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Faculty of Science

Laboratory Manual

Organic Mechanisms in Biology

Bachelor of Biotechnology (Hons.)

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Organic Mechanism in Biology

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

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

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

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

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

INTRODUCTION

1. The Scientific Method

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

2. Practical Exercises

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

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

3. Following Instructions

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

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

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

4. Handling apparatus

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

5. Having Due Regard for Safety

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

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

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

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

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

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

6. Making Accurate Observations

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

7. Recording Results in an Appropriate Form

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

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

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

8. Presenting Quantitative Results

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

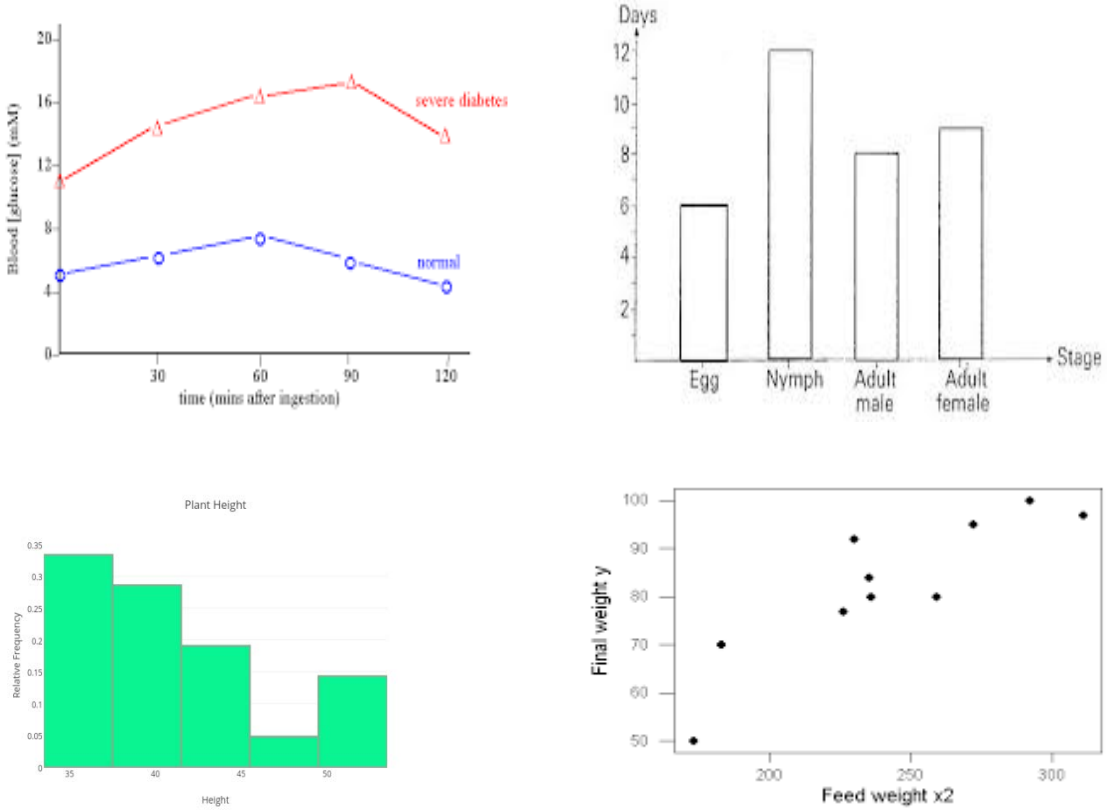

Figure 1: Line graphs, bar charts, histograms and scatter graphs

9. Drawing Conclusions

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

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

10. Writing a Scientific Lab Report

Title

- Communicate the subject investigated in the paper.

Introduction

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

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

Materials and Methods

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

Results

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

Discussion

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

Conclusion

- Restate your conclusion.
- Restate important results.

Literature Cited

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

Acknowledgement

- State any appropriate acknowledgement that you think is necessary.

Practical 1
Title: Optimal enzyme activity

Objectives:
After completing the practical, you will be able:
1. To determine the effect of different factors (such as temperature and pH on enzyme activity)
2. To differentiate the common precipitation and colour-changing in enzyme experiment

Introduction

The use of enzymes in the diagnosis of disease is one of the important benefits derived from the intensive research in biochemistry since the 1940's. Enzymes have provided the basis for the field of clinical chemistry.

