PRACTICAL TEST 1: PLANT ANATOMY AND PHYSIOLOGY

(47 points, 60 minutes)

Task 1 (33 points)

Study of factors affecting the activity of stomata

You should try and complete this task in 30 minutes.

Materials and equipment	**Quantity**
1. Specimens labeled 1 to 8 (in red capped vials)	8
2. Compound binocular microscope	1
3. Glass microslides	8
4. Box of coverslips	1
5. Watchglass	1
6. Forceps	1
7. Brush	1
8. Wash bottle containing distilled water	1
9. Permanent marker pen	1
10. Tissue paper roll	1
11. Container for washing and discard	1

Introduction

Stomata are specialized microscopic structures found in all vascular plants.
These microscopic pores allow exchange of gases between the environment and the plant cells. Stomata are also the sites from where water evaporates from the plant. Various environmental factors such as temperature, humidity and light intensity can affect the opening or closing of the stomata.

Q. 1.1. (3 points) Some statements about stomata are given below. Indicate whether the statements are true or false by putting a tick mark (√) **in the appropriate boxes in Q. 1.1. in the Answer Sheet.**

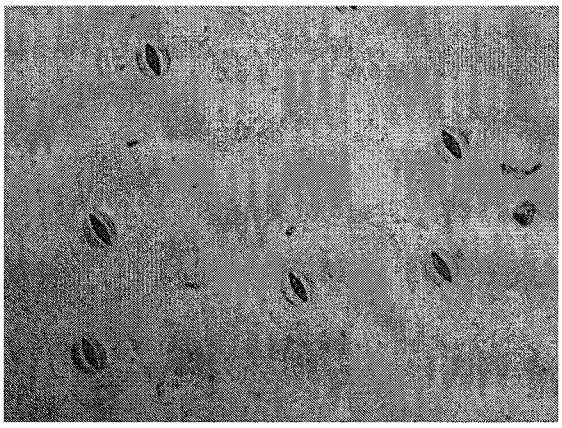
	Statement	True	False
a.	Guard cells are the only epidermal cells that contain chloroplasts.		
b.	The Stomatal Index of any plant species is the ratio of number of stomata in a given area of the leaf to the total number of stomata and other epidermal cells in that same area.		
c.	Stomata are characteristic of angiosperms alone.		
d.	Larger the stomatal pore, greater is the rate of transpiration per unit area of the pore.		
e.	The Stomatal Index is always constant for a given species.		
f.	A plant with stomata only on the upper surface of its leaves is most likely to be a submerged hydrophyte.		

Stomata can be observed by taking an epidermal peel of a leaf. Alternatively, an imprint of the stomata can be obtained, without damaging the leaf tissue, as follows:

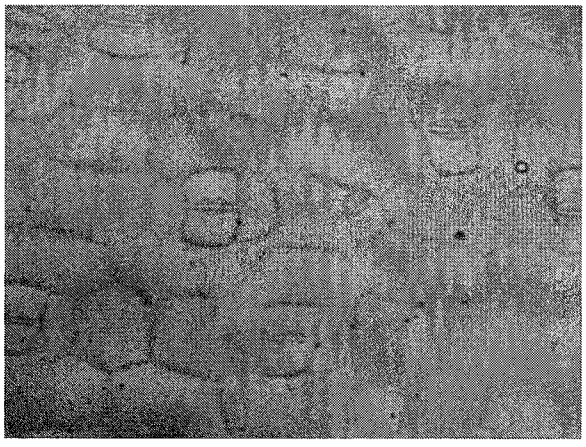
A thin coat of transparent colourless nail polish is applied on the leaf surface. The coat is allowed to dry and peeled off using a pair of forceps. This imprint is placed on a microslide with a drop of water, a coverslip is placed on it and it is observed under the microscope.

Open and closed stomata can be easily distinguished from these imprints. Representative microscopic images of the imprints are given below.

OPEN stomata: (10X)



CLOSED stomata: (10X)



In this task, you will study the effect of various factors on the opening and closing of the stomata. These experiments are conducted by immersing leaves in solutions of different chemicals under specified conditions.

Q. 1.2. (0.5 point) The best choice for such an experiment would be:

a. a mesophyte
b. a hydrophyte
c. a xerophyte
d. a halophyte

Put a tick mark (√) in the appropriate box **in Q. 1.2. in the Answer Sheet.**

a.	b.	c.	d.

Q. 1.3. (2 points) In one such experiment, a solution of pH 4.0 containing 100 mM K^+ and 0.1 mM Ca^{++} is required. Choose the correct way to prepare this solution from the following options and calculate the amount of KCl and volume of 10mM $CaCl_2$ solution you have to add.
Atomic mass of K is 39.1 and of Cl is 35.5.

a. Dissolve X g of KCl in 40 ml citrate buffer of pH 4.0, add Y ml of 10 mM $CaCl_2$, and make up the volume to 50 ml.
b. Dissolve X g of KCl in 40 ml distilled water. Add Y ml of 10 mM $CaCl_2$ to 5 ml distilled water. Mix both the solutions and adjust the pH to 4.0 with HCl. Make up the volume to 50 ml.
c. Dissolve X g of KCl in 40 ml distilled water. To this, add Y ml of 10 mM $CaCl_2$. Make up the volume to 50ml. Adjust the pH to 4.0 with HCl.

Put a tick mark (√) in the appropriate box and give the correct answer for X and Y **in Q. 1.3. in the Answer Sheet**.

a.	b.	c.

X = _____ g KCl

Y = _____ ml 10mM $CaCl_2$

Experiment

In order to study the effect of various factors on stomata, leaves of a plant were treated in eight different ways. The imprints obtained after each of these treatments (1 to 8 given below) are provided in red-capped vials labeled 1 to 8, respectively.
Please note that these treatments have also been described in the following table for your convenience.

Treatment 1: Leaves were immersed in a solution containing 100 mM KCl, 0.1 mM $CaCl_2$, pH 7.0 and kept in light for 2 h.

Treatment 2: Leaves were immersed in a solution containing 10 mM KCl, 0.1 mM $CaCl_2$, pH 7.0 and kept in dark for 2 h.

Treatment 3: Leaves were immersed in a solution containing 0.5 M mannitol, 100 mM KCl, 0.1 mM $CaCl_2$, pH 7.0 and kept in light for 2 h.

Treatment 4: Leaves were immersed in a solution containing 10 mM KCl, 0.1 mM $CaCl_2$, pH 4.0 and kept in dark for 2 h.

Treatment 5: Leaves were immersed in solution containing 10 mM KCl, 0.1 mM $CaCl_2$, pH 7.0 containing an Unknown Chemical and kept in dark for 2 h.

Treatment 6: Leaves were immersed in a solution containing 100 mM KCl, 0.1 mM $CaCl_2$, pH 4.0 and kept in light for 2 h.

Treatment 7: Leaves were immersed in a solution containing 100 mM KCl, 0.1 mM $CaCl_2$, 10 µM abscisic acid, pH 7.0 and kept in light for 2 h.

Treatment 8: Leaves were immersed in a solution containing 100 mM KCl, 0.1 mM $CaCl_2$, 10 µM abscisic acid, pH 4.0 and kept in light for 2 h.

Treatment	**KCl**	**$CaCl_2$**	**pH**	**Light**	**Abscisic Acid**	**Mannitol**	**Unknown Chemical**
1	100mM	0.1mM	7.0	2 h Light	-	-	-
2	10mM	0.1mM	7.0	2 h Dark	-	-	-
3	100mM	0.1mM	7.0	2 h Light	-	0.5 M	-
4	10mM	0.1mM	4.0	2 h Dark	-	-	-
5	10mM	0.1mM	7.0	2 h Dark	-	-	Yes
6	100mM	0.1mM	4.0	2 h Light	-	-	-

7	100mM	0.1mM	7.0	2 h Light	10µM	-	-
8	100mM	0.1mM	4.0	2 h Light	10µM	-	-

Q. 1.4. (8 points): Observation of the imprints

(i) Pick up the imprint gently from the container using a brush. You may need to gently swirl the vial in order to locate the imprint.
(ii) Place the imprint in a drop of water on a glass microslide.
(iii) Place a coverslip and observe under the 10X objective of the microscope. **Note that you will be observing unstained specimens. Hence, make appropriate adjustments in the microscope.**
(iv) Note down the observations **in Table 1.4. in the Answer Sheet**. You need to count at least 20 stomata per imprint.

Table 1.4.

Treatment	Total number of stomata counted	Number of open stomata	Number of closed stomata
1			
2			
3			
4			
5			
6			
7			
8			

Interpretations

Q. 1.5. (10 points)
Based on the results obtained from the given set of experiments, answer the questions **Q. 1.5.A to Q. 1.5.D in the Answer Sheet**. Fill in the blanks with the appropriate serial numbers from the options given below. Use all options, but each option only once.

A. The factor/s that clearly lead to stomatal closure is/are: _____
B. The factor/s that clearly lead to stomatal opening is/are: _____
C. The factor/s that clearly have no effect on stomatal opening/closing is/are: ____
D. The factor/s whose effect cannot be clearly established in this experiment is/are: _____

Options:

1. Light alone
2. Darkness alone
3. Acidic pH
4. Mannitol
5. Unknown Chemical
6. 10 mM KCl alone
7. 100 mM KCl alone
8. $CaCl_2$
9. Abscisic acid alone
10. Abscisic acid and acidic pH

Q. 1.6. (2.5 points) The correct explanation for the observations in Treatments 7 and 8 is:

a. Acidification of guard cells leads to opening of K^+ channels of the plasma membrane. This results in entry of K^+ and water molecules to the guard cells.
b. As the pKa of abscisic acid is close to 5.0, most of the molecules remain undissociated at pH 4.0. This hastens their entry into the guard cells.
c. No effect was observed in either treatment because there was no water stress.
d. Abscisic acid is a strong acid and works best at highly acidic pH.

Put a tick mark (√) in the appropriate box **in Q. 1.6. in the Answer Sheet**

a.	b.	c.	d.

Q. 1.7. (2.5 points) Which of the following correctly explains the effect of mannitol on the stomatal aperture?

a. Mannitol is a highly hydrophilic substance and restricts the entry of water molecules into the guard cells.
b. High concentration of mannitol in the extracellular fluid forces K^+, Cl^- and Ca^{++} to enter the guard cells. This leads to entry of water molecules into the cells as well.
c. Entry of mannitol into guard cells increases their solute potential leading to uptake of water.
d. High solute concentration of mannitol results in withdrawal of water from guard cells.

e. Entry of mannitol in the guard cells is counter-balanced by the efflux of K^+ and Ca^{++} leading to the withdrawal of water from the guard cells.

Put a tick mark (√) in the appropriate box **in Q. 1.7. in the Answer Sheet**

a.	b.	c.	d.	e.

Q. 1.8. (2.5 points) You have already observed the effect of the Unknown Chemical on stomata (Treatment 5. Leaves were immersed in solution containing 10 mM KCl, 0.1 mM $CaCl_2$, pH 7.0 containing an Unknown Chemical and kept in dark for 2 h.). These results suggest that the chemical could be useful for:

a. weed control by increasing the rate of respiration.
b. keeping plant cuttings fresh over long periods by preventing water loss.
c. weed control by acting as a wilting toxin.
d. increasing crop yield in arid lands by increasing rate of photosynthesis.
e. increasing plant growth by reducing photorespiration.

