole and mebendazole
- Commonly preferred method: Percutaneous Aspiration Injection Reaspiration (PAIR) of the cyst.

Diphyllobothrium latum
- Largest tapeworm in human GIT
- Adult is >10 meters with long >3000 proglottids

Diphyllobothrium Latum

- Largest tapeworm in human GIT: Adult is >10 meters with long > 3000 proglottids
- Scolex bears two longitudinal groove called bothria
- Also k/a-fish tapeworm or human broad tapeworm
- Definitive host: Man
- Intermediate host:
  - 1st intermediate host: Cyclops/diaptomus
  - 2nd intermediate host: Fresh water fish
- There are three larval stages: L1 (coracidium), L2 (procercoid) and L3 (plerocercoid)
- Infective form- Plerocercoid: (L3 stage larva)
- Mode: Ingestion of raw fish
- Life cycle: Ingestion of Plerocercoid (L3) in fish → develop to Adult → Eggs released in feces → Eggs transform to coracidium (L1) in feces → Cyclops (forms Procercoid) → ingested by fish (forms pleurocercoid)
- Causes Megaloblastic anemia (adult worm absorbs B_{12})
- Diagnostic form: Operculated eggs in stool.

Diphyllobothrium latum
- Infective form: Plerocercoid (L3 stage larva)

Diphyllobothrium latum
- Causes Megaloblastic anemia (adult worm absorbs B12)

Sparganosis

- Caused by *Spirometra* and other nonhuman *Diphyllobothrium* tapeworms
- Definitive hosts: Dogs and cats (rarely man), 1st intermediate host: *Cyclops* and 2nd intermediate host: Frog, snakes and birds.
- Sparganosis: Sparganum or plerocercoid (L3) larva get deposited in SC tissues, muscles, eyes, lymphatics and visceral organs like brain.

Hymenolepis Nana

- Also called as Dwarf tapeworm
- Egg is infectious to man
- Only one host involved
- Autoinfection seen
- Armed scolex

- Larva form called Cystecercoid larva
- Egg smaller, bile non stained and has polar filament: Diagnostic form.

H. diminuta

- Rat tapeworm
- Mode: Ratflea infected with cystecercoid larva
- Diagnosed by the detection of eggs in the stool: Egg larger and lack polar filament.

Dipylidium Caninum (Double Pored Tapeworm)

- Host: Definitive host—dogs and cats (rarely man), intermediate host—insects (flies)
- Man acquires infection by ingestion of flea containing cysticercoid larva
- GIT symptoms
- Diagnostic form:
  - Eggs in packets
  - Proglottid has two common genital pore
  - Barrel shaped Proglottid.

Treatment of Cestodes

- Praziquantel is the DOC of all cestodes followed by Niclosamide except. Hydatid disease and neurocysticercosis: Albendazole.

TREMATODES

General Features

- Agents:
  - Schistosoma (blood fluke)
  - Fasciola hepatica (liver fluke), Fasciolopsis buski (intestinal fluke)
  - Paragonimus westermani (lung fluke)
  - Clonorchis, Opisthorchis
- Infective form: Metacercaria larva for all except: (Cercaria larva for Schistosoma)
- Definite host: Man
- Intermediate host:
  - 1st – Snail
  - 2nd–Aquatic plants (F. hepatica and F. buski) Cray fish/crab Fish (Paragonimus, Clonorchis, Opisthorchis)
- Mode of transmission:
  - For all: Ingestion of 2nd intermediate host containing Metacercaria larva
  - Schistosoma: Skin penetration by cercaria larva present in contaminated water
- All trematodes are Oviparous (lays eggs)
- Diagnostic form:
  - For all: Demonstration of operculated Eggs
  - Schistosoma: Demonstration of nonoperculated Eggs
- All trematodes are hermaphrodite (except Schistosoma: sexes are separate)
- *DOC:* Praziquantel is DOC of all trematodes except *F. hepatica (*Triclabendazole).

Infective form of Trematodes:
- Metacercaria larva for all except: (Cercaria larva – for Schistosoma)

Mode of transmission of Trematodes
- For all except Schistosoma- Ingestion of 2nd intermediate host containing Metacercaria larva
- For Schistosoma: Skin penetration by cercaria larva present in contaminated water

Schistosoma Hematobium – (Blood Fluke)

- Resides in: Vesical and pelvic venous plexus
- Associated with:
  - Hematuria
  - Hydroureter and hydronephrosis
  - Bladder Carcinoma: Sqamous cell Ca (in high worm burden) > transitional cell Ca (low worm burden)

- Egg has terminal spine
- Antibody detection:
  - HAMA-FAST: ELISA (Falcon assay screening test ELISA) using *S. haematobium* adult worm microsomal antigen (HAMA).
  - HAMA Western blot: Specific
  - Other methods: Cercarial Huller reaction, IFA, IHA
- Antigen detection:
  - Circulating cathodic antigen (CCA) in urine and circulating anodic antigen (CAA) in serum.
  - It indicates recent infection and can be used for monitoring the treatment.

S. Mansoni

- Common in Africa including Caribbean Islands (West Indies), South America
- Resides in mesenteric veins draining sigmoido-rectal region.
- Clinical manifestation:
  - Swimmer's itch (cercarial dermatitis): Type 1 hypersensitivity reaction
  - Dysentery and Eosinophilic diarrhea
  - Acute schistosomiasis (Katayama fever) Serum sickness like illness: Type III hypersensitivity
  - Chronic schistosomiasis: due to fibrosis and granuloma formation as a result of egg deposition in various sites like intestinal wall, liver, spleen and lungs.
  - Secondary bacterial infection especially with *Salmonella* spp.
- Diagnostic form: Egg has lateral spine (feces), *eggs of S. mansoni are acid fast.*
- Quantitations of eggs in stool by Kato Katz thick smear technique.

S. Japonicum

- Resides in mesenteric veins draining the ileocecal region.
- Clinical feature: Similar to S. mansoni but it is **more severe** due to higher egg production and smaller size of the eggs (easy dissemination).
- Diagnostic form: Eggs in stool (has rudimentary lateral spine).

Fasciola Hepatica - Sheep Liver Fluke

- Definitive host: Sheep or man, Intermediate host- 1st -Snail and 2nd -Water cress
- Mode of transmission: Ingestion of aquatic plant contaminated with encysted metacercaria.
- Clinical manifestation:
  - Hepatomegaly
  - Halzoin (laryngeal edema): d/t eating sheep liver
  - Bile duct obstruction
- Diagnostic form: Operculated eggs in feces.

Paragonimus westermani:
- Causes endemic hemoptysis
- Endemic in Manipur

Fasciolopsis Buski

- Intestinal fluke → Largest fluke
- Definite host: Man/pig
- Mode of transmission: Ingestion of aquatic plant contaminated with encysted metacercaria
- GIT symptoms
- Diagnostic form: Operculated eggs in feces.

Paragonimus Westermani: (Lung Fluke)

- Definitive: host man; Intermediate host- 1st –snail, 2nd - Cray/Crab fish
- Mode of transmission: Ingestion of crab/crey fish contaminated with encysted metacercaria
- Cyst in Right lung (granuloma formation due to egg deposition)
- Cerebral and cutaneous paragonimiasis

- Golden brown sputum
- Causes endemic hemoptysis
- Diagnostic form: Operculated eggs in early morning, deeply coughed sputum
- Endemic in Manipur.

Clonorchis Sinensis

- Oriental/Chinese liver fluke
- Definitive: host man; Intermediate host: 1st –snail, 2nd - Cray/crab fish
- Mode of transmission: Ingestion of crab/crey fish contaminated with encysted metacercaria
- Causes:
  - Cholangitis, dilatation of the bile duct and ductal epithelial hyperplasia and fibrosis
  - Cholangiocarcinoma
- Diagnostic form: Flask shaped operculated egg in stool.

MULTIPLE CHOICE QUESTIONS

CESTODES

1. Cysticercus cellulose is larvae of: *(TNPG 2014)*
a. Echinococcus granulosus
b. Taenia saginata
c. Taenia solium
d. Hymenolepis nana

TAENIA

2. T. saginata is differentiated from T. solium by presence of: *(PGI May 2015)*
a. Hooklets are present in scolex (head)
b. 4 large pigmented sucker
c. Uterus is thin and dichotomous
d. Short neck
e. Egg is not infective to man

3. Which of the following is the most common location of intracranial neurocysticercosis: (AIIMS Nov 2005)
a. Brain parenchyma b. Subarachnoid space
c. Spinal cord d. Orbit

4. Cysticercosis is caused by: (PGI June 2005, TN 2005)
a. T. solium b. T. saginata
c. A. duodenale d. E. granulosus
e. E. multilocularis

5. Which of the following is the most common central nervous system parasitic infection? (AIIMS 2003)
a. Echinococcosis
b. Sparganosis
c. Paragonimiasis
d. Neurocysticercosis

6. Cause of epilepsy in up to 50% Indian patients is:
a. Neurocysticercosis *(MHPG 2014)*
b. Cerebral malaria
c. Toxoplasmosis
d. Cerebral hydatid cyst

ECHINOCOCCUS GRANULOSUS

7. Water lily sign is seen in infection due to:
a. Neurocysticercosis *(TNPG 2015)*
b. Schistosomiasis
c. Filariasis
d. Echinococcus infection

8. All are true about hydatid cyst, except: *(TNPG 2015)*
a. Dog is the definitive host
b. Man is the intermediate host
c. Liver is the most common site of infection
d. The egg is labile at extremes of temperature

9. Dog is the host for? *(PGI May 2016)*
a. Toxocara canis
b. Echinococcus granulosus
c. Echinococcus vogeli
d. Taenia solium
e. Taenia saginata

10. Skin test useful in hydatid disease is:
a. Casoni's test *(MHPG 2015; DNB Dec 2011, AI 2000)*
b. Schick test
c. Patch test
d. Dick's test

11. Arc-5 in CIEP is diagnostic for: *(MH 11)*
a. Hydatid disease b. Cysticercosis
c. Cryptococcosis d. Brucellosis

12. Intermediate host for hydatid disease:
a. Man *(AIIMS May 2009)*
b. Dog
c. Cat
d. Foxes

13. Hydatid disease of liver is caused by: *(PGI Dec 2001)*
a. Strongloides
b. Echinococcus granulosus
c. Taenia solium
d. Trichinella spiralis
e. Echinococcus multilocularis

14. PAIR stands for: *(Recent MCQ 2013)*
a. Percutaneous Aspiration Injection and Reaspiration
b. Pervenous Aspiration Injection and Reaspiration
c. Per subcutaneous Aspiration Injection and Reaspiration

DIPHYLLOBOTHRIUM LATUM

15. True about diphyllobothrium: *(NEET Pattern Based)*
a. Man is single host
b. Iron deficiency anemia is seen
c. Operculated egg is diagnostic
d. Fish is the definitive host

16. Second intermediate host to Diphyllobothrium latum is: *(PGI 06)*
a. Cyclops b. Man
c. Snail d. Fresh water fish

17. Helminth implicated in causing pernicious anemia is: *(AIIMS MAY 2016)*
a. Diphyllobothrium latum
b. Ascaris
c. Taenia solium
d. Hymenolepis nana

HYMENOLEPIS NANA

18. Dwarf tapeworm refers to: *(Recent Question 2014)*
a. Echinococcus
b. Loa Loa
c. Hymenolepsis nana
d. Schistosoma mansoni

19. Which of the following egg contains three pairs of hooklets and polar filaments but bile non stained? *(PGI 02)*
a. H. nana
b. T. solium
c. T. saginata
d. E. granulosus
e. D. latum

TREMATODES

20. Which one cannot act as definitive host of Clonorchis sinensis? *(PGI Nov 2016)*
a. Dogs
b. Pigs
c. Cats
d. Cows
e. Goats

21. 15-Year-old complains of loose motion, intermittent abdominal pain of 1-year wet mount of stool shows multiple ova > 100 microns in length. Which of the following agent is responsible: *(AIIMS May 2013)*
a. Fasciola gigantica
b. Gastrodiscoides hominis
c. Ancyclostoma caninum
d. Ophistotichus viverni

22. Effective drugs for Fasciola hepatica: *(PGI Nov 2016)*
a. Bithionol
b. Praziquantel
c. Triclabendazole
d. Metronidazole
e. Niclofolan

23. Water host required for schistosomiasis:
a. Fish *(NEET Pattern Based)*
b. Cyclops
c. Snails
d. Crabs

24. True about trematodes: *(NEET Pattern Based)*
a. Three hosts required
b. Segmented
c. Anus present
d. Body cavity present

25. Which organism can be isolated from stool and sputum? *(NEET Pattern Based)*
a. Paragonimus
b. Fasciola
c. Chlonorchis
d. P. carini

26. Cercariae are infective form of: *(NEET Pattern Based)*
a. S. hematobium
b. P. westermanii
c. F. hepatica
d. T. solium

27. A man after return from complains of pain abdomen, jaundice with increased alkaline phosphatase and conjugated hyperbilirubinemia: USG shows blockade of the biliary tree. What could be the cause?
a. Fasciola buski *(AIIMS 09, AI 2008)*
b. Strongyloides
c. Clonorchis sinensis
d. Gnathostoma spinigerum

28. A 7-year-old presented with intermittent abdominal cramps, loose stool and on stool examination eggs of size 100 µm are seen, which is not the cause:
a. Fasciola gigantica *(AIIMS Nov 2009)*
b. Echinostoma iliocanum
c. Gastrodiscoides hominis
d. Opisthorchis viverini

29. Parasites causing lung infestation are: *(PGI Dec 2003)*
a. H. nana
b. Paragonimus westermanii
c. Taenia saginata
d. E. granulosus
e. E. multilocularis

30. Cholangiocarcinoma is caused by: *(PGI June 2002)*
a. Fasciola infestation
b. Clonorchis infestation
c. Paragonimus infestation
d. Ascaris infestation
e. None of these

31. Pancreatic Ca is caused by: *(AI 2001)*
a. Fasciola
b. Clonorchis
c. Paragonimus
d. None

32. Which is not a liver fluke? *(PGI 10)*
a. Paragonimus
b. Clonorchis sinensis
c. Gnathostoma spinigerum
d. Opisthorchis viverinii
e. Whipworm

33. Katayama fever is seen in:
a. Schistosoma haematobium
b. Sch. mansoni *(NEET Pattern Based, TNPG 2015)*
c. Sch. japonicum
d. Sch. mekongi

34. Terminal spined eggs are seen in: *(PGI 01, 1995)*
a. Schistosoma haematobium
b. Sch. mansoni
c. Sch. japonicum
d. Chlonorchis sinensis

35. Schistosoma japonicum resides in: *(AI 2000, 1992)*
a. Vesical Plexus
b. Splenic vein
c. Systemic Circulation
d. Gallbladder

36. Man-Snail-Crab-Man cycle is seen:
a. Paragonimus wetsermanii *(Recent MCQ 2013)*
b. Fasciola hepatica
c. Schistosoma mansoni
d. Echinococcus granulosus

EXPLANATIONS

CESTODES

1. **Ans. (c) (Taenia solium)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p160
   - Refer chapter review.

TAENIA

2. **Ans. (b, e) (4 large pigmented sucker, Egg...)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p166

	T. saginata	T. solium
Head/scolex	Large and quadrangular	Small and globular
	Four suckers present which may be **pigmented**	Four suckers present—not pigmented
	No rostellum, **No hooklets**	Bears rostellum with hooklets
Neck	**Longer**	Shorter
Uterus	Bears in 15–20 lateral branches	Bears in 7–13 lateral branches
Infective form	**Larva** for intestinal taeniasis	Larva for intestinal taeniasis
		Egg for cysticercosis

3. **Ans. (b) (Subarachonoid space)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p171
   - Though brain parenchyma was thought to be the commonest site of Neurocysticercosis but recent evidences have shown that ***subarachnoid space*** being the commonest site, followed by brain parenchyma.

4. **Ans. (a) (T. solium)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p171
   - Causative agent of cysticercosis: *Taenia solium* (Pork Tape worm)
   - *Human beings are infected by eating undercooked pork containing the cysticerci.*
   - ***Cysticercus cellulosae*** is the larval stage found commonly in Pig, but sometimes in humans by drinking contaminated water or by eating uncooked vegetables infected with egg/autoinfection.

5. **Ans. (d) (Neuro...)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p171

Parasites causing CNS infections:

Protozoan Parasites	Helminths
Acanthamoeba: Granulomatous amoebic encephalitis	***T. Solium***: Neurocysticercosis
Entamoeba histolytica: 2^0 invasion	*Taenia multiceps: Coenurosis*
Naegleria: Primary amoebic meningoencephalitis	*Angiostrongylus cantonensis: Eosinophilic meningitis*
Balamuthia: Granulomatous amoebic encephalitis	*Echinococcus granulosus (hydatid cyst): 2^0 invasion*
Plasmodium falciparum: Cerebral malaria	*Gnathostoma spinigera*
Trypanosoma brucei: African sleeeping sickness	
Toxoplasma gondii in HIV: Encephalitis	

   - Neurocysticercosis (NCC) is the most common form and accounts for 60–90% cases of Cysticercosis.
   - NCC is considered as the most common parasitic CNS infection of man and most common cause of adult onset epilepsy throughout the world.

6. **Ans. (a) (Neurocysticercosis)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p171
   Neurocysticercosis accounts for 50% cases of late onset epilepsy in Indian patients.

ECHINOCOCCUS GRANULOSUS

7. **Ans. (d) (Echinococcus infection)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p181
   Water lily sign is seen in hydatid disease and is due to detached cyst floating within the cavity.

8. **Ans. (d) (The egg is labile...)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p177-81
Echinococcosis, http://www.cfsph.iastate.edu/Factsheets/pdfs/echinococcosis.pdf.
Echinococcus eggs remain viable in water and damp sand for three weeks at 30°C, 225 days at 6°C and 32 days at 10–21°C.

9. **Ans (a, b, c) (Toxocara canis, Echinococcus granulosus, Echinococcus vogeli)** Ref: Apurba Sastry's Essentials of Medical Parasitology/p6-7,184, 249
   - Echinococcus granulosus (dog tape worm), Echinococcus vogeli (Bush dogs are final host).
   - Toxocara canis (also known as dog roundworm) is worldwide-distributed helminth parasite of dogs.

10. **Ans. (a) (Casoni's test)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p182
   - Casoni's test done for hydatid disease
   - Schick test done for diphtheria
   - Patch test done for filariasis
   - Dick's test done for streptococcus.

11. **Ans. (a) (Hydatid disease)** Ref: KD Chatterjee 13/e p165; Apurba Sastry's Essentials of Medical Parasitology 1/e p181
   - Immunodiffusion and counter current electrophoresis (CIEP) are used for hydatid disease: Detecting antibody against antigen-5 (arc-5)
   - Western blot: Detecting antibody against antigen B fragment
   - Antibody methods are useful for seroepidemiological study but cannot differentiate recent and past infection.

12. **Ans. (a) (man)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p177
   - **Causative agent of hydatid disease: *Echinococcus granulosus*/** *dog tape worm/ hydatid worm*
   - Definitive host: Dog and other canine animals
   - **Intermediate host: man**
   - Habitat: Man harbors the larval form, which is found within the hydatid cyst
   - It represents the structure of the scolex of the future adult worm.

13. **Ans. (b) (e) (Echi..., Ech....)** Ref: KD Chatterjee 13/e p165; Apurba Sastry's Essentials of Medical Parasitology 1/e p177
Hydatid disease:
   - Echinococcus granulosus: Causes Hydatid disease—Hepatomegaly (60—70% of cases), then lungs
   - *E. multilocularis*: Causes Alveolar Hydatid disease (90%-liver involvement)
   - E. oligarthrus and E.vogeli: Causes Polycystic Hydatid disease.

14. **Ans. (a) (Percutaneous Aspiration Injection and...)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p182
   - PAIR (Percutaneous Aspiration Injection and Reaspiration) is a semi conservative surgery done for hydatid disease.

DIPHYLLOBOTHRIUM LATUM

15. **Ans. (c) (Operculate...)** Ref: KD Chatterjee 13/e p148; Apurba Sastry's Essentials of Medical Parasitology 1/e p160
   - Diphyllobothrium has three hosts. Man is definitive host, Cyclops are the 1st intermediate host and fish is the 2nd intermediate host
   - It causes megaloblastic anemia (due to absorption of vitamin B_{12} from intestine)
   - Operculated egg is the diagnostic form seen in stool.

16. **Ans. (d) (Fresh water fish)** Ref:Apurba Sastry's Essentials of Medical Parasitology 1/e p160
   - *Diphyllobothrium latum* or fish tape worm or broad tapeworm:
     - Definitive host: man, dog and cat.
     - Intermediate host: 1st Cyclops/diaptomus, 2nd - fresh water fish

17. **Ans. (a) (D. latum)** Ref: Apurba Sastry's Essentials of Medical Parasitology/p161
   - D. latum adult worm sucks vitamin B from intestine leading to Pernicious anemia.

