

NATIONAL JUNIOR COLLEGE, SINGAPORE  
Senior High 2  
Preliminary Examination  
Higher 2

CANDIDATE  
NAME

BIOLOGY  
CLASS

REGISTRATION  
NUMBER

## Biology

**9744/04**

Paper 4 Practical

**3 September 2025**

**2 hours 30 minutes**

### READ THESE INSTRUCTIONS FIRST

Write your name, Biology class, and registration number 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 the brackets [ ] at the end of each question or part question.

Shift			
1	2	3	
Laboratory			
BI23	BI24	CM43	CM44

For Examiner's Use	
1	35
2	20
Total	55

This document consists of **19** printed pages and **1** blank page.

Answer **all** questions.

- 1 Plants can be categorised into sun and shade plants. Sun plants need more sunlight than shade plants for photosynthesis. Sun plants are exposed to high sunlight intensity and hence are adapted against overheating and desiccation, while shade plants have little exposure to sunlight and hence are adapted to be more efficient in absorbing light for photosynthesis.

Chloroplasts were isolated from these two types of plants and suspended in a buffer solution.

You are required to:

- immobilise chloroplast in sodium alginate beads, and
- investigate the effect of immobilised chloroplast from sun and shade plants on the rate of photosynthesis.

During the light-dependent stage of photosynthesis, hydrogen ions and electrons are transferred to hydrogen acceptor molecules, including NADP.

Potassium permanganate can be used as an indicator to monitor the rate of the light-dependent stage of photosynthesis. When potassium permanganate is reduced, it turns from purple-pink to colourless.

You are provided with:

- chloroplast suspensions **S1** and **S2** from the two types of plants
- sodium alginate solution, labelled **A**
- calcium chloride solution, labelled **C**
- dilute sulfuric acid, **H<sub>2</sub>SO<sub>4</sub>**
- potassium permanganate solution, **KMnO<sub>4</sub>**
- distilled water, labelled **W**
- a lamp.

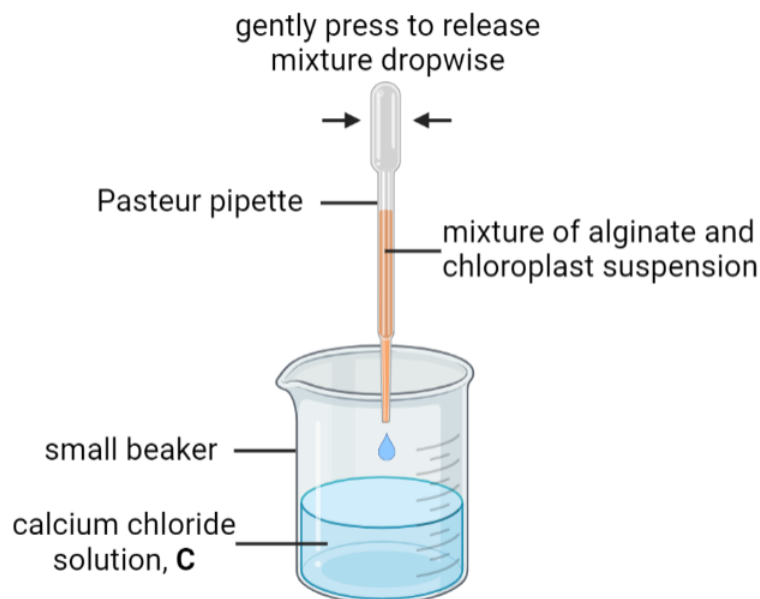
**Dilute sulfuric acid and potassium permanganate are corrosive. If they come into contact with your skin, wash them off immediately under cold water.**

Read steps **1–18** before starting the investigation.

**Proceed as follows.**

- 1 Put 20.0 cm<sup>3</sup> of **C** into a small beaker.
- 2 Put 5.0 cm<sup>3</sup> of **A** into a separate small container.
- 3 Stir the chloroplast suspension in **S1** thoroughly.
- 4 Put 5.0 cm<sup>3</sup> of **S1** into the same container as **A**. Mix well.
- 5 Use a Pasteur pipette to collect about 2.0 cm<sup>3</sup> of the mixture containing **S1** and **A**.
- 6 Suspend the Pasteur pipette over the beaker containing **C**.