Put a tick mark (√) in the appropriate box **in Q. 1.8 in the Answer Sheet.**

a.	b.	c.	d.	e.

Q. 1.9. (2 points) In this task, you studied the effect of various factors on the opening and closing of the stomatal aperture. Similar experiments were performed by scientists and they discovered that light activates zeaxanthin molecules, present in the guard cells, which in turn, activate an ATP-powered proton pump of the guard cell membrane. With this background information and the observations made by you in this task, you have to arrange the sequence of events involved in the response of stomata to light. Fill in the correct options against each step **in Q. 1.9. in the Answer Sheet**.

Mechanism:
Step I: 1
Step II: 2
Step III: _____
Step IV: 6
Step V: _____
Step VI: _____
Step VII: _____

Options:
1) Activation of zeaxanthin by light
2) Activation of ATP-powered proton pump

3) Closing of the stomata
4) Influx of K^+
5) Efflux of K^+
6) Change in membrane potential
7) Efflux of Ca^{++}
8) Efflux of protons
9) Influx of water molecules
10) Efflux of water molecules
11) Opening of the stomata

Task 2 (14 points)
Study of plant anatomy and its correlation with the habitat

You should try and complete this task in 30 minutes.

Materials and equipment	**Quantity**
1. Fresh plant specimens	
(i) Leaf in a Petri dish (labeled X)	1
(ii) Stem in a Petri dish (labeled Y)	1
2. Compound binocular microscope	1
3. Razor blades	2
4. Glass microslides	2
5. Box of coverslips	1
6. Watchglasses	3
7. Safranin staining solution (labeled S)	1
8. Brush	1

Introduction

Plants growing in different habitats exhibit various adaptations. These adaptations can be studied macroscopically as well as microscopically and correlated to their habitats.

In this task, you will study the anatomy of the given specimens using the following method. Both specimens X and Y belong to the same plant.

Method

1. Take thin transverse sections of the leaf specimen X.
2. Stain with Safranin staining solution for about 30 to 60 seconds.
3. Wash the section with distilled water and mount on a clean glass microslide in a drop of water.
4. Place a coverslip and observe under 10X objective of the microscope.
5. Repeat Steps 1- 4 for the stem specimen Y.

Observations on the leaf specimen X:

Observe the leaf section and answer **Questions Q. 2.1. and Q. 2.2.**

Q. 2.1. (4 points) Choose the appropriate letters from the Dichotomous Keys 1 and 2 given in **Annexure 2.1.** and fill in **Q. 2.1.I. and Q. 2.1.II. in the Answer Sheet.**

Note: Schematic representations of some of the plant structures are given in **Annexure 2.2.** for your reference.

I. Trichomes ☐

II. Stomata ☐

Q. 2.2. (4 points) Based on your observations on the leaf section, put a tick mark (√) in the appropriate boxes **in Q. 2.2. in the Answer Sheet**.

	Present	Absent
1. Cuticle	☐	☐
2. Sclerenchyma	☐	☐
3. Collenchyma	☐	☐
4. Aerenchyma	☐	☐
5. Water storage tissue	☐	☐
6. Glands:		
a. Oil gland	☐	☐
b. Salt gland	☐	☐
c. Digestive gland	☐	☐

Observations on the stem specimen Y:
Observe the stem section and put a tick mark (√) in the appropriate boxes in **Q. 2.3. in the Answer Sheet**.

Q. 2.3. (3.5 points)

	Present	Absent
1. Cuticle	☐	☐
2. Sclerenchyma	☐	☐
3. Collenchyma	☐	☐
4. Aerenchyma	☐	☐
5. Water storage tissue	☐	☐
6. Vascular bundle:	Open	Closed
	☐	☐
	Collateral	Bicollateral
	☐	☐

Q. 2.4. (2.5 points) Based on your observations of specimens X and Y, identify the type of plant to which they belong.

a. Mesophyte
b. Succulent xerophyte
c. Submerged hydrophyte
d. Floating hydrophyte
e. Insectivorous mesophyte
f. Parasitic mesophyte
g. Halophyte
h. Freshwater hygrophyte

Put a tick mark (√) in the appropriate box in **Q. 2.4. in the Answer Sheet.**

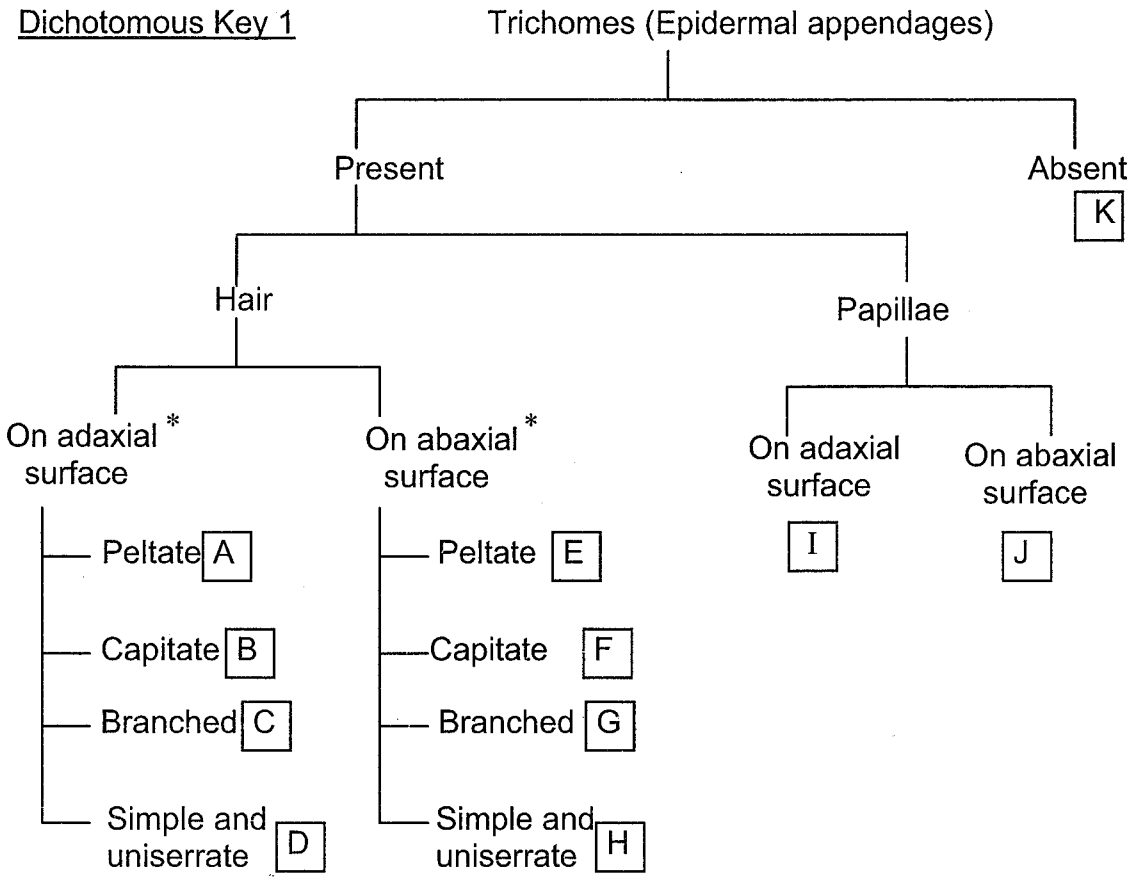
(In this question, the correct interpretation will be given points if it is consistent with your observations.)

a.	b.	c.	d.	e.	f.	g.	h.

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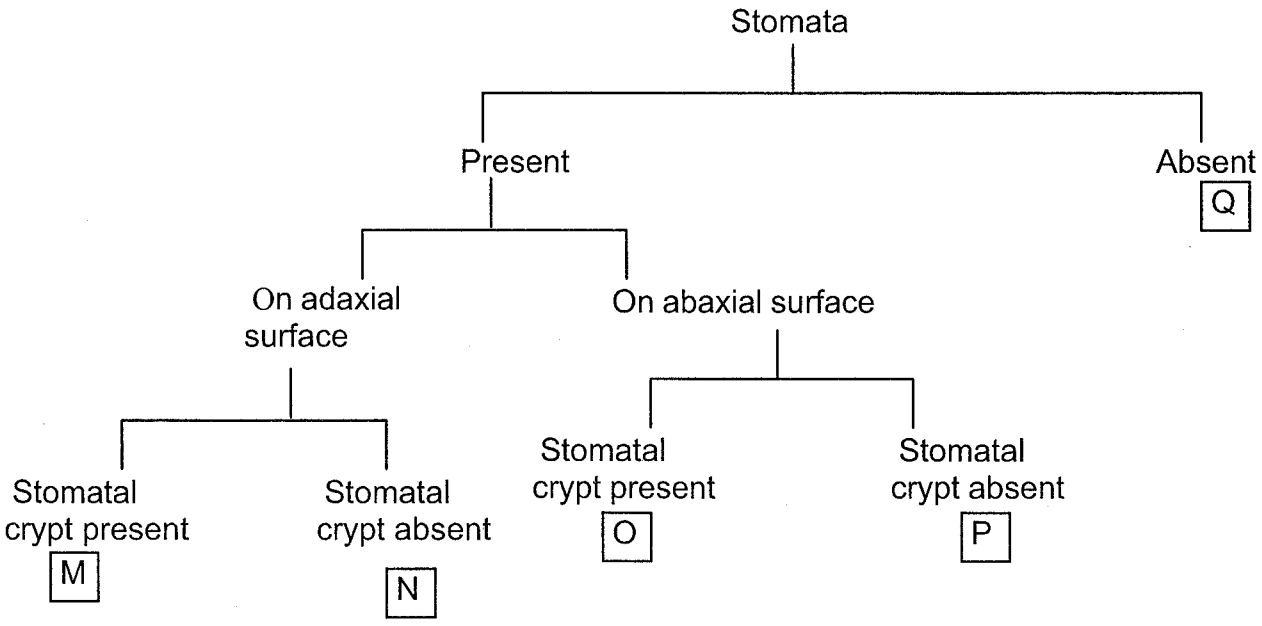
ANNEXURE 2.1

Dichotomous Key 1



* NOTE: Adaxial: facing the stem; abaxial: facing away from the stem

Dichotomous Key 2



ANNEXURE 2.2.

