

MICROBIOLOGY PRACTICAL RECORDS

differentiation.

3. It produces a useful magnification of about 1000-2000 times (x1000- x2000).

Structure of bright field microscope (various parts)

1. Base:
2. Mirror:
3. Condenser:
4. Stage:
5. Arm:
6. Body tube:
7. Adjustment knobs
8. Nose piece:
9. Objectives:
10. Eye piece:

Object Magnification

Objective Magnification	Eye piece Magnification	Total Magnification of the Object
10 x	10 x	100 times
40-45 x	10 x	400-450 times
100 x	10 x	1000 times

How to use a Microscope:

- 1) Place the microscope on a firm bench, so that it does not vibrate.
- 2) Put on the illumination when using artificial light. Use the flat side of mirror to reflect the light up through condenser.
- 3) place the slide to be examined on the stage making sure that the under side of slide is completely dry.
- 4) Place the low power objective 10x in position.

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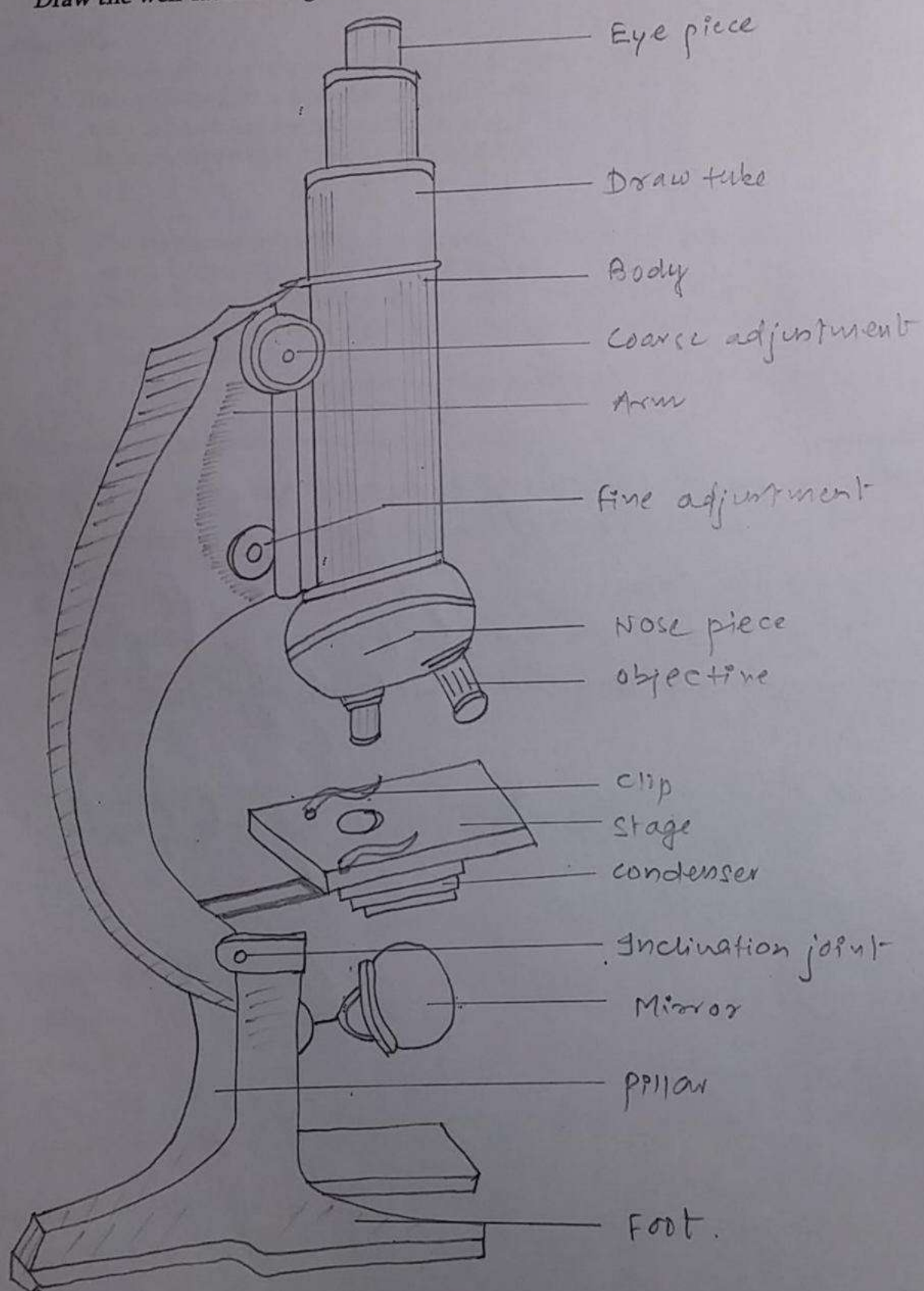
- 5.) Now look through eye piece & adjust illumination and diaphragm for greatest amount of light.
- 6.) Use the fine adjustment.

Care of Microscopes :

- 1.) Use both the hands for carrying microscope. one for grasping the arm & another beneath the base.
- 2.) Never touch the lens.
- 3.) Never leave a slide on microscope when not in use.
- 4.) Always remove oil from oil immersion objective lens after its use.
- 5.) Keep stage of microscope clean & dry.
- 6.) When not in use, keep microscope in cabinet.

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Draw the well-labeled diagram of a Compound Microscope:



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Size of Bacteria:

The unit of measurement used in bacteriology is the micron.

1 μ (micron) equal to 1/1000 of a millimeter.

1 m μ (millimicron) or nanometer (nm) equals to 1/1000 of a micron or one millionth of a millimeter.

1 Å (Angstrom unit) equals 1/10 of a nanometer. Bacteria of medical importance generally measure 0.2-1.5 μ m in diameter and about 3-5 μ m in length.

Shape of the Bacteria:

Depending on their shapes, bacteria are classified into several varieties e.g.

1. Cocci – Spherical or oval in shape
2. Bacilli- rod shaped
3. Vibrios- comma shaped
4. Spirilla- rigid spiral form
5. Spirochetes- flexible spiral form
6. Actinomycetes- branching filamentous form
7. Mycoplasma- cell wall deficient, hence, no stable morphology

Stained Preparations

Structural details of the bacteria can not be much studied under the light microscope due to lack of contrast. Hence, staining techniques have been employed to produce colour contrast. Bacteria have an affinity for basic dyes due to acidic nature of the cytoplasm. The following staining techniques are commonly employed in bacteriology laboratories.

1. Simple stain

- Methylene blue or basic fuchsin are used for simple staining
- They provide colour contrast, but impart some colour to all bacteria.

2. Negative stain:

- Indian ink or nigrosin are emulsified with the sample or organism to provide a uniformly coloured background against which the unstained bacteria stand out in contrast.

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- useful in demonstration of capsule which do not take simple stain.
- very slender bacteria such as spirochaetes that are not demonstrated by simple staining method can be viewed.

3. Impregnation method:

- Bacterial cells & structure that are too thin to be seen under the light microscope, are thickened by impregnation of silver on the surface to make visible.
- eg:- demonstration of bacterial flagella & spirochaetes.

4. Differential stains

a. Gram staining

i. Principle: The gram reaction is dependent on the permeability of bacterial cell wall & cytoplasmic memb. It is also dependent on the integrity of cell wall. Gram +ve cells have more acidic protoplasm that account for retaining basic dye more strongly than gram -ve bacteria.

ii. Procedure:

- 1) Make the smear in fresh grease free glass slide.
- 2) Cover the slide with methyl violet (basic dye). Allow it to stand for 1 min.
- 3) Rinse the smear gently under tap water.
- 4) Cover the smear with gram iodine and allow it to stand for 1 min.
- 5) Decolourise it w alcohol.
- 6) Immediately wash it with water.
- 7) Then add saffranin (for counterstain) & wait for 30 sec, and wash it.

iii. GP and GN organisms:

- * GP organism:- Staphylococcus aureus, Streptococcus sp., Streptococcus pneumoniae
- * GN organism:- E. coli, Vibrio cholerae, N. gonorrhoea.

b. ZN staining (Ziehl-Neelsen)

i. Principle: Acid-fastness is due to the high content of lipids, fatty acids and higher alcohol found in the cell wall of mycobacterium. Mycolic acid (a wax), acid-fast in the free state, is found in all acid-fast bacteria. Besides lipids content, acid-fastness depends also on the integrity of cell wall.

ii. Procedure:

- 1) The smear is stained by a strong solution of carbol fuchsin with application of heat.
- 2) It is then decolourised in 20% H_2SO_4 & counterstained with contrasting dye such as methylene blue.
- 3) Acid fast bacteria retain fuchsin colour while other taken counter stain.

iii. Examples: *Mycobacterium tuberculosis*, Acid fast bacilli

Demonstration

1. GPC in clusters (Staphylococci)
2. GPC in chains (Streptococci)
3. GPC in pairs (*D. pneumoniae*)
4. GNC in pairs (Meningococci/Gonococci)
5. GPB
 - a. *C. diphtheria*- Gram's, Albert, Neissers
 - b. *B. anthracis*
 - c. *Clostridium welchii*
 - d. *Cl. septicum*
 - e. *Cl. oedematiens*
 - f. *Cl. tetani*
6. GNB
 - a. *Escherichia coli*
 - b. *Vibrio cholerae* (comma shaped)
7. AFB
 - a. *Mycobacterium tuberculosis*
 - b. *M. leprae*
 - c. *Nocardia*
 - d. Spores
8. Spirochetes

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a. *Treponema pallidum*

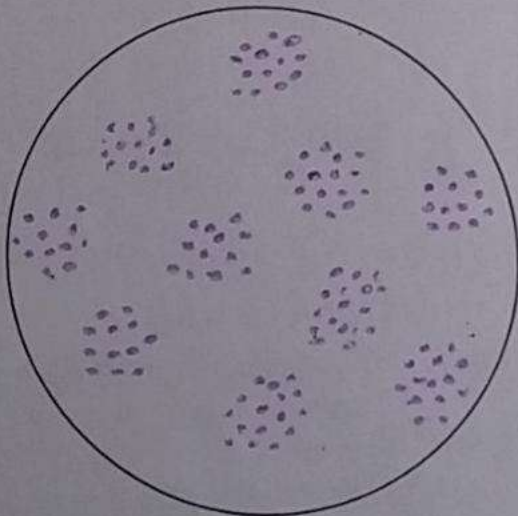
b. *Leptospira*

9. Actinomycetes

10. Demonstration of motility

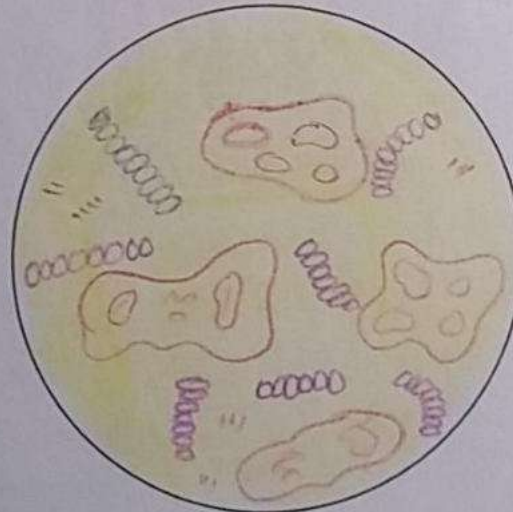
Diagrams

GPC in clusters



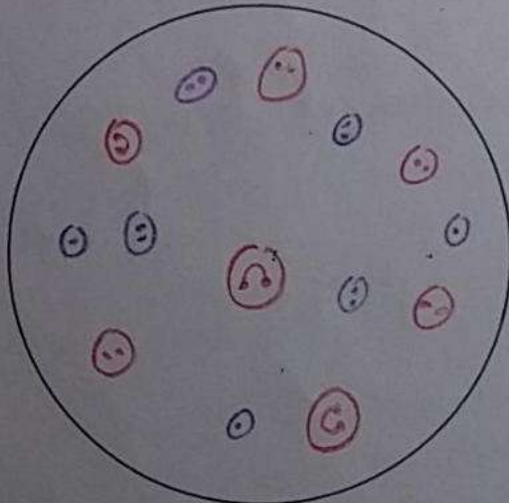
From pus sample showing GPC in clusters and pus cells

GPC in chains



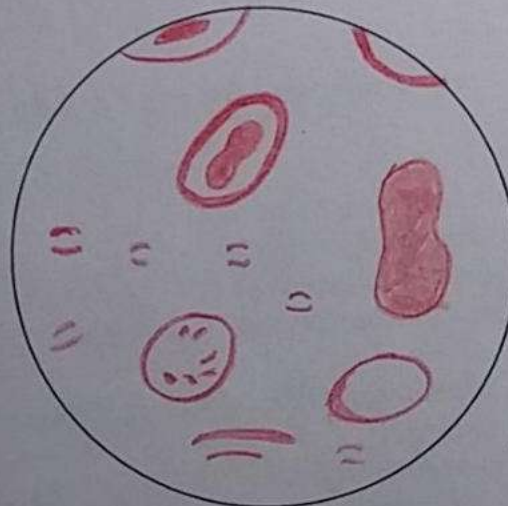
From acute follicular tonsillitis showing GPC in chains & pus cells

GPC in pairs



From sputum sample of a case of pneumonia showing GPC in pairs with capsule and pus cells

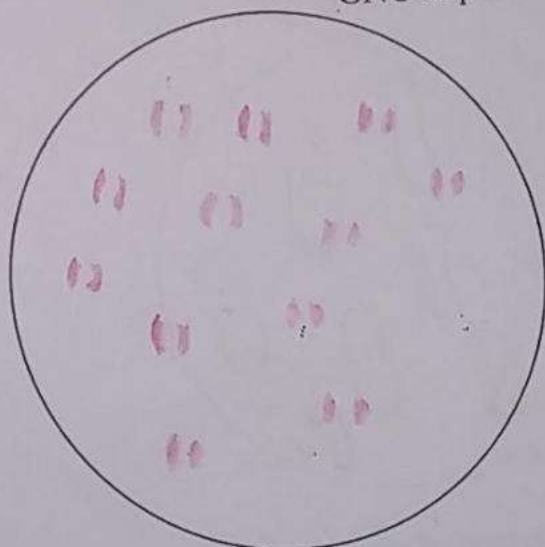
GNC in pairs



From CSF of a case of pyogenic meningitis showing GNC in chains with pus cells

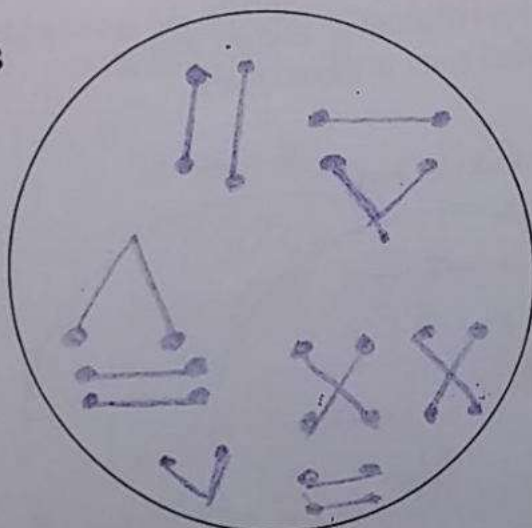
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GNC in pairs



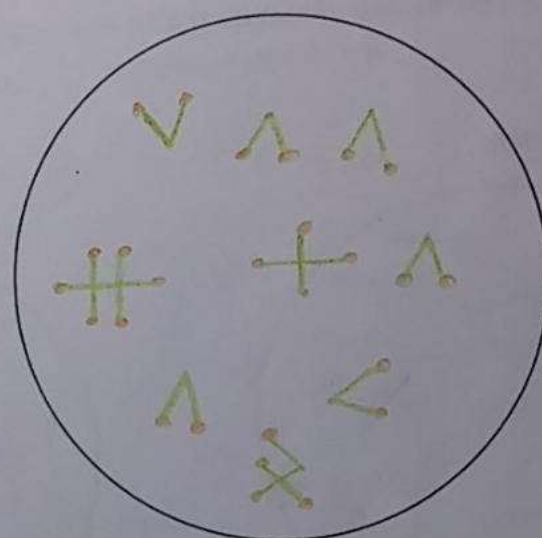
From a urethral discharge of a patient suffering from gonorrhea showing GNC (intracellular) and pus cells

GPB



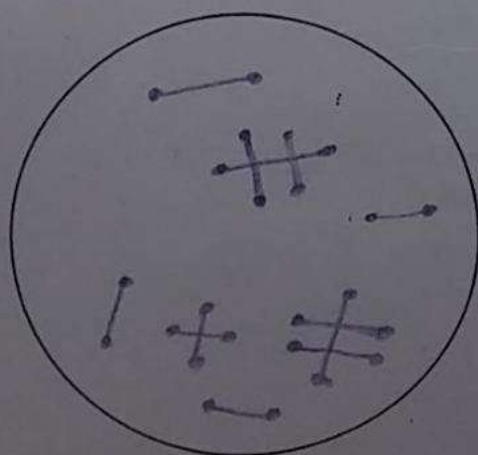
Gram stain

Gram stain- GPB with metachromatic granules and typical arrangement described as cuneiform or Chinese letter arrangement of *C. diphtheriae*.



Albert stain

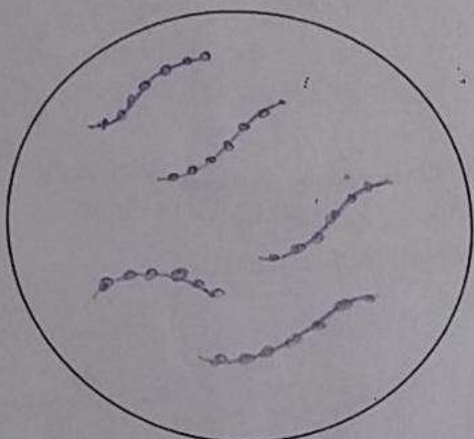
Albert stain- Metachromatic granules – bluish black. Bacillary body- green



Neisser's stain- Bacillus body- blue Granules- pink

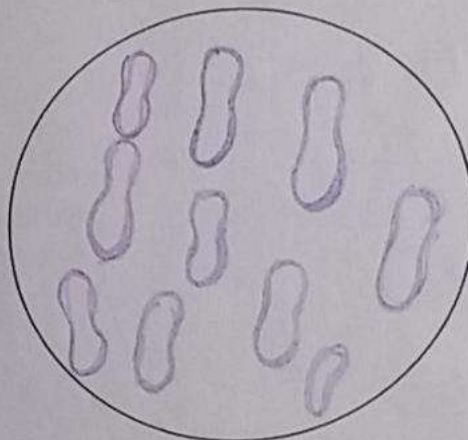
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Bacillus anthracis



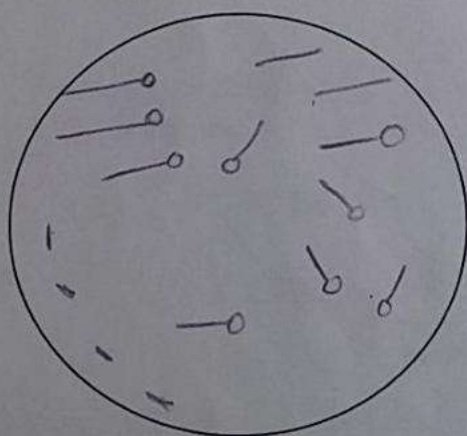
GPB with central spherical spores with typical bamboo stick appearance

Clostridium welchii



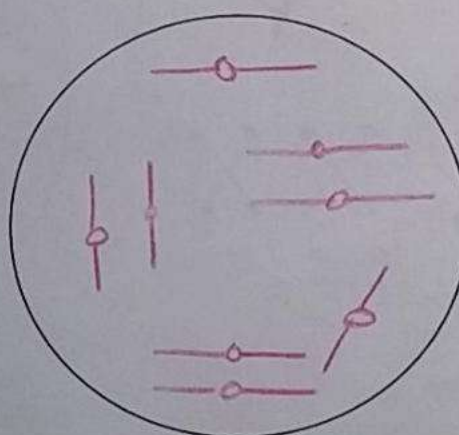
From a case of gas gangrene showing GPB, thick stumpy with truncated ends without spores.

Cl. septicum



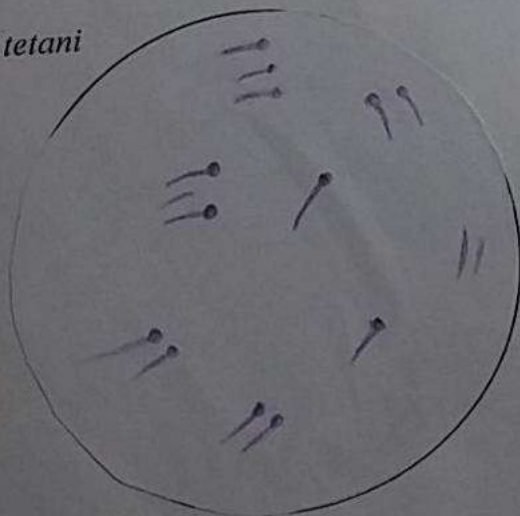
From a case of gas gangrene showing GPB with central spore (Citron body appearance)

Cl. oedematiens



From a case of gas gangrene showing GPB, with subterminal bulging spores

Cl. tetani

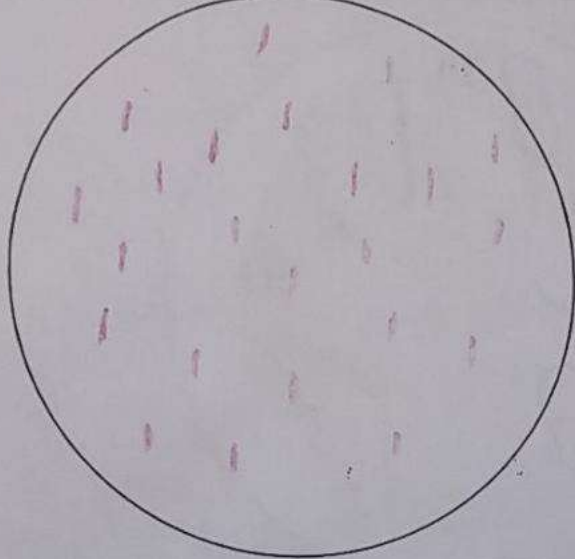


GPB with terminal spherical spore (drumstick appearance)

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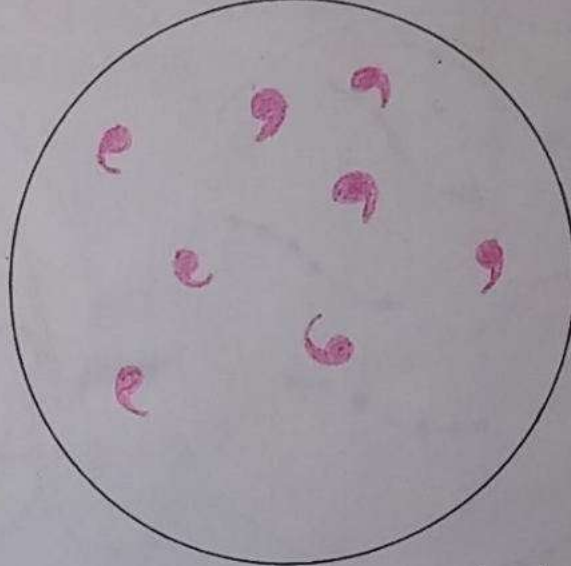
GNB

Escherichia coli



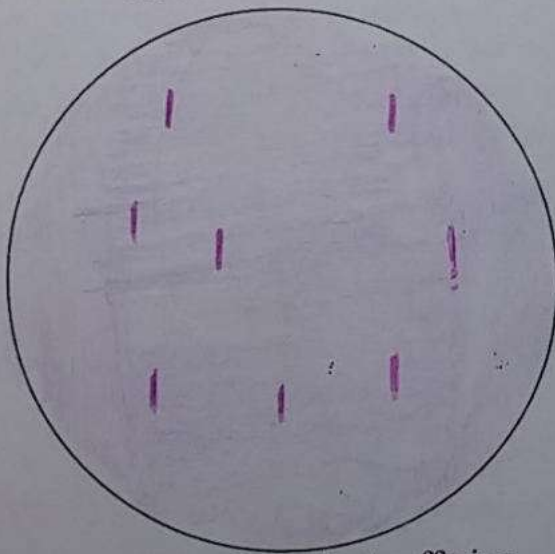
From urine sample of a case of UTI showing pink rods.

Vibrio cholerae



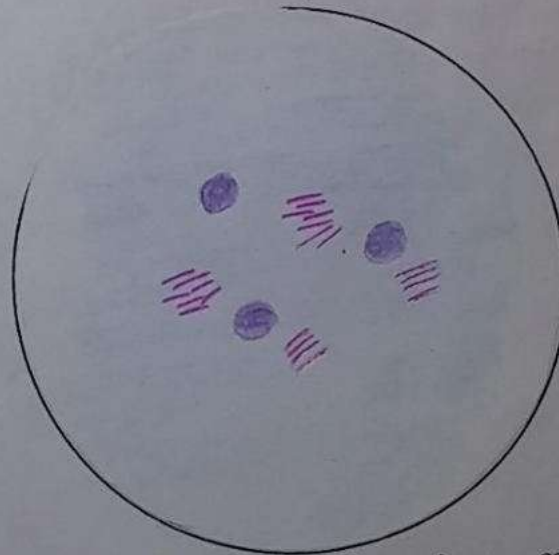
From stool of a case of cholera showing comma shaped rods.

M. tuberculosis



From sputum of a patient suffering from pulmonary tuberculosis showing pink rods

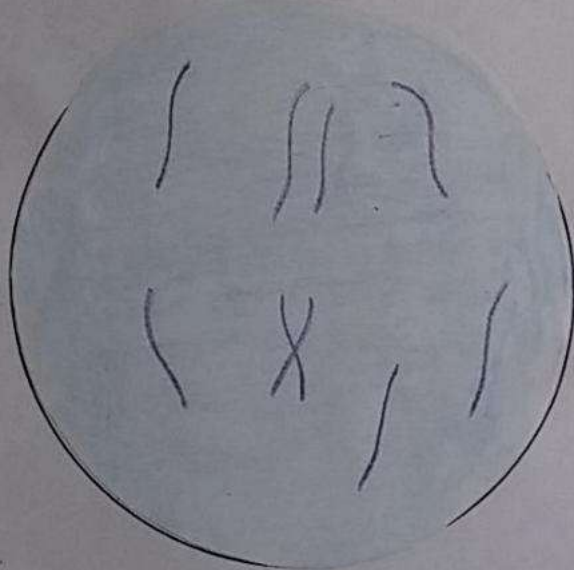
M. leprae



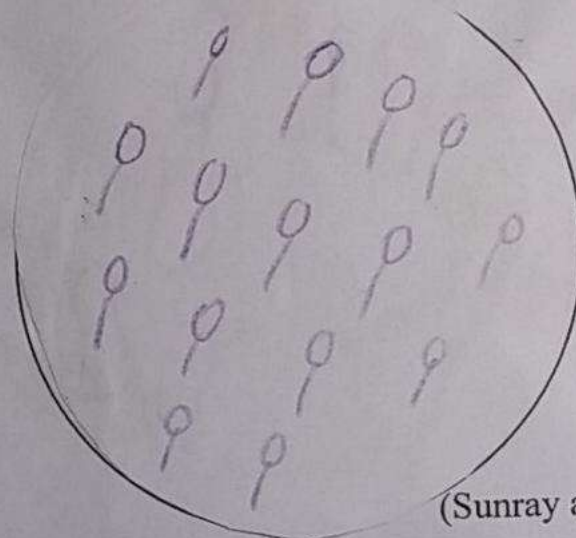
From skin clipping of a patient suffering from lapromatous leprosy showing pink rods in groups (Cigar bundle arrangement)

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Nocardia

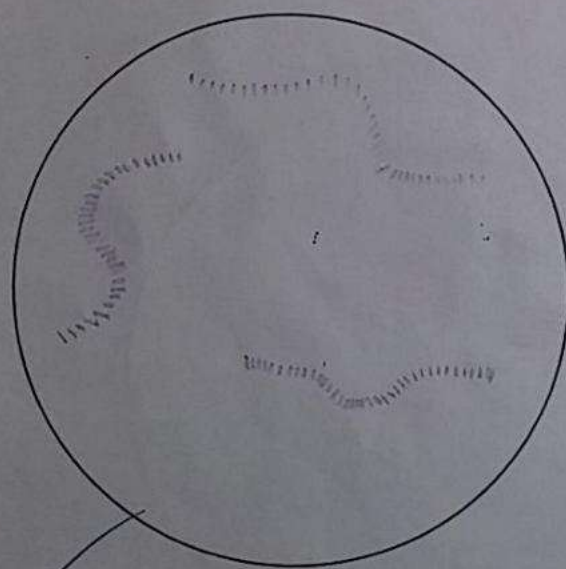


Cl. tetani (spore staining)

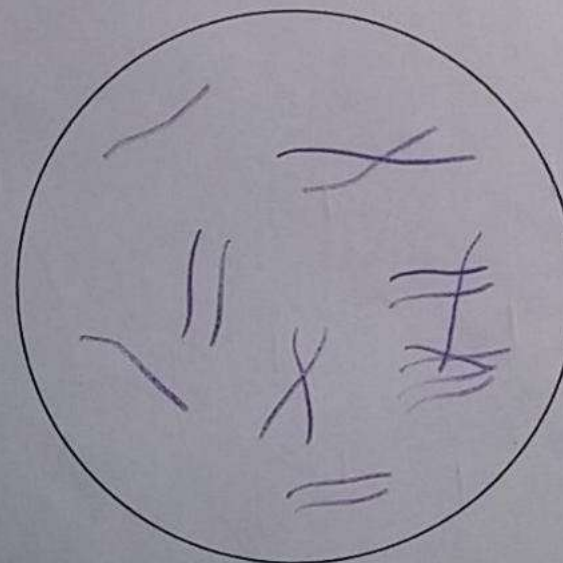


(Sunray appearance)

Spirochetes: Leptospira



Actinomycosis



Exercise

1. To which Kingdom bacteria have been included?
2. What are the differences between prokaryotes and eukaryotes?
3. Which is the unit of measurement of bacteria?
4. What are the different forms of bacteria?
5. What are the different staining methods? Who introduced staining techniques?
6. What are the examples of differential stains?
7. What are the principles of Gram's stain and Z-N stain?
8. Draw and label the following microorganisms
 - a. GPC in clusters, chains and pairs
 - b. GNC in pairs
 - c. *C. diphtheriae* with metachromatic granules
 - d. GPB with spores
 - e. AFB

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B. Chemical agents

i. Alcohols

1. Ethyl alcohol
2. Isopropyl alcohol
3. Trichlorobutanol

ii. Aldehydes

1. Formaldehyde
2. Glutaraldehyde

iii. Dyes

iv. Halogens

v. Phenols

vi. Surface active agents

vii. Metallic salts

viii. Gases

1. Ethylene oxide
2. Formaldehyde
3. Betapropiolactone

Different methods of sterilization by dry heat

Mechanism of killing of microorganisms by dry heat :

1. *Flaming :*

- An inoculating loop or wire, the tip of forceps & tearing spatulas are held in a burner flame till they become red hot.
- Inoculation loops carrying infective material may be dipped in disinfectant before flaming to prevent spattering.

2. *Incineration :*

- This method is used for safely destroying materials such as contaminated cloth, animal carcasses & pathologic materials.
- By this method, infective material is reduced to ashes by burning.
- Incineration is used for this purpose.
- Plastic such as PVC & polythene can be dealt with similarly but polystyrene materials emit clouds of dense black smoke & hence should be autoclaved.

3. **Hot air Oven**: Used to sterilise glasswares, forceps, scissors, scalpels, all glass syringes, swabs & some pharmaceutical products such as liquid paraffin, dusting powder, fats & grease. oven is electrically heated is fitted with a fan to ensure adequate and even distribution of hot air in the chamber. It is also fitted with a thermostat that maintains the chamber air at a chosen temperature.

* Temp. & Time →
160°C for 2 hrs (holding time)
170°C for 1 hrs
180°C for 30 mins.

Different methods of sterilization by moist heat

Mechanism of killing of Microorganism by moist heat

1. Temperature below 100°C

a. Water bath:

- Serum or body fluids containing coagulable proteins can be sterilised by heating for 1 hr. at 56°C in a water bath for several successive days.

b. Pasteurization of milk:

- The milk is heated at either 63°C for 30 min (Holder method) or 72°C for 20 sec. (flash process) followed by cooling quickly to 13°C or lower.
- By these process, all non-spore forming pathogens such as mycobacteria, brucellae and salmonellae are destroyed.
- *Coxiella burnetii* is relatively heat resistant and may survive the holder method.

c. *Inspissator*:

- Media such as Lowerstein-Jensen's & Loeffler's serum are rendered sterile by heating at $80-85^{\circ}\text{C}$ for half an hour on 3 successive days in an inspissator.

d. *Vaccine bath*:

- Vaccines of non-sporing bacteria are heat inactivated in special vaccine bath at 60°C for 1 hr.

2. *Temperature 100°C*

- a. *Boiling*: Vegetative bacteria are killed almost immediately at $90-100^{\circ}\text{C}$ but many spores withstand boiling for considerable time.
- Not recommended for sterilisation of instrument used in surgical procedures. Hard water should not be used. Sterilisation may be promoted by addition of 2% sodium bicarbonate to water.

b. *Koch's/Arnold's steam sterilizer*:

- An atmosphere of free steam is used to sterilise culture media which may decompose if subjected to higher temp.
- Steamer consists of a copper cabinet & the walls suitably lagged.
- The lid is conical enabling drainage of condensed steam.
- Articles are kept on perforated tray above the water level through which steam can pass. A single exposure of 90 min. ensures sterilisation (at atm pressure).

3. *Temperature above 100°C*

Autoclave: Autoclave is a modified pressure cooker/boiler. It consists of a vertical or horizontal cylinder of gun-metal or stainless steel in a supporting iron case.

Principle of autoclaving: steam above 100°C or saturated steam has a better killing power than dry heat. Bacteria are more susceptible to moist heat as bacterial protein coagulates rapidly. Saturated steam can penetrate porous material easily. When steam comes into contact with a cooler surface it condenses to water and liberates its latent heat to that surface.

Working: The cylinder is filled with sufficient water & the material to be sterilised is placed on the tray. The lid is closed & the discharge tap open. The heater is put on. Safety valve is adjusted to the required pressure. After boiling of water, the steam and air mixture is allowed to escape till all the air has been displaced. The discharge tap is now closed. The steam pressure rises inside & when it reaches the desired set level, the safety valve opens & the excess steam escapes from this point. The holding period is counted. After the holding period, the heating is stopped & autoclave allowed to cool till the pressure gauge indicates that the $P_{\text{inside}} = \text{atm pressure}$. Discharge tap is opened and air is allowed to enter autoclave. Lid is now opened.

Uses:

- 1) To sterilise culture media, rubber material, gowns, dressing gloves etc.
- 2) It is particularly useful for materials which cannot withstand the higher temp. of hot air oven.

Filtration

1. **Asbestos:** Made up of Asbestos (magnesium silicate).

- Disposal of single use discs.
- High adsorbing capacity & tend to alkalinise filtered liquids.
- Carcinogen potential of asbestos has discouraged their use.

Eg. - Suz & Sterimat.

2. **Candle:** These have been used widely for purification of water for industrial & drinking purposes. These filters are usually made in the form of hollow candles. The liq. to be filtered is passed through the candle. These are available in diff. grades of porosity. There are of 2 types:-

- 1) Diatomaceous earth filters.
- 2) Unglazed porcelain filters.

3. **Membrane:** Made up of cellulose esters & other polymers.
- Routinely used in water purification & analysis sterilisation & sterility testing & for preparing of solⁿ for parenteral use. for bacterial counts of water also it is used.
4. **Sintered glass filter:** Prepared by heat fusing finely powdered glass. Particles of graded sizes. They have low absorptive property and can be cleaned easily but are brittle and expensive.
5. **HEPA (High Efficiency Particulate Air Filters):**
- Used to deliver clean bacteria free air to a cubicle or a room.
 - Used in the air filtration in laminar air flow system in microbiology lab.
 - Can remove particles of 0.3 μm or larger.

Radiation: 2 types of radiation are used for sterilisation.

- 1) **Ionising radiation:** - Include γ rays, x rays & cosmic rays. Highly lethal to DNA. High penetration power. γ rays used for sterilisation of plastics, syringes, swabs, catheters, animal feeds, cardboard, acts, greases, fabric & metal foils.
- 2) **Non-ionising radiation:** - Em wave of wavelength longer than visible light are used (UV rays & Infrared rays) UV rays used for disinfecting enclosed areas such as OTS, lab, entry ways. Infrared rays used for rapid mass sterilisation of prepared items such as syringes and catheter.

B. Chemical Agents: Selection of the chemical agents depends on-

- a. Nature of the material to be treated e.g. -skin or utensils
- b. Types of Microorganism e.g. bacteria, viruses, spore etc.
- c. Environmental conditions e.g. Temperature, pH, time, concentration, presence of organic material etc

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5. Sulphydryl groups- chlorine and its compounds, ethylene oxide, iodine, BPL, glutaraldehyde, heavy metal salts.
6. Aminoacids- chlorine and its compounds, ethylene oxide, formaldehyde, glutaraldehyde, BPL.

Disinfection/Sterilization of various articles in the hospital/ laboratory

Item	Method of Sterilization
------	-------------------------

1	Hospital linen	Autoclave
2	Surgical gown	Autoclave
3	Gloves	Autoclave
4	Cotton	Autoclave
5	Needles	Autoclave
6	Surgical instruments	Autoclave
7	Glass syringe	Hot air oven
8	Oils	Hot air oven & γ -radiation
9	Dusting powder	Hot air oven
10	Plastic syringe	Infrared & γ -radiation
11	Endoscopes	Glutaraldehydes
12	Cardiac catheters	γ -radiation
13	Antibiotics	Seitz's filter
14	Sera	Seitz's filter

Note-All media are sterilised by autoclave. LJ medium and Loeffler's serum slopes are sterilised by serum inspissator and sugar media by Koch's or Arnold's steam steriliser.

Fumigation: Method of pest control that completely fills an area with gaseous pesticides/ fumigation to suffocate or poison the pest. It is utilised for control of pests in building, soil etc. It is used to prevent transfer of exotic organism.
eg:- formaldehyde gas.

Procedure

- Formaldehyde gas is generated by adding 150 gm of KMnO_4 to 280 ml, formalin for every 1000 cm^3 ft of room volume.
- Reaction produces considerable heat so heat resistant vessels should be used.
- Doors should be sealed & left unopened for 48 hrs. when formaldehyde vapour is generated.

- c. Dorset egg medium
- d. LJ medium
- e. Loeffler's serum slope

3. Enrichment media

For GPC

- a. Glucose broth

For Salmonella

- a. Bile broth
- b. Selenite F broth
- c. Tetrathionate broth

For Vibrio

- a. Alkaline peptone water
- b. Monsur's tauracholate tellurite peptone water

1. Simple Media :

- Nutrient broth is an example of simple medium. It contains peptone water, meat extract 1% when glucose (0.5%) is added to nutrient broth, it becomes glucose broth when 2-3% agar is added to nutrient broth, it becomes nutrient agar. This is the simplest and routinely employed medium in laboratory for diagnostic purposes.

2. Enriched Media :-

These are solid selective media. This media in addition to basic nutrients also contains nutritional supplement like blood, serum or eggs, etc. which favours growth of bacteria.

3. Enrichment Media :

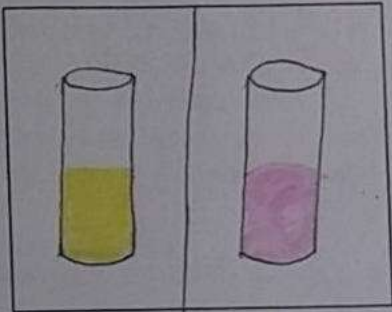
These are liq. selective media. They favour the growth of some bacteria by extending the log phase of other bacteria. (unwanted)
* Media is incorporated with inhibitory substances to suppress the unwanted organism.

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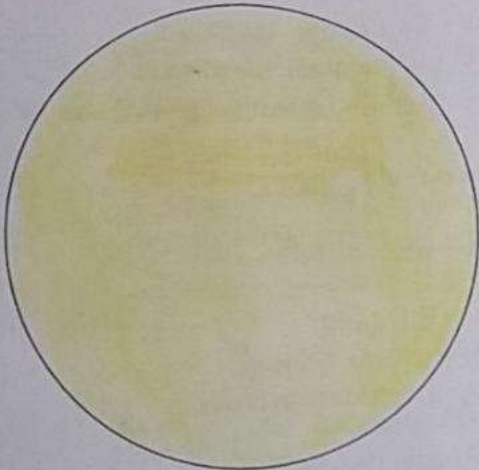
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Simple media



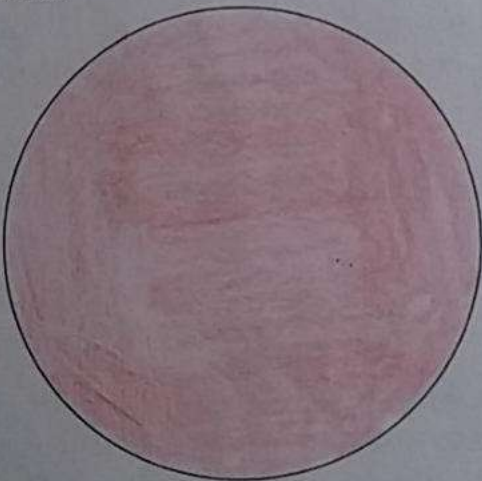
PWB

NB

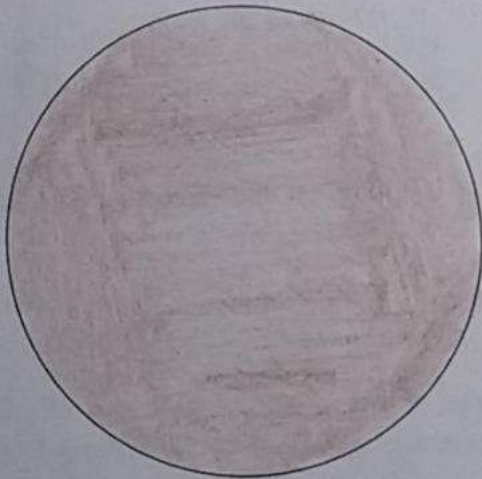


NA

Enriched



BA

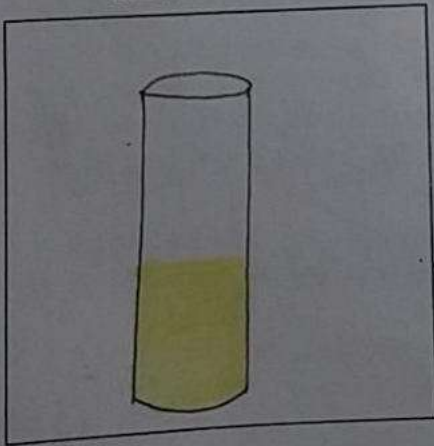


CA

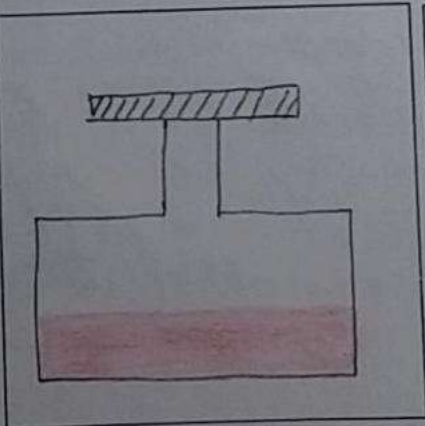


LSS

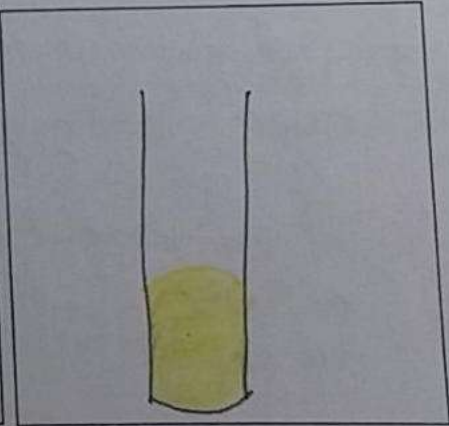
Enrichment



GB



SFB



APW

4. Selective Media

Selective media contain substances that inhibit all but a few types of bacteria and facilitate the isolation of a particular species. These media are used to isolate a particular bacteria from specimens where mixed bacterial flora is expected.

a. Salmonella

- i. Deoxycholate Citrate agar (DCA)
- ii. Wilson and Blair (WB)
- iii. Xylose Lysine Deoxycholate (XLD)
- iv. Salmonella-Shigella (S-S agar)

b. Vibrio

- i. Bile salt agar (BSA)
- ii. Mansur's GTTA
- iii. TCBS

c. *Staphylococcus aureus*

- i. Ludlam's medium
- ii. Salt milk agar
- iii. Mannitol salt agar

d. *C. diphtheriae*

- i. Potassium telurite agar (PTA)

e. *Pseudomonas spp.*

- i. Cetrimide agar

f. *Streptococcus pyogenes*

- i. Blood agar with crystal violet, nalidixic acid, colistin sulphate

g. *B. anthracis*

- i. Media containing PLET

h. *N. meningitidis*

- i. Mueller Hinton starch casein hydrolysate agar
- ii. Modified Thayer Martin medium
- iii. New York city medium

i. *N. gonorrhoeae*

- i. Thayer Martin medium

j. *Mycobacterium tuberculosis*

- i. Lowenstein Jensen medium (LJ)

5. Differential media (Indicator)

A media that has subs. incorporated in it enabling it to bring out differing characteristics of bacteria & thus helping to distinguish betⁿ them. is called a differential medium.

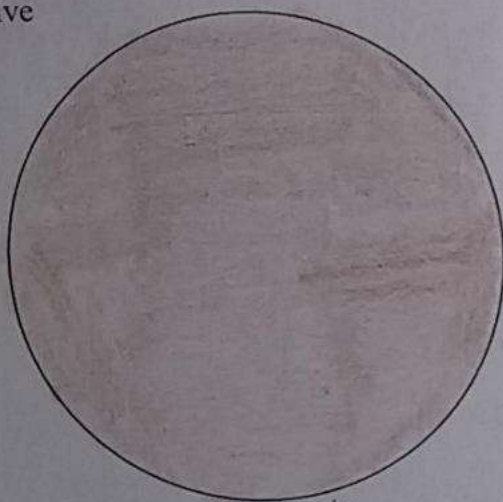
- a. MacConkey's agar. Peptone, lactose, sodium + auro cholate Agar
- b. DCA Nutrient agar, sodium deoxycholate, sod. citrate, lactose neutral Red.
- c. BA Nutrient agar, sheep blood.
- d. PT Nutrient agar, sterile pot. solution, sterile defibrinated blood

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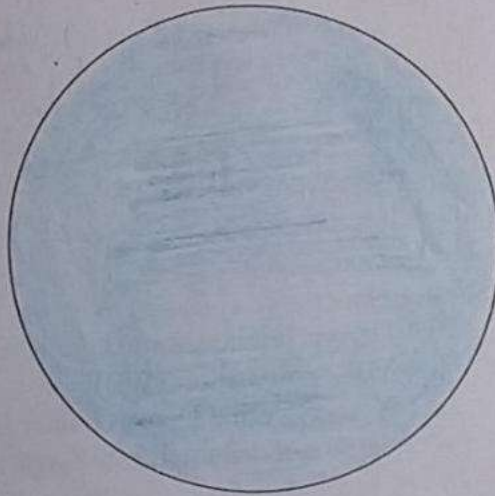
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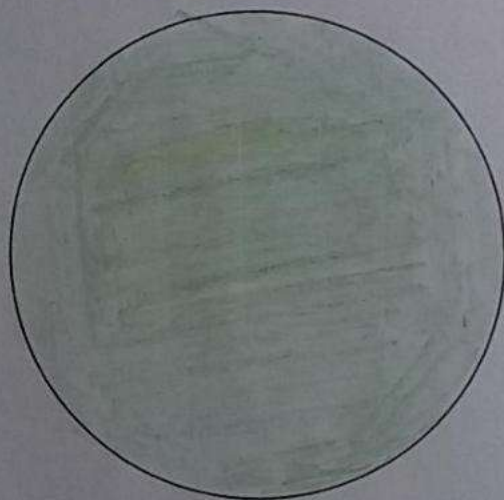
Selective



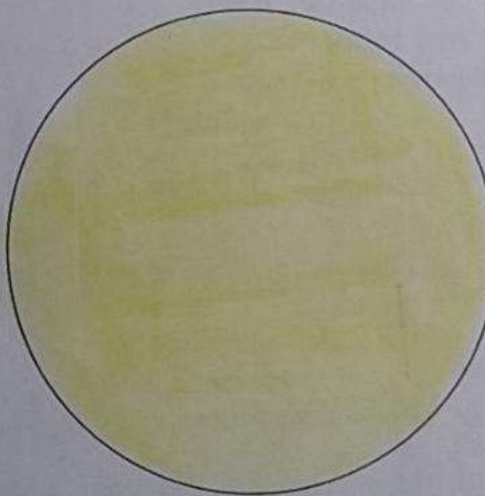
DCA



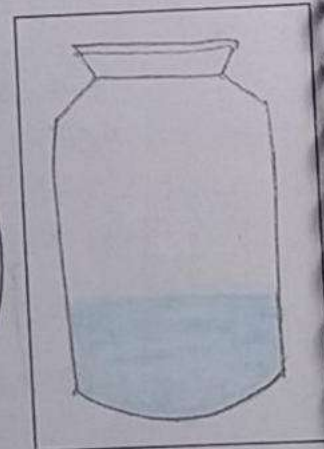
WB



TCBS

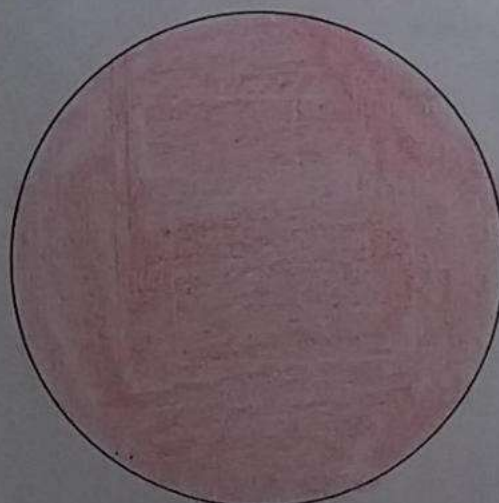


PT



LJ

Differential Media



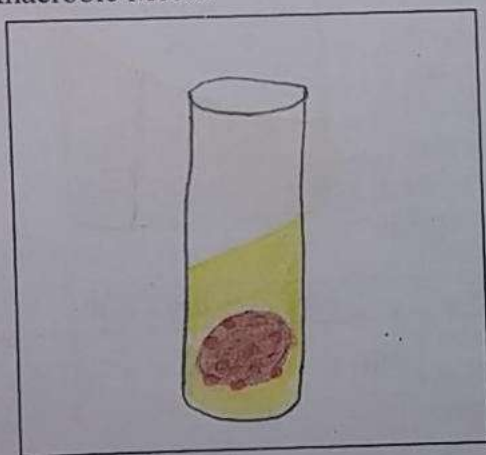
MacConkey

6. Anaerobic media

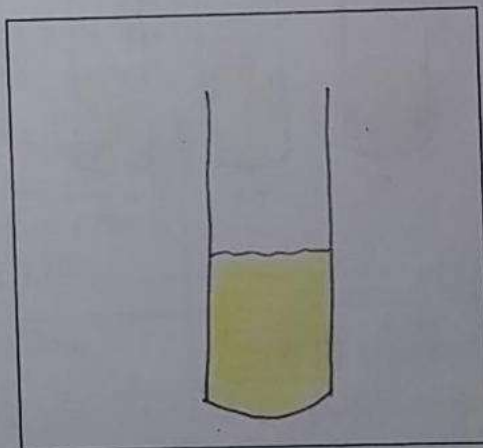
These media are used to grow anaerobic organisms, Robertson cooked meat medium. Various media to test special properties like urease production & composite media for simultaneous demonstration of diff. features have been devised.

