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PRACTICAL EXAMINATION # 1: MOLECULAR LABORATORY

This practical examination is composed of two 45-minute Tasks:

Task 1: Measurement of enzyme activity (20 points)

Task 2: Separation of proteins by chromatography and electrophoresis (20 points)

After 45 minutes, competitors will swap tasks.

Competitors are requested to follow instructions when swapping from one task to another.

There is to be no discussion or exchange of any materials between competitors when changing tasks.

Competitors must not commence the new task until instructed.

THESE INSTRUCTIONS ARE FOR TASK 1
GENERAL INSTRUCTIONS

Competitors are advised to read the examination before commencing.

It is recommended that Competitors proportion their time according to the allotted points for each task and question.

IMPORTANT

All answers must be recorded on the answer sheets provided.

Ensure that your 3 digit code number is written and coded on the top of each page of the answer sheets.

Using the pencil provided, fill in the appropriate circle on the answer sheet.
EXAMINATION # 1: Molecular Laboratory

TASK 1

MEASUREMENT OF ENZYME ACTIVITY

INTRODUCTION

Alcohol dehydrogenase (ADH) is an enzyme that oxidises ethanol to acetaldehyde (ethanal) according to the following reaction.

\[
\text{Ethanol} + \text{NAD}^+ \rightleftharpoons \text{acetaldehyde} + \text{NADH} + \text{H}^+
\]

The cofactor nicotinamide adenine dinucleotide (NAD\(^+\)) is reduced in the reaction. The progress of the reaction can be monitored by measuring the concentration of NADH at a wavelength of 340 nm.

In this practical task you will use a spectrophotometer to measure the activity of this enzyme at various concentrations of ethanol.
MATERIALS AND REAGENTS PROVIDED FOR EACH COMPETITOR

Chemicals

**Ethanol Solutions:** 0.5M, 0.25M, 0.125M, 0.063M

**Reaction Buffer:** 2mM NAD$^+$ in 80mM sodium phosphate buffer pH 7.4 containing 40mM KCl

**Enzyme:** 0.04 mg/mL alcohol dehydrogenase solution

Equipment

- Spectrophotometer with wavelength set at 340nm
- Adjustable pipettors: 1000µL, 100µL and 20µL, plus tips
- Yellow “sharps bin” for tip disposal
- 1mL plastic cuvettes plus holder
- Plastic cuvette stirrers
- Laboratory Timer
- Marking pens
- Safety glasses
- Disposable gloves
- Graph paper with pre-set axes
- Ruler
- Black pen
- Pink card (to attract attention of demonstrator)
- Answer sheet with pencil and eraser
EXPERIMENTAL PROCEDURE

Set up the following duplicate reaction mixtures in 1mL plastic cuvettes, as shown in the Table:

<table>
<thead>
<tr>
<th>Reaction No.</th>
<th>Reaction Buffer</th>
<th>Ethanol solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>0.9 mL</td>
<td>0.1mL of 0.063M</td>
</tr>
<tr>
<td>1b</td>
<td>0.9 mL</td>
<td>0.1mL of 0.063M</td>
</tr>
<tr>
<td>2a</td>
<td>0.9 mL</td>
<td>0.1mL of 0.125M</td>
</tr>
<tr>
<td>2b</td>
<td>0.9 mL</td>
<td>0.1mL of 0.125M</td>
</tr>
<tr>
<td>3a</td>
<td>0.9 mL</td>
<td>0.1mL of 0.250M</td>
</tr>
<tr>
<td>3b</td>
<td>0.9 mL</td>
<td>0.1mL of 0.250M</td>
</tr>
<tr>
<td>4a</td>
<td>0.9 mL</td>
<td>0.1mL of 0.500M</td>
</tr>
<tr>
<td>4b</td>
<td>0.9 mL</td>
<td>0.1mL of 0.500M</td>
</tr>
</tbody>
</table>
Each competitor will be allocated a spectrophotometer. There are two models of spectrophotometer, but they will both give the same results.

Your spectrophotometer has been set up to measure the absorbance at a wavelength of 340 nm. Carry out the following steps to prepare for the enzyme activity measurements:

If you are using an Hitachi U-1100 Spectrophotometer

- Place the cuvette containing the reaction mixture for reaction 1a in position 1 (front) of the cell holder.
- Close the lid.
- To set the absorbance reading to zero, press the 100%T/ 0 ABS button (labelled with red dot).
  The absorbance display should read 0.000.

If you are using an Hitachi U-1800 Spectrophotometer

- Place the cuvette containing the reaction mixture for reaction 1a in position 1 (front) of the cell holder.
- Close the lid.
- To set the absorbance reading to zero, press the “AUTO ZERO” button (labelled with red dot).
  The absorbance display should read 0.000.
Carry out the enzyme activity measurements one at a time, starting with Reaction No. 1a:

(a) Place the cuvette containing the reaction mixture in the spectrophotometer cell.

(b) To start each reaction, add 10 µL of “Enzyme” to the cuvette placed in the spectrophotometer.

(c) Quickly but gently mix the solution in the cuvette with a white plastic stirrer, close the spectrophotometer lid, set the Absorbance to zero, AND immediately start your timer.

(d) Record the absorbance reading exactly 1 minute later. This is the t = 1 min absorbance reading.

This reading is equal to the rate of enzyme reaction (designated “V”), expressed as the change (Δ) in absorbance at 340 nm over the 1 minute period (i.e. ΔA_{340}/min) from t = 0 to t = 1 minute. Enter the values obtained into the Results Table in Column 1.

DATA ANALYSIS AND INTERPRETATION

Complete the Table to determine the average ΔA_{340}/min for each duplicate pair of reactions. Enter these values into Column 2.

For the graphing component of the practical test that follows, also determine the values for 1/V_{average} and enter these in the column of the Table on the right. The values for 1/S (units mM\(^{-1}\)), where S = substrate (ethanol) concentration in mM, have been included for you in the last column. For example, for Reactions 4a and 4b, the actual ethanol concentration in the reactions is 50mM, therefore 1/S = 0.02 mM\(^{-1}\).
<table>
<thead>
<tr>
<th>Reaction No.</th>
<th>Column 1</th>
<th>Column 2</th>
<th>Data for plotting graph</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V A₃₄₀/min</td>
<td>V (Average ΔA₃₄₀/min)</td>
<td>1/Vₐₐᵥₐₑᵣₜₑ</td>
</tr>
<tr>
<td>1a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*ENTER THE VALUES FROM COLUMN 2 ONTO THE ANSWER SHEET*

(Tasks P1.T1.1 – P1.T1.4) (8 points – 2 points each)
If your results were plotted as the enzyme rate (V), expressed as ΔA_{340}/min, versus ethanol substrate concentration (S), a graph of the shape shown below would be obtained. This shows that enzyme rate increases with substrate concentration until a maximum rate (V_{max}) is reached. From this graph, Km, a value equal to the substrate concentration at half-maximal velocity, can be determined.

![Graph showing enzyme rate (V) vs substrate concentration (S)](image)

A more accurate way to determine the Km is using a plot of 1/V against 1/S. The point where the line intersects the X-axis is equal to – 1/Km. **Use the graph with pre-drawn axes provided** to plot a line-of-best-fit for your results.

![Graph showing 1/V vs 1/S](image)

From this plot determine Km.

\[
\text{Km} = \quad \text{mM}
\]

DOUBLE CHECK YOUR Km VALUE (AND UNITS). THEN HOLD UP THE PINK CARD PROVIDED TO ATTRACT THE ATTENTION OF THE DEMONSTRATOR WHO WILL RECORD YOUR ANSWER ON THE ANSWER SHEET. (Task P1.T1.5) (6 points)
Alcohol dehydrogenase assays can be used to determine the levels of ethanol added to motor fuels. Assume that you are a scientist in a testing laboratory and you have been given a 10 mL sample of a fuel sample to test for the level of added ethanol. Following extraction of the sample to remove all traces of solvent, you are left with a 100 mL aqueous sample that contains the ethanol. Using spectrophotometry exactly as before, you test for the ethanol concentration of 0.1 mL of this aqueous sample. The enzyme rate (V), expressed as $\Delta A_{340}$/min, that you obtained is 0.175. Assume that, as before, 0.1 mL of each of the standard ethanol solutions was also tested at the same time, and these measurements allowed you to plot the graph provided. Use the graph to determine the molar concentration of ethanol in the original fuel sample.

**ENTER THIS VALUE ON THE ANSWER SHEET (Task P1.T1.6) (2 points)**

The more usual way of expressing fuel ethanol levels is as a %, i.e. g/100mL. Work out the % fuel ethanol level for this original sample, given that the molecular formula for ethanol is CH$_3$CH$_2$OH and using the following atomic masses (g/mol): C = 12, H = 1, O = 16.

**ENTER THIS VALUE ON THE ANSWER SHEET (Task P1.T1.7) (2 points)**
The light absorption spectra for NAD\(^+\) and NADH are shown in the Figure below.

Which of the following statements (1-7) are correct? (Select from answers on next page.)

1. An absorption peak is seen at 340 nm
2. Only NAD\(^+\) absorbs light at 340 nm
3. Only NADH absorbs light at 340 nm.
4. NAD\(^+\) absorbs light at both 260 nm and 340 nm
5. NADH absorbs light at both 260 nm and 340 nm
6. If the spectrophotometer measurements had been at 330 nm instead of 340 nm the apparent enzyme rates (\(\Delta A/\text{min}\)) would have been lower
7. If the spectrophotometer measurements had been at 350 nm instead of 340 nm the apparent enzyme rates (\(\Delta A/\text{min}\)) would have been higher
The combination with all of the correct statements is:

A) 1, 2, 4, 6
B) 1, 2, 5, 7
C) 1, 3, 5, 6
D) 1, 2, 5, 6
E) 1, 3, 4, 7
F) 2, 4, 5, 6
G) 1, 2, 4, 7
H) none of the above
I) all of the above

ENTER YOUR ANSWER ON THE ANSWER SHEET (Task P1.T1.8) (2 points)

END OF TASK 1

PLACE YOUR ANSWER SHEET ON TOP OF THE REST OF YOUR PAPERWORK AT YOUR WORKSTATION
This practical examination is composed of two 45-minute Tasks:

Task 1: Measurement of enzyme activity (20 points)

Task 2: Separation of proteins by chromatography and electrophoresis (20 points)

After 45 minutes, competitors will swap tasks.

