PART 1

General Microbiology

1.1 Microscopy

LEARNING OBJECTIVES

At the end of the tutorial and practical, the student will be able to:

- List the different methods of microscopy to demonstrate microbes along with respective principles.
- Enumerate the applications of the different microscopes with specific reference to diagnostic microbiology.
- Handle the compound light microscope appropriately.
- Mention the use of various parts and different objectives.
- Explain the following terms: Resolving power of different types of microscopes.

INTRODUCTION

Microscope is an instrument which is used to see small invisible objects. The microscope suitable for use in microbiology laboratory is a binocular compound microscope.

STRUCTURE OF THE MICROSCOPE

Microscope has a heavy stand which is horse-shoe shaped. To it is attached the limb which bears the optical system. The optical system is mounted in the tube. The eyepiece lens which is fitted at the upper end of the tube has a magnification of 10×. The lower end of the tube bears a revolving nose-piece in which three objective lenses are fitted. Length of the tube can be altered by coarse and fine adjustment screws. Coarse adjustment screw is used to focus the object (smear) and the fine adjustment screw is used for accurate focussing.

Microscope has a platform or a stage for keeping object (slide). The slide can be moved from side to side and forwards or backwards. In the centre of the stage there is an aperture through which objects are illuminated from below. Light going to the object can be controlled with the help of a condenser and an iris diaphragm attached below the stage. Below the condenser is a mirror with one surface plane and the other concave (Fig. 1.1.1).

Bacteria of medical importance measures 2–5 µm × 0.2 × 1.5 µm.

The resolving power of unaided eye is about 200 microns.

The resolving power of an ordinary light microscope is approx. 0.2 microns.

Resolving power (R) of a microscope is the ability of the lens system to distinguish two neighbouring objects as separate.

$$R = \frac{0.61 \times \text{wave length of the light used}}{\text{Numerical aperture}}$$

Numerical aperture is defined as the light gathering power of microscope.

Resolution can be adjusted by proper use of condenser and can be further increased by using special medium through which light passes between the object and objective lens. Immersion oil used in 100× objective has a refractive index similar to glass and thus, permit more light which helps in improving the resolving power.

Fig. 1.1.1: Various parts of the microscope

Magnification

Total magnification of the microscope = objective magnification × eyepiece magnification

10 (low power objective) × 10 = 100

40 (high power objective) × 10 = 400

100 (oil immersion objective) × 10 = 1000

Use of different magnifications:

Unstained preparation examined for bacterial motility (hanging drop preparation) with condenser right down — 40×.

Unstained saline preparation used for demonstration of parasitic ova are first screened under – 10× with condenser right down and then changing to 40×.

Stained smears are seen under — 100× with condenser kept in the top position.

CARE OF THE MICROSCOPE

1. Do not tilt the stage of the microscope when wet preparations are examined.
2. After use, oil immersion lens and stage should be properly cleaned with gauze piece.
3. When not in use, it must be protected from dust and covered with a plastic transparent cover.

DEMONSTRATION

- Light microscope and its principle, structure and uses.
- Use, handling and care of the microscope.
- Focusing slides under various objectives and interpreting the same.
- Measurement of objects (relative to size of standard cells like RBC).

QUESTIONNAIRE

1. Define

a. Resolving power:

b. Numerical aperture:

c. Magnification of a microscope:

2. Enumerate different types of microscopes with its uses

3. Draw a well labelled diagram of microscope

Assessment (Based on the Kirkpatrick 4-level model of evaluation)

Sl. No.	*Level*	*Student's performance*	*Score#*			
1.	**Reaction**	Student's prior knowledge about the topic	1	2	3	4
2.	**Learning**	Students level of attention and participation in discussion during SGT session	1	2	3	4
3.	**Behaviour**	Level of professional code of conduct maintained by the students during the teaching–learning session	1	2	3	4
4.	**Results**	Time taken for record book completion activities	1	2	3	4
5.		Able to show evidence of learning the new skills (intellectual/psychomotor)	1	2	3	4
Total score			**/20**			

#**Score 1:** Poor, **2:** Average, **3:** Good, **4:** Very good	**Faculty Remarks/Feedback:**
	Date: **Faculty** **Name and Signature**

Competency achieved	**MI1.1:** Describe the different causative agents of infectious diseases, the methods used in their detection, and discuss the role of microbes in health and disease.