- Liquid- Thioglycolate broth
- Solid- Brain Heart Infusion agar
- Routinely RCM (Robertson's cooked meat) broth is used
- PRAS media (Pre Reduced Anaerobically Sterilized)

Anaerobic Media



RCM

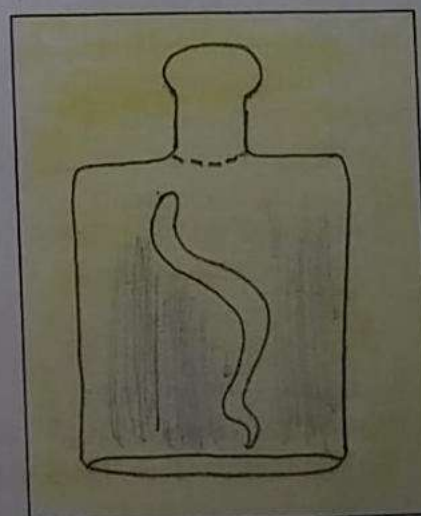


Thioglycolate broth

7. For Fungi

Used for culture of fungi.

- Sabouraud's dextrose agar (SDA)
- Cornmeal agar

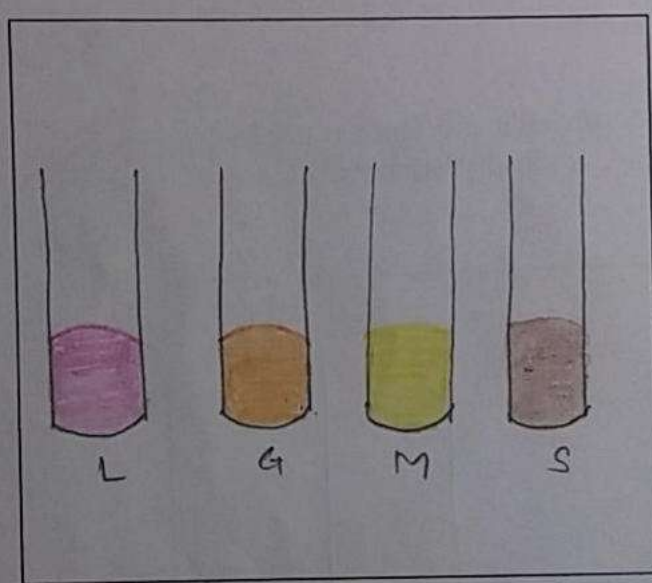


SDA

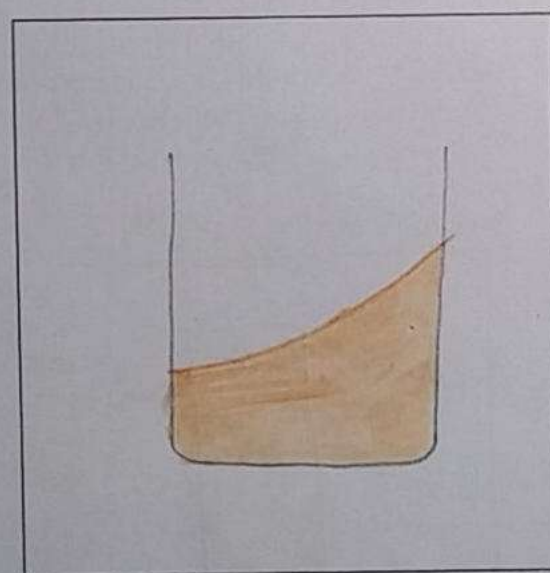
8. Sugar media

- Sugar media denotes an fermentable sub. The usual sugar media consist of 1% sugar in peptone water along with an appropriate indication.
- for organism which are active in their growth requirement its serum sugar are used containing 3% serum.

- Routinely Lactose, Glucose, Mannitol, Sucrose (LGMS) broth
- TSI (Triple Sugar Iron)



Sugars (LGMS)



TSI

9. Transport media

- In case of delicate organisms (gonococci) which may not survive the time taken for transporting the specimen to laboratory or may be overgrown by non-pathogen (such as dysentery, per cholera organisms in faeces). special media are derived for transporting the specimen.

- Salmonella- Carry Blair
- Vibrio- Carry Blair, VR
- Shigella- Carry Blair, Sach's buffered glycerol saline
- Gonococcus- Stuart's medium
- Streptococcus pyogenes- Pike's medium

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Practical Class No. 5

CULTURE METHODS

Introduction

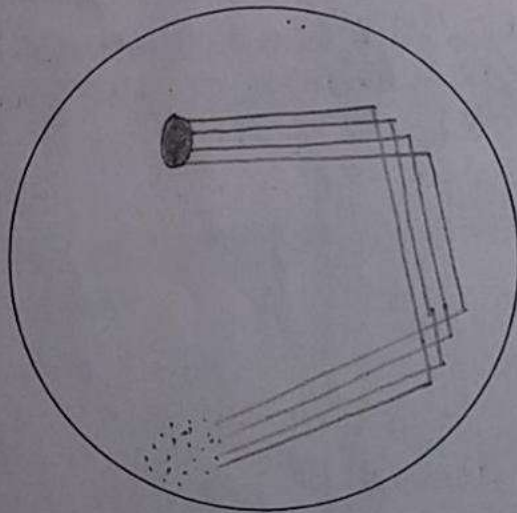
Various culture methods are required to-

1. Isolate bacteria in pure culture.
2. Demonstrate their properties.
3. Determine sensitivity to antibiotics.
4. Estimate viable counts.
5. Maintain stock culture.

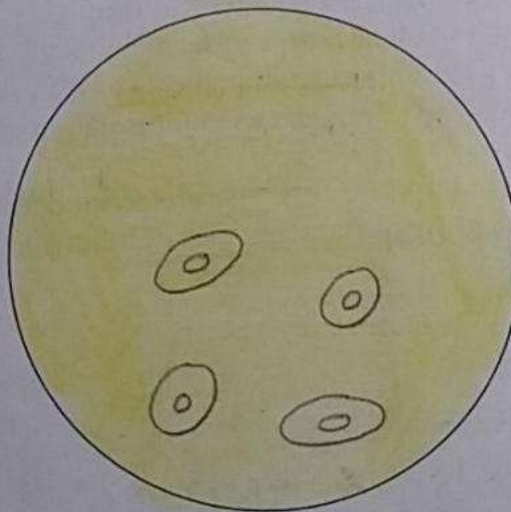
Culture Methods

1. Streak culture
2. Lawn culture (carpet culture)
3. Stroke culture
4. Stab culture
5. Pour plat culture
6. Liquid culture

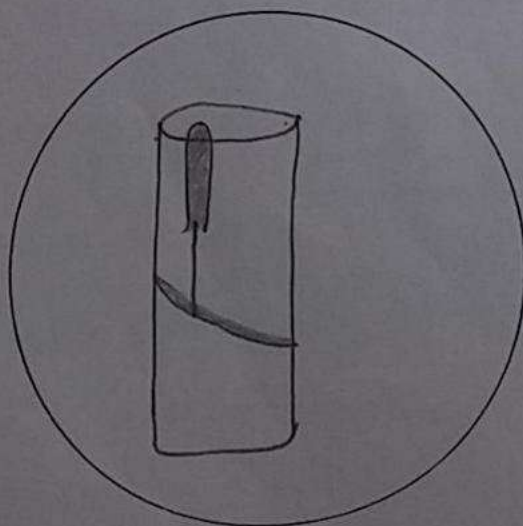
1. *Streak culture*



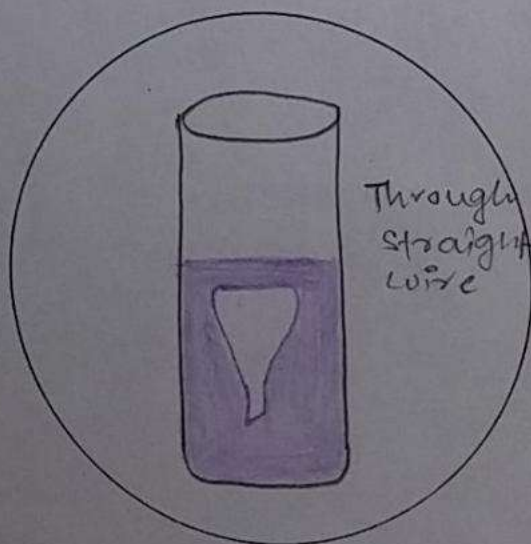
2. *Lawn Culture*



3. *Stroke culture*



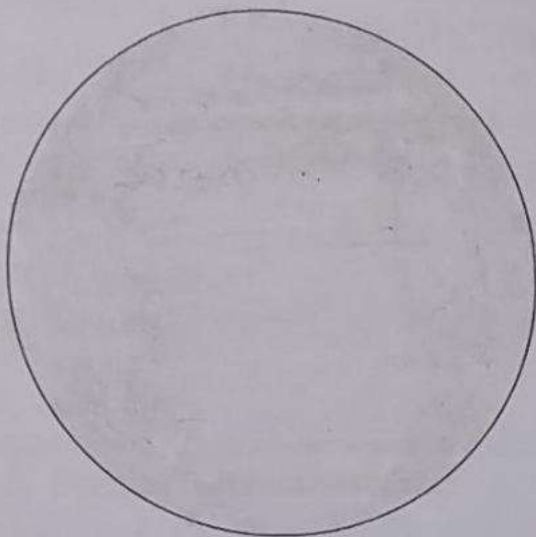
4. *Stab Culture*



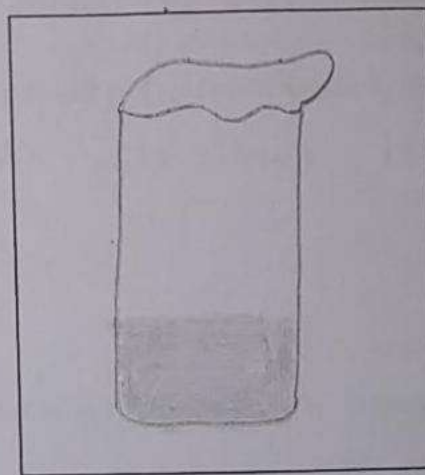
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5. Pour plate Culture



6. Liquid culture



Anaerobic culture Methods

1. MacIntosh-Filde's anaerobic jar

It consists of a metal jar on a glass jar with metal lid which can be clamped air tight. The lid has 2 tubes - gas inlet & gas outlet. The lid has 2 terminals which can be connecting to electrical supply under the lid small grooved porcelain is inoculated. Culture plates are placed inside the jar outlet supplied through inlet (for complete anaerobiosis).

2. Gaspack Jar

contains chemicals which generate H_2 & CO_2 . O_2 addition of water After the inoculated plates kept in jar the capsule envelope & water added is placed inside & lid screwed tight. H_2 & CO_2 liberated & the presence of cold catalyst in the environment produces anaerobiosis.

3. PRAS (pre Reduced Anaerobic system)

for facultative anaerobes, particular for quantitative cultures pre reduced media are available.

4. Bio-bags

Transparent, individual, disposable environmental chamber containing a gas generator consisting mixture of potassium hydroxide & palladium catalyst, indicator containing amphoteric resazurin dr.

5. Closed Glove Technique (Cabinet)

The anaerobic chamber is air tight, glass fronted cabinet. filled with inert gas, with an entry lock for the introduction & removal of materials & gloves for hands on indicator should be used for verifying the anaerobic condition in the jar. Reduced methyl blue is used generally for this purpose. It remains colourless anaerobically but forms blue on exposure of oxygen.

6. Various anaerobic Media

- RCM → Fat free mixed cooked meat in broth.
- Brain-heart Infusion Agar
- Thioglycolate broth. with & vit. K on enriched liq. medium.

Demonstration

- Streak, Stroke, Lawn, Stab, Pour plate and Liquid Culture.
- McIntosh Fildes's Jar.
- Gas pack Jar
- Bio-bags
- RCM, BHI and Thioglycolate broth.
- Photograph of anaerobic cabinet.

Exercise

- Explain the terms- Streak, Stroke, Stab, Lawn, Pour plate, Liquid culture and their uses.
- What are the different methods used for anaerobic culture?
- What is Eh (Redox potential)?
- Give examples of anaerobic indicators.
- Enumerate the anaerobes.

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1. Sugar fermentation

To determine the ability of an organism to ferment -
nt producing acid or acid with gas.

Acid production- change in colour- colourless to pink
Gas production - collected in Durham's tube.
TSI is also used for the same purposes.

2. Biochemical tests (IMViC)

a. Indole

Principle: To determine the ability of an organism to decompose amino acid tryptophan into indole. Tryptophan is decomposed by an enzyme tryptophanase produced by certain bacteria.

Procedure and interpretation

Indole production is detected by inoculating the test bacterium into peptone water (tryptophan rich) and incubating at 37°C for 48 to 96 hours. 0.5 ml of Kovac's Reagent is added to bacterial growth & gently shake.

Indole +ve: A red coloured ring near the surface of medium.

Indole -ve: Yellow coloured ring near the surface of medium.

Examples: Indole +ve: *E. coli*, *Proteus* sp. other than *P. mirabilis*, *Edwardsiella*.

Indole -ve: *Klebsiella* sp. *Proteus mirabilis*.

b. Methyl red test (MR)

Principle: This test detects the production of sufficient acid during fermentation of glucose by bacteria and sustained maintenance of pH below 4.5.

Procedure and interpretation

This test organism is inoculated in glucose phosphate broth & incubated at 37°C for 2-5 days. Then add five drops 0.4% solⁿ of methyl red. Mix well & read the result immediately.

positive - Red colour
negative - yellow colour.

Examples: MR +ve :- *E. coli*, *Persira* sp., *Listeria monocytogenes*.
MR -ve :- *Klebsiella* sp., *Enterobacteria* sp.

c. Voges-Proskauer test (VP test)

Principle: This test depends on the production of acetyl methyl carbinol (Acetoin) from pyruvic acid in the media. In the presence of alkali & at atmospheric oxygen. Acetoin is oxidised to diacetyl which reacts with α -naphthol to give Red colour.

Procedure and interpretation In this test organism is inoculated in glucose phosphate broth & incubated at 37°C for 48 hours. Then add 1 ml of 40% KOH & 3 ml of 95% solⁿ of α -naphthol in Absolute Alcohol.

Interpretation:

+ve pink colour within 2-5 minutes, deepening to crimson colour in 30 minutes.

Examples: VP +ve: *Klebsiella* sp., *Enterobacter* sp., *Enterobacter* sp., *Staphylococcus*.
VP -ve: *E. coli*, *Micrococcus*.

d. Citrate utilization test

Principle: It is the ability of an organism to utilise citrate as the sole source of carbon for its growth, with resulting alkalinity.

Procedure and interpretation

Solid or liq. media can be used & bact. colony is picked up by a straight wire & inoculated into either of these media. These inoculated media are incubated at 37°C for overnight. - Simoni's Citrate medium contains citrate and bromomethyl blue as an indicator original colour is green.

+ve - Turbidity due to growth of bact.

-ve - No turbidity

Examples:

+ve - *Klebsiella* sp, *Salmonella* sp.

-ve - *E. coli*, *Edwardsiella* *Salmonella* type.

e. Urease test

Principle: To determine the ability of an organism to produce an enzyme urease which split urea to ammonia. Ammonia makes the medium alkaline & thus phenol red indicator changes to pink/red in colour.

Procedure and interpretation

This test is done in Christensen's urease medium. The test organism is inoculated on entire slope of medium & incubated at 37°C . & examined after 4 hours & after overnight incubation.

Interpretation: positive - pink colour.

Negative - pale yellow colour.

Examples: Urease +ve - *Klebsiella* sp, *Proteus* sp, *Yersinia enterocolitica*, *H. pylori*.

Urease -ve: - *E. coli*, *Providencia* sp, *Yersinia* sp.

f. Oxidase test

Principle: To determine the +ve of an enzyme cytochrome oxidase which catalyses the oxidⁿ of reduced cytochrome by molecular oxygen.

Freshly prepared solⁿ of 1% tetramethyl para-phenylene diamine dihydrochloride is used.

Procedure and interpretation

There are diff. methods to perform this test.
i) A filter paper strip soaked in the oxidase reagent:- is smeared with test organism. In the oxidase reagent, the smeared area turns deep purple within seconds.

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oxidase +ve control should always be inoculated to find out.

The working of oxidase strip.

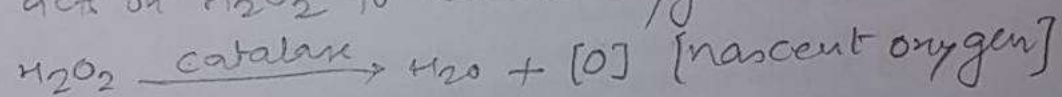
ii) Another method is to pour oxidase reagent on the surface of colonies. The colonies become purple within 10-20 min. The technique is useful to pick up *Nisseria* colonies from mixed growth on culture media. purple coloured colonies can be subcultured to get pure culture.

+ve - deep purple within 10 sec.
-ve - no colour change.

Examples: oxidase +ve - *Pseudomonas* sp., *Vibrio* sp., *Nisseria* sp., *Acetaminos* sp.
-ve - All members of enterobacteriaceae, *Stenotrophomonas*.

g. Catalase test

Principle: certain bacteria have an enzyme catalase which acts on H_2O_2 to release oxygen.



Procedure and interpretation

Pick up few colonies of test bacteria with platinum loop from nutrient agar sloped plate & mix it in a drop of H_2O_2 on clear glass slide. A positive catalase reaction produces gas bubbles immediately. Since blood contains catalase. Culture on blood containing media may result in false +ve reaction.

Examples: +ve test: enterobacteriaceae
-ve test: *Shigella dysenteriae* type 2. *Streptococcus clostridia*.

h. Other tests

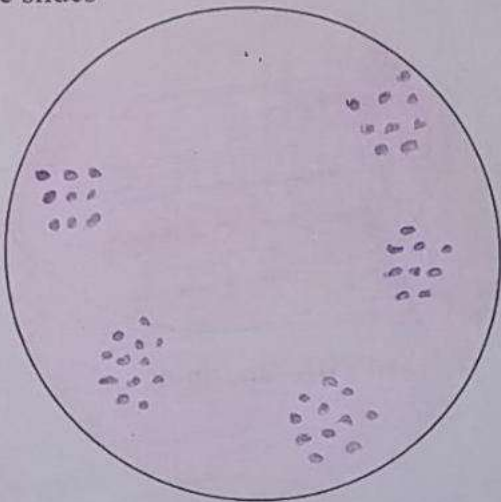
- i. H_2S production
- ii. Nitrate reduction
- iii. Gelatin liquifaction
- iv. PPA test
- v. CAMP test
- vi. Decarboxylase tests
- vii. PYR test
- viii. Bile solubility testing
- ix. Bile esculin test
- x. Bacitracin sensitivity testing
- xi. Optochin sensitivity testing etc.

MICROBIOLOGY PRACTICAL RECORDS

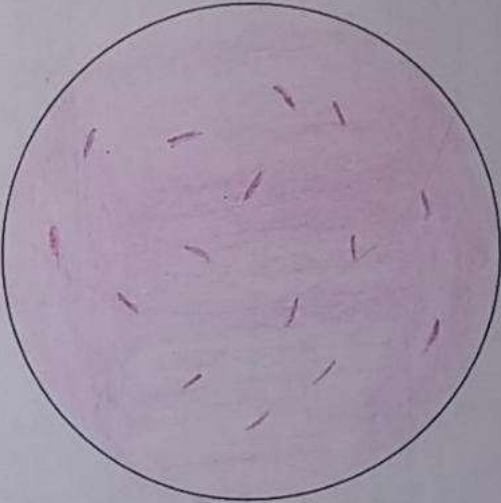
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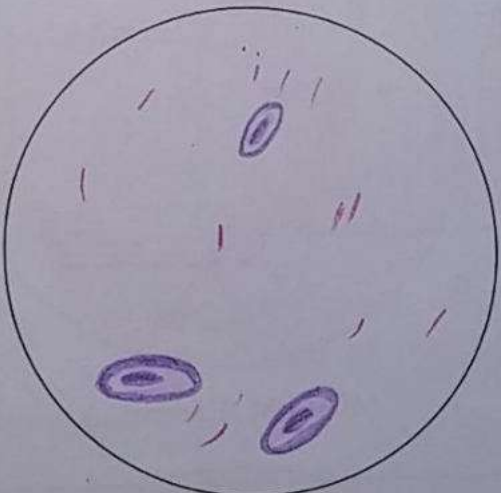
Diagrams
Microscopic slides



GPC in clusters

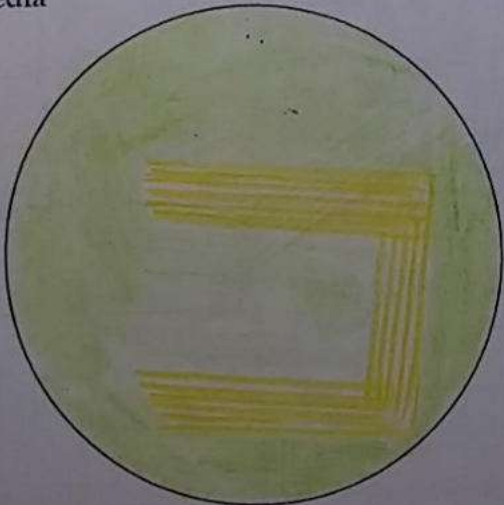


GNB

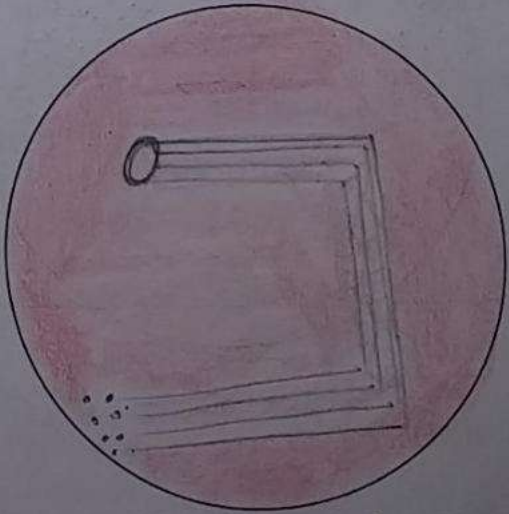


AFB

Culture media



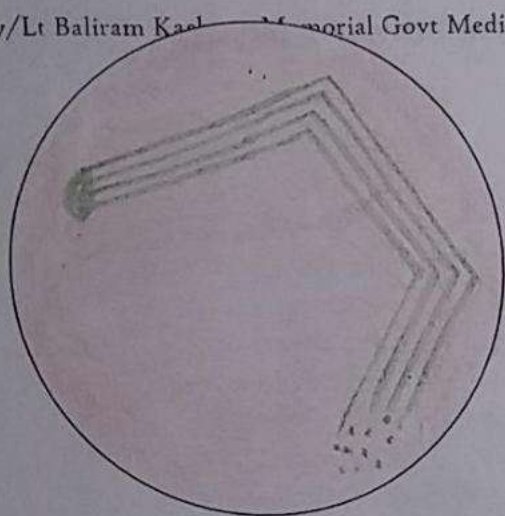
NA- pigmentation
(*S. aureus*)



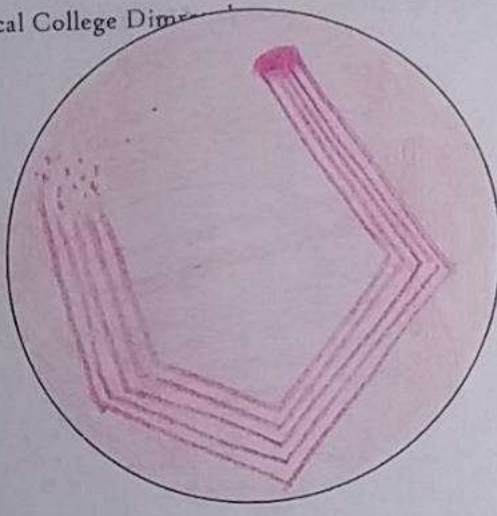
BA- β -haemolysis
(*S. aureus*)

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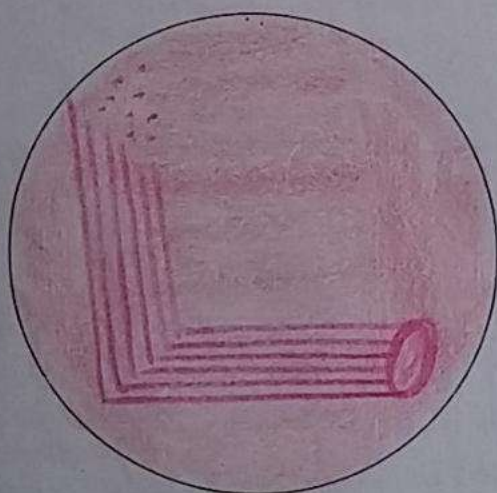
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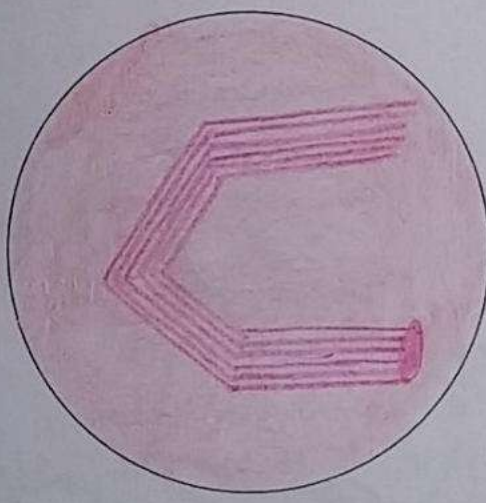
BA- α -haemolysis
(*D. pneumoniae*)



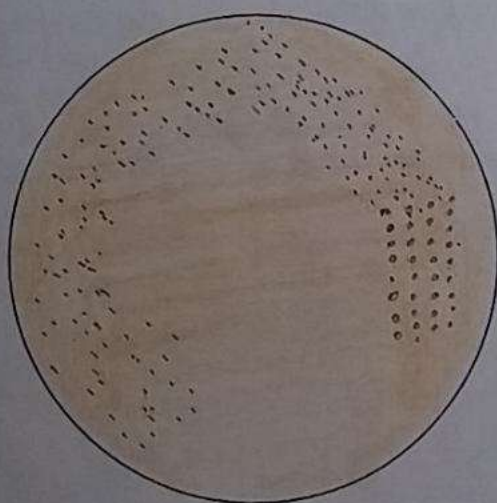
BA-swarming



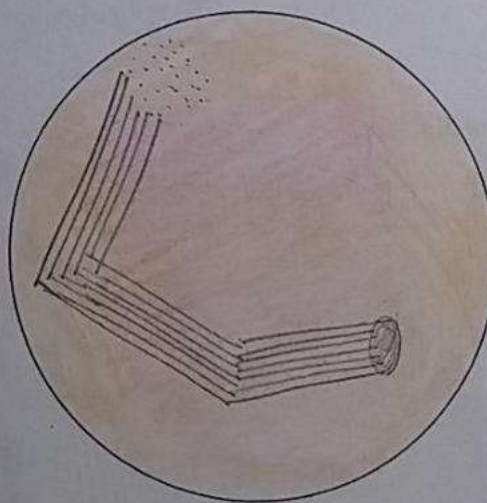
MC-LFF (*E. coli*)



MC-LFM (*Klebsiella*)



PT-black colonies
(*C. diphtheriae*)

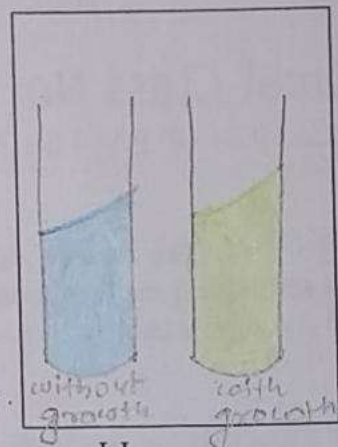


MC-NLF
(*Salmonella*)

MICROBIOLOGY PRACTICAL RECORDS

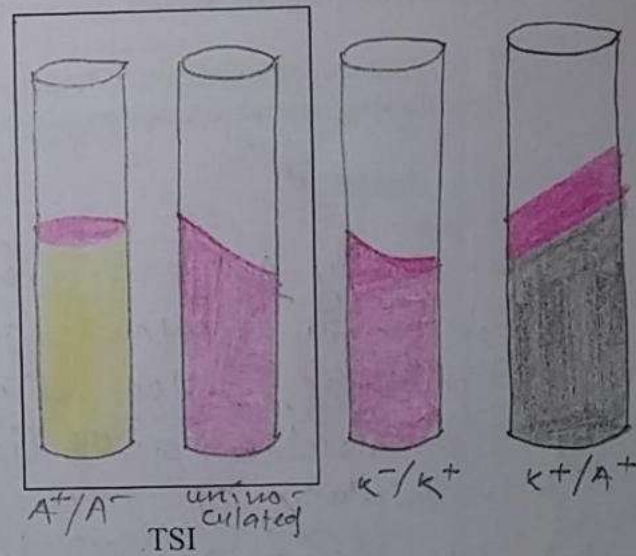
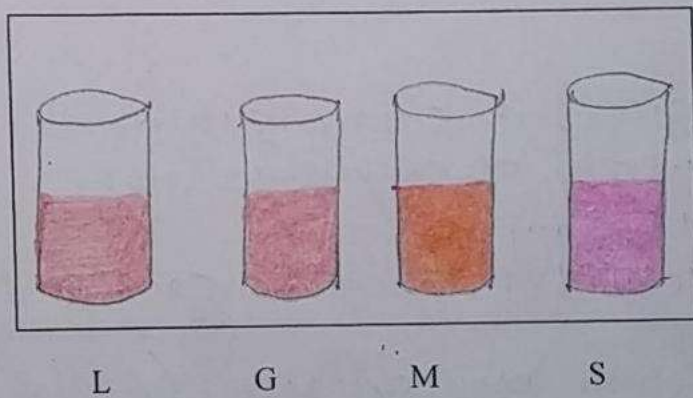
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LJ
(*M. tuberculosis*)

Sugar set
Escherichia coli

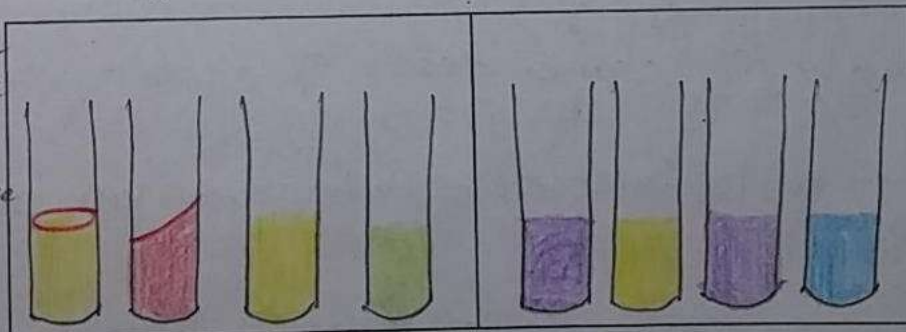


IMViC Set

(i) *Esch. coli*

(ii) *Klebsiella pneumoniae*

Indole +ve
MR +ve
VP -ve
Citrate -ve

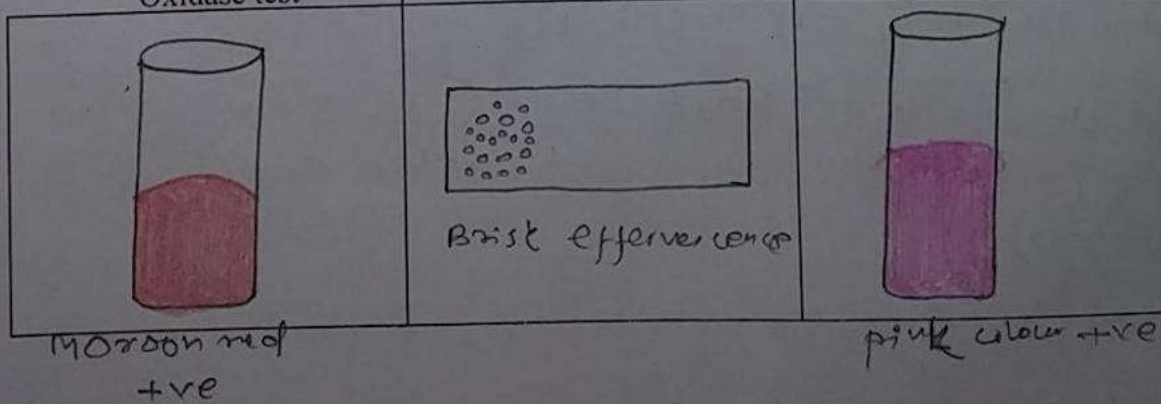


Indole -ve
MR -ve
VP +ve
Citrate +ve

Oxidase test

Catalase test

Urease test



Practical Class No. 7

IDENTIFICATION OF BACTERIA -II

IV Serology

The isolate may be subjected to serology by using antisera (if available). The method is called as slide agglutination test. It is employed for serological confirmation of some bacteria e.g. salmonella, shigella, vibrios. This is a routine method commonly used in bacteriology laboratories.

V Typing

Various typing methods are available especially in research laboratories e.g. phage typing, bacteriocin typing, serotyping, molecular typing, ribotyping etc.

VI Animal pathogenicity testing

The following animals are commonly used in microbiology laboratories

1. Rabbit

Uses

- (i) for testing Antisera (serological) in small quantities.
- (ii) for testing of toxicogenecity of *Corynebacterium diphtheriae*, *Clostridium tetani*.
- (iii) Tubercule bacilli injected into rabbit causing lesions in kidney etc.
- (iv) Identification of *Leistera monocytogene* (Anton's Test) i.e. Addition of few drops of culture broth in the conjunctive of rabbit leads to corneo conjunctivitis.
- (v) *Mycobacterium bovis* if injected intravenously or intracardially it causes death of rabbit within six weeks.
- (vi) If rabies virus is injected subdurally than antibodies are seen in hippocampus.
- (vii) *Treponema pallidum* (Nickle's stain) can be maintained in the testis of rabbit because it cannot grow in artificial medium.
- (viii) for detection of heat labile (HLS toxin & heat stable (HS) toxin for *Escherichia coli* (Rabbit gideal loop).

2. Guinea pig

Uses

1. Most susceptible animal for *Mycobacterium tuberculosis* hence used for its diagnosis.
2. Toxicogenicity testing for *Corynebacterium diphtheria*.
3. For the testing of *Clostridium botulinum*.
4. For the testing of *Clostridium tetani*.
5. Antrax test
6. Seening test
7. For the diagnosis of anthrax.
8. For the diagnosis of plague.
9. To obtain complement for complement fixation test.
10. It is required for diagnosis of endema typhus by wall moxas reaction.

3. Mouse

Uses

1. For differentiation of *Streptococcus viridans* & *Streptococcus pneumoniae*.
2. For confirmation of *Streptococcus pneumoniae*. Mouse dies within 3 days.
3. For the diagnosis of relapsing fever.
4. For the diagnosis of rickettsiae infection.
5. Toxicogenicity testing for *Clostridium tetani* (Robertson cooked meat broth) injected into tail of mouse, within 24 hrs tail become stiff.
6. For isolation of psittacosis after inoculation, after incubation in the emulsi can be.
7. Sucking mouse are used for the growth of coxsackie virus.
8. Leptrae bacilli can be multiply in the foot pad of mice at 20°C.

4. Rat 9. *Clostridium botulinum* infection the sample is injected intraperitoneally animal dies within 3 days if not injected antiserum against it.

Uses

10. Also used for toxigenicity.
 - a) Smeear test uses bacteria to test whether a given chemical can cause cancer. It is quick, convenient method.
 - b) Some eggs of human disorders eg. disease for rats are used as models are as under - hypertension, diabetes, deafness cancer.
 - c) Diagnosis of relapsing fever.
 - d) Diagnosis of rickettsiae infection.

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VII Rapid identification methods

Identification by detection of -

A.

1. Enzymes
2. Toxins
3. Antigens
4. Metabolic product of the isolates by ELISA/RIA/Immunofluorescence

B. Molecular methods

1. DNA probes (hybridization techniques)
2. Polymerase chain reaction (PCR)
3. DNA sequencing

VIII Antibiotic susceptibility test

Once the organism is isolated it is necessary to perform the antibiotic sensitivity testing to avoid the indiscriminate use of antibiotics to avoid drug resistance and to give justice to the patient by giving a proper sensitive drug

Methods:

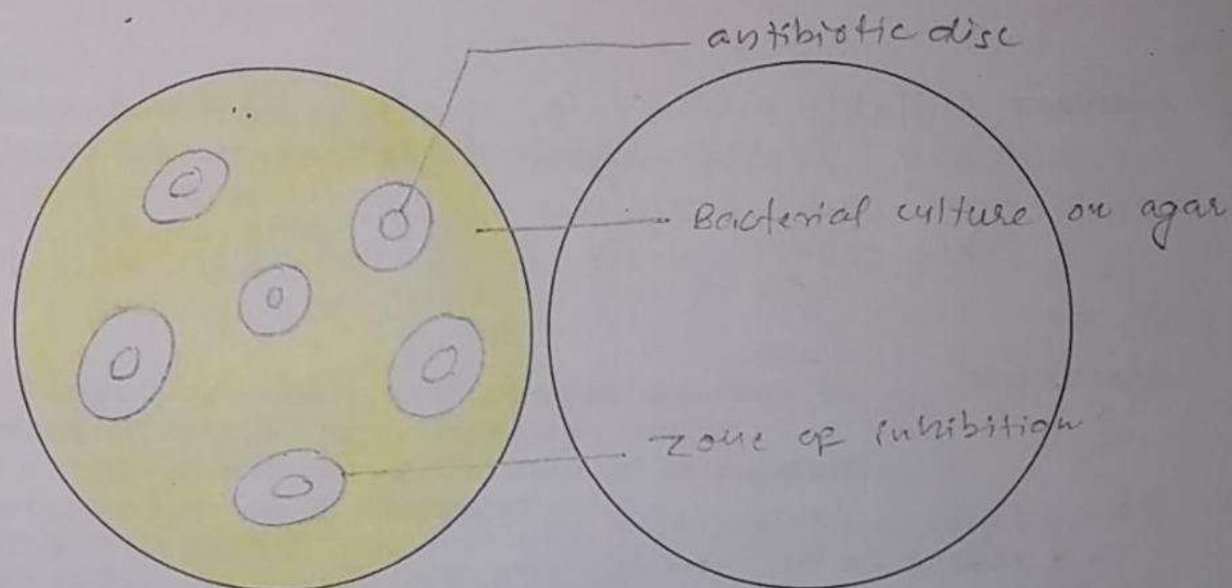
Various methods have been described but for routine following methods have been carried out -

1. Disc diffusion method

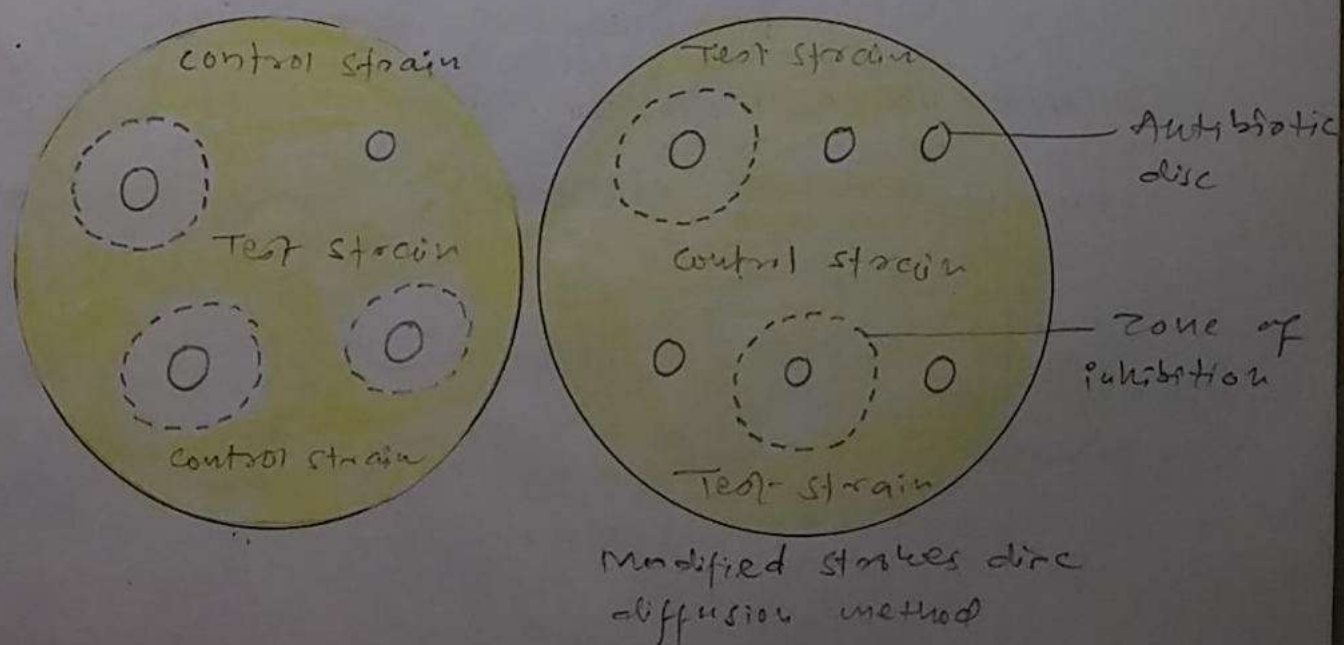
- The drug is allowed to diffuse through a solid medium.
- The disc diffusion method uses filter paper disc 6 mm. diameter changed to appropriate core of the drugs.
- The disc are stored dry in the cold. They may be prepared in the lab or purchased commercially.
- The test bacterium is seeded on the medium & its sensitivity to the drug derived for the inhibition of its growth.

a. Kirby- Bauer Method

- Procedure and interpretation** A suitable dilution (turbidity matching 0.5 Macfarland standard) of a broth culture or a broth/suspension of the test bacterium is inoculated on the surface of solid medium (muller-Hinton agar or nutrient agar) as a lawn by spreading with cotton.
- After drying the plate (37° for 30 min) antibiotic disc (4.6 mm/plate) are applied with sterile forceps.
 - After overnight incubation the degree of sensitivity is determined by measuring the zone of sensitivity of growth around disc.
 - Growth will be inhibited around disc containing antibiotic to which bacterium is susceptible.
 - It is influenced by diffusibility of drug, drug core, nature composition of substance pH so necessary to standardise all variable also necessary to check the potency of disc periodically using control standard.
 - eg:- *Aureus*, *E. coli*, *Pallidum* etc.



b. Stokes method • A standard sensitivity strain of bacteria is incubated in the middle $\frac{1}{2}$ of culture plate. Principle and Interpretation standard strains used are E. coli, ATCC 25922 or P. aureogenosa ATCC 27853 depending on bacterium to be tested. Test is inoculated over upper & lower $\frac{1}{2}$ of plate antibiotic disc are applied to test inocula & standard. comparison of zone inhibition, b/w standard & test bacteria. Indicates sensitivity of latter. Susceptible $\rightarrow$ bacteria growth inhibited at recommended growth. Intermediate susceptible $\rightarrow$ bacterial growth inhibited at higher than normal range dosage. Resistant $\rightarrow$ Bacterial growth is not inhibited even at higher than normal dosage.



Advantages Suitable method for routine use in diagnostic laboratory. Broth control & test organism can be tested on same plate under similar condition & diff. b/w zones. Sizes can be directly measured.

Disadvantages

2. Serial dilution method for MIC

Definition Serial dilution of drug are prepared and inoculated with test bacterium when therapeutic dose is to be regulated accurately on slow growing bacteria when small degrees of resistance are to be demonstrated.

Application when therapeutic doses is to be regulated accurately as in t/t of bacterial endocarditis.

- For test on slow growing bacteria such as tubercle bacilli.
- when small degree of resistance has to be demonstrated.

Definition of MBC

Minimum bacterial concentration is the lowest concentration of drug that kills bacterium.

Application

MICROBIOLOGY PRACTICAL RECORDS

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Demonstration

1. Animals- rabbits, mouse, guineapig, rat
2. Slide agglutination test- salmonella/vibrio/shigella
3. Antibiotic sensitivity testing
 - a. Kirby-Bauer method
 - b. Stokes method

Exercise

1. What are the microbiological uses of rabbit, guineapig, mouse, rat?
2. What are MIC and MBC and what are the applications?
3. What are the differences between Kirby Bauer and Stokes method?
4. What are the advantages of Stokes method?
5. What are ATCC and NCTC stains?
6. What are the applications PCR and DNA probes?

MIC: Minimum inhibitory concentration- is lowest concⁿ of drug that inhibits growth.

Serial dilution of drug in broth are taken in tubes of a standardisation. Suspension of test bacteria inoculated after overnight incubation. MIC is read.

MBC: Minimum bacterial concⁿ is lowest concⁿ of drug that kills bacteria. It can be estimated by subculturing from broth tubes that show no growth in suitable solid media.

3) Kirby-Bauer:

- Test and control have to be tested on separate plates.
- 6 discs applied on 35mm plate.
- zone diameter is measured including disc dia.

4) Stokes method

- tested on same plate.
- 4 disc on 85mm plates & 6 disc by rotatory method.
- zone diameter measured from edge of disc to edge of zone of inhibition disc.

5) ATCC stain (American type culture collection): - A layer collection of microbial strains. Including microbes containing mammalian DNA segment.

NCTC (National collection of type culture) It comprises over 3,000 bacteria culture & 100 mycobacterium cultures are supplied as dried ampoules.

6) PCR:

App → genetic testing
Tissue typing
To study mutation.

DNA probes:

- Diagnosis of infection
- Fragment of DNA/RNA of variable length.

Practical Class No. 8

ANTIGEN-ANTIBODY REACTION

Applications In The Field Of Diagnostic Microbiology

1. **Antigen (Ag)** Molecules that interact specifically with the products of immune response generated by an immunogen. that is, with antibodies B cell receptors (BCRs) and/or T-cell receptors.
 2. **Antibody (Ab)** These are glycoprotein molecules that recognise a particular epitope on an antigen, bind specifically to it & finally facilitate the clearance of that antigen. They are present on the B-cell membrane & are secreted by plasma cells.
 3. **Ag-Ab reaction** In the laboratory, they help in the diagnosis of infections. ② In epidemiological surveys they assist in the identification of infectious agents & non-infectious agents such as enzyme & in screening population for a particular infection.
 4. **Hapten** These are substances that are incapable of inducing antibody formation, but can react specifically with antibodies. But these become immunogenic on combining with a larger molecule carrier.
 5. **Epitope** The smallest unit of antigenicity is known as the antigenic determinant or epitope. It is a small area on the antigen consisting of 4-5 AA or mono-saccharides residues.
 6. **Immunogens** A sub. which induces a detectable immune response. It stimulates the immune reaction, resulting in either the production of antibodies/activation of T-cells finally leading to immune response.
 7. **Precipitation** When a soluble antigen combines with its antibody in the presence of electrolytes at a suitable temperature & pH, the Ag-Ab complex forms an insoluble precipitate.
 8. **Agglutination** Agglutination test is used for the sero-diagnosis of Brucellosis, typhus fever, typhoid (Tube agglutination test).
- ② Streptococcus MC agglutination test for the diagnosis of primary, atypical pneumonia.

9. Zone Phenomenon

(Prozone, postzone, zone of equivalence & lattice formation)

Pre-zone phenomenon: - This is caused by excess antibody in the test system of a visible reaction is due inhibition of lattice formation by the excess antibody.

Zone of equivalence: Here the antigen & antibody are in optimum proportion. Lattice formation and visible reactions are enhanced.

Lattice formation: Marrack proposed the lattice hypothesis. A/c to this concept multivalent antigen combine with bivalent antibodies in varying proportions, depending on the antigen-antibody ratio in the reacting mixture.

10. Infection The lodgement & multiplication of a parasite in or on the tissue of a host constitute infection.

11. Pathogen & Pathogenicity pathogen are micro-organism that are capable of producing disease in the host.

Pathogenicity: - It refers to the ability of a microbe to produce disease or tissue injury but it is important to make a distinction b/w them.

12. Virulence, Attenuation and Exaltation Virulence → The property of having ability in a stream of micro-organism to produce disease.
Enhancement of virulence — is k/a exaltation.
Reduction of virulence is — attenuation.

Introduction:

To produce an infectious disease a pathogen must-

1. Adhere to the body structure
2. Penetrate into the deeper tissue
3. Enter into the lymphatic system and blood
4. Multiplication and production of toxins and toxic products
5. Must overcome the host defense mechanism and produce damage to the host

In this process we can identify the antigen and antibodies by various methods as follows:

1. Precipitation reaction

Ag = slight - ve.
Ab = - - - +ve

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2. Agglutination reaction
3. Complement fixation test (CFT)
4. Immunofluorescence
5. Radio immunoassay (RIA)
6. Enzyme linked immunosorbent assay (ELISA)

For identification of microbes, molecular methods, such as PCR and other amplification procedures coupled with nucleic acid probes carrying specific DNA or RNA based sequence have been developed.

Various Ag-Ab based methods

I Precipitation Reaction

When a soluble antigen combine with its antibody in the +ve of electrolytes (NaCl) at a suitable temp. & pH, the antigen and antibody complex forms an insoluble precipitate.

Application- Precipitation reaction may be carried out in liquid or gel e.g.

