Bacteriology

S.No.	Topic Names	Date	Signature
	Bacteriology		
1.	Staphylococcus Aureus.	10 Sep. 18	
2.	Streptococcus pyogens.	23 Sep. 18	
3.	Pneumococcus	27 Sep. 18	
4.	Corynebacteriea	4 Oct. 18	
5.	Enterobacteriace (E.coli).	7 Oct. 18	
6.	Salmonella.	10 Oct. 18	
7.	Pseudomonas.	15 Oct. 18	
8.	Vibrio.	20 Oct. 18	
9.	Mycobacterium Tuberculosis.	23 Oct. 18	
10.	Clostridium.	12 Nov. 18	
11.	Treponema Pallidum.	18 Nov. 18	
12.	Hemophillus Influenzae	08 Jan 19	
13.	Rickettsia.	04 Jan 19	
14.	Actinomycetes.	05 Jan. 19	
15.	Nocardia.	06 Jan. 19	
16.	Listeria	10 Jan. 19	
17.	Mycoplasma.	12 Jan. 19	
18.	chlamydiae	17 Jan. 19	
19.	Neisseria	15 Jan. 19	
20.	Bacillus Anthrax		
21.	Yersinia		

Paracytology -

1. leishmania donovani
2. Echinococcus granulosus
3. Wuchereria Bancrofti

Mycology -

1. candida
2. Histoplasma
3. Dermatophytes

Staphylococcus Aureus

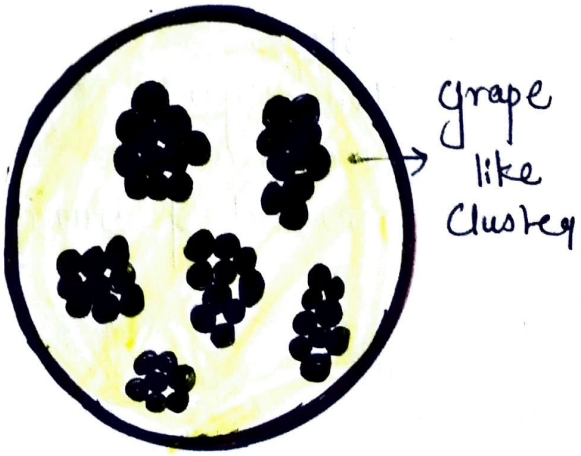
- Introduction
- Morphology
- Pathogenesis
- Laboratory Diagnosis
  - Specimen collection
  - Transportation
  - Direct Microscopy
  - culture characteristics
  - Biochemical Test / Reaction
  - Treatment
  - Prevention / Prophylaxis

Introduction :-

Staphylococci are gram positive cocci arranged in grape-like clusters. They are the commonest cause of suppuration. The genus staphylococcus contains various species but the medically important species are staphylococcus aureus, staphylococcus epidermidis, staphylococcus saprophyticus.

Morphology :-

- Gram positive cocci
- Size - 1 µm



- Shape : grape like clusters

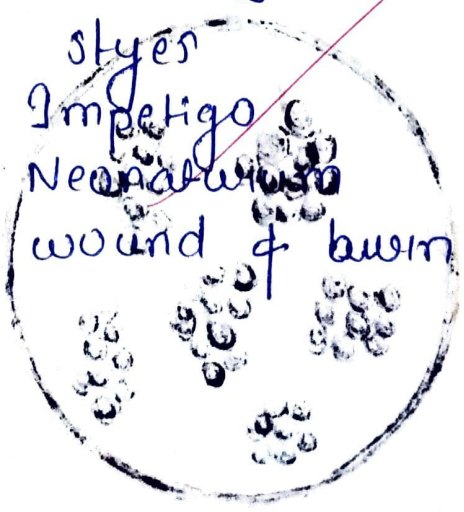
- Non-motile
- Non-sporing
- capsulated

Pathogenesis :-

Staphylococcus aureus is an important pathogenic organism and lessions are localised in nature in contrast to streptococcal lessions which are spreading in nature. Thick creamy pus is formed in staphylococcal infections. Staphylococcal disease may be classified as cutaneous and deep infections food poisioning, Nosocomial infection skin exfoliative disease and toxic shock syndrome (TSS).

→ cutaneous infections :- Superficial infections include —

- pustules
- Boils
- carbuncles
- abscesses
- styes
- Impetigo
- Neonatorum
- wound & burn infections.



↗ Deep / systemic infections :- This include -

- osteomyelitis
- Tonsilitis
- pharyngitis
- sinusitis
- Pneumonitis
- Endocarditis
- Meningitis
- Bacteimia
- Spticaemia
- pyaemia

↗ Food Poisioning :- staphylococcal food poisioning may be follow 2-6 hour after the ingestion of contaminated food which contains performed entrotoxin of staphylococcus Aureus.

↗ Nosocomical Infection (HAI - Hospital Acquired Infection)

- They are important cause of hospital Acquired infection.

↗ Skin Exfoliative disease — This disease are produced by the strain of staphylococcus Aureus that produce exfoliative toxins, stripping of the superficial layer of the skin from the under lying tissue occurs in the various exfoliative syndrome caused by staphylococci (Bullous, impetigo.

pemphigus neonatorum, Ritter's disease). Staphylococcal scalded skin syndrome (SSSS) is one example of exfoliative disease in which toxin spread systemetically.

Toxic Shock Syndrome (TSS) :-

It is caused by toxic shock syndrome toxin (TSST-1) Although TSS become widely known as an association with the use of tempons by menstwiting womens it occuis in other situation also

Laboratory Diagnosis :-

Specimens -

These are collected according to the nature of lessions as follows -

Pus - Superative lessions
Sputum - Respiratory infections
Blood - Bacterimia, sepHicimia
Urine - urinary track infection
CSF - Meningitis
Feeess - food poisioning
Food or Vomet - food poisioning

Collection and Transport :-

- Specimen should be collected in sterile containers under or aseptic conditions.
- In case of urine, midstrain urine should be collected.
- Blood should be collected in blood culture bottles comprising of glucose broath and taurocholate broth.
- Specimens should be transportate immediately to the laboratory and processed.

Direct Microscopy :-

- **Gram staining** Direct microscopy with gram stained smears of pus or wound exudate is useful, where gram positive cocci in clusters may be seen. This is of no value for specimens where mixed bacterial flora are normally present e.g. - sputum.

Culture :-

They grow readily or ordinary culture media within a temprature range of 10-42°C The optimal temprature being 37°C and pH 7.4 - 7.6. They are arobes and facultative anaerobes.

[1] Nutrient Agar :-

After overnight or 24 hours incubation, the colonies are 2-4 mm in diameter, circular, smooth, convex, opaque and easily emulsifiable. Most of the strains produce golden yellow pigment. The pigment is not diffusable into the medium.

[2] Blood Agar :-

colonies are similar to those on nutrient agar and in addition a beta type of haemolysis is seen.

[3] MacConkey's Agar :-

colonies are very small and pink due to lactose fermentation.

[4] Maunitol Salt Agar :-

This is both a selective and an ~~ind~~ indicator medium. It contains nutrient Agar with 1% mannitol, 7.5% sodium cloride and phenol red as indicator, yellow coloured colonies are seen on this medium due to fermentation of mannitol by most strains of Staphylococcus aureus.

[5] Liquid Medium :-

uniform turbidity is produced in peptone water or nutrient broth.

* Biochemical Reaction :-

Staphylococcus Aureus is catalase positive (unlike streptococci) and oxidase negative. It ferments a number of sugars producing acid without gas.

The following characteristics help to distinguish a pathogenic strain of staphylococcus from other non-pathogenic strains.

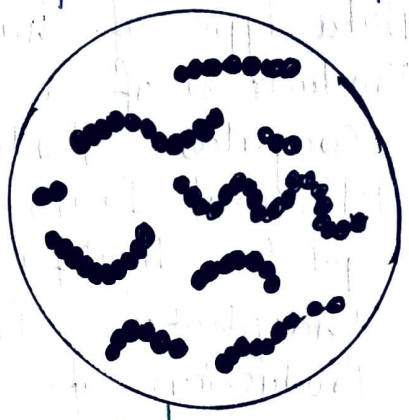
1. Beta type of haemolysis on blood Agar
2. Production of a golden yellow pigment
3. Coagulase Production
4. Mannitol fermentation
5. Gelatin liquefaction
6. Phasphatase Production
7. Production of enzyme dioxyribonuclease
8. Tellurite reduction.

"Streptococcus Pyogens"

Morphology :- The individual cocci are spherical or oval 0.5 to 1 mm in diameter and are arranged in chains.

Chains formation is due to successive cells division occuring in one plane only and daughter cells failing to seprate completely.

→ Capsulated
→ Gram positive
→ Non-motile
→ Non-sporing
→ Arranged in chain shape



Some stains of streptococcus pyogens have capsules composed of hyaluranic acid.

Pathogenesis :- streptococcus pyogens produce pyogenic infection with a tendancy of to spread locally know seprative lessions of local infections include acute glomerulonephritis rheumatic fever.

→ **Pyogenic Infection :-**

[I] Respiratory Infection - Sore throat (acute tonsillities and pharygitis) is the most common of straph. coccus lessions.

[II] Scarlet fever – It consist of a combination of sore throat and generalised erythematous rash. It is caused by a strain producing the erythrogenic toxin.

[III] Skin Infection – Streptococcus pyogenic causes suppurative infections of the skin with a predilection to produce lymphangities and cellulities.

The two typical streptococcal skin infection are impetigo and erysipelas.

These skin infection are the main cause leading to acute glomerulonephritis in childrens in the tropics.

→ Other pyogenic Infections :-

- Puerperal Sepsis – Streptococcus pyogens was an important cause of puerperal sepsis is used to take a heavy tool of before antibiotics become available.
- Sepsis – Infections of skin lessions, wound and burn.
- Pyaemia, Septicaemia, abscess in internal organs (brain, lung, liver & kidney)

- Non - suppurative complications :-

Streptococcus pyogens infections are sometime followed by two important non-suppurative ~~sequelae~~, ~~acute~~ lessions.

→ Rheumatic fever
→ Acute glomerulonephritis

Rheumatic fever is often preceded by sore throat while acute glomerulonephritis by the skin infection.

Laboratory Diagnosis :-

Diagnosis of acute suppurative infections is made by culture, while in the non-supurative complications, diagnosis is mainly based on the demonstration of antibodies.

Specimens :

Specimen is collected according to the site of lessions such as swab, pus, blood or CSF.

Collection and Transportation :

Specimen should be collected in sterile containers under all aseptic conditions. These should be plated immidiately or sent to the laboratory in Pike's

transport medium (1 in 1,000,00 crystal violet and 1 in 16,000 sodium azide).

Direct Microscopy:

- **Gram Staining of smears:** Gram positive cocci in chains is indicative of streptococcal infection. Smears are of no value where streptococci are present as part of the resident flora such as in infections of the throat and genital area.

- **culture:-** The specimen is inoculated on blood agar medium and incubated at 37°C for 18-24 hours.

They are aerobes and facultative anaerobes, growing best at a temprature of 37°C (range 22-42°C). These are most exacting in nutritive requirements, growth occuring in media containing blood, serum or sugars.

- **Blood Agar** - After overnight incubation, the colonies are small (0.5 - 1.0 mm pin point) circular, semitransparent, low convex with a wide zone of β-heamolysis around them.

Growth and heamolysis are promoted by presence of 10% CO_2 in the environment.

↗ selective Media :-

selective media containing 1:500,000 crystal violet (crystal violet blood Agar) permit growth of streptococci but inhibit another bacteria specially staphylococci

↗ Liquid Media :-

In liquid media, such as glucose broth, growth occurs as a granular turbidity with a powdery deposit. Bacterial chains being heavier settle down as deposit.

△ Biochemical Reactions :-

→ catalase Negative (-ve)

△ Toxin and Enzyme :-

↗ Toxin :-

(1) Haemolysins :- streptococci produce two type of haemolysins -

- Streptolysin 'O'
- Streptolysin 'S'

- Streptolysin 'O' :- streptolysin is 'O' is oxygen labile. It is inactivated in the presence of oxygen. It lyses red cell and is also cytotoxic for neutrophills, platlets and cardiac tissue.

- Streptolysin "S" is an oxygen stable heamolysin and is responsible for the haemolysis.

(ii) Pyrogenic erotoxin (erythrogenic toxin) -

This toxin is responsible for the rash of scarlet fever.

Enzymes :-

(i) streptokinase (fibrinolysin)
(ii) dioxyribonucleases (streptodornase)
(iii) Nicotinamide adenine dinucleotidase (NADase)
(iv) Hyaluronidase

Treatment :-

pencillin G is the drug of chaice.

→ Non supperative complication :-

ASO - Anti streptolysin :- O' Ag
aso test is a neutrilisation reaction where austibodies to streptolysin 'O' ASO are neutrilized with streptolysin O Ag. ASO title is usually found in high levels in rematic fever eatin glomeruloriphritis.

"Pneumococcus"

→ Introduction
→ Morphology
→ Pathogenesis
→ Laboratory Diagnosis
→ Biochemical Reactions
→ Treatment.

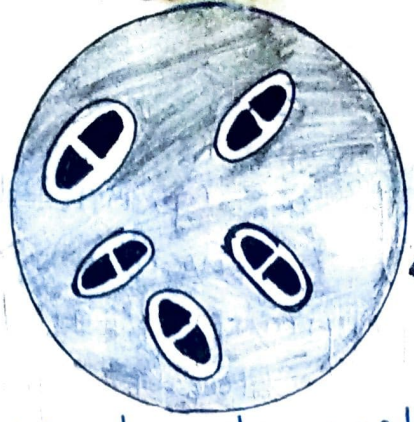
Introduction :-

Pneumococcus are normal commensals flora of the upper respiratory tract. They are important pathogens of pneumonia and otitis media in children.

→ They are reclassified as streptococcus pneumonia.
→ They differ from streptococci in their morphology (diplococci)

Morphology :-

Pneumococcus are gram +ve, small 1 µm in diameter slightly ~~elog~~ elongated cocci arranged in pairs (diplococci) with the broad ends in opposition each cocci has 1 end broad rounded and other pointed (flame shape or lanceolate appearance) They are capsulated and the capsule encloses each pair they are non-motile and non-sporing.



The capsule may be demonstrated in Indian Ink Preparation.

Pathogenesis :-

Streptococcus pneumoniae is one of the most common bacteria causing pneumonia. both lobar and bronchopneumonia.

Streptococcus pneumoniae is the second most important cause of pyogenic meningitis after N. Meningitis (Neisseria).

This disease is more common in children, pneumococcus may be also produced pericarditis, otitis media, empyema, sinusitis conjuctivitis, peritonitis and suppurative arthritis usually as complications of pneumonia.

Laboratory Diagnosis :-

➢ Specimens :- sputum, CSF, Pleural exudate or blood are collected according to the size of lession.

➢ Direct Microscopy :- Gram staining of smear reveals a large no. of polymers, polymorph and typical organism. pneumococcus show bluish purple color after gram staining.

capsule polysaccaride Ag can be demonstrated by counter immuno-electrophoresis. Ag May also be detected by latex Agglutination or co-Agglutination.

Capsule may be demonstrated by Indian Ink Preparation.

➢ culture :- They are aerobes and facultative anaerobes + their growth is improved by 5-10% CO_2. The optimum temprature for growth is 37°C (Range - 25-42°C) and pH 7.8 (6.5-8.3).

→ Blood Agar :- on blood Agar after incubation for 18 hours the colonies are usually small 0.5-1 mm dome shaped with an area of greenish discolouration (α- Hemolysis) around them

on prolonged incubation the colonies become flat, with raised edge and central umbonation (due to autolysis occuring at center).

which creates a draughtsmen appearance.

→ Liquid Media :- In liquid medium such as glucose broth pneumococci produce uniform turbidity.

Biochemical Reactions :-

Pneumococci ferments several sugars with production of acid only.

→ Fermentation is tested in Hiss's serum water.
→ ferment insulin
→ Pneumococci are soluble in bile
→ optochin sensitivity test positive in pneumococcus
→ catalase oxidase negative

Treatment → The antibiotic of choice is parental penicillin, cephalosporin is indicated in case of penicillin resistant strains.

" Bile solubility Test "

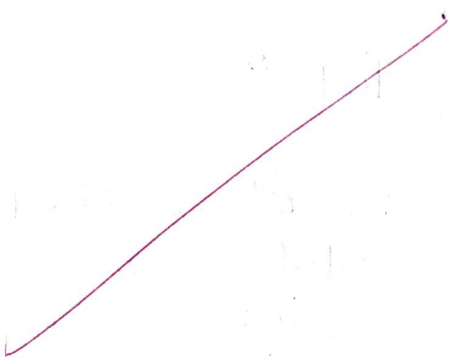
Pneumococci are soluble in bile. when 2% sodium deoxycholate solution is added to a broth culture, the culture clears due to the lysis of the cocci. Alternatively if a loopful of 10% sodium deoxycholate

solution is placed on a pneumococcus colony, lysis of colony occurs within a few minutes. Bile solubility test is an important diagnostic test to differentiate pneumococcus from other streptococci.

"Optochin Sensitivity Test"

pneumococci are delicate organism and are destroyed at 52°C for 15 minutes. They are more sensitive to usual antiseptics.

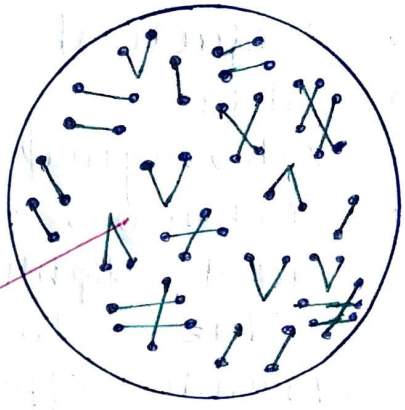
pneumococci are sensitive to optochin in a concentration of 1/500,000. When an optochin disc (5 µg) is applied on a blood agar plate inoculated with pneumo-cocci, a wide zone (14 mm or more) of inhibition occurs on incubation. This is very useful test to differentiate pneumococci from other streptococci which do not show zone of inhibition by optochin disc.



