

Std XI Microbiology Practical Manual

Practicals	Page. No.
Good Laboratory Practices and Laboratory Precautions	3
Cleaning of Glasswares	5
Microscope and its parts	5
Sterilisation by Moist Heat – Autoclave	8
Sterilisation by Dry Heat – Hot Air Oven	9
Lacto Phenol Cotton Blue (LCPB) mount of Fungi	10
Algal wet mount	13
Simple Staining	15
Methylene Blue Reduction Test (MBRT)	18
Media Preparation – Nutrient Agar	21
Spotters	22
Identification of Labwares	22
Petriplate	22
Inoculation loop	23
Glass slide	23
Cavity slide	24
L-Rod	24
Cover slip	24
Identification of Equipment	25
Autoclave	25
Hot air oven	25
Incubator	26
Stained Slides	26
Gram positive cocci in clusters	26
Gram positive cocci in chains	27
Gram positive bacilli in chains	27
Gram negative bacilli in singles	27
Yeast cells	27

Higher Secondary – First Year Practical Examination

Microbiology	
Marking Scheme	
Allotment of Marks	
Internal Assessment	05 marks
External Assessment	15 marks
Total	20 marks
Internal Assessment (Practicals) Marks Break Up	
1. Record Note Book	03 marks
2. Skill of performing Experiments	02 marks
Total	05 marks
External Assessment Mark Break Up	
1. Major Practical	09 marks
2. Spotters	06 marks
Total	15 marks
I. Major Practical (Any one out of 5 questions) $9 \times 1 = 9$ marks	
• Aim	01 mark
• Principle	02 marks
• Procedure	03 marks
• Diagram	01 marks
• Observation	01 marks
• Results	01 marks
Total	09 marks
II. Spotters (Any three – one from each category) $2 \times 3 = 6$ marks	
• Identification	$\frac{1}{2}$ marks
• Two salient points	1 mark
• Diagram	$\frac{1}{2}$ mark
Total	02 marks \times 3 spotters = 6marks

Key for Practical Examination

I. Major Practical (Any one) $9 \times 1 = 9$ marks

1. Perform a wet mount of fungus using lacto phenol cotton blue stain and identify the fungus.
2. Identify the shape and arrangement of bacteria present in given culture/curd/idly batter by performing simple staining method.
3. Determine the quality of the given milk sample by performing methylene blue reduction test.
4. Prepare Nutrient agar medium.
5. Perform wet mount of the given algal sample.

II. Spotters

A. Identify the Labware	2 marks
B. Identify the Equipment	2 marks
C. Identify the Stained Slide	2 marks

1. Good Laboratory Practices and Laboratory Precautions

Good Laboratory practices:

1. A documented safety manual must be available for all staff.
2. Personnel must receive training on the potential hazards associated with the work involved and necessary precautions to prevent exposure to infectious agents.
3. Eating, drinking, smoking, storing either food or personal belongings and applying cosmetics are not permitted.
4. Mouth pipetting is prohibited.
5. Long hair is to be tied back or restrained.
6. Access to laboratory and support areas is limited to authorized personnel.
7. Doors to working areas in laboratories must not be left open.
8. Open wounds and cuts should be covered with waterproof dressings.
9. Laboratories are to be kept clean and tidy.
10. Protective laboratory clothing properly fastened and footwear with closed toes and heels must be worn by all personnel visitors, trainees, and others working in the laboratory.
11. Eye and face protection must be used where there is a known or potential risk of exposure to splashes or flying objects.
12. Glove must be worn for all procedures that may involve direct skin contact with biohazardous material. Gloves are to be removed when a laboratory task is completed and before leaving the laboratory.
13. Protective laboratory clothing must not be worn in non laboratory areas.
14. If known or suspected exposure occurs, contaminated clothing must be decontaminated before laundering.
15. The use of needles, syringes and other sharp objects should be limited strictly. Needles should not be bent, sheared, recapped or removed from the syringe; they should be promptly placed in a puncture resistant sharps container for disposal.
16. Hands must be washed with an appropriate antiseptic soap or rubbed with an alcohol based hand gel after gloves have been removed, before leaving the laboratory, and at anytime after handling materials known or suspected to be contaminated.
17. Work surfaces must be cleaned and decontaminated with a suitable disinfectant at the end of the day and after any spill of potentially biohazardous material.
18. Contaminated materials and equipment that are removed from the laboratory for servicing or disposal must be appropriately decontaminated.
19. Autoclaves used for decontamination should be regularly monitored with biological indicators.

20. All contaminated materials must be decontaminated before disposal or reuse.
21. Leak proof containers are to be used to transport infectious materials.
22. Spills, accidents, breaches of containment, or exposure to infectious materials must be reported immediately to the laboratory supervisor.
23. An effective rodent and insect control program must be maintained.

Rules and Regulations:

There are certain rules and regulations which should be followed in Microbiology. This ensures safety of all the workers in the laboratory. The rules are as follows:

A. General Instructions:

- The working area should be disinfected with suitable disinfectants.
- Infectious materials should not be blown out of pipettes.
- It is important to label the cultures or the samples.
- Before centrifuging the tubes should be inspected for cracks.
- Wetting of cotton plug should be avoided while shaking the broth culture.
- Mixtures of infectious materials should not be prepared by blowing air through the liquid by pipette.
- If a culture is poured or spilled, disinfectants should be poured over the area and clean the area thoroughly.
- Before discarding the bacterial culture it should be sterilised. Used cotton papers, plastics should be disinfected by using swab.

B. Personal Care:

- General body health should be maintained
- It is necessary to wear protective clothings like overcoats, aprons.
- Open cut or wounds should be bandaged.
- Nails should be cut. Hands should be washed preferably with soap or dettol.

2. Cleaning of Glasswares

Cleaning of glasswares is an important process used in microbiology before sterilization.

New glasswares require special attention because of resistant spores which may be present in the straw and other packing materials. They may also contain alkali so they are immersed in 1% HCL overnight and washed several times in distilled water.

Used Glasswares are discarded in the jar containing glassware solution potassium dichromate and allowed to stand overnight. Culture containing glasswares are sterilised

and the medium is drained out. Further immersed in hot soap solution and cleaned with brush and washed in distilled water.

Cleaning of pipettes: Cotton plugs from the pipettes are first removed and water is forced through it. The culture containing pipettes are discarded in disinfectant solution and then washed.

