



## 2025 JC2 PRELIMINARY EXAMINATION

CANDIDATE  
NAME

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CLASS

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INDEX  
NUMBER

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### BIOLOGY

**9744/04**

**Paper 4  
Practical**

**28 August 2025  
Thursday**

Candidates answer on the Question Paper.

Additional Materials: As listed in the Confidential Instructions.

**2 hours 30 minutes**

### READ THESE INSTRUCTIONS FIRST

Write your name and class 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 an HB pencil for any diagrams or graphs.  
Do not use staples, paper clips, 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, fasten all your work securely together.

The number of marks is given in brackets [ ] at the end of each question or part question.

Shift
Laboratory

For Examiner's Use	
1	/ 28
2	/ 13
3	/ 14
Total	/ 55
	%

This document consists of **23** printed pages and **1** blank page.

Answer **all** questions.

**1** Amylase is an enzyme that catalyses the hydrolysis of starch to maltose.

You will investigate the effect of the concentration of amylase on the time taken to completely hydrolyse starch to maltose.

Iodine solution turns from an orange-brown colour to a blue-black when starch is present.

The course of the reaction can be monitored by sampling at intervals and using iodine solution to test for the presence of starch. The end-point is when all the starch has been hydrolysed by amylase.

At the end-point, the colour of the iodine solution may be slightly different compared to the colour of the original iodine solution and there may be some specks of blue-black. These specks of blue-black can be ignored.

- (a)** Describe **and** explain how reducing the concentration of amylase is expected to affect the time taken to reach end-point.

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.....

.....[2]

You are provided with:

- 200 international units  $\text{dm}^{-3}$  ( $\text{U dm}^{-3}$ ) amylase solution **A**, in a container labelled **A**
- distilled water **W**, in a container labelled **W**
- starch solution **S**, in a container labelled **S**
- iodine solution in a container labelled **iodine**.

**Amylase solution A is an irritant and iodine solution is a stain. Suitable eye protection should be worn. If A or iodine solution comes into contact with your skin, wash it off immediately under a tap.**

- (b) (i)** You will carry out proportional dilutions of the  $200 \text{ U dm}^{-3}$  amylase solution **A** to obtain a range of concentrations in which the concentration of the amylase is reduced by  $50 \text{ U dm}^{-3}$  between each successive dilution.

You will prepare  $10.0 \text{ cm}^3$  of each concentration, using **A** and **W**.

Complete Table 1.1 to show how you will prepare the different concentrations of amylase solution.

Table 1.1

Concentration of amylase / U dm <sup>-3</sup>	volume of <b>A</b> / cm <sup>3</sup>	volume of <b>W</b> / cm <sup>3</sup>
200		
0		

[2]

Read steps **1–13** before starting the investigation.

**Proceed as follows.**

- 1 Prepare the concentrations of amylase solution as shown in Table 1.1, in the containers provided.
- 2 Label test-tubes with the concentrations of amylase solution prepared in step 1.

Before proceeding further, use the container labelled **hot water** to collect approximately 300 cm<sup>3</sup> of hot water from where it is provided in the laboratory. You should also fill the container labelled **tap water** with water from a tap.

- 3 The enzyme's optimum temperature is 35°C. Using the hot water and tap water that you have collected, set up a water-bath at 35°C in the beaker labelled **water-bath**. You will need to maintain this temperature throughout the investigation.
- 4 Label the white tile as shown in Fig. 1.1. The numbers indicate the sampling times in seconds.