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Competitors must not commence the new task until instructed.

THESE INSTRUCTIONS ARE FOR TASK 2
GENERAL INSTRUCTIONS

Competitors are advised to read the examination before commencing.

It is recommended that Competitors proportion their time according to the allotted points for each task and question.

IMPORTANT

All answers must be recorded on the answer sheets provided.

Ensure that your 3 digit code number is written and coded on the top of each page of the answer sheets.

Using the pencil provided, fill in the appropriate circle on the answer sheet.
TASK 2

SEPARATION OF PROTEINS BY CHROMATOGRAPHY AND ELECTROPHORESIS

IMPORTANT: There are two Parts to this Task.

Please read Part A so that you can plan your time before commencing.

PART A

ION EXCHANGE CHROMATOGRAPHY

INTRODUCTION

Ion exchange chromatography is a technique used to separate proteins on the basis of overall electric charge. Such a separation depends on the acid-base properties of the relevant proteins and the charge on the chromatographic matrix used to separate them. Since the net charge on a protein is dependent on pH, ion exchange chromatography is only effective under conditions of defined pH. At pH 8.0, cation exchange matrices, such as the Hi-Trap “SP” to be used in this task, carry a negative charge and therefore bind positively charged proteins. Ions in the solution with a like charge to the proteins will compete with those proteins for binding to the matrix. Competing ions in excess prevent or reverse binding of proteins, thereby causing them to be eluted (washed from the column) from the matrix.

In this experiment you will be provided with a protein sample containing two proteins, albumin and cytochrome c. Albumin, a major protein of blood plasma, has a molecular mass of 68,000 Daltons (Da) and consists of a single chain of amino acids. Cytochrome c functions in electron transport in mitochondria and consists of a single chain of amino acids that is bound to an iron-containing heme group that absorbs visible light at a wavelength of 410 nm. Cytochrome c has a total molecular
mass of 12,400 Da. So called “Ribbon” structures of these two proteins are shown below: Albumin (left), cytochrome c (right).

In this practical session you will use ion exchange chromatography to separate albumin and cytochrome c.
MATERIALS AND REAGENTS PROVIDED PER STUDENT

Chemicals

**Protein sample**: 4 mg/mL albumin and 4 mg/mL cytochrome c

**Buffer 1**: 50mM Tris-Cl, pH 8.0 buffer.

**Buffer 2**: 50mM Tris-Cl, pH 8.0 buffer containing 0.5M NaCl

Protein Assay Reagent

Equipment

- Column containing cation exchange matrix (Hi-Trap SP)
- Clamp to hold column
- 2 x 5mL disposable syringe (labelled “Buffer 1” and “Buffer 2”)
- 1 x 1mL disposable syringe (labelled “Protein Sample”)
- Adjustable pipettes plus tips
- Yellow “sharps bin” for tip disposal
- 96 well microtitre plate
- Plastic beaker (labelled “Liquid waste”)
- Safety glasses
- Disposable gloves
- Marker pen
- Blue card (to attract attention of demonstrator)
- Answer sheet with pencil and eraser
PROCEDURE

1. Label the microtitre plate (on the attached tape) with your blue Bench Card Number AND Competitor Number. (For example, if your Bench Card Number is “5” and your Competitor Number is “14-3”, label the plate: 5/14-3).
2. The ion-exchange column is equilibrated with Buffer 1 (50mM Tris-HCl, pH 8.0) and ready for immediate use.
3. Draw up 5mL of Buffer 1 into the 5-mL “Buffer 1” plastic syringe.
4. Remove the black screw-on cap from the outlet plug at the bottom of the column.
5. Attach the syringe to the top of the column by firmly pushing into the black adapter.
6. Load 1mL of buffer onto the column by gently and evenly depressing the syringe plunger. Run the waste into the plastic beaker.
7. Now draw up 0.2mL of the protein sample into a 1mL plastic syringe.
8. Load the protein sample onto the column by gently and evenly depressing the syringe plunger. While loading, start to collect fractions of 4 drops each into each well of Row A of the 96 well microtitre plate.
9. Once the sample has been loaded, replace the syringe with the 5mL syringe containing Buffer 1.
10. Continue to collect 4-drop fractions into each well of Row A.
11. When Row A is complete (Fractions 1-12), replace the screw-on plug to the outlet of the column and remove the syringe from the column.
12. Fill a new 5mL syringe with Buffer 2 (50mM Tris-HCl, pH 8.0 buffer containing 0.5 M NaCl).
13. Attach the new syringe to the column, remove the screw-on plug and continue to collect 4-drop fractions into Row C of the 96 well microtitre plate.
14. When Row C is complete (Fractions 13-24), replace the screw-on plug to the column.
15. Using an adjustable pipette, transfer 20µL from each well in Row A (Fractions 1-12) into the corresponding well in Row B.

16. Similarly, transfer 20µL from each well in Row C (Fractions 13-24) into the corresponding well in Row D.

17. Using an adjustable pipette, add 200µL of the Protein Assay Reagent to each well of Rows B and D. This reagent reacts with protein to yield a blue colour that can be measured by spectrophotometry at 595 nm using a plate reader.

18. Check for and eliminate air bubbles in the wells of your 96-well microtitre plate (do this carefully, using a clean yellow tip).

19. **HOLD UP THE BLUE CARD PROVIDED TO ATTRACT THE ATTENTION OF THE DEMONSTRATOR** to note that your 96-well microtitre plate is ready to be analyzed. The plate reader will measure the absorbance of the fractions at both 595nm and 410nm. A printout of the results will be returned to you by the demonstrator.

| IMPORTANT: MOVE ON TO PART B WHILE YOU ARE WAITING FOR THESE RESULTS |

**Questions**

| ENTER YOUR ANSWERS ON THE ANSWER SHEET |

Q1. Which fraction (1-24) contained the first eluted $A_{595}$ peak *(Task P1.T2.1) (1 point)*

Q2. Which fraction (1-24) contained the second eluted $A_{595}$ peak *(Task P1.T2.2) (1 point)*

Q3. Which fraction (1-24) contained the $A_{410}$ peak *(Task P1.T2.3) (1 point)*
Q4. Subtract the fraction number obtained in answer T2.1 from that obtained in answer T2.2 and enter the value. (Task P1.T2.4) (1 point)

Q5. In which fraction (1-24) did the peak of cytochrome c elute? (Task P1.T2.5) (4 points)

Q6. In your experiment you would have noted that one protein eluted directly from the column but the second protein needed added salt to be eluted. Consider these statements:

1. The salt neutralised the ionic interaction between the matrix and the second protein eluted
2. The protein that eluted first was more positively charged than the protein eluted with salt
3. The protein that eluted first was more negatively charged than the protein eluted with salt
4. The protein that eluted first did so because it was larger than the protein eluted with salt
5. The protein that eluted first did so because it was smaller than the protein eluted with salt

Which combination of statements is correct?

A. 1, 2
B. 1, 3
C. 2, 3, 4
D. 1, 3, 4
E. 2, 3, 4
F. 1, 3, 5
G. 2, 3, 5

ENTER YOUR ANSWER ON THE ANSWER SHEET (Task P1.T2.6) (2 points)
Q7. In another experiment you added a third protein (Protein X) to the protein sample containing albumin and cytochrome c and repeated the ion-exchange chromatography separation and detection exactly as before. The collated results obtained from the plate reader are shown below: The elution peak corresponding to Protein X is labelled. For convenience, the other two proteins are arbitrarily labelled “A” and “B”.

Consider the statements below regarding these results:

1. Protein X has a less positive charge overall than protein B
2. Protein X has a more positive charge overall than protein B
3. Protein X contains a non-polypeptide component
4. Protein X is 100% polypeptide
5. Protein X eluted after protein A because it was larger
6. Protein X eluted after protein A because it was smaller
Choose which combination of statements is correct:

A. 1, 3
B. 2, 3
C. 1, 3, 5
D. 2, 3, 6
E. 2, 3, 5
F. 1, 3, 6

ENTER YOUR ANSWER ON THE ANSWER SHEET (Task P1.T2.7) (4 points)
PART B

TWO-DIMENSIONAL GEL ELECTROPHORESIS

The diagram below shows the results of an experiment where a mixture of albumin, cytochrome c and other, unknown proteins were separated by two-dimensional gel electrophoresis. In this technique, the proteins were separated in the first dimension on the basis of their isoelectric point (pl) followed by separation in the second dimension on the basis of their molecular mass. The isoelectric point is defined as the pH at which the sum of the positive and negative charges on the protein is zero. The isoelectric point (pl) for albumin is 4.9 and for cytochrome c is 10.7. The individual proteins were subsequently detected using a protein stain. Each protein “spot” has been given an alphabetical letter identifier.
Answer the following questions:  

ENTER YOUR ANSWERS ON THE ANSWER SHEET

Q8. Which spot corresponds to albumin?  
(Task P1.T2.8) (2 points)

Q9. Which spot corresponds to cytochrome c?  
(Task P1.T2.9) (2 points)

Q10. Phosphorylation is a relatively common modification of proteins that occurs after they have been synthesised. The proteins affected can have a variable number of attached negatively charged phosphate groups; this also leads to a slight increase in their molecular mass.