Notes/Reflection

1.2 Instruments (Sterilization and Disinfection)

LEARNING OBJECTIVES

At the end of the tutorial and practical, the student will be able to:

- Define the terms:
  - Sterilization
  - Antiseptics
  - Disinfection
  - Decontamination
- Describe various agents and equipment used in sterilization.
- Mention the concentration and contact time for commonly used disinfectants.
- Choose the most appropriate method of sterilization/disinfection for commonly used articles in the hospital and microbiological labs.
- Enumerate the applications of fumigation.
- Enumerate the uses and types of bacterial filters.
- Give the mechanism of action of chemical agents used in antiseptics and disinfectants.

INTRODUCTION

- Sterilization is defined as a process by which an article, surface or medium is freed of all living microorganisms either in the vegetative or spore state.
- Disinfection is the killing, inhibition or removal of microorganisms that may cause disease.
- Antiseptics are chemical agents applied to tissue to prevent infection by killing or inhibiting pathogen growth; they also reduce the total microbial population.
- *Decontamination*: It refers to the reduction of pathogenic microbial population to a level at which items are considered as safe to handle without attire.

DEMONSTRATION

- Autoclave and its use (Fig. 1.2.1).
- Hot air oven and its use (Fig. 1.2.2).
- Sietz filter and its use
- Working of inspissator and biosafety cabinet (Fig. 1.2.3).
- Familiarize the students with the various disinfectants available in the hospital.

Fig. 1.2.1: Autoclave

Fig. 1.2.2: Hot air oven

Fig. 1.2.3: Biosafety cabinet

QUESTIONNAIRE

1. Enumerate the different methods of sterilization by dry heat and moist heat

2. Give the temperature and time of the following methods

a. Autoclave:

b. Hot air oven:

c. Inspissator:

d. Vaccine bath:

e. Steam sterilizer:

3. Write the method of sterilization of the following

a. Glassware:

b. Media:

c. Apron:

d. Endoscope:

e. Gloves:

f. Inoculating loop:

g. Sharp OT instruments:

h. Disposable syringes:

4. Write down the concentration of the following used with its purpose

a. Bleaching powder:

b. Sodium hypochlorite:

c. Ethyl alcohol:

Assessment (Based on the Kirkpatrick 4-level model of evaluation)

Sl. No.	*Level*	*Student's performance*	*Score#*			
1.	**Reaction**	Student's prior knowledge about the topic	1	2	3	4
2.	**Learning**	Students level of attention and participation in discussion during SGT session	1	2	3	4
3.	**Behaviour**	Level of professional code of conduct maintained by the students during the teaching–learning session	1	2	3	4
4.	**Results**	Time taken for record book completion activities	1	2	3	4
5.		Able to show evidence of learning the new skills (intellectual/psychomotor)	1	2	3	4
Total score			**/20**			

#**Score 1:** Poor, **2:** Average, **3:** Good, **4:** Very good	**Faculty Remarks/Feedback:**
	Date: **Faculty** **Name and Signature**

Competency achieved	**MI1.4:** Classify and describe the different methods of sterilization and disinfection. Discuss the application of the different methods in the laboratory, in clinical and surgical practice. **MI1.5:** Choose the most appropriate method of sterilization and disinfection to be used in specific situations in the laboratory, in clinical and surgical practice.

Notes/Reflection

1.3 Morphology of Bacteria and Gram Stain

LEARNING OBJECTIVES

At the end of the tutorial and practical, the student will be able to:

- Mention two important differential staining techniques.
- Identify microscopically the different types of cells in a given stained specimen.
- Identify correctly the morphology of bacteria after staining.
- Give two examples each of the different morphological forms of bacteria.
- Enumerate the methods for demonstrating spores, capsule, flagella.

INTRODUCTION

- Bacteria can be defined briefly as minute microorganisms with a prokaryotic form of cellular organization. They usually measure 0.4 – 1.5 µm having rigid cell walls which cover very closely cytoplasmic membrane and are responsible for maintenance of their shape. The shape may be spherical (coccus), rod shaped (bacillus), comma-shaped (vibrio), spiral (Spirillum and *Spirochaetes*), filamentous (*Actinomycetes*).
- **Staining of bacteria:** Staining is of primary importance for the recognition of bacteria because of its ability to absorb more light and make the bacteria quite distinct.
- **Preparation of smear (done from fresh samples/culture isolated):** On a clean, grease-free slide the smear is prepared in normal saline with the help of loop or swab. The smear is fixed by passing 3–4 times over the burner flame. The fixation kills the vegetative and renders them permeable to stain, prevent autolytic changes and make them physically stick to the surface of the slide.

