

**G.C.E.(A/L)**  
**BIOLOGY**  
**Practical Handbook**  
**(Effective from 2017)**



**Department of Science**  
**Faculty of Science and Technology**  
**National Institute of Education**  
[www.nie.lk](http://www.nie.lk)

**G.C.E.(A/L) Biology**  
**Practical Handbook**

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## **Message from the Director General**

With the intention of realizing the National Educational Objectives recommended by the National Education Commission and with the primary intention of developing common competencies, the content based curriculum which was earlier effective was modernized and the first phase of the new competency based curriculum of an eight year cycle was introduced by the National Institute of Education to the primary and the secondary education system of Sri Lanka in 2007.

Based on the facts revealed by the research and the suggestions by the various sectors on education, a curriculum rationalization process has resulted in the second phase of the curriculum cycle which has commenced its introduction from 2015.

The primary objective of this rationalized new curriculum is to transform the student population into a human resource replete with the skills and competencies required for the world of work, through embarking upon a pattern of education which is more student centered and activity based.

Within this rationalization process, the concepts of vertical and horizontal integration have been employed in order to build up competencies of students, from foundation level to higher levels, and to avoid repetition of subject content in various subjects respectively and furthermore, to develop a curriculum that is implementable and student friendly.

Practical work is part and parcel of the teaching and learning in science. Most practitioners would agree that good- quality practical work can engage students, help them to develop important skills, help them to understand the process of scientific investigation and develop their understanding of concepts.

The new Practical Handbook has been introduced with the aim of providing the teachers with necessary guidance for planning practical activities engaging students effectively in the teaching learning process and to promote students' practical skills in the discipline of Biology.

I wish to make use of this opportunity to thank and express my appreciation to the members of the Council and the Academic Affairs Board of the NIE, the resource persons who contributed to compile this Practical Hand book and other parties for their dedication in this matter.

**Dr. (Mrs.) T. A. R. J. Gunasekara**

Director General

National Institute of Education

## Message from the Director

This Practical Handbook has been developed to support you in advancing your students to fluency in science. We have worked with teachers, university lecturers and curriculum experts to produce this guide the activities presented in this Practical Handbook satisfy curriculum objectives in the areas of Biology. There are three interconnected, but separate, reasons for doing practical work in a course of study they are:

1. To support and consolidate scientific concepts (knowledge and understanding).

This is done by applying and developing what is known and understood of abstract ideas and models. Through practical work we are able to make sense of new information and observations, and provide insights into the development of scientific thinking.

2. To develop investigative skills.

These transferable skills include devising and investigating testable questions, identifying and controlling variables, and analyzing, interpreting and evaluating data.

3. To build and master practical skills.

Such skills include using specialist equipment to take measurements, handling and manipulating equipment with confidence and fluency, recognizing hazards and planning how to minimize risk.

By focusing on the aforesaid reasons for carrying out a particular practical work, I hope, teachers will help their students understand the subject better, to develop the skills of a scientist and to master the manipulative skills required for further study or jobs in STEM subjects (Science, Technology, Engineering, and Mathematics).

In order to make practical work effective, the laboratory should be made a place of learning by doing. Guideline should be laid down by the teacher about the laboratory rules such as the following-

- Work area must be cleared.
- Strict attention should be paid to own work.
- Reagent stoppers should not be left on counter tops.
- Wastage of water, gas, electricity should be strictly avoided.
- Directions should be read and followed very carefully.
- Teachers should allow the student's entry in lab in his/her presence.
- Only those experiments should be done which are recommended by the teacher in-charge.

I take this opportunity to thank university lecturers, teachers, and the other resource persons who contributed to make this effort a success. Finally, I wish practicing biology teachers all the success in their effort to empower our young generation to be successful as members of information-rich as well as technologically advanced societies.

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## PRACTICAL NO.1

### Identification of starch, non-reducing sugars, reducing sugars, proteins and lipids using simple laboratory tests

#### Objectives

##### Students should be able to

- conduct tests to identify the biomolecules of given food materials,
- follow laboratory procedures accordingly,
- conduct experiments with due care,
- record procedures and observations,
- present the obtained results analytically.

#### Materials and equipment

- 1% lactose solution
- 1% fructose solution
- 1% glucose solution
- 1% sucrose solution (Analar sucrose)
- 1% starch solution (corn flour is recommended)
- Coconut oil or Sesame oil
- Egg albumin
- Iodine in Potassium Iodide solution
- Diluted HCl/H<sub>2</sub>SO<sub>4</sub>
- Sodium Hydrogen Carbonate (NaHCO<sub>3</sub>)
- Benedict's reagent
- Sudan III
- 5% Potassium hydroxide solution
- 1% Copper sulphate solution
- pH papers
- Test tubes
- Test tube rack
- Bunsen burner
- Spatula
- 1cm<sup>3</sup> syringe

#### Instructions

- Demonstrate simple laboratory tests to identify starch, non-reducing sugars, reducing sugars, proteins and lipids (appendix I).
- Provide (relevant pure forms of) food materials and equipment for the students.
- Guide students wherever necessary.
- Instruct the students to record the observations and present them analytically.

## PRACTICAL NO.2

### Identification of parts and functions of the light microscope and the use of light microscope to observe specimens

#### Objectives

##### Students should be able to

- name the parts and understand the functions of a light microscope,
- list the functions of a light microscope,
- use the microscope in the correct manner,
- prepare wet mounts of live tissues or cells,
- manipulate the microscope to observe specimens,
- calculate the magnification of objects,
- draw cells proportionately.

##### Materials and equipment

- Light microscopes with low, medium and high power objective lenses
- Clean dry slides and cover slips
- Beaker and watch glasses/ Petri dishes
- Water sample from paddy field, hay infusion, pond water sample, onion/*Rhoeo* epidermal peel, buccal cavity lining.
- Paint brushes
- razor blades
- Mounting needles

##### Instructions

- Instruct the students to follow the guidelines given below.
- Identify the major parts of the microscope (appendix II): The body and base, ocular tube, eyepieces (interchangeable), rotatable nose piece, low, medium and high power objective lenses (which can be screwed in), focusing knobs for coarse and fine focusing, stage with circular opening at center, stage clips, adjustable mirror.
- Instruct students to observe the samples employing proper microscopic techniques.
- Make thin epidermal peels of onion /*Rhoeo (Tradescantia)* and place in water (in a watch glass or Petri dish).
- Transfer a piece of onion *Rhoeo (Tradescantia)* peel into a drop of water on the center of a clean glass slide using a fine paint brush.

Hold the cover slip at the edge of the drop of water, with the help of a mounting needle, and gently lower the cover slip while supporting it with the needle onto the drop of water. Do not allow air bubbles to be trapped under the cover slip.

- Place the slide on the stage of the microscope and observe under low power objective lens.
- Looking through the eye piece, move the slide to bring the object into position for study.
- Adjust the mirror and condenser to give optimum illumination to the object for clear viewing.
- Use the coarse focusing knob to get the image as clear as possible.
- Use the fine focusing knob to improve the quality of the image.
- Study and note the structures visible.
- Rotate the nose piece and bring the medium power into position. Adjust the focusing to get a sharp image.
- Bring the high power into position.
- Use the fine focusing knob only to make the image sharp.
- Study and record what you observe under low, medium and high power.
- Follow the steps given above to observe a drop of water from paddy field, hay infusion, pond water and cells obtained from buccal cavity lining
- Direct them to make notes and draw sketches on their observations.

## **PRACTICAL NO.3**

### **Use of electron micrographs to understand the structure of cellular components**

#### **Objectives**

##### **Students should be able to**

- interpret an electron micrograph,
- identify the cellular components as seen by an electron micrograph,
- draw the cellular components accurately,
- compare the electron micrographs and list down the differences among them.