HYMENOLEPIS NANA

18. **Ans. (c) (Hymenole...)** Ref: KD Chatterjee 13/e p166; Apurba Sastry's Essentials of Medical Parasitology 1/e p184-85
H. nana or *Dwarf tape worm (smallest tapeworm/cestode)* (adult form measures 1–4 cm in length)
Diphyllobothrium latum is the largest cestode affecting man (adult form measures up to 10 meter).

19. Ans. (a) (Hymenolepis nana) Ref: KD Chatterjee 13/e p166; Apurba Sastry's Essentials of Medical Parasitology 1/e p186

- All cestode eggs contain an egg shell and an oncosphere with three pairs of hooklets and bile stained:
  - H. nana eggs contains Polar filaments and is bile nonstained
  - *Diphyllobothrium latum eggs are operculated.*

H. nana eggs

- Eggs are the infective form as well as diagnostic form of the parasite.
- Egg is round to slightly oval, 30–47µm size, Nonbile stained (colorless in saline mount)
- It has two membranes and an oncosphere with six hooklets
- Polar filaments: 4–8 polar filaments emerge from poles and lie between the two membranes.

TREMATODES

20. Ans (d,e) (cows, goats) Ref: Apurba Sastry's Essentials of Medical Parasitology/p207

- The definitive hosts for Clonorchis are fish-eating mammals such as dogs, cats, rats, pigs, badgers, weasels, camels, and buffaloes. Man is an accidental definitive host.
- First intermediate host is snail and second intermediate host is freshwater fish.

21. Ans. (a) (Fasciola Gigantica) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p203-5

Among the options, eggs of Fasciola Gigantica are larger > 100 µm size and Fasciola Gigantica is associated with fever and abdominal symptoms.

- ***Option a: Fasciola Gigantica:***
  - Eggs are oval, bile stained, unembryonated and operculated, larger in size (160–190 µm × 70–90 µm).
  - ***Acute disease*** develops during metacercariae migration (1–2 weeks after infection) and includes fever, right-upper-quadrant pain, hepatomegaly and eosinophilia.
- ***Option b: Gastrodiscoides Hominis***
  - Eggs are operculated, measures 150 µm × 60–70 µm size.
  - Light infection is asymptomatic whereas heavy infection may cause mucus diarrhea and other intestinal symptoms.
- ***Option c: Ancyclostoma Caninum*** causes Cutaneous larva migrans, eggs are not found in humans, found only in canine animals feces. Only the larvae are found in men.
- ***Option d: Opisthorchis Viverrini*-Eggs:** Measure 27 µm × 15 µm, flask shaped with an operculum and a knob.

22. Ans (a,b,c) (Bithionol, Praziquantel , Triclabendazole) Ref: Apurba Sastry's Essentials of Medical Parasitology/p205, Harrison19th/p1429

- Triclabendazole is the DOC for F.hepatica. Bithionol and Praziquantel are the alternative drugs.

23. Ans. (c) (Snail) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p195

- Snails are the intermediate host for schistosomiasis.

24. Ans. (a) (Three....) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e pp/195

- All trematodes need three hosts required except Schistosoma (two hosts)
- Trematodes are Leaf like and unsegmented (Cestodes are segmented and tape like)
- Alimentary canal: Present but incomplete, no anus
- Body cavity absent.

25. Ans. (a) (Paragonimus) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p218

- Eggs of Paragonimus westermanii can be isolated from stool and sputum.

26. Ans. (a) (S. hematobium) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p194

- Metacercaria larva are the infective form for all the trematodes, expect Schistosoma where cercarial larva are the infective form.

27. Ans. (c) (Clonorchis sinensis) Ref: Harrison 18/e p1757; Apurba Sastry's Essentials of Medical Parasitology 1/e p207-8

- Pain abdomen, jaundice with increased alkaline phosphatase and conjugated hyperbilirubinemia. USG shows blockade of the biliary tree. Suggestive of infection by Clonorchis sinensis infection.

Clonorchiasis and opisthorchiasis:

- Low worm burden: asymptomatic.
- Moderate to heavy infection may be associated with vague right-upper-quadrant pain.
- Chronic or repeated infection is associated with manifestations such as Cholangitis, cholangiohepatitis, and biliary obstruction.

- Cholangiocarcinoma is epidemiologically related to C. sinensis infection in China and to O. viverrini infection in Northeastern Thailand.

28. Ans. (d) (Opisthorchis viverrini) **Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p209-10**

- *Fasciola gigantica*: 160 µm
- *Echinostoma iliocanum*: Measures 90–125 µm
- *Gastrodiscoides hominis*: Measures 150 µm
- ***Opisthorchis viverrini:*** **Measures 11–30 µm**

Based on the clue provided in the history the most probable answer is ***Opisthorchis viverrini*** as it is much smaller in size (measures 11–30 µm).

29. Ans. (b) and (d) (Paragonimus.... and E.granulosus...) Ref: Apurba Sastry's essentials of Medical Parasitology 1/e p 216, 183

Parasites causing lung infestation are:

- ***Paragonimus westermanii (Lung fluke)***
- *Ascaris lumbricoides (larva)*
- ***Hook worm (larva)***
- ***Echinococcus granulosus***
- ***Strongyloides stercoralis (larva)***
- *Entamoeba histolytica.*

30. Ans. (b) (Clonorchis...) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p 326

Parasites associated with Malignancy:

Parasites	Malignancy
Schistosoma haematobium	Squamous cell carcinoma of urinary bladder
Clonorchis sinensis	**Cholangiocarcinoma** of liver, bile duct and **Adenocarcinoma of pancreas**
Opisthorchis viverrini	**Cholangiocarcinoma of bile duct**

31. Ans. (b) (Clonorchis) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p207

Refer earlier explanation.

32. Ans. (a) (c) (e) (Parago..., Gnatho... and Whipwor...) Ref: Apurba Sastry's essentials of Medical Parasitology 1/e p 190

Liver fluke

- F.hepatica
- Fascola gigantica
- Clonorchis sinensis
- Opisthorchis viverinii.

33. Ans. (b) (Schistos...) Ref: KD Chatterjee 13/e p177; Apurba Sastry's Essentials of Medical Parasitology 1/e p199

- ***Katayama fever***: In the acute phase of Schistosoma mansoni infection, antigens released from the eggs combine with antibodies and form immune complexes and this serum sickness like illness called ***Katayama fever.*** It is characterized by fever, generalized lymphadenopathy, and hepatosplenomegaly.
- S.mansoni is more common to cause Katayama fever than S.japonicum.

34. Ans. (a) (Schistosoma...) Ref: KD Chatterjee 13/e p177; Apurba Sastry's Essentials of Medical Parasitology 1/e p197

Five species of Schistosomes infecting humans:

- **Eggs with a terminal spine:** ***Schistosoma haematobium***, *Schistosoma intercalatum*
- Eggs with a lateral spine: *Schistosoma mansoni,*
- Eggs with a rudimentary knob: *Schistosoma japonicum, Schistosoma mekongi.*

35. Ans. (b) (Splenic vein) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p201

Habitat

- *S. hematobium:* Vesicle plexus (veins of urinary bladder, pelvis ureter)
- *S. mansoni:* Inferior mesentric plexus
- ***S. japonicum:*** **Superior mesentric plexus and its radicles (splenic vein).**

36. Ans. (a) (Parago...) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p201

- Man-Snail-Crab-Man cycle is seen: Paragonimus wetsermanii
  - Man: Definite host
  - Snail: 1st intermediate host
  - Crab: 2nd intermediate host

CHAPTER

6.7 Nematodes

GENERAL CHARACTERISTICS OF NEMATODES

- Unsegmented, elongated and cylindrical
- Separate sexes
- Buccal capsule present
- GIT is complete
- Body cavity is present
- Size:
  - Small: *Trichinella, Strongyloides, Hookworm, Trichuris*
  - Large: *Dracanculus, Ascaris*
- Body is covered with a tough cuticle.

Feature	Ascaris (Roundworm)	Hookworm	Strongyloides	Trichuris (Whipworm)	Enterobius (Pin/threadworm)
Infective stage	Egg	Filariform larva	Filariform larva	Egg	Egg
Route	Oral	Skin	Skin or autoinfection	Oral	Oral or autoinfection
Location	Small intestine	Small intestine	Small intestine	Large intestine (Cecum, colon)	Large intestine (Cecum, appendix)
Lung stage	Yes	Yes	Yes	No	No
Principal symptoms	GIT symptoms Malabsorption Intussusception Loeffler syndrome	Ground itch Serpiginous tracks Mild pneumonitis GIT symptoms Iron def. anemia	GIT symptoms, Malabsorption, Hyperinfection syndrome.	Dysentery, Iron def. anemia, Rectal prolapse, Growth retardation	Perianal pruritus worse at night
Diagnostic stage	Unembryonated eggs in stool (Fertilized and unfertilized)	Eggs in fresh stool, larvae in old stool	Rhabditiform Unembryonated larva in stool	Unembryonated eggs in stool	Embryonated eggs from perianal skin collected by NIH swab or Cellophane tape
Eggs	***Fertilized egg:*** Round to oval, bile stained, thick albumin coat, floats on saturated salt ***Unfertilized eggs:*** Elongated, rectangular, bile stained thin coat, does not float on saturated salt.	Oval, Bile nonstained Segmented ovum (Four blastomeres)	***Rhabditiform larva:*** Long buccal cavity Genital primordium: Less prominent, small	Barrel shaped Mucus plug at ends, bile stained	Bile nonstained Planoconvex eggs containing larva inside
Fecundity (eggs/day/worm)	***2.4 Lakh***	Acylostoma- 10,000–25,000 Necator- 4000–10,000	5000–10,000	3000–7000	2000
Incubation period	60–75 days	40–100 days	17–28 days	70–90 days	35–45 days
Longevity	1 year	*N. americanus*: 2–5 year *A. duodenale*: 6–8 year	Decades (owing to autoinfection)	5 year	2 months
Treatment	Albendazole	Albendazole	Ivermectin	Albendazole	Mebendazole

INTESTINAL NEMATODES

Classification According to Nematodes Habitat

- Intestinal human nematodes:
  - Small Intestine: *Ascaris lumbricoides*, Hookworm (*Ancylostoma* and *Necator*), *Strongyloides*
  - Large intestine: *Trichuris* and *Enterobius*
- Somatic human nematodes: Filarial worm, *Trichinella spiralis*, *Dracunculus medinensis* (Guinea worm).

Classification According to Whether they Produce Egg/Larva

- ***Viviparous:*** Lay Larva- Filarial worm, *Trichinella and Dracunculus*
- ***Oviparous:*** Lay eggs which hatch out to larva later in the environment- *Ascaris*, Hookworm, *Trichuris* and *Enterobius*
- ***Ovoviviparous:*** Lay eggs containing larva which immediately hatchout, e.g. *Strongyloides.*

Necator vs Ancylostoma

- Egg and Rhabditiform larva—same
- They differ only in filariform larva and in adult worm
- Filariform larva of Necator:
  - Gap between oesophagus and intestine
  - Cuticle: Bears prominent transverse striation
  - Buccal capsule: Larger (15 µm), lumen short.

Ovoviviparous: Lay eggs containing larva which immediately hatchout, e.g. Strongyloides

Adult worm	A.duodenale	N. americanus
Size	Large and thick	Smaller and more slender
Bending of Anterior end	Same direction of body curvature	opposite to body curvature
Buccal capsule	Bears 6 teeth	4 chitinous cutting plate present
Copulatory bursa	• Total no. of rays: 13 • Dorsal ray splits at the tip • 2 spicules present	Total no. of rays: 14 Dorsal ray splits from the base Both spicules fused at the tip
Pathogenicity	• More pathogenic because of: • Larger size, armed with teeth and more migratory • Blood loss: 0.15–0.26 ml/worm/day	Less pathogenic: Except-Ground itch and dermatitis (where it is more severe) Blood loss: 0.03 ml/worm/day

- **Stool Culture** is required for Hookworm and Strongyloides: To demonstrate rhabditiform larva which on further cultivation, transform to filariform larva.
  - Harada Mori technique
  - Baermann funnel technique
  - Charcoal culture method
  - Agar Plate technique (more sensitive).
- ***Chandler's index*** is done for hookworm: eggs per gram of stool:
  - Below 200: Hookworm is not of much significance
  - 200–250: May be regarded as potential danger
  - 250–300: Minor public health problem
  - Above 300: Important public health problem
- *N. americanus* is predominant hookworm in India (and world) and except in Punjab and UP (Ancylostoma is more common)
- **Wakana disease:**
  - Seen only in Ancylostoma but not in Necator
  - Occurs: When L_3 larva is transmitted by oral route (Not by skin penetration)
  - Both GIT and pulmonary symptoms seen.

Necator vs Ancylostoma
- Stool Culture is done to demonstrate rhabditiform larva which on further cultivation, transform to filariform larva

Section 6

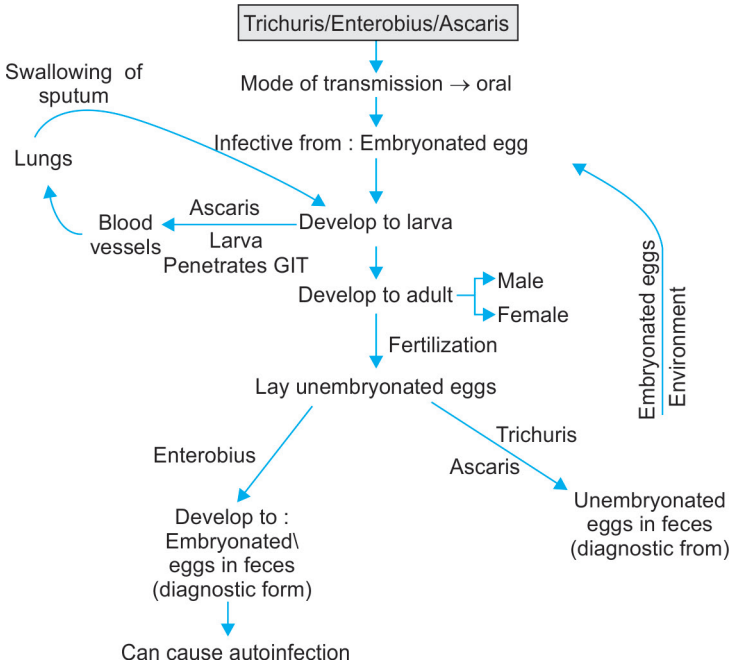


Fig. 6.7.1: Life cycle of trichuris/enterobius/ascaris

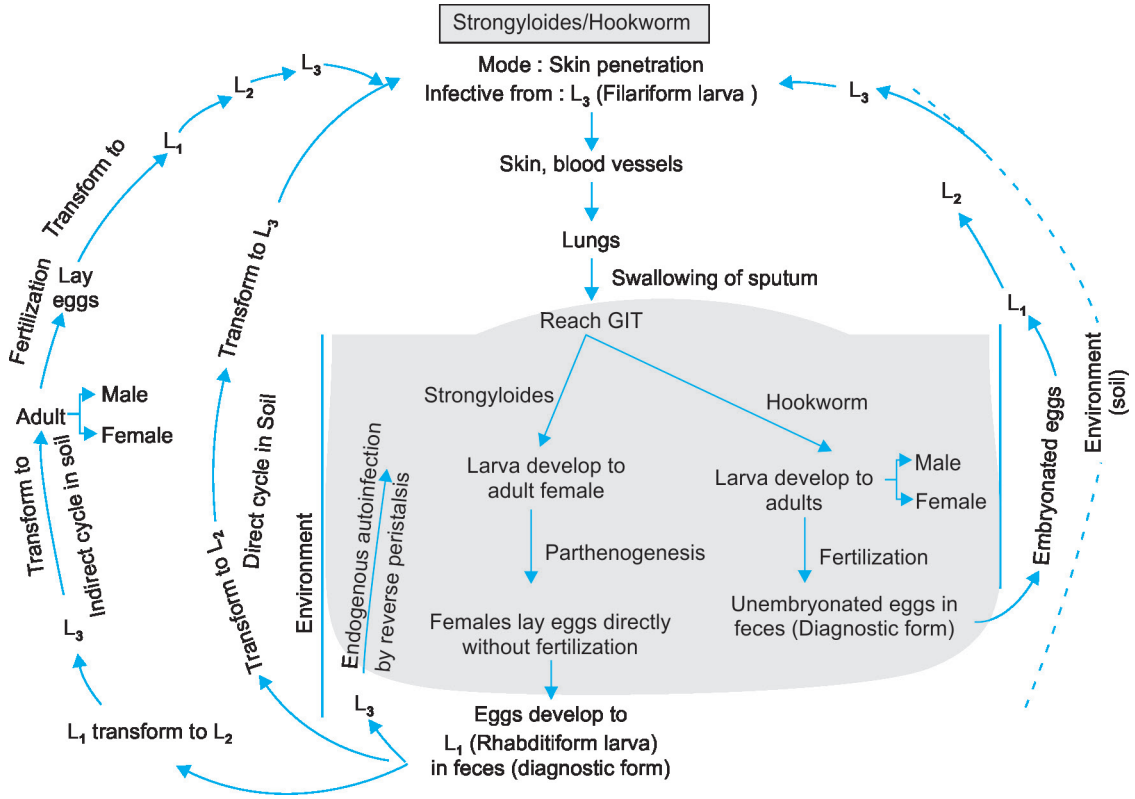


Fig. 6.7.2: Life cycle of strongyloides/hookworm

- **Hyperinfection syndrome:**
  - Repeated autoinfection cycles lead to heavy filariform larvae load
  - Seen in HTLV1 infected people (rarely HIV)
  - Most important risk factor: Glucocorticoid therapy
  - Serum IgE level becomes low
  - Larvae may invade the GIT and migrate to various organs including CNS, peritoneum, liver, and kidneys.
  - Moreover, the passage of enteric flora through disrupted mucosa lead to Gram-negative sepsis.
  - CNS invasion: Brain abscess and meningitis are common. Larvae can be seen in CSF.
- Sometimes, if the stool sample is examined late, the eggs of hookworm may transform to rhabditiform larva which have to be differentiated from that of *Strongyloides.*

Sometimes, if the stool sample is examined late, the eggs of hookworm may transform to rhabditiform larva which have to be differentiated from that of Strongyloides

Rhabditiform larva	Hookworm	Strongyloides
Size	100–150 µm long ×16 µm width	250 µm long ×16 µm width
buccal cavity	Three times longer	Shorter
Genital primordium	Less prominent, small	Prominent and large
Anal pore (subterminal)	80 µm from the posterior end	50 µm from the posterior end

Treatment

- Albendazole is the DOC of all nematodes except
  - Enterobius: Mebendazole
  - Strongyloides: Ivermectin
  - Wuchereria and Brugia: DEC
  - Onchocerca: Ivermectin
  - Dracunculus: Metronidazole.

FILARIAL NEMATODES

Pathogenesis and Life Cycle

- Infective form: Filarial larvae (L3) transmitted by Mosquito bite
- L3 larva migrate through lymphatics to local LN where they transform to adult worm then to L1(microfilaria)

Parasite	Location of Adult	Location of Microfilaria	Microfilaria Periodicity	Vector	Epidemiology
Lymphatic filariasis					
Wuchereria Bancrofti	Lymphatic tissue	Blood	Nocturnal (Mostly)	*Culex*- worldwide *Anopheles*- Rural Africa	Cosmopolitan, (South America, Africa, south Asia)
			Subperiodic (Rare)	*Aedes*	Pacific Islands
Brugia malayi	Lymphatic tissue	Blood	Nocturnal (Mostly)	*Mansonia*	Southeast Asia, Indonesia, India
			Subperiodic (Rare)	*Coquillettidia* and *Mansonia*	Indonesia, Southeast Asia
B. timori	Lymphatic tissue	Blood	Nocturnal	*Anopheles*	Indonesia
Subcutaneous filariasis					
Loa loa	Subcutaneous tissue, conjunctiva	Blood	Diurnal	*Chrysops* (deerflies)	West and Central Africa
Onchocerca volvulus	Subcutaneous tissue	Skin, eye	None	*Simulium* (blackflies)	South and Central America, Africa
Mansonella streptocerca	Subcutaneous tissue	Skin	None	*Culicoides* (midges)	West and Central Africa
Serous cavity					
Mansonella perstans	Body cavities, mesentery	Blood	None	*Culicoides* (midges)	South and Central America, Africa
Mansonella ozzardi	Body cavities	Blood	None	*Culicoides* (midges)	South and Central America
				Simulium (blackflies)	Caribbean islands

- Microfilaria are not pathogenic, but are periodically discharged to blood (diagnostic form)
- Adult female worm: Crucial role in pathogenesis
- Triad of pathogenesis:
  - Dilatation of lymphatic vessel
  - Lymphadenitis
  - Obstruction to lymphnode: Fibrotic degeneration of lymph vessel.