- 7 Gently press the Pasteur pipette to release a drop of the mixture into **C** as shown in Fig. 1.1. The drop should form a bead that will sink to the bottom of the beaker.
- 8 Repeat step 7 to produce at least 40 beads.



**Fig. 1.1**

- 9 Using a spatula, transfer 40 beads to the Petri dish to rinse with distilled water.
- 10 Set up a lamp. Place three test tubes on a test tube rack positioned 10 cm from the lamp. **Do not switch on the lamp yet.**
- 11 Put 4.0 cm<sup>3</sup> of distilled water into each test tube.
- 12 Transfer 20 beads from the Petri dish to each of the two test tubes. The third test tube without any bead will be used as a colour standard.
- 13 Add 0.5 cm<sup>3</sup> of **H<sub>2</sub>SO<sub>4</sub>** to each test tube.
- 14 Add 0.5 cm<sup>3</sup> of **KMnO<sub>4</sub>** to each test tube.
- 15 Turn on the lamp and immediately start the stopwatch. You may use the white tile to assist with colour observation.
- 16 Record in **(a)(i)** the time taken for the purple-pink indicator to become colourless. If the purple-pink indicator solution has not become colourless after ten minutes, record the time as "more than 600".
- 17 Repeat steps 1 to 16 with chloroplast suspension **S2**.
- 18 The rate of photosynthesis can be determined by calculating the reciprocal of the time taken for the purple-pink indicator solution to become colourless.

$$\text{rate of photosynthesis} = 1000/t$$

$$t = \text{time in seconds}$$

- (a) (i) Record your results in a suitable format in the space provided, including the calculation of the mean rate of photosynthesis for the two chloroplast suspensions.

[5]

- (ii) Based on your results for (a)(i), put a tick (✓) in one box to indicate which specimen tube contains chloroplast suspension taken from the sun plant.

Give a reason for your answer.

S1 ☐ S2 ☐

[2]

- (iii) Suggest a suitable control for this experiment to show that it is the chloroplast suspension that causes the decolourisation of the indicator.

[1]

- (iv) One significant source of error in this experiment is identifying when the indicator decolourises.

Complete Table 1.1 to suggest:

- how to make an improvement in identifying when the indicator decolourises
- **one other** significant source of error in this experiment
- how to make an improvement to reduce this other significant source of error.

**Table 1.1**

significant source of error	how to make an improvement
identifying when the indicator decolourises	

[3]

- (b) In another experiment, using sun and shade leaf extracts, a student investigated the effect of varying light intensity on the rate of photosynthesis of these plants, so as to determine their light compensation points.

light intensity =  $1/d^2$ , where d represents the distance from the light source

Compensation points can be investigated using bicarbonate indicator solution. It is very sensitive to changes in carbon dioxide levels. The indicator is red when equilibrated with atmospheric carbon dioxide. The colour changes to yellow when more carbon dioxide is added. The colour changes to purple when more carbon dioxide is removed.

Table 1.2 shows the colour changes corresponding to the pH values.

**Table 1.2**

yellow	orange	red	magenta	purple
pH 7.6	pH 8.0	pH 8.4	pH 8.8	pH 9.2

Absorbance can be measured at 550 nm (wavelength of green light) using a colourimeter. The absorbance of green light increases with increased pH of bicarbonate indicator.

Using this information and your own knowledge, plan an investigation to find out the light intensity at which sun and shade plants reach their light compensation points.

You must plan to use:

- alginate beads containing sun and shade plants leaf extracts
- bicarbonate indicator solution
- colourimeter and cuvettes
- lamp.

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

- normal laboratory glassware, e.g. test-tubes, boiling tubes, beakers, measuring cylinders, glass rods, etc.
- syringes, Pasteur pipettes
- 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
- 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 any analysis of results will be carried out.







