SCHOOL-BASED ASSESSMENT

LABORATORY MANUAL

BIOLOGY

First Edition

4th May 2026

MALAYSIAN INDEPENDENT CHINESE SECONDARY SCHOOLS (MICSS)

Preface

This manual is compiled based on the "Senior Middle Level Biology Curriculum Standards" set by the Biology subject under Unified Curriculum Committee of Malaysian Independent Chinese Secondary School (MICSS) Working Committee, with reference to both national and international secondary biology curricula. Within this framework, experiments are positioned as the core of high school science learning, serving as a bridge between theory and practice. Designed as a tool for school-based assessment by Examination Department, this manual aims to establish a scientific and systematic evaluation system for experimental teaching.

Its key features are as follows:

（1） Guided by the principle of "using assessment to enhance teaching and learning," the manual provides a structured evaluation framework covering the entire experimental process – from operation procedures and data collection to analysis and reasoning. It emphasizes not only the accuracy of data but also the cultivation of scientific thinking and inquiry skills.

（2） The experiments selected align with curriculum standards while reflecting the distinctive characteristics of independent Chinese secondary schools, balancing foundational skills development with opportunities for extended inquiry. Each experiment is designed to holistically assess students' scientific literacy.

We extend our sincere gratitude to the experts, scholars, and experienced teachers who reviewed this manual and provided valuable feedback on its content and design.

We believe that true science education lies at the intersection of hands-on practice and deep intellectual engagement. May this manual guide students in exploring the scientific world, fostering a rigorous and inquisitive mind, and finding joy in discovery and growth.

We welcome any feedback or corrections regarding shortcomings in this manual.

Dong Zong Examination Department

January 2026

Laboratory Safety Guidelines

Introduction

Safety in the laboratory is our highest priority. These guidelines are designed to protect you, your colleagues, and the environment. No experiment is so important that it must be performed at the expense of safety. You are responsible not only for your own safety but also for the safety of those around you. Read, understand, and follow these rules at all times.

Section 1: Personal Safety and Preparation

Personal Protective Equipment (PPE):

- **Safety Glasses/Goggles**: Must be worn at all times in the lab, even if you are not performing an experiment.
- **Lab Coat**: A properly fastened lab coat must be worn to protect your skin and clothing.
- **Gloves**: Wear appropriate, chemically resistant gloves for all procedures involving hazardous chemicals. Inspect them for holes before use. Remove them before touching common surfaces (e.g., door handles, keyboards, phones).
- **Closed-Toe Shoes**: Shoes must completely cover the foot. No sandals, flip-flops, or open-toe shoes are permitted.
- **Appropriate Clothing**: Wear clothing that covers and protects your skin. Avoid loose sleeves, dangling jewellery, and scarves. Tie back long hair.

Personal Hygiene:

- **No Eating or Drinking**: Never consume food, beverages, or chew gum in the laboratory.
- **No Applying Cosmetics**: Do not apply lip balm, makeup, or wear contact lenses.
- **Wash Your Hands**: Wash your hands thoroughly with soap and water after handling chemicals, before leaving the lab, and after removing gloves.

Section 2: Before You Begin an Experiment

Know the Hazards:

- Read the entire experimental procedure and any associated Safety Data Sheets (SDS) **before** starting.
- Identify the location and proper use of all safety equipment: eyewash station, safety shower, fire extinguisher, fire blanket, and first-aid kit.
- Plan your work. Understand the steps and anticipate potential hazards.

Chemical Handling:

- **Never Taste or Smell Chemicals**: To detect odours, use your hand to gently waft the vapours toward your nose.
- **Label Everything**: All containers must be clearly labelled with the contents and hazard warnings.
- **Use the Fume Hood**: Any procedure that produces volatile, toxic, or flammable vapours must be conducted in a properly functioning fume hood.

Section 3: During the Experiment

General Conduct:

- **Work Attentively**: Conduct yourself in a responsible and professional manner at all times. No running, horseplay, or practical jokes.
- **Keep a Clean Workspace**: Clutter leads to accidents. Clean up spills immediately and dispose of waste properly.
- **Minimize Distractions**: Avoid using personal mobile phones for non-work purposes.

Specific Procedures:

- **Heating Substances**: Never heat a closed container. Point the mouths of test tubes being heated away from yourself and others. Use boiling chips or broken porcelain pieces to prevent bumping.
- **Glassware**: Check for chips or cracks before use. Do not use damaged glassware. Learn the proper procedure for inserting and removing glass tubing from stoppers.

- **Waste Disposal**: Dispose of all chemical and biological waste in the appropriately labelled containers. Never pour chemicals down the sink unless explicitly instructed to do so.

Section 4: In Case of Emergency

Know the Emergency Procedures:

- **Spills**: Alert others in the area. For minor, non-hazardous spills, clean them up immediately using the appropriate spill kit. For major or hazardous spills, evacuate the area and notify the instructor/lab manager immediately.
- **Fire**: Alert everyone in the lab. For a small, contained fire (e.g., in a beaker), smother it with a watch glass or use a fire extinguisher if you are trained. For a larger fire, **EVACUATE** immediately and activate the fire alarm.

Chemical Splash:

- **On Skin/Clothing**: Immediately flush the affected area with copious amounts of water in the safety shower for at least 15 minutes. Remove contaminated clothing while under the shower.
- **In Eyes**: Immediately use the eyewash station. Hold eyelids open and flush with water for at least 15 minutes.
- **Injury**: Report all injuries, no matter how minor, to the instructor or lab manager immediately.

Section 5: After the Experiment

Clean-Up:

- Clean all glassware and equipment and return it to its proper storage location.
- Wipe down your bench space with disinfectant or soapy water.
- **Waste Disposal**: Ensure all chemical and biological waste has been disposed of according to the provided instructions.
- **Personal Hygiene**: Wash your hands thoroughly with soap and water before leaving the laboratory.

A Final Reminder:

If you are ever unsure about the safety of a procedure, **STOP** and **ASK** your instructor or lab supervisor. Do not proceed until you are certain it is safe to do so.

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To investigate the nutrient compositions in food

Problem statement

What are the nutrients in food?

Hypothesis

Food are rich in sugar, fats and protein.

Aim

1. To investigate the components of nutrients in food
2. To master the basic procedure of food test using Benedict reagent, Sudan III and Biuret reagent.

Variables

Manipulated variable: Food sample

Responding variable: The content of sugar, fats and protein in food samples

Constant variable: Concentration of reagents

Material and apparatus

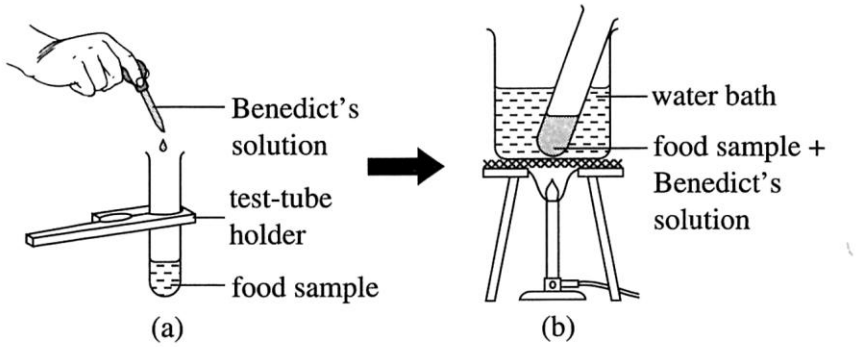
Material and apparatus	Concentration/specification	Volume/number
Test tubes	15 mm x 150 mm	9
Rubber stopper	-	3
Test tube rack	-	1
Bunsen burner	-	1
Retort stand and clamp	-	1
Beaker	250 mL	1
Dropper	-	8
Test tube holder	-	1
Benedict reagent	-	6 mL
Sudan III stain	-	3 mL
Biuret reagent A (Sodium hydroxide solution)	0.1 g/mL	5 mL
Biuret reagent B (Copper sulphate solution)	0.01 g/mL	3 mL

Food sample (At least 3)	-	Small amount
Tripod stand	-	1
Wire gauze	-	1
Stopwatch	-	1
Measuring cylinder	10 mL	5

Procedure

Reducing sugar test

1. Take a test tube and add 2 mL of food sample 1.
2. In the same test tube, add 2 mL of Benedict reagent. Gently swirl to mix well.
3. In a water bath, heat up the test tube till boiling, as shown below.



4. Observe the colour changes carefully. Record and analyse the result.
5. Repeat steps 1 - 4 with food sample 2 and 3.

Lipid test

1. Take a test tube and add 2 mL of food sample 1.
2. In the same test tube, add 5 drops of Sudan III stain.
3. Put a rubber stopper on the test tube and shake vigorously.
4. Put the test tube on a test tube rack and let it sit for 3 minutes.
5. Observe the colour changes carefully. Record and analyse the result.
6. Repeat steps 1 - 5 with food sample 2 and 3.

Protein test

1. Take a test tube and add 2 mL of food sample 1.
2. In the same test tube, add 1 mL of Biuret solution A. Gently swirl to mix well.
3. In the same test tube, add 4 drops of Biuret solution B. Gently swirl to mix well.
4. Observe the colour changes carefully. Record and analyse the result.
5. Repeat steps 1 - 4 with food sample 2 and 3.

Data record and analysis

Food sample	**Test**				**Analysis**
		Reducing-sugar test	Lipid test	Protein test	
Sample 1	Prediction				
	Actual result				
Sample 2	Prediction				
	Actual result				
Sample 3	Prediction				
	Actual result				

Discussion

1. In your observation, did you notice any variations in the colour intensity of the positive reaction? What is the relationship between the colour intensity and the nutritional content of the food?

2. Proteins denature when heated. If a denatured protein undergoes protein test, predict the result.

3. Sugar cane contains high concentration of sucrose. Sucrose is easily extracted and crystallized to make table sugar. If table sugar is used as a sample for reducing-sugar test, predict the result. Explain your answer.

4. Briefly explain how to test for the presence of starch.

To prepare and observe temporary slides of plant and animal cells

Aim

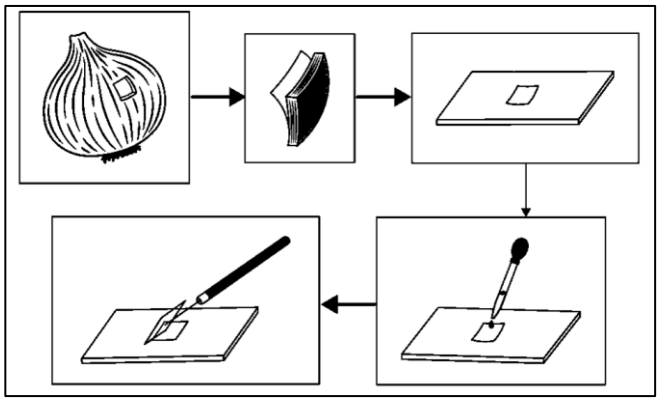
1. To learn how to use high-powered microscope and to prepare temporary slides.
2. To learn the structures of animal and plant cells and to differentiate the similarities and differences between animal and plant cells.