Liquid

1. Ring test- Ascoli's thermoprecipitin test, Lancefield grouping of streptococci
2. Slide test- VDRL- flocculation test
3. Tube test- Kahn flocculation test

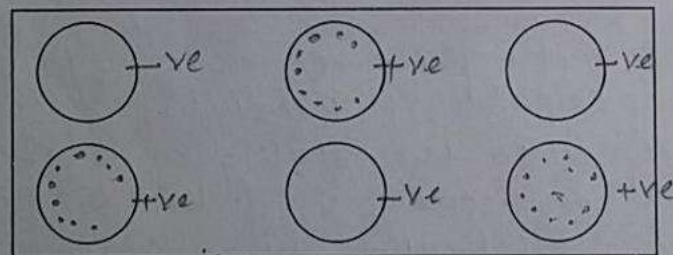


Diagram - VDRL tile showing flocculation

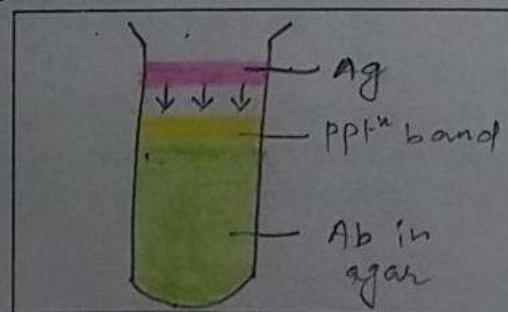
Precipitation in gel-

When, instead of sedimenting the ppt remain suspended as floccules, the rxn is known as flocculation. It can occur in agar, agarose or polyacrylamide.

Precipitation may be carried out in agar, agarose or polyacrylamide gel e.g.

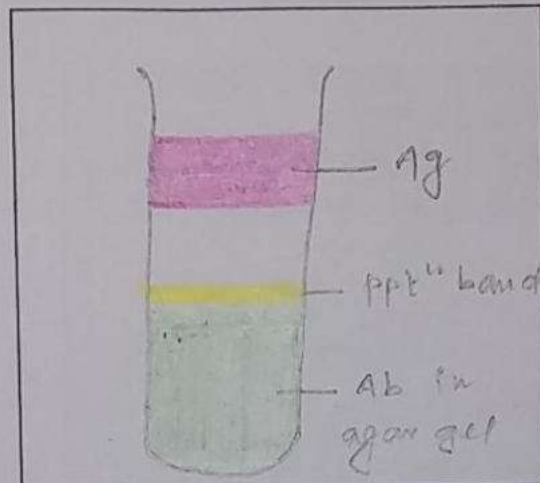
1. Immunodiffusions

- a. Single diffusion in one dimension (Oudin procedure)

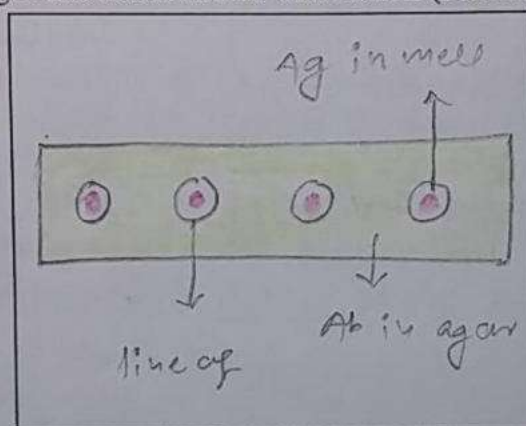


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b. Double diffusion in one dimension (Oakley- Fulthrope procedure)



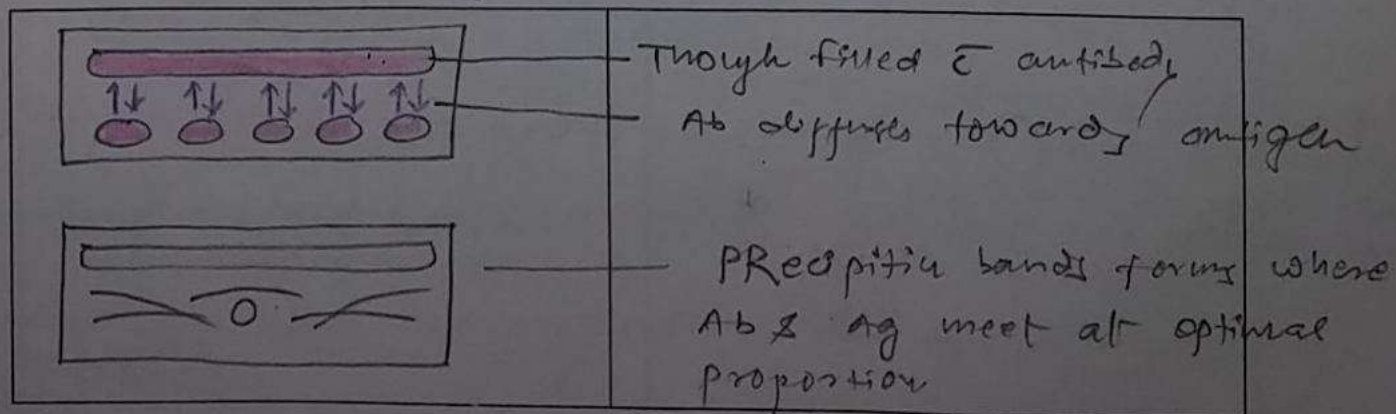
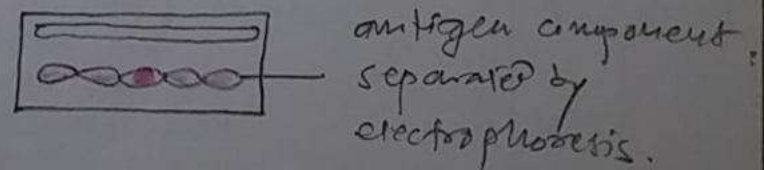
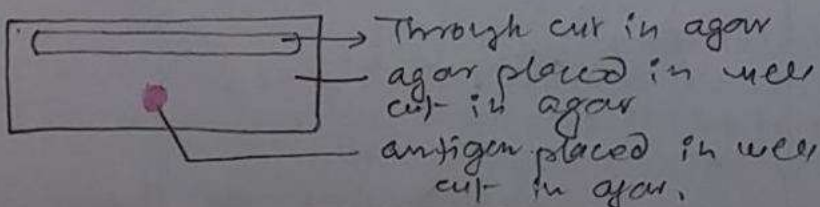
c. Single diffusion in two dimension (Radial immunodiffusion)



d. Double diffusion in two dimension (Ouchterlony procedure)

e. Immunoelectrophoresis

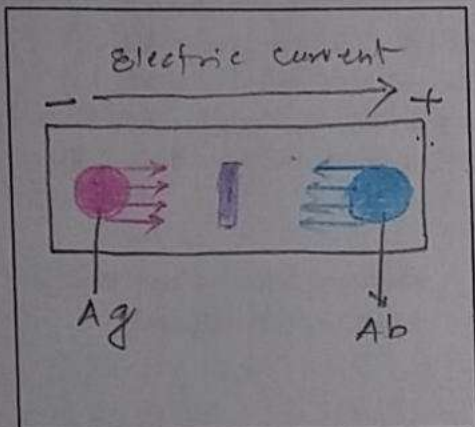
Procedure: This is done on glass slide layered with semisolid agar, a well is cut and Ag. is filled. The 1st step is electrophoresis of Ag for about 1 hr. Rectangular trough is cut in the agar parallel to the dirⁿ of migration of antigen & filled wth Ab diffusion is allowed to proceed for 18-24 hrs. pptⁿ lines develop with each separated component of the antigen.



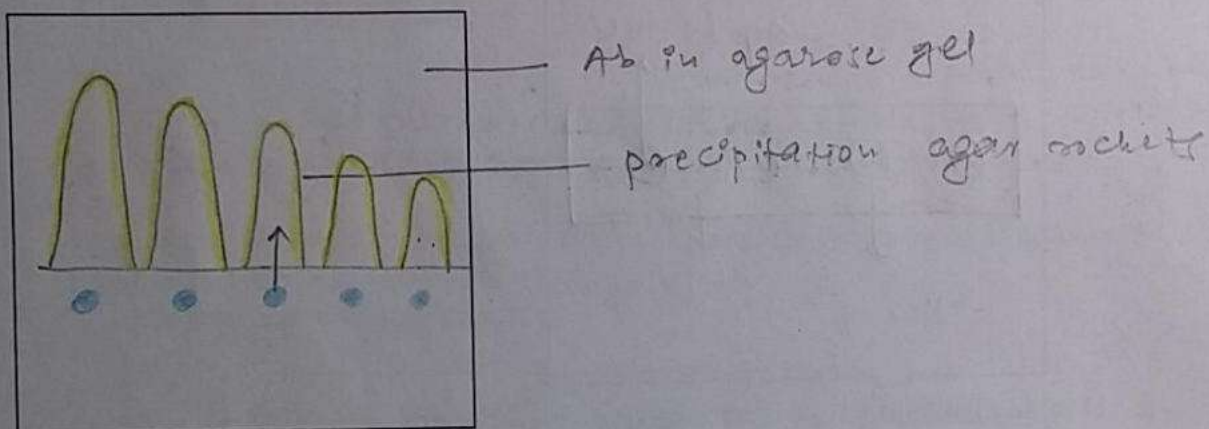
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2. Electroimmunodiffusion

a. Counterimmunoelectrophoresis

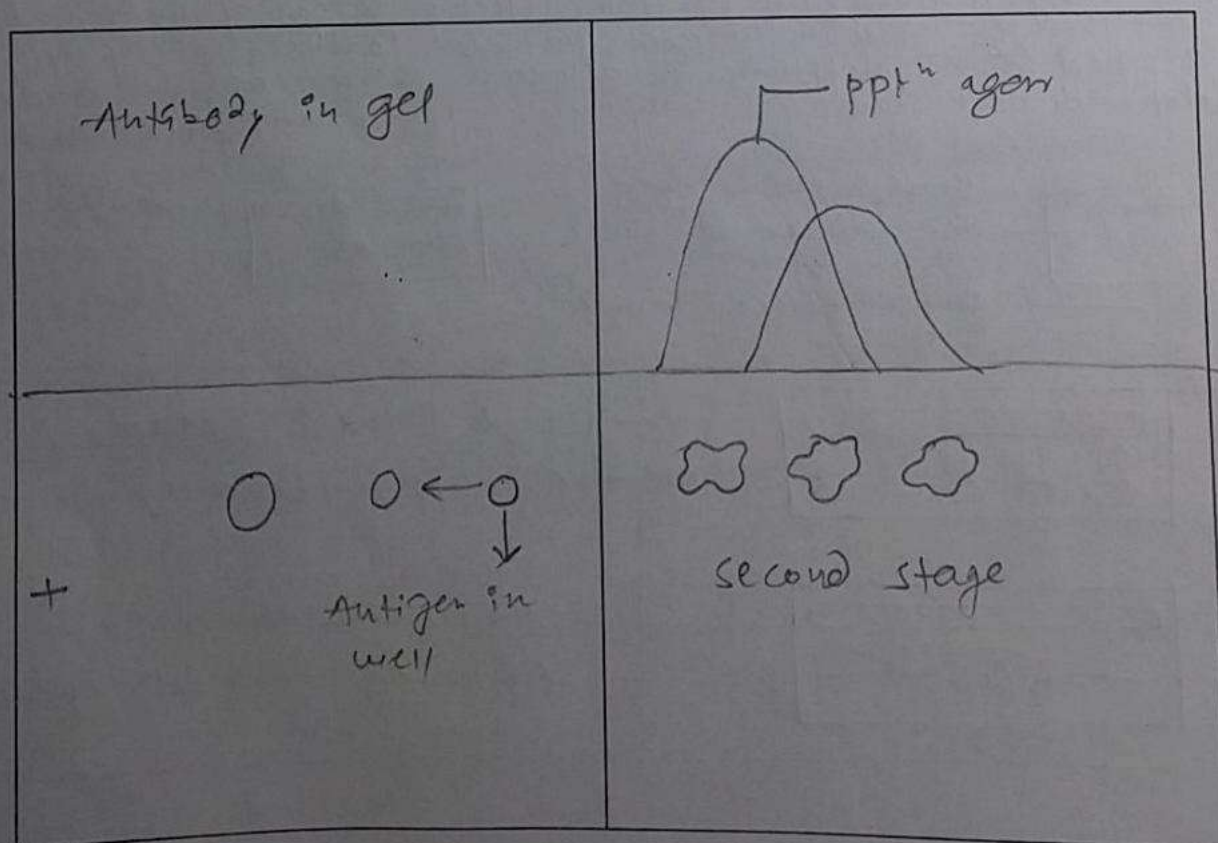


b. Rocket electrophoresis



c. Laurell's modification of rocket electrophoresis

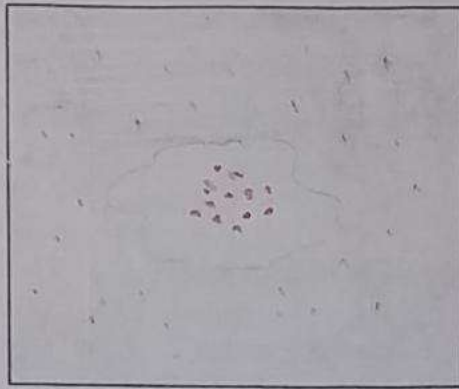
It is used for quantitative estimation of several antigens in a mixture.



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II Agglutination reaction- It is of 2 types-

1. Slide agglutination



2. Tube Agglutination

a. Widal test

b. Agglutination test for Brucellosis and typhus fever

In the widal test used in typhoid two layers of antigens are used. The 'H' or flagellar antigen on combining with its antibody forms large, loose, fluffy clumps resembling cups of cotton wool. The 'O' or somatic antigen forms tight, compact deposits resembling chalk powder. agglutinated bacilli spread out in a disc like pattern at the bottom of the tubes.

Tube agglutination is routinely applied for diagnosis of typhoid, Brucellosis & typhus fever, complications include prozone phenomena & blocking antibodies.

Example of Heterophile agglutination tests-

- I. Weil-Felix reaction- Rickettsial disease
- II. Paul-Bunnell test- Infectious mononucleosis
- III. Streptococcus MG test- Primary atypical pneumonia
- IV. Cold agglutination test- Primary atypical pneumonia

3. Passive agglutination test

The only diff. b/w pptⁿ & agglutination tests is the physical nature of antigen. By attaching soluble antigen to the surface of carrier particles, it is possible to convert precipitation test into agglutination test, which are more convenient & more sensitive for the detection of antibodies. such test are known as passive agglutination test.

The commonly used carrier particles are red cells, latex particles, or bentonite.

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4. *Reverse passive agglutination test*

When instead of antigen, antibody is adsorbed to carrier particles in tests for the estimation of antigen, the technique is known as passive agglutination test. This method is used to diagnose bacterial antigens like *Legionella*, *S. pyogenes* & *N. gonorrhoea* in clinical samples.

5. *Hemagglutination test*

A special type of passive hemagglutination test is the Rose-Wodder test. In rheumatoid arthritis, an antibody CRF factor appears in the serum, which acts as an antibody to gamma globulin. The CRF factor is able to agglutinate red cells coated with globulin. The antigen used for the test is a suspension of sheep erythrocytes sensitised with a sub-agglutinating dose of rabbit & sheep erythrocyte antibody (antiboreceptor).

6. *Hemagglutination Inhibition test* done mainly for detection of Ab.

eg:- Some Avian viruses tend to dump $\bar{c}$ specific Ab $\bar{c}$ blocks RBC to adhere.

III *Complement fixation test*

IV RIA-

Radioimmunoassay depends on the labeling of antibody or antigen with ^{125}I . When Ab and labeled Ag combine a complex is formed. Bound and unbound antigens are separated by centrifugation and the complexed radioactivity is found in the centrifuged pellet where it can be counted by a gamma counter.

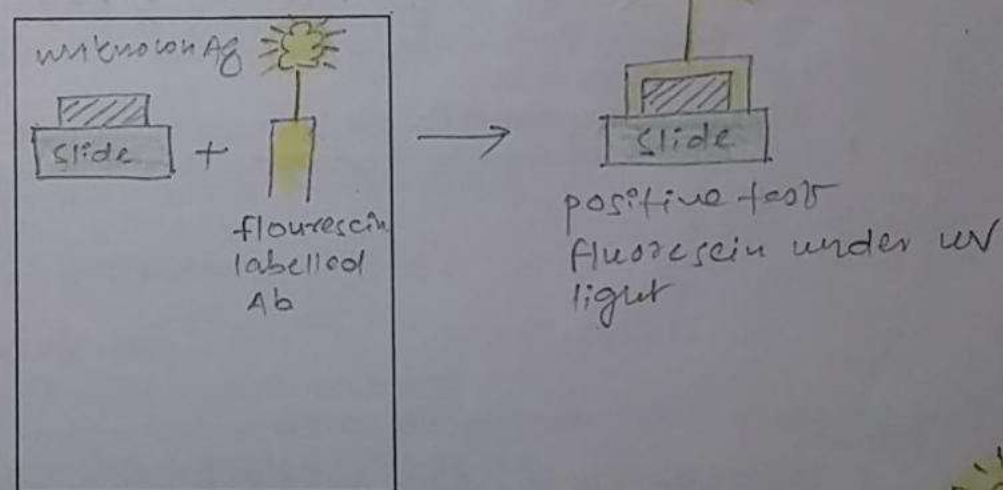
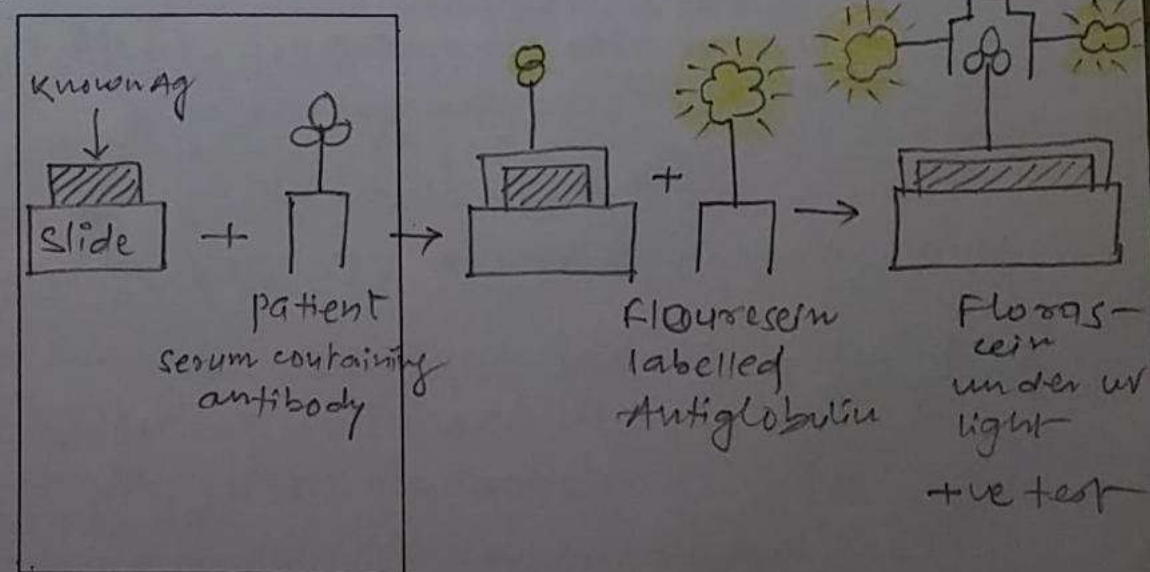
The concentration of Ab or Ag present in the unknown specimen is measured by referring to a standard curve.

Applications-

1. Quantitation of hormones
2. Drugs
3. Tumour markers
4. IgE
5. Viral antigens

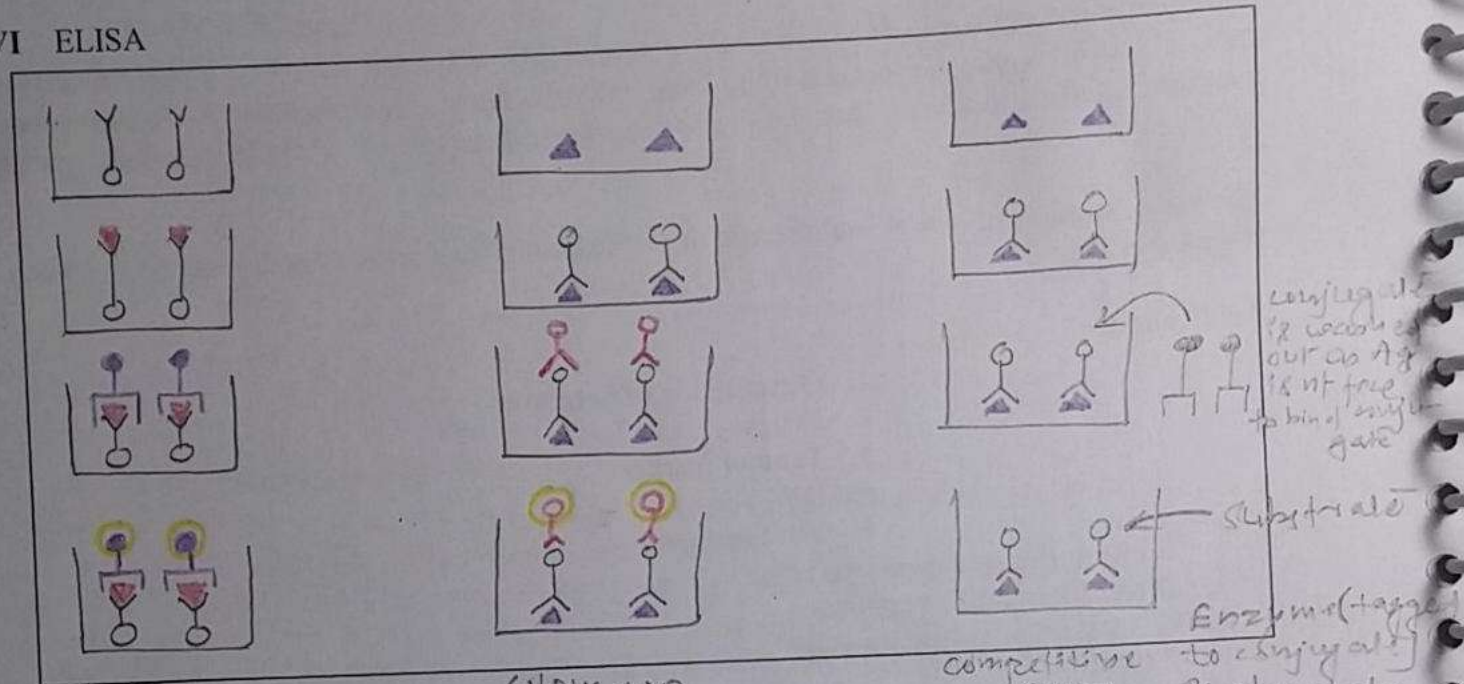
V Immunofluorescence- two types

1. Direct immunofluorescence
2. Indirect immunofluorescence

1. Direct Immunofluorescence**Indirect Immunofluorescence**

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VI ELISA



Colour +ve
Sandwich
ELISA
Demonstration

Colour +ve
Indirect ELISA

Competitive
ELISA

Enzyme (tagged)
to conjugate
is absent to
act on substrate

- Legend: Δ - antigen, Y - conjugate, Y - Ab, Y - conjugate/No colour +ve
1. Various precipitation reactions such as Ring test, VDRL, RIA, Serum electrophoresis, CIEP, Rocket electrophoresis.
 2. Agglutination reaction- Widal test, Latex agglutination test (RA factor, HbsAg)
 3. Demonstration of diagrams of various Ag-Ab reactions (e.g. immunofluorescence, ELISA, CFT)

Exercise

1. Define various terms- Ag, Ab, Ag-Ab reaction, Immunogen, Tolerogen, Hapten, Epitope, Antigenic determinants, Precipitation, Agglutination.
2. Enumerate various precipitation reactions with their applications.
3. Enumerate various agglutination reactions with their applications.
4. Enumerate various advance methods for detection of Ag/Ab/Hormone/Enzymes in reference to the diseases.

Agglutination: When a particulate Ag is mixed with it in the of electrolytes at a suitable temp. eg- pH, the particles are clumped or agglutinated.

Ring test - clinical application.
eg- Ascoli thermoprecipitation test & grouping of streptococci by Lancefield technique.

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Infections caused by *Staph. aureus*

Pyogenic Infection	Toxins Mediated Infection
- Boils, carbuncles, wound infection, abscesses. - Bacteraemia - Osteomyelitis - Pneumonia	- Scalded Skin Syndrome - Pemphigus neonatorum - Impetigo, Bullous impetigo - Toxic Shock syndrome - Food poisoning

Pathogenesis :

It is present in the nose of 30% of healthy people and may be found on the skin. It causes infection when resistance of the host is lowered e.g. damaged skin or mucous membranes.

Virulence factors of the *Staph. aureus*

Virulence Factor	Activity
1. Cell wall polymers a. Cell peptidoglycan c. Capsular polysaccharides b. Teichoic Acid	Inhibits opsonisation. Inhibits inflammatory response, Endotoxin like activity. Phage adsorption, facilitates adhesion
2. Cell surface proteins a. Protein A b. Clumping factor	Reacts with Fc portion of IgG. Binds to fibrinogen
3. Exoproteins a. Haemolysins b. Leucocidins	Lysis of RBCs Lysis of WBC and inhibition of phagocytosis.
c. Epidermolytic toxin	Blistering of skin
d. Enterotoxin	Food poisoning, Toxic shock syndrome
e. Coagulase	Converts fibrinogen in to fibrin, inhibits phagocytosis
f. Hyaluronidase	Degrades hyaluronic acid in connective tissues
g. Staphylokinase	Degrades fibrin
h. Deoxyribonuclease	Degrades DNA
i. Lipases	Degrade lipids
j. Phospholipases	Degrade phospholipids
k. Proteases	Causes proteolysis

Laboratory Diagnosis

Collection of the specimens-

1. Pus from abscess, wound, burns etc. Preferably by syringe and needle than swabs.
2. Sputum from a case of lower respiratory tract infection (pneumonia).
3. Faeces, vomitus or food- in case of food poisoning.
4. Blood – in case of bacteraemia (osteomyelitis, septic shock, Sub-acute endocarditis)
5. Midstream urine- cystitis, pyelonephritis.
6. Nasal and perineal swab for detection of carriers.

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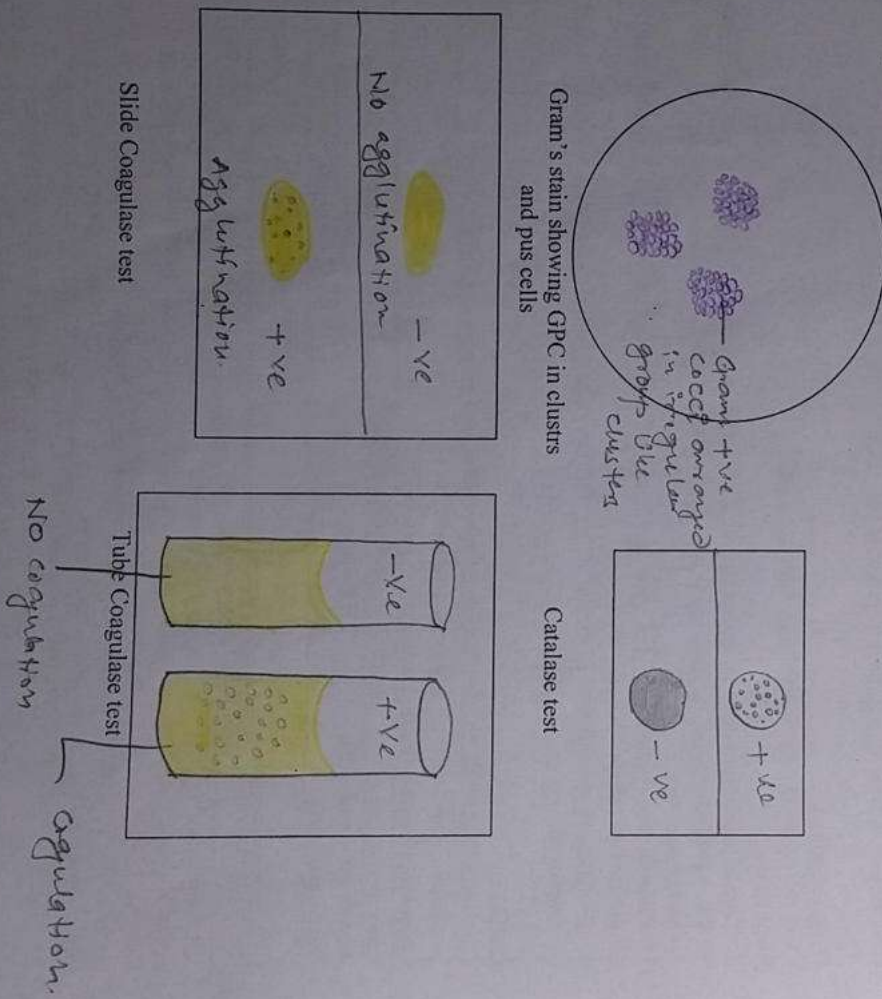
Demonstration

1. Microscopic slides showing Gram positive cocci and pus cells in Gram's staining.
2. Catalase test, slide coagulase, tube coagulase test.
3. Mannitol fermentation anaerobically
4. Growth of *Staphylococcus aureus* on - NA, BA, NA slope.
5. Antibiotic sensitivity testing.

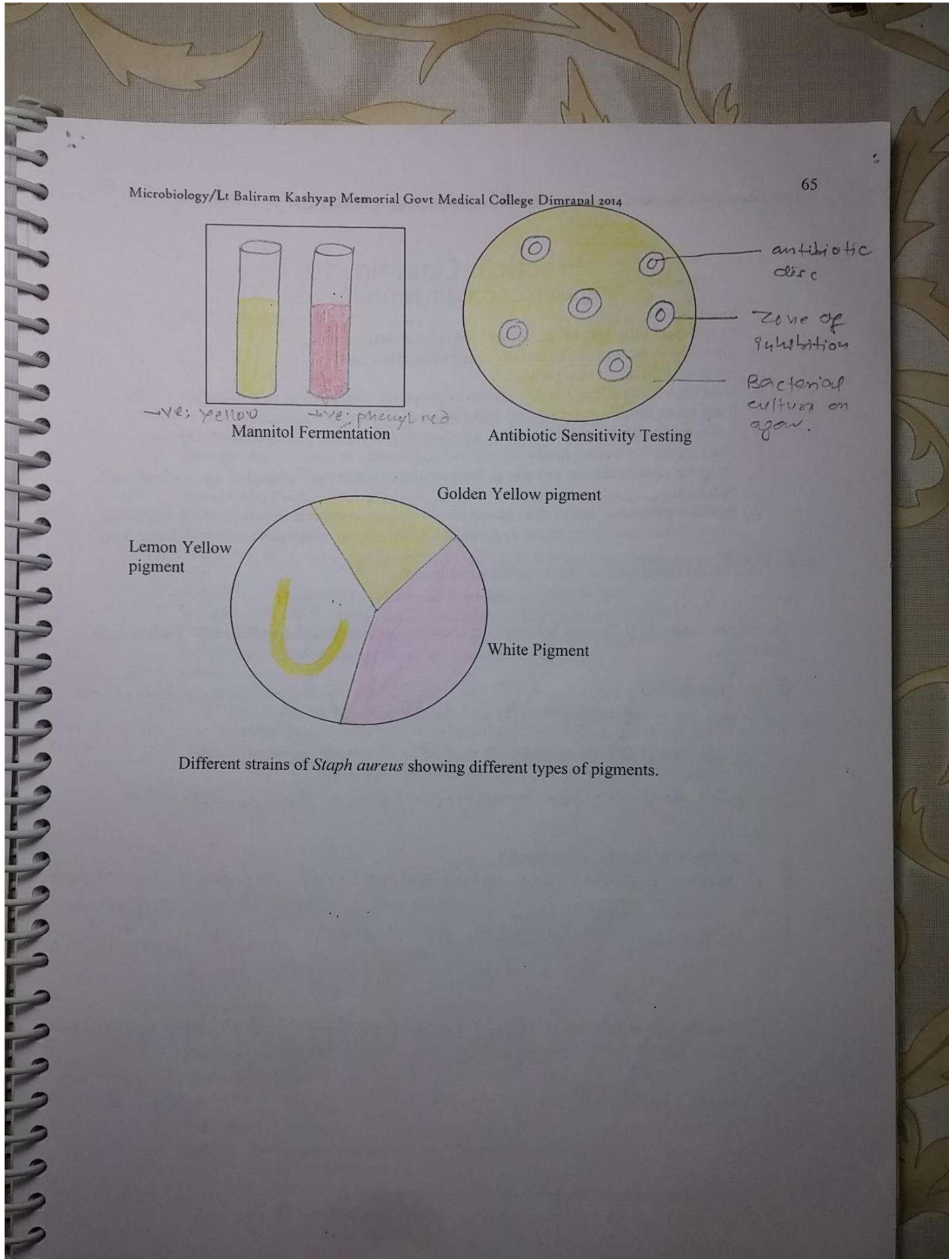
Exercise

1. Perform Gram staining of the smear and mention the result.
2. What are the different lesions produced by *Staphylococcus aureus*.
3. What are the different virulence factors?
4. What are the different mechanisms of drug resistance in *Staphylococcus aureus*?
5. How staphylococci are classified?
6. Describe laboratory diagnosis of pyogenic lesions caused by *Staphylococcus aureus*.

Diagrams:



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Practical Class No. 10

STREPTOCOCCI AND PNEUMOCOCCI

I Introduction

1. Streptococci form part of the normal flora of man and animals.
2. They inhabit various sites specially upper respiratory tract.
3. Some of them are human pathogens.
4. Most important pathogen is *Streptococcus pyogenes*, which has aggressive role.
5. It can cause tonsillitis, erysipelas, impetigo, scarlet fever and septicemia.
6. It also causes nonsuppurative lesions i.e. rheumatic fever and acute glomerulonephritis.
7. The suppurative lesions produced by it are of spreading nature.
8. *Streptococcus agalactiae* (Group B Streptococci) is important causative agent of neonatal meningitis.
9. Peptostreptococcus (anaerobic Streptococci) causes puerperal sepsis, genital infections, wound infections, gangrenous appendicitis, UTI, osteomyelitis and abscess in brain, lung etc.
10. Group D Streptococci are classified into 2 groups-
 - a. Enterococcal Group
 - b. Nonenterococcal Group

Enterococci cause UTI, wound infection, endocarditis, septicemia, intra-abdominal abscess and peritonitis.

II Classification streptococci are classified into 3 various based on their growth in house blood agar.

1. α -Hemolysis: Greenish discoloration, partial hemolysis eg. pneumococci.
2. β -Hemolysis: Colourless zone of hemolysis, complete hemolysis eg. *Streptococcus pyogenes*.
3. γ -Hemolysis: No hemolysis. eg. *Strepto faecalis*.

A STREPTOCOCCUS PYOGENES

1. **Morphology** Cocci are spherical or oval, arranged in chains, non-motile, non-sporing. *Streptococcus pyogenes* have capsules composed of hyaluronic acid.
2. **Cultural Characteristics** They are aerobes & facultative anaerobes.
 - Grow best at temp. 37°C.
 - On blood agar, colonies are small, circular, semi-transparent low convex with clear area of β -hemolysis.
 - On lig. media, granular turbidity with powdery deposit is formed.
3. **Important biochemical Reactions**
 - ferment sugar producing acid.
 - oxidase, catalase negative
 - Not soluble in 10% bile
 - PVR test +ve
 - Do not ferment Ribose.

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4. Bacitracin sensitivity sensitive to bacteriostatic.

III Pathogenesis

Mode of entry:

1. Most common route of entry is upper respiratory tract.
2. Through skin

IV Extracellular Products (Virulence factors)

1. M protein – it is antiphagocytic.
2. Hyaluronidase- spread of infection.
3. DNase (A, B, C, D) – depolymerisation of DNA. Consequently causing death of the host cells.
4. Streptokinase (fibrinolysin) – it breaks fibrin barrier around the lesion.
5. Streptolysin O and S – lysis of red cells and cytotoxic to other cells.
6. Cell wall lipoteichoic acid – adherence to host cell respiratory epithelium.
7. Erythrogenic agents – causes fever, shock and tissue damage.

V Laboratory diagnosis

A. Suppurative Lesion:

- a. Specimen – throat swab, exudate from the wound/lesion may be collected by syringe and needle or by swab.
- b. Microscopy- prepare the smear from the specimen. Perform a Gram staining

Morphology: They are gram +ve cocci arranged in chains.

c. Culture –

Media: pike's medium (Blood agar containing crystal violet & sodium oxide) is used as transportation medium.
Blood agar used for culture.

Growth Characters:

on blood agar, colonies are small circular, semi-transparent, low convex with area of clear hemolysis.

d. Biochemical tests

- i. Catalase negative
- ii. Not bile soluble
- iii. Ribose not fermented
- iv. PYR test positive
- v. Sensitive to bacitracin

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- e. Antibiotic sensitivity- not necessary as *Streptococcus pyogenes* is sensitive to most of the routine antibiotics (e.g. penicillinG, erythromycin etc)

B. Nonsuppurative Lesions (Rheumatic fever and acute glomerulonephritis)

- a. Swabs from the throat and skin – for microscopy and culture may help. But serological tests are more useful for the diagnosis.
b. Serological tests –

i. Antistreptolysin -O test (ASO test)

ASO test higher than 200 is indicative of stre infection. High levels are useful for diagnosis of Rheumatic fever but glomerulonephritis titre are often low.

ii. Antideoxyribonuclease test (anti-DNAase-B test)

Titre higher than 300 are taken significant. Anti DNAase B & Anti hyaluronidase are very useful for the retrospective diagnosis of strepto. pyogenes for which ASO is low.

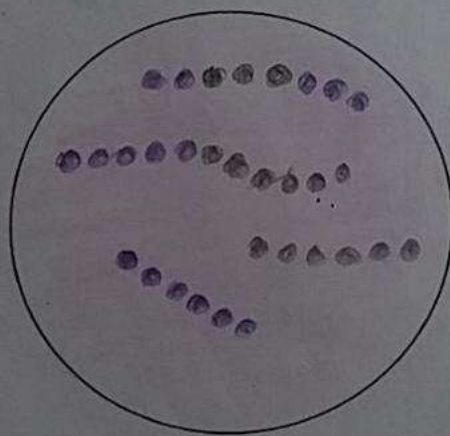
iii. Anti hyaluronidase test

Useful for retrospective diagnosis of stre infection for which ASO is much less.

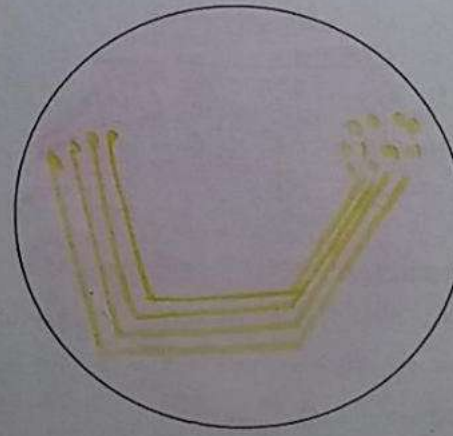
iv. Streptozyme test

Diagrams

1.

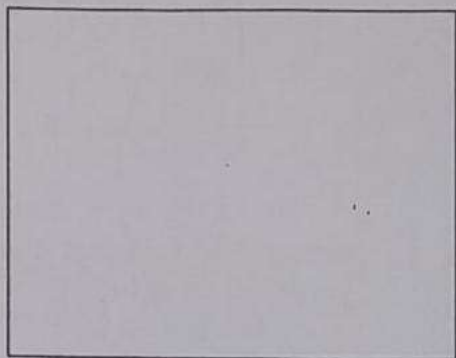


Gram's stain showing GPC in chain with pus cells from a case of follicular tonsillitis



BA showing β hemolytic colonies of *S. pyogenes*

2. ASO test



3. Bacitracin sensitivity

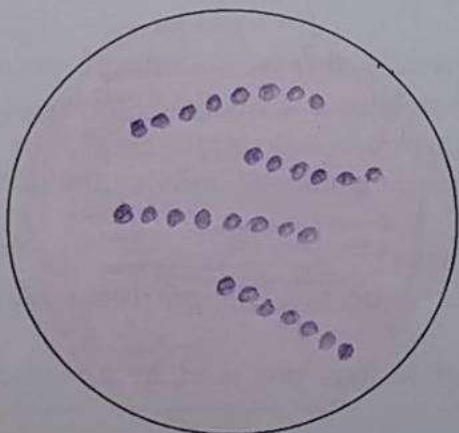


BA

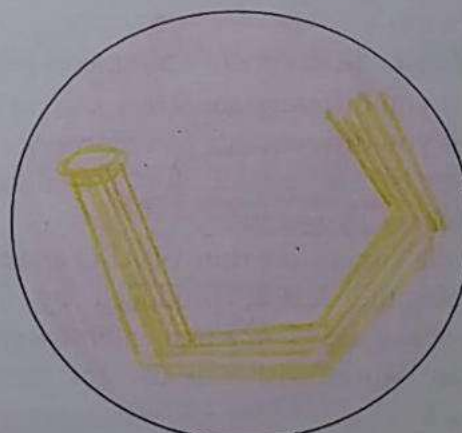
Other Streptococci

1. *Str viridans*

- GPC in chains.
- Shows α -hemolysis on blood agar.
- They are commensals of the mouth and upper respiratory tract.
- They cause dental carries, subacute bacterial endocarditis.



Gram's stain showing GPC in chains

BA showing α -hemolytic colonies
(*Str viridans*)2. Group B Streptococci (*Str agalactiae*)

- They produce bovine mastitis.
- Common cause of neonatal meningitis.
- Confirmed by CAMP test, hippurate hydrolysis (both are positive)

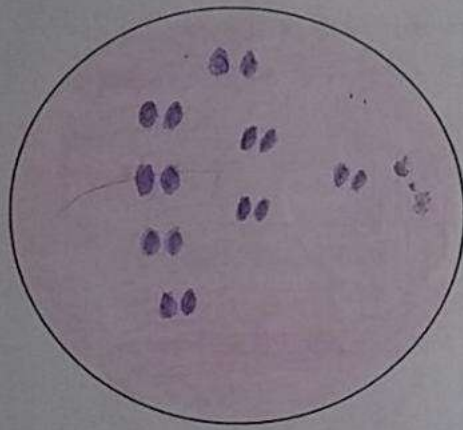
3. Enterococci

- They are Gram positive cocci arranged in pairs at particular angle.
- They are able to grow in 40% bile and 6.5% sodium chloride.
- They are relatively heat resistant.
- They cause endocarditis, UTI, wound infection, hospital infection and infections in immunocompromised host.
- They are resistant to common antibiotics including vancomycin

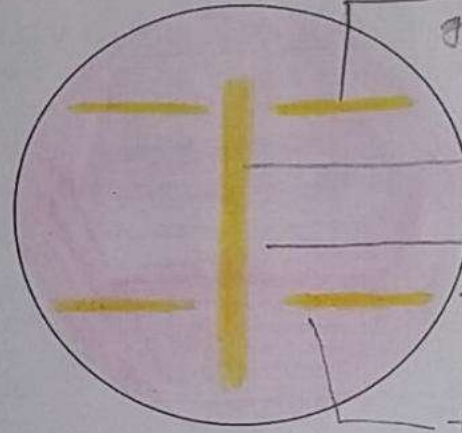
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Gram's stain showing GPC in pairs (Enterococcus)



CAMP test

-ve control
group A streptococci

S. aureus

arrow head

zone of hemolysis

+ve control
group A streptococci

Exercise

1. Perform Gram's staining of a smear prepared from a case of soar throat. Report your findings.
2. Describe morphology, culture characteristics and laboratory diagnosis of *Streptococcus pyogenes*.
3. How will you differentiate *Streptococcus pyogenes* from *Staphylococcus aureus*?
4. How will you differentiate *Streptococcus pyogenes* from other β -hemolytic Streptococci?
5. How will you differentiate *Streptococcus viridans* from *Streptococcus pyogenes*?
6. Which tests are useful for the diagnosis of nonsuppurative lesions produced by *Streptococcus pyogenes*?
7. What is the suggestive time of ASO and antiDNAase tests?
8. Enumerate the lesion produced by *Streptococcus pyogenes*, *Streptococcus viridans*, *Streptococcus agalactiae* and enterococci.
9. Which antibiotic will you use for the treatment of lesions produced by *Streptococcus pyogenes*?
10. Which tests are used for the confirmation of *Streptococcus agalactiae* and enterococci?

B STREPTOCOCCUS PNEUMONAE (*D. pneumoniae*)

I Introduction

1. First identified in the sputum in 1881 by Louis Pasteur.
2. It forms normal oropharyngeal flora.
3. It is an important human pathogen.
4. It causes disease of the middle ear, paranasal sinuses, mastoids and the lung paranchyma.
5. It may spread to the other sites such as joints, peritonium, endocardium and billiary tract and meninges.

II **Morphology:** Gram +ve, small, slightly elongated cocci arranged in pairs with broadends in position, each colonies cocci has one end broad or rounded & other end pointed. They are capsulated & capsule encloses each pair. They are non-motile & non-sporing.

III **Culture Characteristics:**

They are aerobes & facultative anaerobes & their growth is improved by 5-10% CO₂, opt temp. - 37°C & pH. 7-8.

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Blood agar - After incubation for 12 hrs one colonies are small, dome shaped with an area of greenish discoloration. On prolonged incubation, colonies become flat with raised be edges & control combination which creates draughtiness appearance.

Liquid media - uniform turbidity.

Demonstration of the Capsule:

1. India-Ink preparation
2. Nigrosine staining
3. Quellung reaction

Difference between *Streptococcus pneumoniae* and *Streptococcus viridans*

Tests	<i>Str pneumoniae</i>	<i>Str viridans</i>
Growth in liq. media	+ve	-ve
Bile solubility	uniform turbidity	growth turbidity
fermentation	variably +ve	powdery deposit
optochin sensitivity	+ve	variably -ve
Intraperitoneal	+ve	-ve
Incubation in mice.	fatal infection	non-pathogenic

IV Virulence:

1. Capsular polysaccharide- antiphagocytic
2. Pneumolysin - membrane damaging toxin (cytolytic)

V Pathogenesis:

1. Normal oropharyngeal flora of 5-70% of the population.
2. In winter isolation rate is high.
3. Contagious spread results in otitis media, mastoiditis, sinusitis and respiratory infection including lobar pneumonia and broncho-pneumonia.
4. Pneumococcal bacteremia may lead to infections in the heart, peritoneum, joints and meninges.

VI Predisposing factors:

1. Pre-existing respiratory disease
2. Ischemic Heart Disease
3. Diabetes mellitus
4. Chronic renal failure

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5. Immunodeficiency disease

VII Laboratory Diagnosis

1. Lobar pneumonia

Sample- sputum (rusty)

In infants- serum coated laryngeal swabs are used for collecting the sample.

a. Microscopy-

Gram's staining

pneumococcus are gram +ve small, diplococci, flame shaped or lancelet in appearance.

b. Culture

specimen is inoculated on blood agar & at 37°C for 24 hrs in the +ve of 5-10% CO₂. Typical colonies develop & hemolysis, colonies are small with α -hemolysis around them.

c. Biochemical reaction

specimen is inoculated on blood agar.

Bile solubility +ve

Inulin fermentation → +ve

Optochin sensitivity → +ve

Note: if required Capsule can be demonstrated by quelling reaction/India-ink preparation.

d. Animal pathogenicity testing

From specimen where organism are expected to be scanty intra peritoneal inoculation in mice is used. Inoculated media in 24-48 hrs. Heart blood & peritoneal exudate of animal show pneumococcus.

e. Antibiotic sensitivity testing

optochin sensitive

Note: Blood culture may be possible in acute stage of pneumonia but prognosis is bad.

Samples in other conditions

1. Otitis media - fluid aspirated from middle ear.
2. Meningitis- Cerebro Spinal Fluid

Pneumococcal meningitis

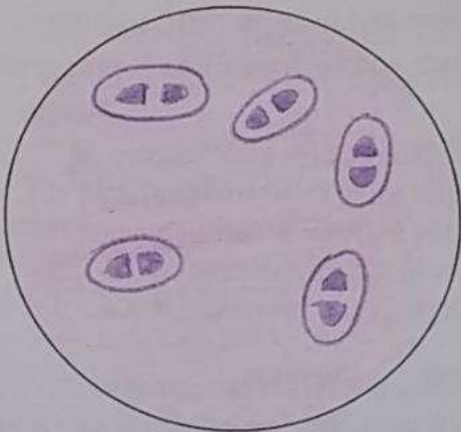
Sample - CSF is collected

It is centrifuged. Prepare the smear from the deposit and perform the Gram's staining. Secondly the deposit is used for the culture. Rest of the procedure is same as above.

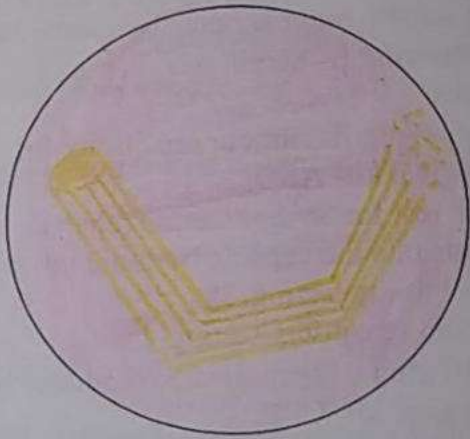
Note: 1. If culture is negative then diagnosis may be made by demonstrating SSS in CSF by precipitation with antisera. 2. Capsular polysaccharide can be demonstrated in the blood, urine and CSF by CIEP. 3. Demonstration of antibodies by following test- A. Agglutination B. Precipitation C. IHA D. Indirect FA E. RIA

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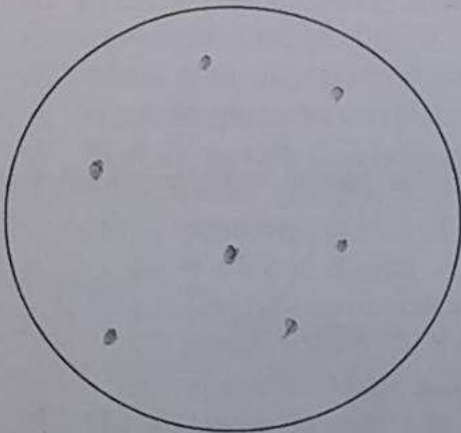
Diagrams



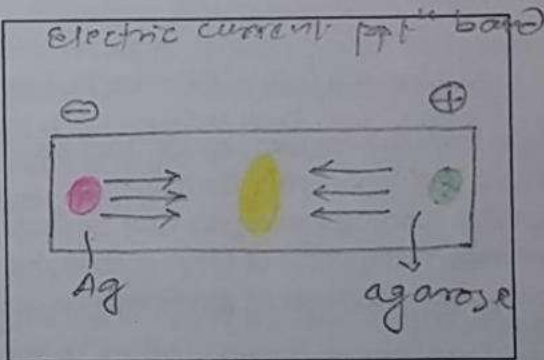
Gram's staining of CSF showing GPC in pairs with capsule (*D. pneumoniae*)



BA showing α -haemolysis (*D. pneumoniae*)



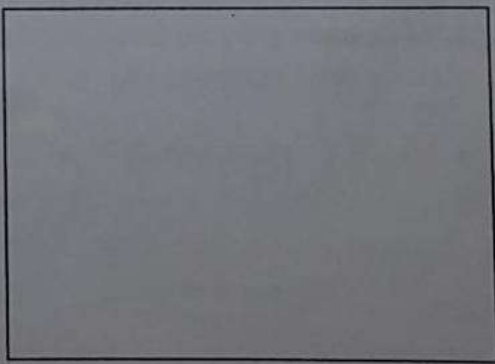
Colonies showing Draughtsman Appearance



CIEP showing line of ppt in the center



Demonstration of capsule (India-ink preparation)



Demonstration of CRP

Practical Class No. 11

AEROBIC GRAM NEGATIVE COCCI- NEISSERIA
(Meningococci and Gonococci)

Introduction

1. The genus Neisseria contains Gram-negative aerobic, nonsporing oxidase positive cocci typically arranged in pairs (diplococci).
2. It consist of two important pathogens-
 - a. *N. meningitidis* (meningococcus)
 - b. *N. gonorrhoeae* (gonococcus)

A. *N. meningitidis* (Meningococcus)

It causes meningococcal meningitis. Cerebrospinal meningitis and meningococcal septicemia are two main types of meningococcal diseases.