Infective form of Filarial – worms-– Filarial larvae (L3)

Lymphatic Filariasis

- Endemic Normal:
  - Asymptomatic, no microfilaria in blood
  - Occurs due to insufficient exposure, immunological resistance, prepatent period at time of detection.
- Asymptomatic Stage:
  - Microfilaria present in blood, but no clinical feature
  - Th1 is down regulated but Th2 is high (IL4↑)
  - After several years, hyporesponsiveness breaks and inflammatory reaction occurs.
- Acute Filariasis:
  - Due to antigens released from female adult worm
  - Filarial fever: High grade fever
  - Lymphatic inflammation (lymphangitis and lymphadenitis)
  - Transient local edema
  - Dermatolymphangitis
- Chronic Filariasis:
  - 10–15 years after acute phase
  - Fibrotic changes occurs (obstructive phase) in lymph vessels
  - Featured by:
    - Lymph varices
    - Hydrocele
    - Elephantiasis of scrotum, leg, arms, breast and vulva (nonpitting edema)
    - Granuloma of female breast
    - Chyluria chyle in urine (d/t obstruction of lymph vessels of kidney and abdomen).

Characters	Classical Filariasis	Occult Filariasis
Causative Agent	Inflammatory changes to adult worm	Hypersensitivity reaction to microfilaria antigen
Diagnostic form	Microfilaria in blood and in fluid	Microfilaria absent in blood
Organs affected	Lymph Nodes and lymphatic vessels	Lungs, liver, spleen
Pathology	Lymphangitis and lymphadenitis	Eosinophilic granuloma
Serology	Antibody not diagnostic	Antibody (IgE) – diagnostic

- Occult filariasis: Also called as Weingarten's Syndrome.
- Tropical Pulmonary Eosinophilia: Also called as *Meyers-Kouwenaar Syndrome*.

- Occult filariasis: Also called as Weingarten's Syndrome.
- Tropical Pulmonary Eosinophilia: Also called as Meyers: Kouwenaar Syndrome

Brugia Malayi

- **Transmission:**
  - Nocturnal strains: Transmitted from Man-Man by bite of Mansonia
  - Subperiodic strains: Transmitted from monkey (Zoonotic): by Mansonia
  - Anopheles and Aedes rarely transmit.
- *Pistia stratiotes plant:* Important for survival for Mansonia
- Clinical feature:
  - Leg below knee: ONLY affected (Contour of knee- normal)

- DEC provocation test: Done to demonstrate microfilaria in day time.

Microfilaria NOT found in peripheral blood:
- Occult filariasis
- Chronic filariasis (some cases)
- Wrong time.

Antigen detection:
- Indicates recent infection
- More sensitive than microscopy
- Can be detected in day time.

Trichinella Spiralis
- Infective stage: First stage (L1) larva.

- Genital and chyluria NOT marked
- MC-acute adenolymphangitis and filarial abscesses.

- Diagnosis: Microfilaria is differentiated from that of W. bancrofti by pointed tail tip, Nuclei column darkly stained, large, coarse, overlapping, extended till the tail tip.
- B. timori: Timor island of Indonesia, Vector-Anopheles barbirostris.

Lab Diagnosis

1. *Blood microscopy:*
- Blood is collected during:
  - Nocturnal: 10 pm to 4 am
  - Subperiodic nocturnal: 8 pm to 10 pm
  - Subperiodic diurnal: 2 pm to 6 pm.
- DEC provocation test: Done to demonstrate microfilaria in day time
- Direct wet mount: To see serpentine movement of microfilaria
- Staining: Thick blood smear stained with Leishman/Giemsa
- Concentration methods: Membrane filtration technique and Knott's centrifugation technique
- QBC: Quantitative Buffy Coat
- Microfilaria NOT found in peripheral blood:
  - Occult filariasis
  - Chronic filariasis (some cases)
  - Wrong time.
- Other samples:
  - Urine microscopy: 10–20 ml early morning chylous urine
  - Hydrocele fluid and LN aspirate microscopy.

2. Demonstration of antibody:
- Methods… IHA , IFA, ELISA, RIA
- Disadvantage:
  - Cross reactivity
  - Unable to discriminate between recent and past infection.

3. Demonstration of antigen:
- Indicates recent infection
- More sensitive than microscopy
- Can be detected in daytime
- Can differentiate current and past infection: Antigen disappears after clinical cure.
- Can detect in urine antigen
- ELISA: Using monoclonal antibody against AD12 antigen detects adult worm only
- ELISA: Using monoclonal antibody against Og4C3 antigen detects adult worm and microfilaria
- No antigen detection methods are available for *Brugia* infection.

4. Molecular methods: PCR detecting as low as 1pg of filarial DNA

5. Imaging methods:
- X Ray: Dead and calcified worm in LN and chest X ray shows pulmonary infiltrate in TPE
- Ultrasound of scrotum: Live adult worms with serpentine movement (Filarial Dance sign).

Treatment

- Diethyl Carbamazine (DEC): DOC
- DEC + Albendazole regimen: In India
- DEC + Ivermectin regimen: In Africa
- DEC acts on both microfilaria and adult where as ivermectin acts only on microfilaria.

Onchocerca Volvulus

- West Africa
- Skin manifestations:
  - Dermatitis (Sowda)
  - Leopard skin
  - Onchocercoma (subcutaneous nodules).
- Ocular involvement:
  - River blindness
  - Punctate keratitis
  - Sclerosing keratitis.
- Lymph Nodes: Hanging groin
- Detection of the microfilariae: Skin snips technique
- Mazzotti skin test (DEC patch test)
- Ivermectin: DOC.

- Bachman intradermal test: Persists for life, hence cannot differentiate past and present infection.

TRICHINELLA SPIRALIS

- Host-Pig: Optimum host and reservoir, man is an accidental host and acts as dead end.
- Infective stage: First stage (L1) larva
- Mode of transmission: By ingestion of raw or uncooked pork
- Larva penetrate intestine and migrate to muscle where it undergoes encystment
- MC muscle: Extraocular muscles followed by the biceps; and the muscles of the jaw, neck
- Diagnosis: Demonstration of larvae in muscle biopsy taken near tendon insertions of deltoid
- Antibody detection: Confirms the diagnosis but cannot differentiate past and present infection.
- Bachman intradermal test: Persists for life, hence cannot differentiate past and present infection.

DRACUNCULUS MEDINENSIS

- Causes Guinea worm disease or dracunculiasis
- It is eliminated from India since 2000 (and also from Pakistan), incidence is reduced in Asia
- Last case was reported from July 1996
- Currently: Limited to few countries in sub-Saharan Africa
- **Host:** Man is the definitive host and Copepods (Cyclops) are the intermediate host.
- **Infective form:** Third stage filariform larvae
- **Mode of transmission**: Drinking fresh water from stagnant pools containing minute fresh water crustaceans (Cyclops) infected with L3 larvae
- Presentation: Starts as painful papule , becomes blister from which the worm emerges
- Seasonal (June–September); Disease is a strong indicator of poor socioeconomic development
- *Microscopic detection of L1 larvae and adults:* On contact with cold water placed on the leg ulcer
- Treatment: Worm removal and symptomatic treatment
- **Reasons for Eradication of Guinea worm disease from India:**
  - d/t National Guinea worm eradication programme (1984)
  - Provision of safe drinking water: Filtration of drinking, installing hand pumps and pipes
  - Cyclops control: By application of Abate larvicide
  - Provision of clean drinking water from boreholes or wells
  - Health education of people in matter related to boiling or filtering of drinking water
  - Treatment of cases.

Parasites causing Malignancy
- **Schistosoma haematobium:** Squamous cell carcinoma of urinary bladder
- **Clonorchis sinensis:** Cholangiocarcinoma of liver, bile duct and Adenocarcinoma of pancreas
- **Opisthorchis viverrini:** Cholangiocarcinoma of bile duct.

Parasites Causing Anemia
- **Hookworm:** Iron deficiency anemia (thrives on Plasma)- Necator: 0.03 ml/day, Acylostoma: 0.2 ml/day
- **Babesia:** Hemolytic anemia
- **Plasmodium spp.:** Autoimmune hemolytic anemia
- **Trichuris trichiura:** Iron deficiency anemia
- **Leishmania donovani:** Autoimmune hemolytic anemia
- **Diphyllobothrium latum:** Vit. B12 Deficiency/Megaloblastic anemia.

LARVA MIGRANS

- The life cycle of most of the human nematodes involves penetration of the skin by the larval stage followed by migration of the larvae to intestine, lungs or other organs.
- However, the larvae of lower animal nematodes when accidentally infect man, they are not able to complete their normal development (because humans are the unusual host for them) and their life cycle gets arrested. The larvae wander around aimless way in the body. This is called as larva migrans.
- Two types of larva migrans exists:
  - Cutaneous larva migrans: Also called as creeping eruption. Larva migration occurs in skin and subcutaneous tissue.
  - Visceral larva migrans: Larva migration takes place in viscera.

Causes of Cutaneous Larva Migrans (CLM)	Causes of Visceral Larva Migrans (VLM)
Important cause: Nonhumans *Ancylostoma* spp. Causes Ground itch • *A. brasiliensis*, • *A. caninum* • *A. ceylanicum.* *Occasional human nematodes may cause:* • *Strongyloides stercoralis: Larva currens* • *Ancylostoma duodenale* • *Necator americanus* *Other rare nematodes:* • *Gnathostoma spinigerum:* Migratory cutaneous swellings • *Uncinaria stenocephala* • *Bunostomum phlebotomum* *Due to nonhelminthic agents:* • *Hypoderma* spp. • *Gastrophilus* spp.	*Important cause:* Toxocara *Other agents:* • *Angiostrongylus cantonensis: causes* eosinophilic meningoencephalitis • *Gnathostoma spinigerum* • *Anisakis* spp. • *Baylisascaris procyonis.*

MISCELLANEOUS TOPICS

- Capillaria philippinensis: Eggs resemble like Trichuris
- Trichostrongylus spp. (Pseudo hookworm): Eggs resemble to that of hookworm. Rhabditiformslarva's tail end has a bead-like swelling.

Non bile stained eggs (NEHA)
- Necator americanus
- Enterobius vermicularis
  - Hymenolepis nana
  - Ancylostoma

Autoinfection is seen in
- Cryptosporidium
- Hymenlepis nana
- Taenia solium
- Strongyloides
- Enterobius.

Does not float in saturated salt solution (ULTO)
- Unfertilized egg of Ascaris
- Larva of Strongyloides
- Taenia egg
- Operculated egg of trematodes.

MULTIPLE CHOICE QUESTIONS

GENERAL

1. What is the most common clinical manifestation when larvae of *Ascaris*, hookworm and *Strogyloides* migrates through the body? *(AIIMS Nov 2016)*
a. Asymptomatic
b. Pneumonitis
c. Acute dermal reaction
d. Anemia

2. Auto infection not seen in? *(AIIMS MAY 2016)*
a. Ascaris
b. Hymenolepis
c. Tenia solium
d. Enterobius

3. All the following parasites enter the human body by penetrating the skin except: *(APPG 2014)*
a. Ancylostoma duodenale
b. Necator americanus
c. Strongyloides stercoralis
d. Trichinella spiralis

4. Small intestine helminthes are: *(PGI June 2011)*
a. Ascaris
b. Necator
c. Trichuris
d. Enterobius
e. Ancyclostoma

5. Autoinfection is seen with: *(AIIMS May 2009, Nov 2001, AI 2000)*
a. Cryptosporodium
b. Strongyloides
c. Giardia
d. Gnathostoma

6. All cause malabsorption except: *(AIIMS May 2009)*
a. Giardiasis
b. Ascaris lumbricoides
c. Strongyloides
d. Capillaria phillipinesis

7. Microcytic hypochromic anemia found in infestation of: *(PGI June 2006)*
a. Ancylostoma
b. Ascaris
c. Necator
d. Diphyllobothrium

8. Nematodes are differentiated from other worms by:
a. Segmentation absent *(PGI Dec 2005)*
b. Separate coelomic cavity
c. Sexes separate
d. They are cylindrical
e. GIT is formed completely

9. Which of the following is viviparous? *(SGPGI 2005)*
a. Strongyloidis stercoralis
b. Trichinella spiralis
c. Enterobius
d. Ascaris

STRONGYLOIDES

10. Rhabditiform larvae is seen in: *(NEET Pattern Based)*
a. Taenia solium
b. Strongyloides
c. D. latum
d. Trichenella

11. Larvae which pass through lung during its life cycle:
a. Strongyloides stercoralis *(PGI May 2012)*
b. Ascaris lumbricoides
c. Necator
d. Trichuris trichiura
e. Wuchereria bancrofti microfilarae

12. Larval form in stool is found in:
a. Strongyloides *(PGI Dec 2001, WBPG 2016)*
b. Ancylostoma duodenale
c. Ascaris lumbricoides
d. Necator americanus

13. Ova in stool are not of diagnostic significance in: *(DNB June 2009)*
a. Strongyloides
b. Entrobius
c. Ankylostoma
d. Trichuris

14. The cause of larva currens: *(TN 2009)*
a. Strongyloides stercoralis
b. Necator americanus
c. Ankylostoma duodonale
d. H. nana

15. Infection with colitis is caused by: *(PGI 2000)*
a. Enterobius vermicularis
b. Trichuris trichura
c. Strongyloides
d. Clonorchis

HOOKWORM

16. The average blood loss in Ancylostomiasis per worm is: *(JIPMER 07)*
a. 0.2 ml/day
b. 2 ml /day
c. 0.33 ml/day
d. 1 ml/day

17. Chandler's index: *(AI 2006, Recent Question 2015)*
a. No. of hookworm eggs/gm of stool
b. No. of hookworm larva/gm of stool
c. No. of E.coli in a water sample
d. No. of failure of contraception for 100 women year exposure

18. **Parasites penetrating through skin for entry into the body are:** *(PGI June 2001)*
    a. Ancylostoma duodenale
    b. Stronglyoides
    c. Round worm
    d. Trichuris trichura

19. **Chandler's index. All are true except:** *(AIIMS Nov 2014)*
    a. It is average no of hookworm eggs per gram of feces for the entire community.
    b. < 200 eggs/gm indicates hookworm infection is of not much significance
    c. > 300 indicate hookworm infection is an important public health problem.
    d. Can be used for monitoring treatment response

20. **A child with 3 years of age presents with weakness with Hb level < 5 g/dL. History of blood loss in stool was present. What would be the causative organism?** *(AIIMS May 2014)*
    a. Hookworm
    b. Roundworm
    c. Whipworm
    d. Pinworm

ENTEROBIUS VERMICULARIS

21. **The nematode which resides in the cecum and appendix is?** *(DNB Dec 2010)*
    a. Ascaris lumbricoides
    b. Mansonellaozzardi
    c. Enterobius vermicularis
    d. All of these

22. **Most common presenting symptom of threadworm infection amongst the following is:** *(AI 2001, 97)*
    a. Abdominal pain
    b. Rectal prolapse
    c. Urticaria
    d. Vaginitis

23. **Eggs of which of the following parasite cause intense pruritus in perianal skin:** *(TNPG 2014)*
    a. Ascaris
    b. Hookworm
    c. Enterobius vermicularis
    d. Trichuris

TRICHURIS

24. **Unsegmented eggs are seen in which parasite:** *(NEET Pattern Based)*
    a. Trichuris trichura
    b. Necator americanus
    c. Ancylostoma
    d. Dracunculus

25. **Chronic dysentery, abdominal pain and rectal prolapse in children is caused by:** *(UP 07)*
    a. Trichuris
    b. Ascariasis
    c. Enterobius
    d. Trichinella

ASCARIS LUMBRICOIDES

26. **Specific diagnosis of Ascaris is made by:** *(PGI Dec 2004)*
    a. Adult worm in stool
    b. Egg detection
    c. Antigen detection
    d. Antibody detection
    e. Skin test

27. **Ascaris causes:** *(MAHE 07)*
    a. Bile duct obstruction
    b. Intestinal obstruction
    c. Appendicitis
    d. All of the above

FILARIAL WORMS

28. **Lymphatic obstruction occurs with which worm?** *(Recent Question 2015)*
    a. Filarial worm
    b. Hookworm
    c. Pinworm

29. **Filarial stage of adult worms responsible for disease in all of the following except:** *(AIIMS May 2015)*
    a. Onchocerca volvulus
    b. Brugia malayi
    c. Wuchereria bancrofti
    d. Mansonella ozzardi

30. **Wuchereria bancrofti all are true except:**
    a. DEC provocation test is used to demonstrate microfilaria during the day *(JIPMER Nov 2015)*
    b. Two nuclei in tail region
    c. Microfilaria is major diagnostic form

31. **Which does not cause lymphatic filariasis?** *(NEET Pattern Based)*
    a. Loa loa
    b. Wuchereria bancrofti
    c. Brugia malai
    d. Brugia timori

32. **Which of the following disease is transmitted by all of these mosquitoes like Anopheles, Aedes and Culex?** *(AI 2012)*
    a. Japanese Encephalitis
    b. Malaria
    c. Filariasis
    d. Yellow Fever

33. **Which among the following does not affect eyes?** *(DNB Dec 2010)*
    a. Onchocerca volvulus
    b. Trypanosoma
    c. Loa loa
    d. Trichuris

34. **Life cycle of Wuchereria bancrofti in mosquito is described as:** *(AP 2005)*
    a. Cyclopropagative
    b. Cyclodevelopmental
    c. Propagative
    d. None

35. Incubation period of filariasis: *(TN 2003)*
a. 10–20 years
b. 3–6 months
c. 6–12 months
d. 8–16 months

36. Sheathed microfilaria is/are:
a. W.bancrofti *(PGI Dec 2004, TNPG 2015)*
b. Loa loa
c. M.perstans
d. M.malayi

37. The organism most commonly causing genital filariasis in most part of Bihar and Eastern U.P. is: *(AI 2003)*
a. Wuchereria bancrofti
b. Brugia malayi
c. Onchocerca volvulus
d. Dirofilaria

38. In which stage of filariasis are microfilaria seen in peripheral blood? *(AI 2001)*
a. Tropical eosinophilia
b. Early adenolymphangitis stage
c. Late adenolymphangitis stage
d. Elephantiasis

39. A child from Bihar comes with fever. Blood examination shows sheathed microfilaria with nuclei up to tail tip. The diagnosis is: *(AI 2000)*
a. B. malayi
b. W.bancrofti
c. Loa loa
d. Onchocerca volvulous

40. Kallu a 30-year-old man, presented with subcutaneous itchy nodules over the left iliac crest. On examination they are firm, nontender and mobile. Skin scrapping contains microfilaria and adults worms of:
a. Loa loa *(AIIMS May 2001, JIPMER 2002)*
b. Onchocerca volvulus
c. Brugia malayi
d. Mansonella perstans

41. Calabar swelling is produced by: *(AI 2001)*
a. Onchocerca volvulus
b. Loa loa
c. Burgia malayi
d. Wuchereria bancrofti

42. Mayer Kouwenaar syndrome is a synonym for:
a. Tropical pulmonary eosinophilia
b. Occult filariasis *(NEET Pattern Based)*
c. Cutaneous Larva migrans
d. Visceral Larva migrans

43. Which of the following parasite causes myocarditis?
a. Trichenella spiralis *(DNB June 2010, Dec 2011)*
b. Trichuris trichura
c. Ancyclostoma duodenale
d. Tienea solium

44. Prepatent period in filariasis is: *(Recent MCQ 2013)*
a. Entry of the parasite to appearance of first symptom
b. Entry of the parasite to detection of first microfilaria in blood
c. Entry of the parasite to the vector till the vector becomes infective to man.

