Indira Gandhi National
Open University
School of Sciences

CLTL-102
BASIC EXPERIMENTS IN BIOLOGY

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IMPORTANT

Attendance is compulsory in the Laboratory Course work held generally at the Study Centre.

- The Laboratory Course is worth **2 credits** to be completed over **5 days** duration:
  - **4 days** of **Guided** Laboratory work
  - **1 day** for the **Unguided** Laboratory work
- To successfully complete the laboratory course you will have to pass (at least **35% marks**) in the Guided and Unguided components separately.

BASIC EXPERIMENTS IN BIOLOGY : INTRODUCTION

Experimentation is an integral component of any scientific study. This is particularly true of biological sciences because of wide diversity in its areas of study. Starting from investigation on evolution of life, experiments have played an important role in the growth and development of our knowledge. The revolutionary discoveries in medical science owe a lot to refinements in instrumentation and here lab technicians have always played a vital role.

This laboratory course is worth 2 credits. Most of the experiments are based on the units you have studied in two blocks of CLT-102 course. These experiments have been designed to develop your practical skills in organising and maintaining a school/college biology laboratory and ensuring its smooth and safe running. You will get 'hands on' experience in operating various equipments, acquiring and arranging biological specimens in a specific order; preparing solutions, stains and reagents.

In Experiment 1, you will perform three activities i.e. plan the organisation of a laboratory, write general instructions for the use of laboratory and label specimens, slides and reagents. In Experiment 2, you will learn to use a pressure cooker, autoclave, hot air oven, water bath and laminar air flow system.

In Experiment 3, you will learn how to procure plant material for experiments. You will learn how to collect algae, bryophytes, pteridophytes and also collect and preserve angiosperm specimens to make a herbarium.

In Experiment 4, you will learn to set-up apparatus for demonstration experiments related to physiological processes taking place in plants. These experiments require special equipments which are not used by students individually, but require assistance from the lab technical staff.

In Experiment 5, you will learn to work, care and maintain stereo and compound microscopes. In Experiment 6, you will prepare some important plant and animal fixatives, reagents and stains. In Experiment 7, you will prepare temporary slides using smear and squash techniques and also prepare whole mounts of protozoans.

In Experiment 8, you will prepare the media and raise a microbial culture. You will also do the disposal and sterilization of the glassware used in culture experiments. In Experiment 9, you will be able to extract DNA from strawberries using a simple procedure.

Objectives

After performing the experiments given in this course, you should be able to:

- plan the organisation of a typical biology laboratory,
- operate, care and maintain stereo and compound microscopes and other basic equipment found in a biology lab taking safety precautions,
- procure plant and animal materials required for conducting experiments and arrange biological specimens in a specific order in a museum or herbarium,
- set-up demonstration of some physiological experiments for students,
- prepare some simple slides and important fixatives, stains, reagents and microbial culture medium required for biological experiments,
- extract and observe DNA.

Study Guide

- Before you enter the laboratory you should have read each experiment and the related theory units carefully. While reading, spend adequate time on the methodology to be followed in each experiment.
- Underline the important steps in the lab manual.
- Keep the working place in the lab as clean as possible. Be careful and cautious while handling the chemicals and the instruments in the laboratory. Follow the safety precautions given in the manual.
- You should keep an observation notebook for recording all your observations, results, doubts, if any, and difficulties experienced in carrying out the experiments.
- Besides the observations notebook, you are expected to prepare a record notebook which you will have to submit to the counsellor everyday for evaluation. You should record your experimental data and discuss your findings.
- Try to make the best use of your time with your counsellor during the period spent in doing the lab course.

Time Required for Exercises

The laboratory course consists of practicals worth 2 credits and would require four days. Each day you have to work for two sessions consisting of four hours each. The examination for the practicals will be held on the fifth day.

Guidelines for Doing Exercises

While doing practicals you must have the two blocks of the course CLT-102 with you. In many of the experiments, you'll find references to the materials given in Blocks 1 and 2 of the course CLT-102.

EXPERIMENT 1

LABORATORY ORGANISATION

Structure

1.1 INTRODUCTION

In Unit 1 of the CLT-102 course, you have learnt about lab organisation and day-to-day management. In principle a well designed, organised, and aesthetically appealing lab is attractive to everyone. Besides, it is convenient to use, easy to clean and maintain. It elevates the mood, increases the enthusiasm of students and teachers alike, and is conducive for working. Unfortunately, many of us tend to neglect this aspect and do not make an effort in this direction.

Whether you will be allowed to reorganise a lab or not, however, through this exercise you will become aware of the approach and guidelines used for organising it. Besides, you may get a few tips for organising other places such as – home, work place, factory etc. and we would like that whichever place you are allowed to implement your ideas you can take the opportunity and do so.

In most jobs a well-organised place is primary for efficient functioning. Organising a place is almost always a collective activity and a lab is also organised by the joint effort of people using it. Though there are a set of

guidelines common for most laboratories and do's and don't are well-defined, but each lab is unique in its set-up and reflects the talent of the users.

It is seen that many people are uncomfortable at the thought of rearranging a place because it is quite demanding and involves extra effort. Therefore, they simply put up with any amount of disorder and lack of cleanliness. This attitude is undesirable and we should strongly overcome it.

In this exercise you will be doing the following activities with regard to laboratory organisation:

Activity 1 : To plan the organisation of a laboratory

Activity 2 : To display general instructions for laboratory use

Activity 3 : To label specimens, slides and reagents

Expected Learning Outcomes

After doing this exercise you should be able to:

- state the need for an aesthetically organised, clean and well maintained lab;
- describe how to organise and maintain a laboratory;
- display instructions for the users of a lab so that it could be used effectively, conveniently, and safely;
- display instructions and necessary precautions required for using sophisticated instruments; and
- prepare labels for biological specimens, slides and reagent bottles.

1.2 ACTIVITY 1 : TO PLAN THE ORGANISATION OF A LABORATORY

It is important for a technician to participate in lab organisation since he/she is given the responsibility for its day-to-day management. He/she is expected to know about everything that is present in the lab and its purpose. In this activity you will learn how to plan the organisation of a lab.

To save your time we have listed the things commonly available in most labs. First read the list of items given in subsections 1.2.1 to 1.2.10. Examine the lab in which you are working or doing the practical and find out what is available. Add the missing things to complete the list and strike off what is not available. With regard to each item it is important to consider the size, number and suitable place for keeping them. You should classify small items and put them together.

1.2.1 Accommodation

No. of Main labs

Preparation room

Store

Office

Museum

Any other (animal house, green house, herbarium etc.)

.....

.....

.....

1.2.2 Persons to be Accommodated

Number of Permanent staff

Number of Teachers

Number of supporting staff

Number of Research students

Number of Batches of students

Number of classes

Any other

.....

1.2.3 Furniture including Computer and its Accessories

Computer

Printer

Scanner

Teacher's table and chair

Work Tables/ benches

Revolving stools

Almirah for storage

Filing cabinet

Slide cabinet

Open shelves

Office tables

Any other

.....

.....

1.2.4 Apparatus/Equipment

Dissecting Microscopes

Compound Microscopes

Microtomes

Spectrophotometer

Incubators

Refrigerator

Laminar Flow unit

Centrifuge

Hot air oven

Hot plate

Water bath

Magnetic stirrer

Physical balance

Fine electronic balance

Distillation plant/RO (Reverse osmosis) unit

Knife sharpener

Shaker

Autoclave

Pressure cooker

Safety cabinet

pH meter

Any other

.....

.....

1.2.5 Glassware

Beakers

Test tubes

Graduated test tubes

Conical flasks

Round bottom flasks

Flat bottom round flasks

Erlenmeyer flask

Distillation flasks

These glassware are also available in heat resistant Corning glass

Pipettes

Burettes

Volumetric flasks

Measuring cylinders

Jars

Specimen jars with lids

Bell jar

Dessicators

Troughs

Petri plates

Watch glasses

Cavity blocks

Microslides/glass slides

Cavity slides

Cover slips

Any other

.....

.....

It is important to know the quantity in each category in order to be able to estimate the space requirement.

1.2.6 Chemicals

Solid chemicals

Liquid chemicals

Stock of solutions, stains and culture media

Try to prepare a list of the chemicals available in the lab and estimate the space required.

1.2.7 Specimens, Charts and Models

Write the details of each and estimate the space requirement and appropriate place for each.

1.2.8 Miscellaneous Items

Bunsen burners

Spirit lamps

Iron stands

Tripod stands

Wire gauges

Dissecting kits

Test tube holders

Test tube stands

Any other

.....

Complete the list and try to categorise them.

1.2.9 Provision for Disposal

Garbage cans

Incinerators

Contract with authorised firm for garbage disposal

1.2.10 Files, Records and Catalogues

Determine the quantity, storage units and space available.

Once a comprehensive list has been prepared by you, then you should be adequately ready and prepared to examine and if need be re-organise the lab. See if there is scope for improvement. It is also likely that you may come up with a completely new plan of organisation. It may happen that you may be unable to implement your idea for organising the lab due to practical considerations. In any case whether you are allowed or not to implement your ideas for this lab put down your suggestions in Table 1.1.

You must consider the following questions before giving and listing your suggestions:

1. Is the present arrangement satisfactory?
2. Who all use the lab and for what purpose?
3. Is the furniture arranged in a manner that staff and students would be able to move around easily?
4. What items are to be used everyday?
5. Which of the experiments are routinely performed?
6. What precautions are required while placing electrical appliances?
7. Which is the best place for storing glassware?
8. Where could the different chemical (acids/photosensitive/heat sensitive) be safely stored.
9. Is the provision for storage most appropriate?
10. Are the provisions for safety adequate?
11. Is the arrangement of chemicals and equipment adequate for independent student activity?
12. Is the arrangement of furniture, equipment, glassware, etc. convenient and serviceable?
13. Has the area for conducting certain experiments been identified?
14. Is it possible for the students to study the displayed charts, models and specimens?
15. Is there a convenient arrangement for frequent washing of the glassware by the students?
16. Is the arrangement for disposal satisfactory?
17. Are the provisions for good management adequate?

Now complete the Table 1.1 given below:

List of Items	Placement		Suggestions
	Appropriate	Not Appropriate	

After completing this exercise discuss your suggestions with your peer group and counsellor. Remember organising a place is a collective effort.

Remarks:

1.3 ACTIVITY 2: TO DISPLAY GENERAL INSTRUCTIONS FOR LABORATORY USE

1.3.1 Materials Required

Coloured chart paper

Scissors

Coloured pens

Adhesive tape

1.3.2 Procedure

Displaying instructions /rules is very important for good management, convenience, ease and above all the safety of a lab. Preparing a display is partly a creative exercise since it requires a little bit of artistic ability. A display should be conspicuous, legible, neat, and attractive.

Here we have listed some general laboratory safety guidelines. Choose any 7 instructions that you consider more important than the rest for the display. Cut a chart paper of an appropriate size, write the instructions neatly using a colour pen that can highlight them. Try to make the instructions attractive without missing the purpose. When the instructions are properly written on the chart paper, display them at an appropriate place.

1. Keep the lab scrupulously clean and free of unnecessary things.
2. While entering the lab, place all books, notebooks and purses in the designated areas and not on the working benches.
3. Do not eat, drink or smoke in the laboratory.
4. Do not make a noise in the lab.
5. Do not wear short or loose clothes in the lab.
6. Always wear a lab coat.
7. Wear lab coats, safety glasses, gloves or other protective devices whenever necessary.
8. Wear disposal gloves whenever necessary.
9. Do not perform unauthorised experiments.
10. Do not use equipment without reading the instructions.
11. Report all spills and accidents to your instructor immediately.
12. Never leave heat sources unattended.
13. Keep containers of alcohol, acetone, and other inflammable liquids away from flame.
14. Leave the laboratory clean and organised for the next batch of students.
15. Wash your hands with soap and disinfect them with a liquid sanitizer prior to leaving the laboratory.
16. Upon completion of laboratory exercises, place all discarded materials in the disposal area designated by your counsellor.

17. Do not allow any liquid to come into contact with electrical cords. Handle electrical connectors with dry hands. Do not attempt to disconnect electrical equipment that crackle, snap, or smoke.
18. Wash skin immediately and thoroughly if exposed to chemicals or micro-organisms.
19. Never pipette by mouth. Use mechanical pipetting devices.
20. Use bandage on cuts or scrapes on the skin before attending the lab.
21. Do not taste the chemicals.
22. Do not lick the labels.
23. Decontaminate work surfaces after any spill of potentially dangerous chemicals/micro-organisms
24. Turn off gas, electricity and water before leaving the lab.

1.4 ACTIVITY 3: TO LABEL SPECIMENS, SLIDES AND REAGENTS

In this activity we have given you the type of information that should be put on the labels of biological specimen jars, slides and reagent bottles. For each of these prepare **two** labels so that they can be pasted on two sides of the specimen jars, slides and reagent bottles.

1.4.1 Labels for Specimens

Biology laboratories have a collection of a variety of plants and animals. In Unit 5 of CLT-102 you have learnt how to collect and preserve biological specimens. After the collection it is essential that such specimens are appropriately and attractively labelled. Likewise, certain slides of tissues, organs or micro-organisms prepared by the teacher or students may be essential or valuable for permanent record. These need to be appropriately labelled for proper identification.

Fig. 1.1 shows specimen jars with labels. All such labels should carry the following essential information:

1. Common name of organism
2. Kingdom
3. Phylum
4. Class
5. Order
6. Family
7. Genus
8. Species
9. Place from where it is collected (optional)
10. Name of the collector, date

Example of a Label

Sea cucumber

Kingdom – Animalia

Phylum – Echinodermata

Subphylum – Echinozoa

Class – Holothuroidea

Genus – *Holothuria*

Collected by: Neha Dhyani – 30/3/2016

Now prepare a label for any two specimens of your choice.

Fig. 1.1: Labelled animal specimen jars.

1.4.2 Labels for Slides

Slides should carry the following information (Fig.1.2):

1. The name of the organism – if the whole organisms is mounted then the slide can be marked WM = whole mount or E = entire. Examples : *Plasmodium vivax* WM and Frog blastula WM
2. The part of the organism used. Examples : Liver of Frog and Onion root tip.
3. The type of preparation, whether permanent or temporary like smear or squash. Example: Squash of onion root tip and human blood smear.
4. Type of section whether TS = transverse section or VS = vertical section or LS = Longitudinal section. Examples: TS of kidney and VS of phloem.
5. Other specifications like type of cell division (mitosis, meiosis), stage of cell division (prophase/metaphase/anaphase/telophase).

6. The following information is desirable but not essential:

   Stain(s) used: Example H.E (Haematoxylin, eosin).

   If the slide is prepared 'in house' then it should be:

   Dated

   Initialled

It is common to use two labels, one on each end of the slide. Self-adhesive or gummed slide labels pre-printed with lines are available from lab suppliers. You will find it easier to write the label before you stick it on the slide and remember if the label is gummed (rather than self-adhesive) you must not lick it, instead use a wet sponge.

Prepare at least one label and stick it to a slide.

Example of a Label

Onion root tip,
squash preparation,
Mitosis: prophase stage
Prepared by: Charu Gupta
30th March, 2016

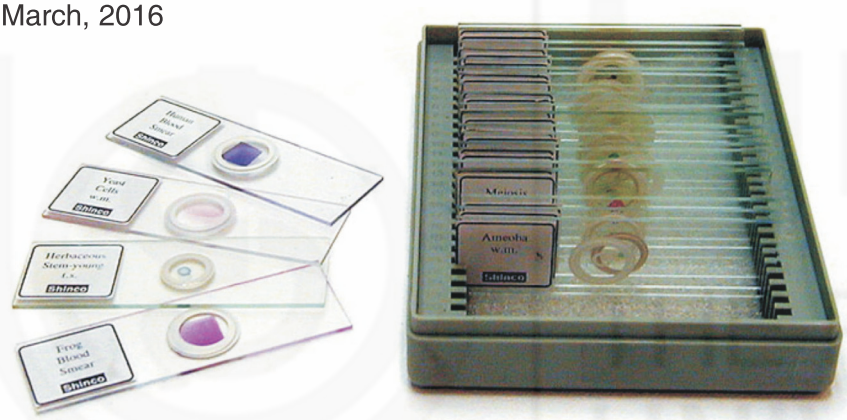


Fig.1.2: Showing labelled glass slides.

1.4.3 Labels for Reagents

We also need to routinely label prepared stock solutions, stains and culture media for identification. These labels should carry the following information:

Name of the Chemical(s)

Percentage/molarity

Aqueous/any other solvent

Date

Initial

Example of a Label

5% $CuSO_4$ (aqueous)

Prepared by: Deepmala,

1/4/2016

EXPERIMENT 2

TECHNIQUES USED FOR STERILIZATION OF COMMON LABORATORY EQUIPMENT

Structure

2.1 INTRODUCTION

Each science lab i.e. Physics, Chemistry and Biology is equipped with equipment as practicals are essential components of science. In this Experiment, you will study about the equipments which are necessary for a biology lab. A lab technician should know about various types of equipments and their uses. As a lab technician you should also know about the working and maintenance of equipment.

Expected Learning Outcomes

After performing this experiment, you should be able to:

- use a pressure cooker and autoclave for sterilisation;
- operate hot air oven and water-bath; and
- operate laminar air flow system.

2.2 MATERIALS

Autoclave

Pressure cooker

Hot air oven

Incubator, Water bath

Laminar air flow

2.3 PRESSURE COOKER

In Unit 2 of CLT-102, you studied the working principle of a pressure cooker. In this experiment you will study about the procedure to use a pressure cooker.

Procedure

1. Pour 300 ml water into a cooker.
2. Place the container with materials to be sterilized inside the cooker and close the lid.
3. Lock the cooker making sure that it is sealed properly.
4. Place the cooker on a burning stove.
5. See that the air is expelled from inside the cooker and a clear and continuous steam comes out from the vent tube. Fit the vent weight on the vent tube immediately.
6. A hissing sound is heard in about 5 minutes and the vent weight (pressure regulating device) will produce a whistle. If there is no hissing sound after 5-7 minutes and the steam is seen escaping around the rim, it means the lid has not been properly fitted. In this condition take the cooker off the stove, adjust the lid with thumbs pressing down slightly.
7. Now the steam will lift the vent weight and will be expelled from the vent tube producing a loud hissing sound. This will indicate that the full cooking pressure (sterilizing pressure) has been reached.
8. Allow the cooker to remain on the stove for atleast 15-20 minutes for sterilization.
9. Later, take the cooker off the stove and allow it to cool of its own for a sufficient period without removing the lid or vent weight (Fig. 2.1).

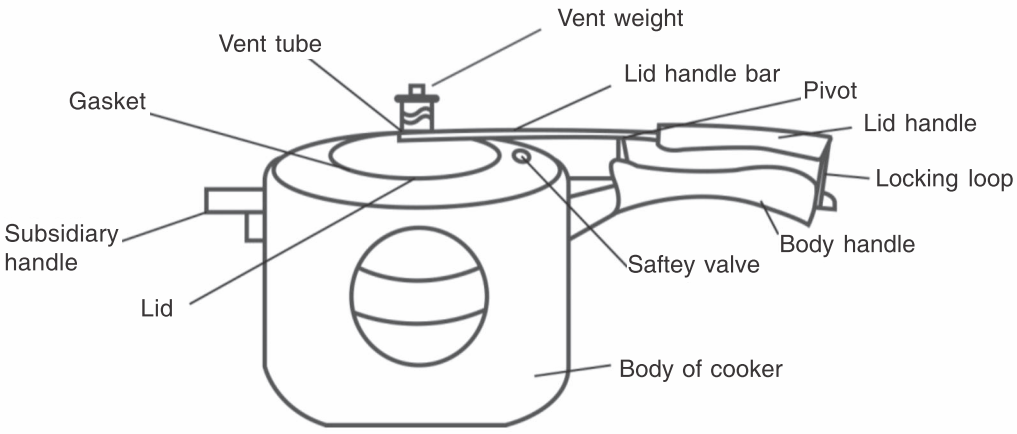


Fig. 2.1: Pressure Cooker.