I Pathogenesis

1. They are strict human parasites inhabiting the nasopharynx.
2. In normal population 5-10% are the carriers of meningococci among which half the carriers carry capsulated strains.
3. Where outbreaks are seen the carriage rate of epidemic strain increases up to 90%.
4. Smokers appear to carry meningococci more often than nonsmokers.
5. Route of spread- it is not clear but it is suggested that-
 - a. From nasopharynx to subarachnoid space through Cribriform plate along the, perineural sheath of olfactory nerve.
 - b. Via blood stream
 - c. Through conjunctivaOn reaching the CNS a suppurative lesion of the meninges is set up involving surface of spinal cord and base and cortex of the brain (meningococci are found in CSF).
6. The disease is common in infants and young children from 3 months to 3 yrs of age (in which protective antibody is usually absent).
7. Predisposing factors-
 - a. Over crowding
 - b. Complement deficiency (C5-C9 of the components)
 - c. Absence of protective group specific antibody
8. It may lead to purulent arthritis and skin involvement, pericarditis, endocarditis, pneumonia.
9. A fatal complication called as meningococemia (Waterhouse-Friderichsen syndrome) may develop which is characterized by shock, disseminated intra-vascular coagulation and multi-system failure, which is usually fatal.

II **Morphology** Gram -ve oval or spherical cocci, 0.6-0.8 μ m in size. arranged typically in pairs with adjacent sides flattened. In smears from lesions, cocci are more regular & generally intracellular. They are non-motile, non-sporing.III **Culture characteristics** Growth occur in media enriched in blood, serum which promote by neutralising certain inhibitory subs. in culture media. On solid media, after incubation of 18-24 hrs, colonies are small, translucent, round, convex, bluish grey with smooth glistening surface & with entire edges. Colonies are lenticular in shape, but texture is consistency & easily emulsifiable. In liq. media, granular turbidity is seen with little growth.

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IV Biochemical reactions

Catalase & oxidase +ve
ferments glucose & maltose with acid production & no gas
Do not ferment lactose & sucrose. Indole +ve → -ve

V Classification

classified on the basis of capsular polysaccharide antigen into 9 serogroups A, B, C, X, Y, Z, E, 29, W, 135, I, K, L, J. serogroups are classified into serotypes on the basis of outer membrane protein.

VI Laboratory Diagnosis

Specimen:

1. CSF

Procedure

collection of specimen is done under sterile condition by lumbar puncture for CSF specimen should be transported immediately CSF should never be refrigerated. Influenzae may die at cold temp.

Microscopy

CSF is divided into 3 portions. One portion is centrifuged & smear is prepared from deposit for gram staining. Meningococci as seen as gram -ve diplococci. Suspend fluid used for detection of meningococci. 2nd portion is directly cultured. 3rd portion is incubated overnight after adding equal vol. of glucose broth & then subcultured on blood agar or chocolate agar.

Culture characteristics

Centrifuged deposit is inoculated in blood agar & chocolate agar plate is incubated at 35-36°C for 18-24 hrs under 5-10% CO₂ colonies. agar are gram stained.

Biochemical tests

Catalase, oxidase → +ve

Glucose, Maltose → fermented with acid production, No gas.

2. Blood- blood cultures are often positive in case meningococemia

Blood collection is done by venipuncture & then injected into blood culture bottles through hole. Blood culture are +ve in meningococci & in early cases of meningitis. Bottles are incubated at 35-36°C for 24 hrs, sub cultures are made from the bottles into blood agar/chocolate agar. These plates are again incubated at 35-36°C for 18-24 hrs. under 5-10% CO₂. Identification is done by colony morphology, gram staining, biochemical test.

3. Nasopharyngeal swab

specimen is collected using sterile swab, it should be held into a transport medium until it is inoculated on a culture medium. These are inoculated on blood or chocolate agar.

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4. *Petechial lesion* They are aerobic but grow anaerobically along growth occurs. best at temp. 35-36°C pH 7.4-7.6. Grow well on chocolate/late/MHA. TM Medium is selective with inhibit most contaminants. Colonies are round, small, translucent, convex or slightly umbonate with finely granular surface. They are soft & easily emulsifiable.

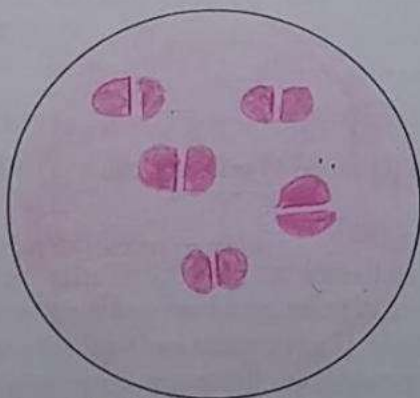
5. Autopsy

6. Retrospective diagnosis -detection of antibodies by:

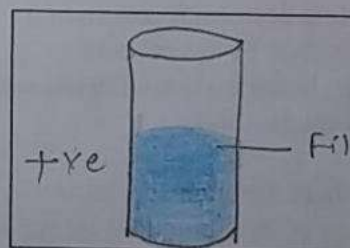
- Precipitation
- Agglutination
- Immunofluorescence
- CFT
- ELISA/RIA

Demonstration

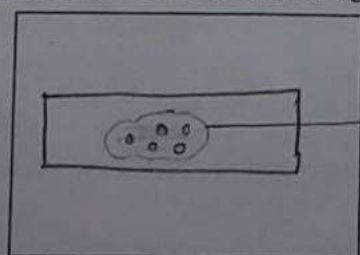
- Microscopic slide of Gram's stain of the smear from CSF of a case of meningococcal meningitis.
- Oxidase test
- Fermentation of glucose and maltose with acid only.
- Media used for isolation of meningococci (BA, CA, TM)



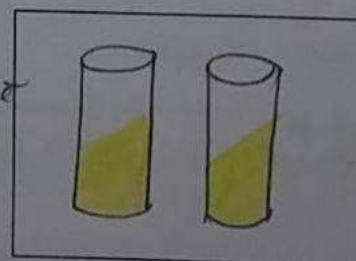
Gram's stain showing GNC in pairs from CSF of a case of meningitis



Oxidase test



Catalase test



Glucose & maltose fermentation with acid only

Exercise

- What is Waterhouse-Friderichsen syndrome?
- Describe the pathogenesis of meningococcal meningitis.
- Enumerate the media used for isolation of meningococci.

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Culture Characteristics They are aerobe but grow anaerobically also. Growth occurs best at temp. $25-36^{\circ}\text{C}$ & pH 7.4-7.6. Grow well on chocolate / MHA. TM medium is selective which inhibit most constituents. colonies are round, small, translucent, convex or slightly umbonate with finely granular surface. They are soft & easily emulsifiable.

Biochemicalscatalase $\rightarrow$ +veoxidase $\rightarrow$ -ve

ferment only glucose.

Laboratory Diagnosis

Collection of sample Urethral/discharge & cervical discharge collected in acute urethritis. In chronic urethritis urethral discharge is observed only in few cases. In these cases exudate obtained from prostatic massage, or morning drops of secretion is examined.

Procedure

In male Meatus is first cleaned with a gauze soaked in saline. A sample of urethral discharge is then collected with platinum loop or by sterile swab. Calcium alginate & some cotton swabs are inhibitory to gonococci.

In female In women, cervical swab is collected in addition to urethral discharge. High vaginal swab are not satisfactory.

Microscopy examination**Preparation of smear**

Gram -ve diplococci are found in smear of atleast 95% cases of acute gonococcal urethritis in men. In female diagnosis of gonorrhoea by smear examination is unreliable as some of normal genital flora or have similar morphology.

Gram's staining**Morphology**

on solid media colonies are small, round, grey, translucent, convex with finely granular surface. They are easily emulsifiable. They are gram -ve diplococci with adjacent sides concave.

Culture

specimen should be inoculated directly on preheated plates. Immediately on collection chocolate agar is used for culture. Incubated at $35-36^{\circ}\text{C}$ under 5-10% CO_2 for 48 hrs. In chronic cases when mixed infection is usual & lesion like proctitis, selective media like TMT medium is used.

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Biochemicals

Oxidase $\rightarrow$ +ve

Ferment only glucose with Acid production.

Antibiotic sensitivity testing

Susceptible to sulphonamides & penicillin However penicillin resistant strain have been identified.

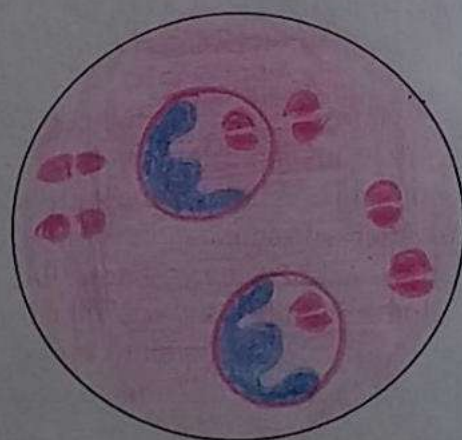
Demonstration

1. Microscopic slide of smear prepared from a case of gonorrhoea and stained with Gram's stain showing GNC in pairs mostly intracellular with pus cells.
2. Media used for isolation of gonococci (BA, Chocolate agar, TM media).
3. Fermentation of glucose with acid only but not maltose.

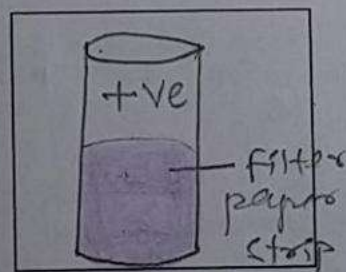
Exercise

1. Define the term venereal disease.
2. What are the complications of gonorrhoea in males and females?
3. What are the different media used for isolation of gonococci?
4. Describe the laboratory diagnosis of gonorrhoea.
5. Give the examples of nonvenereal form of gonococcal infection.
6. Comment on the serological diagnosis of gonorrhoea.

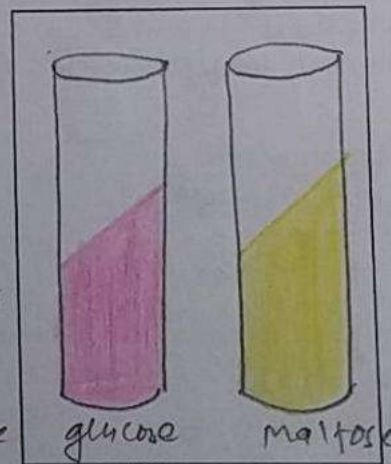
Diagram



GNC (intracellular) in pair with pus cells (gonococci)



turned purple
Blue colour
Oxidase test



glucose +ve
maltose -ve
Fermentation of glucose & maltose

MICROBIOLOGY PRACTICAL RECORDS

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Practical Class No. 12

CORYNEBACTERIA

Introduction

1. Coryneforms (or diphtheroids) are defined as pleomorphic Gram positive rods which stain irregularly, arranged in "V" or "L" forms. Nonmotile, catalase positive nonacid fast, nonbranching and form only acid from carbohydrates.
2. The group includes *Corynebacterium diphtheriae*. It is an important pathogen causing diphtheria.
3. Diphtheria is a serious illness where localized inflammation of the throat with grayish white adherent exudate (formation of pseudomembrane) with generalised toxæmia is seen. This is because of powerful exotoxin which is produced by *C. diphtheriae*. All the clinical manifestations are due to exotoxin.
4. The exotoxin is absorbed in the blood and toxæmia is produced. The toxin has specific action on heart muscle, nerve ending and the adrenal glands. The toxin acts by inhibiting protein synthesis. The toxin is neutralized by specific antitoxin. Hence, early clinical diagnosis and prompt treatment with antitoxin and antibiotics (penicillin, erythromycin, tetracycline, clindamycin, rifampicin) is necessary. If delayed it may be fatal.

Note: erythromycin has advantage that it eliminates the carrier state.

5. Pseudomembrane:

The toxin causes localised necrotic changes and resulting fibrinous exudate together with disintegrating epithelial cells, RBC, WBC and bacteria constitute the pseudomembrane. It is tough and leathery in consistency and when tried to remove it, bleeding occurs.

6. *C. diphtheriae* is originally nontoxicogenic but when it gets infected with β -phage it becomes toxigenic i.e. it starts producing exotoxin.
7. In laryngeal diphtheria, the formation of pseudomembrane (even a small patch) causes obstruction in air passage leading to asphyxia and death. In other types of diphtheria, the death is toxin mediated causing damage to cardiac muscles and heart failure.

8. Types of *C. diphtheriae*

No	Gravis	Intermedius	Mitis
1	Sever	Intermediate	Mild
2	Paralytic complications Hemorrhagic manifestations	Hemorrhagic manifestations	Obstructive

9. Sites:

- a. Faucial- (common)
- b. Laryngeal
- c. Nasal
- d. Cutaneous
- e. Conjunctival
- f. Otitic
- g. Genital- vulval

1 Morphology

1. *Gram's stain* They are thin, slender, gram +ve bacilli, pleomorphic. They are club shaped due to the presence of metachromatic granules. Bacilli are seen in angular fashion resembling letter V or L. Bacilli look green & metachromatic granules look bluish black when Albert stain is used. They are non-capsulated, non-motile, non acid fast.

2. Albert's stain Bacilli look green & metachromatic granules look bluish black.

3. Neisser's stain have been used for demonstrating granules clearly.

4. Metachromatic granules stained with Loeffler's methylene blue granules take up a bluish purple colour & hence k/s metachromatic granules. These granules are composed of polymetaphosphate & represent energy storage depots.

II Cultural Characteristics

1. BA useful for differentiating staphylococcus & streptococcal pharyngitis which may stimulate diphtheria.

2. Loeffler's serum slope grows very rapidly & colonies can be seen in 6-8 hrs, long before other bacteria grow. colonies are 1st small, circular disc white opaque discs but enlarge on continue incubation & may acquire a distinct yellow tint.

3. Potassium tellurite medium

Colonies appear grey-black colour after 2-3 days of incubation.

Advantages/disadvantages-

- Tellurite inhibit most of other bacteria, acting as a selective agent
- Diphtheria bacilli reduce tellurite to metallic tellurium which is incorporated in colonies giving them a grey or black colour.

Disadvantages:

- growth of bacteria may be delayed on Tellurite media & colonies may take 2 days to appear.

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Biochemicals - ferment glucose, galactose, maltose & dextrin.

Glu

Gla

Mal

Dex

|

|

|

|

|

Production of acid without gas.

Lactose, mannitol and sucrose are not fermented.

Differentiation of the types

Types	Glucose	Glycogen	Starch
Gravis	Acid	Acid	Acid
Intermedius	Acid	Neg	Neg
Mitis	Acid	Neg	Neg

Note: Intermedius is always nonhemolytic and mitis is usually hemolytic.

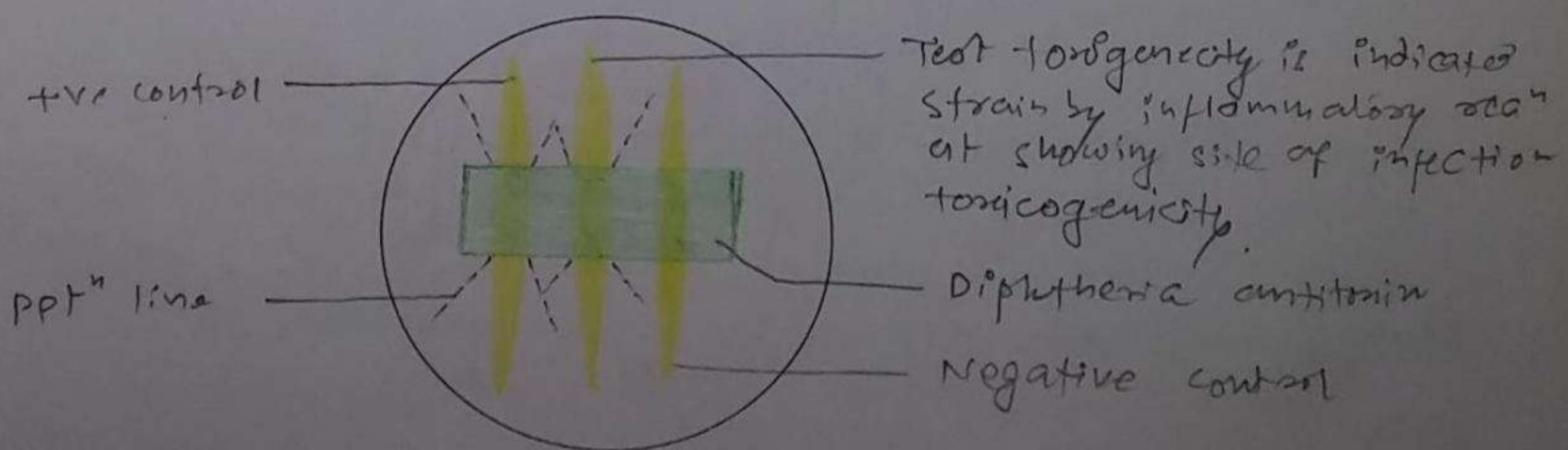
Toxicogenicity testing

There are *in vivo* and *in vitro* tests as below-1. *In vivo* test- These are carried out in guinea pigs or rabbits. These are-

- Subcutaneous
- Intracutaneous

Subcutaneous: The growth from an overnight culture on Loeffler's slope is emulsified in 2-4 ml broth & 0.8 ml of emulsion is injected subcutaneously in into 2 guinea pigs. one of which has been protected with 500 units of diphtheria antitoxin 18-24 hrs previously. If strain is virulent, unprotected animal will die within 4 days.

Intracutaneous: Broth emulsion of culture is inoculated intracutaneous into 2 guinea pigs each receive 0.1 ml in 2 diff. sites. Control animal should receive 500 units of antitoxin previous day other receives 50 units of antitoxin intraperitoneally.

2. *In vitro* test (Elek's gel ppt test)- (carried out in laboratory)

Gleick's gel precipitation Test:

A rectangular strip of filter paper impregnated with diphtheria antitoxin (1000 units/ml) is placed on surface of 20% normal horse serum in agar in a petri dish while medium is still fluid. When agar is set surface is dried & narrow streaks of strain are made at right angle to filter paper strip. A +ve & -ve control should be put up.

plate is incubated at 37°C for 24-28 hrs. Toxin produced by bacterial growth will diffuse in agar & where it meets antitoxin at optimal concentration will produce a line of precipitation.

Test is very convenient & economical some brands of peptone & some samples of serum do not give satisfactory results.

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Passive Immunization:

Diphtheria antitoxin is used

Diphtheria antitoxin

- emergency measure when susceptibles are exposed to infection as when a case of diphtheria is admitted to gen. paediatric ward.
- consists of subcutaneous administration of 500-1000 units of antitoxin antidiphtheric serum (ADS).

Active Immunization

DPT is given

Immunization schedule:

- ① 3 doses of DPT given at intervals of atleast 4 weeks preferably 6 weeks or more.
- ② 4th dose about a year afterward.
- ③ Booster dose given at school entry.

Laboratory Diagnosis

1. Collection of sample

Throat swab

specimen one or two swabs from lesions are collected under ~~the~~ vision using a tongue depressor.

2. Processing:

a. Microscopy

I Gram's staining

II Albert's staining, or

III Neisser's staining.

Demonstration of metachromatic granules purple blue granules in green bacilli.

b. Culture

BA for differentiating streptococcal & staphylococcal laryngitis.

Loeffler's serum slope The serum slope may show growth in 4-8 hrs if it is incubated for 24 hrs.

P.T. medium it is incubated for atleast two days before considered - as it is important in isolation of bacilli from convalescents, contact & carriers is in these cases.

c. *Biochemical Reactions*

They ferment glucose, galactose, maltose and dextrose with production of acid.

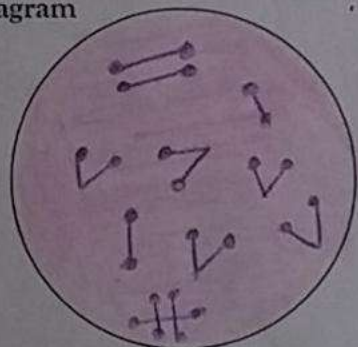
d. *Toxigenicity Testing.*

- Vivo test (Rabbit/ G.P.)
- Vitro test (Elek's gel precipitation test)

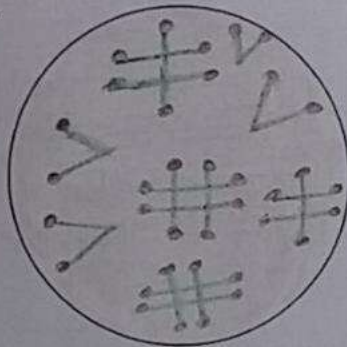
Demonstration -

1. Demonstration of *C. diphtheriae* in various staining procedure

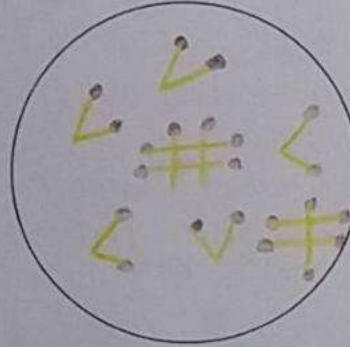
Diagram



Gram's stain
C. diphtheriae



Albert's stain
C. diphtheriae



Neisser's stain
C. diphtheriae

2. Elek's gel precipitation test (Chart).
3. Demonstration of DPT vaccine
4. Demonstration of uninoculated and inoculated media with *C. diphtheriae* (BA, Loeffler's serum slope, P.T.)

Exercise:

1. Which specimen is collected for laboratory diagnosis of diphtheria?
2. Which media are used for isolation of *C. diphtheriae*?
3. Describe morphology of *C. diphtheriae*.
4. What are the metachromatic granules?
5. Mention Adv/Disadv. of P.T. medium.
6. Mention advantage of Loeffler's serum slope.
7. How will you carry out toxigenicity testing of *C. diphtheriae*?
8. Mention passive and active immunization against diphtheria.
9. Why the name diphtheria is given to the disease?
10. Mention the pathogenesis of diphtheria.
11. What are the complications of diphtheria?
11. How the carrier state of diphtheria can be eliminated?
12. Elek's test - Mention type of reaction.

Practical Class No. 13

BACILLUS

Spore bearing rod shaped bacteria are classified in to two groups –

- I Bacillus (aerobic)
- II Clostridia (anaerobic)

I Bacillus

Introduction

1. The genus Bacillus includes large Gram positive, spore forming aerobic rods.
2. They form heat resistant spores.
3. The genus includes psychrophilic, mesophilic and thermophilic species.
4. Most of the species are saprophytic.
5. They are frequently encountered as contaminants of culture media in the laboratory.
6. *Bacillus anthracis* is an important pathogen causing anthrax in animals and humans. Anthrax is a zoonotic disease.
7. *Bacillus cereus* is causative agent of food poisoning.
8. Some other important species.
 - a. *B. stearothermophilus*- biological indicator for testing autoclave.
 - b. *B. subtilis*- test organism for ethylene oxide.
 - c. *B. pumilis*- to test efficiency of ionizing radiation for sterilization.
 - d. *B. polymyxa* – Source of antibiotic i.e. polymyxin.

C. Anthracis

1. **Historical Importance** It is the 1st pathogenic bacteria observed under microscope.
 - first communicable disease transmitted by inoculation in blood agar – first bacillus isolated in pure culture.
 - first bacteria used for prepare of an attenuated vaccine.
2. **Morphology and McFadyean's Reaction**
When blood film containing anthrax bacilli is stained with polychrome methylene blue for few second and examined under microscope and amorphous purplish material is noticed around bacilli.

3. Cultural characteristics

- a. **Nutrient Agar** Irregular rounded colonies 2-3 mm in diameter raised due to opaque grayish white with a fresh glass appearance.
- b. **Blood Agar**
Non-haemolytic
Long-interlacing chain of bacilli resembling locks of matted hairs.

c. *Selective Media (PLET)* consist of polymyxin, lysozymes, ethylene diamine tetraacetate acid & thallus acetate added to heart infusion agar, has been used to isolate *B. anthracis* from other spore bearing bacilli.

4. *String of Pearls Reaction.* When *B. anthracis* is grown on surface of solid medium containing 0.05-0.5 unit of penicillin/ml in 3-6 hr the cells become large spherical & occur in chains on the surface of agar resembling a string of pearls.

5. *Gelatin liquefaction.* with stab culture, a characteristic inverted fine free appearance is seen & slow liquifaction commencing from the top.

6. Biochemical Tests.

1. Fermentation of sugars

Glucose	Maltose	Sucrose
+	+	+

2. Catalase Test - Positive

3. Nitrates are reduced to nitrites.

4. Resistance destroyed at 60°C in 30 min.

spore are highly resistance to physical & chemical agents. Isolated from infused solid or long or 60 yrs. Resist dry heat at 140°C for 1-3 hr & survive in 5% phenol for weeks.

5. Duckering

Destruction of spores in formaldehyde 2% solⁿ at 36-40°C for 20 min.

6. Pathogenesis infect herbivores

— Virulence factor.

1. capsular polysaccharide - inhibitor phagocytosis.
2. Anthrax toxin
 - edema factor (ef or factor I)
 - protective antigen (pa or factor II)
 - lethal factor (lf or factor III).

Anthrax

1. Anthrax is a zoonotic disease. Man acquires infection from infected animal.
2. Large number of bacilli are shed in the discharges of infected animals
3. The disease may be:
 - a. Cutaneous
 - b. Pulmonary
 - c. Intestinal (rare)

a. Cutaneous Anthrax & Malignant Pustule (Hide Porter's disease):

Followed by entry of infection through skin resolve spontaneously. If untreated leads to septicaemia lesions start or peripore afterward becomes vesicular, whole area is congested and oedematous.

b. Pulmonary Anthrax (Wool sorter's disease):

- Due to inhalation of dust from infected wool.
- haemorrhagic pneumonia with high fatality rate

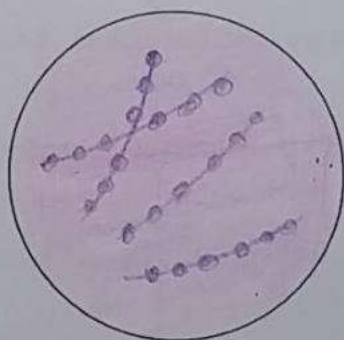
c. Intestinal Anthrax:

- rare
- mainly seen in primitive countries.
- A violent enteritis & diarrhoea occurs.
- Industrial - in workers of industrial such as meal packing or wool factories.
- Non-industrial - occupational dfg
 - veterinarians butchers and farmers

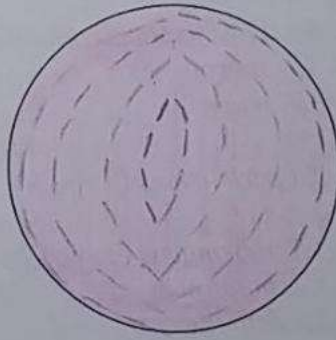
3. When large amount of rice is cooked and allowed to cool, the spores germinate and the vegetative cells produce toxin. When food containing the toxin is ingested it leads to food poisoning.
4. The disease is of two distinct types-
 - a. Emetic form
 - Associated with fried rice
 - Nausea vomiting abdominal cramps, rarely diarrhoea
 - b. Diarrhoeal form
 - Associated with meat
 - Profuse diarrhoea, abdominal pain and cramps
 - c. Other infections- *B cereus* may cause
 - Eye infections (keratitis etc)
 - Associated with local as well as systemic infections such as endocarditis, osteomyelitis pneumonia etc

Demonstration

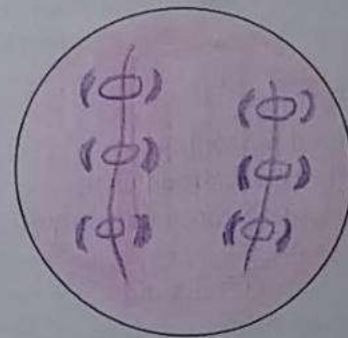
1. Smear of *B. anthracis*
2. Smear of *B. subtilis*
3. Smear showing McFadyean's reaction



Gram's stain
(*B. anthracis*)



Gram's stain
(*B. subtilis*)



McFadyean's reaction

Exercise

1. Enumerate the important species belonging to genus *Bacillus*.
2. Describe various lesions produced by *B anthracis*.
3. What is McFadyean's reaction?
4. What is String of Pearls reaction?
5. What is malignant pustule?
6. What is Duckering?
7. What is Wool Sorter's Disease and Hide Porter's Disease?
8. What is the pathogenesis of anthrax?
9. How will you carry out laboratory diagnosis of anthrax in man and animal?
10. What is Ascoli's thermoprecipitation test?
11. Define the terms psychrophilic, mesophilic and thermophilic bacteria.
12. What is the historical importance of *B anthracis*?
13. How will you differentiate *B anthracis* from *B cereus*?
14. What are the types of food poisoning caused by *B cereus*?

MICROBIOLOGY PRACTICAL RECORDS

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Findings

1. Presence of large number of thick Gram positive bacilli without spores is strongly suggestive of *Cl. perfringens* infection.
2. Presence of 'Citron' bodies and boat or leaf shaped pleomorphic bacilli with irregular staining is suggestive of infection with *Cl. septicum*.
3. Presence of large stout pleomorphic Gram positive bacilli with large, oval subterminal spore is suggestive of presence of *Cl. oedematiens*.

II Culture

1. RCM inoculated with the sample is kept in the water bath at 45°C / 4 to 6 hours for enrichment. At this temperature the generation time of *Cl. perfringens* is about 10 min. Subcultures are made on following media.
 - a. Horse blood agar- two plates are inoculated and one is incubated aerobically and the other one anaerobically.
 - b. Blood agar with neomycin (selective medium) : Incubate it anaerobically . After overnight incubation the plates are examined and colony charcters are studied. *Cl. perfringens* shows 'target hemolysis' on horse blood agar and blood agar containing sheep/rabbit/human blood.
2. The isolate is confirmed by Gram's stain, saccharolytic activity (meat turns pink), litmus milk test (stormy fermentation) and Nagler's reaction (production of α toxin).
 - a. Other tests
 - i. Fermentation of various sugars with acid and gas production.
 - ii. Indole, MR, VP negative.
 - iii. H_2S is produced.
 - iv. Nitrates are reduced to Nitrites
 - b. The isolated pathogens of gas gangrene are separated by following methods
 - i. Gram's staining (morphological characteristics)
 - ii. Saccharolytic activities
 - iii. Proteolytic activities.
 - iv. Biochemical tests

How gas gangrene is differentiated from anaerobic streptococcal myositis?

1. Gram stain $\rightarrow$ gram +ve bacilli shows pleomorphism
2. H_2S is produced.
3. Saccharolytic $\rightarrow$ RCM turned to pink.
4. Milk test
5. Nagler's Rxn

Spacial feature

1. Morphological characteristics
 - a. *Cl. perfringens*
 - i. It is Gram positive bacillus ($4-6\mu \times 1\mu$)
 - ii. It is a plump (stout) with straight parallel sides and rounded or truncated ends
 - iii. Capsulated and nonmotile.
 - iv. Spores are central or subterminal oval or spherical.

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Note: Sores are not seen in artificial culture or in materials from pathological lesions.

- b. *Cl. septicum*
 - i. Pleomorphic, Gram positive bacilli ($3-8 \mu \times 0.6 \mu$)
 - ii. Oval, central or subterminal spore
 - iii. Motile with peritrichate flagella
- c. *Cl. oedematiens* (*Cl. novyi*)
 - i. Large, stout, pleomorphic Gram positive bacilli
 - ii. Spores are large, oval, subterminal

2. Target hemolysis

After overnight incubation on blood agar (rabbit/sheep/human) colonies of most of the strains shows target hemolysis resulting from a narrow zone of complete hemolysis due to ϕ toxin and wider zone of incomplete hemolysis due to α toxin.

3. Saccharolytic activity

Some clostridia have the ability to attack the carbohydrates. In RCM meat is turned pink but not digested. The culture has an acid reaction and a sour odour.

4. Proteolytic activity

Some clostridia have the ability to digest protein. In RCM the meat becomes black and the odour is foul smelling.

5. Litmus milk test (stormy fermentation)

In this fermentation of lactose leads to formation of acid which is indicated by the change in the colour of litmus from blue to red, the acid coagulates the casein from the milk (acid clot) and the clotted milk is disrupted due to the production of vigorous gas. The paraffin plug is pushed up and shreds of clot are seen sticking to the side of the tube. This is known as 'stormy fermentation'.

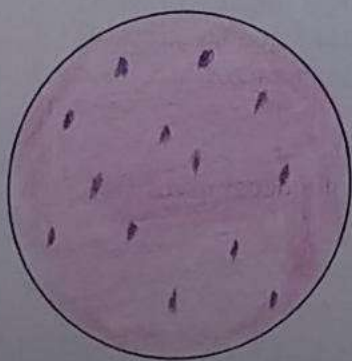
6. Nagler's reaction

α - toxin is produced by *Cl. perfringens*. It is a major toxin. It has lecithinase activity. It acts on lecithin in the presence of calcium and magnesium ions and split it into phosphoryl choline and diglyceride. This activity is seen as an opalescence on the medium containing serum or egg yolk which is specifically neutralized by antitoxin.

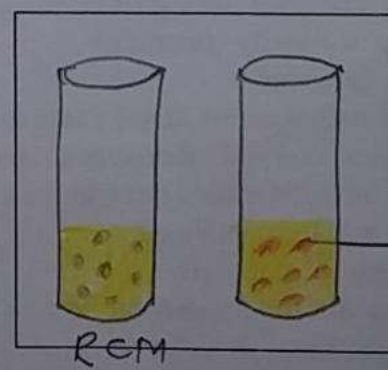
Procedure: When *Cl. perfringens* is grown on a medium containing 6% agar, 5% Field's peptic digest of sheep blood and 20% human serum with the antitoxin spread on one half of the plate, colonies on the other half without the antitoxin will be surrounded by a zone of opacity, but there will be no opacity around the colonies on the half of the plate with the antitoxin due to specific neutralization of the α -toxin.

Note: human serum may be replaced by 5% egg yolk. Incorporation of neomycin sulphate makes it more selective.

Diagram

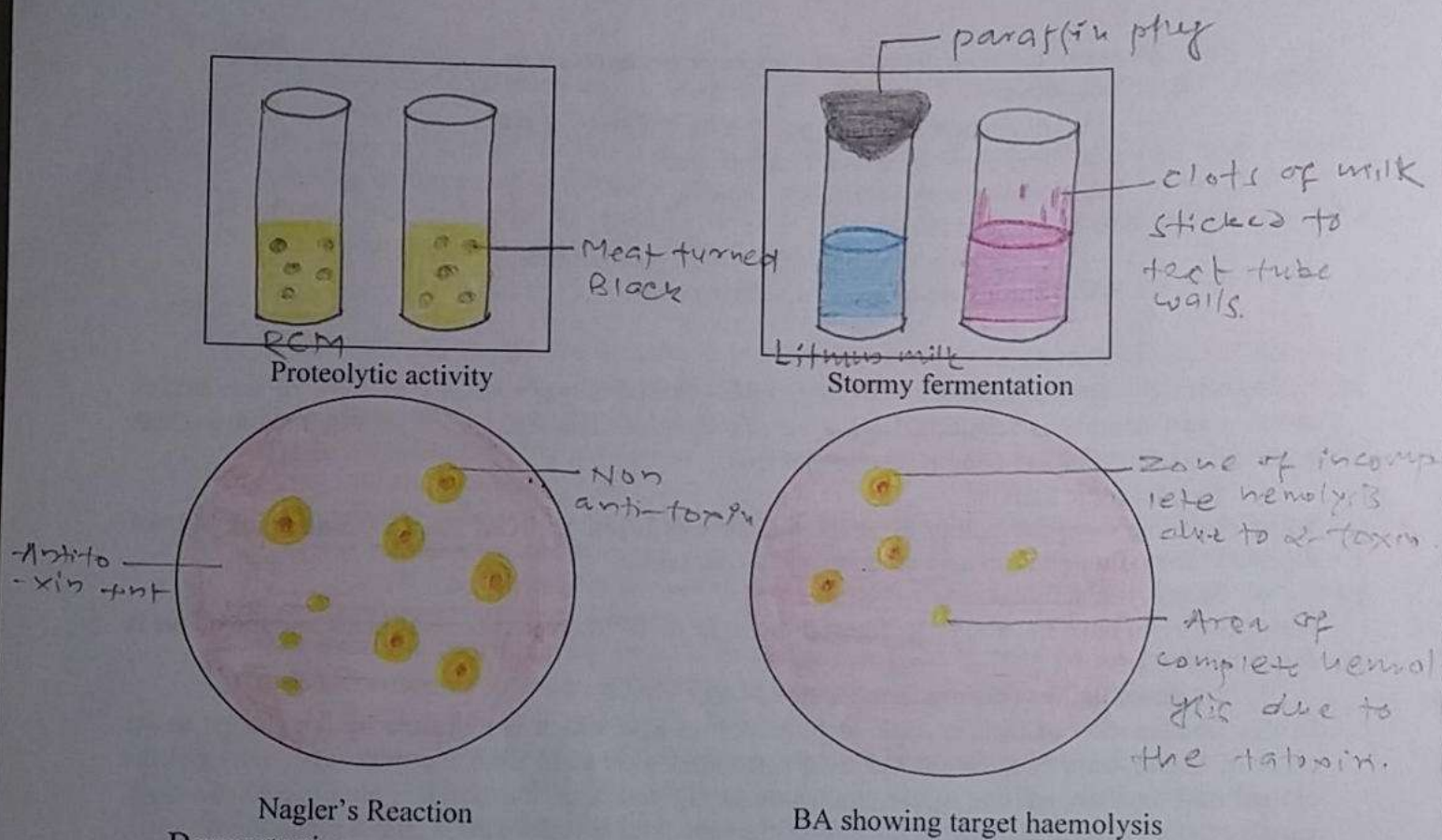


Gram's stained smear from a case of gas gangrene



Saccharolytic activity

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Nagler's Reaction Demonstration

1. Gram's staining of a smear prepared from the specimen from the deeper part of wound occurred in road accident.
2. Saccharolytic activity
3. Proteolytic activity
4. Stormy fermentation (litmus milk)
5. Nagler's reaction (culture plate)
6. RCM
7. RCM with growth
8. McIntosh Filde's jar
9. Gas pack jar
10. BA showing target haemolysis
11. AGS

Exercise

1. What are established pathogens of gas gangrene?
2. How will you classify clostridia?
3. Define gas gangrene.
4. What is the pathogenesis of gas gangrene?
5. How will you carry out laboratory diagnosis of gas gangrene?
6. How will you differentiate gas gangrene from anaerobic streptococcal myositis?
7. What is stormy fermentation?
8. What is Nagler's reaction?
9. What are the different anaerobic culture methods?
10. What is target haemolysis?
11. Comment on utility of AGS.
12. Which drugs will you prefer to use for the treatment of gas gangrene?

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CLOSTRIDIUM TETANI

It is a causative agent of tetanus.

Introduction

Tetanus occurs in animals and man when a wound is infected with *Cl. tetani*. The organism is widely distributed in soil and the intestine of man and animals.

Deep puncture wounds, crush injuries, thorn pricks, ear piercing, umbilical stump, burns, surgical sutures etc. specially when contaminated with ionised calcium, soil and other foreign bodies provide good growth conditions for multiplication of *Cl. tetani*.

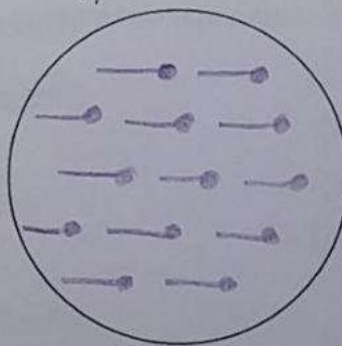
Exotoxin (tetanospasmin) secreted by the growing bacilli is transported along the motor neurons to CNS.

The toxin causes synaptic block of inhibitory control on motor neurons. The abolition of spinal inhibitor causes uncontrolled spread of impulses initiated anywhere in the CNS.

This results in muscle rigidity and spasms due to simultaneous contraction of agonists and antagonists in the absence of reciprocal inhibition.

The disease is characterized by increased muscle tonus and exaggerated muscular response to the stimuli leading to violent spasms.

Diagram



Cl. tetani showing typical drumstick appearance

Demonstration

1. Gram stained smear of *Cl. Tetani* (typical drumstick appearance).
2. TT, DPT, DT.

Exercise

1. Mention about the active and passive immunization against tetanus.
2. Describe various types of tetanus.
3. Describe pathogenesis of tetanus.
4. Name the toxins produced by *Cl. tetani*.

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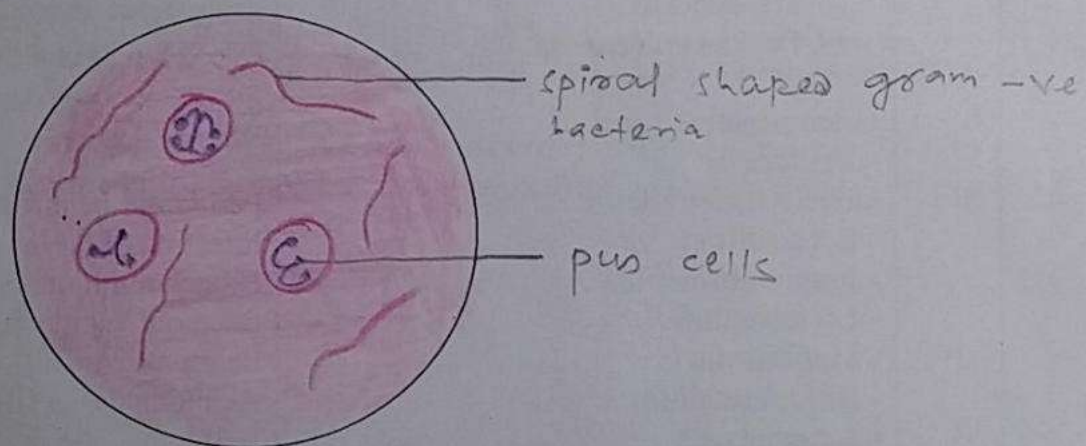
Lab. Diagnosis

1. Sample should be collected (aspirated) with airtight syringe and needle, after plunging it into sterile rubber cork to seal it and sent to the laboratory.
2. The sample is processed immediately with all anaerobic precautions as follows –
 - a. Smear and Gram's staining.
 - b. Examination in U.V. Light (to observe pigmentation).
 - c. Culture—on blood agar containing neomycin, yeast extract, haemin, Vit K.
 - d. Incubate anaerobically for 24-48 hrs.
 - e. Examine the plate, study growth characters and confirm by Gram's staining, biochemicals, sugar fermentation and report.

Demonstration

1. Gram stained smear of pus from a case of Vincent's angina.
2. Gram stained smear of pus from a case of anaerobic infection showing multimicrobial aetiology.

Diagrams



Gram stained smear of pus from a case of Vincent's angina
(*F. fusiforme*)

Exercise

1. Enumerate nonsporing anaerobes.
2. Enumerate the disease caused by *Peptostreptococcus*, *B. fragilis*, *Actinomyces*, *Fusobacterium fusiforme* and *Mobiluncus*.
3. Mention the characteristics of nonsporing anaerobes.

Practical Class No. 16

MYCOBACTERIA

Introduction

1. These are slender rods sometimes showing branching, filamentous forms resembling mycelium in liquid cultures. They form a mould like pellicle. Hence, the name 'mycobacteria'.
2. They do not stain readily but once stained, resists decolourization with dilute mineral acids. Hence, they are called as 'Acid Fast Bacilli' (AFB).
3. Lepra bacilli were identified by Hensen in 1868.
4. Mammalian tubercle bacilli were isolated by Robert Koch in 1882.

Classification

1. Tubercle bacilli - *M. tuberculosis*, *M. bovis*, *M. africanum*
2. Lepra bacilli - *M. leprae*.
3. Mycobacteria causing skin ulcers - *M. ulcerans*, *M. hemophilum*, *M. marinum* or *battei*.
4. Atypical mycobacteria = non-tuberculous = paratubercle = most other than tuberculous.
5. John's bacillus = *M. paratuberculosis*.

1 *M. tuberculosis*

1. **Morphology** straight or slightly curved rods $3 \times 0.3 \mu m$ singly or in pairs mammalian tubercle, stained by Ziehl Neelsen fluorescent dyes auramine O, rhodopsine. can't be classified as gram +ve/-ve. acid fastness is due to unsaponifiable wax - mycolic acid or to a semipermeable membrane.

2. Cultural Characteristics

Generation time 14-15 hrs. Löwenstein-Jensen media Loeffler potassium sodium pyruvate enhances growth media are used to cultivate. on solid media - dry, rough, raised irregular colonies & a wrinkled surface they are creamy white.

3. Biochemical Reactions

Niacin +ve
peroxidase +ve
catalase +ve
Nitrate reduction test +ve.

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4. Pathogenesis Source of infection - open case of T.B.
Mode of infection - 1. inhalation of aerosolized bacteria.
2. ingestion through infected milk.
3. inoculation.

Inhaled bacilli reaching lungs are ingested by alveolar macrophages. But they have a ability to survive & multiply in macrophages. - effective specific immune mechanism is cell mediated immunologic lesions called tubercles are produced in tissue.

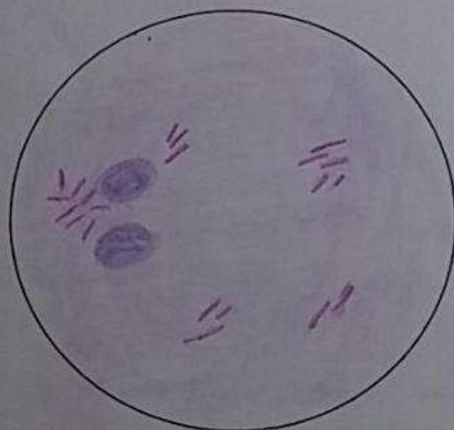
Laboratory Diagnosis

1. Pulmonary tuberculosis
2. Renal tuberculosis

Pulmonary Tuberculosis

Microscopic Examination

- a. Collection of the sample
 - ① specimen - sputum
Sputum is best collected in morning before any meal if sputum is scanty or not available.
 - ② laryngeal swabs.
 - ③ bronchial washing.
 - ④ gastric lavage may be collected.
- b. Smear preparation and Z-N staining



Z-N staining of sputum sample showing AFB

New slides should be used for smears & they should not be reused. Smears should be prepared from thick purulent part of sputum. Smears are dried heat fixed & stained.

c. Grading

10 or > 10 / F	4+
1-9 / F	3+
1-9 / 10 F	2+
1-9 / 100 F	1+

d. Fluorescent technique

- smears are stained w/ auramine phenol or auramine rhodamine,
- fluorescent dyes - on examⁿ under UV light bright rods against background.

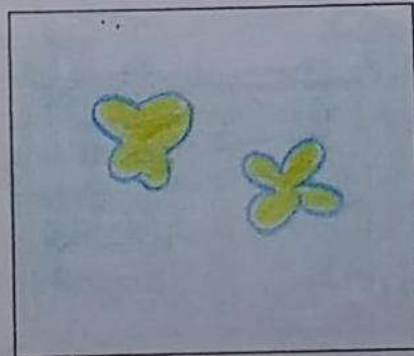
e. Concentration methods

Useful microscopy - treatment w/ antifoaming, NaOH & detergent like tergitol, floatation methods using hydrocarbons & autoclave method.

Petroff's Method

sputum is incubated w/ equal vol. of 4% NaOH solⁿ at 37°C with frequent shaking till it becomes clear, on an avg. for 20 minutes. Then it is centrifuged at 2000 rpm for 20 minutes & sediment is neutralised with N/10 HCl.

f. Culture



LJ medium

Animal inoculation

Guinea pig

- concentrated material is inoculated in- into thigh of 2 healthy guinea pigs about 12 weeks old.
- animals are weighed before & after inoculation.
- Loss of wt. is an indication of infection.
- after 4 & 8 weeks respectively, each guinea pig is killed & autopsied & checked for caseous necrosis.

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Sensitivity techniques aid in t/t.

I. absolute concn method - a no. of media containing serial concn of drugs are inoculated.

II. Resistance ratio method.

2 sets of media containing graded concn of drugs & inoculated one set with test strain & other with standard strain of known sensitivity.

III. Proportion method - indicates avg. sensitivity of strain considering any population will contain cells with varying degrees of sensitivity.

Advance techniques for diagnosis

Nucleic acid technique

PCR

LCR

RLFP

IS fingerprinting

Test for rifampicin resistance.

II Renal tuberculosis

Collection of the sample-

Urinary excretion of sample is intermittent.

It is advisable to test 3-6 morning samples of urine each sample is centrifuged for 3000 rpm for 30 min.

Sediment is used for culture.

Tuberculin test / Mantoux test:

1 ml PPD containing 5 TU is injected intradermally on flexor aspect of forearm with a tuberculin syringe raising a wheal.

Site is examined after 48-72 hrs. Induration is measured along transverse axis, >10 mm is +ve.

≤ 5 mm -ve

6-9 mm - equivocal

BCG vaccination

Bacille Calmette Guérin Vaccine.

This is a strain of M. Bovis attenuated by 239 serial subculture in glycerine-bile potato medium over 13 yrs.

- Immunity lasts for 10-15 yrs, gives protection to infants & young children.

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Demonstration

1. Smear prepared from sputum sample of patient suffering from pulmonary tuberculosis and stained with Z-N staining.
2. LJ medium (uninoculated).
3. LJ medium (with growth of *M. tuberculosis*).
4. LJ medium (with growth of Atypical mycobacteria)
5. Guinea pig
6. Fluorescent microscopy (photograph).

Exercise

1. Why they are called as mycobacteria?
2. Why they are called as AFB?
3. How will you classify mycobacteria?
4. How much time is required for the growth of *M. tuberculosis*?
5. Which medium is used for its culture? How is it sterilized and what are the ingredients of it?
6. How will you grade the direct smear and why?
7. What are the complications of BCG vaccine?
8. Interpret the results of tuberculin test.
9. Which animal is most susceptible to *M. tuberculosis*?
10. What are the examples of saprophytic mycobacteria?
11. How will you differentiate *M. tuberculosis* from *M. Bovis* on morphological characteristics?
12. What are the different concentration methods?
13. What are much granules?
14. What is Koch's phenomenon?
15. What is cord factor?
16. What do you understand by the term Eugonic and Dysgonic?
17. What do you know about extrapulmonary tuberculosis?
18. What is MDR *M. tuberculosis*?
19. What is DOTS programme?

