GCE A LEVEL

1400U50-1D

S25-1400U50-1D

To be opened on WEDNESDAY, 19 MARCH 2025 by TEACHERS / TECHNICIANS

BIOLOGY – A2 unit 5
Practical Examination

Experimental Task
TESTS 1 and 2

SETTING UP INSTRUCTIONS

Confidential

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This document must not be stored within the science department when not in use by the teacher / technician.
Its contents should not be divulged except to those concerned with the preparation of the assessment.

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TEST 1

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

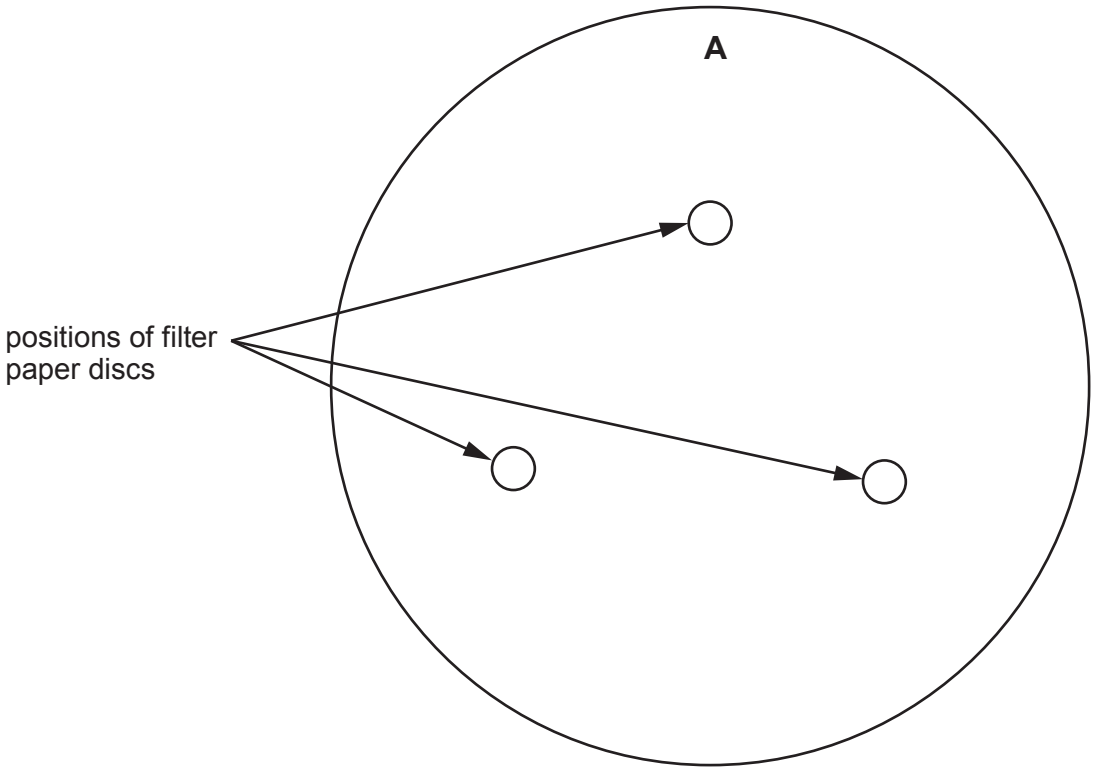
YOUR TEACHER WILL BE OBSERVING YOUR EXPERIMENTAL TECHNIQUE.

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**.

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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**.

The remainder of the examination paper is not needed for the purpose of checking the setting up of the task.

2. TEST 2

The same as Test 1.

For Tests 1 and 2:

Making amylase solution
To make sample **A**:

- dissolve 1 g of amylase powder in 100 cm^3 of pH7 phosphate buffer

OR

- mix 1 cm^3 of amylase solution with 99 cm^3 of pH7 phosphate buffer.

To make sample **B** add 5 cm^3 of sample **A** to 95 cm^3 of pH7 phosphate buffer.

Each student will need approximately 0.5 cm^3 of both sample **A** and sample **B**. These can be provided to the students in any suitable small containers.

This quantity will provide enough of both samples **A** and **B** for 150+ students.

For each candidate two starch-agar plates are required:
Starch-agar 0.2 % soluble starch, 2 % agar.

1. Mix 1 g of agar powder and 0.1 g soluble starch with 50 cm^3 of hot water.
2. Boil gently for a few minutes.
3. Pour approximately half into each Petri dish.
4. Allow to cool.

Iodine / potassium iodide solution – this can be commercially bought or freshly made.
To make 1 dm^3 of fresh iodine / potassium iodide solution:

3 g iodine crystals
6 g potassium iodide
Dissolve potassium iodide in about 200 cm^3 distilled water and then add iodine crystals.
Make the solution up to 1 litre with distilled water.

It is essential to prepare the iodine/potassium iodide solution 24 hours before it is required, as iodine is slow to dissolve.
It is important that the iodine / potassium iodide solution is made no more than five days before the examination as the solution darkens over time which could affect students' results.

In exceptional circumstances, where an individual has been unable to generate any data, the teacher should provide data in an unstructured format for analysis. The work should be annotated to this effect.

Please ensure that the "Information required from centres" sheet is completed and given to the exams officer to be sent to the examiner with the completed examination papers.

Awarding of teacher-assessed marks

Setting up of agar plate	Award 1 mark if the starch-agar plates have been labelled on base and filter paper discs arranged with enough space for diffusion of amylase

It is recommended that teachers complete the teacher-assessed mark after candidates have completed the practical.

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INFORMATION REQUIRED FROM CENTRES

Centre Number .....

(Please detach and send with the completed examination papers to the **examiner**.)

TEST 1

One set of teacher results for each unknown sample

Unknown sample	Maximum diameter of clear zone / mm			Mean maximum diameter of clear zones / mm
	Trial 1	Trial 2	Trial 3	
A				
B				

TEST 2

One set of teacher results for each unknown sample

Unknown sample	Maximum diameter of clear zone / mm			Mean maximum diameter of clear zones / mm
	Trial 1	Trial 2	Trial 3	
A				
B				

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