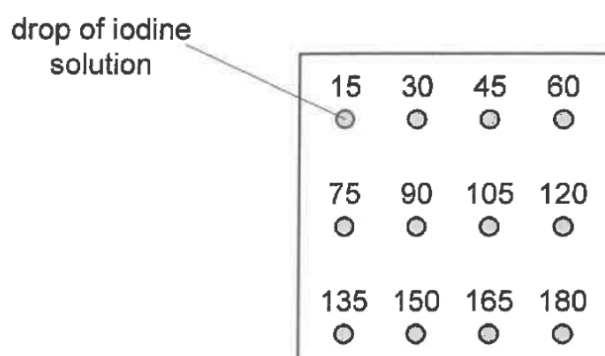


Fig. 1.1

- 5 Put one drop of iodine solution on the white tile at each labelled sampling time, as shown in Fig. 1.1.
- 6 Put 3.0 cm<sup>3</sup> of **S** into each of the test-tubes labelled in step 2.
- 7 Put 2.0 cm<sup>3</sup> of 200 U dm<sup>-3</sup> amylase solution into the test-tube labelled with this concentration. Use a glass rod to mix the amylase solution with **S**. Proceed to step 8 without delay.
- 8 Put the test-tube with the mixture of amylase solution and **S** into the water-bath prepared in step 3. Start timing immediately.
- 9 After 15 seconds, and every 15 seconds from then on, use the glass rod to transfer one drop of the mixture from the test-tube onto the drop of iodine solution on the white tile that corresponds to the correct sampling time. After each transfer, clean the glass rod with a paper towel.
- 10 Continue testing further drops of the mixture from the test-tube, as described in step 9, until the end-point is reached. If the end-point has **not** been reached within 180 seconds, do **not** test further samples from the test-tube.
- 11 Record, in **(b)(ii)**, the time taken to reach the end-point.  
  
If the end-point has **not** been reached after 180 seconds, record the result as 'more than 180' **and** note the colour of the mixture in brackets, e.g. 'more than 180 (blue-black)'.
- 12 Rinse the white tile with running water from a tap and wipe dry with a paper towel. If the labels are no longer visible, you will need to label the white tile again, as shown in Fig. 1.1.
- 13 Repeat steps 7–11 with each of the other concentrations of amylase that you prepared in step 1. Before testing each of the concentration of amylase, make sure that the white tile is clean by carrying out step 12.

- (ii)** Record your results in an appropriate table.

**[4]**

The urine of healthy people contains a low concentration of amylase. Some medical conditions can cause variation in the concentration of amylase in urine. Amylase concentrations in the urine can be tested as part of a health check.

You will be testing solutions that represent urine samples collected from three different people as part of a health check, **U1**, **U2** and **U3**. They are **not** actual urine samples. The samples are provided in containers labelled **U1**, **U2** and **U3**.

You are required to test each sample and estimate the concentration of amylase in each sample.

Read steps **14–18**.

**Proceed as follows.**

- 14** Ensure that the water-bath prepared in step **3** is still at a temperature of 35 °C. You will need to maintain the water-bath at this temperature.
- 15** Label three clean test-tubes **U1**, **U2** and **U3**.
- 16** Put 3.0 cm<sup>3</sup> of **S** into each of the test-tubes labelled in step **15**.
- 17** Repeat steps **7–10** with each of the unknown concentrations of amylase: **U1**, **U2** and **U3**. Before testing each of the unknown concentrations of amylase, make sure that the white tile is clean by carrying out step **12**.
- 18** Record, in **(b)(iii)**, the time taken to reach the end-point for **U1**, **U2** and **U3**.

If the end-point has **not** been reached after 180 seconds, record the result as 'more than 180' **and** note the colour of the mixture in brackets, e.g. 'more than 180 (blue-black)'.

**(iii)** Record your results for **U1**, **U2** and **U3**.

result for **U1** .....

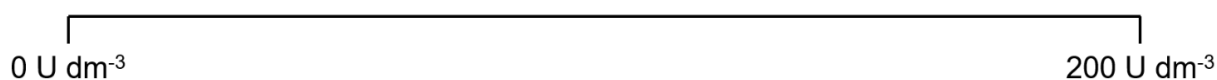
result for **U2** .....

result for **U3** .....

[2]

**(iv)** The line shown in Fig. 1.2 can be used as a linear scale to represent the amylase concentrations used in this investigation. The positions for 200 U dm<sup>-3</sup> and 0 U dm<sup>-3</sup> are shown on the line.

Complete Fig. 1.2 to show the positions on the linear scale of the other amylase concentrations you prepared in step 1, as detailed in Table 1.1.



**Fig. 1.2**

[1]

- (v) Use your results in (b)(ii) and (b)(iii) to estimate the amylase concentrations of **U1**, **U2** and **U3**.

Show your estimates of amylase concentration by drawing **one** arrow ( $\downarrow$ ) at the correct position on the scale in Fig. 1.2 for each of **U1**, **U2** and **U3**. Label the arrows **U1**, **U2** and **U3**.