B Atypical Mycobacteria

Introduction

1. Micobacteria other than human or bovine tubercle bacilli which may occasionally cause human disease resembling tuberculosis, have been called 'atypical or anonymous or unclassified mycobacteria'.
2. They are also called as paratubercle, tuberculoid and MOTT (Mycobacteria Other Than Tubercle bacilli).

Classification- It is based on pigment production and rapidity of growth.

1. Photochromogens

1. *M. marinum* → swimming pool granuloma
2. *M. kansasii* → pulmonary tuberculosis

⇒ produce orange yellow pigment & when exposed to light.

2. Scotochromogens

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- produce yellow orange red pigment even in the dark.
→ *M. scrofulaceum* & *M. goodii* > cervical lymphadenitis mostly
contaminant from tap water.

3. Nonphotochromogens

No pigment even on exposure to light.

*M. avium**M. intracellulare* > Disseminated d/s } In AIDS pt.
pulmonary lesions }

4. Rapid growers

Growth within 7 days

M. chelonae, *M. phlei* - chronic abscess.

Pathogenesis

1. Lymphadenitis
2. Skin lesion
3. Post injection abscesses
4. Swimming-pool granuloma
5. Buruli ulcer
6. Pulmonary disease
7. Disseminated diseases

Treatment

It is resistant to most of the antitubercular drugs. However, isoniazid, rifampicin, ethambutol are effective.

Demonstration

1. LJ medium showing yellow-orange pigment (photochromogen)
2. LJ medium showing yellow- orange- red pigments (scotochromogen)

Exercise

1. Classify atypical mycobacteria.
2. What is MOTT?
3. Enumerate the infections caused by atypical mycobacteria.

Practical Class No. 17

MYCOBACTERIUM LEPRAE

Introduction

1. It is the causative agent of leprosy.
2. Leprosy is known since Vedic times in India and from Biblical times in the Middle East.
3. The lepra bacilli was first observed by Hensen in 1868.
4. It can't be cultured on artificial culture media (exception to Koch's postulate).

Morphology:

Straight or slightly curve rod, $1-8 \times 2-5 \mu\text{m}$ in size
gram +ve
acid fast but in 5% H_2SO_4 .

Morphological Index:

- % of uniformly stained bacilli out of total no. of bacilli counted.
- method for assessing progress of chemotherapy.

Bacteriological Index:

No. of total bacilli in tissue.
for calculating BI, a min. 4 skin lesion, a nasal swab & both ear lobes are to be examined.

Cultivation:

- Not yet been grown on artificial culture media or tissue culture.
- Following animals have been used for experimental infection mouse, Nine-Banded armadillos, chimpanzees, muntjac.

Resistance:

Survive in warm humid environment for 9-16 days
& in moist soil for 46 days

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Leprosy Classification-
Madrid Classification, 1953 -

Ridely and Jopling Classification 1966-

Tuberculoïd TT
Borderline tuberculoïd BT
Borderline BB
Borderline lepromatous BL, Lepromatous LL

Leprosy and Cell Mediated Immunity:

Leprosy infection induces both humoral & CMI.
patient with deficient CMI develop lepromatous.
when CMI is adequate tuberculoïd type is developed.

Lepromin Test:

- 0.1 mL of lepromin is injected intradermally.

Response → Early Rxⁿ of Fernandez - erythema & indurⁿ developing in 24-48 hrs & remains for 3-5 days lesion consists of exudate with lymphocyte infiltration.

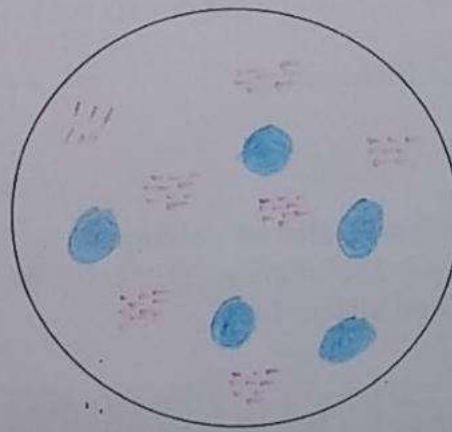
→ Late Rxⁿ of Mitsuda - appears 1-2 wks injection reaching a peak in 4 weeks. it appears in the form of a nodule & ulcerate it takes a few weeks to heal at exudate consists of lymphocytes, epithelioid cells & giant cells.

Laboratory Diagnosis

Specimen collected from nasal mucosa, skin lesions, & ear lobules.
slit & scrape method is used.
samples from skin should be obtained from edges of lesion.
A cut about 5 mm long is made with scalpel, deep enough to get into the infiltrated layers.

Demonstration

Z-N staining of a smear prepared from the material obtained from an ear lobule by skin clipping of a case of lepromatous leprosy.



Lepra bacilli in skin clipping with 'Globi'

Exercise

1. Which specimens are collected from a patient for laboratory diagnosis of leprosy?
2. Enumerate the differences between the tuberculoid and lepromatous leprosy.
3. What are the animal models used for culture of *M leprae*?
4. What is morphological and bacteriological indices?
5. What is the use of lepromine test?
6. Mention complications of lepromatous leprosy.
7. Mention about the classification of leprosy.
8. Comment on vaccination against leprosy.
9. What is lepra reaction?
10. Mention the role of CMI in leprosy.
11. Mention drugs used in the treatment of leprosy.

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- UTI
- Pyogenic infection. It is an important cause of neonatal meningitis.
- Septicemia

Morphology

Gram -ve bacillus
Motile by peritrichate flagella.
Non-sporing & non-capsulated.

Cultural Characteristics

1. NA: circular, moist, smooth & entire margin & non-mucoid.

2. BA:

β hemolysis

3. MC:

Lactose fermenting - pink colour.

Biochemicals tests

IMViC ++--

Others

1. Urease -ve
2. Ferment lactose & mannitol, but not sucrose

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2.

3.

Virulence

1. Surface Ag
 - a. Surface O Ag
 - b. Fimbriae
2. Toxin
 - a. Enterotoxin
 - b. Haemolysins

Laboratory diagnosis

1. UTI

Sample- midstream urine, catheter specimen, supra pubic aspiration.

Microscopy (wet mount)

pus cells, RBC, epithelial cells, & bacteria are examined.

Culture-

Standard loop technique.

Biochemicals-

1. Griess nitrite test
2. TTC - Triphenyltetrazoline chloride test
3. Catalase test
4. glucose test paper
5. polymorpho nuclear neutrophil.

Serology-

6. Leucocyte esterase.

Antibiotic sensitivity testing- Done by streaks diffusion method.

2. Diarrhoea

E. coli - 5 groups - cause diarrhoea.

1. Enteropathogenic
2. Enterotoxigenic
3. Enteroinvasive
4. Enterohemorrhagic
5. Enteraggregative.

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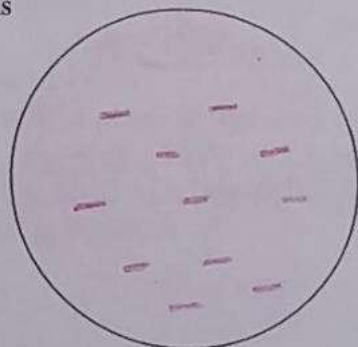
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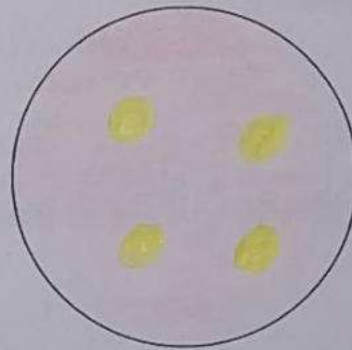
Demonstration

1. Gram's stain smear of *E. coli* (GNB).
2. BA- showing growth of *E. coli*.
3. MC- showing growth of *E. coli*.
4. Antibiotic sensitivity testing of *E. coli* isolated from urine sample.
5. Biochemical sets and sugar fermentation sets of *E. coli*.
6. Demonstration of motility.

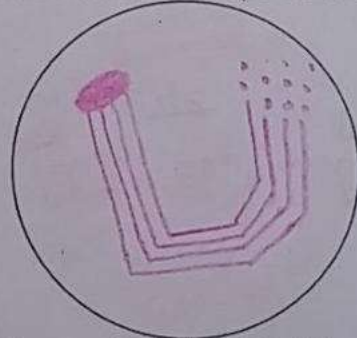
Diagrams



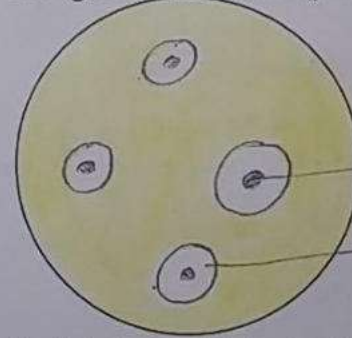
Gram's stain smear (*E. coli*, GNB)



BA- growth of *E. coli* (β haemolysis)



MC- growth of *E. coli* (pink colour)



Antibiotic sensitivity testing

Antibiotic disc
Zone of inhibition

Motility: *E. coli* are motile organisms.

Biochemical set (*E. coli*)

Sugar set (*E. coli*)

Indole	MR	VP	Citrate	Urease	H ₂ S
+	+	-	-	-	-

L	G	M	S
+	AG	+	-

Exercise

1. What are the general characters of Enterobacteriaceae?
2. What is the importance of lactose in MC medium?
3. Describe growth of *E. coli* on BA & MC.
4. Which antibiotics will you ask for antibiotic sensitivity testing of *E. coli* isolated from a case of UTI?
5. What are the virulence factors seen in *E. coli*?
6. How heat labile enterotoxin acts and how is it detected?
7. Enumerate the causative agents of neonatal meningitis.
8. What is traveler's diarrhoea?
9. What is Sereny test?
10. Enumerate the screening tests used for diagnosis of UTI.
11. What is significant and insignificant bacteriuria?
12. Mention different types of *E. coli* causing diarrhoea.
13. Describe quantitative and semiquantitative methods used for diagnosis of UTI.

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KLEBSIELLA

Introduction

1. The genus Klebsiella consists of Gram negative, nonmotile, capsulated rods.
2. They grow on ordinary media and form large, dome shaped, mucoid colonies with stickiness.

Classification

They are classified into three species.

- *Klebsiella pneumoniae*
- *K rhinoscleromatis*
- *K ozaenae*

Morphology

short, plump gram -ve, capsulated non-motile bacilli.

Culture Characteristics

1. NA

- Large, mucoid
- mucoid nature of colony is due to capsular material produced by organism.

2. BA

3. MC

Lactose fermenting - pink colouration.

Biochemicals

I M V C - - + +

Urease +

ferment lactose, mannitol & sucrose.

Pathogenicity

1. *K pneumoniae* → broncho pneumonia, UTI, nosocomial infections, wound infection, septicemia, meningitis.
2. *K rhinoscleromatis* → granulomatous dts of nose & pharynx - rhinoscleroma.
3. *K ozaenae* → atrophic rhinitis - Ozaena.

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Laboratory Diagnosis

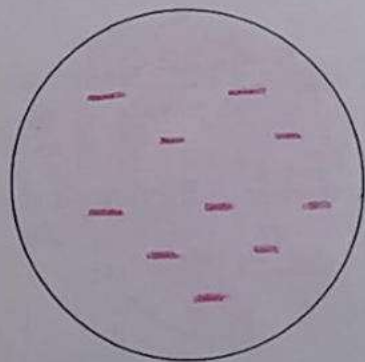
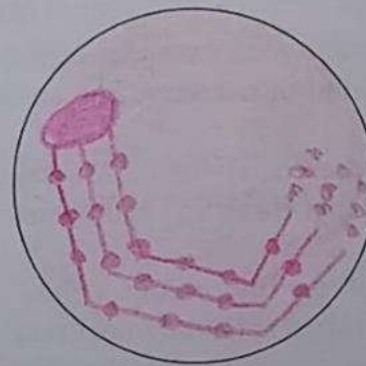
Appropriate sample is collected depending upon the nature of infection

Identification is done by –

1. Microscopy
2. Isolation by culture
3. Confirmation by biochemical tests
4. AST is routinely performed

Demonstration

1. Gram's stain smear showing typical morphological features of Klebsiella.
2. MC- showing growth of Klebsiella (pink, dome, shaped, mucoid colonies)
3. Biochemical tests (set of IMViC) and sugar fermentation (set of LGMS)
4. Motility (nonmotile)

Diagram

 Gram's stain smear
(Morphology of Klebsiella)

 MC showing pink colour
domed shaped mucoid colonies

Biochemical set (Klebsiella)

Indole	MR	VP	Citrate	Urease	H ₂ S
–	–	+	+	+	

Sugar set (Klebsiella)

L	G	M	S
+	AG	+	+

Motility: nonmotile

Exercise

1. Describe the growth of Klebsiella on the MacConkey's medium.
2. What are the different species and the lesions produced by them?
3. Comment on its role in causing hospital infections.
4. Why antibiotic sensitivity testing is essential in *Klebsiella pneumoniae*?

PROTEAE (Tribe)
Introduction

1. They are Gram negative pleomorphic, noncapsulated, motile rods
2. They do not ferment lactose.

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3. They are normal intestinal commensals and act as opportunistic pathogens.
4. As they are pleomorphic and Greek God Proteus has ability to assume any shape, in his honour the name proteus has been given to these organisms.

Classification

Proteeae (Tribe)

Proteus (genus)

Pt. vulgaris
Pt. mirabilis

Morganii (genus)

Morg. morganii

Providencia (genus)

Prov. stuartii
Prov. rettgerii
Prov. alcalifaciens

This shows that genus proteus consist of two important species i.e. *Pr. vulgaris* and *Pr. mirabilis* and belongs to the tribe Proteaeae.

They are PPA positive (this test differentiates them from other members of Enterobacteriaceae which are PPA negative).

Genus Proteus

1. Widely distributed in nature as saprophytes.
2. They are found in decomposing animal material, sewage, manured soil, human and animal faeces.
3. Frequently present on the moist area of skin.
4. They are opportunistic pathogens and cause UTI, pyogenic infections and hospital infections.
5. It consists of two important species.
 - a. *Pr. vulgaris* (Indole positive)
 - b. *Pr. mirabilis* (Indole negative)

Morphology

gram -ve bacilli
Non-capsulated.
Non-sporing
actively motile

Cultur Characteristics

1. NA emits putrefactive (fishy/seminal) odour & swarming motility.
2. BA (swarming) swarming motility.
3. MC smooth, pale & colourless (NLF)
No swarming motility.

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Biochemical Tests

The identification of the species requires different biochemical tests e.g.-

1. Indole
2. Urease
3. Ornithine decarboxylation
4. Fermentation of adonitol and trehalose

Genus *Morganella*

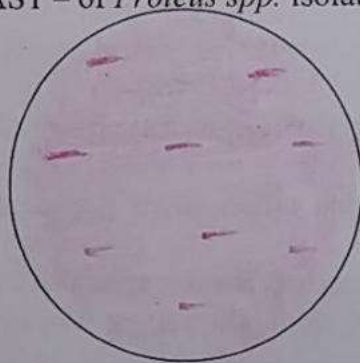
1. Consists of only one species (*Morg. morganii*)
2. Does not produce swarming
3. Causes UTI and hospital infection

Genus *Providencia*

1. It contains three species
2. They cause diarrhoea, UTI, hospital infection, infection in burns and wounds.

Demonstration

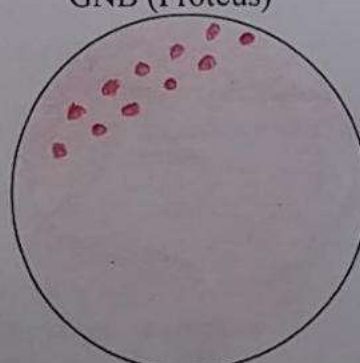
1. Gram stained smear of GNB (*Proteus*) isolated from a case of wound infection.
2. BA- with swarming growth.
3. MC- NLF colonies.
4. AST – of *Proteus spp.* isolated from a clinical sample.



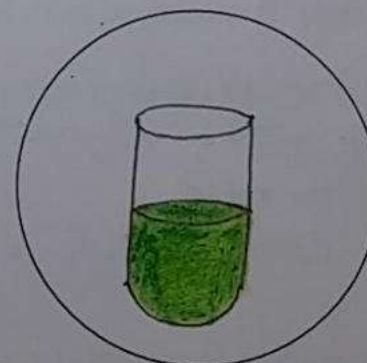
Gram's stain smear
GNB (*Proteus*)



BA showing swarming



MC showing NLF colonies



PPA test

Exercise

1. What is Diene's Phenomenon?
2. Why the name *Proteus* is given?
3. Describe the growth of *Pr. vulgaris* on blood agar.
4. How will you prevent swarming?
5. What are the examples of the species included in the Tribe Proteace?
6. What are the infections produced by *Pr. vulgaris* and *Pr. mirabilis*?
7. What do you understand by the terms 'Hauch' and 'Ohne Hauch'?
8. Which property of *Proteus* is utilized in 'Weil Felix reaction'?
9. What is heterophilic agglutination? Give the examples of heterophilic agglutination reaction.

Practical Class No. 19

SHIGELLA

Introduction

It is the causative agent of bacillary dysentery.

Classification

It is classified into four types-

1. *Sh dysenteriae*- it consists of 10 serotypes.

Serotype I is called as *Shigella shiga*

It is indole negative and catalase negative. It causes the more severe form of bacillary dysentery.

Serotype II (*Shigella schmitzi*)

It is indole positive.

Afterwards serotypes III to VII were added which were described by Large-Sachs groups. After that again 3 serotypes were added making total 10 serotypes.

2. *Sh. flexneri*

It was described by Flexner (from Philippines in 1900). It consists of 6 serotypes. *Sh. flexneri* is common type in India.

3. *Sh. boydii*

It was described by Boyd from India in 1931. It consists of 15 serotypes.

4. *Sh sonnei*

It was described by Sonne in 1915 in Denmark. It is late lactose fermenter. It causes the mild form of bacillary dysentery. It consists of many colicin types.

Morphology

- short, Gram -ve bacilli

Non-motile

Non-capsulated & non-sporing.

Culture Characteristics

1. NA

circular, convex, smooth & translucent

2. MC

- colourless

But pink colonies are formed in case of *Sh. sonnei*

3. DCA

colourless.

4. XLD agar

Red without black centers, selective media.

Biochemical reactions

I	M	Vi	C
Variable	-	-	-

Pathogenesis

Infective dose- 10-100 bacilli

Ingestion (food)

Stomach (resist gastric acidity and survive)

Large intestine (infect the epithelial cells of villi & multiply inside them)

Spread laterally to involve adjacent cells

Penetrate the lamina propria (inflammatory reaction develops leading to capillary thrombosis)

Necrosis of patches of epithelium

Sloughs off leaving behind transverse ulcers

Bacillary dysentery

Dysentery is a clinical condition characterized by frequent passage of blood stained mucopurulent stools. In bacillary dysentery the tenesmus is common.

Laboratory diagnosis

Sample - stool

Characteristics-

1. No offensive smell
2. Amount is small. Large quantity of mucous with freshly passed blood (red current jelly) and fecal matter is absent or scanty.
3. Frequency over 10 motions per day.
4. Tenesmus is more.

Transport media-

1. Cary - Blair medium
2. Sach's buffered glycerol saline

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Macroscopic and Microscopic examination of stool

Macroscopic	Microscopic
1. Blood & mucous, but no faeces	1. Discrete RBC
2. small amount	2. Numerous pus cells.
3. Bright red	3. Macrophage laden E RBC
4. odourless	4. Numerous ghost cells.
5. Adherent to container	5. very low eosinophils.

Isolation by Culture;

BA-

MC- Colourless - NLF

DCA- - Colourless

XLD agar- Red without black centers

Colony characters are studied, then smear is prepared from the colony and Gram's staining is performed and morphological characters are studied. Then the isolate is subjected for biochemical tests.

Biochemical tests-

The growth is confirmed by the various biochemical tests as described above, in this way various types are identified. If antisera are available then *Sh. dysentery*, *Sh. flexneri*, *Sh. boydii* are confirmed by serology. Colicin typing may be performed to *Sh. sonnei*, if facilities are available. The isolated strain is subjected to AST and may be sent to the reference laboratory i.e. NICED Kolkata.

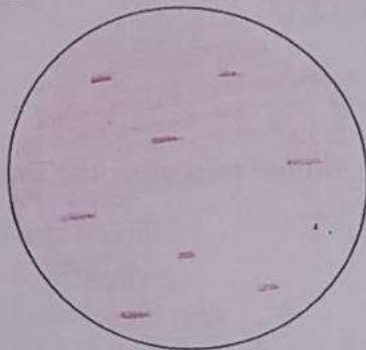
Demonstration

1. Gram stained smear prepared from the fecal sample of a case of bacillary dysentery.
2. Growth of *Shigella* on - NA, BA, MC, DCA, XLD agar.
3. Biochemical tests of *Shigella*.
4. Serology by antisera.
5. Cary-Blair medium.

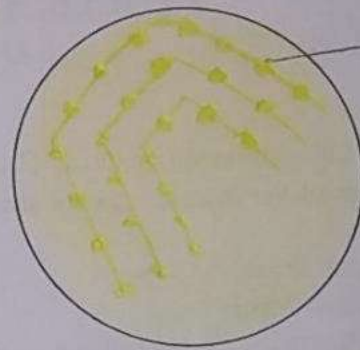
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Diagram

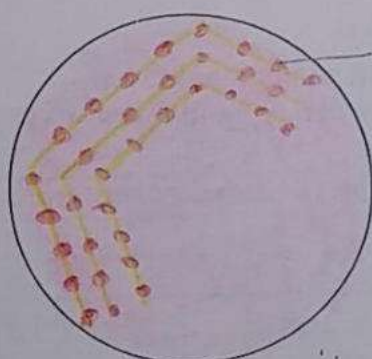


Gram's stained smear of fecal matter of a case of bacillary dysentery



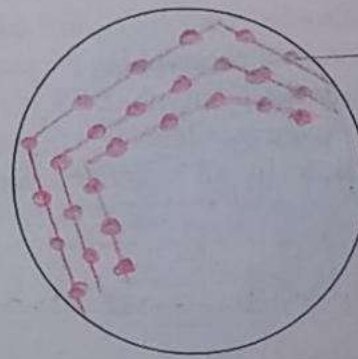
circular, convex, smooth, translucent colonies

NA (growth of Shigella)



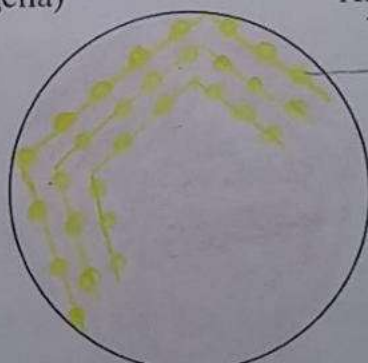
No pink colouration

MC (growth of Shigella)



Red colonies

XLD agar (growth of Shigella)



colorless colonies

DCA (growth of Shigella)

Exercise

1. Define the term dysentery.
2. Classify Shigella organisms.
3. How will you diagnose a case of bacillary dysentery?
4. Enumerate the differences between amoebic and bacillary dysentery.
5. Which Shigella is common in India?
6. Which Shigella causes severe and mild form of bacillary dysentery?
7. What are the characteristics of *Shigella shiga*?
8. What are the different media for isolation of Shigella organism?
9. What are the characteristics of bacillary dysentery?
10. What is Shigellosis?
11. What are the other agents causing dysentery?

Practical Class No. 20

SALMONELLA

Introduction

1. The genus salmonella consists of Gram negative bacilli which parasitise the intestine of a large number of vertebrate species and infect human beings.
2. They cause
 - Enteric fever.
 - Gastroenteritis
 - Septicemia with or without suppuration
 - Carrier state
3. The genus consists of more than 2000 serotypes.
4. The most important members are
 - a) *Salmonella typhi* (*S. typhi*)- causing typhoid fever
 - b) *Salmonella paratyphi A* (*S. paratyphi A*)- causing paratyphoid fever
 - c) *Salmonella paratyphi B* (*S. paratyphi B*)- causing paratyphoid fever
5. All salmonella are motile except *S. gallinarum-pullorum*.
6. H_2S is produced by all salmonellae except *S. paratyphi A*, *S. choleraesuis* and some other serotypes.

Morphology: Gram -ve bacilli
motile & peritrichate flagella

Cultural Characteristics:

NA- Large, circular, low convex & smooth, translucent

MC- Non-lactose fermenting.

DCA- Black heads are formed due to H_2S production

WB Agar-

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XLD Agar- Xylose lysine deoxycholate
Black heads due to H_2S production.

Enrichment Media

- Bile broth
- Selenite F broth
- Tetrathionate broth

These are commonly used.

Biochemical tests:

	Indole	MR	VP	Citrate	Urease	H_2S	L	G	M	S
<i>S. typhi</i>	—	+	—	+	—	+	—	A		—
<i>S. paratyphi</i> A	—	+	—	+	—	—		AG		—
<i>S. paratyphi</i> B	—	+	—	+	—	—		AG		—

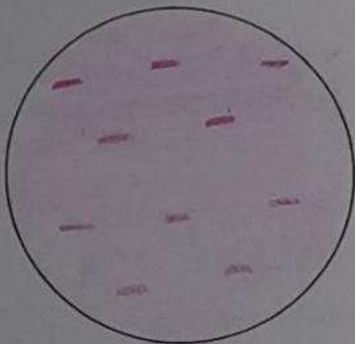
Decarboxylase Test: -

	Lysine	Ornithine	Arginine
<i>S. typhi</i>			
<i>S. paratyphi</i> A			
<i>S. paratyphi</i> B			

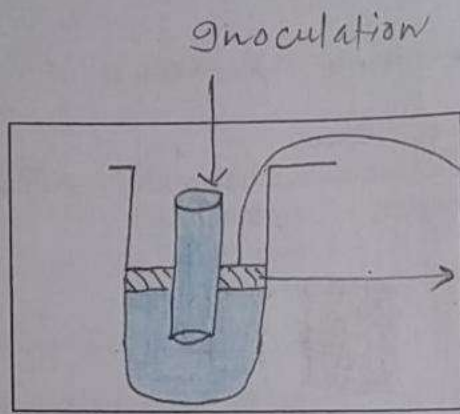
Mode of Infection: - Ingestion of contaminated food and drink, milk and milk products, shellfish from contaminated water, dried or frozen eggs of infected fowls, meat and its products of infected animals.

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Diagrams

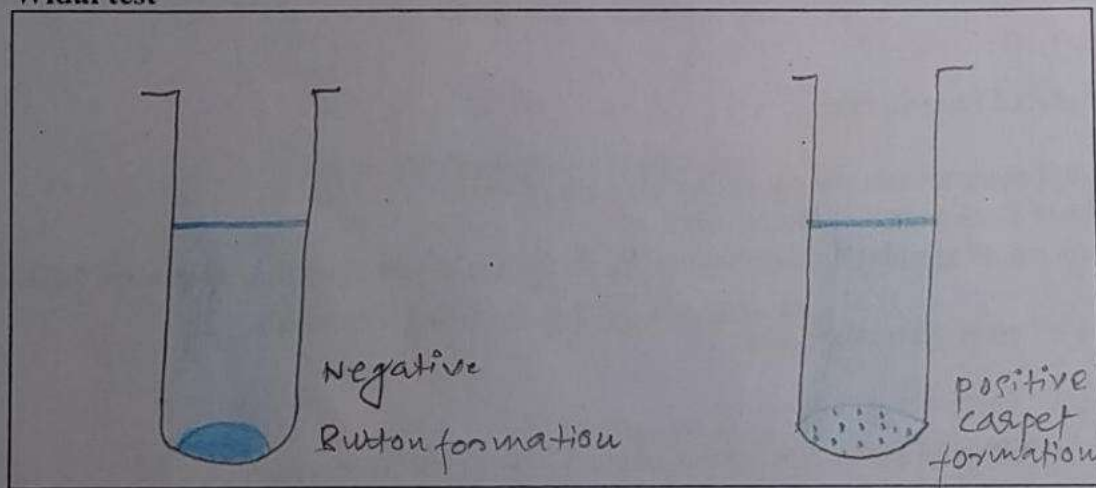


Gram's stained smear of Salmonella isolated from blood culture

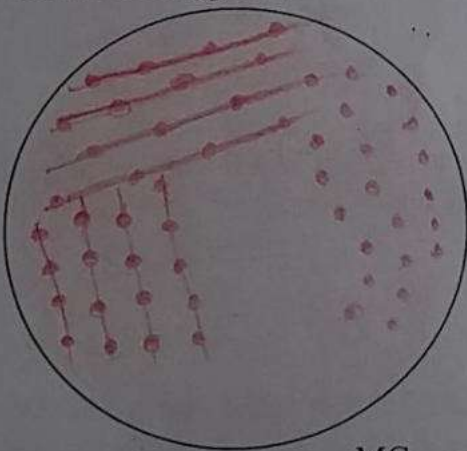


Craigie's tube

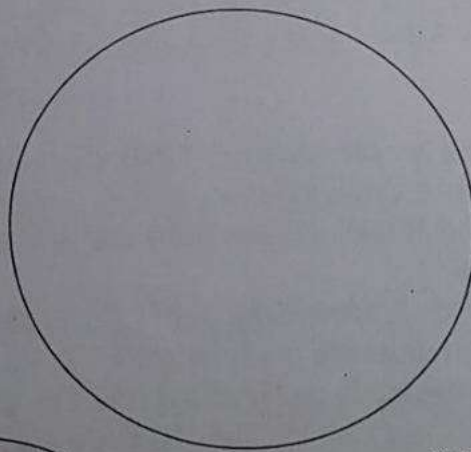
Widal test



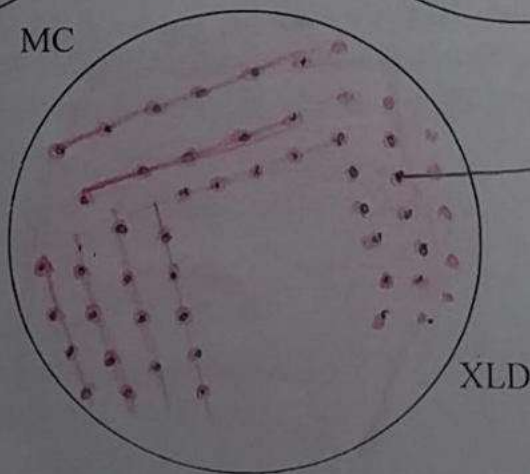
Growth of *S. typhi* on-



MC

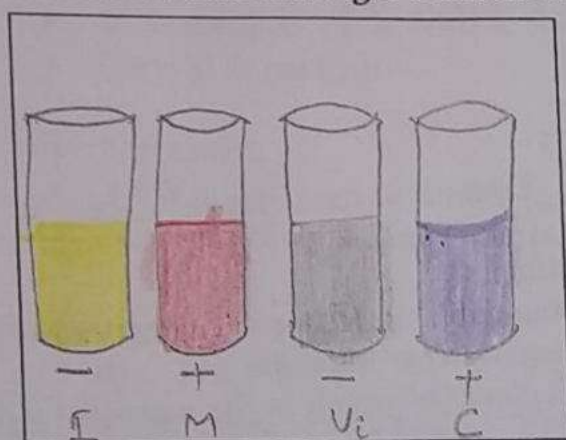


WB

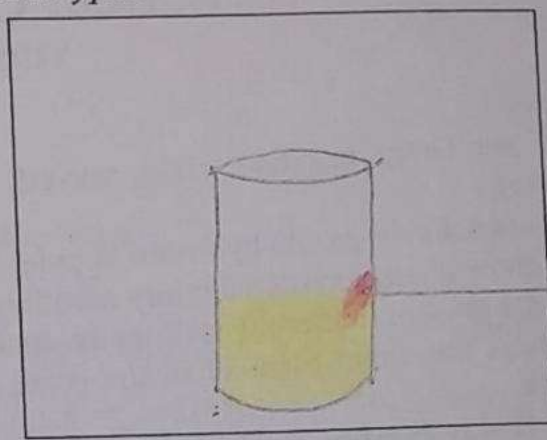


XLD

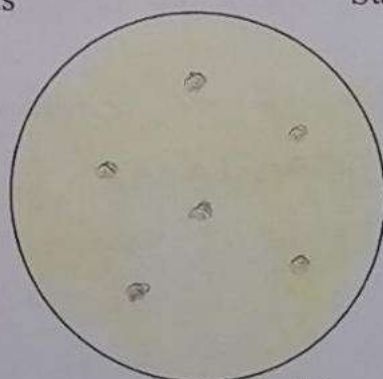
Black heads due to H_2S production

Biochemical tests and sugar fermentation of S. typhi -

Biochemical tests



Sugar fermentation

AST (*S. typhi*)**Exercise**

1. How will you carry out laboratory diagnosis of enteric fever?
2. Which salmonella is nonmotile?
3. What is the use of Craige's tube?
4. What is Kauffmann-White scheme of classification of salmonella?
5. What are the salmonella carriers? How will you detect them?
6. What are the drawbacks of Widal test?
7. What are the diagnostic O and H agglutinin titers of Widal test?
8. What is the advantage of clot culture over blood culture?
9. What is the advantage of stool culture in enteric fever?
10. What are the different antigens seen with salmonella?
11. Why Vi antigen is not included in Widal test?
12. Describe the growth of *S. typhi* and *S. paratyphi* A on WB and XLD agars?
13. Mention the enrichment media for isolation of salmonella and how enrichment is done?
14. Which species of salmonella cause gastroenteritis (food poisoning)?
15. What are the complications, which may occur in a case of enteric fever?
16. Which drugs will you select for the treatment of enteric fever?
17. Give the examples of GNB, which are motile and nonmotile.
18. Give the examples of GNB producing pink coloured and colourless colonies on MC medium.
19. What do you know about Arizona group?
20. Which salmonella are common in India?
21. Comment on vaccination against typhoid fever.
22. What is the mechanism of development of multidrug resistance in salmonella?
23. Mention the tests used for detection of Salmonella Ag in the early stages of enteric fever.

Practical Class No. 21

VIBRIOS

Introduction

1. They are Gram negative, rigid, curved rods (Comma shaped, hence, called as *Vibrio comma*).
2. They are actively motile by means of polar flagellum.
3. They show characteristic vibratory motility (Vibrae- vibrate). Hence the name Vibrio.
4. They are present in marine environment and surface water worldwide.
5. The most important member of the genus is *V. cholerae* which is the causative agent of cholera.
6. It was first isolated by Koch (1883) in Egypt from a case of cholera.

Morphology

Gram -ve, curved or comma shaped, non-sporing, non-capsulated.

Motility

Darting motility $\bar{c}$ a single polar flagella.

Cultural Characteristics

NA-

Moist, translucent, round disks, $\bar{c}$ a bluish tinge in transmitted light.

BA-

No hemolysis

E1 to ∞ biotype produces hemolysis

MC-

colourless.

TCBS- yellow colour colonies due to sucrose fermentation.

Large & convex.

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Transport Media

- V. R. medium
- Cary-Blair medium

Enrichment media

- APW
- Monsur's taurocholate tellurite peptone water

String test

A loopful of growth is mixed in a drop of 0.5% sodium deoxycholate or saline on a slide & the suspension loses turbidity becomes mucoid & forms string.

Biochemical test

1. Sugar fermentation

- ferment glucose, mannitol, sucrose & maltose.

2. Indole production +ve

3. Nitrate reduction +ve

4. Cholera red reaction *V. cholera* is indole +ve & it reduces nitrite to nitrate. When a few drops of con. H_2SO_4 is added to peptone water culture - a reddish pink colour is produced due to formation of nitroso indole.

5. Catalase test and oxidase test

Both are +ve.

6. Decarboxylase test

Vibrio decarboxylate lysine & ornithine. But do not use arginine.

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Differentiation between Classical & El Tor Vibrios

Sr. No.	Test	Classical	El Tor
1.	Hemolysis of Sheep RBC	-	+
2.	Agglutination of chick RBC	-	+
3.	Voges-Proskauer VP R ⁺	-	+
4.	Polymyxin B sensitivity	+	-
5.	Susceptibility to Mukherjee phage IV	+	-
6.	Modified CAMP Test	-	+

Classification

Vibrios are classified by –

1. Heiberg classification (based on sugar fermentation)
2. Gardner and Venkatraman's classification.

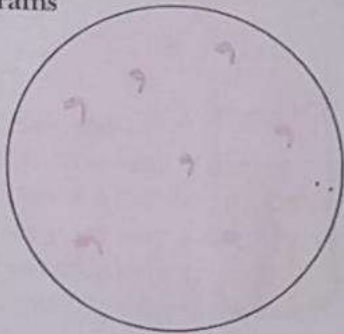
Note: Gardner and Venkatraman scheme of classification is based on the antigenic structure of vibrios (O and H Ag).

By this they are classified into –

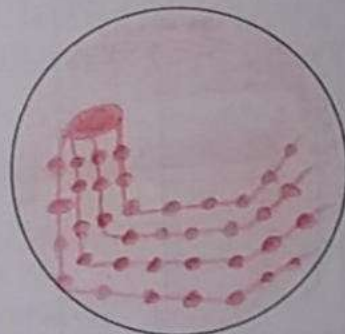
1. Classical vibrios (nonhemolytic) having 3 serotypes i.e. Inaba, Ogawa, Hikojima.
2. El Tor vibrios (hemolytic) having 3 serotypes i.e. Inaba, Ogawa, Hikojima.

- Both Classical and El Tor vibrios show agglutination with O subgroup 1 antisera hence called as 'O1' serovar or serogroup.
- First six pandemics were caused by classical vibrios and seventh pandemic was caused by El Tor vibrios. Now the cases of cholera which occur are due to -
 - El Tor vibrios and by non-O1 types (O139).
 - El Tor vibrios were first isolated at Tor quarantine, hence the name El Tor vibrios.
 - El Tor vibrios cause cholera but the severity is less than Classical vibrios.

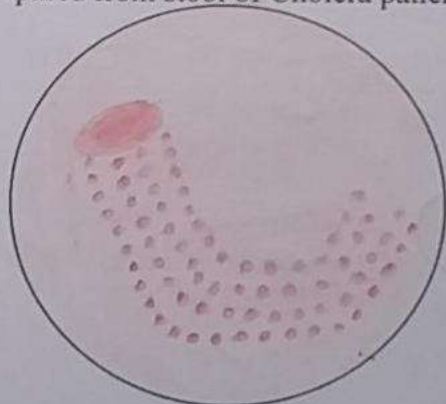
Diagrams



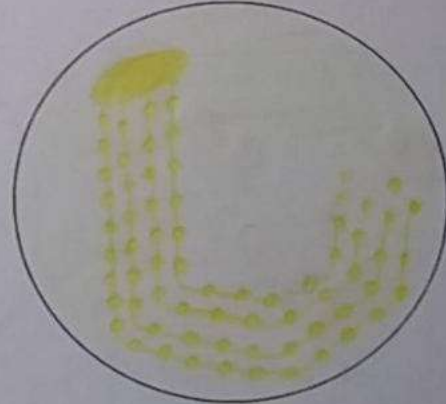
Gram stain showing comma shaped GNB (*Vibrio*) prepared from stool of Cholera patient



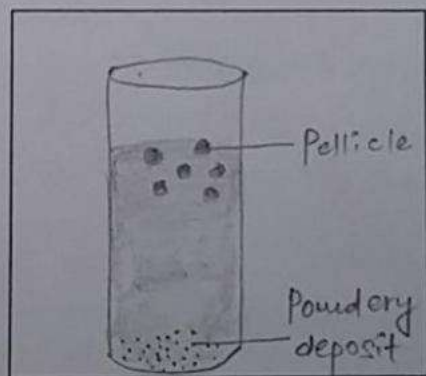
BA (hemodigestion)



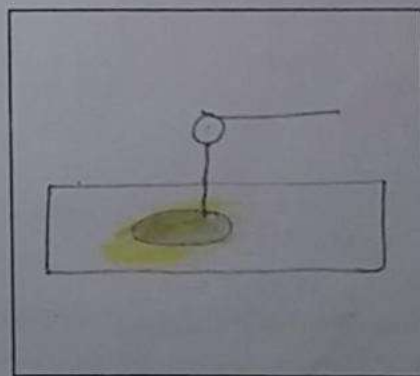
MC (NLF)
(*V. cholerae* is late lactose fermenter)



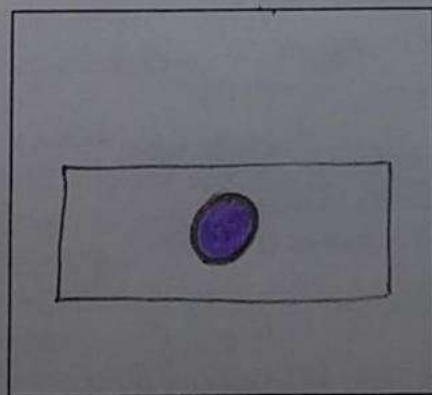
TCBS (yellow colour colonies)



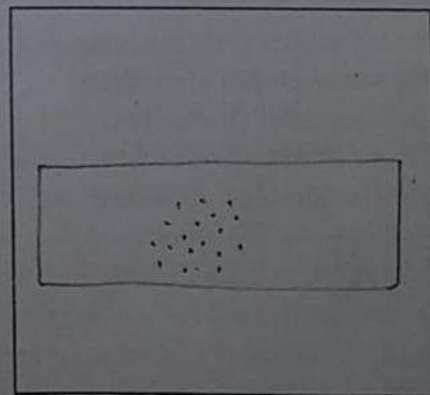
APW



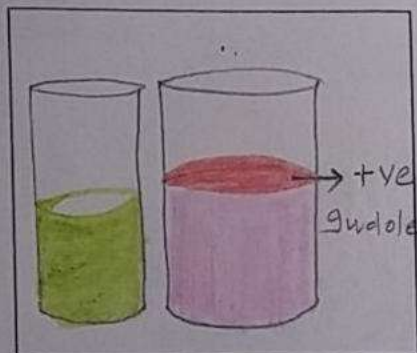
String test



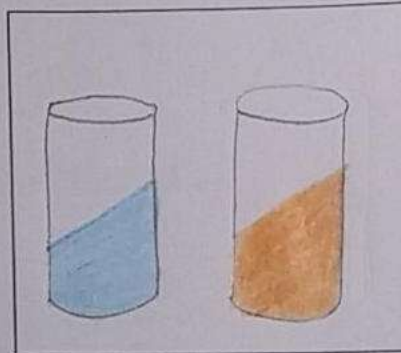
Oxidase Test



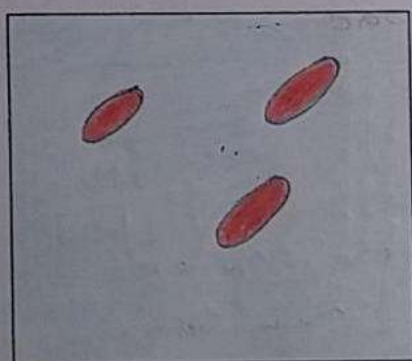
Catalase Test



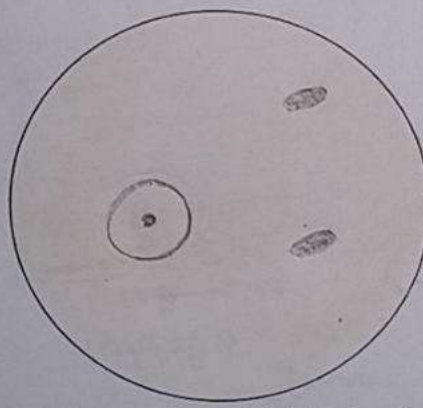
Cholera red reaction



Serology



Chick red cell agglutination Test



Polymyxin B sensitivity Testing

Exercise

1. Why the name *V. cholerae*?
2. Describe the motility of *V. cholerae*.
3. How will you differentiate Classical and El Tor Vibrios?
4. What is Gardner and Venkatraman's scheme of classification of Vibrios?
5. What is string test?
6. Mention three important tests for identification of *V. cholerae*.
7. Comment on present status of cholera.
8. What is the pathogenesis of cholera?
9. Give examples of transport, enrichment and plating media used for *V. cholerae*.
10. What is the role of serology in identification of cholera vibrios?
11. How will you carry out the laboratory diagnosis of a case of cholera?
12. What is cholera red reaction?
13. Comment on vaccination against cholera.
14. What are halophilic vibrios? Give example.
15. What is the role of antibiotics in treating the case of cholera?
16. What do you understand by the term rice water stool?
17. What are the characteristics of cholera stool?

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Practical Class No. 22

PSEUDOMONAS

Introduction

1. Pseudomonas is a large group of aerobic, nonsporing GNB.
2. They are motile by polar flagella.
3. They are seen everywhere, saprophytic, found in water, soil and other moist environment.
4. Few species cause human infections.
5. Most important species is – *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa (Ps. pyocyanea)

Morphology: slender, gram -ve bacillus.
Non-capsulated

Cultural characteristics and pigments:

Strict aerobe grows well on ordinary media
produces - pyocyanin, pyoverdine, pyorubin & pyomelanin
pigments -
These diffuse into surrounding medium.

Biochemical tests:

1. catalase +ve
2. oxidase +ve
3. Reduce Nitrate.

Pathogenesis

It causes opportunistic infections in the cases where the host resistance is lowered (immunocompromised host) e.g. in AIDS, malignancies etc.

Pathogenicity

It causes-

1. Hospital infections
2. UTI
3. Suppurative otitis media
4. Wound infection and infections in the burns
5. Bedsore, eye infections
6. Iatrogenic meningitis
7. Post - tracheostomy pulmonary infection
8. Septicemia, endocarditis
9. Ecthyma gangrenosum and skin lesion in the case of malignancies, leukemia, and in immunocompromised host.

Laboratory Diagnosis

Collection of the sample e.g. in burns, wounds - pus

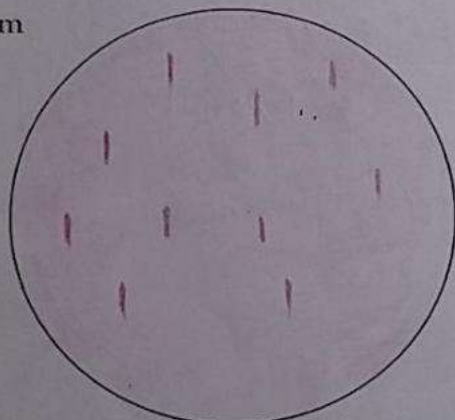
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1. Microscopy (Gram's staining)
2. Culture (BA, MC)
3. Biochemical tests (oxidase test etc)
4. Pyocin typing (in research laboratory)
5. The organism is confirmed and subjected to antibiotic sensitivity testing by using following antibiotic discs-
 - a. Gentamicin
 - b. Tobramycin
 - c. Polymyxin B
 - d. Carbenicillin
 - e. Cephalosporins (Cephataxime etc.)
 - f. Fluoroquinolons (Ciprofloxacin etc.)

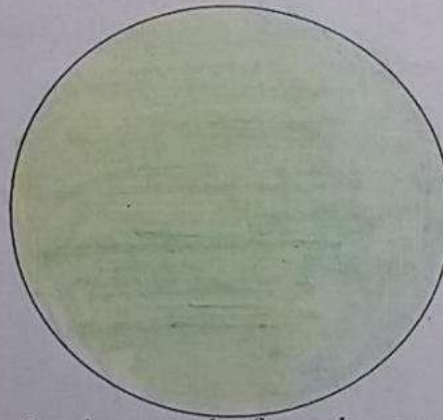
Demonstration

1. Gram stained smear of a pus sample from a case of burns showing GNB (pseudomonas)
2. NA – showing blush green pigmentation, greenish yellow pigmentation.
3. MC- NLF
4. AST (G, Car, Tob, Cipro, Poly, Cefotaxime)
5. Oxidase test

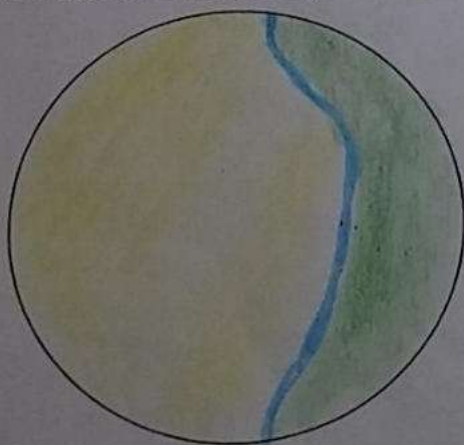
Diagram



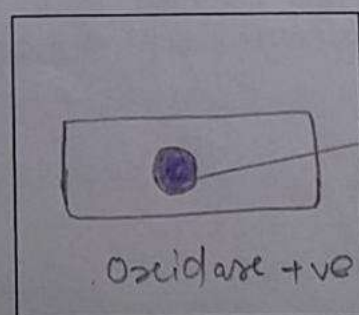
Gram's stained smear prepared from pus of a case of burn (GNB)- *Pseudomonas*



NA showing growth of pseudomonas with blue green pigments (pyocyanin)



NA- showing growth of pseudomonas with greenish yellow pigment (fluorescin)

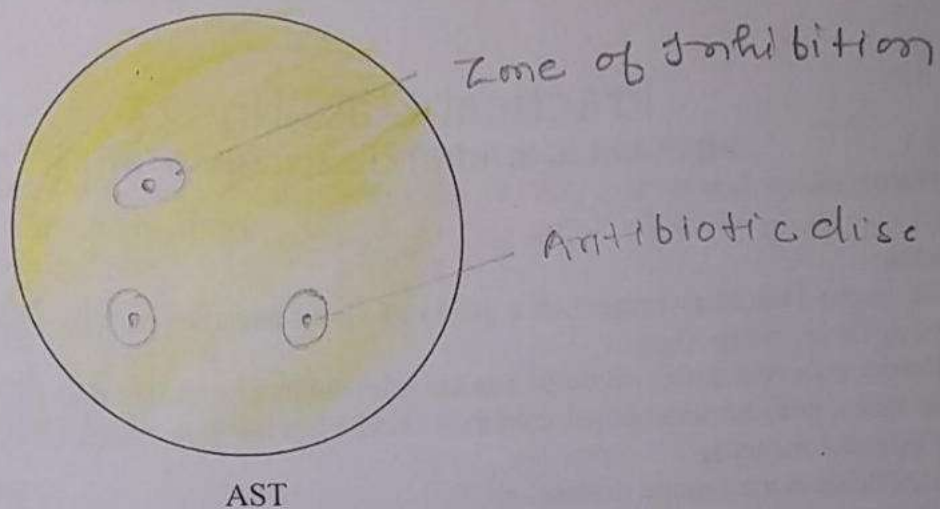


Oxidase disc

Oxidase +ve

Oxidase test

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Exercise

1. What are the lesions produced by *Ps. aeruginosa*?
2. What are the different pigments produced by *Ps. aeruginosa*?
3. Is there any relation between the pigment production and virulence?
4. How will you control hospital infection in regard to *Ps. aeruginosa*?
5. Which antibiotic will you offer to treat the infection with *Ps. aeruginosa*?
6. Enumerate other organisms causing hospital infections.
7. What is pyocin typing?
8. What is the role of pyocyanin?
9. What are the sources of hospital infections?

