DEEPSHIKA KALA SANSTHAN

ESTD, 1976

DEEPSHIKHA

COLLEGE OF TECHNICAL EDUCATION

ZOOLOGY MANUAL

Building Careers, Building the Nation

DEEPSHIKHACOLLEGE OF TECHNICAL EDUCATION, JAIPUR

MANUAL FOR ZOOLOGY LABORATORY

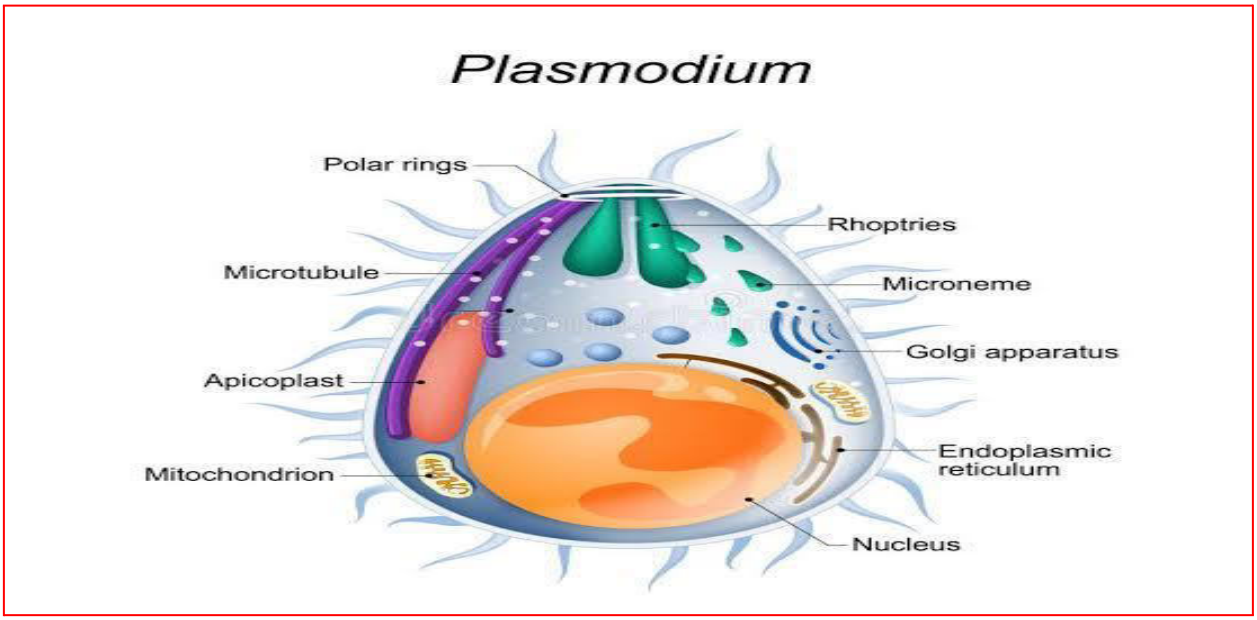
Foundations and Laboratory Essentials

- **Laboratory Safety**: Chemical safety, emergency protocols, and personal protective equipment (PPE) guidelines.
- **Instrumentation & Techniques**: Care and handling of compound and stereomicroscopes, preparing wet mounts, and staining techniques.
- **Dissection Box Instruments**: Proper identification and use of blunt and sharp forceps, needles, scalpels, scissors, and droppers.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

2. Microscopic & Cellular Analysis

- **Animal Cells and Tissues**: Microscopic study of epithelial, connective, muscular, and nervous tissues via permanent slides.
- **Protists**: Observations of single-celled, animal-like organisms such as *Amoeba*, *Paramecium*, *Euglena*, and *Plasmodium*.



3. Invertebrate Zoology (Taxonomy & Spotters)

This section covers classification, general characteristics, and key adaptive structures of major phyla:

- **Porifera & Cnidaria**:
- Sponges and specimens like *Hydra*.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

CHARACTER	INFERENCE
• Body bears a canal system with many ostia and osculum. • Fixed and incapable locomotion. • Non tissue grade multi cellular organism. • Spongocoel is lined by flagellated choanocyte cells. • Diploblastic cylinder shaped body supported by spicules or spongin or both.	Hence, Phylum – Porifera
• Skeleton of calcareous spicules. • Spicules of calcium carbonate. • Spicules are monaxon, triaxon, tetraxon type.	Hence, Class - Calcarea
• Triaxon spicules usually have one long ray.	Hence, Subclass - Calcaronea
• Body shape is vase like. • Body wall is thick form which spicules projected. • Canal system is syconoid type. • Apical ends of each cylinder bears osculum guarded by monaxon spicules. • Group of ostia present at the surface of the body.	Hence, the specimen seems to be *Sycon* sp.

