H

RAFFLES INSTITUTION
2024 Year 6 Preliminary Examination

Higher 2

CANDIDATE NAME											
CIVICS GROUP	2	4	S	0	3		INDEX NUMBER						

BIOLOGY

9744/04

Paper 4 Practical

15th August 2024
2 hours 30 minutes

Candidates answer on the Question Paper.
Additional Materials: As listed in the Confidential Instructions.

READ THESE INSTRUCTIONS FIRST

Write your index number, CT group and name on all the work you hand in.
Give details of the practical shift and laboratory, where appropriate, in the boxes provided.
Write in dark blue or black pen.
You may use a 2B pencil for any diagrams or graphs.
Do not use staples, paper clips, highlighters, glue or correction fluid.

Answer **all** questions in the spaces provided on the Question Paper.

The use of an approved scientific calculator is expected, where appropriate.
You may lose marks if you do not show your working or if you do not use appropriate units.

At the end of the examination, hand in the Question Paper containing your answers.
The number of marks is given in brackets [ ] at the end of each question or part of the question.

Shift
Laboratory

For Examiner's Use	
1	
2	
3	
Total	/55

This document consists of **18** printed pages.

Raffles Institution
Internal Examination

Answer **all** questions.

1 You are advised to read the whole of the question before starting the practical work, as you will need to make decisions about how to obtain high quality results using the apparatus and materials provided.

You are going to investigate the clotting of milk.

Young mammals are fed on milk. To enable efficient digestion of milk it is clotted in the stomach using the protease enzyme, rennin. Rennin converts the soluble protein caseinogen in the milk to an insoluble form called casein.

The manufacture of cheese also requires the clotting of milk. This can be done in two ways by:

- acidifying the milk
- using the enzyme, rennin

You are provided with the materials shown in Table 1.1.

Table 1.1

labelled	contents	hazard
M	100% milk	none
E	1% rennin	harmful irritant
A	1 mol dm^{-3} hydrochloric acid	corrosive

If **E** or **A** comes into contact with your skin, wash off immediately under cold water.

It is recommended that you wear suitable eye protection.

In **Part 1** you will observe the clotting of milk using two methods:

Method 1 involves the use of **A** and **Method 2** involves the use of **E**.

In **Part 2** you will investigate the effect of various temperatures on the clotting activity of **E**.

Part 1 – observing clotting

Method 1 – using acid

1. Add 5 cm^3 of **M** to a test-tube.

2. Add 1 cm^3 of **A** into the same test-tube.

3. Put a bung into the test-tube and mix the contents by inverting the test-tube three times.

4. Tilt the tube and rotate it slowly such that the contents touch all of the internal glass surfaces as shown in Fig. 1.1. Do **not** shake the tube.

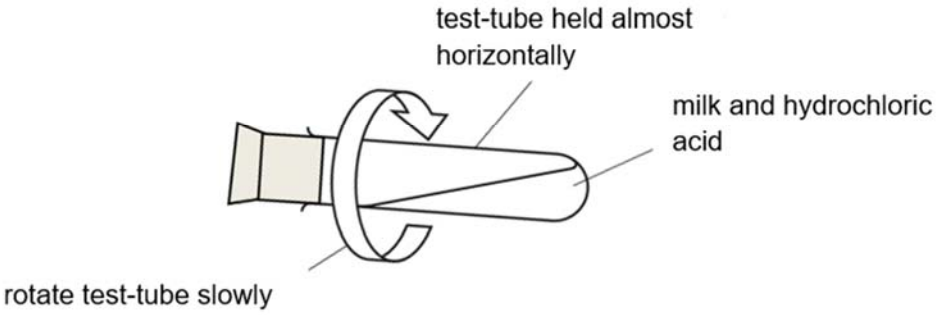

Fig. 1.1

(a) Record your observations.

……

……

……

……[2]

5. Keep the test-tube for later reference.

Method 2 – using rennin

1. Prepare and maintain a water-bath at 55 °C.

2. Put 5 cm^3 of **M** into a test-tube.

3. Put 1 cm^3 of **E** solution into a separate test-tube.

4. Place both test-tubes in the 55 °C water bath for 3 minutes. It is important to maintain the temperature of the water bath during the entire 3 minutes.

5. Add the 5 cm^3 of **M** into the test-tube containing **E**.

6. Put a bung into the test-tube and mix the contents by inverting the test-tube three times and immediately start the stopwatch.

7. Tilt the tube and rotate it slowly such that the contents touch all of the internal glass surfaces as shown in Fig. 1.1. Do **not** shake the tube.