Practical Class No. 23

BRUCELLA, BORDETELLA, HAEMOPHILUS

Introduction

Brucella

Introduction

1. The genus Brucella consists of a group of Gram-negative bacilli, which are pathogens of goats, cattle, sheep, pigs.
2. Human infection arises through contact with infected animals due to handling of infected carcasses, consumption of infected milk and its products, handling of excreta and secretions of infected animals.
3. Brucellosis is a zoonotic disease.

Morphology:

gram -ve
Non motile
Coccobacilli
Nonsporing and Noncapsulated

Cultural characteristics:

Strict aerobe
On solid media - small, moist and translucent.
Growth is slow (scanty), improved by addition of Glucose.

Classification-

The genus consists of three main species-

1. *B. melitensis*- infects goats and sheep
2. *B. abortus*- infects cattle
3. *B. suis*- infects pigs

These three species can be easily identified by -

1. CO₂ requirement
2. H₂S production
3. Growth in presence of basic fuchsin, thionine, methyl violet
4. Urease activity
5. Agglutination with monovalent antiserum

Pathogenesis

1. All three species are pathogenic to man.
2. I.P.- 10 to 30 days.
3. Brucella enters the body through skin abrasion, mucous membrane of alimentary or respiratory tract and conjunctiva. They reach the blood stream via regional lymphatics. At this stage, the infection may persist for some period leading to undulant fever with vague symptoms. Subsequently the organisms localize in various parts of RE system with formation of granulomatous lesions causing complications.

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Acute Brucellosis

It is characterised by –

1. Bacteraemia and irregular fever
2. Muscular and articular pains, asthmatic attacks, sweating, exhaustion, anorexia, constipation, irritability, chills.
3. Complications include:
 - a. Articular
 - b. Osseous
 - c. Visceral
 - d. Neurological

Chronic Brucellosis

It is a low-grade infection.

1. Chronic hypersensitive condition.
2. Sweating, lassitude, joint pains with low-grade fever.
3. Illness remains for years.

Laboratory Diagnosis

1. Blood culture:

– 5-10 ml blood is inoculated into 50-100ml of serum Dextrose both subcultures are made on selenite media every 3-4 days for 8 weeks.

Castaneda's method can be used.

2. Serological tests:

a. Standard agglutination test

Tube agglutination test. equal volume of serial 2 fold dilution of Pts serum and standardised ag. Single titre of $> 1:160$ is +ve.

b. Mercaptoethanol test

c. CFT

Detects Both IgG and IgM more useful in chronic disease.

d. Coomb's test

This test to detect incomplete antibodies.

*e. Brucella skin test***Vaccination-**

Vaccination of man is not recommended. In animals (cattle at 6 to 8 month's age) vaccination (with *B. abortus*) can protect them from abortion for 4-5 pregnancies. It can not protect completely but still it is useful and limits the spread.

Bordetella**Introduction**

1. The genus *Bordetella* consists of a group of very thin, ovoid or rod shaped GNB (Parvobacteria)
2. It consists of two important species which are human pathogens-
 - a. *Bordetella pertussis* (per- tussis means sever cough)
 - b. *B. parapertusis*

They produce most frequently serious bacterial respiratory infection of childhood called as 'whooping cough'.

Whooping Cough (the pertussis syndrome)

Child suffers many bouts of paroxysmal coughing each day without any pause for air intake. During the paroxysms tongue is protruded fully, secretions from the nose, eyes and mouth appear. The face becomes cyanotic. Ultimately final cough clears the secretions and with massive respiratory effort air is sucked through the narrow glottis producing a long, high-pitched whoop. Hence, the name 'whooping cough'.

Morphology:

Small ovoid
Gram -ve coccobacille.
Capsulated
Bipolar, metachromatic +ve granules can be seen on staining with toluidine blue.

Cultural Characteristics:

Aerobic
Bordet Gengou is commonly used medium
Do not grow in ordinary medium.

Pathogenesis

Humans are the only natural host. It is a noninvasive infection of the respiratory mucosa. Incubation period is 1-2 weeks which is followed by –

1. Catarrhal phase- simple cough, no distinctive feature (for about 1 week)
2. Paroxysmal phase- Paroxysmal coughs with increasing severity and frequency (duration- many weeks)
3. Convalescent phase- prolonged duration.

In the initial preventable stage large number of bacteria colonizes the ciliated epithelium of bronchi and trachea (surface agglutinogens of *Bordetella* have role in attachment).

B. pertussis produces a tracheal cytotoxin. It paralyses the cilia and leads to paroxysms of coughing, the mean by which increased mucous is removed.

Another bacterial product is pertussis toxin (PT) responsible for lymphocytosis in uncomplicated cases of whooping cough.

Secondary infections may occur causing increase in the number of neutrophils with fever suggesting bronchopneumonia. Blockage of airways may cause lung collapse, anoxia, and convulsions.

Treatment – Erythromycin is the drug of choice.

Laboratory diagnosis

It consists of –

1. Bacterial culture:

Typical Pearl like colonies of *B. Pertussis* appear.

2. Detection of bacterial antigens:

3. Detection of *Bordetella* antibody:

Prophylaxis

DPT: Pertussis vaccine is generally administered in combination with diphtheria and tetanus toxoid.

Erythromycin:

Drug be choice.

Hemophilus influenzae**Introduction**

1. *H. influenzae* is the major pathogen included in the genus *Hemophilus*.
2. It causes variety of invasive infections such as meningitis, epiglottitis, pneumonia, and septic arthritis and localized disease of the respiratory tract such as bronchitis and otitis media.
3. *H. aegyptus* cause epidemic conjunctivitis and Brazilian purpuric fever.
4. *H. ducreyi* causes chancroid (soft sore).
5. *H. parainfluenzae*, *H. aphrophillus* and *H. paraphrophilus* cause infective endocarditis, dental infection, lung abscess and brain abscess.

Morphology:

Small
Gram -ve, Non Motile bacillus
Non-sporing.

Capsule:

Virulent strains are capsulated.

Cultural Characteristics:

- Fastidious growth requirements.
- Requires enriched media such as blood/chocolate agar because accessory growth factors known as X and V are present in blood.

Growth factor

1. X factor - Heat stable protoporphyrin IX haemin or iron.
2. V factor - Heat labile +nt in RBC either NAD/NADP.

Satellitism

Staph. aureus induce hemolysis resulting in breakdown of RBC and Release of V factor.

Hena colonies near *Staph. aureus* are large because they get both X and V growth factor.

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Biochemicals

Catalase and oxidase +ve
Ferment glucose and galactose.
Reduce nitrite to nitrate.

Pathogenesis

- Invasive infections are usually caused by capsular type 1.
- The capsular polysaccharide is the major virulence factor for *H. Influenzae* type b. It is responsible for invasive lesion.
- Infections are rare in the first two months of life due to presence of maternal antibodies to the capsular polysaccharide. The most of the infections occur after the age of 2 months.

Invasive lesion include-

- Meningitis
- Pharyngitis
- Septic arthritis
- Pneumonia
- Cellulitis

Other virulence factors-

- Fimbriae -attachment to epithelial cells
- IgA proteases -involved in colonization
- Outer membrane compounds -facilitate invasion

Note: Noncapsular types may also cause meningitis and septicaemia in neonates. Noninvasive lesions- include-

- Localised lesions
- Otitis media
- Sinusitis etc.

Laboratory diagnosis

It consists of -

1. Direct examination gram stain - gram -ve Coccobacilli antigen detection, Coagglutination, Latex agglutination Counter, immunoelectrophoresis.

2. Sputum/throat swabs Culture

homogenised by t/t with Pancreation or by shaking with water

3. Blood culture

4. Antigen detection Type b capsular ag is detected.

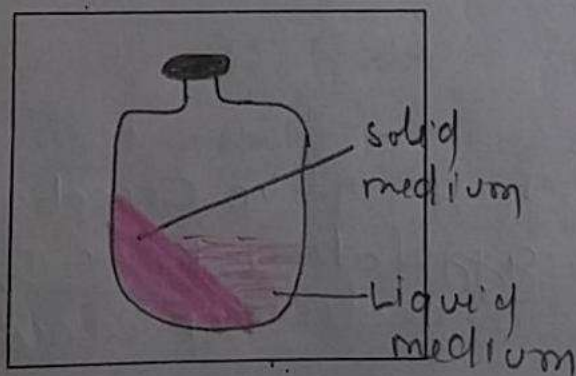
- Latex agglutination.
- Coagulation
- Counter immunoelectrophoresis.

5. Molecular techniques

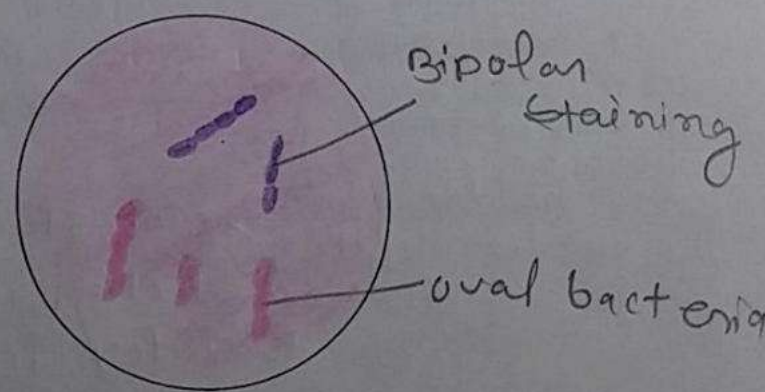
Demonstration

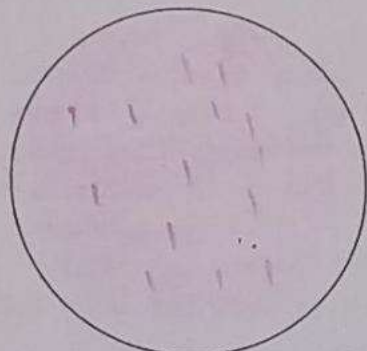
1. Castaneda's method of blood culture for Brucella.
2. *H. ducreyi* smear from a case of chancroid.
3. Gram's stained smear of *H. influenzae* showing coccobacillary and pleomorphic bacilli.
4. West's post-nasal swab.
5. DPT vaccine.
6. *H. influenzae* growth on blood agar showing satellitism.

Diagram

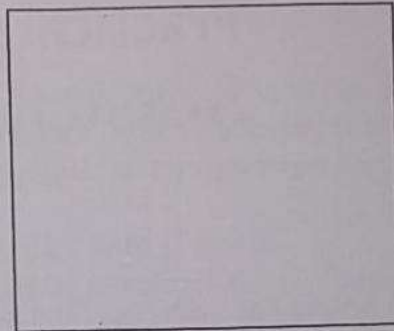


Castaneda's method of blood culture for Brucella

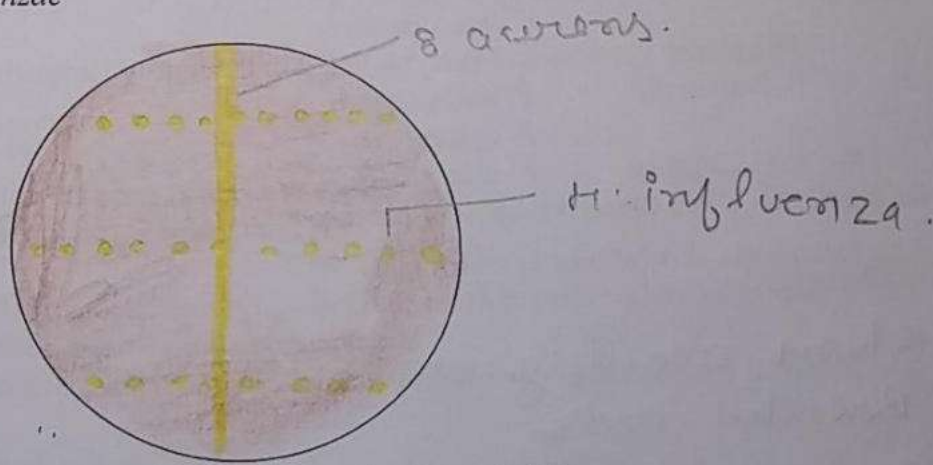
*H. ducreyi* smear from a case of chancroid



Gram stained smear of
H. influenzae



West's postnasal swab



Growth of *H. influenzae* showing satellitism

Exercise

1. How the infection is transmitted to man from animal in case of Brucella?
2. Which sample will you collect for the laboratory diagnosis of Brucellosis?
3. Mention the media used for isolation of Brucella. → SDA
4. What is the pathogenesis of Brucellosis?
5. Mention serological tests used for diagnosis of Brucellosis.
6. What are blocking antibodies?
7. Mention the test by which blocking antibodies are detected.
8. Define zoonosis.
9. What is the advantage of Castaneda's method? → To avoid frequent subcultures.
10. Mention important species of Hemophilus and lesions produced by it.
11. Describe laboratory diagnosis of meningitis caused by *H. influenzae*.
12. Mention the rapid diagnostic tests for the diagnosis of meningitis.
13. How CSF is handled before sending it to culture?
14. Which type of *H. influenzae* is common in causing meningitis?
15. Comment on vaccination against *H. influenzae*. → OPT
16. Mention about the prophylaxis against 'whooping cough'.
17. Why the name whooping cough is given to the disease caused by Bordetella?
18. Describe in brief – laboratory diagnosis of whooping cough. → PCR
19. Which drug will you prefer for prophylaxis/ treatment of whooping cough? → Erythromycin
20. Mention about the prophylaxis against whooping cough.
21. What is cough plate method?
22. Mention the medium used for isolation of Bordetella. Bordet Gengou

Practical Class No. 24

YERSINIA & ACTINOMYCOSES

Introduction

Introduction

1. The group Yersinia has been classified into 3 genera.

- a. Yersinia
 - *Y. pestis*
 - *Y. pseudotuberculosis*
 - *Y. enterocolitica*

The genus Yersinia has been included in the family Enterobacteriaceae.

- b. Pasturella
 - *P. maltocida*
 - *P. aviseptica*

- c. Francisella
 - *F. tularensis*

Yersinia pestis - it is a causative agent of plague.

Morphology:

Short arod gram -ve bacilli

Rounded ends

Occur singly, in short chain or singly.

Cultural Characteristics:

Aerobic, facultative anaerobic

Nutrient agar - Small, delicate, transparent.

Blood agar - Non haemolytic.

Dark brown due to absorption of haemin pigment.

M.O.C. - Colourless.

Biochemical tests:

Catalase +ve

1 m vic

- + - -

Ferment Glucose mannitol and mannose.

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Exercise

1. Classify the group Yersinia
2. What are the different forms of human plague?
3. Describe the appearance of *Y. pestis* when stained with methylene blue.
4. Describe the growth characteristic of *Y. pestis* growth in Ghee broth culture.
5. What do you understand of the term 'stalactite growth'?
6. Mention about the prophylaxis against plague.
7. What is the pathogenicity of the following?
 - i. *Y. pseudotuberculosis*
 - ii. *Y. enterocolitica*
 - iii. *P. maltocida*
 - iv. *F. tularensis*

→ hangs down
from under
surface of oil
Stalactite Growth

Actinomycetes

These are true bacteria having superficial resemblance to fungi. They are Gram positive, nonmotile nonsporing, noncapsulated filaments which breakup into bacillary and coccoid elements. Most are free living particularly in the soil. They are sensitive to antibiotics.

Classification-

Medically important genera-

1. Actinomyces (anaerobic or microaerophilic and nonacid fast)
2. Nocardia (aerobic and acid fast)
3. Streptomyces (rarely cause disease but these are major source of antibiotics)

Actinomyces

This genus includes following species

1. *A. israelii* - causes human actinomycoses
2. *A. bovis* - actinomycosis in cattle
3. *A. naeslundii* and other may cause infections in the oral cavity including dental infections.

Actinomycosis

Gram +ve Non motile, Non sporing
organism appear in pus as granules.

Laboratory Diagnosis

Microscopy:

Dense Network of thin gram +ve filaments.
Surrounded by radiating gram -ve clubs presenting
a sandy appearance.

Culture:

A. israeli produces spider colonies that resemble molar teeth.

Nocardia

All species are Gram positive and some species are acid fast- e.g.

-*N. asteroides*

-*N. braziliensis*

1. Frequently found in soil
2. Cause cutaneous, subcutaneous or systemic lesions in man.
 - Cutaneous lesions- lead to abscesses, cellulitis or lymphocutaneous lesions.
 - Subcutaneous lesions- actinomycotic mycetoma.
 - Systemic nocardiosis- pulmonary disease, pneumonia, lung abscess etc.

Actinomycotic mycetoma

Chronic granulomatous d/s characterised by

1. Multiple abscesses.
2. Tissue destruction
3. Fibrosis
4. Formation of multiple sinuses.

Property	Actinomyces	Nocardia
O ₂ requirement	Anaerobic / microaerophilic	Strict aerobic
Temp. range	33 - 37°C	wide range
Habitat	Oral commensals	Saprophytic of environment.
Acid fastness	-	Weakly
Mode of infection	Endogenous	Exogenous.

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Demonstration

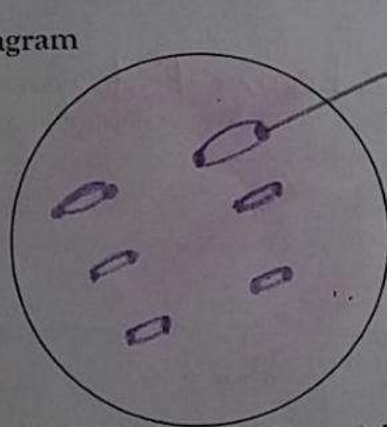
1. A specimen of actinomycotic mycetoma
2. A microscopic slide prepared from sulphur granules appearance'
3. A microscopic slide showing acid fast Nocardia
4. Brain heart infusion (BHI) agar
5. Thioglycolate broth
6. Photograph of a case of actinomycotic mycetoma

showing 'sunray

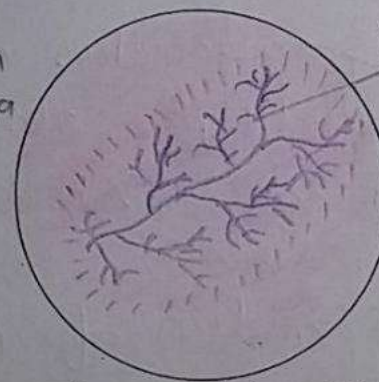
Exercise

1. What are the characteristics of actinomycetes?
2. What is actinomycosis?
3. What are the common sites of actinomycosis? → cervicofacial, Thoracic, abdominal and pelvis.
4. Why the name actinomyces?
5. How will you differentiate actinomycotic mycetoma from eumycotic mycetoma?
6. What is botryomycosis?
7. What is the basic difference between actinomyces and nocardia?
8. Which media are used for isolation of actinomyces? → on brain heart infusion agar, BHI agar or thioglycolate and eumycotic
9. How will you carry out laboratory diagnosis of actinomycosis?
10. Which are the causative agents of actinomycotic mycetoma mycetoma?
11. Which drugs will you prefer for treating actinomycotic mycetoma?

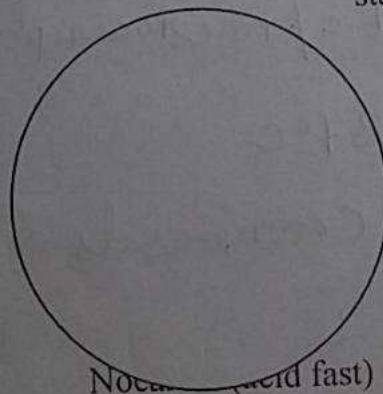
Diagram



Y. pestis (safety pin app) in Methylene blue staining



Actinomycosis (sunrays appr.) slide prepared from sulphur granules and stained with Gram's stain



Practical Class No. 25

SPIROCHETES

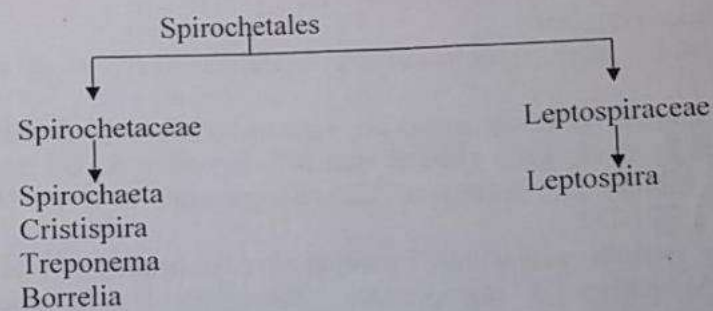
Introduction

1. They are elongated, motile, flexible bacteria twisted spirally along the long axis. (Spiral coil, chaite- hair).
2. They possess endoflagella, which are polar flagella, wound along the helical protoplasmic cylinder and situated between the outer membrane and cell wall.
3. Classification

Order

Family

Genus



Members of the genus Spirochetaceae are saprophytes found in water and sewage. Cristispira are found in molluscs. The members of Treponema, Borrelia and Leptospira consist of important human pathogens, which are considered in this practical.

Treponema

Treponemes cause the following diseases in humans.

1. Venereal syphilis- by *T. pallidum*
2. Endemic syphilis- by *T. pallidum* subspecies endemicum
3. Yaws -by *T. pertenue*
4. Pinta- by *T. carateum*

Now, *T. pallidum* is considered to include 3 subspecies-

- Subspecies pallidum causes venereal syphilis
- Subspecies endemicum causes endemic syphilis
- Subspecies pertenue causes yaws

T. pallidum subspecies pallidum

It is the causative agent of syphilis and first isolated from syphilitic lesion in 1905. Infection usually acquired by sexual contact (common age group 15-30 years). Congenital syphilis usually occurs following vertical transmission of *T. pallidum* from infected mother to the foetus *in utero*. Neonates may be infected during passage through the infected birth canal during delivery.

Morphology:

Gram -ve
Thin, delicate with tapering ends.
actively motile.

Cultivation: Pathogenic stain can't be cultivated

Syphilis

Pathogenesis

Venereal syphilis is acquired by sexual contact. The *T. pallidum* enters the body through minute abrasions on the skin or mucosa (ID-60 Treponemes). It multiplies at the site of entry. Clinical disease sets up after one month (IP).

The clinical manifestation fall in three stages-

1. **Primary lesion** (syphilis)

It is the chancre (hard chancre) at the site of entry (genital). Other sites are mouth and nipples.

Hard chancre- Painless, relatively avascular, circumscribed, superficially ulcerated lesion. The chancre is covered by thick, glary exudate rich with *T. pallidum*. The regional lymph glands are swollen, discrete, rubbery and nontender. The chancre heals in about 10-40 days even without treatment leaving a thin scar.

2. **Secondary syphilis-** sets within 3 months after the healing of primary lesions. During this interval the patient is asymptomatic. Secondary lesions occur due to widespread multiplication of *T. pallidum* and dissemination through blood.

Manifestations-

Includes

- Roseolar or papular skin rashes.
- Mucous patches in the oropharynx and condylomata at the mucocutaneous junction.
- T. pallidum* are abundant in the lesions.
- Patients are most infectious at this stage.

After the secondary lesions disappear there is a period of quiescence called as 'latent syphilis'. This is diagnosed only by 'serological tests'. In many cases this is followed by natural cure but in some cases after several years patient goes into 'Tertiary stage'.

3. **Tertiary stage-** This consists of cardiovascular lesions including aneurysms, chronic granulomata and meningovascular manifestation. In few cases neurological manifestations such as Tabes dorsalis or general paralysis of the insane develop.

Laboratory Diagnosis

Microscopy:

Dark ground microscopy.
Slender, spiral organism showing rotational and
flaccid and extension movements
Concentration $\times 10^4/\text{ml}$ +ve.

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Serological test

1. Nonspecific tests

1. VDRL
2. RPR

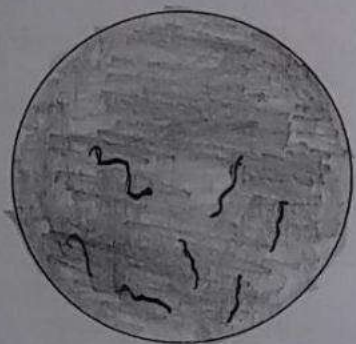
2. Specific (treponemal) tests

1. FTA, FTAABS (using killed T. Pallidum)
2. TPHA, MHA-TP, TP-PA (using T pallidum antigen)
3. TPI test (using live T. Pallidum).

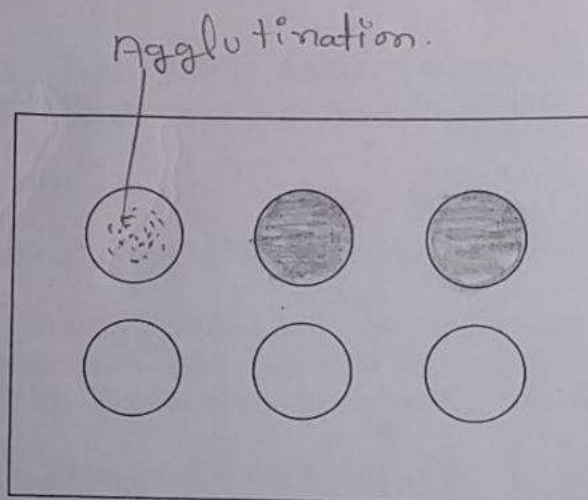
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Demonstration

1. *T. pallidum* – dark ground illumination.
2. VDRL tile
3. VDRL antigen
4. VDRL rotator
5. Dark ground microscope.



T. pallidum
(Dark ground illumination)



VDRL tile

Exercise

1. Mention two procedures for the demonstration of *T. pallidum*.
2. Mention examples of syphilitic lesions from which *T. pallidum* can be demonstrated.
3. What is hard chancre?
4. Enumerate nonspecific (standard) tests for diagnosis of syphilis. → RPR, VDRL
5. Enumerate specific (treponemal) tests for syphilis.
6. What is the long form of VDRL?
7. Enumerate the conditions, which give BFP result in VDRL test.
8. Why serum sample is inactivated before subjecting it to standard tests for syphilis? How?
9. Which is the most specific test for diagnosis of syphilis?
10. What is Nichols' strain and Reiter's strain?
11. What are the complications of tertiary stage? → Anorexia, chronic, granuloma
12. Which stage of syphilis is most infectious? → Secondary
13. What is latent syphilis?
14. Why the name 'syphilis' has been given to this disease? → tabes dorsalis Paralysis.
15. What is endemic syphilis?
16. What is Yaws?
17. What is Pinta?
18. How will you diagnose a case of neurosyphilis?

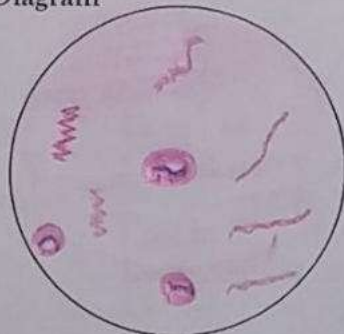
Borrelia

1. Large, motile, refractile spirochetes with regular, open coils.

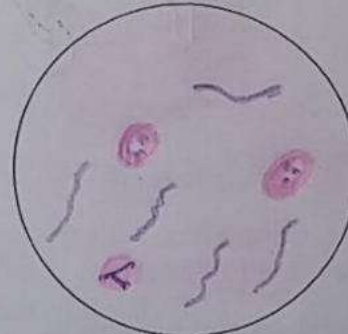
2. They are usually 10-30 μ long and 0.3-0.7 μ wide and Gram negative.
3. Several species of *Borrelia* occur as commensals on the buccal and genital mucosa.
4. Medically important *Borrelia*-
 - a. *B. recurrentis*- relapsing fever
 - b. *B. vincenti* – fusospirochetosis (Vincent's angina)
 - c. *B. burgdorferi*- Lyme's disease.

Demonstration

1. Pus smear from a case of Vincent's angina
2. White mice
3. Body louse and tick
4. Diagram – *B. recurrentis* in PBS.

Diagram

Pus smear from a case of
Vincent's angina (Fusospirochetosis)



B. recurrentis in PBS

Leptospira

1. *Leptospira* are actively motile, delicate and possess large number of closely wound spiral with characteristics hooked ends.
2. They are too thin to be observed under light microscope and can be visualised under dark ground illumination.
3. Several species are saprophytic.
4. The genus *Leptospira* has been now classified into 2 species-
 - a. *L. interrogans*-contain pathogenic leptospira
 - b. *L. biflexa* –contain saprophytic leptospira
5. *L. interrogans* is again classified into several serogroups.
6. *Leptospira* are 6 to 20 μ long and 0.1 μ thick.

They can be demonstrated by –

- a) Dark ground illumination
- b) Electron microscopy
7. They can be stained with Giemsa and silver impregnation method.
8. Can grow in the media containing rabbit serum e.g. Korthof's.
9. Infection occurs when leptospira in water contaminated with urine of a carrier animal (rat) enters the body through cut or abrasion in the skin and mucosa of mouth, nose or conjunctiva. It causes Weil's disease, which is characterised by – acute onset of fever, rigor, vomiting, headache, intense injection of eyes. Jaundice appears on about third day. Purpuric hemorrhages may occur. Albuminuria is constant feature. Leptospirosis may be icteric or nonicteric.

Laboratory Diagnosis

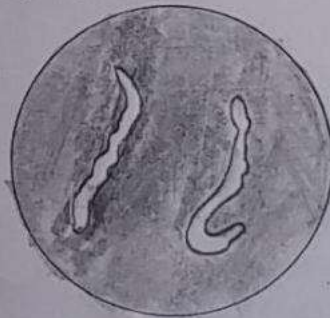
1. Demonstration of leptospira in blood and urine by microscopy (dark ground illumination)
2. Isolation in culture (EMJH)

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3. Animal inoculation (Guinea pig)
4. Serology

Demonstration

1. Guinea pig
2. Diagrams showing leptospira (dark ground illumination)



Leptospira (dark ground illumination)

Exercise

1. Enumerate different species of Borrelia
2. What is Vincent's angina?
3. What are the lesions produced by *B. vincenti*?
4. What is Lyme's disease?
5. What is the mode of transmission in relapsing fever?
6. Explain the use of mice in the diagnosis of relapsing fever.
7. What is the source of infection in leptospirosis? → direct / Indirect Contact with water
8. Which samples will you collect for laboratory diagnosis of leptospirosis? Contaminated by
9. How will you demonstrate leptospira? - culture, animal inoculum Using of carriers
10. Enumerate the serological tests for diagnosis of leptospirosis. blood, urine, serology, animal test
11. Explain the use of guinea pig in the diagnosis of leptospirosis. - animal inoculation.

Practical Class No. 26

VIROLOGY

Defination

1. Virion - *extracellular infection virus Partiale.*

2. Viroides - *Protein free, low mol. wt. RNA resistant to heat and organic solvent but sensitive to nucleases.*

3. Prions - *As small Particles without any detectable nucleic acid, resistant to heat, UV rays and Nucleases and sensitive to proteases.*

Viruses

1. Do not have cellular organisation.
2. Contain only one type of nucleic acid (either DNA or RNA, never both).
3. Obligate intracellular parasites.
4. Lack in enzyme required for protein and nucleic acid synthesis.
5. Dependent for replication on the synthetic machinery of host cells.
6. Multiply by a complex and not by binary fission.
7. Unaffected by antibacterial antibiotics.

General Properties

1. Size- Vary widely in size e.g.

- Largest- Pox viruses (300nm)
- Smallest - Parvoviruses (20nm)

Various methods to estimate size of the viruses e.g. -

- a) Pushing them through colloidal membrane filters
- b) Ultracentrifugation
- c) Electron microscopy

2. Structure and shape

The virion consists of:

- a) Capsid- a protein coat which surrounds the nucleic acid.
- b) Nucleocapsid - capsid with nucleic acid.
- c) Capsomeres - capsid is composed of large number of capsomeres (morphological unit)
- d) Capsid may be icosahedral or helical.
- e) The virion may be enveloped or nonenveloped (naked). The envelope is lipoprotein in nature where the lipid is of host cell origin and protein is virus coded.
- f) Peplomer- these are the projecting spikes on the surface of envelop e.g. influenza virus. It has two types of peplomers i.e. hemagglutinin and neuraminidase. Enveloped viruses are susceptible to ether, chloroform and bile salts.
- g) Chemical units of capsid are polypeptide molecules.

3. Chemical Properties

- a) Viruses contain only one type of nucleic acid (RNA or DNA).
- b) Viruses contain proteins, which make the capsid.

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- a. Inoculation into animals
- b. Embryonated eggs
- c. Tissue culture.

1. Animal Inoculation

used for - ① 1st isolation of certain virus.
② to study pathogens of viral ds.
③ to study viral oncogenesis.

Infant (suckling) mice are used. Inoculation can be done by several routes - intracerebral, intra-peritoneal, intranasal. Then animals are observed for sign and symptoms.

2. Embryonated eggs:

Embryonated hens egg 7-12 yrs old are inoculated by one of several routes - such as - ① CAM ② allantoic cavity ③ amniotic sac ④ yolk sac.

Then egg are incubated for 2-9 days.

3. Tissue culture

- a. Organ culture
- b. Explant culture
- c. Cell culture

Cell Culture

a. Primary cell culture

1. Rhesus monkey kidney cell culture.
2. Human amnion cell culture
3. chick embryo fibroblast Cell culture

b. Diploid cell strains

1. WI - 38
2. HL - 8

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c. Continuous cell lines

1. Hela
2. HEP-2
3. Vero
4. McCoy
5. BHK-21

Detection of viral growth in cell culture
Detected by following methods-

1. Cytopathic effects
2. Metabolic inhibition
3. Haemadsorption
4. Interference
5. Transformation
6. Immunofluorescence

List of DNA and RNA viral families of medical importance

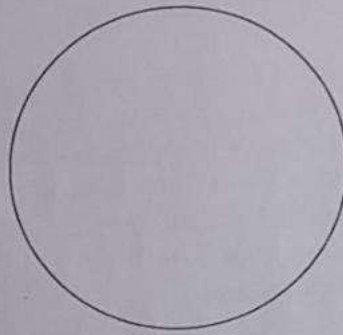
DNA			RNA			
ds		ss	ds	ss (-) sense	ss (+) sense	
Enveloped	Necked				Enveloped	Necked
Herpesviridae	Adenoviridae	Parvoviridae	Reoviridae		Orthomyxoviridae	Picornaviridae
Hepadnaviridae	Papoviridae				Paramyxoviridae	Eelaciviridae
					Togaviridae	Astroviridae
					Flaviviridae	
					Bunyaviridae	
					Arenaviridae	

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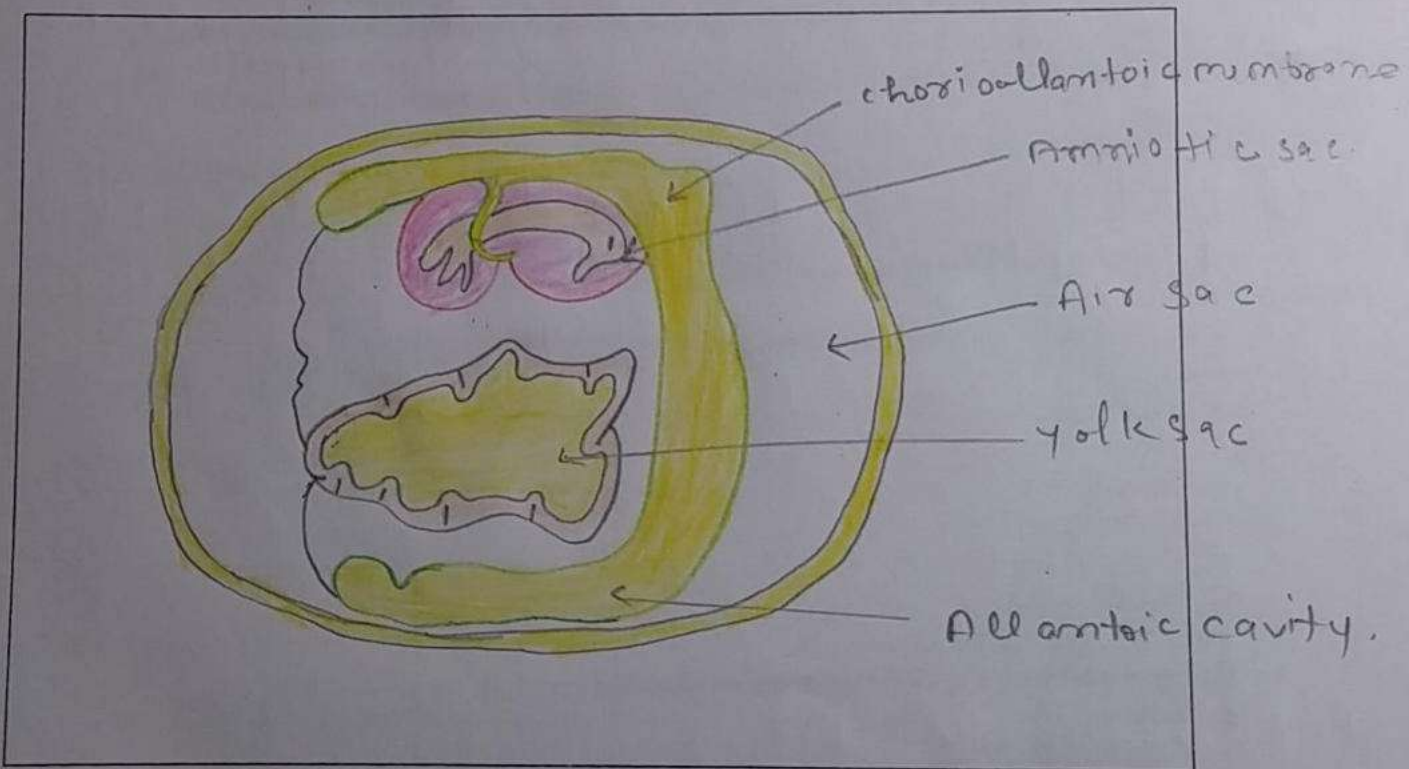
Diagrams



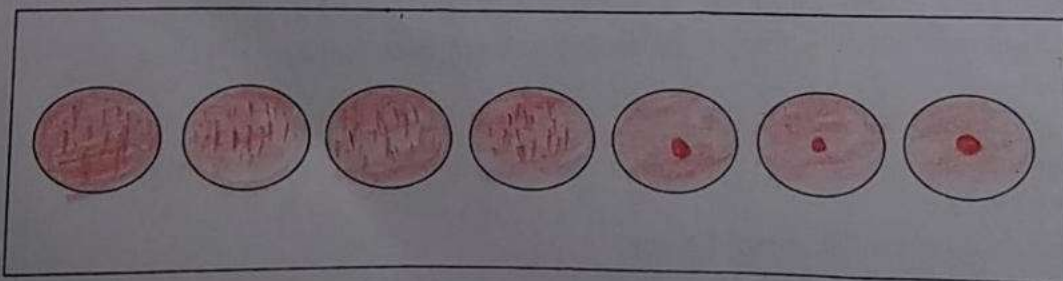
Negri bodies



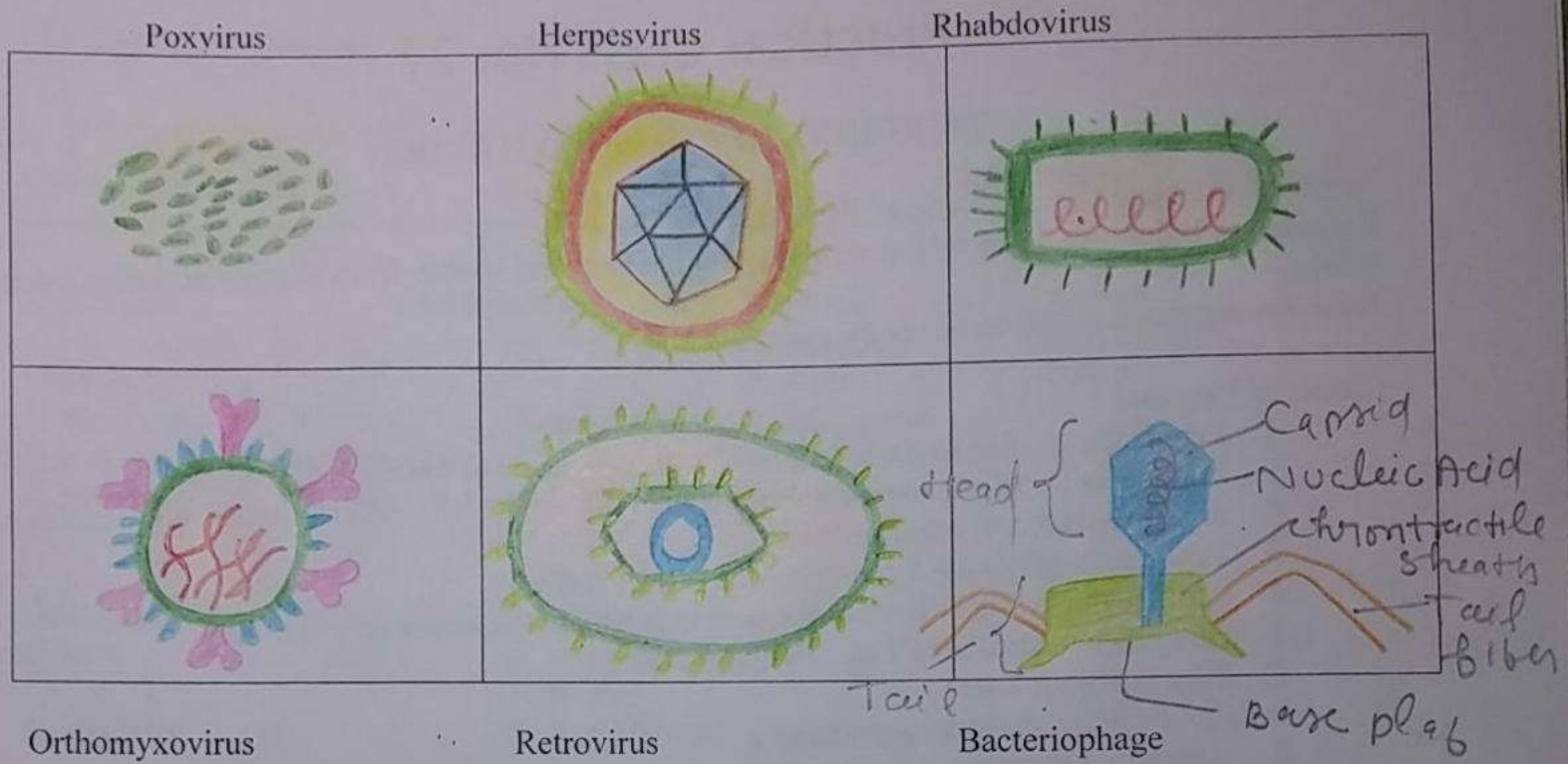
Molluscum contagiosum



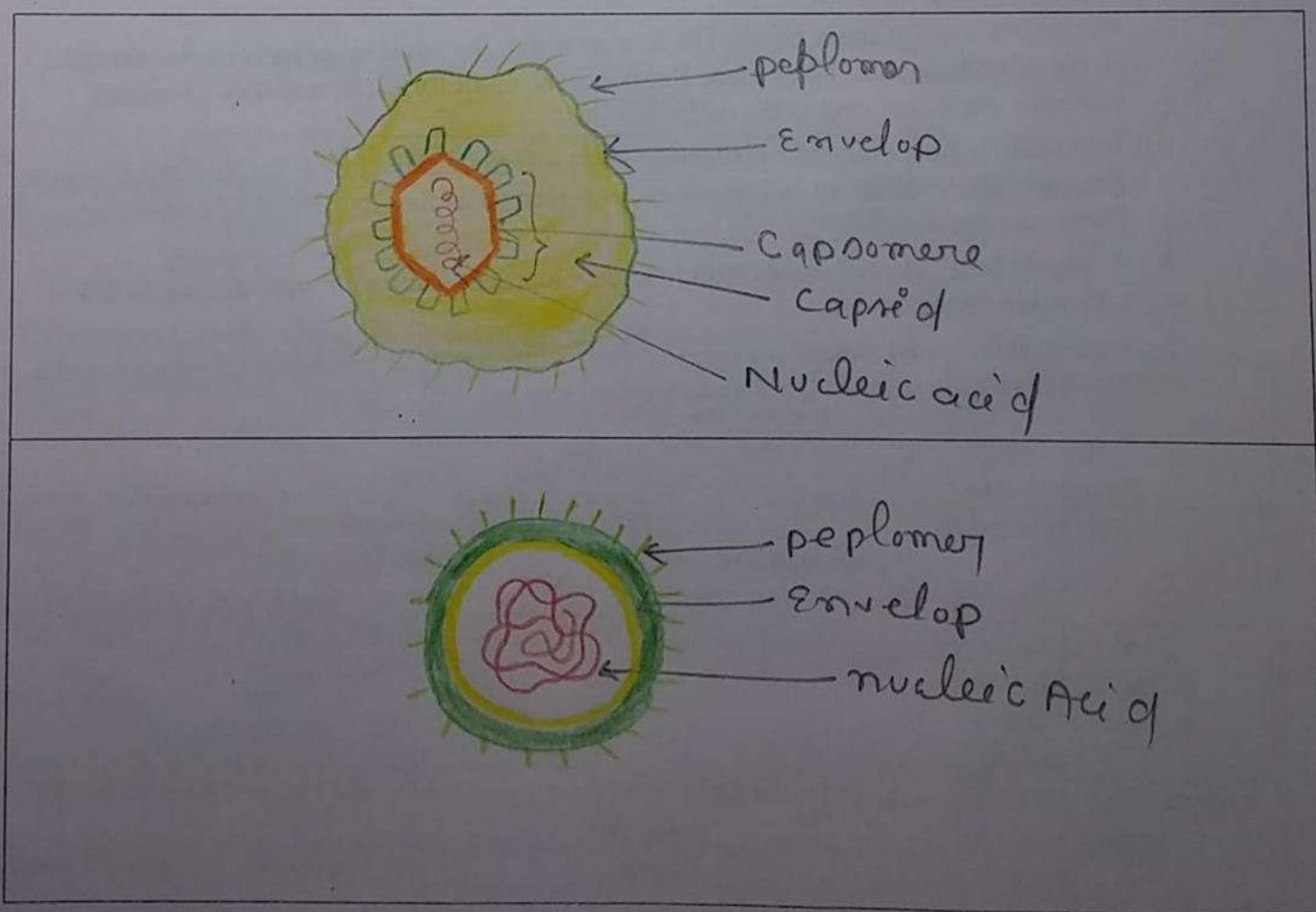
Embryonated hen's egg



Viral haemagglutination


Design and structure of Virion

Enveloped icosahedral (cubical)



Enveloped helical

Prophylaxis

1. Avoid faecal contamination of food and water.
2. Use boiled water for drinking.
3. Handwashing after defaecation and before eating.
4. Improvement of general sanitation by proper disposal of faeces.

Entamoeba coli

It occurs in three stages- in Trophozoites, Precyst and Cyst. Trophozoites of *E. coli* is the largest amoeba in the large intestine of man. It's cytoplasm never contain RBCs but contains large number of bacteria. Mature cyst contains 8 nuclei.

***Giardia lamblia* (*Lamblia intestinalis* / *Giardia duodenalis*)**

G. lamblia causes diarrhoea. It occurs in 2 stages i.e. trophozoite and cyst. Man is the only host in its lifecycle. Ingestion of cyst produces infection. The trophozoites live in the duodenum and upper portion of small intestine. The trophozoite attaches itself to jejunal mucosa and prevents absorption of fats. It leads to steatorrhoea and malabsorption. This infection is common in children.

Laboratory diagnosis involves the examination of fresh stool specimen.

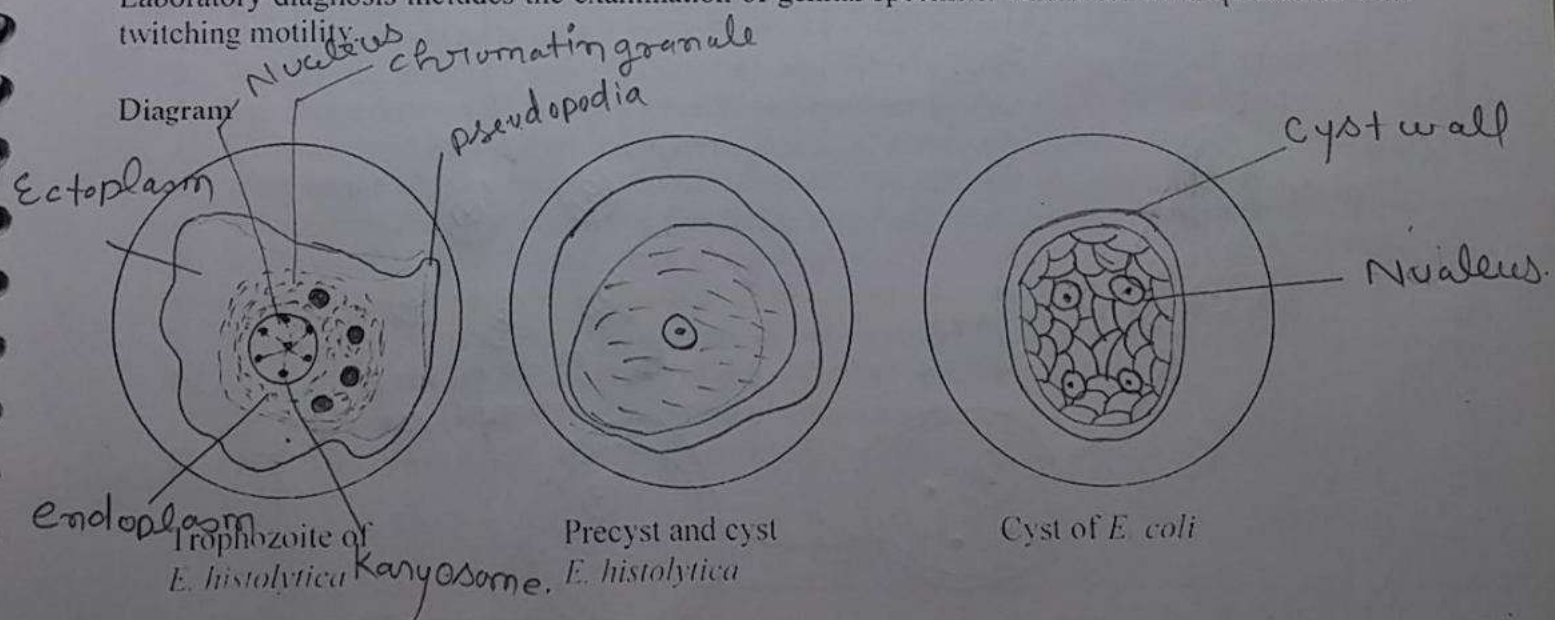
Acute disease- actively motile trophozoites in saline mount.

Chronic disease- typical cyst in iodine mount.