Corynebacteria

[corynebacterium diphtheriae]

Morphology :-



- Gram (+ve) positive bacilli
- Thin, slender
- Measure 3-6 µm X 0.6-0.8 µm
- Pleomorphic
- Club-shaped
- Chinese letter / cuneiform pattern
- Non-capsulated
- Non-acid-fast
- Non-motile

The bacilli look green and metachromatic granules appears bluish black when Albert stain is used.

Pathogenesis :-

diphtheria is most commonly seen in childrens of 2-10 years infection is confirmed to humans only the incubation period is 3-4 days. but may on occasion be as short as one day.

Infection occurs by way of droplet spray. diphtheria may be following clinical types depending upon the site of infection.

Corynebacteria

→ faucial
→ laryngeal
→ Nasal
→ conjuctival
→ otitic
→ vulvovaginal
→ cutaneous mainly around mouth and nose. facial diphtheria is the commonest type. The toxin has both local as well as systemic effects.

Laboratory Diagnosis :-

Specimen :-
→ scrapping of pseudomembrane
→ conjuctival fluid
→ sputum
→ Nassal discharge
→ swab from mouth

Laboratory diagnosis consist of molation of organism & demonstration of its toxicity by virulence test.

Isolation of Organism :-

→ collection of specimen two swabs from the lesions (throat, Nose, larynx, ear, conjuctiva, vagina or skin) are collected

one swab is used for smear examination and other for culture.

→ Swabs are collected prior to start of antibiotics and applications of antiseptic in form of gargles.

Direct Microscopy :- smear are stain with both gram & albert stain. Diphtheria bacilli show beaded slender green rods in typical chinese letter pattern on albert staining.

→ **Culture characteris :-**

C-diphtheriae are grown based on media enriched with blood serum or egg. growth is scanty on ordinary media. They are aerobic & facultative anaerobe optimum temprature - 37°C (Range 15-40°C) pH - 7.2

> **Hiss's serum Water :-** This is liquid medium containing serum growth is seen as a turbidity and pelicle formation.

> **Loffler's serum slope :-** diphtheria bacilli grow on this medium very rapidly, colonies appear after 6-8 hours of incubation, long before other bacteria grow the colonies are

circular white or creamy glistening.

➢ Tellurite Blood Agar Medium :-

It contains potassium tellurite (0.02%) which inhibits most other bacteria & act as a selective agent the organism grow slowly on this medium & form grey or black colour colonies due to reduction of pottassium tellurite to tellurium.

➢ Biochemical Test :-

— Hiss's serum water is used for testing fermentation of carbohydrates.

— They ferment glucose & maltose with the production of acid but without gas.

➢ Virulence Test :→ Subcutaneous test
→ Intracutaneous test

"Subcutaneous Test"

→ The growth from an overnight culture on Loeffler's serum tope is emulsified in 2.5 ml of growth & 0.8 ml of this emulsion is injected subcutaneously in two guinea pigs.

→ one of which has recieved an intramuscular injection of 500 units of diphtheria antitoxin 18 - 24 hours previously. (This protected animal act as a control)

→ If the strain is virulent, the unprotected animal will die within 2-3 days with evidance of hemorrhage in the adrenal gland which is pathogenic fever.

"Intracutaneous Test"

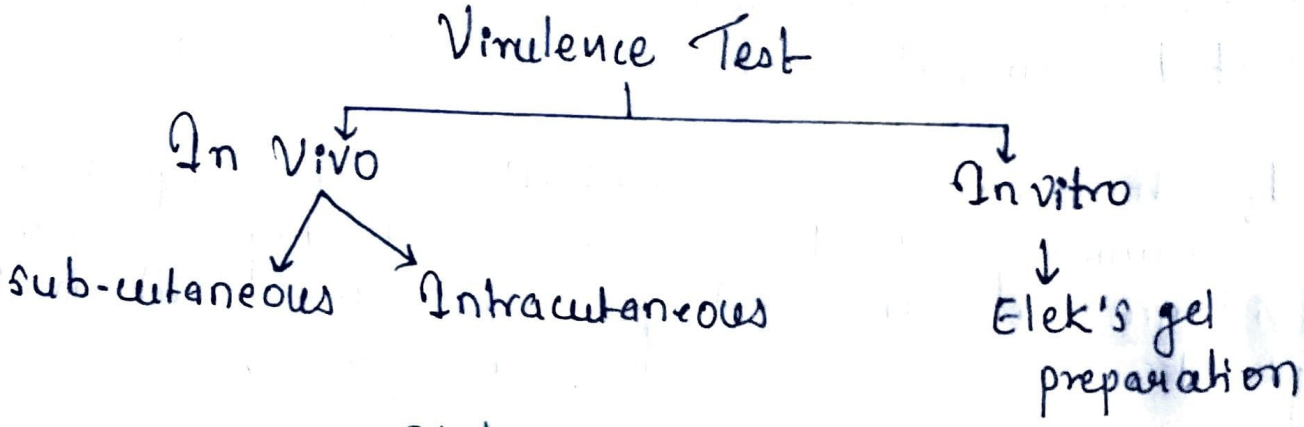
→ Two guinea pigs (or rabbits) are injected intracutaneously with 0.1 ml emulsion from growth on loeffler's serum slope.

→ one of these animals is protected with 500 unit antitoxin the previous day (control) and the other is given 50 unit of antitoxin intraperitonealy 4 hours after the skin test, in order to prevent death.

→ If the strain is toxigenic the inflammatory reaction at the site of injection progress to necrosis in 48-72 hours in the test animal but there is no change in the controll animal.

→ An advantage in the intracutaneous test is that 8-10 strains can be tested at a time

on a pair of animals and the animal do not die.

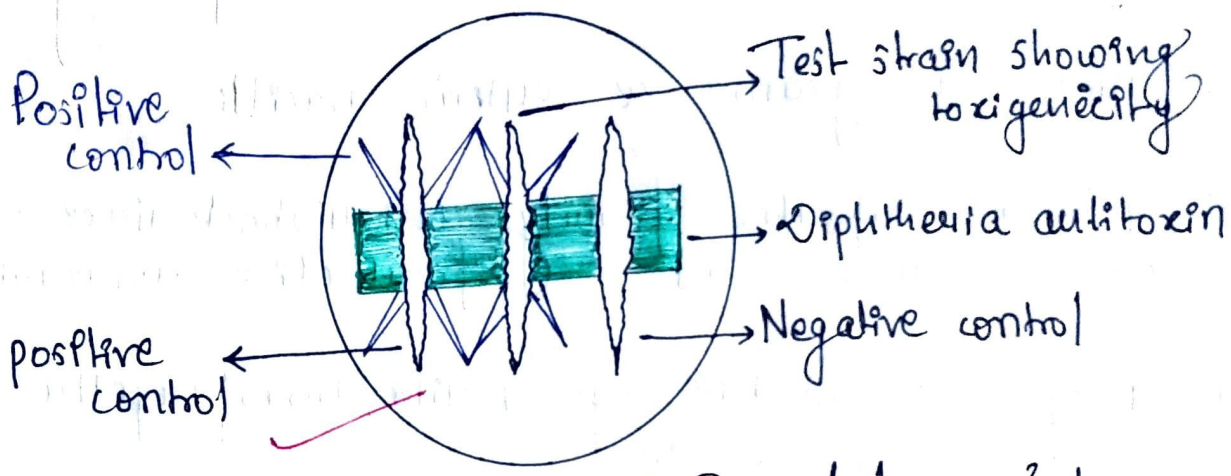


" Elek's Gel Precipitation "

- This is an immunodiffusion test
- A rectangular strip of filter paper soaked in diphtheria antitoxin (1000 unit/ml) is placed on the surface of a 2% horse serum agar plate while the medium is still fluid.
- When the Agar solidifies, the test strain is streaked at right angle to the filter paper strip.
- The positive and negative controls are also put up.
- The plate is incubated at 37°C for 24 - 48 hours.
- The toxin produced by the bacterial growth diffuse in the agar to produce a line

of precipitation where it meets the antitoxin at optimum ~~temperature~~ concentration.

→ Non-toxigenic strain will not produce any precipitation line.



Elek's gel Precipitation Test

"Enteriobacteriaceae"

- Shalmonella
- shigella
- Proteus
- E. coli
- klebsilla

Features of enteriobacteriaceae family :-

(i) They are gram -ve enteric bacilli

(ii) Member of the family enteriobacteriaceae are aerobic and facultative anaerobic.

(iii) They are motile by peritrichous flagella or are non-motile.

(iv) They grow on ordinary media ferment glucose with the production of acid or gas.

(v) They reduce nitrates to nitrites.

(vi) They are oxidase negative and catalase positive except shigella dysentriae.

E. Coli

Morphology :

- E. coli is gram -ve bacilli.
- Measuring 1-3 μm X 0.4-0.7 μm
- Most strains are motile
- It is non-sporing & non-capsulated

Pathogensis :- E. coli forms apart of normal intestinal flora of the men & animals.

There are four major type of clinical syndrome which are caused by E. coli.

(i) Urinary Tract Infection :- E. coli is the commonest organism responsible for the UTI. E. coli is that causes UTI often originates in the intestine of the patient.

(ii) Diarrhoea :- E. coli causing diarrhoeal disease are of four groups. They produces diarrhoea with different pathogenic mechanism.

- Diarrhoea
  - (EPEC) entero-pathogenic E. coli
  - (ETEC) entero-toxigenic E. coli
  - (EIEC) entero-invasive E. coli
  - (EHEC) entero-Heamorrhagic E. coli

enterocyte - intestinal lining

→ **Enteropathogenic E. coli**

EPEC adhere tightly to enterocytes leading to inflammatory reactions + epithelial degenerative changes.

→ **Enterotoxigenic E. coli**

These are the strains that form Heat labile entrotoxin (LT) or a heat stable entrotoxin or both.

They are now known to be a major cause of diarrhoe in children in developing countries and are the most important cause of travellers diarrhoe.

→ **Enteroinvasive E. coli**

Some strains of E. coli invade the intestinal epithelial cells as do dysentry bacilli and produce disease identical to shigella dysentry.

These have been named enteroinvasive E. coli (EIEC) cause keratoconjunctivitis the diagnostic test for EIEC is called Sereny Test.

→ **Enterohaemorrhagic E. coli or verocytotoxin producing E. coli (VTEC) :-**

These strains cause heamorrhagic colitis (HC) and heamolytic uraemic syndrome (HUS) Toxin responsible is called 'verotoxin' because of its effect on vero cells in culture.

(iii) Pyogenic Infections :-

E. coli may cause wound infection, peritonsitis cholecystitis and neonatal meningitis. It is an important cause of neondtal meningitis.

(iv) Septicaemia :-

E. coli is very common cause of septicemia in many hospitals. This condition usually occurs in debiliated patients and mortality is very high.

▷ Laboratory Diagnosis :-

→ Specimen :- urine
stool
pus
Blood
CSF

* Catheter Specimen :- urine should be collected directly from the catheter and not from the collection bag.

The catheter should not touch the container.

→ Transport :- As urine is a good culture medium, specimens after collection should reach the laboratory with minimum delay, if it is not possible, the specimen is to be refrigerated at 4°C.

Direct Microscopy :- urine is centrifuged and deposit is examined under the microscope for detecting pus cells, RBC, epithelial cells and bacteria.

'Gram staining'.

Culture characteristics :- It is an aerobe and facultative anaerobe and grow on ordinary culture medium at optimum temprature of 37°C (Range - 10 to 40°C) in 18 to 24 hours.

→ MacConkey Agar - on macConkey agar medium the colonies are pink colour due to lactos fermentation.

→ Nutrient Agar - After overnight incubation the colonies are on nutrient agar circular, moist, smooth with entire margin and non-mucoid unlike colonies are klebsiella which are mucoid.

→ Liquid Media - In liquid medium growth occurs as a uniform turbidity.

Biochemical Test :-

- They ferments most of the sugars (glucose, lactose, maltose, mannitol) with production acid and gas.

- Indole Positive
- MR - Methyl Red ~~positive~~ ~~Negative~~ (+ve)
- VP ~~Negative~~ ~~positive~~ (-ve) Voges Proskauer
- Citrate - Negative
- Urea - Negative

"Salmonella"

Introduction :- The salmonellae are primarily intestinal parasite of vertebrates and which infect man, leading to enteric fever, gastroenteritis and septicaemia.

Morphology :- Salmonellae are gram negative bacilli measuring 1-3 µm x 0.5 µm. They are motile, non-sporing and non-capsulated. Motality is due to the presence of peritrichous flagella except S. gallinarum and S. pullorum which are non-motile.

Pathogenesis :-

S. typhi, S. paratyphi A and usually S. paratyphi B are confined to human beings.

Salmonellae cause three types of clinical syndrome in human beings - enteric fever
septicaemia
gastroenteritis.

→ **Enteric fever** :-

The term enteric fever includes typhoid fever (S. typhi) and paratyphoid fever (S. paratyphi A, B, C). Infections due to S. typhi and S. paratyphi A are prevalent in India.

(i) Typhoid fever :- The infection is acquired by ingestion through contaminated food and water. The incubation period is usually 7-14 days. The clinical course may vary from a mild pyrexia to a fatal fulminating disease. The characteristic features are hepatosplenomegaly, step-ladder pyrexia with relative bradycardia and leucopaenia, skin rashes known as rose-spots may appear during the second or third week.

(ii) Paratyphoid fever :- paratyphoid fever resembles typhoid fever but is milder. S. paratyphi A, B and C cause paratyphoid fever.

→ Septicaemia :- Salmonella septicaemia is commonly caused by S. Cholerae-suis or S. paratyphi C and occasionally by other Salmonellae.

→ Gastroenteritis :- Salmonella gastroenteritis or food poisioning is caused by ingestion of food like meat, milk, egg contaminated by certain Salmonellae which are primarily animal pathogens.

The incubation period is 12 to 24 hours.

Laboratory Diagnosis :-

Bacteriological diagnosis of enteric fever consists of -
1. Isolation of bacilli
2. Demonstration of antibodies

⇒ Isolation of Bacilli -

This may be done by culture of specimens like blood, faeces, urine, aspirated duodenal fluid etc.

Relevance of examination of different specimens at different phases of enteric fever

Duration of disease	Specimen examination	% positivity
1st Week	Blood culture	90
2nd Week	Blood culture Faeces culture Widal ~~culture~~ test	75 50 100 titre
3rd Week	Widal test Blood culture Faeces culture	80-100 60 80

⇒ Culture characteristics :-

Salmonella grow on ordinary culture media at optimum temprature of 37°C (Range -

15-41°C) pH 6-8 and are aerobic & facultative anaerobic.

▷ Nutrient Agar :- They are produce colonies on n/A 2-3 mm in diameter circular, translucent, low convex & smooth.

▷ MacConkey / DCA (Deoxycholate citrate Agar) :-

on macConkey's agar & DCA colonies are colourless due to non-lactose fermentation (NLF).

▷ Wilson-Blair bismuth sulphite Medium :-

Jet-black colonies with metallic sheen are form due to formation of hydrogen sulfide. S. paratyphi A and other species which do not form H_2S produce green colonies.

▷ Xylose lysin deoxycholate Agar (XLD) :-

XLD Agar is another medium used for isolation of these organism. most strains of salmonella produce red colonies with black centre.

▷ Selenite F Broth and tetrathionate Broth (TTB)

Selenite F Broth and TTB are commonly used enrichment media for inoculation of specimen specially faeces.

> Biochemical Reactions :- Salmonellae are Catalase ~~negative~~ +ve, oxidase negative, nitrate reduction positive and ferment glucose, mannitol but not lactose or sucrose. S. typhi ferments glucose and mannitol with production of acid only but paratyphoid bacilli (S. paratyphi A, B and C) form acid and gas.

→ Demonstration of Antibodies :-

Widal Test :- It is an agglutination test for detection of agglutinins (H and O) in patients with enteric fever. Salmonella antibodies start appearing in the serum at the end of first week and rise sharply during the third week of enteric fever. Two specimens of sera at an interval of 7 to 10 days are preffered to demonstrate a rising antibody titre.

"Pseudomonas"

Introduction :-

The genus pseudomonas belongs to the family pseudomonadaceae which contains over 200 species. The most important among these Ps. aeruginosa.

"Pseudomonas Aeruginosa"

Morphology :-

It is slender, Gram -ve bacilli, 1.5 - 3 μm X 0.5 μm in diameter, non-capsulated, non-sporing & is actively motile by a polar flagellum.

Pathogenesis :-

- It causes infections more commonly in patients with Neutropenia, cystic fibrosis, burn & those on ventilators.
- It is the most important agent causing nosocomial infections.
- It is due to its resistance to common antibiotics and antiseptics that is established itself widely in hospitals.
- equipments such as respirators and endoscopes articals such as bed pans, antiseptic or

"Pseudomonas"

disinfectant solution may be frequently contaminated

→ Urinary Tract infection
→ Acute Meningitis
→ Post Tracheostomy pulmonary infection
→ Septicaemea
→ Wound & burn infection
→ eye infections
→ Infantile diarrhoea

Laboratory Diagnosis –

Specimen :- Pus
Wound swab
Urine
Sputum
Blood
CSF

Direct Microscopy :-

Gram staining

Culture characteristics – It is a strict aerobe and grows well on ordinary media like nutrient broth and N/A

The optimum temprature for growth is 37°C

→ Nutrient Agar – colonies are smooth, large translucent, low convex, 2-4 mm in diameter. The organism produce a sweetish aromatic ~~and~~ odour. There is greenish blue pigment which diffuses in to the medium.

