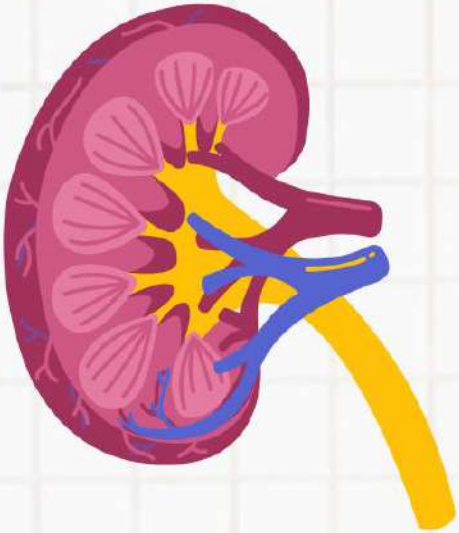
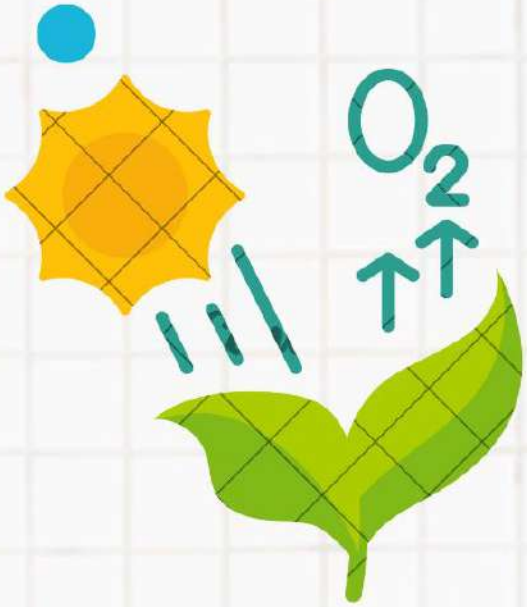
Pusat Asasi Sains dan Perubatan UniSZA
UniSZA Science and Medicine Foundation Centre

LABORATORY MANUAL
BIOLOGY II

SEM II 2023/2024

1. Identifying Bacteria Using Gram Stain
2. Plant Diversity - Bryophyta & Pteridophyta Animal Diversity - Invertebrates
3. Cellular Respiration by Yeast
4. Photosynthesis
5. Kidney and Urine





UniSZA SCIENCE AND MEDICINE FOUNDATION CENTRE ,
UNIVERSITI SULTAN ZAINAL ABIDIN,
GONG BADAK, TERENGGANU

PAA 20104: BIOLOGY II

Format of Laboratory Report

1.	**Title**	:	State the title of the experiment.
2.	**Objective**	:	State the objective of the experiment.
3.	**Apparatus**	:	State the instruments used.
4.	**Theory**	:	Explain briefly the theory.
5.	**Procedures**	:	Describe the steps and methods to perform the experiment.
6.	**Data/Results**	:	Present the data/results appropriately.
7.	**Discussion**	:	Explain your results and discuss.
8.	**Conclusions**	:	Indicate what is measured, the uncertainties and the sources of uncertainties
9.	**Reference**	:	List the references either from books and journals.

PUSAT ASASI SAINS DAN PERUBATAN UNISZA,
UNIVERSITI SULTAN ZAINAL ABIDIN,
GONG BADAK, TERENGGANU

LABORATORY REPORT

PAA 20104: BIOLOGY II

TITLE OF EXPERIMENT:

Name	**:**	
Student ID	**:**	
Lecture's Group	**:**	
Date of Experiment	**:**	
Date of Submission	**:**	
Lecturer's name	**:**	
Group Members	**:**	**1)** **2)** **3)** **4)** **5)** **6)** **7)**

LIST OF PRACTICALS (BIOLOGY SEMESTER 2)

NO.	TITLE	PAGE
1.	Identifying Bacteria Using Gram Stain	1 – 3
2.	(i) Plant Diversity I - Bryophyta & Pteridophyta (ii) Animal Diversity – Invertebrates	4 – 11
3.	Cellular Respiration by Yeast	12 – 14
4.	Photosynthesis (Chromatography)	15 – 17
5.	Kidney and Urine : Urinalysis	18 – 23

PRACTICAL 1 : IDENTIFYING BACTERIA USING GRAM STAIN

Objectives

1. To introduce the Gram staining technique to the students.
2. To identify Gram positive and Gram negative bacteria.
3. To identify different shape/ morphology of the bacteria.

Learning Outcomes

At the end of this practical the students are able to:

1. Apply the basic principle of a Gram staining technique.
2. Identify Gram positive and Gram negative bacteria.
3. Distinguish the type of shapes/ morphology of bacteria.

Hypothesis

Gram staining is a method used to stain bacteria for identification.

Introduction

Gram stain is a widely used method of staining bacteria as an aid to their identification. It was originally devised by Hans Christian Joachim Gram, a Danish doctor. Gram stain differentiates two major cell wall types. Bacterial species with walls containing small amount of peptidoglycan and characteristically, lipopolysaccharide, are Gram negative whereas bacteria with walls containing relatively large amount of peptidoglycan and no lipopolysaccharide are Gram positive. Apart from Gram staining technique, the identification of bacteria can also be based on shapes. The three most common shapes are spheres, rods and spirals.

Apparatus

Microscopes, Oil immersion lenses, slides and cover slips, wash bottle.