From the data presented in the figure above, choose which group of proteins best represents the situation where a “parent” protein has been modified to generate a number of phosphorylated species that are less abundant. List the proteins in this group in order, starting with the “parent” protein through to the most phosphorylated protein.  
(Task P1.T2.10) (2 points)

END OF TASK 2

PLACE YOUR ANSWER SHEET ON TOP OF THE REST OF YOUR PAPERWORK AT YOUR WORKSTATION
PRACTICAL EXAMINATION # 2: CELL BIOLOGY LABORATORY

This practical examination is composed of 3 Tasks:

Task 1: Differential leucocyte count (16 points)

Task 2: Blood group analysis (11 points)

Task 3: Single radial Immunodiffusion antigen analysis (13 points)

Total Points available: 40

Total time available: 90 minutes
GENERAL INSTRUCTIONS

Competitors are advised to read the examination before commencing.

It is recommended that Competitors proportion their time according to the allotted points for each task and question.

IMPORTANT

All answers must be recorded on the answer sheets provided.

Ensure that your 3 digit code number is written and coded on the top of each page of the answer sheets.

Using the pencil provided, fill in the appropriate circle on the answer sheet.
TASK 1: Differential Leucocyte Count.

Requirement
In this task, you are required to perform a differential leucocyte count and answer two supplementary questions.

Material and equipment
1. Binocular microscope - with 10X, 40X, 100X (oil immersion) objective lens and 10X eyepiece lens.
2. Microscope oil immersion lens
3. Oil for oil immersion microscopy
4. Cell maturation charts (provided).
5. Stained blood smear (Wrights stain).

Procedure
You are supplied with a prepared blood smear that has been stained with Wright’s stain. Wright’s stain is a standard haematological stain for blood smears. This blood film was collected from a patient who has a persistent cough and fever. The patient is a male adult and had a total white cell count (WCC) of 15.0 x 10^9/L. You are required to complete a differential leucocyte count and to record your results in the table supplied. The cell maturation charts will help you identify the leucocytes.
How to perform a differential leucocyte count

(i) It is **recommended** that you use a 100X oil immersion objective lens. Focus on 10X objective. Put a drop of oil on the slide. Turn the turret carefully to bring the 100X objective into the oil. Focus.

(ii) **Identify and count 100 consecutive leucocytes** in a longitudinal strip from the tail end towards the head of the smear as shown in Figure 1a, recording the occurrence of each cell.

(iii) The lateral edges of the smear must be avoided. It may not be possible to count 100 consecutive leucocytes in a single longitudinal strip due to the thickness of the smear and the subsequent difficulty in cell identification. If this situation occurs, adopt the technique of counting from the tail to the head end and back again as shown in Figure 1b.

**FIGURES 1a and 1b**

---

Legend
A = tail of the blood film
B = body of the blood film
C = head of the blood film
(iv) Record the results of the differential count on the answer sheet (Table 1), taking care that the results are expressed as percentages, and that the total recorded adds up to 100%. An example is given in Figure 1c.

FIGURE 1c: Example of a completed differential count

<table>
<thead>
<tr>
<th>LEUCOCYTES</th>
<th>%</th>
<th>Absolute count (10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>60</td>
<td>6.0</td>
</tr>
<tr>
<td>(total of band form and segmented)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>30</td>
<td>3.0</td>
</tr>
<tr>
<td>Monocytes</td>
<td>8</td>
<td>0.8</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Basophils</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>
## TABLE 1: Results of differential leucocyte count.

<table>
<thead>
<tr>
<th>Type of leucocyte</th>
<th>Occurrence (%)</th>
<th>Absolute count $\left(10^9/L\right)$</th>
<th>Reference Range $\left(10^9/L\right)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td>2.0 - 7.5</td>
</tr>
<tr>
<td>(total of band form and segmented)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td>1.5 – 4.0</td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td>0.2 – 0.8</td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
<td>0.04 - 0.4</td>
</tr>
<tr>
<td>Basophils</td>
<td></td>
<td></td>
<td>0.0 - 0.1</td>
</tr>
<tr>
<td>Total WCC</td>
<td>100</td>
<td>15.0</td>
<td>4.0 - 10.0</td>
</tr>
</tbody>
</table>

Enter results on answer sheet. (14 points)
Questions

P2.T1.1 How could you improve the accuracy of your differential leucocyte count?

A. Count 50 cells.
B. Count 200 cells.
C. Only include cells that are easily identified.
D. Only use the x40 objective.
E. Count all red blood cells in each field.

(1 point)

P2.T1.2 In the differential leucocyte count, calculation of the absolute count from the percentage of each cell type is an important step because of which of the following factors?

A. Absolute counts provide an indication of anaemia.
B. Percentage counts do not vary with the type of infection.
C. A reference range (normal range) for each cell type can be determined.
D. Leucocyte numbers cannot be validated from a blood smear.
E. All of the above.

(1 point)
TASK 2: Blood Group Analysis.

Background

Column agglutination blood grouping cards are used to determine the blood group of individuals in term of the ABO and Rhesus blood groups.

Material and equipment

1. Images of 12 column agglutination blood grouping cards. Ten are labelled with patient identification numbers.

2. Examples of two column agglutination blood group cards (provided).

Procedure and requirement

You are provided with the images of ten (10) blood group cards, each with a unique patient identification number. You are required to interpret the ABO and Rh D (Rhesus) blood group for each patient and record the results in the table provided on the answer sheet. Please refer to Figure 2 for the ABO grouping reactions table and Figure 3 for the Rhesus group reaction table. Individuals with the D-antigen are described as Rhesus positive (Rh +) and those without the D-antigen as Rhesus negative (Rh NEG).
Additional notes on column agglutination cards for blood grouping.

- The cards use the principle of column agglutination. If there is a reaction between the cells and an antibody the cells agglutinate, and become trapped in the column.
- A column may contain antisera, e.g. anti-A, anti-B or in the case of the control no added antisera.
- Columns contain micro glass spheres so they trap agglutinated but not single red blood cells.
- A **positive reaction** is indicated by trapped red blood cells at the top.
- A **negative reaction** is indicated by red blood cells not being trapped and passing completely through the column.
- The columns on the card from left to right are patient cells added to anti-A, patient cells added to anti-B, patient cells added to anti-D, control column (patient cells only), A_1_ cells (strongest form of A cells) added to patient serum, B cells added to patient serum.
- The control has just cells added to allow for the detection of spontaneous autoagglutination – if this occurs, it makes the results invalid.
- For a card to be valid the control column must read negative.
- For all cards that are invalid, write **INVALID** in the ABO Blood Group columns.

Be sure to transfer your answers from Table 2 to the answer sheet.
EXAMINATION #2: Cell Biology Laboratory

Figure 2: ABO grouping reactions table

<table>
<thead>
<tr>
<th>PHENOTYPE</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A1 Cells</th>
<th>B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>POS</td>
<td>NEG</td>
<td>NEG</td>
<td>POS</td>
</tr>
<tr>
<td>B</td>
<td>NEG</td>
<td>POS</td>
<td>POS</td>
<td>NEG</td>
</tr>
<tr>
<td>AB</td>
<td>POS</td>
<td>POS</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>O</td>
<td>NEG</td>
<td>NEG</td>
<td>POS</td>
<td>POS</td>
</tr>
</tbody>
</table>

Figure 3: Rh D (Rhesus) grouping reactions table

<table>
<thead>
<tr>
<th>Rh Phenotype</th>
<th>Anti-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh POS</td>
<td>POS</td>
</tr>
<tr>
<td>Rh NEG</td>
<td>NEG</td>
</tr>
</tbody>
</table>

TABLE 2: Results of Patient Blood Grouping

<table>
<thead>
<tr>
<th>Patient identification number</th>
<th>RESULTS – POS or NEG</th>
<th>ABO blood group (A,B,O or AB)</th>
<th>Rh D (POS or NEG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Anti-) (Anti-) (Anti-)</td>
<td>A1 (cells) B (cells)</td>
<td></td>
</tr>
<tr>
<td>P 942715</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 945857</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 942675</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 974199</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 926723</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 976348</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 923413</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 981342</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 917300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 981398</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Enter your results on the answer sheet.
Questions

P2.T2.1 If a person’s blood group is O Rh POS which of the following ABO antigens are present on their red blood cells?
   A. A antigens only.
   B. B antigens only.
   C. Both A and B antigens.
   D. Neither A nor B antigens.
   E. A₁ antigens.

(1 point)

P2.T2.2 A person who has the blood group A Rh NEG has which combination of (non red blood cell stimulated) ABO antibodies?
   A. anti-B.
   B. anti-A.
   C. anti-A,B.
   D. anti-H.
   E. None.

(1 point)

P2.T2.3 On the basis of the blood grouping reactions you have recorded in Task 2, which of the patients is the most likely to have been transfused?
   A. Patient P 942715
   B. Patient P 945587
   C. Patient P 942675
   D. Patient P 974199
   E. Patient P 926723
   F. Patient P 976348
   G. Patient P 923413
   H. Patient P 981342
   I. Patient P 917300
   J. Patient P 981398

(1 point)
TASK 3: Single radial immunodiffusion antigen analysis.

Background

Single radial immunodiffusion (SRID) is used to measure the concentration of immunoglobulins in blood. It is normally carried out by incorporating an antibody in an agarose gel at a known concentration and placing samples containing an antigen into the standardised wells in the gel. At completion of immunodiffusion a stable precipitate forms, with the diameter squared ($D^2$) having a linear relationship with the antigen concentration.

A standard curve of diameter squared ($D^2$) versus antigen concentration plotted on ordinary graph paper can then be used to estimate the concentration of a number of unknowns. It is normal for just three standard points to be used to construct the standard curve.

