H

NANYANG JUNIOR COLLEGE
PRELIMINARY EXAMINATIONS
Higher 2

CANDIDATE NAME

CLASS

BIOLOGY

Paper 4 Practical

9744/04

22 August 2024

Candidates answer on the Question Paper

Additional Materials: As listed in the Confidential Instructions

2 hour 30 minutes

READ THESE INSTRUCTIONS FIRST

Write your name and CT 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, highlighters, glue or correction fluid.
DO NOT WRITE IN ANY BARCODES.

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.

Shift
Laboratory

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.

For Examiner's Use	
1	
2	
Total	

This document consists of **19** printed pages and **0** blank pages.

[Turn over

Answer **all** the questions.

1 **J1** is a slide of a stained transverse section through a plant leaf.

(a) (i) Draw a large plan diagram of the whole section on **J1**. Use a sharp pencil. Use **one** ruled label line and label to identify the epidermis.

[5]

(ii) The leaf section on **J1** is from a plant that lives at high altitudes in very cold conditions. In the winter the ground is often frozen and plants are unable to take up water.

Suggest **one** observable feature of the leaf section on **J1** which enables it to survive these conditions.

.....

.....

.....

[1]

(iii) Observe the cells in the epidermis of the section on **J1**.

Select a line of four adjacent cells that make up this tissue.

Each cell must touch at least one of the other cells.

- Make a large drawing of this line of **four** cells.
- Use **one** ruled label line and label to identify the cell wall of **one** cell.

[5]

(b) Fig. 1.1 is a photomicrograph of a stained transversed section through a different type of leaf from **J1**.

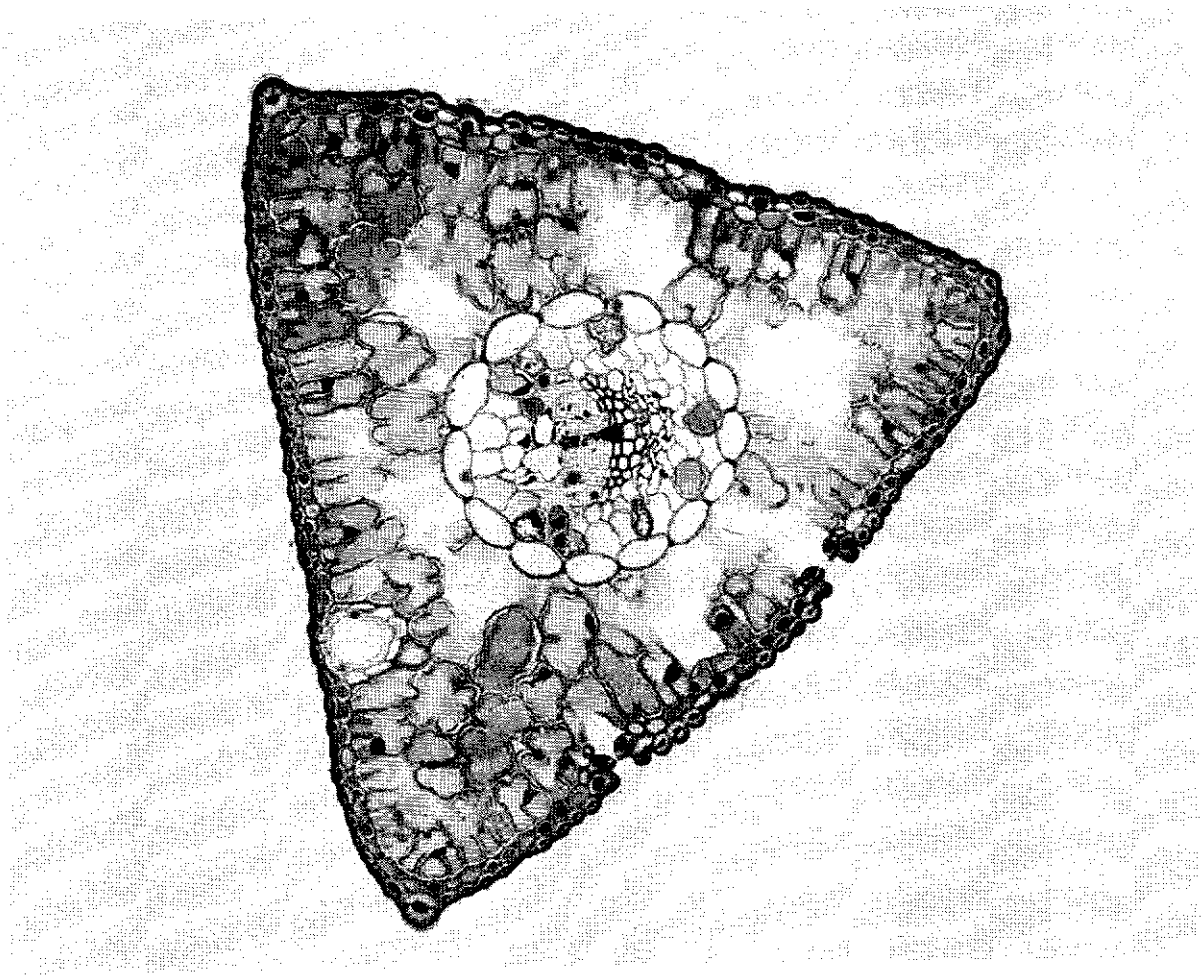

Fig. 1.1

Identify three observable differences between the leaf section on **J1** and the leaf section in Fig. 1.1. Record these three observable differences in Table 1.1.

Table 1.1

Feature	J1	Fig 1.1

[3]

(c) Fig. 1.2 is the same photomicrograph as that in Fig. 1.1, with the line **X – Y** drawn across its width.