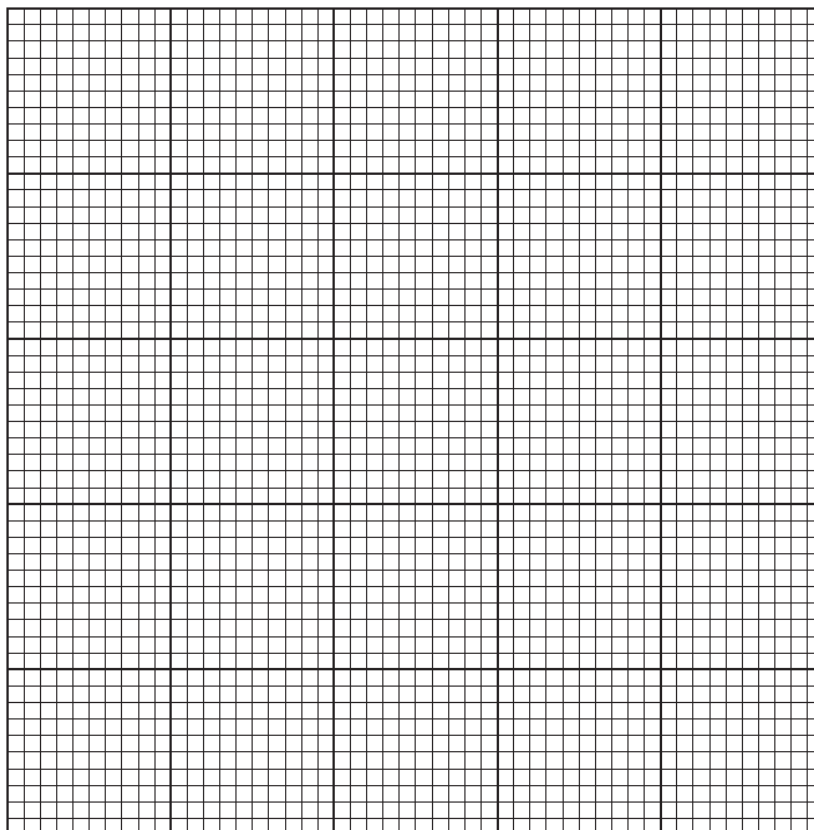
**Question 1(c) continues on page 10.**

- (c) The student conducted another experiment by varying light intensity using different light filters which allow different percentages of light to be transmitted to the experiment tubes. The results obtained are shown in Table 1.3.

**Table 1.3**

percentage of light transmitted to the experiment tube	absorbance of bicarbonate indicator at 550 nm for sun plant	absorbance of bicarbonate indicator at 550 nm for shade plant
100	0.58	0.34
75	0.35	0.33
50	0.17	0.31
25	-0.03	0.15
0	-0.18	-0.08

- (i) Plot a graph to display the students' results in the grid below.



[5]

- (ii) With reference to your graph in (c)(i), state what it is meant by light compensation point and identify the light compensation point of sun and shade plants.

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..... [2]

- (iii) Besides the difference in light compensation point, describe **two other** differences between the results for sun and shade plants.

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..... [2]

- (iv) Suggest **two** reasons for the differences between the results for sun and shade plants.

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..... [2]

- (d) Another student investigated the light compensation point of sun and shade plants. The light compensation point was measured in eleven samples of a sun plant and eleven samples of a shade plant.

Table 1.4 shows the results of the investigation.

**Table 1.4**

sample number	light compensation point / $\mu\text{mol m}^{-2} \text{s}^{-1}$	
	sun plant	shade plant
1	144	140
2	142	137
3	149	138
4	141	135
5	143	142
6	145	134
7	144	136
8	146	139
9	141	132
10	147	140
11	141	141
mean ( $\bar{x}$ )	144	138
standard deviation ( $s$ )	2.7	3.1
variance ( $s^2$ )		

- (i) Complete Table 1.4 by calculating the variance ( $s^2$ ) for the light compensation point of the sun plant and the shade plant.

[1]

- (ii) A  $t$ -test can be used to determine whether the light compensation point of the sun plant is higher than the light compensation point of the shade plant.

Calculate the value of  $t$  and the number of degrees of freedom, using these formulae:

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

key to symbols

$s$  = standard deviation

$\bar{x}$  = mean

$n$  = sample size (number of observations)

$v$  = degrees of freedom

Show your working.

value of  $t$  = .....

number of degrees of freedom = .....

[2]

- (iii) For this  $t$ -test, the student proposed the null hypothesis:

“There is no difference in the light compensation point of the sun plant and the light compensation point of the shade plant.”

Table 1.5 shows the critical values for  $t$  at several different probabilities and degrees of freedom.