Material and apparatus

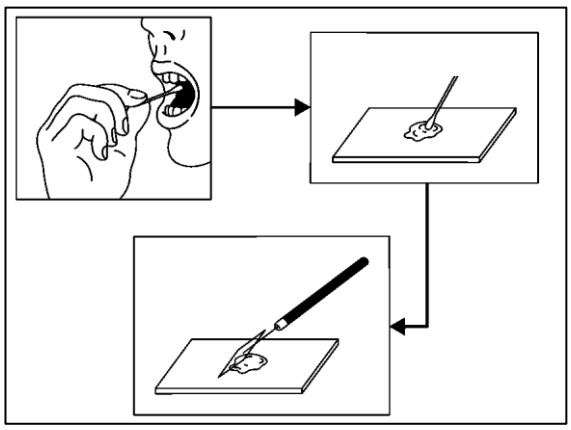
Material and apparatus	Concentration/specification	Volume/number
Onion	-	1
Oral mucosal cell	-	-
Human blood	-	1 drop
Microscope slides	-	4
Cover slips	-	4
Distilled water	-	1 bottle
Dropper	-	2
Forceps	-	1
Scalpel	-	1
Microscope	-	1
Iodine solution	-	1 bottle
Methylene blue	-	1 bottle
Wright's stain	-	1 bottle
Filter paper	-	2
Toothpick	-	1
Alcohol pad	-	1

Procedure

1) Making temporary slides
   a) Onion scale epidermal cell
      1. Cut out a small section of the onion.
      2. Remove the translucent epidermis from the inside of the onion using forceps.
      3. Add a drop of distilled water at the centre of the slide and lay the onion epidermis on the water. Make sure the epidermis is not folded up.
      4. Add a drop of iodine solution to the onion epidermis.
      5. Gently place the cover slip on one side, and avoid making air bubbles. Gently absorb excess water around the cover glass by using a filter paper.



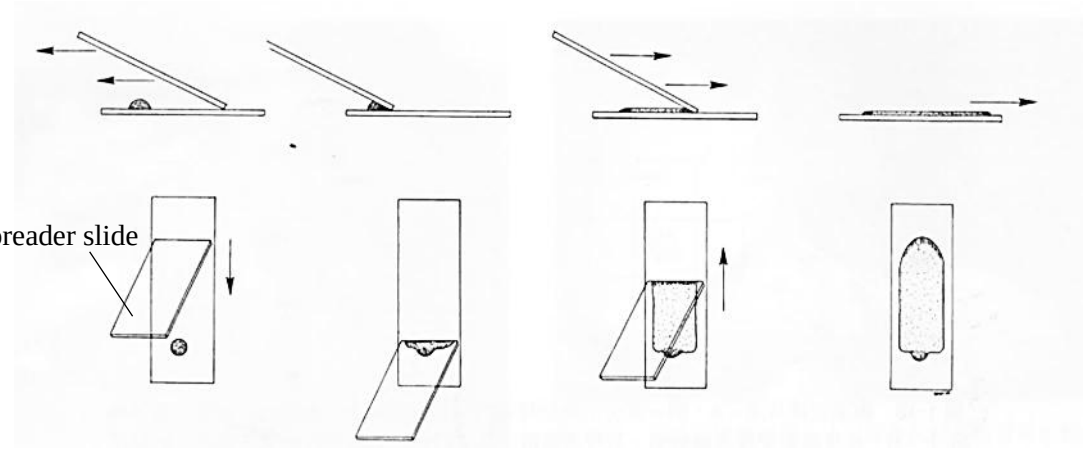
   b) Oral mucosal cell
      1. Rinse your mouth with cool boiled water before collecting the sample to remove food debris.
      2. Gently scrape the inner membrane of your mouth with the blunt end of a clean toothpick.
      3. Add a drop of distilled water at the centre of the slide and stir the scrapings into the water.
      4. Gently place the cover slip on one side, and avoid making air bubbles.
      5. Stain the cheek cells with methylene blue solution by adding the methylene blue solution on one side of the cover slip. Place the filter paper at the opposite end of the cover slip to spread the methylene blue solution evenly.
      6. Gently absorb excess water around the cover glass by using the filter paper.



c) Blood smear

1. First, massage the area where you want to draw blood (e.g., fingertip) to improve blood flow. Then disinfect the area with an alcohol swab.
2. Prick the area with a sterile needle. Place a drop of blood approximately 4-5 mm from one side of the slide.
   Note: Dispose of the sterile needle after use, do not share the needle .
3. Using another rounded-corner slide as spreader slide, place one side of the spreader slide in front of the drop of blood and move the spreader slide towards the blood. Once the spreader slide comes into contact with the blood, quickly and evenly spread the blood at the point of contact between the spreader and the slide.
4. Hold the spreader slide and microscope slide at 30° - 40° angles. Steadily and evenly spread the blood film towards the other side of microscope slide. Let the blood smear slide dry naturally.
5. After the blood has dried naturally, add 10 drops of Wright's stain until the stain completely covers the blood film.
6. After 3 to 5 minutes, add an equal volume of distilled water.
7. After another 3 to 5 minutes, gently wash away the stain.

Note: If Wright's stain is unavailable, methylene blue solution can be used. However, methylene blue solution will not stain red blood cells, so the red blood cells will appear almost colourless.



2) Observing temporary slides
   Observe under low power objective lens, select a region within the field of view where the cells are not too crowded and the structures are complete. Then, change to high power objective lens and observe.

3) Draw a diagram of the cell structure and label the observed organelles.

Data record and analysis

Draw and label the cell structure of onion epidermal cells, oral mucosal cells, and blood cells as you observe them under a microscope, and indicate "cell sample" and "magnification".

Discussion

1. Why does the field of view become smaller and darker under high power objective lens?

2. When using microscope, why is it necessary to start observing the specimen using low power objective lens first, and only change to high power objective lens after moving the desired part of the specimen to the middle of the field of view?

3. Why is blood smear needed? Why can’t the blood be observed just as a droplet?

4. Based on your observation, what are the similarities and differences between the structure of animal cell and plant cell?

5. When putting on the cover slip, why is it necessary to avoid forming air bubbles?

To investigate the factors that affect the rate of substances passing through selectively permeable membrane

Problem statement

How does the size of a molecule affect the rate at which a substance passes through a cell membrane?

Aim

To investigate the factors affecting the rate of substances passing through selectively permeable membrane

Principle

Red blood cells are completely permeable to water but only semi-permeable to most other substances. When red blood cells are placed in hypotonic solution, a large amount of water molecules will diffuse in, causing the red blood cells to expand and eventually burst. The haemoglobin will spread out from the cell. This phenomenon is known as haemolysis. Different solutes diffuse into the cells at different rate, and thus the time taken for haemolysis to occur is different too. Hence, we can estimate the permeability of the membrane to various substances based on the time taken for haemolysis to happen.

Material and apparatus

Material and apparatus	Concentration/specification	Volume/number
Small beaker	50 mL	4
Test tubes	-	9
Test tube holder	-	1
Graduated pipette	-	3
Syringe	-	1
Stopwatch	-	1
Chicken blood (with sodium citrate to prevent coagulation)	-	10 mL
Ethane-1,2-diol aqueous solution ($C_2H_6O_2$)	0.3%	10 mL
Glycerol aqueous solution ($C_3H_8O_3$)	0.3%	10 mL
Glucose solution	0.3%	10 mL

Procedure

1. In three labelled test tubes, add in 2 mL of 0.3% ethane-1,1-diol, 0.3% glycerol, and 0.3% glucose solution respectively.
2. Add in 2 drops of blood into each of the test tubes. Gently shake the test tubes evenly and leave it in room temperature. Observe the time taken for haemolysis to happen and the changes in each test tube.
3. Repeat steps 1 and 2 two more times to get 3 sets of data in order to increase the reliability of the test
4. Fill in the result in the table.

Data record and analysis

Solution	**Molecular weight (g/mol)**	**Time taken for haemolysis to occur (s)**			**Average**
		First set	**Second set**	**Third set**	
Ethane-1,2-diol	62				
Glycerol	92				
Glucose	180				

Discussion

1. Does the result support the hypothesis? Explain your answer.

2. What other factors will affect the semi-permeability of the cell membrane?

3. Why the test tube should not be shaken vigorously after the blood is added?

4. Plant cells have cell membrane too. Is this experiment suitable to investigate the effect of the size of the molecules to the diffusion rate of substances through plant cell membrane? Explain your answer.

To investigate the relationship between the concentration of the extracellular fluid of plant cells and plasmolysis

Problem statement

What is the relationship between the concentration of the extracellular fluid of plant cells and plasmolysis.

Aim

To study the relationship between the concentration of the extracellular fluid of plant cells and plasmolysis.

Principle

The cell membrane, tonoplast, and the cytoplasm between the two membranes is known as protoplasm. Protoplasm is a selectively permeable membrane. When the cell sap concentration is lower than the concentration of extracellular fluid, the water in the cell sap will diffuse out of the cell through protoplasm. The size of the vacuole will decrease. At the same time, cell wall and protoplasm will shrink to a certain extent. However, protoplasm is more flexible than cell wall. As the cell lose more and more water, cell wall and protoplasm gradually separate. This phenomenon is known as plasmolysis. When the cell that is undergoing plasmolysis is soaked into distilled water again, water from outside the cell will enter the cell again. The vacuole will become bigger and the protoplasm will return to its original state. This phenomenon is known as deplasmolysis.

Material and apparatus

Material and apparatus	Concentration/specification	Volume/number
Purple onion	-	1
Petri dish	-	5
Marker pen	-	1
Knife	-	1
Forceps	-	1
Dropper	-	5
Microscope slides	-	5
Cover slips	-	5
Filter paper	-	1
Microscope	-	1

Sucrose solution	0.1 g/mL, 0.2 g/mL, 0.3 g/mL, 0.4 g/mL, 0.5 g/mL	10 mL each

Procedure

1. Take 5 petri dishes and labelled them 1 - 5, and add the 5 different concentration of sucrose solution into each petri dish respectively.
2. Peel the epidermal cells of onion bulb scale and immerse them into the petri dishes with different concentration of sucrose solution.
3. After 10 minutes, take out the epidermal cells from the petri dish and make temporary slides. Add a drop of sucrose solution of the corresponding concentration on each of the slide.
4. Use the low power objective lens to observe the size of the purple vacuole of the epidermal cells of the onion bulb scale. Observe the position of the protoplasm too. In the field of view, calculate and record the cells that undergo plasmolysis and cells that did not.
5. Calculate the percentage of plasmolyzed cells:

   Number of cells that undergo plasmolysis (A)
   Number of cells that didn't undergo plasmolysis (B)
   Total number of cells (N) = A + B
   Percentage of plasmolyzed cells = $\frac{A}{N} \times 100\%$

Result

Sucrose solution concentration (g/mL)	0.1 g/mL	0.2 g/mL	0.3 g/mL	0.4 g/mL	0.5 g/mL
Number of cells that undergo plasmolysis (A)					
Number of cells that didn't undergo plasmolysis (B)					
Percentage of plasmolyzed cells (%)					

Discussion

1. Based on the data collected from the experiment, plot the graph of the percentage of plasmolyzed cells against sucrose solution concentration. (The x-axis represents the sucrose solution concentration, and the y-axis represents the percentage of plasmolyzed cells.)