In this task you are required to construct the standard curve for two immunoglobulins (IgG and IgA), and then determine the immunoglobulin concentration for two patients. There are also three supplementary questions on the technique.

Requirement

You are required to construct standard curves for two (2) sets of SRID reactions (IgG and IgA), and then to determine the concentration of the immunoglobulin from each patient.

Material and equipment

1. 2 x images of SRID (IgG and IgA) plates with standards and unknown.
2. Reading ruler.
3. Graph paper.
**Procedure**

You are provided with two SRID plates. These plates have been loaded with standards of varying immunoglobulin concentrations, a control serum and serum from a patient. The diffusion has been allowed to come to completion.

For each plate, measure the diameter (D) of the precipitation rings (standards, controls and unknowns) using the reading ruler provided. (*Hint:* place the gel over the ruler, aligning the centre of the well with the central line of the ruler). Move the gel until the outer rim of the precipitin circle **just touches** the inside of both divergent lines. Read to 0.1 mm accuracy.

Record the measurements in the table provided.

Plot the square of the diameter (D²) of the precipitin rings against the immunoglobulin concentration of the standards. For the plot use the graph paper provided, with immunoglobulin concentration on the horizontal (x) axis and ring diameters squared (D²) on the vertical (y) axis. A line of best fit is to be drawn through the three points. (*Hint:* The y-intercept should be in the range of 10 mm² to 12 mm²).

Interpolate the IgG and IgA concentrations of the patient serum from the graphs obtained and record your results on the answer sheet.

There are three supplementary questions
TABLE 3: Results from the analysis of the IgG plate.

<table>
<thead>
<tr>
<th>Well Number</th>
<th>Description</th>
<th>IgG Concentration (g/L)</th>
<th>Diameter (D) (mm)</th>
<th>D² (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Standard 1</td>
<td>2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Standard 2</td>
<td>9.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Standard 3</td>
<td>17.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>14.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Patient A</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The IgG concentration of Patient A is _________________g/L

(5 points)

Enter this value on the answer sheet.

TABLE 4: Results from the analysis of the IgA plate.

<table>
<thead>
<tr>
<th>Well Number</th>
<th>Description</th>
<th>IgA Concentration (g/L)</th>
<th>Diameter (D) (mm)</th>
<th>D² (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Standard 1</td>
<td>1.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Standard 2</td>
<td>3.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Standard 3</td>
<td>5.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>2.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Patient B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Patient B (1/4 dilution)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The IgA concentration of Patient B is _________________g/L

(5 points)

Enter this value on the answer sheet.
Questions

P2.T3.1 Why is it that the plot of the line does not pass through the origin?
   
   A. The technique is designed for only low concentrations of antibody.
   B. The technique is designed for only low concentrations of antigen.
   C. The size of the well introduces a zero error.
   D. The gel system expands during incubation, introducing an error.
   E. Deformation of the gel due to sample application introduces an error.

   (1 point)

P2.T3.2 What could cause a poor (non-linear) calibration curve in this technique?
   
   A. Omission of the control sample.
   B. Cloudy gel.
   C. Patient serum too dilute.
   D. Patient serum too concentrated.
   E. Incomplete diffusion.

   (1 point)

P2.T3.3 How could you improve the accuracy of this technique?
   
   A. Use a thicker agarose gel.
   B. Use concentrated antibodies in the wells.
   C. Heat the gels in a dry oven at 37 degrees Celsius.
   D. Adjust the antibody concentration in the gel.
   E. None of the above.

   (1 point)
PRACTICAL EXAMINATION # 3: ORGANISMAL LABORATORY

This practical examination is composed of 4 Tasks:

Task 1: Dissection of the mouthparts of a grasshopper. (10 points)

Task 2: Relationship between form, function and ecology in some insect groups. (10 points)

Task 3: Identification of insects to species using a dichotomous key. (14 points)

Task 4: Vector efficiency of Anopheles mosquitoes in the transmission of malaria. (6 points)

Total Points available: 40

Total time available: 90 minutes
GENERAL INSTRUCTIONS

Competitors are advised to read the examination before commencing.

It is recommended that Competitors proportion their time according to the allotted points for each task and question.

IMPORTANT

All answers must be recorded on the answer sheets provided.

Ensure that your 3 digit code number is written and coded on the top of each page of the answer sheets.

Using the pencil provided, fill in the appropriate circle on the answer sheet.
TASK 1. Dissection of the mouthparts of a grasshopper. (10 points)

Introduction

Grasshoppers are examples of insects with chewing mouthparts. For this task you will be required to complete three activities.

i. Identify, dissect out, and display the individual components of the grasshopper mouthparts and arrange them as shown in Figure 1 below. (5 points)

ii. Label the dissected mouthparts with the numbered pins according to the numbered names in Table 1. (3.5 points)

iii. Identify the functions of some of the mouthparts. (1.5 points)

Materials and Equipment

1. Grasshopper (*Valanga irregularis*)

2. A set of instruments (2 pairs of forceps, 2 dissecting needles, 1 pair of scissors)

3. Dissecting dish

4. Unnumbered pins for holding the specimen in place in the dissecting dish

5. Pins marked I – VII

6. Latex gloves

7. Dissecting microscope

8. A piece of white foam on which to display mouthparts

9. Piece of paper to indicate competitors number
Table 1. Names of mouthparts

<table>
<thead>
<tr>
<th>Code</th>
<th>Name of mouthpart</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Mandible</td>
</tr>
<tr>
<td>II</td>
<td>Labial palp</td>
</tr>
<tr>
<td>III</td>
<td>Labrum</td>
</tr>
<tr>
<td>IV</td>
<td>Hypopharynx</td>
</tr>
<tr>
<td>V</td>
<td>Maxilla</td>
</tr>
<tr>
<td>VI</td>
<td>Maxillary palp</td>
</tr>
<tr>
<td>VII</td>
<td>Labium</td>
</tr>
</tbody>
</table>

Figure 1. Labelling of grasshopper mouthparts
TASK P3.T1.1

1. Remove the head from the grasshopper body – (Please Note, you are allocated only one grasshopper for this task.)

   With a pin secure the head, anterior down, in the wax dissecting dish.

   Identify the most posterior component of the mouthparts. Insert your forceps underneath and remove the part at its base.

   Working forwards, remove each component in turn by grabbing it at its base with your forceps (as close to the head capsule as possible) and pulling it off.

   NOTE. Your dissection and display will be photographed, assessed and recorded on a special control sheet by an attendant. The correctness of the mouthparts preparation and presentation will be scored. Points will be lost for damage to parts or failure to remove all parts.

   If the attendant is busy with another participant, continue with the next task while waiting to have your dissection assessed.

2. Arrange the parts on the piece of white foam as shown in Figure 1.

3. Label the dissected parts with the numbered pins according to the number code in Table 1.

4. Write your competitor number on the piece of paper pinned to the foam.

5. Display the tick [✓] on the green card to indicate to the attendant that you have completed this task.

6. Place your dissection to the side of your bench for assessment.

   (5 points)
**TASK P3.T1.2** Identify the parts A – G of Figure 1 using the number code for the appropriate part from Table 1.

<table>
<thead>
<tr>
<th>Labelled Mouthpart</th>
<th>Code for name of mouthpart</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td></td>
</tr>
</tbody>
</table>

(3.5 points)

Enter your answer on the answer sheet.

**TASK P3.T1.3** By studying each of the mouthparts, determine the primary function. Use the code number from Table 1 to complete the table below.

<table>
<thead>
<tr>
<th>Primary function</th>
<th>Code for name of mouthparts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grinding and crushing of food</td>
<td></td>
</tr>
<tr>
<td>Acting as a tongue</td>
<td></td>
</tr>
<tr>
<td>Acting as a top lip to form part of the mouth cavity</td>
<td></td>
</tr>
</tbody>
</table>

(1.5 points)

Enter your answer on the answer sheet.
TASK 2. Relationship between form, function and ecology in some insect groups. (10 points)

INTRODUCTION

In this task you will investigate the relationship between form, function and ecology in some insect
groups. The task is divided into two parts, Task 2A and Task 2B

Task 2A Determination of the function of insect legs (5 points)

Introduction

In Task 2A you will study the relationship between the function and structure of the legs of
different insects.

Materials and Equipment

1. A board with 8 insect specimens labelled I – VIII
2. Dissecting microscope
3. Slide with plasticine to hold specimens

TASK P3.T2.1 Study the pinned insect specimens provided. To examine each insect, pin the
specimen into the mound of plasticine on the glass slide and place under the dissecting microscope.
Change the position of the pin to view the insect from different angles. To study the underneath of
the specimen, turn the pin upside down and insert the head of the pin into the plasticine. The insect
specimens are labelled I – VIII. These insects belong to a number of different orders and have hind
or fore legs modified for special functions. Table 2a below presents a list of these special functions
(Codes A – E) and Table 2b provides list of leg modifications necessary to perform the special
functions (Codes a – e). Figure 2 explains the terminology used in Table 2b.
Table 2a. Leg functions

<table>
<thead>
<tr>
<th>Code</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fossorial (digging)</td>
</tr>
<tr>
<td>B</td>
<td>Raptorial (for seizing prey)</td>
</tr>
<tr>
<td>C</td>
<td>Saltatorial (jumping)</td>
</tr>
<tr>
<td>D</td>
<td>Gressorial (walking)</td>
</tr>
<tr>
<td>E</td>
<td>Natatorial (swimming)</td>
</tr>
</tbody>
</table>

Table 2b. Modifications of leg structure

<table>
<thead>
<tr>
<th>Code</th>
<th>Modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Legs flattened with fringes of hairs/setae</td>
</tr>
<tr>
<td>b</td>
<td>Legs with long, narrow coxae, femora with strong spines on ventral surface</td>
</tr>
<tr>
<td>c</td>
<td>Legs short, thickened, spined</td>
</tr>
<tr>
<td>d</td>
<td>Legs long with muscular femora</td>
</tr>
<tr>
<td>e</td>
<td>All legs similar in shape and size</td>
</tr>
</tbody>
</table>

Figure 2 Terminology of Insect Leg

By closely observing the insect specimens, for each insect leg function (A-E), select one insect specimen (I-VIII) that has such legs and the type of modification (a-e)

<table>
<thead>
<tr>
<th>Leg Function</th>
<th>Insect specimen</th>
<th>Leg modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Fossorial (digging)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Raptorial (for seizing prey)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Saltatorial (jumping)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. Gressorial (walking)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. Natatorial (swimming)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(5 points)

Enter your results on the answer sheet.
Task 2B. Relationship between the external morphology and ecology of two ectoparasites

(5 points)

Introduction

Fleas and lice are both external insect parasites of vertebrates. A louse is an example of a parasite that spends its entire life cycle on its host. A flea is an example of a parasite that does not spend its entire life cycle on its host. Each possesses morphological adaptations to suit its respective feeding style and host-associated habitat. This Task examines some of these morphological adaptations and how they relate to the biology of these insects.

