SECOND YEAR MBBS RECORD

MICROBIOLOGY

Name : **Subject :**

Course : **Batch :** **Roll No.**

University Regn. No.:

Certified that this is a bonafide record of work done by the above name student in the subject of MICROBIOLOGY.

Professor / HOD
Department of Microbiology

Instructions for Maintaining the
RECORD NOTE BOOK

1. Records should be written neatly on the right hand pages only, left hand pages being reserved for diagrams and observations

2. Record should contain :

   i) The date
   ii) Number and Name of the Expt.
   iii) The aim of the Expt.
   iv) A list of the apparatus required
   v) A description of the apparatus
   vi) The Theory of the Expt. in brief
   vii) Observations (to be entered in neat tabular forms)
   viii) The Result

Instructions to the Students for Laboratory Works and Conduct

A) **General**

1. Always wear clean white coat for all laboratory sessions

2. Each student must provide himself / herself with
   a) glass marking pencils
   b) a box of colour pencils including eosin & haematoxylin pencils.
   c) a six inch plastic ruler with mm scale
   d) a record note book

3. Keep your work bench neat, clean and orderly. Replace reagent in their proper places after use.

B) Special

1. Do not put anything (e.g., pencils, labels, fingers, food, etc.) in mouth during laboratory work.

C) Care of Microscope

The microscope is a valuable instrument and should be handled with care.

1. It should be placed only on the work bench and should not be taken to any other place.
2. After use, clean the eye piece, oil immersion and other objectives with a soft linen.
3. Do not use xylol on the lens.
4. Remember to clean the oil on the lens always.
5. In case of any problem, take assistance from the instructor or technician.

D) **Record**

1. All experimental procedures adopted and the results of the experiments should be carefully recorded in your note book.
2. Each days's work should be completed in the class room and approved by the instructor under his signature.

CAUTION

Throughout this course you will be working with potentially pathogenic organisms and infected materials which are capable of causing infections to yourself and even you may carry them to your homes. Therefore, you should adopt strict ASEPTIC precautions while working.

C O N T E N T S

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Practical : A Compound microscope is provided. Describe and make a diagram of the microscope indicating the parts.

M i c r o s c o p y

What is a simple Microscope ?

What is a compound Microscope ?

List the factors on which the magnification of a compound microscope is dependent on

What is the function of oil when used with a oil immersion objective ?

What is the resolving power of a microscope ?

List the type of microscopes generally used to visualize microbial agents?

Describe the procedure used to focus the microscope :

Focussing

Low Power

High Power

Oil immersion

List the do’s and don’ts on the care of the Microscope ?

Preparation of Smears and Staining of Bacteria
Study of Morphology

Live Preparation :

Methods used for study of bacterial morphology & motility using live bacterial smear preparations are :

1. Hanging drop preparation
2. Wet cover slip preparation
3. Phase contrast microscopy
4. Dark-ground microscopy

Demostration of Motility by Hanging Drop Method

Practical : Prepare a Hanging drop of the given suspension. Focus under high power and note down your findings. Show the preparation to the instructor.

Procedure for Hanging Drop Preparation

1. Take a clean cover slip apply a minimal amount of vaseline to the four corners with a thin glass rod or art applicator stick.
2. Using a sterile loop place a loopful of the given suspension at the centre of the cover slip.
3. Invert a cavity slide over the cover slip so that the drop of suspension is in the centre of the cavity.
4. Quickly and carefully turn the slide over so that the cover slip is uppermost with the drop hanging from the same into the cavity!
5. Focus the edge of the drop under low power of the microscope after reducing the light using the condenser.
6. Turn the high power objective, identify the edge and carefully focus the edge with fine adjustment. Observe the motility and other possible morphological features of the microbes.

Questions

1. List the advantages of phase contrast and dark-ground microscopy over the first two methods.

2. Name the Organelle which helps in the motility of the organism.

3. Describe the arrangement of flagellae found among bacteria.

4. Define motility of Bacteria.

Preparation of Smears and Staining of Bacteria
Study of Morphology

Study of morphology of bacteria helps in its identification. Apart from wet preparation wherein living organisms are studied, stained preparations are of great value in the study of morphology. The staining methods was first introduced by Robert Koch. Aniline dyes are generally used for staining bacteria.

Simple Stain

Practical : Prepare two smears from the given suspension, fix and stain each using
(1) Loeffler's Methylene Blue and (2) Dilute carbol-fuchsin.

Procedure for Preparation and Fixation of Smear

1. Place loopful of given suspension on the centre of a clean slide and spread it to form a thin uniform film of about one centimeter in diameter.
2. Allow the smear to dry in air.
3. Fix the smear by passing the slide through bunsen burner flame two or three times so as to become just warm enough but not hot when placed on the dorsum of the hand.
4. Mark on the back of the slide around the margin of the smear.
5. Cool the slide to room temperature before staining.

Procedure for Simple Staining

1. Keep the slide on the slide rack fixed onto the sink or enamel tray
2. Pour the stain (Loeffler's Methylene Blue or dilute carbolfuchsin) over the smear so as to just cover the same and allow to act for 30 seconds.
3. Wash the slide with tap water, keeping the slide in a slanting position
4. Blot to dry using blotting paper.
5. Observe under oil immersion objective and note your findings. Show the Preparation to the instructor.