Slides and coverslips are boiled in a detergent solution and washed in tap and distilled water. With the help of forceps, slides are transferred to the split container and finally cleaned with cloth before use.

3. Microscope and its parts

Aim: To study the parts of microscope

Theory and Principle:

Microbiology is one of the biological sciences that deals with the study of microorganisms that cannot be seen with naked eye. They can be seen only through a microscope. A microscope is an instrument consisting of lens or combination of lenses which magnifies a minute object. The commonly used microscope is called the bright field microscope which uses bright light source to examine microorganisms.

A compound microscope has two sets of lenses, one known as the objective and the other known as the eyepiece mounted in a holder commonly known as the body tube. The lens system nearest to the object called objective, magnifies the object a definite number of times. The second lens system called the eyepiece magnifies the image formed by the objective further. The image seen by the eye has the magnification equal to the product of the magnification of the two systems.

A compound microscope consists of three parts.

They are: 1. The Stand, 2. The body, 3. The series of optical lenses.

The stand comprises of heavy foot to give stability and a limb hinge bears the optical system. The limb is attached to the foot hinge joints or fused directly.

The optical system is mounted in the tube which is usually in two parts, an external tube which bears at its lower end a revolving nose piece, in which interchangeable objective lenses of various magnifications are fitted and the inner draw tube which carries eye piece at its upper end. The whole assembly is held in position by the body which houses two mechanisms the coarse and the fine adjustments, whereby the height of the tube can be adjusted in such a way to focus the object by the lens systems. The condenser housed below focuses a cone of light on the specimen. The amount of light entering the condensor can be adjusted by the iris diaphragm.

Principle involved in the magnification of the object:

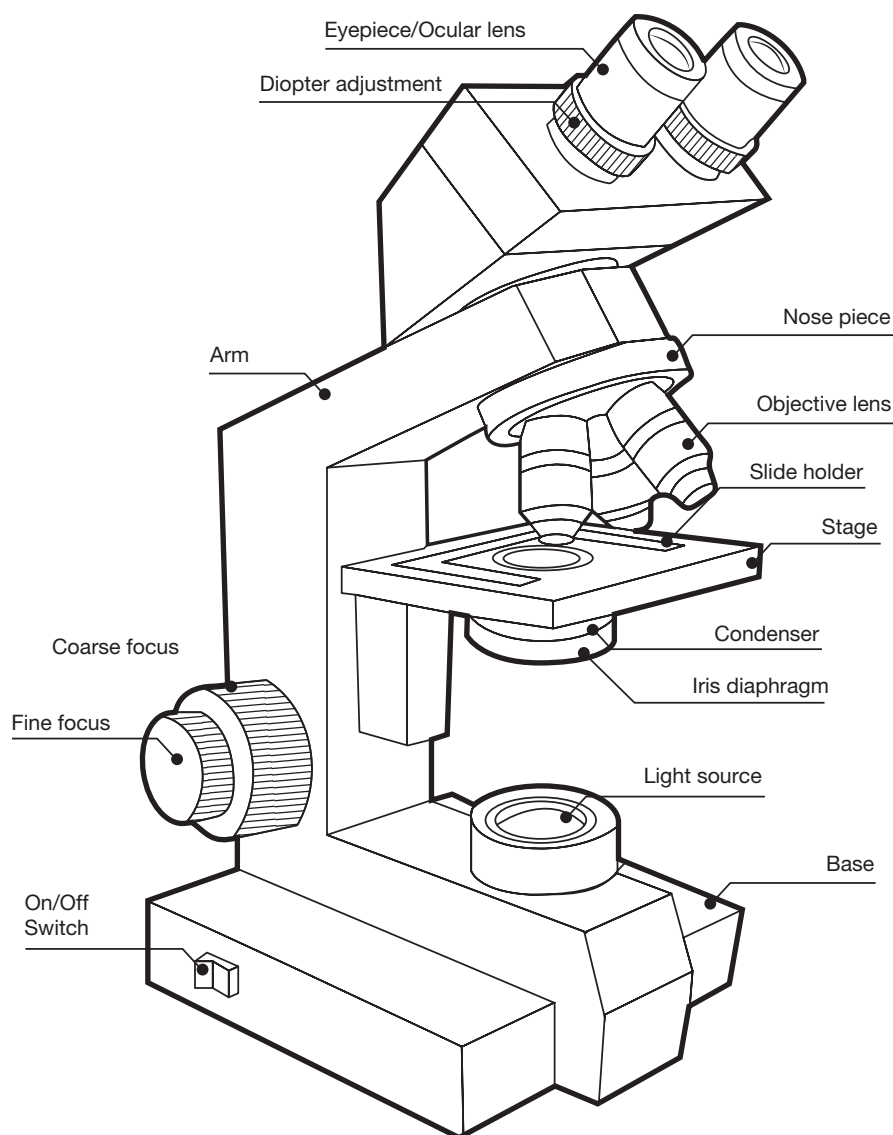
In biconcave lens if the object is placed between f (focal length) and $2f$, the image is enlarged. This image is real and can be projected onto a screen. If the object is placed

between the f and the lens then an enlarged virtual image which cannot be projected onto a screen is produced. In the compound microscope the object is placed between the f and $2f$ of the objective lens. The objective produces the primary image. The primary image is real, inverted and magnified. The eye piece consists of the two lenses, a field lens and an eye lens which is near the eye, and a diaphragm between the two lenses. The field lens of the eyepiece brings the real image to focus at the plane of the diaphragm. This is within the focal length(f) of the eye lens(i.e. between the f and the lens). This eye lens produces the virtual magnified image that is seen by the eye.

Magnification of the object depends on the eyepiece and objective magnification.