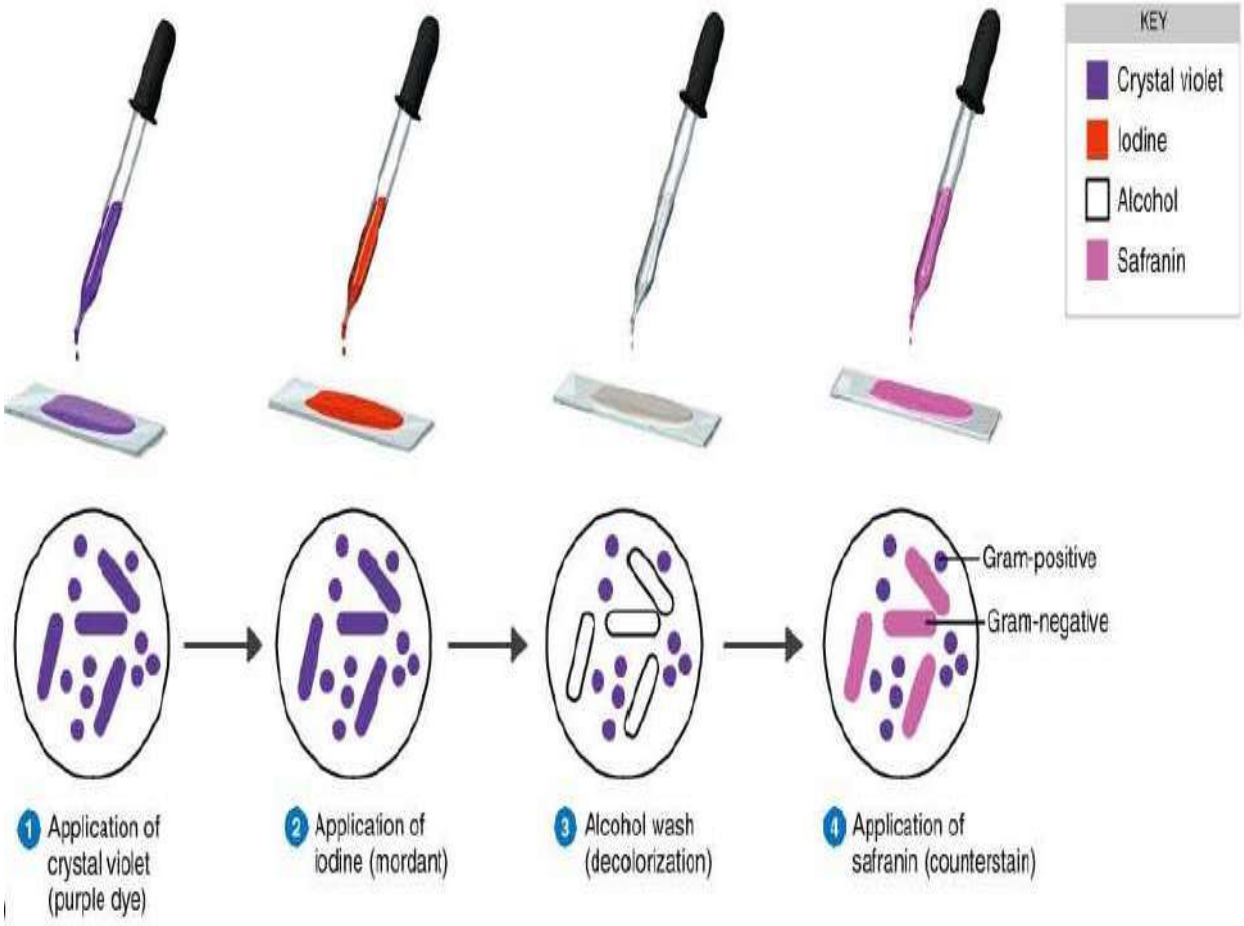
Materials

Bunsen burner, bacterial loops, cultures of bacteria (*Escherichia coli* and *Staphylococcus aureus*), immersion oil, petri dish, safranin, crystal violet, 95% ethanol, iodine, prepared slide of different types of bacteria, forceps.

Procedures and Observation

1. Observe the colour, surface and margin of the colonies in the bacterial cultures.
2. Place a drop of sterile distilled water/ saline on a clean slide and put a small amount of bacterial colony.
3. Gently heat the slide to fix the bacteria onto the slide.
4. Place the slide on the staining rack. Flood the smear with single drop of crystal violet and wait for I minute.
5. Gently, rinse the slide with slow running water.
6. Flood smear with 2 drops of iodine. Rotate and tilt the slides to allow the iodine to drain. Then cover again with iodine for 1 minute. Since the iodine does not mix well with water, this procedure ensures that the iodine will be in contact with the cell walls of the bacteria on the slide.
7. Rinse the slide with water as in step 6.

8. Place several drops of 95% alcohol (decolouriser) evenly over the smears, rotate and tilt the slide. Continue to add alcohol until most of the excess stain is removed and the alcohol running from the slide appear clear. If the smears are too thick or if the alcohol is kept on the slide for too long or too short of time, the results will not be accurate. Although there is no recommended time for this step, it usually takes 5 – 10 seconds to decolourise if exposed to a sufficient amount of decolouriser.
9. Add safranin and leave it for approximately 45 seconds. Colourless Gram negative cell will readily accept the light red safranin stain, while the already dark coloured Gram positive cell will undergo no change at all.
10. Rinse off with water and blot dry with filter paper.
11. Observe the slide under 40X magnification then, 100X magnifications and describe your observation in terms of types of bacteria, shape, colour and whether it is Gram positive or Gram negative.



Results

Table : Observation results on the type of bacteria, shape, colour and Gram positive or Gram negative.

Bacteria	Shape	Colour	Gram +ve/-ve

Questions

1. Why is it necessary to excessively rinse the smear with water?
2. Why are Gram positive bacteria purple in colour, while Gram negative are red?
3. How does thickness of peptidoglycan affect the outcome of Gram stain experiment?

PRACTICAL 2 :

(i) PLANT DIVERSITY – BRYOPHYTA & PTERIDOPHYTA

Objectives

1. To investigate the diversity of species in Bryophyta & Pteridophyta.
2. To examine the unique characteristics of different Bryophyta & Pteridophyta classes.

Learning Outcomes

At the end of this practical the students are able to:

1. Identify the plants species in Bryophytes and Pteridophyta.
2. Describe the characteristics of different species of plant in Bryophyta and Pteridophyta.
3. Draw the important structures of plants in both phylum species.

Hypothesis

Liverworts and mosses (non-vascular tissues plant) are grouped in the phyla Bryophyta and ferns (non-flowering seedless plants possessing vascular tissues) are grouped in the phyla Pteridophyta.

Introduction

There are three main classes of Bryophyta, namely Musci (Mosses), Hepaticae (Liverworts), and Anthocerotae (Hornworts).

Liverworts and mosses are grouped under in the phyla Bryophytes, which is the most primitive among the terrestrial plants. They are non-vascular and are confined to moist areas because they lack well-developed tissues for transportating water and nutrients. Bryophytes are green in colour and have a root-like structure, which is called rhizoid and stem and leaf-like structure called the thallus. Bryopytes is characterized by the clear alternation of generation in its life cycle where the gametophytes generation is dominant. The male reproductive organ is called antheridium and produces flagellate sperms (antherozoids). The sperm fertilizes the egg (oosphere), which is produced by the archegonium that is the female reproductive organ.

After fertilization, the zygote develops in the archegonium to produce sporophyte, which grows out from the gametophyte. The sporophyte produces haploid spores, which will eventually give rise to mature gametophytes.

Ferns (Pteridophyta) are the only non-flowering seedless plants possessing vascular tissues –xylem and phloem. This enables pteridophytes to achieve larger size than the bryophytes. In the tropics, ferns may grow up to 18 m (60ft). A major difference between pteridophytes and bryophytes is that the diploid sporophyte generation is dominant in pteridophytes. The gametophyte generation retains two traits that are reminiscent of the bryophyte. Firstly, the small gametophytes lack conducting vessels. Secondly, as in bryophytes, the ciliated sperms (antherozoids) require water medium to reach the egg (oosphere), so pteridophytes still depend on the presence of water for sexual reproduction. Ferns have true stems with vascular tissues, and also true roots and leaves.

Apparatus
Compound Microscope, Dissecting Microscope, Dissecting set.