→ Blood Agar – colony characters are similar to those on nutrient Agar. Many strains are hemolytic on blood Agar.

→ MacConkey Agar – colonies are pale or colourless due to non-lactose fermentation.

→ Cetrimid Agar – It is selective media for Ps. aeruginosa.

→ Peptone Water – It forms a turbidity with a surface pelide. pseudom. Being are strict aerobe tends to collect at the surface for more oxygen hence forming surface pelide.

Pigment Production :- pseudomonas aeruginosa produces a number of pigment which diffuse into surrounding medium.

→ Pyocyanine – It is a bluish green pigment It is not produced by other species of the genus. Hence, it is diagnostic of Ps. aeruginosa.

→ Fluorescin (pyoverdin) – It is a greenish yellow pigment. It may be produced by many other species also.

→ Pyorubin – It is a Redish brown pigment

Biochemical Reactions –

oxidase positive, glucose fermentation with acid only, citrate positive.

→ AST

"Vibrio"

Vibrios are gram negative (-ve) oxidase positive short rigid curved rods that are actively motile by a polar flagellum.

The most important member of the genus is vibrio cholerae.

"Vibriocholerae"

Morphology :-

→ Gram negative
→ curved or comma shaped rod
→ Non - sporing
→ Non - capsulated
→ 1.5X0.2 - 0.4 µm in size
→ motile due to presence of polar flagellum and movement is named 'Darting Motility'.

Pathogenesis :- vibriocholerae both (01 and 0139) causes an acute diarrhoel disease known as cholerae and it occurs only in man.

Source of Infection - The human infections occurs by ingestion of contaminated food and water.

Mechanism of Entrotoxin

- The ingested organism pass through the acid barrier of the stomach and multiply in the alkaline medium of Intestine.
- Vibrio become adherent to the epithelium.
- Then vibrios produces entrotoxin (Its a heat labile, Protein in nature 90,000 molecular weight).

Entrotoxin → A (Active subunits)
→ B (Binding subunits)

- B subunit binds specifically to the GM_1 gangliosides receptors on the intestinal epithelial cells.
- A active subunit the enters the cell and is clared to its fragements → A_1 A_2
- A_2 fragment stimulates adenyl cyclase activity of the epithelial cells.
- This is turn convert ATP into cAMP
- The cAMP concentration within the cells cause -

(i) Hypersecretion of water & electrolytes with in intestinal lumen.
(ii) Inhibition of reabsorption of Na, Cl by cells which result in purging diarrhoea. (Rice water stev.).

Laboratory Diagnosis :-

Specimen – Klatery Blood
Rectal Swab

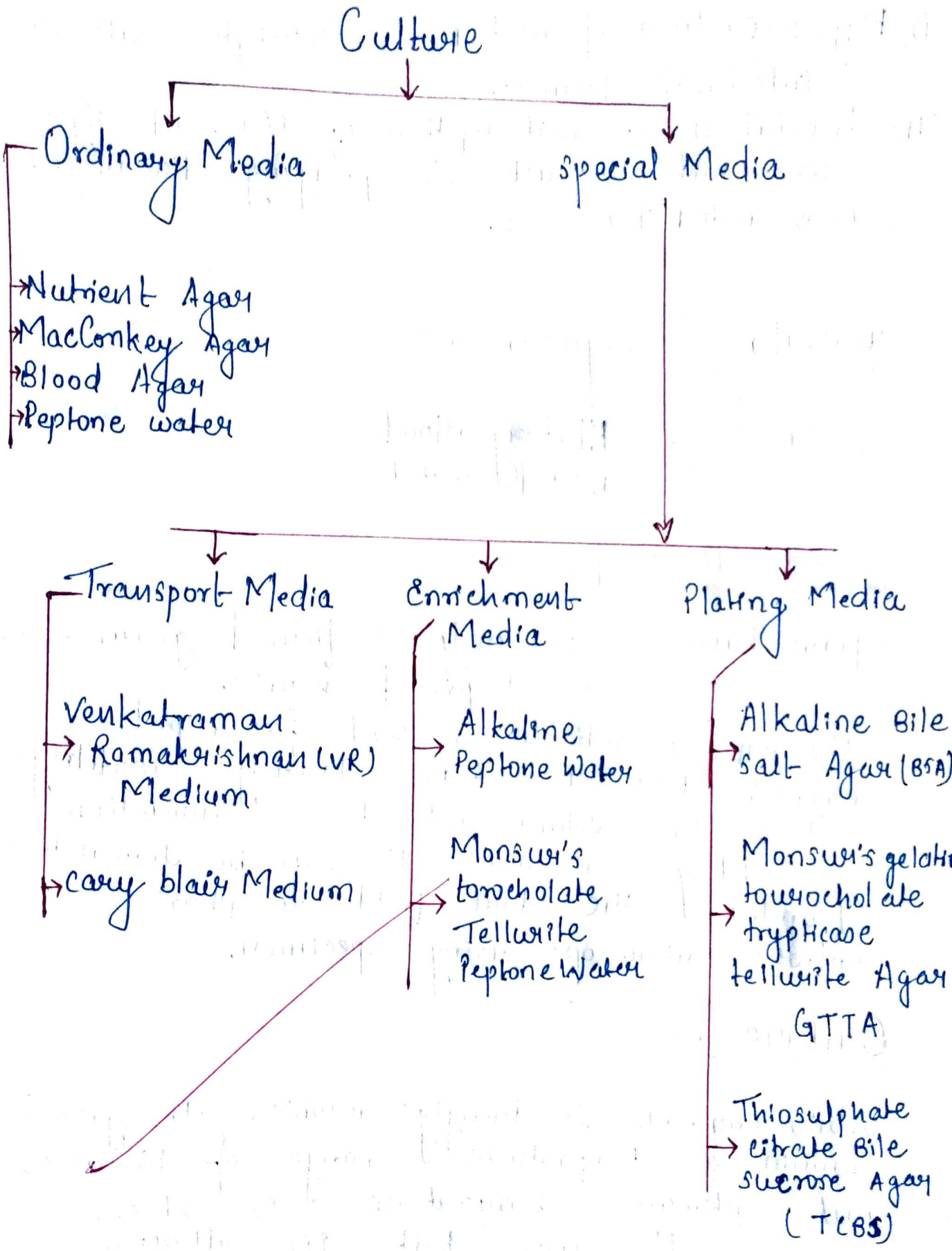
Direct Microscopy –

Gram stain – Its only confirmed gram –ve or shape of vibrio.

Its not a reliable method for rapid diagnosis the characters darting motility of the vibrio and its inhibition by adding antibiotic It can be demonstrated under the dark field or phase contrast microscope using specimen.

Culture :-

Vibrio cholerae is strongly aerobic. It grows within a temprature range of 16-40°C but optimum temprature is 37°C. It grows best in alkaline media. the optimum pH being 8.2 (pH range 7.4 to 9.6)


Culture
Ordinary Media
Special Media
Nutrient Agar
MacConkey Agar
Blood Agar
Peptone water
Transport Media
Enrichment Media
Plating Media
Venkatraman Ramakrishnan (VR) Medium
Cary blair Medium
Alkaline Peptone Water
Monsur's tetracholate Tellurite Peptone Water
Alkaline Bile salt Agar (BSA)
Monsur's gelatin tetrachlorate trypticase tellurite Agar GTTA
Thiosulphate citrate Bile sucrose Agar (TCBS)

Ordinary Media :-

- **Nutrient Agar** – After overnight incubation the colonies are moist, translucent round disk, 1-2 mm in diameter with a bluish ting is transmitted light.
- **MacConkey Agar** – The colonies are colourless or pale at first raddish or pink on prolonged incubation due to late fermentation of lactose.
- **Blood Agar** – vibrio cholerae classical bio type doesn't produce heamolyse some strains produce greenish discolouration(γ) around colonies which later becomes clear due to heamodigestion.
- **Peptone Water** – It grows as a surface pellicle because of its aerobic nature.

Special Media :-

- **Transport or holding Media** –

→ **Venkatraman Radhakrishanan(VR) Media** –

20 gm – salt
5 gm – Peptone
1000 ml – d/w
pH – 8.6-8.8

Vibrios donot multiply but remain viable for several weeks.

→ Cary blair Medium :- This medium is prepared by adding -

- disodium Phosphate
- sodium thioglycollate
- sodium chloride +
- calcium chloride
- pH is adjust at 8.4

If a transport medium is not available, a strip of thick blotting paper is soaked in the feacal matter then placed in a sealed plastic bag and send to the laboratory.

o Enrichment Media :-

→ Alkaline Peptone Water (APW) - It is peptone water at pH 8.6 besides enrichment media, It is also an excellent transport Media.

→ Monsur's tourocholate Tellurite Peptone Water :- It contains peptone sodium chloride sodium tourocholate in 1 litre of distilled water and pH is adjust at 9.2. To this medium sterile pottassium tellurite solution is added like APW its not only a good enrichment media but is transport medium as well.

- Plating Media :-

→ Alkaline Bile Salt Agar (BSA) - pH 8.2

It is modified nutrient agar medium containing 0.5% sodium taurocholate (bile salt). The colonies are similar to those on nutrient Agar.

→ Monsur's gelatin taurocholate tryptcase tellurite Agar (GTTA) -

The colonies are small, translucent with a greyish black centre and a turbid halo around the colonies due to hydrolysis of gelatin. The colonies become 3-4 mm in size after 48 hour incubation.

→ Thiosulphate citrate bile sucrose (TCBS) Agar -

It is the most widely used selective medium for isolation of vibrios. It contain sodium thiosulphate, sodium citrate, bile salts, sucrose bromothymol blue (indicator), yeast extract, peptone, sodium chloride, ferric citrate & water. Vibrio cholerae forms yellow colonies due to sucrose fermentation.

- Biochemical Reactions - carbohydrate breakdown is fermentative, producing acid, but no gas. It is catalase and oxidase positive. It ferment glucose, mannitol, sucrose, maltose and mannose, but not lactose, though lactose may be split very slowly.

→ Indole positive
→ Methyl Red (MR) Negative
→ urease negative
→ Voges-proskauer (VP) reaction and haemolysis of sheep erythrocyte are positive in EL Tor biotype and both these tests are negative in classical biotype.

Kukreni
27 Oct 18

"Mycobacterium Tubeculosis"

Morphology :-

- Mycobacterium Tuberculosis is a slender, straight or slightly curved bacillus with rounded ends, occuring singly, in pair or in small clumps.
- Size :- It measures 1 - 4 μm X 0.2 - 0.8 μm (average 3 μm X 0.3 μm) in size.
- This bacilli are Acid fast, Non-sporing, Non-capsulated & non-motile
- Ziehl-Neilson staining is useful to study the morphology of these organism.
- Tubercle bacilli may also be seen/stain with the flowrocent dye (Auramine O, rhodamine) & appear yellow luminous bacilli under the flowrescent microscope.
- They are gram positive but are difficult to stain with the gram stain.

Pathogenesis -

Source of infection - The infection is commonly acquired by inhilation of infected droplets, coughed or sneezed into

"Mycobacterium Tuberculosis"

the air by a patient with pulmonary Tuberculosis.

- In Bovine Tuberculosis infected cows develope lessions in the udder & bacilli are excreted in the milk which can then infect people who drink it raw.

Tuberculosis may be involve lungs (pulmonary) or sites other then lungs (extra pulmonary)

Tubercle bacilli are engulfed by macrophages but they survive & multiply in the macrophages.

The cell mediate immunity (CMI) plays a major role to intract with these macrophages whereas humoral immunity appears to be irrelevent.

Explore

exposure to source → Aerosolization of droplet nuclei → Inhalation of Bacteria

Bacteria reach lungs enter into macrophages

Bacteria multiply in macrophages

Granulomatous lesions begins to form (caseous necrosis)

Bacteria cease to grow lesion calcifies (95%)

lesion liquefies

Bacteria coughed up in sputum

Immuno suppression

Spread to Blood Organs

Death

Reactivation

Laboratory Diagnosis :-

Specimen - specimen collection depends on the site of involvement. Tuberculosis may involve lungs (pulmonary) specimen is - sputum or sites other then lungs (extra pulmonary).

(i) Pulmonary Tuberculosis - sputum is the most common specimen. It is collected in a clean wide mouthed container.

A morning specimen may be collected on three consecutive days.

If sputum is scanty, A 24 hour specimen may be collected. When sputum is not available laryngeal swab or bronchial washings are collected. In children gastric washing may be examined as they tend to swallow sputum.

(ii) Meningitis - Cerebrospinal fluid (CSF) from tuberculous meningitis (TBM) often forms a spider web clot on standing examination of which may be more useful than of fluid.

(iii) Renal Tuberculosis - Three consecutive days morning samples of urine are examined.

(iv) Bone & joints tuberculosis – Aspirated fluid

(v) Tissue – Biopsy of Tissue

Culture Character :- colonies of these organisms appear in about 2 to 8 weeks, since they grow slowly. optimum temprature is 37°C and optimum pH is 6.4 to 7.0.

Mycobacterium Tuberculosis is an obligate aerobe & mycobacterium bovin in microaerophilic (on primary isolation and become aerobic in subculture).

Lowenstein - Jensen :- After incubation on this media mycobacterium forms dry, raised rough, irregular colonies. Initially the color of colonies is milky white and it becomes buff colored or yellowish on further incubation.

Composition - Beaten egg
Melachite green
Asparajine

Petroff's Method - Sputum or any other specimen is incubated with an equal volume of 4% (w/v) sodium hydroxide

at 37°C with constant shaking till it becomes clear (in about 15-20 minute).

→ Sodium hydroxide acts as a decontaminator and as an emulsifier reagent.

→ It is centrifuged at 3000 RPM for 20 minutes supernatant is decanted.

→ The alkaline sediment is first neutralized with an equal quantity of 0.1 N HCl and then used for smear culture + animal inoculation.

→ Neutralization of the sediment should be carried out as early as possible, since mycobacterium are susceptible to prolonged exposure to 2% (v/v) sodium hydroxide.

Clostridium

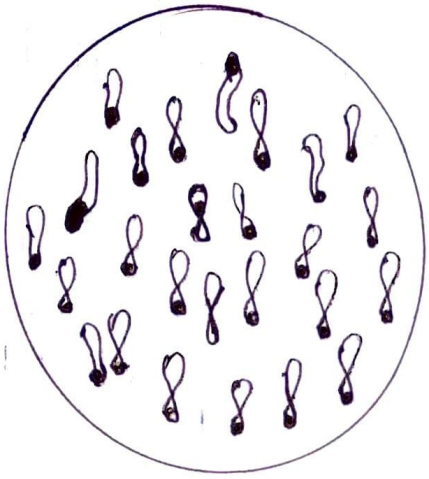
Classification - clostridia of medical importance may be classified on the basis of disease they produce.

	Disease	Bacterial species
A.	Tetanus	Cl. Tetani
B.	Gas gangrene	
	Established	Cl. perfringens
	Pathogens	Cl. septicum
		Cl. Novyi
C.	Food poisoning	
	Gastroenteritis	Cl. perfringens
	Necrotising enteritis	Cl. perfringens
	Botulism	Cl. botulinum
D.	Acute colitis	Cl. difficile

Clostridium Perfringens -

Cl. perfringens is a commensal in the large intestines of man & animals.
The spore are commonly found in soil & dust.

Morphology - It is a large stout, gram positive bacillus measuring 4-6 µm x 1 µm, with subterminal spore. It is capsulated & non-motile.



Pathogenesis - Cl. perfringens is the most important & common aetiological agent of gas gangrene. It also produces food poisioning & nacrotising enteritis in man.

Laboratory Diagnosis - The specimen to be collected are exudates from wound nacrotic tissue & muscle fragments.

Direct Microscopy - Gram stained smears give presumptive diagnosis. large number of gram positive bacilli are seen in the field.

Culture characteristics –

It is anaerobic & grows over a pH range of 5.5 to 8.0 & wide temprature range of 20°C to 50°C the optimum temprature for growth is 37°C.

It grows on blood agar cooked meat broth (CMB) & thioglycollate broth within 24 to 48 hours.

1. Blood Agar – On blood agar colonies of most strains show a target heamolysis resulting from a narrow zone of complete heamolysis caused by theta (θ) toxin & a much wider zone of incomplete heamolysis due to alpha toxin.

2. Robertson cooked meat broth – In CMB are the meat pieces turn pink but are not digested.

Biochemical Reaction –

→ It ferments glucose, lactose, sucrose & maltose with the production of acid & gas.

→ In litmus milk, lactose fermentation leads to formation of acid, which changes the color of litmum from blue to red. The acid coagulates the casein (acid clot) and the clotted milk is disrupted

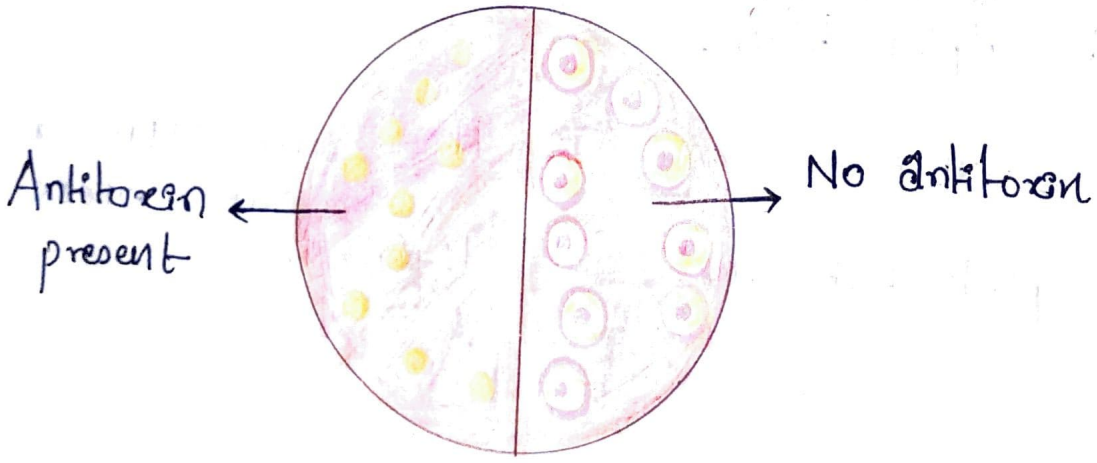
due to vigorous gas production & this is known as stormy fermentation.