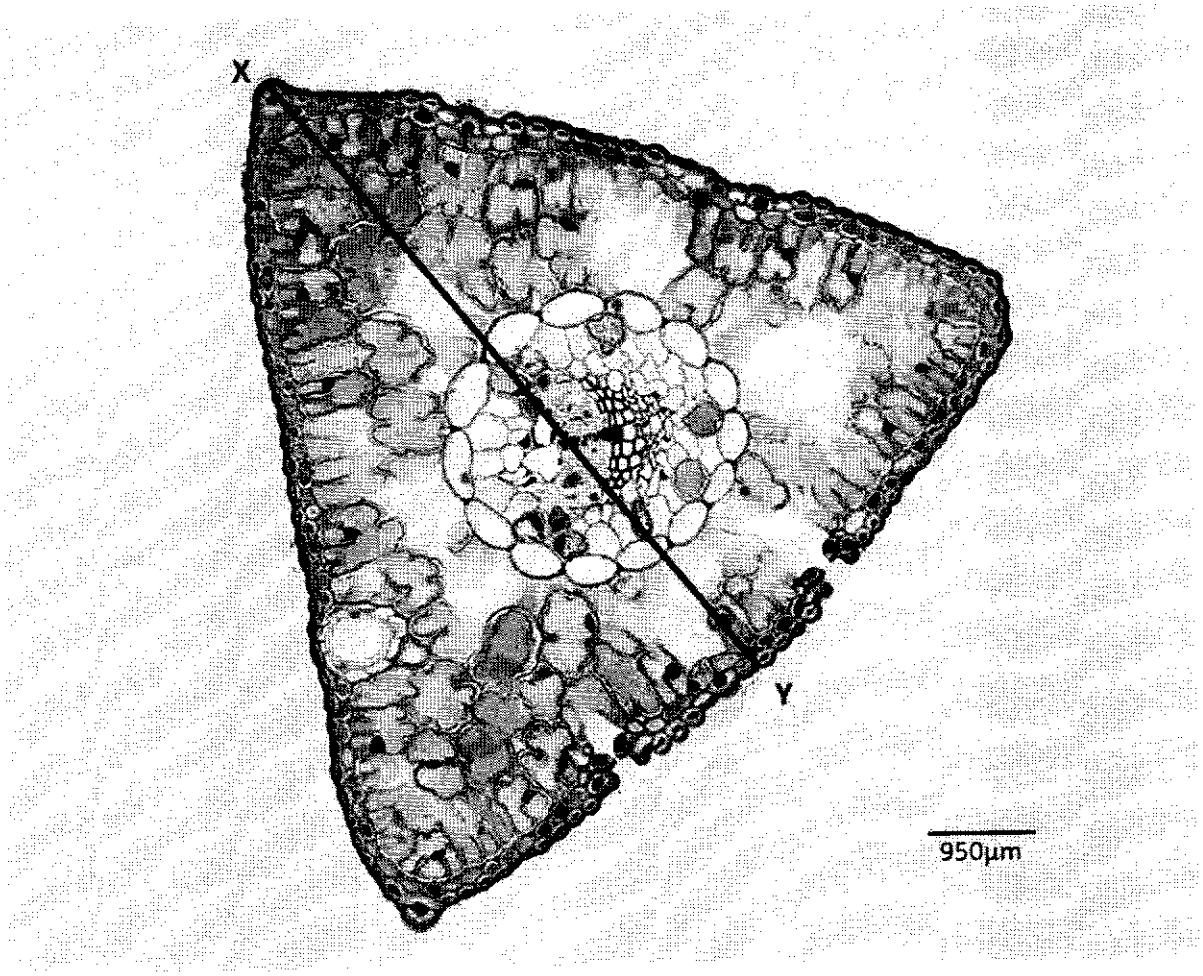

Fig. 1.2

In Fig. 1.2 the line **X – Y** is drawn across the width of the leaf. Use the line X – Y and the scale bar to calculate the actual width of the leaf.

Show your working.

actual width of leaf = ... [4]

Several studies have suggested that shifts in stomatal densities in pine leaves can be attributed to rising atmospheric carbon dioxide concentrations and climate change.

A student decided to investigate the differences by observing 6 pine leaves from the *Pinus* genus under a light microscope.

Table 1.2 shows the student's results.

Table 1.2

	P. taeda	*P. ponderosa*
Mean stomatal density	114.64 ± 10.51	52.11 ± 3.35

The formula for t-test is:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}} \qquad v = n_1 + n_2 - 2$$

Key to symbols

s = standard deviation
$\bar{x}$ = mean
n = sample size (number of observations)
v = degrees of freedom

(i) Complete the calculation to find the value of t for the stomatal densities. Show your working.

t = $\dfrac{\text{-}}{\sqrt{\dfrac{}{6} + \dfrac{}{6}}}$

= $\dfrac{}{4.50}$

t = _____ [3]

Table 1.2 shows the critical values for the *t*-test.

Table 1.2

Degrees of freedom	Significance level					
	20% (0.20)	10% (0.10)	5% (0.05)	2% (0.02)	1% (0.01)	0.1% (0.001)
1	3.078	6.314	12.706	31.821	63.657	636.619
2	1.886	2.920	4.303	6.965	9.925	31.598
3	1.638	2.353	3.182	4.541	5.841	12.941
4	1.533	2.132	2.776	3.747	4.604	8.610
5	1.476	2.015	2.571	3.365	4.032	6.859
6	1.440	1.943	2.447	3.143	3.707	5.959
7	1.415	1.895	2.365	2.998	3.499	5.405
8	1.397	1.860	2.306	2.896	3.355	5.041
9	1.383	1.833	2.262	2.821	3.250	4.781
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11	1.363	1.796	2.201	2.718	3.106	4.437
12	1.356	1.782	2.179	2.681	3.055	4.318
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15	1.341	1.753	2.131	2.602	2.947	4.073
16	1.337	1.746	2.120	2.583	2.921	4.015
17	1.333	1.740	2.110	2.567	2.898	3.965
18	1.330	1.734	2.101	2.552	2.878	3.922
19	1.328	1.729	2.093	2.539	2.861	3.883
20	1.325	1.725	2.086	2.528	2.845	3.850
21	1.323	1.721	2.080	2.518	2.831	3.819
22	1.321	1.717	2.074	2.508	2.819	3.792
23	1.319	1.714	2.069	2.500	2.807	3.767
24	1.318	1.711	2.064	2.492	2.797	3.745
25	1.316	1.708	2.060	2.485	2.787	3.725
26	1.315	1.706	2.056	2.479	2.779	3.707
27	1.314	1.703	2.052	2.473	2.771	3.690
28	1.313	1.701	2.048	2.467	2.763	3.674
29	1.311	1.699	2.043	2.462	2.756	3.659
30	1.310	1.697	2.042	2.457	2.750	3.646
40	1.303	1.684	2.021	2.423	2.704	3.551
60	1.296	1.671	2.000	2.390	2.660	3.460
120	1.289	1.658	1.980	2.158	2.617	3.373
∞	1.282	1.645	1.960	2.326	2.576	3.291

(ii) State and explain the meaning of the results.

.....

.....

.....

.....

.....

[2]

[Total: 23]

2 You are required to investigate the effect of concentration of hydrochloric acid (independent variable) on the rate of diffusion.
The investigation involves placing agar cubes containing an indicator into dilute hydrochloric acid. As the acid diffuses into the agar cubes the indicator changes colour.

You are provided with the materials shown in Table 2.1.

Table 2.1

labelled	contents	hazard	volume / cm^3
H	1.0 mol dm^{-3} hydrochloric acid	irritant	100
A	agar	none	–
W	distilled water	none	60

It is recommended that you wear suitable eye protection.

If **H** comes into contact with your skin, wash it off immediately under cold water.

You must not touch the agar with your hands. Use the blunt forceps and paper towel to handle the agar.

You will need to carry out a trial test (step 1 to step 4) before you start your investigation.

Read step 1 to step 4 before proceeding.

1. Cut 3 cubes from the agar, **A**, as shown in Fig. 2.1. Each cube should be approximately 5 mm × 5 mm x 5 mm but they must all have the same dimensions. Cut the cubes from **A** on the white tile provided.

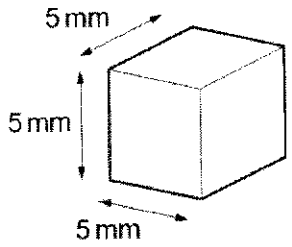

Fig. 2.1

2. Put 10 cm^3 of 1.0 mol dm^{-3} hydrochloric acid, **H**, into a beaker.

3. Put the 3 cubes you cut in step 1 into the beaker containing **H**, using blunt forceps. Start timing.

   As the acid diffuses into the agar cubes they change colour. The end-point is reached when the blue-green colour disappears and the **whole cube** has changed colour from blue-green to pink.