Materials and Equipment

1. 2 slide-mounted specimens
   i) a cat flea (*Ctenocephalides felis*) and
   ii) a poultry louse (*Menopon gallinae*)

2. A compound microscope

**TASK P3.T2.2** Using the microscope, examine the flea and louse specimens and determine if the characteristics in the table below are **present (+)** or **absent (-)** in each specimen.

<table>
<thead>
<tr>
<th>Character/Modification</th>
<th>Flea</th>
<th>Louse</th>
</tr>
</thead>
<tbody>
<tr>
<td>body dorsoventrally compressed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tarsal claws</td>
<td></td>
<td></td>
</tr>
<tr>
<td>comb-like row of spines on head</td>
<td></td>
<td></td>
</tr>
<tr>
<td>body with distinct bristles/setae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>elongate mouthparts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>obvious eyes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(3 points)

Enter your results on the answer sheet.
QUESTION P3.T2.3 On the basis of your observations, which of the following combinations of characters would be most important for a parasite that spends its entire life cycle on its host?

(1 point)

A. Eggs scattered throughout host’s hair/feathers; legs modified for gripping; body dorso-ventrally compressed; compound eyes reduced/absent

B. Eggs cemented onto host’s hair/feathers; legs modified for jumping; body dorso-ventrally compressed; compound eyes well developed

C. Eggs scattered throughout host’s hair/feathers; legs modified for gripping; body laterally compressed; compound eyes reduced/absent

D. Eggs cemented onto host’s hair/feathers; legs modified for gripping; body dorso-ventrally compressed, compound eyes reduced/absent

E. Eggs cemented onto host’s hair/feathers; legs modified for jumping; body laterally compressed; compound eyes well developed.
QUESTION P3.T2.4. Which combination of characters would most likely be found in an adult parasite that feeds only on blood?

(1 point)

A. Piercing and sucking mouthparts; chewing mandibles absent; digestive tract with specialised area for grinding; muscular pumps to suck blood

B. Non-piercing mouthparts; chewing mandibles absent; digestive tract not modified for grinding; muscular pumps to suck blood

C. Piercing and sucking mouthparts; chewing mandibles absent; digestive tract not modified for grinding; muscular pumps to suck blood

D. Non-piercing mouthparts; chewing mandibles present; digestive tract not modified for grinding; no pumps to suck blood

E. Piercing and sucking mouthparts; chewing mandibles present; digestive tract not modified for grinding; muscular pumps to suck blood
TASK P3.T3 Identification of ants to species using a dichotomous key. (14 points)

Introduction

Ants are an important part of most terrestrial ecosystems. They occur in large numbers and are found in soil, on the surfaces and on vegetation. They can occur around homes where they may be considered pests but they are gaining increasing significance as bioindicators. For these reasons, their accurate identification is often required.

Materials and equipment

1. A tray with 10 species of ants in ethanol numbered I - X.
2. A dissecting microscope
3. A dichotomous key
4. A set of instruments (2 pairs of forceps, 2 dissecting needles, a ruler)
5. 3 glass dishes for studying ants under the microscope
6. Plastic pipette

Task P3.T3.1

You are provided with 10 specimens of ants (numbered I to X) and a dichotomous key to ant species, including the species provided. Figure 3 explains the terminology used in the key.

Identify the ants using the key. You may remove the ants from the vials and place in the glass dishes for viewing under the microscope. When you have identified each specimen, enter your answer on the answer sheet by filling in the letter code corresponding to the species identified.
Figure 3: Ant anatomy and terminology.
IDENTIFICATION KEY TO ANT SPECIES

1. Head and gaster with distinct metallic green or purple lustre; surface of head, trunk and petiole pitted and rough.......................... Rhytidoponera metallica

Head and gaster not with distinct metallic green or purple lustre;
surface of head, trunk and petiole not pitted and rough........................... 2.

2. Colour mainly black or dark brown.................................................................. 3.

Colour mainly yellow-brown or distinctly black and orange.............................. 6.

3. Ant length no more than about 3-4 mm............................................................... 4.

Ant length more than 5 mm................................................................................. 5.

4. No node on abdominal petiole; tarsi pale yellowish, distinctly paler than femora .......................................................... Technomyrmex albipes

Abdominal petiole with a single node; tarsi brown........................................... Ochetellus glaber

5. Propodeum smooth and rounded, without spines.......................... Camponotus aeneopilosus

Propodeum with distinct spines........................................................................... Polyrhachis sp.

6. Abdominal petiole 1-segmented; funiculus without a distinct elongate terminal 3-segmented club.................................................. 7.

Abdominal petiole 2-segmented; funiculus usually with a distinct elongate terminal 3-segmented club............................................. 9.

7. Gaster pale yellow-green................................................................. Oecophylla smaragdina

Gaster black........................................................................................................ 8.

Please refer to next page for couplets 8 to 11
8. Gaster and head black; trunk and petiole orange/brown.........*Camponotus consobrinus*
   Gaster and petiole black; head and trunk orange/brown...........*Iridomyrmex purpureus*

9. Propodeum with distinct spines or teeth-like projections .............................................. 10.
   Propodeum without distinct spines or teeth-like projections ........................................... 11.

10. Head and gaster greyish black.................................................................*Pheidole sp.*
    Head and gaster pale brown ...............................................................*Pheidole megacephala*

11. Funiculus with a distinct terminal 3-segmented club ..........*Monomorium pharaonis*
    Funiculus without a distinct terminal 3-segmented club..........*Monomorium destructor*

(14 points)
TASK 4. Vector efficiency of *Anopheles* mosquitoes in the transmission of malaria

(6 points)

Introduction

Malaria is regarded as one of the most prevalent and destructive diseases in the tropics, with over 40% of the world’s population being at risk of infection. The disease is transferred between humans by mosquitoes, with the main vectors belonging to the genus *Anopheles*. More than 422 species of *Anopheles* have been described, of which 68 have been identified as vectors of malaria. Species differ in their efficiency as vectors, with some being primary or main vectors, and others acting as secondary or less important vectors. Factors that determine efficiency as vectors include distribution, feeding and habitat preference, the time of biting and malarial stability.

Table 4a shows the influence of time of biting and habitat preference on vector efficiency.

**Table 4a: Influence of time of biting and habitat preference on vector efficiency**

<table>
<thead>
<tr>
<th>Species</th>
<th>Time of Biting</th>
<th>Habitat Preference</th>
<th>Vector Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anopheles 1</em></td>
<td>10pm - 4am</td>
<td>exophilic</td>
<td>low</td>
</tr>
<tr>
<td><em>Anopheles 2</em></td>
<td>10pm - 4am</td>
<td>endophilic</td>
<td>high</td>
</tr>
<tr>
<td><em>Anopheles 3</em></td>
<td>9am – 4pm</td>
<td>endophilic</td>
<td>medium</td>
</tr>
</tbody>
</table>
**Glossary of terms:**

**Anthropophilic:** Likes to feed on humans

**Zoophilic:** Likes to feed on animals

**Endophilic:** Likes to feed and rest indoors

**Exophilic:** Likes to feed and rest outdoors

**Malaria Stability:** the chance of the mosquito surviving long enough for the malarial parasite to become infective. A low value represents unstable malaria, meaning the mosquito dies before it is capable of spreading infection.

**TASK P3.T4.1** Based on the information in Table 4a, the glossary above and the Table below, rank the six species of *Anopheles* (*Anopheles a – Anopheles f*) on the answer sheet in rank order where rank 1 is the **most efficient malaria vector** and rank 6 is the **least efficient malaria vector**.

<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution</th>
<th>Feeding Preference</th>
<th>Habitat Preference</th>
<th>Time of Biting</th>
<th>Malaria Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anopheles a</em></td>
<td>wide</td>
<td>highly anthropophilic</td>
<td>endophilic &amp; exophilic</td>
<td>9am - 4am</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Anopheles b</em></td>
<td>wide</td>
<td>moderately anthropophilic</td>
<td>exophilic</td>
<td>9am - 4pm</td>
<td>1.8</td>
</tr>
<tr>
<td><em>Anopheles c</em></td>
<td>wide</td>
<td>zoophilic</td>
<td>exophilic</td>
<td>9am - 4pm</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Anopheles d</em></td>
<td>restricted</td>
<td>moderately anthropophilic</td>
<td>endophilic</td>
<td>9am - 4pm</td>
<td>1.5</td>
</tr>
<tr>
<td><em>Anopheles e</em></td>
<td>wide</td>
<td>highly anthropophilic</td>
<td>endophilic</td>
<td>10pm - 4am</td>
<td>1.8</td>
</tr>
<tr>
<td><em>Anopheles f</em></td>
<td>wide</td>
<td>zoophilic</td>
<td>exophilic</td>
<td>9am - 4pm</td>
<td>1.2</td>
</tr>
</tbody>
</table>

(3 points)
**TASK P3.T4.2** Figure 4 below is a map of several proposed sites for the construction of a tourist camp site in an area where *Anopheles* mosquitoes are found. Table 4b summarises the climatic conditions of each site. Table 4c lists the five common species of *Anopheles* (*AnophelesI – AnophelesV*) found in the vicinity of the five sites. All five species are known vectors of malaria.