##### **Materials and equipment:**

- Electron micrograph of a bacterial cell
- Electron micrograph of an animal cell
- Electron micrograph of a plant cell.

##### **Instructions**

- Allow the students to observe the electron micrographs of the following; a bacterial cell, a plant cell and an animal cell
- Advise them to identify /recognize cellular components/organelles.
- Guide students to draw their observations.
- Allow students to compare the above electronmicrographs and guide them to list down the differences.

## PRACTICAL NO.4

### Identification of different stages of mitosis and meiosis using microscopic slides

#### Objectives

##### Students should be able to

- identify the major/main stages of cells in the process of mitosis and meiosis,
- observe and record the behavior of chromosomes during the two types of cell division,
- state the observable differences between mitosis and meiosis from their microscopic observations.

#### Materials and equipment

- Light microscope
- Onion root tips (permanent/ temporary slides) for studying mitosis
- Anther of *Rhoeo/ Tradescantia* (permanent/ temporary slides) for studying meiosis
- Computer animations

#### Instructions

- Let the students observe each of the slides under low, medium and high powers of the microscope respectively.
- Ask them to identify the cells which show the main stages of mitosis and meiosis using the positions and shapes of the chromosomes.
- Direct students to identify, carefully the various positions and shapes of the chromosomes and the changes that take place.
- Direct students to draw the observed stages of mitosis and meiosis in correct sequence.
- Assist students to describe major events of cells undergoing mitosis and meiosis using computer animations.

## PRACTICAL NO.5

**Laboratory experiment to demonstrate enzyme activity and to determine the effect of temperature on the rate of enzymatic reaction (starch - amylase)**

### Objectives

#### Students should be able to

- set up the starch-amylase reaction,
- record the time taken for the reaction,
- tabulate the results and observations,
- conduct the experiment-set different temperatures,
- interpret the observation analytically.

#### Materials and equipment

- 1% (w/v) amylase solution
- 1% (w/v) starch solution
- Iodine solution (I<sub>2</sub>/KI)
- Stop watch
- White porcelain tile
- Thermometer
- Pipettes
- Water bath
- Boiling tubes and test tubes

#### Instructions

- Instruct students to set up the experiments as given below.
  - Measure definite volumes (5 ml) of amylase solution and (10 ml) of starch solution into separate test tubes.
  - Allow the solutions to attain the same temperature.
  - Mix up the two solutions and start the stop watch (starch to amylase).
  - Test a drop of reaction mixture with a drop of Iodine solution on the white porcelain tile at 2 minute intervals.
  - Continue the test until the dark blue colour will not appear.
    - Observe the time taken.
    - Tabulate the results indicating time elapsed and change of the colour.
    - Repeat the above procedure for different temperatures (5 °C, room temperature, 40 °C, 60 °C- Temperature can be maintained by adding cold or hot water to the water bath).
    - Assist students to plot a graph using the results obtained (1/t vs temperature).
    - Guide them to interpret their findings analytically.

## PRACTICAL NO.6

### Determination of rate of photosynthesis by the amount of oxygen released using Audus apparatus(for different CO<sub>2</sub> concentrations and light intensities)

#### Objectives

#### Students should be able to

- set up the Audus apparatus according to the instructions using *Hydrilla* or *Elodea*,
- determine the rate of photosynthesis by measuring the volume of oxygen released. assuming that the gas evolved within the setup is oxygen, and proportionate to the rate of photosynthesis
- conduct the experiment at different light intensities,
- draw conclusions from the results obtained,
- suggest an experimental method to show the effect of concentration of carbon dioxide on the rate of photosynthesis.

#### Materials and equipment

- Aquatic plants such as *Hydrilla* or *Elodea*
- Audus photosynthesis apparatus ( micro burette)
- 0.01% solution of Sodium hydrogen carbonate
- Test tube, glass funnel, table lamp, thermometer, stop watch, ruler

#### Instructions

- Direct the students to set up the Audus apparatus. Make sure that the micro burette is completely filled with water. Place a table lamp close to the aquatic plants to provide adequate light (appendix II).
- Let them observe the oxygen bubbles released due to photosynthesis and how oxygen bubbles get collected at the bend of micro burette.
- Instruct them to measure the volumes of oxygen released by plant using a syringe at Definite intervals.
- Direct them to determine the rate of photosynthesis at various conditions such as changing the intensity of light, (by changing the distance of the table lamp).
- Direct them to record and analytically interpret the results.

**Note :** Guide students to suggest an appropriate experimental set up to demonstrate the effect of carbon dioxide concentration on the rate of photosynthesis.

## **PRACTICAL NO.7**

**Microscopic observation of cross sections of C<sub>3</sub> and C<sub>4</sub> plant leaves with special reference to the adaptations for photosynthesis**

### **Objectives**

#### **Students should be able to**

- use the light microscope to observe the permanent slides with cross sections of C<sub>3</sub> and C<sub>4</sub> plant leaves,
- identify the differences between the cross sections of C<sub>3</sub> and C<sub>4</sub> leaves,
- draw suitable labeled diagrams to show the cross sections of C<sub>3</sub> and C<sub>4</sub> plant leaves,
- relate the differences of cross sections of C<sub>3</sub> and C<sub>4</sub> leaves to the functions based on their adaption for photosynthesis.

#### **Materials and equipment**

- Microscopes.
- Prepared slides of cross sections of C<sub>3</sub> and C<sub>4</sub> plant leaves.

#### **Instructions**

- Allow students to examine slides under the low power of the microscope.
- Direct them to observe the differences in the cross sections of C<sub>3</sub> and C<sub>4</sub> plant leaves.
- Let suitable labeled diagrams to show the observed characters under low power of the microscope.
- Let the students correlate the observed structures with the adaptations of C<sub>3</sub> and C<sub>4</sub> plants for photosynthesis.

## PRACTICAL NO. 08

### Determination of rate of respiration and respiratory quotient using germinating seeds

#### Objectives

#### Students should be able to

- set up the apparatus to determine the rate of respiration of germinating seeds,
- make accurate observations and measurements,
- determine the rate of respiration by measuring the volume of oxygen used or the volume of carbon dioxide released,
- determine the respiratory quotient.

#### Materials and equipment

- Green gram seeds
- Two respire meters
- KOH solution
- Ignition tubes
- Stop watches
- Balances
- Water bath
- Vaseline / clay

#### Instructions

- Guide the students to germinate the green gram seeds, by soaking in water for at least 8 hours and spreading on a wet paper for one day.
- Guide the students to set up two respire meters (appendix II) and to follow the instructions given below.
  - Add equal weights (25 g) of germinating seeds to each.
  - Fill one ignition tube with KOH solution and the other one with equal volume of water.
  - Insert these ignition tubes into the two respire meters separately.
  - Make the apparatus as airtight using Vaseline or clay.
  - Keep the flasks of the respire meters in a water bath.
  - Level the coloured liquid columns in A and B using C stopper.
  - Note the initial positions of the water column in each of the tubes.
  - Start the stop watch.
  - Observe and record changes in the water column after two hours.
  - Calculate the volume of O<sub>2</sub> used and the volume of CO<sub>2</sub> released and determine the rate of respiration and the respiratory quotient.

## **PRACTICAL NO.09**

### **Observing and distinguishing bacteria and cyanobacteria under light microscope**

#### **Objectives**

#### **Students should be able to**

- observe characteristic features of bacteria and cyanobacteria under the microscope,
- distinguish bacteria and cyanobacteria,
- draw diagrams showing features of bacteria and cyanobacteria

#### **Materials and equipment**

- Permanent slides/ temporary preparations of *Anabaena*, *Lyngbia* and *Microcystis*
- Light Microscopes

#### **Instructions**

- Let students observe and identify the characteristic features of above mentioned bacteria and cyanobacteria using light microscopes.
- Let students to record their observations using appropriate diagrams.