   Putting the beaker on the white card provided may help you to see the colour changes more clearly.

4. Measure the time taken for each cube to reach the end-point and record the times in **(a)(i)**. If any cube remains blue-green after 3 minutes, record as 'more than 180'.

(a) **(i)** Record your results in an appropriate table.

[2]

(ii) Calculate the mean time taken for the cubes to reach the end-point.

mean time = ... [1]

The student investigated the effect of concentration of hydrochloric acid on the rate of diffusion and suggested the hypothesis:

> Lowering the concentration of hydrochloric acid below 1.0 mol dm^{-3} will have no effect on the rate of diffusion of acid into the agar cubes.

You will need to use simple (proportional) dilution of the 1.0 mol dm^{-3} hydrochloric acid, **H**, to prepare four further concentrations of hydrochloric acid.

You need to prepare 10.0 cm^3 of each concentration.

(iii) Draw a table to show how you will prepare these concentrations.

Table 2.2

volume of 1.0 mol dm^{-3} hydrochloric acid / cm^3	volume of distilled water, **W** / cm^3	final concentration of hydrochloric acid / mol dm^{-3}

[2]

Read step 5 to step 10 before proceeding.

5. Prepare the concentrations of hydrochloric acid, as shown in Table 2.2, in the containers provided.

6. Cut three cubes, each approximately 5 mm × 5 mm × 5 mm, from **A**. Cut the cubes on the white tile provided.

7. Put the 3 cubes into the container containing 1.0 mol dm^{-3} hydrochloric acid, **H**, and immediately start timing. Do **not** stir.

8. Measure the time taken for each cube to reach the end-point and record the times in **(a)(iv)**. If any cube remains blue-green after 3 minutes record as 'more than 180'.

9. Repeat step 6 to step 8 for each of the other concentrations of hydrochloric acid you have prepared.

10. Calculate the mean time taken for each concentration and record the mean times in **(a)(iv)**.

(iv) Record your results and mean times in an appropriate table.

[3]

(v) Using your mean times, calculate the rate of diffusion when the concentration of hydrochloric acid is 1.0 mol dm^{-3} **and** in the lowest concentration of hydrochloric acid you have investigated.

rate in 1.0 mol dm^{-3} hydrochloric acid ..... s^{-1}

rate in the lowest concentration ..... s^{-1}

[1]

The student's hypothesis stated that:

Lowering the concentration of hydrochloric acid below 1.0 mol dm^{-3} will have no effect on the rate of diffusion of acid into the agar cubes.

(vi) State whether you **support** or **reject** this hypothesis.
Explain how your results provide evidence for this decision.

support or reject

.....

explanation

.....

.....

[1]

(vii) Explain **two** significant sources of error in this investigation.

.....

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[4]

(viii) Describe **two** modifications to this investigation which would improve the confidence in your results.

.....

.....

.....

[2]

(b) A scientist carried out an experiment to investigate whether a chemical, **C**, extracted from the flowers of a plant, was able to inhibit the reproduction of pathogenic bacteria.

The scientist prepared 5 Petri dishes containing agar (agar plates) which had each been inoculated with a different type of pathogenic bacterium.

A filter paper disc, soaked in chemical **C**, was put onto each agar plate. Chemical **C** diffused from the filter paper disc into the agar. The agar plates were incubated at 25 °C to allow the bacteria in the agar to reproduce.

A clear zone, called a zone of inhibition, is observed around the filter paper disc if the chemical is effective at preventing bacteria from reproducing, as shown in Fig. 2.2.

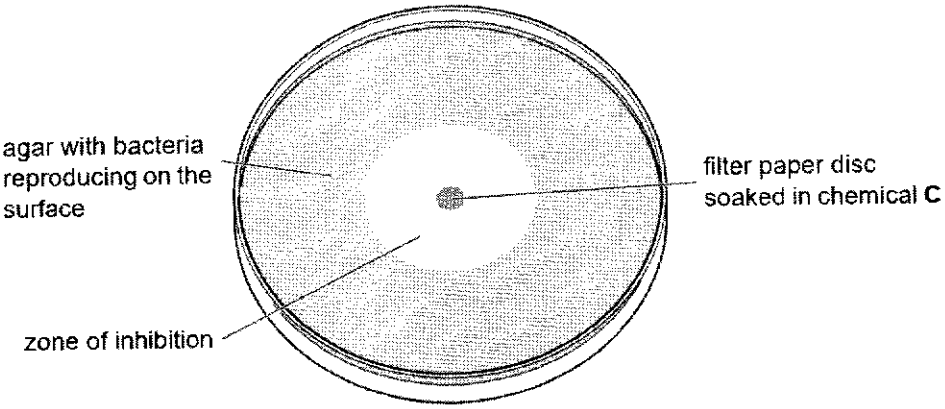

Fig. 2.2

The scientist measured the diameter of the zone of inhibition produced in the agar for each of the 5 different types of pathogenic bacterium.

The results are shown in Table 2.3.

Table 2.3

type of pathogenic bacterium	diameter of zone of inhibition /mm
P	7.0
Q	24.0
R	18.0
S	15.5
T	19.0

(i) Draw a bar chart of the data in Table 2.3 on the grid in Fig. 2.3.
Each bar should be separated for each type of pathogenic bacterium.

Use a sharp pencil for drawing bar charts.

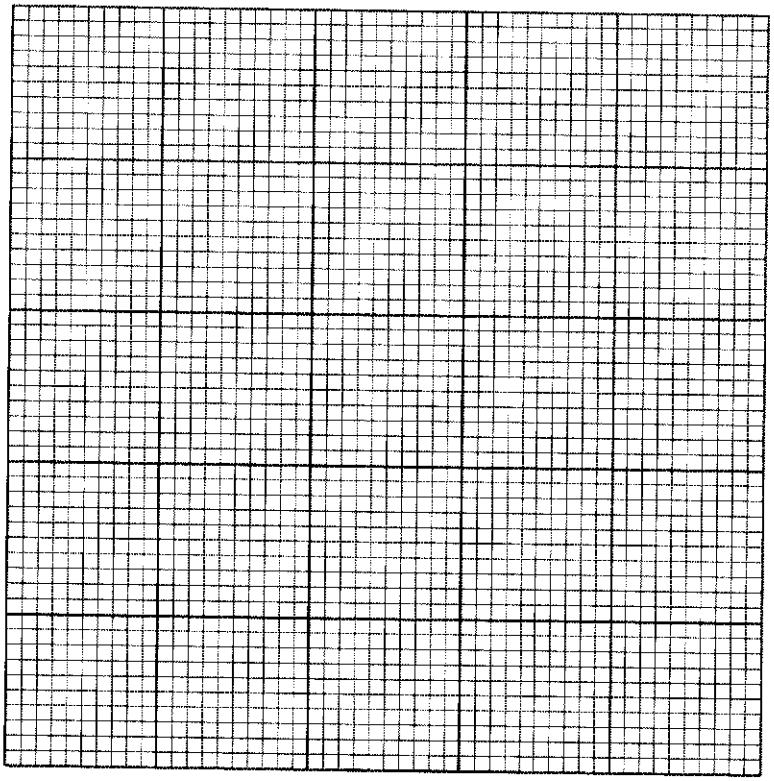

Fig. 2.3

[4]

(ii) Suggest how chemical **C** may act as an antibiotic.