Diagram:

Parts of a microscope

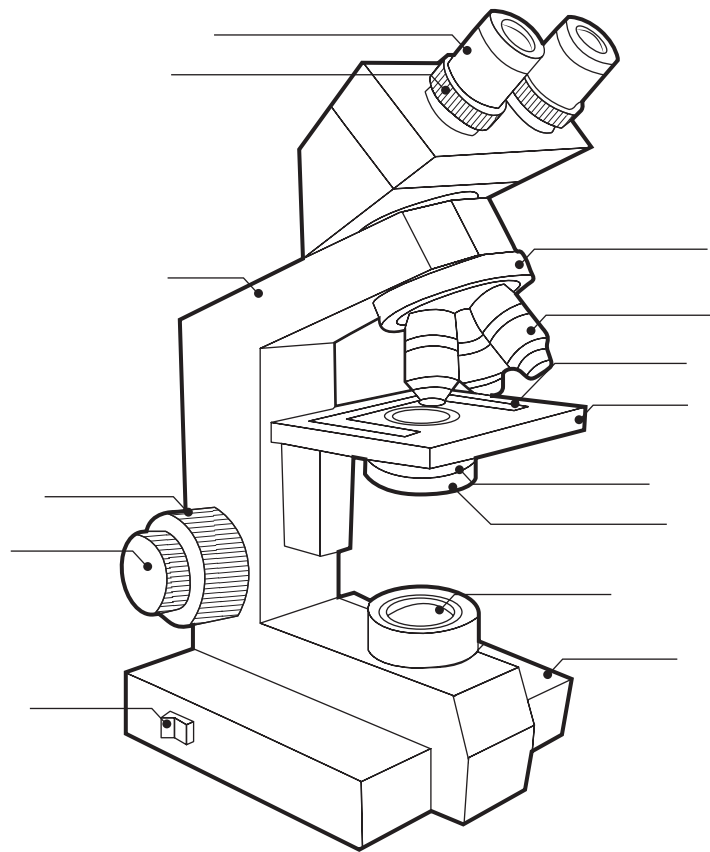


Result:

The parts of compound microscope were studied.

Aim:

Diagram:



Result:

4. Sterilization by Moist Heat – Autoclave

Aim: To study the physical process of sterilization using moist heat and to sterilise culture media and glasswares.

Theory and Principle:

Sterilisation is freeing of an article from living organisms. Physical method using moist heat is one of the methods used for sterilization.

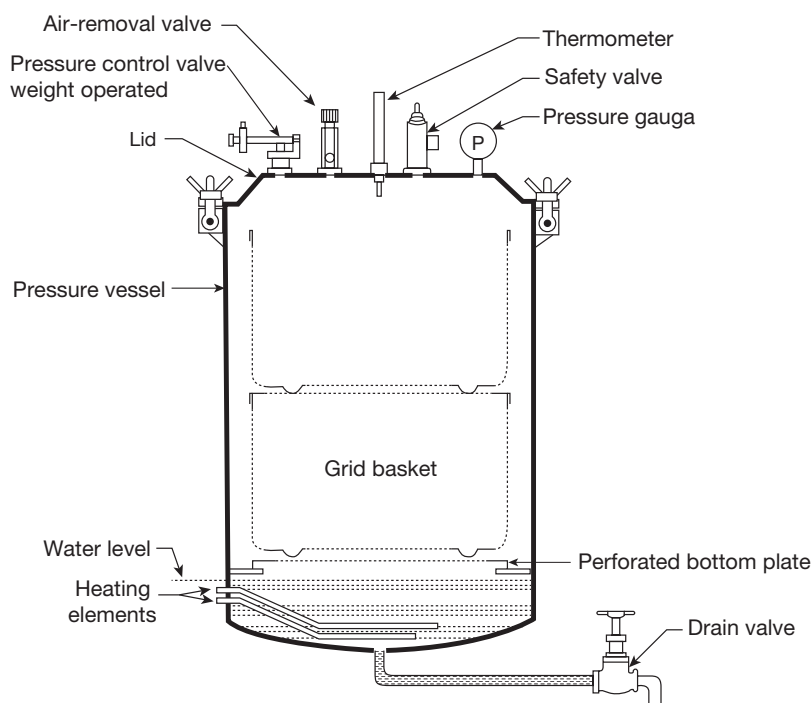
Autoclave: When water is boiled in a closed vessel at increased pressure, the temperature at which it boils and that of the steam it forms, will rise above 100°C. This principle is employed in the autoclave.

Moist heat denatures proteins and cause destruction of membranes and DNA. Presence of moisture, significantly speeds up heat penetration.

Pressure cookers are used generally to sterilize small quantities of media. When large quantities of media and other materials are to be sterilised, the use of autoclave is recommended.

Procedure: (Autoclaving)

1. The glassware are washed dried and covered with paper.
2. Media are prepared in testtube, flasks and covered with cotton plugs and paper.
3. Pipettes are wrapped in paper and placed in canisters.
4. Water is poured into the autoclave until the thermostat level is reached.
5. Things are placed in the stainless steel drum of autoclave.
6. The lid of the autoclave is closed tightly.
7. Current is switched on.
8. The steam valve is closed after steam starts leaving the steam outlet.



9. The sterilization is carried out for 15–20 minutes after the pressure guage reading reaches 15 psi.
10. Switch off the current.
11. Cool down and slowly release the lid knobs.
12. Remove the things from the autoclave for further use.

Result:

Physical method using moist heat of sterilization were studied and glasswares and media were sterilised using autoclave.

5. Sterilization using Dry Heat – Hot Air Oven

Aim: To study the physical process of sterilization using dry heat and to sterilise oils, powders and glasswares.

Theory and Principle:

Sterilisation is freeing of an article from living organisms. Physical method using dry heat is one of the method used for sterilization.

Hot air oven employs dry heat at a temperature of 160°C for one hour. Glasswares, syringes, metal instruments and paper wrapped goods which are not sensitive to high temperature are sterilised in hot air oven.

This technique requires longer exposure time and higher temperatures than moist heat sterilization. Sterilizing by dry heat is accomplished by conduction. Dry heat does most of the damage by oxidizing molecules.

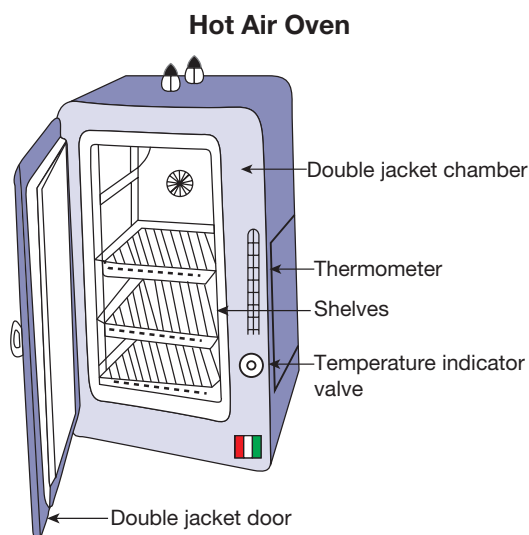
Moisture impermeable materials like anhydrous fat, oils and powders are sterilised by hot air oven.