It is, however, only within the recent past few decades that interest in diagnostic enzymology has multiplied. Many methods currently on record in the literature are not in wide use, and there are still large areas of medical research in which the diagnostic potential of enzyme reactions has not been explored at all.

Enzymes are affected by changes in pH. The most favorable pH value - the point where the enzyme is most active - is known as the optimum pH. This is graphically illustrated in below (Figure 1).

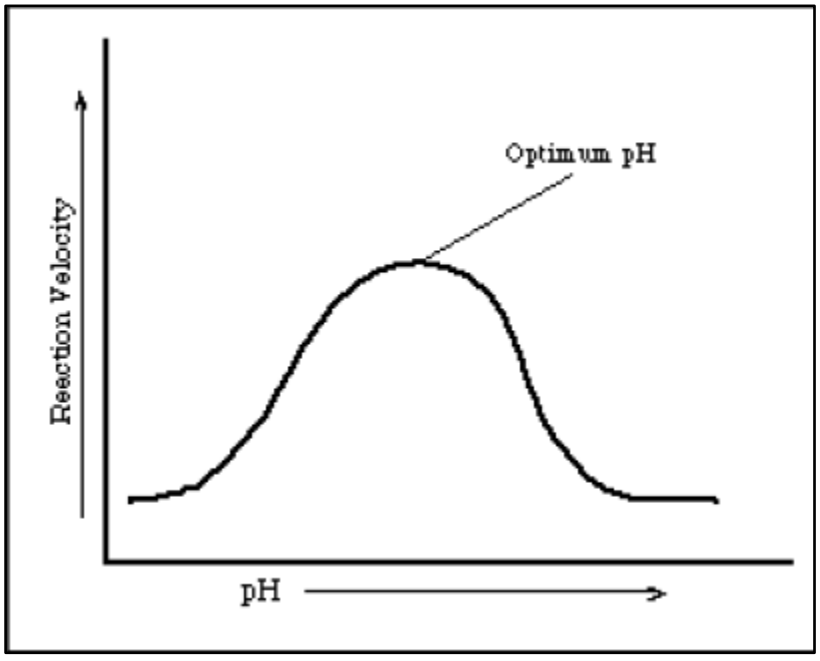

Figure 1: Effect of pH on enzyme activity

Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes. As with activity, for each enzyme there is also a region of pH optimal stability.

Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised. A ten degree Centigrade rise in temperature will increase the activity of most enzymes by 50 to 100%. Variations in reaction temperature as small as 1 or 2 degrees may introduce changes of 10 to

20% in the results. In the case of enzymatic reactions, this is complicated by the fact that many enzymes are adversely affected by high temperatures. As shown in Figure 2, the reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature. Because most animal enzymes rapidly become denatured at temperatures above 40°C, most enzyme determinations are carried out somewhat below that temperature.

Over a period of time, enzymes will be deactivated at even moderate temperatures. Storage of enzymes at 5°C or below is generally the most suitable. Some enzymes lose their activity when frozen.