Gram Stain

Purpose:

a. To differentiate between Gram-positive and Gram-negative bacteria (Figs 1.3.1 to 1.3.4).
b. To study the bacterial morphology (cocci/bacilli)
c. Presumptive diagnosis

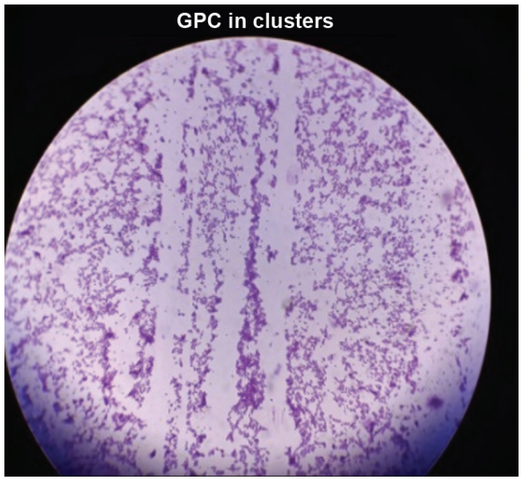


Fig. 1.3.1: Gram-positive cocci

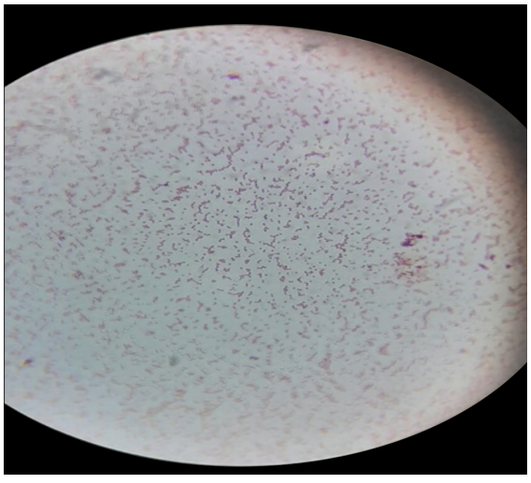


Fig. 1.3.2: Gram-negative cocci

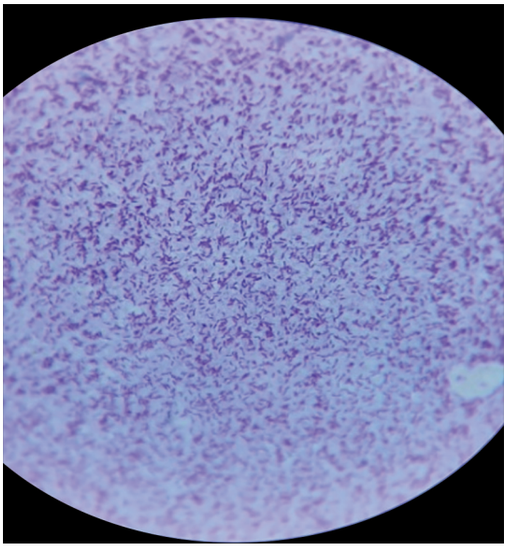


Fig. 1.3.3: Gram-positive bacilli

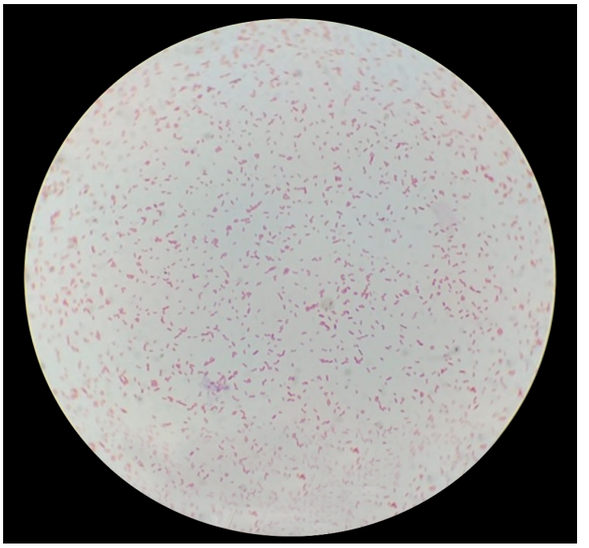


Fig. 1.3.4: Gram-negative bacilli

Principle: Gram-positive bacteria are stained with certain aniline dyes such as crystal violet and are subsequently treated with iodine which acts as a mordant forming a dye-iodine complex. They resist subsequent decolourization treatment with acetone and are seen as violet colour. Gram-negative bacteria, however, after similar treatments are readily decolourized by acetone to become colourless and they take up the colour of counterstain (safranin) to become pink.