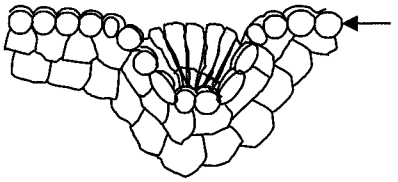


Figure 1: Salt Gland

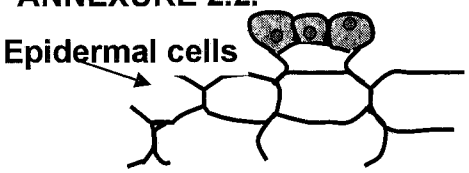


Figure 2: Digestive Gland

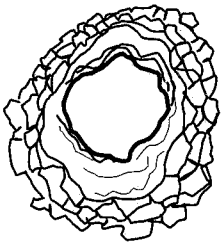


Figure 3: Oil Gland

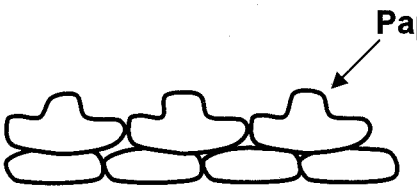


Figure 4: Papillose Epidermis

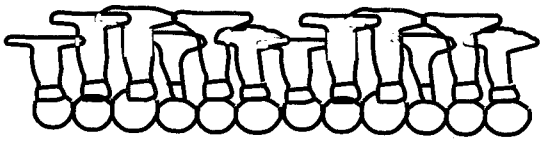


Figure 5: Peltate Hair

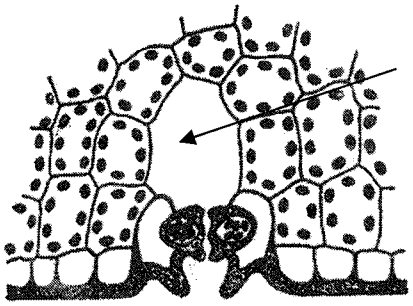


Figure 6: Sunken Stoma

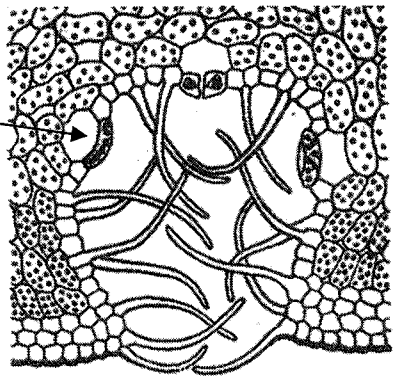


Figure 7: Stomatal Crypt

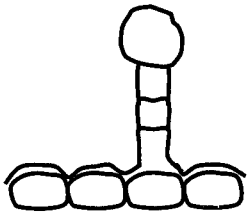


Figure 8: Capitate Hair

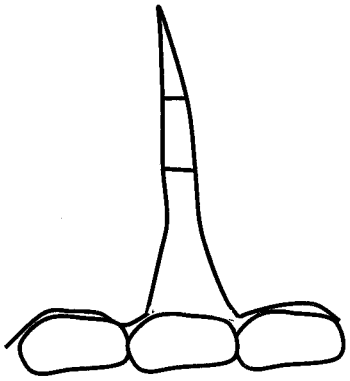


Figure 9: Uniserrate Trichome

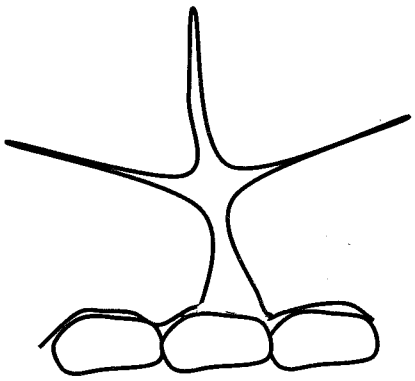


Figure 10: Branched Trichome

**********END OF PRACTICAL TEST 1**********

PRACTICAL TEST 2: ANIMAL ANATOMY AND PHYSIOLOGY

(66 points, 60 minutes)

Task 1 (54 points)

Study of animal skeletal systems

You should try and complete this task in 45 minutes.

Materials and equipment	**Quantity**
12. Set of skeletal specimens labeled 1 to 9 in sealed boxes **(Please do not open the boxes!)**	9
13. A set of photographs of three skulls labelled 1A, 2A and 3A	1
14. Magnifying hand-lens	1

Introduction

The skeletal system provides physical support and a scaffold for the body and defines its architecture in animals. The three types of skeletal systems include an external (exoskeleton), internal (endoskeleton) and a fluid-based (hydrostatic skeleton) system.

The internal skeleton in vertebrates determines its body shape, provides support for its weight and offers sites for muscle attachment. Although structural modifications in the skeleton may occur in different groups of animals, the basic plan by and large remains the same.

In this task, you will observe and compare the internal skeletal systems of three present-day vertebrates. The models of the skeletal parts provided to

you include the skull, the vertebral column and the limb bones. At the end of the task, you will match these parts to form the complete skeletal system of each of the three vertebrates.

Part A: Comparative study of skulls

(i) Types of skull:

The skull of vertebrates is a bony structure that serves as the general framework for the head. Structurally, the skull comprises four regions – frontal, parietal, occipital and temporal (Figure 1). There are various openings in different regions of the skull, including the nostrils, eye sockets and the temporal openings. The placement of the eyes with respect to each other determines the field of vision of the animal.

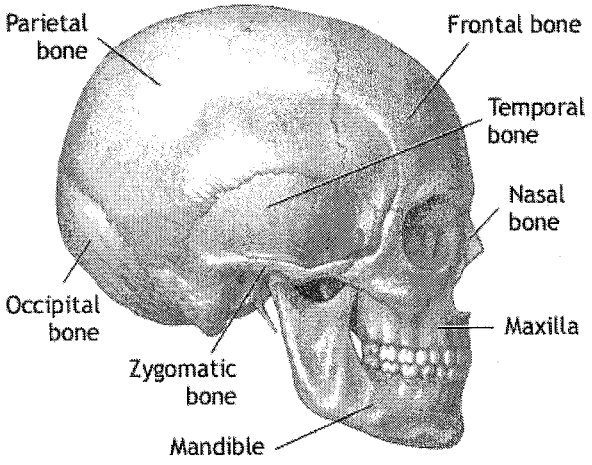


Figure 1

The number of temporal fenestrae (openings) and the position of these openings are used to broadly classify vertebrate skulls into the following four major categories:

(A) Anapsid skull: Anapsids get their name from the fact that they have no additional openings in their skulls apart from their eye sockets and nostrils. The temporal region is covered completely by bone. This type of skull is characteristic of fishes, amphibians, and early reptiles (Figure 2).

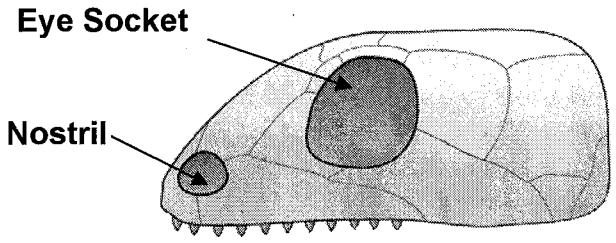
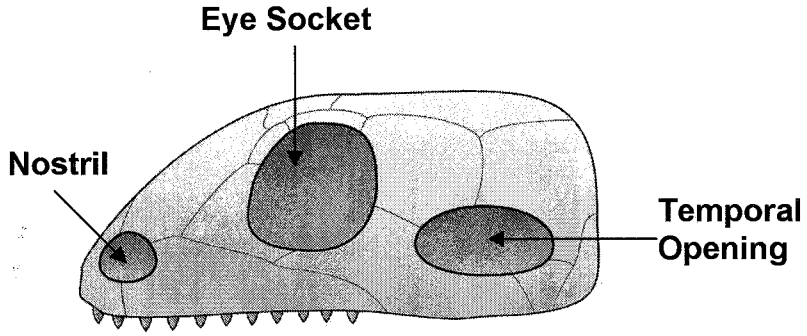


Figure 2

(B) Synapsid skull: It has a single pair of temporal openings. It was found in mammalian ancestors and represented an early divergence from the anapsids. The skull of present day mammals represents a modified synapsid pattern (Figure 3).



(C) Diapsid skull: It is characterized by two pairs of temporal openings. This type diverged from the anapsids and has undergone extensive modification. It is found in pterosaur and dinosaur fossils, as well as in birds and all living reptiles. One of the highly modified forms of the diapsid skull is found in lizards, where the lower temporal opening is not as distinct as the upper one (Figure 4).

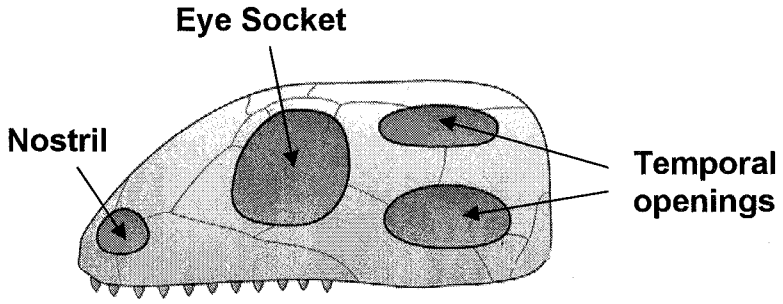


Figure 4

(D) Euryapsid skull: It has a single pair of temporal openings. The euryapsid skull seems to be derived from diapsid ancestors by loss of the lower temporal openings. Two groups of Mesozoic marine reptiles (plesiosaurs and ichthyosaurs) possessed this type of skull (Figure 5).

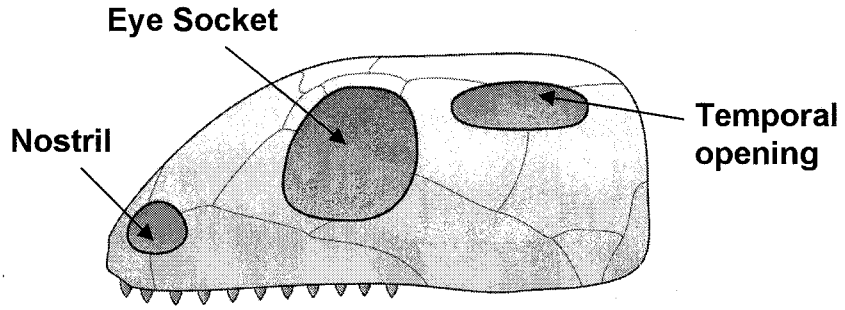
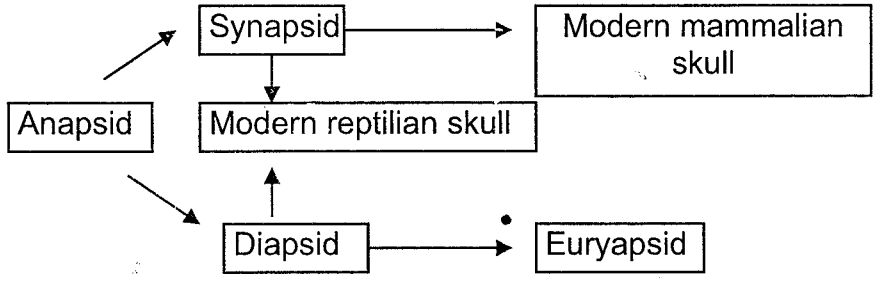


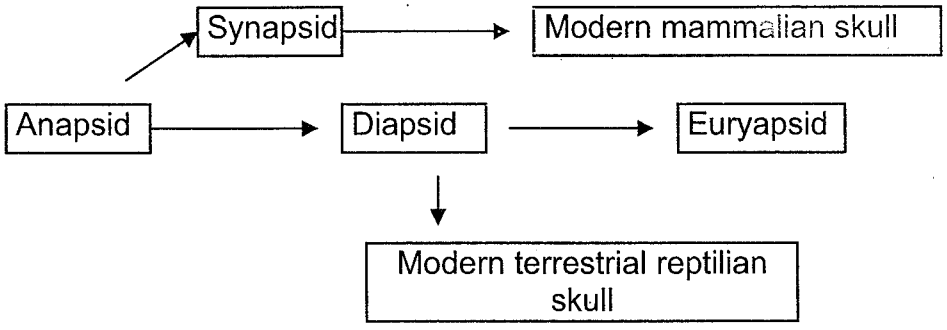
Figure 5

Q. 1.A.1. (2 points): Based on the information provided earlier, choose the cladogram that most likely depicts the evolution of skulls by putting a tick mark (√) in **Q. 1.A.1. in the Answer Sheet**.