2. Based on the graph, deduce the concentration of the cell sap of the plant cell.

3. Predict the result of the experiment if onions of different freshness were used.

4. Based on the principle of this experiment, explain the effect of overfertilization on crops.

To investigate the effect of pH on catalase activity

Problem statement

What is the effect of pH on the catalase activity?

Aim

To investigate the effect of different pH on catalase activity and to understand the factors affecting the rate of enzyme-catalyzed reactions.

Principle

Hydrogen peroxide (H_2O_2) is the by-product of certain chemical reaction in the cells and it has strong oxidizing properties. Accumulation of H_2O_2 will kill the cells if it is not removed or broken down in time. Catalase in cells can catalysed the breakdown of H_2O_2. However, catalase is affected by factors such as pH and temperature.

Material and apparatus

Materials and apparatus	Concentration/specification	Volume/number
Test tube	15 mm x 150 mm	3
Test tube holder	-	1
Measuring cylinder	10mL	1
Wooden splinter	-	1
Lighter/matches	-	1
pH paper/pH meter	-	3/1
Standard colour charts	-	1
Hydrogen peroxide solution	2%	30 mL
Hydrochloric acid solution	5%	10 mL
Sodium hydroxide solution	5%	10 mL
Potato	-	1
Knife	-	1
Distilled water	-	2 mL
Ruler	15 cm	1
Rubber stopper	-	3

Procedure

1. Use pH paper/pH meter to measure the pH of distilled water, hydrochloric acid, and sodium hydroxide solutions.
2. Take a fresh potato and cut out 9 small pieces of potato, 1cm^3 each.
3. Carry out the experiment according to the following steps.

Steps	Test tube 1	Test tube 2	Test tube 3
a)	Add 5 mL of 2% hydrogen peroxide into each test tube respectively.		
b)	Add 2 mL of distilled water	Add 2 mL of 5% hydrochloric acid	Add 2 mL of 5% sodium hydroxide solution
c)	Add 1 cm^3 of potato piece into each test tube respectively. Immediately close the test tubes with rubber stoppers.		
d)	Immediately observe the vigour of the reaction and measure the height of air bubbles releases. Record your observation.		
e)	Take out the rubber stopper and insert a glowing wooden splinter into the test tube, above the solution. Observe and record the condition of the reignition.		

4. Repeat step 3 three times to obtain 3 sets of data in order to increase the reliability of the experiment.

Data record and analysis

Test tube	The height of bubbles produced (cm)				The condition of glowing wooden splinter		
	Set 1	Set 2	Set 3	Average	Set 1	Set 2	Set 3
Test tube 1							
Test tube 2							
Test tube 3							

Discussion

1. Why does the potato used in this experiment need to be fresh? Explain your reasoning.

2. Why are air bubbles produced in this experiment? What are the air bubbles? Explain your answer.

3. Can step 3b and step 3c be switched? Will the result still be the same? Explain your answer and predict the result if step 3b and step 3c is switched.

4. Human liver also produces catalase. Predict the optimum temperature for human catalase. Explain your answer.

To investigate the factors that affect photosynthesis

There are many external factors that affect the rate of photosynthesis, including light intensity, carbon dioxide concentration, temperature and water. Choose one of the factors and design an experiment to investigate the effect of that particular factor on the rate of photosynthesis.

Problem Statement

What is the effect of light intensity/carbon dioxide concentration/temperature/ towards the rate of photosynthesis?

Hypothesis

_____

Aim

_____

Variables

Manipulated variable: _____

Responding variable: _____

Constant variable: _____

Material and apparatus

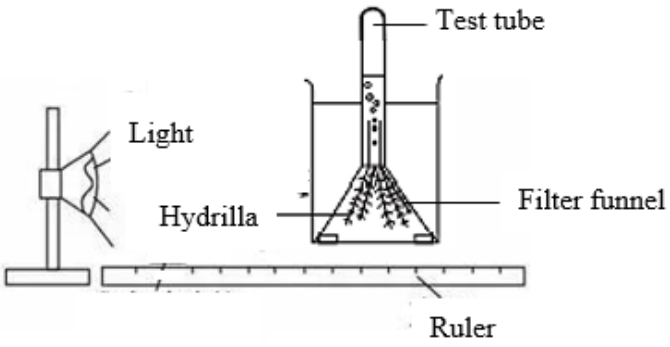
Listed below are the materials and apparatus needed for this experiment. Based on the experiment you designed, list down the concentration/specification and the volume/number needed. What other material and apparatus are needed?

Material and apparatus	Concentration/specification	Volume/number
Hydrilla sp.	-	
Distilled water	-	
Sodium hydrogen carbonate solution	0.2%	
Scissors	-	
Light bulb	60W	
Meter ruler	1m	

Stopwatch	-	
Filter funnel	-	
Test tube	10-15 mm diameter	
Retort stand and clamp	-	
Beaker	500 mL	

Procedure

According to the set-up below, design and write down your experimental procedure. Carry out the experiment according to your procedure. Observe the result of the experiment and record your observation.



Result

Design a table and record the data in the table. Use the following formula to calculate the rate of photosynthesis.

$$\text{Rate of photosynthesis} = \frac{\text{Volume of } O_2 \text{ produced}}{\text{Time}}$$

Discussion

Based on the recorded data, plot a graph to show the relationship between the manipulated variable and responding variable. Does the graph support your hypothesis? Explain.

Conclusion

Based on the experiment conducted and the data collected, what conclusion can be made?

To investigate the factors that affect transpiration

In nature, there are many factors that affect transpiration, such as temperature, humidity, light intensity and air movement. Choose one of the factors and design an experiment to investigate the effect of that particular factor on the rate of transpiration.

Problem statement

_____

Hypothesis

_____

Aim

_____

Variables

Manipulated variable：_____

Responding variable：_____

Constant variable: _____

Material and apparatus

First, decide which experimental method you want to use to investigate transpiration. The most common methods are the weighing method and the potometer method. Different methods require different material, apparatus and also experimental procedure. According to the experimental method you chose, list down the material and apparatus needed, along with the concentration/specification and volume/number.

Procedure

According to the aim of the experiment and the experimental method you have chosen, design specific experimental procedure. During the experiment, you should:

1. Strictly follow the procedure you designed to ensure the accuracy and reliability of the experimental data.
2. Carefully observe the experimental phenomena and record the observed changes, such as decrease in weight, distance travelled by the bubbles, etc.
3. Think about how to calculate the rate of transpiration using the experimental data and state the formula in the lab report.

Result

Design a table and record the data collected from the experiment in the table. Use the formula you have written down to calculate the rate of transpiration.

Discussion

1. Based on your calculation of the rate of transpiration, does your result support your hypothesis? Explain.

2. Based on your understanding on transpiration, predict the rate of transpiration of cactus compared to normal plants. Explain.

Conclusion

Based on the experiment conducted and the data collected, what conclusion can be made?

To observe the structure of kidney

Problem statement

What are the structures of a kidney?

Aim

1. To be able to correctly identify the blood vessels and ureter connected to the kidney.
2. To be able to describe the morphology of the kidney and its longitudinal sectional structure in detail.
3. To learn how to dissect a kidney

Material and apparatus

Material and apparatus	Concentration/specification	Volume/number
Animal kidney	-	1
Dissecting knife	-	1
Dissecting tray	-	1
Disposable gloves	-	2
Magnifying glass	-	1

Procedure

1. Observe the external shape of the animal's kidney.
2. If the kidney is encased in a layer of fat, carefully remove the fat layer.
3. Carefully cut the kidney longitudinally with a dissecting knife to obtain a cross-sectional structure.
4. Observe the internal structure of the kidney using a magnifying glass.
5. Draw the structures you observed.

Data record and analysis

Draw and label the internal structures of the kidney you observed

Discussion

1. What are the components of the renal cortex? Why is this structure red in colour?

To detect the pH, glucose and protein in urine

Problem statement

Is healthy urine acidic or alkaline? Does urine contain substances such as glucose and protein?

Aim

1. To learn the basics of pH, glucose and protein detection in urine.
2. To be able to make reasonable analysis and judgement based on the observation and the data collected in the experiment.
3. To be able to accept or reject the hypothesis based on evidence

Material and apparatus

Material and apparatus	Concentration/specification	Volume/number
Urine sample A	-	2 mL
Urine sample B	-	2 mL
Urine sample C	-	2 mL
Glucose solution	0.5%	2 mL
Albumin solution	0.5%	2 mL
Distilled water	-	2 mL
Test tubes	-	6
Test tube holder	-	1
Glass rod	-	6
Dropper	-	8
Watch glass	-	1
Marker pen	-	1
White tissues	-	1
pH standard colour charts	-	1
pH paper	-	6
Glucose test strips	-	6
Glucose standard colour charts	-	1
Biuret solution A (NaOH solution)	0.1 g/mL	12 mL
Biuret solution A ($CuSO_4$ solution)	0.01 g/mL	5 mL

Procedure

1. Preparation.
   Take 6 clean test tubes, labelled them 1 to 6. Then, add 2 mL of the following solution into each test tube respectively: distilled water, glucose solution, albumin solution, urine sample A, urine sample B, and urine sample C. Put a clean dropper in each test tube.

2. Detection procedures
   a) pH test
      Tear a pH paper and put it on the watch glass. Use the dropper to take a drop of solution from test tube 1 and add it to the pH paper. Then compare the test strip with the pH standard colour chart to determine its pH. Record the results in the table. Repeat the steps for test tubes 2 – 6.

   b) Glucose test
      Tear a glucose test strip and put it on a clean tissue. Use the dropper to take a drop of solution from test tube 1 and add it to the glucose test strip. Observe the colour changes on the glucose test strip and compare it with the glucose standard colour chart. Record the observation in the table. Repeat the steps for test tubes 2 – 6.

   c) Protein test
      Add 2 mL of biuret solution A into test tube 1 and shake gently to mix well. Then, add 5 drops of biuret solution B and shake gently to mix well. Observe the colour change and record the observation in the table. Repeat the steps for test tubes 2 – 6.

Result

Tests	pH	Glucose	Protein
Test tube 1 (Distilled water)			
Test tube 2 (Glucose solution)			
Test tube 3 (Albumin solution)			
Test tube 4 (Urine sample A)			
Test tube 5 (Urine sample B)			
Test tube 6 (Urine sample C)			

Discussion

1. What does changes in urine pH indicate about a person’s health?

2. What might the presence of glucose in urine suggest? Why is glucose normally not found in urine?

3. Does a healthy person's urine contain protein? What does the presence of protein in urine mean?

4. What are some limitations of using urine tests for diagnosing health problems?

To observe mitosis in root apical meristem cells

Problem statement

Does mitosis occur in root apical meristem cells?