CHARACTER	INFERENCE
• Body soft, biradialy symmetrical, tissue grade. • Diploblastic animal with cnidoblast cell. • Presence of nematocysts. • Presence of gastrovascular cavity. • Ectoderm and endoderm separated by mesoglea. • Polyp and medusoid forms. • Presence of tentacles around the mouth.	Hence, Phylum - Cnidaria
• Medusoid cnidaria. • Presence of endodermal gastric filament. • Mesoglea thick and noncellular. • Presence of oral arms. • Velum absent. • Sense organs are tentacles.	Hence, Class - Scyphozoa
• Highly branched radial canal. • Four horse-shoe shaped and four intra radial gastricpouches present at the surface of the body. • Umbrella shaped body and mouth drawn into 8 long oral arms. • Sub umbrella contains 8 pairs of mesoglea tentacles. • Inner side of oral arms have ciliated groove.	Hence, the specimen seems to *Aurelia* sp.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

- **Platyhelminthes & Nematoda**:

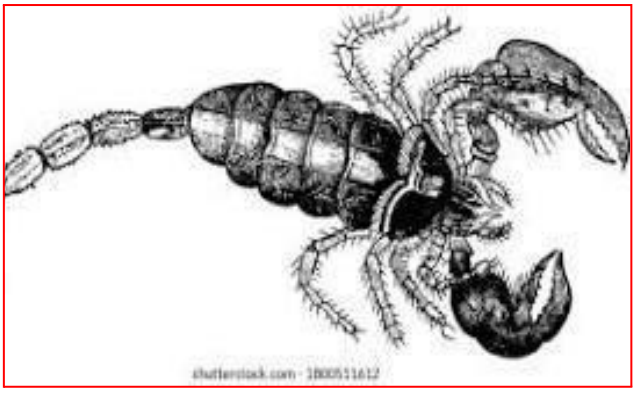
- Parasitic worms including Liver Flukes and *Ascaris*.

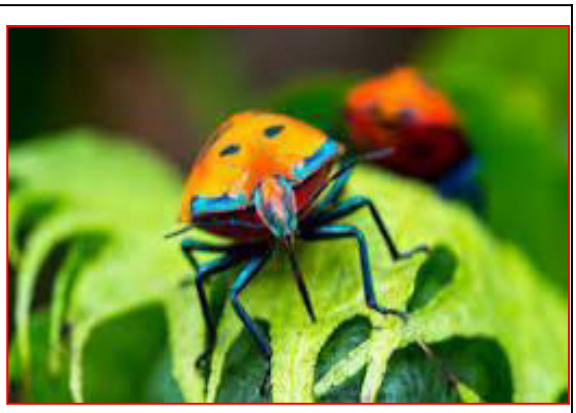
Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

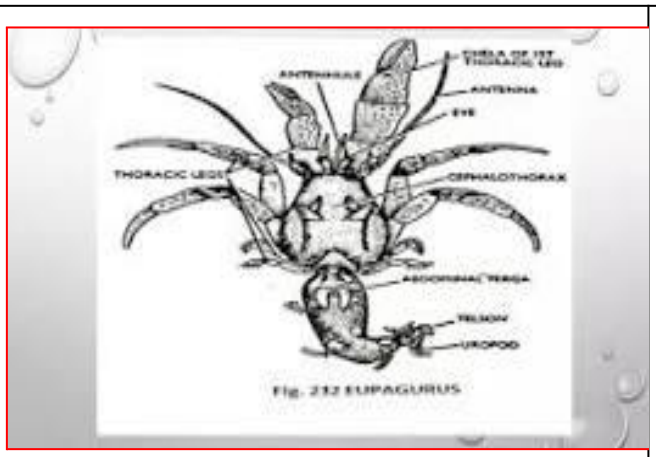
- **Annelida**: Segmented worms such as Earthworms and Leeches.