45. Unsheathed microfilaria are seen in: *(MHPG 2014)*
a. Wuchereria bancrofti
b. Brugia malayi
c. Loa loa
d. Onchocerca volvulus

DRACUNCULUS MEDINENSIS

46. Dragon or serpent worm is: *(AIIMS 2001)*
a. Enterobius
b. Trichuris
c. Dracunculus
d. T. solium

47. All of the following regarding dracunculiasis are true except: *(NEET Pattern Based)*
a. Eliminated from India
b. Niridazole prevents transmission of disease
c. Limited to tropics and subtropical region
d. No animal reservoir

48. Guinea worm was declared eradicated from India in:
a. 1999
b. 2000 *(Recent MCQ 2013)*
c. 2001
d. 2002

49. True about dracunculiasis: *(PGI Nov 2012)*
a. Eliminated from India
b. Transmitted by the soil
c. Chigger transmitting disease
d. Waterborne disease

LARVA MIGRANS

50. Most common cause of visceral larva migrans. *(AI 2011)*
a. Ancylostoma
b. Visceral leishmaniasis
c. St. stercoralis
d. Toxocara canis

MISCELLANEOUS

51. Protozoa associated with megaesophagus: *(NEET Pattern Based)*
a. Trypanosoma
b. Ameba
c. Giardia
d. Gnathostoma

52. Eosinophilic meningoencephalitis is caused by:
a. Gnathostoma spinigerum *(PGI 2000)*
b. Naegleria
c. Toxocara canis
d. Angiostrongylus cantonensis

53. Eggs of all the following helminthic worms float in a saturated salt solution EXCEPT: *(MHPG 2014)*
a. Eggs of Necator americans
b. Eggs of Enterobius vermicularis
c. Eggs of Hymenolepis nana
d. Eggs of Taenia solium

EXPLANATIONS

GENERAL

1. **Ans. (a) A (Asymptomatic)** **Ref: Apurba Sastry's Essentials of Medical Parasitology /p227-244**
   - Most of infected individuals with Ascaris, hookworm and Strogyloides are asymptomatic.
2. **Ans. (a) (Ascaris)** **Ref: Apurba Sastry's Essentials of Medical Parasitology/p5**
   - Autoinfection is seen by: CHEST (Cryptosporidium parvum, Hymenolepis nana, Enterobius vermicularis, Strongyloides stercoralis and Taenia solium)
3. **Ans. (d) (Trichinella spiralis)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p286
   Trichinella spiralis is transmitted by consumption of contaminated pig's meat containing L1 stage larvae.
4. **Ans. (a) (b) (e) (Ascaris, Necator, Ancylostoma)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p223
   Refer chapter review
5. **Ans. (b) (Strongyloides)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p5
   - Auto infection **-** Refer chapter review
6. **Ans. (None of the above)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p325
   - *Giardiasis*: Malabsorption syndrome and weight loss are complications both in adults and children.
   - *Ascaris lumbricoides:* Heavy infection causes impairment of host nutrition leading to malabsorption of proteins, carbohydrates and vitamins.
   - *Strongyloides stercoralis* infection in children causes prolonged malabsorption of fat and protein in severe cases.
   - *Capillaria phillipinesis:* Adult cause protein loosing enteropathy and severe malabsorption.
7. **Ans. (a) and (c) (Ancylo..., Necator)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p325
   - Parasites causing Anemia- Refer chapter review
8. **Ans. (b), (c), (d) and (e) (Separate coelomic cavity, Sexes are separate, They are cylindrical and GIT is formed completely)** Ref: KD Chatterjee 13/e p143; Apurba Sastry's Essentials of Medical Parasitology 1/e p320
   Differences between Cestodes, Trematodes and Nematodes: Refer chapter review of 6.6
9. **Ans. (b) (Trichinel...)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p221
   - ***Viviparous:*** Worms that lay Larva directly, without egg formation, e.g. Filarial worms, *Trichinella and Dracunculus*

STRONGYLOIDES

10. **Ans. (b) Strongyloides)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p122
    - Rhabditiform larvae is the diagnostic form of Strongyloides
11. **Ans. (a) (b) (c) (e) (Strongyloides stercoralis, Ascaris lumbricoides, Necator, Wuchereria bancrofti microfilarae)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p122
    - Pulmonary migration is seen in the life cycle of:
      - Ascaris: Causes Loffler's pneumonia
      - Hookworm: Causes mild pneumonitis
      - Strongyloides: Causes mild pneumonitis
      - Wuchereria bancrofti microfilarae: Cause Tropical pulmonary eosinophilia
12. **Ans. (a), (b) and (d) (Strongyloides, Ancylostoma duodenale and Necator americanus)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p241
    - **Strongyloides is ovo-vivparous,** hence **larva** found in stool.
    - If passed feces is allowed to stand for several hours, the **Rhabditiform larvae** of Ancylostoma duodenale and Necator americanus will hatch out and even they can be seen while performing stool examination.

13. **Ans. (a) (Strongyloides stercoralis)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p241
    - **Strongyloides is ovoviviparous,** hence the eggs hatch out immediately to produce rhabditiform larva which is the diagnostic form found in stool.
14. **Ans. (a) (Strongyloides stercoralis)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p240
    - Strongyloides larva produces a condition in an individual who has been already sensitized called larva currens.
    - This is caused by external auto-infection and local allergic reaction to the larvae penetrating skin.
    - Migrating filariform larva moves rapidly in a short line at the rate of 3-4 cm in an hour, hence is termed as larva currens.
15. **Ans. (c) (Strongyloides)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p240

    Intestinal Strongyloidiasis: Intestinal invasion by adult Strongyloides worm produces following symptoms:
    - Midepigastric pain like peptic ulcer.
    - Nausea, Diarrhea, GIT bleeding, **Mild chronic colitis,** Weight loss.

HOOKWORM

16. **Ans. (a) (0.2 ml/day)** Ref: KD Chatterjee 13/e p217; Apurba Sastry's Essentials of Medical Parasitology 1/e p232
    - Refer earlier explanation.
17. **Ans. (a) (No. of hookworm eggs/gm...)** Ref: Park 22/e p221, Apurba Sastry's Essentials of Medical Parasitology 1/e p236
    - Details about Chandler's index refer chapter review.
18. **Ans. (a) and (b) (Ancy..., Strong...)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p232,238

    Parasites enter through penetration of skin:
    - From Soil-*Hookworm, Strongyloides*
    - From Water-*Acanthamoeba, Schistosoma spp.*
19. **Ans. (d) (Can be....)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p230

    Chandler's index is an endemicity marker of hookworm infection in the community, it is not a prognostic marker, hence cannot be used for monitoring treatment response.
20. **Ans. (a) (hookworm)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p235

    Among the given options, Hookworms cause chronic intestinal blood loss that result in iron deficiency anemia. Blood loss may also be seen following whipworm infection but NOT severe.

ENTEROBIUS VERMICULARIS

21. **Ans. (c) (Enterobius vermicularis)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p227-9
    - Ascaris lumbricoides: Resides in small intestine
    - Mansonella ozzardi: Resides in serous cavity
    - Enterobius vermicularis: Resides in large intestine.
22. **Ans. (a) (Abdominal pain)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p226
    - Most patients infected of thread worm/pin worm/***seat worm*** /Enterobius vermicularis are asymptomatic.
    - Most common complaint: **Perianal and perineal pruritus** (pruritus ani)- nocturnal/early morning.
    - Other complaints: **Abdominal pain**, irritability and restlessness.
    - All these symptoms caused by the female worms laying eggs on anal area.
    - Severe infections: Sleep disturbances, neurosis, nail bite, grinding teeth at night.
    - Adult worms can cause appendicitis.
23. **Ans. (c) (Enterobius vermicularis)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p227
    - Refer chapter review.

TRICHURIS

24. **Ans. (a) (Trichuris)** Ref: Harrison 18/e p1743; Apurba Sastry's Essentials of Medical Parasitology 1/e p226
    - Trichuris trichura has unsegmented, barrel shaped eggs with mucus plugs at the ends.
    - Eggs of Hookworm (Necator americanus and Ancylostoma) are segmented and oval.
    - Dracunculus does not have egg stage.

25. Ans. (a) (Trichuris) Ref: Harrison 18/e p1743; Apurba Sastry's Essentials of Medical Parasitology 1/e p225-6

- **Clinical feature of Trichuriasis:**
  - Heavy infections may result in abdominal pain, anorexia, and bloody or mucoid diarrhea resembling inflammatory bowel disease.
  - Rectal prolapse can result from massive infections with Trichuris in children, who often suffer from malnourishment and other diarrheal illnesses.
  - Moderately heavy Trichuris burdens also contribute to *growth retardation.*

ASCARIS LUMBRICOIDES

26. Ans. (a), (b) and (d) (Adult worm in stool, Egg detection and Antibody detection) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p245

Laboratory diagnosis of Ascariasis

Parasitic diagnosis:

- Demonstration of characteristic **Ascaris eggs in the stool**: Eggs are passed in large numbers.
- Concentration of stool by Salt floatation and Formalin-ether method- light infections
- Demonstration of egg in bile (duodenal intubation)
- Demonstration of **adult worm in stool/ vomitus**- after treatment with antihelminthics.
- Demonstration of larvae in gastric aspirate, sputum or bronchial aspirates – pulmonary infections
- Serodiagnosis- In case extraintestinal Ascarisis

Antibody detection by

- Microprecipitation on the larvae
- Indirect hemagglutination test
- Immunofluorescent antibody test.

27. Ans. (d) (All of the above) Ref: Harrison 18/e p1739; Apurba Sastry's Essentials of Medical Parasitology 1/e p245

Features of Ascariasis

- Lung phase of larval migration: Causes eosinophilic pneumonitis (Löffler's syndrome) and fever
- Intestinal phase: In heavy infections, particularly in children:
  - A large bolus of entangled worms can cause pain and small-bowel obstruction, sometimes complicated by perforation, intussusception, or volvulus.
  - A large worm can enter and occlude the biliary tree, causing biliary colic, cholecystitis, cholangitis, pancreatitis, or (rarely) intrahepatic abscesses.
  - In highly endemic areas, intestinal and biliary ascariasis can induce acute appendicitis and gallstones as causes of surgical acute abdomen.

FILARIAL WORMS

28. Ans. (a) (Filarial worm) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p268

29. Ans. (d) (Mansonella ozzardi) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p278

- For lymphatic filariasis (Wuchereria and Brugiya): The adult female worm is the pathogenic form and is responsible for disease manifestation; whereas the microfilaria (larva form) is responsible for allergic manifestations seen in occult filariasis.
- In Onchocerca volvulus infection, skin nodules or onchocercoma (occurs due to adult worms) and other manifestations (cutaneous and ocular involvement) are due to hyper reactive immune response to the microfilarial antigens.
- The pathogenicity of M.ozzardi needs further research. Although the adult worms live in the body cavities and the mesentery, they seem to cause little or no harm to their human hosts.

30. Ans. (b) (Two nuclei in tail region) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p264

- The tail tip of microfilaria of *Brugiya malayi* is blunt and has two nuclei; whereas tail tip of Wuchereria bancrofti is pointed without nuclei.
- Microfilaria is the diagnostic form for lymphatic filariasis, but not for occult filariasis.
- DEC provocation test is used to demonstrate microfilaria during the day- microfilaria comes to peripheral blood within 30 minutes of DEC intake.

31. Ans. (a) (Loa loa) Ref: Harrison 18/e p1745, Apurba Sastry's Essentials of Medical Parasitology 1/e p263

- Agents of lymphatic filarisis: Wuchereria bancrofti, Brugia malai and Brugia timori

32. Ans. (c) (Filariasis) Ref: Harrison 18/e p1745, Apurba Sastry's Essentials of Medical Parasitology 1/e p315

- *Wuchereria bancrofti*: Transmitted by
  - Nocturnal strains- Culex, Anopheles (mosquitoes),
  - Subperiodic strains- *Aedes* (mosquitoes)
- Japanese Encephalitis: Transmitted by Culex tritaeniorhynchus
- Malaria: Transmitted by Female Anopheles mosquito
- Yellow Fever: Transmitted by Aedes.

33. Ans. (d) (Trichuris) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p325

- Onchocerca volvulus – Causes River blindness, Punctate keratitis and Sclerosing keratitis
- Trypanosoma cruzi- Romana's sign (periorbital edema)
- Loa loa: Causes Calabar swelling.

34. Ans. (b) (Cyclodevelopmental...) Ref: Park 22/e p713, Apurba Sastry's Essentials of Medical Parasitology 1/e p267

- Propagative transmission- the multiply extensively without change of form, e.g. Arboviruses,
- Cyclopropagative transmission: Pathogen undergoes a developmental cycle (changes from one stage to another) as well as multiplication in the body of the arthropod, e.g. Malaria
- Cyclodevelopmental transmission: The pathogen undergoes developmental changes from one stage to another, but does not multiply, e.g. Filaria.

35. Ans. (d) (8–16 months) Ref: Park 22/e p247, 21/e p246

Clinical Incubation period of filariasis: 8–16 months

36. Ans. (a) and (b) (W.bancrofti and Loa loa) Ref: Parija's Parasitology 3/e p328; Apurba Sastry's Essentials of Medical Parasitology 1/e p263

- Sheathed microfilaria is covered by hyaline sheath, in which larva moves backwards and forwards within the sheath, e.g. Wuchereria, Brugia and Loa loa

37. Ans. (a) (Wucher...) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p265

- The nocturnal periodic strains of Wuchereria bancrofti is most wide spread in India.
- Mainly along seacoast and riverbanks.
- **Heavily infected areas – *Uttar Pradesh, Bihar***, Andhra Pradesh, Orissa, Tamil Nadu, Kerala and Gujarat.

38. Ans. (b) (Early ade...) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p272

- Microfilaria are also **demonstrated in peripheral blood** in early stages of filariasis:
  - *Acute filariasis (lymphangitis, lymphadentis)*
  - *Asymptomatic stage*
- Microfilariae are not found in the peripheral blood:
  - Occult filariasis such as *tropical pulmonary eosinophilia*
  - Chronic filariasis *(Elephantiasis)*
  - *Wrong time collection*

39. Ans. (a) (B.malayi) Ref: Rajesh Karyakarthe 1/e p180; Apurba Sastry's Essentials of Medical Parasitology 1/e p265,272

- Microfilaria having nuclei up to tail tip: ***B. malayi***, *B. timori*, ***Loa loa***, *M.perstans* and *M.streptocerca*
- Microfilaria with tail tip free from nuclei: **W.bancrofti**, M.ozzardi and **Onchocerca volvulus**
- **Loa loa** is restricted to **tropical Africa** (African eye worm).
- As in the provided history patient is residence of Bihar, rules out Loa loa infection, hence *B.malayi* is the appropriate option.

40. Ans. (b) (Onchocerca volvulus) Ref: Harrison 18/e p1748, 17/e p1327; Apurba Sastry's Essentials of Medical Parasitology 1/e p278-9

Clue for diagnosis

- Subcutaneous firm, nontender and mobile, itchy nodules over the left iliac crest.... indicates Oncocercoma
- Skin scrapping contains microfilaria and adults worms
- Detail: Refer text of Oncocerca

41. Ans. (b) (Loa loa) Ref: KD Chatterjee 13/e p253; Apurba Sastry's Essentials of Medical Parasitology 1/e p276-7

- Calabar Swelling: Localized angioedema and erythema usually on the extremities, characterized by fugitive, swollen lumps of subcutaneous tissue caused by a parasitic filarial worm (Loa loa) endemic to Central and West Africa.
- The swollen areas migrate with the worm through the body at a speed of about 1 cm per minute and may become as large as a small egg.

42. Ans. (a) (Tropical...) Ref: KD Chatterjee 13/e p244; Apurba Sastry's Essentials of Medical Parasitology 1/e p271
- Occult filariasis: Also called as Weingarten's Syndrome.
- Tropical Pulmonary Eosinophilia- Also called as *Meyers- Kouwenaar Syndrome*.

43. Ans. (a) (Trichenella spiralis) Ref: Harrison 18/e p1735; Apurba Sastry's Essentials of Medical Parasitology 1/e p286-7
- Myocarditis with tachyarrhythmias or heart failure—and, less commonly, encephalitis or pneumonitis—may develop and accounts for most deaths of patients with trichinellosis.

44. Ans. (b) (Entry...) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p93,94,95
- Incubation period: Entry of the parasite to appearance of first symptom
- Prepatent period: Entry of the parasite to detection of first microfilaria in blood
- Extrinsic Incubation period: Entry of the parasite to the vector till the vector becomes infective to man.

45. Ans. (d) (Onchocerca volvulus) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p264
- Microfilaria: Sheathed in Wuchereria bancrofti, Brugia malayi and Loa loa
- Microfilaria: Unsheathed in Onchocerca volvulus and Streptocerca

DRACUNCULUS MEDINENSIS

46. Ans. (c) (Dracunculus) Ref: KD Chatterjee 13/e p254; Apurba Sastry's Essentials of Medical Parasitology 1/e p282-3
Dracunculus medinensis also called as:
- *Guinea worm*
- ***Serpent worm***
- ***Dragon worm***
- *Medina worm.*

47. Ans. (b) (Niridazo...) Park 22/e p223-24,21/e p223-24; Apurba Sastry's Essentials of Medical Parasitology 1/e p282-4
- No drug is effective in treating dracunculiasis. Gradual extraction of the worm by winding of a few centimeters on a stick each day remains the common and effective practice. Worms may be excised surgically.
- Dracunculiasis is restricted to the tropics of Africa
- Eliminated from India since 2000 (Last case was seen in July 996)
- No animal reservoir is there for dracunculiasis.

48. Ans. (b) (2000) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p282-85
- Guinea worm was declared eradicated from India in- 2000
- Last case detected in India- 1999

49. Ans. (a) (d) (Eliminated..., Waterborne...) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p282-85.
Refer chapter review for detail

LARVA MIGRANS

50. Ans. (d) (Toxocara canis) Ref: Parija's Parasitology 3/e p323; Apurba Sastry's Essentials of Medical Parasitology 1/e p250
- *Toxocara canis* and *Toxocara catis* are the commonest cause of Visceral larva migrans.

MISCELLANEOUS

51. Ans. (d) (Gnathostoma) Ref: Parija's Parasitology 3/e p359; Apurba Sastry's Essentials of Medical Parasitology 1/e p255
- Gnathostoma has a mega esophagus, surrounded by four salivary glands.

52. Ans. (d) (Angiostrongylus cantonensis) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p252
- Angiostrongylus cantonensis or rodent lung worm Causes Eosinophilic meningoencephalitis in man. Cases in India are reported from Mumbai.

53. Ans. (d) (Eggs of Taenia solium) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p230
Eggs of all the following helminthic worms float in a saturated salt solution EXCEPT
- ULTO- Unfertilized eggs of Ascaris, Larva of Strongyloides, Taenia eggs and Operculated eggs of trematodes.

NOTE

NOTE

SECTION 7

Applied Microbiology

CHAPTER OUTLINE

Clinical Microbiology (Infective Syndromes)

CHAPTER 7.1

URINARY TRACT INFECTIONS

	Lower UTI	Upper UTI
Sites involved	Urethra and bladder	Kidney and ureter
Symptoms	Local manifestations- dysuria, urgency, frequency	Local and systemic manifestations (fever, vomiting, abdominal pain)
Route of spread	Ascending route	Both ascending (common) and descending route
Occurrence	More common	Less common

Microorganisms causing UTI

Bacterial agents:	Other agents:
Gram-negative bacilli: • ***Escherichia coli-*** Commonest agent of UTI • *Proteus mirabilis* • *Klebsiella pneumoniae* • *Pseudomonas aeruginosa* • *Acinetobacter* species • *Enterobacter* species • *Serratia* species	**Fungus:** *Candida albicans* **Parasites:** • *Schistosoma haematobium* • *Trichomonas vaginalis*
Gram-positive cocci: • *Staphylococcus saprophyticus* • *Staphylococcus aureus* • *Staphylococcus epidermidis* • *Enterococcus spp.*	**Viruses:** • Herpes simplex virus • Adenovirus • JC and BK virus • Cytomegalovirus

Most organisms are acquired by ascending route. Organisms acquired by descending route include - *Staphylococcus aureus, Salmonella, Mycobacterium tuberculosis, Leptospira* and *Candida*.

DIARRHEA, DYSENTERY AND FOOD POISONING

Table 7.1.1: Infectious agents of acute diarrhea and the underlying mechanism

Mechanism	Features	Examples of pathogens involved	
Non-inflammatory	**Location:** Proximal small bowel **Illness:** Watery diarrhea **Stool findings**: • No fecal leukocytes • Fecal lactoferrin-not increased	**Bacteria:** **(Mostly enterotoxin mediated)** • *Vibrio cholerae* • *Escherichia coli* ○ Enteropathogenic ○ Enterotoxigenic ○ Enteroaggregative • *Clostridium perfringens* • *Bacillus cereus* • *Staphylococcus aureus,* • *Aeromonas hydrophila* • *Plesiomonas shigelloides*	**Viruses:** • Rotavirus • Norovirus • Enteric adenoviruses **Parasites:** • *Giardia lamblia* • *Cryptosporidium* • *Cyclospora* species • Microsporidia

Contd...

Contd...