If any estimate falls between two concentrations, you may place the arrow anywhere in between the two concentrations.

[2]

- (vi) Medical conditions including acute pancreatitis, cirrhosis of the liver, kidney disease and perforated ulcer can all affect the amylase concentration in the urine.

Acute pancreatitis and a perforated ulcer often result in increased amylase concentrations in the urine. Cirrhosis of the liver and kidney disease often result in reduced amylase concentrations in the urine.

Using your estimates from (b)(v) and the information given, complete Table 1.2 to identify medical conditions that would be consistent with the estimated concentrations for **U1**, **U2** and **U3**.

Although there is wide variation, in this question **you should assume that the concentration of amylase in the urine of healthy people is approximately  $100 \text{ U dm}^{-3}$ .**

Put one or more ticks ( $\checkmark$ ) in each row of Table 1.2 to identify **every** medical condition that could be consistent with the estimated concentrations for **U1**, **U2** and **U3**. If the estimated concentration in a sample is **not** consistent with any of the medical conditions, put a tick ( $\checkmark$ ) in the column 'no medical conditions' for that sample.

**Table 1.2**

sample	no medical conditions	acute pancreatitis	cirrhosis of the liver	kidney disease	perforated ulcer
<b>U1</b>					
<b>U2</b>					
<b>U3</b>					

[3]

- (vii) One source of error in the method is the difficulty in maintaining the water-bath at a consistent temperature of 35 °C.

Identify **one other** source of error in the method that affects the temperature at which the hydrolysis of starch is carried out in this investigation.

Suggest an improvement to the method that will reduce the effect of this error.

error

.....

.....

improvement

.....

.....

[2]

- (viii) Suggest **one** limitation in accurately measuring the time to the end-point.

Suggest an improvement to the method that will increase the accuracy of measuring the time to the end-point.

limitation

.....

.....

improvement

.....

.....

[2]



- (c) A student suggested that it would be more efficient to estimate the concentration of amylase present in urine samples by adding each sample to a starch solution and measuring the concentration of starch remaining after a short time interval.

You are provided with three solutions, **R1**, **R2** and **R3**. These represent urine samples that have been collected and prepared for testing in exactly the same way. They are **not** actual urine samples.

During preparation of the samples, each was mixed with a dilute solution of starch and incubated at 37°C for 30 seconds. After 30 seconds, a dilute solution of copper(II) sulfate was added.

- (i) Suggest why a dilute solution of copper(II) sulfate was added after 30 seconds.

.....  
 .....  
 .....[1]

In addition to the three samples, **R1**, **R2** and **R3**, you are provided with:

- iodine solution in a container labelled **iodine**
- distilled water, W in a beaker labelled **W**
- a white tile
- syringes
- test-tubes.

**Iodine solution is a stain. Suitable eye protection should be worn. If iodine solution comes into contact with your skin, wash it off immediately under a tap.**

Using the materials provided, work out how to carry out a standardised semi-quantitative test to rapidly identify the sample with the highest concentration of amylase.

When you have decided on a method, use your method to test each of the three samples **R1**, **R2** and **R3**.

Record your results in (c)(iii).

- (ii) Outline the method that you carried out for the standardised semi-quantitative test.

.....  
 .....  
 .....  
 .....[1]

(iii) Record your results for **R1**, **R2** and **R3**.

[1]

(iv) Identify which sample **R1**, **R2** and **R3**, contains the highest concentration of amylase.

sample .....[1]