→ Clostridium tetani has slight proteolytic but no saccharolytic property.

→ Gelatin liquefaction occurs very slowly.

Nagler Reactions

6%. Agar
5%. Elides peptic digest of sheep blood
20%. human serum
5%. egg yolk



α- toxin (lecithinase c) splits lecithin into phosphoryl choline & a diglyceride (lipid).

"Treponema Pallidum"

Morphology -

(i) It is a thin delicate spirochates with tapering ends having about 10 regular spirals.

(ii) It is about 10 µm long (Range 4-14) 0.1 - 0.2 µm width.

(iii) It is actively motile, showing rotation round the long axis, backward & forward movements & flexion of the whole body.

(iv) It does not take ordinary stain.

(v) It can be identify by -ve stain with India ink. It's morphology & motality of phase contrast microscopy.

(vi) It can be stain with silver impregnation method

(vii) The Treponemes reduce silver nitrate to metallic silver that is deposited on the surface enlarging the diameter of the organism.

Pathogenesis — (i) Natural infection with Treponema pallidum occurs only in human beings. venereal syphilis is acquired by

sexual contact.

(ii) The Treponema enters the body through minute abrasion on the skin or mucosa.

Incubation Period → 10 - 90 days

Stages of Syphilis - Primary syphilis
- Secondary syphilis
- Congenital syphilis

↗ Primary Syphilis : A papule appear on the genital area that ulcerates forming a classical chancre of primary syphilis called hard chancre.

→ It is Painless.

→ The chancre heals within 10-14 days, even without treatment having a thin scars.

↗ Secondary syphilis : After healing of primary lesions, the patient remain Asymptomatic for 4-6 months. then secondary syphilis sets in.

→ It is painful.

→ The secondary lesions are due to wide spread multiplication of the treponems & there disamination through the blood.

→ Congenital syphilis – The Treponems can cross the placenta barrier infection in fetus usually occurs from primary & secondary syphilis of the mother.

Laboratory Diagnosis :

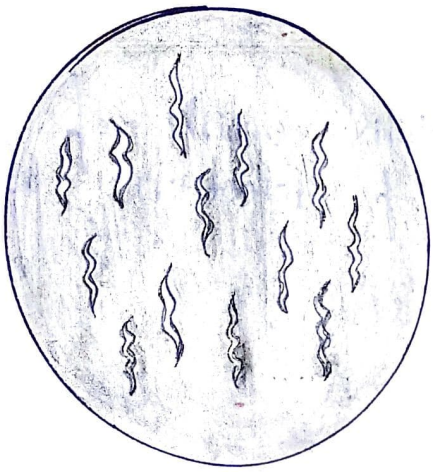
The diagnosis of syphilis consists of demonstration of treponemes & detection of antibodies by serological tests.

Diagnostic method in syphilis

Demonstration of Treponemes	1. Dark ground Microscopy 2. Direct fluorescent Ab staining for T. pallidum T. in tissue. 3. (i) silver impregnation method (ii) immunofluorescence staining
Serological Tests – Non-treponemal tests	1. VDRL 2. RPR
– Treponemal tests	1. FTA, FTA-ABS (using killed T. pallidum) 2. TPHA (using T. pallidum extract) 3. TPI Test

→ Demonstration of Treponemes :-

↗ **Dark ground microscopy** - Treponema pallidum, appears as a slender, spiral organism showing rotational as well as flexion & extension movements.



↗ **Serological Tests** - These tests form the mainstay of laboratory diagnosis. Depending upon the antigen used, serological tests for syphilis are divided into non-treponemal tests & treponemal tests.

Non - Treponemal Test :-

→ In the standard tests for syphilis (STS), reagin antibodies are detected by cardiolipin antigen.

→ cardiolipin antigen is an alcoholic extract of beef heart tissue to which lecithin & cholesterol are added.

→ The STS includes venereal disease research lab. (VDRL) Test, Rapid Plasma Reagin (RPR), kahn test & wassermann reaction.

→ The wassermann reaction is no longer in use. similarly kahn test is rarely done.

(a) VDRL (Venereal Disease research laboratory) Test -

It is the most widely used simple & rapid serological test. It is a slide flocculation test.

(b) RPR (Rapid plasma Reagin) Test -

It is almost similar to VDRL Test. Finely divided carbon particles are added to cardiolipin Ag.

Treponemal Tests :→

Tests using Reitter's Treponemes -

Reiter's protein complement fixation (RPCF) Test - The principle of the test is same as that of wassermann reaction i.e. complement fixation test (CFT).

Tests using T. pallidum (Nichol's strain) -

(i) Using live T. pallidum -

Treponema pallidum immobilisation (TPI) Test - This test employs live T. pallidum. The test serum is mixed

mixed with actively motile Nichol's strain of T. pallidum & incubated anaerobically. If antibodies are present, the treponemes are immobilised, when examined under dark ground illumination.

(ii) Using killed T. pallidum -

(a) Treponema Pallidum Agglutination Test (TPA) -

A suspension of T. pallidum, inactivated by formalin is mixed with the test serum & examined under dark ground microscopy. The treponemes are found agglutinated in the presence of Ab.

(b) Treponema Pallidum immune adherence (TPIA) Test -

A suspension of inactivated T. pallidum is mixed with the test serum, complement & fresh heparinized whole blood from a normal individual & incubated. The Treponemes will be found to adhere to the erythrocytes in the presence of Ab. Both TPA & TPIA are not used in diagnostic laboratories.

(iii) Using an extract of T. pallidum -

Treponema pallidum heamagglutination Assay (TPHA) -

Tanned sheep erythrocytes are sensitised with an extract of T. pallidum. When these sensitised erythrocytes are mixed with patient's serum containing anti-Treponemal Ab. the erythrocytes clump together.

"Haemophillus"

The genus Haemophilus contains non-motile, non-sporing, Gram -ve bacilli and require one or both of two necessary growth factors (X & V) present in blood.

Haemophilus Influenzae

Morphology →
- 1.5 µm X 0.3 µm
- Gram -ve bacilli
- Non-motile
- Non-sporing
- Non-capsulated
- Non-Acid fast
- showing pleomorphism

Pathogenesis →

* Its obligate human parasite.
* Its commensal flora of Nasopharynx or Oropharynx

- **Source of Infection** – the organisms enters by respiratory route.

[1] Meningitis – This is the most serious disease occuring in children of 2 months to 2 year of age. Majority of the cases are due to Heamophilus B strains.

[2] Acute Epiglottitis – It is the 2nd most common infections caused by H. Influenzae this is an acute inflammation of epiglottitis with obstructive laryngeal oedema, seen in children over 2 years old.

[3] Pneumonia – its typically occurs in infants.

[4] Bronchitis – H. Influenzae is an Important pathogen in the acute exacerbations of chronic bronchitis.

[5] supurrative lessions – Septic arthritis
Endocarditis
Pericarditis
otitis Media

Laboratory Diagnosis –

→ Specimen – CSF, Blood
Throat swab
Sputum
Pus
Aspirates from joints, middle ear or sineses.

→ Collection & Transport –

- Collect in sterile container
- H. Influenzae is very sensitive to ↓ temprature Therefore, clinical specimens should never refrigerated.

→ Direct Microscopy -

1. Gram Stain - In meningitis Gram stained smear of CSF show pleomorphic gram negative coccobacilli.

2. Immunofluorescence & quelling Reaction -

These can be employed for direct demonstration of H. Influenzae after mixing with specific type B Antiserum.

3. Antigen Detection - Type B capsular Ag can also be detected in patients serum CSF urine, pus by latex agglutination ~~coagulation~~ coagglutination & counter immuno-electrophoresis (CIE).

→ Culture Characteristics -

- H. Influenzae has fastidious growth requirement
- It grow aerobically than anaerobically it requires enriched media such as blood agar or chocolate agar, because the accesory growth factors known as X or V factor present in blood are essential for growth.

OPTIMUM TEMPRATURE - 35-37°C

X factor - It necessary for the synthesis of catalase & other enzymes cytochrome 'C' cytochrome oxidase. • Involved in aerobic respiration.

V factor – It present in red blood cells. ordinary blood agar is not suitable for the growth of H. Influenzae where growth is scanty as the V factor is not freely available.

V factor is released from RBC in chocolate agar heated Blood agar on 80-90°C.

* V factor is also synthesised by staphylococci.

Colony Characters – small translucent & non-Hemolytic of Blood Agar.

Satellitism :-

- Although blood Agar contains X & V factors, colonies of H. influenzae are small due to non-availability of V factor.
- After inoculating suspected H. Influenzae on a B/A plate, Staph. aureus is streaked across the same plate
- Incubate at 37°C for 18-24 hours.
- the colonies suspected of H. Influenzae will be large & well developed alongside the streak staphylococci.

· Rickettsia ·

Morphology – Rickettsiae are pleomorphic
- coccobacilli
- 0.3 - 0.6 µm X 0.8 - 2.0 µm in size
- Non - motile
- Non - capsulated
- Gram negative
- organisms stain bluish purple with giemsa stain

Culture – Rickettsiae are unable to grow in cell free media. they are cultivated in yolk sac of chick embryo.

OPTIMUM TEMPRATURE - 32 - 35°C

They can also grow on Hela, HEp2 & other continuous cell line.

Pathogenesis – Rickettsiae are generally transmitted to humans by the bite or by the feces of an infected arthropod vector.

– Rickettsiae include - typhus fever
Spotted fever

* **Typhus fever Group** – The group consist of epidemic typhus & its recrudescent infection (Brill - Zinsser disease) & endemic (murine) typhus.

Weil-Mooser or tunica Reaction –

When male guinea pigs are injected subperitoneally with blood from a case of endemic typhus or with a culture of R. mooseri, they develop fever and a characteristics scrotal inflammation.

* Spotted fever Group –

The disease of this group include rocky mountain spotted fever (RMSF), boutonneuse fever, Australian tick typhus, siberian tick typhus & rickettsial pox.

Laboratory Diagnosis –

Laboratory diagnosis of rickettsial diseases may be carried out by – Isolation of rickettsia
– Serology

→ Isolation of rickettsiae –

Blood clot ground in skimmed milk is inoculated intraperitoneally in male guinea pigs or mice. The inoculated animals are to be observed for 3 to 4 weeks. The response of animal to different rickettsial infections vary.

for, demonstration of rickettsiae, smears from peritoneum, tunica & spleen of infected animals may be stained by giemsa or Gimenez methods.

* Serology –

Serological diagnosis may be done by
– weil-felix reaction
– specific test using rickettsial antigens.

(i) Weil-Felix Reaction –

It is a heterophile agglutination test which detect anti-rickettsial Ab that cross react with certain non-motile proteus strains OX 19, OX 2 & OX K. The basis of the test is the sharing of an alkali stable carbohydrate antigen or some rickettsiae with non-motile strains of proteus, P. vulgaris OX 19, and OX 2. The proteus organism are used as antigens.

In Brill-zinsser disease the test in negative or weakly positive. In spotted fever both OX19 & OX 2 are agglutinated. OXK agglutinins are found only in scrub typhus.

(ii) Specific tests using rickettsial antigens –

serology method using rickettsial antigens are specific which include CFT, LA & enzyme immunoassay.

Weil - Felix Reaction in Diagnosis of Rickettsial Disease -

Disease	Agglutination with Proteus strain		
	OX 19	OX 2	OXK
Epidemic typhus	+++	±	-
Brill - Zinsser disease		-	-
Epidemic typhus	+++	±	-
Spotted fever group	++	++	-
Scrub typhus	-	-	+++
Rickettsial pox	-	-	-

Treatment -

Tetracyclines or chloramphenicol are used for treatment of rickettsial disease.

"Actinomycetes"

Actinomycetes contains two medically important genera
- Actinomyces
- Nocardia

- Actinomyces -

Morphology
- Gram positive
- Non-motile
- Non-sporing
- Non-~~sporin~~ acid fast
- Mycelial forms
  (break up into coccal & bacillary forms)

Pathogenesis -

* The actinomyces cause the disease know as actinomycosis. It is chronic granulomatous disease characterised by multiple abscesses tissue distruction, fibrosis & formation of multiple sinuses.

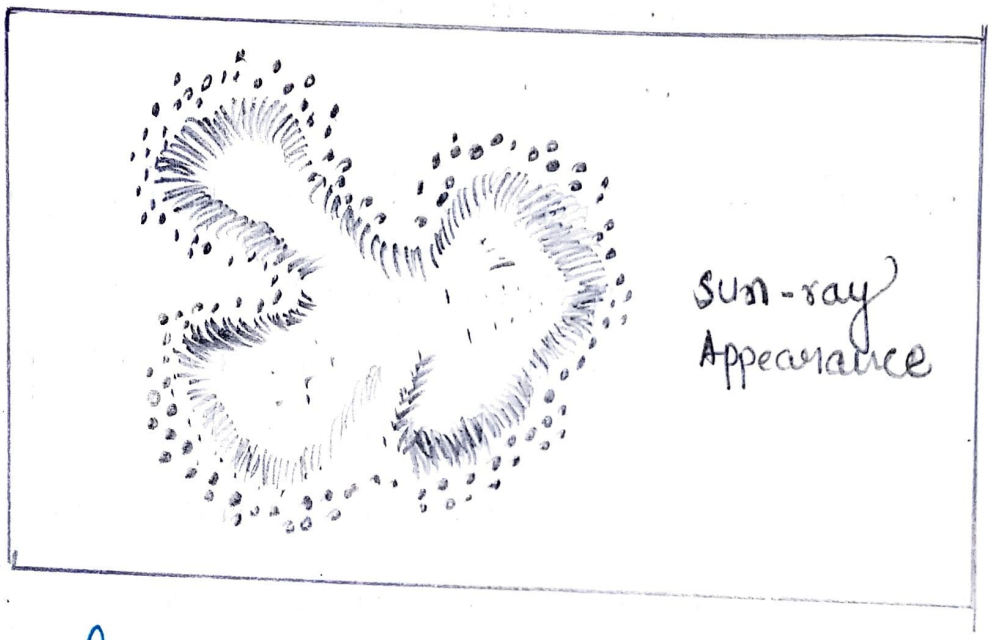
* Actinomyces have also been incriminated to cause gingivitis & periodontitis (inflammatory disease of gums)

Microscopically, it is a painless indurated swelling with multiple discharging sinuses. The pus contains usualy yellow coloured sulphur granules. Actinomycosis may also present as mycetoma.

Laboratory Diagnosis —

- **Specimen** – Pus from lesions or sinuses
  Discharge from fistula
  Sputum in pulmonary disease
  Tissue in biopsy
- **Microscopy** – Pus is shaken along with some saline in a test. tube & the mixture is allowed to settle.
  The sulphur granules sediment is withdrawn with a capillary pipette. Granules are crushed b/w two slides & smears are prepared. one smears is stained by gram stain & other by acid-fast stain (decoloration with 1% sulphuric acid).
  → Gram staining shows a sun-ray appearance
  → Acid fast staining shows central part as non acid fast surrounded by acid fast 'clubs'.

Culture – They grow best under anaerobic or microaerophilic conditions at optimum temprature of 37°C under 5-10% CO_2. They can be grown on brain heart infusion agar, blood agar or thioglycollate ~~agar~~ broth.



Sun-ray Appearance

Treatment –

Surgical removal of affected tissue along with penicillin therapy are effective.

'Nocardia'

Nocardiae resemble actinomyces morphologically but are strictly aerobic. It contains a total of 85 species.

Species – N. asteroides
N. brasiliensis
N. caviae

Morphology → Gram positive bacteria, Aerobic
→ mycelium form (fragments into rod shaped & coccoid element)
→ Nocardia resembles actinomyces
→ some species are acid-fast & few are non-acid fast.
→ non-motile

Pathogenesis → Non-capsulated
→ Size 0.5–1.2 mm in diameter
→ Filamentous morphology

Pathogenesis –

Nocardiae are usually produce opportunistic pulmonary disease known as nocardiasis.

One notable exception is N. basiliensis which is typically considered an obligatory pathogen.

→ Man acquires infection by inhalation of the bacteria from environmental sources. (soil)

→ The basic type of human disease caused are -
- pulmonary disease
- Neural
- systemic nocardiasis
- pneumonia, lung abscess

→ symptoms -
- cough
- fever
- dyspenea

Laboratory Diagnosis -

Specimen - Pus
Purulent sputum

Microscopy - The smears are stained with gram staining & Zn technique using decolourisation with 1% sulphuric acid. Gram positive filamentous bacteria can be seen on gram staining. Acid fast bacilli are detected on Zn technique though some species are non-acid fast.

Culture –

The specimen are inoculated on nutrient agar SDA & BHI agar & incubated at 37°C for 3 weeks. colony morphology is seen & bacteria are identified by staining.

Colony - dry granular wrinkled with white yellow. Pink to red colour.

Treatment → cotrimoxazole (3 month or more)

→ Nocardiae are also susceptible to
nalidixic Acid
Amikacin
Tobramycin
Vancomycin.