Hypothesis

Mitosis occurs in root apical meristem cells.

Aim

1. To observe mitosis in onion root tip cells and identify the characteristics of each stage of mitosis.
2. To deepen the understanding of the main characteristics of each stage of mitosis through drawing.

Material and apparatus

Material and apparatus	Concentration/specification	Volume/number
Light microscope	-	1
Knife	-	1
Forceps	-	1
Dropper	-	1
Microscope slide	-	1
Cover slip	-	1
Filter paper	-	1
Petri dish	-	1
Onion root tip	-	1
Dissociating solution	1:1 ratio of 95% alcohol and 15% concentrated HCl	10 mL
Distilled water	-	1 bottle
Acetocarmine solution	-	Small amount

Note: Acetocarmine is corrosive; inhalation or contact can irritate the respiratory tract, skin, and eyes.

Procedure

1. Cut a 2 - 3 mm section of an onion root tip and place it in a petri dish containing dissociating solution for approximately 3 - 5 minutes to allow the cells of the root tip to separate. Stop the dissociation when the root tip becomes transparent and soft.
2. Remove the root tip with a forceps and rinse it in a glass dish filled with clean water for approximately 10 minutes.
3. Place the rinsed root tip on a glass slide and stain it with one drop of acetocarmine solution for 3 - 5 minutes.
4. Place a coverslip over the stained root tip and gently press with your thumb to form a uniform, thin layer of cells.
5. Observe the cells of the onion root tip using a low-power objective lens to locate the root apical meristem (this area contains small, square cells that are densely packed, as shown in the image below).

Meristematic zone

Onion root tip under low-power objective lens

6. After finding the meristematic zone, switch to a high-power objective lens for observation.
7. Find cells at different stages of mitosis.
8. Draw the cells you have observed and based on what you've learned, arrange them into a mitosis flow chart.

Data record and analysis

Draw the cells at different stages of mitosis that you observed under the microscope and arrange it into a flowchart.

Discussion

1. In a field of view, cells in which phase of cell division is the most abundant? Explain your answer.

2. Besides mitosis phase, you can also observe interphase under high-power objective lens. Draw the interphase that you observed.

To investigate the effect of alcohol temperature on DNA extraction

Problem statement

What is the effect of alcohol temperature on the amount of DNA extracted from banana?

Aim

To investigate the effect of alcohol temperature on the amount of DNA extracted from banana.

Principle

DNA is water-soluble, but its solubility is extremely low in high-concentration alcohol at low temperatures. When alcohol is slowly added into an aqueous solution containing DNA, the DNA precipitate from the aqueous phase at the interface, forming a visible white, flocculent precipitate. Meanwhile, most other soluble substances in the cell (such as proteins, carbohydrates, and small molecule metabolites) remain in the aqueous or alcoholic solution, thus making the precipitated DNA relatively pure. Using this principle, DNA with fewer impurities can be extracted from banana cells.

Material and apparatus

Material and apparatus	Concentration/specification	Volume/number
Marker pen	-	1
Mortar and pestle	-	1
Filter funnel	-	1
Filter paper	-	2
Beaker	100 mL	1
Test tube	-	3
Bunsen burner	-	1
Tripod stand	-	1
Thermometer	-	1
Banana	-	100 g
Detergent	-	3 mL
Sodium chloride (solid)	-	1 g
Alcohol	95%	30 mL

Distilled water	-	100 mL
Glass rod	-	1
Measuring cylinder	10 mL	3
Measuring cylinder	100 mL	1

Procedure

1. Mix 3 mL of detergent solution, 1 g of solid sodium chloride, and 97 mL of distilled water to make an extract.
2. Weigh 100 g of bananas, place them in a mortar, and mash until fine. Add 10 mL of the extract. Mix thoroughly, then filter through filter paper into a beaker.
3. Pour the filtrate evenly into three test tubes and label them as A, B, and C.
4. Prepare alcohol at three temperatures: 0 °C, 25 °C, and 50 °C. (Note: 50 °C alcohol needs to be heated through water bath instead of directly)
5. Slowly pour 10 mL of alcohol of the corresponding temperature down the wall of each test tube. Be careful not to stir, allowing the alcohol to float on top of the extract to form distinct layers.
6. Wait for 5 – 10 minutes then observe for the appearance of DNA precipitate at the boundary between the alcohol and the extract.
7. Observe and record the thickness of the DNA layer.
8. Using a glass, spool (twirl) the white strands and collect onto a filter paper.
9. Observe and record the amount of visible DNA.

Result

Group	Temperature	Thickness of DNA layer and the amount of visible DNA
A	0 °C	
B	25 °C	
C	50 °C	

Discussion

1. What is the function of detergent in this experiment?

2. Why is the alcohol added slowly along the side of the test tube?

3. Predict the result if strawberry or kiwi are used instead of banana.

To investigate and analyse the effect of pH on the growth of bacteria

Problem statement

What is the effect of pH on the growth of *Escherichia coli* (*E. coli*)?

Aim

To investigate and analyse the effect of pH on the growth of *E. coli*, and determine the optimum pH.

Material and apparatus

Material and apparatus	Concentration/specification	Volume/number
Nutrient agar powder	-	100 mL
Escherichia coli	-	Small amount
Hydrochloric acid	0.1M	10 mL
Sodium hydroxide	0.1M	10 mL
Petri dish	-	3
Inoculating loop (or cotton swab)	-	1
Incubator (if available)	-	1
pH paper/pH meter	-	2/1
pH standard colour charts	-	1
Alcohol (or bleach)	70%	Small amount
Glass rod	-	2
Beaker	250 mL	3
Thermometer	-	1
Bunsen burner	-	1
Parafilm (or masking tape)	-	1
Marker pen	-	1
Dropper	-	2

Procedure

Nutrient agar plates preparation

1. Take 300 mL of nutrient agar solution. Heat till boiling as simple sterilization.
2. Carefully divide the nutrient agar solution into three beakers, labeled pH 4, pH 7, and pH 9.
3. In the pH 4 beaker, slowly add 0.1 M hydrochloric acid dropwise to the nutrient agar solution while stirring. Take a small sample and measure the pH of the solution using pH paper/pH meter until it reaches pH 4.
4. In the pH 9 beaker, slowly add 0.1 M sodium hydroxide dropwise to the nutrient agar solution while stirring. Take a small sample and measure the pH of the solution using pH paper/pH meter until it reaches pH 9.
5. Take 3 petri dish and labeled pH 4, pH 7, and pH 9 at the bottom of each petri dish respectively.
6. When the nutrient agar solution has cooled to about 50 – 55 °C, pour it into the respective Petri dish until it just covers the bottom. Be sure to perform this step quickly in a laminar flow cabinet or near a Bunsen burner to maintain sterile conditions as much as possible.
7. Leave the lid slightly ajar until the nutrient agar in the petri dish has completely solidified.

***E. coli* inoculation**

1. Sterilize the inoculating loop and metal rod by heating them over the flame of a Bunsen burner until the loop glows red.
2. After the inoculating rod has cooled, use it to collect the *E. coli* culture solution and spread it evenly on the agar plate.
3. Seal the agar plate with parafilm.
4. Repeat steps 1 – 3 for the other two agar plates with different pH.
5. Invert all plates (lids facing down) and place in a dark area in the laboratory for 24 – 48 hours to incubate.
6. After the incubation period, observe the number and growth of colonies on each plate. If the colonies are independent and do not touch each other (as shown in Figure 1), you can choose to record the number of colonies. If the colonies grow into a continuous layer and cannot be distinguished (as shown in Figure 2), then you can choose to record the colony coverage area. The counting method is as follows:

Figure 1

Figure 2

Number of colonies	Colony coverage area
Place the petri dish against a piece of white paper and count the number of colonies. Lightly mark the counted colonies on the bottom of the dish with a marker pen to avoid double counting.	Consider the whole petri dish as 100% of the surface area. Estimate the percentage of the entire surface covered by colonies (e.g., 10%, 50%, 80%).

Result

pH of the agar plate	Number of colonies/colony coverage area
4	
7	
9	

Discussion

1. What is the optimum pH for *E. coli*? Does the result support your hypothesis?

2. Some *E. coli* strains can make human sick by causing diarrhea and stomach pain. The most common way to get infected by *E. coli* is through ingesting contaminated food. Based on your understanding of the optimum pH for *E. coli* growth, explain how to use this knowledge to help prevent *E. coli* infection?

3. Is your agar plate contaminated? How can you prevent contamination when growing bacteria culture?

To investigate the effect of antibiotics on the growth of bacteria

Problem statement

What is the effect of penicillin on the growth of different bacteria?

Hypothesis

Penicillin will inhibit the growth of *Staphylococcus aureus* more effectively than *Escherichia coli*.

Aim

To investigate the effect of antibiotics on the growth of bacteria.

Variables

Manipulated variable: The type of bacteria

Responding variable: Diameter of the zone of inhibition (mm) around the penicillin disc

Constant variable: Concentration of penicillin, incubation temperature, incubation time, amount of bacterial culture, type of agar medium

Material and apparatus

Material and apparatus	Concentration/specification	Volume/number
Sterile nutrient agar plates	-	3
Escherichia coli	-	Small amount
Staphylococcus aureus	-	Small amount
Penicillin antibiotic discs	-	3
Forceps	-	1
L-shaped spreader	-	1
Dropper	-	1
Incubator (if available)	-	1
Ruler	-	1
Alcohol	70%	Small amount
Beaker	250 mL	1

Bunsen burner	-	1
Marker pen	-	1
Filter paper	-	1
Parafilm (or masking tape)	-	1

Procedure

1. Label the three agar plates for each bacterial species.
2. Use a dropper to take 0.1 mL of *E. coli* solution and add it to the surface of the corresponding agar plate.
3. Immerse the L-shaped spreader in a beaker containing alcohol. Sterilize the spreader by heating it over a flame until it glows red. After cooling for 8-10 seconds, use the spreader to evenly spread the bacterial solution onto the surface of the agar plate. Rotate the agar plate during spreading to ensure even distribution of the bacterial solution.

4. Repeat steps 2 - 3 with the other 2 bacteria.
5. Sterilize the forceps over a flame, place one penicillin disc (small round filter paper discs containing a certain amount of penicillin) in the center of each plate. Gently press down to ensure contact with the agar surface.
6. Seal each plate with parafilm or masking tape, invert the plates (lids facing down) and place in a dark area in the laboratory for 24 – 48 hours to incubate.
7. After incubation, observe and measure the zone of inhibition (clear area with no bacterial growth) around each disc in millimeters. Record the results for each bacterial species.

Result

Types of bacteria	**Diameter of the zone of inhibition (mm)**
Escherichia coli	
Staphylococcus aureus	

Discussion

1. Which bacterial species showed the largest and smallest zones of inhibition? What does this indicate about their sensitivity or resistance to penicillin?

