# ***Microbiology and Parasitology***

## 1. Microbiology

a. Bacteriology

**i. Collection, Transport, Processing and Staining of Specimen**

1. First thing tobe done for collection of sputum sample – gargle with water
2. Acid fast stain in tissues – kinyoun
3. AFB stains – Red
4. Non-acid fast bacteria stains – blue
5. Critical step in gram stain – decolorizer
6. Nonspecific staining of cellular structures – Fluorochroming
7. Nasopharyngeal swabs are for – Neisseria, H, influenza, B. pertussis
8. Late chlamydia specimen must be – rejected

**ii.Culture Media**

1. Preferred medium for isolation of B. pertussis – Regan-Lowe/Charcoal cephalexin Blood Agar
2. K Tellurite – gray black colony
3. Cystine tellurite – C. Diptheriae
4. Cystine glucose – F. tularensis
5. Significant colony count in urine – 100,000

**iii. Bacteria (Aerobes)**

1. Morphology and staining characteristics

2. Cultural characteristics

a. Golden yellow colonies in BAP – S. aureus
b. Alpha-prime – S. aureus
c. S. Saprophyticus – Cystitis
d. C. amycolatum in nasopharynx – Normal flora
e. Commonly isolated in ICU – P. aeruginosa
f. P. aeruginosa – Growa in 42 and 35 degress Celsius
g. Flat, serrated colonies with confluent growth on BAP – P. Aeruginosa
h. Salmonella bacterial culture – 2-3 specimen (blood) within 24 hours
i. Whipple disease – Trophyrema

3.Work-up for identification: biochemical, differential and confirmatory tests

a. Clumping factor – Coagulase
b. 30% $H_2O_2$ – Superoxol test
c. MR and VP reaction – Opposite
d. Chromogenic B-lactamase result – Color formation
e. Demonstrate Streptolysin O – Anaerobic culture
f. Differentiate S. aureus and S. epidermidis – Coagualse, DNAse
g. Negative CAMP test – No enhancement of hemolysis
h. Bile solubility – S. pneumonia
i. Simmilar to C. diptheria – C. ulcerans
j. Shigella – Biochemically inert
k. Acetamide Test – P. aeruginosa (35C for 7 days)
l. Bordetella oxidase & urease (+) – Bronchiseptica
m. Requires V factor – H. parahemolyticus
n. requires X factor – H. ducreyi

4. Serology/molecular tests

a. Not common in microbiology – PCR
b. Lancefield – Detects carbohydrates in Streptococcus group
c. Quellung – Capsular swelling
d. Kauffman white – Salmonella serotyping

5. Susceptibility tests

a. Not an antibiotic – Sulfonamide
b. Penicillin – Inhibit cell wall synthesis
c. Vancomycin – Inhibit cell wall synthesis
d. Gentamycin – Inhibit protein synthesis
e. Clindamycin – Inhibit protein synthesis
f. ESBL – Extended spectrum Beta-Lactamase

6. Bacteriologic examination of water, food, milk and utensils

a. Red milk – S. marcescens
b. Blue milk – P. aruginosa
c. Stormy fermentation of milk – peptostreptococcus, peptococcus

**iv. Bacteria (Anaerobes)**

1. Pseudomembranous colitis – C. difficile
2. Common gut flora – Bacteriodes
3. Gram-positive anaerobes – peptostreptococcus, peptococcus

**v. Mycobacteria**

1. AFB smear measures – 2-3cm
2. MPT 64 – M. tuberculosis
3. Niacin and nitrate positive – M. tuberculosis
4. Niacin and nitrate negative – M. bovis
5. Tween 80 positive – M. Kansasii

**vi. Other bacteria with unusual growth requirements (spirochetes, Chlamydia, Mycoplasma, Ricketssia)**

b. Mycology

**i.Collection, transport and examination of clinical specimens**

1. Basic, branching, intertwining structure of clinical specimens
2. Stain for sharp delineation of fungal elements by fluorescent microscopy – Calcoflour white
3. Presumptive test for candida that uses serum – germ tube

4. Positive hair baiting test – V shape penetration of the hair shaft
5. Ascospore – Saccharomyces
6. Farmers lung disease – Aspergillus fumigatus
7. Macroconidia absent – M. Audouinii
8. Microconidia absent – E. floccosum
9. Epidermophyton – skin, nails
10. Microsporum – skin, hair
11. Tricophyton – skin, hair, nails
12. T. mentragophytes – Positive hair baiting test
13. T. rubrum – Red pigment, teardrop shaped conidia

**ii. Culture**

1. AMAN medium stain – Lactophenol cotton blue
2. Cornmeal agar – Chlaamydospores
3. Czapek – Aspergillus
4. Rice agar – M. canis
5. Urease media – Cryptococcus neoformans
6. Birdseed – Phenol oxidase

c. Virology

**i. General characteristics, transmission and diseases**

1. 1st step in viral replication – Adsorption/Attachment and Penetration
2. Part of virus where envelop is acquired – Nuclear or cytoplasmic membrane
3. ssDNA virus – Parvovirus
4. dsRNA – Reovirus
5. Largest virus – Poxvirus
6. Largest RNA virus - Paramyxovirus
7. Virus that causes acute central nervous system disease in humans and animals – rabies
8. Acid sensitive – Rhinovirus
9. Ether sensitive – Herpes virus