Listeria - Miscellaneous Bacteria

Morphology :-
- Listeria are small
- Gram positive coccobacilli
- rod shaped
- Measuring 1-3 μm X 0.5 μm
- non-sporing
- Non-capsulated
- Non-Acid Fast
- motile at 25°C but non-motile at 37°C

Pathogenesis :- All the 8 species may cause disease in animals. only Listeria monocytogenes & very rarely L. ivanovii are likely to be associated with human disease.

source of Infection - Human infections result from contact with infected animals, inhalation of contaminated dust or ingestion of contaminated milk or food.
→ Hospital-Acquired infections are also reported.

L. Monocytogenes can be divided into following groups -
1. Neonatal infections
2. Adult Infections

➢ Neonatal Infections - Infection from pregnant women to foetus is transmitted either

transplacentally or intrapartum. It may lead to absorption or stillbirth or neonatal disease. meningitis or septicaemia may occur in neonates.

Adult Infections –

→ Meningitis accounts for the most cases of listeriosis.

→ Listeriosis include – encephalitis,
brain abscess
spinal abscess
septecemia
endocarditis

→ cutaneous infection ~~Butchers~~ due to the direct contact with infected animal or birds.

Laboratory Diagnosis –

- Specimens – Blood
CSF
Amniotic fluid
Pus
Swabs from cervical & vaginal secretions
Meconium
cord blood

- Direct Microscopy – In gram stained smears of CSF sediments, the organisms may be seen as gram positive coccobacilli.

Leucocytes in csf are raised, of which 40-60% may be lymphocytes. Protein content of csf is raised & glucose is slightly lowered.

Culture – Specimen should be inoculated on
- Blood agar
- chocolate agar

~~Blood agar glucose~~
These specimens may also be added to nutrient broth & incubated at 35-37°C for 5 days followed by subculture on solid media.

Blood agar shows small colonies surrounded by a narrow zone of β-haemolysis. The bacteria are actively motile when grown at 25°C.

Colonies – 1-2 mm in diameter, round, smooth translucent, easily emulsifiable & non-pigmented.

Treatment – Ampicillin, amoxycillin, gentamicin

Biochemical Reactions – Listeria produce acid for glucose.
- catalase positive
- oxidase, urease, indole -ve

"Mycoplasma"

General characters -

→ Mycoplasmas differ from other bacteria in that they lack a rigid cell wall resulting in the plasticity of the organisms.

→ Due to lack of rigid cell wall, they are extremely pleomorphic.

→ The cytoplasm is surrounded by a single triple layered membrane.

Morphology – Mycoplasm are the smallest free living microorganisms. They can pass through bacterial filters.

→ They present as small spherical shapes (125-250 nm in diameter) longer branching filaments (500-1000 nm in size).

→ Gram Negative, but are better stained by Giemsa stain.

→ Non-sporing

→ Non-flagellated

→ Non-motile

Pathogenesis – Mycoplasma causes two type of disease in man – pneumonia & genital infections.

1. Mycoplasma Pneumoniae –

M. pneumoniae causes primary atypical pneumonia (Mycoplasmal pneumonia). Transmission is by droplet of nasopharyngeal secretions.

2. Mycoplasma hominis –

It may be isolated from 20-25% cases with non-gonococcal urethritis (NGU). This has also been incriminated in postpartum sepsis, proctitis, acute salpingitis, pelvic inflammatory disease, cervicitis & vaginitis. It is transmitted by sexual contact.

Laboratory Diagnosis –

Laboratory diagnosis may be carried out by isolation of the organism or by serological test.

Isolation – Mycoplasma can be recovered from throat swab, nasopharyngeal swab respiratory secretions, sputum or urethral secretions, prostatic secretions, cervical swabs urine.

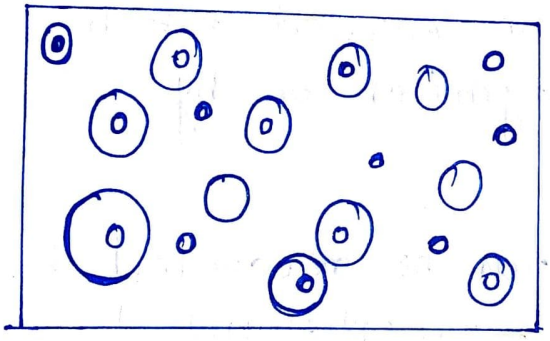
Culture – Culture media should be inoculated immediately after collection of specimen. If inoculation is not possible immediately then specimen may be kept at

4°C up to 24 hours. In case of delay more than 24 hours, the specimen should be frozen at -70°C.

Enriched Media - containing 20% horse or human serum & yeast extract.
A medium widely used for the isolation of mycoplasmas is PPLO broth. This medium can be made solid by the addition of agar.

Colonies - appear after (48-72) hours.

→ Typical tiny fried egg colonies
→ Size varies from 200-500 µm (large colonies)



fried egg colonies

* Serological Test -

(i) Detection of antigen -

→ direct Immunofluorescence
→ counter Immunoelectrophoresis (CIEP)
→ enzyme Immunoassay (EIA)

(ii) Detection of antibody -

→ Imunofluoreseence
→ enzyme Immunoassay
→ Indirect haemagglutination assay

Non specific tests are :

- Streptococcus MG Agglutination test
- cold agglutination Test

Treatment -

→ Tetracyclines
→ erythromycins

Chlamydiae → Gram (-ve), non-motile, non-flagellated NS, Non-capsulated

Species – Genus – Chlamydia
species – C. trachomatis, C. psittaci, C. pneumoniae, C. pecorum.

Morphology – Chlamydiae exist in two forms-

→ Elementary Body (EB) – It is spherical particle measuring 200-300 nm in diameter. It is the extracellular infective form.

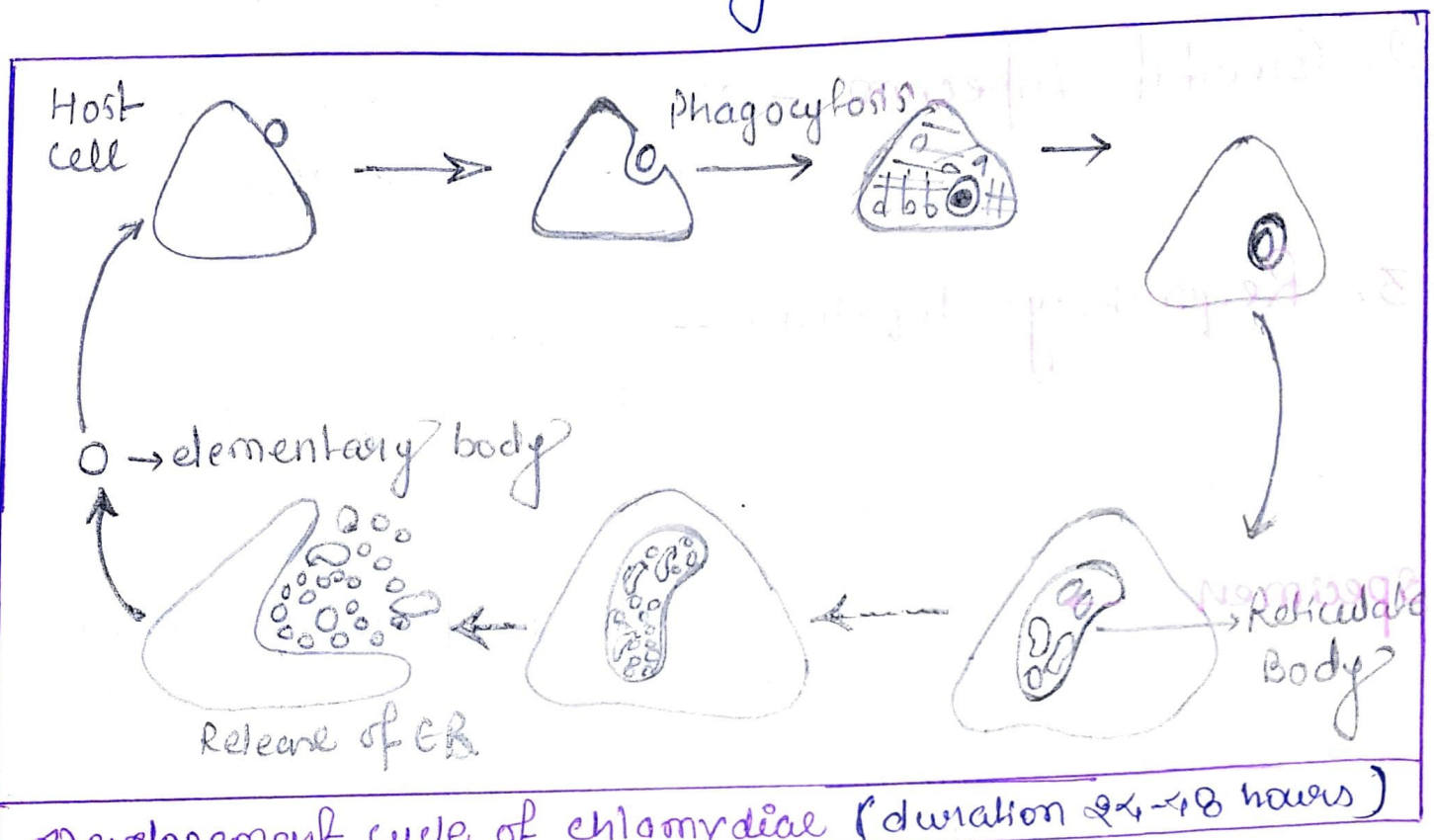
→ Reticulate body (RB) → non-infectious in nature
→ intracellular infective step
→ 500–1000 nm in size

Culture :- Chlamydiae can be isolated by
→ Animal Inoculation
→ Yolk sac inoculation
→ Tissue culture

→ Animal Inoculation – Mice are inoculated by intranasal, intraperitoneal inoculation. The mice die within 10 days & smears from various tissue.

→ Yolk sac Inoculation – yolk sac of chick embryo is inoculated & the organisms can be detected in impression smears.

→ Tissue culture – MacCoy cells treated with cycloheximide are the most commonly used cell line. The organisms in the tissue culture can be detected by staining for inclusion or elementary bodies.



Development cycle of chlamydiae (duration 24–48 hours)

The infectious particle (EB) enters the host cell by phagocytosis. It enlarges to form reticulate body 500–1000 nm in size. This is the reproductive form which grows in size & divides repeatedly by binary fission to produce a large number of EB. The newly formed infectious particle (EB) on liberation from host cell, may infect new cells & cycle is repeated.

Pathogenesis –

Chlamydial Infections in man occur in three forms –

1. ocular Infection – Trachoma
   Inclusion conjuctivitis
   ophthalmia neonatrum

2. Genital Infection – Non-gonococal urethritis
   lymp granuloma Venereum

3. Respiratory Infections – Pneumonia
   Psittacosis & ornithosis

Laboratory Diagnosis –

Specimen → ocular, urethrial, vaginal & cervical scrapping
blood
Sputum
Pus
respiratory secretions

1: Direct detection of Antigens –

→ Light Microscopy – Chlamydia infections to conjuctivita, urethra & cervix may be diagnosed by demonstrating inclusion bodies in the smears stained with Giemsa, Castaneda or machiavello methods,

→ Immunofluorescence (IF)
→ ELISA

2. Isolation –

Chlamydiae may be isolated by inoculation into mice, yolk sac of chick embryo or in tissue cultures.

3. Serology of Antibody detection –

→ complement fixation test (CFT)
→ Microimmunofluorescence

Treatment – Azithromycin
tetracycline

(Paracyte in blood cause allergic)
— eosinophills

Parasitology

"Leishmania Donovani"

→ Introduction
→ Morphology
→ life cycle
→ Pathogenesis / clinical symptoms / features
→ Laboratory Diagnosis

Introduction - This parasite causes visceral leishmaniasis or kala-azar. It is academic in many places in india. It is a parasite of reticuloendothelial system.

Morphology -

The parasite exists in two forms -

Amastigote - occurs in man.

Promastigote - occurs in guts of sandfly & artificial culture.

(1) Amastigote culture Stage -

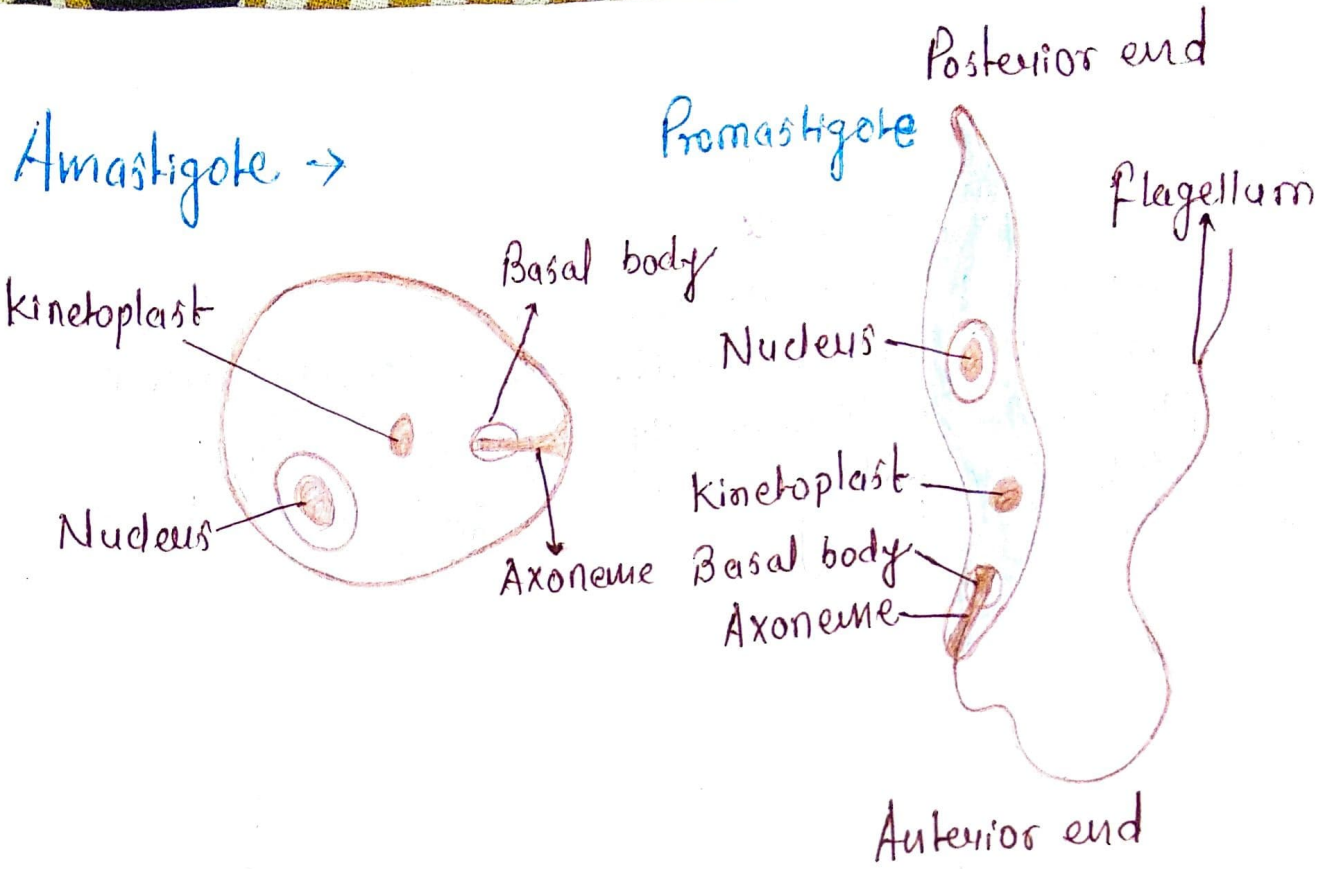
→ It is a Aflagellar stage.

→ It is present in the cells of reticuloendothelial system of vertebrate hosts (man, dog etc.)