Procedure:

1. Switch on the hot air oven.
2. Set the required temperature and other conditions.
3. When the temperature is reached place the glasswares and other things for sterilization.
4. Switch off the oven after an hour.
5. Cool and remove the glasswares.

Result:

Physical methods of sterilization were studied and glasswares, oils and powders were sterilized using hot air oven.



6. Lacto Phenol Cotton Blue (LCPB) mount of Fungi

Aim: To identify the fungus by wet mount method using lactophenol cotton blue stain.

Theory and Principle:

Filamentous fungi are reliably identified by their characteristics microscopic morphology such as shape, size and arrangement of spores and hyphae. Fungi are eukaryotic and range from unicellular yeast to multicellular molds. They reproduce by producing spores.

Common fungi are *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*. They are filamentous and collectively form mycelium. The morphology of the hyphae and spores can be identified using a simple wet mount technique using lactophenol cotton blue stain.

The organisms suspended in the stain are killed due to the presence of phenol. Lactic acid preserves fungal structures and cotton blue stains the fungal cell wall.

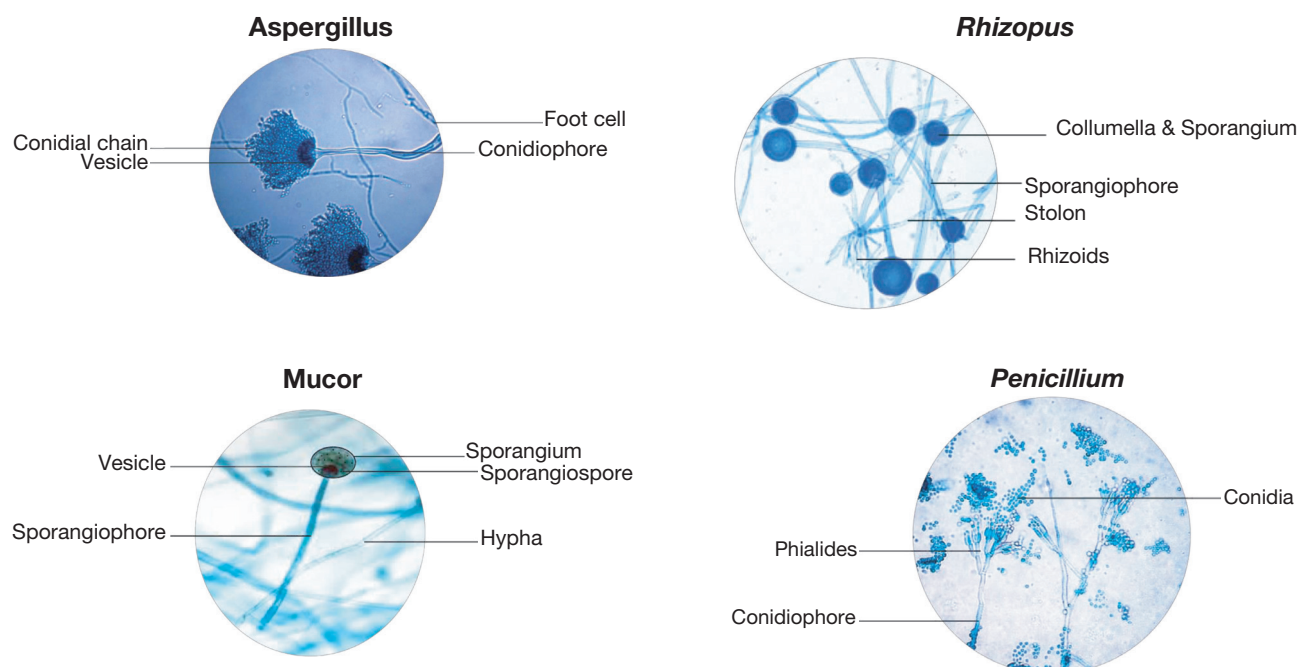
Requirements:

1. Clean grease free slide
2. Coverslip
3. Forcep
4. Teasing needle
5. Distilled water
6. Lacto Phenol Cotton Blue

Procedure:

1. Take a clean slide.
2. Place a drop of water on the slide.
3. With the help of forceps transfer mycelium from a sample (Spoilt Bread).
4. Tease it with needle to separate the filaments (hyphae).
5. Add a drop of lacto Phenolcotton blue.
6. Gently place a coverslip avoiding air bubble formation.
7. Observe under low power and high power objective lens.
8. Record the observations and interpret.

Diagram:



Fungi	Characteristics of Hyphae	Spores borne in
<i>Aspergillus sp.</i> and <i>Penicillium sp.</i>	Septate	Conidiophore bear conidia
<i>Mucor</i> and <i>Rhizopus sp.</i>	non-septate	Sporangiospore bear sporangium containing sporangia.

Observation

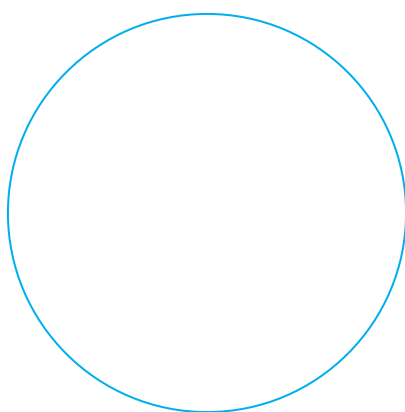
Filamentous hyphae bearing sporangia were observed.

Result:

Wet mount using lactophenol cotton blue was carried to identify the fungus sample. Hyphae with sporangium bearing sporangiospores were observed. It is likely to be of *Mucor* species.

Aim:

Diagram:



Observation:

Result:

7. Algal wet mount

Aim: To perform a wet mount of algal sample and determine its morphology.

Theory and Principle:

Algae are protists. They possess the characteristics of plants. They are found in aquatic environments. Algae are eukaryotic organisms containing chloroplast and have the capacity to carry out photosynthesis. Some algae possess flagella, centrioles and capable of feeding organic materials from the environment where they live. The size varies from single cell to very large multicellular species. They can live in different environments like salt water, fresh water, wet soil or wet rock.

Examples: Euglena, Diatoms, Dinoflagellates, Chlamydomonas, Spirogyra, Gelidium.