Materials
Fresh specimens:
Polytrichum sp. (Phylum Bryophyta)
Marchantia sp. (Phylum Hepatophyta)
Lycopodium sp. (Phylum Lycopodiophyta)
Selaginella sp. (Phylum Lycopodiophyta)
Dryopteris sp. (Phylum Pteridophyta)

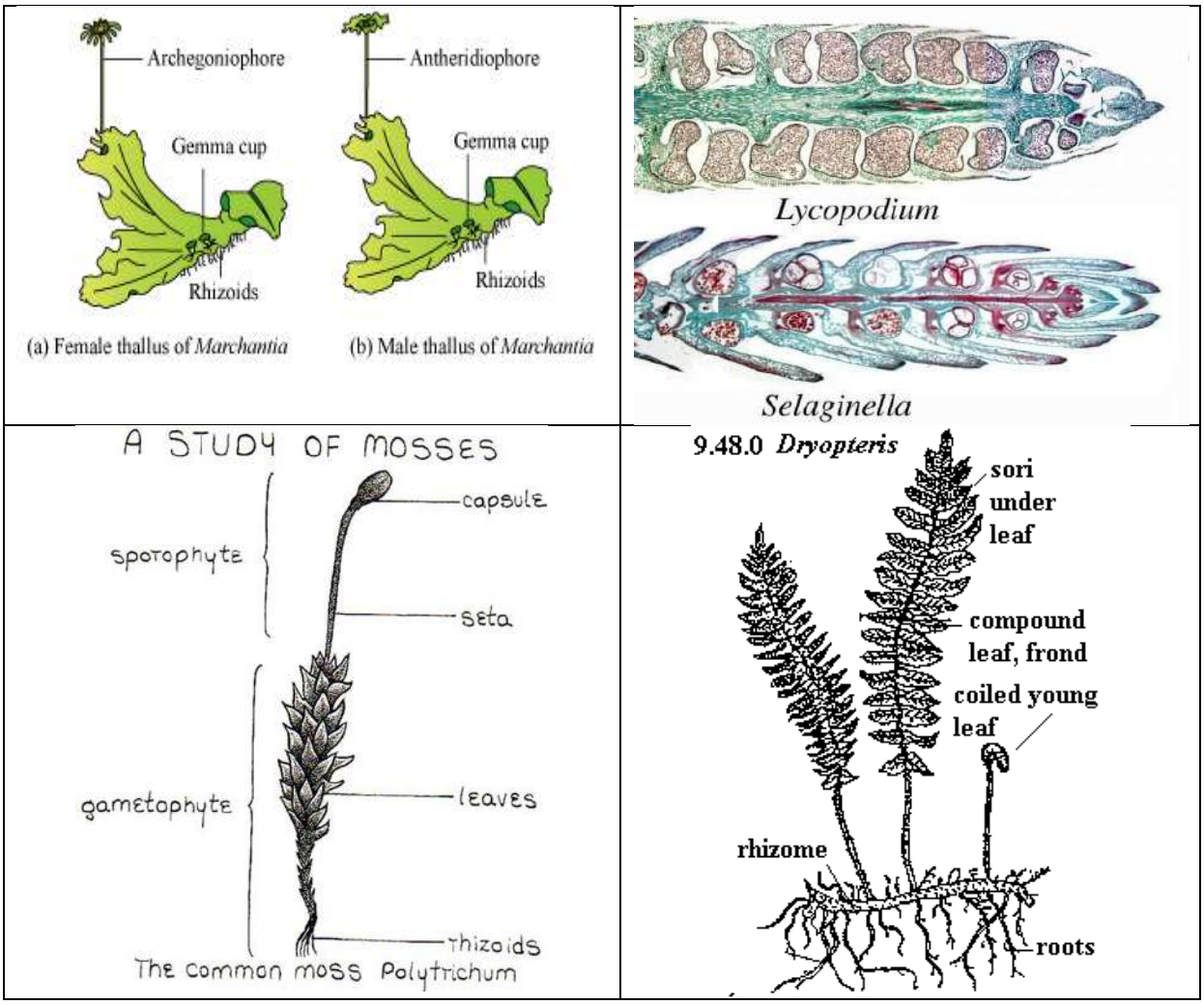
Prepared slides:
Marchantia sp. – capsule (LS)
Marchantia sp. – male gametophyte (LS)
Marchantia sp. - female gametophyte (LS)
Lycopodium sp. – strobilus (LS)
Selaginella sp. – strobilus (LS)
Dryopteris sp. - sorus

Procedures and Observation
Merchantia sp.
1. Examine *Merchantia sp.* under the dissecting microscope. Observe the "y" shaped growth that is the dichotomous branching of the thallus. Observe the gemma cups and the gemmae on the dorsal surface and the rhizoids on the ventral surface of the thallus.
2. Draw and label the thallus, antheridiophore, archegoniophore, rhizoid and gemma cup.
3. Examine the slide, which shows the longitudinal section of *Merchantia sp.* capsule, antheridium and archegonium.

Polytrichum sp.
4. Observe *Polytrichum sp.* under the dissecting microscope.
5. Draw and label the diagram of thallus, rhizoid, male gametophyte and female gametopyte. Identify the sporophyte.
6. Draw and label the diagram of the male gametophyte and female gametopyte.
7. Examine the longitudinal section slide of *Polytrichum sp.*
8. Draw and label the operculum, spore, peristome, annulus, calyptra, seta and capsule.

Lycopodium sp. and *Selaginella sp.*
9. Examine the specimens of *Lycopodium sp.* and *Selaginella sp.* Observe the dichotomous branching, types and arrangement of sporophyll and strobilus.
10. Examine the slides showing longitudinal sections of strobilus of *Lycopodium sp.* and *Selaginella sp.*
11. Draw and label sporophyll, sporangium and spore.

Dryopteris sp.
12. Observe the frond morphology *of Dryopteris sp.* Their fronds are subdivided into pinnae and pinnules. The young fronds are circinnate.
13. Draw and label the arrangement and shape of the sori on the undersurface of the spore producing pinnules.



Questions

1. What is the function of elater found in the capsule *Merchantia sp*.?
2. Explain the importance of gemma cups to *Merchantia sp*.?
3. How is the gametophyte of *Merchantia sp*. differs from the gametophyte of *Polytrichum sp.?*
4. What is the process involved in spore formation?
5. What is the ecological role of liverworts and mosses to the terrestrial environment?
6. Compare the spores and leaves of Lycopodium sp. and Selaginella sp.
7. Fern sporophytes have an underground stem called rhizomes. How do you distinguish that rhizomes are stems and not roots?

(ii) ANIMAL DIVERSITY – INVERTEBRATES

Objectives

1. To investigate the diversity of invertebrates.
2. To examine the unique characteristics of the given phyla in the invertebrates.

Learning Outcomes

At the end of this practical the students are able to:

1. Classify the diversity of invertebrates.
2. Describe the characteristics of different species in the phylum of invertebrates.
3. Identify the different species of invertebrates.

Hypothesis

Invertebrates are classified as multicellular animals without vertebral column.