## **PRACTICAL NO.10**

**Use of electron micrographs to study morphological features of bacteria and cyanobacteria**

### **Objectives**

**Students should be able to**

- identify characteristic features of bacteria using an electron micrograph,
- identify characteristic features of cyanobacteria using an electron micrograph,
- distinguish bacteria and cyanobacteria based on their electron micrographs.

### **Materials and equipment**

- Electron micrographs of bacteria.
- Electron micrographs of cyanobacteria.

### **Instructions**

- Allow students to examine the electron micrograph of bacteria.
- Allow students to examine the electron micrograph of cyanobacteria.
- Let students to compare and contrast bacteria and cyanobacteria.

## PRACTICAL NO.11

### Observation of morphological features of *Euglena*, *Paramecium*, *Amoeba*, *Ulva*, Diatoms, *Sargassum*, *Gelidium*

#### Objectives

##### Students should be able to

- Identify *Euglena*, *Paramecium*, *Amoeba*, *Ulva*, Diatoms, *Sargassum* and *Gelidium* using diagrams / slides / specimens,
- List morphological features of the above organisms,
- record the observations.

#### Materials and equipment

- Diagrams/permanent slides/specimens of *Euglena*, *Paramecium*, *Amoeba*, *Ulva*, Diatoms, *Sargassum*, *Gelidium*.
- Light Microscopes
- Slides and cover slips

#### Instructions

- Allow students to observe the diagrams/ slides/ specimens of *Euglena*, *Paramecium*, *Amoeba*, *Ulva*, Diatoms, *Sargassum*, *Gelidium*.
- Let the students list characteristic morphological features of above mentioned organisms and identify each of them
- Let students record the observations.

## PRACTICAL NO.12

**Observation of morphological features of organisms of phyla Hepatophyta, Bryophyta, Anthoceroophyta, Lycophyta, Pterophyta, Cycadophyta, Gnetophyta, Coniferophyta, Anthophyta and classes Monocotyledoneae and Dicotyledoneae**

### Objectives

#### Students should be able to

- Identify *Marchantia*, *Pogonatum*, *Anthoceros*, *Selaginella*, *Nephrolepis*, *Cycas*, *Gnetum*, *Pinus* and flowering plants using specimens/ diagrams,
- list morphological features of above mentioned organisms,
- record the observations.

#### Materials and equipment

- Specimens/ diagrams of *Marchantia*, *Pogonatum*, *Anthoceros*, *Selaginella*, *Nephrolepis*, *Cycas*, *Gnetum*, *Pinus* and flowering plants-a monocot and a dicot
- Hand lenses

#### Instructions

- Allow students to observe the diagrams /specimens of *Marchantia*, *Pogonatum*, *Anthoceros*, *Selaginella*, *Nephrolepis*, *Cycas*, *Gnetum*, *Pinus* and flowering plants-a monocot and a dicot.
- Let the students list characteristic morphological features of above mentioned organisms and identify each of them
- Let students record their observations.

**Note :** Arrange a field study to observe above organisms.

## PRACTICAL NO.13

### Observation of characteristic features of organisms of phyla Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota

#### Objectives

##### Students should be able to

- Identify *Allomyces*, *Mucor*, *Aspergillus* and *Agaricus* using diagrams/ slides / specimens,
- list characteristic features of above mentioned organisms,
- record the observations.

##### Materials and equipment

- Diagrams/slides/specimens of *Allomyces*, *Mucor*, *Aspergillus* and *Agaricus*
- Light Microscopes
- Slides and cover slips

##### Instructions

- Allow students to observe the diagrams/ slides/ specimens of *Allomyces*, *Mucor*, *Aspergillus* and *Agaricus*.
- Let the students list characteristic morphological features of above mentioned organisms and identify each of them
- Let students record their observations.

##### Note

- Fungal growth rate is higher in dark places.
- Mycelia of *Mucor* can be obtained
  - by making a thin layer of moistened flour on a glass slide and keeping the slide inside a Petri dish
  - or by keeping moistened bread covered with a glass jar.

## PRACTICAL NO.14

### Observation of morphological features of organisms of phyla Cnidaria, Platyhelminthes, Nematoda, Annelida, Mollusca, Arthropoda and Echinodermata

#### Objectives

##### Students should be able to

- Identify common organisms of phyla Cnidaria, Platyhelminthes, Nematoda, Annelida, Mollusca, Arthropoda and Echinodermata using morphological features,
- record the observations,
- develop and use dichotomous keys to distinguish animals.

##### Materials and equipment

- Diagrams/ slides/ specimens of *Hydra*, *Planaria*, *Ascaris*, Earthworm, Garden snail, Starfish
- Light Microscopes

##### Instructions

- Allow students to observe organisms listed above
- Let the students to list characteristic morphological features of above mentioned organisms and identify each of them
- Let students to record their observations
- Let the students to prepare a dichotomous key to distinguish the above mentioned organisms.

## PRACTICAL NO.15

### Observation of characteristic features of organisms of classes Osteichthyes, Chondrichthyes, Amphibia, Reptilia, Aves and Mammalia

#### Objectives

##### Students should be able to

- Identify shark/skate, tuna, toad/salamander/*Ichthyophis*, lizard/cobra/crocodile, crow, a common mammal using specimens/ diagrams,
- list characteristic morphological features of above mentioned organisms,
- record the observations.

#### Materials and equipment

- Specimens/ diagrams of shark/ skate, tuna, toad/salamander/ *Ichthyophis*, lizard/ cobra /crocodile, crow, a common mammal

#### Instructions

- Allow students to observe the diagrams/ specimens of shark/ skate/ tuna/ toad/ frog/ salamander/ *Ichthyophis*, lizard/ cobra /crocodile, crow, a common mammal
- Let the students list the characteristic morphological features of above mentioned organisms and identify each of them
- Let the students record their observations.

## PRACTICAL NO.16

### Identification of special characters of cell types of plant tissues using light microscope

#### Objectives

##### Students should be able to

- identify plant tissues namely parenchyma, collenchyma, sclerenchyma (sclereids, fibers), xylem and phloem using light microscope,
- draw suitable diagrams of observed plant tissues as seen through the light microscope proportionately,
- differentiate the plant tissues according to the characters of each tissue.

#### Materials and equipment

- Light Microscopes
- Prepared slides of cross sections of stem, root and leaf of *Helianthus*
- Other suitable prepared slides containing major plant tissues (cross section of *Nymphaea* leaf petiole, leaf epidermis of monocot and dicot, material macerated from flesh of Guava/ *Annona* fruits, and wood of stem cuttings, etc.)
- Slides and coverslips  
(If prepared slides are not available make suitable wet mounts in the classroom.)

#### Instructions

- Allow students to examine the slides under low power of the microscope.
- Direct them to identify the areas /zones which show the distribution of different tissues.
- Let students identify the characteristics of each tissue under medium and high powers.
- Provide students with other suitable prepared slides for further identification of a variety of plant tissues.
- Let students make suitable diagrams to show the observed characteristics of the tissues.

## PRACTICAL NO. 17

### Observation of transverse sections of primary stem and primary root of a monocot and a dicot

#### Objectives

##### Students should be able to

- cut thin sections of parts of plants,
- identify the arrangement of different tissues in primary roots and primary stems under the light microscope,
- distinguish anatomical differences between transverse sections of a monocot and a dicot,
- Draw and label line diagrams of transverse sections of monocot and dicot observing under light microscope.