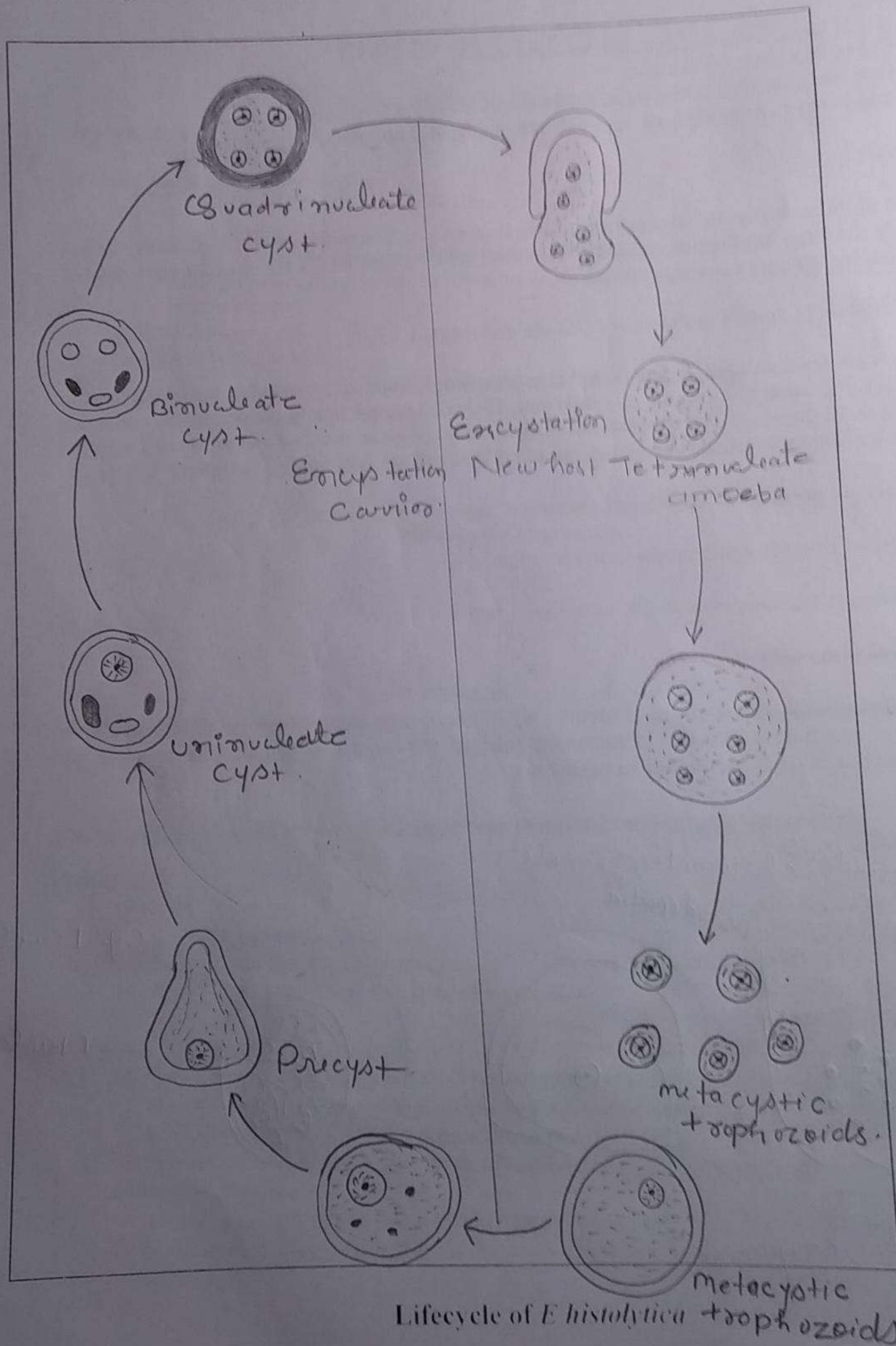
Trichomonas vaginalis

T. vaginalis causes trichomoniasis. It occurs only in trophozoite stage which is the infective form. Infection is transmitted sexually. Trophozoite inhabits the vagina in female, the prostate and seminal vesicle in male and urethra in both sexes.

Laboratory diagnosis includes the examination of genital specimen which shows trophozoites with twitching motility.

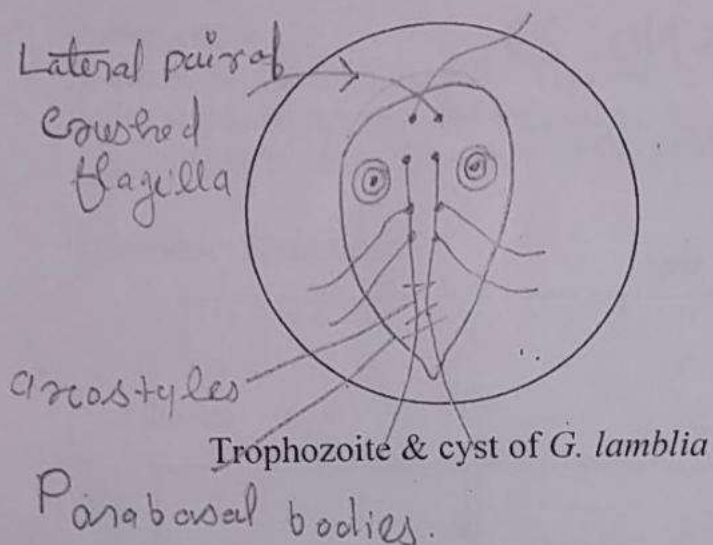


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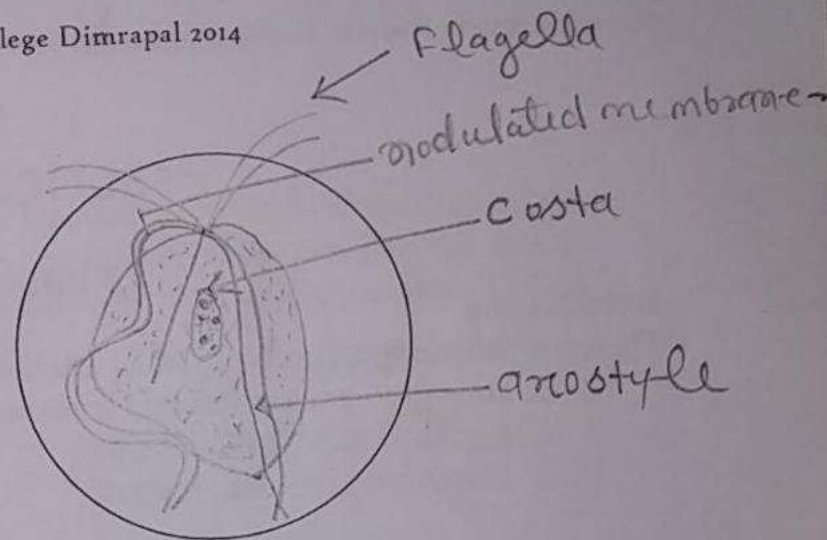


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Trophozoite & cyst of *G. lamblia*



Trophozoite of *T. vaginalis*

Demonstration

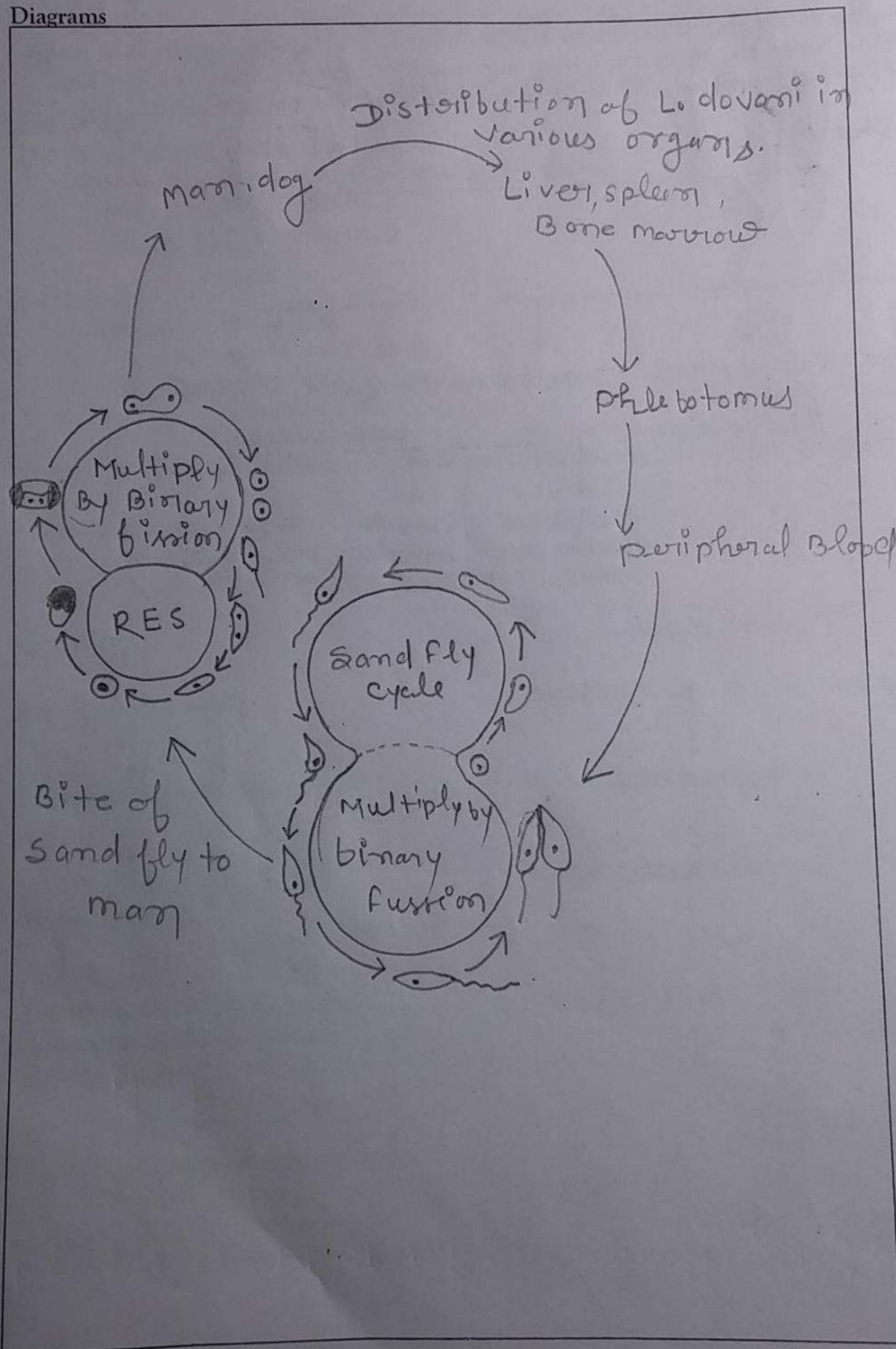
1. Saline/iodine mount of stool sample showing trophozoites and cysts of *E. histolytica*.
2. Amoebic liver abscess and intestinal amoebic ulcer (specimens).
3. Lifecycle of *E. histolytica* (chart).
4. Saline/iodine mount of stool sample showing trophozoites and cysts of *G. lamblia*.
5. Trophozoites, cysts of *E. histolytica* and *G. lamblia* (chart).
6. Trophozoite of *T. vaginalis* (chart).
7. Free living amoebae (soil amoebae) (chart).

Exercise:

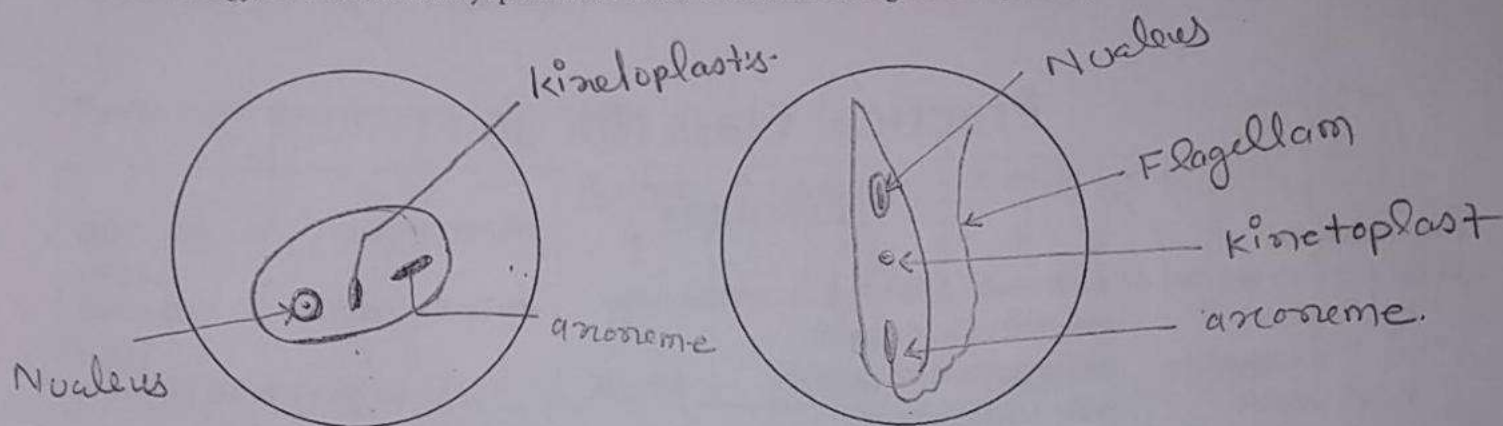
1. Which amoeba is pathogenic? *Entamoeba histolytica*.
2. Which is the infective form of *Entamoeba histolytica*? Quadrinucleate cysts.
3. Enumerate differences between amoebic and bacillary dysentery.
4. What are the extraintestinal lesions produced by *E. amoeba histolytica*? Hepatic amoebiasis.
5. What are the characteristics of amoebic liver abscess pus? Anchovy Subphrenic abscess.
6. What are the lesions produced by free-living amoebae? Scurfey Liver abscess.
7. Which medium is for the culture of *E. histolytica*?
8. Describe the motility of trophozoite of *E. histolytica*, *G. lamblia* and *T. vaginalis*.
9. Which drugs are used for treating a case of amoebic liver abscess?
10. Comment on serological diagnosis of extraintestinal amoebiasis.
11. Mention drug of choice in the treatment of trichomoniasis.
12. Suggest measures of prevention of amoebiasis.

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Diagrams

Lifecycle of *L. donovani*

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Amastigote of *L. donovani*Promastigote of *L. donovani***Demonstration:**

1. PBS showing amastigote forms of *L. donovani*.
2. Chart showing amastigote and promastigote forms of *L. donovani*.
3. Lifecycle of *L. donovani* (chart)
4. A case of dermal leishmaniasis (chart)

Exercise

1. What is the pathogenicity of *L. donovani*?
2. Mention reservoir host for Indian leishmaniasis.
3. Mention the endemic states of visceral leishmaniasis in India.
4. Mention the sites from where LD bodies can be demonstrated.
5. Comment on serological diagnosis of Kala azar.
6. Mention the name of the vector in the transmission of Leishmaniasis.
7. Mention control measures of Kala azar.
8. What is PKDL?
9. What is Delhi boil (Oriental sore)?
10. Mention treatment of Kala azar.

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Practical Class No. 30

PLASMODIA

Introduction

There are four species of Plasmodia causing infection in man-

- *P. vivax*- benign tertian malaria.
- *P. falciparum*- malignant tertian malaria.
- *P. ovale*- ovale tertian malaria.
- *P. malariae*- quarten malaria.

Mode of Infection- Transmission by the bite of female *Anopheles* mosquito.

Lifecycle- It occurs in two different hosts.

- Mosquito- definitive host (sexual stage- completion of gametogony and sporogony).
- Man- intermediate host (asexual stage- schizogony and beginning of gametogony).

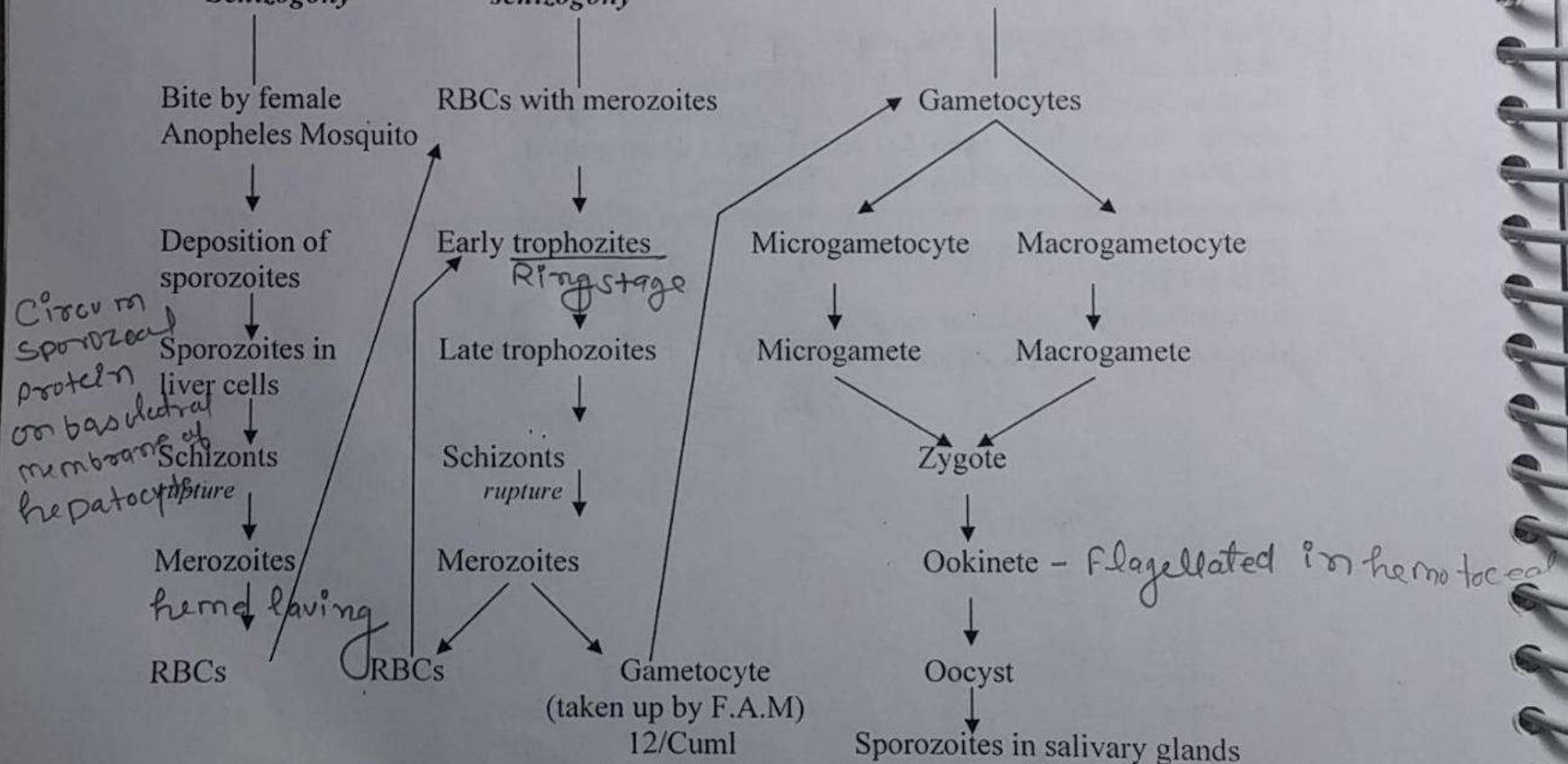
Human' phase

Mosquito phase

1. Preerythrocytic Schizogony

2. Erythrocytic schizogony

3. Sporogony



hemozoites → End product of asexual multiplication. motile haploid

Note: Merozoites infect the new RBCs and the cycle repeats. After some cycles they get differentiated into sexual forms i.e. gametocytes (male and female), which are taken by FAM in the blood meal, and further development occurs in FAM (completion of gametogony and sporogony).

P. vivax and *P. falciparum* - merozoites again attack liver cells.

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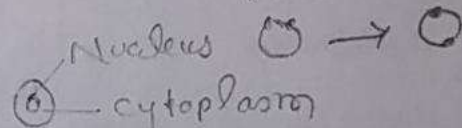
Products of Preerythrocytic Schizogony

	<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. ovale</i>	<i>P. malariae</i>
Duration of preerythrocytic stage	8	5.5 (6) 4.8 hrs.	9	15
Diameter of mature schizonts (μm)	45	60	60	55
Number of merozoites	10,000	30,000	15,000	15,000

Products of Erythrocytic Schizogony

1. Trophozoites
 - Early trophozoite- ring form
 - vs.
 - Late trophozoites- amoeboid form
2. Schizonts
3. Gametocytes (male, female)

Nuclear division.



	<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. ovale</i>	<i>P. malariae</i>
Early trophozoite	2.5 (μm), Cytoplasm opposite to the nucleus is thicker	1.5 (μm), Cytoplasm fine & regular in outline, often with 2 nuclei. Accole forms seen.	Same as <i>P. vivax</i>	Same as <i>P. vivax</i>
Late trophozoite	Actively amoeboid. Vacuoles present.	Compact. Pigment collected into single mass.	Slightly amoeboid. No ribbon shape	Slightly amoeboid, Band like.
Schizonts	9-10 (μm) in size. Completely fills an enlarged RBC	5 μm in size. Fills 2/3 of RBC which is not enlarged.	6.2 μm in size. Fills about 3/4 of a RBC which is slightly enlarged	6.5-7 μm in size. Fills a normal RBC
No. of Merozoites	12-24	18-24	6-12	6-12
Malaria pigments	Yellowish brown fine granules	Black. One or 2 solid blocks	Dark yellowish brown, coarser than <i>P. vivax</i>	Dark brown coarse granules
*Gametocytes	Spherical, larger than RBC. Host cell enlarged with Schuffner's dots	Crescentic, larger than normal RBC. Maurer's clefts seen.	Ovale. Size of RBC slightly enlarged with Jame's dots	Round or ovale, RBC not enlarged

* Female- cytoplasm stains blue, nucleus small, compact.
Male- cytoplasm stains pale blue or reddish, nucleus large and diffuse.

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Pathogenesis

P. vivax- during infection with *P. vivax*, only 1-2% young RBCs are affected. Typical picture of benign tertian malaria includes periodic bouts of fever with rigor, followed by anaemia and splenomegally. When schizonts rupture, large amount of endogenous pyrogens are liberated. This leads to elevation in temperature (febrile paroxysms).
1-2% young RBCs Reticulocyte because
Nuclear pinpoints are not

Periodicity is 48 hours in tertian and 72 hours in quartan malaria

Quotidian Periodicity- fever occurs at 24 hours interval due to 2 broods of tertian parasites maturing on successive days or due to mixed infection.

P. falciparum

During falciparum malaria 2% to 50% RBCs are infected. Erythrocytic schizogony occurs in the capillaries of internal organs like spleen, bone marrow, brain, kidney, intestine, heart and placenta.
25-40% Younger and older are infected

P. falciparum infection leads to cytoadherence which results in holding back (sequestration) of mature parasites in vital organs like brain, heart and cause interference with metabolism of host.
stick infected RBC to non infected

RBCs size not effected.
 Complication of *P. falciparum* includes

- Black water fever- here massive intravascular haemolysis occurs which leads to haemoglobinuria and kidney failure.
male gametocyte banana shaped
female gametocyte - Nuclei at periphery
splenomegaly
- Pernicious malaria- this is referred to as a series of phenomena which, if not effectively treated, threatens the life of patients within 1-3 days. This is the result of capillary blockage.
indirect bilirubin - H₂O soluble

Clinical Types

- Cerebral malaria
- Algid malaria (algid-chilly, cold)
- Septicaemic malaria

Laboratory Diagnosis

It includes -

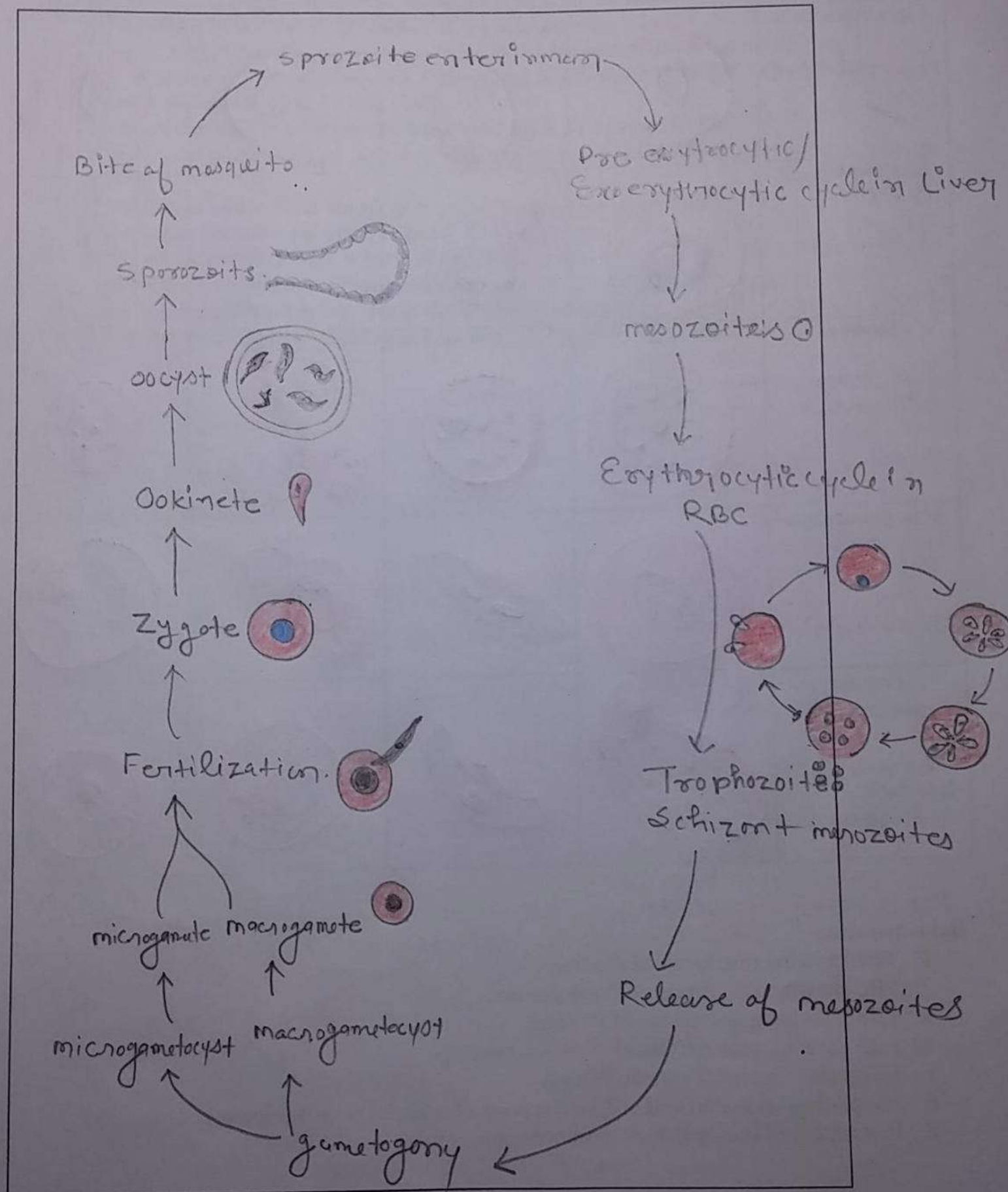
- Examination of peripheral blood smear (PBS). Stained by Giemsa or Leishman stain.
 - Thick smear- useful for rapid diagnosis
 - Thin smear- useful for species identification

Note: in case of *P. vivax*, *P. malariae* and *P. ovale* all erythrocytic forms are seen in PBS while in *P. falciparum* only the rings or gametocytes are seen.

- Serological tests- not routinely used.

Prophylaxis-

Diagrams



Lifecycle of malarial parasite

1. Morphological forms of malarial parasites

	<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. ovale</i>	<i>P. malariae</i>
Early trophozoite				
Late trophozoite				
Schizont				
Male gametocyte				
Female gametocyte				

Demonstration

1. PBS showing ring forms of *P. vivax*.
2. PBS showing ring forms of *P. falciparum*.
3. PBS showing gametocytes of *P. vivax*.
4. PBS showing gametocytes of *P. falciparum*.
5. Lifecycle of malarial parasite (chart)
6. Morphological features of different species of malarial parasites (chart).
7. Preparation of thin and thick smear (demonstration).

Exercise

1. What are the different species of malarial parasite?
2. Why the disease is named as malaria?

P. vivax, *falciparum*, *ovale*, *malariae*
 Malaria = Bad air
 People thought that d/s is caused by foul air in marshy areas.

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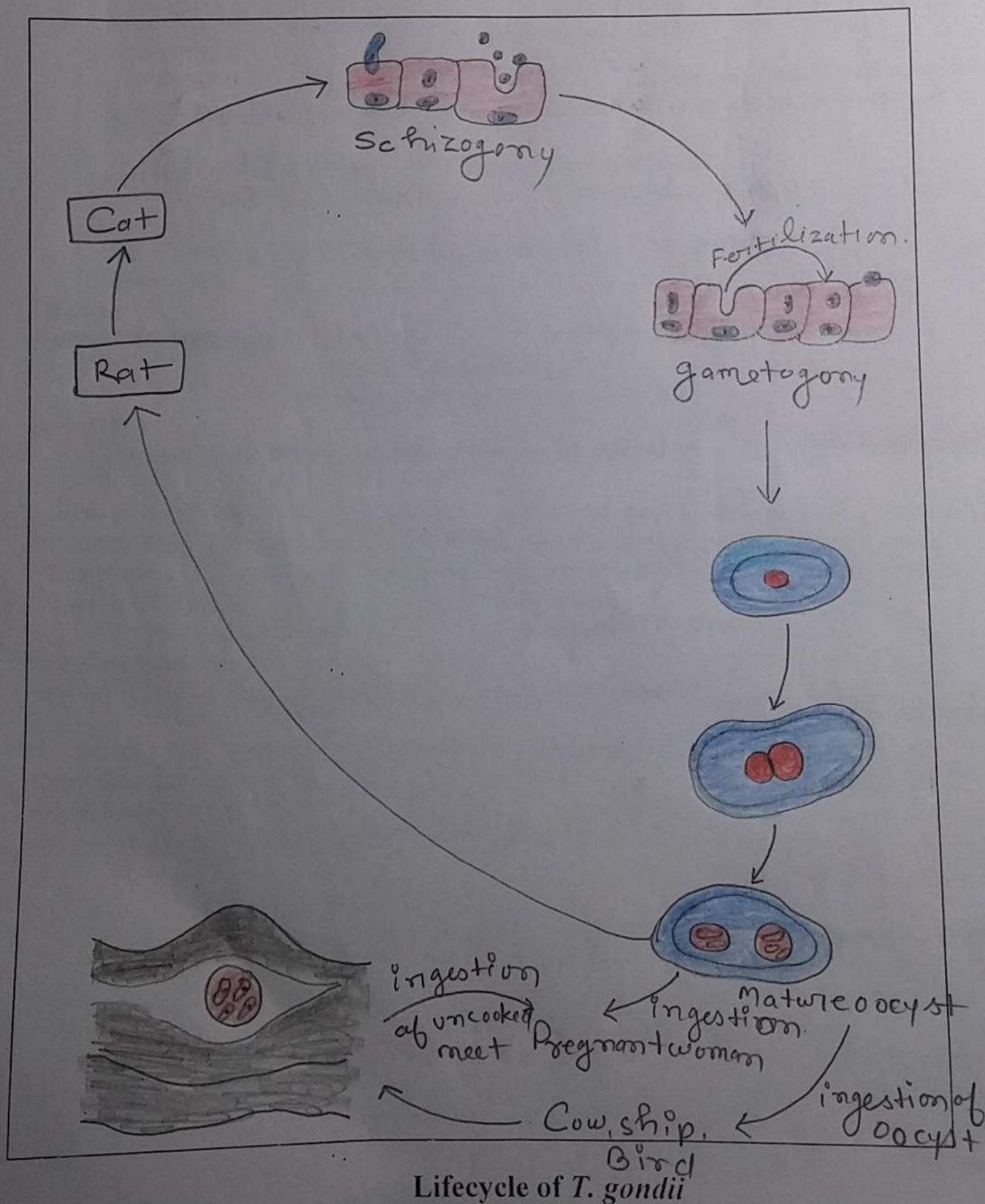
Cytosporidium parvum

It causes chronic life threatening diarrhoea in immunocompromised individuals. The infection is acquired by ingestion of oocyst in contaminated food or drink. Oocyst contains 4 sporozoites. Diagnosis is made by demonstration of oocyst (which is acid fast) in faeces.

Isospora belli

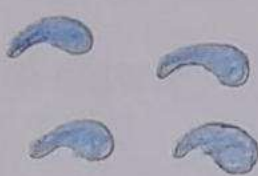
It is a coccidian parasite which can cause diarrhoea in man. Infection is acquired by ingestion of mature oocyst in food or drink.

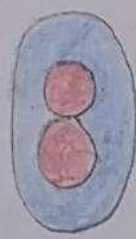
Diagrams



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Morphological stages of *T. gondii*:

Trophozoite	Pseudocyst	Tissue cyst	Oocyst
			

Oocyst of <i>I. belli</i>	Oocyst of <i>C. parvum</i>
  Immature. Mature	  Thin walled thick walled oocyst

Demonstration

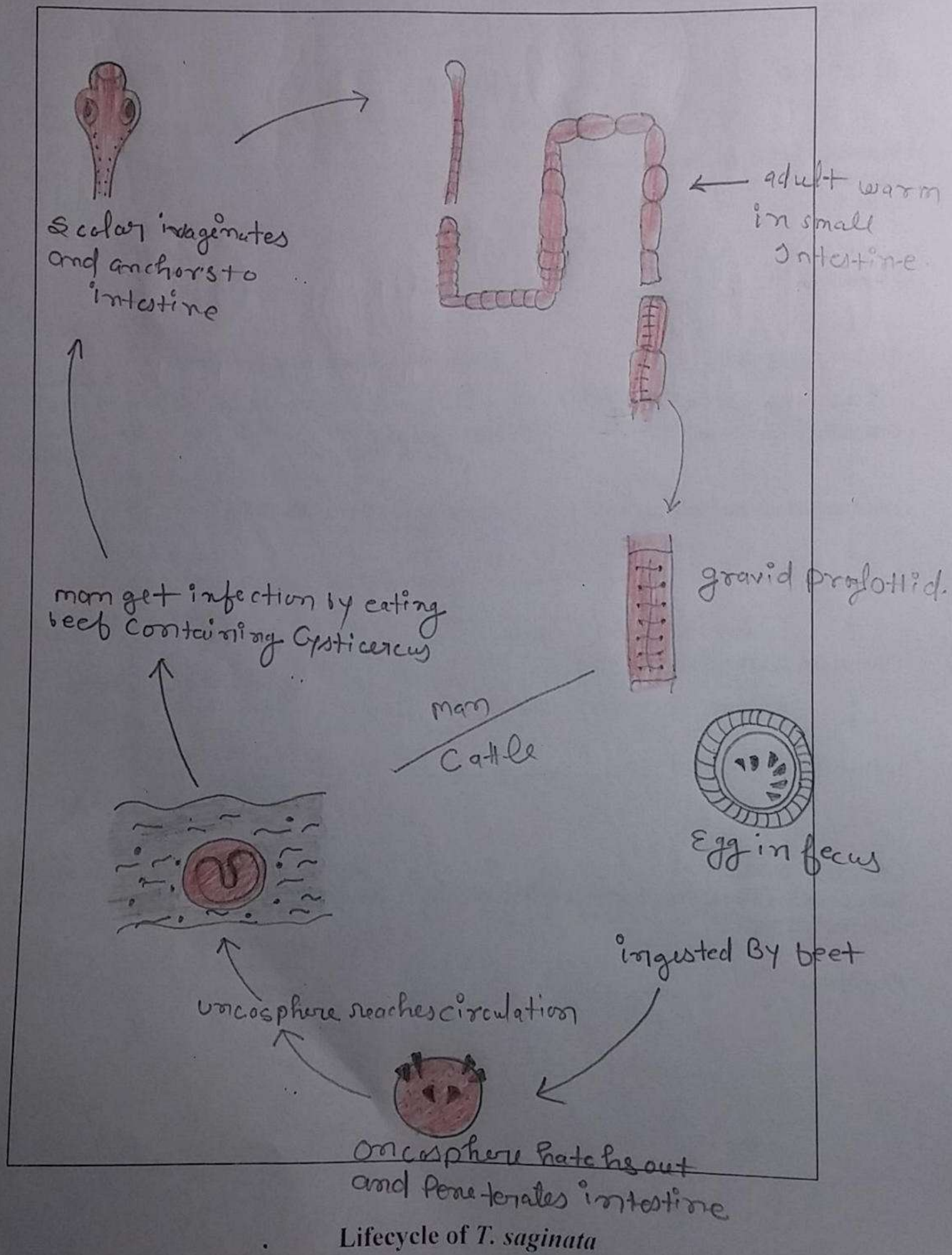
1. Smear showing trophozoites of *T. gondii*.
2. Lifecycle of *T. gondii* (chart).
3. Morphological stages of *T. gondii* (chart).
4. Lifecycle of *C. parvum* (chart).
5. Lifecycle of *I. belli* (chart).
6. Oocyst of *C. parvum* and *I. belli* (chart).

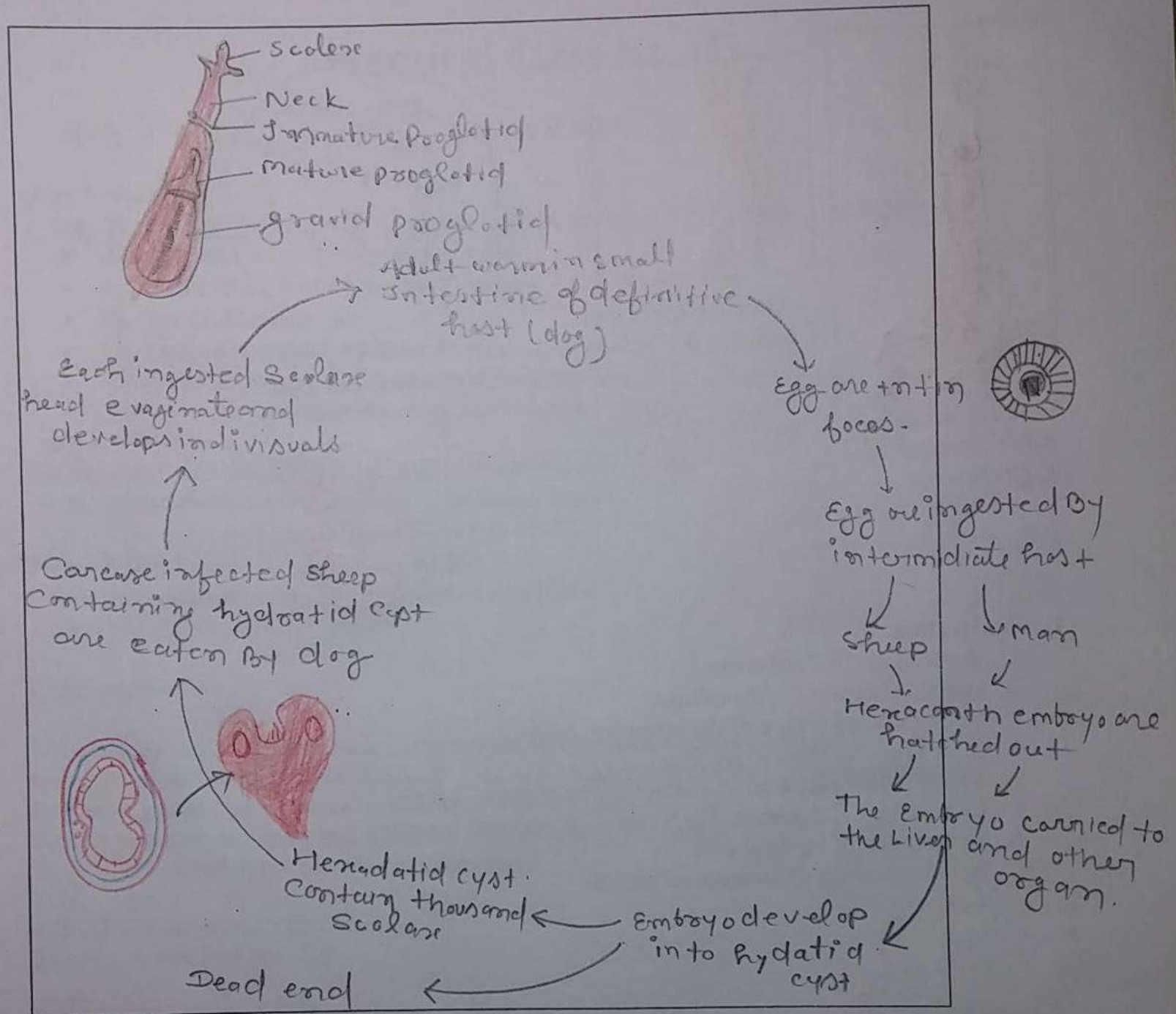
Exercise

1. What are the pseudocyst, tissue cyst and oocyst?
2. What is the pathogenesis of Toxoplasmosis?
3. Enumerate serological tests used for diagnosis of Toxoplasmosis.
4. What are TORCH agents?
5. Mention drug of choice for treatment of Toxoplasmosis.
6. What do you know about Sabin-Feldman dye test?
7. Mention the diseases produced by *T. gondii*.
8. Mention about prevention of infection by *T. gondii*.
9. What is the pathogenicity of *C. parvum*?
10. What is the staining characteristic of oocyst of *C. parvum*?
11. What is the pathogenicity of *I. belli*?
12. What are the different opportunistic Protozoal infections in AIDS?

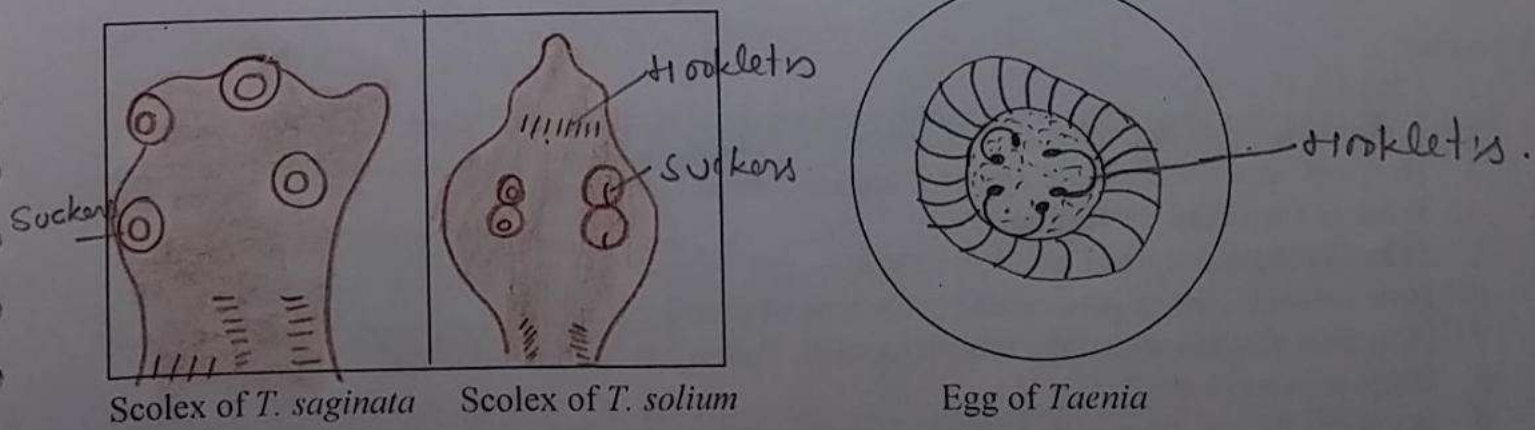
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Diagram



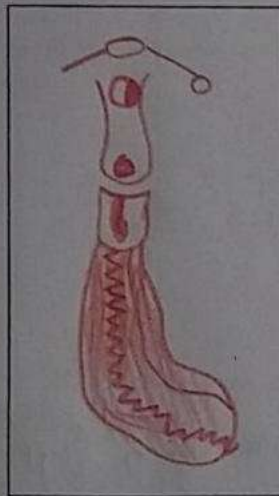
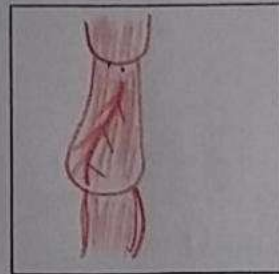
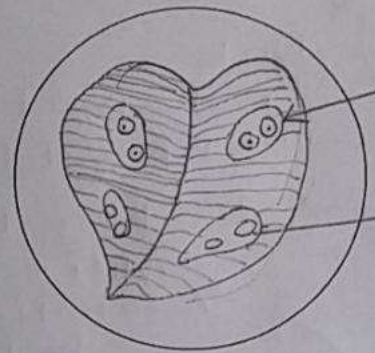


Lifecycle of *E. granulosus*



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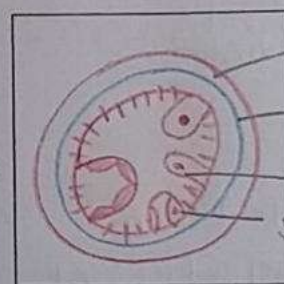
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Adult worm of
E. granulosusGravid segments of
T. saginata & *T. solium*

Cyst

Muscle.

Cysticercus cellulosae



Hydatid cyst

ectocyst

endocyst

brood capsule

scolex

Demonstration

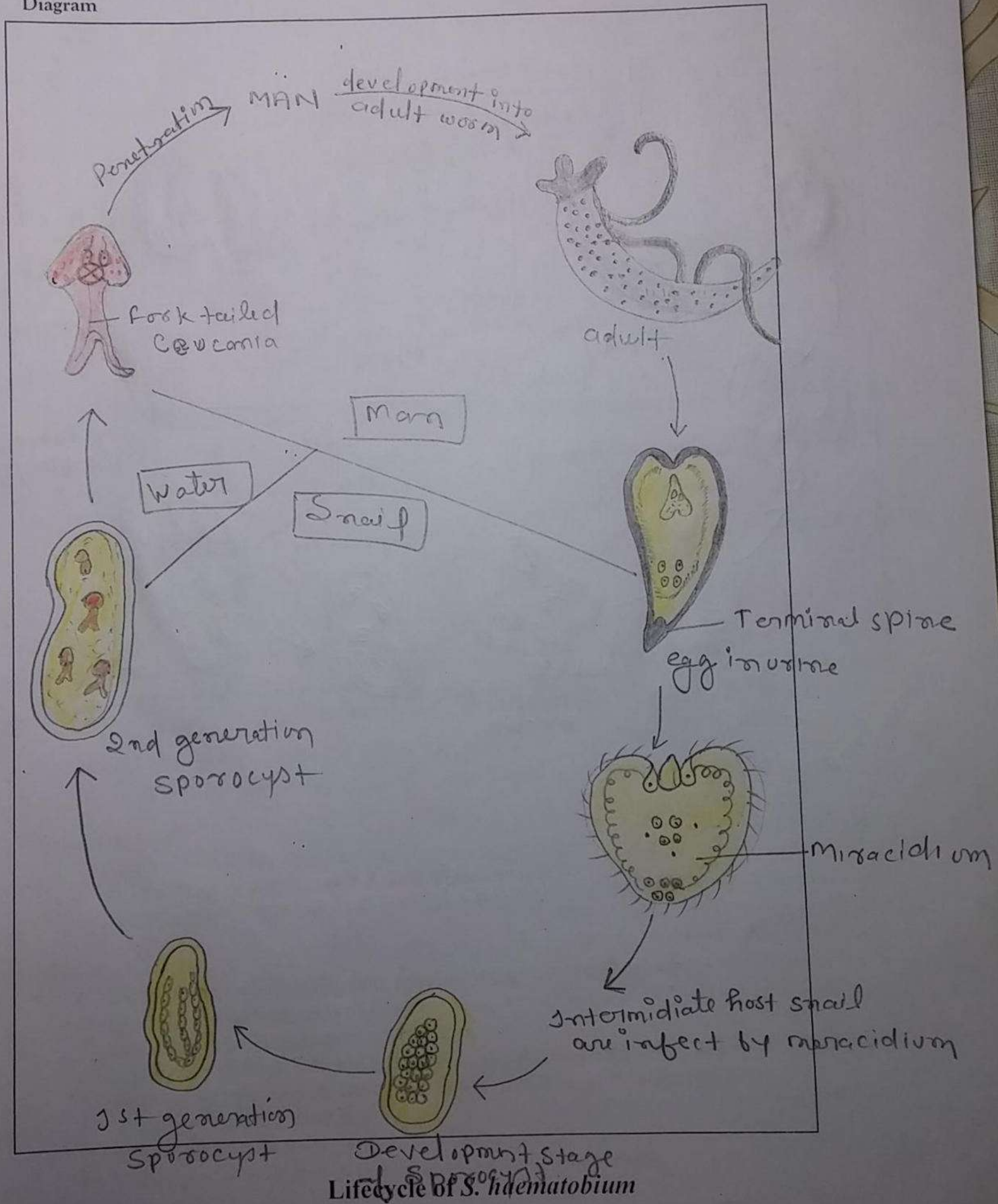
1. A specimen of Tapeworm.
2. A slide showing *E. Granulosus*.
3. Slide showing scolex of the tapeworm /chart.
4. Slide showing proglottids /chart.
5. Saline/iodine preparation of stool showing egg of Taenia.
6. Lifecycle of *T. saginata*, *T. solium*, *E. granulosus* (chart).
7. A specimen of Hydatid cyst.
8. A slide showing cysticercus cellulosae.

