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Faculty of Science

Laboratory Manual

Cell Biology

Bachelor of Biotechnology (Hons.)

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Cell Biology

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LINCOLN UNIVERSITY COLLEGE
FACULTY OF SCIENCE (DEPARTMENT OF BIOTECHNOLOGY)
LABORATORY SAFETY RULES

The following rules must be obeyed by all students in the science laboratory of the faculty. Wilful or repeated in advertent non-compliance may result in dismissal or suspension from the laboratories

- No entry without permission:
  - Outsiders are not allowed to enter the laboratory without permission.
  - No student is allowed to enter the laboratory unless permission has been given by a laboratory assistant or a lecturer.

- At work in the laboratory:
  - No experiment may be attempted without the knowledge and permission of a lecturer.
  - Students must wear shoes in the laboratory. Students wearing slippers or sandals are not allowed to work in the laboratory.
  - Lab coat must be worn at all times during practical work in the laboratory.
  - Do not mouth pipette chemicals.
  - Do not eat or smoke in the laboratory.
  - Do not taste any chemicals, including dilute solutions. If any acid or alkali accidentally enters your eyes or mouth, wash immediately with plenty of tap water. Inform your lecturer, and seek medical attention if necessary.
  - Paper should be used to light up the Bunsen burners.
  - Used match sticks, filter papers, and other solid waste must never be thrown into the sinks. They must be thrown into the dustbins provided. Lighted match sticks and smoldering materials must be extinguished with tap water before thrown in to the dustbins.
  - Any equipment broken or damaged must be reported to the laboratory assistant.

- Before leaving the laboratory:
  - All the equipment and benches must be cleaned at the end of each practical session.
  - Wash hands and arms with soap and water before leaving the laboratory.
  - No student is allowed to take away any chemicals, equipment or other property of the laboratory.

INTRODUCTION

1. The Scientific Method

- Making observations
- Generating hypotheses
- Making predictions
- Designing and carrying out experiments
- Constructing scientific models

2. Practical Exercises

To get the most out of the practical exercises, you need to follow carefully the instructions given. These instructions have been designed to provide you with the experience in the following skills:

- Following instructors
- Handling apparatus
- Having due regard for safely
- Making accurate observations
- Recording results in an appropriate form
- Presenting quantitative results
- Drawing conclusions

3. Following Instructions

Instructions are provided in the order in which you need to carry them out. We would advise that before carrying out the instructions, you read through the entire exercise. This will help you to remember what you have learned.

Each practical exercise in the book begins with a few lines describing its purpose in most cases the following headings are also used:

- Procedure-numbered steps that need to be carried out.
- For consideration -some questions to help you think carefully about the results you have obtained.
- Materials-a list of the apparatus, chemicals and biological materials you need.

4. Handling apparatus

Biologists need to able to use many different types of apparatus, for example, photometers (to measure water uptake by plants), respirometers (to measure oxygen uptake or carbon dioxide production), Petri dishes (for plating out bacteria and other microorganisms) and the light microscope (to magnify specimens). Many of the practical exercises are designed to help you derive the maximum benefit from a piece of apparatus.

5. Having Due Regard for Safety

Surveys have been shown that science laboratories are among the safest places to be. Nevertheless, this is no cause for complacency.

- Always move slowly and carefully in a laboratory.
- Never put your fingers in your mouth or eyes after using chemicals or touching biological specimens until you have washed your hands thoroughly with soap and warm water, and dried them.
- Make sure glass objects (e.g, thermometers, beakers) cannot roll off tables or be knocked onto the floor.
- Wear safely goggles whenever there is a risk of damage to the eyes.

<u>Situations of risk include:</u>

- Heating anything with a Bunsen burner (even heating water has its dangers')
- Handling many liquids, particularly those identified as corrosive, irritant, toxic or harmful

- Handling corrosive or irritant solids
- Some dissection work
- Allow Bunsen burners, tripods, gauzez and beakers to cool down before handling them.
- Never allow your own body fluids (especially blood and saliva) to come into contact with someone else, or theirs into contact with you.
- Keep long hair tied back and do not wear dangly earrings.
- Do not allow electrical equipment to come into contact with water.
- If you are unsure how to carry out a scientific procedure, ask.
- Make sure you understand why you are going to do something before you do it.
- Wear a lab coat when using chemicals or handling any biological specimens.
- Follow exactly agreed procedures with regard to cuts, burns, electric shocks and other accidents (e.g. with chemicals).
- Follow exactly all specific safely instructions given in this book or provided by your teacher for particular practical exercises (e.g. use of gloves, disinfection)

With practice, these procedures should become second nature to you. They will enable you to carry out practical work in safety.