##### Materials and equipment

- Transverse section of a dicot root taken from a bean seedling or other similar plant.
- Transverse section of an onion root or any other similar plant.
- Transverse section of a dicot stem taken from a plant like *Tridax*.
- Transverse section of a monocot stem taken from a grass or other similar plant.
- Razor blades, slides, cover slips, small paint brushes, watch glasses.
- Light Microscopes

##### Instructions

- Guide students to cut thin transverse sections and transfer them to the water in a watch glass.
- Instruct them to mount a thin section to a drop of water on a glass slide and cover it with a cover slip.
- Ask them to observe the prepared wet mounts under the light microscope.
- Let them observe the structure and distribution of the different types of tissues and cells.
- Direct them to identify epidermis, cortex, endodermis, pericycle, xylem, phloem and pith of the prepared thin sections.
- Instruct students to make line diagrams to demarcate the important structures studied.
- Ask them to label the above mentioned tissues in their diagrams.

## PRACTICAL NO.18

### Microscopic and macroscopic examination of secondary structure of dicotyledonous wood using transverse sections

#### Objectives

##### Students should be able to

- identify different tissues in a mature dicot stem,
- identify the growth rings of a dicot stem,
- prepare wet mounts of thin transverse sections of a dicot stem.

#### Materials and equipment

- Part of a secondary thickened dicot plant stem (*Stachytarpheta /Vernonia/ Helianthus*)
- Slice of a stem of a matured woody plant
- Watch glasses with water, slides and cover slips
- Razor blades and small paint brushes
- Aniline sulphate solution
- Light Microscopes
- A slice of a stem of matured woody plant

#### Instructions

- Instruct students to cut thin transverse sections of the given stem and transfer them in to a watch glass filled with water.
- Instruct students to stain the stem sections with Aniline sulphate solution.
- Ask them to mount sections in a drop of water, on a slide and cover it with a cover slip.
- Let the students to observe prepared wet mounts under low power of light microscope and select a thin section where secondary xylem and secondary phloem has just begun to form.
- Direct them to observe under high power and note the distribution of different tissues.
- Guide the students to observe a slice of a stem of a matured woody plant and identify important structures such as bark, sap wood, heart wood and growth rings (macroscopic observation).
- Instruct the students to record their observations.

## PRACTICAL NO.19

### Observation of the structure of stomata and lenticels through light microscope

#### Objectives

#### Students should be able to

- identify the structure of stomata,
- Identify the structure of lenticels,
- Draw and label diagrams of stomata and lenticels.

#### Materials and equipment

- Epidermal peel taken from a fresh leaf of betel/ *Rhoeo (Tradescantia)*.
- A cross section taken from a secondary stem of *Stachytarpheta*, etc.
- A permanent slide of a transverse section of a secondary stem.
- Light microscopes.

#### Instructions

- Allow students to get an epidermal peel of a fresh leaf of betel/ *Rhoeo (Tradescantia)*.
- Allow them to get a thin cross section of a secondary stem.
- Let them observe an epidermal peel of betel/ *Rhoeo* and cross section of a secondary stem under low power of light microscope.
- Let them observe stomata and lenticels under the high power of light microscope.
- Instruct them to record their observations.

## PRACTICAL NO.20

### Determination of solute potential of epidermal peels of *Rhoeo (Tradescantia)*

#### Objectives

##### Students should be able to

- prepare solutions of known concentrations using stock solutions,
- differentiate between the status of flaccid, turgid and incipient plasmolysis of cells in *Rhoeo (Tradescantia)* epidermal peels through microscopic observations,
- determine percentage plasmolysis of the tissue by making accurate observations under light microscope
- plot a graph to illustrate the data obtained,
- determine solute potential of cells in *Rhoeo (Tradescantia)* epidermal peels using the values obtained by the graph.

#### Materials and equipment

- Fresh leaves of *Rhoeo (Tradescantia)*
- Six Petri dishes with lids (labeled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- Six test tubes (labeled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- A test tube rack
- Two 10.00 ml graduated pipettes
- A beaker with distilled water
- A beaker with 1M sucrose solution
- Fine forceps, razor blades and paint brushes
- Light microscopes
- Slides and cover slips
- Graph papers

#### Instructions

- Instruct the students to prepare 20 ml of sucrose solutions of different molarities as given (0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M) in each of the labelled test tubes using the graduated pipettes, 1M sucrose solution and distilled water.
- Direct them to pour the prepared solutions from test tubes into Petri dishes.
- Ask students to take small fragments from the lower epidermis (purple coloured) of *Tradescantia* and place a few (2-3) fragments in each of the sucrose solutions in Petri dishes.

- Instruct them to set the Petri dishes aside with their lids closed at least for 20 minutes for the cells to achieve osmotic equilibrium.
- Direct the students to mount fragments of each of the epidermal peels on slides using paint brushes in a drop of the sucrose solution from which the peels were immersed.
- Let students examine under the low power of microscope and select a clear field of cells and turn to the medium power.
- Instruct the students to count the number of cells that have undergone plasmolysis and total number of cells within that particular microscopic field.
- Ask them to calculate the percentage plasmolysis.
- Instruct the students to plot a graph with percentage of plasmolysis on Y axis versus concentration of sucrose solution on X axis.
- Direct students to determine the molarity of the sucrose solution that would give 50 % plasmolysis, from the graph. Calculate the solute potential of the sucrose solution from the Table provided.
- Discuss the results obtained.
- Guide students to observe turgid (in distilled water), flaccid and incipient plasmolysis conditions of plant cells by placing pieces of epidermal peel in petri dishes containing appropriate solutions.

**Solute potentials of sucrose solutions at 20 °C**

<b>Concentration of sucrose solution (molarity)</b>	<b>Solute potential/kPa</b>	<b>Solute potential/atm</b>
0.05	-130	-1.3
0.10	-260	-2.6
0.15	-410	-4.0
0.20	-540	-5.3
0.25	-680	-6.7
0.30	-820	-8.1
0.35	-970	-9.6
0.40	-1 120	-11.6
0.45	-1 280	-12.6
0.50	-1 450	-14.3
0.55	-1 620	-16.0
0.60	-1 800	-17.8
0.65	-1 980	-19.5
0.70	-2 180	-21.5
0.75	-2 370	-23.3
0.80	-2 580	-25.5
0.85	-2 790	-27.5
0.90	-3 010	-29.7
0.95	-3 250	-32.1
1.00	-3 510	-34.6
1.50	-6 670	-65.8
2.00	-11 810	-116.6

## PRACTICAL NO.21

### Determination of water potential of petioles of *Alocasia*/ potato strips

#### (A)Determination of water potential of *Alocasia* petioles

#### Objectives

#### Students should be able to

- measure the curvature of *Alocasia* petiole strips,
- plot a graph using the percentage of change in curvature (in Y axis) verses concentrations of sucrose solutions (in X axis),
- interpret experimental results,
- determine the water potential of *Alocasia* petioles using the graph.

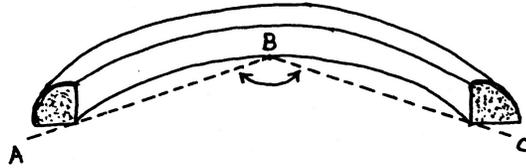
#### Materials and equipment

- Fresh petioles of *Alocasia*
- Six Petri dishes with lids (labeled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- Six test tubes (labeled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- A test tube rack
- Two 10.00 ml graduated pipettes
- A beaker with distilled water
- A beaker with 1M sucrose solution
- Fine forceps, razor blades
- Graph papers
- Protractors
- Blotting papers

#### Instructions

- Instruct students to prepare 20 ml solutions of different concentrations as given above and pour them into labeled petri dishes.
- Direct the students to follow the instructions given below.
  - Take six pieces of 6 cm long *Alocasia* petioles having uniform diameter and mark the centre of each piece.
  - Split each of them radially in to 4 strips of equal size.

- Place each piece on blank paper and mark the three points as given in the diagram.