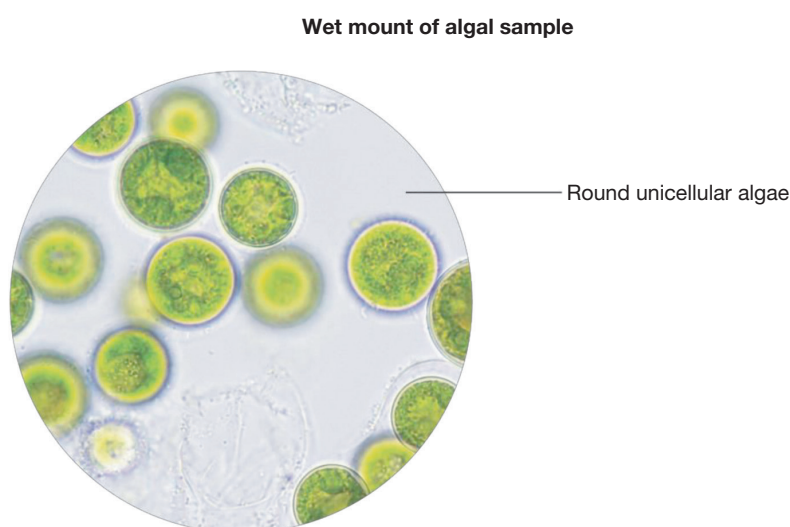
Requirements:

1. Algae containing water sample
2. Glass slide
3. Coverslip
4. Pasteur pipette

Procedure:

1. Place a drop of algal sample on a slide.
2. Gently place a coverslip over it.
3. Observe under low power objective.

Diagram:



Observation:

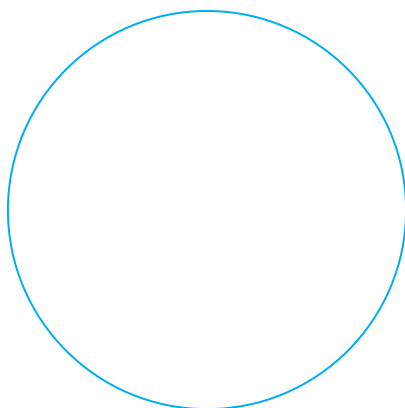
Spherical, unicellular and colonial forms of algal cells were observed.

Result:

Wet mount of algal sample revealed the presence of single, spherical algal cells.

Aim:

Diagram:



Result:

8. Simple Staining

Aim: To identify the shape and arrangement of bacteria present in the given bacterial culture/idly batter/curd by simple staining technique.

Theory and Principle:

Stains are used to observe bacteria clearly with contrast. In simple staining or monochrome staining only one stain is used. Basic stains like methylene blue, crystal violet, safranin, malachite green and basic fuchsin are commonly used. Basic stains are positively charged which binds to negatively charged bacteria. The process of simple staining is used to illustrate the shape and arrangement of bacterial cell.

Requirements:

1. Clean grease free slide
2. Nichrome loop
3. Given culture
4. Basic Stains eg: Crystal Violet.

Procedure:

1. Take a loopful of the given culture and place on the slide.
2. Prepare a smear and heat fix it.
3. Apply any one basic stain (eg., Crystal Violet) for one minute.
4. Wash gently with water.
5. Air dry.
6. Observe the slide under high power and oil immersion objectives.
7. Record your observations.

Observation Table: (any one shape and stain)


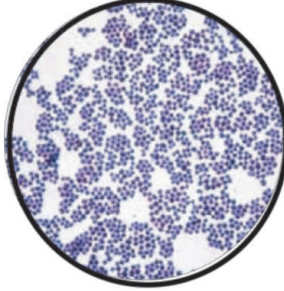

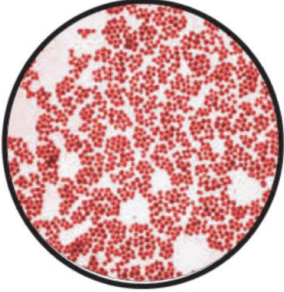
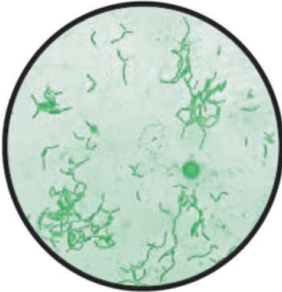
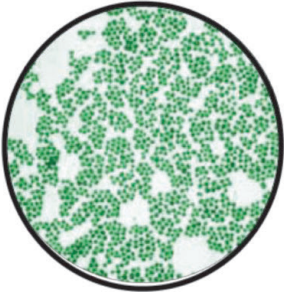
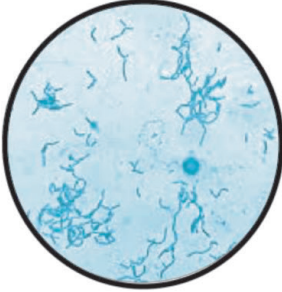
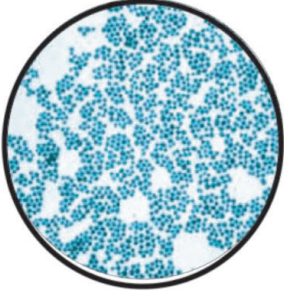
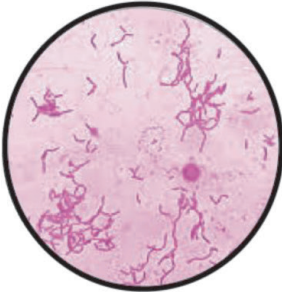
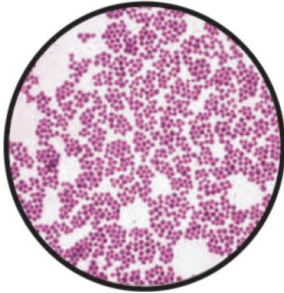
Sr. no	Stain used	Colour of cytoplasm	Colour of Background	Shape	Arrangement
1.	Crystal violet	violet	colourless	Rod (bacilli)	Singles and chains
2.	Malachite green	green	colourless	Spherical (cocci)	clusters

Result:

The shape and arrangement of the bacteria present in the given culture was found to be rod (bacilli) in chains. Simple staining of the bacteria revealed violet coloured bacteria as the stain used was crystal violet.