Mechanism	Features	Examples of pathogens involved	
Inflammatory (invasion or cytotoxin)	**Location:** Colon or distal small bowel **Illness:** • Dysentery or • Inflammatory diarrhea **Stool findings**: • Fecal pus cells (polymorphonuclear leukocytes)–increased • Fecal lactoferrin–increased	**Predominantly dysentery:** • *Shigella* species • *Campylobacter jejuni* • Enterohemorrhagic *E. coli* • Enteroinvasive *E. coli* • *Vibrio parahaemolyticus* **Predominantly inflammatory diarrhea-** • *Salmonella* species • *Yersinia enterocolitica* • *Listeria monocytogenes* • *Clostridium difficile* • *Aeromonas hydrophila* • *Plesiomonas shigelloides* • *Klebsiella oxytoca*	**Parasite** (predominantly dysentery): • *Entamoeba histolytica,* • *Balantidium coli*
Penetrating	**Location:** Distal small bowel **Illness:** Enteric fever **Stool findings**: Fecal mononuclear leukocytes	*Salmonella* Typhi (enteric fever) *Yersinia enterocolitica*	

Table 7.1.2: Infectious agents causing food poisoning

Organisms	Symptoms	Common food sources
1–6 h incubation period		
Staphylococcus aureus	Nausea, vomiting, diarrhea	Ham, poultry, potato or egg salad, mayonnaise, cream pastries
Bacillus cereus	Nausea, vomiting, diarrhea	Fried rice
Clostridium botulinum	Nausea, vomiting, diarrhea	Canned food
8–16 h incubation period		
Clostridium perfringens	Abdominal cramps, diarrhea (vomiting rare)	Beef, poultry, legumes, gravies
B. cereus	Abdominal cramps, diarrhea (vomiting rare)	Meats, vegetables, dried beans, cereals
> **16 h incubation period**		
Vibrio cholerae	Watery diarrhea	Shellfish, water
Enterotoxigenic *E. coli*	Watery diarrhea	Salads, cheese, meat, water
Enterohemorrhagic *E. coli*	Bloody diarrhea	Ground beef, roast beef, salami, raw milk, raw vegetables, apple juice
Salmonella species	Inflammatory diarrhea	Beef, poultry, eggs, dairy products
Campylobacter jejuni	Inflammatory diarrhea	Poultry, raw milk
Shigella species	Dysentery	Potato or egg salad, lettuce, raw vegetables
Vibrio parahaemolyticus	Dysentery	Mollusks, crustaceans

Table 7.1.3: Agents of Traveller's diarrhea

Etiologic agent	Comments
Bacteria (50–75%)	
Enterotoxigenic *Escherichia coli* (10–45%)	Single most important agent
Enteroaggregative *E. coli* (5–35%)	Emerging enteric pathogen with worldwide distribution
Campylobacter jejuni (5–25%)	More common in Asia
Shigella	Major cause of dysentery

Contd...

Contd...

Etiologic agent	Comments
Salmonella	Common in India
Others	*Aeromonas, Plesiomonas*, and *Vibrio cholerae*
Viruses (**< 20**%)	
Norovirus (< 10%)	Associated with cruise ships
Rotavirus (< 5%)	Common among children
Parasites (**0–10** %)	*Giardia lamblia*, *Cryptosporidium*, *Entamoeba histolytica*, *Cyclospora*

Table 7.1.4: Pathogenic mechanisms of diarrheal agents

Toxins	Other mechanisms

Enterotoxins:
• Cholera toxin
• *Vibrio parahaemolyticus*
• *E.coli:*
  ○ LT and ST of ETEC
  ○ EAST(enteroaggregative heat-stable enterotoxin) of EAEC
  ○ VT of EHEC
• *Clostridium difficile* (toxin A)
• *Aeromonas*
• *Campylobacter jejuni*
	Attachment within or close to mucosal cells:
	• *E. coli:*
	  ○ Enteropathogenic *E. coli* (EPEC)
	  ○ Enterohemorrhagic *E. coli* (EHEC)
	• *Cryptosporidium* species
	• *Cyclospora* species
	• Rotavirus
	• Norovirus

Cytotoxins • *Shigella dysenteriae* type 1 • Enterohemorrhagic *E.coli* (EHEC) • *Clostridium difficile* (toxin B)	**Invasion of intestinal epithelium:** • *Shigella* species • Enteroinvasive *E. coli* • *Campylobacter jejuni* • *Yersinia enterocolitica* • *Plesiomonas shigelloides* • *Entamoeba histolytica* • *Balantidium coli*
Neurotoxins: • *Staphylococcus aureus* enterotoxin • *Bacillus cereus* toxin • *Clostridium botulinum* toxin	

MENINGITIS

Table 7.1.5: Causes of meningitis (pyogenic and aseptic)

	Pyogenic meningitis	Aseptic meningitis
Neonates or infants of 0–2 months	• *Escherichia coli* • Group B streptococcus (*S. agalactiae)* • *Listeria monocytogenes* • Other Gram-negative bacilli (like *Klebsiella pneumoniae*)	**Viruses** Enteroviruses (Polioviruses, echoviruses, Coxsackie viruses): The most common agents Herpes simplex virus 1 and 2 Other Herpes group: Varicella zoster, CMV, EBV Myxoviruses: Influenza A and B, parainfluenza virus, and mumps virus Arboviruses, and adenoviruses, Rubella viruses and HIV
2–20 years	• *Neisseria meningitidis:* Most common agent • *Haemophilus influenzae* • *Streptococcus pneumoniae*	
> 20 years (adults)	*Streptococcus pneumoniae:* Most common agent *Haemophilus influenzae* *Neisseria meningitidis*	**Bacteria:** *Treponema pallidum,* and *Leptospira* **Parasites**-*Naegleria* species, *Acanthamoeba* species and *Toxoplasma gondii*
Overall	Most common agent is *Streptococcus pneumoniae*	**Fungi:** *Cryptococcus neoformans*

Table 7.1.6: Cytological and biochemical parameters in CSF of normal individuals and in different types of meningitis

Character	Normal individual	Pyogenic meningitis	Tuberculous meningitis	Viral meningitis
CSF pressure (mm of water)	Normal (50–150)	Highly elevated (>180)	Moderately elevated	Slightly elevated/normal
Total leukocyte count (per mm^3)	0–5	100–10,000	10–500	25–500
Predominant cell	Lymphocytes	Neutrophils	Lymphocytes	Lymphocytes
Glucose (mg%)	40–70	(< 40 mg/dL) (Decreased to absent)	20–40 mg/dL (Slightly ↓)	Normal
Total proteins (mg%)	15–45	> 45 mg/dL (usually > 250) (markedly ↑↑↑)	100–500 mg/dL (moderate to markedly ↑↑)	20–80 mg/dL (Normal or slightly elevated)

BLOODSTREAM INFECTIONS

Table 7.1.7: Agents of endocarditis

Agents of endocarditis	Most common agent in specific types of endocarditis
• Streptococci (Viridans streptococci and others) • Pneumococci • Enterococci • *Staphylococcus aureus* • Coagulase-negative staphylococci • (e.g. *Staphylococcus epidermidis)* • Fastidious Gram-negative coccobacilli (HACEK group) • Gram-negative bacilli • *Candida* species • Diphtheroids • Culture-negative endocarditis: such as *Bartonella, Coxiella*	***Native valve endocarditis*** – *Staphylococcus aureus*
	Prosthetic valve endocarditis: It occurs following cardiac valve replacement • Early prosthetic valve endocarditis (occurs within 12 months of valve replacement)–*Staphylococcus epidermidis* is the commonest agent • Late prosthetic valve endocarditis (occurs after 12 months of valve replacement)–Viridans streptococci are the commonest agent
	Endocarditis in IV drug abusers: Young males are the most common victims. The skin is the commonest source of infection. • Right sided–Most common agent is *Staphylococcus aureus* • Left sided–Most common agent is *Enterococcus* followed by *S.aureus* • Over all–Most common agent is *Staphylococcus aureus*
	MC cause of Subacute endocarditis – Viridans streptococci

Fever of Unknown Origin

Petersdorf and Beeson classification (1961) was traditionally used for defining PUO.

- Temperatures of > 38.3°C (>101°F)
- For a duration of > 3 weeks; and
- Failure to reach a diagnosis despite 1 week of inpatient investigation.

This classification has stood for more than 30 years, but later in 1990s, it was revised as Durack and Street classification. There after it is further modified in 2015. According to Harrison 19th edition, FUO is now defined as:

1. Fever > 38.3°C (101°F) on at least two occasions
2. Illness duration of > 3 weeks
3. No known immunocompromised state
4. Diagnosis that remains uncertain after a thorough history-taking, physical examination and the obligatory investigations.
   Infections (36%) accounts for majority of FUO cases followed by Neoplasms (19%).

OTHER INFECTIVE SYNDROMES

Table 7.1.8: Microorganisms causing URTI (upper respiratory tract infections)

Rhinitis or common cold	Pharyngitis (sore throat), and tonsillitis	Laryngitis	Laryngotracheobronchitis (or croup)
Mostly caused by viruses: • Rhinovirus • Coronavirus • Adenovirus • Influenza virus • Parainfluenza virus • Human metapneumovirus • Respiratory syncytial virus **Sinusitis:** **Agents of acute sinusitis:** Viruses (most common cause): • Rhinoviruses, • Influenza viruses, • Parainfluenza viruses Bacterial agents • *Streptococcus pneumoniae* • *Haemophilus influenzae* • *Moraxella catarrhalis* • *Pseudomonas* and other Gram-negative bacilli (nosocomial sinusitis) **Agents of chronic sinusitis:** • Obligate anaerobes • *Staphylococcus aureus*	**Viruses-** (most common cause) • Influenza virus • Parainfluenza virus • Coxsackievirus A • Rhinovirus • Coronavirus • Epstein-Barr virus • Adenoviruses **Bacteria:** • *Streptococcus pyogenes* (MC bacterial cause) • *Streptococcus* C and G • *Arcanobacterium* species • *Neisseria gonorrhoeae* • *Corynebacterium diphtheriae* and *C.ulcerans* • *Mycoplasma pneumoniae* • Vincent angina • *Treponema vincentii* • *Leptotrichia buccalis* **Fungal:** *Candida albicans*	**Mostly viral agents:** • Influenza virus • Parainfluenza virus • Rhinovirus • Adenovirus • Coronavirus • Human meta-pneumo-virus **If membrane or exudate present:** • *Streptococcus pyogenes* • *C.diphtheriae* • Epstein-Barr virus	Age: Children, < 3 years **Agents:** • Parainfluenza virus (most common) • Influenza virus • Respiratory syncytial virus • Adenoviruses **Epiglottis** **Most common agent**: *Haemophilus influenzae* b

Table 7.1.9: Comparison of STDs producing genital ulcer

Feature	Syphilis	Herpes	Chancroid	LGV	Donovanosis
Incubation period	9–90 days	2–7 days	1–14 days	3 days–6 weeks	1–4 weeks (up to 6 months)
Genital ulcer	Painless, indurated, single	Multiple, painful	Painful, soft Single or multiple	Painful, soft	Painless
Lymphadenopathy	Painless, hard, moderate swelling (no bubo)	Absence or moderate swelling (no bubo)	Painful, soft, marked swelling leads to *bubo formation*	Painless	Absent (*pseudo bubo* may be present due to subcutaneous swelling)

Congenital Infection

Vertical transmission (spread of infections from mother-to-baby) may occur by transplacental route (congenital infection), during labor and delivery, or after delivery.

Congenital infection (transmission from placenta to fetus): They often lead to defects in fetal development or even death.

Examples include TORCH syndrome:

- Toxoplasmosis
- Other infections (congenital syphilis, hepatitis B, Coxsackievirus, Epstein-Barr virus, varicellazoster virus, Plasmodium falciparum and human parvovirus)
- Rubella
- Cytomegalovirus (CMV)
- Herpes simplex virus

Perinatal infections (during labor and delivery): CMV, Gonococcus, Chlamydia, HSV, HPV and Group B streptococci

Postnatal infections (after delivery): CMV, HIV and Group B streptococcus.

Emerging and Re-Emerging Infection

Emerging infection: Incidence in human increased in last two decades (newly appearing disease or those spreading newly to a geographical area). Example include:

- Crimerian congo hemorrhagic virus in India (Ahmedabad- Jan 2011)
- Plasmodium knowlesi
- H1N1 -2009 and Avian flu (H5N1)-2005,
- SARS-2003 (did not affect India)
- HGV, HEV, HHV8, Sin Nombre

Re-emerging infection: Incidence was brought down in past but again increased due to breakdown in public health measure including emergence of drug resistance

- India: Plague in Gujrat (1994), Chikungunya (2005), Dengue (North), V.cholerae O139 (south east costal)
- MRSA, MDRTB, Leptospira (South, Andaman)

Table 7.1.10: Bioterrorism and classification of bioweapons (CDC)

Bioterrorism is a form of terrorism (unlawful use of weapon against mankind) where there is intentional and deliberate release of biological agents (bacteria, viruses, or their toxins) to cause mass illness or death of people, animals, or plants	
Category A: Agents are the highest-priority pathogens which pose the greatest risk to national security	
• Can be easily disseminated or transmitted from person to person • Result in high mortality rates and have the potential for major public health impact • Might cause public panic and social disruption • Require special action for public health preparedness.	• Anthrax (*Bacillus anthracis*) • Botulism (*Clostridium* botulinum toxin) • Plague (*Yersinia pestis*) • Tularemia (*Francisella tularensis*) • Smallpox (Variola major) • Hemorrhagic viruses • Arenaviruses: Lassavirus • Bunyaviridae: Crimean-Congo virus, • Filoviridae: Ebola, Marburg virus
Category B: These agents are the second highest priority pathogens	
• Moderately easy to disseminate,	• Melioidosis (*Burkholderia pseudomallei*) • Glanders (*Burkholderia mallei*)
• Result in moderate morbidity rates and low mortality rates, • Require specifically enhanced diagnostic capacity.	• Brucellosis (*Brucella* species) • Psittacosis (*Chlamydophila psittaci*) • Q fever (*Coxiella burnetii*) • Typhus fever (*Rickettsia prowazekii*) • Toxin: Ricin, *S.auerus* Enterotoxin B, Epsilon toxin of *Clostridium perfringens* • Viral encephalitis [alphaviruses (e.g., Venezuelan, eastern, and western equine encephalitis)] • Food threats-*Salmonella, Shigella*, *E.coli* O157 • Water threats: *Vibrio cholerae*, *Cryptosporidium*
Category C: These agents are the third highest priority pathogens. They are the emerging pathogens, to which the general population lacks immunity	
• These agents could be engineered for mass dissemination in the future because of availability, ease of production, and ease of dissemination. • They have a potential for high morbidity and mortality rates	• Nipah virus • Hantavirus • SARS coronavirus, • Pandemic influenza virus • MDRTB • Yellow fever virus

Table 7.1.11: Risk-based classification of agents causing laboratory-acquired infections

Group	Definition	Bacteria	Virus	Fungi	Parasite
Group-1	Biological agents that are unlikely to cause human disease	- No pathogenic organisms	-	-	-
Group-2	Biological agents that can cause human disease and may be hazard to workers; but are unlikely to spread to community; effective treatment or prophylaxis is usually available	*Bacillus* species (except *B.anthracis)* *Clostridium* species *Corynebacterium diphtheriae* Enterobacteriaceae *Staphylococcus* *Streptococcus Mycobacterium* (except *M.tuberculosis*)	Adenovirus Calcivirus *Coronavirus* (not SARS Co.V) Herpesvirus Influenza virus	*Cryptococcus* *Candida* Dermatophytes *Aspergillus*	All clinically important parasites
Group-3	Biological agents that can cause severe human disease and are a serious hazard to workers; they may spread to the community; but effective treatment or prophylaxis is usually available	*B.anthracis* *Brucella* species *Coxiella burnetii* *Francisella tularensis, M.tuberculosis*	Prion LCM virus (Lymphocytic choriomeningitis) Hantavirus SARS Co.V Encephalitis virus such as- St.Louis Japanese West Nile Western equine		
Group-4	Same as group 3 except that effective treatment or prophylaxis is usually not available		Lassa virus Ebola virus Marbug virus Herpes simiae virus		

MICROBIOLOGICAL PROFILE OF OSTEOMYELITIS

1. MC cause of osteomyelitis - S. aureus in all sites (hematogenous long-bone and Vertebral and sternal). (Harrison 19th/p838-844)
2. Sickle cell disease-Salmonella osteomyelitis is frequently cause
3. IV drug users- *P. aeruginosa* is common (Harrison 19th/p844)
4. Diabetic foot osteomyelitis - Polymicrobial (MC is *S. aureus* followed by anaerobes and Gram negative like *E.coli*) (Harrison 19th/p845)
5. Implant associated osteomyelitis- Coagulase-negative staphylococci (*S. epidermidis*) are the second most common etiologic agents (after *S. aureus*). (Harrison 19th/p842)
6. Streptococcus agalactiae is common in children next to *S. aureus*

Ref: Harrison 19th/p 838-844, Other Journals and e Books

MULTIPLE CHOICE QUESTIONS

URINARY TRACT INFECTIONS

1. **Most common cause of UTI in neonate:** *(Recent Question 2015)*
   a. E coli
   b. S. aureus
   c. Enterococcus

2. **Route of infection for renal tuberculosis:** *(West Bengal 2016)*
   a. Ascending route
   b. Hematogenous route
   c. Direct

3. **Most common nonlactose fermenting organism that causes UTI, in patient with catheter in situ:** *(Recent Question 2015)*
   a. E. coli
   b. Proteus
   c. Enterococcus
   d. Pseudomonas

SEXUALLY TRANSMITTED DISEASES

4. **A young male patient presented with UTI, on urine examination pus cells were found but no organisms. Which method would be best used for culture:** *(AIIMS Nov 2007, 2005, AI 2006)*
   a. McCoy cell line
   b. Thayer Martin medium
   c. LJ medium
   d. Levinthal medium

5. **A man presents to a STD clinic with urethritis and urethral discharge. Gram stain shows numerous pus cells but no microorganism. The culture is negative on the routine laboratory media. The most likely agent is:** *(AIIMS Nov 2007, 2002)*
   a. Chlamydia trachomatis
   b. Hemophilus ducreyi
   c. Treponema pallidum
   d. Neisseria gonorrhoeae

6. **A 30-year-old male patient presents with urethritis. All of the following can be the causative agent except:** *(AIIMS Nov 2004)*
   a. Neisseria gonorrhoeae
   b. Chlamydia trachomatis
   c. Trichomonas vaginalis
   d. Hemophilus ducreyi

7. **All of the following are sexually transmitted, except:** *(AI 2002)*
   a. Candida albicans
   b. Echinococcus
   c. Molluscum contagiosum
   d. Group B streptococcus

8. **Treatment of partner is required in all infections except:** *(PGI 2000)*
   a. Candida
   b. Herpes
   c. Trichomonas
   d. Gardnerella

GASTROENTERITIS

9. **Food poisoning occurs in 6–7 hours by all Except:** *(DNB June 2011)*
   a. Staphylococcus
   b. E. coli
   c. Clostridium
   d. Bacillus cereus

10. **Food poisoning in canned food is caused by:** *(DNB Dec 2011)*
    a. Staphylococcus
    b. Salmonella
    c. Clostridium botulinum
    d. Bacillus cereus

11. **Food poisoning that does not presents within 6 hours is due to:** *(DNB Dec 2011, AIIMS Nov 2001)*
    a. Staphylococcus
    b. Salmonella
    c. Clostridium botulinum
    d. Bacillus cereus

12. **Microorganisms invading the GIT causing gastroenteritis:** *(PGI Dec 2007)*
    a. EHEC
    b. Shigella
    c. Vibrio parahemolyticus
    d. Campylobacter
    e. Salmonella

13. **Traveller's diarrhea is caused by:** *(AIIMS Dec 2007, PGI DEC 2008)*
    a. Shigella
    b. E. coli
    c. E. histolytica
    d. Giardiasis

14. **Thirtyeight children consumed eatables procured from a single source at a picnic party. Twenty children developed abdominal cramps followed by vomiting and watery diarrhea 6–10 hours after the party. The most likely etiology for the outbreak is:** *(AI 2003)*
    a. Rotavirus infection
    b. Entero toxigenic E. coli infection
    c. Staphylococcal toxin
    d. Clostridium perfringens infection

15. **A cook prepares sandwiches for 10 people going for picnic. Eight out of them develop severe gastroenteritis within 4-6 hrs of consumption of the sandwiches. It is likely that on investigations the cook is found to be the carrier of:** *(AIIMS Nov 2002)*
    a. Salmonella typhi
    b. Vibrio cholerae
    c. Entamoeba histolytica
    d. Staphylococcus aureus

16. Heat stable enterotoxin causing food poisoning is caused by all the following except: *(AI 02)*
a. Bacillus cereus
b. Yersinia enterocolitica
c. Staphylococcus
d. Clostridium perfringens

NORMAL MICROBIAL FLORA OF HUMAN BODY

17. Common natural flora of skin are:
a. Streptococcus *(PGI June 2009, PGI Dec 2008)*
b. Staphylococcus aureus
c. Candida albicans
d. Bacteroides fragilis
e. Propionibacterium acne

18. Transient colonization is caused by: *(PGI Dec 2008)*
a. HSV
b. Trichomonas vaginalis
c. H. influenzae
d. N. gonorrhoea
e. Staphylococcus aureus

19. Which of the following is the main colonizer of sebaceous gland? *(PGI June 2007)*
a. Propionibacterium acnes
b. Diphtheria
c. Strept. pyogenes
d. Staph. aureus
e. Candida

20. In the gut, anaerobic bacteria outnumber the aerobes by a ratio of: *(AIIMS May 2006)*
a. 10:1 b. 100:1
c. 1000:1 d. 10,000:1

21. It is true regarding the normal microbial flora present on the skin and mucous membranes that: *(AI 2005)*
a. It cannot be eradicated by antimicrobial agents
b. It is absent in the stomach due to the acidic pH
c. It establishes in the body only after the neonatal period
d. The flora in the small bronchi is similar to that of the trachea