- Measure the initial curvature as the angle.
- Immerse four such strips in each of the sucrose solutions and set aside with the lid closed for at least one hour to achieve osmotic equilibrium.
- Remove the strips from the solutions. Blot the excess solution using a blotting paper and place on a sheet of paper.
- Draw the outlines of each strip to record the curvature again and measure the angle.
- Determine the change in curvature of each strip.
- Plot a graph against the percentage change in curvature (in Y axis) verses concentrations of sucrose solutions (in X axis)
- Determine the concentration of the solution which caused no change in curvature from the graph.
- Determine the water potential of *Alocasia* tissue using the given table in practical number 20.
- Comment on their observations.

## **(B) Determination of water potential of cells of potato strips**

### **Objectives**

#### **Students should be able to**

- measure the change in length of potato strips,
- plot a graph with percentage of change in length of potato strips (in Y axis) verses molarity of sucrose solutions (in X axis),
- interpret experimental results,
- determine the water potential of potato tuber cells using the graph.

## Materials and equipment

- Fresh potato tubers
- Six Petri dishes of relevant sucrose solutions covered with lids (labelled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- Six test tubes (labelled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- A test tube rack
- Two ( 10 cm<sup>3</sup> or 25 cm<sup>3</sup> ) graduated pipettes
- A beaker with distilled water
- 1M sucrose solution
- Cork borer and razor blades
- Two 100 cm<sup>3</sup> beakers
- Graph papers

## Instructions

- Direct the students to follow the instructions given below.
  - Prepare 20ml of sucrose solutions of different molarities as given above.
  - Peel potato tubers.
  - Cut 12 cylinders from potato tubers using the cork borer.
  - Keep a graph paper below each petri dish and place two cylinders (5cm in length) of potato tubers.
  - Add appropriate sucrose solutions to each petri dish until the cylinders are completely immersed.
  - Immediately measure their lengths against the graph paper seen through the bottom of the Petri dishes.
  - Leave in covered Petri dish for 30 minutes to 60 minutes (depending on the diameter of the tubers) to achieve osmotic equilibrium.
  - Measure the lengths again and calculate the mean percentage change in length. Then plot a graph of the mean percentage change in length (Y axis) versus molarity of the sucrose solution (X axis).
  - Determine the concentration of the solution which caused no change in length from the graph.
  - Determine the water potential of potato tissue using the given Table for the Practical No. 20.

## **PRACTICAL NO.22**

### **Determination of rate of transpiration from leaves and shoots using a potometer**

#### **Objectives**

##### **Students should be able to**

- set up experiments according to the instructions,
- use a potometer to determine the rate of transpiration,
- use a potometer to show the effect of environmental factors on transpiration,
- interpret their observations analytically.

#### **Materials and equipment**

- a shoot from healthy plant cut under water
- Ganong's potometer or any other potometer
- Vaseline/ clay

#### **Instructions**

- Guide the students to set up the potometer as follows:
  - Cut and fix a shoot underwater to the Ganong's potometer.
  - Apply Vaseline/ clay on the rubber stopper to make it air tight.
  - Introduce an air bubble into the capillary tube of potometer.
  - Keep the potometer at a place when there is sufficient light.
- Record the time taken for the air bubble to travel a particular distance in the capillary tube.
- Correlate the rate of movement of the air bubble to the rate of transpiration.
- Change the environmental factors (wind speed, light intensity and humidity) and note the changes in rate of movement of the air bubble.
- Comment on the influence of above environmental factors on the rate of transpiration.
- Suggest appropriate experimental set ups for demonstrating the influences of other environmental factors on the rate of transpiration.

## **PRACTICAL NO.23**

### **Microscopic observation and identification of different types of animal tissues**

#### **Objectives**

##### **Students should be able to**

- observe major animal tissues using a light microscope,
- to draw suitable diagrams of animal tissues as seen through the light microscope proportionately,
- differentiate the animal tissues according to their characteristic features.

#### **Materials and equipment**

- Light Microscopes
- Prepared slides of epithelial tissues, smooth, striated and cardiac muscles, connective tissues such as loose, dense, cartilage, bone and human blood cells

#### **Instructions**

- Allow students to examine the slides of epithelial tissues, smooth, striated and cardiac muscles, connective tissues such as loose, dense, cartilage, bones and human blood cells under a light microscope.
- Let students draw suitable diagrams to show the observed characteristics of above tissues.
- Instruct the students to record their observations highlighting the identified features of each tissue.

## **PRACTICAL NO.24**

### **Explaining the structure of human digestive system using diagrams and models**

#### **Objectives**

#### **Students should be able to**

- relate the functions of each part of the alimentary canal of man,
- relate the functions of each part to its structure.

#### **Materials and equipment**

- Chart / model/computer animations to illustrate clearly the entire alimentary canal.
- Prepared slides containing T.S. of liver and T.S. of pancreas.
- Light microscopes

#### **Instructions**

- Provide students with wall charts/ models/computer illustrations to observe the major parts of the alimentary canal.
- Direct students to examine the gross external morphology of the stomach, small intestine, large intestine and rectum.
- Let students to observe charts/slides/ models /computer illustrations of T.S of liver and T.S of pancreas.
- Instruct students to make appropriate short notes and illustrative sketches in respect of all above observations.

## PRACTICAL NO. 25

### **Describing the organization of circulatory system of man using specimens/models/ diagrams**

#### **Objectives**

##### **Students should be able to**

- Describe the location and gross external structure of the human heart, its blood supply and related major arteries and veins,
- describe the major features of the internal structure of heart,
- explain the human heart as an example of a mammalian heart with complete double circulation.

#### **Materials and equipment**

- A model/chart / computer illustrations of heart showing gross external morphology including pericardium, main vessels entering and leaving the heart and the main coronary vessels.
- A model/chart showing gross internal structure of heart in sectional view including chambers, valves, origin of main vessels, position of pacemaker and Bundle of His.
- A model/chart / computer illustrations/ computer animations showing the cardiac cycle, directions of blood flow, pattern of transmission of neuro- muscular impulse.
- Charts showing the main pattern of arterial and venous circulation and diffusion in capillary beds.

#### **Instructions**

- Instruct students to study the external and internal structure of the heart using the models/ computer illustrations and charts.
- Direct them to relate the cardiac cycle to the transmission of neuro- muscular impulses.
- Instruct the students to record their observations.

## PRACTICAL NO. 26

**Explaining the structure of human respiratory system using models or diagrams and observations of effects of exercise on respiratory rate and pulse rate**

### Objectives

**Students should be able to;**

- describe the gross structure of human respiratory system.
- describe the location of lungs in the thoracic cavity.
- relate the structure of major components of the respiratory system to the function of each component.
- measure pulse rate and respiratory rate.
- determine the effect of exercise on respiratory rate and pulse rate.

### Materials and equipment

- Models/charts/computer illustrations of the human respiratory system.
- Stop watch

### Instructions

- Allow students to observe the model or chart and note the relative positions and gross structure of different components of respiratory system.
- Let students to observe the status of the thorax during full inspiration, full expiration and during normal uncontrolled breathing.
- Instruct students to hold the back of their hand immediately below their nostrils to count the number of expirations during normal breathing over a period of five minutes.
- Ask them to count pulse during one minute at rest.
- Instruct students to stand up and step-march to a rhythm set by the teacher for a period of three minutes.
- Direct students to determine pulse rate over a period of one minute and breathing rate over a period of three minutes.

## PRACTICAL NO. 27

### **Explaining the major types of excretory structures in animals using diagrams and charts**

#### **Objectives**

##### **Students should be able to**

- describe the structure and location of a body surface, contractile vacuoles, flame cells, nephridium, malphigian tubules, green glands, sweat glands and salt glands,
- explain the structure of human kidney, ureters, bladder, urethra and their locations,
- explain the gross internal structure of human kidney,
- draw and label diagrams of observed structures.