2. Penicillin inhibit the growth of gram-positive bacteria more effectively than gram-negative bacteria. Based on your result, classify *Escherichia coli* and *Staphylococcus aureus* into gram-positive and gram-negative bacteria.

To investigate the nutrient compositions in food

*Teachers could choose other food test such as Fehling test or ethanol emulsion test, according to the chemicals available at school. Teachers could change the procedure in this manual.

Result

*****Teachers could adjust the answers based on the food sample used

Test	Positive result	Negative result
Reducing sugar test	Benedict solution will turn from blue solution to brick red precipitate	Benedict solution will remain a blue solution
Lipid test	There's an orangish yellow layer	There is no orangish yellow layer
Protein test	The Biuret solution change from blue colour to purple	The Biuret solution remains blue colour

Food sample	**Test**				**Analysis**
		Reducing-sugar test	Lipid test	Protein test	
Sample 1	Prediction				Sample 1 contains reducing sugar but not lipid and protein
	Actual result				

Discussion

1. In your observation, did you notice any variations in the colour intensity of the positive reaction? What is the relationship between the colour intensity and the nutritional content of the food?

   In food test, the intensity of the colour in the test result reflects the content of the corresponding nutrient in the sample. For example, in reducing-sugar test, light green colour means low content of reducing sugar. Orange colour means higher content of reducing sugar. And brick-red colour means very high content of reducing sugar.

2. Proteins denature when heated. If a denatured protein undergoes protein test, predict the result.
   This experiment uses biuret reagent. Biuret reagent detects peptide bonds in protein molecules. Peptide bonds do not break upon heating; only the protein structure unfolds. All denatured proteins will still show a positive result.

3. Sugar cane contains high concentration of sucrose. Sucrose is easily extracted and crystallized to make table sugar. If table sugar is used as a sample for reducing-sugar test, predict the result. Explain your answer.

   The Benedict solution will remain blue colour. This is because Benedict solution tests for the presence of reducing sugar and table sugar is a non-reducing sugar.

4. Briefly explain how to test for the presence of starch.

   The iodine test can be used to test for the presence of starch. Add a drop of iodine solution on the food sample. If the food sample contains starch, the iodine solution will change from yellowish-brown colour to dark blue.

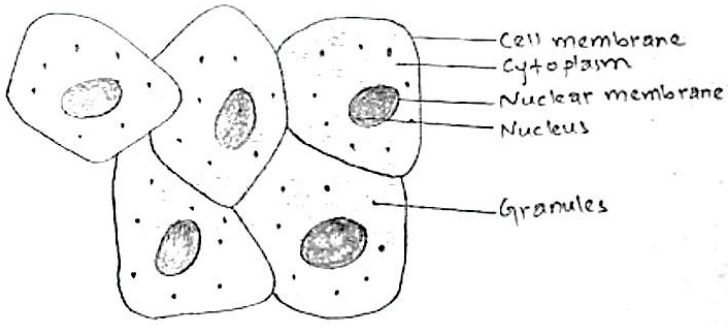
Conclusion

Benedict solution can be used to test for the presence of reducing sugar, Sudan III for fats and Biuret solution for protein. Sample 1 contains _____, sample 2 contains _____ and sample 3 contains _____.

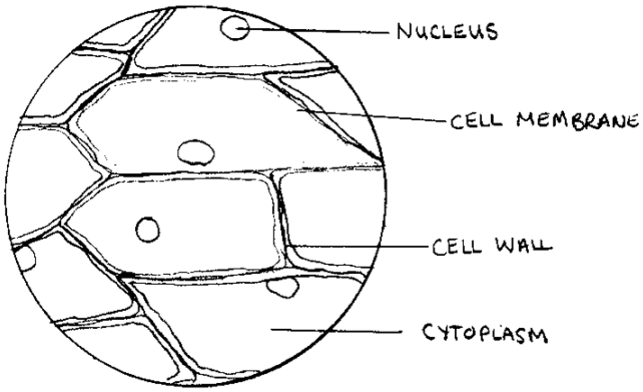
To prepare and observe temporary slides of plant and animal cells

Data record and analysis

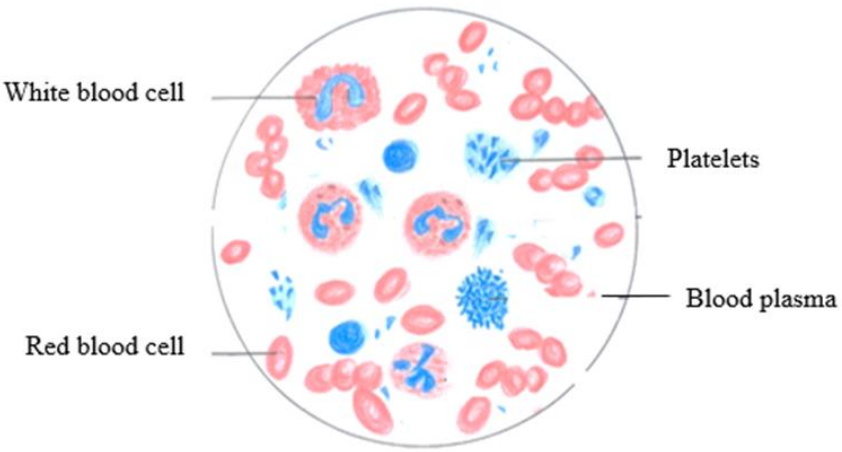
Oral mucosal cell



Onion cell



Blood cell



Discussion

1. Why does the field of view become smaller and darker under high power objective lens?

   The field of view becomes smaller because high power objective lens has higher magnification. As the field of view becomes smaller, less light can pass through compared to low power objective lens. Hence, the field of view becomes darker too.

2. When using microscope, why is it necessary to start observing the specimen using low power objective lens first, and only change to high power objective lens after moving the desired part of the specimen to the middle of the field of view?

   When changing from low power objective lens to high power objective lens, the field of view becomes smaller. If the part of the specimen we want to observe is not in the middle of the field of view, it would be difficult to find the specimen as part of the specimen might not be in the field of view anymore.

3. Why is blood smear needed? Why can't the blood be observed just as a droplet?

   Blood droplet is too thick, it contains many overlapping cells. Light can't pass through well, making it impossible to focus on individual cells. In a smear, the blood is spread into a thin, even layer where cells lie mostly in a single layer, allowing clear viewing of individual cells.

4. According to your observation, what are the similarities and differences between the structure of animal cell and plant cell?

   Both onion cells and oral mucosal cells have cell membrane, nucleus and cytoplasm. Onion cells are more regular and rectangular in shape and their cell walls are visible while oral mucosal cells are irregular in shape and does not have cell wall.

5. When putting on the cover slip, why is it necessary to avoid forming air bubbles?

   Air bubbles will block the view, make the image blurry and affect the observer to observe the cell structure clearly.

Conclusion

Onion cells will appear more regular and rectangular in shape, and have a visible cell wall, while oral mucosal cells will appear irregular in shape and without a cell wall.

To investigate the factors that affect the rate of substances passing through selectively permeable membrane

Hypothesis

The larger the molecular size of the substance, the slower the rate of substances passing through cell membrane

Variables

Manipulated variable: The size of the molecule of the substance

Responding variable: The rate of substance passing through the cell membrane

Constant variable: Concentration of substance, type of blood

Discussion

1. Does the result support the hypothesis? Explain your answer.

   The result supports the hypothesis. Ethane-1,2-diol has the smallest molecular mass, so the rate of ethane-1,2-diol passing through the cell membrane is the highest. Glucose has the largest molecular mass, so the rate of glucose passing through the cell membrane is the lowest.

2. What other factors will affect the semi-permeability of the cell membrane?

   Temperature, pH, polarity, lipid-solubility of substance.

3. Why the test tube should not be shaken vigorously after the blood is added?

   To avoid mechanical haemolysis. The cell membrane of red blood cell is very fragile. Vigorous shaking will break the cell membrane. This will lead to haemolysis that is not caused by the factor investigated by the experiment, and it will affect the result.

4. Plant cells have cell membrane too. Is this experiment suitable to investigate the effect of the size of the molecules to the diffusion rate of substances through plant cell membrane? Explain your answer.

   Not suitable. Because plant cells have cell wall, so the plant cells will become turgid but it will not burst.

Conclusion

The larger the molecular size of the substance, the lower the rate of the substance passing through cell membrane, the slower haemolysis occurs.

To investigate the relationship between the concentration of the extracellular fluid of plant cells and plasmolysis

Hypothesis

The more concentrated the extracellular fluid of plant cells, the more cells undergo plasmolysis.

Variables

Manipulated variable: Solution concentration
Responding variable: The percentage of plasmolyzed cells
Constant variable: observation time, solution temperature, volume of solution

Result

To calculate the percentage of plasmolyzed cells
Number of plasmolyzed cells (A)
Number of non-plasmolyzed cells (B)
Total number of cells (N) = A + B

$$\text{Plasmolyzed percentage} = \frac{A}{N} \times 100\%$$

Discussion

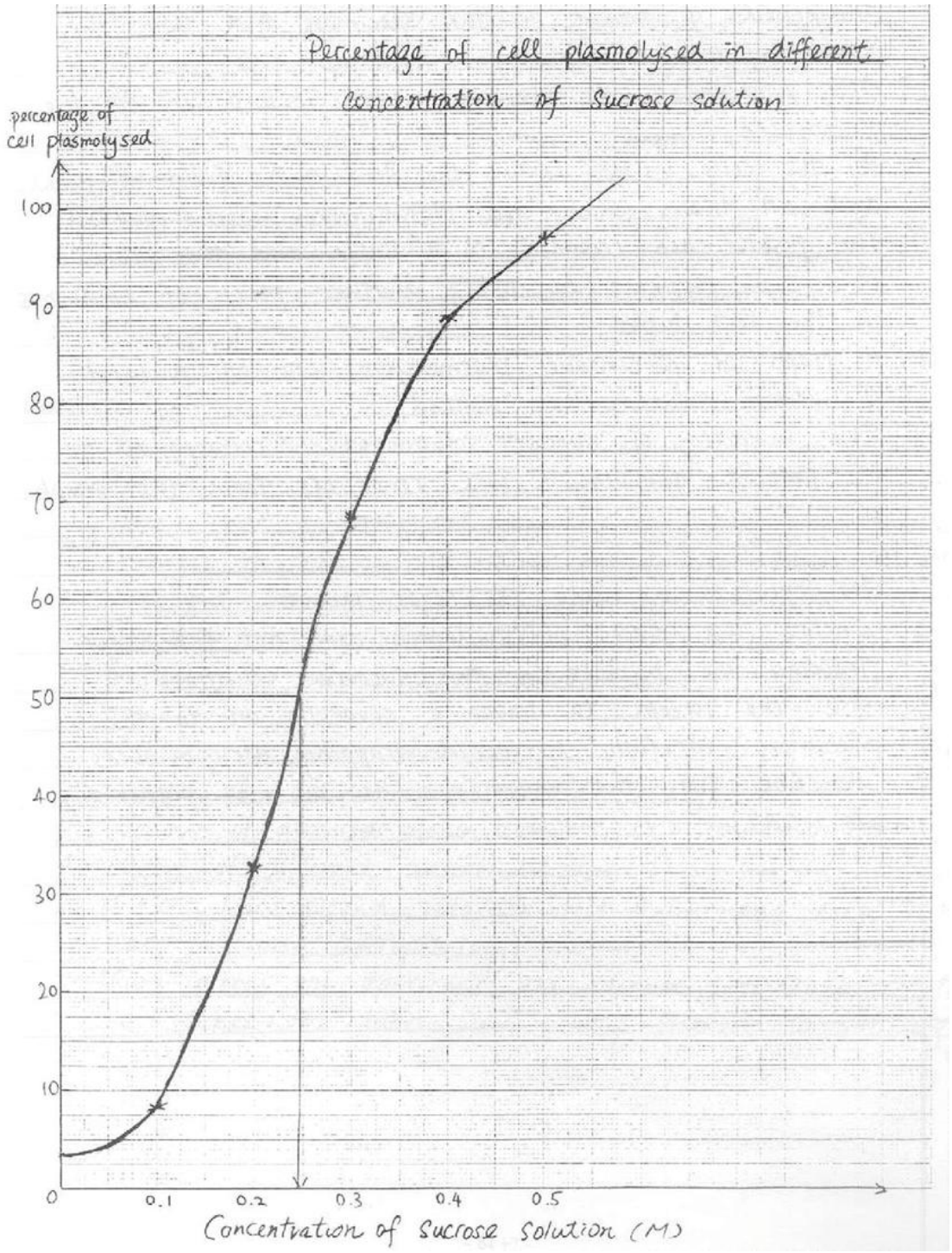
1. Based on the data collected from the experiment, plot the graph of the percentage of plasmolyzed cells against solution concentration.