CHARACTER	INFERENCE
• Body bilaterally symmetrical, metamerically segmentedand elongated triploblastic. • Body covered with thin cuticle. • Body coelomate and anus & mouth terminal.	Hence, Phylum - Annelida
• Body segmentation well marked. • Each segment bears one pair of muscular parapodia. • Head distinct and bears pulp, cirri, sometime eyes.	Hence, Class - Polychaeta
• Prostomium distinct and bears appendages.	Hence, Subclass - Errantia
• Body cylindrical and metamerically segmented. • One pair of cirri present. • Head composed of 1. Prostomium, with tentacles, pulp, eyes. 2. Peristomium, with four pairs of tentacles. • Parapodia present in each segment except head and anul segment.	Hence, the specimen seems to be *Neries* sp.

- **Arthropoda**: Social insects and organisms like Honeybees, Cockroaches, and Prawns/Squilla.







Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

CHARACTER	INFERENCE
• Body segmented and covered by chitinous cuticle. • Each segmented bears paired externally jointed appendages. • Exoskeleton are two types – 1. Chitinous cuticle, thick, tough and covers the body. 2. Arthroidal membrane, soft thin, flexible and connect the segmental exoskeleton.	Hence, Phylum - Arthropoda
• Body divided into three parts – head, thorax and abdomen. • Head bears two or one paired of antennae two pairs of maxillae and one pair of mandible.	Hence, Subphylum - Mandibulata
• Dorsal surface covers by carapace. • Thorax and head fused to form cephalothorax. • Head bears two pairs of pre oral and three pairs of post oral appendages.	Hence, Class - Crustacea
• Mandible with pulp. • Presence of with eyes.	Hence, Subclass - Malacostraca
• Asymmetrical body divided into broad cephalothorax and an abdomen. • Abdomen soft fleshy and coiled. • Right chela is larger than other. • Telson reduced. • Uropods hook like.	Hence, the specimen seems to be *Eupagurus* sp.

- **Mollusca & Echinodermata**: Soft-bodied animals like Apple Snails and marine animals like Starfish.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

CHARACTER	INFERENCE
• Body soft and unsegmented in adult. • Body divided into head, ventral muscular foot anddorsal visceral mass. • Dorsal visceral mass enclosed by mantle. • External body covered by exoskeleton calcareous shell.	Hence, Phylum – Mollusca
• Body elongated and bilaterally symmetrical. • Pedal and pallial nerve cord present with cross connectives. • Head reduced.	Hence, Class - Amphineura
• Presence of shell valve with insertion plate and teeth. • External gills present on mentle groove. • Dorsal surface is convex and ventral part bears a broad foot.	Hence, Subclass - Polyplacophora
• Dorsal part is convex, ventral part is flat. • Eyes and tentacles absent. • Lines of growth present on valves. • Labial pulp present. • Shall valves overlapping.	Hence, the specimen seems to be *Chiton* sp.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

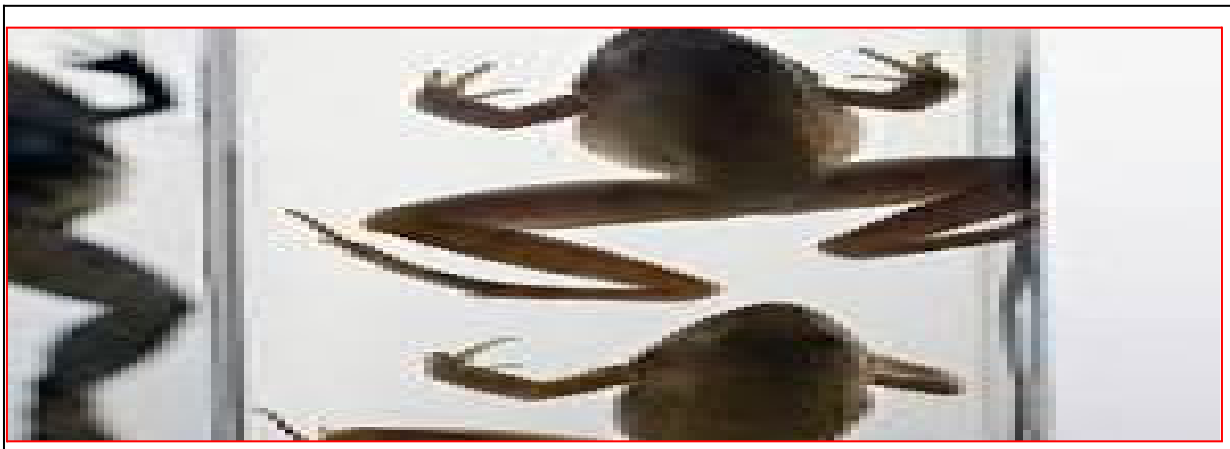
CHARACTER	INFERENCE
• Pentamerically symmetrical body with distinct oral & aboral surface. • Exoskeleton with calcareous spine or ossicles. • Present of five intervening ambulacral on body surface.	Hence, Phylum - Echinodermata
• Mouth on oral and anus on aboral surface. • Ambulacral grooves are covered.	Hence, Subphylum - Echinozoa
• Ambulacral groove meridional in position. • Ambulacral plates bear pores through which tube feet project.	Hence, Class - Echinoidea
• Tube feet with terminal suckers. • Madreporite on aboral surface.	Hence, Subclass - Euechionoidea
• Body nearly spherical with a flat oral and domed aboral surface and covered by spine. • Test rigid, ambulacral and inter-ambulacral zones clearly distinguished. • Presence of Aristotle's lantern, projected from mouth. • Pdeicellariae and sphaeridia present between the spines. • The madreporite and gonopore are present near arms.	Hence, the specimen seems to be Sea-urchin