6. Making Accurate Observations

In most cases the practical exercise will make it clear what you need to observe, e.g. the time taken for a certain volume of gas to be evolved or the width of a sample cells. Ensure that you know how to use any necessary equipment before starting practical. Think carefully about the precision with which you will make your observations.

7. Recording Results in an Appropriate Form

Results can be recorded in various ways. Often it is helpful to record raw data in a table. Most data will be in the form of numbers, i.e. they will be quantitative data (also known as numerical data). However, some data, e.g. flower colour, will be qualitative data.

One form in which some biological findings can be recorded is a drawing. You don't need to be professional artist to make worthwhile biological drawings. If you follow the following guidelines, a drawing can be of considerable biological value:

- Ensure that your completed drawing will cover at least a third of A4 page.
- Plan your drawing so that the various parts are is proportion and will not be drawn too small. Small marks to indicate the length and breadth of the drawing are a great help in planning and a faint outline can be rapidly drawn to show the relative positions of the parts.
- The final drawing should be made with clean, firm lines using a sharp HB pencil and, if needed, a good quality eraser (not a fluid).If important details are too small to be shown in proportion, they can be put in an enlarged drawing at the side of the main drawing.
- Avoid shading and the use of colour unless you are an excellent artist and they really help, for example when drawing soil profiles.
- When drawing structures seen with the naked eye or hand lens, use two lines to delineate such things as blood vessels and petioles. This will help you to indicate the relative widths of such structures.
- When drawing low power plan drawings from the light microscope, do not attempt to draw individual cells-just different tissues.
- When drawing plant cells at high power under the light microscope, use two lines to indicate the width of cell walls, but a single line to indicate a membrane.
- Always put a scale on each drawing.

8. Presenting Quantitative Results

Presentation of data is all about using graphs or other visual means to make it easier to see what your results tell you. The following four ways of presenting data are the most frequently used in biology: line graphs, bar charts, histograms and scatter graphs (Figure 1).