Gram's Stain

Practical : Prepare a smear from the given bacterial suspension and stain by Gram's Method.

Procedure for Gram's Stain

1. Cover the smear with crystal violet/gentian violet and keep for one minute and wash with water.
2. Drain excess water and cover the smear with Gram's lodine for one minute.
3. Wash the smear with alcohol (or acetone) until the violet colour is just about to disappear and wash with water.
4. Drain the excess water on the slide and cover the smear with safranine (or dilute carbon fuchsin, or neutral red) for one minute and wash with water.
5. Blot dry with filter paper.
6. Observe under oil immesion objective and note down your findings. Show the focussed smear to the instructor.

	Point to be noted	**Observation**	**Inference**
1.	Colour		
2.	Shape		
3.	Arrangement		

Staining procedures generally followed are :

1. Simple Stain

2. Differential Stain

   (1) Gram Stain

   (2) Ziehl - Neelsen's Stain

3. Special stain to bring out certain specific morphological features of bacteria

   (1) Albert's Stain for C. diphtheriae - metachromatic granules

   (2) Capsule stain for capsulated organism eg. Streptococcus pneumoniae.

   (3) Spore stain for Clostridium and Bacillus species of bacteria.

4. Flagellar staining - Silver impregnation method for spirochetes

5. Negative staining by India ink method or nigrosin method for Cryptococcus neoformans or Streptococcus pneumoniae.

6. Fluorescent staining - Rhodamine, auramine & stain for mycobacterium tuberculosis.

7. Define simple stain

8. Define differential stain

9. State the function of (a) lodine (b) Alcohol or (Acetone) In Gram Stain procedure

10. List the possible explanations of why some bacteria are Gram positive and others Gram negative

Cultivation of Bacteria

To study bacteria in the laboratory one must be able to grow them in pure culture. For this purpose, conditions satisfactory for their growth should be provided as bacteria have a great diversity of nutritional and physical requirements. All bacteria require sources of energy, carbon, nitrogen, sulphur, phosphorus, some metalic elements and water for their living and multiplication. In the laboratory they are grown on Culture Media.

Demonstration of Media

Liquid Media

1. Nutrient broth
2. Peptone water
3. Glucose broth
4. Serum broth
5. Alk. Peptone water
6. Selenite F broth
7. Tetrathionate F broth
8. Robertson's cooked meat medium
9. Brain heart infusion broth
10. Thioglycollate broth

Solid Media

1. Nutrient Agar (NA)
2. Mueller Hinten Agar (MHA)
3. Blood Agar (BA)
4. Sabouraud's Dextrose Agar (SDA)
5. MacConkey Medium (MAC)
6. Deoxycholate citrate agar
7. Salmonella-Shigella agar (SS)
8. Thiosulphate citrate Bill-salts sucrose
9. Chocolate agar (CA)
10. Potassium Tellurite Agar (PTA)
11. Loeffler's Medium (LF)
12. Novy, McNeal-Nicolle Medium (NNN)
13. Triple Sugar Iron (TSI)
14. Simmon's Citrate Medium
15. Christensen's Urease Medium

Q : What are the advantages of solid media over liquid media?

Q: Based on their energy and nutritional requirements, list the different types of bacteria

Q: Based on their temperature requirements for growth list the different types of bacteria

Q : Based on oxygen requirement for their growth, list the different types of bacteria

Q: Classify culture media based on their consistency

Q: Classify culture media based on composition

Q : What is simple medium ?

Q: What is an enriched medium ?

Q: What is an enrichment medium ?

Q : What is transport medium or preservation medium ?

Q: Define selective medium ?

Q: What is an anaerobic medium ?

Sterilisation and Disinfection

Micro-Organisms infect humans and animals producing diseases which range in seriousness from mild infection to death. They are transmitted to human beings or animals by food, water and air or directly through skin or mucous membrane resulting in infection. It is imperative that procedures are available to control microbial contamination. The term control refers to the inhibition of multiplication, killing or removal of micro-organisms.

Q: Define the following terms :

(1) Sterilisation (2) Disinfectant (3) Antiseptic (4) Anti microbial agent

Q : List the agents generally used for sterilisation and disinfection

Q: List the conditions influencing anti-microbial action of the above agents?

Q: List the mode of action of anti-microbial agents

Q : List methods generally used to destroy micro-organisms by application of heat

Q : Is Moist heat is more rapid and effective in killing micro organisms than dry heat ?

Q: What is the thermal death point of a micro-organism ?

Q: Define the term ‘pasteurisation’ and describe the methods used

Q : List the articles generally sterilised in autoclave

Q : List the articles generally sterilised in hot air oven

Q : List the types bacterial filters generally used for bacteria free filteration

Q: State the application of filtration for sterilisation purpose

Q: List other physical agents used for sterilisation ?

Chemical Methods :

The chemicals are of limited value in the disinfection process except a few.

1. Antiseptics of phenol group are used for disinfection of instruments and discarded cultures 0.5% of phenol (carbolic acid) is used for preservation of sera and vaccines.

2. Metallic salts or organic compounds of metals such as mercuric chloride are used as disinfectants.

3. Ethylene oxide is widely used for sterilisation of plastics and rubber materials.

4. Formaldehyde is used for fumigation of rooms & operation theatres.

Q: List the characteristics of an ideal disinfectant ?

Q: List the major groups of chemicals used for control of micro-organism.