.....

.....

.....

.....

[2]

(c) Due to the increasing problem of antibiotic resistance, the scientist wanted to learn more about the effects of chemical **C**. He wanted to find out the most effective concentration of chemical **C** that can inhibit the growth of this bacterium, which is determined by the size of the zone of inhibition.

Bacterium will be spread onto the agar plate and incubated at 25°C to produce an evenly distributed growth of the bacteria (a bacterial lawn).

Design an experiment to determine the **lowest concentration** of chemical **C** that will give the largest zone of inhibition.

You are required to decide on an appropriate dilution method.

In your plan you **must** use:

- prepared agar plates with bacteria lawn
- $30cm^3$ 100mg cm^{-3} chemical C
- $100cm^3$ distilled water
- 5mm filter paper disc

You may select from the following apparatus and plan to use appropriate additional apparatus:

- sterile swabs and forceps
- disinfectant solution
- normal laboratory glassware, e.g. test-tubes, boiling tubes, beakers, measuring cylinders,
- graduated pipettes, glass rods, etc.
- incubator
- autoclave (a pressurised oven for heating sterilizing apparatus and materials)
- Bunsen burner
- Parafilm
- ruler
- syringes
- timer, e.g. stopwatch

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it
- be illustrated by relevant diagrams, if necessary
- identify the dependent variable and the independent variable
- identify the variables you will need to control
- use the correct technical and scientific terms
- indicate how the results will be recorded and analysed
- indicate the safety measures to minimize the risks

[10]
[Total: 32]

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Higher 2

CANDIDATE NAME **ANSWERS**

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BIOLOGY **9744/04**

Paper 4 Practical **22 August 2024**

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For Examiner's Use	
1	**23**
2	**32**
Total	**55**

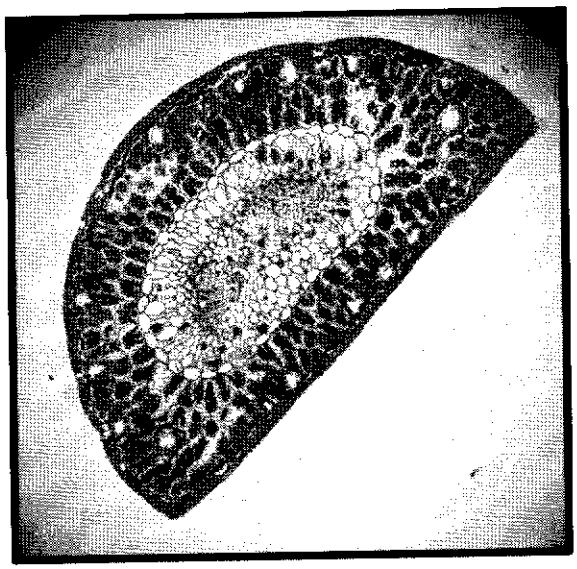
This document consists of **16** printed pages and **0** blank pages.

[Turn over

Answer **all** the questions.

1 **J1** is a slide of a stained transverse section through a plant leaf.

(a) (i) Draw a large plan diagram of the whole section on **J1**. Use a sharp pencil. Use **one** ruled label line and label to identify the epidermis.



1 label line and label to epidermis ;
2 uses most of the available space and no shading (2/3 of space given) ;
3 whole leaf section drawn and no cells drawn ; i resin chambers drawn
4 correct shape of the leaf ;
5 4 layers;
6 draws stomata opening into an air space;

[5]

(ii) The leaf section on **J1** is from a plant that lives at high altitudes in very cold conditions. In the winter the ground is often frozen and plants are unable to take up water.

Suggest **one** observable feature of the leaf section on **J1** which enables it to survive these conditions.

Cuticle;
Few/ sunken stomata;
.....
[1]

(iii) Observe the cells in the epidermis of the section on **J1**.

Select a line of four adjacent cells that make up this tissue.

Each cell must touch at least one of the other cells.

- Make a large drawing of this line of **four** cells.
- Use **one** ruled label line and label to identify the cell wall of **one** cell.

1. uses most of the available space and all lines sharp and continuous ;
2. draws only four whole cells and each cell touches at least one other cell
3. cell wall drawn as two lines around each cell and three lines where cells touch ;
4. correct cell shape/ proportion
5. label line and label to one cell wall ;
6. draws inclusions in at least two cells ;

[5]

(b) Fig. 1.1 is a photomicrograph of a stained transversed section through a different type of leaf from **J1**.

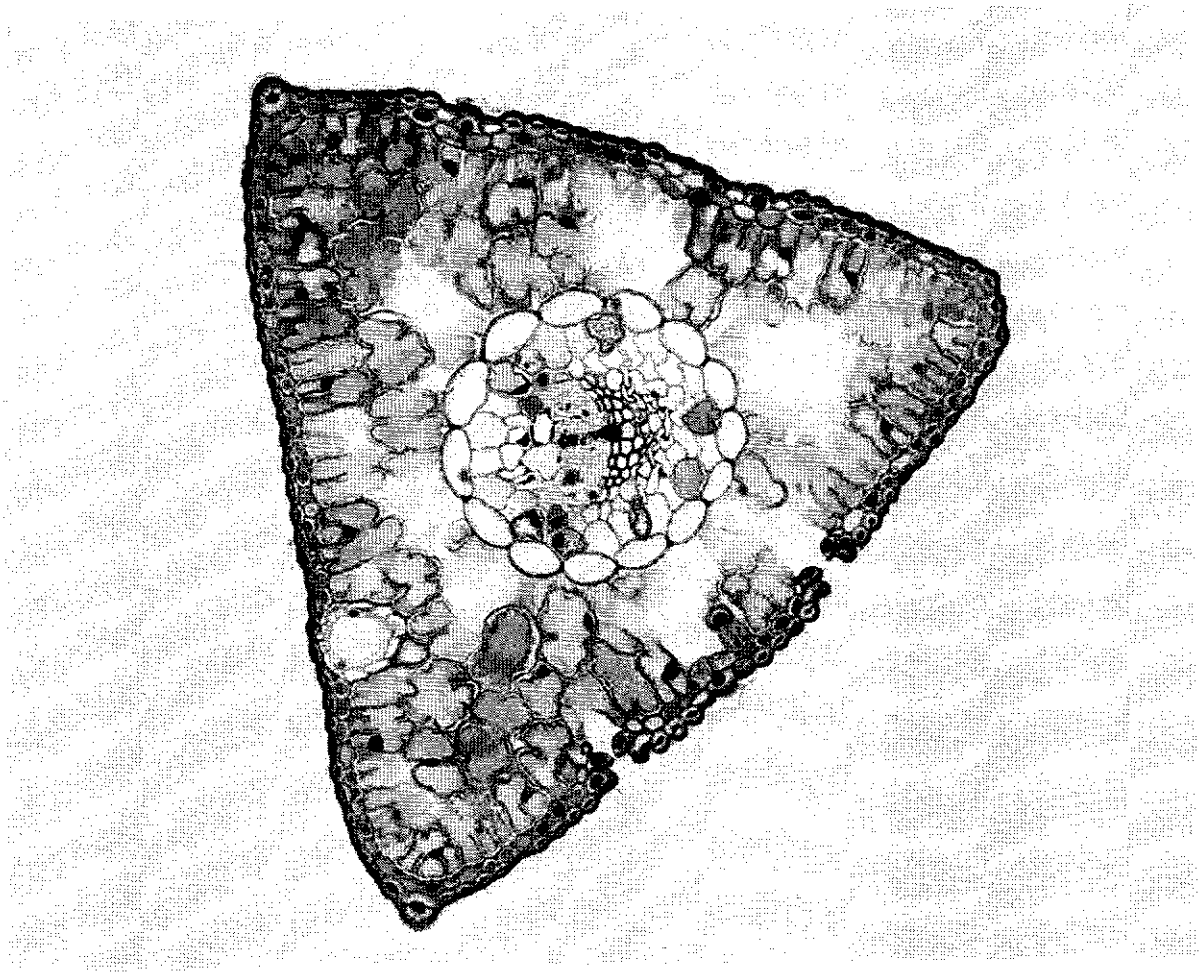
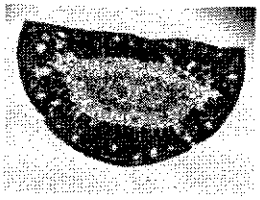
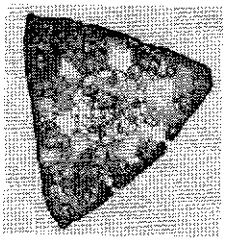