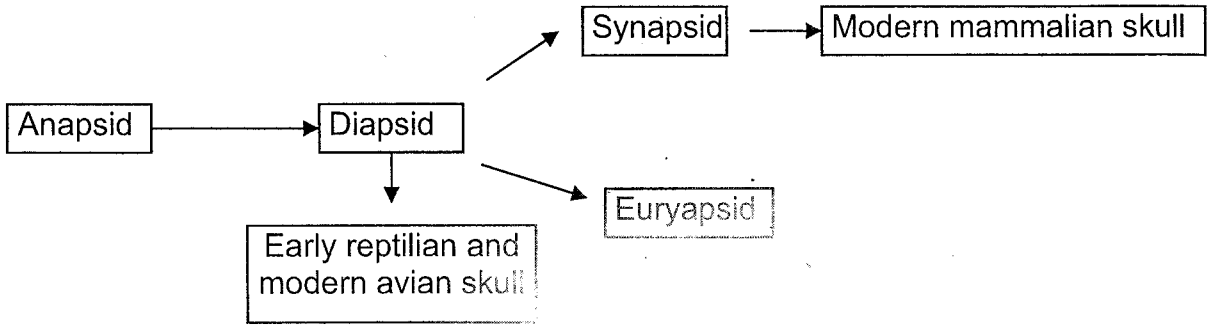
a.



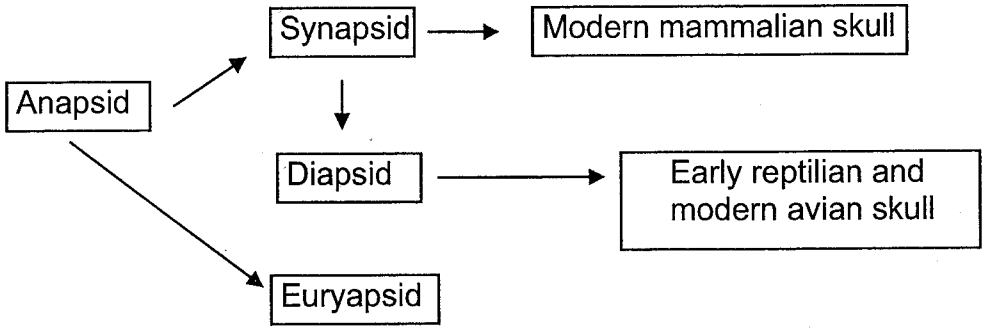
b.



c.



d.



a.	
b.	
c.	
d.	

(ii) Dentition: Dentition refers to the type and arrangement of teeth in an animal and is an adaptation to its feeding habit. Based on the dentition, vertebrates can be broadly classified as homodont or heterodont. Depending on the number of times the teeth are replaced during the life span of an animal, they can be further classified as diphyodont or polyphyodont.

Q. 1.A.2. (6 points) Observe the Specimens 1, 2 and 3 for the type of skull and the respective photographs 1A, 2A, and 3A for their dentition. Put tick marks (√) in the appropriate boxes in **Table 1.A.2. in the Answer Sheet**.

Table 1.A.2.

Character		1	2	3
Type of skull	Anapsid			
	Diapsid			
	Synapsid			
	Euryapsid			
Type of dentition	Homodont			
	Heterodont			

Q. 1.A.3. (6 points) Observe the specimens for position of orbit (the eye sockets), and for types of teeth. Fill in the **Table 1.A.3. in the Answer Sheet** by putting tick marks (√) in the appropriate boxes.

Table 1.A.3.

Features		1	2	3
Vision	Predominantly stereoscopic vision			
	Predominantly non-stereoscopic vision			
Feeding habit	Predominantly carnivorous			
	Predominantly herbivorous			

Part B: Comparative study of vertebral columns and ribs

The vertebral column and ribs are components of the axial skeletal system.

The vertebral column defines the major body axis and comprises a series of separate bones (vertebrae) joined to form a backbone (Figure 6).

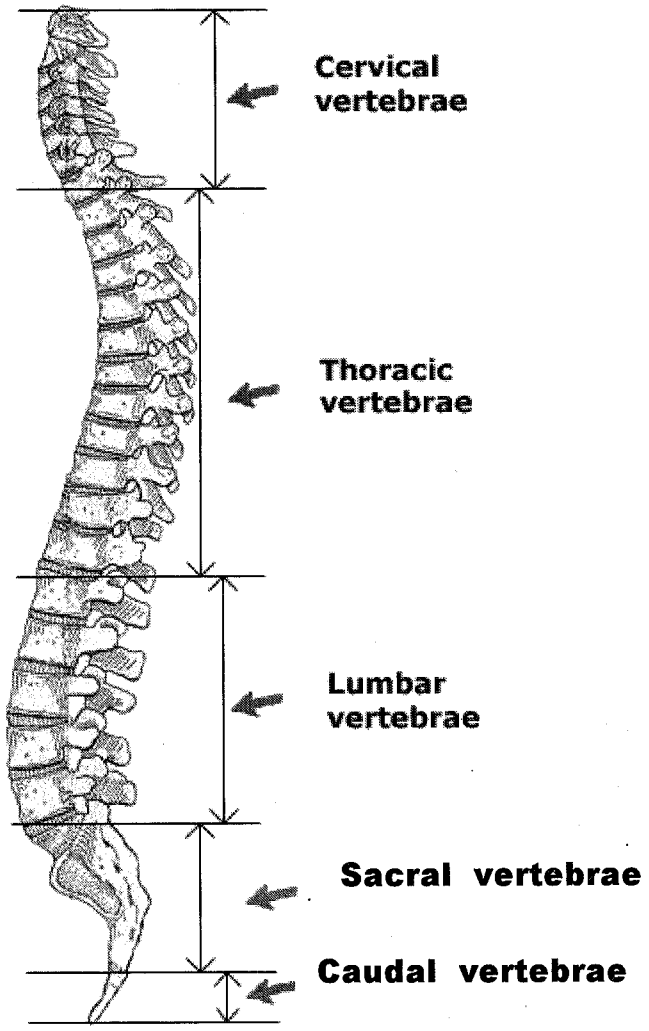


Figure 6

Cervical vertebrae, the first set of vertebrae, are characterized by highly reduced transverse processes in contrast to the following set of vertebrae, the thoracic vertebrae. The number of cervical vertebrae is usually correlated with the degree of neck movement.

In higher animals, the thoracic vertebrae are important because they articulate with the ventral sternum and ribs to form a rib cage.

Ribs also provide sites for secure muscle attachment, help suspend the body, form a protective case around the viscera and sometimes serve as accessory breathing devices (Figure 7).

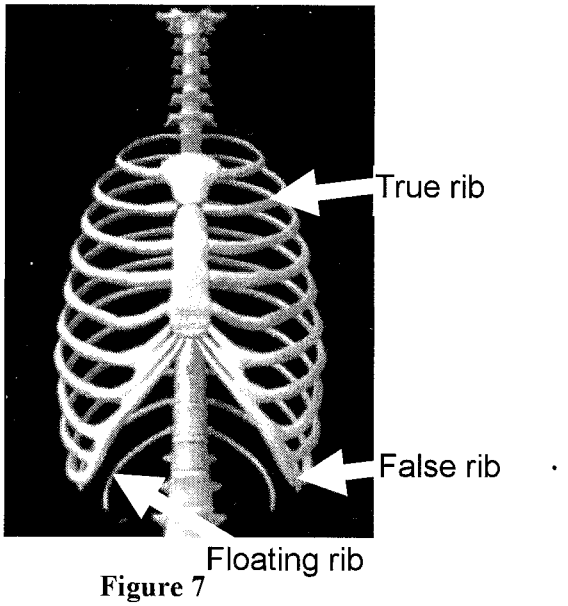


Figure 7

The sternum is a mid-ventral skeletal structure that offers a site of origin for chest muscles and secures the ventral tips of true ribs to complete the protective rib cage. A well-developed rib cage is characteristic of mammals.

The classification of ribs in tetrapods is based on the type of association they establish with the sternum. The three kinds of ribs are:

True ribs – these ribs meet ventrally with the sternum.
False ribs – these ribs articulate with each other but not with the sternum.
Floating ribs – these ribs do not articulate with the sternum or any other structure. These ribs, when present in large numbers offer flexibility to the body during locomotion.

Q. 1.B.1. and Q.1.B.2. (8 + 3 = 11 points) Observe the Specimens 4, 5 and 6 and put tick marks (√) in the appropriate boxes in **Tables 1.B.1. and 1.B.2. in the Answer Sheet**.

Table 1.B.1.

Characters		4	5	6
Ribs	Present			
	Absent			
Major type of ribs	True			
	False			
	Floating			
Tail	Present			
	Reduced /Absent			

Table 1.B.2.

Feature		4	5	6
Neck movement	Restricted			
	Free			

Part C: Comparative study of limb bones

The transition of vertebrates from aquatic to terrestrial and from terrestrial to aerial has had an impact upon the design and redesign of the appendicular system. The appendicular skeleton includes the paired fins or limbs and the
girdles. Schematic figures of representative limb arrangements are given below (Figures 8 and 9).

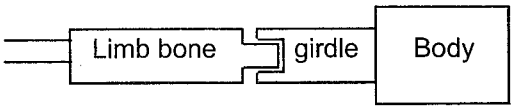


Figure 8: Schematic representation of the articulation of a sprawled limb

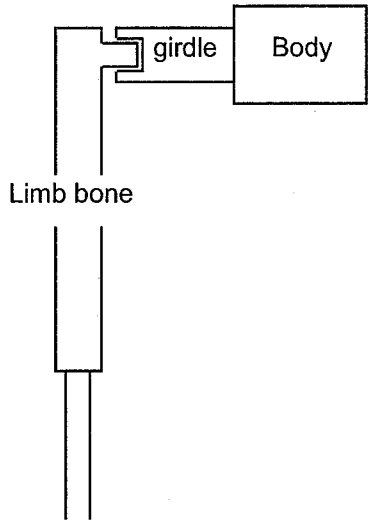


Figure 9: Schematic representation of the articulation of an underneath limb

Q. 1.C.1. (12 points) Carefully study the Specimens 7, 8 and 9, and fill in **Table 1.C.1. in the Answer Sheet** by putting tick marks (√) in the appropriate boxes.