8. Continue to rotate the tube and record the time taken for clotted milk particles to **first start to form** on the walls of the tube as shown in Fig. 1.2 .

(b) Time taken for the milk to clot. ……s [1]

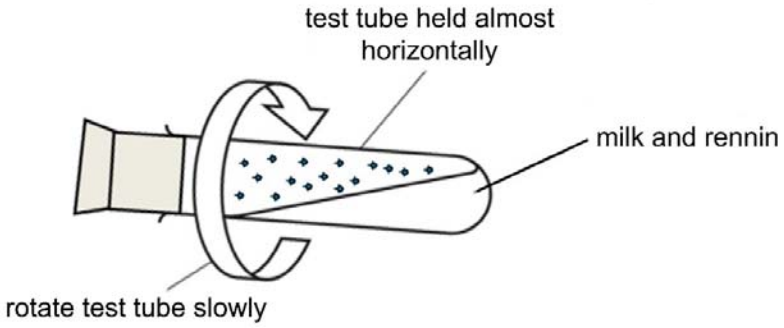

Fig. 1.2

9. Keep the test-tube for later reference.

(c) Suggest the advantage of adding the milk to the rennin solution rather than adding the rennin solution to the milk.

……

……[1]

(d) Use the universal indicator paper to test the pH of:

- milk
- milk with acid (use the test-tube from Method 1 step 5)
- milk with rennin solution (use the test-tube from Method 2 step 9)
- rennin solution

Record your results in the space below and explain the advantage of carrying out this test before investigating the effect of concentration of rennin on the clotting of milk.

pH of milk:……..……..

pH of milk with acid:……..……

pH of milk with rennin:……..

pH of rennin: …….

Explanation: ……..…

……..……[2]

(e) Caseinogen molecules are hydrophilic and therefore soluble in water. When formed, they are incorporated, along with certain other molecules and ions, into spherical structures called micelles. Each micelle has a hydrophobic core and a stabilising outer layer of hydrophilic caseinogen molecules.

During clotting the micelles are broken open and their contents clump together to form clots.

Suggest why the contents tend to clump together.

……..……

……..……[1]

(f) Suggest why the time taken for clotting was different in the two methods.

……..……

……..……

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……..……

……..……[3]

Part 2 – investigating the effect of temperature on the rate of clotting of milk

(g) Carry out the steps in **Method 2** for all 3 temperatures at 35^0C, 45^0C, and 55^0C.

Record your results for all 3 temperatures in a suitable table in the space below to show the effect of temperature on the rate of clotting of milk.

If the end-point has not been reached by 180 seconds, stop timing and record this as 'more than 180'.

[4]

(h) The descriptions of precautions that should have been taken when conducting the experiment are in the table below.

Complete table 1.2 by writing explanations for the descriptions of the precautions that should have been taken to ensure high quality results.

Table 1.2

	description of precaution	explanation
1	All test tubes and syringes were labelled clearly.	
2	The experiment was carried out on one tube at a time.	
3	Syringes of appropriate volume were used.	

[3]

(i) Complete Table 1.3 by describing an improvement to reduce the effect of each error that may have affected your results.

Table 1.3

sources of error	improvement
No replicates were done.	Three replicates could have been conducted to identify anomalies;
Judging the end point or when little specks of clotted milk particles to first start to form on the walls of the test-tube was difficult.	
Measurement of volumes could be inaccurate due to the volume of syringes used.	
It was difficult to maintain temperature of water bath during equilibration time.	

[3]

(j) A scientist carried out an investigation into the effect of enzyme concentration on the coagulation of milk. The scientist calculated the activity of the enzyme for each concentration of enzyme.

The results are shown in Table 1.4.

Table 1.4

enzyme concentration /%	activity of enzyme /arbitrary units (au)
0.05	19
0.10	34
0.15	50
0.20	65
0.30	96

(k) Plot a graph of the data in Table 1.4 on the grid in Fig. 1.3.

Use a sharp pencil.