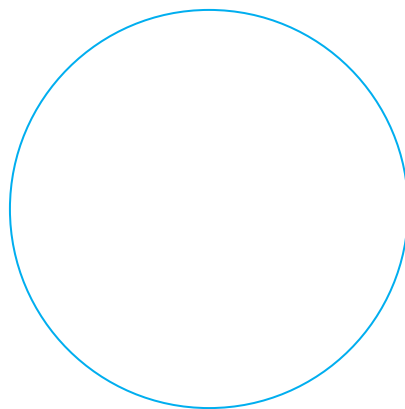
Diagram: (Any one Diagram)

MONOCHROME STAINING

STAIN USED	Rod shaped Bacteria (Bacilli)	Spherical shaped Bacteria (Cocci)
Crystal violet		
Safranin red		
Malachite green		
Methylene blue		
Basic fuchsin		

Aim:

Diagram:



Observation:

Result:

9. Methylene Blue Reduction Test (MBRT)

Aim: To determine the quality of milk sample by using methylene blue reduction test.

Theory and Principle:

Milk is a good medium for the growth of microorganisms. Actively growing organism present in the milk reduce the oxidation reduction potential of milk medium due to the exhausted oxygen by the microorganisms. The dye methylene blue, an indicator losses its blue colour in anaerobic environment and is thought to be reduced. The speed at which reduction occurs following addition of methylene blue to sample of milk indicates the quality of milk.

Methylene Blue reduction time	Quality of Milk
<2 hours	POOR
2-6 hours	FAIR
6-8 hours	GOOD

Requirements:

1. Milk Sample
2. Methylene Blue (1:25000)
3. Screw capped tubes
4. Pipettes
5. Water bath / Incubator

Procedure:

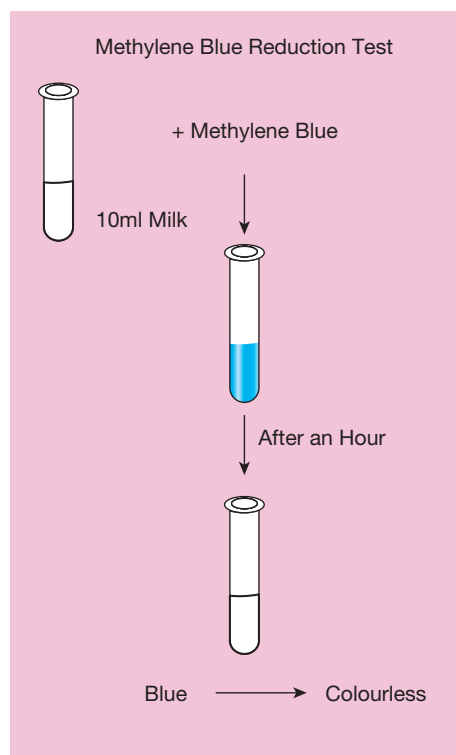
1. Add 10 ml of milk sample to a test tube using pipette.
2. Add 1ml of methylene blue into the test tube.
3. Close the tubes and gently invert for proper mixing.
4. Place the tubes in water bath or incubator at 37°C.
5. Remove the tubes from the water bath every half hour until the end of the experiment upto 8 hours and observe the change in colour and note the duration.

Observation:

Methylene blue dye reduction time	Inference
1 hour	Poor quality milk

Result:

The quality of the given milk sample was determined as POOR since methylene blue dye reduction time was within an hour.



Aim:

Observation:

Result:

10. Media Preparation – Nutrient Agar

Aim: To prepare nutrient agar medium for cultivation of microorganisms.

Theory and Principle:

A culture medium is an environment which supplies necessary nutrients for the growth of microorganisms. Nutrient agar is a basal medium which supplies the basic requirements of carbon, nitrogen and mineral source for growth of microorganisms. It is a general purpose medium which allows the growth of wide range of nonfastidious microorganisms.

Composition of Nutrient Agar

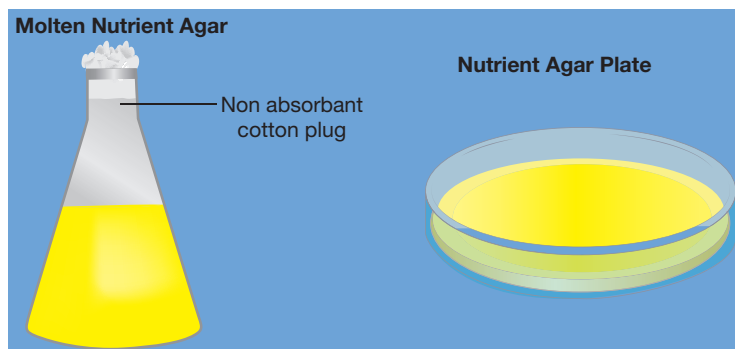
1. Peptone: 1gm
2. Beef Extract: 0.5gm
3. Sodium Chloride: 0.3gm
4. Agar: 2.8gms
5. Distilled Water: 100ml
6. pH: 7.4 (neutral)

Requirements:

1. Peptone
2. Beef Extract
3. Sodium Chloride
4. Agar
5. Distilled Water
6. Measuring Cylinder
7. Petridishes
8. Non Absorbant Cotton
9. Rubber bands and Newspaper

Procedure:

1. Weigh the ingredients and transfer it to 250ml conical flask.
2. With the help of measuring cylinder measure 100ml distilled water.
3. Dissolve the weighed ingredients in 50ml of distilled water and make up the volume to 100ml.



4. Adjust the pH to 7.4 using sodium hydroxide with the help of pH meter or paper.
5. Cover the flask with cotton plug and paper and label it.
6. Sterilise the medium in an autoclave.
7. Cool the medium to 45°C and pour the medium into petriplates, or tubes as per the requirement.

Observation:

Nutrient agar medium is in the molten state after autoclaving. It solidifies after cooling to form a solid medium to be further used for isolation and growth of microorganisms.

Result:

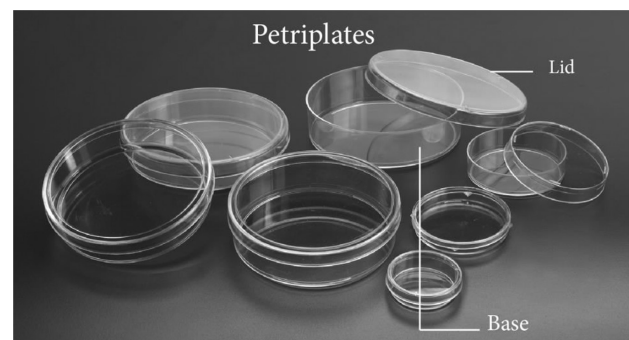
Nutrient agar medium was prepared and sterilised. Further Nutrient agar plates and slants were made for isolation of microorganisms.