Introduction

This practical explores the diversity of invertebrate animals (e.g., sponges, jellyfish, flatworms, molluscs, annelids, nematodes, arthropods, and echinoderms), focusing on the special attributes and biological requirements of different groups, how they function in their natural environments, and what makes each group vulnerable to human-based exploitation. Labs emphasize recognition of major groups, and use living organisms where possible, but involve no invasive procedures.

About 98% of animal species found in the world belonged to invertebrates. They are multicellular animals without vertebral column. Many invertebrates such as jellyfish or worm have a fluid-filled hydrostatic skeleton that function as a support mechanism. Other invertebrates such as insects and crustaceans possess hard outer shell for protection.

Materials

Fresh specimens:

i)	Phylum Porifera	- sponge (*Leucosolenia* sp.)
ii)	Phylum Coelenterata/Cnidaria	- hydra (*Obelia* sp.)
iii)	Phylum Platyhelminthes	- planarian (*Dugesia* sp.)
iv)	Phylum Nematoda	- roundworm (*Ascaris lumbricoides*)
v)	Phylum Annelida	- earthworm (*Pheretima* sp.)
vi)	Phylum Mollusca	- garden snail (*Achatina fulica*)
vii)	Phylum Echinodermata	- star fish (*Asterias* sp)
viii)	Phylum Arthropoda	(a) Class Crustacea –crab
		(b) Class Chilopoda – centipede
		(c) Class Arachnida – spider
		(d) Class Merostomata – horseshoe crab
		(e) Class Insecta – grasshopper
		(f) Class Diplopoda - millipede

Procedures and Observation

1. Observe the morphological characteristics of the given specimens.
2. Draw and label the unique characteristics of your specimens.

(b) Phylum Porifera

Example: sponge (*Leucosolenia* sp.)

Characteristics:

- No definite symmetry.
    - Body multicellular, few tissues, no organs.
    - Cells and tissues surround a water filled space but there is no true body cavity.
    - All are sessile, (live attached to something as an adult).
    - Reproduce sexually or asexually, sexual reproduction can be either gonochoristic or hermaphroditic.
    - Has no nervous system.
    - Has a distinct larval stage which is planktonic.
    - Lives in aquatic environments, mostly marine.
    - All are filter feeders.
    - Often have a skeleton of spicules.

(c) Phylum Coelentrata

Example: hydra (*Obelia* sp.)

Characteristics:

- Lower stage multicellular animal
- Radial symmetry
- Diploblastic (2 germ layers)
- Nematocyst
- Nervous system consist of network of nerve cells, tentacles around the manubrium (mouth), alternation of generation between polyp and medusa stage.

(d) Phylum Platyhelminthes

Example: planarian (*Dugesia* sp.)

Characteristics:

- Bilaterally symmetrical.
- Body having 3 layers of tissues with organs and organelles.
- Body contains no internal cavity.
- Possesses a blind gut (i.e. it has a mouth but no anus)
- Has Protonephridial excretory organs instead of an anus.
- Has normally a nervous system of longitudinal fibres rather than a net.
- Generally dorsoventrally flattened.
- Reproduction mostly sexual as hermaphrodites.
- Mostly they feed on animals and other smaller life forms.
- Some species occur in all major habitats, including many as parasites of other animals.

(e) Phylum Nematoda

Example: roundworm (*Ascaris lumbricoides*)

Characteristics:

- Bilaterally symmetrical, and vermiform.
- Body has more than two cell layers, tissues and organs.

- Body cavity is a pseudocoel, body fluid under high pressure.
- Body possesses a through gut with a subterminal anus.
- Body covered in a complex cuticle.
- Has a nervous system with pharyngeal nerve ring.
- Has no circulatory system (no blood system)
- Reproduction normally sexual and gonochoristic.
- Feed on just about everything.
- Live just about everywhere, many species are endoparasites.

(f) Phylum Annnelida
Example: earthworm (*Pheretima* sp.)
Characteristics:
- Bilaterally symmetrical and vermiform.
- Body has more than two cell layers, tissues and organs.
- Body cavity is a true coelom, often divided by internal septa.
- Body possesses a through gut with mouth and anus.
- Body possesses 3 separate sections, a prosomium, a trunk and a pygidium.
- Has a nervous system with an anterior nerve ring, ganglia and a ventral nerve chord.
- Has a true closed circulatory system.
- Has no true respiratory organs.
- Reproduction normally sexual and gonochoristic or hermaphoditic.
- Feed a wide range of material.
- Live in most environments

(g) Phylum Mollusca
Example: garden snail (*Achatina fulica*)
Characteristics:
- Bilaterally symmetrical.
- Body has more than two cell layers, tissues and organs.
- Body without cavity.
- Body possesses a through gut with mouth and anus.
- Body monomeric and highly variable in form, may possess a dorsal or lateral shells of protein and calcareous spicules.
- Has a nervous system with a circum-oesophagal ring, ganglia and paired nerve chords.
- Has an open circulatory system with a heart and an aorta.
- Has gaseous exchange organs called ctenidial gills.
- Has a pair of kidneys.
- Reproduction normally sexual and gonochoristic.
- Feed a wide range of material.
- Live in most environments

(h) Phylum Echinodermata
Example: star fish (*Asterias* sp)
Characteristics:
- Possess 5-rayed symmetry, mostly radial, sometimes bilateral.
- Body has more than two cell layers, tissues and organs.

- Body cavity a true coelom.
- Most possesses a through gut with an anus.
- Body shape highly variable, but with no head.
- Nervous system includes a circum-oesophageal ring.
- Has a poorly defined open circulatory system.
- Possesses a water vascular system, which hydraulically operates the tube feet or feeding tentacles.
- Without excretory organs.
- Normally possesses a subepidermal system of calcareous plates
- Reproduction normally sexual and gonochoristic.
- Feeds on fine particles in the water, detritus or other animals.
- All live marine environments

(i) Phylum Arthropoda
Characteristics:
- Bilaterally symmetrical (in most cases).
- Body has more than two cell layers, tissues and organs.
- Body cavity a true coelom.
- Most possesses a through straight gut with an anus (in most cases).
- Body possesses 3 to 400+ pairs of jointed legs.
- Body possesses an external skeleton (in most cases).
- Body is divided in 2 or 3 sections.
- Nervous system includes a brain and ganglia.
- Possesses a respiratory system in the form of tracheae and spiracles (in most cases).
- Possesses a open or lacunnar circulatory system with a simple heart, one or more arteries, and no veins, (in most cases).
- Reproduction normally sexual and gonochoristic, but can be parthenogenetic.
- Feed on everything.
- Live everywhere.