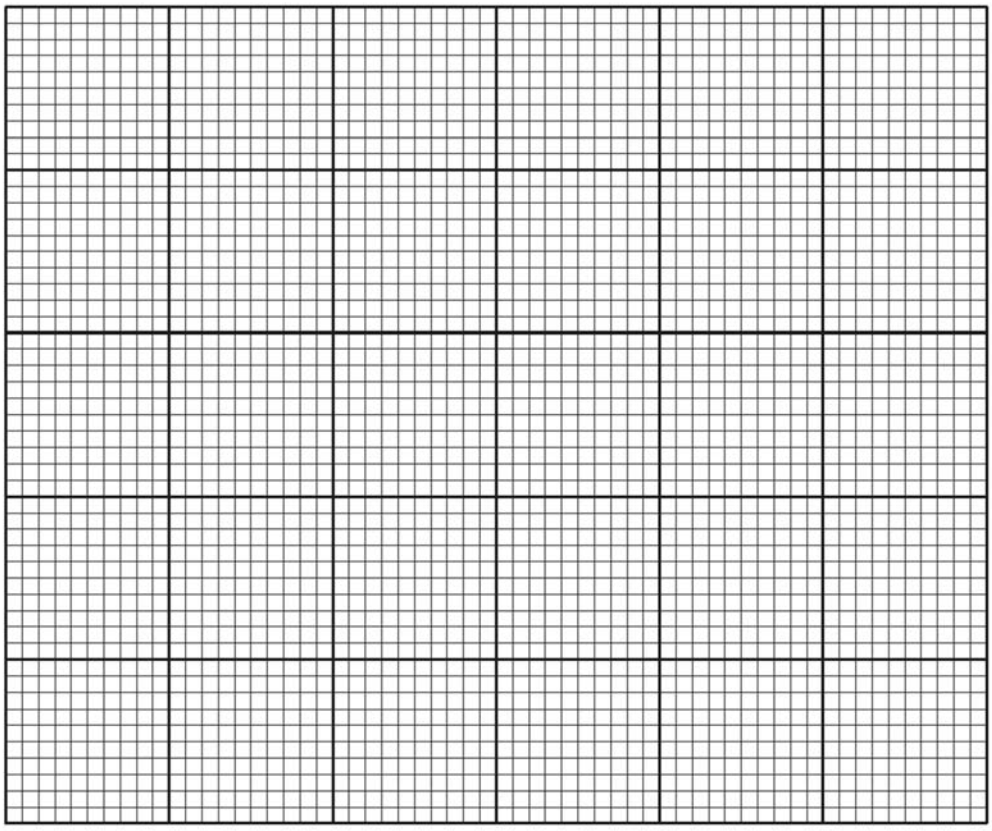


Fig. 1.3 [3]

(l) Use your graph to determine the activity of the enzyme when the enzyme concentration is 0.25%.

Show on your graph how you determined this value.

activity of enzyme : ……[1]

[Total: 24]

2 Yeast cells contain enzymes that catalyse respiratory reactions. Different sugars have different effects on the respiratory reactions in yeast.

Two students had a discussion on whether galactose or sucrose will produce a faster respiratory reaction with yeast.

Jason claimed that galactose would produce a faster reaction, while **Kate** claimed that it should be sucrose. The two students decided to investigate.

One way to investigate the progress of enzyme-catalysed respiratory reactions in yeast is to test for the release of carbon dioxide.

A sample of yeast cells, **Y**, can be added into a piece of Visking tubing, **V**, which can then be placed inside a beaker containing 100 cm^3 distilled water, as shown in Fig. 2.1.

The Visking tubing membrane can contain between 10.0 cm^3 to 12.0 cm^3 of liquid. It is selectively permeable, hence any carbon dioxide molecules produced by the yeast cells when sugars are added will diffuse through the membrane of the tubing into the water surrounding the Visking tubing.

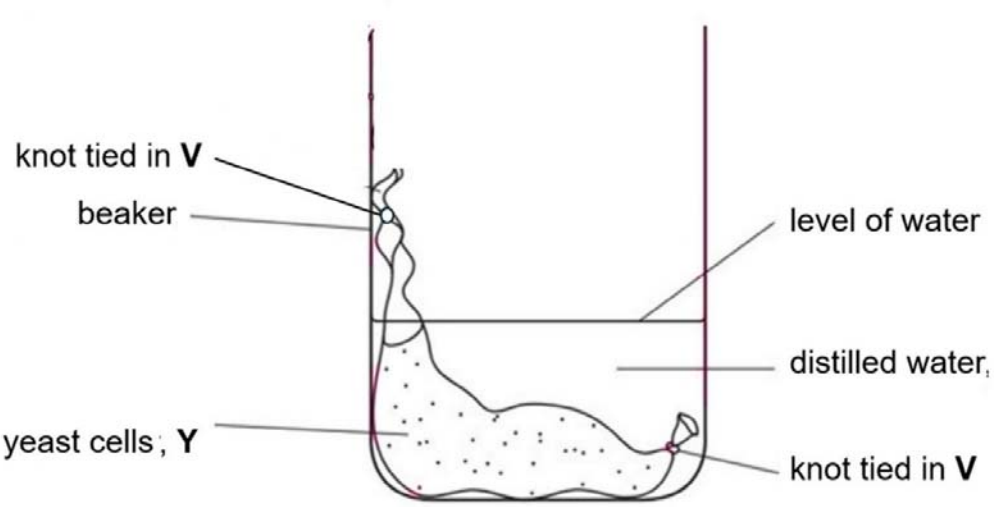
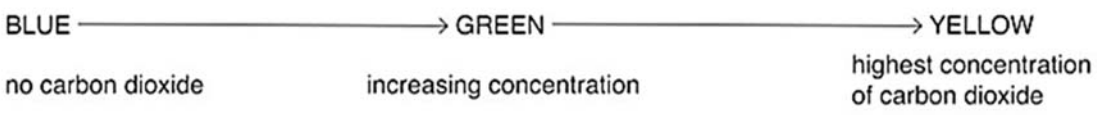