2.4 AUTOCLAVE

These days sterilization is very conveniently done in the laboratories with the aid of an autoclave. These are of different types such as: (1) *Simple autoclave* (2) *Steam – jacketed autoclave* and (3) *Automatic autoclave*.

You have studied the structure and design of autoclaves in Unit 2 of CLT-102 course. In this experiment you will know how to use an autoclave.

2.4.1 Simple Autoclave

The following steps are to be carried out at the time of using an autoclave:

Procedure

1. Pour a sufficient amount of water in the autoclave.
2. Set the safety valve, put the articles to be sterilized in the container and load it inside the autoclave.
3. Open the steam outlet for passing it out.
4. Lock the door by tightening the bolts diagonally.
5. Set the autoclave pressure.
6. Place the autoclave on heat source ensuring that heat is maximum.
7. See that air is expelled from within the body of the autoclave and a continuous stream of steam comes out from the steam outlet. Now shut the steam outlet.
8. Allow the autoclave to come to the required pressure and adjust the amount of heat so that the needed pressure is maintained and then time it.
9. Let the materials remain at a necessary pressure for a definite time. Later cut the heat source and allow the autoclave to cool.
10. Open the steam outlet when the needle reaches zero in the pressure gauge.
11. Before opening the door of the autoclave and taking out articles which were sterilized, let the autoclave cool down for a considerable time (complete cooling is necessary).

Precautions

1. Do not open the valve before zero is reached because the boiling liquid will dampen the cotton wool plugs and there will be chances of contamination by bacteria which may enter through the moisture film upto the medium.
2. Do not open the valve when a vacuum has developed within because the air will rush suddenly inside the autoclave and will carry loose fitting plugs into the autoclave.
3. Do not allow the vacuum to remain for a long time because it will take out moisture from the medium kept inside the autoclave for sterilization.

2.4.2 Steam-jacketed Autoclave

Procedure

1. Allow circulation of steam through jacket continuously at a required pressure.
2. Put the articles inside the autoclave when the jacket has attained a working temperature.
3. Shut the door, lock it in position and see that discharge outlet, provided at the bottom of barrel, is open.
4. Close the discharge outlet when required temperature is reached. (In automatic jacketed autoclaves the discharge outlet is thermostatically controlled and automatically closes, when predetermined temperature is reached). Any drop in temperature due to condensation of steam during sterilization helps in opening of outlet. The condensed steam goes to waste allowing the fresh steam to enter inside the barrel and predetermined temperature is reached again.
5. Allow steam to enter from steam jacket through baffle (a device to restrain the flow of stam) fitted at the back of barrel.
6. Count the time needed for sterilization after required temperature has been reached and discharge outlet valve has closed (15-20 minutes are needed for sterilization of media).
7. Cut down the supply of steam to barrel after 15-20 minutes of sterilization and allow it to cool.
8. Open the door of autoclave when the pressure inside has become equal to atmospheric pressure and take out the sterilized articles (Fig. 2.2).

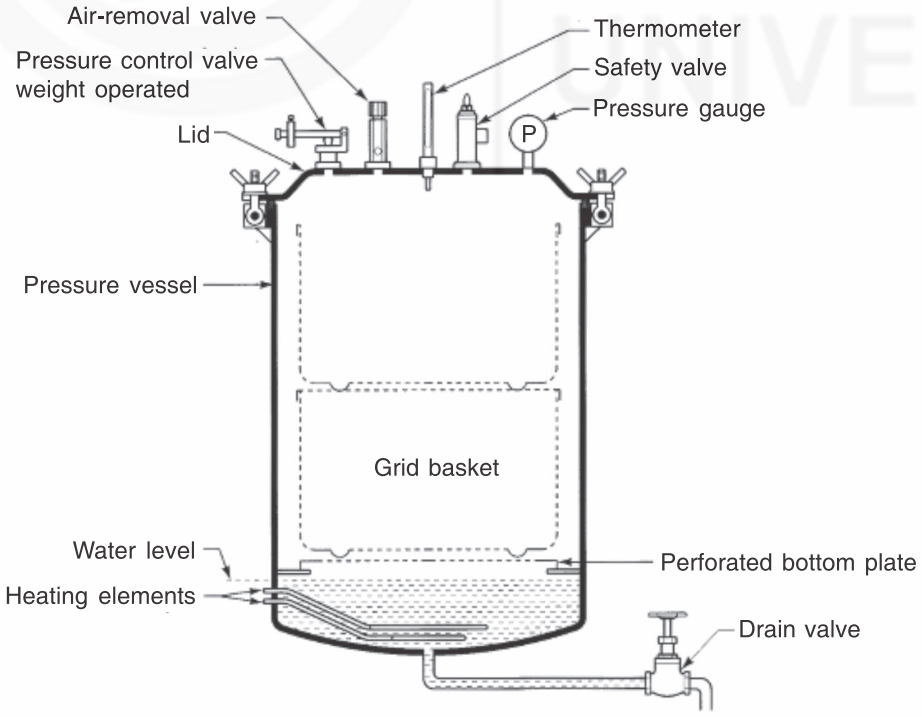


Fig. 2.2: Diagrammatic representation of simple autoclave.

2.5 HOT AIR OVEN

You have studied about the structure of a hot air oven in Unit 2 of CLT-102 course. In this experiment you will learn how to use one. Fig. 2.3 shows a hot air oven and its working mechanism.

Procedure

1. Sterilize only dry petridishes singly or together in a metal can. If a metal can is not available, wrap the petridishes with paper. Do not bring out the sterile petridishes from can or unwrap them till they are used.
2. Flasks and tubes should be dried and plugged with cotton wool before sterilization. The tubes should be placed together in iron, steel container or heat-resistant glassware e.g. enamel tray, test tube stand and beaker.
3. Pipettes should be plugged with cotton wool at the mouth end after air drying and placed in an oven in a closed metal cylinder.
4. Do not exceed the prescribed temperature.
5. Do not overload. These may prevent air circulation and the glassware may not be properly sterilized.
6. Allow the temperature to rise upto 160°C and continue sterilization at this temperature for one hour.
7. Do not open the door of the oven immediately after the sterilization because glass may develop cracks due to sudden fall in temperature.
8. Use a towel to remove the glassware.

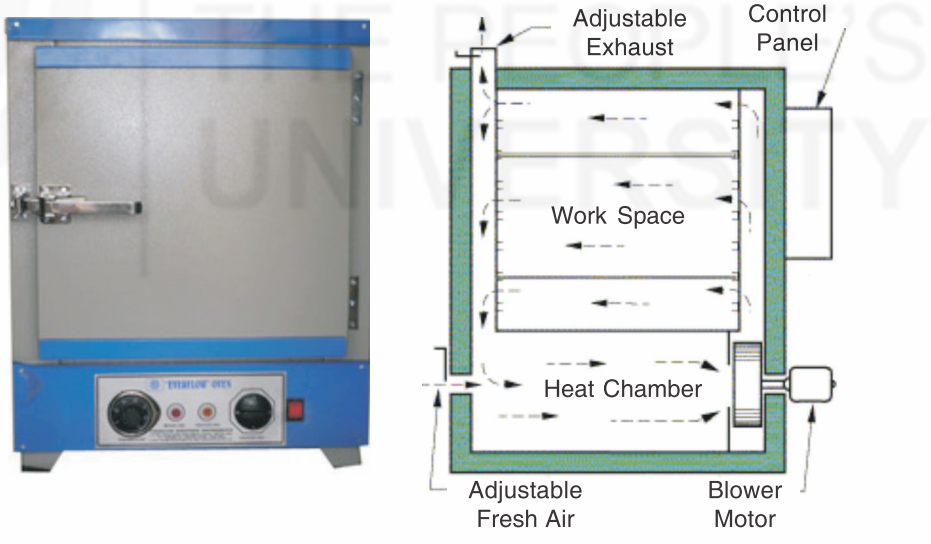


Fig. 2.3: a) Hot air oven, b) Working mechanism of hot air oven.

2.6 INCUBATOR

It is an electrically operated equipment designed to provide a controlled temperature for the growth and development of microorganisms in culture media. Its construction and operation are more or less the same as those of a

hot-air oven. Only the operational range of temperature is lower in an incubator which lies between room temperature to a temperature of 50°C (Fig. 2.4)

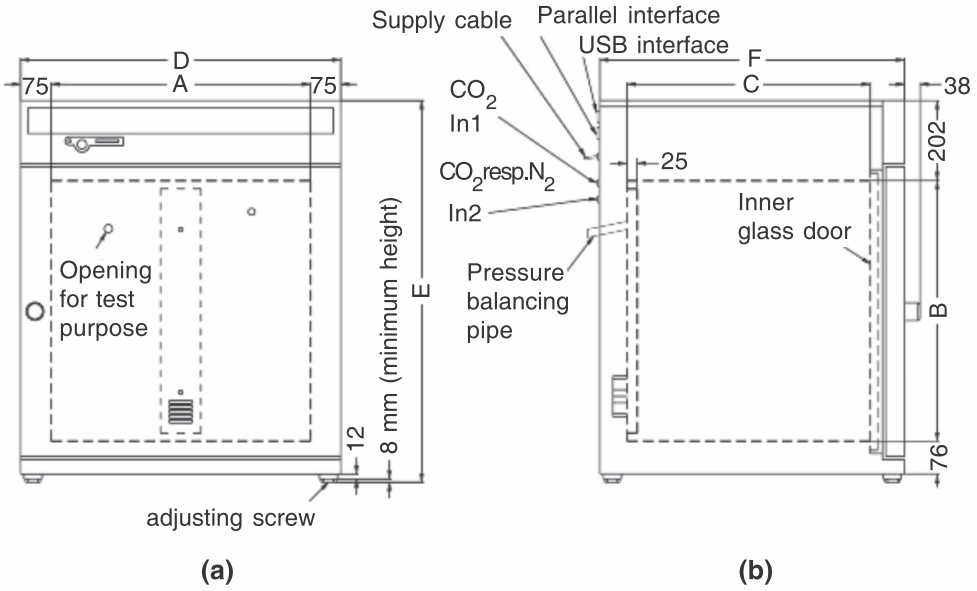


Fig. 2.4: a) Diagrammatic representation of an incubator b) Working mechanism of an incubator.

Incubators, hot air ovens and water baths require accurate temperature control. Required temperature in such apparatus is maintained by a thermostatic system.

2.7 WATER BATH

The liquid contents of tubes or flasks kept in a water-bath are raised to the required temperature much more easily and much more rapidly than in an incubator. The difference in the level of water in the water-bath and that of the liquid in the tube or flask causes a convection current which makes the liquid of the tube mix well and hasten reactions. A water-bath is equipped with thermostat, stirrers and cooling device. It is advisable to use distilled water in a water-bath to avoid chalky deposits on tubes.

2.8 LAMINAR AIRFLOW SYSTEM

Handling of microorganisms under a bacteriological safety cabinet may not always be suitable for many practical reasons.

To keep such a room free of all microbe carrying particles, a new kind of technology has been developed. This technique is known as laminar airflow technique. In this technique, air of a closed room or cabinet is allowed to pass through a high efficiency particulate air (HEPA) filter pack and the filtrate becomes free of all particles above 0.3 μm dimension. The technique involves sucking in room air and blowing it out through a pack of filters with uniform velocity and in parallel flow line used in microbiological and pharmaceutical laboratories and in aerospace industries.

The advantage of the system is that in an operation involving inoculation, transfer of culture and in opening of lyophilized culture, no closed chamber is necessary, instead, the operation can be done on a platform provided with the laminar airflow unit making it easier for handling material (Fig. 2.5).

Fig. 2.5: Laminar airflow unit.

2.9 SELF ASSESSMENT QUESTIONS

1. How will you use a simple autoclave?
2. What precautions should be taken while using hot air oven?
3. What is the correct procedure to use pressure cooker?

EXPERIMENT 3

PROCURING PLANT MATERIAL AND BIODIVERSITY SURVEY

Structure

3.1 INTRODUCTION

Laboratory work is an integral part of learning science. In biological sciences living or preserved organisms have to be provided for the study of anatomy, physiology, taxonomy etc. As lab technical staff, you will have to supply various plant materials to the students for experiments, for this you have to collect lower and higher plants from the field. In this experiment we are going to describe all the techniques which will help you in collecting plants. For collecting the plants you should know what type of bottles, fixatives, solutions and equipments are needed. You can start by collecting the plants with the teacher who will help you to identify them. In due course of time you will also be able to perform this activity independently.

Expected Learning Outcomes

After studying this experiment you will be able to:

- grow root tips of a few plant species for squash preparation;

- fix the root tip;
- estimate plant diversity through quadrate method;
- collect specimens of algae, bryophytes, pteridophytes and gymnosperms; and
- collect, press, dry mount, label, store, and preserve angiosperm specimens to make a herbarium.

STUDY GUIDE

Before starting this experiment you should study Unit 5 and Unit 6 of Block 2 of CLT-102.

3.2 GROWING OF MATERIAL FOR SQUASH PREPARATION

The root tips may be obtained by allowing seeds to germinate on a wet blotting paper disc or/sand free from soil and debris. The seeds of *Medicago* (Alfa-alfa) have been found more suitable because these germinate soon and their chromosomes are also bigger in size. We generally grow root tips from onion so we are going to discuss both of them one by one.

3.2.1 Material Required

Onion bulbs

Seeds of *Medicago* (Alfa-alfa)

Coplin jars or wide mouth bottles

100 ml beakers

Scalpel

3.2.2 Procedure

1. Soak Alfa-alfa seeds overnight and then place them in sterilized wet filter paper cover them and leave them to germinate.
2. Cut the root tip, as soon as they are visible between 7-9 a.m. because during this time the cells are actively dividing and the chromosomes also duplicate and separate at the same time.
3. These root tips are ready for squash preparation.

Onion Bulbs

1. Take an onion and scrape off the dry roots from the bulbs to expose the disc.

2. Fill a coplin jar with tap water and place the onion bulb on it in such a way that the disc touches the water.
3. Place this near the window to get enough light for three to four days. Roots will start growing and the roots tips can be clearly seen.
4. The technique for squash preparation will be explained to you later in Experiment 7 of this course.

3.3 BIODIVERSITY SURVEY

Biological diversity or biodiversity is that part of nature which includes the differences in genes among the individuals of a species; the variety and richness of all the plant and animal species at different scales in space – locally, in a region, the country and the world; and the types of ecosystems, both terrestrial and aquatic, within a defined area.

We human derive food, shelter, several medicines and industrial products from biological diversity, apart from these it also provides ecological services that makes life livable on earth. Biodiversity also plays an important role in sustaining environment.

Biodiversity surveys are undertaken to find out what organisms/plant exist in a given area. The data that is gathered from these surveys can be used for numerous purposes.

Here we are going to describe how you can estimate plant population density by quadrate method which is used most commonly in ecological studies.

3.3.1 Material Required

Cotton thread or nylon thread (five meters)

Four nails

Hammer

3.3.2 Procedure

1. First you should select an area for the study. Then make a square of 1m×1m with the help of thread and nails, the quadrate is ready. You should see that nails are firmly placed on the ground. You should be cautious in laying quadrate so that vegetation is not damaged.
2. Now list the names of the plant species which you can see in the quadrat if, you do not know the name of the species mark them as species A or B etc. If the same species is seen in another quadrat assign them the same alphabet.
3. Count the numbers of individuals of each species present the quadrat and record the data as shown in the Table 3.1 which is given as an example.

Table 3.1: Density studies of the given vegetation

Plant species	Number of quadrats and No. of individuals in each quadrat					Total No. of individuals (S)	Total no. of Quadrats studies (Q)	Population Density (D)
	I	II	III	IV	V			
A	4	2	3	5	10	24	5	24/5=4.8
B	3	1	4	10	3	21	5	21/5=4.2

4. Now make four more quadrats randomly at the site of study and record the names and number of individuals of each species.

Density values are significant because they show relative importance of each species. The results can also help in conservation of plants of the surveyed area.

3.4 COLLECTION OF LOWER PLANTS

The collection of plant material is a simple job but one should take much care of collected plants or their parts so that they are preserved without any damage. The plants may be collected in vasculum, polythene bags or in bottles. You will need a pair of secateurs which are special type of scissors for cutting hard material, a sharp knife for cutting soft parts, pick for digging out underground parts like roots and rhizomes, scalpel and forceps for separating those plants which grow attached to the barks of trees and rocks.

The stems and roots are cut into pieces of size about 3 cm long with the aid of sharp razor or knife so that the tissues at cut ends do not get macerated. Bryophytes are made free from soil particles and debris before storing in some preservative. The smaller leaves can be preserved as such and larger ones can be cut in pieces, and then preserved.

We will now describe how to collect algae, bryophytes, pteridophytes and gymnosperms specimens one by one.

3.4.1 Algae

Sources: The algae occur widely in nature viz. on the soil surface and below it, on the bark of trees, in fresh water, sea water, and a variety of other habitats.

Collection from Bark

i) In case of bark algae you should pick up the algal patches from the tree trunk with the help of iron spatula.

ii) Before picking the algal patches you should sterilize spatula by swirling it in spirit and then flaming it to avoid contamination of any kind.

iii) Store various samples collected in separate sterilized bottles after fixing and labeling in their designated lab shelves.

Collection from Fresh Water

i) Collect the fresh water algae at the spot in sterilized specimen tubes containing some habitat water.

ii) Never fill the container more than a quarter of its capacity with half water from which alga is collected keeping the last quarter free for aeration, thus avoiding suffocation.

iii) Fix the material, label them and keep in designated place in the lab.

Collection from Sea Water

i) In India western coast is best for marine algae collection during low tides of winter season as during this period these are mostly in their reproduction stages. By collecting algae in this season you will get reproductive organs as well.

ii) Collect marine algae in large bottles along with sea water.

iii) Fix it and label them and keep them in their designated shelves in the lab.

3.4.2 Bryophytes

Now we will describe collection of bryophytes. Normally bryophytes occur in nature attached to wet soil, rocks, and bark of living and dead trees, wood and humus rich organic substances.

- Scrape the bryophytes from the place of occurrence with the help of a sharp scalpel or knife and keep them in polythene bags within which they remain alive for a number of days.
- Keep these bags loosely tied and in damp condition in laboratory.
- Wash the soil from the sample species with ordinary water to remove soil particles and dirt attached to plant.
- Keep the bags under illumination at 0°C - 5°C to keep the plant alive for a longer duration.
- Fix the material, label it and store it.

3.4.3 Pteridophytes

Pteridophytes are commonly known as vascular cryptogams, these are spore-producing vascular plants. They possess the vascular tissues: xylem and phloem. They grow in variable habitats. Most of the pteridophytes are terrestrial which thrive well in moisture and shade, while others are found growing in xeric conditions. A few are epiphytes and some of them are also found in aquatic habitats.

- Collect the pteridophytes from natural habitats in mature spore producing stage.
- Collect the plants with or without strobili or mature sporophyll in polythene bags loosely tied at mouth.

- If the material is large cut them into pieces, fix, label and keep them in a jar before storing.