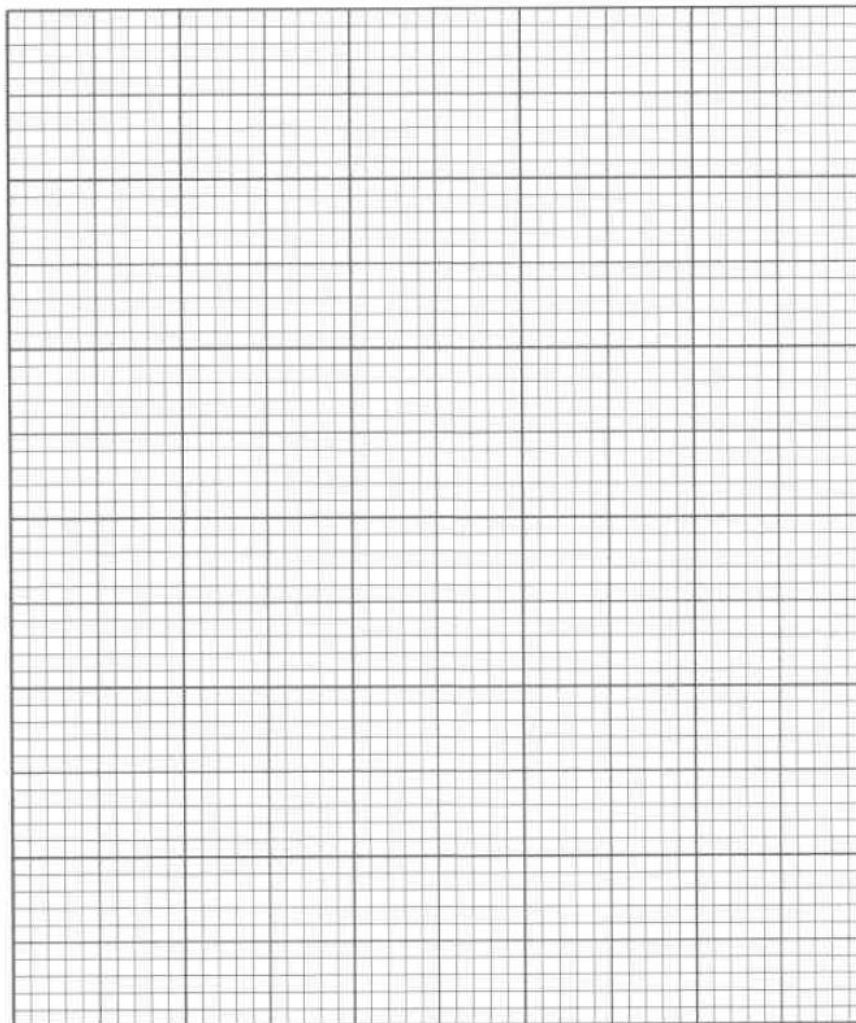
Amylase activity in blood can also be used as an indicator of particular medical conditions.

- (d) Table 1.3 shows the amylase activity in arbitrary units (au) found in the blood of five people with different medical conditions.

**Table 1.3**

medical condition	amylase activity in blood / au
mumps (MP)	1110
liver failure (LF)	20
pancreatic cyst (PC)	630
cholecystitis (CH)	120
chronic pancreatitis (CP)	40

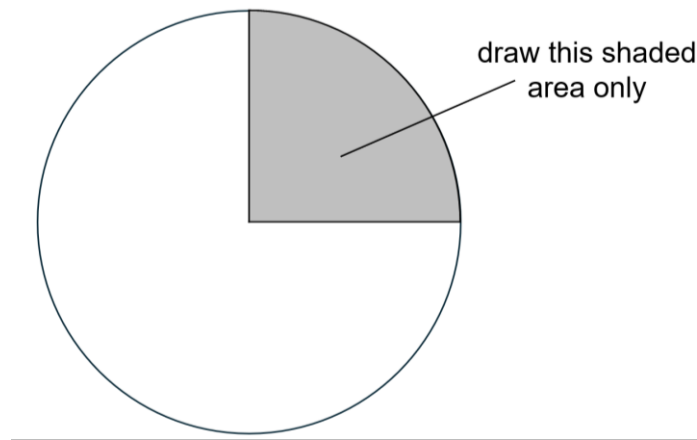
Use the grid provided to display the results shown in Table 1.3 in an appropriate form.



[4]

[Total: 28]

- 2 During this question you will require access to a microscope and slide **Y**. **Y** is a slide of a stained transverse section through a plant stem.
- (a) (i) Draw a large plan diagram of the part of the stem on slide **Y** shown by the shaded area in Fig. 2.1.



**Fig. 2.1**

A plan diagram shows the arrangement of different tissues. Your drawing should show the correct shapes and proportions of different tissues.

No cells should be drawn.

Labels are **not** required.