Fig. 2.1

The students found out that mixing the yeast cell suspension with any sugars in a 1:1 volume ratio would result in immediate activation of the yeast.

To test for the release of carbon dioxide, a sample of the water (1.0 cm^3) surrounding the Visking tubing can be taken and added to two drops of an indicator, **B**. As the concentration of carbon dioxide increases, **B** changes from blue to green to yellow.



(a) Explain why the indicator changes from blue to yellow.

……..

…….[1]

The students also found that the difference in the results of the yeast cells with the different sugars can be determined between 3 to 10 min.

To follow the progress of this reaction you will need to take samples from the water surrounding the Visking tubing at different times up to a suitable time. If the colour change to yellow takes more than 10 min, record as "more than 600 seconds".

You are provided with the following which you **must** use:

labelled	contents	hazard
Y	yeast cells in sucrose solution	none
B	bromothymol blue indicator solution	harmful
W	to collect distilled water from teacher's bench	none
S	sucrose solution	none
G	galactose solution	none
V	two 15 cm length of Visking tubing in a container containing distilled water	none

You are also provided with the following which you may use:

	contents	number of items
1	pasteur pipette	2
2	10 cm^3 syringes	4
3	1 cm^3 syringe	4
4	spotting tile	1
5	glass rod	1
6	100 cm^3 beakers	2
7	paper towels	8
8	glass marker pen	1
9	stopwatch	1
10	suitable eye protection	1

If any liquids come into contact with your skin, wash off immediately under cold water. It is recommended that you wear suitable eye protection.

(b) State:

the time interval when you will remove the samples ……

the total volume that you will put into **V** …… [1]

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(c) Describe the steps that you will take to investigate whether **Jason** or **Kate** is correct, including steps to ensure reliability of results. You are not required to plan for replicates.

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....[6]

(d) Record your results in an appropriate format.

[3]

(e) State which student is correct and suggest why the sugar stated by the student results in a faster rate of reaction.

……

……

……

……[2]

[Total: 13]

3 Starch grains from different types of plant differ in size and shape. Some starch grains have rings on their surfaces.

You will now observe and draw starch grains from one type of plant.

1. Put one **clean** and **dry** microscope slide onto a paper towel.

2. Using a pipette, put a few drops of **P** onto the slide. **P** is a suspension of starch grains.

3. Cover the drops of **P** on the slide with a coverslip and use a paper towel to remove any excess suspension.

4. Use the microscope to find and observe the starch grains on the slide.
You may need to reduce the amount of light entering the microscope and adjust the fine focus to observe the starch grains clearly.

5. Select, from a single field of view, **four** starch grains that show different sizes and features.

(a) Make a large drawing of the **four** starch grains that you have selected.

[4]

(b) Remove the slide from the microscope and place it on a paper towel.

Fig. 3.1 shows some different types of starch grains and patterns on the surface of the starch grains.

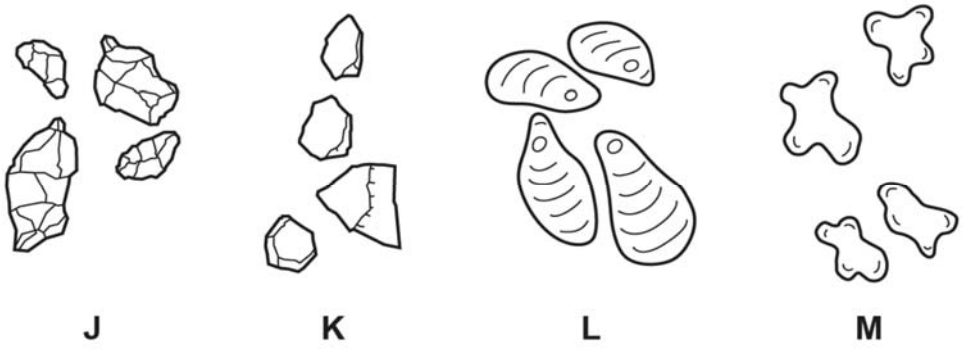

Fig. 3.1

(i) Use the diagrams in Fig. 3.1 to identify which of the starch grains, **J**, **K**, **L** or **M**, matches most closely the starch grains drawn in **(a)**.