#### **Materials and equipment**

- Diagrams/charts/ slides of body surface as excretory surface, contractile vacuoles, flame cells, nephridium, malphigian tubules, green glands, sweat glands and salt glands
- Charts/models of human excretory system and slides of L.S of mammalian kidney to study the- gross internal structure, diagram of nephron
- Light microscopes

#### **Instructions**

- Allow students to examine body surface as excretory surface, contractile vacuoles, flame cells, nephridium, malphigian tubules, green glands, sweat glands and salt glands using diagrams/charts.
- Instruct students to observe the kidney, ureters, urinary bladder and urethra of man using diagrams/models/ specimens/ slides.
- Make them to observe L.S of kidney under a light microscope and recognize cortex, medulla, distribution of nephrons and parts of a nephron
- Instruct them to draw and label line diagrams of observed structures.

## **PRACTICAL NO.28**

### **Explaining the organization of the human nervous system using diagrams or models**

#### **Objectives**

##### **Students should be able to**

- describe the general organization of human nervous system,
- identify central nervous system and peripheral nervous system,
- identify the main parts of human brain,
- illustrate the human brain with labeled diagram.

##### **Materials and equipment**

- Models/ diagrams of human nervous system.

##### **Instructions**

- Allow students to study the general organization of human nervous system.
- Instruct them to identify central nervous system and peripheral nervous system.
- Guide students to identify the main parts of the human brain.
- Instruct them to draw and label a diagram of human brain.

## **PRACTICAL NO.29**

### **Describing sensory structures of human using diagrams/ models/ charts**

#### **Objectives**

##### **Students should be able to**

- identify different human sensory receptors,
- illustrate the structure of each sensory receptor.

#### **Materials and equipment**

- Models/ diagrams/ charts of taste receptors, olfactory receptors, thermoreceptors, photoreceptors, mechanoreceptors and pain receptors of man.

#### **Instructions**

- Provide students with models/ diagrams/ charts of the human sensory receptors and allow them to observe.
- Instruct them to make appropriate sketches of the observed sensory receptors.

## **PRACTICAL NO. 30**

**Describing the structures of human eye and ear using diagrams/ models/ charts**

### **Objectives**

#### **Students should be able to**

- draw appropriate sketches of the human eye and the ear,
- describe the location and the structure of human eye and ear.

#### **Materials and equipment**

- Charts/models of entire human eye and sagittal sections
- Charts /models of human ear; external, middle and inner ear

#### **Instructions**

- Allow students to observe the location and structure of human eye and ear.
- Direct the students to relate the main parts of the human eye and ear to their functions.

## **PRACTICAL NO.31**

### **Identifying main components of male reproductive system using models/ diagrams**

#### **Objectives**

##### **Students should be able to**

- identify the structural components of male reproductive system,
- relate the structure to the functions performed by each component of male reproductive system,
- identify different stages of sperm development in human testis.

#### **Materials and equipment**

- Charts/diagrams/computer illustrations/models of vertical sectional view of lower abdominal region of male showing the reproductive system as well as the urinary system.
- Charts/diagrams/computer illustrations of transverse and/or longitudinal sections of human testis.
- Prepared histological sections of human testis
- A detailed diagram/ picture/ prepared slide of a human sperm
- Light microscopes

#### **Instructions**

- Allow the students to study the given material carefully and understand the structure and relative positions of each component of the male reproductive system.
- Guide them to observe the T.S and/or L.S of testis, to note the various stages of the germinal epithelium, the sperms and their relative arrangements, Leydig cells and sertoli cells.
- Lead a discussion on the relationship of structure of each component of male reproductive system to its functions.

## **PRACTICAL NO.32**

### **Identifying main components of female reproductive system using models/ diagrams**

#### **Objectives**

##### **Students should be able to**

- Identify structural components of female reproductive system,
- relate the structure to the functions performed by each component of female reproductive system,
- identify follicles of different stages in human ovary.

#### **Materials and equipment**

- Charts/diagrams/computer illustrations/models of vertical sectional view of the lower abdominal region of a female showing the reproductive system
- Charts/diagrams/computer illustrations of transverse and/or longitudinal sections of human ovary.
- Prepared histological sections of human ovary
- Light Microscopes

#### **Instructions**

- Allow the students to study the given materials carefully and understand the structure and relative positions of different organs of the female reproductive system.
- Guide them to observe the T.S and/or L.S of ovary to note the germinal epithelium and various development stages of follicles
- Lead a discussion on the relationship of structure of each component of female reproductive system to its functions.

### **PRACTICAL NO. 33**

#### **Describing the gross structure of the human skull and vertebral column in relation to functions of various parts using specimens/ models/ diagrams**

#### **Objectives**

##### **Students should be able to**

- describe the morphology of the skull and vertebral column,
- relate the structure of the skull to its functions,
- analyze the structure and articulation of the vertebral column in relation to weight bearing and erect posture,
- draw appropriate diagrams and sketches to highlight prominent and distinctive features of the skull and the various parts of the vertebral column.

#### **Materials and equipment**

- Diagrams/models/charts/specimens/computer illustrations of the human skull and vertebral column with articulations

#### **Instructions**

- Make the students to observe the following features in the skull:-
  - a. Shape, smooth surface and volume
  - b. Frontal view with prominent forehead, flattened face, forwardly directed orbits, well-formed chin.
  - c. Mandibles and its articulations with skull and dentition.
  - d. Inferior, superior, posterior and anterior views of the skull, position of foramen magnum, occipital condyles and articulation with atlas vertebra
  - e. Location of auditory apparatus
  - f. Nasal region and turbinals
- Ask students to make observations on themselves and on other students and note
  - a. three dimensional range of mobility of head and how it moves in relation to the atlas and axis vertebrae
  - b. range of movement of mandible and its movements during mastication of solid food material
- Instruct them to observe the following features of the vertebral column
  - a. The curvatures of the vertebral column as seen in lateral view
  - b. The increase in size of vertebrae from the superior to the inferior part of the vertebral column

- c. Vertebrae in the cervical, thoracic, lumbar and sacral regions and the coccyx and the number of vertebrae in each region
  - d. The relationship of the thoracic vertebrae to the ribs and the nature of the articulation of each rib to the corresponding vertebra
  - e. The inter – vertebral discs
  - f. The sacral vertebrae and their relationship to the pelvic girdle
- Instruct students to draw appropriate diagrams and sketches.

## **PRACTICAL NO.34**

### **Describing the human appendicular skeleton using specimens/ models/ diagrams**

#### **Objectives**

##### **Students should be able to**

- relate the skeletal structure of upper limb to the range of functions performed,
- relate skeletal structure of joints and bones of lower limb to erect body posture, bearing of body weight and walking.

##### **Materials and equipment**

- Charts /models/specimens/ illustrations/computer illustrations showing the bones of the upper arm, forearm, wrist and hand.
- Charts/models/specimens/illustrations/computer illustrations/ computer animations/ of pronation and supination and opposability of thumb and fingers.
- Charts / models/specimens/illustrations/computer illustration of bones of thigh, shank, ankle and foot.
- Charts/models/specimens/illustrations/computer illustrations/ computer animations/ to show involvement of lower limb in maintenance of erect posture, bearing of body weight and walking
- Charts/models /specimens/illustrations/computer illustration of complete human skeleton.

##### **Instructions**

- Allow students to observe and study upper limb.
- Direct the students to study and record the movement of the limbs including joints, pronation, supination and opposability.
- Lead a discussion on weight bearing & bipedalism and structure of the foot.
- Highlight the movements of the leg, joints, heel and toe during walking.