Sensitivity of Bacteria to Antimicrobial Drugs

It should be noted that strains of bacteria are not alike in their susceptibility to antimicrobial drugs. Therefore, the reliable guide to the therapeutic use of antimicrobial agents is an In -Vitro sensitivity test. There are several in-vitro tests available of which two methods are generally in use.

Disc Diffusion Test : Six mm discs made out of Whatman Filter Paper are impregnated with antbiotics and preserved at 4^{o} C in a refrigerator. The discs are impregnated with required quantity of the drugs which is usually the concentration of the same in blood. The medium used is Mueller-Hinton Agar, on which a young culture is uniformly spread and individual discs are placed. The plates are incubated overnight and inhibition of growth around the discs are measured and sensitivity of the organism to each drug is estimated.

Record the drug sensitivity of the Organism Demonstrated
Make the Diagram of the Results

Estimation of Minimum Inhibitory Concentration : This is done by diluting the drug in test tube broth into different concentrations and to each tube 0.1 ml. of 4-6 hours old culture is added and the tubes are incubated. The highest dilution (minimum concentration of drug) in which the organism does not show growth after overnight incubation, is considered as the minimum inhibitory concentration (MIC) of the drug for the particular strain of bacterium. To treat such infection blood concentration should be higher than the MIC which should not be toxic to the Patient.

Serological Reactions

Serology - The study of reaction between antigen & antibody . This is applied in clinical medicines.

1. To identify antigens or antibodies
2. To estimate the relative quantity of these reactants
3. For diagnosis of an infection by detection of antibody rise against an antigen.
4. For diagnosis of an infection by detection of circulating antigen of the organism.
5. "Matching" of red blood cells against the serum of a potential receipient
6. To study hypersensitivity states.
7. For transplantation procedures, Tissue typing in transplantation.
8. For study of tumour immunology

Collection of Blood : Dry sterile syringe is used and blood is collected in a dry sterile test tube. Generally about 3-5ml. of blood is collected. After the clot is formed and retracted serum is collected and kept at $4^{o}C$ until it is tested. The serum is inactivated at $56^{o}C$ for 30 minuted to destroy complement at the time of test if required.

Titre : The serum may be tested undiluted or at a given dilution for detection of antibody and this is a qualitative test. It is generally tested at a series of doubling dilution in a buffer or normal saline and the result is expressed as titre, which is the highest dilution of serum at which the antigen-antibody reaction is detectable.

Antigen - Antibody reactions : Antigen-antibody reaction can be classified as primary, secondary and tertiary reactions.

Primary Reactions : Is the specific recognition and combination of the antigenic determinant with the binding site of the corresponding antibody.

Common tests done are : Immunofluorescence, Radio immunoassay and enzyme immunoassays.

Primary tests are more sensitive than secondary or tertiary reactions.

Secondary Reactions : This follows the primary interaction. Includes preciptation in solution or in gel, direct agglutination or haemagglunation, flocculation and complement fixation.

Tertiary Reactions : Occur as biologic reaction examples are phagocytosis, opsonization, chemotaxis and immune adherence.

Agglutination

Precipitation

Complement Fixation Test

Neutralization Test

Fluorescent antibody Test

ELISA Test

Radioimmunoassay

Section I
SYSTEMATIC BACTERIOLOGY

Ziehl-Neelsens' Method of Acid Fast Staining

Procedure :

1. Heat fix Smears

2. Staining and Mordanting

3. The smear is well covered with strong carbol fuchsin and a non-luminous flame is shown gently on the under surface of the slide until stream rises. Once the steam is seen the flame is immediately withdrawn to avoid boiling. Wait until the steam subsides and again flame until steam rises, take care to see that fresh stain is added each time to keep the smear completely covered.

   Repeat the process for 5 to 7 mts.

4. Washing

   The excess of the stain drained and the smear is washed well under the gentle stream of running water for 2 minutes keeping the slide inclined.

5. Decolourisation and counter staining

   After washing the smear cover it well with Gabbot's methylene blue and leave it for 2 minutes after which time the stain is poured off and again washed with water.

   Dry between folds of blotting paper.

Rationale :

a) The strong carbol fuchsin contain basic fuchsin acts as the basic stain and carbolic cid is the Mordant.

b) The acidfast bacilli which possess a rich lipid material when subjected to heat liquefy and the organisms is further strengthened by the Mordant carbolic acid.

c) If the stain is not replenished each time it will dry up and form a scum over the smear thus spoils the whole preparation and the same happens when the stain boils.

Washing is an important procedure and a good preparation depends on it . A continuous stream of running tap water was h e s o f f t h e e x c e s s s t a i n o r a n y deposit over the smear.

Gabbot's methylene blue contains a deco-l o u r i s e r, 2 0 % s u l p h u r i c a c i d a n d a counterstain Loeffler's methylene blue. Since 20% sulphuric acid is a powerful decolouriser, all except the acid fast bactilli are decoulourised and take up the counter stain methylene blue.

Hence the name acid fast bacilli since these bacilli resist decolourisation with acid.

1. Place a drop of oil over the smear and examine by oil immersion objective. The acid fast bacilli appear as Red rods with the entire background appearing blue. If the given smear is that of sputum other cocci, bacilli and pus cells will appear blue.

2. For staining Mycobacterium leprae, instead of 20% sulphuric acid, 5% to 10% of sulphuric acid is used as a decolouriser.