Questions

1. (a) What is the advantage of the folded body walls in sponges?
   (b) What is the function of spicule other than to support?
   (c) Which characteristic that make spongia important/essential for domestic use?
2. Phylum Coelenterata shows polymorphism. Name the 2 types of polymorphism.
3. List some adaptations possessed by Platyhelminthes as endoparasites.
4. (a) What is the meaning of metameric segmentation?
   (b) Stage the advantages of metameric segmentation in Annelids.
   (C) What is the function of clitellum?
5. Give the function of radula.
6. Compare the members of the classes under phylum Arthropoda based on the following characteristics:
   (a) Parts of the body
   (b) Number of appendages
   (c) Number of antennae
   (d) Type of respiratory organ
7. What is the function of the vascular water system in star fish?

PRACTICAL 3 : CELLULAR RESPIRATION BY YEAST

Objectives

1. To understand the conditions that influence growth and cellular respiration of yeast (*Saccharomyces cerevisiae*).
2. To investigate the effect of sucrose concentration on the rate of cellular respiration in yeast.

Learning Outcomes

At the end of this practical the students are able to:

1. Describe the cellular respiration of yeast (*Saccharomyces cerevisiae*).
2. Describe the effect of sucrose concentration on the rate of cellular respiration in yeast.
3. Apply the knowledge on cellular respiration by yeast.

Hypothesis

If yeast is added to water that has a higher concentration of sucrose then the rate of cellular respiration in the yeast will rise because glycolysis will have more sugar to turn to ATP and glycolysis will react faster because it would be in a more habitable environment.

Introduction

All living cells, including the cells in your body and the cells in yeast, need energy for cellular processes such as pumping molecules into or out of the cell or synthesizing needed molecules. ATP is a special molecule which provides energy in a form that cells can use for cellular processes. Cellular respiration is the process that cells use to transfer energy from the organic molecules in food to ATP. The following equation summarizes the chemical changes that occur in cellular respiration of the monosaccharide glucose when oxygen is available.

$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O + ATP$

Apparatus

Erlenmeyer flasks (500 ml), Balloons, 30 cm thread, ruler

Materials

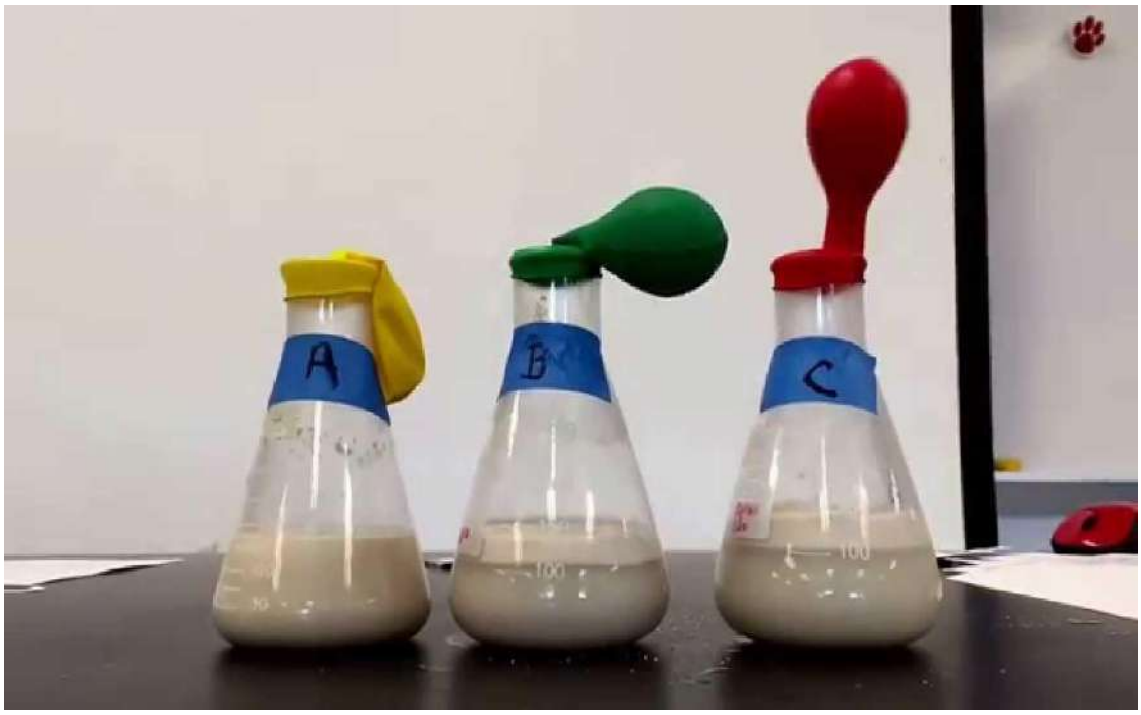
Yeast, sucrose solution 1.0 M, plain water

Procedure

1. Prepare 100 ml of sucrose solution with different molarities using the dilution method. The molarities required are (0M, 0.05M and 0.2M). The volume of the sucrose solution (1.0M) and plain water must be recorded into the table below. Label each of the Erlenmeyer flask.

Molarity	0M	0.05M	0.2M
Volume of 1.0 M sucrose (ml)	0	5	20
Volume of plain water (ml)	100	95	80
Final volume of sucrose solution (ml)	100	100	100

2. Add 4 g of yeast to each Erlenmeyer flask.
3. Put a balloon on the top of each Erlenmeyer flask and seal it.
4. Shake each Erlenmeyer flask until the yeast is dissolved.
5. Measure the circumference of balloon by using a piece of thread to the length of 30 cm by using a ruler.
6. Record the circumference of the balloon in intervals of 10 minutes for the duration of about 40 minutes. Record the measurements in the table.
7. Plot the graph regarding to the circumference of the balloon against the time.



Results

Concentration of sucrose solution (M)	Time (minutes)	Circumference of balloon (cm)	Description of balloon
0	**0 (start)**		
	10		
	20		
	30		
	40 (end)		
0.05	**0 (start)**		
	10		
	20		
	30		
	40 (end)		
0.2	**0 (start)**		
	10		
	20		
	30		
	40 (end)		

Questions:

1. Is yeast a living cell? How do you know?
2. Which organelle does cellular respiration occur in?
3. What is the purpose of the balloon in this experiment?
4. Did your experimental results as expected? Explain.
5. How to increase the rate of cellular respiration? Explain.

PRACTICAL 4 : PHOTOSYNTHESIS (CHROMATOGRAPHY)

Objective

1. To identify the pigment that presence in the leaves.

Learning Outcome

At the end of this practical the students are able to:

1. Describe the process of photosynthesis.
2. Point out the factors that influence photosynthesis process.
3. Apply the knowledge in the theory through the practical process.

Hypothesis

All of the plant tissues tested will contain photosynthetic pigments.

Identification of Pigment Presence in Leaves

Introduction

Photosynthesis is the process of converting light energy to chemical energy and storing it in the bonds of sugar. This process occurs in plants, algae, certain other protists and some prokaryotes. Plants are autotrophic, which means they can make their own food (sugar). The capability of making its own food (sugar) is mainly influenced by the present of chlorophyll (a pigment molecule), light energy, carbon dioxide (CO_2) and water (H_2O). There are 2 stages of photosynthesis, light reactions (light dependent reaction) and Calvin cycle (light independent reaction) which give rise to the overall chemical reaction:

$$6\ CO_2 + 6\ H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6\ O_2$$

The above chemical reaction formed simple sugar and oxygen. This is the source of the oxygen we breathe, and thus, a significant factor in the concerns about deforestation.