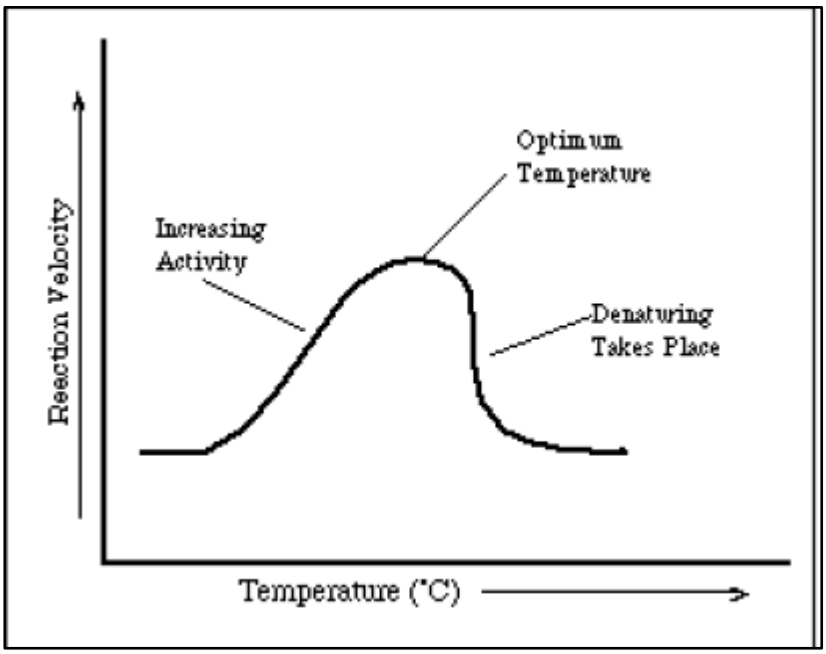

Figure 2: Effect of temperature on enzyme activity

Materials

- Test tubes
- Beaker
- Ice
- Water bath (37 and 70°C)
- Bunsen burner

Reagents

- Distilled water
- 0.5% sodium chloride (NaCl)
- 2% Starch
- Benedict reagent
- Alkaline solution
- Acidic solution
- Neutral solution (normal saline)

Preparing enzyme solution

I. In a beaker, collect about 1 ml of your own 'saliva'
II. Add 9 ml of distilled water and 60 ml of 0.5% sodium chloride solution

III. Gently mix.
IV. Use this 'enzyme solution' to carry out the following four experiments:

(a) Effect of temperature on the activity of salivary amylase:

Principle:

Salivary amylase is protein in nature; the optimum temperature for its activity is 37°C. In extreme temperatures (cold and heat), the activity of the enzyme is inhibited (high temperature causes denaturation of the protein structure of the enzyme).

Procedure

1. Get 3 test tubes and number them '1', '2' & '3'.
2. Put 2 ml of the previously prepared enzyme solution in each of the three test tubes.
3. To each test tube, add 2 ml of 2% starch solution and mix well.
4. Immediately put:

- Test tube '1' on ice
- Test tube '2' in a 37°C water bath
- Test tube '3' in a 70°C water bath each for 20 minutes

5. Remove each tube to room temperature
6. Carry out Benedict's test to check for the presence of the starch hydrolysis product 'maltose' as follows:

- Add 2 ml of Benedict's reagent to the reagent to the enzyme-starch mixture in each tube
- Put in a boiling water bath for 5 minutes

7. After 5 mins, place them in a test tube rack.
8. Wait for few minutes or until the colour change is complete
9. Observe the colour changes. Record all results in the data table.

(b) Effect of pH on the activity of salivary amylase:

Principle:

The optimum pH for the activity of salivary amylase is the neutral pH (about 7). In the acidic pH of the stomach, the activity of salivary amylase stops. Pancreatic amylase, on the hand, has an optimum activity in alkaline medium.

Procedure:

1. Get 3 test tubes and number them '1', '2' & '3'.
2. Put 2ml of the previously prepared enzyme solution in each of the three test tubes.
3. To each test tube, add 2 ml of 2 % starch solution and mix well.
4. For:

Test tube '1', add 2 ml of acidic solution
Test tube '2' add 2 ml of neutral solution
Test tube '3' add 2 ml alkaline solution
*Put the test tubes in a 37°C water bath for 10 minutes

5. Remove each tube to room temperature

6. Carry out Benedict's test to check for the presence of the starch hydrolysis product 'maltose' as follows:

- Add 2 ml of Benedict's reagent to the content of each tube
- Put in a boiling water bath for 5 minutes

7. After 5 minutes, place them in a test tube rack
8. Wait for few minutes or until the colour change is complete
9. Observe the colour changes. Record all results in the data table.

(a) Effect of temperatures on amylase activity

Temperature	Amylase activity (colour changes)
0	
37°C	
70°C	

(b) Effect of pH on amylase activity

pH	Amylase activity (colour changes)
Acidic	
Neutral	
Alkaline	

Practical 2

Title: Blood glucose test by using glucometer

Objective:

After completing the practical, you will be able:

1. To determine the glucose level in blood using glucometer

Introduction

Glucose determination was done to detect level of glucose in patient. It is important to see whether the level of glucose can cause consequences to the patient or not. It is either hyperglycaemia or hypoglycaemia. When hyperglycaemia occurs it is condition occurs it is condition which the level of blood glucose is higher than normal range, while hypoglycaemia is low blood glucose. Both conditions can give consequences to the patient.