   Tubercle bacillus is Acid Fast and Alcohol Fast. Leprae bacillus is Acid Fast but not Alcohol Fast.

Tuberculosis & Leprosy

Fixed smear made from the sputum of a case of pulmonary tuberculosis is given

1. Direct smear : Stained by Ziehl Neelsen's (Acid fast) methods Record your findings with suitable diagram

(1) What are acid fast bacilli? eg.

(2) Why are they acid fast ?

(3) What is the difference between staining of M. tuberculosis & M. leprae?

(4) What are atypical mycobacteria ?

(5) How do you identify them ?

(6) What are pathogenic AFB and what are saprophytic AFB?

(7) What are the other structures seen in sputum of AFB smear?

2. Culture : on Lowenstein Jensen Medium (LJ)
Record the colony characteristics

3. Smear from a colony from LJ medium stained by acid fast method is focussed Record your findings with suitable diagram

Slit skin smear from a suspected case of Hansen's disease stained by acid fast method focussed?

Record your microscopic finding with suitable diagrams

(1) What are the sites from which leprabacilli can be demonstrated?

(2) How are leprabacilli culture maintained ?

Ponder's Staining method for Metachromatic Granules of Corynebacterium diphtheriae

1. Heat fix the smear

2. Cover the smear well with the Ponder's stain and apply gentle heat. Do Not Boil. Once the steam appears withdraw the flame immediately.

3. Keep the stain for 10 Minutes.

4. Pour off the excess stain and wash with gentle stream of running tap water or distilled water

5. Air dry or dry between the folds of filter paper and examine with oil immersion objective

The Metachromatic granules present in the bacilli appear as purplish red dots, where as the bacilli appear Pale Blue. This method of staining with one dye showing different colour is known as methahromasia. Hence the granules are known as Metachromatic granules.

Composition of Ponder's Stain

Toludine blue	0.02 gram
Acetic Acid (glacial)	1 ml.
Alcohol (95 percent ethanol)	2 ml.
Distilled water	100 ml.

Albert's Method for Staining Metachromatic Granules

Albert's Stain :

Toludine blue	1.5 gm.
Malachite Green	2.0 gm
Glacial acetic acid	10 ml.
Alcohol (95 percent ethanol)	10 ml
Distilled water	1000ml

Dissolve the dyes in alcohol and add to the water and acetic acid. Allow to stand for one day and then filter.

Albert's lodine :

lodine	6 gm.
Potassium lodide	9 gm.
Distilled water	900 ml.

The lodine solution used in Jensen's modification of Gram's method works equally well.

Procedure :

1. Make film dry in air, and fix by heat
2. Cover, the slide with Albert's stain and allow to act for 3-5 minutes
3. Cover, the slide with Albert's lodine and allow to act for 1 minute
4. Wash and blot dry

By this method the granules stain bluish black, the protoplasm green and other organisms mostly light green.

DIPHTHERIA

Excercise No. (2)

Clinical History

1. Throat Swab from 5 yr old boy with white patches on tonsils is taken. Stain the smears from swab by Gram's and Albert's method view under microscope Record your findings

2. Swab was inoculated onto Blood Agar, Potassium tellurite agar and Loeffler's medium.

   Study the colony characteristics and record your findings.

3. Smears from a colony on Loeffler's medium is stained by Ponder's, Albert's and Gram's staining Methods to be focussed under the microscope & record your findings.

4) Relevant Biochemical tests are put up. Identify the organism

Section II
APPLIED MICROBIOLOGY

(1) ABSCESS

Clinical History

Pus obtained from a deep muscle abscess in thigh from a 5 year old boy. Smear from the pus stained by Gram's method and focussed under the microscope. Record your findings and give the morphological identification of the organism.

Direct Smear

Culture

Pus inoculated onto Blood Agar and MacConkey Agar, Describe the colony characteristics.

Smear from Colony

Smear from the colony is stained by Gram's method and focussed under microscope. Record your findings.

Relevant Identification tests and Anitbiogram are done . Record your findings and Identify the organism.

(2) PHARYNGITIS

Clinical History

Throat swab from a three year old boy with white patches on the pharyngeal wall is taken. Smear made from one of the two swabs was stained by Gram's method and focussed under Microscope. Observe and record your findings. The other swab was inoculated on the Blood Agar and Chocolate Agar.

Study the colony characteristics and record you findings.

Direct Smear

Culture

Relevant Identification tests are put up. Identify the organism and given the antibiotic sensitivity pattern.

(3) PNEUMONIA

Clinical History

Sputum from a 15 year old boy clinically diagnosed as having lobar pneumonia is bacteriologically investigated.

Direct Smear : stained by Gram's Method. Record your findings.

1. **Direct Smear**

2. Culture

Cultured on Blood Agar
Chocolate Agar
} Record the colony characteristics of Predominantly grown organism

3. Smear from colony on Blood agar stained by Gram's method and focussed under the microscope. Record your findings

4 . Relevant identification test and antibiotic sensitivity tests are demonstrated . Record your findings, identify the pathogen and record the sensitivity reports

Animal Inoculation

(4) BACTERIAL ENDOCARDITIS

Clinical History

Blood Culture is done in a patient diagnosed as having subacute bacterial endocarditis.