   x-axis – solution concentration
   y-axis - the percentage of plasmolyzed cells
   This should be a sigmoid curve graph.

2. Based on the graph, deduce the concentration of the cell sap of the plant cell.

   Students should look for the point on the y-axis where the percentage of plasmolyzed cells is at 50% and draw a horizontal line. Find the intersection between the sigmoid curve and the horizontal line. Draw a vertical line downward from the intersection point to x-axis. The corresponding value on the x-axis is the concentration of the cell sap of the plant cell.

   *Refer to the graph below as a reference


Percentage of cell plasmolysed in different Concentration of Sucrose solution
percentage of cell plasmolysed
100
90
80
70
60
50
40
30
20
10
0
0.1
0.2
0.3
0.4
0.5
Concentration of Sucrose Solution (M)

3. Predict the result of the experiment if onions of different freshness were used.

   The percentage of plasmolyzed cells would be higher. An onion that is not fresh will experience water loss. So, the cells would appear to be undergoing plasmolysis even at a lower solution concentration. Besides, the permeability of a senescence cell will decrease, thus affecting the movement of water.

4. Based on the principle of this experiment, explain the effect of overfertilization on crops.

   Fertilizers contain a lot of soluble salt. This cause the solution concentration of the soil to be more concentrated than the cell sap concentration of the root. The root cells in hypertonic condition. Water will diffuse out of the cells, causing plasmolysis. The crops will wilt and die.

Conclusion

The more concentrated the extracellular fluid of plant cells, the more cells undergo plasmolysis.

To investigate the effect of pH on catalase activity

Hypothesis

The optimum pH for catalase is neutral.

Variables

Manipulated variable: pH

Responding variable: The catalase activity

Constant variable: Substrate concentration, reagent concentration, the size of test tubes, order of experiment, and temperature

Discussion

1. Why does the potato used in this experiment need to be fresh? Explain your reasoning.

   Fresh potato contains a lot of catalase. However, catalase is very sensitive to temperature. After the potato is frozen, the rate of reaction of catalase becomes very slow. This would affect the result of the experiment.

   Or

   Old potatoes have lower catalase activity because the enzyme protein ages or decomposes over time. The cells of old potatoes become dehydrated, and enzymes require a fully hydrated environment to function optimally, dehydration renders them inactive.

2. Why are air bubbles produced in this experiment? What are the air bubbles? Explain your answer

   The catalase in the potato will react with hydrogen peroxide solution to produce water and oxygen. The air bubbles in the experiment is oxygen.

3. Can step 2 and step 3 be switched? Will the experiment result still be the same? Explain your answer and predict the experiment result if step 2 and step 3 is switched.

   No, the experiment result will be different. Because step 1 is adding hydrogen peroxide solution into the test tube. If it's followed by step 3, which is adding the fresh potato, the chemical reaction would begin immediately. Thus, the experiment couldn't accurately investigate the effect of pH on the rate of reaction on catalase. Test tubes 2 and 3 will produce more gas bubbles and the glowing wooden splinter will reignite.

4. Human liver also produces catalase. Predict the optimum temperature for human catalase. Explain your answer.

   37°C because this is the normal human temperature.

Conclusion

The optimum pH for catalase is neutral/pH 7, condition that are too acidic or alkaline will reduce the rate of reaction for catalase.

To investigate the factors that affect photosynthesis

Hypothesis

The higher the light intensity/the higher the carbon dioxide concentration/the higher the temperature, the higher the rate of photosynthesis.

Aim

To investigate the effect of light intensity/carbon dioxide concentration/temperature on the rate of photosynthesis.

Variables

Manipulated variable: The distance between the light source and Hydrilla sp./the carbon dioxide concentration/temperature

Responding variable: The number of air bubbles released in 5 minutes

Constant variable: The type and size of *Hydrilla* sp., the voltage of the bulb

Material and apparatus

*For reference only, adjust according to actual situation

Material and apparatus	Concentration/specification	Volume/number
Hydrilla sp.	-	1
Distilled water	-	1 bottle
Sodium hydrogen carbonate solution	0.2%	300 ml
Scissors	-	1
Light bulb	60 W	1
Ruler	1 m	1
Stopwatch	-	1
Filter funnel	-	1
Test tube	10 – 15mm in diameter	1
Retort stand and clamp	-	1
Beaker	500 mL	1

Procedure

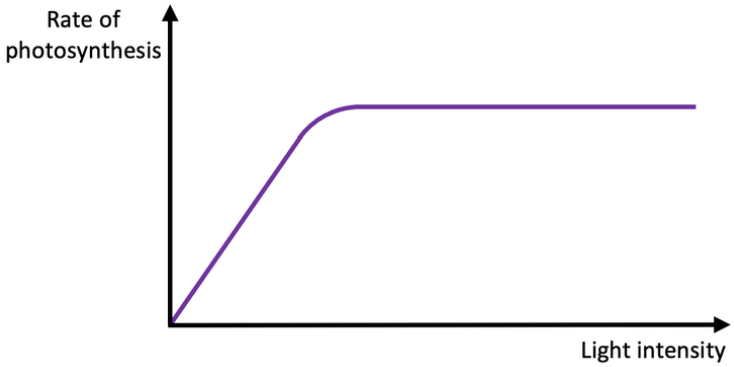
*As long as students come up with procedure that works and can investigate the effect of their chosen factor on photosynthesis.

Result

Distance of the light source (cm)	Number of air bubbles released in 5 minutes			Average
	1	2	3	
20				
30				
40				
50				
60				

*The table above is only an example, students can design their own table. The table needs to complete, with clear title, the manipulated variable and responding variable.

Discussion



*The graph should be a curve graph, like the graph above. The x-axis and y-axis and their labels should be correct.

**Students should explain what is shown in the graph, as light intensity increase (or other factors), the rate of photosynthesis increase, up to a point. After that, the rate levels off and reaches a plateau. This is because other factors become the limiting factor.

Conclusion

The higher the light intensity/the higher the carbon dioxide concentration/the higher the temperature, the higher the rate of photosynthesis, up to a point.

To investigate the factors that affect transpiration

Problem statement

What is the effect of temperature/humidity/light intensity/air movement on transpiration?

Hypothesis

The higher the temperature/the lower the humidity/the stronger the light intensity/the faster the air movement, the higher the rate of transpiration.

Aim

To investigate the effect of temperature/humidity/light intensity/air movement on transpiration.

Variables

Manipulated variable: Temperature/humidity/light intensity/air movement

Responding variable: Loss in mass (for weighing method)/the distance travelled by the air bubble (for potometer method)

Constant variable: Type of plants, time intervals

Material and apparatus

*For reference only

Weighing method

Material and apparatus	Concentration/specification	Volume/number
Potted plant	-	1
Electronic balance	-	1
Plastic wrap, plastic bag or aluminium foil (to cover the soil)	-	1
Stopwatch	-	1
Fan, lamp, thermometer, hygrometer (To control the environmental factor)	-	1

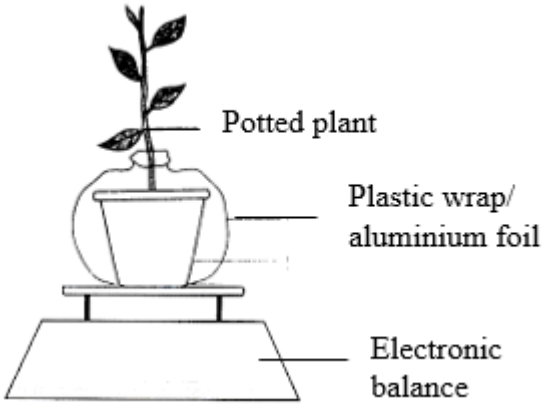
Potometer method

Material and apparatus	Concentration/specification	Volume/number
Potometer	-	1
Fresh twig with leaves	-	1
Distilled water	-	1 bottle
Scissors	-	1
Stopwatch		
Fan, lamp, thermometer, hygrometer (To control the environmental factor)	-	1

Procedure

Weighing method

1. Select a healthy potted plant.
2. Water the plant until the soil is moist, but not dripping from the bottom.
3. Cover the soil surface completely with plastic wrap or aluminium foil to prevent evaporation. Leave only the stem and leaves of the plant exposed to air.
4. Use an electronic balance to weigh the total mass of the plant and pot, recording this as the initial mass.



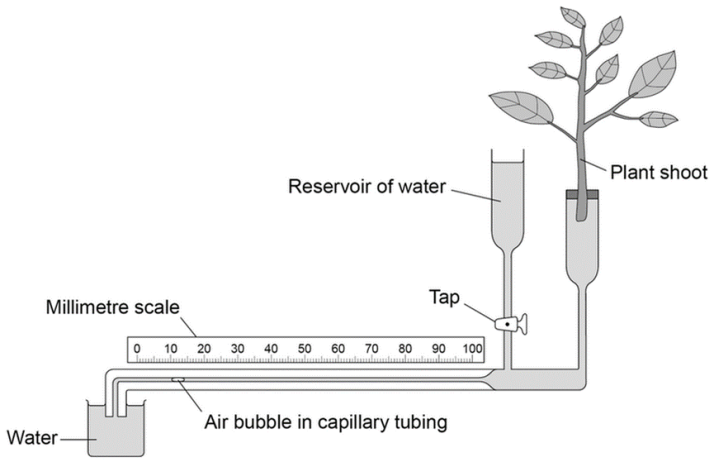
5. Place the setup in controlled conditions (e.g., under a light source, at a known temperature).
6. Allow the plant to transpire for 30 minutes.
7. After 30 minutes, weigh the plant again and record the final mass.
8. Calculate the rate of transpiration using the formula

$$\text{Rate of Transpiration} = \frac{\text{Initial Mass} - \text{Final Mass}}{\text{Time}}$$

9. Repeat steps 1-8. Observe the effects of varying environmental factors such as light, temperature, and humidity on the transpiration rate.