Characters		7	8	9
Position of limb with respect to body	Sprawled			
	Underneath			
Length of fore- and hindlimbs	Similar			
	Fore limbs longer			
	Hind limbs longer			
Claws	Present			
	Absent			
Modifications	Tibia and fibula completely fused			
	Tibia and fibula partially separate			

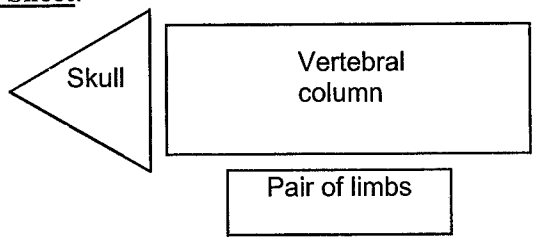
Q. 1.C.2. (8 points) Based on your observations, fill in **Table 1.C.2. in the Answer Sheet** by putting tick marks (√) in the appropriate boxes.

Table 1.C.2.

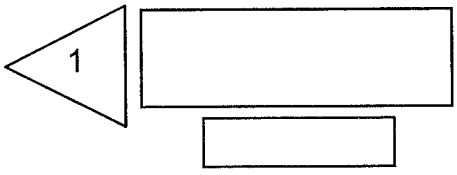
Features		7	8	9
Limb movement during locomotion	Swinging (rotational movement)			
	Pendulum-like			
Habit of the animal	Saltatorial (jumping)			
	Cursorial (walking)			
	Fossorial (digging)			

Part D: Assembly of the skeletal systems

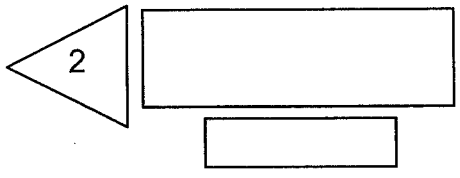
Q. 1.D.1 (6 points) The nine specimens (three skulls, three vertebral columns and three sets of limb bones) belong to three different animals (I, II, and III). In the schemes given below, write the respective specimen numbers (4 to 9) in the appropriate boxes to construct the three animals in **Q. 1.D.1. in the Answer Sheet**.



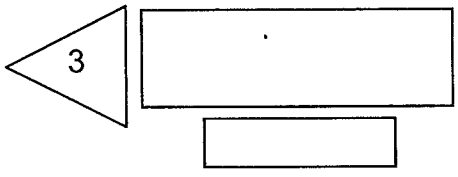
Animal I



Animal II



Animal III



Q. 1.D.2 (3 points): Assign each of the three animals to the most probable class. Choose from the options given below and write appropriate letter in **Q. 1.D.2. in the Answer Sheet.**

Animal I: Class: _____

Animal II: Class: _____

Animal III: Class: _____

Options:
A. Mammalia
B. Reptilia
C. Aves
D. Amphibia
E. Pisces

Task 2 (12 points)

Semi-quantitative estimation of nitrogenous waste products

You should try and complete this task in 15 minutes.

	Materials	**Quantity**
1.	Porcelain spot plates, each with 6 cavities	3
2.	Toothpicks	20
3.	Permanent marker pen	1
4.	Tissue paper roll	1
5.	Container for wash and discard	1
6.	Reagents (given in a plastic box)	1 bottle each

Label	**Reagent**
A	Phosphotungstic acid
B	Sodium carbonate (20% w/v)
C	Uric acid (standard solution)
D	Ehrlich's reagent (mildly corrosive)
E	Urea (standard solution)
F	Sodium nitroprusside
G	Oxidizing solution
H	Phenol solution (mildly corrosive)
I	Ammonia (standard solution)
S1	Simulated Sample 1
S2	Simulated Sample 2
S3	Simulated Sample 3
H_2O	Distilled water

Introduction

Vertebrates have evolved different modes of excretion of nitrogenous wastes, which are mostly derived from degradation of proteins and nucleic acids. They use different ways of excretion of these wastes during their transition from an aquatic to terrestrial mode of life. The three major forms of these wastes are ammonia, urea and uric acid. While ammonia is highly soluble in water, uric acid is the least soluble. Ammonia, being most toxic, needs to be excreted in a highly diluted form. Uric acid is mostly excreted as semisolid crystals.

Three simulated samples (S1, S2 and S3), representing nitrogenous wastes from three groups of animals, are provided. Follow the protocols given below to find out the relative levels of uric acid, urea and ammonia in these samples.

General Instructions

1. ***For each test, run a positive control and a negative control using the standard solutions and distilled water, respectively.***
2. ***Grade the colour of positive control as '+++' and that of negative control as ' – '.***
3. ***Please note that the recording of the results for the positive and negative controls carries NO points.***

Protocols for estimation

1. Estimation of uric acid by phosphotungstic acid reduction method

Principle

Under alkaline conditions, uric acid reduces phosphotungstic acid to give a blue- coloured product.

Method

(i) Put three drops each of Samples S1, S2 and S3 in separate cavities of a given spot plate.

(ii) Add one drop each of solutions A followed by B to each cavity. Mix with separate toothpicks and observe the developed colour.

(iii) Grade the colour of the positive control as '+++' and that of the negative control as ' – '.

Q. 2.1.1. (3 points) Record the results in **Table 2.1. in the Answer Sheet** by putting '+++', '++' or '+' for positive results depending on the intensity of the colour developed and '–' for negative results.

2. Estimation of urea using Ehrlich's reagent

Principle

Under strong acidic conditions, urea reacts with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde) to form a yellow-coloured dye (protonated Schiff's base).

Method

(i) Put three drops each of Samples S1, S2 and S3 in separate cavities of a given spot plate.

(ii) Add one drop of solution D to each cavity. Mix with separate toothpicks.

Q. 2.1.2. (3 points) Record your results **immediately** in **Table 2.1. in the Answer Sheet** by putting '+++', '++' or '+' for positive results depending on the intensity of the colour developed and '-' for negative results. For comparison, grade the colour of the positive control as '+++' and that of the negative control as ' – '.

3. Estimation of ammonia by indophenol blue method

Principle

In an alkaline solution, ammonium ions react with oxidizing solution to form monochloramine. In the presence of phenol and an excess of oxidizing solution, the monochloramine forms a blue-coloured product, indophenol, when nitroprusside is used as a catalyst.

Method

(i) Put three drops each of Samples S1, S2 and S3 in separate cavities of a given spot plate.

(ii) Add one drop each of solutions F, followed by G and finally H to each cavity. Mix with separate toothpicks.

Q. 2.1.3. (3 points) Record your results **after two minutes** in **Table 2.1. in the Answer Sheet** by putting '++++', '+++', '++' or '+' for positive results depending on the intensity of colour developed and '-' for negative results. For comparison, grade the colour of positive control as '+++' and that of negative control as ' – '.

Table 2.1.

Samples	Uric acid test	Urea test	Ammonia test
S1			
S2			
S3			
Positive control			
Negative control			

Q. 2. 2. (3 points): Based on the results obtained, match each of the samples with the appropriate class of vertebrates listed below. Fill in your answer by putting the appropriate letter in **Q. 2.2. in the Answer Sheet**.

Answer: _____

a.	S1: Pisces	S2: Mammalia	S3: Reptilia
b.	S1: Amphibia	S2: Aves	S3: Pisces
c.	S1: Mammalia	S2: Reptilia	S3: Aves
d.	S1: Mammalia	S2: Pisces	S3: Aves
e.	S1: Aves	S2: Pisces	S3: Mammalia
f.	S1:Reptilia	S2: Amphibia	S3: Mammalia
g.	S1: Aves	S2: Reptilia	S3: Amphibia

********** END OF PRACTICAL TEST 2 **********

PRACTICAL TEST 3: BIOCHEMISTRY AND CELL BIOLOGY

(43 points, 60 minutes)

Task 1

PART A (35 points)

Study of β-lactamase activity and its inhibition

Materials and equipment	Quantity
1. Colorimeter, with a set of seven cuvettes	1
2. Test tubes	8
3. Test tube stand	1
4. Micropipette (10 – 100 µl capacity)	1
5. Micropipette (100 – 1000 µl capacity)	1
6. Micropipette tips (10 – 100 µl capacity)	20
7. Micropipette tips (100 – 1000 µl capacity)	20
8. Photographs of Petri plates	6
9. Permanent marker	1
10. Tissue paper roll	1
11. Wash bottle containing distilled water	1
12. Container for wash and discard	1
13. Graph paper	1

Reagents (please see the next page)

Label	Reagent	Container
A	β – Lactamase enzyme (1.85 mg/ml)	Vial
B	Inhibitor (100 mM)	Vial
C	Penicillin G (0.54 mM)	Blue-stoppered tube
D	Sodium phosphate buffer, pH 7.0 (10 mM)	Blue-stoppered tube
E	$CuSO_4$-Neocuproine reagent	Blue-stoppered tube
F	HCl (2 M)	White-stoppered tube

Handling of micropipette:

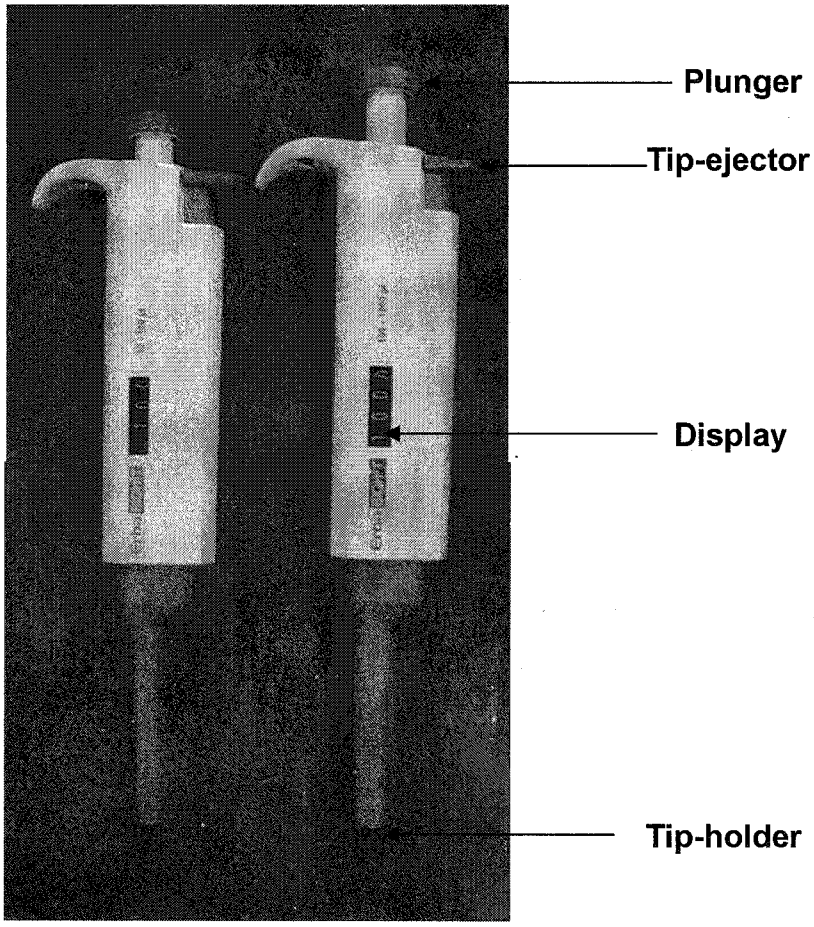


Figure 1

Adjustment method

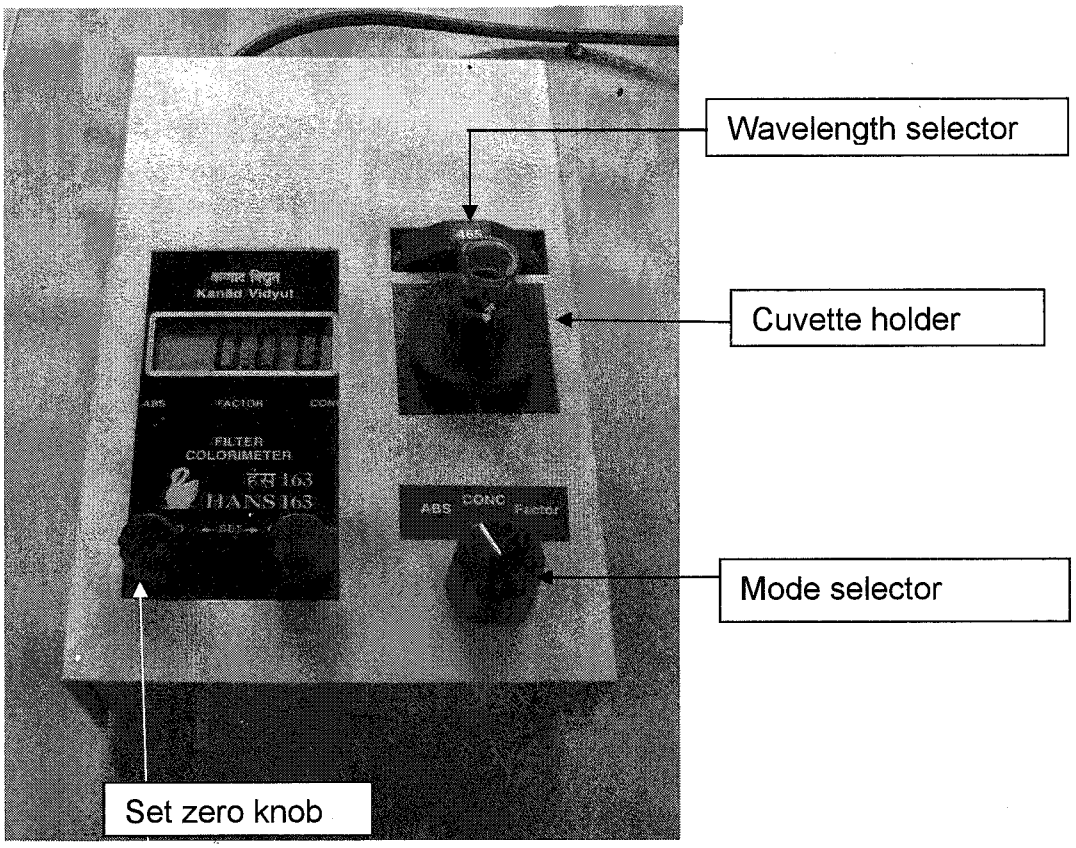
Turn the plunger (Figure 1) to set the value to the desired volume, which can be seen in the display.

Remember that each micropipette has a fixed range of volumes as indicated on the pipette. DO NOT CROSS THE LIMITS OF THIS RANGE.

Usage method

Secure the pipette tip to the tip holder (Figure 1). Gently push down the plunger to the first stop, hold, and dip the tip into the solution vertically to a depth of 2 - 4 mm. Release the plunger slowly and make it return to the original position. Remove the pipette from the liquid and transfer the contents to the desired tube. Make sure that the tip is close to the inner wall of the tube. Push the plunger to the first stop and then push further to discharge the solution completely from the tip. Remove the pipette from the tube. Eject the used tip into the discard container by pressing the tip-ejector.

Operating Instructions for the colorimeter



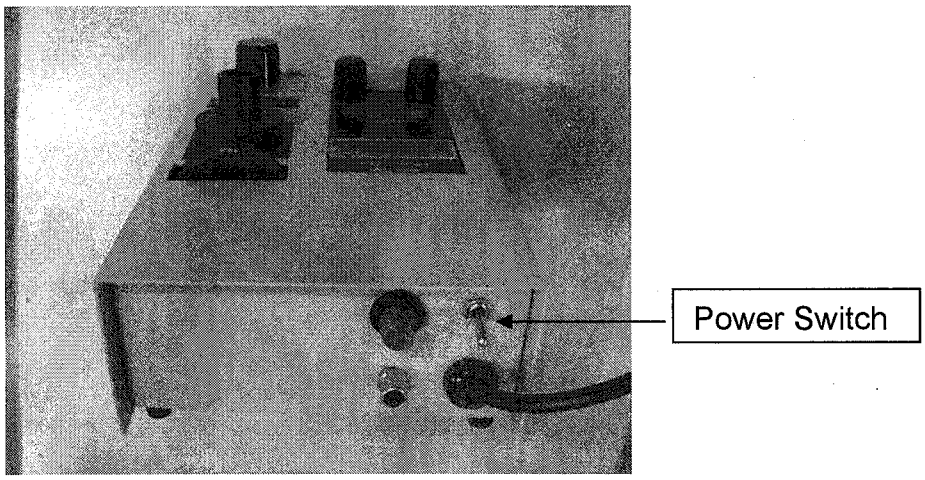


Figure 2: Rear view of colorimeter

1) Turn the power switch (Figure 2) of the colorimeter ON.
2) Set the instrument to Absorbance mode ("ABS") using the mode selector.

3) Set the wavelength to 465 nm using the wavelength selector.
4) Put the blank solution in a cuvette. Clean the outside surface of the cuvette with tissue paper and insert it into the cuvette holder. Gently push the cuvette all the way down.
5) Rotate the 'set zero' knob to set the reading to zero. The instrument is now ready for measuring the absorbance of the test solutions.

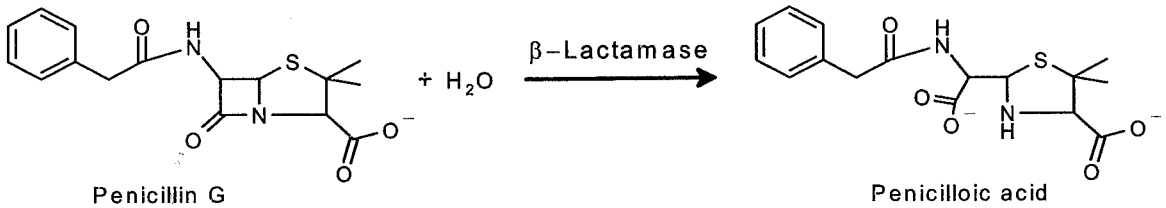
Introduction

Penicillins are antibiotics with a characteristic β-lactam ring in their structure. This antibiotic kills bacteria by inhibiting the cell wall synthesis. However, these molecules are rendered inactive by some bacteria, which synthesize an enzyme called β-lactamase. These bacteria, which produce β-lactamases, are resistant to penicillins. Due to this, penicillin treatment is ineffective in patients infected with such resistant bacteria. One approach to overcome this problem is to develop effective β-lactamase inhibitors.

The effectiveness of a β-lactamase inhibitor can be evaluated by determining its IC_{50} and K_i values. The IC_{50} of an inhibitor is defined as the concentration of the inhibitor required to inhibit the enzyme activity by 50 percent. The K_i of an inhibitor is a measure of its binding affinity for the enzyme.

Principle of β-lactamase assay

β-Lactamase inactivates penicillin by catalyzing the following reaction:



Penicillin G

Penicilloic acid

The penicilloic acid generated is complexed with $CuSO_4$ in the presence of neocuproine. The yellow-colored product formed can be monitored by measuring its absorbance at 465 nm using a colorimeter.

In this task, you will:

- determine the IC_{50} value of a given inhibitor by generating a dose-response curve, and
- calculate the K_i value for the inhibitor.

A dose-response curve for the inhibitor is generated by measuring the activity of β-lactamase in the presence of varying concentrations of the inhibitor at a fixed concentration of the substrate.

Q. 1.A.1. (18 points) Follow the protocol given below and enter the absorbance values **in Table 1.A.1. in the Answer Sheet**.

I. Prepare the following reaction mixtures:

Test tube	Sodium phosphate buffer, pH 7.0	Inhibitor (100 mM)	β-lactamase enzyme	Distilled water
1	1.48 ml	-	20 µl	-
2	1.46 ml	20 µl	20 µl	-
3	1.44 ml	40 µl	20 µl	-
4	1.42 ml	60 µl	20 µl	-
5	1.40 ml	80 µl	20 µl	-
6	1.38 ml	100 µl	20 µl	-
Blank	1.43 ml	50 µl	-	20 µl

II. Mix gently and incubate at room temperature for 5 minutes. **You may use the wall clock or your wrist watch to keep track of the incubation time.**
III. Add 1 ml of penicillin G (0.54 mM) to each tube and mix gently. Incubate at room temperature for 10 minutes.
IV. Add 1.5 ml of the $CuSO_4$-neocuproine reagent to each tube and mix gently. Incubate at room temperature for 5 minutes.
V. Stop the color development by adding 100 µl of HCl to each tube and mix gently.
VI. Set the colorimeter to 465 nm.
VII. Use the Blank to set the absorbance to zero.
VIII. Measure the absorbance values of the solutions in Test tubes 1 to 6, and enter these values in the table. **You should get any one absorbance reading countersigned by the supervisor. To call the supervisor, raise the yellow card.**

Table 1.A.1.

Test tube	Absorbance
1	
2	
3	
4	
5	
6	

Data analysis and interpretation

Q. 1.A.2. (6 points)

I. Calculate the concentrations (in mM) of the inhibitor [I] in 2.5 ml of the enzyme reaction in Test tubes 1 to 6 and enter these values **in Table 1.A.2. in the Answer Sheet.**

II. Consider the absorbance values to be the rates of hydrolysis of penicillin G. Now calculate v_i/v_0, where:

v_0 is the rate of hydrolysis of penicillin G by β-lactamase in the absence of the inhibitor, and v_i is the rate of penicillin G hydrolysis in the presence of the inhibitor. Note that for Test tube 1, $v_i = v_0$.

Enter these values (up to two decimals) **in Table 1.A.2. in the Answer Sheet.**

Table 1.A.2.

Test tube	[I] (mM)	v_i/v_0
1		
2		
3		
4		
5		
6		

Q. 1.A.3. (5 points) Plot a graph of v_i/v_0 versus [I] in the **Graph Paper attached to the Answer Sheet**.

Determination of the IC_{50} and K_i value of the inhibitor

Q. 1.A.4. (3 points) Determine the IC_{50} value by interpolation of the data points in the graph. Write the value (up to two decimals) in the box **in the Answer Sheet.**

IC_{50} = _____ mM

Q. 1.A.5. (3 points) Calculate the dissociation constant K_i of the inhibitor using the equation:

$$IC_{50} = K_i\left(1+\frac{[S]}{K_m}\right)$$

where K_m is the Michaelis-Menten constant of β-lactamase for penicillin G and [S] is the initial concentration of substrate (penicillin G) present in the enzyme reaction mixture.
Assume the K_m of β-lactamase for penicillin G to be **0.05 mM**. Write down your answer (up to two decimals) in the box **in the Answer Sheet**.