1. Name the liquid media used for collection of blood

2. Sub-cultures from Blood culture bottle medium onto Blood agar, chocolate agar and MacConkey's agar for aerobic growth are done

   Record the colony characteristics on these media

3. Smear from alpha haemolytic colonies stained by Gram's Method is focussed under Microscope. Record your findings.

4. Relevant identification tests and antibiotic sensitivity tests on the isolated organism are provided, Record the results. Identify the Organism, suggest the antibiotics which may be given to this patient.

(5) URINARY TRACT INFECTION

Case I Escherichia coli

Clinical History

A 25 year old female complaints of frequency of micturition, burning sensation on micturition and continuous fever of two days duration . Culture for urine is put up . Record the bacteriological characteristics and identify the isolated organism . Report the anitibiotic sensitivity.

1. **Direct Smear**

2. Culture

3. **Biochemical Tests**

4. Antibiogram :

(1) How is urine collected for culture ?

(2) What is colony count ? What is its importance ?

(3) What is meant by significant bacteriuria ?

(4) What are the common organisms that cause urinary tract infections ?

Case II Klebsiella

Clinical History

A 5 year old female child seen in the O.P.D. with continuous fever for 3 days duration. Patient complaints of lower abdominal pain . Culture for urine is put up . Record the bacteriological characters and identify the isolated organism . Report the antibiotic sensitivity.

Case III Proteus

Case IV Pseudomonas

Case V Enterococci

(6) ENTERIC FEVER

A twelve year old boy is admitted with complaints of intermittent fever for 5 days duration. Also complaints of headache, abdominal discomfort. Tongue is coated. Temparature is 101 ^{o} F, spleen is palpable. Blood culture is done for this patient. Clinically diagnosed as Enteric fever.

Inoculated Blood culture bottle is provided.

1. Name the liquid medium used for blood culture.

2. Name the medium generally used for isolation of enteric group of organisms.

Subculture from blood culture medium onto Blood Agar and MacConkey's Agar is done.

Record Colony characteristics on these media.

1. Any other special media used for culturing enteric group of organisms ?

Smear from the colony on MacConkey’s Agar Stained by Gram’s method and focus under microscope.

Record your findings.

Note : The organism is motile.

Relevant identification tests & antibiotic tests on the isolated organism are provided.

Record the results. Identify the organism, Suggest the antibiotics which may be given to this patient.

What further test will you do to confirm the identification of the organism?

What test for the same patient has been done on the 10th day of illness . Read and record the results and interpret the same.

1. How will you prove the carrier state of a person ?

C u l t u r a l a n d B i o c h e m i c a l c h a r a c t e r i s t i c s o f t h e f o llow i n g o r g a n i s m s a r e demonstrated. Record the reactions

i) Salmonella Paratyphi (A)

ii) Salmonella Paratyphi (B)

1. What are the different specimens to be collected for isolation and diagnosis of S. typhi and at what stage of the disease ?

2. Which of the salmonella produce food poisoning?

(7) ACUTE GASTRO ENTERITIS

Clinical History

A watery stool from 7 year old boy having acute gastroenteritis inoculated into MacConkey's Agar & Thiosulphate citrate Bile salt sucrose Agar. (TCBS)

Name the enrichment media used in this case.

How will you demonstrate the motility of the bacilli from a specimen ? What are the other media used for isolation of Vibrio cholerae ?

A smear from a colony on TCBS agar is stained by Gram's method and focussed under the microscope. Record your findings.

Biochemical reactions are put up . Identify the organism . What will you do to confirm the identification of the organism.

Note : The organism is motile.

How do you differenciate between classical and Eltor vibrios ?

What are NAG Vibrios ?

What are the pathogenic vibrios isolated from sea food ?

(8) BACILLARY DYSENTERY

Stool specimen from a 15 year old boy with acute dysentery. Microscopy shows pus cells, RBCs and a few epithelial cells. No amoebae seen.

Specimen inoculated onto MacConkey agar and Salmonella - Shigella agar.

1. Name the enrichment media used for the suspected organism in this case ?

Record the Colony characteristics on the solid media.

Smear from the pale colony on the MacConkey medium stained by Gram's method and focussed under microscope. Record your findings. Organism is non motile. Biochemical reactions slide agglutination and antibiotic sensitivity tests are demonstrated. Identify the organism and report the antibiotic sensitivity pattern.

What are the different species of shigella ?

(9) ANAEROBIC WOUND INFECTION

Excercise I Clostridium welchiior Clostridium perfringens

Clinical History

P u s c o l l e c t e d f r o m t h e m u s c l e t i s s u e o f t h e t h i g h i n a 2 2 y e a r o l d m a n w h o w a s involved in a road accident. Direct smear stained by Gram's method, focussed under the microscope.

Record your finding

1. What are the organisms suspected ?

2. What are the methods to grow anaerobic bacteria ?

Inoculated onto 2 Blood agar plates. MacConkey's agar and Robertson's cooked meat medium. One BA plate is incubated aerobically and the other anaerobically.

Smear from colony on BA incubated anaerobically is made, stained by Gram's method and focussed under microscope. Record your findings with suitable diagram.

What are the relevant Identification tests to be done to identify the organism ?

Excercise II Clostridium tetani

Clinical History

A swab obtained from a patient with a stab wound has been inoculated into blood Agar, thioglycollate & RCM. Incubated anaerobically.