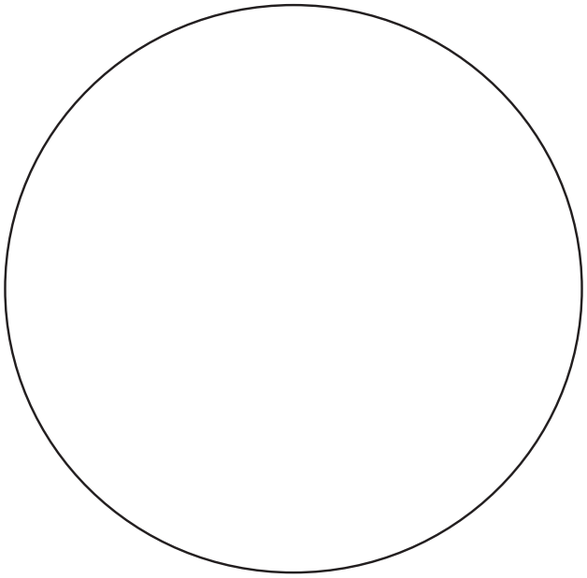
DEMONSTRATION

- Smear from pus showing pus cells and Gram-positive cocci in clusters.
- Smear of urine showing pus cells and Gram-negative bacilli from a case of UTI.
- Smear from a case of gonococcal pus showing Gram-negative diplococci.
- Smear from sputum showing pus cells and Gram-positive cocci in pairs.
- A smear showing comma-shaped organism.
- Albert's stain of diphtheria bacilli showing metachromatic granules.

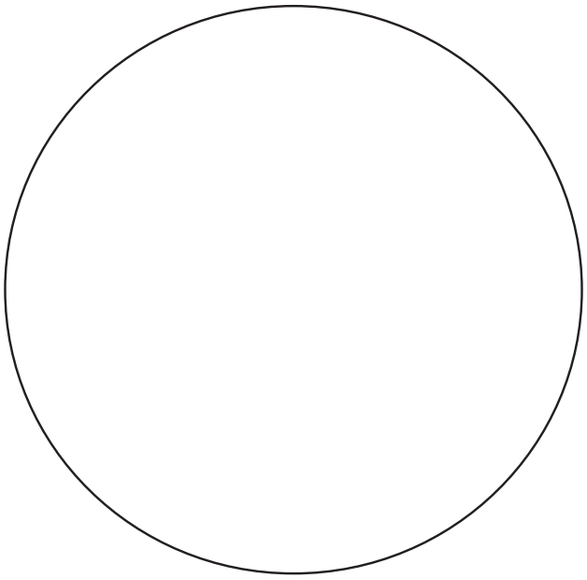
QUESTIONNAIRE

Note: **Please draw your findings in the space provided and label appropriately.**

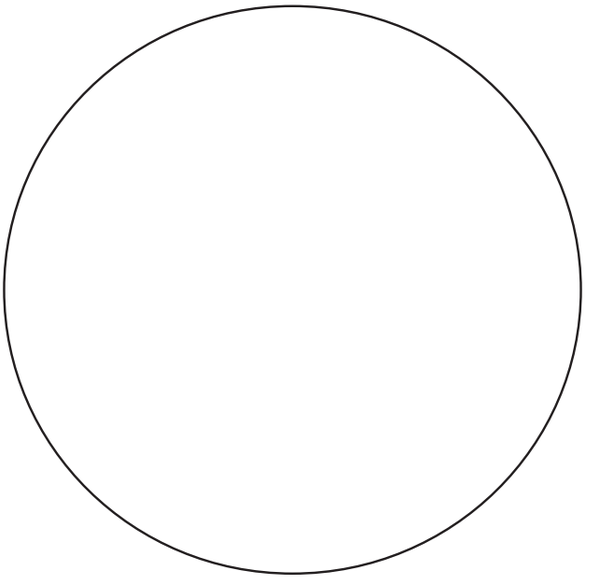
1. **Stain the given smear by Gram's technique and record your finding with the suitable diagram.**