## PRACTICAL NO. 35

### **Sterilization of water, culture media, glassware, heat labile substances and inoculating needles**

#### **Objectives;**

#### **Students should be able to**

- identify suitable technique for sterilization of a given material
- describe the procedure for different sterilization techniques

#### **Materials and equipment**

- Autoclave/ Pressure cooker
- Oven
- Culture media
- Inoculating needles
- Cotton wool
- Pipettes
- Conical flasks
- Petri dishes
- Beakers

#### **Instructions**

- Instruct the students to follow the techniques used in sterilization.
  - a) Sterilization by dry heat (using direct flame)
    - i For inoculating needles, loops and such materials which will not be damaged by heat. Hold in flame of Bunsen burner until red hot.
    - ii. In the case of scalpels, metal spatulas and glass rods dip in methylated spirits or ethyl alcohol. Allow excess spirit to drip off and flame the instrument in the Bunsen flame.
  - b) Sterilization by dry heat (in the hot air oven)

For sterilization of dry glassware such as Petri dishes, flasks and pipettes.  
Prepare glassware for sterilization as follows: -

    - Wash glassware, clean and wipe dry thoroughly.
    - Wrap the glassware in Aluminum foil or wrap in a paper or store in a suitable container(canister).
    - For conical flasks, plug the mouth with clean cotton wool and cover the plugs with Aluminum foil.

- For pipettes, plug mouth with cotton wool and burn the cotton fibres sticking out using a Bunsen flame.
- Wrap the pipettes individually in Aluminium foil or paper or store in a suitable container (canister).
- Store all prepared glassware in an oven, at a temperature of 170 °C for 1-2 hrs depending on the amount of glassware in the oven. Keep the oven door tightly closed.

c) Sterilization in an autoclave (wet heat).

For sterilization of water/ culture media

- Prepare the glassware for autoclaving according to the procedure outlined above.
- Place the prepared liquid culture media or water in test tubes, flasks or bottles as appropriate.
- Plug the containers with cotton wool and cover with Aluminium foil or paper.
- If bottles with screw caps are used, loosen the screw cap slightly.
- Place the containers/ glassware in the autoclave.
- Close the lid of the autoclave tightly and open the valve.
- Set the pressure at 15 lb / sq inch and heat
- Close the valve when water vapour is released.
- Autoclave for 15 - 20 minutes at 121°C

d) Sterilization by filtration using membrane filter apparatus.

For sterilization of heat labile substances.

- Sterilize the components of the membrane filter apparatus separately.
- Filter the liquid using membrane filters.
- Collect the filtrate into sterile bottles/test tubes

• Direct the students to record their observations highlighting the following:

- different types of apparatus used in sterilization.
- procedures followed

Guide the students

- comment on the selection of suitable technique for sterilizing different materials

## PRACTICAL NO. 36

### Preparation of a microbiological culture medium (Nutrient Agar/ Potato Dextrose Agar), inoculation with a sample of toddy and yoghurt and observation of microbial colonies

#### Objectives

##### Students should be able to

- prepare a microbiological culture medium,
- inoculate the culture medium with an inoculum,
- distinguish various types of colonies of microorganisms after incubation.

#### Materials and equipment

- 150 ml flask with screw cap or cotton wool plug
- 100 ml graduated cylinder
- Sterilized glass rod
- Sterilized Petri dishes
- Inoculating loop
- Bunsen burner
- Autoclave
  

• For nutrient agar		• For potato Dextrose agar	
• Peptone	10 g	• Potato	200g
• Beef extract	10 g	• Glucose	20g
• Sodium chloride	05 g	• Agar	15g
• Agar	15 g	• Distilled water	1000ml
• Distilled water			
1000 ml			

  - Toddy and yoghurt sample

#### Instructions

- Let students prepare Nutrient agar and PDA from the given material.
- Let students sterilize the solutions by autoclaving at 121 °C for 15 min (15 lb/sq in.)
- Assist students to prepare nutrient agar and PDA plates as given below.

- Pour about 15 ml of the sterilized Nutrient Agar and PDA into sterilized Petri dishes, using aseptic techniques.
- Set aside to solidify.
- Inoculation of the plates:
  - Label the bottom of each agar plate using a marker pen.
  - Flame the inoculating loop to redness, allow it to cool and aseptically obtain a loop full of the sample. eg. toddy and yoghurt
  - Place a loop full of sample on the agar plate near the edge of the dish and streak on the agar surface in a zig zag pattern
  - Incubate for 24-48 hr. at room temperature
  - Observe the colonies on incubated plates
  - Instruct the students to record the practical highlighting the following.
    - Preparation of microbiological culture medium
    - Inoculation and incubation of culture plates
    - Observation of colony types

## PRACTICAL NO. 37

### Staining of microorganisms found in toddy and yoghurt using a simple stain (Methylene Blue)

#### Objectives

#### Students should be able to

- prepare smears from solid and liquid samples,
- stain the smear using a simple staining technique,
- observe the stained microorganisms under the microscope.

#### • **Materials and equipment**

- Toddy and yoghurt /curd sample
- Methylene Blue (dilute solution)
- Slides and cover slips
- Inoculating loops
- Bunsen burner
- Distilled water
- Light microscopes
- Marking pen or wax pencil

#### Instructions

- Instruct the students to carry out the following procedure.
  1. Preparation of smear
    - Clean slides with cleanser, rinse and air dry
    - Handle the clean slides by their edges, preferably using a pair of forceps
    - Use marker pen or pencils to label each slide according to the sample used (toddy, yoghurt / curd).  
A - for yoghurt/ curd
    - Place 1 or 2 loops full of distilled water on the centre of one slide using the sterilized inoculating loop.
    - Heat the loop until it is red hot and allow to cool.
    - Scrape a small amount of the sample using the cooled loop.
    - Emulsify the scrapings in the drop of water and spread the suspension in the shape of a circle (the smear should be very thin)

B - For toddy.

- Do not use water as the microorganisms are already suspended in water.  
Follow other steps as above

- Let the smear air dry

Common to both A and B

- Heat fix the smear by passing the slide through a flame two or three times.
- (Do not heat fix until the smear is completely air dried)
- Flood the prepared, heat – fixed smear with 2 or 3 drops of Methylene Blue and allow 30-60 seconds for the stain to act
- Wash with tap water to remove the excess stain and gently blot the smear with blotting paper and let it dry.
- Examine the stained smears under the microscope
- Make the students to observe and note the colour of the stained bacteria and yeast
- Instruct them to make appropriate diagrams of bacteria and yeast.
- Direct the students to distinguish between bacteria and yeast

## PRACTICAL NO.38

### Use of alcohol and other disinfectants to control microorganisms

#### Objectives

##### Students should be able to,

- develop the skills to control microorganisms
- explain what disinfectants are

#### Materials and equipment

- Nutrient agar medium.
- Sterilized petri dishes.
- Bunsen burner.
- Phenol/ Lysol/ chlorine compound.
- Alcohol

#### Instructions

Assist students to do the following;

- Prepare two sets of sterilized petri dishes with solidified nutrient agar medium.
- Expose them to the air for about 10 minutes.
- Close one set of petri dishes after rinsing with alcohol/ phenol/ Lysol/ chlorine compounds
- Other set without detergent should be kept closed.
- Observe both for number of colonies after 24-48 hours of incubation
- Ask students to record their observations and comment.

### Test for Carbohydrates

1) Test for reducing sugars

#### Benedict's test

Add 2 cm<sup>3</sup> of a solution of a reducing sugar. Add equal volume of Benedict's solution. Shake and bring to boil.

2) Test for non reducing sugars

Add 2 cm<sup>3</sup> of sucrose solution to 1 cm<sup>3</sup> dil. HCL. Boil for one minute. Neutralize with NaHCO<sub>3</sub> and check with pH paper. Carry out Benedict's test.

3) Test for starch

Add 2 cm<sup>3</sup> , 1% starch solution in a test tube and add a few drops of I<sub>2</sub>/KI solution.

### Test for Lipids

Add 2cm<sup>3</sup> oil to 2cm<sup>3</sup> of water in a test tube. Add a few drops of Sudan III and shake.

### Test for Proteins

#### Biuret test

Add 2cm<sup>3</sup> protein solution to equal volume of 5% KOH solution and mix. Add two drops of 1% CuSO<sub>4</sub> solution and mix.