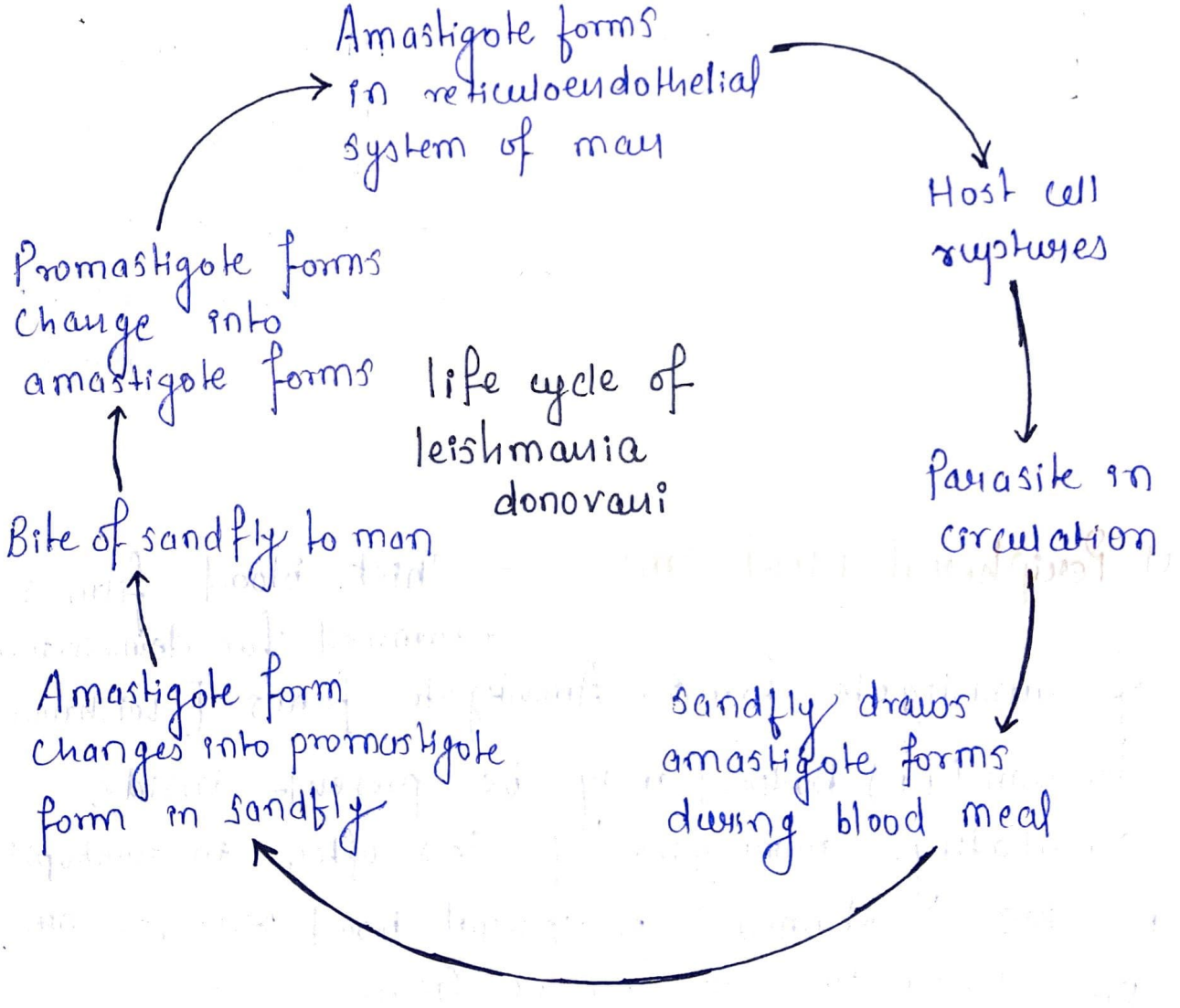
→ It is a round or oval body. (2 to 4 µm)
→ Nucleus is oval or round & is usually situated in the middle of the cells.
→ kinetoplast lies at right angle of the nucleus.
→ Axoneme extends from the kinetoplast to the margin of the body. It represents the root of the flagellum.

(ii) Promastigote stage –

→ It is a flagellar stage.
→ It is present in insect vectors (sandflies) & in cultures.
→ It is long, slender, spindle-shaped body (15-20 µm X 1-2 µm)
→ Nucleus is situated centrally.
→ kinetoplast lies near the anterior end.
→ Flagellum may be of the same length as the body or even longer.


Amastigote →
Kinetoplast
Basal body
Nucleus
Axoneme
Promastigote
Posterior end
Flagellum
Nucleus
Kinetoplast
Basal body
Axoneme
Anterior end

Life Cycle :-


Amastigote forms in reticuloendothelial system of man
Host cell ruptures
Parasite in circulation
Sandfly draws amastigote forms during blood meal
Amastigote form changes into promastigote form in sandfly
Bite of sandfly to man
Promastigote forms change into amastigote forms
life cycle of leishmania donovani

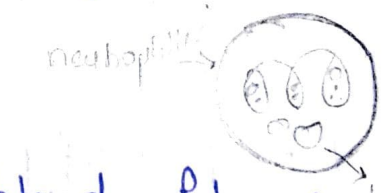
Pathogenesis –

Leishmani donovani produces the disease kala azar or visceral leishmaniasis. It is characterised by pyrexia, splenomegaly, hepatomegaly, lymphadenopathy + general features of anemia.

Laboratory Diagnosis –

- **Direct Evidences**
  - Peripheral Blood smear
  - Blood culture (N.N.N.) [Novy MacNeal, Nicolle]
  - Bone marrow Aspiration
  - Splenic puncture
  - Antigen detection
- **Indirect Evidences**
  - Blood cell count
  - Hb estimation
  - Non-specific serological Test
  - specific test
  - Rapid immunochromatophagic strip test

+ Direct Evidence –



(a) Peripheral Blood smear – Thick blood film is examined for demonstration of amastigote forms. Amastigote forms [Leishmano-donovan (LD) bodies] may be present inside circulating monocytes + less often in neutrophills On the stained peripheral blood film, smears are stained by leishman or Giemsa stain.

(b) Blood culture – Blood culture can be done in N.N.N. [Novy, MacNeal Nicolle] medium & incubated at 22°-24°C. Promastigote forms can be demonstrated.

(c) Bone Marrow Aspiration – Bone-marrow aspiration is a useful method for diagnosis. Amastigote forms can be demonstrated in a stained film. The promastigote forms can be demonstrated when this material is cultured in N.N.N. Medium.

(d) Splenic puncture – It is one of the most valuable methods for diagnosis. The amastigote are found in stained films & promastigotes in culture. The only risk of splenic puncture ~~wound resulting~~ is that bleeding may continue from the puncture wound resulting in death.

(e) Antigen Detection – ELISA using species specific monoclonal Ab & DNA probes are very useful in the direct detection of leishmania Antigen.

* Indirect evidences –

(a) Blood count → There is progressive leucopenia.
→ The proportion of leucocytes to erythrocytes is greatly altered.

(b) Heamoglobin estimation – It reveals anaemia.

(c) Non-specific serological test –

→ Napier's Aldehyde test : This test indicates increased serum gamma globulins & thus is non-specific. It is positive only when the disease is of at least three months duration.

→ Chopra's Antimony test : This test also depends upon increased serum gamma globulin. It is less reliable than aldehyde test.

→ complement fixation test with W.K.K. Antigen –

→ It detects antibodies in sera of kala-azar patients.

→ the antigen used was prepared from human tubercle bacillus by Wilebsky, Kingenstein & Kuhn, hence named as W.K.K. Antigen.

→ The test is considered non-specific since the Ag is not prepared from L. donovani.

(d) Specific tests –

Indirect heamagglutination Assay (IHA), indirect fluorescent antibody test (IFAT) & ELISA are more specific tests.

(e) Rapid Immunochromatographic strip Test –

It has been developed for detection of antibodies against rk 39 Ag of L. donovani. It indicates active kala-Azar.

"Echinococcus Granulosus"

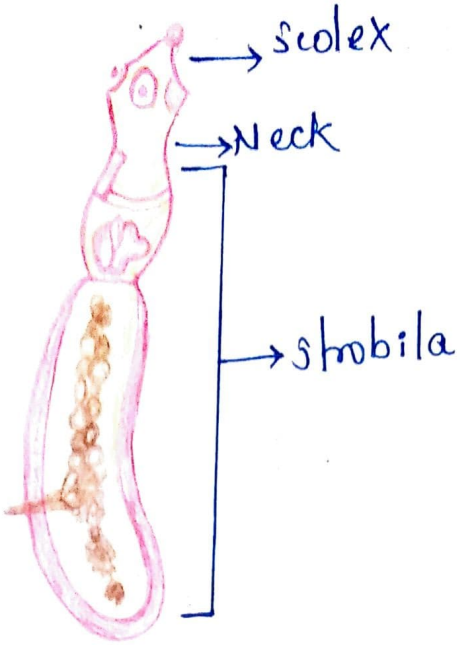
It is a cestode & is the causative agent of the hydatid disease. Man harbours the larval form of the parasite, while the adult worm is found in small intestine of dog & other canine animals.

Morphology -

→ Adult Worm -

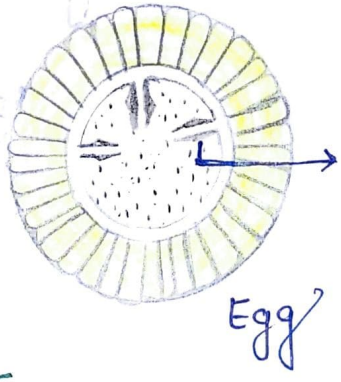
- It measures 3-6 mm in length.
- It contain scolex (head), neck & strobila, strobila consist of three segments. occasionally, the fourth segment is also present.
- The first segment is immature, the second one is mature & the third one is gravid. If fourth segment is present, that will also be gravid.
- The terminal segment is the biggest among all the segments.
- The neck is short & thick.
- The scolex bears four suckers & a rostellum which contains two circular rows of hooks.

Echinococcus Granulosus — Adult Worm



→ Egg -

- It is ovoid in shape & measures 32-36 µm X 25-32 µm.
- It contains hexacanth embryo with 3 pairs of hooks.

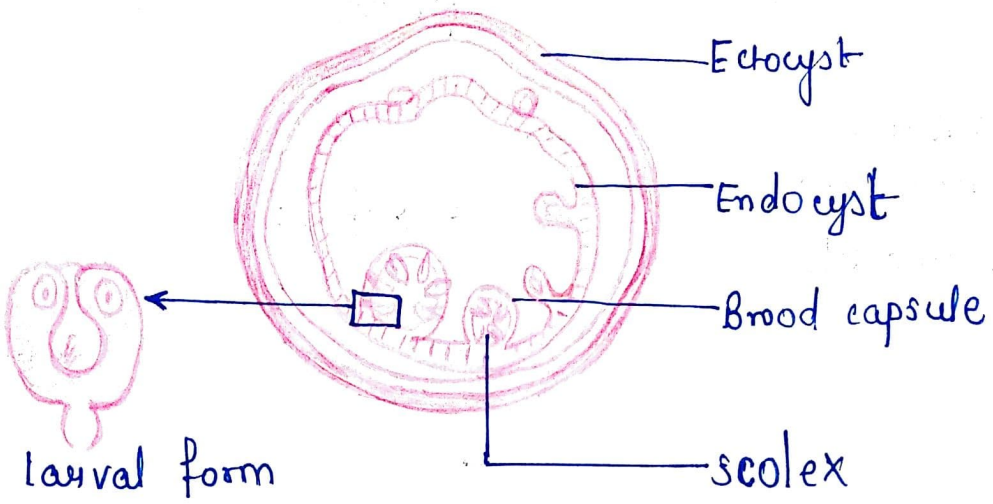


Egg

→ larval form -

- This is found within the hydatid cyst & represents the scolex of the future adult form.
- It remains invaginated within a vascular body.
- When it enters the definitive host, the scolex with four suckers & hooklets, becomes evaginated & develops into an adult worm.

" HYADATID CYST "



Life Cycle :-

Adult worm in small intestine of the definitive host (dog)

Eggs passed in faeces

Eggs ingested by the intermediate host (man)

Hexacanth embryos are hatched out

The embryos are carried to the liver & other organs

Embryo develops into hyatid cyst

Hyatid cyst contains thousand of scolices

Hyatid cysts ingested by the dog

Each scolex head evaginates & develops into adult worm

The worm passes its life cycle in two hosts i.e. definitive host & intermediate host.

→ Definitive hosts - The adult worm live in small intestine of these animals & discharge eggs in their feces. The dog is the optimum definitive host but wolf, fox & jackal may also act as definitive host.

→ Intermediate Hosts - The larval stage is present in these animals including man, giving rise to hydatid cyst. sheep, pig, cattle, horse, goat & man may act as intermediate host.

Pathogenesis & clinical features -

In man, the larval form causes unilocular hydatid disease.

→ Mode of infection -

Ingestion of eggs by man may occur in the following ways:

1. By direct contact with infected dogs.
2. By allowing the dog to feed from the same dish.
3. By uncooked vegetables contaminated with infected animal faeces.

Infection is generally acquired in childhood due to their intimate association with dogs.

→ Hydatid cyst -

The cyst wall secreted by embryo consists of two layers
- outer layer (ectocyst) &
- inner or germinal layer (endocyst)

Ectocyst - It is elastic but when incised or ruptured, it curls on itself thus exposing the inner layer containing the brood capsules & daughter cysts.

Endocyst - It is inner or germinal layer. It forms the outer layer (ectocyst) & give rise to broad capsules & scolices on inner side. It also secrets hydatid fluid.

Hydatid cyst may involve liver, lung, brain, heart kidney, spleen, bone, muscles etc. liver is the commonest side. The disease remains symptomless for many years.

Laboratory Diagnosis -

It consist of the following tests:

→ Casoni Test -

→ It is an immediate hypersensitivity skin test introduced by casoni. Sterile hydatid fluid is used as antigen. ~~The hydatid fluid is~~

→ The hydatid fluid is drawn from unilocular hydatid cysts from human cases (removed by operation) or from animals.

→ It is filtered & made sterile.

→ 0.2 ml of this antigen is injected intradermally in one arm.

→ Sterile normal saline, 0.2 ml, is injected intradermally in the other arm which acts as control.

→ In positive case, tested site develops a large wheal (measuring 5 cm in diameter or more) with multiple pseudopodia.

→ This test has a low sensitivity, thus, gives false positive reaction in other cestode infections.

△ Blood Examination - Eosinophillia (20-25%) may be present.

△ Serological test - ELISA, CFT, IHA & LA are used for diagnosis of hydatid cyst.

"Wuchereria Bancrofti"

Introduction – Wuchereria bancrofti is mainly confined to the tropics & subtropics in India.

It is distributed along the sea coasts & also along the banks of big rivers.

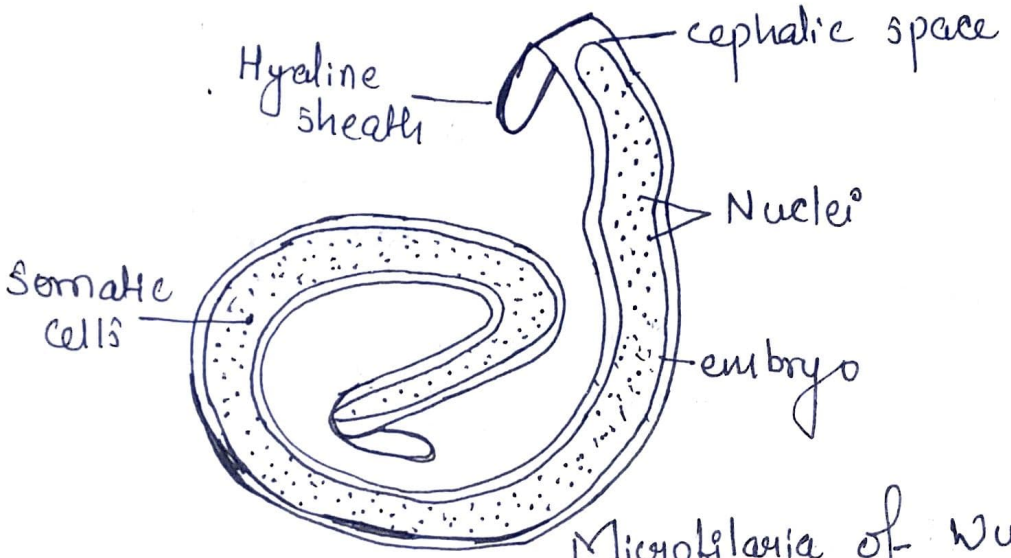
Morphology –

→ Adult Worm :

- Adult worm are transparent, long hair-like structures.
- They are often creamy-white in colour.
- They are filiform in shape & both ends are tapering.
- The size of male & female are 2.5 – 4.0 cm (length) X 0.1 mm (thickness) & female are 0.2 – 0.3 mm (thickness) X 8-10 cm (length) respectively.
- The female is ovo-viviparous (laying eggs with embryos). The life span of adult worm is several years (5 to 10 years).

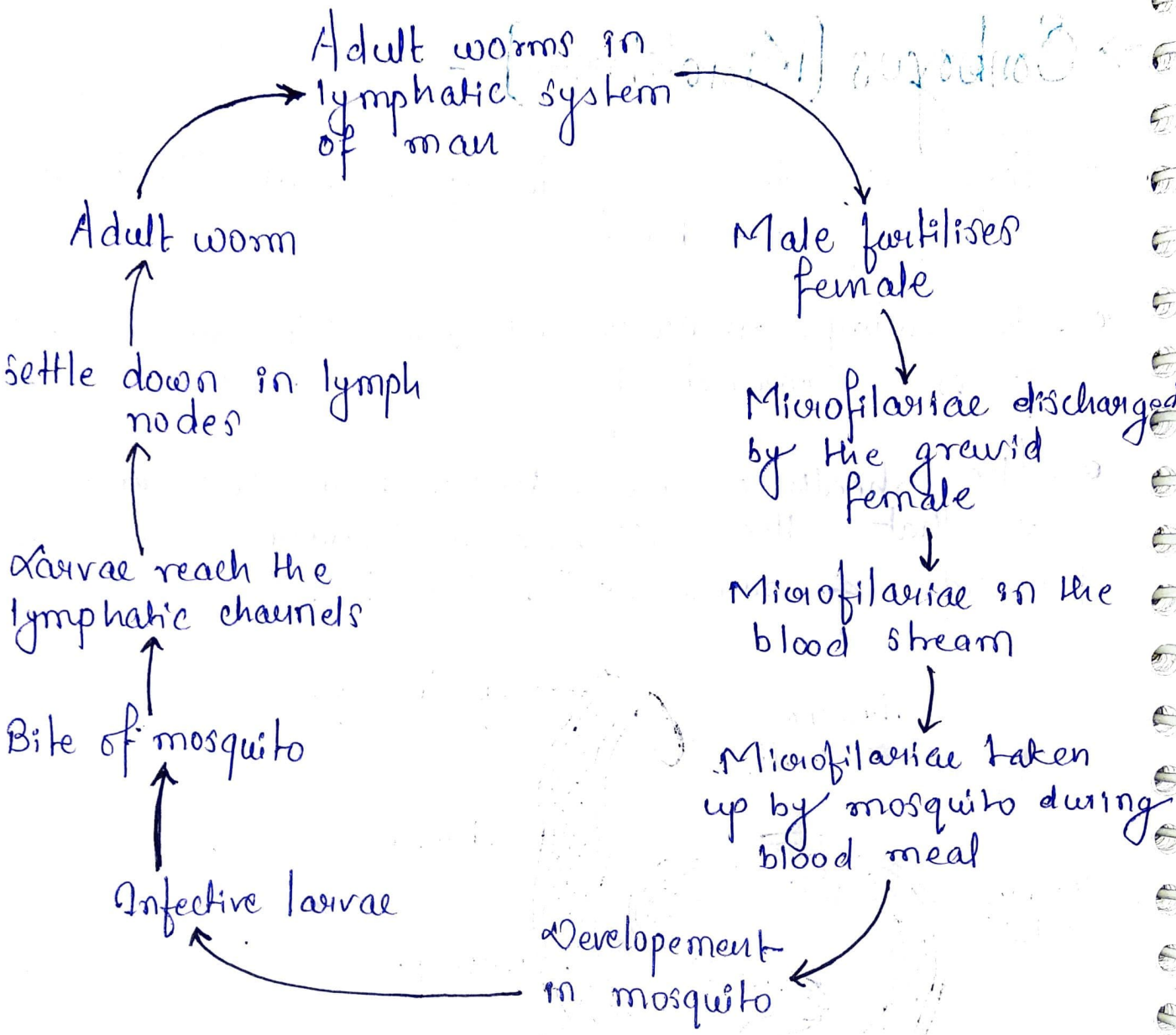
→ Embryos (Microfilariae) -

- They are colourless & transport with blunt heads & pointed tails.
- The embryo measures 290 µm X 6-7 µm in size & is covered by a hyaline sheath.
- The sheath is much longer than the embryo so that the microfilariae (embryo) can remove forwards & backwards within it.