Record your findings

Smear from RCM and thioglycollate has been made after 5 days of incubation and stained with Gram's method. Focus under microscope and record your findings?

What are the different stains for staining spores ?

What are the relevant identification tests to be done ?

(1) How do you detect that the Isolated organism is toxigenic ?

(2) What is the prophylaxis for Tetanus ?

(3) What are the small gram negative non-sporing anaerobic bacteria ?

(10) MENINGITIS

Clinical History

Cerebrospinal fluid obtained from a two year old child clinically diagnosed as having acute meningitis.

D i r e c t S m e a r s t a i n e d b y G r a m ' s m e t h o d i s f o c u s s e d u n d e r m i c r o s c o p e . M a k e a suitable diagram and record your findings.

(1) Waht are the commom organisms that cause meningitis in children?

(2) What are the viruses and fungi that cause meningitis ?

(3) What are the specimens in addition to CSF to be collected in a case of meningitis ?

i) Collection of C.S.F. & Processing

ii) Rapid diagnostic methods for meningitis

Hemophilus influenzae

Pneumococcus

Meningococcus

Streptococcus

(11) SEXUALLY TRANSMITTED DISEASES

A 35 yr. old male attended OPD with complaint of purulent urethral discharge for 3 days.

The purulent material was collected. Smear was made. Gram's staining was done and focussed under the microscope. Record your findings.

(1) Name the sexually transmitted diseases.

(2) Waht are the sites from which specimen can be collected to identify the organism in gonorrhoea?

Specimen was investigated bacteriologically. Name the media used for isolation of Neisseria gonorrhoeae from urethral discharge. Record the colony characteristics.

Smear from transparent colony is stained by Gram's technique, focussed under microscope. Record your findings.

What are the relevant identification tests to be done to identify the organism ?

(12) PYREXIA OF UNKNOWN ORIGIN (PUO)

Section III
DIAGNOSTIC VIROLOGY (General Principles)

ACUTE VIRAL RESPIRATORY INFECTIONS

1. (a) How do you diagnose viral infections ?

(b) What are the different methods of cultivation of viruses ?

2. Which are the Viruses transmitted through Blood transfusion ?

3. Which are the Viruses that are Congenitally transmitted?

4. Which are the Viruses that causes acute respiratory infection ?

5. Which are the Viruses that affect the nervous system ?

6. Which are the Viruses that cause acute diarrhoea ?

7. Which are the Viruses that are sexually transmitted?

8. What are the oncogenic viruses ?

9. Name some viral vaccines ?

10. Name the Antiviral agents

Section IV
MYCOLOGY

MYCOLOGICAL TECHNIQUES

1. KOH (Potassium Hydroxide) Preparation

Principle :

Fungal materials in tissues cannot be visualised, unless treated with clearing agents. The clearing agent digests the tissue material and makes the fungal material visible.

The commonly used clearing agent is KOH (Potassium Hydroxide) in the concentration of 10% for materials like skin-scrapings, hair teased tissue and sputum. Some times 20% concentration is used for hard materials like nail scraping, bone etc.

Ingredients	10%	20%
Potassium hydroxide	10 gm	20 gm
Dist. water	80 ml	20 ml
Glycerol	20 ml	20 ml

(Glycerol prevents crystallisation occuring in the solution)

Procedure :

1. Place a drop KOH in the centre of a clean glass slide

2. Place a fragment of tissue in KOH with teasing needles. Tease the material well enough to give a thin preparation

3. Mount with a cover slip and gently heat the preparation by passing over Bunsen burner 2 to 3 times. DO NOT BOIL

(or)

Alternatively allow the preparation to stay 15 to 20 minutes for clearing before examination

4. Focus under dry lens low power and then in high power.

(2) Lactophenol Cotton Blue (LPCB) Mounting

Principles :

Filamentous Fungi and spores do not take up Grams stain. The LPCB Mounting, is used to study the fungal cultures particularly from slide cultures. The filaments and spores are stained blue.

Ingresients :

Phenol crystal	20 gm	mordanting agent / fungicidal
Lactic acid	20 ml.	preserves morphology
Glycerol	40 ml.	Prevents drying
Cotton blue	0.05 gm.	Stain
Dist. water	20 ml.	

Procedure :

1. Place a drop of LPCB mounting fluid on a clean glass slide.
2. Using sterile techniques, remove a small portion of fungus growth with bent nichrome wire.
3. Place the fungal material in LPCB drop
4. Using 2 sterile teasing needle, tease the material to give a thin preparations
5. Place the cover slip over the preparation
6. Examine under low power objective and then under high power objective.

(3) Slide Culture Techniques

Principle :

This technique facilitates the microscopic observation of the fungi in natural state for identification.