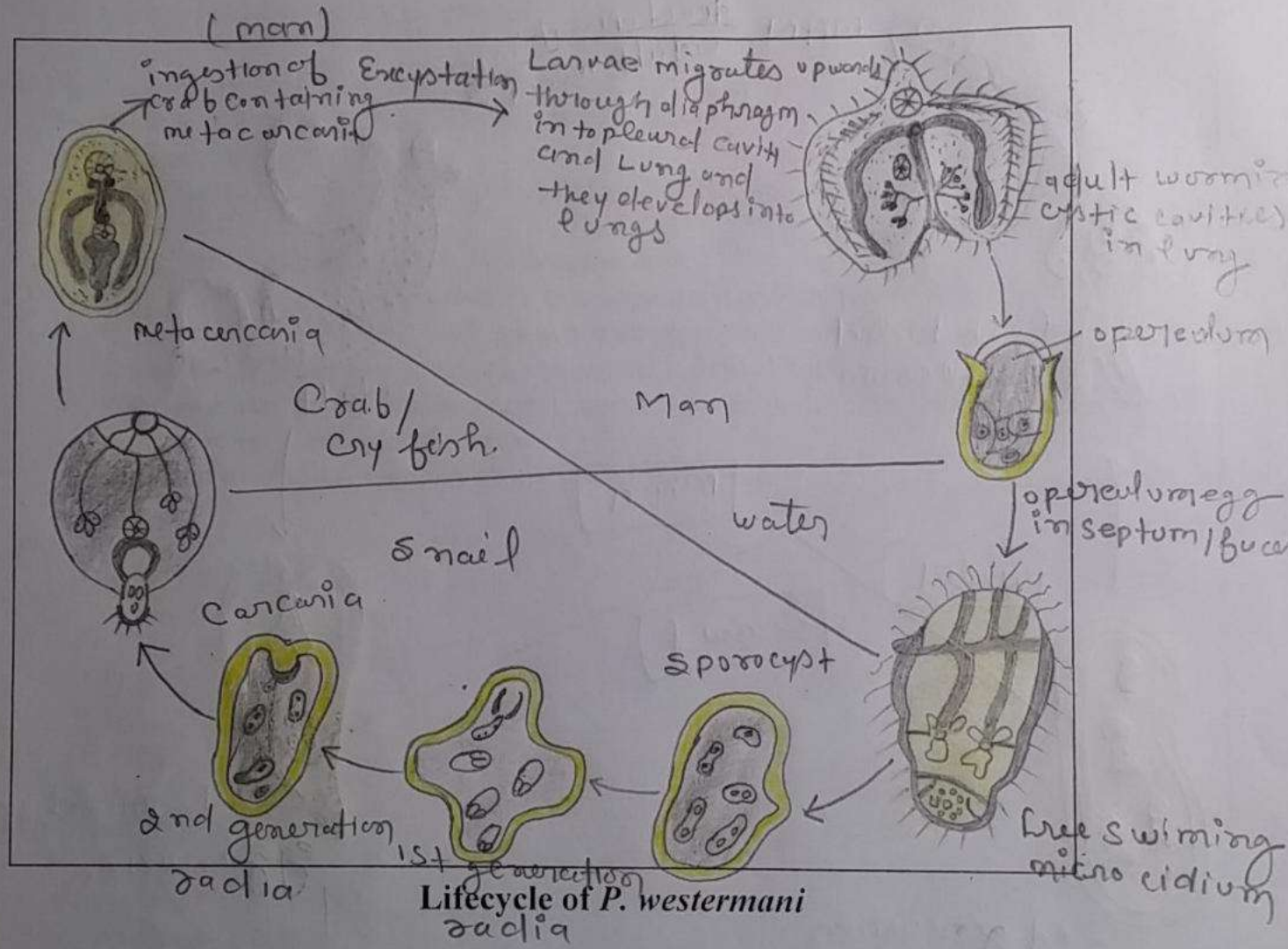
Exercise

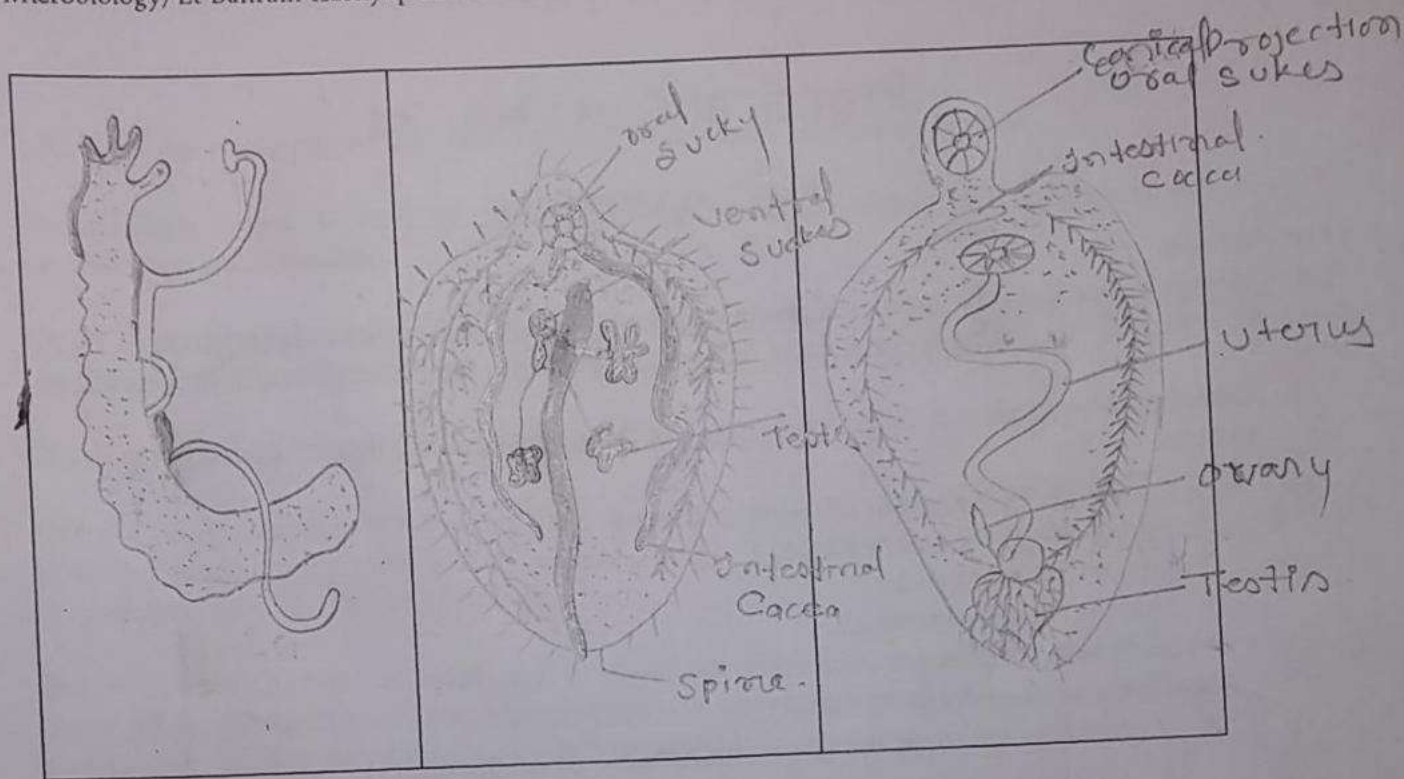
1. Classify Cestodes.
2. Mention the pathogenicity of *T. saginata*, *T. solium*, *E. granulosus*.
3. How will you differentiate egg of *T. saginata* from *T. solium*?
4. What is Cysticercus cellulosae?
5. Mention the example of smallest Cestode.
6. How Casoni's test is performed? What type of reaction is it?
7. How will you know that the infestation with Taenia has been eliminated?
8. What is brood capsule?
9. What are the common sites of Hydatid cyst in the human body?
10. Write the drug of choice for elimination of Taenia infestation.
11. How will you prevent Tapeworm infestation in human beings?
12. What are the examples of bile stained eggs?

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Diagram



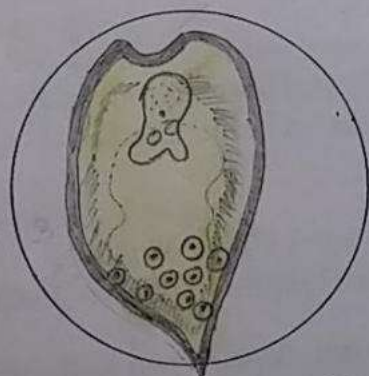




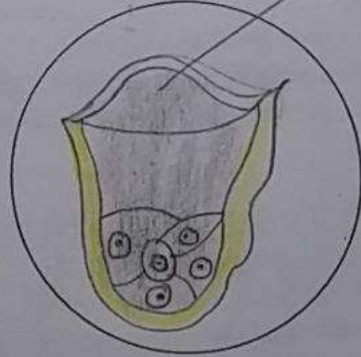
Adult worms of
S. haematobium

Adult worms of
P. westermani

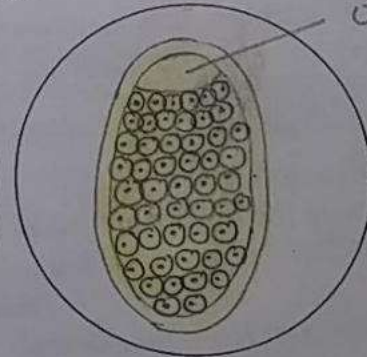
Adult worms of
F. hepatica



Egg of *S. haematobium*



Egg of *P. westermani*



Egg of *F. hepatica*

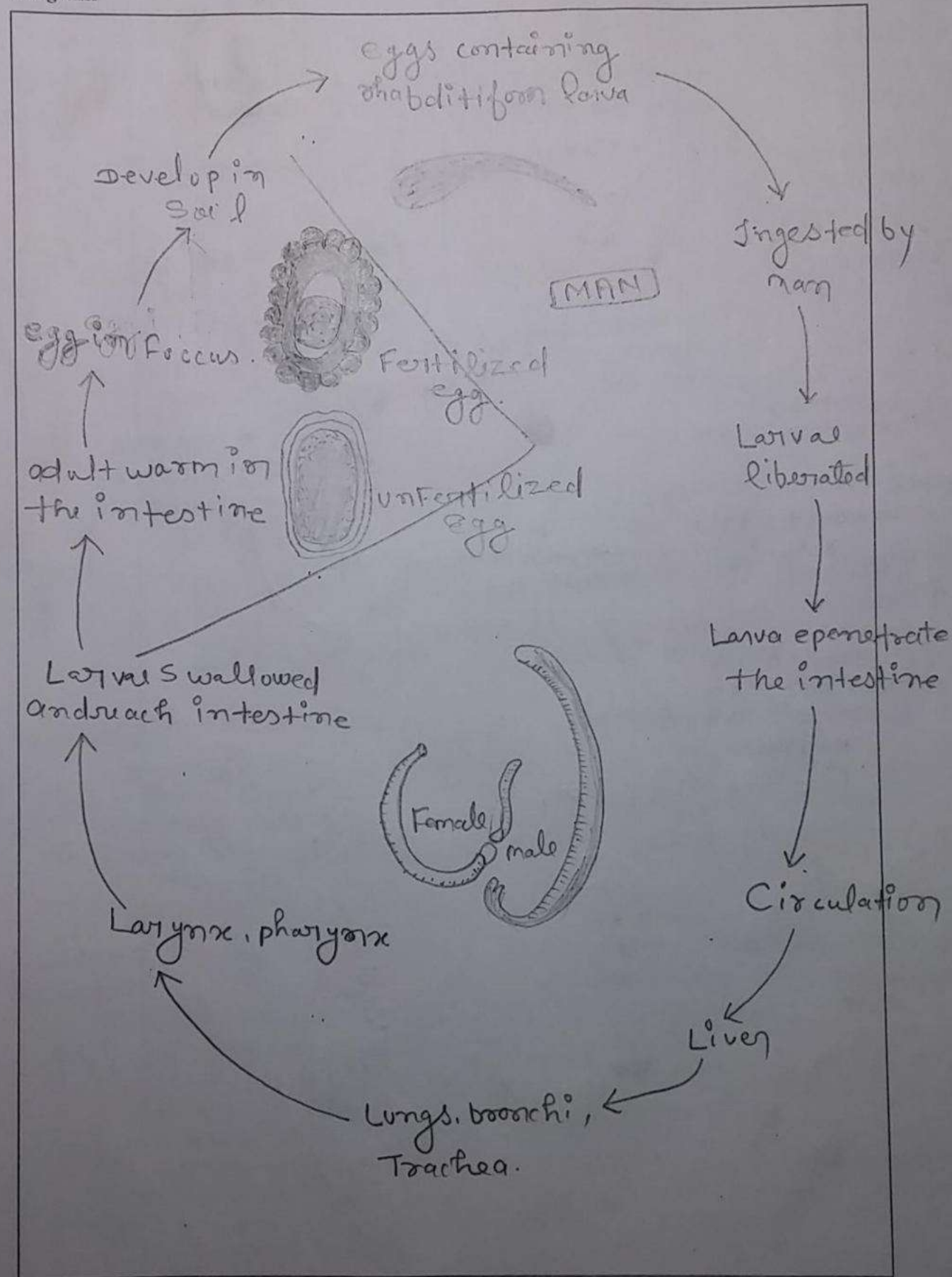
Demonstration

1. Lifecycles of *S. haematobium* and *P. westermani* (charts).
2. Eggs of *S. haematobium* and *P. westermani* (charts).
3. A specimen of Liver Fluke/ slide of Liver Fluke.

Exercise

1. Describe the pathogenicity of *S. haematobium*.
2. Describe the pathogenesis of *P. westermani*.
3. Describe the pathogenesis of Liver Fluke.
4. Is there any relation between Schistosomiasis and Salmonella carriers and Ca bladder?
5. Mention the intermediate hosts for *S. haematobium* and *P. westermani*.
6. What is swimmer's itch?
7. What is Kotayama fever?
8. Write mode of infection in *S. haematobium* and *P. westermani*.

Diagram

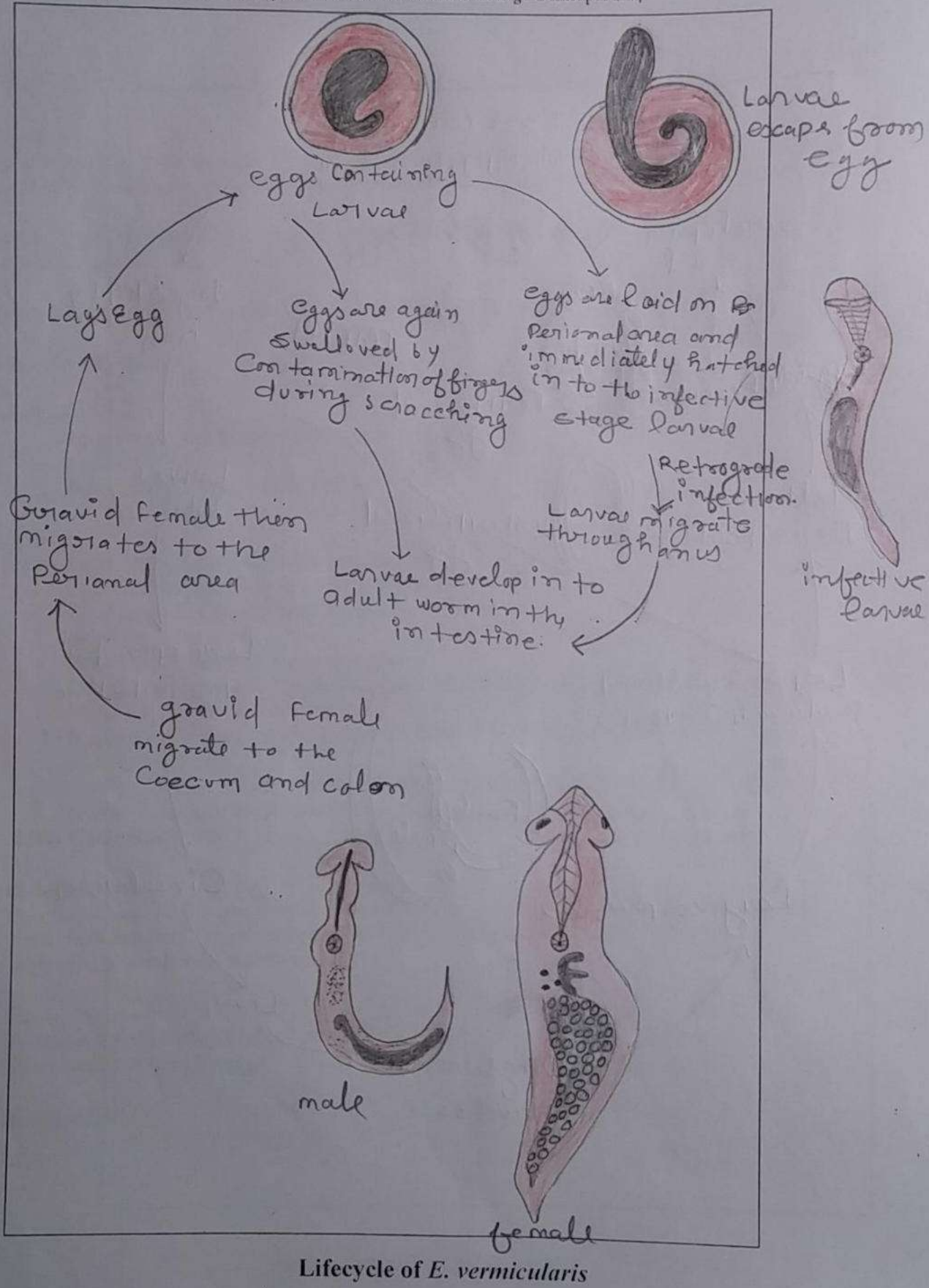


Lifecycle of *A. lumbricoides*

MICROBIOLOGY PRACTICAL RECORDS

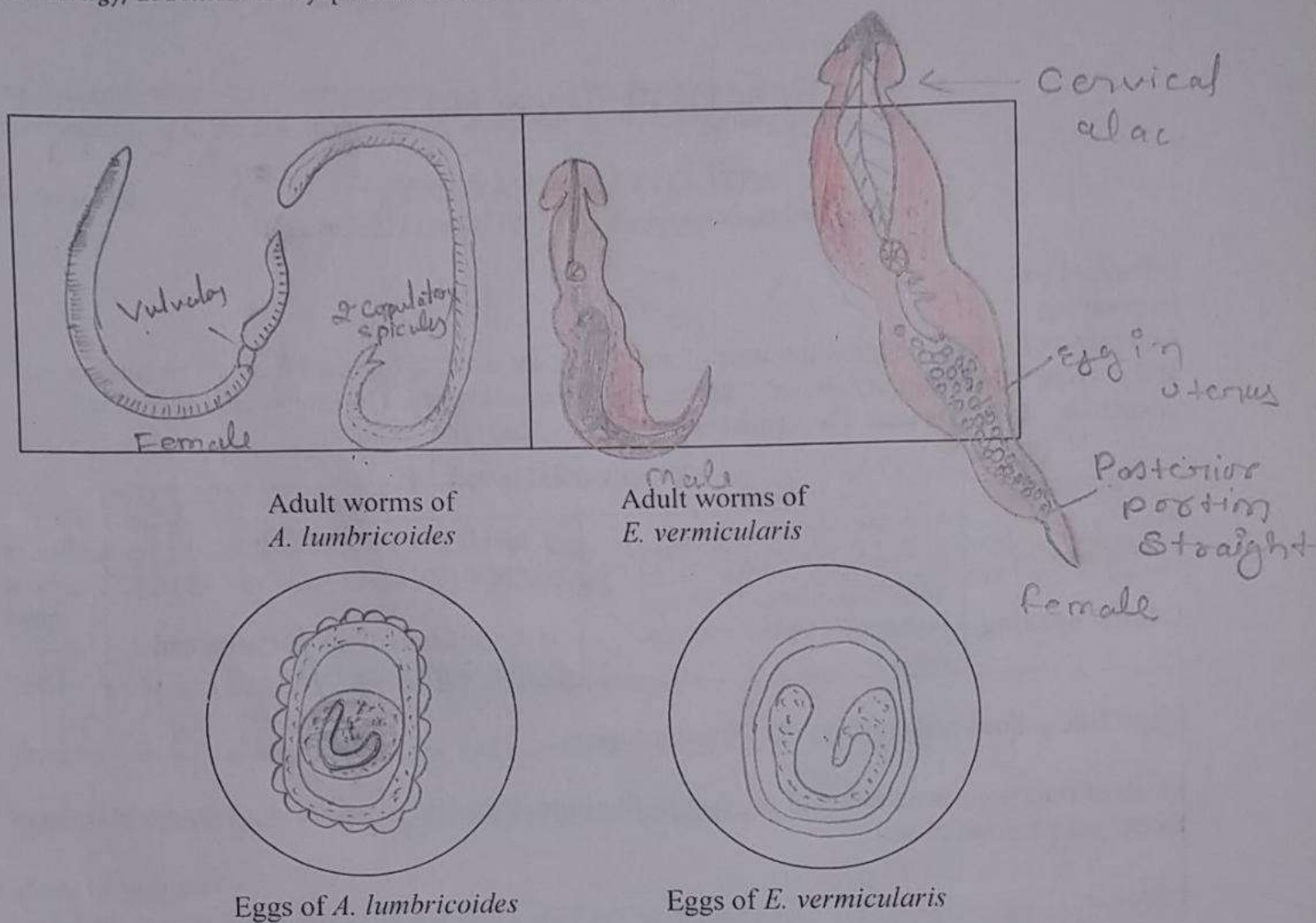
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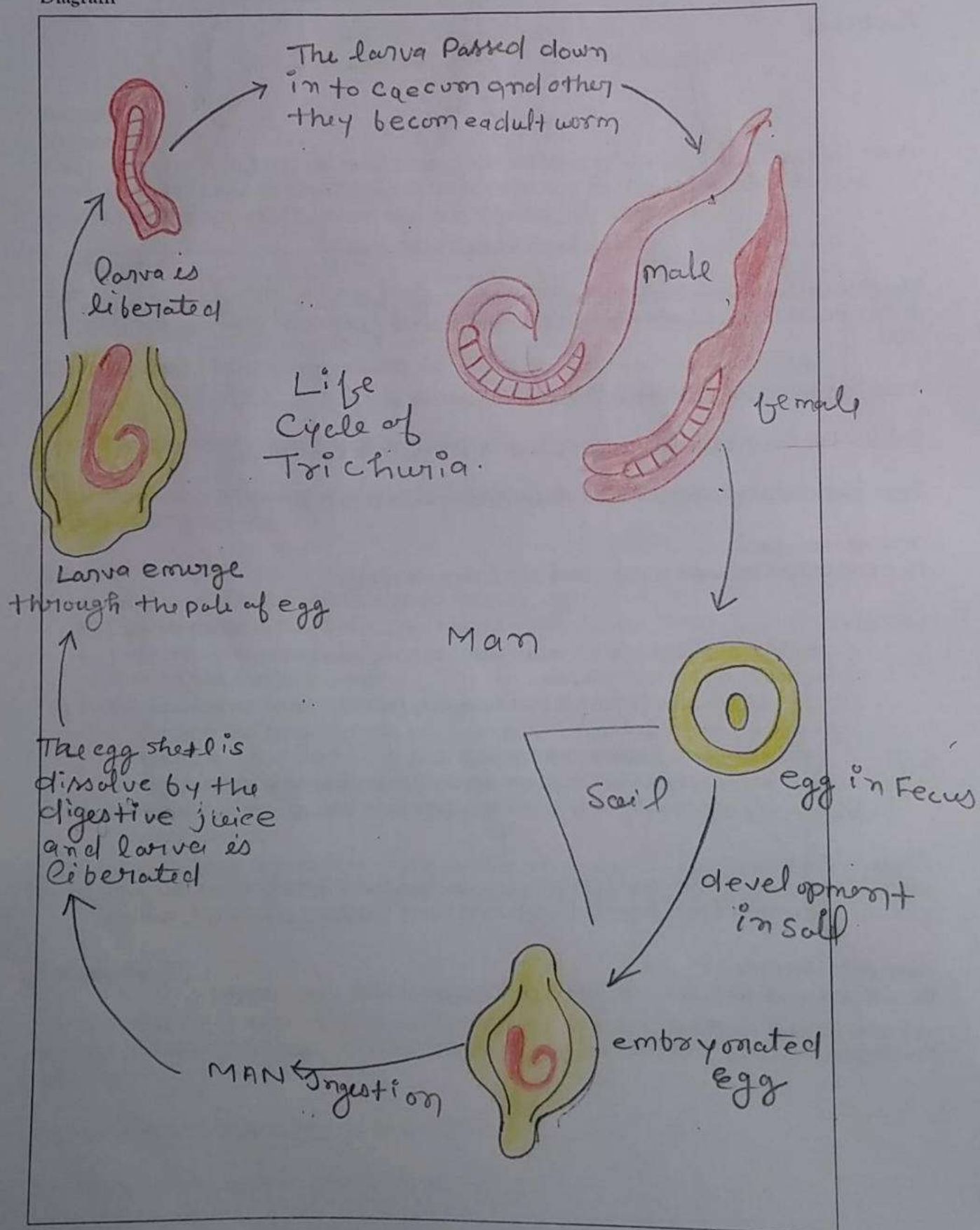
**Demonstration**

1. A specimen of *A. lumbricoides* (Male, Female).
2. A specimen of *E. vermicularis*.
3. Lifecycle of *A. lumbricoides* and *E. vermicularis* (chart)
4. Saline and iodine preparation of a sample of stool containing eggs of *A. lumbricoides* and *E. vermicularis*.
5. Diagrams of eggs of *A. lumbricoides* and *E. vermicularis* (chart).
6. NIH swab.

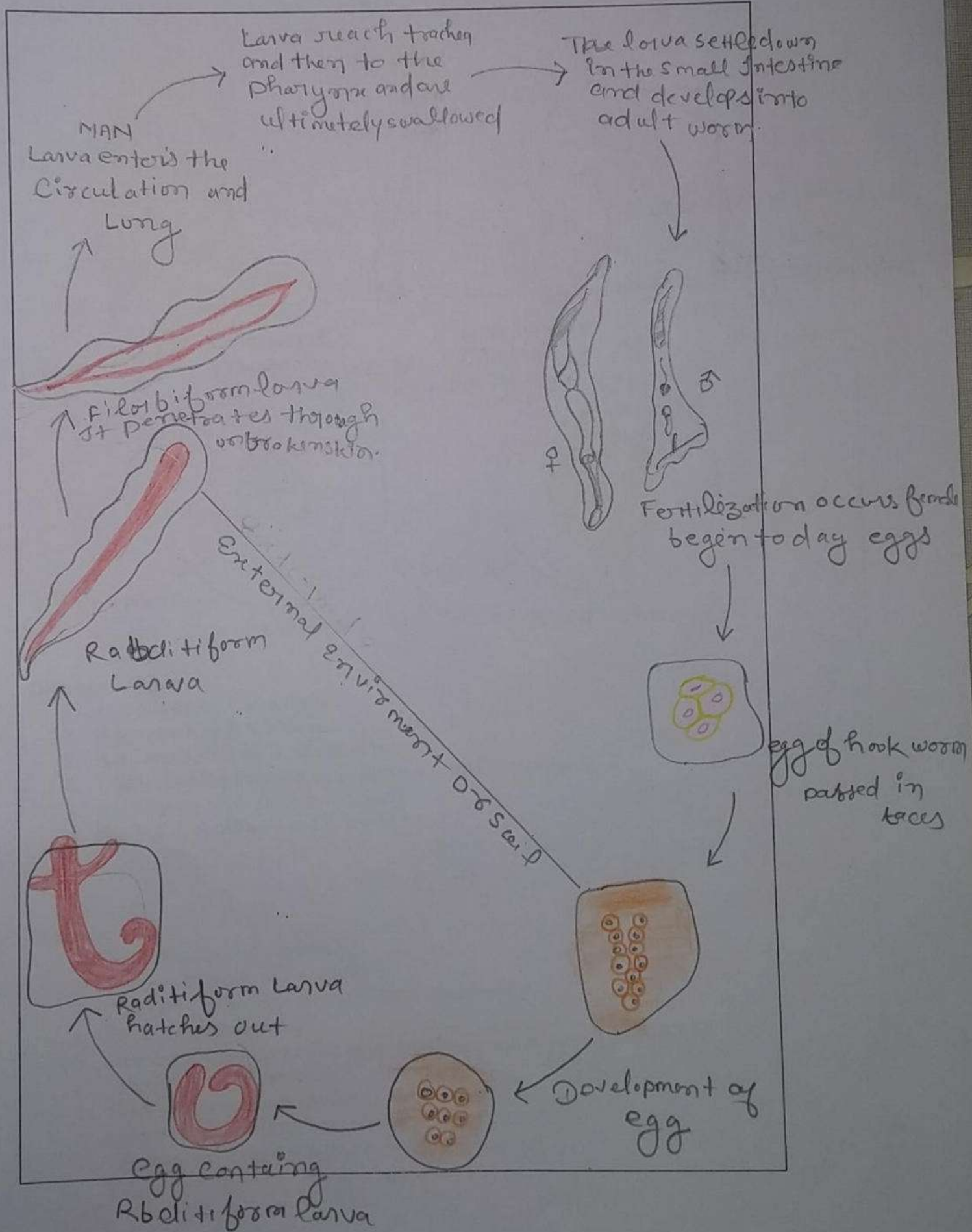
Exercise

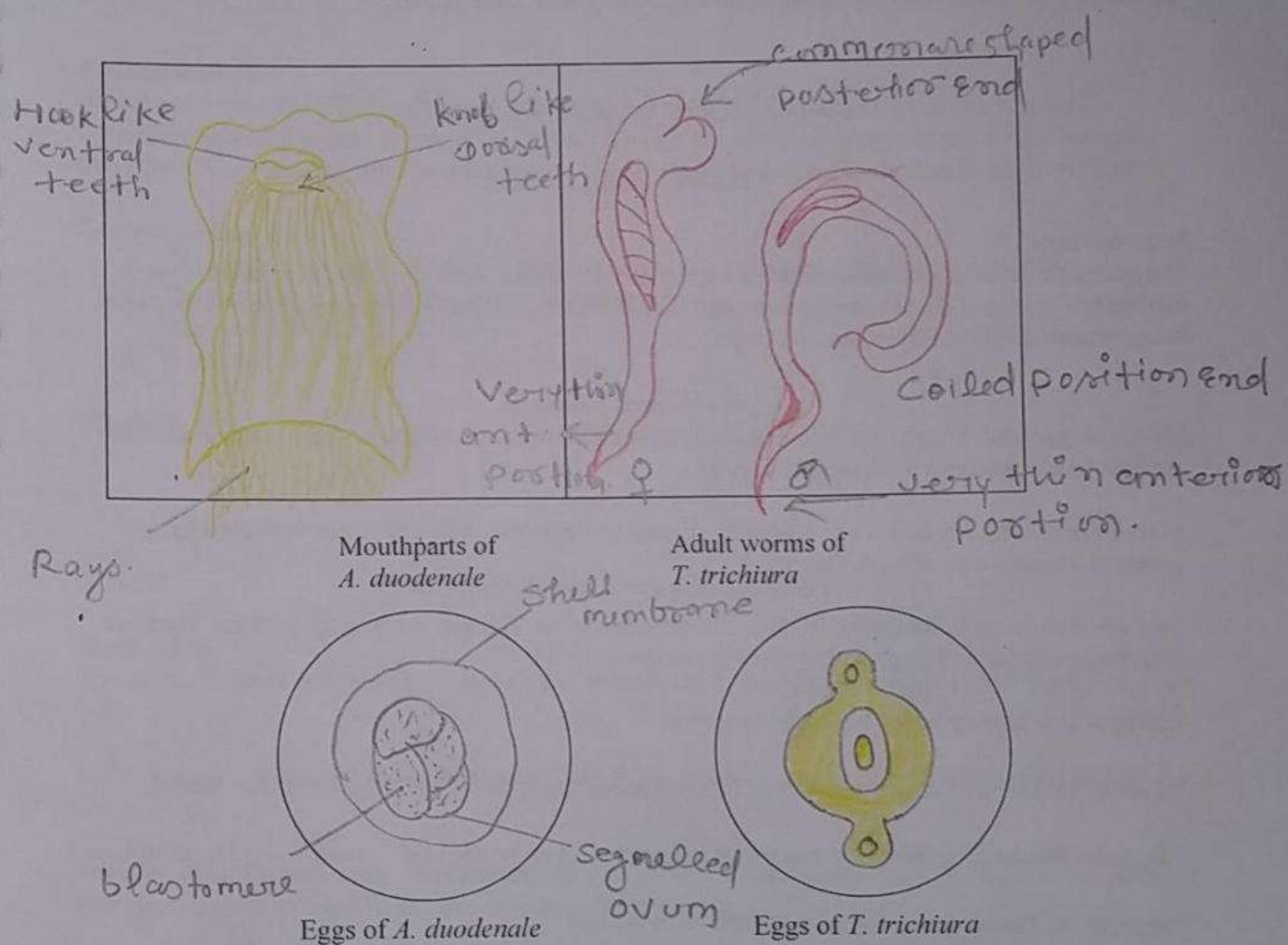
1. Write mode of infection in case of *A. lumbricoides* and *E. vermicularis*.
2. Which Vitamin deficiency occurs in *A. lumbricoides* infestation?
3. What is Loeffler's pneumonia? Which stage of the parasite is responsible for that?
4. What are the complications of Ascariasis?
5. What is the use of NIH swab?
6. What measures will you take prevent the infection of *A. lumbricoides* and *E. vermicularis*?
7. Which sample will you collect to demonstrate the larva of *A. lumbricoides*?
8. What is autoinfection and retrograde infection in case of *E. vermicularis*?

Diagram



Lifecycle of *A. duodenale*



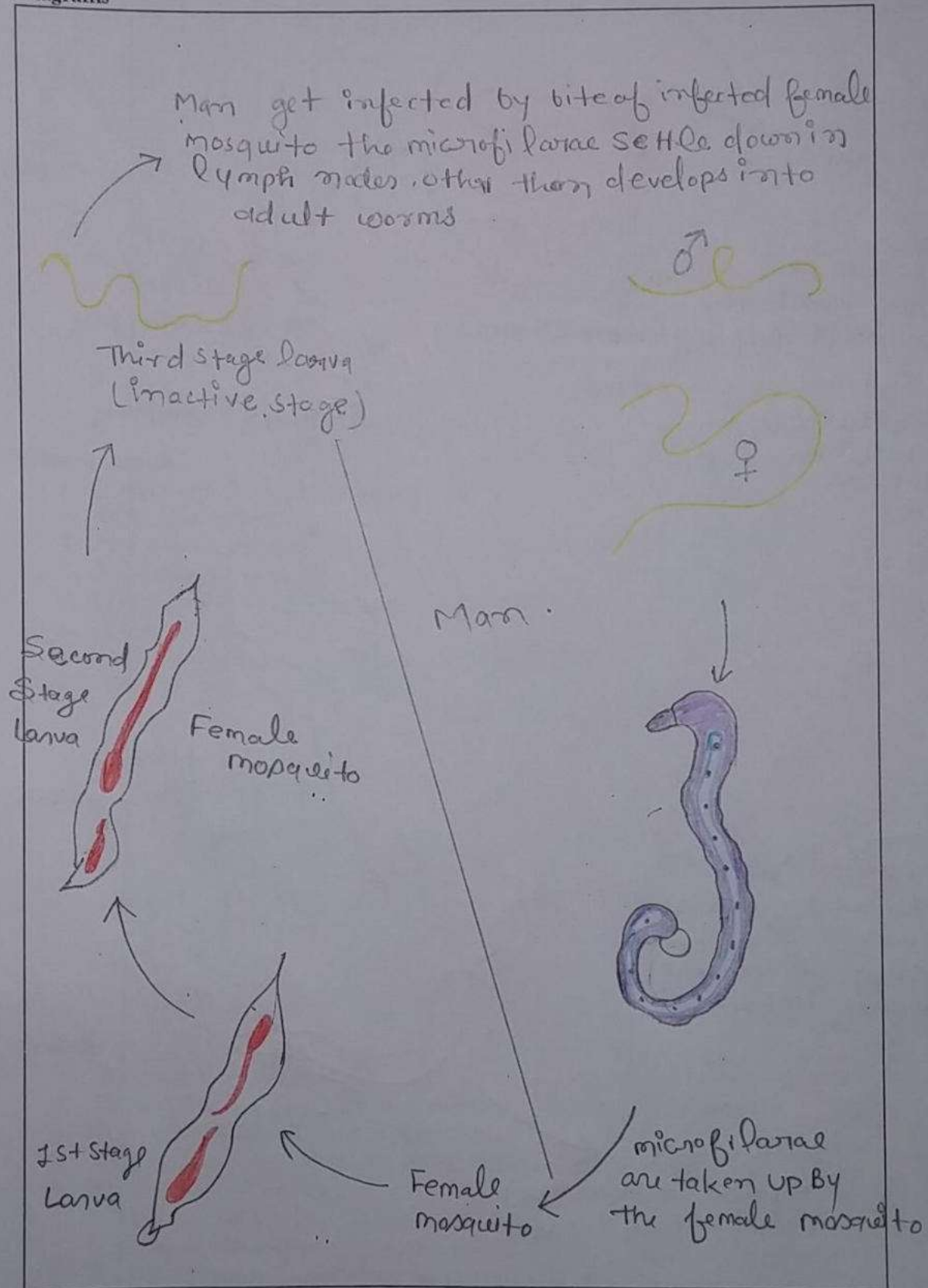
**Demonstration**

1. A specimen of *A. duodenale*.
2. A specimen of *T. trichiura*.
3. A mounted slide of *A. duodenale*.
4. Charts showing lifecycle of *A. duodenale* and *T. trichiura*.
5. Mouth parts of *A. duodenale* (chart).
6. Eggs of *A. duodenale* and *T. trichiura*.

Exercise

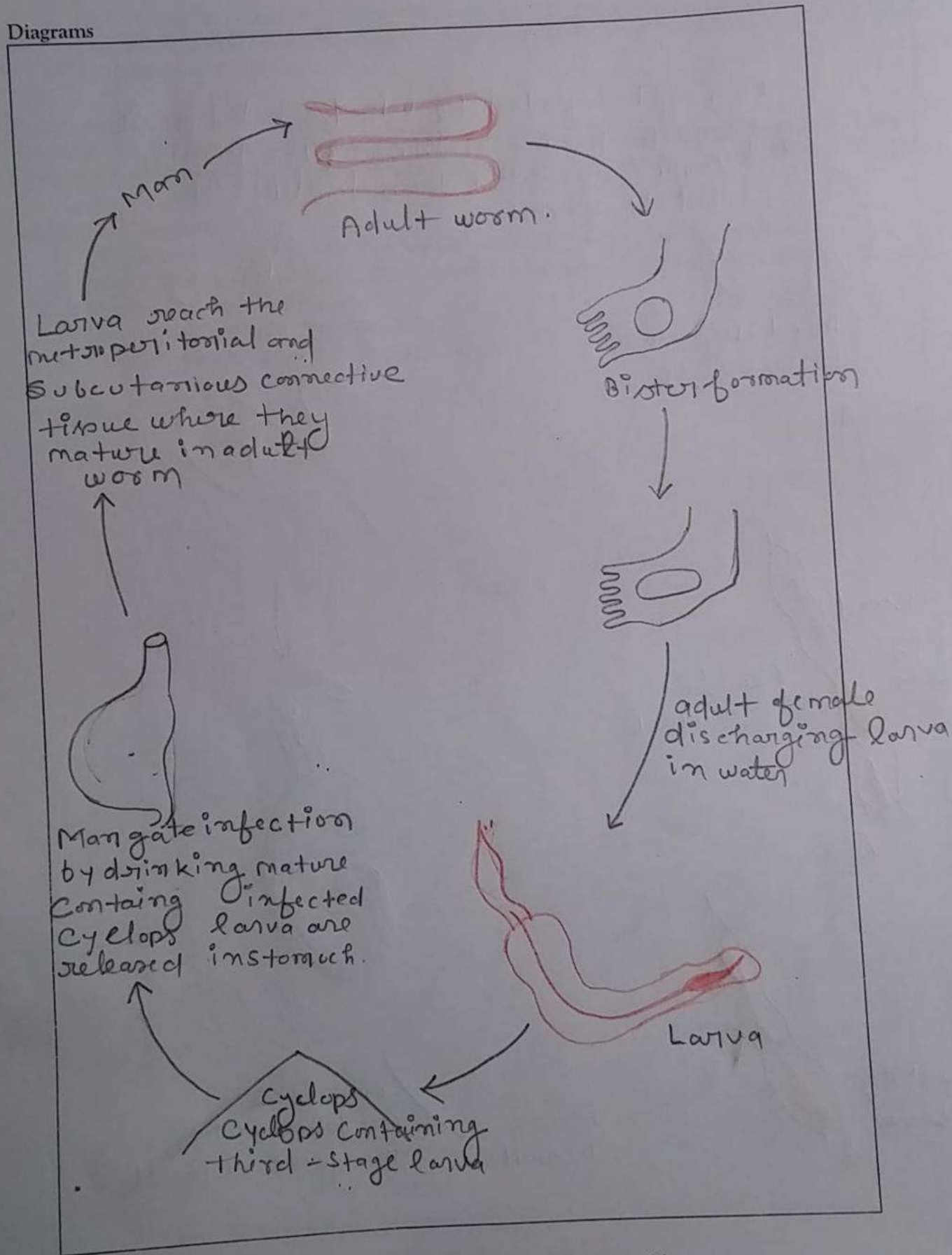
1. Why the name Hookworm?
2. What is the mode of infection?
3. What are the diseases produced by *A. duodenale*?
4. What is larva migrans?
5. How much blood is sucked by *A. duodenale* and *N. americanus* daily?
6. Why the name Whipworm?
7. What are the diseases caused by Whipworm?
8. How will you prevent Ankylostomiasis and Trichuriasis?
9. Enumerate the concentration methods for the stool.

Diagrams



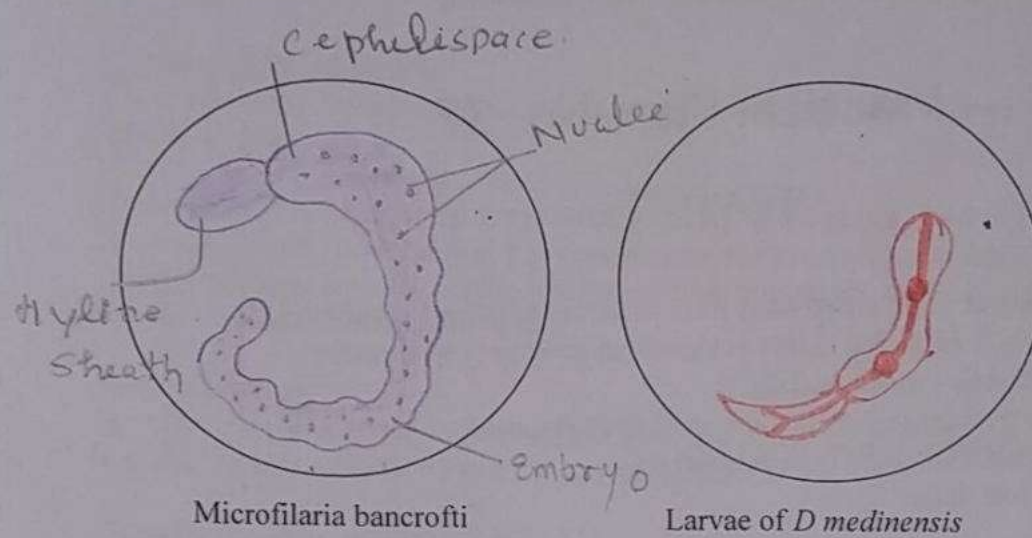
Lifecycle of *W. bancrofti*

Diagrams



Lifecycle of *W. bancrofti*

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Demonstration

1. Lifecycle of *W. bancrofti* (chart).
2. Microfilaria (chart)
3. PBS showing Microfilaria
4. A specimen of *D. medinensis*.
5. Lifecycle of *D. medinensis*(chart).
6. A slide showing cyclops.
7. A case of Elephantiasis (chart).
8. A case of Dracunculiasis (chart).

Exercise

1. Enumerate the filarial worms.
2. Mention the pathogenicity of *W. bancrofti*.
3. How will you demonstrate the microfilaria?
4. What is the provocative test for demonstration of microfilaria?
5. Write prevention of Filariasis.
6. What is the status of Dracunculosis in India?
7. Describe the movement of *D. medinensis*.
8. What is the pathogenesis behind the formation of blister in dracunculosis?
9. How dracunculosis is prevented?

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c. Colonizing aspergillosis (aspergilloma)- fungus grows in preexisting pulmonary cavities growing into large balls.

2. Disseminated aspergillosis- involves the brain, kidneys and other organs.

Laboratory diagnosis is made by-

1. Microscopical examination
2. Culture- Aspergillus has septate hyphae. Asexual conidia are arranged in chains, carried on elongated cells called as sterigma, born on the expanded ends (vesicles of conidiophores).

Mucormycosis

Mucormycosis is an invasive disease caused by Phycomycetes mainly by species Rhizopus, Mucor and Absidia. It is an opportunistic infection occurring when general resistance is extremely low. The primary infection is in the upper respiratory tract, the mycelia invading the adjacent tissues.

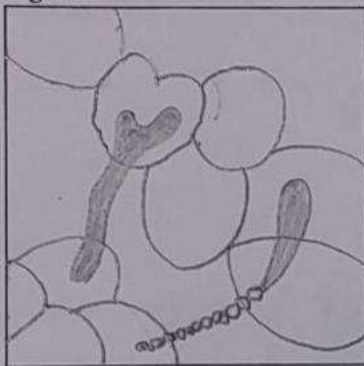
Laboratory diagnosis is made by-

1. Histological examination of autopsy material- shows broad, long septate mycelia in tissues.
2. Culture on SDA.

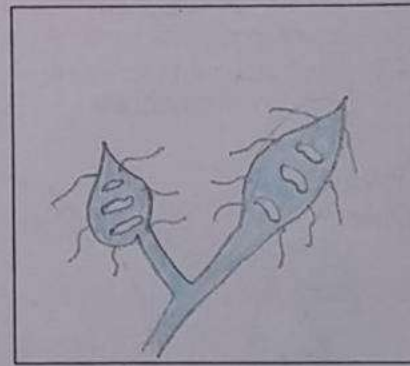
Rhizopus- sporangiophores arise in groups directly above the rhizoids.

Mucor- branched sporangiophores arising randomly along aerial mycelium. Rhizoid absent.

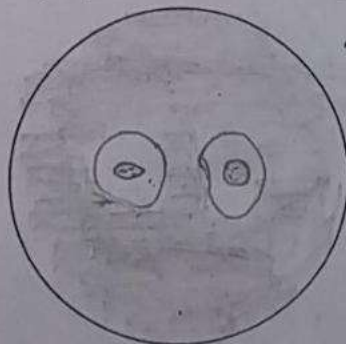
Diagram



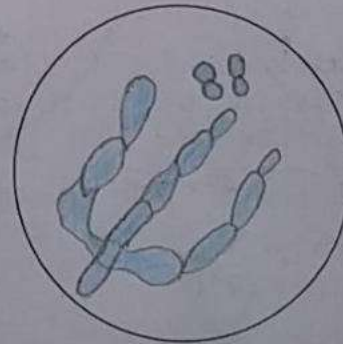
KOH preparation of skin scraping



Macroconidia

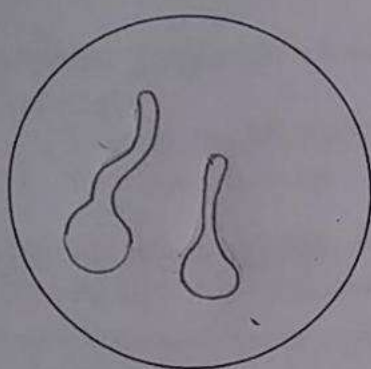


C. neoformans (negative stain)

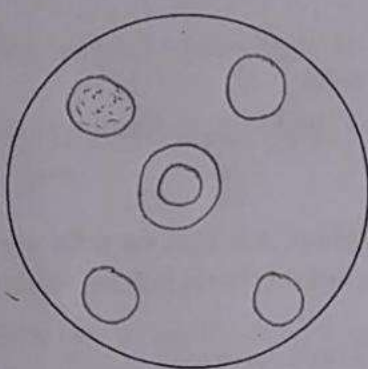


Gram stain of *C. albicans*

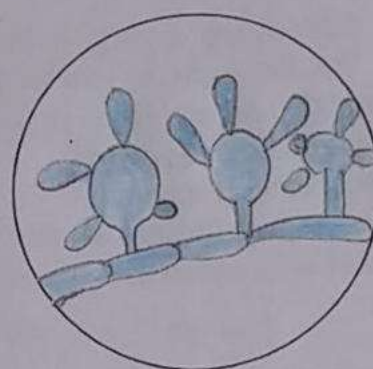
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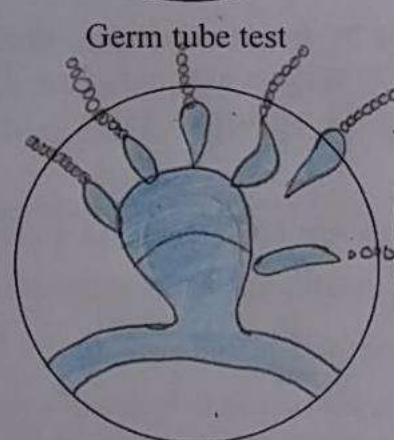
Germ tube test



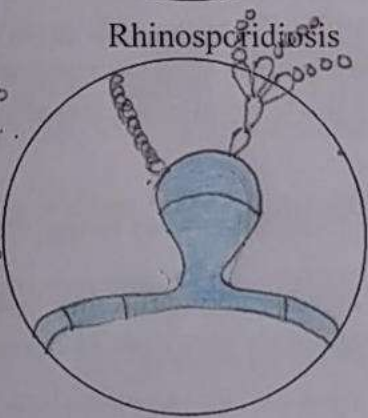
Rhinosporidiosis



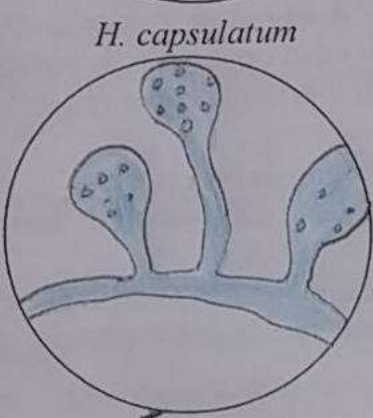
H. capsulatum



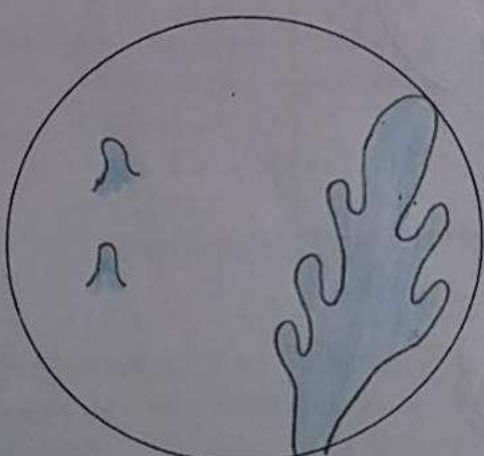
Aspergillus



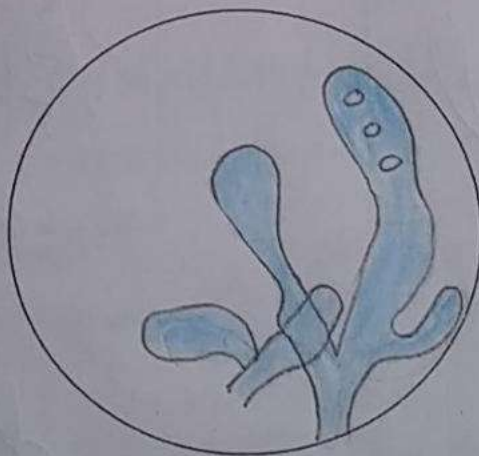
Penicillium



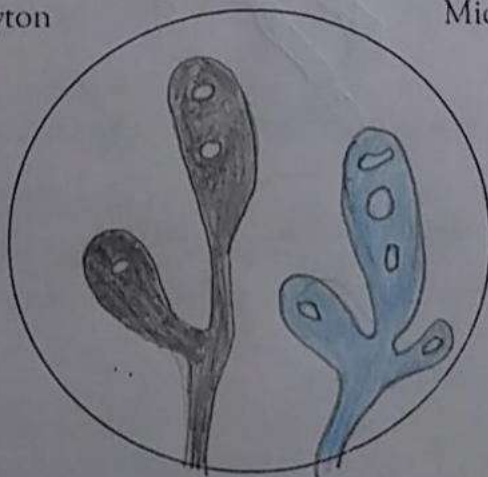
Mucor



Trichophyton



Microsporum



Epidermophyton