22. MC commensal gut flora in adult: *(PGI 2000)*
a. Lactobacillus b. Bacteroides
c. E. coli d. Klebsiella

MISCELLANEOUS MICROBIOLOGY

23. IP < 4 weeks is for: *(PGI May 2016)*
a. Gonorrhea b. Hepatitis B
c. Babesiosis d. Tertiary syphilis
e. Filariasis

24. MC organism to cause acute osteomyelitis: *(Recent Question 2015)*
a. Staphylococcus aureus
b. E. coli
c. Pseudomonas
d. Streptococcus pyogenes

25. In which of the following condition, the spread of the rash is centrifugal? *(Recent Question 2015)*
a. Dengue b. Smallpox
c. RMSF d. Erythema multiforme

26. Arthropod borne disease is/are: *(PGI May 2012)*
a. Brucellosis b. Lyme's disease
c. Borrelia recurrentis d. Malaria
e. Plague

27. Tick is vector for: *(PGI May 2012)*
a. Crimean Congo fever
b. Rocky Mountain spotted fever
c. Epidemic typhus
d. Endemic typhus
e. Scrub typhus

28. Which of the following virus is least likely to cross placenta? *(AI 2011)*
a. Rubella b. Herpes simplex
c. HIV d. HBV

29. Most potential agent that can be used in bioterrorism are a/e: *(AI 2011)*
a. Plague b. Smallpox
c. TB d. Clostridium botulinum

30. Atypical pneumonia is caused by all except: *(DNB Dec 2011)*
a. Klebsiella b. Adeno virus
c. Chlamydia d. Hemophilus

31. A 30-year-old person has fever and headache from 20 days. CSF values are following: glucose 38 mg/dl, protein 60 mg/dl, lymphocytes pleocytosis with 20 cells/mm^3. Initial diagnosis should include:
a. Indian ink smear of CSF *(PGI June 2011)*
b. AFB
c. Toxoplasmosis
d. HSV detection

32. Which of the following do not have a non-human reservoir? *(PGI June 2009)*
a. Salmonella typhi b. N. gonorrhoea
c. E. coli d. Clostridium tetani
e. Treponema pallidum

33. Probiotics are useful for: *(AI 2008)*
a. Necrotizing enterocolitis
b. Breast milk jaundice
c. Hospital acquired pneumonia
d. Neonatal seizures

34. Biosafety precaution grade III is practiced in all of the following organisms except: *(AIIMS Nov 2008)*
a. Human influenza virus
b. St. Louis encephalitis virus
c. Coxiella burnetii
d. Mycobacterium tuberculosis

35. All of the following cause hemolytic uremic syndrome except: *(AIIMS May 2007, PGI Dec 2007)*
a. Shigella
b. Campylobacter
c. EHEC
d. Vibrio cholerae

36. Loeffler's syndrome occurs in all except: *(AIIMS May 2007)*
a. Toxocara
b. Strongyloides
c. Ascaris
d. Giardia

37. Vaccination causing Intussusception: *(PGI Dec 2007)*
a. Rotavirus
b. Parvovirus
c. Poliovirus
d. BCG
e. Measles

38. A veterinary doctor had pyrexia of unknown origin. His blood culture in special laboratory media was positive for gram-negative short bacilli which was oxidase positive. Which one of the following is the likely organism grown in culture? *(AI 2006)*
a. Pasteurella spp
b. Francisella spp
c. Bartonella spp
d. Brucella spp

39. The single most common cause of pyrexia of unknown origin is: *(AIIMS May 2006)*
a. Mycobacterium tuberculosis
b. Salmonella Typhi
c. Brucella sp
d. Salmonella paratyphi A

40. Acute intravascular hemolysis can be caused by infection due to all of the following organisms except:
a. Clostridium tetani *(AIIMS Nov 2003, AI 2003)*
b. Bartonella bacilliformis
c. Plasmodium falciparum
d. Babesia microti

41. A neonate develops encephalitis without any skin lesions. Most probable causative organisms is: *(AIIMS May 2002)*
a. HSV I
b. HSV II
c. Meningococci
d. Streptococci

42. Zoonotic diseases are all except: *(PGI Dec 2001)*
a. Typhoid
b. Anthrax
c. Rabies
d. Q fever

43. Post disaster, which of the following does not spread as epidemic? *(AIIMS May 2014, AIIMS Nov 2013)*
a. Leptospirosis
b. Food poisoning
c. Leishmaniasis
d. Rickettsia
e. Acute respiratory infections

44. Incubation period less than 5 days: *(PGI Nov 2014)*
a. Salmonella
b. Yersinia pestis
c. Vibrio parahemolyticus
d. Staphylococcus aureus
e. Measles

45. Community acquired pneumonia is caused by:
a. Staphylococcus aureus *(PGI Nov 2014)*
b. Streptococcus pyogenes
c. Mycoplasma pneumoniae
d. Chlamydiapneumoniae
e. Legionella pneumophila

46. Which of the following agent-disease combinations are correct? *(PGI Nov 2014)*
a. Lymes-Boreliaburgdoreferi
b. Catscratch- Bartonellahensale
c. Glanders– Burkholderia mallei
d. Rat bite fever- Borreliarecurrentis
e. Trench fever- Bartonellabaciliformis

47. Relative bradycardia is a feature of all except: *(TNPG 2014)*
a. Typhoid
b. Malaria
c. Brucella
d. Leptospira

48. Quantitative cultures are necessary for laboratory diagnosis in infection of: *(MHPG 2014)*
a. Urinary tract
b. Blood stream
c. Lungs
d. Small intestine

49. Population doubling time in coliform bacilli is: *(MHPG 2014)*
a. 20 seconds
b. 20 minutes
c. 20 hours
d. 20 days

50. Zoonotic diseases are all except: *(PGI Nov 2014)*
a. Leptospirosis
b. Guinea worm
c. Rabies
d. Plague
e. Japanense encephalitis

51. Class C biological terrorism is by: *(PGI May 2016)*
a. Hanta virus
b. Ebola virus
c. Crimean Congo virus
d. HIV
e. Marburg virus

52. New infectious agents are:
a. Nipah virus *(PGI Dec 2007, PGI Dec 2004)*
b. Pneumocystic jieruveci
c. Corona virus
d. SARS
e. Prion

EXPLANATIONS

URINARY TRACT INFECTIONS

1. **Ans. (a) (E. coli)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p586
2. **Ans. (b) Hematogenous route** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p273
   M.tuberculosis enters through descending or Hematogenous route.
3. **Ans (d) (Pseudomonas)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p340
   Pseudomonas is the MC organism to cause UTI in catheterized patients and it produces NLF colonies on MacConkey agar.

SEXUALLY TRANSMITTED DISEASES

4. **Ans. (a) (McCoy cell lines)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p403, Harrison 18/e p1097
   This is a case of non-gonococcal urethritis due to Chlamydia trachomatis.
   Chlamydia trachomatis *can be successfully cultivated in* ***McCoy and HeLa cells treated with cycloheximide*** *and grown in monolayer on cover slip in shell vials* …..Jawetz 24/e p360
5. **Ans. (a) (Chlamydia trachomatis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p403, Harrison18/e p1097
   - This is a case of STD with history of urethritis and organism does not grow in culture and not seen by Gram stain.
   - Chlamydia trachomatis is the most appropriate answer
   - Neisseria gonorrheae: can be diagnosed by Gram staining and also by Culture techniques.
6. **Ans. (d) (Hemophilus...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p343, Ananthanarayan 8/e p230
   - Hemophilus ducreyi can cause genital ulcer but not urethritis.
7. **Ans. (b) (Echinococcus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p604, Harrison 18/e p1095, 16/e p763
   - Refer chapter review for detailed explanation.
8. **Ans. (a) and (d) (Candida and Gard...)** Ref: Apurba Sastry's Microbiology 1/e p604, Harrison 18/e p1110-11, 17/e p828
   Treatment of sexual partner is required for:
   - Chlamydia trachomatis
   - Neisseria gonorrhoea
   - Trichomonas vaginalis
   - Herpes simplex virus
   - Mycoplasma genitalium.

   Treatment of sexual partner is not required:
   - Vulvovaginal Candidiasis: Usually treatment of sexual partner is not required, topical treatment if candidal dermatitis of penis is detected
   - Gardnerella vaginalis: No treatment is required.

GASTROENTERITIS

9. **Ans. (b) (E. coli)** Ref : Harrison 18/e p1088, 17/e p877-78 and Apurba Sastry's Essentials of Medical Microbiology 1/e p589
   - Staphylococcus aureus, Bacillus cereus (emetic type) and Clostridium botulinum are the causative agents for Toxic type of food poisoning, where symptoms manifests with in 6 hours.
   - E. coli and Salmonella spp cause infective type of food poisoning wherein symptoms manifest after 16 hours.
   - Detailed explanation refer chapter review.
10. **Ans. (c) (Clostridium botulinum)** Ref: Park 22/e p217
    - The most common food associated with botulism are home preserved foods such as home canned vegetables, smoked or pickled fish, homemade cheese and similar low acid food.
11. **Ans. (b) (Salmonella)** Ref: Harrison 18/e p1088 and Apurba Sastry's Essentials of Medical Microbiology 1/e p588
    - Already explained.

12. **Ans. (a), (b), (c), (d) and (e) (EHEC, Shigella, Vibrio parahemolyticus, Campylobacter and Salmonella)**

Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p588-90

Microorganisms invading the GIT causing Dysentery:

- Bacteria: Shigella, E. coli (EIEC, EHEC), Y. enterocolitica, Campylobacter jejuni, V. parahemolyticus, Salmonella
- Protozoa: E. histolytica, Balantiduim coli.

13. **Ans. (b) (Escherichia coli)** Ref: Harrison 18/e p1087, 17/e p816

Causative agents of traveler's diarrhea: Refer chapter review

'In all areas, Enterotoxigenic and Enteroaggregative E. coli are the most common isolates from persons with the classic secretory traveler's diarrhea syndrome'.

14. **Ans. (d) (Clost...)** Ref: Harrison 18/e p1088, 17/e p877-78 and Apurba Sastry's Essentials of Medical Microbiology 1/e p589

The onset of food poisoning with in **6–10 hours** of consumption of food is mainly due to

- Clostridium perfringens
- Bacillus cereus (diarrheal type)

About other options already explained.

15. **Ans. (d) (Staphylococcus aureus)** Ref: Harrison 18/e p1088, 17/e p877-78 and Apurba Sastry's Essentials of Medical Microbiology 1/e p589

The onset of food poisoning with in **4–6 hours** of consumption of food is mainly due to:

- Staphylococcus aureus
- Bacillus cereus (emetic type)
- Clostridium botulinum.

16. **Ans. None of the above**

- Bacillus cereus can secrete both heat stable (emetic type) and heat labile (diarrheal type) Enterotoxins Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p256
- Yersinia enterocolitica produces heat stable enterotoxin Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p315
- Staphylococcus aureus produces heat stable enterotoxin Ref: Ananthanarayan 8/e p247, Apurba Sastry's Essentials of Medical Microbiology 1/e p212
- Clostridium perfringens food poisoning can be caused by both heat resistant and heat labile spores which germinate and produce enterotoxin Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p259

NORMAL MICROBIAL FLORA OF HUMAN BODY

17. **Ans. (a), (b), (c) and (e) (Sterptococcus, Staphylococcus aureus, Candida albicans and Propionibacterium acne)**

Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p581-83, Ananthanarayan 8/e p588 and p268

Refer chapter review for skin flora.

18. **Ans. (a), (b) and (e) (HSV, Trichomonas vaginalis and Staphylococcus aureus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p581-82

The skin and mucous membranes have 2 types normal flora:

- **Resident flora:** Consists of relatively fixed types of microorganisms regularly found in a given area at a given age; if disturbed, it promptly reestablishes itself and cannot be removed permanently.
- **Transient flora:** Consists of nonpathogenic or potentially pathogenic microorganisms that inhabit the skin or mucous membranes for hours, days, or weeks; it is derived from the environment, does not produce disease, and does not establish itself permanently on the surface.
  - Transient flora is of little significance so long as the normal resident flora remains intact.
  - If the resident flora is disturbed, transient microorganisms may colonize, proliferate, and produce disease.
  - Members of transient flora: Microorganisms other than coagulase-negative Staphylococci, Corynebacterium spp., Micrococcus spp., Bacillus spp. Candida spp., Trichomonas vaginalis, Neisseria meningitides, CMV, HSV, Malassezia, Enterococcus, Bacteroides, M. smegmatis, Fusobacterium.

19. **Ans. (a) (Propionibacterium acnes)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p582

- Propionibacterium acnes are the commonest agent causing acne in teenagers as it has affinity for sebaceous glands.
- Lesions in acne develop with in the sebaceous follicle.

20. **Ans. (c) (1000:1)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p582-83, Ananthanarayan 8/e p265
    - In the human intestines, Anaerobes out number aerobic bacteria a thousand fold.
21. **Ans. (a) (It cannot be eradicated by antimicrobial agents)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p581, Ananthanarayan 8/e p633
    - Resident Normal flora: Consists of relatively fixed types of microorganisms regularly found in a given area at a given age; if disturbed, it promptly reestablishes itself and cannot be removed permanently.
22. **Ans. (b) (Bacte...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p581-83, Ananthanarayan 8/e p636 and p265
    - In the human intestines, Anaerobes out number aerobic bacteria a thousand fold
    - Among anaerobes Bacteroides are in predominant number in human gut flora.

MISCELLANEOUS MICROBIOLOGY

23. **Ref: (a, c) (Gonorrhea, Babesiosis)** Ref: Apurba Sastry's Essentials of Medical Microbiology/p239,374, parasitology/p115,269
    - IP of Gonorrhea- 2-7 days, hepatitis B- 30-180 days, babesiosis- 1-6 weeks,
    - IP of tertiary syphilis- 10-30 years, filariasis- 8-16 months
24. **Ans. (a) (Staphylococcus aureus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p215
25. **Ans. (d) (Erythema multiforme)** Ref: Internet source

    Spread (means direction of movement of rash), Distribution (means area where rashes are predominant)
    - Centripetal spread = Rashes appear first on periphery, spread towards trunk, e.g. Smallpox
    - Centrifugal spread = Rashes appear first on trunk/face, spread towards periphery, e.g. Chickenpox
    - Centripetal distribution = Rashes are mainly on trunk, e.g. Chickenpox
    - Centrifugal distribution = Rashes are mainly on extremities, e.g. Smallpox.

    For most rashes, centripetal/fungal terms are described for spread of rash; where as in chickenpox and smallpox usually they are described in terms of distribution of rash.

    Rashes with centrifugal spread (body/face first, then spread towards periphery):
    - **Erythema multiforme**, Epidemic typhus, Endemic typhus
    - Measles, Rubella, Herpes
    - Chickungunya
    - Chickenpox (Centrifugal spread, but Centripetal distribution).

    Rashes with Centripetal spread (periphery first, then spread towards body/face):
    - Coxsackie Virus, Eczema herpeticum/Kaposi sarcoma
    - Syphilis (secondary), **Dengue fever** and **Rocky mountain spotted fever**
    - **Smallpox** (Centripetal spread, but centrifugal distribution).
26. **Ans.(b) (c) (d) (e) (Lyme's..., Borrelia..., Malaria, Plague)** Ref: Apurb Sastry's Essentials of Parasitology 1/e p315
27. **Ans. (a) (b) (Crimean Congo fever, Rocky Mountain spotted fever)** Ref: Apurb Sastry's Essentials of Parasitology 1/e p315
    Refer table in the appendix.
28. **Ans. (b) (Herpes Simplex)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p604
    Among the given option, HSV is the most appropriate answer.
    Agents causing congenital infection by transplacental transmission: Refer chapter review.
29. **Ans. (c) (TB)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p627, Patrick R Murray 9/e p108
    Classification of Agents of Bioterrorism: Refer chapter review
    - Category A (e.g. of Smallpox, plague, Cl. botulinum) agents are most potential agents that can be used as bioweapons.
30. **Ans. (a) (Klebsiella)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p600
    - Klebsiella infects alveoli and can cause lobar/typical pneumonia.
31. **Ans. (a) (b) (d) (Indian ink smear of CSF, AFB, HSV detection)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p594, Harrison 18/e p3418

- The combination of unrelenting headache, stiff neck, fatigue, night sweats, and fever with a CSF lymphocytic pleocytosis and a mildly decreased glucose concentration is highly suspicious for tuberculous meningitis. Harrison 18/e p3426
- So if it was a single response question, I would prefer to go with tuberculous meningitis
- But in a multi choice format we should analyze further.

32. **Ans. (a), (b) and (e) (Salmonella typhi, N.gonorrhoea and Treponema pallidum)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p631, Ananthanarayan 8/e p288 and p373
    - S. Typhi, N. gonorrhea and Treponema pallidum are exclusively human parasites

33. **Ans. (a) (Necrotizing enterocolitis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p584
    - Probiotics are substances secreted by one organism which stimulates the growth of another.

    Probiotics are useful in following conditions:
    - Rota virus diarrhea
    - Antibiotic associated diarrhea
    - Radiation induced diarrhea
    - Traveller's diarrhea
    - Inflammatory bowel disease
    - Cancers.

34. **Ans. (a) (Human...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p630, Patrick R Murray 9/e p98

    Risk based classification of microorganisms. Refer chapter review.

    Human influenza virus is Biosafety grade II virus.

35. **Ans. (d) (Vibrio cholerae)** Ref: Harrison 17/e p963, 967 and 1814

    Causative agents of hemolytic uremic syndrome:
    - Entero Hemorrhagic E. coli- MC
    - Shigella dysenteriae
    - Campylobacter
    - Salmonella spp.

36. **Ans. (d) (Giardia)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p245
    - Loeffler's syndrome occurs due larval migration through lungs during its life cycle.
    - It is observed in following parasitic infections:
        - Strongyloides stercoralis
        - Ascaris lumbricoides
        - Toxocara: Larva migrans.

37. **Ans. (a) (Rotavirus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p526, Jawetz 25/e p511, 24/e p506
    - An oral live attenuated rhesus based vaccine was licensed in U.S. in 1998 for vaccination of infants
    - But was withdrawn a year later because of reports of Intussusception (bowel blockages).

38. **Ans. (d) (Brucella spp)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p356, Ananthanarayan 8/e p340-343

    Points in favor of Brucellosis:
    - Veterinary doctor: Occupational exposure to infected animals
    - Organism could grow only in special laboratory media
    - Oxidase positive Gram-negative short bacilli
    - Brucellosis is one of the common cause of PUO.

39. **Ans. (a) (Mycobacterium tuberculosis)** Ref: P Apurba Sastry's Essentials of Medical Microbiology 1/e p597

    Infectious causes of pyrexia of unknown origin (PUO)- refer chapter review

40. **Ans. (a) (Clostridium tetani)** Ref: Harrison 17/e p712
    - Organisms that parasitize RBCs can directly lyse them and can cause intravascular hemolysis.
    - Intracellular parasites; reside within RBCs: Plasmodium spp., Babesia spp., Bartonella baciliformis
    - Clostridium tetani is not intracellular parasite; it can cause hemolysis indirectly by production of tetanolysin toxin, but it not intravascular hemolysis.

41. **Ans. (b) (HSV-II)** Ref: OP Ghai 6/e p526-27
    - Among the given options HSV-II and Meningococcus can cause meningoencephalitis

- Meningococcal meningitis will be associated with skin rashes and it rarely occurs in neonates as they are protected by maternal antibodies
- HSV-II encephalitis can occur in neonates, it can manifest with or without skin lesions.

42. Ans. (a) (Typhoid) Ref: B Apurba Sastry's Essentials of Medical Microbiology 1/e p631, and PSM Park 19/e p642

Causative agents of Zoonosis - Refer chapter review

Bacteria	Viruses	Parasites
Bovine tuberculosis	Rabies	Leishmanaisis
Bubonic plague	JE	Babesiosis
Q fever	Monkey pox	Toxoplasmosis
Brucellosis	Yellow fever	Taeniasis
Bacillus anthrax	Cow pox	Hydatid disease
Salmonella food poisoning	Fungus	Trichinellosis
Leptospirosis	Microsporum canis	Schistosomiasis
	Trichophyton verrucosum	Trypanosomiasis

- Enteric fever causing Salmonellae like S. Typhi and S. Paratyphi are exclusively human Pathogens.

43. Ans. (c) (Leishmaniasis) Ref: Park 22/e p742, WHO Website- Communicable diseases following natural disasters.

- Diseases associated with natural disaster:
- Waterborne diseases: E. coli, V. cholerae, Hepatitis A and E and Leptospirosis
- Over-crowding: Measles, Meningitis and Acute respiratory infections
- Vector-borne diseases: Disaster can affect vector breeding sites and vector-borne disease transmission, e.g. Malaria and Dengue
- Zoonoses: Rickettsiosis, Equine encephalitis and Rabies
- Other associated diseases: Tetanus, Coccidiomycosis.