Fig. 1.1

Identify three observable differences between the leaf section on **J1** and the leaf section in Fig. 1.1.
Record these three observable differences in Table 1.1.

Table 1.1

Feature	**J1**	**Fig 1.1**
		
Vascular tissue/ number of bundles	Larger in proportion to the whole leaf; 2	smaller in proportion to the whole leaf; 1
Endothelium/ middle tissue structure	Oval	Circle
Air spaces	Few/ Smaller in proportion to leaf section	More/ Larger in proportion to leaf section
Shape of leaf	Semi-circle/ half circle;	Triangle;
Stomata	More	Fewer;
Epidermal cell shape	Squarish	rounded

[3]

(c) Fig. 1.2 is the same photomicrograph as that in Fig. 1.1, with the line **X – Y** drawn across its width.

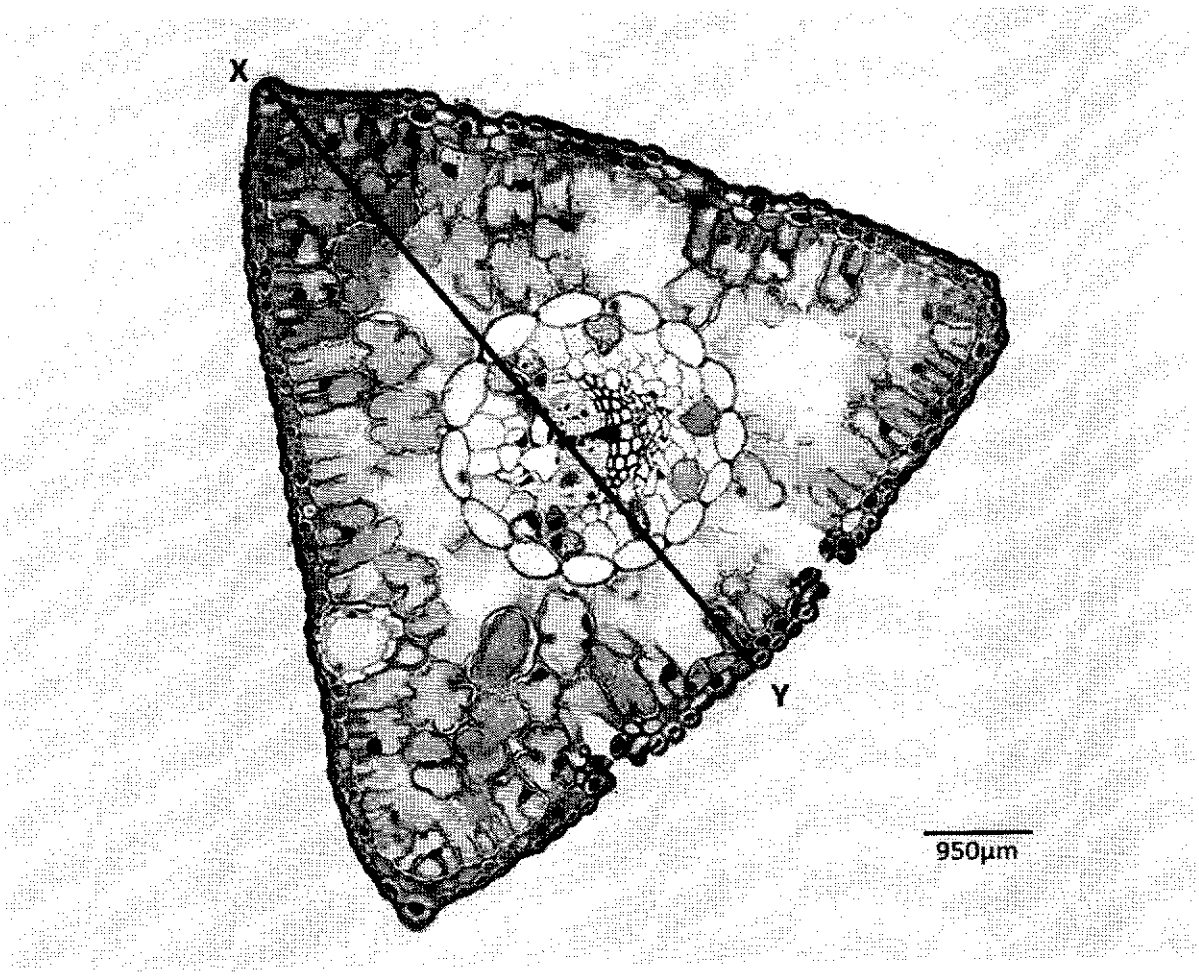


Fig. 1.2

In Fig. 1.2 the line **X – Y** is drawn across the width of the leaf. Use the line X – Y and the scale bar to calculate the actual width of the leaf.

Show your working.

Scale bar = 1.5cm;
X—Y = 10.5cm

(10.5 / 1.5) x 950um = 6650um or 950/1.5 x 10.5

1. measures and records the correct length of the scale bar ; **(1.5cm)**
2. shows the length of scale bar divided by 950 ; **(1.5/950)**
3. shows the length of line **X.Y** divided by the answer to mp2 ; **(10.5 / (1.5/950))**
4. records the correct width of the leaf and units (µm) ; **(6650µm)**

@ 10.6; 6713µm (whole no.)
@ 10.7; 6776µm (whole no.)

actual width of leaf = ... **[4]**

Several studies have suggested that shifts in stomatal densities in pine leaves can be attributed to rising atmospheric carbon dioxide concentrations and climate change.

A student decided to investigate the differences by observing 6 pine leaves from the *Pinus* genus under a light microscope.

Table 1.2 shows the student's results.