[4]

- (ii) Observe the outermost layer of cells on the surface of the stem on slide **Y**. This outermost layer is called the epidermis and is one cell thick. Select a group of four touching cells comprising two cells from the epidermis and two cells from the layer below the epidermis.

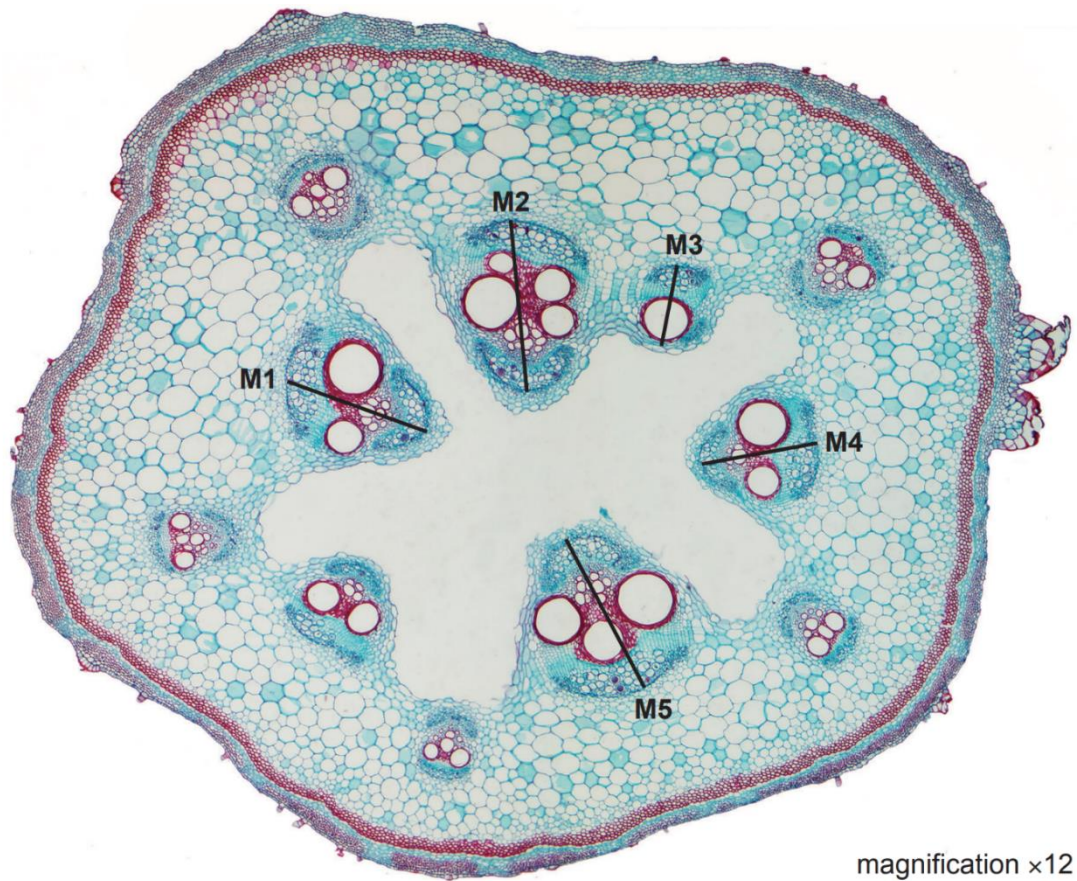
Each cell must touch at least two other cells in the group.

Make a large drawing of this group of four touching cells.

Labels are **not** required.

[3]

(b) Fig. 2.2 is a photomicrograph of a stained transverse section of a stem from a different plant.



**Fig. 2.2**

- (i) Measure the length of the vascular bundles using the lines **M1**, **M2**, **M3**, **M4** and **M5** in Fig. 2.2 and calculate the mean actual length of the vascular bundles in mm.

Show your working.

**M1 =**

**M2 =**

**M3 =**

**M4 =**

**M5 =**

mean actual length ..... [4]

- (ii) A student suggested that the mean actual length of the vascular bundles calculated in (b)(i) was not accurate for the whole plant.

Describe **two** modifications to the method used in (b)(i) that would allow a more accurate mean length of the vascular bundles for the whole plant to be calculated.

.....

.....

.....

.....

.....