44. Ans. (b) (c) (d) (Yersinia pestis, Vibrio parahaemolyticus, Staphylococcus aureus) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p313,319,214 Ananthanarayan 9/e p206, 322, 311,295

Incubation period

- Salmonella: 10–21 days
- Yersinia pestis: Bubonic plague- 2–7 days, Pneumonic plague- 1–3 days
- Vibrio parahaemolyticus: 3 days (4 h to 4 days)
- Staphylococcus aureus food poisoning: 1–6 hrs
- Measles 10 days.

45. Ans. (a), (c),(d),(e) (Staph.., Mycoplasma…, Chlamydia…, Legionella..) Ref: Harrison 18/e Table 257-2

46. Ans. (a) (b) (c) (Lymes-Borelia burgdoreferi, Cat scratch—Bartonella hensale, Glanders – Burkhulderia mallei) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p631, Ananthanarayan 9/e p380, 412, 317, 398

- Rat bite fever: Streptobacillus moniformis
- Trench fever: Bartonella quintana.

47. Ans. (c) (Brucella) Ref: Journal: Significance of relative bradycardia, Clinical Microbiology and Infection/2000.

Brucella is an intracellular Gram-negative organism but is not associated with relative bradycardia.

Causes of relative bradycardia

- Infectious causes: Legionella, Psittacosis, Q fever, Typhoid fever, Typhus, Babesiosis, Malaria, Leptospirosis, Yellow fever, Dengue fever, Viral hemorrhagic fevers, Rocky Mountain spotted fever
- Non-Infectious causes: β-blockers, CNS lesions, Factitious fever, Lymphomas, Drug fever.

48. Ans. (a) (Urinary tract) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p586, Ananthanarayan 9/e p162

As few number of bacilli may be there in urine sample due to contamination with periurethral flora, hence quantitation of bacteria in urine is MUST for the diagnosis of UTI.

49. Ans. (b) (20 minutes) Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p26,Ananthanarayan 9/e p22

- Population doubling time or the generation time of:
  E. coli- 20 min, M. tuberculosis- 20 hr and M. leprae- 20 days

50. Ans. (b) (Guinea worm) Ref: Apurba Sastry's Essentials of Medical Parasitology 1/e p282

Guinea worm infection is transmitted to Man by drinking fresh water from stagnant pools containing minute fresh water crustaceans (Cyclops) infected with L3 larva.

51. Ans. (a) (Hanta virus) Ref: Apurba Sastry's Essentials of Medical Microbiology/p628, Harrison 19th/261e

- Class C biological terrorism include: Emerging infectious diseases threats such as Nipah, hantavirus, SARS or MERS coronavirus, and pandemic influenza.
- Ebola, Crimean Congo and Marburg viruses belong to Class A.

52. Ans. (a), (c), (d) (Nipah virus, Corona virus, SARS) Ref: Apurba Sastry's Essentials of Medical Microbiology/p625, Harrison 18/e p1007,17/e p749 and B. Arora's Microbiology 3/e p689-691

- Emerging infectious diseases are defined by WHO
- Those infections, the incidence of which in humans has either increased during the last 2 decades or threatens to increase in near future
- These are newly appearing infectious diseases or those spreading to new geographical areas.

Hospital Acquired Infection, Biomedical Waste, Bacteriology of Water, Air and Milk

CHAPTER 7.2

HOSPITAL ACQUIRED INFECTIONS

Hospital acquired infections or nosocomial infections can be defined as: the infections acquired in hospital by a patient:

- Who was admitted for a reason other than that infection
- In whom the infection was not present or incubating at the time of admission
- Symptoms should appear at least after 48 hours of admission
- This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the hospital care facility.

Microorganisms Implicated in HAIs

ESKAPE pathogens—They are the multidrug resistant isolates present in a hospital, such as ***E****nterococcus faecium,* ***S****taphylococcus aureus,* ***K****lebsiella pneumoniae,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa,* ***E****nterobacter* species

Other common infections that can spread in hospitals include:

- *Escherichia coli,* Nosocomially acquired *M. tuberculosis, Legionella pneumophila, Candida albicans*
- *Clostridium difficile* diarrhea
- Blood borne infections (BBIs) transmitted through contaminated needle prick injury or mucocutaneous exposure of blood include- HIV, hepatitis B and C viruses.

Modes of Transmission of HAIs

Contact transmission		• Direct skin to skin contact is the most important and frequent mode of transmission. • Indirect contact through (1) Contaminated dressings, or gloves, instruments, (2) Parenteral transmission through-Needle or sharp prick injury, splashes
Inhalational mode		
	Droplet transmission	Droplets of > 5 µm size can travel for shorter distance (< 3 feet). Agents of bacterial meningitis, diphtheria, RSV are transmitted by this route
	Airborne transmission	Airborne droplet nuclei (≤ 5 µm size) or dust particles that remain suspended in the air for long time and can travel longer distance: • This is more efficient mode than droplet transmission. • *Legionella*, *Mycobacterium tuberculosis*, measles and varicella viruses
Other modes: Vector borne and Common vehicle (food, water)		

Types of HAIs

In any hospital, the four most common HAIs encountered are UTI (33%), Pneumonia (15%), Surgical site infections (15%) and Bloodstream infections (13%)

Prevention of HAIs

Standard (Routine) Precautions

Standard precautions are a set of infection control practices used to prevent transmission of diseases that can be acquired by contact with blood, body fluids, non-intact skin (including rashes), and mucous membranes

Components of standard precautions include (indicated while handling all patients, specimens, and sharps):

- Hand hygiene
- Personal protective equipments (PPEs): Gloves, mask, gown, shoes, eye cover
- Spillage cleaning by 10% sodium hypochlorite

- Disinfection usage
- Waste handling and sharp handling: All sharps should be handled with extreme care.

Hand Hygiene

Hand hygiene is the most important measure to avoid the transmission of HAIs. Types of hand hygiene methods available:

- **Hand rub:** Alcohol (70–80% ethyl alcohol) plus chlorhexidine (2–4%) based hand rubs is ideal. The duration of contact has to be at least for 20–30 seconds.
  - Advantage: After a period of contact, it gets evaporated of its own hence drying of hands is not required separately.
  - Indications (**Five Moments of Hand Hygiene, WHO):** Hand rub should be done in the FIVE situations/moments during routine round in ward or ICU, such as: (1) Before touching the patient, (2) Before an aseptic procedure, (3) After an aseptic procedure/body fluid exposure risk, (4) After touching the patient, (5) After touching the patient's surrounding.
- **Handwash:** Antimicrobial soaps (liquid, gel or bars) are available. Duration of contact has to be at least for 40–60 seconds. Handwashing is indicated in the following situations:
  - When the hands are visibly soiled with blood, excreta, pus, etc.
  - Before and after eating
  - After going to toilet
  - Before and after shift of the duty.

Specific Precautions

Additional precautions are needed for preventing specific modes of transmission, such as Airborne precautions, Droplet precautions and Contact precautions.

HAI Surveillance

The Hospital Infection Control Committee (HICC) maintains surveillance of hospital acquired infections. The four key parameters used for HAI surveillance are:

- CA-UTI (Catheter associated urinary tract infection)
- CLABSI (central line associated bloodstream infection)
- VAP (ventilator associated pneumonia)
- SSI (surgical site infections).

BIOMEDICAL WASTE MANAGEMENT

Biomedical Waste Management in India is followed according to New BMW Rule 2016. The earlier 1998 BMW rule is obsolete now.

Table 7.2.1: Biomedical Waste Management Rule 2016

Category	Type of waste	Type of bag/container	Treatment/ Disposal options
Yellow	Human anatomical waste	Yellow coloured non chlorinated plastic bags	Incineration/ Plasma pyrolysis/ deep burial
	Animal anatomical waste		
	Soiled waste		Incineration/ Plasma Pyrolysis/ deep burial/ autoclaving or hydroclaving + shredding/mutilation
	Expired/ discarded medicines-pharmaceutical waste, cytotoxic drugs	Yellow coloured containers/ non chlorinated plastic bags	Incineration (cytotoxic drugs at temperature > 1200°C)
	Chemical waste	Yellow coloured containers/ non chlorinated plastic bags	incineration or Plasma pyrolysis or Encapsulation
	Chemical liquid waste	Separate collection system leading to effluent treatment system	Pre-treated before mixing with other wastewater
	Discarded linen contaminated with blood/ body fluids	Non-chlorinated yellow plastic bags/ suitable packing material	Non- chlorinated chemical disinfection followed by incineration/ plasma pyrolysis

Contd...

Contd...

Category	Type of waste	Type of bag/container	Treatment/ Disposal options
	Microbiology, other clinical lab waste, blood bags, live/ attenuated vaccines	Autoclave safe plastic bag/ container	Pre-treat to sterilize with non-chlorinated chemicals on-site as per NACO/ WHO guidelines + Incineration.
Red	Contaminated Waste (Recyclable)	Red coloured non-chlorinated plastic bags or containers	Autoclaving/ micro-waving/ hydroclaving + shredding Mutilation/ sterilization+ shredding. Treated waste sent to registered or authorized recyclers or for energy recovery or plastics to diesel or fuel oil or for road making,
White (Translucent)	Waste sharps including metal sharp	Puncture proof, Leak proof, tamper proof containers	Autoclaving/ dry heat sterilization+ shredding/ mutilation Encapsulation in metal container or cement concrete Sanitary landfill/ designated concrete waste sharp pit
Blue	Glassware Metallic body implants	Cardboard boxes with blue colored marking	Disinfection (by soaking the washed glass waste after cleaning with detergent and Sodium Hypochlorite treatment)/ through autoclaving/ microwaving/ hydroclaving + recycling

Table 7.2.2: Biomedical Waste Management Rules 1998 and 2016

Biomedical Waste Management Rules 1998	Biomedical Waste Management Rules 2016
• The waste were categorized into ten categories and were given overlapping colour coded containers	The waste are categorized into four categories, each having a specific color coded container
• Chemical pretreatment was with 1% sodium hypochlorite.	Chemical pretreatment with 10% sodium hypochlorite.
• The minimum limit for the release of furans from incinerator have not been specified.	The minimum limit of furans from incinerator has been clearly specified.
• Outsourcing of biomedical waste for final treatment has not been mandatory.	Outsourcing is strongly recommended.
• The methods of disposal were incineration/autoclaving/ microwaving /mutilation and shredding.	Apart from those of 1998, newer methods introduced such as- i) plasma pyrolysis, ii) hydroclaving, iii) encapsulation, iv) inertization, v) sharp pit.
• Cytotoxic drugs were to be discarded in black colour bag.	Cytotoxic drugs to be discarded in yellow bag.
• Chemical solid waste are to be discarded in black bag.	Chemical solid waste are to be discarded in yellow bag.
• Waste sharps/ metal sharps are to be discarded in blue/ white bag.	Waste sharps/metal sharps are to be discarded in transparent puncture proof box.
• Metallic body implants are to be discarded in blue/ white bag	Metallic body implants are to be discarded in transparent transparent puncture proof box.
• Majority of the rules were for discarding the biomedical waste.	Majority of the waste disposal are directed for recycling.

BACTERIOLOGY OF WATER, MILK AND AIR

Bacteriology of Water

Indicator organisms of fecal pollution of water

Indicator organisms	Interpretation (Presence in water indicates)
Coliform (other than *E.coli*)	Remote contamination—either fecal (presumptive) or soil and vegetation
Fecal (thermotolerant) *E.coli*	Most sensitive indicator, confirms recent fecal contamination of water
Fecal streptococci	Confirms remote fecal pollution
Clostridium perfringens	Remote fecal contamination
Pseudomonas aeruginosa	Least reliable indicator, useful in hospitals and food establishments
Bacteriophages	Phage specific for *E.coli*: Indicate fecal pollution of water indirectly indicates viral pollution

Methods of Water Analysis

Presumptive coliform count (Multiple tube method): This detects the probable number of coliform bacilli in water.

- It is done by calculating as the most probable number (MPN) of coliform organisms in 100 ml water
- Medium: MacConkey purple broth (double strength and single strength) in is the standard medium of choice

- Detection of coliform bacteria does not always indicate fecal contamination as some of them may be found in environment. Hence, it is further tested by differential coliform count to detect the fecal *E. coli*.

Eijkman test: It is done to confirm that the coliform bacilli detected in the presumptive test are fecal thermo tolerant *E.coli* which grows at 44 °C with indole and gas production and lactose fermentation. Brilliant green bile broth is used.

Other methods

- *Clostridium perfringens* detection
- Enzyme detection: β galactosidase (coliform bacilli specific enzyme) and β glucuronidase (fecal *E.coli* specific)
- Membrane filtration method
- Examination for specific water borne pathogens such as *Salmonella* Typhi and *Vibrio cholerae*

Bacteriology of Milk

Table 7.2.4: Milk borne diseases (By Joint FAO/WHO Expert committee on Milk Hygiene, 1970)

Diseases primarily of animal origin that can be transmitted to man by milk:	
Primary Importance: • Tuberculosis (*M.bovis*) • Brucellosis • Salmonellosis • *Coxiella burnetii* (Q fever) • Staphylococcal food poisoning • Streptococcal infections	**Lesser Importance:** • Cow Pox • Hand Foot Mouth Disease • Anthrax • Leptospirosis • *Campylobacter jejuni* infection • Tick borne encephalitis viruses
Diseases primarily of human origin that can be transmitted by milk	
Primary Importance: • Typhoid and paratyphoid fever • Shigellosis • Cholera • Enterohemorrhagic *E.coli* (EHEC) infection	**Lesser Importance:** Nondiarrheal diseases: • Streptococcal infection • Staphylococcal food poisoning • Diphtheria, Tuberculosis, Enteroviruses, Viral Hepatitis

Methods for Bacteriological Examination of Milk

- Colony count: Viable count (by plate dilution method) and Coliform count
- Chemical tests:
  - Methylene blue reduction test (more economical)
  - Phosphatase test (test the effectiveness of pasteurization of milk)
  - Turbidity test (definitive test for checking the sterilization of milk)
- Detection of specific pathogens such as Tubercle bacilli and *Brucella.*

Bacteriology of Air

Methods for measuring bacterial content of air- fall into two broad categories.

- Settle plate method-measure bacteria carrying particles settle down by gravity from air on to the exposed surface
- Slit sampler method and air centrifuge method: Count the number of bacteria carrying particles in a given volume of air.

Acceptable Limit of air pollution:

The upper limits of the bacterial count in air in various areas are as follows:

- 50 per cubic feet in factories, offices and homes
- 10 per cubic feet in general operation theater
- 1 per cubic feet in operation theater for neurosurgery

MULTIPLE CHOICE QUESTIONS

HOSPITAL ACQUIRED INFECTIONS

1. Most common species of Pseudomonas causing intravascular catheter related infections is: *(AIIMS Nov 2008)*
a. P. cepacia b. P. aeruginosa
c. P. maltophila d. P. mallei

2. Commonest cause of nosocomial infection: *(JIPMER 2008)*
a. Pseudomonas b. Staphylococci
c. Klebsiella d. Enterobacteriaceae

3. Most common catheter related blood stream infection is: *(AIIMS May 2007)*
a. Candida
b. Gram negative organisms
c. Coagulase positive staphylococci
d. Coagulase negative staphylococci

4. The most common pathogens responsible for nosocomial pneumonias in the ICU are: *(AI 2005)*
a. Gram-positive organisms
b. Gram-negative organisms
c. Mycoplasma
d. Virus infections

5. All the following are true about nosocomial infections except: *(AI 2004)*
a. May manifest within 48 hours of admission
b. May develop after discharge of patient from the hospital
c. Denote a new condition which is unrelated to the patient's primary conditions
d. May already present at the time of admission

6. In a postoperative intensive care unit, five patients developed postoperative wound infection on the same wound. The best method to prevent cross infection occurring in other patients in the same ward is to: *(AI 2003)*
a. Give antibiotics to all other patients in the ward
b. Fumigate the ward
c. Disinfect the ward with sodium hypochlorite
d. Practice proper handwashing

7. Most effective method of protection from infected body fluids is: *(JIPMER Nov 2015)*
a. Gown b. Goggles
c. Gloves d. Mask

8. Most cost effective method to prevent hospital acquired infections: *(AIIMS Nov 2015)*
a. Alcohol based hand rub b. Handwash
c. Disinfectant use d. Use of antibiotics

9. Best method of preventing further transmission in MRSA infection in ward is: *(Recent Question 2015)*
a. Handwash b. Antibiotics

BIOMEDICAL WASTE MANAGEMENT

10. What is the most preferred method of management of the blood stained cloth? *(Recent Question 2015)*
a. Autoclaving b. Chemical treatment
c. Deep burial

11. A known HIV positive patient is admitted in an isolation ward after an abdominal surgery following an accident. The resident doctor who changed his dressing the next day found it to be soaked in blood. Which of the following would be the right method of choice of discarding the dressing: *(AIIMS Nov 2005)*
a. Pour 10% hypochlorite on the dressing material and send it for incineration in appropriate bag
b. Pour 5% hypochlorite on the dressing material and send it for incineration in appropriate bag
c. Put the dressing material directly in an appropriate bag and send for incineration
d. Pour 2% hypochlorite on the dressing material and send it for incineration in appropriate bag

12. All of the following categories of biomedical waste can be disposed by Incineration Except: *(MHPG 2014)*
a. Category 1 b. Category 2
c. Category 3 d. Category 4

13. Yellow bag contains: *(PGI Nov 2012, APPG 2012, MHPG 2014)*
a. Incineration ash b. Sharps
c. Human anatomical waste
d. Solid waste

14. The most suitable disinfectant for decontamination of HIV contaminated endoscope is: *(MHPG 2014)*
a. 10% sodium hypochlorite
b. 2% glutaraldehyde
c. 5% phenol
d. 70% ethanol

15. All are disposed by incineration/deep burial except: *(JIPMER Nov 2015)*
a. Solid waste b. Animal waste
c. Cytotoxic waste d. IV set

16. Which of the following is disposed in red bag? *(JIPMER Nov 2015)*
a. Plastic waste b. Cytotoxic waste
c. Sharps
d. Radioactive waste generate from lab

17. All are true except: *(AI 2011)*
a. Human anatomical waste is disposed in a yellow bag
b. Red bag contents can be a source of contamination
c. Black bag is used for incineration ash
d. Blue bag contents are always disposed in secure landfill

BACTERIOLOGY OF WATER, AIR AND MILK

18. The following organisms are indicative of fecal pollution of water Except: *(APPG 2015)*
a. Halophilic vibrio
b. Fecal Streptococci
c. E. coli
d. Clostridium perfringens

19. Coliform count is done by: *(NEET Pattern Based)*
a. VR medium
b. McConkey's agar
c. Multiple tube method
d. None

20. Which of the following is not true regarding surveillance of water pollution? *(AIIMS May 2011)*
a. Multiple tube method is used to calculate the number of bacteria
b. E. coli can be tested by Indole test at 44 °C
c. Sodium thiosulfate is used to neutralize chlorine
d. Presence of corynebacterium indicates recent contamination

21. Pasteurized milk is most commonly tested by: *(NEET Pattern Based)*
a. Phosphatase test
b. Coliform test
c. Catalase test
d. Oxidase test

22. Milk borne diseases are: *(PGI June 2011)*
a. Salmonellosis
b. E. coli
c. Streptococcus
d. Tuberculosis
e. Q fever

EXPLANATIONS

HOSPITAL ACQUIRED INFECTIONS

1. **Ans. (b) (P. aeruginosa)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p340, Ananthanarayan 8/e p316
   Pseudomonas aeruginosa is most troublesome agent of nosocomial infections

2. **Ans. (a), (c), (d) (Pseudomonas, Klebsiella, Entero...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p606
   - Controversial MCQ, no where there is clear cut reference for MC agent of HAI
   - Initially hospital outbreaks were caused by Gram positive organisms like Streptococcus pyogenes and the methicillin resistant Staphylococcus aureus (MRSA)
   - Presently Gram negative enteric pathogens (Enterobacteriaceae) including Escherichia coli, Klebsiella and Enterobacter species have emerged as an important cause
   - Pseudomonas and Acinetobacter species are also common nosocomial pathogens and are extremely resistant to common antibiotics and antiseptics.

3. **Ans. (d) (Coagulase negative Staphylococcus)** Ref: Harrison 18/e p1116, 17/e p839
   - Intravascular devices cause up to 50% of nosocomial bacteremia
   - Central vascular catheters (CVCs) account for 80–90% of these infections
   - Catheter-related bloodstream infections derive largely from the cutaneous microflora of the insertion site, usually during the first week after insertion
   - Most common pathogens isolated from vascular device–associated bacteremia include:
   - Coagulase-negative staphylococci (S.epidermidis) (commonest).

4. **Ans. (b) (Gram-negative organisms)** Ref: Harrison 18/e p1115,17/e p838
   - Pneumonia accounts for 15–20% of nosocomial infections
   - Mortality due to ventilator-associated pneumonia - 6–14%
   - Precipitating factors for mortality include
     - Co morbidities and inadequate antibiotic treatment
     - Involvement of specific pathogens (particularly Pseudomonas aeruginosa and Acinetobacter, which are Gram-negative organisms).