**Table 4b: Summary of climatic conditions at each proposed campsite**

<table>
<thead>
<tr>
<th>Site</th>
<th>Altitude</th>
<th>Daily Max Temp.</th>
<th>Daily Min Temp</th>
<th>Monthly Rainfall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>650m</td>
<td>20°C</td>
<td>8°C</td>
<td>150mm</td>
</tr>
<tr>
<td>2</td>
<td>200m</td>
<td>25°C</td>
<td>13°C</td>
<td>100mm</td>
</tr>
<tr>
<td>3</td>
<td>50m</td>
<td>28°C</td>
<td>17°C</td>
<td>300mm</td>
</tr>
<tr>
<td>4</td>
<td>100m</td>
<td>27°C</td>
<td>15°C</td>
<td>&lt;50mm</td>
</tr>
<tr>
<td>5</td>
<td>50m</td>
<td>27°C</td>
<td>17°C</td>
<td>300mm</td>
</tr>
</tbody>
</table>
Table 4c: Species information for *Anopheles*

<table>
<thead>
<tr>
<th>Species</th>
<th>Larval Habitat</th>
<th>Extra Information</th>
<th>Vector Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anopheles I</td>
<td>swamps and stagnant pools of water</td>
<td>high mortality in temperatures &lt;15°C</td>
<td>0.71</td>
</tr>
<tr>
<td>Anopheles II</td>
<td>water collected in tree holes and stems of plants</td>
<td>high altitude species (&gt;400m) tolerates temperatures &lt;10°C</td>
<td>2.49</td>
</tr>
<tr>
<td>Anopheles III</td>
<td>fast flowing water</td>
<td>high larval mortality in warm, unshaded waters</td>
<td>0.22</td>
</tr>
<tr>
<td>Anopheles IV</td>
<td>fast flowing water</td>
<td>can tolerate arid conditions and high temperatures</td>
<td>6.54</td>
</tr>
<tr>
<td>Anopheles V</td>
<td>swamps and stagnant pools of water</td>
<td>larvae shelter and feed on submerged vegetation</td>
<td>1.36</td>
</tr>
</tbody>
</table>
Figure 4: Location and vegetation layout of proposed camp sites.

LEGEND

▲▲ Forested areas

w w w Swampy area

III Low growing, semi-submerged aquatic vegetation

→ Fast flowing water e.g. stream/river

\text{.yahoo} Open grassland
Based on the information provided for each species of Anopheles (Anopheles I – Anopheles V), select the site (Site1- Site 5) where it is most likely to occur. There is only one correct site for each species of Anopheles. (2 points)

Enter your answer on the answer sheet.

TASK P3.T4.3 Using the information provided select the best site (Sites 1 to 5) to locate a tourist camp site where the risk of contracting malaria would be the lowest, assuming that each species is confined to the one camp site and the maximum distance species can infect within is 10km. (1 point)

Enter your answer on the answer sheet.
This practical examination is composed of 2 Tasks:

Task 1: Plant responses to nitrogen nutrition and carbon dioxide levels (20 points)

Task 2: Interactions between two aquatic plants (16 points)

Total Points available: 36

Total time available: 90 minutes
GENERAL INSTRUCTIONS

Competitors are advised to read the examination before commencing.

It is recommended that Competitors proportion their time according to the allotted points for each task and question.

IMPORTANT

All answers must be recorded on the answer sheets provided.

Ensure that your 3 digit code number is written and coded on the top of each page of the answer sheets.

Using the pencil provided, fill in the appropriate circle on the answer sheet.
TASK 1: Plant responses to nitrogen nutrition and carbon dioxide levels.  (20 points)

INTRODUCTION

Forty plants were grown in a mixture of fine sand and sandy loam topsoil, in controlled temperature enclosures in a glasshouse with natural illumination. The temperature regime (25°C day, 20°C night) was near the optimum for the species concerned and the relative humidity was maintained above 75%. Nutrients were supplied as half-strength Hoagland solution which provided a sufficient and balanced supply of essential macro- and micro-nutrients except for nitrogen. Five nitrogen treatments were applied, containing nitrate at concentrations of 0, 1, 2, 4 or 8 mM. Nutrient solutions were supplied to the soil surface each day until solution drained from the base of the pot. Carbon dioxide concentrations in the chambers were regulated to either 350 or 700 parts per million by volume (ppm) by scrubbing CO$_2$ from the air stream entering the chambers and then adding CO$_2$ at the required rates.

The plants were grown for 20 weeks and the five youngest fully expanded leaves were harvested.

TASKS

You have been provided with a picture of the five youngest fully expanded leaves from one plant at the final harvest of the treatment that supplied nitrate at 8 mM and a CO$_2$ concentration of 350 ppm. Please do not write on or mark the photograph.
**Question P4.T1.1** From the images of the leaves you have been given, indicate in your answer sheet the plant group to which this species belongs:

- A. Monocotyledon (Monocot)
- B. Gymnosperm (Conifer)
- C. Pteridophyte (Fern)
- D. Bryophyte (Moss)
- E. Dicotyledon (Dicot)

(1 point)

**Task P4.T1.2** For each leaf image, use the measuring scale to measure the length of the leaf from the apex to the point where the leaf is attached to the petiole, and the maximum width of the leaf measured at right angles to the direction of the measurement of leaf length. Record your measurement to the nearest 1 mm in your answer sheet.

<table>
<thead>
<tr>
<th>Leaf number</th>
<th>Maximum Length (mm)</th>
<th>Maximum Width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(2 points)
Task P4.T1.3 For another plant from a different treatment, the leaf length and width is given in Table 1.

**Table 1:** Length and width of five leaves from one plant from another treatment in the experiment.

<table>
<thead>
<tr>
<th>Leaf number</th>
<th>Leaf length (mm)</th>
<th>Leaf width (mm)</th>
<th>Leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>112</td>
<td>72</td>
<td>Value a</td>
</tr>
<tr>
<td>2</td>
<td>107</td>
<td>71</td>
<td>Value b</td>
</tr>
<tr>
<td>3</td>
<td>104</td>
<td>68</td>
<td>Value c</td>
</tr>
<tr>
<td>4</td>
<td>99</td>
<td>64</td>
<td>Value d</td>
</tr>
<tr>
<td>5</td>
<td>86</td>
<td>57</td>
<td>Value e</td>
</tr>
<tr>
<td>Mean</td>
<td>Value f</td>
<td>Value g</td>
<td>Value h</td>
</tr>
</tbody>
</table>

An estimate of the area of each leaf in Table 1, can be obtained by using the equation:

\[
\text{Area (cm}^2) = [0.0079 \times \text{length (mm)} \times \text{width (mm)}] - 0.252
\]

Calculate the values a to h in Table 1 to the nearest 0.1mm or 0.1 cm² and record them on your answer sheet. The value h should be calculated by taking the mean of values a to e.

(2 points)

Task P4.T1.4 Calculate the mean leaf area of the plant in Task P4.T1.3 by using the mean values for leaf length (value f) and leaf width (value g) in the formula given for estimating the area of a leaf.

Enter the value (to the nearest 0.1 cm²) on the answer sheet.

(1 point)
**TASK P4.T1.5**—Compare the values for mean leaf area as calculated by the mean of the area of each individual leaf with that calculated by using the mean length and mean width values. Indicate which of the following statements is correct:

A. The values are equal
B. The values are unequal because of an unbalanced data set.
C. The values are unequal because of an error in calculation.
D. The values are unequal because of a normal distribution of leaf area data about the mean.
E. The values are unequal because of a symmetrical distribution of leaf length data about the mean.
F. The values are unequal because of an irregular distribution of values within the data sets.

(2 points)

**TASK P4.T1.6**—Table 2 shows the mean leaf area for the five youngest fully expanded leaves of plants grown for 20 weeks under two ambient carbon dioxide concentrations and five nitrate nutrition regimes.
Table 2: Mean leaf area (cm$^2$) for the five youngest fully expanded leaves on plants grown under two ambient CO$_2$ concentrations and five nitrate concentrations.

<table>
<thead>
<tr>
<th>Nitrate (mM)</th>
<th>Ambient CO$_2$ concentration (ppm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>350</td>
<td>700</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>19.8</td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>33.0</td>
<td>31.6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>44.4</td>
<td>45.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>50.2</td>
<td>51.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>39.5</td>
<td>53.2</td>
<td></td>
</tr>
</tbody>
</table>

On the graph paper provided, prepare a graph comparing mean leaf area against nitrate concentration in the nutrient solution for each of the two CO$_2$ treatments. From these plots, which of the following statements is/are most likely to be correct?

I—At both 350 and 700 ppm CO$_2$ and at nitrate concentrations in the nutrient medium between 0 and 2 mM, leaf area is limited by nitrogen availability.

II—At 700 ppm CO$_2$, photon flux density is likely to limit leaf size at nitrate concentrations in the nutrient medium higher than 4 mM.

III—This evidence proves that, at 350 ppm CO$_2$, total plant leaf area is reduced when nitrate supply increases from 4 to 8 mM.

A. I only
B. III only
C. II and III only
D. I and II only
E. I, II and III

(2 points)
TASKS P4.T1.7 to P4.T1.10 relate to information in Tables 3a and 3b.