Table 1.2

	P. taeda	*P. ponderosa*
Mean stomatal density	114.64 ± 10.51	52.11 ± 3.35

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$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}} \qquad v = n_1 + n_2 - 2$$

Key to symbols

s = standard deviation
$\bar{x}$ = mean
n = sample size (number of observations)
v = degrees of freedom

(i) Complete the calculation to find the value of t for the stomatal densities. Show your working.

Source: https://academic.oup.com/jxb/article/52/355/369/558373
9700 w23 qp 53
9700_w10_qp_51

$$t = \frac{\mathbf{114.64 - 52.11}}{\sqrt{\frac{(10.51)^2}{6} + \frac{(3.35)^2}{6}}}$$

$(10.51)^2$ /6 + $(3.35)^2$ / 6;

62.53/ (4.50);

t = **13.896**
(3 dp follow t table)

$$= \frac{\mathbf{62.53}}{4.50}$$

t = _____ [3]

Table 1.2 shows the critical values for the *t*-test.

Table 1.2

Degrees of freedom	Significance level					
	20% (0.20)	10% (0.10)	5% (0.05)	2% (0.02)	1% (0.01)	0.1% (0.001)
1	3.078	6.314	12.706	31.821	63.657	636.619
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9	1.383	1.833	2.262	2.821	3.250	4.781
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22	1.321	1.717	2.074	2.508	2.819	3.792
23	1.319	1.714	2.069	2.500	2.807	3.767
24	1.318	1.711	2.064	2.492	2.797	3.745
25	1.316	1.708	2.060	2.485	2.787	3.725
26	1.315	1.706	2.056	2.479	2.779	3.707
27	1.314	1.703	2.052	2.473	2.771	3.690
28	1.313	1.701	2.048	2.467	2.763	3.674
29	1.311	1.699	2.043	2.462	2.756	3.659
30	1.310	1.697	2.042	2.457	2.750	3.646
40	1.303	1.684	2.021	2.423	2.704	3.551
60	1.296	1.671	2.000	2.390	2.660	3.460
120	1.289	1.658	1.980	2.158	2.617	3.373
∞	1.282	1.645	1.960	2.326	2.576	3.291

(ii) State and explain the meaning of the results.

(at 10 degrees of freedom and p=0.05)

1. Calculated t value of 13.89 is greater than critical value of 2.228;
   OR the probability at which the calculated t value lies is less than 0.001;

No mark awarded if both points are written but they contradict each other

2. the difference between the means is significant and not due to chance;

[2]

[Total: 23]

2 You are required to investigate the effect of concentration of hydrochloric acid (independent variable) on the rate of diffusion.

The investigation involves placing agar cubes containing an indicator into dilute hydrochloric acid. As the acid diffuses into the agar cubes the indicator changes colour.

You are provided with the materials shown in Table 2.1.

Table 2.1

labelled	contents	hazard	volume / cm^3
H	1.0 mol dm^{-3} hydrochloric acid	irritant	100
A	agar	none	–
W	distilled water	none	60

It is recommended that you wear suitable eye protection.

If **H** comes into contact with your skin, wash it off immediately under cold water.

You must not touch the agar with your hands. Use the blunt forceps and paper towel to handle the agar.

You will need to carry out a trial test (step 1 to step 4) before you start your investigation.

Read step 1 to step 4 before proceeding.

1. Cut 3 cubes from the agar, **A**, as shown in Fig. 2.1. Each cube should be approximately 5 mm × 5 mm x 5 mm but they must all have the same dimensions. Cut the cubes from **A** on the white tile provided.

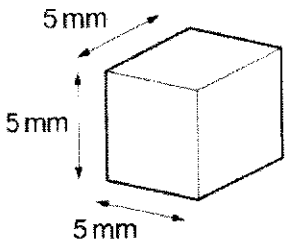


Fig. 2.1

2. Put 10 cm^3 of 1.0 mol dm^{-3} hydrochloric acid, **H**, into a container.

3. Put the 3 cubes you cut in step 1 into the container containing **H**, using blunt forceps. Start timing.

   As the acid diffuses into the agar cubes they change colour. The end-point is reached when the blue-green colour disappears and the **whole cube** has changed colour from blue-green to pink.

   Putting the container on the white card provided may help you to see the colour changes more clearly.

4. Measure the time taken for each cube to reach the end-point and record the times in **(a)(i)**. If any cube remains blue-green after 3 minutes, record as 'more than 180'.

(a) **(i)** Record your results in an appropriate table.

T - records 3 times under 180s ;
P - seconds recorded as whole numbers ;

[2]

(ii) Calculate the mean time taken for the cubes to reach the end-point.

calculates mean time correctly using results from (a)(i) + units (s / seconds) R sec ;

mean time = ... [1]

The student investigated the effect of concentration of hydrochloric acid on the rate of diffusion and suggested the hypothesis:

> Lowering the concentration of hydrochloric acid below 1.0 mol dm^{-3} will have no effect on the rate of diffusion of acid into the agar cubes.

You will need to use simple (proportional) dilution of the 1.0 mol dm^{-3} hydrochloric acid, **H**, to prepare four further concentrations of hydrochloric acid.

You need to prepare 10.0 cm^3 of each concentration.

(iii) Draw a table to show how you will prepare these concentrations.

Table 2.2

volume of 1.0 mol dm^{-3} hydrochloric acid / cm^3	volume of distilled water, **W** / cm^3	final concentration of hydrochloric acid / mol dm^{-3}

[2]

V - correct volumes of HCl : DW (10/0, 8/2, 6/4, 4/6, 2/8);
C - 5 Conc: 1.0, 0.8, 0.6, 0.4, 0.2

Read step 5 to step 10 before proceeding.

5. Prepare the concentrations of hydrochloric acid, as shown in Table 2.2, in the containers provided.

6. Cut three cubes, each approximately 5 mm × 5 mm × 5 mm, from **A**. Cut the cubes on the white tile provided.

7. Put the 3 cubes into the container containing 1.0 mol dm^{-3} hydrochloric acid, **H**, and immediately start timing. Do **not** stir.

8. Measure the time taken for each cube to reach the end-point and record the times in **(a)(iv)**. If any cube remains blue-green after 3 minutes record as 'more than 180'.

9. Repeat step 6 to step 8 for each of the other concentrations of hydrochloric acid you have prepared.

10. Calculate the mean time taken for each concentration and record the mean times in **(a)(iv)**.

(iv) Record your results and mean times in an appropriate table.

[H] headings are concentration of hydrochloric acid / mol dm^{-3} + time to reach end-point / s ;
[M] records 4 mean times for 4 concentrations of hydrochloric acid ;
[T] correct trend with the time for the highest concentration recorded as the shortest time ;

Concentration of HCl / $moldm^{-3}$	Time taken for each cube to reach end-point / s			
	Trial 1	Trial 2	Trial 3	Mean

[3]

(v) Using your mean times, calculate the rate of diffusion when the concentration of hydrochloric acid is 1.0 mol dm^{-3} **and** in the lowest concentration of hydrochloric acid you have investigated.

Using values from the candidates' results in **(a)(iv)** +
calculates correct rate of diffusion in 1.0 mol dm^{-3} hydrochloric acid **and** correct rate of diffusion for the lowest concentration of hydrochloric acid ;

rate in 1.0 mol dm^{-3} hydrochloric acid ..... s^{-1}

rate in the lowest concentration ..... s^{-1}

[1]

The student's hypothesis stated that:

Lowering the concentration of hydrochloric acid below 1.0 mol dm^{-3} will have no effect on the rate of diffusion of acid into the agar cubes.