.....[2]

[Total: 13]

- 3 Catalase is an enzyme that is found in many plant cells and animal cells. Catalase breaks down hydrogen peroxide, which is a waste product from cell metabolism.

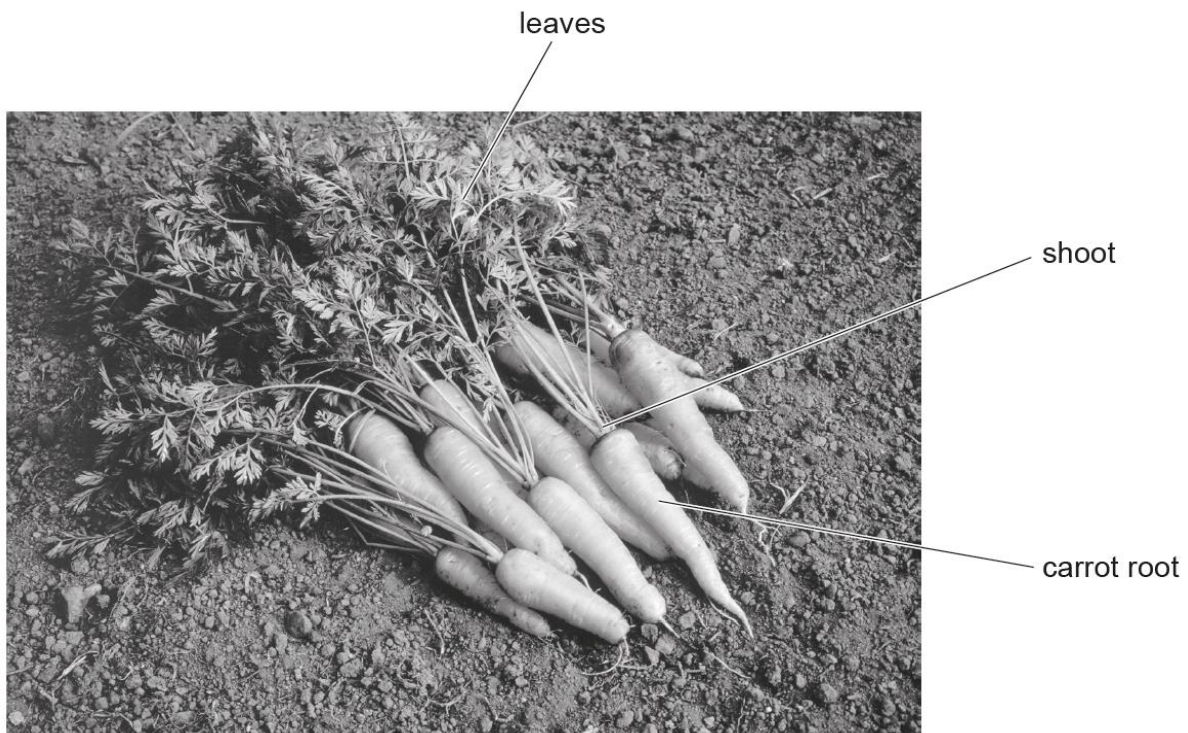
Fig. 3.1 shows the breakdown of hydrogen peroxide by catalase.



**Fig. 3.1**

A student decided to investigate how catalase activity varies along the length of a carrot root.

Fig. 3.2 shows a photograph of some carrots.