4. Vertebrate Zoology & Comparative Anatomy

Chordate Classification: Identification of specimens across Fish

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

Amphibians (e.g., frogs),



Reptiles (e.g., lizards)

, Birds

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

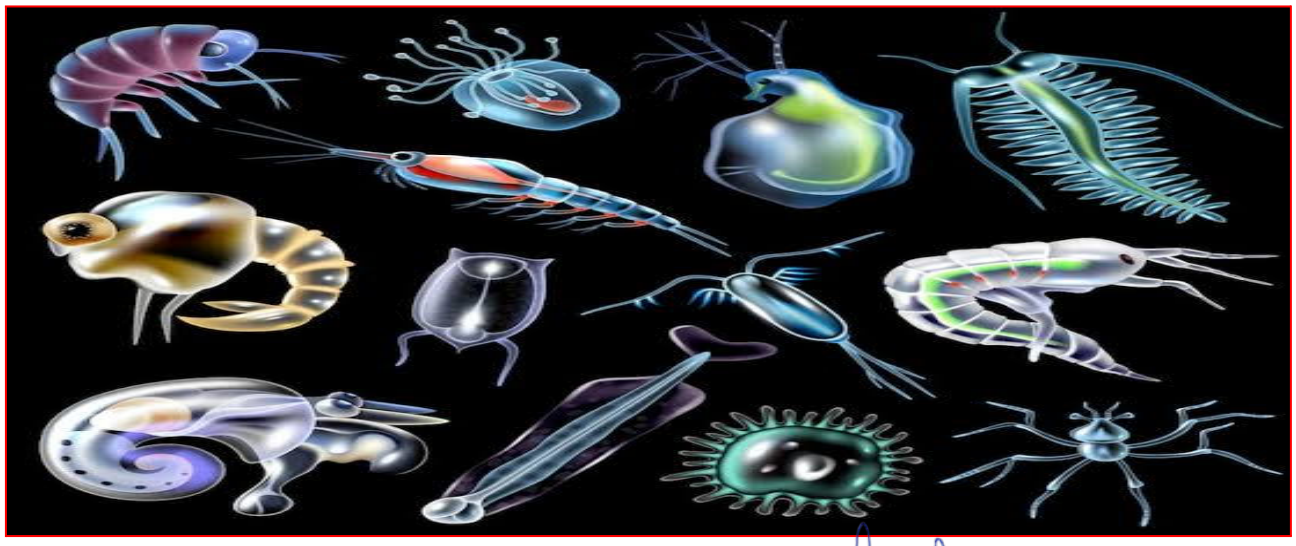
Mammals (e.g., rabbits).

- **Organ Systems & Models**: Morphological and anatomical studies of digestive, nervous, and circulatory systems using 3D models, charts, or digital simulations.
- **Osteology**: Comparative study of the skeletal structures of different vertebrate classes.