Blood sugar monitoring devices called glucometer provide with instant feedback and let me know immediately what the blood sugar is. This can give valuable information about whether the blood sugar is too low, too high or in a good range.

Materials

- Alcohol prep pad
- Lancet
- Test strip
- Glucometer

Methods

1. First, set out the glucometer, a test strip, a lancet and alcohol prep pad.
2. Wash your hands to prevent infection.
3. Decide where you are going to obtain the blood from, usually a finger. Some of the newer monitors let you use your forearm or another less sensitive place.
4. Sometimes it helps to warm your hands to make the blood flow easier. You can rub your hands together briskly or run them under warm water.
5. Turn o glucometer and place a test strip in the machine when the machine is ready. Watch the indicator for placing the blood to the strip.
6. Make sure your hand is dry and wipe the area you've selected with an alcohol prep pad and wait until the alcohol evaporates.
7. Pierce your finger tip on the soft, fleshy pad and obtain a drop of blood. His type of drop of blood is determined by the type of strip you are using (some use a 'hanging drop' of blood versus a small drop for strips that draw blood in with a capillary action).
8. Place the drop of blood on or at the side of the strip.
9. The glucometer will take a few moments to calculate the blood sugar reading. Follow your doctor's orders for whatever blood sugar reading you get.
10. You may use the alcohol prep pad to blot the site where you drew the blood if is still bleeding.
11. Write down your result. Keeping a record makes it easier for you and your doctor to establish a good treatment plan. Some glucometers can store your results in a memory, for easier record keeping.

Expected result:

Diagnosis blood glucose concentration (mmol/L)		
Diagnosis	Sample	Plasma glucose concentration
Normal	Fasting	<6.1
Impaired fasting glycaemia	Fasting	>6.1 and <7.0
	2h post glucose	<7.8
Impaired glucose tolerance	Fasting	<7
	2h post glucose	>7.8 and <11.1
Diabetes mellitus	Fasting	>7.0
	2h post glucose	> 11.1

Practical 3
Title: Urine dipstick test

Objectives:

After completing the practical, you will be able:

1. To screen urine components using dipstick method which provides results for specific gravity, pH, protein, glucose, and blood in the urine
2. To obtain information of metabolic state, nutritional status, kidney function and acid-base balance

Introduction:

Urinalysis test strips are easy to use reagent strips for the detection of key diagnostic chemical markers in human urine. The reagent pads react with the sample urine to provide a standardized visible colour reaction within 30 seconds to one minute depending on the specific panel screen. The colour is then visually compared to the included colour chart to determine the level of each chemical factor. Test results may provide useful information regarding carbohydrate (sugar) metabolism (diabetes), kidney function, acid-base balance, bacteriuria, occult blood, high leukocytes (infection) and other conditions of overall health.

Materials:

1. Urine dipstick
2. Urine container
3. Gloves
4. Tissue paper or absorbent paper

Methods

1. Collect fresh urine specimen into a clean and dry container.
2. Remove one strip from the bottle and replace cap immediately. Do not touch the test pads of the strip.
3. Briefly (no longer than one second) immerse all strip/reagent areas into the urine.
4. Wipe off excess urine on the rim of the container or using the edge of absorbent paper.
5. Compare the reagent areas to the corresponding colour chart on the bottle label at the time specified. 'Hold strip in vertical position'.
6. Hold the strip close to the colour blocks and match carefully. Proper reading times are critical for optimal results.
7. Avoid laying the strip directly on the colour chart.
   From time dipped, after:

- 30 seconds: glucose
- 60 seconds: protein, nitrite
- 2 minutes: leukocytes

8. Record results in table.

Dipstick Test Result (Normal)

Dipstick Parameter	Observed result: Test pad colour or reading	Negative/Positive	Indication or Discussion

Dipstick Test Result (Abnormal)