3.4.4 Gymnosperms

Gymnosperms belong to seed plants but the seeds are naked with a very conspicuous and independent sporophyte which is the main plant and have very reduced gametophyte dependent on the sporophyte. They also have xeric characteristics.

i) Collect the root, stem, and leaves, male and female gametophytes of the plant.

ii) Cut the material into small pieces of 3 cm, fix and label them before storing them

iii) You can collect the dry fruits and cones of gymnosperms and preserve them as such.

3.5 COLLECTION OF HIGHER PLANTS FOR MAKING A HERBARIUM

In this section you will study about preserving plants for a herbarium because as a technician you have to make and maintain a herbarium. Some plants are also grown in a botanical garden or specially maintained in green house for this purpose. Before doing this exercise you should study Unit 6 of this course (CLT-102).

3.5.1 Collection of Plants

1. You should remember one important thing about making plant collection **– Do not pick plants haphazardly**. Choose only those plants whose flowers are large and whose organs are easily seen.
2. If possible collect complete plants with flowers and fruits (sometimes they are necessary for recognising species of some families such as Cruciferae, Umbelliferae, Asteraceae).
3. The underground parts (root, bulbs, and rhizomes) are often interesting. Try to collect them. If the plant is large take just a branch with leaves, flower and fruits.

3.5.2 Carrying the Specimens

1. Carry each plant specimen in separate bag or container.
2. Place the plant flat between newspapers and place in a cardboard folder and fastened in a strap or keep the plant in the vasculum as you have already read in Unit 6 of this course.
3. Attach a label to each plant stating details of its habitat, date, name of place, name of collector, flower colour and any other interesting feature.

3.5.3 Examining the Plant

1. Identify the name of the plant with the help of your counsellor and in due course of time you will be able to name the plant by yourself.
2. You can also keep a plant with some labelling with it and it could be identified with the help of different Floras.
3. Make a few sketches to point out the characteristics which helped you in identification of the plant.

3.5.4 Drying

1. Spread each plant carefully in between blotting paper or newspaper (Unit 6, Section 6.5 of CLT-102).
2. Then place the plant in the plant press with its label.
3. After a few days (the next day, if the plants are very moist) check that the plants are properly laid out and change the paper.

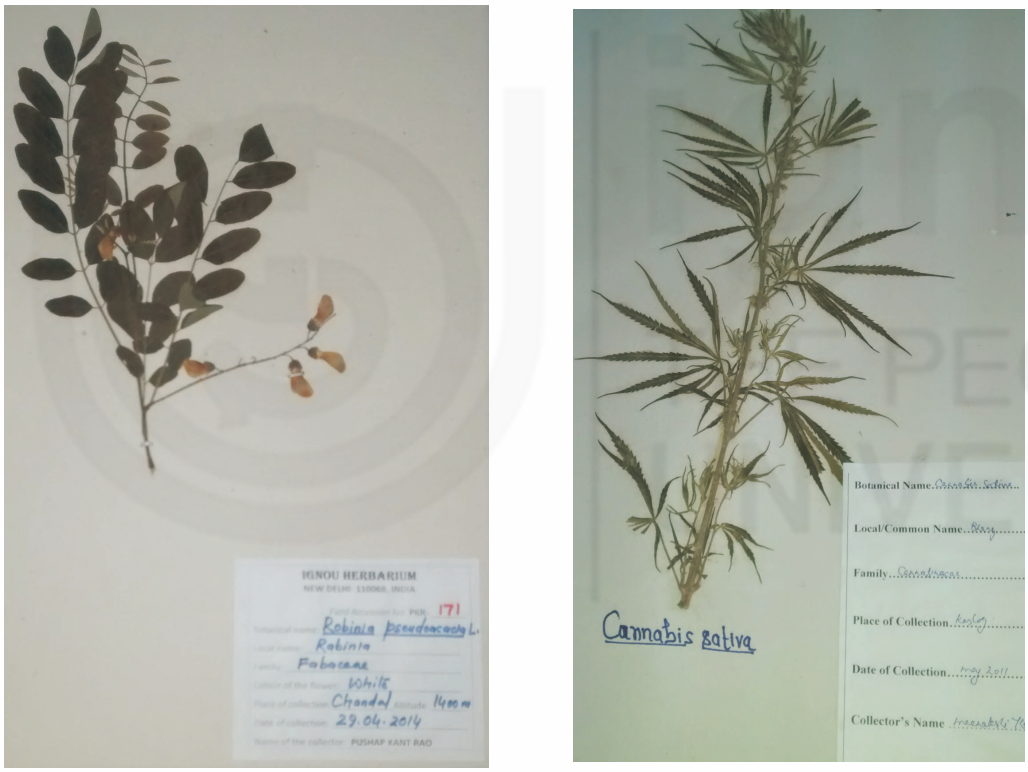


Fig. 3.1: Mounted specimens.

4. Check the specimens and if they are properly dried, place them in your herbarium.
5. Place the plant on a herbarium sheet (a fairly large piece of special paper or on any fairly thick paper), fix the dried plants in their natural position with some cellotape.
6. If the plant is too big, break the stem cleanly or arrange fragments of the leafy stem, the floral stem and the fruits.

7. Rewrite the label which will be placed in the bottom right hand corner of the page as given below. Copy out the notes you have already made on it. (It is not necessary that you should identify the family and name of a plant). The name and family of the plant can be identified with the help of the counsellor at your study centre.

   i) *Heading* : Country, state or province name, name of the institution.

   ii) *Scientific name* of the specimen followed by the author's name and the name of the family.

   iii) *Locality:* Specific locality should be mentioned, so that if another person wants to collect the same specimen, he should be able to reach the exact site without much difficulty.

   iv) *Habitat:* Vegetation type, moisture content of soil and atmosphere, soil type, elevation, direction of slope, etc. should also be mentioned.

   v) *Date of collection* should include the exact month and year to indicate when the specimen was collected.

   vi) *Name of collector* ……

   vii) *Collection number* : The literature on plant systematics identifies and refers to the specimen by the collector's name and collection number. Hence, the collection number is a must for any collector.

8. You can arrange the herbaria according to their habitats (aquatic, from dunes, from mountain rocks) or according to their uses (edible, medicinal, ornamental, fodder plants ……; harmful plants, poisonous plants etc.).

Acitivity 1: Collect two plants of your choice and prepare herbarium sheets. Submit these to your counselor when you come for the lab session. You will be evaluated on this work.

EXPERIMENT 4

STUDY OF PHYSIOLOGICAL PROCESSES IN PLANTS

Structure

4.1 INTRODUCTION

Certain experiments require special equipment and are tedious to set up. Instead of asking students to perform such experiments individually, demonstrations are set up for the class in such a way that each student can record, interpret and analyse the readings and present the results. The job of demonstrating experiments is assigned to a technician. In this experiment, you will study how to set up demonstrations for the following three physiological processes:

1. Transpiration
2. Respiration
3. Photosynthesis

Expected Learning Outcomes

After doing this exercise you should be able to:

- list and procure equipments and materials required to set up a given demonstration;
- set up the demonstrations for transpiration, respiration and photosynthesis;

- improvise suitable alternative equipment and material for these demonstrations, if need be;
- identify faults in the set-up and correct them; and
- help students in recording the readings.

4.2 TO SET A POTOMETER

The loss of water by a plant through stomata or other pores is called transpiration. The instrument used to show the process and measure its rate is called a potometer. In this exercise you will set up a potometer.

4.2.1 Materials Required

Potometer

250 ml beaker

Iron stand with a clamp

2 ml graduated pipette

Razor, sharp knife or scissors for cutting a branch

Rubber bung

A piece of rubber tubing (about 6 cm)

Stop watch

Eosin or any other water soluble dye

4.2.2 Method

Potometer is generally available in most labs. If it isn't, you can assemble one as shown in Fig. 4.1. To set up the equipment you should follow the steps given below:

1. Bring a small branch of a plant which has soft leaves from the field. It is important to see that the plant material used is in healthy condition; therefore while you cut the branch minimum damage is done. It is best to cut the part to be used for the experiment under water. So keep a water container ready with you and place the cut end of the twig quickly in it. Also remember to make a second cut above 2 cm or more from the end of the twig, while **under water** before you fix it in the rubber bung.
2. Fit the lower end of the branch into a non-flexible rubber tube and insert it tightly through the hole in the rubber bung. If you need to drill a hole use a cork-borer. Both the bung and the cork-borer must be kept wet all the time. You can drill better if water plus a trace of *surfactant* (any detergent) is used.
3. To set up a potometer choose a place on a clean bench close to a window, clamp it to the stand and assemble it carefully as shown in Fig. 4.1. The essential part of a potometer is a *graduated capillary tube which shows very small losses of water by transpiring leaves.*

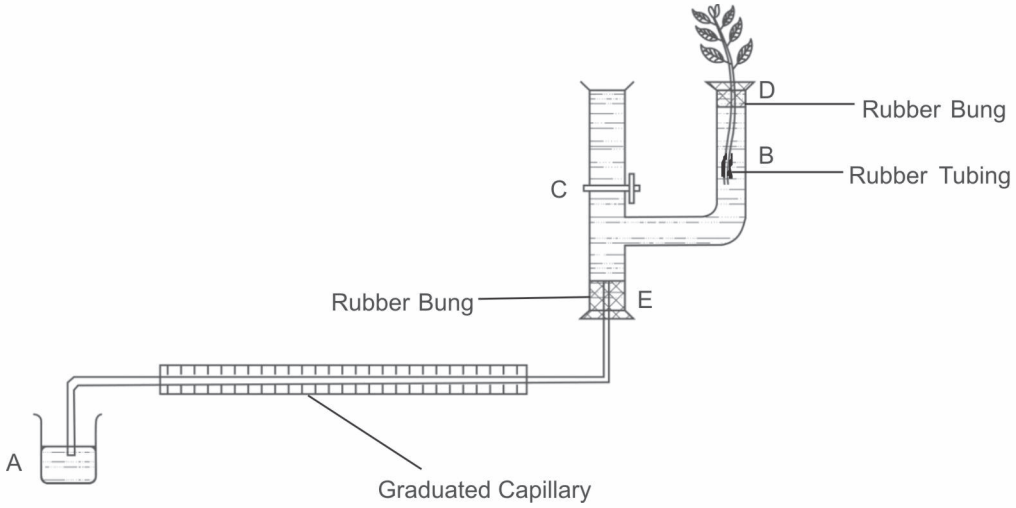


Fig. 4.1: A Potometer.

4. Pour eosin coloured water into the beaker (A). You must ensure that when the capillary is dipped in the beaker containing coloured water there isn't a trapped air bubble. Fill the tube B with tap water and then close the tap C. Also make sure that the two rubber bungs (D and E) are sealed well.

5. Now lift the capillary end out of the beaker carefully and hold it till a bubble of air is introduced. Then re-immerse it so that the bubble of air is caught. Since the twig is transpiring the bubble will gradually travel along the capillary followed by coloured water. When the bubble proceeds beyond the marked area it may be driven back by opening the tap C.

Now your potometer is ready, and you can demonstrate to the students transpiration of water from leaves, and they can measure its rate. *The rate of transpiration is measured as distance moved by the bubble per unit time*. To compare the rates under different environmental conditions place the apparatus under the fan, in light and in dark.

4.3 TO SET A RESPIROMETER

Respiration involves gaseous exchange of O_2 and CO_2 for this a manometer (*a manometer is a device for measuring gas pressure*) is used and the commercial available instrument is called Warburg manometer after the scientist who first devised it. Since this is an expensive instrument, a simple manometer also called respirometer is assembled in most labs for routine demonstrations to the students. In this exercise you will learn to assemble a respirometer to measure respiration rates.

4.3.1 Materials Required

500 ml conical flask

25 ml test tube

T - tube

Pinch clip

A rubber bung

A piece of rubber tube

1 mm diameter graduated pipette

KOH pellets

Stop-clock

Wire gauze

Thermometer

Sprouted mung bean

4.3.2 Method

1. You can assemble a simple respirometer as shown in Fig. 4.2. Take a T-tube, fix its one end in the rubber bung and slip a piece of rubber tubing and pinch clip to the other end.
2. Now fix the end with the rubber bung to a test tube.
3. Wrap 2 pellets of KOH in a piece of wire gauze and place them at the bottom of tube. Add a few drops of water.
4. Now insert carefully a wad of cotton to partition KOH from the specimen (see Fig. 4.2). Make sure that the cotton is well above the wire gauze.
5. Weigh 20 germinating mung beans and place them on the cotton wad.
6. Join a 2 ml graduated pipette to the central arm of the T with a piece of rubber tube.
7. Assemble the apparatus and place it in a 500 ml conical flask containing water at room temperature or in any other suitable water bath. Place the thermometer in it to record the temperature. Observe the marking on the 2 ml graduated pipette of manometer and calculate in μl the minimum volume change it indicates. If 2 ml is divided into 20 fractions each fraction will be equal to 100μl (1 ml = 1000 μl).
8. Loosen the pinch clip and leave it to equilibrate for 5 to 10 minutes. Then tighten the pinch clip.
9. Insert a drop of colour solution (eosin) in the pipette. It will start moving to the left. Adjust it to extreme right upto a certain mark by carefully loosening the pinch clip.
10. Now the student can start the stop-watch and take the reading at zero and after an interval of 5, 10, 15, 20 and 25 minutes and they can calculate the volume changes (distance moved by the colour drop) in minute.

The unit should be air-tight. You must ensure that when you place an organism (seeds) it must not be in contact with KOH which is corrosive.

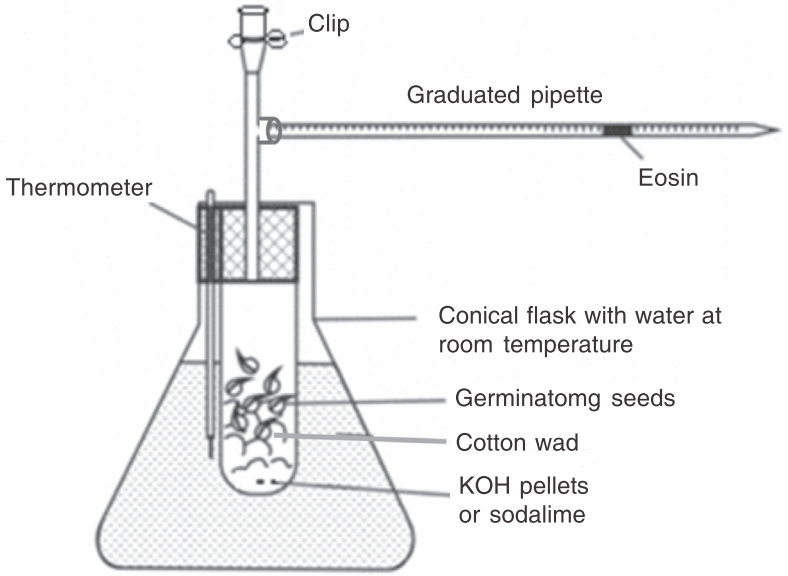


Fig. 4.2: A simple pipette manometer.

4.4 TO SET UP DEMONSTRATION EXPERIMENTS FOR PHOTOSYNTHESIS

Photosynthesis is a process by which green plants make carbohydrates, sugar and starch from CO_2 and H_2O using light energy absorbed by chlorophyll. This process can be demonstrated by evolution of gas bubbles from a leaf or the *Hydrilla* a water plant in the presence of sunlight. In this exercise you will set up this demonstration and also learn how to improvise it for measuring the effect of quantity of light.

4.4.1 Materials Required

250-500 ml beaker

15-25 ml test tube

Hydrilla plant or leaves of any terrestrial plant

Cork borer

Table lamp with 100 W bulb

Iron stand with a clamp

Stop watch

0.2% $NaHCO_3$

Green, blue and red cellophane paper

4.4.2 Method

The simple pipette manometer used for measuring the rate of respiration can be used for the demonstration of the following experiments on photosynthesis:

A. Measurement of Rate of Photosynthesis

1. Clean the manometer thoroughly and dry it before use.
2. Using a dropper pour 2 ml of 1% sodium bicarbonate solution into the tube. Make sure that it does not touch the sides of the test tube. Now insert a cotton wad into the tube to partition bicarbonate solution.
3. Cut several discs of leaves with 1 cm diameter cork borer and keep them moist in a Petri dish in the dark. Insert one piece in the tube and place it vertically on the cotton wad.
4. Assemble the apparatus and place it in a 500 ml flask containing water at room temperature. Cover the flask with black paper thoroughly. Let it equilibrate for 5 to 10 minutes.
5. Introduce a drop of eosin dye in the pipette and adjust it towards a mark to extreme right.
6. Start the stop-watch and note the readings on the pipette immediately and after an interval of 5 to 10 minutes.
7. Now adjust the black paper to make a vertical slit so that the leaf can be exposed to the light source. Keep the light source 10 cm away from the apparatus.
8. Again adjust the marker drop to the extreme left upto a mark.
9. Readings can be taken at zero time and after an interval of 5, 10, 15, 20 and 25 minutes.

B. Effect of Light Quality on the Rate of Photosynthesis

Cover the light source with green, blue or red cellophane paper. Take readings as in the previous experiment.

EXPERIMENT 5

MICROSCOPE HANDLING AND MAINTENANCE

Structure

5.1 INTRODUCTION

This experiment is based on Unit 3 of Block 1of this course (CLT-102). In this unit you have learnt that microscopes are optical instruments that provide magnified images of objects or specimens with the help of light and a set of eyepiece and objective lenses. In this experiment you would be practically handling two types of light microscopes – stereo and compound. You would learn to set-up, use, care and maintain these microscopes. As a study guide you are advised to go through Unit 3 of CLT-102 to refresh your memory and for the best learning outcomes of this experiment.

Expected Learning Outcomes

After completing this experiment, you should be able to:

- identify the different parts of stereo and compound microscopes;
- set-up and use stereo and compound microscopes; and
- explain and demonstrate the ways of care and maintenance of the stereo and compound microscopes.

5.2 STEREO MICROSCOPE

Stereo or dissecting microscope is an essential instrument in school, college and research laboratories. It is widely used in various ways and wherever the three-dimensional images of objects are required to be seen, as for example, in watch making, manufacture of circuit boards and many such precision jobs. The parts of a stereo microscope are described in the following subsection.

5.2.1 Parts

The three main components of a stereo microscope are: head, body and base (see Figs 5.1 to 5.3).

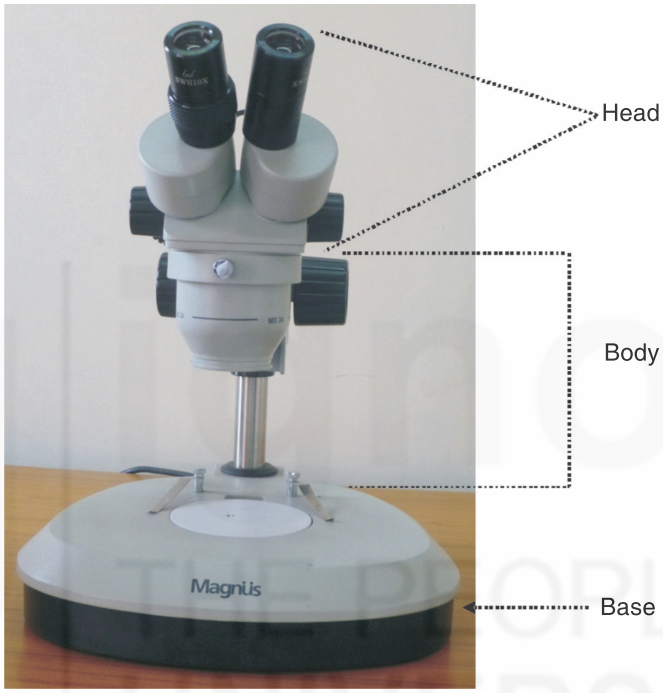


Fig. 5.1: A stereo microscope in front view.