Procedure :

1. Place a U-shaped glass rod, 2 glass slide & 2 cover slips, in a Petridish, sterilize and keep it ready for use.
2. Prepare Sabouraud's Dextrose Agar in a Petridish (Corn meal Agar (or) Potato dextrose Agar may also be used)
3. Cut agar block of 1cm square and 2-3 mm deep from the agar plate.
4. Place the block of agar, using sterile techniques on a sterile slide in the already sterilised Petridish.
5. Pick out small piece of colony to be tested, using a straight nichrome wire.
6. Inoculate centre and four sides of agar block with the fungal material
7. Cover the inoculated agar block with sterile cover slip.
8. With sterile technique, add 8 ml of sterile distilled water to which 10% glycerine has been added to the bottom of petridish to use the set up as a moist chamber
9. Incubate at 25^{o}C (B.O.D.) for 18 hours to few days
10. When spores appear carefully lift off the coverslip (and lay the coverslip with fungus growth upward)
11. Lift agar block from the slide and discard in Sodium hypochlorite solution.
12. Place a drop of LPCB on the slide and cover with a clean coverslip.
13. Obtain clean slide, place a drop of LPCB and cover with coverslip with mycelial surface down.
14. Blot away excess mounting fluid from coverslips of the two preparation
15. Seal the edges with nail polish.
16. Examine under low power and high power dry objective to delineate the morphology of the fungus with spores in situ.

Mycological Techniques

1. **Stains**

i. KOH Preparation

ii. Gram's Stain

iii. Acid Fast Stain

iv. Lactophenol Cotton Blue

v. India ink

vi. Tissue Stains - PAS, methanamine silver stain.

2. **Media**

3. Slide Culture Technique

4. Opportunistic Fungi

(i) **Aspergillus flavus**

(ii) Aspergillus fumigatus

(iii) **Aspergillus niger**

(iv) Penicillium species

(v) **Mucor**

(vi) Rhizopus

(vii) **Candida**

(viii) Cryptococcus

5. Dermatophytes

6. **Mycetoma**

7. Rhinosporidium

8. Antifungal Agents

9. **Use of fungi to mankind**

10. What are mycotoxins ?

Section V
ENVIRONMENTAL MICROBIOLOGY

Parasitology

Although medically significant micro organisms are considered parastic, traditionally, Parasitology has been concerned with protozoans, Helminths and arthropods.

Protozoology

Protozoa meaning “first animal” - are eukaryotic protista; occur as single cells. A typical protozoan cell is enclosed by a cytoplasmic membrane with many having a well differentiated ectoplasm and endoplasm which contains most structures. Every cell has atleast one nucleus; many species, however, have multiple nuclei through out the greater part of their life cycle. Many protozoans form cysts which is essentially a protective form for the trophozoites against adverse conditions . Protozoans reproduce by a variety of sexual and asexual processes. Some have complex
r e p r o d u c t i v e c y c l e s i n w h i c h o n e p a r t o f t h e l i f e c y c l e i n V e r t e b r a t e h o s t s a n d t h e o t h e r p a r t i n s o m e o t h e r h o s t s e g . i n s e c t s .

The phylum protozoa is classified into four major classes based on the type of locomotion.

1. Sarcodina - typically amoeboid
2. Mastigophora - flagellates
3. Sporozoa-No external locomotor appendages. Show

   gliding movements and / or non-motile.

   Have a complex Sexual and asexual and reproductive phase.
4. Ciliophora-Ciliates.

Normal Constituents of Faeces

Practical: Make a saline and iodine preparations of faeces on a slide. Demonstrate microscopically the cysts of amoeba. Draw the morphological characteristics of the cysts.

Demonstration : Examine the microscopic preparation provided - identify the same illustrating the typical characters. Draw the morphological characters.

Intestinal or Faecal Parasites

Faecal specimens are best collected in water tight cardboard containers. Specimen should not be mixed with urine, oil or any chemical as the morphology of vegetative forms is likely to be altered. Consistency, colour and nature of faeces should be recorded.

I. Macroscopic Examination :

1. Colour-Black, Brown or Yellow
2. Consistency-Hard, formed, loose or watery
3. Nature-Fibrous, Colloidal, Mucoid or Blood tinged.

II.Microscopic Examination :

(a) **Saline wet mount procedure :**

1. Place 1-2 drops of physiological saline on a slide.
2. With an applicator stick, select 1-2 mg of the given sample (1mg=1mm^3) if mucous flakes are present they must be included in the preparation.
3. Make a homogeneous suspension of the faecal matter; remove coarse fibres, seeds, etc.
4. Cover with 22x22 mm coverslip. A proper smear is said to be made if a newsprint kept under the slide is just visible.
5. Examine the preparation under microscope, first under low power and when any abnormality is seen, it is examined under high power for final identification.

(b) **Iodine wet mount preparation - procedure :**

Procedure is same as above, except instead of saline, Lugol's lodine is used.

Protozoology

Q List out the names of amoebae that are parasitic to man

Q : List in a tabular form the differentiating features of trophozoite Entamoeba histolytica and Entamoeba coli.

Q : List the intestinal and genital flagellates causing infections in man

1. Name the stages seen in Entamoeba histolytica

2. How does amoebae move ?

3. Differentiate between the cysts of E. histolytica and E. coli

4. After a swim a person complaints of sudden severe headache? What will you suspect and what will be the causative agent ?

5. What are the lesions seen in intestinal amoebiasis ?

Haemoflagellates

Describe the morphology of haemoflagellates and draw suitable diagrams

Haemoflagellates

1. Name the trypanosomes and the diseases caused in man.

2. Give reasons for lowered resistance in a person suffering from African trypanosomiasis.

3. Name the definitive and intermediate hosts of T: brucei

4. Name the infective stage of T.brucei

5. Name the transmitting agents of T.brucei (rhodesiense)

6. List the specimens to be collected for examination in a suspected case of trypanosomiasis

7. Name the habitat of T. cruzi

8. Name the transmitting agent of T. cruzi

9. Name three human pathogens of the Genus Leishmania and the diseases caused by them.

10. Classify Leishmaniasis on clinical grounds

11. Name the vector and definitive host in case of Leishmania donovani

12. Name the medium used for the cultivation of L.donovani

13. Name the vector that transmit L. donovani in India

14. List the serological tests for kala-azar

Leishmanstain

JSP stain

Malaria

Demonstration: Microscopicpreparationsareprovided.Describe the morphological features of malarial parasites seen . Draw diagrams . Identify the morophological stage of the parasite.