Microfilaria of Wuchereria

Life cycle -



Pathogenesis -

The infection with this parasite is named wuchereiasis of filariasis. The disease is of two types -

- Classical filariasis (caused by adult worm)
- occult filariasis (caused by embryos).

→ Classical filariasis :-

It leads to lymphangitis, lymphadenitis, lymph-oedema with hypertrophy of affected part (elephantiasis), lymphangiovarix, hydrocele & chyluria.

→ Occult filariasis :-

It is due to hyper-sensitivity reaction to microfilarial antigens. Patient develops massive eosinophillia (30-80%) hepato-splenomegaly, generalised lymphadenopathy & pulmonary symptoms. Microfilariae are not found in the peripheral blood. classical features of lymphatic filariasis are absent.

Laboratory Diagnosis -

It depends on direct evidences (to search for microfilariae & adult worm) & indirect evidences (serological test).

(i) Direct evidence -

Demonstration of microfilariae in peripheral blood film, chylous urine, exudate of lymph varix & hydrocele fluid. The microfilariae appear in large numbers in peripheral blood at night. Hence, blood film should be made in night b/w 10 P.M. & 2 A.M.

(ii) Indirect evidences –

Eosinophillia (5 to 15%) can be detected due to allergic reaction to antigens. serological tests like enzyme linked immunosorbent assay (ELISA).

IFA - Indirect fluorecent antibody
IHA - Indirect Heamagglutination Assay

can also be used but these teste have low sensitivity & specificity.

Mycology

"Candida"

Candida is a genus of yeasts & is the most common cause of fungal infections worldwide.

Genus - Candida

Family - Saccharomycetaceae

class - Saccharomycetes

Kingdom - Fungi

Species - C. albicans, C. ascalaphidarum, C. argentea, C. atlantica, C. auris, C. marina,

Candidiasis -

→ causative fungus - candida albicans (80-90% of cases)

→ Candidiasis is an infection of skin, mucosa, and internal organs, caused by yeast like fungus candida albicans, & occasionally by other candida species.

→ Candida albicans is the normal inhabitant of skin, gastrointestinal tract, oral & vaginal cavities.

Morphology - C. albicans is an ovoid or spherical budding yeast cell, 3-5 µm in diameter.

Pathogenesis –

→ candidiasis is an opportunistic endogenous infection.

→ Lessions caused by candida are as follows –

(a) Mucocutaneous lesions –

Oral thrush
Vulvovaginitis
Balanitis
Conjuctivitis
Keratitis

(b) Skin + nail infections –

~~Skin~~ –

(c) systemic candidiasis –

urinary tract infections
Pulmonary candidiasis
Endocarditis
Meningitis
Septicaemia
Intestinal candidiasis

(d) oral Manifestations –

Various manifestations or oral candidiasis are as follows – Thrush
Chronic oral candidiasis
chronic mucocutaneous candidiasis
Circumoral candidal dermatitis

Laboratory Diagnosis -

Direct Microscopy :- Gram stained smears & KOH Mounts from lesions of skin, nail or mucous membranes show budding gram positive yeast cells.

Culture -

→ Candida species grow well on SDA & ordinary bacteriological culture media e.g. - blood agar.

→ They grow at 25-37°C within 24 hours.

→ Cream coloured, smooth, pasty colonies appear

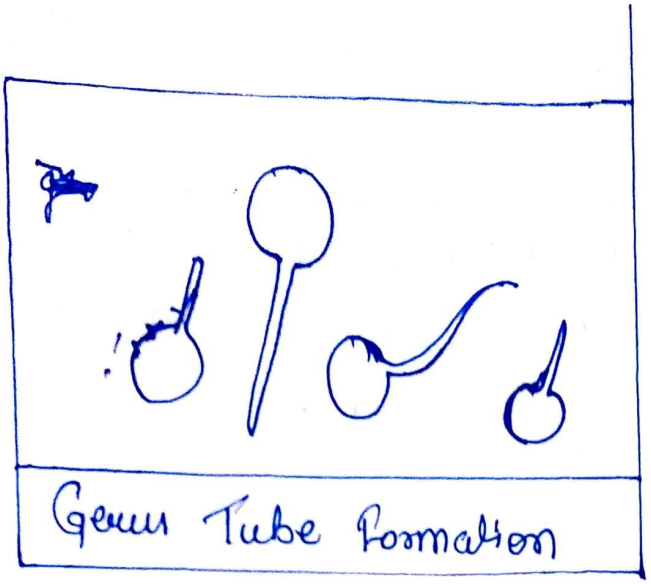
→ Gram stained smear from colonies shows gram positive budding yeast cells.

Identifications -

To differentiate C. albicans from other species, the following tests are done -

➢ Germ tube Test - C. albicans has ability to form germ tubes within two hours when incubated in human serum at 37°C (Reynold-Braude phenomenon)

~~Antigen~~ -



Germ Tube Formation

Treatment -

→ Predesposing factors are to be removed in all cases.

→ Imidazole (miconazole , clotrimazole) or polyene

→ Amphotericin B

Histoplasma -

Histoplasmosis –

→ Causative fungus - Histoplasma capsulatum, a dimorphic fungus

→ It is primarily a disease of reticuloendothelial system.

→ H. capsulatum is an intracellular parasite.

Source of infection -

- The fungus is present in the soil enriched with excreta of birds or bats.
- Human infection results from inhalation of spores.

Clinical features –

→ The large majority of infections are asymptomatic.

→ Some individuals develop pulmonary disease which resembles tuberculosis.

→ Disseminated histoplasmosis develop only in a minority of infected individuals.

→ Involvement of reticuloendothelial system results in lymphadenopathy, hepatosplenomegaly, fever anaemia & high rate of fatality.

→ Granulomatous & ulcerative lesions may develop on the skin or mucosa.

Oral Manifestations –

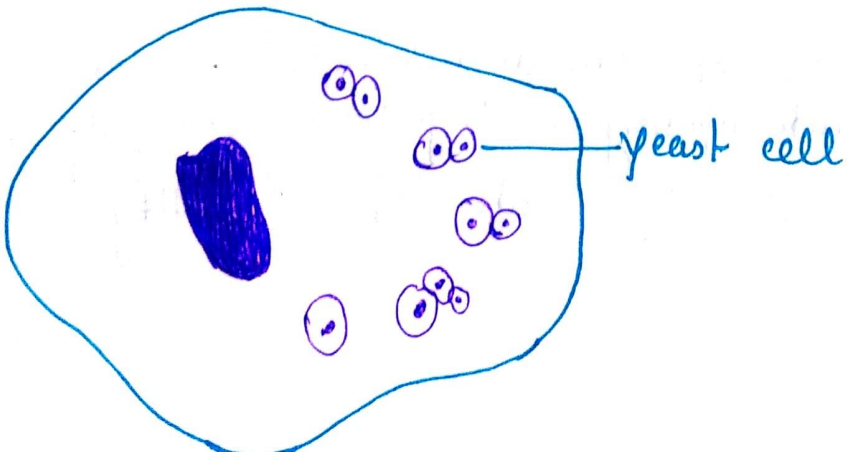
The nodular, ulcerative or vegetative ~~test~~ oral lesions may be present on the buccal mucosa, lips, gingiva, tongue to palate. The ulcerative areas are indurated & are usually covered by a grey membrane.

Laboratory Diagnosis –

Specimen – Sputum
Bone-Marrow aspirate
Peripheral blood
Scraping from dermal of mucosal ulcers
Biopsies of lymph nodes & other organ

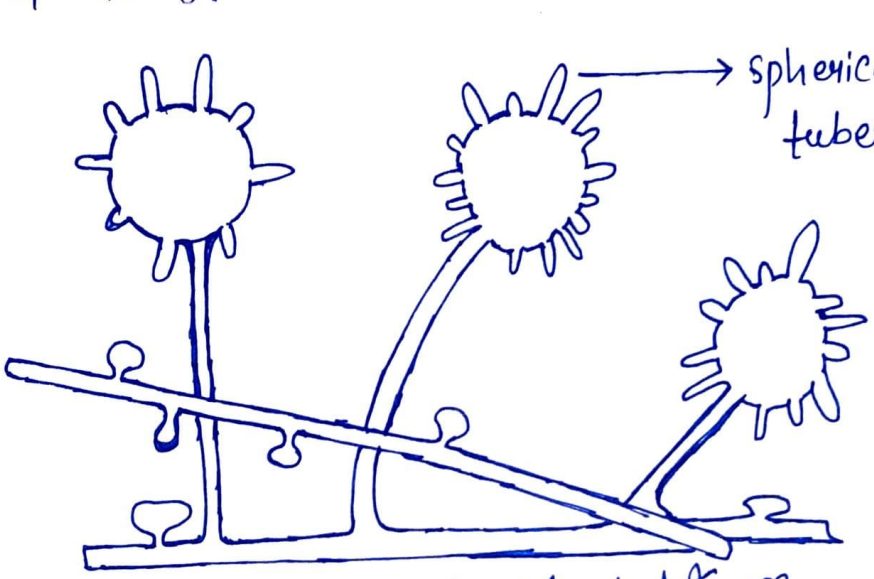
Direct Examination –

Smears of sputum or pus are stained with Geimsa or wright stains. On microscopy, H. capsulatum appears as small oval yeast cell (2-4 μm in diameter) packed within the cytoplasm of macrophages or monocytes.



Culture –

- SDA (sabouroud dextose agar) or BHI (brain-heart infusions agar with cyclo-heximide & chloramphenicol are inoculated.
- The yeast phase is formed in cultures at 37°C white cottony mycelial growth containing large (8-20 µm) thick walled, spherical spores with tubercles or finger like projections appear at 25°C.



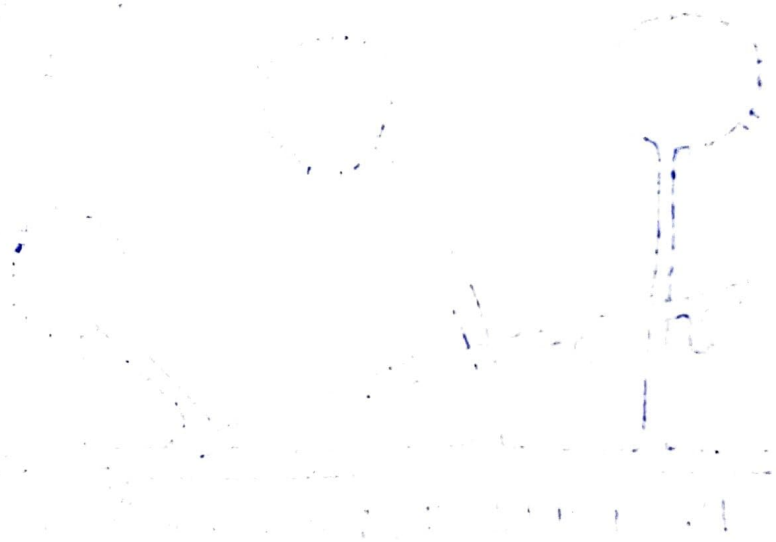
H. CAPSULATUM : Mycelial form

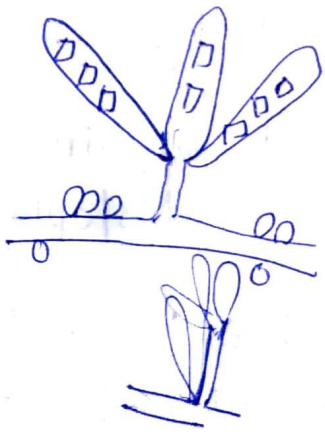
* Histoplasmin Skin Test –

- Delayed hypersensitivity test
- The test is similar to tuberculin test but antigen used in histoplasmin.
- A positive 'histoplasmin skin test' indicates past or present infection, but does not differentiate active & past infections.

Treatment –

Amphotericin B has been found useful in treatment of histoplasmosis.





"Dermatophytes"

Introduction :-

Derma - skin
phyton - plant

* Dermatophytes are a group of fungi that infect only superficial keratinised tissue (skin, hair & nails) without involving the ~~utilise~~ living ~~keratin~~. tissue.
* They break down & utilise keratin.
* They are incapable of penetrating subcutaneous tissue.
* They cause dermatophytoses, also known as tinea or ringworm.

Classification -

Dermatophyte are classified into three genera as follows :

Trichophyton - Hair, skin, nail
Microsporum - Hair, skin
Epidermophyton - skin, Nail

→ Trichophyton -

(Pencil shape)
Trichophyton is a genus of fungi. Trichophyton fungi are molds characterised by the developement

of both smooth walled macroconidia & microconidia.

Macroconidia – Are mostly borne laterally directly on the hyphae or on short pedicels.

→ 

Thin or thick walled clavate to fusiform 4-3 to 8-50 µm.

Microconidia – Are spherical pyriform to ~~cato~~ clavate or of irregular 4 2-3 to 2-4 µm.

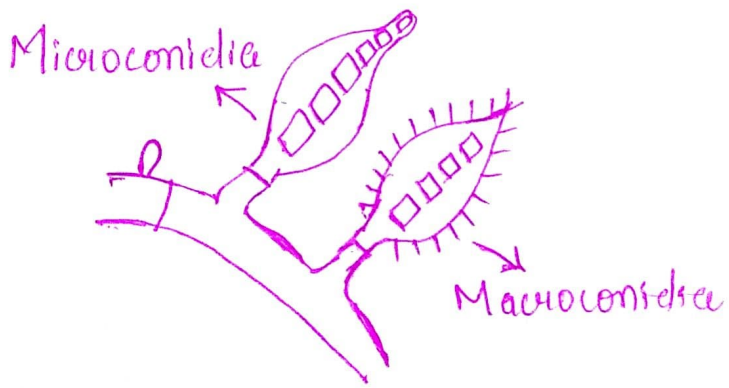
→ 

Infection – The anthrophilic varieties cause forms of dermatophytosis that's fungal infection of skin, Nail.

→ Microsporum –

(spindle shaped)
Microsporum is a genus of fungi that cause –

- Tenea capitis
- Tenea corporis
- Ringworm



Microsporum from both macroconidia & microconidia.

* Macroconidia – large asexual reproductive structures
  - Macroconidia are hyaline multiseptate variable inform fusiform spindle shaped 7-20 by 30 - 160 µm.

* Microconidia – smaller asexual reproductive structures on short conidiospores
  - Hyaline single celled
  - pyriform to clavate
  - smooth walled
  - 2.5 - 3.5 by 4-7 µm

→ M. gypseum
→ M. canis
→ M. cookei
→ M. Nanum

→ Epidermophyton – (E. floccosum)
(club shaped)
→ E. is a genus of fungus causing superficial & cutaneous mycoses causes -

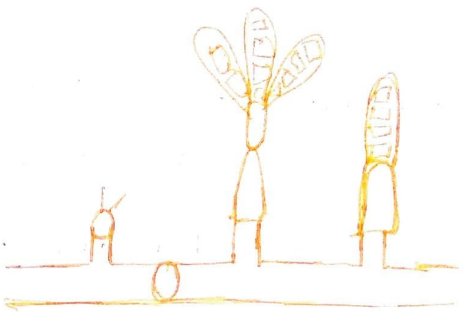
Tinea corporis (ring worm)
Tinea cruris (jock itch)
Tinea pedis (Athlete's foot)
Tinea unguium (fungal infection of the nail bed)

Microconidia – Absence

Macroconidia → Are borne singly or in cluster of 2 to 3, 20–40 µm in length X 7–12 width.

→ contain 1–9 septa

→ club shaped, broad



(Macroconidia)

Pathogenesis –

Source of infection – Dermatophytes are fungi that require keratin for growth. These can cause superficial infection of the skin, nail, hair.

Spread by –

Anthropophilic → preffering humans to animals
→ Blood sucking

Zoophilic – preffering animals to human

Geophilic – soil living to animals humans

Infection - clinically ringworm can be classified depending on the site involvement these include

Tinea capitis → scalp or skin
Tinea corporis → Non-hairy skin of the body
Tinea cruris → groin
Tinea pedis → Athlet's foot infection

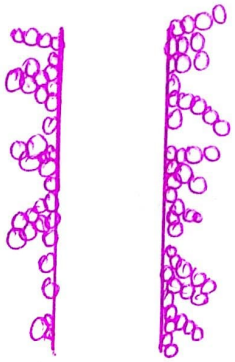
Tinea Barbae (barber's itch) → bearded areas of the face + neck

Favus - Is a chronic type of ringworm involving the hair follicles. It leads to alopecia + scarring.