**ii. Collection, transport and examination of clinical specimen**

1. CMV isolation is recommended using – Human embryonic fibroblasts
2. Grape-like cluster – Adenovirus

d. Equipment and instrumentation

**i. Manual**

a. How to prepare agar – Add agar to water
b. RPM for centrifugation of bacteria – 3500-5000RPM for 10mins

**ii. Automated**

e. Quality assurance and safety

**i. Collection of specimen**

a. Lyophilization of pure culture – freeze at 20 to 30C
b. Mineral oil – Anaerobes

**ii. Quality control**

a. Setting of rpm marked on the face of the rheostat control on the centrifuge should be checked – monthly
b. Oxidase, Catalase, coagulase – tested each day, when vial is first opened

**iii. Safety – patient/staff**

a. BSC II – Laminar flow
b. Sterilize needles for sputum – Dip in 70% alcohol + sand

**iv. Safety – workplace/environment**

1. AFB is skilled by – Boiling 10mins, Autoclave
2. Autoclave – 121C, 15 psi (lbs/in2), 15mins
3. Not killed by sterilization

**2. Parasitology**

a. Parasites – life cycle, morphological characteristics, epidemiology, prevention and control, manner of reporting, counting

b. Nematodes

1. First stage of nematodes – Rhabditiform
2. Viviparous – Produces larva
3. Oviparous – Produces egg
4. Parasite most prevalent in orphanage – Unholy Three
5. Larvae that passes through the lungs – Ascaris, stronglyloides, Hookworm
6. Roundworm that inhabits the small intestine and is usually demonstrates as rha bditiform larvae in fecal specimen – Threadworm
7. Ascaris egg lacking its mamillated coat – Decorticated
8. A. lumbricoides vector – Cockroach
9. Resembles Trichiuris – C. philippinensis
10. S. stercoralis – chinese lantern
11. Adult trichinella – Intestine
12. Unsheathed microfilariae – O. volvulus
13. Longest nematode – D. medinensis
14. Internal autoinfection – S. stercoralis
15. External autoinfection – E. vermicularis

**ii. Trematodes**

1. 1st IH of flukes – Snail
2. 2nd IH of P. westermani – Fresh water crabs
3. 2nd IH of Echinostoma – Snail
4. 2nd IH of fasciola/fasciolopsis – aquatic vegetation
5. Parasite found in sheep/cattle, not common in PH – F. hepatica
6. Eggs with abopercular thickening – P. westermani
7. Small lateral spine – S. japonicum
8. Prominent lateral spine – S. mansoni
9. Terminal spine – S. haemotabium
10. Schistosomule – cercaria minus tail
11. Swimmers itch – Schistosoma
12. C. sinensis – old fashioned light bulb
13. Mode of transmission of Clonorchis – Ingestion of metacercaria

**iii. Cestodes**

1. Head of tape worm – scolex
2. Body of tapeworm – strobila
3. Finger like uterine branches – T. solium
4. Tree like uterine branches – T. saginata
5. 3rd Taenia specie – Taenia Asiatica
6. Hexacanth embryo in a radially striated shell – Taenia
7. Hexacanth embryo that lacks polar filaments – H. diminuta
8. Egg of D. latum – Operculated
9. 1st IH of D. latum – operaculated
10. 2nd IH of D. latum – Fresh water fish
11. Spirometra – may resemble D. latum
12. Found in IH of E.granulosus – Hydatid cyst
13. Double-pored tapeworm – D. caninum

**iv. protozoa**

1. Motile, reproducing, feeding stage – Trophozoite
2. Organ most often involved in extraintestinal amoebiasis – Liver
3. E. histolytica – Ingest RBC
4. Differentiates hartmanni and histolytica – Size
5. E. gingivalis – Ingests WBC
6. E. nana – Cross-eyed cyst
7. Often mistaken for cyst of amoeba – B. hominis
8. Largest intestinal protozoa – B. coli
9. Undulating membrane – Trichomonas, trypanosoma
10. Intestinal flagellate is described as – Pear shaped
11. T. vaginalis – jerking, tumbling motility
12. Pingpong disease – T. vaginalis
13. Vector of African sleeping sickness – glossina species
14. DH for plasmodium species – female anopheles' mosquito
15. Principal vector for malaria – Flavirostris
16. Plosmodium species that can cause relapse – P. vicax, P. ovale
17. Not recommended for venipuncture – malaria, babesia, hemoflagellates
18. Blood specimen preferred for protozoa – finger puncture
19. 90% cases of malaria caused by – P. vivax and falciparum
20. Toxoplasma gondii – cat

**v. Ectoparasites**

1. Crabs – Ectoparasites

c. Parasitologic Technique

**i. Routine**

1. Iodine – Destroys trophozoites
2. Stain to demonstrate uterine arrangement of Taenia species – India ink
3. Chromatoid bodies on Trichrome stain is colored as – Bright to red
4. Stain for Naegleria, Acanthamoeba – H&E, wrights
5. To detect stippling, prepare blood films – 30 mins to 1 hour
6. Reagent for kato-thick smear – Malachite green, glcerine, cellophane

**ii. Concentration**

1. Zinc sulfate specific gravity – 1.18
2. Flotation techniques – Operculated eggs and eggs with spines not recovered

**iii. Others**

1. Sheathers sugar flotation – cryptosporidium
2. Baermann funnel – strongyloides

d. Quality assurance

**i. Collection and preservation of specimen**

a. Stool for more than 1hr is stored at – Refrigerator

b. Stool preservative – polyvinyl alcohol, schaudinn