Surname	Centre Number	Candidate Number
First name(s)		2

GCE A LEVEL

1400U50-1A

S25-1400U50-1A

WEDNESDAY, 26 MARCH 2025

BIOLOGY – A2 unit 5
Practical Examination

Experimental Task
TEST 1

2 hours

For Teacher's use only Award a mark of 0 or 1 for the following	
Setting up an agar plate	

For Examiner's use only	
Mark Awarded	
Total	

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01

ADDITIONAL MATERIALS

A calculator and a ruler.

INSTRUCTIONS TO CANDIDATES

Use black ink or black ball-point pen. Do not use gel pen or correction fluid.
Pencil may be used to draw tables and graphs.
Write your name, centre number and candidate number in the spaces at the top of this page.
Write your answers in the spaces provided in this booklet.

INFORMATION FOR CANDIDATES

The total number of marks available for this task is 20.
Your teacher will directly assess your practical skills.
The number of marks is given in brackets at the end of each question or part-question.
You are reminded of the necessity for orderly presentation in your answers.

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1. Enzymes are used in industry to catalyse the conversion of one molecule to another. Often, the enzymes are extracted from cultures of bacteria which produce varying concentrations of enzymes depending on the culture conditions.

Before harvesting microbial enzymes from the culture medium, it is important to determine the concentration of enzyme present. You are going to determine the concentration of amylase in two samples of unknown concentrations.

Follow these instructions carefully

You are provided with:

2 × starch-agar plates
6 × filter paper discs
1 × forceps
1 × sample **A** containing an unknown concentration of amylase
1 × sample **B** containing an unknown concentration of amylase
1 × dropping bottle of iodine / potassium iodide solution
1 × 30 cm ruler
small beaker of distilled water for washing forceps
stopclock
marker pen
paper towels
beaker for waste iodine solution

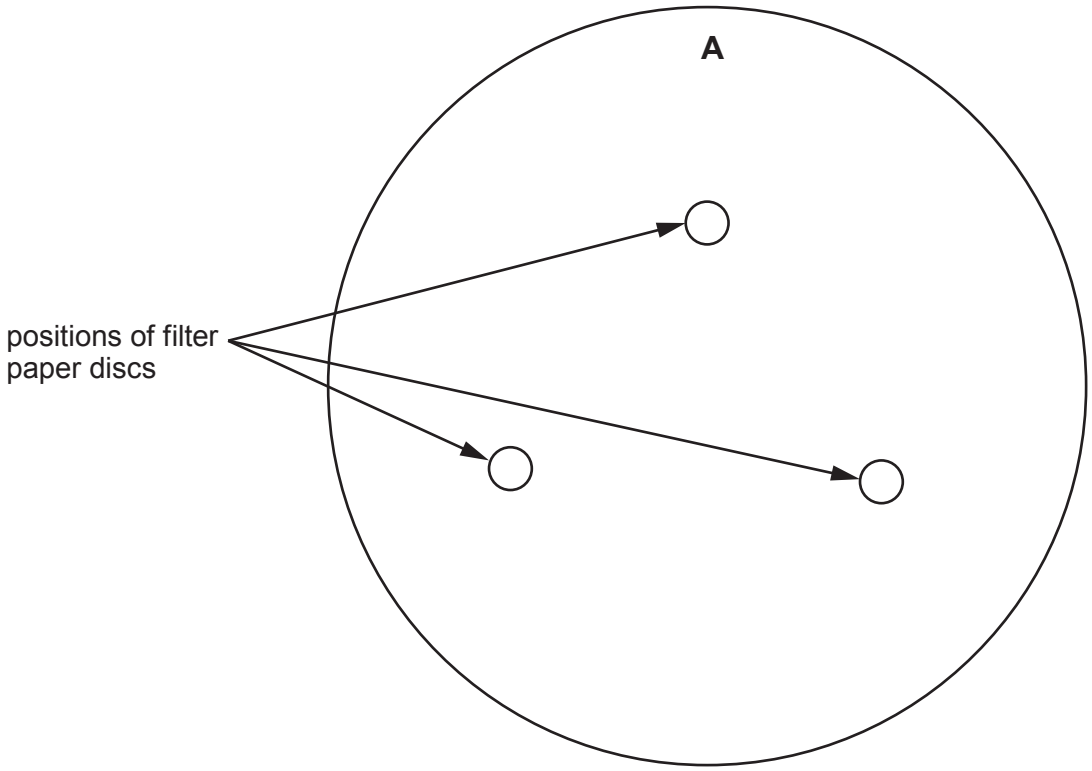
YOUR TEACHER WILL BE OBSERVING YOUR EXPERIMENTAL TECHNIQUE.