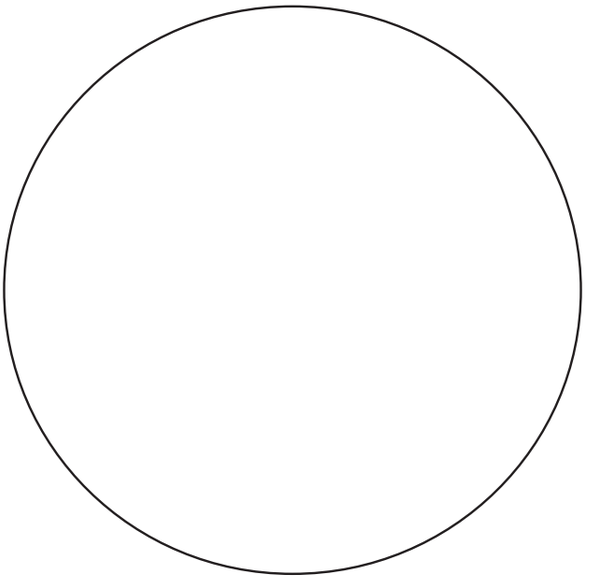
2. **Gram stained smear of pus.**



3. Gram stained smear of sputum having history of pneumonia.



4. Gram stained smear from throat swab having history of follicular tonsillitis.



Checklist for the Gram stain—OSPE Station (Sample)

S. No.	*Steps of the procedure*	*Marks allotted*	*Marks achieved*
1.	Student picks up the slide and confirms the side of slide on which the smear is on		
2.	Put the primary stain (crystal violet/methyl violet/gentian violet) on the smear area for 1 min		
3.	Washes the smear with tap/distilled water		
4.	Put the mordant (Gram's Iodine) on the smear area for 1 min		
5.	Washes the smear with tap/distilled water		
6.	Decolorises the smear with ethyl alcohol for 20–30 sec or with acetone 2–3 sec or acetone alcohol for 10 sec		
7.	Washes the smear with tap/distilled water		
8.	Puts safranin/dilute carbol fuchsin on the smear for 30 sec		
9.	Washes the smear with tap/distilled water		
10.	Air dries the smear and visualizes the smear under oil immersion microscope		

Assessment (Based on the Kirkpatrick 4-level model of evaluation)

Sl. No.	*Level*	*Student's performance*	*Score#*			
1.	**Reaction**	Student's prior knowledge about the topic	1	2	3	4
2.	**Learning**	Students level of attention and participation in discussion during SGT session	1	2	3	4
3.	**Behaviour**	Level of professional code of conduct maintained by the students during the teaching–learning session	1	2	3	4
4.	**Results**	Time taken for record book completion activities	1	2	3	4
5.		Able to show evidence of learning the new skills (intellectual/psychomotor)	1	2	3	4
Total score			**/20**			

#**Score 1:** Poor, **2:** Average, **3:** Good, **4:** Very good	**Faculty Remarks/Feedback:**
	Date: **Faculty** **Name and Signature**

Competency achieved	**MI1.1:** Describe the different causative agents of Infectious diseases +A208, the methods used in their detection, and discuss the role of microbes in health and disease. **MI1.2:** Perform and identify the different causative agents of Infectious diseases by Gram stain, ZN stain and stool routine microscopy. **MI6.2:** Identify the common etiologic agents of upper respiratory tract infections (Gram stain). **MI6.3:** Identify the common etiologic agents of lower respiratory tract infections (Gram stain and acid fast stain.)

Skill Certification

MI1.1: Describe the different causative agents of Infectious diseases+A208, the methods used in their detection, and discuss the role of microbes in health and disease.
MI1.2: Perform and identify the different causative agents of Infectious diseases by Gram stain, ZN stain and stool routine microscopy.
MI6.2: Identify the common etiologic agents of upper respiratory tract infections (Gram stain).
MI6.3: Identify the common etiologic agents of lower respiratory tract infections (Gram stain and Acid fast stain).