Malarial Parasites

Questions :

1. Which are the intermediate and definitive host of the malarial parasite?

2. Name the stages of human cycle of Malarial parasites.

3. Are parasites found in the peripheral blood in pre erythrocyte schizogony ?

4. There is no relapse in P. falciparum infection. Why ?

5. How do P. vivax and P. falciparum differ in their affinity towards RBC ?

6. Differentiate between the ring stages of P.vivax and P. falciparum

7. What are all the changes seen in peripheral blood ?

8. Tabulate the changes in the RBC in P. vivax and P. falciparum infection.

9. What are the stages of the parasite seen in the peripheral blood in case of P. falciparum infection.

10. Diagrammatically represent the gametocytes of P. falciparum.

The Trematodes

The Trematodes or flukes constitute one class of Phylum platyhelminthes. Adullt trematodes are parasites of vertebrates, dorsoventrally flattened . They are hermaphrodites except schistosomes. Common flukes causing infection in man are:

Intestinal flukes :

Fasciolopsis buski

Liver flukes :

Clonorchis sinensis

Ophistorchis felineus

Fasciola hepatica

Blood flukes :

Schistosoma haematobium

Schistosoma mansoni

Schistosoma japonicum

Lung flukes :

Paragonimus westermani

Demonstration :

Draw suitable diagrams and label the parts.

Trematodes

Questions :

1. Which is the definitive and intermediate host of Liver fluke?

2. Name the larval stage which is infective.

3. Where do adult worms live ?

4. What is the name of the larvae escaping from the snail ?

5. What is ‘metacercaria ?

6. What is schistosomule ?

7. What is the name of the lung fluke and how is it diagnosed?

8. Where do S. haematobium and S. japonicum develop in the body ?

9. How will you differentiate the egg of S. japonicum, S. mansoni&S. haematobium?

10. What is Swimmer’s itch ?

11. What is “Pipe-Stem fibrosis?

Cestodes

Cestodes or tapeworms constitute a class of the phylum Platyhelminthes.The adult tapeworms have body consisting of an anterior attachment organ called scolex followed by a chain of proglottids also know as strobila and inhabits the small intestine of man and animals. The cestodes commonly infecting man are:

Diphyllobothrium latum

Taenia saginata

Taenia solium

Hymenolepis nana

Hymenolepis diminuta

} Adult worms in Intestine

Echinococcus granulosus

Echinococcus multilocularis

} Larval forms in organs

Demonstration : Specimens and microscopic preparations are demonstrated; Write down your findings, draw suitable diagrams and label the parts.

Cestodes

Questions :

1. Tabulate the differences between Cestodes and Trematodes.

2. Tabulate the morphological differences between T.solium and T.saginata

3. How does the scolex of D. latum appear?

4. In which tapeworm the egg is operculated?

5. Draw the diagram of Dog tapeworm (E.granulosus)

5. What is hydatid cyst ?

6. What is hydatid sand ?

7. In which tapeworm infection multilocular cysts are seen ?

8. What is ‘sparganosis’?

9. What is ‘Cysticercus cellulosae’?

10. What is ‘Cysticercus bovis’ ?

Nematodes

Nematodes, belonging to the Phylum Nemathelminthes are non-segmented generally cylindrical in shape, tapering at both ends covered by a cuticle, sexes are separate, have complete digestive system with both oral and anal opening . Males are generally smaller than females. Common nematodes causing human infection are:

Intestinal nematodes

Ascaris lumbricoides

Enterobius vermicularis

Ancylostoma duodenale

Necator americanus

Trichuris trichiura

Strongyloides stercoralis

Blood and tissue nematodes :

Wuchereria bancrofti

Brugia malayi

Brugia timori

Loa loa

Mansonella ozzardi

Onchocerca volvulus

Dracunculus medinensis

Trichinella spiralis

In addition several other nematodes which cause cutaneous and visceral larva migrans.

Practical : Prepare wet microscopic preparation of faeces provided, examine under the microscope. Describe the parasitic ova, make diagrams and identify them.

Demonstration : Specimen and microscopic preparation are provided. Describe th suitable diagrams and identify them.

Nematodes

Questions :

1. Name the optimum and alternative hosts of Trichinella spiralis

2. Differentiate between filariform and rhabditiform larvae.

3. Differentiate between male and female worms of Trichuris trichiura?

4. What are the diagnostic measures of trichinellasis ?

5. Name the infective stage, portal of entry and site of localization of Strongyloides stercoralis.

6. List the difference between male and female worms of Ancylostoma duodenale.

7. What are the major differences between male and female Ascaris ?

8. What is ‘Cutaneous larva migrans’ and Visceral larva migrans ?

9. Explain the pathologenesis of tropical eosinophilia in filariasis.

10. What is ‘D.E.C. Provocative test ?