answer ..... [1]

A student calibrated the eyepiece graticule in a light microscope using a stage micrometer scale.

The calibration was:

1 eyepiece graticule unit = 16 µm

The student used the microscope to observe and draw three starch grains to the same scale. The student drew a line across the length of each drawing of a starch grain. The student's drawings are shown in Fig. 3.2.

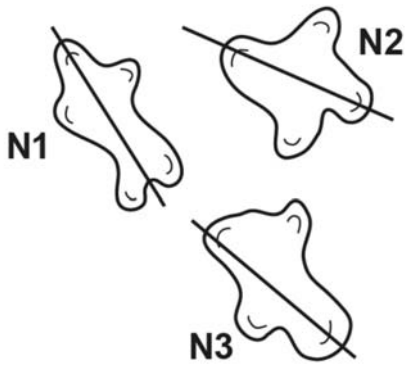

Fig. 3.2

(ii) When viewed using the microscope, the student found that starch grain **N1** measured 4 eyepiece graticule units along the position of the line drawn in Fig. 3.2.

Calculate the mean **actual** length of the three starch grains in Fig. 3.2.
Show all the steps in your working and use appropriate units.

mean actual length = ...
[3]

(c) **J1** is a slide of a stained transverse section through a part of a plant.
Draw a large plan diagram of the region of the root on slide **J1** indicated by the shaded area in Fig. 3.3. Use a sharp pencil.

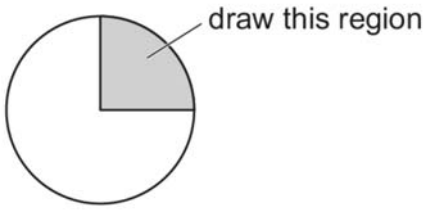

Fig. 3.3

Use **one** ruled label line and label, with the letter **X**, the tissue that contains most of the starch grains.

[5]

(d) Fig. 3.4 is a photomicrograph of a transverse section through a stem of a different type of plant.

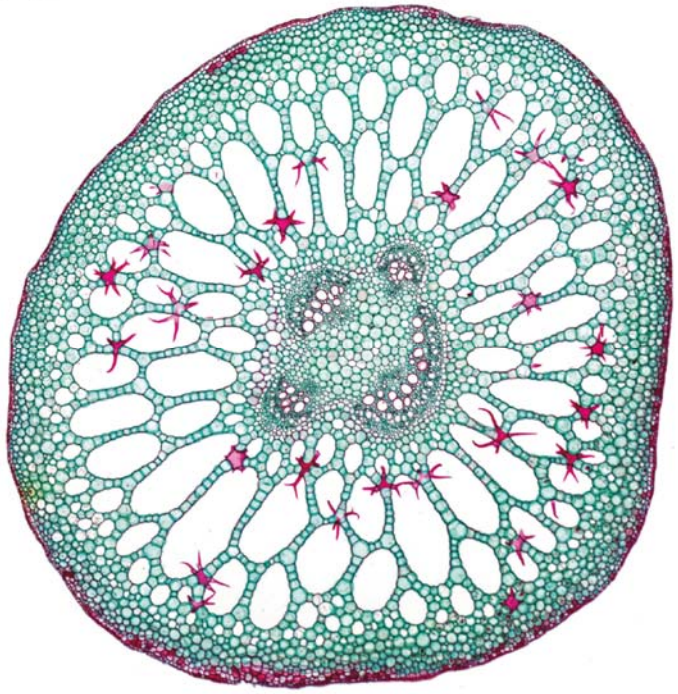

Fig. 3.4

Identify **two** observable differences, other than size and colour, between the specimen on slide **J1** and the plant part in Fig. 3.4 in your table.

[3]

(e) Suggest a habitat where the plant in Fig. 3.4 might grow and explain using one observable feature shown in Fig. 3.4, which enables it to live in this habitat.

Habitat

.....

explanation

.....

.....[2]

[Total: 18]

End of Paper