Dipstick Parameter	Observed result: Test pad colour or reading	Negative/Positive	Indication or Discussion

Practical 4
Title: Determination of presence of sugar, ketones and protein in urine

Objective:
After completing the practical, you will be able:
1. To learn how to analyse glucose, ketone and protein of unknown concentration urine sample

Introduction:

Benedict's reagent is used as a test for the presence of reducing sugar. This includes all monosaccharides and the disaccharides, lactose and maltose. Even more generally, Benedict's test will detect the presence of aldehydes (expect aromatic ones), and alpha-hydroxy-ketones, including those that occur in certain ketones. The three main ketone bodies are acetone, acetoacetic acid (diacetic acid) and beta-hydroxybutyric acid.

To presence of chemical species within urine, with their respective concentrations, has routinely been used as a diagnostic tool for both illness and death. Nurses and doctors commonly request a urine or blood sample in an attempt to diagnose health condition, while toxicologist regularly analyze blood and urine to aid medical examiners in determination of cause of death.

High levels of proteins, glucose and ketones in urine usually occur within six potentially fatal medical conditions. Sometimes, elevated levels for pregnancy also shown. Protein will be high urine for certain cases such as heavy metal poisoning, kidney failure, diabetes-related and heart failure. While ketones concentration will be elevated in dehydration cases, starvation and diabetes related. Glucose level in urine will be increase in kidney failure, diabetes-related and pregnancy related.

Materials
- Bunsen burner
- Test tube
- Test tube racks
- Graduated cylinder
- Urine glucose sample
- Urine protein sample
- Urine ketone sample

Reagent

- Benedict solution
- Sodium nitropusside
- Concentrated ammonium sulfate

Procedure:

Part A

Obtain test tubes and a test tube rack. Using a graduated cylinder measure out 1 ml of water into a test tube. Mark the water level with a wax pencil or a marker. If all your test tubes are the same, used the marked tube as reference and mark all the others. If the test tubes are different you will need to calibrate each tube separately.

1. Obtain standard simulated urine samples of protein, glucose, and ketones. Label your samples if this has not already been done.

(a) Test for proteins

2. Dispense approximately 1-2 ml of standard simulated urine protein and 1-2 ml of blank simulated urine sample into two separates tubes.
3. Prepare and light your Bunsen burner. Hold the urine protein test tube over the flame of the Bunsen burner. Follow all precautions when heating a test tube in a open flame. Remove the test tube from the flame just as it begins to boil. The precipitate formed in this experiment appears as 'soapy' bubbles or froth on the inside of the test tube.
4. Record your observations.
5. Turn off your Bunsen burner. Dispose of your samples in an appropriate waste container and rinse your test tubes clean.

(b) Test for glucose (Benedict's Test)

6. Set up apparatus similar into part A, step 11; however, the water will need to begin boiling for this procedure.
7. Add 5-10 drops of standard simulated urine glucose sample and 5-10 drops of blank simulated urine into two separate tubes. Additionally, using graduated disposable pipette, add 2 ml of Benedict's solution to each test tube.
8. After the water prepared in step 6 has begun to boil, place the test tubes into the boiling water and allow it to heat for minutes. Remove from heat and allow it to cool.
9. Record your observation.
10. Turn off your Bunsen burner. Dispose of your samples in an appropriate waste container and rinse your test tubes clean.

(c) Test for the ketones (Rothera's Test)

11. Dispense approximately 1-2 ml of standard simulated urine ketone sample and 1-2 ml of blank simulated urine sample into two separates tubes. Additionally, add 1 ml of sodium nitropusside solution to each.
12. In a fume hood, slowly add approximately 1ml of concentrated ammonium hydroxide to each test tube, so as to form a separate layer on top of the solutions.
13. After 1-2 minutes, record your observation focusing on the interface between the two layers.
14. Dispose of your samples in an appropriate waste container and rinse your test tubes clean.

Expected result:

	Standard sample	Blank sample
Urine sample	Foamy formation	Clear
Urine glucose (BENEDICT'S TEST)	Red or green or yellow precipitate obtained	Clear
Urine ketones (ROTHERA'S TEST)	Purplish layer form	No colour form

Notes

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