(vi) State whether you **support** or **reject** this hypothesis.
Explain how your results provide evidence for this decision.

support or reject _____

explanation _____

_____

_____

[1]

correct statement concerning the rejection or supporting the hypothesis based on the results +

correct explanation of how results provide evidence for rejection or supporting the hypothesis ;

.....

(vii) Explain **two** significant sources of error in this investigation.

.....

.....

.....

..... [4]

1. Range of HCl concentrations is too narrow + insufficient to observe for significant changes in rate of diffusion;
2. Visual determination of the decolourisation of dye / colour disappearance + is subjective/difficult to judge;
3. Three pieces of agar put in at different times/ only started timing when last piece of agar was put in + thus timing might be inaccurate ;
4. Sizes of the agar pieces may vary slightly due to manual cutting method + leading to different surface areas of agar exposed to HCl;
5. Agitation/stirring of the pieces of agar in HCl varies/not the same + affects speed of decolourisation;
6. Pieces of agar with stained side down/ stick together + ascorbic acid not reaching indicator

(viii) Describe **two** modifications to this investigation which would improve the confidence in your results.

.....

.....

..... [2]

1. Wider or different or larger intervals between concentrations used; (cie: at least 5 different conc)
2. Do a positive control (i.e. agar piece that has completely turned pink) to determine end-point more accurately);
3. Stagger start time for the different concentrations (to prevent timing error / increase accuracy of measuring time taken for colour change);
4. Cut agar block on graph paper using grid lines as a guide (to ensure same dimensions of agar blocks) / OWTTE
5. Use electric or automatic shaker (to standardise stirring method);

(b) A scientist carried out an experiment to investigate whether a chemical, **C**, extracted from the flowers of a plant, was able to inhibit the reproduction of pathogenic bacteria.

The scientist prepared 5 Petri dishes containing agar (agar plates) which had each been inoculated with a different type of pathogenic bacterium.

A filter paper disc, soaked in chemical **C**, was put onto each agar plate. Chemical **C** diffused from the filter paper disc into the agar. The agar plates were incubated at 25 °C to allow the bacteria in the agar to reproduce.

A clear zone, called a zone of inhibition, is observed around the filter paper disc if the chemical is effective at preventing bacteria from reproducing, as shown in Fig. 2.2.

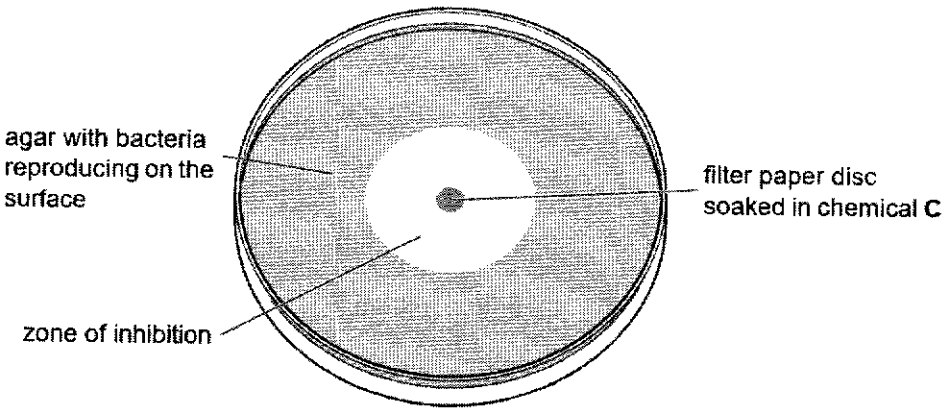


Fig. 2.2

The scientist measured the diameter of the zone of inhibition produced in the agar for each of the 5 different types of pathogenic bacterium.

The results are shown in Table 2.3.

Table 2.3

type of pathogenic bacterium	diameter of zone of inhibition / mm
P	7.0
Q	24.0
R	18.0
S	15.5
T	19.0

(i) Draw a bar chart of the data in Table 2.3 on the grid in Fig. 2.3.
Each bar should be separated for each type of pathogenic bacterium.

Use a sharp pencil for drawing bar charts.

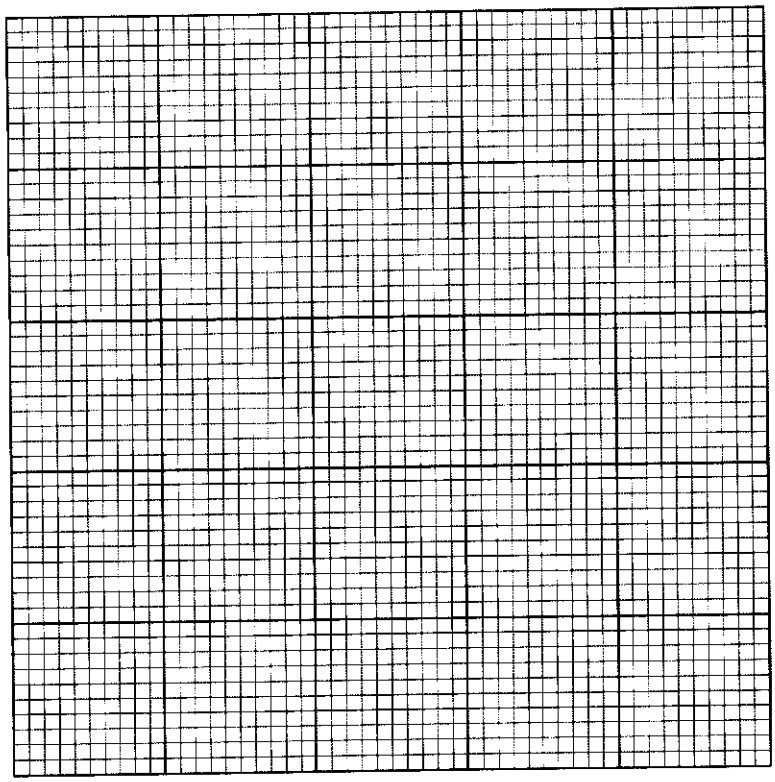


Fig. 2.3

[4]

[A]	label on *x*-axis as "type of pathogenic bacteria "+ label on *y*-axis as "diameter of zone of inhibition /mm" ;
[B]	even width of bars + scale on *y*-axis is 5 to 2 cm + labelled each 2 cm ;
[P]	correct plotting of 5 bars ;
[L]	separate bars drawn with vertical lines meeting horizontal lines + labelled **P**, **Q**, **R**, **S**, **T** ;

(ii) Suggest how chemical **C** may act as an antibiotic.

1 correct reference to cell, wall / membrane ;
2 cell / bacterial lysis or cells / bacteria burst ;
3 idea of inhibition of transcription / translation / protein synthesis ;
4 idea of inhibition of cell division ;
5 acts as an enzyme inhibitor ;
idea of inhibiting DNA replication / synthesis ; [Max 2]

.....

.....

.....

.....

[2]

(c) Due to the increasing problem of antibiotic resistance, the scientist wanted to learn more about the effects of chemical **C**. He wanted to find out the most effective concentration of chemical **C** that can inhibit the growth of this bacterium, which is determined by the size of the zone of inhibition.