5. **Ans. (d) (May already...)** Ref : Apurba Sastry's Essentials of Medical Microbiology 1/e p606, Ananthanarayan 8/e p677
   - Hospital associated or hospital-acquired infection or nosocomial infection is applied to infections contracted after admission to a hospital for treatment of a different illness
   - These infections are neither overtly present nor are in incubation period in the patients at the time of admission, and are often due to organisms endemic in the institution
   - Approximately 5% of hospitalized patients experience a nosocomial infection.

6. **Ans. (d) (Practice...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p609, and Harrison 18/e p1113
   - MC way of spread of infection in hospital: From hands of hospital staffs
   - Hence, thorough handwashing after any procedure involving nursing care or close contact with the patient is the most effective preventive measure.

7. **Ans. (c) (Gloves)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p610
   Hand hygiene is the most important as hands will be in contact first. So gloves will be the most effective method among the options given here.

8. **Ans. (b) (Handwash)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p610
   Handwash is the single most important method to prevent hospital acquired infections.

9. **Ans. (a) (Handwash)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p610

BIOMEDICAL WASTE MANAGEMENT

10. **Ans. (c) (Deep burial)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p613

11. **Ans. (a) (Pour 10% hypo....)**
Ref: Apurba Sastry's Essentials of Medical Microbiology 1/p613, PSM, Park 20/e p696-699
    - According to new BMW 2016, Dressing material soaked with HIV contaminated blood are discarded in 10 % hypochlorite and then sent for incineration

12. **Ans. (d) (Category 4)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p613, Park 20/e p696
    - This answer is according to BMW 1998 rule, which is not applicable now.

13. **Ans. (c), (d) (Human anatomical waste, Solid waste)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p613, Park 22/e p739, 21/e p734-35
    - Yellow bag contains item of category -1, 2, 3 and 6. Refer chapter review for details.

14. **Ans. (b) (2% glutaraldehyde)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p613, Ananthanarayan 9/e p34
    - All Scopes are sterilized best by glutaraldehyde (CIDEX 2%).

15. **Ans (d) (IV set)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p613
    - Incineration/deep burial (yellow bag) is recommended for Anatomical waste (human and animal), Microbiological waste (nonplastic, nonreusable), Solid waste (nonplastic) and Cytotoxic drug (black bag)
    - Autoclave (red bag) is recommended for Microbiological waste (plastic, or reusable) and Solid waste (plastic).

16. **Ans. (a) (Plastic waste)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p613
    - Plastic infectious waste are discarded in red bag.

17. **Ans. (d) (Blue bag...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p613

BACTERIOLOGY OF WATER, AIR AND MILK

18. **Ans (a) (Halophilic vibrio)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p616
    - Halophilic vibrios are not indicator of fecal pollution of water.

19. **Ans. (c) (Multiple tube method)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p615-18, Ananthanarayan 9/e p626-27, 8/e p591
    - Presumptive coliform count is calculated by Multiple tube method
    - Differential coliform count is done by Eijkman test.

20. **Ans. (d) (Presence of Corynebacterium indicates recent contamination)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p615-18, Ananthanarayan 9/e p626-27, 8/e p591
    - Corynebacterium is not an indicator for water contamination
    - **Indicator of fecal contamination of water:**
        - Thermotolerant E. coli (recent contamination): Most definite
        - Coliform count
        - Fecal streptococci
        - Clostridium perfringens

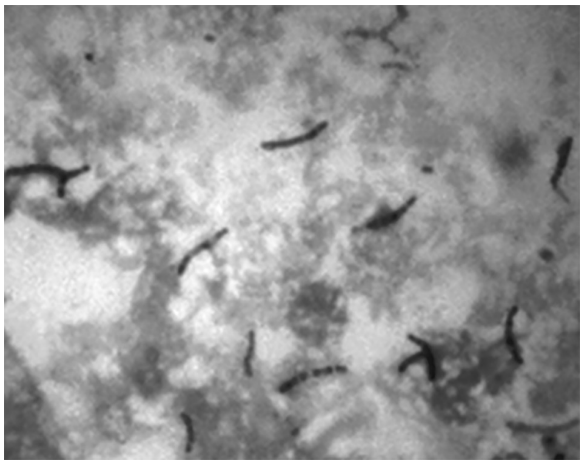
21. **Ans. (a) (Phosphatase...)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p620 Ananthanarayan 9/e p631, 8/e p596
    - ***Phosphatase test: Used to check adequacy of pasteurization of milk***
        - Enzyme phosphatase is normally present in raw milk and will be inactivated by pasteurization
        - ***Its residual presence following pasteurization indicates, pasteurization has not been adequate.***
    - ***Other tests used for bacteriological examination of milk: Refer text.***

22. **Ans. (a) (b) (c) (d) (e) (Salmonellosis, E. coli, Streptococcus, Tuberculosis, Q fever)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1/e p 619
Refer chapter review

Recent Questions 2016

1. **False about Listeria:**
   a. Gram negative coccobacilli
   b. Cold enrichment
   c. Tumbling motility
   d. Meningitis in neonates
2. **MCC of catheter induced UTI:**
   a. E.coli
   b. Klebsiella
   c. Pseudomonas
   d. Staphylococcus
3. **Oxidase positive:**
   a. Pseudomonas
   b. Klebsiella
   c. Staphylococcus
   d. E.coli
4. **The colonies grown in MacConkey agar belongs to:**
   a. E.coli
   b. Pseudomonas
   c. Klebsiella

5. **Intracellular fungus**
   a. Histoplasma
   b. Candida albicans
   c. Cryptococcus
   d. Sporothrix
6. **Intra uterine infection is indicated by presence of which antibody?**
   a. IgM
   b. IgG
   c. IgA
   d. IgE
7. **Intestinal immunity is due to which Ig?**
   a. IgM
   b. IgG
   c. IgA
   d. IgE
8. **Safety pin appearance is not seen in:**
   a. Burkholderia
   b. Brucella
   c. Donovaniasis
   d. Vibrio
9. **Most common parasitic cause of recurrent diarrhea in HIV patient is……**
10. **Most common organism in smokers:**
    a. Coxiella burnettii
    b. Burkholderia cepacia
11. **Disposable glass syringes are best sterilized by:**
    a. Autoclave
    b. Hot air oven
    c. Ethylene oxide
    d. Glutaraldehyde
12. **Which can differentiate E.histolytica from E.dispar?**
    a. 170 kDa antigen
    b. Cyst
    c. Trophozoite
13. **Common feature of Rickettsia and virus:**
    a. Obligate intracellular
    b. Only DNA present
    c. Facultative intracellular
14. **Rota virus belong to which family?**
15. **Rabies virus belong to which family?**
16. **A patient with history of pneumonia. Sputum examination shows the following picture. Which stain is used here?**



17. **Patient had bronchopneumonia and gastroenteritis stool examination showed motile larvae diagnosis is:**
    a. Ascaris
    b. Enterobius
    c. Strongyloides
    d. Trichinella

18. Most common manifestation of primary HSV infection:
a. Asymptomatic
b. Orolabial ulcers
c. Gingivo stomatitis

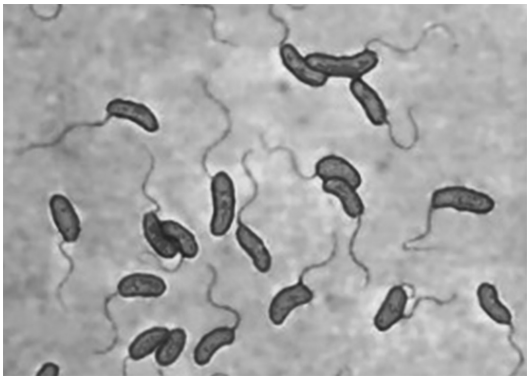
19. Aquatic plants are second intermediate host for which of following trematode?
a. Fasciola hepatica
b. Paragonimus
c. Clonorchis sinensis
d. Schistosoma

20. Most common nosocomial infection:
a. UTI
b. Surgical site
c. Lower respiratory tract
d. Skin and soft tissues

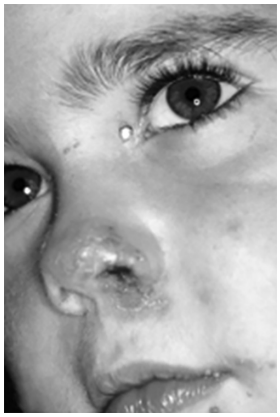
21. Virulence of Cryptococcus is due to:
a. pf65
b. Glycocalyx
c. Capsule

22. Watery diarrhea of cholera is disinfected by:
a. Lysol
b. Cresol
c. Chlorine

23. A lady who was admitted in the hospital for diarrhea after attending Kumbh Mela. The organism picture is given. What type of motility the organism exhibits?
a. Tumbling motility
b. Darting motility
c. Stately motility
d. Corkscrew motility



24. Honey comb crusting – impetigo. What is the most common cause?
a. Staphylococcus
b. Pseudomonas



25. Reduvid big transmits which disease?

26. Difference between type 2 and type 3 hypersensitivity:
a. Ag-ab complex formation
b. Immediate
c. Delayed

27. Vaccine trial RTS, S/AS01 is carried out for:

28. Motile and non-motile bacteria differentiated by all except:
a. Gram stain
b. Craigie tube
c. Flagellar stain
d. Hanging drop

29. Organism causing wool sorter disease?

30. Bile Esculin hydrolysis test is done for?

31. TCBS media is used for?

32. Triple sugar iron test detects all the following properties except:
a. Gas
b. H_2S
c. Sugar fermentation
d. Citrate utilization

33. Case history of pneumonia. Sputum staining reveled partially acid fast branching bacillus. Identify the pathogen?

34. Rapid diagnostic test for meningococcus:
a. Oxidase test in CSF
b. CSF culture
c. CSF latex agglutination

35. History of perianal pruritus and planoconex eggs?

36. Hair perforation test is done for?

37. Dark field microscope is used in identifying which organism?
a. Treponema
b. Chlamydia
c. Rickettsia

38. In spleen removal of old RBC is carried out by which cells?
a. WBC
b. Macrophages

39. Orientia tsutsugamushi causes?

40. Weil Felix reaction is diagnostic of ?

41. Ebola virus belong to which family?

42. Hepatitis B virus belong to which family?

43. Patient of HIV with CD4 less than 200. Tested negative for tuberculosis diagnosis. Organism was partially acid fast. Identify the pathogen:
a. Nocardia
b. M.kansasii
c. Actinomyces

44. VAPP is MC associated with Polio serotype?
a. Type 1
b. Type 2
c. Type 3

45. A 25 year boy presented with deep injury and abrasions of left shoulder, thigh and leg with immunisation status unknown. What is to be given now?
a. DTaP only
b. DTaP + Ig
c. dT only
d. dT + Ig

46. Identify the parasite?

47. Which parasite has no cyst stage; only trophozoite present?

48. Pathology of Mycoplasma pneumoniae is located in ?
a. Interstitium
b. Intra-alveolar

49. The capsule of S.pneumoniae is made up of:
a. Polypeptide
b. Lipopolysaccharide
c. Polysaccharide
d. Glycopeptide

50. Specific site of antigen that reacts with antibody:
a. Epitope
b. Paratope
c. Iditope
d. Isotope

51. Complement constitutes how much of total serum protein?
a. 5% b. 10%
c. 15% d. 20%

52. Large granular lymphocytes are:
a. T cell
b. B cell
c. Plasma cell
d. NK cell

53. Delayed type of hypersensitivity is mediated by:
a. B cell
b. T cell
c. Plasma cell
d. Macrophages

54. Staphylococcus aureus causes:
a. Scarlet fever
b. Rheumatic fever
c. Erysipelas
d. Food poisoning

55. A four year old boy had three episodes of cervical lymphadenitis; which were incised and drainage was done. Last episode was two months back. Child also has hepatic abscess which was also drained two years back. Pus culture shows Staphylococcus aureus. The nitroblue-tetrazolium (NBT) test was found to be positive. Most probable diagnosis is:
a. Tuberculosis
b. Kostman disease
c. Chronic granulomatous disease

56. A 10 year old boy had developed lobar pneumonia. Sputum microscopy revealed lanceolate shaped diplococci and culture on blood agar grew alfa haemolytic colonies. The probable identification is:
a. Staphylococcus aureus
b. Streptococcus pyogenes
c. Streptococcus pneumoniae
d. Neisseria meningitides

57. Cholera toxin attaches to which receptor?
a. GD1 b. GM1
c. GM2 d. GD2

58. A young man is presented with very painful vesicular ulcer in penis with ragged margin and painful inguinal lymphadenopathy. The most probable diagnosis:
a. Syphilis b. Chancroid
c. Herpes genitalis

59. Chlamydia psittaci causes:
a. Childhood pneumonia
b. Avian pneumonia

60. True about Actinomycetes
a. Produces branching
b. Produces pseudo branching
c. Produces hyphae
d. Produces psuedohyphae

61. Azole group of antimicrobial agents are used in?
a. Chlamydia b. Candida
c. Trichomonas d. Gardnerella

62. Partially dsDNA is present in?
a. Parvovirus b. Herpesvirus
c. Hepadnavirus

63. Atypical lymphocytes are found in which infection?
a. EBV b. CMV
c. Both

64. Most common infectious cause of nasopharyngeal carcinoma is

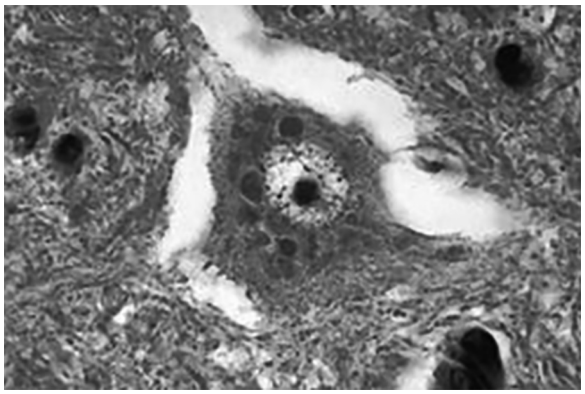
65. Epidemic pleurodynia is caused:
a. Echovirus b. Coxsackie virus
c. Adenovirus

66. Reverse transcriptase is encoded by:
a. gag gene b. pol gene
c. env gene d. tax gene

67. Intermediate host for Taenia saginata is:
a. Cattle
b. Pig
c. Man

68. Which fungi is present in reticuloendothelial cells?

69. Image belongs to which virus?
a. Rhabdovirus
b. Myxovirus
c. Picornavirus



70. Vi vaccine can be given at what age?
a. 6 months
b. 12 months
c. 24 months

71. Protective titre of HBV is____ IU/ml:
a. 0.1
b. 1
c. 5
d. 10

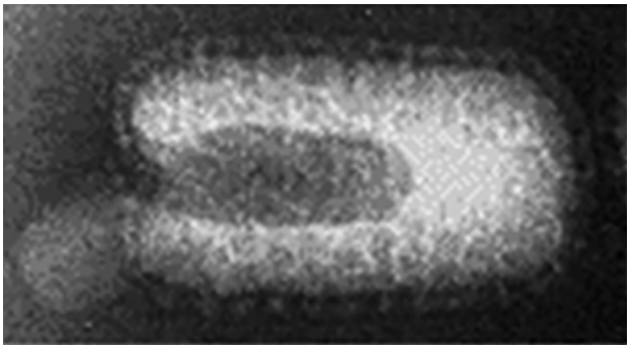
72. IL2 is?
a. Th1 cell inhibitor
b. T cell growth factor
c. B cell growth factor

73. Causative agent of Q fever?

74. Causative agent of cat scratch disease?

75. Causative agent of RMSF?

76. The following image belongs to which virus?



77. Inspissation is heating:
a. At 85 deg C for half an hour on 3 consecutive days
b. 85 deg C for an hour
c. At 100 deg C for half an hour on 3 consecutive days

78. Best way to prevent MRSA infection in hospital staff:
a. Mupirocin treatment
b. Screening of all health care workers
c. Hand hygiene by washing and alcohol scrub

79. Late lactose fermenting Shigella:
a. S.sonnei
b. S.flexneri
c. S.boydii
d. S.dysenteriae

80. Agent causing Rheumatic fever:
a. Alfa hemolytic Streptococci
b. Beta hemolytic Streptococci
c. Gamma hemolytic Streptococci
d. Staphylococcus aureus

EXPLANATIONS

1. **Ans (a) (Gram negative coccobacilli)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p296
2. **Ans (a) (*E.coli*)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p587
3. **Ans (a) (Pseudomonas)**
4. **Ans (c) (Klebsiella)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p309
5. **Ans (a) (Histoplasma)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p563
6. **Ans (a) (IgM)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p113
7. **Ans (c) (IgA)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p114
8. **Ans (d) (Vibrio)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p328
9. **Ans (Cryptosporidium parvum)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1st/p129
10. **Ans (b) (Burkholderia cepacia)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p343
11. **Ans (b) (Hot air oven)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p32
12. **Ans (a) (170 kDa antigen)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1st/p36
13. **Ans (a) (Obligate intracellular)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p388
14. **Ans (Reoviridae)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p414
15. **Ans (Rhabdoviridae)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p414
16. **Ans (Ziehl Neelson)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p276
17. **Ans (c) (Strongyloides)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1st/p241
18. **Ans (c) (Gingivo stomatitis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p435
19. **Ans (a) (F.hepatica)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1st/p204
20. **Ans (a) (UTI)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p607
21. **Ans (c) (Capsule)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p569
22. **Ans (c) (chlorine)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p38,336
23. **Ans (b) (darting motility)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p333
24. **Ans (a) (Staphylococcus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p215
25. **Ans (Trypanosoma cruzi)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1st/p79
26. **Ans (a) (Ag-ab complex)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p178
27. **Ans (Malaria)** Ref: Internet -PLoS Med. 2014 Jul 29;11(7):e1001685. doi: 10.1371/journal.pmed.1001685. eCollection 2014
28. **Ans (a) (Gram)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p15
29. **Ans (Bacillus anthracis)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p251
30. **Ans (Enterococcus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p230
31. **Ans (Vibrio)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p334
32. **Ans (d) (Citrate utilization)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p57
33. **Ans (Nocardia)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p295.
34. **Ans (c) (CSF latex agglutination)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p237.
35. **Ans (Enterobius)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1st/p227.
36. **Ans (T.mentagrophytes)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p557.
37. **Ans (a) (Treponema)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p11.
38. **Ans (b) (Macrophages)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p154.

39. **Ans (Scrub typhus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p389.
40. **Ans (Scrub typhus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p389.
41. **Ans (Filoviridae)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p519.
42. **Ans (Hepadnaviridae)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p530.
43. **Ans (a) (Nocardia)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p293.
44. **Ans (c) (Type 3)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p479.
45. **Ans (d) (dT+Ig)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p263.
46. **Ans (Giardia)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1st/p50.
47. **Ans (Trichomonas)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1st/p55.
48. **Ans (a) (Interstitium)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p406.
49. **Ans (c) (Polysaccharide)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p232.
50. **Ans (a) (Epitope)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p105.
51. **Ans (a) (5%)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p138.
52. **Ans (d) (NK cell)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p153.
53. **Ans (b) (Tcell)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p179.
54. **Ans (d) (Food poisoning)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p213.
55. **Ans (c) (Chronic granulomatous disease)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p191.
56. **Ans (c) (S.pneumoniae)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p232.
57. **Ans (b) (GM1)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p330.
58. **Ans (b) (Chancroid)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p348.
59. **Ans (b) (Avian pneumonia)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p401.
60. **Ans (a) (Branching)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p293.
61. **Ans (b) (Candida)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p569.
62. **Ans (c) (Hepadna)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p530.
63. **Ans (c) (Both)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p440.
64. **Ans (EBV)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p442.
65. **Ans (b) (Coxsackievirus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p480.
66. **Ans (b) (Pol gene)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p504.
67. **Ans (a) (Cattle)** Ref: Apurba Sastry's Essentials of Medical Parasitology 1st/p167.
68. **Ans (Histoplasma)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p562.
69. **Ans (a) (Rhabdovirus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p499.
70. **Ans (c) (24 months)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p326.
71. **Ans (d) (10)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p536.
72. **Ans (b) (T cell growth factor)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p160.
73. **Ans (Coxiella burnettii)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p394.
74. **Ans (Bartonella henselae)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p395.
75. **Ans (Rickettsia rickettsii)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p391.
76. **Ans (Rabies virus)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p496.
77. **Ans (a) (At 85 deg C for half an hour on 3 consecutive days)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p32.
78. **Ans (c) (Hand hygiene by washing and alcohol scrub)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p219.
79. **Ans (a) (S.sonnei)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p305.
80. **Ans (b) (Beta haemolytic streptococci)** Ref: Apurba Sastry's Essentials of Medical Microbiology 1st/p226