Method – Part 1

1. Mark the underside of one of the starch-agar plates with the letter **A**.

2. Using the forceps, dip a disc of filter paper in unknown sample **A**, tap to remove excess solution from the filter paper disc and then place carefully on the starch-agar plate.

3. Repeat step 2 twice more so that three filter paper discs soaked in sample **A** are placed on the starch-agar plate as shown in the diagram below.



4. Wash the forceps in the distilled water and dry them before following the next steps.

5. Using a different starch-agar plate, repeat step 1 but mark the plate with the letter **B**.

6. Repeat steps 2 and 3 using unknown sample **B**.

7. **Leave the plates for 45 minutes**.

During this time, read the information and then complete the tasks and questions on pages 4, 5, 6 and 7.

The method is continued on **page 8**.

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Three starch-agar plates were set up with filter paper discs soaked in increasing concentrations of amylase. The plates were left for 45 minutes. The filter paper discs were then removed and the plates flooded with iodine solution. The excess iodine was then poured off and photographs taken of each plate.

Images 1, **2** and **3** on pages 4 and 5 show the appearance of the plates after 45 minutes. The concentrations of amylase used are marked on the plate in **Image 1**. The **same concentrations** were tested in the **same positions** on the plates in **Images 2** and **3**.

Using the plates in **Images 1**, **2** and **3** measure the **maximum diameter** of the clear zone for each concentration of amylase to the **nearest mm**. There is space to record these readings on page 5.