Student's performance	***Max. marks (05)***	***Marks scored***
Performs skill by following all the steps correctly	04	
Focusses the stained slide appropriately	02	
Identifies the structures correctly and interprets.	02	
Draws colored labelled diagram of the microscopic field and writes the report	02	
	Score	
Rating as per CBME Below expectations (B) (score: 1–4) Meets expectations (M) (score: 5–8)* Exceeds expectation (E) (score: 8–10)	**Rating**	
Certification (**Students should secure 'M' or 'E' to be able to get certification in a given skill*)	**NO**	**YES**

Date: **Faculty Name and Signature**

Notes/Reflection

1.4 Culture Media

LEARNING OBJECTIVES

At the end of the tutorial and practical, the student will be able to:

- Enumerate the characteristics of ideal culture medium.
- List the growth requirements of bacteria.
- Explain the terms:
  a. Isolation
  b. Inoculation
  c. Incubation
  d. Nutrient medium
  e. Colony
- Classify various types of culture media based on their nutritional factors.
- Enumerate the methods used for isolating bacteria in pure culture.

INTRODUCTION

- The process of growing organisms helps in obtaining organisms in pure form (isolation) and in undertaking further testing to confirm their identity. The food material on which the organism is grown is known as culture medium (Fig. 1.4.1) and the growth of organism is known as culture.

Fig. 1.4.1: Different culture media

DEMONSTRATION

- Uninoculated media: (a) Nutrient agar, (b) blood agar and (c) MacConkey agar
- Mueller Hinton agar: (a) Chocolate agar, (b) TCBS, (c) triple sugar iron agar, (d) RCM and (e) LJ media
- Alkaline peptone water: (a) Selenite F broth
- Inoculated media:
  - Pigmented colonies of *Staphylococcus* sp. on nutrient agar
  - MacConkey agar showing LF and NLF colonies
  - Blood agar showing alpha and beta hemolysis
  - TSI medium showing various reactions.

QUESTIONNAIRE

1. Define the terms:

a. Simple medium:

b. Enriched medium:

c. Enrichment medium:

d. Selective medium:

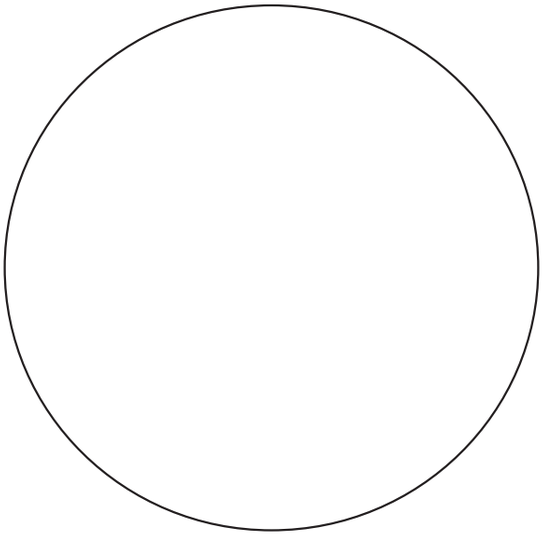
e. Differential medium:

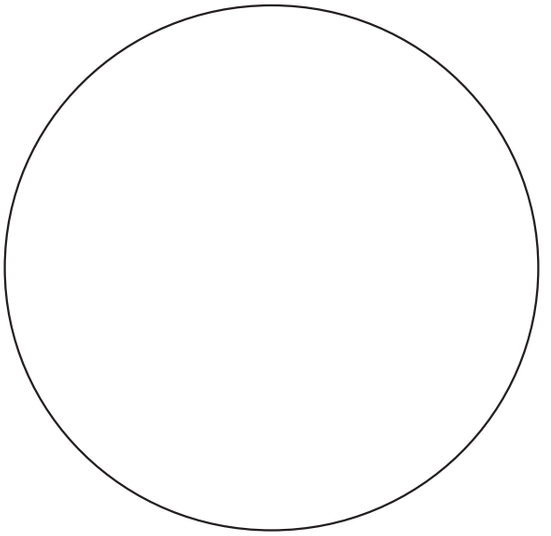
f. Transport medium:

2. Draw the following media with appropriate colours

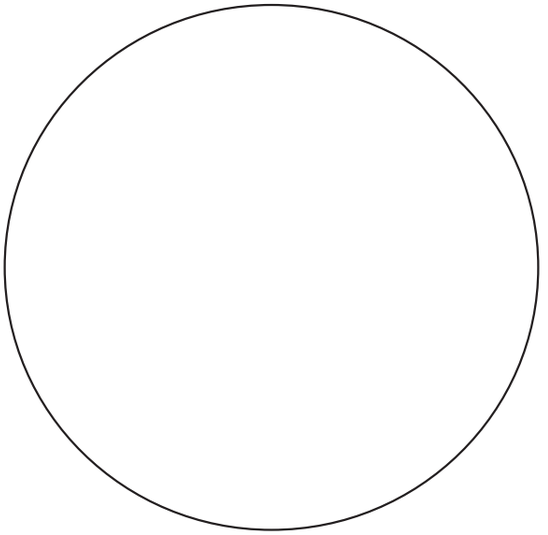
a. Chocolate agar

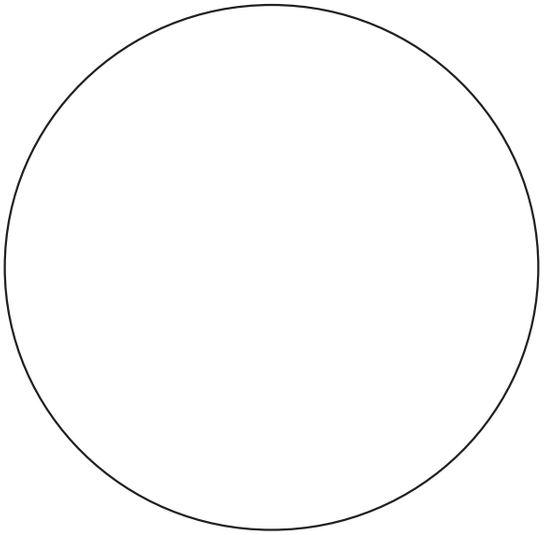
b. Blood agar





c. MacConkey agar with LF and NLF colonies.





3. Describe the contents with labelled diagram of the following media:

a. RCM

b. TSI

Assessment (Based on the Kirkpatrick 4-level model of evaluation)

Sl. No.	*Level*	*Student's performance*	*Score*[#]			
1.	**Reaction**	Student's prior knowledge about the topic	1	2	3	4
2.	**Learning**	Students level of attention and participation in discussion during SGT session	1	2	3	4
3.	**Behaviour**	Level of professional code of conduct maintained by the students during the teaching–learning session	1	2	3	4
4.	**Results**	Time taken for record book completion activities	1	2	3	4
5.		Able to show evidence of learning the new skills (intellectual/psychomotor)	1	2	3	4
Total score			**/20**			

[#]**Score 1:** Poor, **2:** Average, **3:** Good, **4:** Very good	**Faculty Remarks/Feedback:**
	Date: **Faculty Name and Signature**

Competency achieved	**MI1.1:** Describe the different causative agents of infectious diseases, the methods used in their detection, and discuss the role of microbes in health and disease.

Notes/Reflection

1.5 Culture Methods

LEARNING OBJECTIVES

At the end of the tutorial and practical, the student will be able to:

- Enumerate the Indications for culture of bacteria.
- Define Inoculation of culture media
- Enumerate the type of culture methods
- Define the terms: Aerophilic, anaerobic and microaerophilic incubation.
- Explain aerobic culture methods.
- List automated culture methods.
- Differentiate laminar air flow cabinet and biosafety cabinets with their indications.

INTRODUCTION

- The methods used for growing microorganisms are known as culture methods. Different culture methods are available for culture of bacteria based on the purpose for which they are intended.
- Inoculation is seeding a medium with a specimen so as to obtain growth of bacteria present in that specimen.
- Streaking is the commonest method of culture of a clinical sample wherein a loopful of sample is applied and spread on the surface of a solid medium with the help of a loop. The idea is to obtain growth of separate colonies of individual bacteria present in that sample after incubation on the surface of that media at necessary temperature and after needed time.