Hair Infection

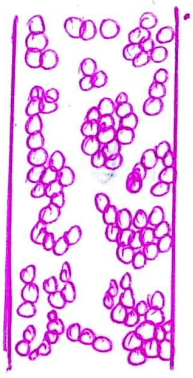
Ectothrix

A sheath of arthospores is present on the surface of hair shaft.



Endothrix

while the arthospore formation occurs entirely within the hair shaft in endothrix



Laboratory Diagnosis –

Specimens – Skin scrapings
Hair clippings
Nail

Direct Microscopy – Direct 10% KOH Mount may show fungal hyphae

Culture → SDA agar + SDA c antibiotics are used.

→ Incubates at 25 to 30°C for 3 weeks

* colony – creamy powdery or cottony colonies pigmented on reverse (Red) T.rubrum

* Macroconidia + microconidia

Microscopy – lectophenol cotton blue for the detection of macroconidia + microconidia

T – more microconidia, very few macroconidia

M – Predominant Macroconidia

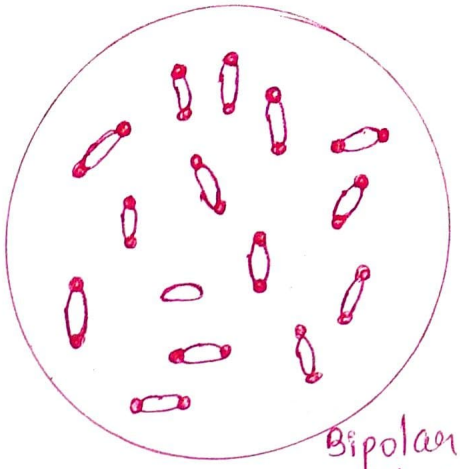
E – Macroconidia

Treatment of dermatophytoses –

oral griseofulvin is the drug of choice.

Yersinia

(species - Y. Pestis, Y. Pseudotuberculosis)



Bipolar staining

Morphology :-

→ Gram -ve bacillus
→ 1.5 µm X 0.7 µm in size
→ Non - motile
→ Non - Acid Fast
→ Non - sporing
→ Safety pin appearance (stained with methylene blue)
→ Slime layer (envelope or capsule)

Pathogenesis :-

Y. Pestis is a natural pathogen of rodents & causes Zoonotic disease called plague.

Infection is transmitted from one animal to another by the bite of flea.

Source of Infection - bite of infected rat flea

Incubation Period - 2-6 days

In man, plague occurs in three forms - Bubonic plague
- Pneumonic plague
- Septicaemic plague

Laboratory diagnosis -

Specimen - Pus or fluid aspirated ~~from~~ bubonic plague
Sputum & blood - pneumonic plague
Blood - septicaemic plague
CSF - meningeal plague

Direct Microscopy –

Sputum & aspirates from lymph nodes are stained with gram's staining & methylene blue.

Gram staining shows → gram-ve coccobacilli

Methylene Blue → typical biopolar staining

Culture –

The organism is aerobic & faultatively anaerobic. The optimum temprature for growth is 27°C. It grows on ordinary media.

- Nutrient Agar → colonies are small, delicate, transparent after 24-48 hours incubation.
- Blood Agar → colonies are non-heamolytic & dark brown & other are same as N/A.
- Maclonkey Agar → colourless colonies are formed.

~~Bacillus~~ Neisseria

The genus Neisseria consist of gram -ve, aerobic oxidase positive, non motile, diplococci.
The two pathogenic species are - N. meningitidis
- N. gonorrhoeae

Neisseria Meningitidis [Meningococcus]

Morphology - Gram -ve
spherical or oval cocci
0.6-0.8 μm in size
Non-motile

Pathogenesis - N. meningitidis causes pyogenic meningitis in all ages, but is most common in childrens & young adults.

Maningococci are strict human pathogens. The infection is acquired by droplet spread via the carriers & the cases.

Human nasopharynx is the reservoir of N. meningitidis

Incubation Period - 3 days

Maningococcaemia presents as acute fever with petechial rash. A few develop fulminant meningo-coccaemia characterised by shock, disseminated intravascular coagulation & multisystem failure. It is usually a fatal condition.

Laboratory Diagnosis -

Specimen → CSF
Blood
Petechial lesions
Nasopharyngeal swab

Collection & Transport → collection of specimen is done under sterile conditions by lumbar puncture for CSF & by venepuncture for blood.

→ Blood is injected into blood culture bottles.

→ Nasopharyngeal ~~swab~~ is collected by using a sterile swab.

Direct Microscopy → Gram staining
meningococcus are seen as gram -ve diplococci

Antigen detection - counter current Immunoelectrophoresis (CIEP)
Coagglutination & LA

Culture → Blood Agar
Chocolate Agar

(i) Indole Positive Bacteria - E. coli
vibrio cholerae
Klebsiella oxytoca
Proteus sp.
Enterobacter fecalis

Indole Negative Bacteria - Streptococcus pyogens
Enterobacter aerogenes

(ii) Methyl Red Positive - E. coli
Proteus vulgaris

Methyl Red Negative - vibrio cholerae
enterobacter aerogenes

(iii) Gram ~~+ve~~ -ve Cocci - Neisseria
Moraxella
veillonella

Gram +ve cocci - Staphylococcus
Streptococcus
Pneumococcus

(iv) Gram +ve Bacilli - Corynebacteria
Mycobacterium Tuberculosis
clostridium, Listeria

Gram -ve Bacilli - E. coli
Shallmonella
shigella
pseudomonas

(v) Hemoflagellates – Leishmania donovani
Trichomonas vaginalis
Trypanosomes cruzi

[Hemoflagellates live in blood & tissues of human host
They are obligate parasites]

(vi) Blood Paracyte – Plasmodium species
Toxoplasma gondii
Mycoplasma

(vii) Cestode – . Taenia solium (tapeworm)
. Echinococcus granulosus (cause hydatid disease)
. Diphyllobothrium latum (fish or broad tapeworm)
. H. Nana

(viii) Vector-borne disease -
vector borne disease are human illness caused by parasite, virus & bacteria that are transmitted by mosquitoes, sandflies etc.

→ Aedes Mosquitoes – chikungunia, Dengue, yellow fever
→ Anopheles mosquitoes – Malaria, lymphatic filariasis
→ Sandflies – Leishmaniasis, kala azar
→ Ticks – Rickettsial disease, Relapsing fever
→ Black flies – onchocerciasis (river blindness)

(IX) Definition of diarrhoea –

Diarrhoea is the passage of 3 or more loose or liquid stools per day, or more frequently than is normal for the individual.

diarrhoea cause a liquid diet, food intolerance stress, anxiety.

Causative agent – Salmonella & vibrio parahemolyticus

(X) Definition of dysentery –

Dysentery is an inflammatory disease of the intestine, especially of the colon, which always result in severe diarrhoea & abdominal pains.

* symptoms – Bloody (mucous) diarrhoea, abdominal pain & fever

Causative Agent – shigella or entamoeba histolytica.

(XI) VP Positive bacteria – Enterobacter cloacae
Klebsiella pneumoniae

VP Negative bacteria – Proteus mirabillis
Escherichia coli

(xii)

MacConkey Agar -

Macconkey agar is an indicator, a selective & differential culture medium for bacteria designed to selectively isolate gram -ve & enteric bacilli & differentiate them based on lactose fermentation.

Pink colour - due to lactose fermentation
colourless - Non-lactose fermentation

The medium was developed by "Alfred Theodore maclonkey" while working as a bacteriologist for the 'Royal' commission on sewage disposal.

(xiii) Nutrient Agar -

Nutrient agar is a general purpose medium supporting growth of a wide range of non-fastidious organisms.

N/A is popular because it can grow a variety of types of bacteria & fungi.

* organisms that grow on N/A Plate - Bacillus
streptococcus
E.coli
fungi

* composition - 0.5% Peptone
0.3% beef extract/yeast extract
1.5% Agar
0.5% sodium chloride
d/w

(xiii) Candida species –

There are more than 17 different candida species are known to be aetiological agent of human infection.

However more than 90% of invasive infections are caused by candida albicans.

→ Candida Albicans
→ candida globrata
→ Candida tropicalis
→ candida krusei
→ Candida argentea
→ candida atlantica
→ candida atmosphaerica

(xiv) Sabouraud Dextrose Agar (SDA) –

SDA is used for the isolation, cultivation & maintenance of non-pathogenic & pathogenic species of fungi & yeasts.

* SDA was formulated by Sabouraud in 1912 for culturing dermatophyl.

* composition :- Dextrose - 40 gm
Peptone - 10 gm
Agar - 15 gm
d/w - 1000 ml

(xv) Coagulase Test – Coagulase is an enzyme needed to make blood clot.

This enzyme is present in staphylococcus aureus bacteria.

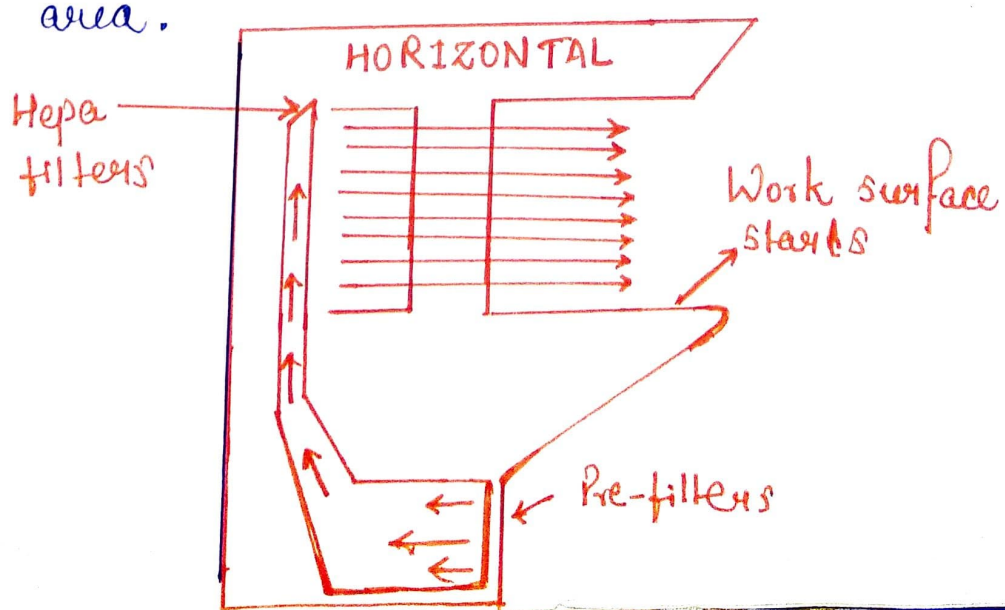
* Coagulase positive - Staphylococcus aureus
* Coagulase Negative - Staph. epidermidis
  Staph. saprophytics.

(xvi) Laminar Air Flow -

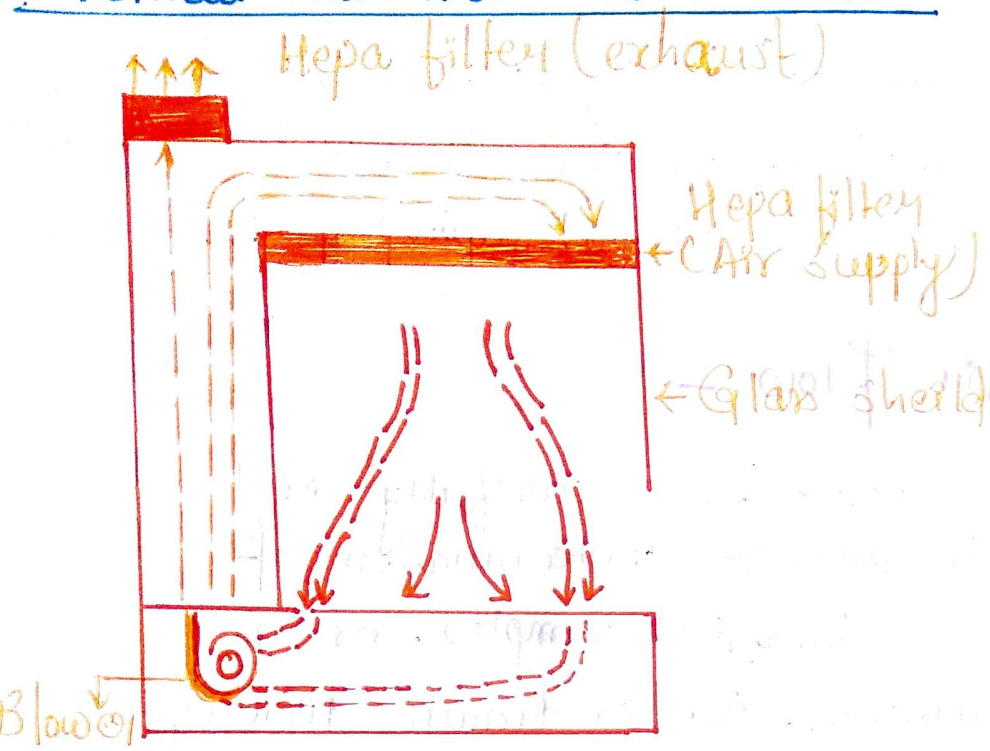
Laminar air flow cabinet is a carefully enclosed bench, designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive materials. Air is drawn through a HEPA filter & blown in a very smooth, laminar flow towards the user.

* Laminar air flow cabinet, are normally made of stainless steel with no gaps or joints thereby preventing the build-up of bacteria from collecting anywhere in the working zone.

* Principle - The critical principle of using laminar air flow is that nothing interrupts the flow of air b/w the HEPA filter & the sterile object the space b/w the HEPA filter & the sterile object is known as the critical area.



Vertical Laminar flow cabinets -



Function equally well as horizontal laminar flow cabinets.

Subcutaneous Mycoses –

Eumycetoma, Actinomycetoma — chronic granulomatous infection
Mycetoma Mycosis

Chromomycosis – Dematiaceae family
Sporotrichosis – Sporothrix schenckii
Rhinosporidiosis – Rhinosporidium seeberi

Systemic Mycoses –

Histoplasmosis – Histoplasma capsulatum
Blastomycosis – Blastomyces dermatitidis
Paracoccidioidomycosis – Paracoccidioides brasiliensis
Coccidioidomycosis – coccidioides immitis
Cryptococcosis – cryptococcus neoformans

Opportunistic Mycoses –

Candidiasis – candida Albicans
Aspergillosis – Aspergillus fumigatus
Mucormycosis (zygomycosis) – Mucor, Rhizopus & leichtheimia

Superficial Mycoses –

Dermatophytes – dermatophytoses (tinea or ringworm)

Germ Tube

→ Introduction — It is differential test for candida. It is a special test use to detect candida albicans.

Because here is candida albicans have special features that show formation in germ tube.

→ Principle → When human serum is added to ~~bacterial~~ Fungal colonies & incubate at 37°C for ~~2 hour~~ minimum 2 hour. If candida albicans present there is formation in germ tube.

→ Specimen → • Colonies of candida ~~albicans~~
• human serum use as a reagent.

→ ~~Procedure~~ → (i) Requirements — Test tube
— Incubator
→ Reagent (human serum)
→ nichrome loop

→ Procedure — (i) Firstly take a clean & dry test tube.

(ii) 3-4 ml human serum fill in the test tube.

(iii) then ~~bacterial~~ Fungal colonies of candida albicans are added in test tube with the help of nichrome loop.

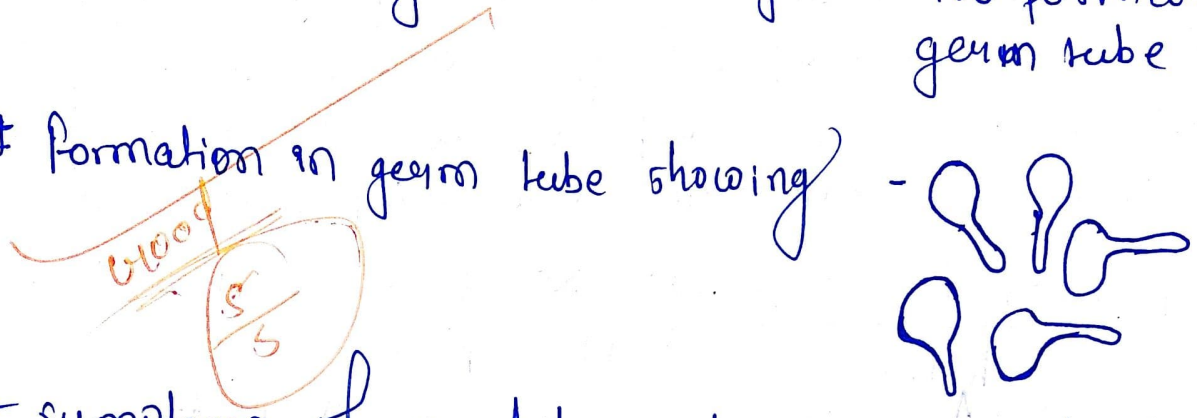
(iv) then mix both in the test tube carefully & ~~4~~ 4.

(v) Incubate at 37°C for minimum required time 2 hour (~~Incubate~~ if required, incubate for 24h)

(vi) then observe the test-tube

Observation - germ tube positive - formation in germ tube
germ tube Negative - No formation in germ tube

formation in germ tube showing -



symptoms of candida albicans (candidiasis).

- urinary tract infection
- Nosocomial
→ superficial
- systemic
- vaginal