Tables 3a and 3b show the values of leaf mass of the five youngest fully expanded leaves from one plant grown at 350 ppm CO\(_2\) and one plant grown at 700 ppm CO\(_2\). You are asked to determine whether the values for leaf mass at the two CO\(_2\) concentrations are significantly different using unpaired values (Table 3a) and paired values (Table 3b). The appropriate test is Student’s \(t\)-test. Instructions for the use of this test and a table of values of \(t\) can be found in Appendices A and B. Please also refer to the abbreviations given at the end of Table 3b. To assist, some of the data has been processed. You may write on the exam paper but ensure you provide the appropriate answers on the answer sheet.
Table 3a: Leaf mass for plants grown at 350 ppm and 700 ppm CO₂. Calculations for unpaired values

<table>
<thead>
<tr>
<th>Leaf Number</th>
<th>350 ppm CO₂ (X)</th>
<th>700 ppm CO₂</th>
<th>(X - X)²</th>
<th>(Y - Y)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf mass (mg) (X)</td>
<td>X²</td>
<td>X - X</td>
<td>Leaf mass (mg) (Y)</td>
<td>Y²</td>
</tr>
<tr>
<td>1</td>
<td>448</td>
<td>619</td>
<td>383161</td>
<td>104</td>
</tr>
<tr>
<td>2</td>
<td>428</td>
<td>593</td>
<td>351649</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>415</td>
<td>484</td>
<td>234256</td>
<td>-31</td>
</tr>
<tr>
<td>4</td>
<td>386</td>
<td>479</td>
<td>229441</td>
<td>-36</td>
</tr>
<tr>
<td>5</td>
<td>370</td>
<td>400</td>
<td>160000</td>
<td>-115</td>
</tr>
</tbody>
</table>

Sum (ΣXᵢ, Σ(Xᵢ²), etc.) 2575 1358507 0 32382

Mean (X, Y, D) 515.1

(ΣXᵢ)²/n 6630625

SD = \([\sum(Xᵢ²) - (\sum Xᵢ)²/n]/(n-1)\] 0.5 90.0

Difference between means

S = \([\sum(Xᵢ-Xᵢ)² + \sum(Yᵢ-Yᵢ)²]/(nₓ+nᵧ-2)\] 0.5

\(t = (X-Y)/(S[2/(nₓ+nᵧ)])^{0.5}\)
Table 3b: Leaf mass for plants grown at 350 ppm and 700 ppm CO$_2$. Calculations for paired values

<table>
<thead>
<tr>
<th>Leaf number</th>
<th>Leaf mass (mg)</th>
<th>350 ppm CO$_2$ (X)</th>
<th>700 ppm CO$_2$ (Y)</th>
<th>D = (Y–X)</th>
<th>D - D</th>
<th>(D – D)$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>448</td>
<td>619</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>428</td>
<td>593</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>415</td>
<td>484</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>386</td>
<td>479</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>370</td>
<td>400</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum (Σ$X_i$, etc.)</td>
<td>2575</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (X, Y, D)</td>
<td>515.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Difference between means

SD = ([(Σ(D–D)$^2$)/(n-1)]$^{0.5}$)/(n$^{0.5}$)

t = (D - (Σ(D–D))/ SD

Abbreviations:

n, number of individuals in sample

S, sum of variates

t, Student’s t

D, difference between pairs of variates

D, mean of differences between pairs of variates

df, degrees of freedom

P, probability of a significant difference

SD, Standard deviation
**TASK P4.T1.7** Calculate the standard deviation about the mean leaf mass for the 350 ppm CO₂ treatment in the unpaired value comparison and enter the value (to the nearest 0.1 mg) in your answer sheet.  

**TASK P4.T1.8** Use the Student’s t-test to determine the level of probability that the leaf mass at 350 ppm CO₂ is not significantly different from the leaf mass at 700 ppm CO₂ in the unpaired test (Table 3a) is:

- A. Greater than 0.99
- B. 0.95 to 0.99
- C. 0.05 to 0.10
- D. 0.01 to 0.05
- E. Less than 0.01

**TASK P4.T1.9** Use the Student’s t-test to determine the level of probability that the leaf mass at 350 ppm CO₂ is not significantly different from the leaf mass at 700 ppm CO₂ in the paired test (Table 3b) is:

- A. Greater than 0.99
- B. 0.95 to 0.99
- C. 0.05 to 0.10
- D. 0.01 to 0.05
- E. Less than 0.01

(1 point)
TASK P4.T1.10 Indicate which of the following statements is correct:

A. The two tests produce the same level of significance of the difference between the means.

B. The variation in significance of the difference between means in the two tests is due to an error in the formula.

C. The variation in significance of the difference between means in the two tests is due to random variation in one sample.

D. The variation in significance of the difference between the means in the two tests is due to non-random variation in one sample.

E. The variation in significance of the difference between the means in the two tests is due to non-random but matching variation in both samples.

(1 point)

TASKS P4.T1.11 to P4.T1.12 relate to information in Table 4.

**Table 4:** Leaf mass of plants grown under two ambient CO₂ concentrations and five concentrations of nitrate in the nutrient medium.

<table>
<thead>
<tr>
<th>Nitrate (mM)</th>
<th>Leaf mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[CO₂] 350 ppm</td>
</tr>
<tr>
<td>0</td>
<td>118</td>
</tr>
<tr>
<td>1</td>
<td>214</td>
</tr>
<tr>
<td>2</td>
<td>310</td>
</tr>
<tr>
<td>4</td>
<td>401</td>
</tr>
<tr>
<td>8</td>
<td>316</td>
</tr>
</tbody>
</table>
On the graph paper provided, plot the mean leaf mass for each treatment against the nitrate concentration in the nutrient solution.

Compare the graphs you have plotted for:

1. leaf area vs. nitrate concentration, and
2. leaf mass vs. nitrate concentration.

**TASK P4.T1.11** From the relationships between leaf area, leaf mass and nitrogen concentration in your graphs, indicate on your answer sheet which of the following statements is most likely to be correct:

I. At nitrate supply concentrations between 0 and 2 mM, there is a positive interaction between nitrate concentration in the soil solution and CO$_2$ concentration in the atmosphere in their effects on mean leaf mass.

II. Climate change that results in a doubling of the ambient CO$_2$ concentration is very likely to increase mean leaf mass significantly in this plant species at all nitrate concentrations in the root medium between 0 and 8 mM.

III. At 700 ppm CO$_2$, there is no change in leaf mass per unit area at nitrate supply concentrations between 4 and 8 mM.

A. I only
B. III only
C. I and II only
D. I and III only
E. I, II and III

(1 point)
TASK P4.T1.12 Indicate which of the following statements are correct:

I. Between 0 and 2 mM NO$_3^-$, neither leaf area nor leaf mass is affected by the concentration of CO$_2$ in the air.

II. The evidence from this study indicates that climate change that results in a doubling of the ambient CO$_2$ concentration is likely to result in more uniform plant growth over the surface of the earth.

III. Between 4 and 8 mM NO$_3^-$, there is a negative interaction between increasing NO$_3^-$ and CO$_2$ concentrations in their effects on leaf mass.

A. I only  
B. II only  
C. III only  
D. I and III only  
E. I, II and III

(1 point)
Task 2: Interactions between two aquatic plants

INTRODUCTION
Lemna is a monocotyledon plant and azolla is a fern. Both are floating plants and reproduce by vegetative means. Azolla has a cyanobacterium associated with its root system that fixes nitrogen in water with low nitrogen concentrations. The growth of colonies of these plants can be indicated by counting leaves (lemna) or fronds (azolla), or by measuring their dry weights at different times. Both of these procedures have difficulties, and a common ecological expression of the importance of species in a community is the proportion of land or water surface that they occupy. The following questions relate to the estimation of the area occupied by two species in a mixed population. In most ecological studies, it is impossible to measure every individual, and sampling must be used. The following problem requires you to evaluate a sampling procedure.

TASKS
To complete TASKS P4.T2.1 to P4.T2.5, you have been provided with a photograph of an area of fresh water supporting lemna (small bright green leaves) and azolla (the darker green fern). For reference, we have provided you with a petri dish with samples of lemna and azolla. You are required to design in part a sampling procedure to estimate the percentage of area occupied by different components of this plant community. Sampling may be carried out by (1) random points, (2) randomly placed transect lines, or (3) randomly placed sampling areas. Please do not write on or mark the photograph.
Ten randomly located transect lines (each approximately 50 mm long) have been drawn on the photograph. Each line has a number that represents the order of its selection and corresponds to a row in Table 5. Make your measurements in the order indicated by the line number.

**NOTE:** You may not need to measure every line. Analyse your data progressively, so that after you have measured four lines, you calculate the mean and other values as indicated. With each additional line, your sample size increases, and you should complete another calculation.

For each transect line that you select, place your measuring scale on the line with the zero end closest to the line number. **Make your measurements on the side of the line closest to the number.** Measure the total length of the line that lies over:

1. lemna
2. azolla

Light coloured areas on the photograph represent open water. Dark coloured areas on the photograph represent submerged azolla, which should be measured.

**Use these values to calculate the percentage of the transect line occupied by each species.** Record the percentage cover values in your work sheet. For each sample size greater than three (that is four or more sample lines), calculate:

1. the mean percentage cover of lemna
2. the standard deviation and confidence limit (probability 0.05) of the mean percentage cover of lemna. Use the formulae in Appendix A.
3. the coefficient of variation, which is the ratio (confidence limit)/(mean percentage cover of lemna).
(4) the mean percentage cover of azolla.

(5) the standard deviation and confidence limit (probability 0.05) of the mean percentage cover of azolla. Use the formulae in Appendix A.

(6) the coefficient of variation, which is the ratio (confidence limit)/(mean percentage cover of azolla).