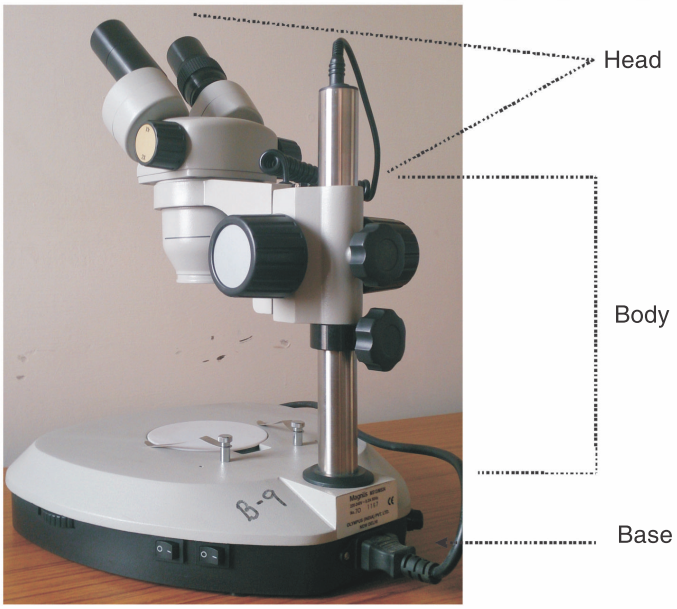


Fig. 5.2: Back side view of a stereo microscope.

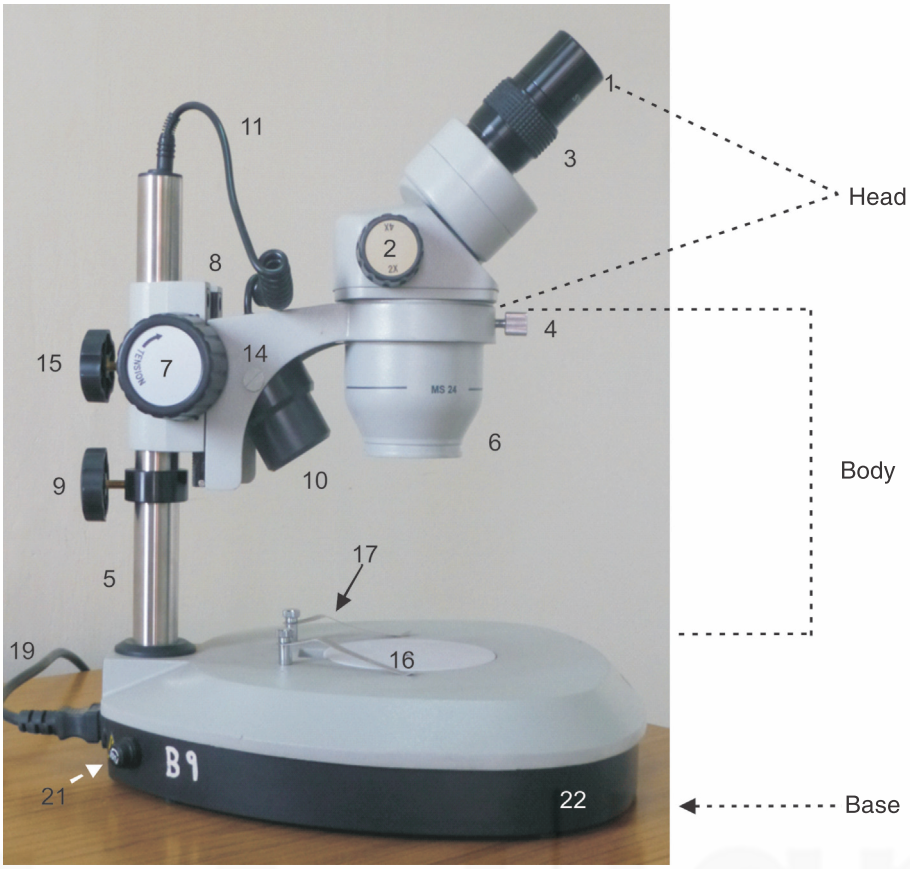


Fig. 5.3: A stereo microscope in side view.

The numbers in the figures and the text refer to a particular part of the microscope.

i) HEAD (Figs 5.3 & 5.4) comprises two ocular lenses or eyepieces (1), zoom magnification knobs (2), diopter (3), and a head-objective lens unit holding screw (4).

Fig. 5.4: A portion of the head of stereo microscope in front view showing two eyepieces (1). The eyepiece on the right hand side has a ring-like structure – the diopter (3).

ii) BODY (see Figs 5.1 to 5.3) consists of an arm (5), objective lens unit (6), focus knobs (7), rack and pinion arrangement (8, Fig. 5.5), rack stop (9), a top light (10), electric supply for top light (11), on-off switch for top light (12, see Fig. 5.6), light intensity control for top light (13, Fig. 5.6), sleeve for the head objective lens unit (14), and head-body assembly holding knob (15).

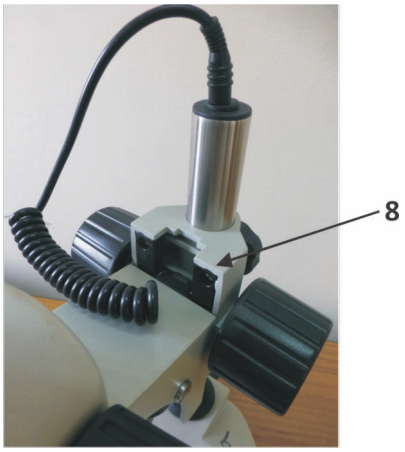


Fig. 5.5: Rack and pinion arrangement in a stereo microscope as seen from top and tilted view.

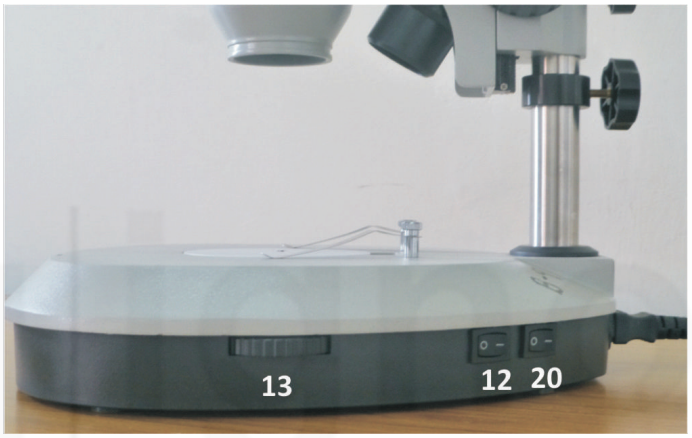


Fig. 5.6: Base of the stereo microscope in side view showing on-off switch for top light (12), on-off switch for base light (20) and light intensity control for top light (13).

iii) BASE (Figs 5.1 to 5.3) contains stage plate (16, Fig. 5.7), stage clips (17, Fig. 5.7), mirror/stage light (18, Fig. 5.8), electric supply cable (19), switch each for top (12) and stage light (20, Fig. 5.6), light intensity control for top light (13), fuse (21), and stand (22).

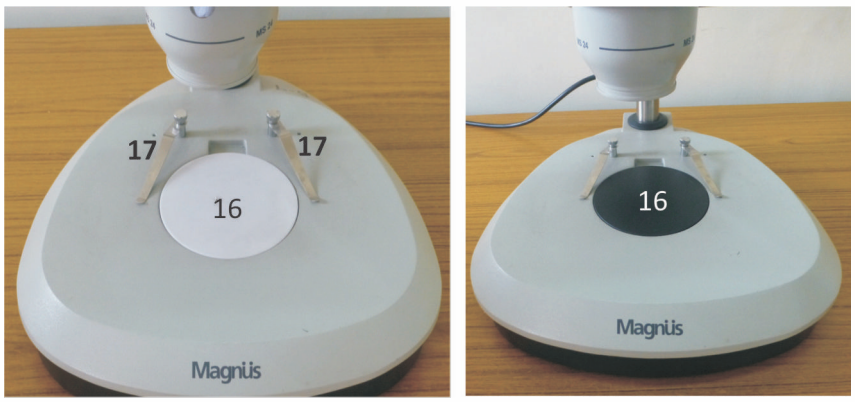


Fig. 5.7: Base of the stereo microscope in front view showing both the sides of the stage plate – white (left hand side figure) and black coloured (right hand side figure).

Fig. 5.8: Base of the stereo microscope in front view showing stage light (18).

5.2.2 Steps in Using Stereo Microscope

1. Set the microscope on a table top or a flat stable surface with some space to work comfortably.
2. Familiarize yourself with the stereo microscope and its different parts. If familiar, check its different parts. Thereafter, plug in the cable for electric supply.
3. Switch on the top or stage light or both the light sources as per requirement.
4. Centre the specimen on the stage plate. If the specimen is thin and flat or if its edges tend to curl up the stage clips can hold it in place.
5. Adjust the eyepieces so that you can look through the microscope comfortably and without straining the eyes. The diopter ring on one or both the eyepieces can be moved clockwise or anticlockwise to make up for focusing differences between the eyes.
6. Set contrast background for the object to be observed under the microscope. For light coloured translucent specimens such as salt crystals, the black side of the stage plate, if it is reversible, is the best. For darker objects the white plate would provide a good contrast.
7. Bring the specimen just below the objective lens. To determine the magnification of the microscope, multiply the magnification value of the eyepiece lens, say 2X by that of the objective lens say 10X. The magnification in this case would be 20X ($2 \times 10 = 20$).
8. For focusssing the specimen, slowly turn the focus knobs while looking through the eyepieces until the specimen comes into view. Once the outline of the specimen is visible, turn the knobs slowly clockwise or anticlockwise to have a sharp focus of the specimen.
9. After completing the use of microscope, turn off the light switch, remove the specimen, take out the electricity plug from power supply, clean the microscope with a clean soft cloth and cover it with a dust cover.
10. Store the microscope in a safe place protected from extreme hot, cold temperatures and moist conditions.

5.2.3 Care and Maintenance

Lenses in eyepiece and objective are important components of the stereo microscope. They need to be regularly cared for and maintained.

To clean the exterior side of eyepiece or objective lenses, use a non-solvent lens cleaning solution. First, remove dust on the lens with a soft brush or a small blower. Then moisten a piece of dry, lint-free tissue or lens paper and clean the surface of lens in a circular motion. Repeat with a second piece of paper moistened with lens cleaning solution if necessary. Repeat once again with a dry lens paper until the lens is clean and dry.

For cleaning the interior side of lenses, a bulb-type duster or a compressed gas canister used for cameras and other optical instruments is utilized. Do not use the procedure employed for cleaning the outer side of the lens in this case. Take the help of a specialist for this purpose. To clean the body of the microscope a clean, soft, dry or damp cloth is used.

After cleaning the microscope, cover it with a dust cover to keep it clean and dust free.

5.3 COMPOUND MICROSCOPE

Compound microscopes are high power microscopes with objective lenses of varied magnification values and one or two eyepieces. As compared to stereo microscope the compound microscope provides images of specimens at much higher magnifications and with clarity. The microscope with single or two eyepieces are called as monocular (Fig. 5.9) and binocular (Fig. 5.10)

Fig. 5.9: A monocular compound microscope in side view.
Source: https://www.google.co.insearch?q=student+microscope&biw.

compound microscope respectively. A binocular microscope having a port for camera at the top is referred to as trinocular microscope. The modern compound microscopes have digital cameras and are connected to desktop computers, televisions and printers. These are known as digital microscopes.

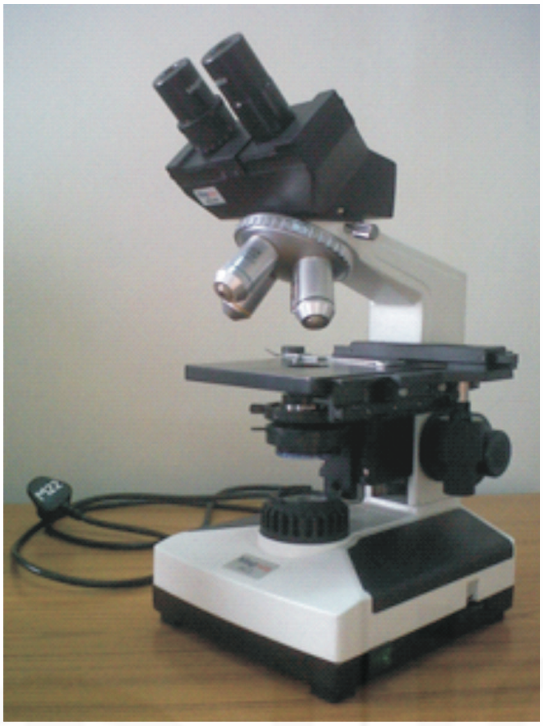


Fig. 5.10: A binocular compound microscope in angular view.

5.3.1 Parts

A compound microscope is typically composed of a head, body and base (see Fig. 5.11).

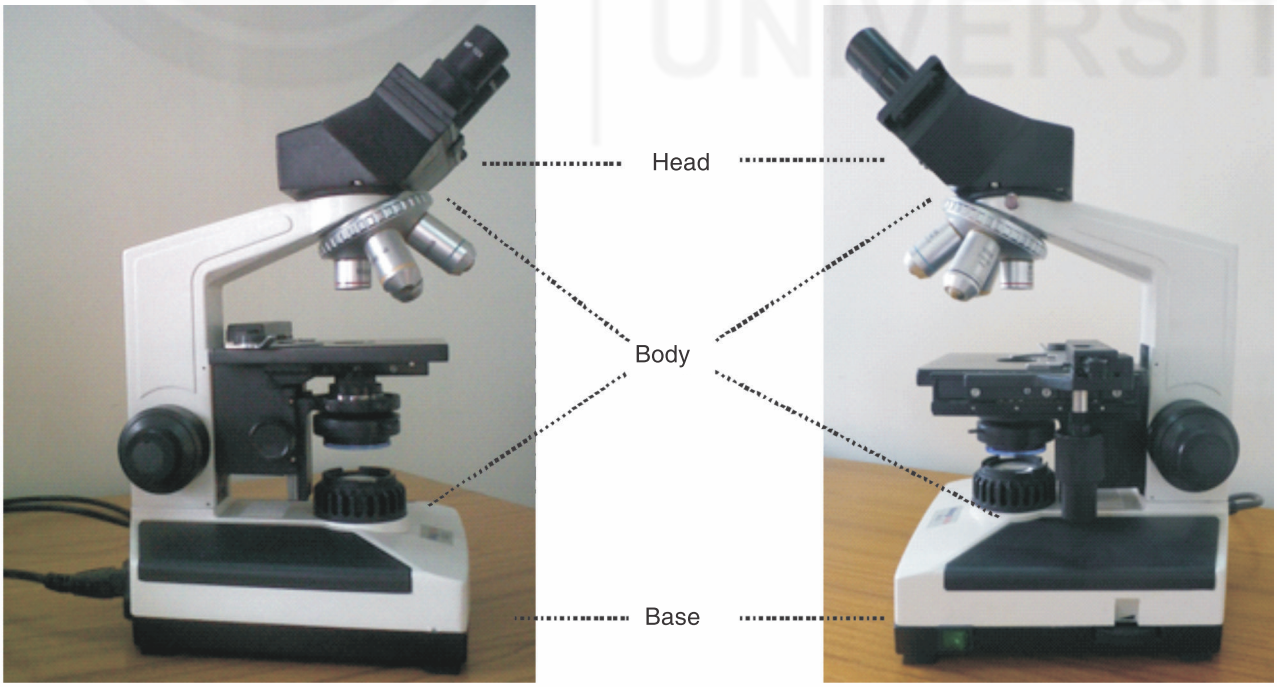


Fig. 5.11: Side views of a binocular compound microscope. The microscope is composed of three main components – head, body and base.

i) HEAD comprises of one or two ocular lenses or eyepieces (1), screw for fixing the head to the microscope body (2, Fig. 5.12), ocular slide (3, Figs 5.14 & 5.15) and diopter (4, Fig. 5.13).

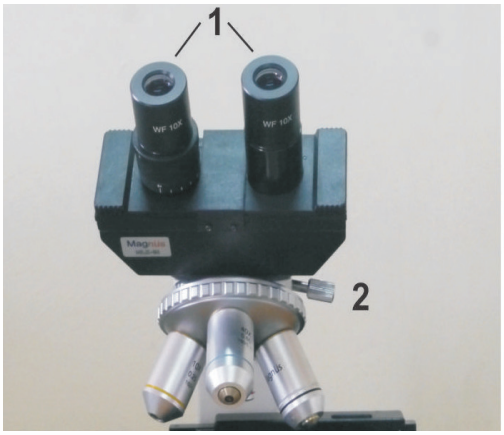


Fig. 5.12: The head portion of the binocular microscope in front view showing two eyepieces, screw for fixing the head to the microscope body.

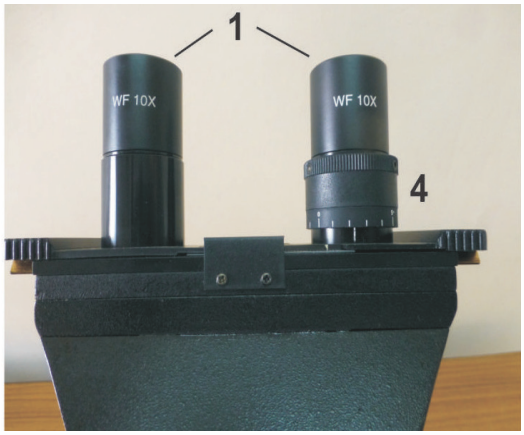


Fig. 5.13: A portion of head of binocular compound microscope in front view showing two ocular lenses. The ocular on right hand side has a ring-like structure known as the diopter.

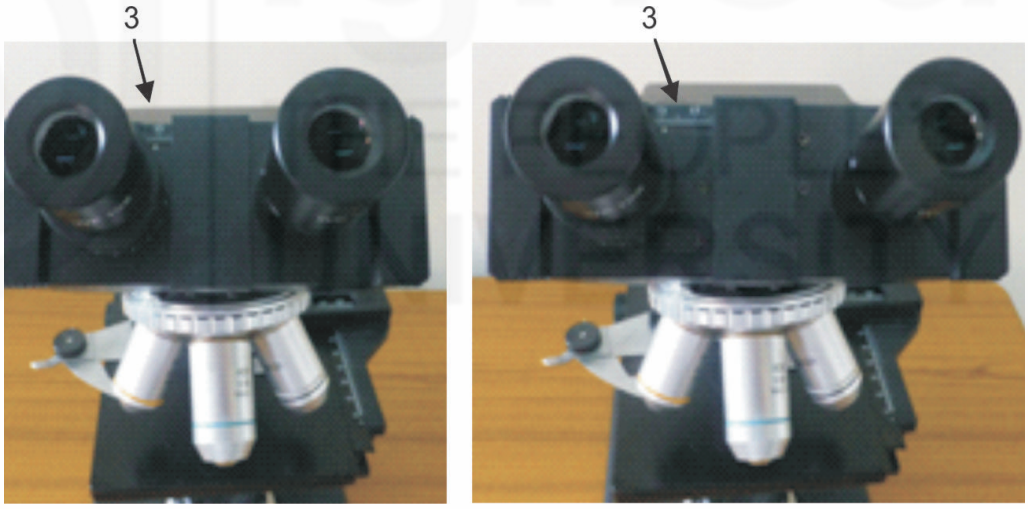


Fig. 5.14: A portion of head of binocular compound microscope in front view showing an ocular slide in a closed position (left-hand) and in opened position (right hand).

ii) BODY (Figs 5.15 & 5.16) of the compound microscope consists of an arm (5), turret with objective lenses (6), stage (7), stage clip (8), slide moving knobs (9), condenser lens assembly (10, also see Fig. 5.17 and 5.18), stage moving or coarse focus knob (11), fine focus knob (12), rack and pinion arrangement (13), and rack stop (14).