Potometer method

1. Select a healthy, pest-free, leafy branch.
2. Use a scissors to cut the base of the branch diagonally in water.
3. Insert the branch into the potometer with water, ensuring the device is completely filled with water and free of bubbles.
4. Create a small bubble in the capillary tube; this will serve as a marker for water flow.



5. Place the setup in controlled conditions (e.g., under a light source, at a known temperature).
6. Record the starting position of the bubble.
7. Allow the plant to transpire for 30 minutes.
8. After 30 minutes, record the position of the bubble on the graduated tube.
9. Calculate the rate of transpiration using the formula

$$\text{Rate of Transpiration} = \frac{\text{Distance moved by air bubble}}{\text{Time taken}}$$

10. Repeat steps 1 - 9. Observe the effects of varying environmental factors such as light, temperature, and humidity on transpiration rate.

Result

Environmental factor	Loss in mass (g)/ Distance travelled by air bubble (cm)	Rate of transpiration
High light intensity		
Medium light intensity		
Low light intensity		

*The table above is only an example, students can design their own table. The table needs to complete, with clear title, the manipulated variable and responding variable.

Discussion

1. Based on your calculation of the rate of transpiration, does your result support your hypothesis? Explain.

   The result supports the hypothesis. Under high light intensity, the loss in mass is the highest/distance travelled by air bubble is the furthest, hence the rate of transpiration in the highest. This is because as light intensity increases, rate of photosynthesis increases. In order to absorb more carbon dioxide, the stoma on the leaves will open wider. This cause the water to evaporate faster.

2. Based on your understanding on transpiration, predict the rate of transpiration of cactus. Explain.

   Cactus's transpiration rate is much lower than normal broadleaf plants. This is because cactus does not have leaves, only thorns. Thorns have small surface area with very little stomata. Transpiration almost doesn't occur. Besides, the cactus's stomata only open at night. The stomata is closed during the day to prevent water loss under hot weather. At night, when the temperature is lower and the humidity is higher, the cactus will open the stomata to absorb carbon dioxide to prevent water from evaporated.

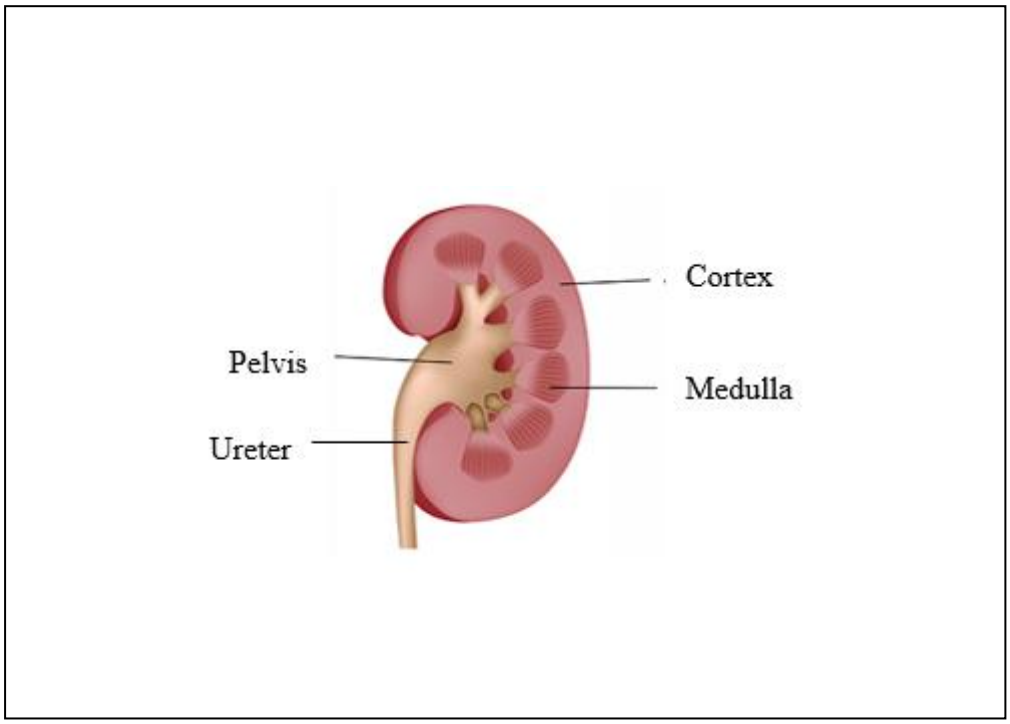
Conclusion

The higher the temperature/the lower the humidity/the stronger the light intensity/the faster the air movement, the higher the rate of transpiration.

To observe the structure of kidney

*Teachers can exchange this experiment with other dissection experiment to assess students. Teachers need to change the procedure and the discussion question accordingly.

Result



Discussion

1. What are the components of the renal cortex? Why is this structure red in colour?
   The renal cortex is composed of glomeruli, Bowman's capsules, proximal convoluted tubules, and distal convoluted tubules. The cortex appears red because it contains a large number of capillaries and glomeruli, resulting in a very rich blood supply. The blood contains haemoglobin, which gives the cortex a red or dark red colour. In addition, the renal cortex is the main site of blood filtration, so it requires an adequate blood supply to carry out its filtering function.

To detect the pH, glucose and protein in urine

Hypothesis

Healthy urine is acidic and does not contain protein and glucose.

Variables

Manipulated variable: Urine samples

Responding variable: The pH, glucose and protein content in urine

Constant variable: Detection method

Discussion

1. What does changes in urine pH indicate about a person's health?

   Changes in urine pH can indicate different health conditions: a very acidic urine (low pH) might suggest diabetes or starvation, while alkaline urine (high pH) could indicate urinary tract infections or kidney stones.

2. What might the presence of glucose in urine suggest? Why is glucose normally not found in urine?

   Glucose in urine (glucosuria) usually suggests high blood sugar levels, often linked to diabetes. Normally, kidneys reabsorb glucose back into the bloodstream, so it doesn't appear in urine unless blood glucose is too high for the kidneys to handle.

3. Does a healthy person's urine contain protein? What does the presence of protein in urine mean?
   In healthy individuals, urine generally does not contain protein. Proteins cannot pass through the glomerular membrane. Even if a very small amount of protein enters the glomerular filtrate/preliminary urine, it will be reabsorbed by the renal tubules. Therefore, the presence of protein in urine usually indicates that the kidney's ultrafiltration or reabsorption function may be abnormal.

4. What are some limitations of using urine tests for diagnosing health problems?

   Urine tests provide information about kidney function and metabolic status but can be influenced by many factors like diet or medications. They might not detect all health issues and sometimes need confirmation by blood tests or imaging.

Conclusion

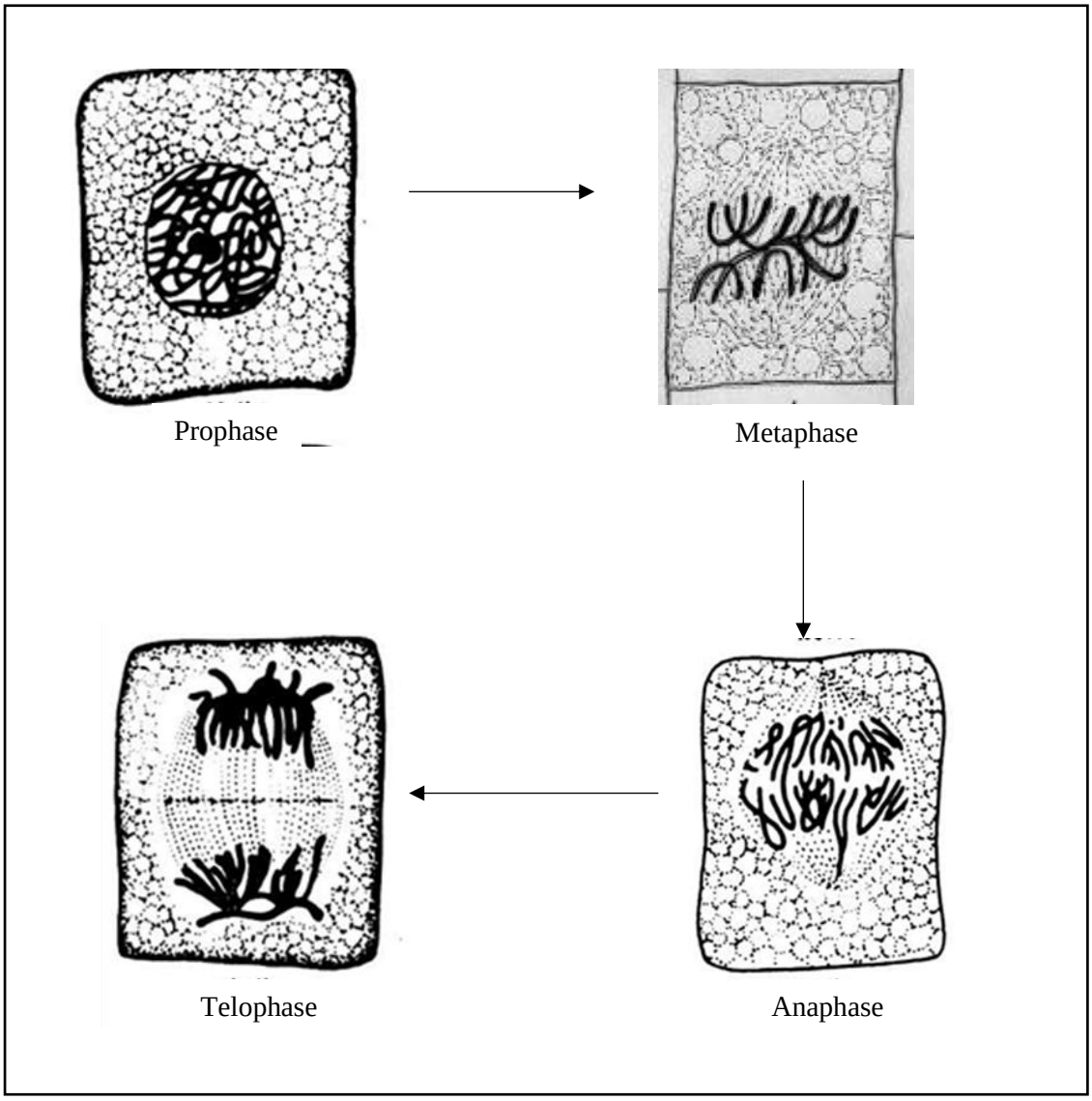
Healthy urine is acidic and does not contain protein and glucose.