K_i = _____ mM

PART B (4 points)

Correlating β-lactamase expression to resistance

When penicillin-resistant bacteria are grown in liquid culture media, β-lactamase is secreted into the medium. The supernatant of such a medium can be assayed for β-lactamase activity. Culture supernatants from four different organisms (P, Q, R and S), which are suspected to be penicillin-resistant, were obtained and 20 µl of each was assayed for β-lactamase activity. The corresponding absorbance values were measured at 465 nm and are given in the table below.

Organism	Absorbance
P	0.090
Q	0.450
R	0.075
S	0.220

These four organisms were tested for their resistance to penicillin G by the disc diffusion plate assay as follows:

1. Each organism was separately inoculated into warm growth medium and poured into a sterile Petri plate. On cooling, the medium solidified.
2. Filter paper discs impregnated with varying concentrations of penicillin G were then placed on the surface of the medium.
3. The plates were incubated allowing penicillin to diffuse and organisms to grow.
4. Organisms sensitive to penicillin will not be able to grow in the vicinity of the antibiotic disc and hence, a clear zone will be obtained around the disc.

You have been given labeled photographs of six plates I - VI.

Plate I is a control plate showing uniform mat growth of organisms in the absence of penicillin G.
Plate II is also a control plate that contains media without the growth of any organism.
Plates III to VI show the growth of the four organisms in the presence of penicillin G. 2.5, 5, 7.5, 10 and 12.5 are the micrograms of penicillin G present in the respective discs.

Q. 1.B.1. (4 points) Observe these plates and infer which organism is growing in each plate. Write your answers **in Table 1.B.1. in the Answer Sheet**.

Table 1.B.1.

Plate	Organism
III	
IV	
V	
VI	

PART C (4 points)

Correlating K_i values of pesticides to bacterial growth

Four pesticides P1 to P4 are reversible inhibitors of an enzyme E that is essential for the growth of a bacterium B. Their K_i values are given in the table below. Each of these four pesticides is used in four geographically different
regions R1 to R4. The residual concentrations of these four pesticides in the respective regions are also shown in the table below:

Region	R1	R2	R3	R4
Pesticide	P1	P2	P3	P4
K_i for the enzyme E	1 nM	5 nM	0.45 μM	0.55 μM
Residual concentration	60 nM	100 pM	30 nM	5.5 μM

Q. 1.C.1. (4 points) Indicate whether the bacterium B would grow or not in each of the four regions by putting tick marks (√) in the appropriate boxes **in the Table 1.C.1. in the Answer Sheet**.

Table 1.C.1.

Region	R1	R2	R3	R4
Bacterium B grows				
Bacterium B does not grow				

********** END OF PRACTICAL TEST 3 **********

PRACTICAL TEST 4: ANIMAL BEHAVIOR

(49 points, 60 minutes)

Task 1 – Part A (6 points)

Study of the olfactory response of *Drosophila melanogaster* larvae

Experimental design

You have been given Part A of this task. You have to answer this part in 10 minutes after which the buzzer will ring and the Answer Sheet for this part will be collected from you. Only then will the rest of the paper be given to you.

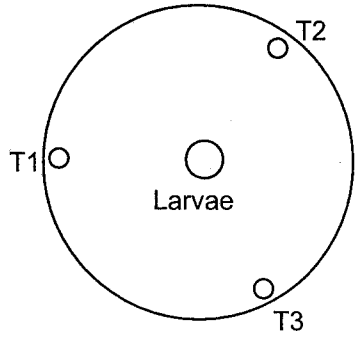
Introduction

Insects have a strong sense of smell. Adult moths, for example, can find their mates by smelling pheromone molecules at very low concentrations. The sense of smell is associated with a discriminatory behavior as well. This is evident from the fact that insects are able to choose their food by odor. The nature of an odor stimulus can be categorized into three types: (1) attractive, (2) repulsive, and (3) neutral.

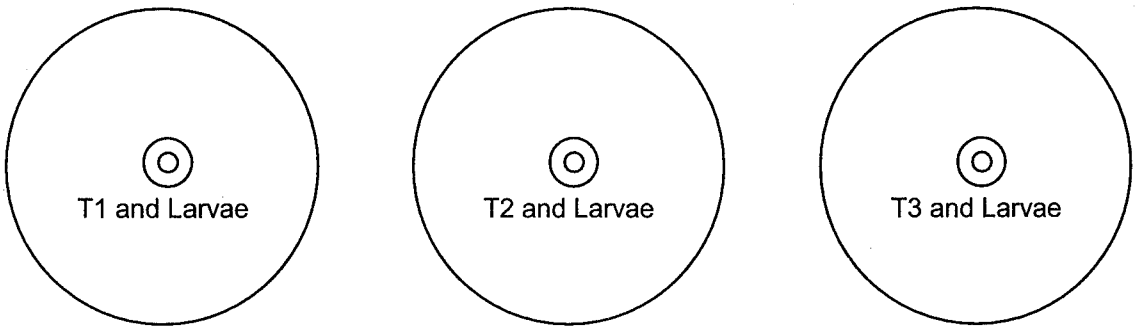
The odor discriminatory behavior of insects such as *Drosophila melanogaster*, the common fruit fly, can be assessed using either the adults or the larvae. *Drosophila* larvae respond to odor stimuli by crawling either towards or away from them. It is, therefore, possible to design an experiment to test the larval response towards different chemicals on a Petri plate.

Q. 1.A.1. (3 points) Suppose you want to determine the response of *Drosophila melanogaster* larvae to three chemical odorants T1, T2, and T3. Five possible experimental designs for this purpose are given below:

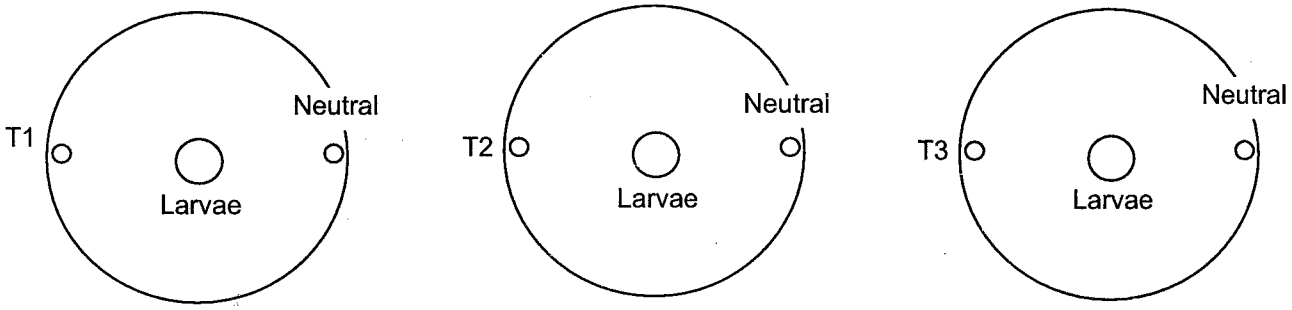
Design I: All the three chemicals are placed at equidistant positions on the periphery of a Petri plate and the larvae are introduced at the centre.



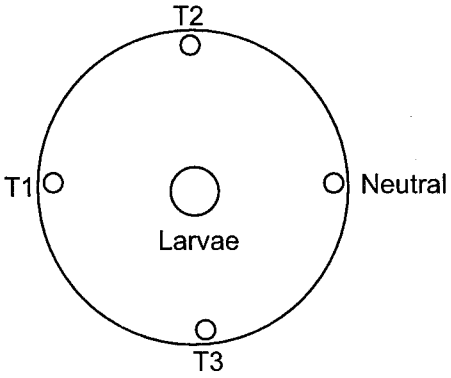
Design II: One of the chemicals and the larvae are placed together at the centre of a plate. Three such plates are set up for the three chemicals.



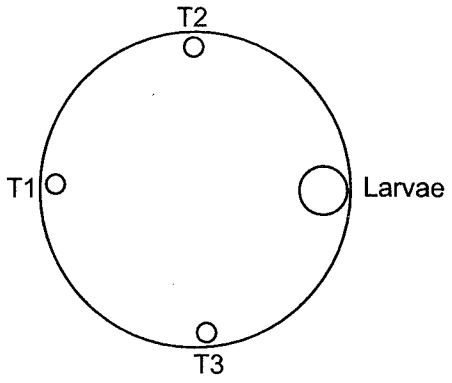
Design III: One of the chemicals and an odor-free (neutral) chemical are placed at two ends of a plate. Larvae are introduced in the centre. The test is repeated for the remaining chemicals.



Design IV: The three test chemicals and a neutral chemical are placed at equidistant positions on the periphery of a plate and the larvae are introduced at the centre.



Design V: The three test chemicals and the larvae are placed at equidistant positions on the periphery of a plate.



Choose the most appropriate experimental design and put a tick mark (√) against it **in Q. 1.A.1. in the Answer Sheet.**

Design I	
Design II	
Design III	
Design IV	
Design V	

Please note that the next question (Q. 1.A.2.) will be evaluated only if your answer to this question (Q. 1.A.1.) is correct.

Q. 1.A.2. (4 points) Mark the following statements as TRUE or FALSE in accordance with your choice of the experimental design.

STATEMENT	TRUE	FALSE
I. It allows the larvae to choose between two or more different chemicals presented simultaneously and thus acts as a discriminatory test.		
II. It can clearly distinguish between attractants and repellants by testing them one at a time against the neutral chemical.		
III. The entire experiment (i.e., testing all the chemicals) can be completed using a single test and thus inter-experimental variation can be avoided.		
IV. It can clearly distinguish the repulsive and attractive nature of the stimuli as each can enhance the response of the larvae to the other(s).		

V. There will not be any mixing of the odors and hence more reliable results will be obtained.		
VI. All the chemicals can be tested against the same control in a single plate.		
VII. Amongst the designs presented, it is the one in which the effect of the weakest odorants can be tested.		
VIII. Larvae can disperse in any direction without any hindrance.		

**********END OF PART A**********

PRACTICAL TEST 4

ANIMAL BEHAVIOR

Task 1 – Parts B and C

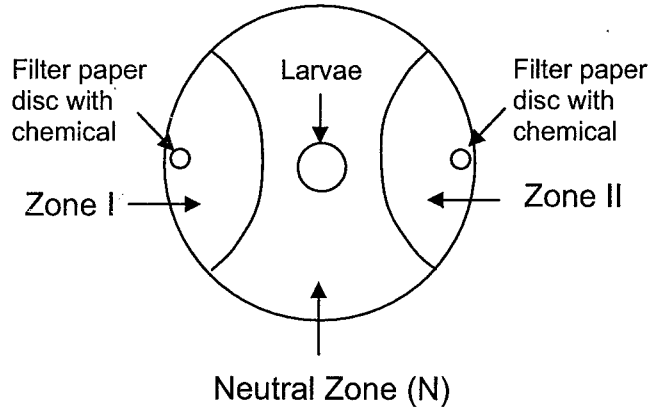
Study of the olfactory and phototactic responses of *Drosophila melanogaster* larvae

You should try and complete Parts B and C of this task in 35 minutes.

Part B (18 points)
Larval plate test

Design

Five experiments were conducted to test the response of *Drosophila melanogaster* larvae to chemical and light stimuli. Four chemical odorants, A, B, C, and D were used in the tests. Of these, D was known to be a neutral chemical while A, B, and C could be an attractant, repellent or a neutral chemical. The design for the experiment is as shown in the figure:



Method

Third instar larvae were used in these experiments. These larvae were obtained by washing the 6-day-old *Drosophila* culture with 15% sucrose solution. The larvae that float in this solution were washed free of sucrose and immediately used in the tests. The tests were performed in Petri plates containing a layer of 1% agarose.