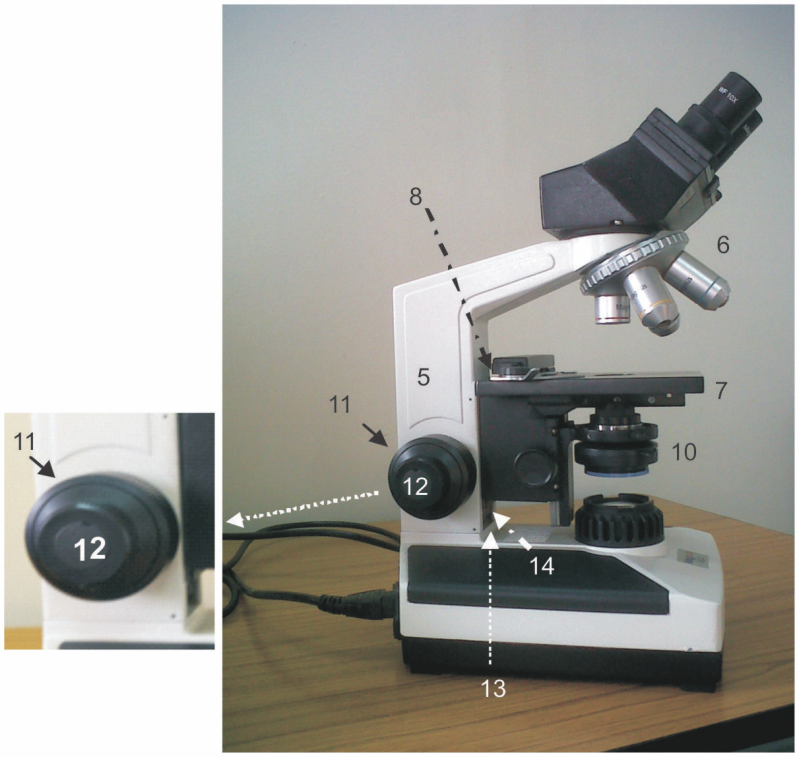


Fig. 5.15: A binocular compound microscope in side view showing arm (5), turret with objective lenses (6); stage (7), condenser lens assembly (10), coarse focus knob (11, also see its enlarged view) and fine focus knob (12).

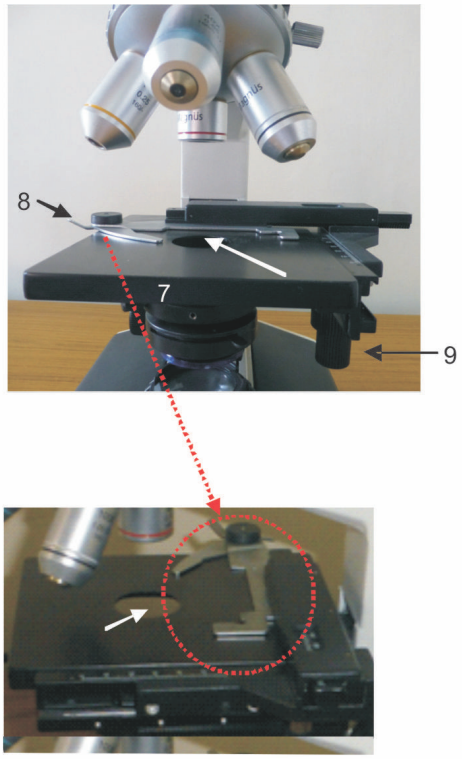


Fig. 5.16: A portion of body of compound microscope in front view showing stage (7), stage clip (8). Note, a hole in stage (arrow) for transmission of light from the source to the objective lens, and the slide moving knobs (9).

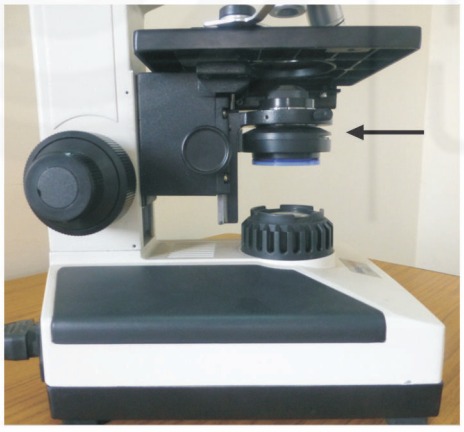


Fig. 5.17: A binocular compound microscope in side view showing condenser lens assembly (arrow) near the stage.

Fig. 5.18: A binocular compound microscope in an angular, front view showing condenser lens assembly unit in a lowered position.

iii) BASE (Figs 5.19 & 5.20) contains light source (15), light on-off switch (16), light intensity control (17), and electricity input cable (18).

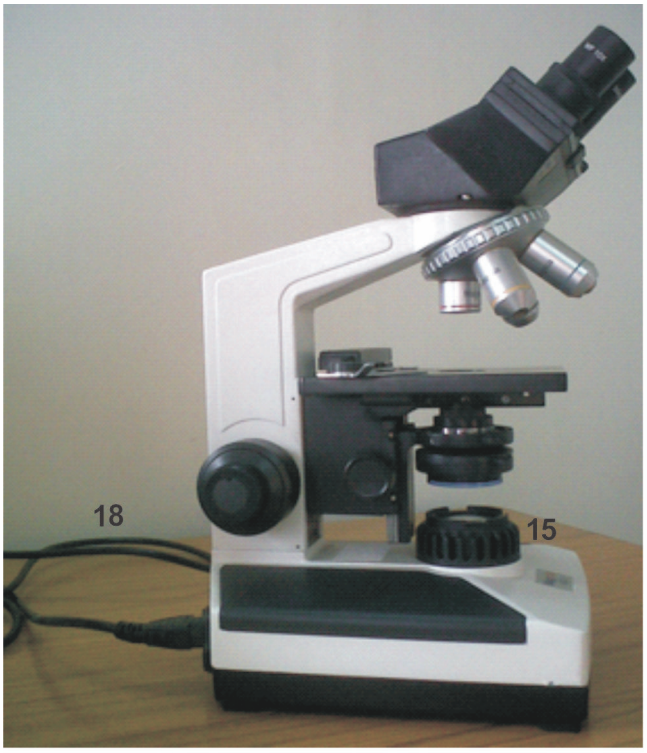


Fig. 5.19: A binocular compound microscope in side view showing light source (15), and an electricity input cable (18).

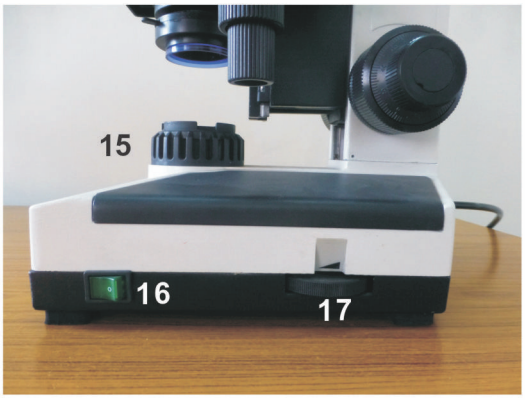


Fig. 5.20: A binocular compound microscope in side view showing light source (15), a light on-off switch (16), and light intensity control (17).

5.3.2 Steps in Using Compound Microscope

Compound microscope is a sensitive scientific instrument. It is to be handled with care.

1. Lift the microscope with one hand on its arm and the other hand for support under its base. Place it on a clean, level and stable surface near an electric outlet.
2. Familiarize yourself with the microscope and check all parts of the microscope that you are going to use so that its operation is smooth.
3. For focusing the microscope, first switch on its light source, next revolve the turret with objective lenses and bring the lowest power objective into position. This objective is the easiest to focus. Center the light in the field of view.
4. Place the slide on the stage and secure it with stage clip.
5. Look through the ocular lenses, also known as eyepieces, and gradually turn the coarse focus knob so that the stage moves upwards. Move the stage up till the specimen comes into focus. Care has to be taken that the objective does not touch the coverslip or the slide in the focussing process.
6. Next, look through the oculars and adjust the condenser with condenser focus knob and diaphragm for the desired quality of light and to attain the clearest image of the specimen.

7. The fine focus knob may then be moved so that the specimen is in sharp focus and its details are clearly seen.
8. The slide can be moved manually or with the help of slide moving knobs on the stage to bring the desired part of the specimen below the objective lens. When working with higher magnification a mechanical stage in used.
9. To observe the specimen under higher magnification, lower the stage a little bit with the coarse focus knob. Revolve the turret with objective lenses and bring the objective of desired magnification value over the slide. Make the necessary focus adjustments first with coarse focus knob and then with fine focus knob. Take care that the objective lens does not touch the slide.
10. When the observation work is completed, lower the stage and bring the low power objective lens into position and remove the slide from the stage.
11. If the microscope is to be kept at another place then switch off electricity and take out the electric plug from the board.
12. Clean the microscope with a soft, dry or damp cloth.
13. Carry the microscope with both hands. Grasp the arm with one hand and place the other hand under the base.

General Advice

i) While moving the stage upwards care should be taken that it does not touch the objective lens.

ii) Always use microscope slide with a cover glass or coverslip over the specimen. This will protect the objective lens if at all it touches the slide.

iii) When using a monocular compound microscope the best technique to view the specimen is to look through the eyepiece with one eye and keep the other eye open. As a reflex action, one tends to close the other eye. By keeping both the eyes open there won't be much strain on the eyes.

iv) You may recall the formation of image with a compound microscope (Fig. 3.26 of Unit 3). The image is formed through multiple lenses. As a result the image is upside down and back-to-front so when the slide is moved to the right the image moves to the left and vice versa.

5.3.3 Care and Maintenance

Some basic steps for the care and maintenance of the compound microscope are as follows:

i) Switch off electric supply of the microscope when it is not in use.

ii) Avoid touching the glass part of the lenses.

iii) Use only lens cleaning paper to clean the external surface of the lenses. For cleaning the inner surface of lenses professional help may be taken.

iv) After completing work with the microscope, clean its stage with a clean, dry, soft cloth, especially when it is used for viewing a temporary slide.

v) Lower the stage and bring the objective lens of low magnification value in focus position.

vi) Cover the microscope with a dust cover.

vii) When not in use for extended period, place the microscope in its box or in an almirah.

EXPERIMENT 6

PREPARATION OF NORMAL SALINE, REAGENTS AND STAINS

Structure

6.1 INTRODUCTION

In this laboratory exercise you will familiarize yourself with the proper procedures required for handling chemicals and also for preparing as well as handling chemical solutions and stains. You will also be able to learn how to prepare vertebrate physiological saline which is a simple chemical solution. The vertebrate physiological saline is used for a short period of time for rinsing animal organisms and tissues. You will also learn to prepare a few reagents or stains that are needed for colouring live or dead small animals as well as sections of plant and animal tissues. The stained animal organisms as well as sections of plant and animal tissues are mounted on glass slides for study under the microscope as you will learn to do in the next experiment.

In the present experiment we will expect you to be able to remember how to handle chemicals, chemical solutions and stains properly as well as how to accurately prepare a few important chemical solutions and stains which will be required by you to prepare temporary slides in Experiment 7 of this course.

Expected Learning Outcomes

After completing this laboratory experiment you will be able to:

- demonstrate the proper method of handling chemicals; preparing and handling chemical solutions and stains;
- prepare normal physiological saline for vertebrates; and
- prepare four stains — Aceto-orcein, Aceto-carmine, Methylene blue, modified Noland's solution.

6.2 GENERAL RULES FOR HANDLING CHEMICALS AND PREPARING AND HANDLING CHEMICAL SOLUTIONS

Proper techniques are required for preparing solutions and stains accurately, as well as for handling chemicals, solutions and stains properly as these will improve an experiment's safety and chances for success. The proper techniques required for handling chemicals, chemical solutions and stains are thus given below.

6.2.1 Instructions for Handling Chemicals, Chemical Solutions and Stains

1. The laboratory should be well ventilated so that noxious or poisonous fumes and gases can easily be removed.
2. Use clean containers for preparing solutions.
3. Distilled water should be used in preparing solutions unless otherwise specified.
4. When diluting acids always add the acid to the solvent/diluent, never the solvent to the acid. The acid should be added slowly.
5. All chemicals, solutions and stains should be kept in clearly and correctly labelled containers.
6. Strong acids and bases must be handled with care.
7. Avoid inhalation of acid and other fumes, particularly osmic acid or mercuric chloride powder.
8. Volatile fluids such as xylol (xylene), toluene (toluol), dioxane, chloroform, ether, etc. should be kept covered as much of the time as possible.
9. Flammable solutions should be kept away from open flames and sparks. Be particularly careful with ether and with ether-alcohol-celloidin mixtures that are flammable.
10. Many fixatives and other solutions are poisonous or corrosive and should be handled while wearing gloves.

11. Always wash your hands before smoking or eating and before and after working in the laboratory.
12. When some quantity of solution is taken out from the stock solutions then it should never be poured back into the stock bottle. If you take out too much solution it is better to discard the excess rather than risk contaminating the entire supply by attempting to conserve a small amount.

6.2.2 Accuracy Required in Preparing Solutions

1. A weighing balance that weighs upto one-hundredths of a gram is ideal for the biology laboratory. Unless milligrams are specified, an analytical balance is not required for routine preparations used in microscopic techniques.
2. Graduated pipettes should be used to measure volumes of 1 ml and less.
3. A small graduated, measuring cylinder should be used for measuring small quantities from 1 to 10 ml. A graduated cylinder of 10 ml, should be used instead of a 1000 ml graduated cylinder for measuring small quantity of liquids.
4. For most solutions used in biological techniques such as preparing fixatives and staining solutions, ordinary graduated cylinders provide the necessary degree of accuracy.

6.2.3 Method for Preparation of Solutions

Many biological experiments require the preparation of chemical solutions, that are prepared by mixing together in known quantities two or more chemical substances in which one substance has to be a liquid. The procedure for preparing a solution by diluting a solute is shown in Fig. 6.1. In a solution, one pure substance is homogenously dissolved in another pure substance. For example, in a sugar and water solution, the solution has the same concentration throughout, i.e. homogenous.

A solution is prepared by weighing a precise amount of solid or dry material called solute and dissolving it in a small amount of the measured quantity of a suitable liquid, called solvent. The rest of the measured amount of solvent is added gradually, while shaking the flask in order to dissolve the solute. Water or alcohol are often used as solvents.

In some cases the solute is a liquid rather than a solid. In such a case the required amount of the liquid solute is measured accurately and transferred directly into the volumetric flask containing some of the measured quantity of solvent. Rest of the measured solvent as in the earlier preparation is added gradually, while shaking the flask in order to make a homogeneous solution.

In order to note the reading of the amount of liquid solution contained in the flask or measuring cylinder you should know that in the case of a clear solution, a concave meniscus is formed on the surface of the liquid when it is poured into a graduated flask or cylinder. The bottom of the curved surface of

the meniscus is read at eye level and the volume measurement is read to the proper number of significant digits.

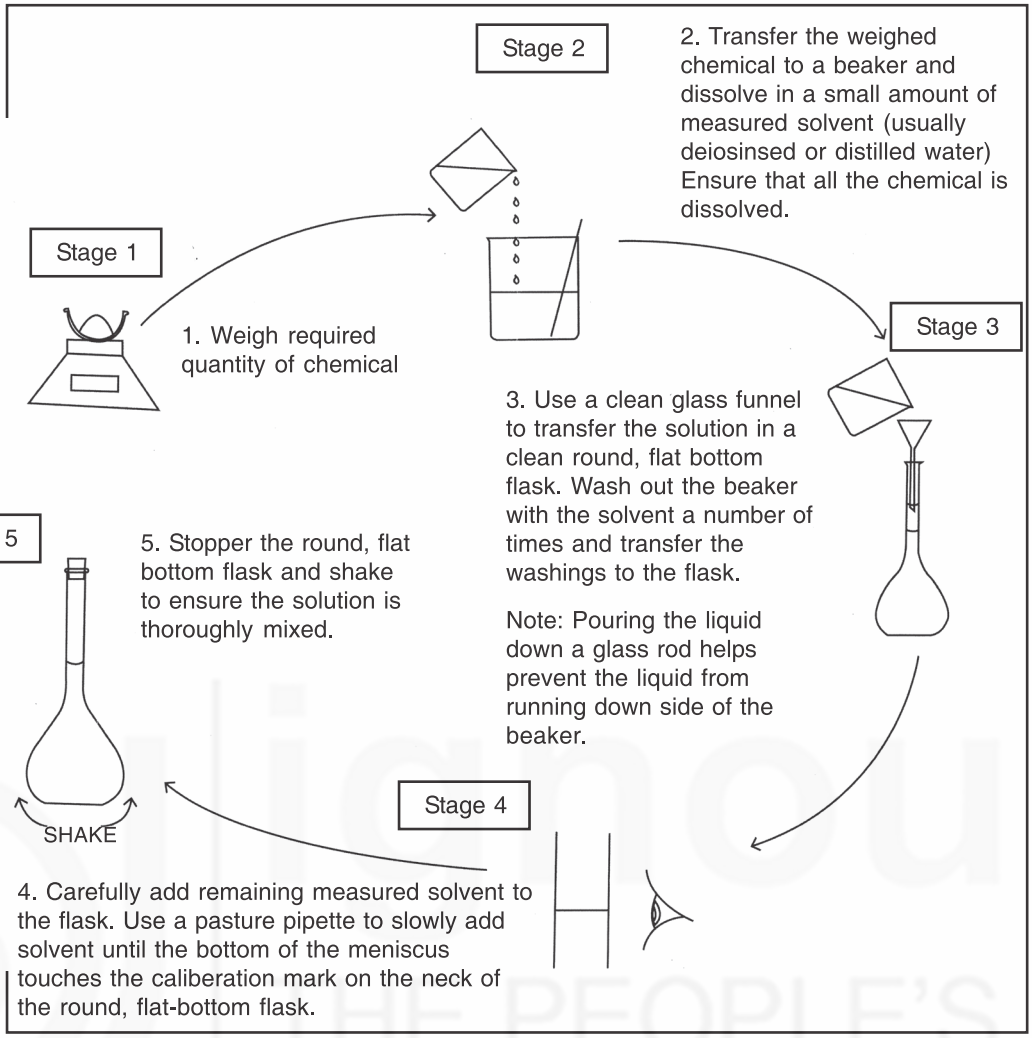


Fig. 6.1 : Steps required in preparing a solution.

6.3 USE OF CHEMICALS

Acids

The acids most commonly used in preparation of fixatives and staining techniques are acetic and nitric acids. Hydrochloric acid is used in decalcifying and destaining solutions. When percentages are indicated they are based upon dilutions of the acid for example glacial acetic acid (99 per cent); concentrated nitric acid (about 70 per cent), and concentrated hydrochloric acid (about 39 per cent). Thus, 1 per cent hydrochloric acid for destaining refers to a solution prepared by adding 1 ml of concentrated hydrochloric acid to 99 ml of alcohol or water as specified. The exact percentages of the concentrated acids vary slightly with different suppliers and grades and this must be taken into account when a “normal” solution is specified.