To observe mitosis in root apical meristem cells

***Teacher should grow the onion root tip in advance**

1. Fill a conical flask with water
2. Place an onion on top of the conical flask, make sure the base of the onion touches the water
3. New white roots will form in 3-4 days.

Data record and analysis

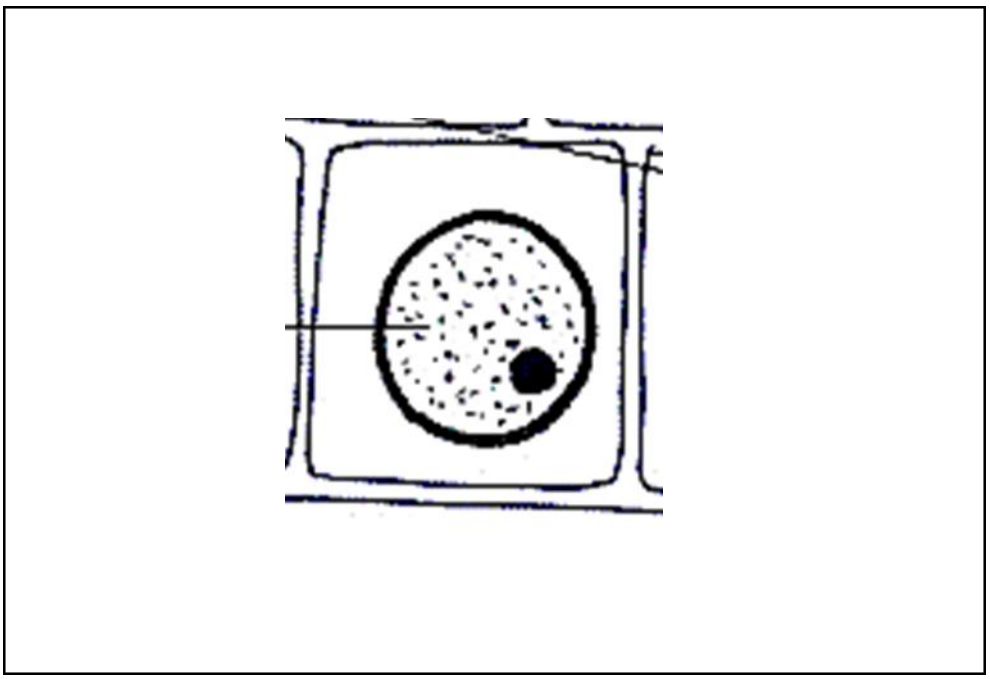


Discussion

1. In a field of view, cells in which phases of mitosis is the most abundant？Explain your answer.

   Interphase because prophase is the longest phase in mitosis, cells in prophase are the most abundant.

2. Besides mitosis phase, you can also observe interphase under high-power objective lens. Draw the interphase that you observed



Conclusion

Mitosis occurs in root apical meristem cells. The stages in mitosis can be observed under microscope.

To investigate the effect of alcohol temperature on DNA extraction

Hypothesis

The higher the temperature of the alcohol, the lesser the amount of DNA extracted from the banana.

Variables

Manipulated variable: Alcohol temperature

Responding variable: Amount of DNA extracted

Constant variable: Volume and concentration of alcohol

Result

*Based on the teacher's answer. DNA has very low solubility in alcohol at 0°C, which promotes precipitation of DNA into white strands. Higher alcohol temperatures increase DNA solubility, making it difficult to precipitate into white strands. There should be almost no noticeable precipitation in the 50°C group.

Discussion

1. What is the function of detergent in this experiment?

   Dishwashing liquid contains surfactants that can dissolve the lipids in the cell membrane and nuclear membrane, thereby breaking down the cell structure and releasing the DNA.

2. Why is the alcohol added slowly along the side of the test tube?

   This allows the alcohol and the water layer to form clear layers, and the DNA will precipitate at the interface between the two layers. If the alcohol is poured in directly, the layers will mix, and the DNA will not precipitate effectively.

3. Predict the result if strawberry or kiwi are used instead of banana.

   Strawberry and kiwi cells have larger nuclei and higher DNA content, so they usually yield more DNA with a more visible precipitate. Strawberries also contain a polyploid genome, which makes their DNA content even greater.

Conclusion

The DNA layer is the thickest and the amount of visible DNA extracted is the highest when the alcohol is at 0°C. The lower the temperature of the alcohol, the higher the amount of DNA extracted from the banana.

To investigate and analyse the effect of pH on the growth of bacteria

Prepare the nutrient agar solution

The teacher or lab assistant should prepare the nutrient agar solution in advance.

- Weigh the nutrient agar powder according to the instructions (e.g., 28 g/L distilled water). Each group will need approximately 100 mL of nutrient agar solution.
- Weigh out nutrient agar powder according to the instructions (adjust based on the amount needed). Pour 1 L (adjust based on the amount needed) of water into the flask and add the powder while stirring to mix thoroughly (a slightly cloudy solution is normal).
- Gently heat the solution, stir until the agar powder is completely dissolved and slightly translucent.
- Seal the flask with aluminium foil or tightly cap the bottle.
- If available, sterilize the agar solution in an autoclave or pressure cooker at 121°C for 15–20 minutes. (If sterilization equipment is unavailable, boil for a few minutes instead, but this sterilization is less effective.)

Hypothesis

E. coli grow best at a near-neutral pH (around 7).

Variables

Manipulated variable: The pH of the agar medium

Responding variable: Growth of *E. coli*

Constant variable: The type and concentration of the agar medium, incubation time

Discussion

1. What is the optimum pH for *E. coli*? Does the result support the hypothesis?

   The optimum pH for *E. coli* is 7 because it grows best at pH 7. The result support the hypothesis.
   *If the students' answer is not pH 7, they need to explain possible reason for their result.

2. Some *E. coli* strains can cause diarrhea and stomach pain. The most common way to get infected by *E. coli* is through ingesting contaminated food. Based on your understanding of the optimum pH for *E. coli* growth, explain how to use this knowledge to help prevent *E. coli* infection?

   E. coli's growth is inhibited in acidic or alkaline environments. Foods preserved with acidity, such as kimchi, yogurt, and pickled foods, can effectively inhibit or kill *E. coli*. Furthermore, using alkaline cleaners like bleach to clean kitchen surfaces, cutting boards, fruits and vegetables can disrupt the environment in which *E. coli* thrives.

3. Is your agar plate contaminated? How can you prevent contamination when doing bacteria culture?
   - Bacterial cultures can be performed in a laminar flow cabinet. If this is not possible, work near the flame of a bunsen burner (to create an updraft) to reduce the chance of airborne microorganisms being dropped into the culture dish.
   - Wipe the workbench with 75% alcohol before working.
   - All culture media, solutions, and equipment must be sterilized. Autoclave machine sterilization (121°C for 15–20 minutes) is a common method. Small tools (such as inoculating loops) can be heated red-hot with the flame of a bunsen burner before use.
   - Keep the petri dish open for as short a time as possible during work, opening it only slightly to prevent air from entering.

Conclusion

E. coli grow best at a near-neutral pH (around 7).

To investigate the effect of antibiotics on the growth of bacteria

Prepare the nutrient agar plates

The teacher or lab assistant should prepare the nutrient agar plates in advance.

- Weigh the nutrient agar powder according to the instructions (e.g., 28 g/L distilled water). Each group will need 3 nutrient agar plates and each agar plate need approximately 10 mL of nutrient agar powder solution.
- Weigh out nutrient agar powder according to the instructions (adjust based on the amount needed). Pour 1 L (adjust based on the amount needed) of water into the flask and add the powder while stirring to mix thoroughly (a slightly cloudy solution is normal).
- Gently heat the solution, stir until the agar powder is completely dissolved and slightly translucent.
- If available, sterilize the agar solution in an autoclave or pressure cooker at 121°C for 15–20 minutes. (If sterilization equipment is unavailable, boil for a few minutes instead, but this sterilization is less effective.)
- When the nutrient agar solution has cooled to approximately 50–55°C, pour it into the Petri dish until it just covers the bottom. Be sure to perform this step near a Bunsen burner to ensure sterile conditions.
- Leave the lid slightly ajar until the nutrient agar in the petri dish has completely solidified.

Result

* According to the teacher's answer, but according to the principle, *Staphylococcus aureus* will have the largest inhibition zone diameter, while *Escherichia coli* will have the smallest.

Discussion

1. Which bacterial species showed the largest and smallest zones of inhibition? What does this indicate about their sensitivity or resistance to penicillin?

   Staphylococcus aureus had a large zone of inhibition, indicating that it was sensitive to penicillin. In contrast, *Escherichia coli* showed a much smaller or no inhibition zone, suggesting greater resistance.

2. Penicillin inhibit the growth of gram-positive bacteria more effectively than gram-negative bacteria. Based on your result, classify *Escherichia coli* and *Staphylococcus aureus* into gram-positive and gram-negative bacteria.

   Staphylococcus aureus is gram-positive bacteria while *Escherichia coli* is gram-negative bacteria.

Conclusion

Penicillin will inhibit the growth of *Staphylococcus aureus* more effectively than *Escherichia coli*.

Name:_____ Date of completion: _____

Class: _____ Teacher: _____

Experiment Title

A concise, descriptive and informative phrase used to clearly state the subject of an experiment.

Problem Statement

A clear and concise description of a problem or challenge that must be resolved to achieve the intended outcomes

Hypothesis

A testable prediction about an observed phenomenon, which serves as a preliminary answer to an experimental question and can be supported or refuted through experimentation.

Objectives

1. A clear and concise statement describing the intended goal of an experiment, designed to guide the experimental process and maintain project focus.

Variables

Manipulated variable: Factor that is deliberately altered or controlled in an experiment to observe its effects on other variables.

Responding variable: Factor that is measured and observed in an experiment to examine whether it is influenced by the manipulated variable(s).

Controlled variable: Factor that remains constant and unchanged throughout the experiment.

Materials and Apparatus

Materials/Apparatus	Concentrations/Specifications	Volumes/Quantities

Precautionary Steps (If there is)

1. Safety measures and error control strategies planned before and during the experiment, aimed at preventing harm to students, equipment or the environment while ensuring accurate and reliable results.

Procedures (Can be presented as a schematic diagram)

1. A detailed, step-by-step description of the procedure used to test a hypothesis. It typically includes the definition of variables, a list of materials, a sequential outline of operational steps and specifies data collection and analysis methods to ensure the experiment's reproducibility and reliability.
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Results

X	Y

Discussion

1. Why is a Discussion section necessary in a lab report?

   The Discussion section of a lab report requires an analysis of the experimental results: interpreting their meaning, comparing them with the initial hypothesis and existing knowledge, identifying sources of error and experimental limitations and proposing directions for future research.

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Conclusion

A concise summary of experimental outcomes, evaluation of hypothesis testing, analysis of potential error sources and limitations, along with the broader implications revealed by the study.