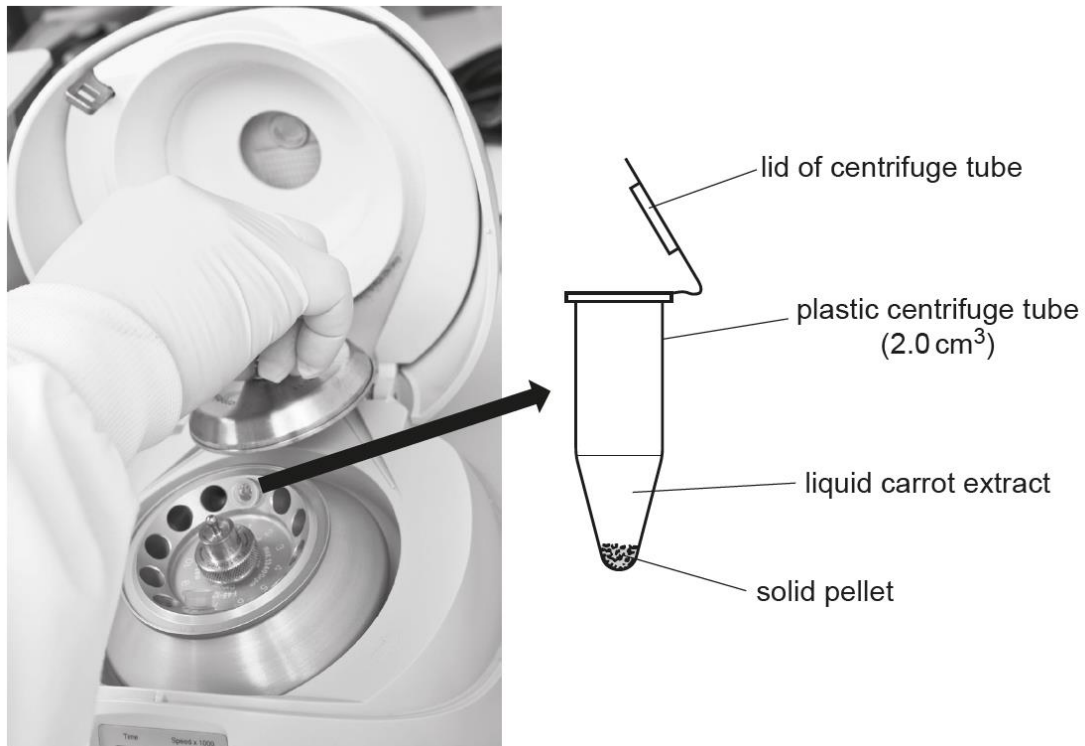
**Fig. 3.2**

The student extracted catalase from different parts of the carrot root tissue.

- Carrot root tissue was mixed with a small volume of pH7 buffer and some fine sand.
- The mixture was ground with a pestle and mortar.
- The mixture was placed into a centrifuge tube and spun in a variable speed centrifuge for a few minutes.
- This caused the mixture to be separated into a solid pellet at the bottom of the centrifuge tube and a liquid carrot extract at the top, as shown in Fig. 3.3.
- The liquid carrot extract was poured into a clean test-tube. This liquid contained catalase.



Fig. 3.3 shows a centrifuge and centrifuge tube with its contents, after spinning in the centrifuge for a few minutes.



**Fig. 3.3**

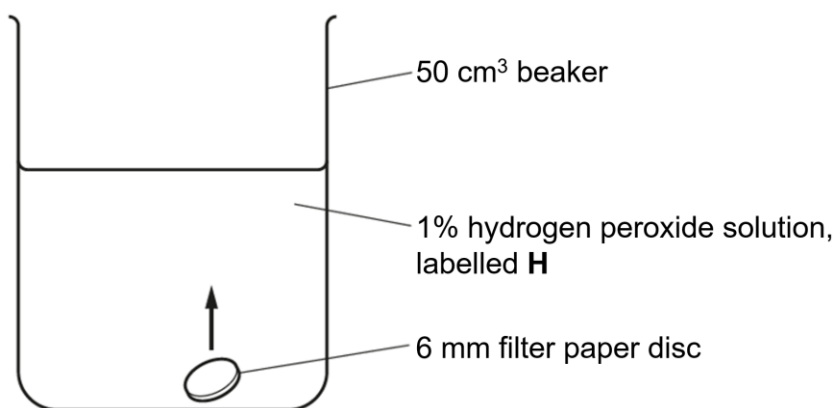
- (a) State one variable that should be standardised in the procedure to obtain the liquid carrot extract, **other than** the carrot tissue, fine sand and apparatus used.

.....  
 .....[1]

The student carried out the following steps to investigate the activity of catalase in the different samples of carrot root tissue.

- The total volume of 1% hydrogen peroxide provided, labelled **H** was poured into a 50 cm<sup>3</sup> beaker.
- A disc of filter paper of 6 mm diameter was dipped into the liquid carrot extract, labelled **C** using forceps.
- Using the forceps, the filter paper disc was then placed at the bottom of the beaker of **H**.