Apparatus

250 ml beaker, 4 chromatography paper / filter paper, coin, ruler, pencil, straw/ bamboo stick

Materials

Leaves –spinach, hibiscus leaf, purple cabbage and lettuce, solvent – isoprophyl alcohol 70%

Procedures

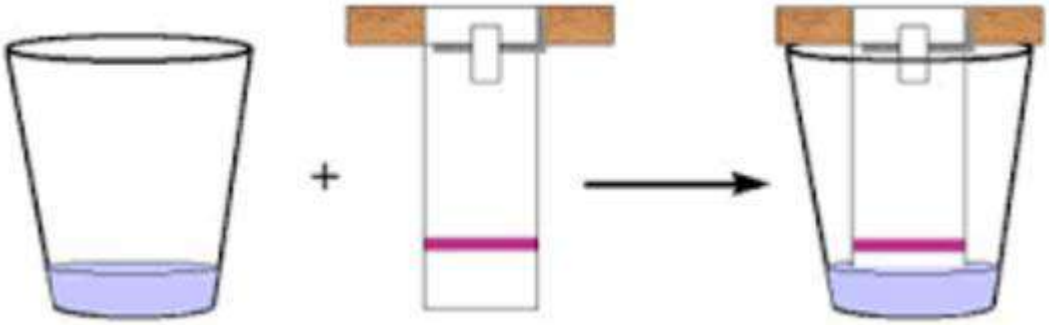
1. With a pencil, draw a line on the filter paper, 2 cm from one end.
2. By using the coin, rub the spinach leaf onto the line that you made in step 1 by placing the leaf on top of the filter paper. Keep rubbing until a significant amount of pigment has been transferred on the filter paper. Push down hard enough to leave a green smear on the paper on top of the original pencil mark. Repeat steps 1 and 2 for hibiscus leaf, purple cabbage and lettuce.
3. Fill beaker to a depth of 1 cm with isopropyl alcohol 70%.

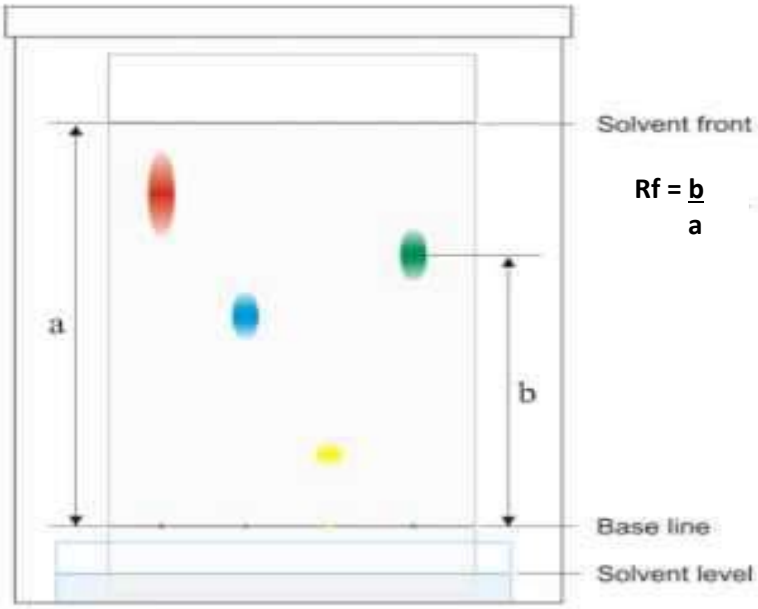
4. Lay the stick across the mouth of the beaker like a bridge from one edge to the other. Tape the 4 filter paper strips to the stick so that the paper is held in place just touching the alcohol.
5. Gently and carefully lower the filter paper strips, green smear end in first, into the beaker until the bottom just touches the alcohol. Make sure that the green smear does not touch the alcohol, only the tip of the paper should be in contact with the alcohol,
6. At this time, the alcohol gradually moves up the paper, bringing many of the pigments along with it. The alcohol is highly flammable and emits potentially harmful fumes.
7. Observe the solvent as it is drawn upward toward the leaves extract. Wait 15 to 20 minutes for the chromatograms to develop.
8. Remove the stick and paper when the alcohol has almost reached the stick. Mark how far the alcohol traveled with a pencil (should be colourless but wet). This is called the solvent front.
9. Let the filter paper dry for 1 minute before measuring. Mark the highest point of each pigment (colour) by using a pencil. Measure the distance (cm) between the line (that you draw earlier on the filter paper) and the highest point of each pigment. Record the data.
10. Measure the distance between the pigment initial locations (the line that you drew earlier on the filter paper) and the solvent front (the highest point the solvent traveled) in cm. Record the data.
11. Calculate the Rf value for each pigment for each leaf using the formula given.
    Rf : a value of calculation which is use in determination of the relative distance moved by a particular kind of molecule via chromatography. If the molecules move half as far as the solvent traveled, the Rf = 0.5. If the molecules moved ¼ the distance, the Rf = 0.25. Therefore, the maximum value for Rf is 1.0.

    Pigment Identification
    Chlorophyll a – blue green
    Chlorophyll b – olive green
    Xanthophyll – yellow
    Carotene – orange yellow

    Formula to calculate Rf

$$Rf = \frac{\text{Distanced moved by pigment from original spot}}{\text{Distanced moved by solvent from original spot}}$$





Questions

1. Which layer of the leaf contains the majority of chloroplasts?
2. Which of these molecules is produced during the light dependent stage of photosynthesis?
3. In the overall reaction of photosynthesis, which starting molecules will get oxidized?
4. What are the wavelength colours will be absorbed by the pigment of Chlorophyll a?

PRACTICAL 5 : KIDNEY AND URINE (URINALYSIS)

Objectives

1. To understand the anatomy of a kidney and its function.
2. To examine the urinalysis technique.

Learning Outcome

At the end of this practical the students are able to:

1. Illustrate the importance of urinary system.
2. Identify the main structures of a kidney and their functions.

Hypothesis

Experiment A:

The kidney is the site of urine manufacture, the waste products eliminated from the bloodstream by the filtration processes that occur within these organs. The ureter, bladder and urethra are structures for collecting urine and transporting it from the body.

Experiment B:

A urinalysis is a test of urine. A urinalysis is used to detect and manage a wide range of disorders, such as urinary tract infections, kidney disease and diabetes. A urinalysis involves checking the appearance, concentration and content of urine.

Introduction

The major function of the urinary system is to remove waste products from our bodies and help regulate the volume and composition of blood by the production and excretion of urine. The urinary system consists of two kidneys, two ureters, one bladder, and one urethra.