Bacterium will be spread onto the agar plate and incubated at 25°C to produce an evenly distributed growth of the bacteria (a bacterial lawn).

Design an experiment to determine the **lowest concentration** of chemical C that will give the largest zone of inhibition.

You are required to decide on an appropriate dilution method.

In your plan you **must** use:

- prepared agar plates with bacteria lawn
- $30cm^3$ 100mg cm^{-3} chemical C
- $100cm^3$ distilled water
- 5mm filter paper disc

You may select from the following apparatus and plan to use appropriate additional apparatus:

- sterile swabs and forceps
- disinfectant solution
- normal laboratory glassware, e.g. test-tubes, boiling tubes, beakers, measuring cylinders,
- graduated pipettes, glass rods, etc.
- incubator
- autoclave (a pressurised oven for heating sterilizing apparatus and materials)
- Bunsen burner
- Parafilm
- ruler
- syringes
- timer, e.g. stopwatch

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it
- be illustrated by relevant diagrams, if necessary
- identify the dependent variable and the independent variable
- identify the variables you will need to control
- use the correct technical and scientific terms
- indicate how the results will be recorded and analysed
- indicate the safety measures to minimize the risks

[10]

[Total: 32]

Mark Scheme

IV	Independent variable: States **5 concentrations** of C / mg cm^{-3} (ie. 100, 50, 25, 12.5, 6.25/ 100, 80, 60, 40,20)
DV	Diameter of zone of inhibition / mm, measured with a ruler
CV1 CV2 CV3	Variables to be kept constant: [max 3] **(a)** time of soaking filter paper discs in chemical C / Vol of Chemical C, **(b)** number of filter paper discs, **(c)** duration of incubation for all 5 petri dishes being the same, 24/ 48h; **(d)** incubation at 25°C in incubator; **(e)** concentration and volume of bacterial culture, **(f)** concentration and volume of agar used, **(g)** type of bacteria
S1 S2	Serial dilution table drawn with: **(a)** Correct headers: "Volume of chemical C from previous concentration/ cm^3" **(b)** Correct dilution volumes + 1d.p *Award also for mp (b) if student has correct volumes indicating simple dilution*
A1 A2	Aseptic Techniques: [max 2] **(a)** Using disinfectant solution/ 10% bleach solution and paper towels, wipe the work area. **(b)** Turn on the Bunsen burner, (ref to) idea of working near the flame; **(c)** (ref to) use of sterile apparatus: Using a sterile forceps, gently place the singe paper disc from 100 mg cm^{-3} in the centre of the plate. **(d)** Seal the plates with Parafilm before leaving in incubator
C	For control, add a filter paper disc into 10.0 cm^3 of sterile distilled water for 10 minutes and repeat step 6.
R	Repeat steps 3-9 twice to get total of 3 results to ensure reproducibility/ to calculate mean
T	Table of results drawn with appropriate headers (ECF wo mean)
G	Graph of "mean diameter of clear zone" against "concentration of chemical C" sketched (ECF wo mean)
M	Indicate on graph, lowest concentration of chemical C that gives maximum diameter of clear zone.
R1 R2	Risks and Precautions: [Max 2] **(a)** Wear disposable sterile gloves and goggles **to prevent / protect the skin and eyes** from bacteria contact. **(b)** **To prevent any contact** with bacteria, wipe down any spillages with disinfectant solution. **(c)** To protect against **bacteria growth and potential health hazard**, there should be proper disposal and treatment of contaminated materials or equipment using sterilizer/autoclave. **(d)** Turn off Bunsen burner when not in use, to reduce the incidence of **burns**. **(e)** Handle sharps (e.g. forceps) with care to avoid cuts.

	(f) Wear heat-resistant gloves /safety glasses / lab coat. Be sure arms are covered by a lab coat and longer heat-resistant gloves to prevent burns from heat and steam from the autoclave machine.

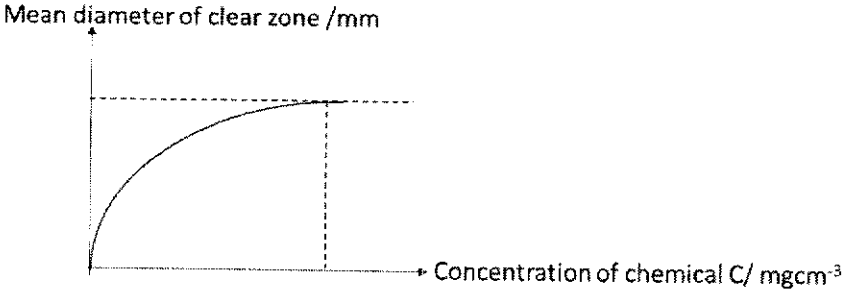
Suggested Procedure

1. Using disinfectant solution/ 10% bleach solution and paper towels, wipe the work area.
2. Prepare chemical C of 5 different concentrations using serial dilution according to the table below. Label the beakers accordingly.

Concentration of chemical C / mg cm^{-3}	Volume of chemical C from previous concentration / cm^3	Volume of distilled water / cm^3	Total volume / cm^3
100	10.0	0.0	10.0
50	5.0	5.0	10.0
25	5.0	5.0	10.0
12.5	5.0	5.0	10.0
6.25	5.0	5.0	10.0

3. Place one filter paper disc into each of the beakers to soak for 10 minutes.
4. Label one agar plate as '100 mg cm^{-3}' on the base of the agar plate using a marker.
5. Turn on the Bunsen burner. Put the agar plate near the flame.
6. Using a sterile forcep, gently place the singe paper disc from 100 mg cm^{-3} in the centre of the plate.
7. Repeat steps 4-6 for the other concentrations of chemical C.
8. For a control setup, add a filter paper disc into 10.0 cm^3 of sterile distilled water for 10 minutes and repeat step 6.
9. Seal the plates with Parafilm and leave in incubator set at 25°C for 24/48 hours.
10. Repeat steps 3-9 twice to get total of 3 results to ensure reproducibility/ to calculate mean.
11. Without opening the lid, measure the diameter of the clear zone around each disc using a ruler.
12. Calculate the mean diameter of the clear zone for each concentration of chemical C.
13. Record the results in a table and plot a graph of 'mean diameter of the clear zone' against 'concentration of chemical C'.

Concentration of chemical C / mg cm^{-3}	Diameter of clear zone /mm			
	1	2	3	Mean
100				
50				
25				
12.5				
6.25				



14. Obtain the concentration of the effective concentration of chemical C from the graph. The concentration before the graph plateau off is the lowest concentration of chemical C that will give the largest zone of inhibition.

Risks and Precautions

Wear disposable sterile gloves and goggles to prevent protect the skin and eyes from bacteria contact.

To prevent any contact with bacteria, wipe down any spillages with disinfectant solution.

To protect against bacteria growth and potential health hazard, there should be proper disposal and treatment of contaminated materials or equipment using sterilizer/autoclave.

Turn off Bunsen burner when not in use, to reduce the incidence of burns.

Handle sharps (e.g. forceps) with care to avoid cuts.

Wear heat-resistant gloves /safety glasses / lab coat. Be sure arms are covered by a lab coat and longer heat-resistant gloves to prevent burns from heat and steam from the autoclave machine.