**Table 1.5**

degrees of freedom	probability, $p$ , for one-tailed test			
	0.25	0.05	0.025	0.005
	probability, $p$ , for two-tailed test			
	0.5	0.1	0.05	0.01
1	1.00	6.31	12.71	63.66
2	0.82	2.92	4.30	9.92
3	0.76	2.35	3.18	5.84
4	0.74	2.13	2.78	4.60
5	0.73	2.02	2.57	4.03
6	0.72	1.94	2.45	3.71
7	0.71	1.89	2.36	3.50
8	0.71	1.86	2.31	3.36
9	0.70	1.83	2.26	3.25
10	0.70	1.81	2.23	3.17
11	0.70	1.80	2.20	3.11
12	0.70	1.78	2.18	3.05
13	0.69	1.77	2.16	3.01
14	0.69	1.76	2.14	2.98
15	0.69	1.75	2.13	2.95
16	0.69	1.75	2.12	2.92
17	0.69	1.74	2.11	2.90
18	0.69	1.73	2.10	2.88
19	0.69	1.73	2.09	2.86
20	0.69	1.72	2.09	2.85

Use Table 1.5 and your answers to (d)(ii) to decide whether the null hypothesis suggested by the student should be accepted or rejected.

Explain your answer.

accept or reject null hypothesis .....

explanation .....

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[2]

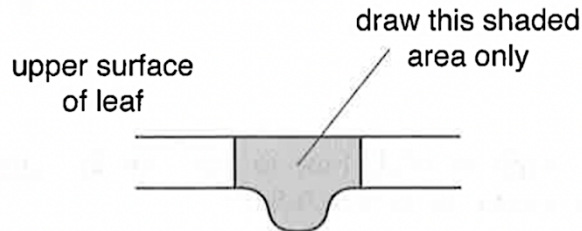
[Total: 35]

- 2 During this question you will require access to a microscope and slides **P1** and **P2**.

Slides **P1** and **P2** are slides of the transverse section of the leaves taken from sun and shade plants respectively.

You are **not** expected to be familiar with these specimens.

- (a) Use the microscope to observe the different tissues in the midrib region of slide **P1** shown in the shaded area in Fig. 2.1.



**Fig. 2.1**

- (a) (i) Draw a large plan diagram of the part of the leaf on slide **P1** shown by the shaded area in Fig. 2.1.

A plan diagram shows the arrangement of different tissues.

Your drawing should show the correct shapes and proportions of the different tissues.

No cells should be drawn.

Labels are **not** required.

[4]

- (ii) Describe how an accurate measurement of the actual thickness of the midrib on slide **P1** can be obtained. State any additional piece(s) of apparatus that you might need.

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[3]

- (iii) Assume that the actual thickness of the midrib you have drawn in **(a)(i)** is 812  $\mu\text{m}$ . Use this information to calculate the magnification of your drawing in **(a)(i)**. Show all the steps in your calculations, including the appropriate units.

magnification = x ..... [3]



- (iv) The outer layer of cells on the upper surface of the leaf on slide **P1** is called the upper epidermis. Underneath the upper epidermis is a layer of tightly packed cylindrical cells which are stained pink on slide **P1**. This layer of cells is called the palisade mesophyll.

Select a group of cells consisting of **two** adjacent palisade mesophyll cells that are touching **two** adjacent upper epidermal cells.

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

Labels are **not** required.

[4]

(b) Examine slides **P1** and **P2** carefully using your microscope.

- (i) Using the eyepiece graticule fitted in your microscope, make the measurements shown in Table 2.1. All measurements should be taken with the **same** objective lens and from the **same** positions on the leaves **avoiding the veins**.

Record your measurements in the form of graticule units in Table 2.1.

**Table 2.1**

feature	thickness / graticule units	
	<b>P1</b>	<b>P2</b>
total leaf thickness		
upper epidermis		
palisade mesophyll		

[3]

- (ii) Apart from the measurements that you have made in (b)(i), use a suitable table to describe **two** other observable structural differences between the palisade mesophyll layer of the leaves on slides **P1** and **P2**.

[2]

- (iii) Sun and shade leaves have a number of structural differences due to their adaptation to exposure to different light intensities.

Suggest **one** advantage for the difference in thickness of the mesophyll layers of the leaves on slides **P1** and **P2**.

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..... [1]

[Total: 20]