Alcohol

A reference to “alcohol” in tissue staining technique procedures means ethyl alcohol unless otherwise specified. Isopropyl alcohol may be substituted for ethyl alcohol in the dehydration and hydration series during preparation of

permanent slides. Methyl alcohol is preferred for the fixation of smears before staining with Giemsa stain.

In this exercise however, we will not be preparing series or grades of alcohol, since we are not going to prepare permanent slides in this laboratory course.

WARNING: METHYL ALCOHOL IS HIGHLY POISONOUS ON DRINKING.

6.4 PREPARATION OF NORMAL PHYSIOLOGICAL SALINE FOR VERTEBRATES

Physiological saline is used for rinsing debris and blood from tissues before placing them in the fixative. Physiological saline for invertebrates and poikilothermal vertebrates is different from that of warm blooded vertebrates due to the proportion of sodium chloride present in their body. The physiological saline for various groups within the warm blooded animal vertebrates also varies. In this exercise you will learn to prepare the physiological normal saline for mammals by following the given steps.

6.4.1 Materials Required for Preparing 0.9% Physiological Saline for Mammalian Vertebrates

Analytical sodium chloride (NaCl)

Distilled water

Graduated measuring cylinder of 100 ml

Round, flat bottom flask of more than 100 ml capacity

Analytical balance

6.4.2 Procedure

1. Weigh **0.9 gms** of analytical sodium chloride and put it in the round, flat bottom flask.
2. Measure **100 ml** of distilled water in the graduated measuring cylinder and pour it gradually into the flat bottom round flask, containing the measured quantity of sodium chloride. Keep shaking the flask while gradually pouring in the distilled water in order to dissolve and mix the NaCl in the water.
3. Always use freshly prepared physiological mammalian vertebrate saline whenever required.

6.5 PREPARATION OF FOUR STAINS

Staining techniques are most difficult to master initially as they depend both on method of preparation of stain and the fact that staining effects of the stains vary with different tissues. Staining is also affected by the type of

fixative used, by the time period of storage of the stain and the different types of dyes used.

In this exercise you will learn to prepare those stains which you will use in Experiment 7 of this course. The stains which you will learn to prepare are:

a) Aceto-orcein

b) Aceto-carmine

c) Noland's solution

d) 1% Aqueous Methylene blue

6.5.1 Preparation of Aceto-Carmine Stain

Aceto-Carmine stain is one of the most commonly used stain for chromosomal studies

Material Required

Dry Carmine stain

Glacial acetic acid

Distilled water

Round, flat bottom, corning flask of more than 100 ml capacity

Graduated measuring cylinder of 100 ml measure capacity

Analytical balance

Bunsen burner

Filter paper

Stoppered stain bottle-1

Procedure of Preparation of Aceto-Carmine Stain

1. Weigh **0.5 g of dry carmine** stain.
2. Measure **45 ml of glacial acetic acid** in a graduated measuring cylinder and pour into a round, flat bottom, corning flask of more than 100 ml capacity
3. Measure **55 ml of distilled water** in the measuring cylinder and gradually add to the round, flat bottom, corning flask containing the glacial acetic acid and mix the two.
4. Heat the round, flat bottom, corning flask containing the distilled water and glacial acetic acid to boiling.
5. Add the weighed amount of **0.5 g of carmine stain** to the boiling mixture contained in the round, flat bottom, corning flask and shake well to mix.
6. Cool the mixture and filter by the filter paper. Your stain is now prepared.
7. Pour the cooled, filtered stain into a stain bottle. Stopper the bottle properly.

6.5.2 Preparation of Aceto-Orcein Stain

Aceto-orcein stain is also an important stain used for chromosomal studies. It helps to distinguish chromosomes according to their sizes and centromere positions. Orcein is extracted from two species of lichens, *Rocella tinctoria* and *Lecanora parella*. Orcein is also available in a synthetic form, but the natural form is preferred for chromosome analysis, because it gives better contrast.

Material Required

Orcein stain

Glacial acetic acid

Distilled water

Round, flat bottom, corning flask of 100 ml capacity

Round, flat bottom, corning flask of more than 100 ml capacity

Graduated measuring cylinder of 100 ml measure capacity

Analytical balance

Bunsen burner

Filter paper

Stoppered stain bottle-1

Procedure

1. Weigh **1.0 g of orcein stain** and put in a flat bottom, round corning flask of more than 100 ml capacity.
2. Measure **45 ml of glacial acetic acid** in the graduated measuring cylinder and pour it into another round, flat bottom corning flask of 100 ml capacity. Stopper the flask containing the acetic acid with cotton wool and heat it to boiling.
3. Pour slowly the boiling glacial acetic acid over **1 g orcein powder** contained in the round, flat bottom corning flask
4. Allow the orcein stain and glacial acetic acid mixture to cool and then add **55 ml distilled water** to it.
5. Filter this prepared stain into a stain bottle. Stopper the stain bottle.
6. Aceto-orcein stain solution is unstable and should be prepared fresh and stopper the bottle.

6.5.3 Preparation of Aqueous Methylene Blue Stain

Material Required

Methylene blue powder

Distilled water

Graduated measuring cylinder of 100 ml measure capacity

Round, flat bottom flask of 100 ml capacity

Analytical balance

Filter paper

Stopperd stain bottle-1

Procedure

1. Weigh **1.0 g of methylene blue** powder and put into a round, flat bottom, corning flask of 100 ml capacity.
2. Measure **100 ml of distilled water** in a measuring cylinder and add gradually to the round, flat bottom, corning flask containing the methylene blue stain.
3. Shake the flask gently while adding the distilled water gradually, in order to dissolve the methylene blue stain in it.
4. Filter and pour the mixture into stain bottle for use. Stopper the bottle.

6.5.4 Preparation of Modified Noland's Stain Solution

Material Required

Phenol (crystals liquefied by heating)

Gentian violet

Distilled water

Glycerine (glycerol)

Graduated measuring cylinder of 100 ml measure capacity

Corning tube of 10 ml capacity

Brown paper covered, round, flat bottom corning flask of 100 ml capacity

Brown paper covered, round, flat bottom corning flask of more than 100 ml capacity

Analytical balance

Filter paper

Brown paper covered or brown coloured, stoppered stain bottle of more than 100 ml capacity-1

Procedure

1. Weigh **5 gm phenol crystals** and put in a 10 ml corning tube. Gently heat this tube in order to liquefy the phenol crystals. Measure and put 5 ml of the liquefied phenol into the brown paper covered, round, flat bottom corning flask of 100 ml capacity.
2. Measure **100 ml of distilled water** and add gradually to the brown paper covered, round, flat bottom corning flask, containing the liquefied phenol. Keep shaking the flask gently while adding the distilled water so that the liquefied phenol and distilled water mix properly.
3. Measure **10 ml glycerol** in a measuring cylinder.
4. Weigh **25 mg of gentian violet** and put into another brown paper covered, round, flat bottom, corning flask of more than 100 ml capacity.

5. Add the distilled water and phenol mixture into the round, flat bottom, corning flask containing the weighed 25 mg of gentian violet. Also add the measured amount of glycerine. Shake the mixture gently till a homogeneous stain solution is prepared.

6. Gently pour the prepared stain solution into a brown paper covered bottle or brown stain bottle of 100 ml capacity. Stopper the bottle and keep it well closed and protected from light.

WARNING: DO NOT HANDLE PHENOL CRYSTALS WITH BARE HANDS. DEATH HAS RESULTED FROM AS LITTLE AS 1.5 GRAM ABSORPTION. NEVER APPLY TO LARGE PORTIONS OF BODY SURFACE.

EXPERIMENT 7

PREPARATION OF TEMPORARY SLIDES

Structure

7.1 INTRODUCTION

You have learnt in Block 2, Unit 4 of this course (CLT-102) that to observe cells clearly under a microscope, the cells need to be spread in a mono layer. This mono layer of cells is then fixed and stained and observed as a temporary preparation or a permanent slide is made which can be stored and observed later.

In this experiment you will learn to make temporary slide preparations of cell suspensions using the smear technique and of soft tissue like onion root tip, using the squash technique. You will also acquire skills to make temporary slides of whole mounts of single celled protozoans and phytoplankton and demonstrate stomata from a leaf epidermal layer and germination of pollen grain from a flower.

Before starting this practical you should read Unit 4 of the Biology course CLT-102 again to recollect the methods used for cleaning and labelling slides.

Expected Learning Outcomes

After completing this practical exercise, you should be able to:

- make a temporary slide of a given cell suspension using smear technique;
- make a temporary slide of soft tissue using squash technique;
- prepare whole mounts of *Paramecium, Volvox, Chlamydomonas*;
- make temporary slides of leaf epidermis peel to depict stomata; and
- demonstrate germination of pollen grains in a cavity slide.

7.2 GENERAL METHOD OF MAKING A TEMPORARY PREPARATION

A temporary preparation of cells is usually observed as a wet mount which is prepared by placing a cell suspension in liquid on the slide or if the material to be studied is dry, by placing it directly on the slide and adding water, glycerol or stain to it. The material is then covered by a cover slip. You have to be careful not to trap air bubbles while lowering the cover slip over the material as it will cause interference in observing the material. The procedure is shown in Fig. 7.1.

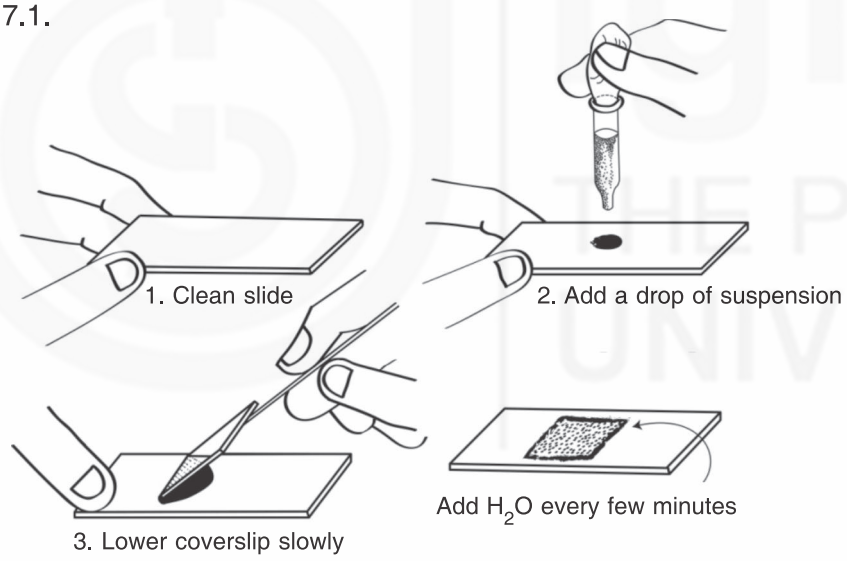


Fig. 7.1: The technique for lowering the cover slip over the slide.

7.3 SMEAR TECHNIQUE FOR CHEEK SCRAPINGS

In this exercise you will learn to make a temporary mount of cheek epithelial cells by following the given steps.

7.3.1 Materials Required

1. Cover slips, slides, slide labels
2. Disposable spatula or tooth pick with a flat end

3. 9% NaCl (physiological saline)
4. Methylene blue stain
5. Filter paper

7.3.2 Procedure

1. Rinse your mouth well with water.
2. Using the flat end of the toothpick or a disposable spatula, gently scrape the inside of your cheek about 8-10 times.
3. Add a drop of 0.9% NaCl or physiological saline (you have learnt how to make physiological saline for mammals in Experiment 6) on a clean slide and spread the cells on the slide. Add a drop of methylene blue.
4. Cover with a cover slip and gently press it to flatten the cells. Alternately you can introduce the stain by irrigation method (see Fig. 7.2). In this method you can place a drop of the stain from one end of the cover-slip and place a small blotting paper on the other end. The stain will be drawn inside through capillary action and excess stain is taken up by the blotting paper. Clean the excess stain from the slide.
5. Put the slide under high power in a microscope. Make sure that your counsellor sees the slide. He/she would wish to assess its quality to award marks for it.

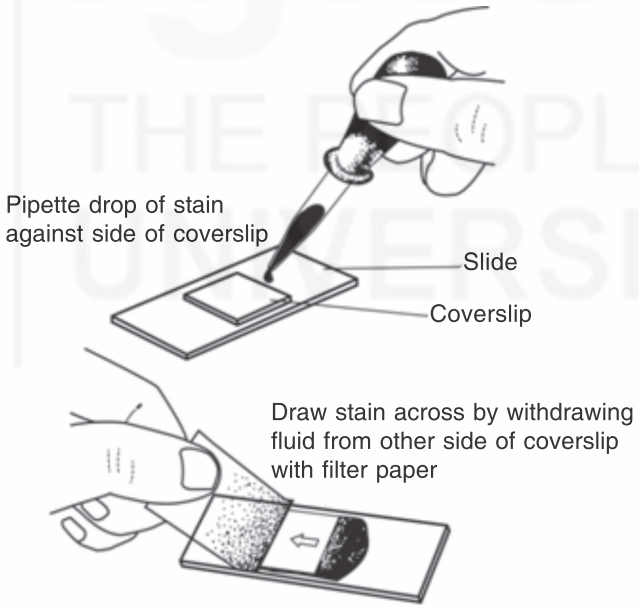


Fig. 7.2: Technique of irrigation.

7.3.3 Observations

Locate a single cell under high power. Many of the cells will be crumpled and irregular in outline because the cell membrane is extremely thin and delicate. The nucleus will be stained dark blue in the centre of each cell.

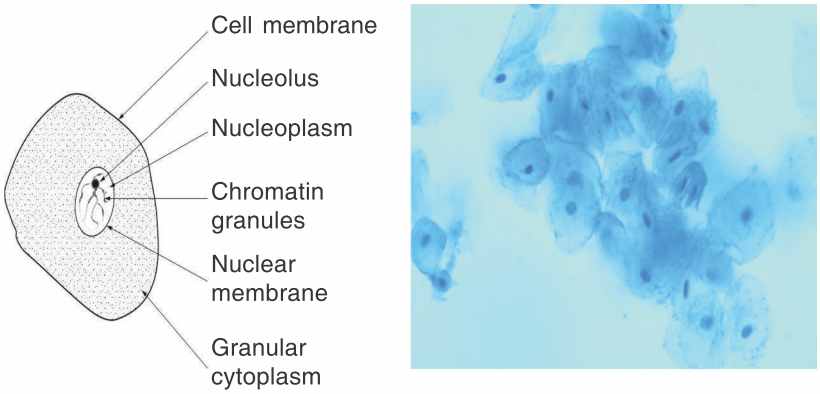


Fig. 7.3: Cheek cells as seen under the high power (400X) after staining. (*Source*: www.scienceprofonlione.com)

If you don't let in too much light through the microscope you will be able to observe the cell structures better. Compare what you see with Fig. 7.3. In epithelial cells obtained from females a distinct darkly staining body attached to the nuclear membrane can be seen. This is known as Barr body.

CAUTION: Certain infectious diseases can be transmitted through saliva. Avoid any contact with another person's saliva. Do not share a spatula with anyone else.

7.4 SQUASH TECHNIQUE FOR ONION ROOT TIP

Squash technique is a simple method widely used for the study of chromosomes. This technique consists of applying gentle pressure on a small piece of stained tissue to spread the chromosomes in the cells. This technique is used to study dividing cells showing either mitosis or meiosis and suitable tissues for this are onion root tip, grasshopper testis or anther buds.

In this exercise you will learn to prepare a temporary slide of onion root tip showing mitosis, which can later be made into a permanent slide. You have learnt how to prepare the material in this case onion root tip in Experiment 3.

The onion root should remain in the acetic alcohol solutions for 12-24 hours. After fixation the tissue can be transferred to 70% alcohol.

7.4.1 Materials Required

1. Onion root tips
2. 70% alcohol
3. Forceps and dissecting needles
4. Pipette, glass dropper, watch glass, beaker
5. 2N Hydrochloric acid
6. Acetocarmine/aceto-orcein stain
7. Slides, cover-slips
8. Acetic acid

9. Filter paper
10. Nail polish

7.4.2 Procedure

1. Transfer the roots from the fixative or storage solution (70% alcohol) in a watch glass.
2. Wash in water until the roots sink.
3. Drain off the water using a pipette and add a few drops of 2N HCl and leave for 10 minutes at room temperature or move the watch glass over a spirit lamp flame quickly for 2-3 times. While hydrolysing over the flame, move the watch glass over the flame so that it does not overheat. Alternately you can warm the watch glass over a beaker of boiling water till the liquid steams.
4. After hydrolysis drain off the HCl and wash root tips in water.
5. Remove water and add 1% acctocarmine or aceto-orcein to the root tips for staining. The staining requires 10-15 minutes.
6. Transfer 2-3 root tips on a slide, cut 2 mm above the pointed end. Discard the rest.
7. Place a drop of 45% acetic acid on the root tip and carefully place a cover-slip. Remove excess acetic acid by the edge of a filter paper.
8. Place the slide between 2 layers of filter paper and gently tap the cover-slip by the back of a pencil to get an even spread of chromosomes.
9. If air bubbles get trapped in the cover-slip add a few drops of glycerol or acetic acid to the edge of the cover-slip.
10. Seal the edges of the cover-slip by applying nail polish so that the fluid evaporation is minimum and observe the slide under a microscope.
11. Label the slide appropriately and show to your counsellor.

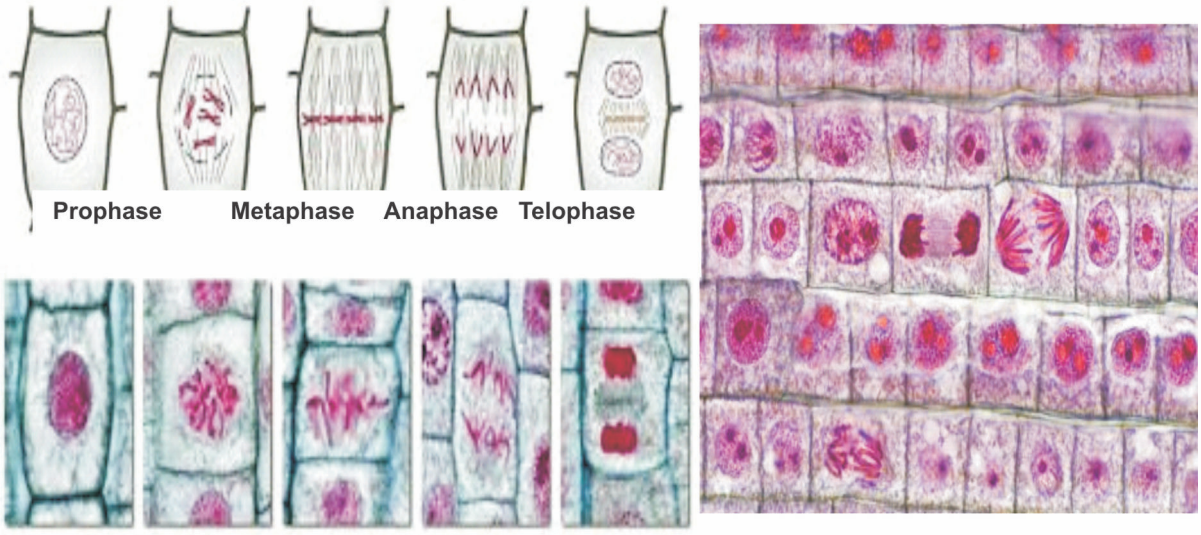


Fig. 7.4 : Stages of mitosis from an onion (*Allium*) root tip. (*Source*: www.scienceprofonline.com)

7.4.3 Observations

In a squash preparation of the onion root tip you may find that a majority of cells will be in interphase (non-dividing cells). The interphase nucleus will appear as a darkly stained body with acetocarmine or aceto-orcein. The nuclear membrane is intact and the chromosomes form a fine network. You will have to look for dividing cells if you use aceto-orcein as only the nucleus and chromosomes will be stained. Acetocarmine will stain the whole cell, use a textbook or the illustration of Fig. 7.4 to identify different phases of division. You could also consult your counsellor for more information.