Record these values in your work sheet (Table 5).

For each species, plot the coefficient of variation against the sample size.

**Repeat the sampling process until the coefficient of variation for the percentage of area occupied by azolla is less than 0.25.** When you have reached this point, enter the following values in your answer sheet:

**TASK P4.T2.1** How many transect lines do you need to measure to obtain a coefficient of variation for percentage cover of azolla which is less than 0.25?

(2 points)

**TASK P4.T2.2** The mean percentage of the area of the photograph occupied by lemna (to the nearest 1 per cent).

(2 points)

**TASK P4.T2.3** The mean percentage of the area of the photograph occupied by azolla (to the nearest 1 per cent).

(2 points)

**TASK P4.T2.4** The confidence limit (P = 0.05) of the mean percentage of the area of the photograph occupied by lemna (to the nearest 0.1 per cent).

(2 points)

**TASK P4.T2.5** The confidence limit (P = 0.05) of the mean percentage of the area of the photograph occupied by azolla (to the nearest 0.1 per cent).

(2 points)
TASK P4.T2.6 Indicate which of the following statements are correct:

I  The confidence limit of the mean area of each species is independent of sample size.

II  The confidence limit of the mean area for azolla decreases with increasing sample size.

III  For a given sample size, the coefficient of variation for the area of lemna is greater than the coefficient of variation for the area of azolla because lemna plants become separated and are redistributed during vegetative growth but azolla remains in larger colonies.

IV  For a given sample size, the coefficient of variation for the area of lemna is greater than the coefficient of variation for the area of azolla because the area occupied by lemna is smaller than the area occupied by azolla.

V  Sampling by random line transect is completely unreliable.

A.  I only
B.  V only
C.  II and IV
D.  II and III
E.  II, III and IV

(1 point)
Table 5: Percentage Areas (A) of lemna and azolla in a mixture

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<th>Sample number</th>
<th>Area (%) (A)</th>
<th>Mean</th>
<th>$A^2$</th>
<th>$\Sigma A^2$</th>
<th>$(\Sigma A)^2$</th>
<th>SD</th>
<th>CL</th>
<th>CV</th>
<th>Area (%) (A)</th>
<th>Mean</th>
<th>$A^2$</th>
<th>$\Sigma A^2$</th>
<th>$(\Sigma A)^2$</th>
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Abbreviations: SD, standard deviation; CL, confidence limit (P = 0.05); CV, coefficient of variation

Formulae to describe these parameters are included in Appendix A or in the text.
**TASKS P4.T2.7 to P4.T2.10** Ecological studies are often undertaken in order to predict the future characteristics of plants or plant associations. The data in Table 6 describe the change in fresh biomass (g) of a colony of lemna growing in a pond with a high nutrient concentration.

**Table 6**: Biomass of lemna on selected days after initiation of a culture.

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<tr>
<th>Time (days)</th>
<th>Biomass of plants (g)</th>
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<td>8</td>
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</table>

**TASK P4.T2.7** By graphing the data in Table 6 (after transforming the data if necessary), extrapolate the relationship to estimate the biomass of the colony on Day 24 of the experiment.

**Enter the value (to the nearest gram) on the answer sheet.**

(2 points)

**TASK P4.T2.8** From the data in Table 6, calculate the Relative Growth Rate (RGR) of the lemna colony between Day 0 and Day 8, using the formula given below. Enter the value (to the nearest 0.001 g g⁻¹ day⁻¹) in your answer sheet.

(1 point)
RGR = \frac{(ln W_2 - ln W_1)}{(t_2 - t_1)}

where RGR is Relative Growth Rate (g g\(^{-1}\) day\(^{-1}\)), W_2 is weight (g) at time \(t_2\) (days), and W_1 is weight (g) at time \(t_1\) (days)

**TASK P4.T2.9** From the data in Table 6, calculate the Relative Growth Rate of the lemna colony between Day 12 and Day 20. Enter the value (to the nearest 0.001 g g\(^{-1}\) day\(^{-1}\)) in your answer sheet.

**(1 point)**

**TASK P4.T2.10** From the graph of lemna biomass vs. time, indicate which of the following statements are correct:

I Lemna relative growth rate increased between Day 8 and Day 20

II The lemna colony grew exponentially between Day 0 and Day 20

III Lemna growth was not limited by nutrient availability.

IV Lemna growth was limited by space.

A. I only

B. II and III

C. III only

D. II and IV

E. I, III and IV

**(1 point)**
**TASKS P4.T2.11 to P4.T2.12** Lemna and azolla have different maximum growth rates, and when grown in mixture, one species or the other may dominate the site. The data in Table 7 indicates the initial biomass values for an experiment where different mixtures of lemna and azolla with a total mass of 10 g were distributed in samples of a medium with a constant level of nutrition.

**Table 7:** Distribution of biomass between lemna and azolla in three populations established as cultures.

<table>
<thead>
<tr>
<th>Per cent initial Azolla biomass</th>
<th>Biomass (g)</th>
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<tr>
<td></td>
<td>Azolla initial</td>
<td>Lemna initial</td>
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<tr>
<td>0</td>
<td>0</td>
<td>10</td>
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<tr>
<td>25</td>
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</table>

After three weeks of growth, the plants were separated and weighed, with the results presented in Table 8.
Table 8: Biomass of lemna and azolla grown for three weeks in three cultures as described in Table 7.

<table>
<thead>
<tr>
<th>Per cent initial Azolla biomass</th>
<th>Final Azolla biomass (g)</th>
<th>Final Lemna biomass (g)</th>
<th>Final total biomass (g)</th>
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<td>100</td>
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On the same graph, plot the final azolla biomass, final lemna biomass and final total biomass after three weeks of growth against the percentage of initial azolla biomass.

Tasks P4.T2.11—Indicate which of the following statements most completely describes the evidence in the graph:

I. The growth of azolla is not affected by the presence of lemna.

II. A species that is less vigorous in monoculture should show a relative depression in growth when grown in mixture with a species that is more vigorous in monoculture.

III. This experiment proves that azolla releases nitrate ions into the water.

IV. The proportion of lemna in the pond may be expected to increase progressively with time.
A. I only
B. II only
C. II and IV
D. II, III and IV
E. I and IV

(2 points)

TASKS P4.T2.12 Indicate which of the following statements is correct:

I. A positive interaction between species is indicated by a curve relating total biomass to different proportions of the two species (from Table 7) that is convex in shape.

II. If a species is aggressive in a mixture its final proportion of biomass is greater than the initial proportion. This characteristic may be independent of the vigour of the species in a monoculture.

III. This experiment does not demonstrate that the two species interact in their use of resources during growth.

IV. Lemna derives some benefit from the presence of azolla, but azolla does not derive any benefit from the presence of lemma.

A. III only
B. IV only
C. I and IV only
D. II and IV only
E. I, II and IV only

(2 points)
APPENDIX A

A1.1 Determination of standard deviation of a mean

The standard deviation is calculated from the following attributes of a sample:

\[ \sum X_i^2 \], the sum of the squares of each value of the variable \( X_i \) where \( i \) has values from 1 to \( n \).

\( n \) is the number of values of the variable \( X \) in the sample

\[ \left( \sum X_i \right)^2 \], the square of the sum of all values of the variable \( X_i \) where \( i \) has values from 1 to \( n \).

\[
SD = \sqrt{\frac{\sum X_i^2 - \left(\frac{\sum X_i}{n}\right)^2}{n - 1}}
\]

A1.2 Confidence limit of a mean

The confidence limit (CL) is derived from the standard deviation by

\[
CL = \frac{t \times SD}{\sqrt{(n-1)}}
\]

Where \( t \) is the value of the distribution of Student’s \( t \) (Appendix B) for the desired level of probability

\( (P = 0.05) \) and the number of degrees of freedom \( (df) \) where \( n \) is the number of samples in the analysis. The number of degrees of freedom is \( (n - 1) \).
A1.3 Significance of difference between two sample means

Differences between two samples (variable $X$ and variable $Y$) can be tested using the Student’s $t$-distribution, which is calculated from the differences between individual values ($X_i$ and $Y_i$) and the mean values ($\bar{X}$ and $\bar{Y}$) for the two samples where $n_x$ and $n_y$ are the numbers of individuals in the two samples. This can be done for either unpaired or paired values from the two sample groups. If the calculated value of $t$ is greater than the value of $t$ for a particular level of probability in the table for the degrees of freedom applicable to the sample, then it can be concluded that the difference between two mean values is real (that is, significant) at that level of probability.

A1.3.1 Unpaired samples

If the collection of data for the two samples was completely independent, each value from one sample value must be compared with all values from the other sample. The procedure can be summarised as

$$s = \sqrt{\frac{(X - \bar{X})^2 + (Y - \bar{Y})^2}{(n_x + n_y - 2)}}$$

and

$$t = \frac{(\bar{X} - \bar{Y})}{s \cdot \sqrt{\frac{2}{n_x + n_y}}}$$
The value of $t$ is compared with the value in the Table of distribution of $t$ (Appendix B), for the level of significance that you want and the number of degrees of freedom, $df$, where $df = (n-1)$.

**A1.3.2 Paired samples**

If the collection of data for the two samples was arranged so that pairs of variables were measured, the differences between the means can be assessed as follows. The difference ($D$) between the two values ($X$ and $Y$) of each pair enables the mean difference ($\bar{D}$) to be calculated. The algebraic sum of ($D - \bar{D}$) for all pairs will equal zero. The procedure can be summarised as

$$S = \sqrt{\frac{\sum (D - \bar{D})^2}{(n-1)}}$$

$$t = \frac{D - \sum (D - \bar{D})}{S}$$

The value of $t$ is tested in the same way as described above for unpaired samples.
### APPENDIX B

#### Table III. Distribution of $t$

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