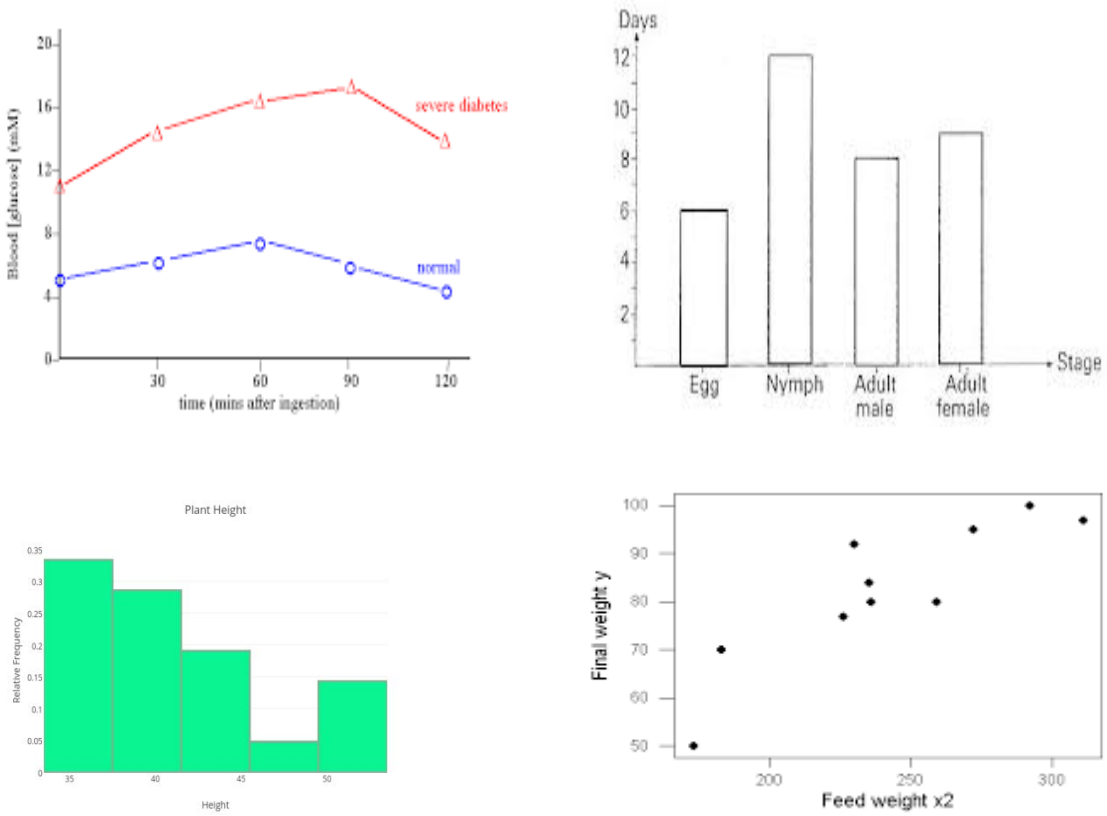

Figure 1: Line graphs, bar charts, histograms and scatter graphs

9. Drawing Conclusions

Finally, you will need to draw conclusions. If your practical exercise has involved the testing of a hypothesis, for example that the enzyme pepsin works better at low pH than in neutral or alkaline conditions, your conclusion should indicate whether the hypothesis has been refuted (i.e. shown not to be the case) or supported. Of course, even if your hypothesis has been supported, it doesn't mean that it has been confirmed with 100% certainty- in other words it isn't proved. Science proceeds more by showing that certain ideas are wrong than by showing that others are right (think about that!). Your conclusion might therefore include further ways of testing the original hypothesis, or might raise new possibilities to be investigated.

Often you will only be able to arrive at your conclusions after statistically analysing your data.

10. Writing a Scientific Lab Report

Title

- Communicate the subject investigated in the paper.

Introduction

- State the hypothesis.
- Give well-defined reasons for making the hypothesis.
- Explain the biological basis of the experiment.
- Cite sources to substantiate background information.

- Explain how the method used will produce information relevant to your hypothesis.
- State a prediction based on your hypothesis. (If the hypotheis is supported, then the results will be.)

Materials and Methods

- Use the appropriate style.
- Give enough detail so the reader could duplicate your experiment
- State the control treatment, replication and standardized variables that were used.

Results

- Summarize the data (do not include raw data).
- Present the data in an appropriate format (table or graph).
- Present tables and figures neatly so they are easily read.
- Label the axes of each graph completely.
- Give units of measurement where appropriate.
- Write a descriptive caption for each table and figure.
- Include a short paragraph pointing out important results but do not interpret the data.

Discussion

- State whether the hypothesis was supported or proven false by the results, or else state that the results were inconclusive.
- Cite specific results that support your conclusions.
- Give the reasoning for your conclusions.
- Demonstrate that you understand the biological meaning of your results.
- Compare the results, with your predictions and explain any unexpected results.
- Compare the results to other research or information available to you.
- Discuss any weaknesses in your experimental design or problems with the execution of the experiment.
- Discuss how you might extend or improve your experiment.

Conclusion

- Restate your conclusion.
- Restate important results.

Literature Cited

- Use the proper citation form in the text.
- Use proper citation form in the Literature Cited section.
- Refer in the text to any source listed in this section.

Acknowledgement

- State any appropriate acknowledgement that you think is necessary.

Practical 1
Title: Preparation of blood smear

Objective:

After completing the practical, you will be able:

1. To identify the different blood cell types in human blood smear

Introduction:

Humans have a closed circulatory system which delivers oxygen, nutrients, hormones, and other essentials throughout our body. As its name suggests, a "closed" system means that all of our blood flows through arteries, veins, and capillaries. This means that our blood isn't just sloshing around inside of us, instead, our heart is forcefully pumping blood throughout our blood vessels. When you suffer from broken blood vessels, such as a cut or scrape, this is called a hemorrhage. An important ability of our body's blood is its ability to clot, or to create a blockage that stops the blood from hemorrhaging, giving the body time to regenerate cells and heal the injury. You may also hear the circulatory system referred to as the cardiovascular system. The cardiovascular system is just a more focused term, referring primarily to the heart (cardio) and blood vessels (vascular). Our blood is made up of four components: red and white blood cells, platelets, and plasma.

Materials:

1. Microscope
2. Glass slides
3. Spirit lamp
4. Sterilized needle
5. A drop of blood
6. Giemsa stain
7. Distilled water

Procedure:

Preparation of blood smears:

1. Obtain 2 clean microscope slides, alcohol wipes, and lancet.
2. Clean a finger with an alcohol and puncture with lancet.
3. Place a small drop of blood at the end of one slide.
4. Use the second slide to make a thin blood film as directed below:
   a. Place the second slide at a 30 degree angle and touch the slide with the blood drop.
   b. Move the spreader slide to touch the blood drop allowing the drop to spread by capillary action along the edge of the slide.
   c. Immediately pull/push the slide away from the blood drop, making a thin smear that should dry quickly as you move away from the drop.
   d. A perfect smear will have a “feathered” edge and separated RBCs when you view it with the microscope.

Staining the blood smear (Horizontal staining procedure):

1. Place thoroughly dried smear on horizontal staining rack.
2. Flood smear with Fixative for 10 seconds, (fixes cells to slide/prepares cells for dyes) drain.
3. Flood smear with dye for 10 seconds, drain.
4. Rinse the smear with distilled water for 1 minute.
5. Air dry and examine under the microscope, using low power first, then high power.
6. Observe as many different types of blood cells as possible.