- Preparation of Iodine solution

Dissolve 7 1.0 g of Iodine crystals and 2.0 g of Potassium Iodide in 300 cm<sup>3</sup> distilled water.

- Preparation of Formalin to preserve specimens

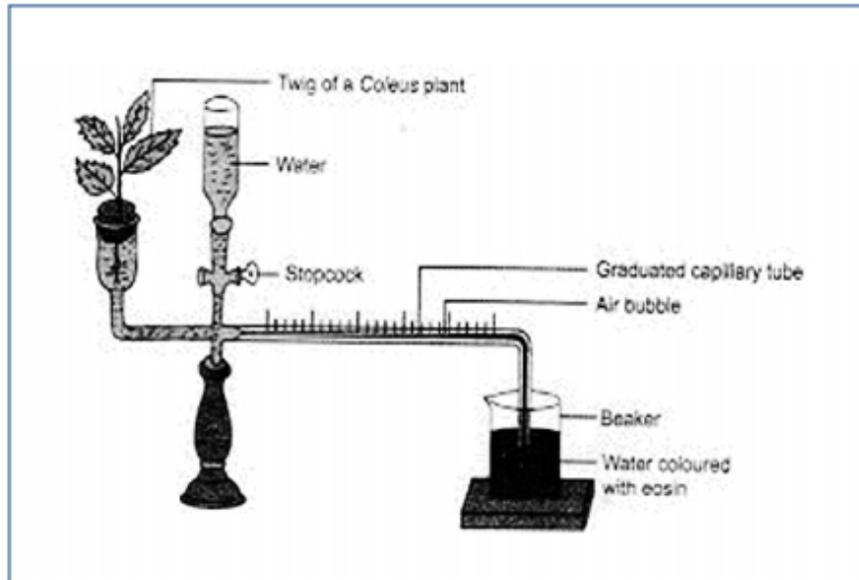
Add 10 cm<sup>3</sup> of commercial Formalin to 90 cm<sup>3</sup> of distilled water.

- Preparation of macerated material

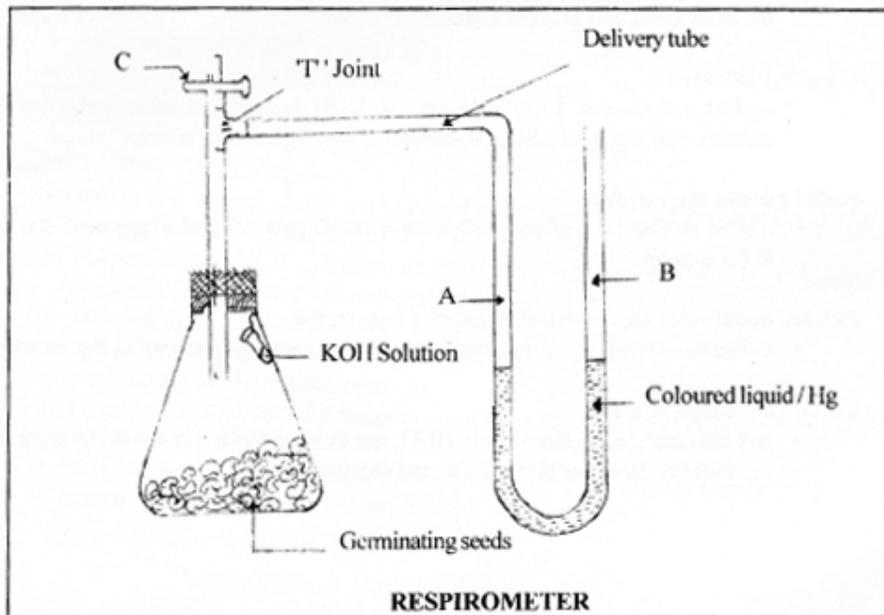
Add Conc. HNO<sub>3</sub> to plant material. Boil for about five minutes in a water bath.

Check the consistency with glass rod.

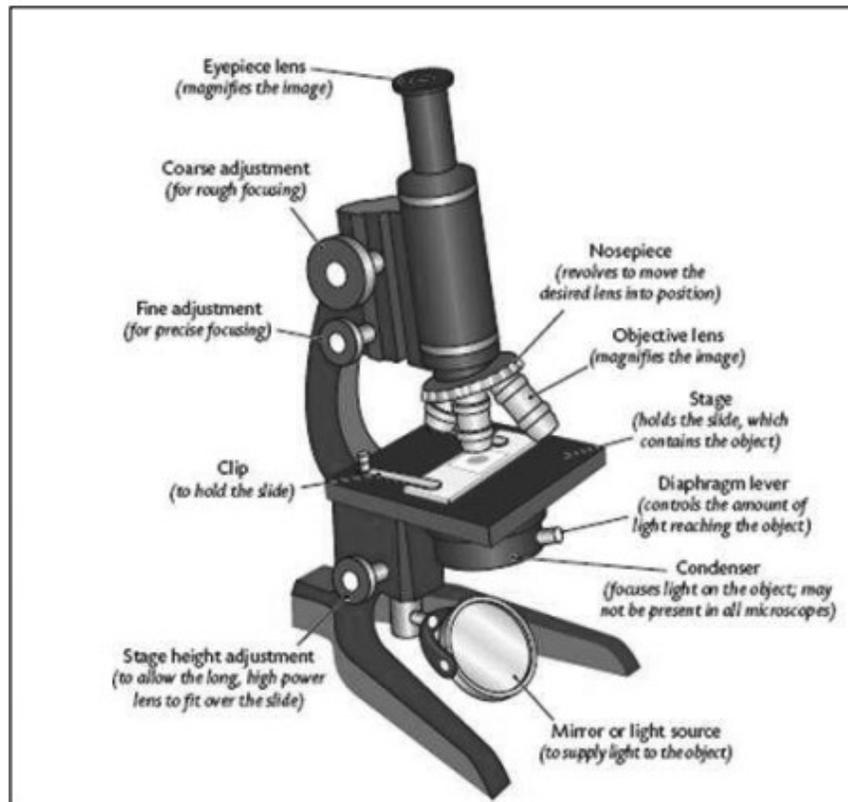
### GANONG'S POTOMETER



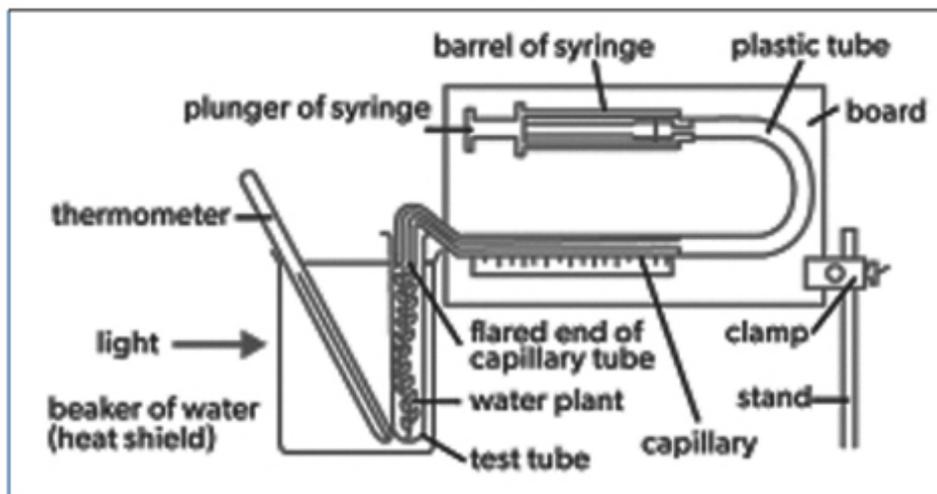
### RESPIROMETER



## Compound Light Microscope



## Apparatus for measuring the rate of Oxygen evolution by a water plant during photosynthesis



## References

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