7.5 WHOLE MOUNTS OF UNICELLULAR ORGANISMS

A unicellular organism has to carry out all the functions within one cell that in a multicellular organism are performed by different cells and tissues. Accordingly it possesses a high degree of internal organisation, which can be observed by making a whole mount of the organism. In this exercise you will learn to make whole mounts of three protists – *Paramecium* an animal like protist, *Chlamydomonas* an autotrophic protist, and *Volvox* which is an example of a colonial protist.

7.5.1 Materials Required

1. *Paramecium* culture, *Volvox*, *Chlamydomonas*
2. Methyl cellulose
3. Methyl green in ethanoic acid/acetocarmine
4. Acetic acid
5. Albumin, glycerin
6. Petridishes, Beakers, Slides, Cover-slips, Slide labels
7. Noland's solution
8. Iodine solution

7.5.2 Procedure

A. Temporary Whole Mount of *Paramecium*

1. Put a drop of *Paramecium* culture on a clean slide and cover with a cover-slip. Since paramecia move very rapidly their movement can be slowed down by adding an equal amount of methyl cellulose to a drop of the culture.
2. Irrigate your slide with either 1% methyl green in ethanoic acid or acetocarmine. Both fix the organism and stain the nuclei green in case of the first and red in the case of the second.
3. Then observe under high power in the microscope. Label and show the slide to your counsellor.

B. Whole Mount of *Chlamydomonas* and *Volvox*

Chlamydomonas and *Volvox* are phytoflagellates that are found in freshwater ponds and ditches. Sometimes they are so abundant as to produce a green scum on ponds. *Chlamydomonas* are single-celled green flagellates while *Volvox* is an example of colonial phytoflagellate that forms a hollow spheroid with several thousand cells embedded in its mucilaginous wall. You would be supplied with specimens of these phytoflagellates collected from ponds and ditches or cultured in the laboratory. The method of preparing a temporary mount is the same for both species.

1. Place a drop of culture or a drop of water containing the flagellates on a clean slide and add a drop of 10 per cent methyl cellulose which will restrict the movement of the organism and allow you to study the organism.
2. Place a cover-slip on the drop and observe under a microscope. By cutting down the light you will be able to observe the flagellar movement better.
3. You could stain the slide by the irrigation method using acetocarmine to see the nucleus, Noland's solution to see the flagella and iodine solution to see the starch grains.
4. Show your slides to your counsellor.

7.5.3 Observations

Under high power of the microscope try and observe as much of the internal organs. In *Paramecium* you will find it difficult to see the micro nucleus as it is generally covered by the macro nucleus. You may be able to see the contractile vacuole discharging before you stain and fix the mount. Use the help of the given diagram (Fig. 7.5) to identify the structures in the *Paramecium*.

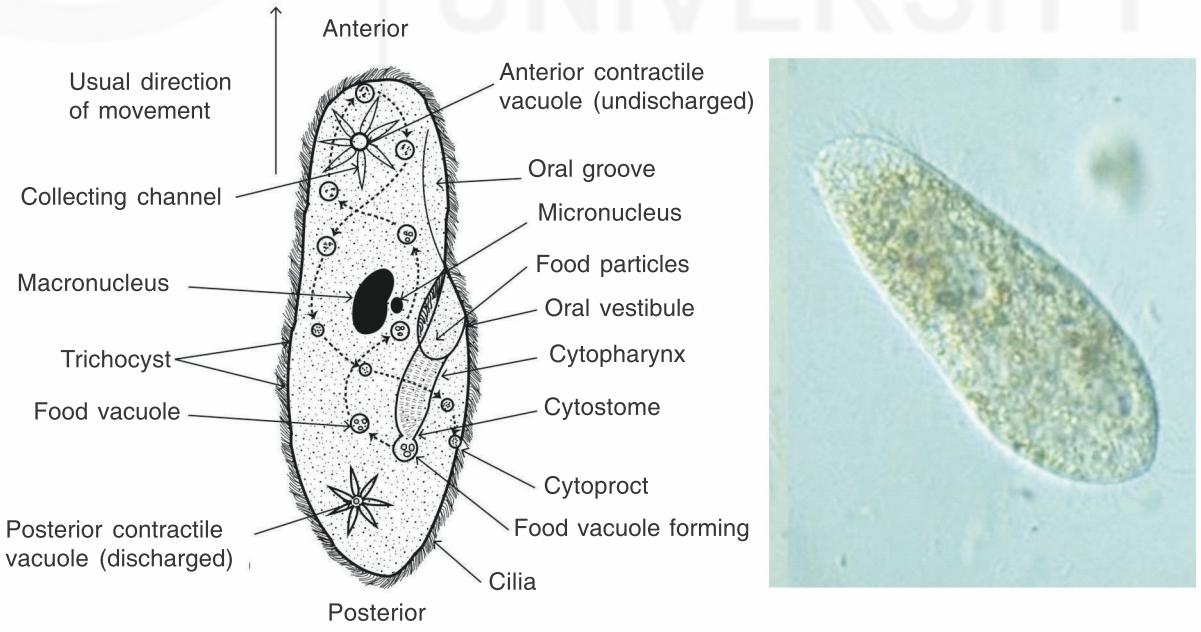


Fig. 7.5 : *Paramecium*. By adjusting the light in the microscope all the structures given in the line drawing can be seen. (*Source*: http://www.fcps.edu)

Under high power you will observe that *Chlamydomonas* has two flagella, cellulose cell wall, two small contractile vacuoles, cytoplasm and cup shaped chloroplast, pigment spot and pyrenoid. You can compare your slide with Fig. 7.6.

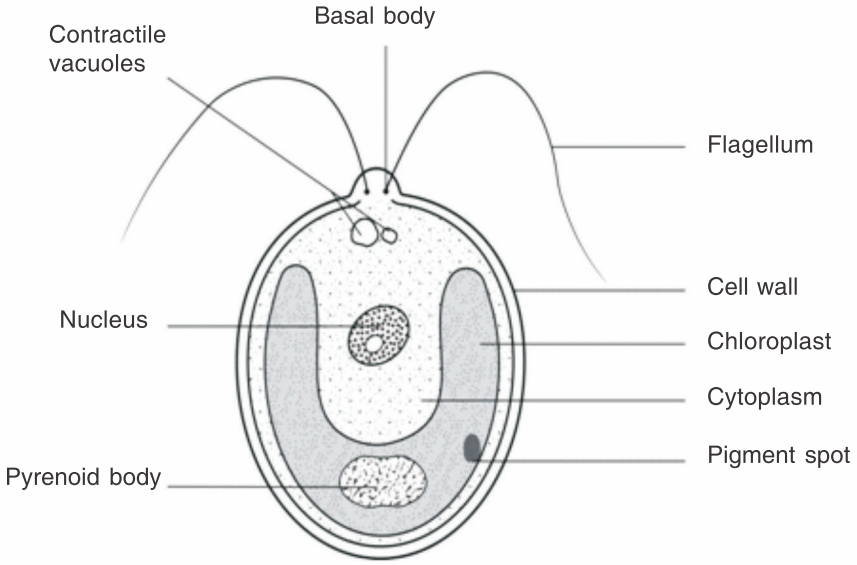


Fig. 7.6 : *Chlamydomonas*, a common green unicellular algae.

In colonial flagellate *Volvox*, notice that the flagellar beat of the individual cells is coordinated with each other so that the colony moves in an oriented fashion. Compare your slide with Fig. 7.7. You may also observe daughter colonies inside individual colonies.

Fig. 7.7 : *Volvox* with daughter colonies. Each cell of the colony looks very much like the single cell of *Chlamydomonas*.

7.6 WHOLE MOUNT OF STOMATA

Stomata are tiny pores in the epidermis of the leaf. The opening and closing of the stomata is controlled by two bean shaped guard cells. Unlike other epidermal cells the guard cells have chloroplasts containing chlorophyll. These openings allow gases to move in and out of the leaf. In most plants stomata open in the presence of light to allow carbon dioxide to enter and oxygen to go out during photosynthesis. They also release the extra water from the leaf though transpiration. Stomata close in the dark. Plants can control water loss by opening and closing of the stomata. Stomata are present either on both sides of the leaf or only on one side of the leaf. The number of

stomata on the leaf can tell you about the habitat of the plant. In dicot plants the number of stomata is greater on the lower surface than on the upper surface of the leaf. On the other hand, monocot plants have the same number of stomata on both surfaces of the leaf. In the case of floating plants, stomata is found only on the upper epidermis.

7.6.1 Materials Required

1. Fresh leaf from Balsam/Bryophyllum/Petunia/Periwinkle
2. Needle, fine brush and dropper
3. Watch glass
4. Distilled water
5. Slide
6. Cover-slip
7. Safranin stain

7.6.2 Procedure

1. Take fresh leaf and peel the thin membrane like epidermal layer.
2. Place a piece of the epidermis in a watchglass in which a few drops of distilled water are already added. Using the fine brush transfer the piece in another watch glass to which a few drops of safranin stain has been added. Leave the tissue in it for about 30 seconds.
3. Remove the extra stain by washing the tissue with distilled water, and place it on a clean glass slide. Add a few drops of glycerin and cover with a cover slip.
4. Observe under scanning power as well as high power to observe the stomata.

7.6.3 Observations

You will see that the epidermis is made up of tightly packed cells with very small intercellular spaces. You can see the stomata as tiny openings at 40X magnification but on 100X and 400X you will be able to see the complete structure. The stoma or the opening will be seen as a slit or wide opening surrounded by two bean shaped cells – guard cells.

Compare what you observe with the structures given in Fig. 7.8.

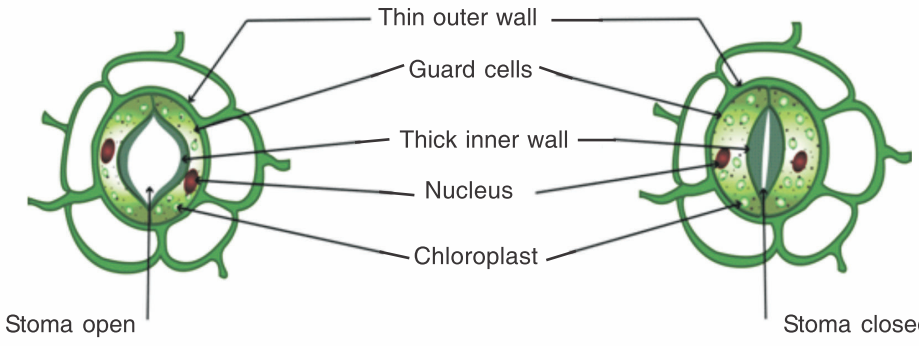


Fig: 7.8: Structure of stomata from tomato leaf. (Redrawn after www.bbc.co.uk)

7.7 WHOLE MOUNT OF POLLEN GRAIN GERMINATION

Pollen dust or grains form the male gametophyte in a flower. When they come in contact with the stigma or female reproductive part of the flower they stick to it because of the sugary or sticky secretions of the stigma. Here the pollen grain germinates forming a pollen tube and digests its way through the tissue of the style to reach the egg in the ovary. Of the two male nuclei or male gametes, one gamete fuses with the egg to form the seed.

7.7.1 Materials Required

1. Cavity slides and cover slips
2. Vaseline
3. Sucrose powder
4. Distilled water
5. Needle
6. Periwinkle flowers (*Vinca rosea*). You can try the pollen from other flowers as well.

7.7.2 Procedure

1. Take approximately 10 g sucrose, add 100 ml water Stir to make a 10% solution.
2. Take the cavity slide; smear a layer of petroleum jelly around the cavity on the slide.
3. Add 2-3 drops of sucrose solution in the cavity on the slide using the glass rod .
4. Take the periwinkle flower and tease out pollen using the needle. You may use as many flowers you want to obtain the pollen grains
5. Put the collected pollen grains in the cavity and cover with a cover slip. The petroleum jelly will seal the cover slip.
6. Wait for at least ½ hour to allow the pollen grain to germinate and observe under the high power of a microscope. If you have time then examine the slide after half hour intervals for 3 hours.

7.7.3 Observations

The pollen grains can be seen under 100X magnifications. Compare your slide with the diagram given in Fig. 7.9. You can see the two male nuclei. You will be able to see the pollen grains with varying lengths of pollen tubes depending on the time interval in which you see the slide. You may see two male nuclei and a vegetative nucleus in the pollen tube of some of the grains.

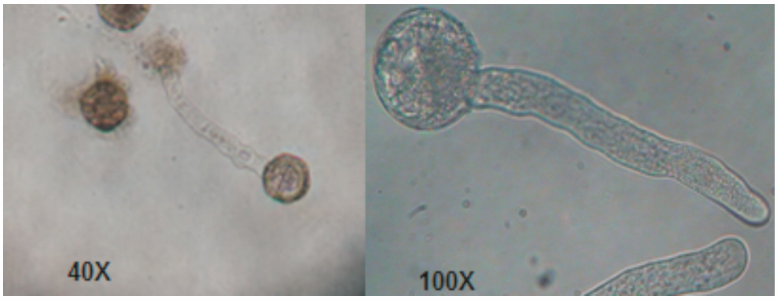


Fig. 7.9: Stages in germination of pollen grain.

7.8 SELF ASSESSMENT QUESTIONS

1. The technique of lowering the cover slip shown in Fig. 7.1 is used to:
   i) Make organelles more visible
   ii) Make specimen transparent
   iii) Reduce formation of air bubbles
   iv) Keep the specimen moist
2. In the squash preparation of the onion root tips what stage/stages of division were seen in your slides?
3. Why do we add methyl cellulose to the culture of paramecium or to the specimen containing *Volvox*?
4. Why do we make a sucrose solution for germination of pollen grains?

EXPERIMENT 8

TECHNIQUES FOR MICROBIAL CULTURE AND GRAM'S STAINING

Structure

8.1 INTRODUCTION

Cultivation of micro-organisms involves the provision of the correct nutrients and physical conditions in the laboratory so as to enable the organisms to grow in an environment similar to their natural one. In this laboratory exercise you will practise culturing micro-organisms such as fungi and bacteria in the laboratory. You will also learn to stain bacteria by means of Gram's staining technique which is done for categorizing bacteria on the basis of their response to the Gram's stain. Bacteria are categorized as Gram positive or Gram negative bacteria on basis of the Gram's staining technique.

Expected Learning Outcomes

After performing this experiment, you should be able to:

- prepare solid culture medium required for culturing microbes namely fungi and bacteria;
- sterilize the culture medium and glassware in order to minimize contamination;
- describe ways of streaking the inoculum to get the isolated colonies; and
- stain and categorize bacteria by using Gram's staining method.

8.2 FUNGAL CULTURE

Most of the fungi can be grown on various kinds of culture media that are also used for culturing the bacteria. Culture medium can be of two types i) synthetic and ii) non-synthetic. **Synthetic culture media are also referred to as "defined media"** since the chemical composition of the constituents are known. Czapek's constituents Dox medium, Richard's solution, Sabourand agar, Starch casein agar are some of the synthetic media that are used for growing fungi while Trypticase soy sugar agar, Nutrient agar or Luria agar are some of the synthetic media that are used for growing bacteria in the lab. Specific synthetic culture medium is generally used to culture specific microorganisms. **Non-synthetic media, are also referred to as "complex media"** since they consist of complex natural products whose exact composition is not known. Non-synthetic media are of plant or animal origin and include extracts of fruits, vegetables, milk, egg, blood, meat, yeast, malt etc. Along with this some known chemical substances are also added to the non-synthetic media. Since the non-synthetic media are of complex nature, they can support large varieties of microbes like fungi and bacteria and so are useful for routine laboratory cultures. In the present laboratory experiment, we will use a non-synthetic solid medium made from potatoes for culturing a non-pathogenic fungi. The same medium can also be used for culturing bacteria.

8.2.1 Materials Required

Corning Beaker–150 ml

Corning Erlenmeyer flask–500 ml

Sterile corning Petri dishes of 15 cm diameter with lids

Graduated, corning test tubes of more than 30 ml capacity

Aluminum Foil

Gloves

Sterile cotton swabs

Metal inoculating loop (usually of nichrome which is a nickel-chromium alloy) or a single-use disposable plastic loop, which is discarded rather than re-sterilized

Permanent marking pen

Bunsen burner lamp

pH meter/pH paper

Pressure Cooker/Autoclave

Laminar air flow cabinet/lab bench

Electronic Balance

Water bath

Media components:

Potatoes	-	50 gm
Dextrose	-	4 gm
Agar	-	3.75 gm
Distilled water	-	250 ml

HCl 1 Normal
NaOH 1 Normal } For pH adjustment of medium prior to addition of agar

Stock fungal culture tube with cap/cotton wool plug that contains a non-pathogenic fungal culture of any one of the following fungi: *Rhizopus oligosporus/Rhizopus sexualis/ Rhizopus stolonifer/Aspergillus niger*.

8.2.2 Procedure

1. Before starting the laboratory experiment wear your lab coat and gloves.
2. First sterilize the corning Petri plates along with their cover in a pressure cooker/autoclave for 15 minutes at 15 lb pressure.
3. Remove the sterilized corning Petri plates along with their covers from the cooker/autoclave after cooling in order to label them. Label these Petri plates on the bottom rather than on the lid. Write near the edge of the bottom of the plate so that you get an adequate area to observe the plate after it has been incubated with the fungus. Labelling should include the name of the organism, type of agar, date of media, and your name or initials.
4. Weigh 3.75 gm (1.5%) of agar and 4 gm of dextrose separately and keep aside.
5. Peel, cut and boil potatoes (50 gm) in 100 ml distilled water in a Corning beaker of 150 ml capacity.
6. Make a thick paste of the boiled potatoes and add about 50 ml of distilled water to the paste.
7. Filter the diluted potato paste through a muslin cloth into a 500 ml capacity Corning Erlenmeyer flask.
8. To the potato filtrate in the Corning Erlenmeyer flask, add the previously weighed 4 gm dextrose. After this add distilled water to the filtrate in order to make the volume up to 250 ml.
9. Adjust the pH of the 250 ml potato filterate solution containing dextrose to pH 6-6.2, by using pH meter or pH paper. If need arises, adjust the pH by using 1N HCl or 1N NaOH as the requirement may be.
10. Once the pH of the 250 ml potato filtrate solution containing dextrose is adjusted, then add the already weighed 3.75 gms (1.5%) of agar to it. The potato agar culture medium is now prepared.
11. Cover the Erlenmeyer flask that contains the potato culture medium with a cap or cotton plug. Wrap aluminum foil around the cap.

12. Now sterilize the prepared potato culture medium, contained in the capped, Erlenmeyer flask by using an autoclave/pressure cooker for 15 minutes, at 15 lb pressure. Sterilization is complete when the pressure of the autoclave/pressure cooker drops to zero.

13. Wear a fresh pair of gloves and take out the Erlenmeyer flask, containing the sterilized potato culture medium and place in a water bath that has been set at 50°C temperature. This temperature will enable the medium to cool but prevent it from setting. You can also keep the medium outside and let it cool till you can hold it in your hands. However, if the medium is kept in the Erlenmeyer flask at lower than 50°C temperature then it will solidify and will not be useful.