In each experiment, two chemicals were spotted on separate filter paper discs that were placed in two Zones, Z I and Z II (marked as semicircular areas) at two diametrically opposite ends of a Petri plate. Approximately 30-40 larvae were placed at the centre of each plate and their movement over the next five minutes was recorded. Five such experiments were conducted. The recordings of these experiments have been provided to you as video films. Experiments No. 1, 2, and 4 were conducted under uniform light conditions. In Experiments No. 3 and 5, half the plate was covered with black paper and the remaining half was left exposed to light.

Q. 1.B.1. (10 points): Observation of video films

1. Double click on the video file labelled **1** on the computer monitor to observe the movement of larvae.

2. The duration of the video film is 5 minutes, compressed to 2.5 minutes. You may forward or rewind the video film, if required.
3. At the end of the experiment, count the number of larvae in Zone I (N_{ZI}) and Zone II (N_{ZII}).
4. Record your readings **in Table 1.B.1. in the Answer Sheet**.
5. Repeat steps 1 to 4 for the video files labelled **2** to **5**.

Table 1.B.1.

Experiment	Chemical in ZI	Number of larvae in ZI (N_{ZI})	Chemical in ZII	Number of larvae in ZII (N_{ZII})	$\frac{N_{ZI}}{N_{ZI}+N_{ZII}}$	$\frac{N_{ZII}}{N_{ZI}+N_{ZII}}$
1	B		C			
2	A		B			
3	A (in dark)		B			
4	B		D			
5	B (in dark)		C			

Q. 1.B.2. (3 points) What is the likely nature of the three chemicals A, B and C? Put a tick mark (√) in the appropriate box **in the Answer Sheet**.

Chemical	Attractant	Repellant	Neutral chemical	Nature cannot be determined
A				
B				
C				

Q. 1.B.3. (5 points) Based on your observations, mark whether the following statements are true or false by putting a tick mark (√) in the appropriate box **in the Answer Sheet**.

a. Larvae exhibit a stronger positive movement towards the attractant odorant tested than towards darkness.
b. Light is a stronger repelling stimulus for the larvae than the repellant odorant tested.

c. The positive phototaxis shown by the larvae is stronger than the movement towards the attractant odorant.
d. In the presence of light, the larvae do not exhibit chemotaxis.
e. The repellant odorant has a stronger influence on the larvae than does darkness.

	True	False
a.		
b.		
c.		
d.		
e.		

Part C (11 points)

Study of olfactory adaptation in *Drosophila melanogaster* larvae

Continuous stimulation of the olfactory system with a given odor tends to result in adaptation, also known as desensitization. As a result, the larvae fail to respond to the odor to which they have been adapted. A researcher working on olfaction in *Drosophila melanogaster* larvae wanted to study adaptation in these larvae. She selected the following odorants for her study:

1. Ethyl acetate
2. Pentyl acetate
3. Hexyl acetate
4. Heptyl acetate

Pre-stimulation experiment: In each experiment (except in Experiment 1), the larvae were pre-stimulated by placing them in a Petri plate containing 40 microlitres of one of the above odorants for 25 min. The same larvae were then picked up from this plate and tested for their response to the same or different odorants using the protocol described earlier in Part B of Task 1.
The data obtained from these tests are tabulated below.

Data from the pre-stimulation experiment

Experiment	Pre-stimulation	Test odorant							
		Experiment A		Experiment B		Experiment C		Experiment D	
		Ethyl acetate	*	Pentyl acetate	*	Hexyl acetate	*	Heptyl acetate	*
		N_{ZI}	N_{ZII}	N_{ZI}	N_{ZII}	N_{ZI}	N_{ZII}	N_{ZI}	N_{ZII}
1	None	21	3	18	5	14	12	8	13
2	Ethyl acetate	14	11	15	11	13	10	9	15
3	Pentyl acetate	16	15	12	11	9	19	9	14
4	Hexyl acetate	17	9	17	14	16	13	8	13
5	Heptyl acetate	15	10	13	5	8	13	10	13

N_{ZI} and N_{ZII} are the number of larvae in Zone I and Zone II, respectively.

*** Zone II in all the experiments contained a neutral chemical.**

The data given in the table are a set of average responses. The actual numbers varied up to 10% on either side of the average.

Q. 1.C.1. (5 points) Calculate the Response Index (RI) for each experiment according to the formula:

$$RI = \frac{N_{ZI} - N_{ZII}}{N_{ZI} + N_{ZII}} \times 100$$

Fill in the RI values **in Table 1.C.1. in the Answer Sheet.**

Table 1.C.1.

Experiment	Pre-stimulation	Test odorant			
		Experiment A	Experiment B	Experiment C	Experiment D
		Ethyl acetate	Pentyl acetate	Hexyl acetate	Heptyl acetate
		RI	RI	RI	RI
1	None				
2	Ethyl acetate				
3	Pentyl acetate				
4	Hexyl acetate				
5	Heptyl acetate				

Q. 1.C.2. (2 points) To which odorant have the larvae adapted the most?
Put a tick mark (√) in the appropriate box **in the Answer Sheet.**

Ethyl acetate	
Pentyl acetate	
Hexyl acetate	
Heptyl acetate	

Q. 1.C.3. (2 points) To which odorant have the larvae adapted the least?
Put a tick mark (√) in the appropriate box **in the Answer Sheet.**

Ethyl acetate	
Pentyl acetate	
Hexyl acetate	
Heptyl acetate	

Q.1.C.4. (2 points) In which one of the experiments do you find that larval sensitivity to the odorant has been reversed?
Put a tick mark (√) the appropriate box **in the Answer Sheet.**

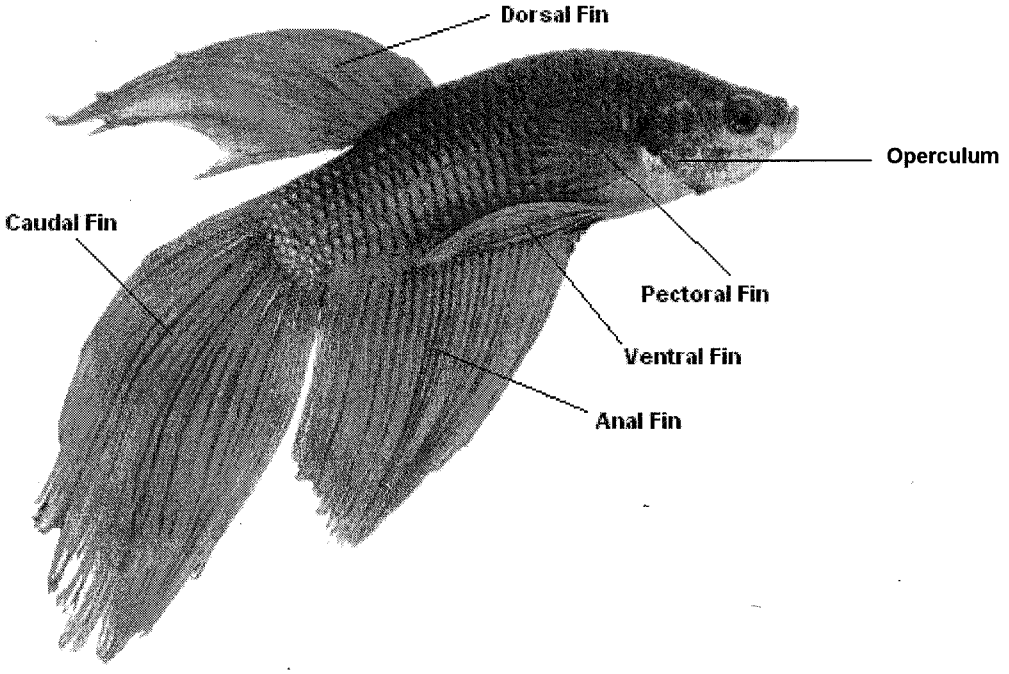
Experiment	Experiment			
	A	B	C	D
1				
2				
3				
4				
5				

Task 2 (13 points)
Study of fish behavior

You should try and complete this task in 15 minutes.

Introduction

The Siamese fighting fish, *Betta splendens,* is one of the most popular species of freshwater aquarium fish. This fish shows varied responses when exposed to different stimuli.



You have been provided with a video recording of an experiment using a male Siamese fighting fish.

Q. 2.1. (11 points) Double click on the video file **6** on the computer monitor and observe the behavior of the fish before and after introduction of a mirror.
After observing the film, write a '+' for the particular behaviors that were displayed by the fish and '-' for those that were not displayed. Record your observations **in Table 2.1. in the Answer Sheet.**

Table 2.1.

Number	Behavior	Before the introduction of the mirror	After the introduction of the mirror
1.	Pectoral fin beating		
2.	Rapid zigzag movement of the body and the caudal fin		
3.	Appearance of horizontal stripes on body		

4.	Pecking at the base of the aquarium		
5.	Brightening of body coloration		
6.	Erection of dorsal, anal and caudal fin		
7.	Appearance of vertical stripes on body		
8.	Operculum display (opening of the operculum)		
9.	Bleaching of body color		
10.	Lateral display*		
11.	Gasping for air		

*The lateral display is a behavior where the fish exhibits the lateral surface of its body, expands its dorsal and caudal fins and vibrates/quivers its body.

Q. 2.2. (1 point) The differences in behavior of the fish that you observed before and after the mirror was introduced into the aquarium could be due to:

a. an apparent increase in the size of the territory that the fish now has to defend.
b. an urge to display courtship behavior towards a conspecific individual that the fish now perceives in its territory.
c. an urge to establish dominance over a conspecific individual that the fish now perceives in its territory.
d. a startle response displayed by the fish when confronted with a mirror.

Put a tick mark (√) in the appropriate box **in Q. 2.2. in the Answer Sheet.**

a.	b.	c.	d.

Q. 2.3. (1 point) Different behaviors in animals have certain benefits and costs associated with them. For example, prolonged extension of the gill cover or operculum display may indicate its physical strength but may also severely limit the ability of the fish to ventilate its gills. In the light of your observation, what could be the rationale for the experimental fish displaying or not displaying this particular behavior?

a. Fish always prefer to maintain regular opercular movement without any display, independent of the presence or absence of another conspecific individual, to maintain the oxygen supply for the body at its optimum.

b. Fish will exhibit the operculum display advertising its ability to tolerate oxygen stress in presence of another conspecific individual to establish its dominance.
c. Operculum display, being an energetically costly behavior, is usually not exhibited by a fish under most circumstances. Males of this species, however, may display this behavior in the presence of a conspecific female because the potential reproductive success that it will acquire will more than compensate for the energetic cost of the display.
d. Operculum display is likely to be determined only by abiotic environmental factors such as level of dissolved oxygen in the water. Thus, fish in sufficiently aerated water will always show this response so as to declare its territory and maintain its dominance.

Put a tick mark (√) in the appropriate box **in Q. 2.3. in the Answer Sheet.**

a.	b.	c.	d.

********** END OF PRACTICAL TEST 4 **********