Spotters

A. Identification of Labwares

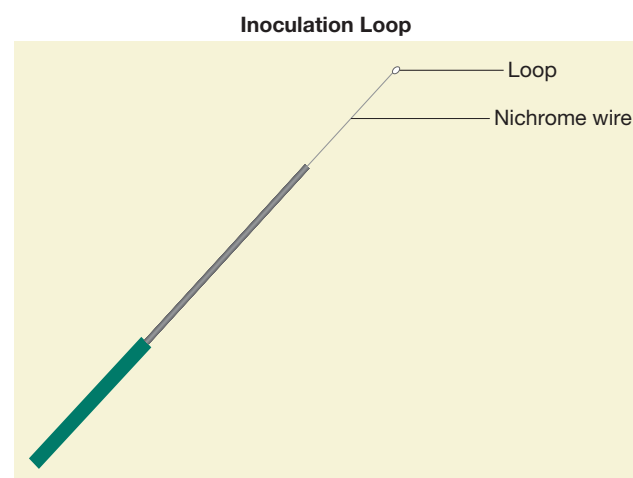
1. Petriplate

- Petriplate or petridish is named after the bacteriologist Richard Petri.
- It is a shallow cylindrical glass or plastic dish with lid used to culture cells.
- Often used to set solid agar medium.
- They are incubated upside down to lessen risk of contamination and prevent accumulation of any water contamination.



2. Inoculation loop

- An inoculation loop is also called smear loop or microstreaker is a simple tool used mainly by microbiologists to retrieve an inoculum from a culture of microorganisms.
- It is used to transfer inoculum for streaking and hence to isolate the microorganisms on media.
- It is sterilised by flaming to red heat.
- It is easy to sterilize and reuse because nichrome wire resists deterioration with repeated heat/cooling cycles.



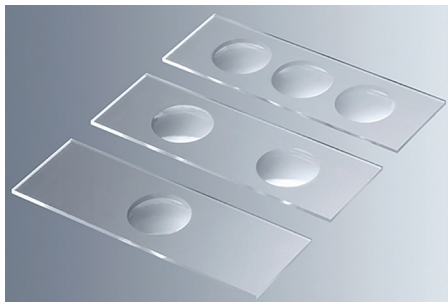
3. Glass slide

- A glass slide or a microscopic slide is a thin flat piece of glass.
- It is usually 15 by 26mm and about 1mm thick.
- It is used to hold objects for examination under a microscope.



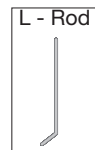
4. Cavity slide

- These slides are glass slides which have polished round depressions.
- They are useful for observing hanging drops and hence motility of bacteria.



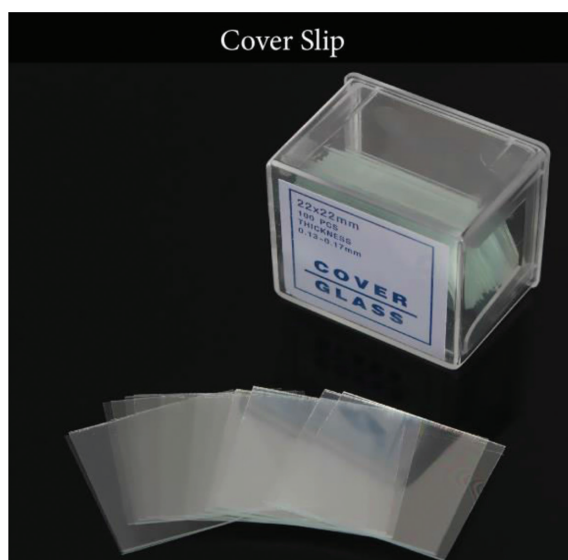
5. L-Rod

- L-shaped glass rod is used for spreading samples on agar surfaces.
- They are also used for mixing the chemical or liquid for laboratory purpose.



6. Cover slip

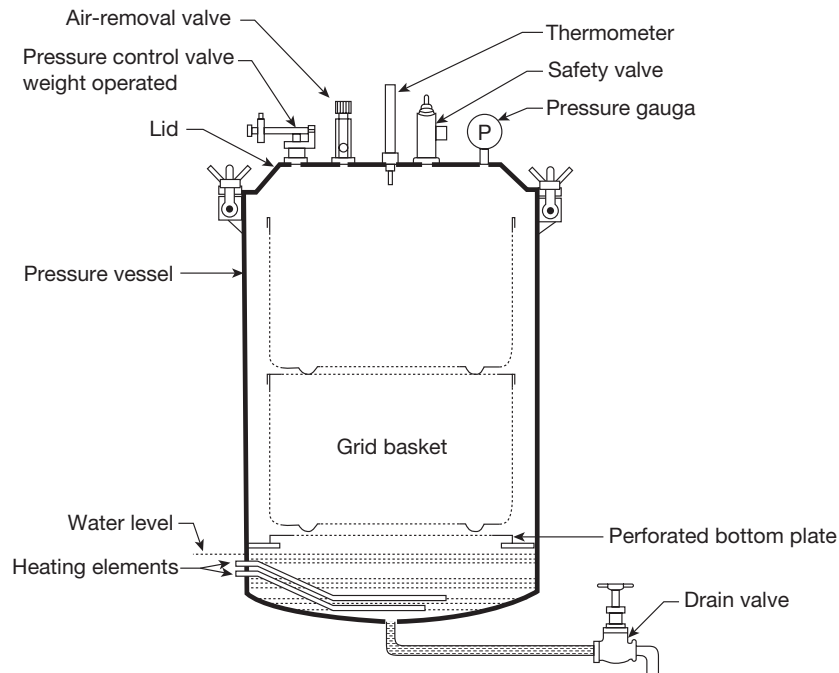
- It is a thin flat piece of transparent material, usually square, rectangular or circular in shape
- It is made up of non-corrosive borosilicate glass.
- It is placed over smears or wet mounts to protect the objective lens.