The composition of urine can vary greatly and constantly fluctuates with dietary intake (food and water) and metabolic activity. Urine consists mostly of water with various organic and inorganic substances such as urea, uric acid, creatine, sodium chloride, ammonia, sulfates, and phosphates as its principal ingredients. A clinical examination of urine can provide a convenient, cost effective and non-invasive means of assessing kidney function and providing an overall assessment of our body's health.

Typically, a complete urinalysis involves an examination of the physical characteristics of urine, a chemical analysis and a microscopic examination of urine sediment. Urine should be collected in a clean container, stored in a cool place, and tested as soon as possible. A proper care during the collection of urine is vital to prevent contamination by commensal flora especially in female and children. There are many types of urine specimens such as mid-stream urine (MSU), catheterized urine, bag urine and suprapubic aspirate urine, received in the laboratory. Most preferable technique is mid-stream urine (MSU) which discarding the first part of the stream before collection in an appropriate sterile container.

In this practical, several physical analysis (color, odor, transparency, pH, specific gravity and urinary solids), chemical analysis (protein content and glucose content) and

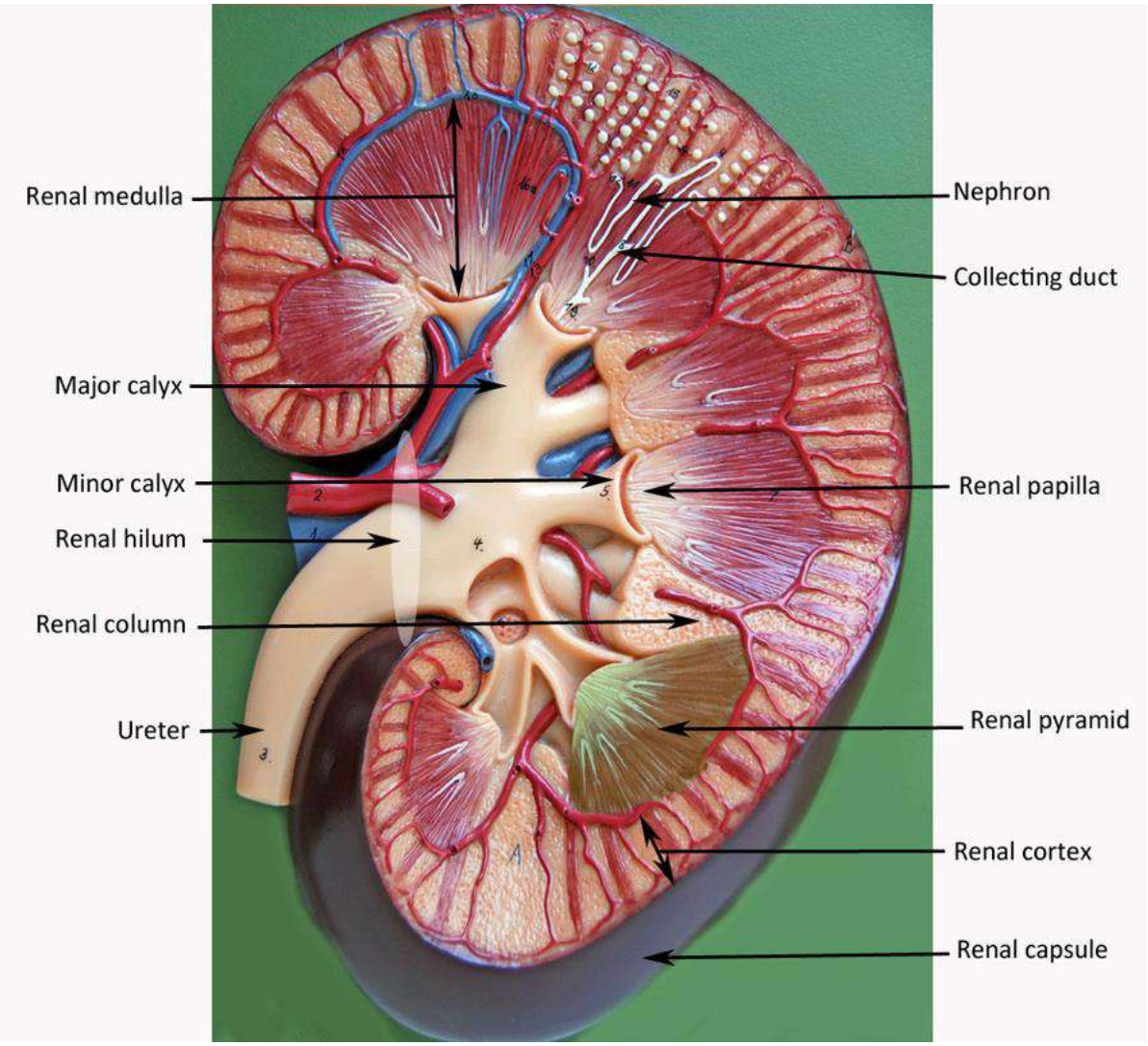
microscopic examination to identify suspended insoluble materials that are often present in the urine.

A. Anatomy of a kidney

Apparatus
Kidney's model

Procedure

1. Study the model of a kidney provided.
2. Draw the basic anatomy of the human kidney and urinary system.
3. In your diagram, label the following anatomical feature: abdominal aorta, inferior vena cava, renal artery and vein, renal pelvis, ureter, ureteral orifice, urinary bladder, ejaculation orifice, urethra, renal capsule, cortex, medulla, renal pyramids, renal columns, major and minor calyces and renal papilla.



B. Urinalysis

(a) Urine collection

Materials
Urine container, wooden swab stick

Procedures

1. A specimen of urine may be collected at any time; urine voided within 3 hours after meal, however, may contain abnormal constituents. For this reason the first voiding in the morning is preferred.
2. Urine collection should be done using a mid-stream technique in a sterile container. A mid-stream sample is essential to avoid contamination from the xternal genitalia, and to avoid the presence of pus cells and bacteria that are normally found in the urethra.

(b) Physical Characteristics of Urine

The physical characteristics of urine include observations and measurements of color, turbidity, odor, specific gravity, pH and volume. Visual observation of a urine sample can give important clues as to evidence of pathology.

i) Colour
The color of normal urine is usually light yellow to amber. Generally the greater the solute volume, the deeper the color. The yellow color of urine is due to the presence of a yellow pigment, urochrome. Deviations from normal color can be caused by certain drugs and various vegetables such as carrots, beets, and rhubarb.

ii) Odour
Slightly aromatic: characteristic of freshly voided urine. Urine becomes more ammonia-like upon standing due to bacterial activity.

iii) Turbidity
Normal urine is transparent or clear; becomes cloudy upon standing. Cloudy urine may be evidence of phosphates, urates, mucus, bacteria, epithelial cells, or leukocytes.

iv) pH
pH ranges are from 4.5 - 8.0. Average is 6.0, slightly acidic. High protein diets increase acidity. Vegetarian diets increase alkalinity. Bacterial infections also increase alkalinity.

v) Specific Gravity
The specific gravity of urine is a measurement of the density of urine - the relative proportions of dissolved solids in relationship to the total volume of the specimen. It reflects how concentrated or dilute a sample may be. Water has a specific gravity of 1.000. Urine will always have a value greater than 1.000 depending upon the amount of dissolved substances (salts, minerals, etc.) that may be present. Very dilute urine has a low specific gravity value and very concentrated urine has a high value. Specific gravity

measures the ability of the kidneys to concentrate or dilute urine depending on fluctuating conditions. Normal range 1.005 - 1.035, average range 1.010 - 1.025.