14. Pour 30 ml each of the liquid potato culture medium, contained in the Erlenmeyer flask into 5 sterilized, graduated test tubes each having 30 ml capacity.

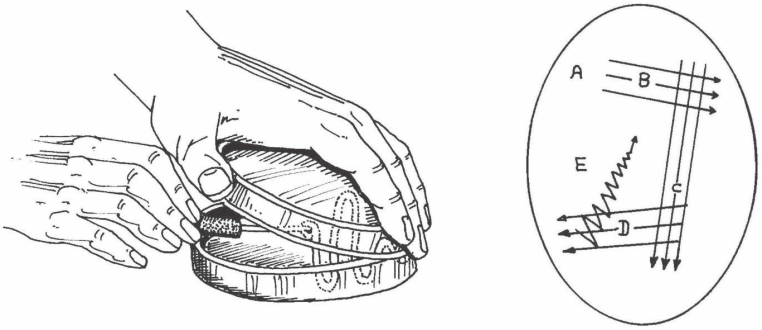
15. Do the rest of your experiment in a laminar air flow cabinet if available or on a clean lab bench. Prepare your work area by disinfecting it thoroughly. Keep the covered culture tubes containing the fungal culture in a test tube stand on the work area table of the laminar air flow cabinet/ clean lab bench. Also place on the work area, the sterilized Petri plate dishes, a Bunsen burner and inoculating needles.

16. Lift the lid of the sterile Petri dish slightly, with the left hand and pour 30 ml of the sterile, molten potato agar culture medium from the graduated test tube into it. Replace the lid. Repeat the process of pouring 30 ml of medium into four more Petri plate dishes (Fig. 8.1). Allow the Petri dishes to cool and the potato agar culture medium to set. Keep the Petri plates containing the potato agar medium on the work area of the Laminar flow cabinet/lab bench.

Fig. 8.1: Pouring medium into Petri plates.

17. Remove any visible moisture whether present on the potato agar culture medium contained in the Petri plate or around the inner rim of the Petri plate by means of sterile cotton swabs.
18. Light the Bunsen burner and properly adjust its flame. The flame should appear like a small blue cone.
19. Now use the metal inoculating loop for preparing a streak plate. The metal inoculating loop is used for the progressive dilution of the fungal inoculum over the surface of the solidified potato agar culture medium contained in the Petri plate dish. Streaking is done in such a way so that fungal colonies grow well separated from each other.
20. Before inoculating or streaking the potato agar culture medium with the fungal culture, first sterilize the metal inoculating loop by flaming it.
21. In order to sterilize the inoculating loop, place it in the light blue area of the flame of the Bunsen burner, just above the tip of the inner flame, until it becomes red-hot.
22. After the inoculating loop is sterilized, allow it to cool by holding it still. Do not wave it around to cool it or blow on it. If you are using plastic disposable loops then remove from the packaging just before streaking so that contamination is avoided. Discard the plastic, disposable loop after one time use, into an appropriate container.
23. For inoculating the fungal culture onto the potato agar culture medium kept in the Petri plate, hold the inoculating loop in the right hand in the same way you hold a pencil.
24. Now lift the stock culture test tube containing the fungal inoculum from the test tube stand, with your left hand and loosen its cap. Remove the cap/cotton wool plug of the culture test tube with the little finger of the right hand. Always keep the cap of the culture tube in your right hand. Never set it on the table as it could pick up contaminants.
25. Flame the neck of the stock culture test tube containing the fungal culture by passing the neck of the tube forward and backward through the Bunsen burner flame.
26. Insert the sterilized loop into the stock culture test tube containing the fungal culture. Withdraw the loop that would also have some of the fungal culture containing the spores. At all times, hold the loop as still as possible.
27. Replace the cap/cotton wool plug on the culture test tube by using the little finger and place the culture test tube back on the test tube stand.
28. For the purpose of streaking which is shown in Fig. 8.2 partially lift the lid of the Petri plate dish and insert the inoculating loop containing the fungal culture onto the surface of the solid potato agar culture medium, contained in the Petri dish. .
29. You can streak several plates from the same stock culture. You can do the streaking of the solid potato agar medium contained in the Petri plates in the following ways:

    i) As shown in Fig. 8.2(a) and (b), take and spread a loopful of culture



(a) (b)

Fig. 8.2: Streaking method for culturing microbes on the solid medium: (a) use of metal loop for streaking the inoculum (fungal spores), (b) streaking directions on agar plate.

over area A without touching the sides of the plate. (Before this inoculation process, ensure that the agar surface is completely set).

ii) Area A will contain the highest concentration of fungi and is referred to as the pool. Streak the culture over areas B, C, D and E one after the other, according to the directions shown in the Fig 8.2(b). This streaking method effectively dilutes the concentration of spores in a stepwise manner and so it becomes possible to produce isolated colonies. Before each inoculation, sterilize the inoculating loop by using the same method as given in the steps 21 and 22.

30. You can streak the Petri dish in different patterns as shown in Fig. 8.3. You must streak by moving the loop in the forward direction only. Never make backward movements with the loop otherwise you will not be able to get isolated colonies, as mixing of spores will occur.

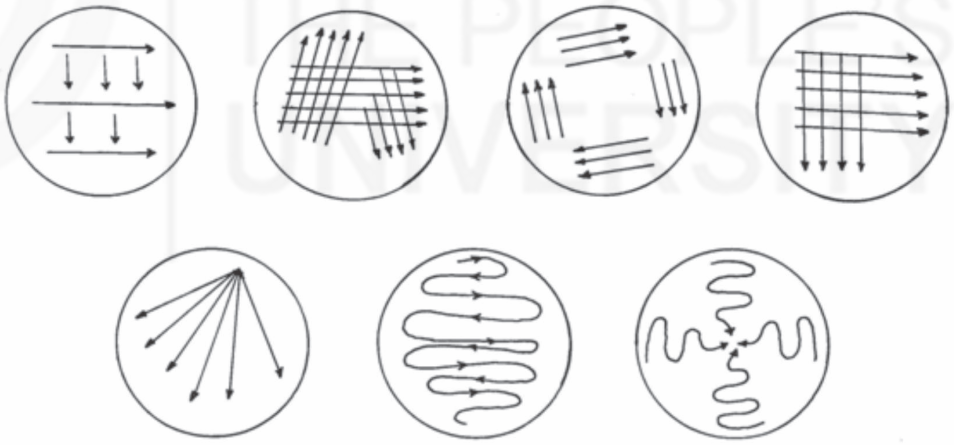


Fig. 8.3: Different ways of streaking on solid medium.

31. Seal the Petri plate dishes by securing their lids with adhesive tapes (Fig.8.4) and place them in an inverted position in the incubator at 37°C for 3 to 4 days. Observe the plates after 48 hours and see the separated colonies growing.

32. These isolated colonies would have grown from a single spore. You can re-culture these isolated colonies on other solid medium plates and store as stock culture.

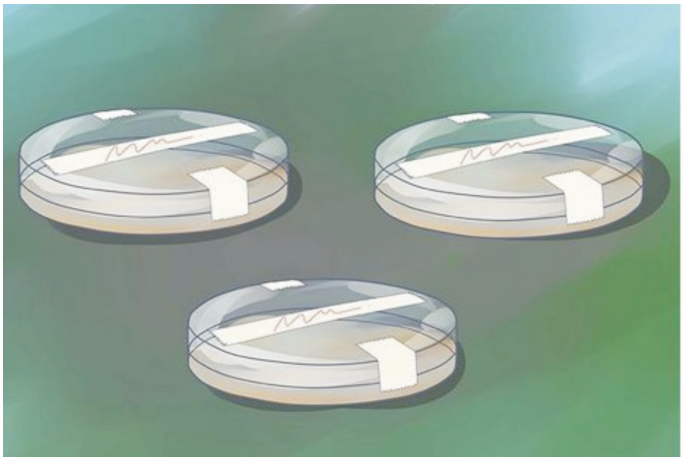


Fig. 8.4: Petri plates, secured with adhesive tape to prevent microorganisms from air contaminating the culture, prior to being placed in the incubator.

8.2.3 Precautions

1. All the glassware and the loop must be thoroughly washed, dried and sterilized before use.
2. The medium should be poured and culture should be streaked on the laminar flow bench. In case you don't have the laminar flow bench in your lab, perform these activities on a normal bench. The bench should be first cleaned by spirit or any other disinfectant. Light a spirit lamp and keep it on the bench before you start your work. (Preferably use two spirit lamps, one on each side on the bench). A spirit lamp is used to sterilize the surrounding air by heating it.
3. Operate the laminar flow unit, autoclave, oven and incubator with the help of your counsellor.
4. Thoroughly wash your hands with soap and clean with disinfectant before and after doing the experiment.
5. Clean the bench with disinfectant after finishing your experiment.
6. Write with the help of a permanent marker pen on the bottom of the Petri plate for the culture identification. Write your name, the type of culture medium used for culturing, the name of the fungus to be cultured and the date of the inoculation.
7. Sterilize the cultured material before disposal in order to prevent contamination. Also sterilize the used glassware before washing them.

8.3 GRAM'S STAINING OF BACTERIA

8.3.1 Materials Required

Crystal violet stain

Gram's iodine

Safranin stain

95% Ethyl alcohol

Blotting paper

Glass slides/microslides

Distilled water

Wash bottles

Pasteur pipettes

Inoculating loop (metal loop)

Cultures of non-pathogenic bacteria like *Escherichia coli, Pseudomonas* etc.

8.3.2 Procedures

1. Sterilise the inoculating loop before using it, by heating it in the flame of a Bunsen burner lamp and then cooling it as you have already learnt in subsection 8.2.2.of this laboratory exercise.
2. Transfer a loopful of bacterial culture provided to you by your counsellor on a clean glass slide. After transfer of the culture on the slide again re-sterilise the loop before setting it down on the work bench.
3. Make a bacterial culture smear by spreading the bacterial culture on the slide with the help of another slide. Allow the smear to air-dry. Pass this air dried smear through the Bunsen burner flame, 4 to 6 times in order to heat fix the bacteria. Because of heat fixing the bacterial enzymes are denatured and so autolysis is prevented. Also the bacterial cells adhere to the slide because of heat exposure.
4. With the help of a Pasteur pipette add a drop of crystal violet stain solution (called primary stain) on the fixed bacteria smear. After one minute, wash off the excess stain with tap water. You will observe that the bacteria are stained purple.
5. Next apply by means of a dropper, a drop of iodine solution (Gram's iodine also called mordant) to the fixed the bacterial smear that has earlier been stained with crystal violet stain. Iodine solution intensifies the ionic bond between the crystal violet stain and the bacteria. After one minute wash off the excess stain with tap water.
6. After this with the help of a dropper apply a drop of 95 % ethyl alcohol that act as decolorizing agent for the primary stain i.e. crystal violet. After one minute wash off the excess alcohol with tap water.
7. The decolorising agent may wash out the crystal violet stain from bacterial smear (decolorizing it). If decolorsation occurs then this indicates that the smear contains gram negative bacteria. However if decolorisation does not occur and the bacterial smear retains the deep violet colour due to crystal violet stain then this indicates that the smear is unaffected by the decolouring agent and contains gram positive bacteria.

8. The decolorised smear consisting of gram negative bacteria is further stained. For this purpose apply with help of a dropper a drop or two of the safranin stain which is called a secondary stain or counter stain. After forty-five seconds wash off the excess stain with tap water. Blot dry the slide with blotting paper. The decolorised bacterial smear consisting of gram negative bacteria will be stained red due to counter staining.

9. You can observe your slides under a compound microscope under oil immersion to see the difference in the staining of bacteria.

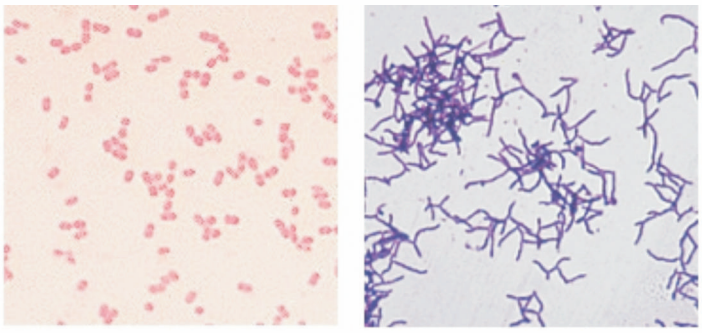


Fig 8.5: Showing: a) gram negative; b) gram positive bacteria after staininig
(*Source*: https://www.tumblr.com/search/gram%20%20stain)

EXPERIMENT 9

DNA EXTRACTION

Structure

9.1 INTRODUCTION

Have you ever wondered how scientists extract DNA from an organism? All living organisms have DNA, which is short for deoxyribonucleic acid and is their genetic material, in some viruses the genetic material is RNA (ribonucleic acid) instead of DNA. DNA is basically the blueprint for everything that happens inside an organism's cells. Overall, DNA tells an organism how to develop and function, and is so important that this complex organic compound is found in virtually every cell. DNA is the genetic instruction manual for making an organism. All processes in the body are controlled by DNA. The chromosomes that we see in a cell are a complex of DNA and associated protein and each gene is a sequence of DNA.

DNA is a molecule in the form of a double helix – two spirals twisting around each other. These spirals are the backbone of the DNA, and are made up of sugars and phosphates. The spirals are connected by chemicals known as bases, which stretch between the spirals like the rungs of a ladder. DNA has four types of bases: adenine (A), thymine (T), guanine (G) and cytosine (C). A always binds with T while G binds with C.

You will be able to extract crude DNA from strawberries and can see it by naked eye. Strawberries are soft and easy to pulverize and have large genomes; they are octaploid, which means they have eight of each type of chromosome in each cell. Thus, strawberries are good choice as a fruit to be used for DNA extraction. If strawberries are not available you can use banana or kiwi.

Expected Learning Outcomes

After doing this experiment, you would be able to:

- extract crude DNA from strawberry; and
- observe crude DNA coil through naked eye.

9.2 Materials

- 70% alcohol
- Measuring cylinder (100 ml)
- Weighing balance and Weights
- NaCl (Salt)
- Distilled water
- Dishwashing liquid
- Beaker (50 ml, 100 ml)
- Cheesecloth
- Funnel
- Three strawberries (approx.10-12 gm each)
- Resealable plastic sandwich bag
- Glass rod

9.3 METHOD

- Prepare 70% alcohol, for this you have to take 70 ml of absolute alcohol (95% ethanol) and then add distilled water to make 95 ml of total volume. You can also use, USP/Surgical spirit, B.P which is a liquid prepared and used primarily for topical application). Keep the prepared alcohol in the freezer as cooled alcohol is necessary for extraction.
- Mix 5 gm of NaCl, 50 ml of distilled water and 9ml of dishwashing liquid in a beaker. Set the mixture aside. This is your extraction liquid. Extraction liquid help's in breaking the cell wall and nucleus and releasing the DNA from the cell of strawberries. The detergent breaks the cell membrane and nuclear membrane by breaking lipids and proteins interaction.
- Now line the funnel with cheesecloth so it is covered completely. Insert the funnel tube into another empty 100 ml beaker.
- Remove and discard the green sepals from the strawberries and wash them with distilled water. Put the strawberries into a resealable plastic sandwich bag and push out all of the extra air. Seal the bag tightly.
- Now squeeze and smash the strawberries for two minutes with your

fingers. Add 9 ml of the extraction liquid which you have prepared, into in the bag with smashed strawberries. Again push out all of the extra air and reseal the bag. Squeeze the strawberry mixture with your fingers for one more minute.

Fig. 9.1: Squeezed and smashed strawberries

- Pour the strawberry mixture from the bag into the funnel lined with cheese cloth. Let it drip through the cheesecloth and into 100 ml beaker until there is very little liquid left in the funnel.

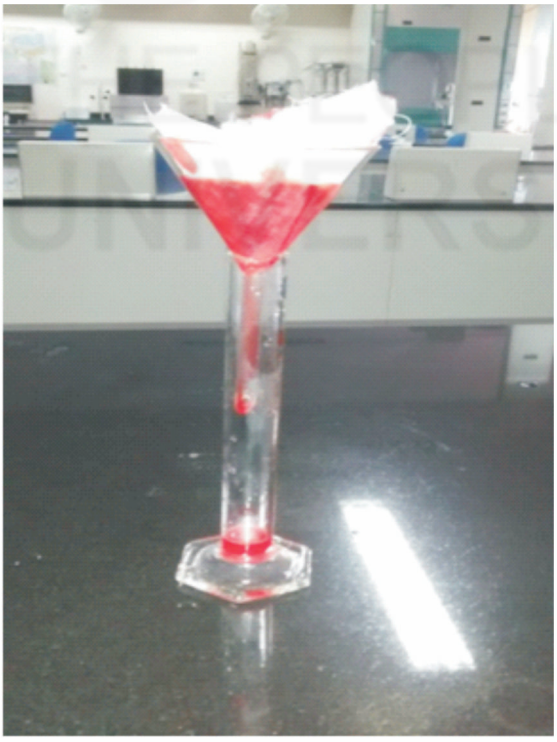


Fig. 9.2: Straining of strawberries.

- Now measure out 50 to 60 ml cold alcohol which you have kept in freezer. Tilt the beaker and very slowly pour the cold alcohol down its side. Pour until the alcohol has formed approximately a one-inch-deep

layer on top of the strawberry liquid. You may not need all of cold alcohol to form the one-inch layer. You have to take precaution so that the strawberry liquid and alcohol does not mix.

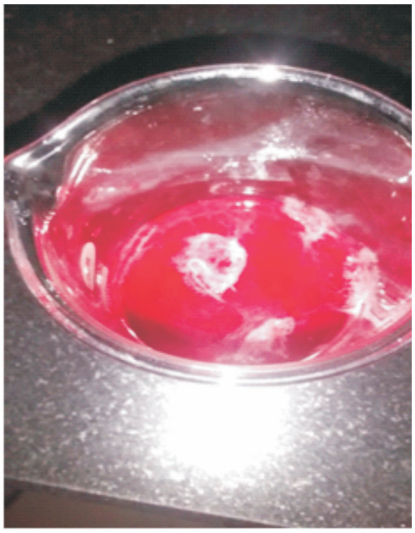


Fig. 9.3: After pouring the cold alcohol.

- Now look at the mixture inside of the jar. Do you see something sticky white?
- This is DNA. When it looks very stringy, place a glass rod into the beaker so that the end of the rod is just below the upper layer of liquid (alcohol) and try to spool the DNA.
- Using a glass stirring rod, gently but quickly twirl the rod into and out of the two layers. Gently lift the rod out of the tube and observe any substance attached to the rod.

9.4 OBSERVATION

- You can see some stringy substance precipitated out.

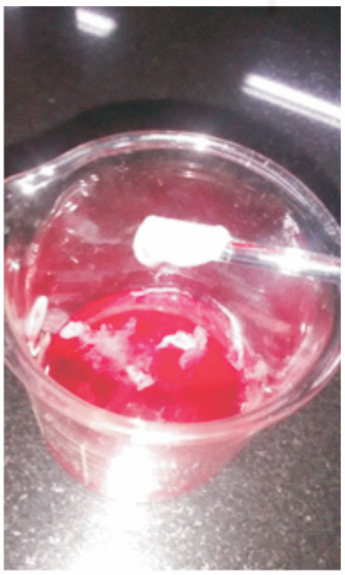


Fig. 9.4: Spooled DNA.

- It looks very clear and glistens around the glass rod. What you see is crude DNA with associated proteins.

Fig. 9.5: DNA extracted.