Observation/ Results:

Questions:

1. Differentiate between plasma and serum.
2. Explain the characteristics of different white blood corpuscles.

Practical 2

Title: Preparation of buccal smear and identification of Bar Body

Objective:

After completing the practical, you will be able:

1. To determine and the presence of Barr body in the female buccal cavity

Introduction:

In human being the sex can be identified by observing the nucleus of their resting cells (the interphase nucleus) in the cells of female. A darkly stained chromatin matrix is observed on the slide in the interphase nucleus. This is known as sex chromatin or Barr body after the name of its discoverer Murray Barr in 1940.

Principle:

Buccal epithelial cells especially have Barr body structure, which are considered to play a major role for sex determination. This small round Barr body is located either in the border of nuclear membrane or sometimes inside of nucleus. This Barr body may be single or more in number in some cases. These structures are present only in the female sex.

Materials:

1. Pre-cleaned slides
2. Methylene blue
3. Epithelial cells (sample)
4. Microscope

Methods:

1. Wash your mouth with sterile water to prepare mucous.
2. Take a sterilized slide and scrap epithelial cells superficially from the inner side skin of the mouth.
3. Keep the sample on the centre of the pre-sterilized glass slides and dry it for few minutes.
4. Then add few drops of Leishman stain on the smear and incubate for 5-10 minutes.
5. After incubation, wash it or remove the excess stain using water by keeping the in slant position.
6. Eventually, blot the water using tissue paper.
7. The smear is now ready for microscopic observation.

Observation/Results:

Questions:

1. Define Bar Body.
2. What is the significance of interphase?

Practical 3

Title: Preparation of microscope slide for dicot leaf section

Objective:

After completing the practical, you will be able:

1. To assess different cell types in dicot leaf in leaf cross section

Introduction:

Plants are present in a wide range around us. It is one of a large kingdom of the organism. Plants have different types of species. One of their classifications is as monocot and dicots. Monocots and dicots differ from each other in four structures: leaves, stems, roots and flowers. This difference begins from the seed and remains throughout the life cycle. Monocots have one cotyledon while dicots have two cotyledons. Monocots include all grasses and grass-like plants while dicots include all our trees, bushes, etc. Monocots leaves mostly have parallel veins whereas, dicot leaves have net-veins. Dicot leaves have thick cuticle at the upper layer and thin cuticle at lower layer whereas monocot leaves have uniform cuticle on both the surfaces. Another main difference in monocot and dicot leaf is that monocot leaf has an equal number of stomata on either side, but dicot has more stomata at its lower surface.

Materials:

1. Leaf sample
2. Blade or scalpel
3. Glass slides
4. Needle
5. Cover slips
6. Microscope

Procedure:

1. Place the specimen (leaf, stem, root cross section) on the centre of a clean slide.
2. Add a drop of water or designated stain if required. (Note: liquid cultures do not require adding water)
3. Place one edge of the cover slip on the slide near the specimen (This is done by holding the cover slide at a 45º angle). Gently lower the cover slip on top of the specimen. Try to avoid trapping air bubbles.
4. Blot an excess fluid with lens paper before you place the slide on the stage of the microscope.

Observation/ Results:

Questions:

1. Explain the features of different cell types in dicot leaves.
2. Differentiate between monocot and dicot leaves?

Practical 4
Title: Preparation of permanent slides

Objective:

After completing the practical, you will be able:

1. To prepare permanent slides using the given sections like stem, root and leaf

Introduction:

Dehydrating preserves the cells and protects them from decaying. There are various dehydrating agents but in this experiment, we will employ the simplest using ethyl alcohol. Specimens that are already dry (like paper or cloth) do not need dehydrating, when dehydrating a specimen with alcohol; the objective is to slowly replace the water in the cell with alcohol. Since pure alcohol will harden the cell wall and make an impenetrable barrier, it must be done gradually.

Materials

1. Pre-cleaned glass slides
2. Glass cover slips
3. Lab brush
4. Dissecting needle
5. Compound microscope
6. Ethyl alcohol
7. Nail polish (cementing agent)

Procedure:

1. Prepare a thin stem section using new blade or scalpel without damaging the tissue.
2. Take a pre-cleaned watch glass adds 3 drops of distilled water and 1 drop of ethyl alcohol (3:1) and transfer the stem sections into watch glass and incubate for 15 minutes.
3. After incubation remove the stem section and transfer to new watch glass containing 2 drops of ethyl alcohol and 2 drops of distilled water, leave it for 15 minutes.
4. Follows again transfer sections into new watch glass containing 3 drop of ethyl alcohol 1 drop of distilled water, leave it for 15 minutes.
5. Again transfer sections into another watch glass containing 4 drops of ethyl alcohol incubate for 15 minutes.
6. Eventually, transfer the dehydrated section into centre of the pre-cleaned glass slide and add a drop of safranin and cover with cover slip then seal or cement the coverslip with nail polish.

Observations/Result:

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