DEMONSTRATION

- Structure and use of:
  a. Incubator
  b. Gas-pak system
  c. McIntosh and Fieldes' jar
  d. Candle jar and its principle

QUESTIONNAIRE

1. Draw well labelled diagram of McIntosh and Fieldes' jar.

2. Enumerate the methods of obtaining pure culture.

Assessment (Based on the Kirkpatrick 4-level model of evaluation)

Sl. No.	*Level*	*Student's performance*	*Score#*			
1.	**Reaction**	Student's prior knowledge about the topic	1	2	3	4
2.	**Learning**	Students level of attention and participation in discussion during SGT session	1	2	3	4
3.	**Behaviour**	Level of professional code of conduct maintained by the students during the teaching–learning session	1	2	3	4
4.	**Results**	Time taken for record book completion activities	1	2	3	4
5.		Able to show evidence of learning the new skills (intellectual/psychomotor)	1	2	3	4
Total score			**/20**			

#**Score 1:** Poor, **2:** Average, **3:** Good, **4:** Very good	**Faculty Remarks/Feedback:**
	Date: **Faculty Name and Signature**

Competency achieved	**MI1.1:** Describe the different causative agents of infectious diseases, the methods used in their detection, and discuss the role of microbes in health and disease.

Notes/Reflection

1.6 Antimicrobial Susceptibility Test (AST)

LEARNING OBJECTIVES

At the end of the tutorial and practical, the student will be able to:

- Explain the importance of antibiotic susceptibility testing on all clinical samples.
- Enumerate different tests performed for assessing antibiotic susceptibility.
- Define MIC and MBC.
- Explain ATCC strains and their use.
- Enumerate the limitations of *in vitro* susceptibility tests.
- Interpret Kirby-Bauer disk diffusion tests.

INTRODUCTION

- The antimicrobial susceptibility testing *in vitro* is routinely employed in the clinical laboratory to determine:
  a. The susceptibility of given microorganism to known drug concentration.
  b. The potency of an antimicrobial agent.
  c. The concentration of antimicrobial agent in body fluids or tissues.

Fig. 1.6.1: Antibiotics sensitivity

DEMONSTRATION

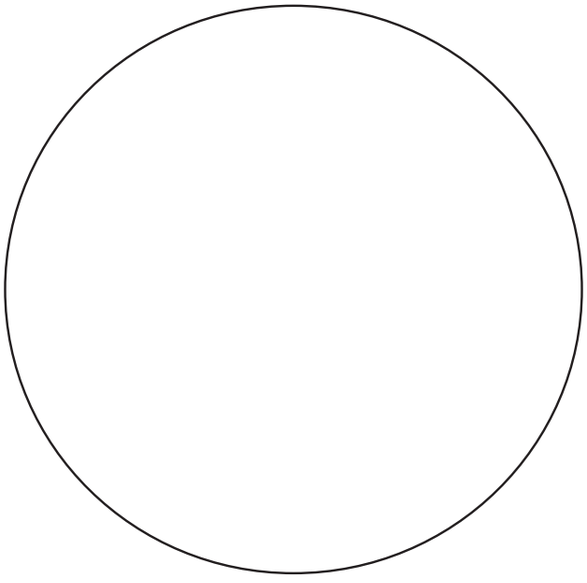
- Mueller-Hinton agar medium showing disc diffusion test (Fig. 1.6.1).
- Zone measuring scale.

QUESTIONNAIRE

1. Enumerate 2 culture media used for AST.

2. Draw and label disc diffusion test demonstrated to you and interpret the result:

Antimicrobial agent	*Zone size*	*Interpretation*



Assessment (Based on the Kirkpatrick 4-level model of evaluation)

Sl. No.	*Level*	*Student's performance*	*Score#*			
1.	**Reaction**	Student's prior knowledge about the topic	1	2	3	4
2.	**Learning**	Students level of attention and participation in discussion during SGT session	1	2	3	4
3.	**Behaviour**	Level of professional code of conduct maintained by the students during the teaching–learning session	1	2	3	4
4.	**Results**	Time taken for record book completion activities	1	2	3	4
5.		Able to show evidence of learning the new skills (intellectual/psychomotor)	1	2	3	4
Total score			**/20**			

#**Score 1:** Poor, **2:** Average, **3:** Good, **4:** Very good	**Faculty Remarks/Feedback:**
	Date: **Faculty Name and Signature**

Competency achieved	**MI1.6:** Describe the mechanisms of drug resistance, and the methods of describe the mechanisms of drug resistance, and the methods of antimicrobial susceptibility testing and monitoring of antimicrobial therapy.

Notes/Reflection