Image 1

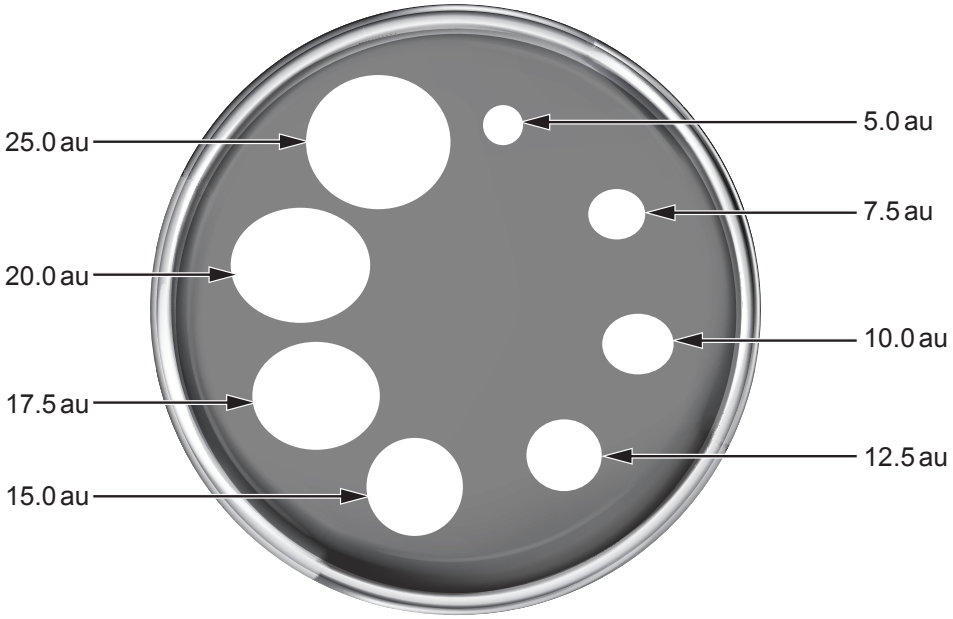

Image 2

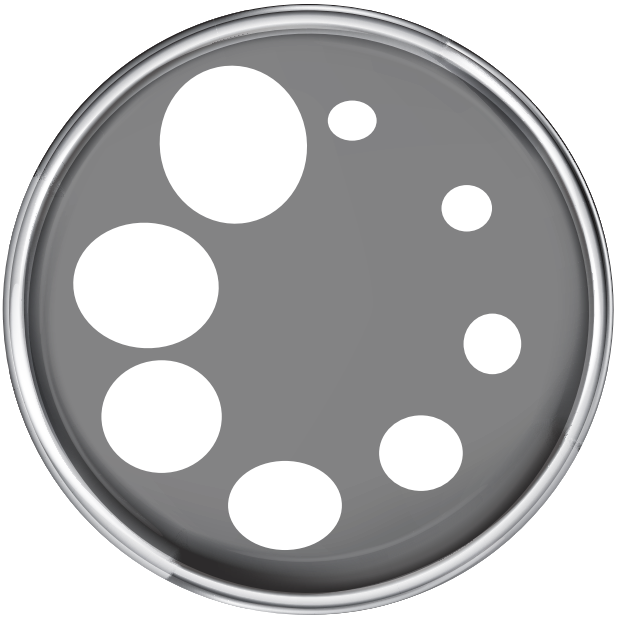

Image 3

Measurements of maximum diameters from Images 1, 2 and 3

You may use the space below to record the maximum diameter of each clear zone for each concentration of amylase in each image and to calculate the mean maximum diameter of clear zones for each concentration. (This will not be assessed.)

Rough results

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Examiner only

(a) Construct a suitable table to include the maximum diameter of clear zones at **each concentration of amylase** from **each Image** and the calculated mean for each concentration. **Your table should include a value of 0 mm at a concentration of 0 au amylase.** [4]

(b) Plot the **mean** maximum diameter of clear zones from the table drawn in part (a) against concentration of amylase on the graph opposite.
Draw a **straight line of best fit** through the plotted data. (The line should pass through the origin, 0,0.)
Range bars are not required. [7]

Examiner only

Examiner only

Method – Part 2

1. After 45 minutes use the forceps to remove the filter paper discs from both plates.

2. Flood both plates with iodine / potassium iodide solution. Make sure that the iodine solution covers the whole surface of the plate.

3. Leave the plates until clear zones appear around where the filter paper discs had been placed.

4. Pour off excess iodine into the waste beaker.

5. Turn both plates upside down on a paper towel and measure and record the **maximum** diameter of each clear zone to the **nearest mm**.

6. Calculate the mean maximum diameter of the clear zones around the three discs for both sample **A** and sample **B**. Use these means and your graph in part (b) to estimate the concentration of amylase in samples **A** and **B**.

(c) Record your measurements and the results of your calculations in the table below. You should **show on your graph** how you estimated the concentration of amylase in samples **A** and **B**. [2]

Unknown sample	**Maximum diameter of clear zone / mm**			**Mean maximum diameter of clear zones / mm**	**Estimated concentration of amylase in the sample / au**
	Trial 1	**Trial 2**	**Trial 3**		
A					
B					

Examiner only

(d) (i) Explain why clear zones appeared around the filter paper discs. [2]

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(ii) The temperature was not controlled in this investigation.
Explain how this could have affected the results of the investigation. [2]

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(iii) Explain why using the **maximum diameter** of clear zone could be a limitation when estimating the amylase concentration of an unknown sample. [2]

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