Fig. 3.4 shows the set-up used to measure the time taken for the filter paper disc to rise.



**Fig. 3.4**

- (b) (i) You are required to decide on the method that you will use to determine the rate at which the filter paper rises in the set-up in Fig. 3.4.

The method should use the apparatus available, take no longer than five minutes and allow an assessment of the degree of confidence in the results to be made.

Describe the method that you plan to use.

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.....[4]

- (ii) Create the set-up in Fig. 3.4 and carry out the method you have described in (b)(i) to collect results.

Record your results in a suitable form in the space below.

[2]

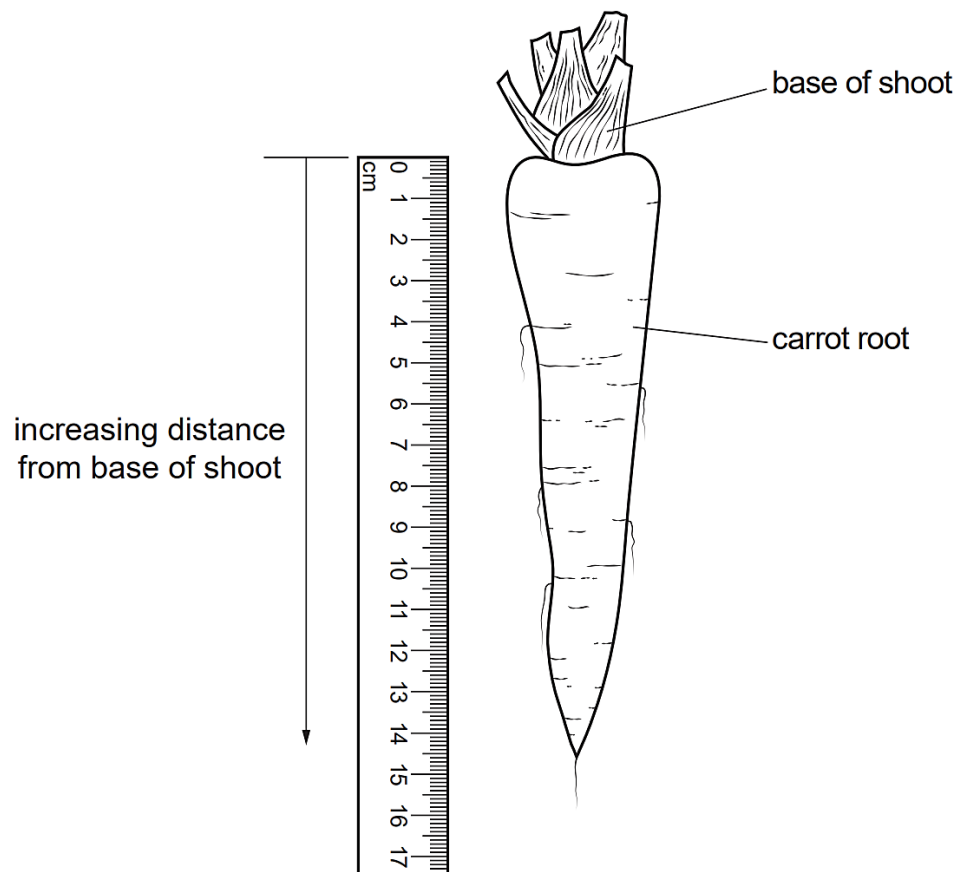
- (iii) Describe a suitable control for this investigation.

.....  
.....  
.....[1]

- (iv) Predict the result for the control described in (b)(iii).

.....  
.....  
.....[1]

- (c) The student decided to investigate how catalase activity varies along the length of a carrot root, as measured in Fig. 3.5.



**Fig. 3.5**

The student made the prediction:

“As the distance from the base of the shoot increases, the catalase activity of carrot root tissue increases.”

- (i) Identify the **independent** variable in this investigation.

.....[1]

- (ii) The later parts of the student's investigation involved using the centrifuge to prepare the liquid carrot extract, and the method you proposed in **(b)(i)**.

Describe a method that the student could use to first obtain root tissue from the carrot in Fig. 3.5 for this investigation.

You are to include reference to a safety measure to minimise one risk associated with the proposed method.

.....[4]

[Total: 14]