B. Identification of Equipment

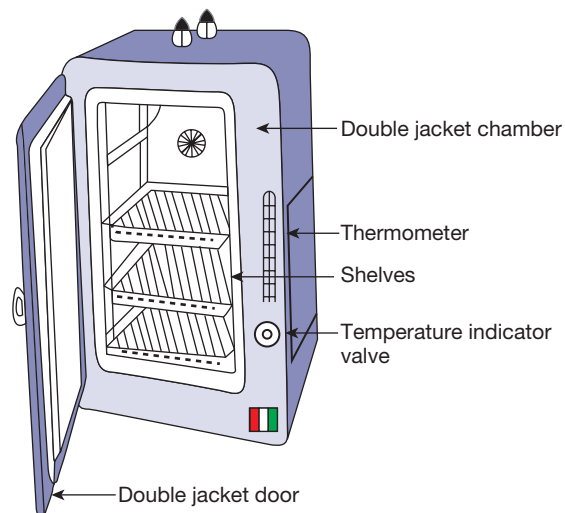
1. Autoclave

- It is an equipment used for sterilisation
- It utilises moist heat (temperature 121°C) and pressure (15 psi).
- Used to sterilise culture media, rubber materials, gowns, dressings, gloves and glasswares.



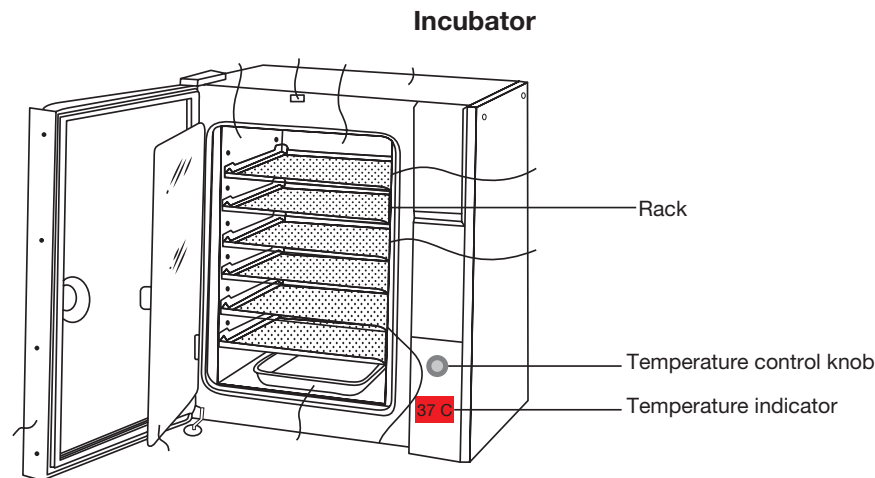
2. Hot air oven

- It is an electrical device which uses dry heat for sterilization.
- Generally, they can be operated from 50°C to 300°C using a thermostat to control the temperature.
- The double layer walled insulation keeps the heat in.
- An air circulating fan helps in uniform distribution of heat.



3. Incubator

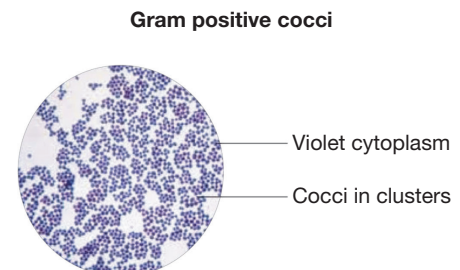
- An incubator is a device used to grow and maintain microbiological cultures.
- The incubator maintains optimum temperature, humidity and other conditions such as CO₂ and O₂ content of atmosphere inside.
- The simplest incubators are insulated boxes with an adjustable heater, typically going upto 60°C to 65°C.
- Types: shaker incubators, gas incubators.



C. Stained Slides

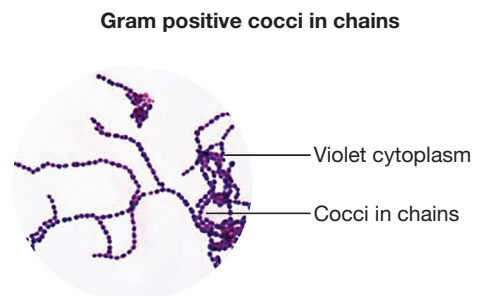
1. Gram positive cocci in clusters

- They are spherical shaped bacteria.
- Division in random planes produces a cluster like arrangement.
- They retain the primary stain in Gram's staining and stain violet.
- Their cell wall is made up of thick peptidoglycan layer.
- Example: *Staphylococcus aureus*.



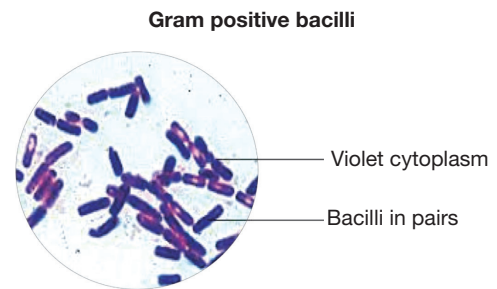
2. Gram positive cocci in chains

- They are spherical shaped bacteria.
- Division in one plane produces chain arrangement.
- They retain the primary stain in Gram's staining and stain violet.
- Their cell wall is made up of thick peptidoglycan layer.
- Example: *Streptococcus faecalis*.



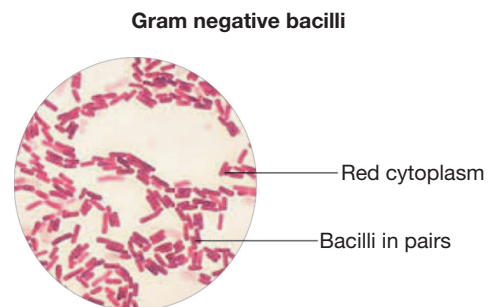
3. Gram positive bacilli in pairs

- They are rod shaped bacteria.
- They tend to divide in one plane and remain attached to appear as pairs.
- They retain the primary stain in Gram's staining and stain violet.
- Their cell wall is made up of thick peptidoglycan layer.
- Example: *Bacillus subtilis*.



4. Gram negative bacilli in singles

- They are rod shaped bacteria.
- They do not retain the primary stain after decolorisation and take up the counterstain and stain pink to red.
- Their cell wall have thin peptidoglycan layer and surrounded by a lipid outer membrane.
- Example: *Escherichia coli*.



5. Yeast cells

- They are unicellular, oval eukaryotic cells.
 - They are used in bakery industry
- Example: *Saccharomyces cerevisiae*.