Low specific gravity is associated with conditions like diabetes insipidus, excessive water intake, diuretic use or chronic renal failure. High specific gravity levels are associated with diabetes mellitus, adrenal abnormalities or excessive water loss due to vomiting, diarrhea or kidney inflammation. A specific gravity that never varies is indicative of severe renal failure. Specific gravity can be determined by either of two methods using a refractometer or a urinometer.

Materials

Urine container, wooden swab stick, urine sample, pH paper, thermometer and distilled water.

Procedures

a) Colour, odour and transparency
   1. Examine the urine sample for color and odor. Record the observation.
   2. To determine the transparency, cover the container and shake your urine sample and observe.
   3. Observe the degree of cloudiness. Record the observation.

b) Hydrogen ion concentration (pH)
   1. Dip a strip of pH paper into urine sample 3 constitutive times.
   2. Shake off any excess urine.
   3. Let the pH paper sit for 1 minute and read the result.

(c) Chemical test :

Benedict test - Glucose content

Benedict's solution is commonly used to detect reducing sugars in urine and it is not specific for glucose only.

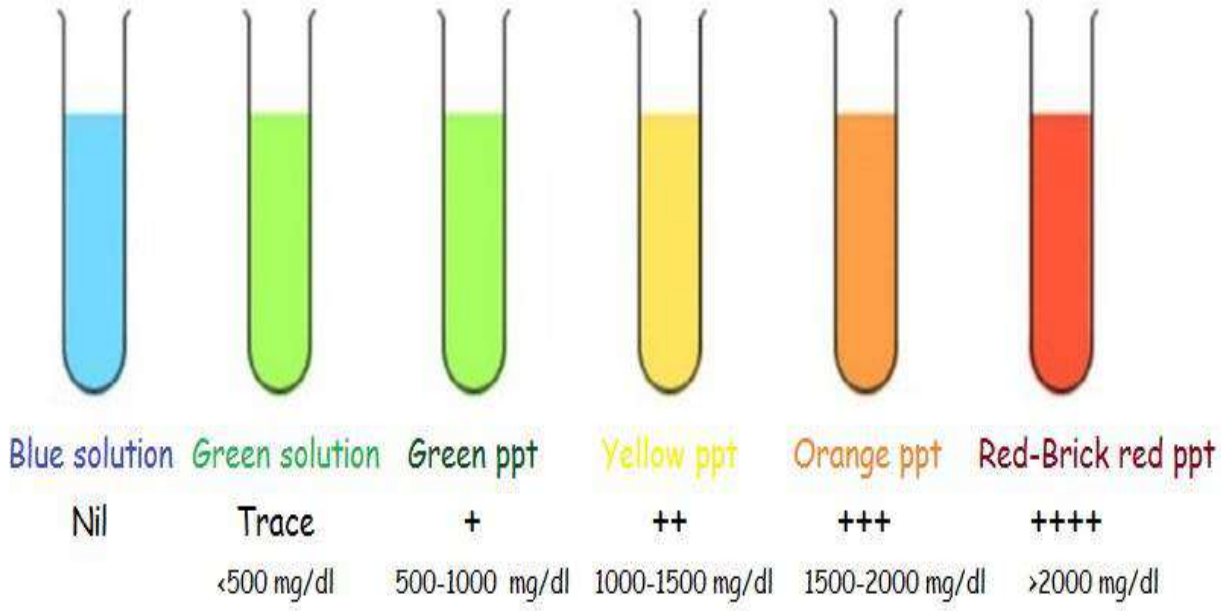
Materials

Benedict's solutions, pipette, urine sample, test tubes, test tubes holder, water bath, 2% acetic acid

Procedures

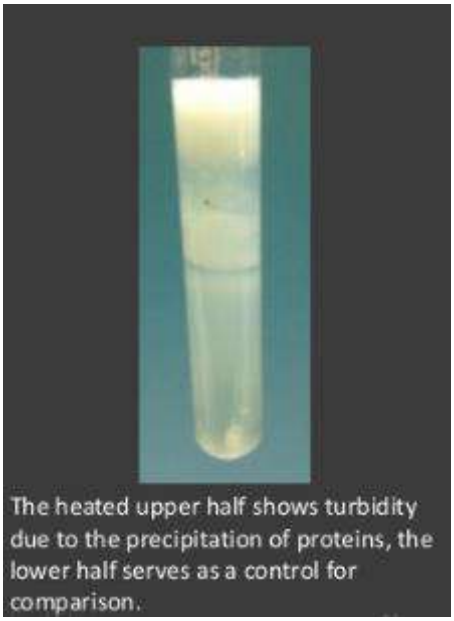
1. In a test tube, combine 10 drops of urine with 5ml of Benedict's solution. Mixed the solution.
2. Using a test tube holder, place the tube in a boiling water bath for 5 minutes. Make sure that the mouth of the test tube is pointed away from you.
3. Using a test tube holder, remove the test tube from the water bath.
4. After the solution has cooled, look for a colored precipitate that would indicate the presence of sugar. Record the color changes.
5. Record whether the test that is performed indicates the presence or absence of reducing sugar.

Color	Results
Blue	Negative
Greenish yellow	1 + (0.5 g/100 ml)
Olive green	2 + (1 g/100 ml)
Orange-yellow	3 + (1.5 g/100 ml)
Brick red (with precipitate)	4 + (>2 g/100 ml)



Protein content (Heat Coagulation Test)

1. Prepare 2 test tubes and label 1 test tube as “unheated” and the other test tube as “heated”.
2. Fill both test tubes with 5 ml of urine.
3. Place the test tube labeled “heated” in a boiling water bath.
4. Remove the test tube from the water bath and compare the clarity of the solutions in test tube labeled “heated” to the test tube label “unheated”. The formation of white precipitate is due to protein or to insoluble phosphates.
5. Add 5 drops of 2% acetic acid. Phosphate dissolved in the acidified solution, while the heat-coagulable proteins remain as a precipitate. Do not add excess acid, as the protein may also dissolve.
6. Record your result.



The heated upper half shows turbidity due to the precipitation of proteins, the lower half serves as a control for comparison.

Questions

1. Explain how the consumption of antacid (sodium bicarbonate) affect urine pH?
2. Explain why the protein is not normally found in urine?
3. Write the expected conditions that show glucose (glycosuria) in their urine specimens.