5. Physiology, Ecology, and Development

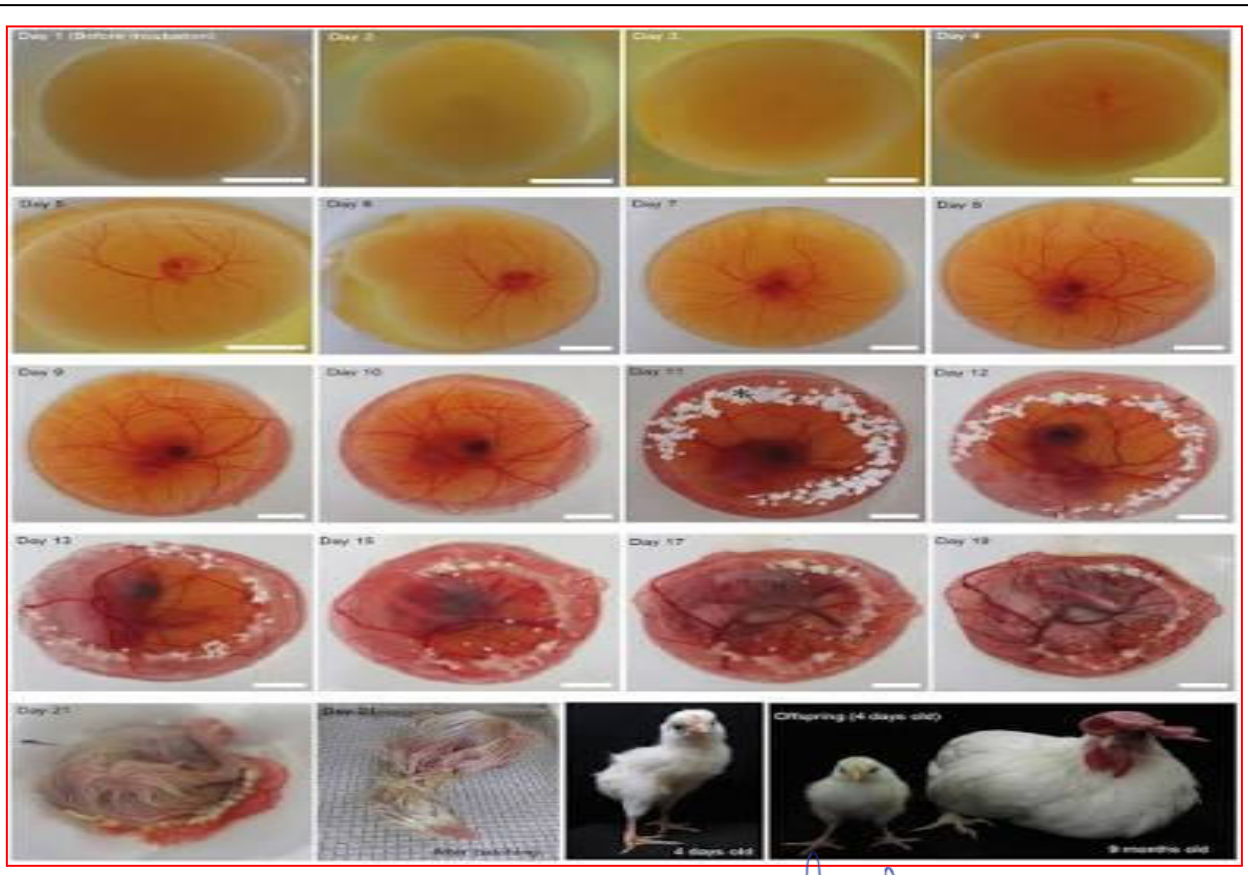
- **Physiology Experiments**: Testing enzyme activities (like salivary amylase), demonstrating protein digestion, or identifying macromolecules.

- **Ecological Analysis**: Studying local plankton populations, water/soil quality analysis, or local animal collections.



Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

- **Developmental Biology**: Observation of different embryological stages (e.g., chick embryo or frog development stages)



Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

GENERAL LABORATORY INSTRUCTIONS

1. Never enter and work alone in the laboratory without prior knowledge and permission of the instructor.
2. Never use any laboratory equipment without instruction and authorization from the instructor. Report any damaged or broken equipment to your instructor immediately.
3. Do not engage in any rowdy, playful, or unprofessional activities in the laboratory.
4. Use all chemicals with caution. Do not taste or inhale and avoid direct touch to your skin. In case of any chemicals splashing in eyes or skin, immediately go to the nearest sink, flush and wash the affected place.
5. Report ANY and ALL accidents, spills, BREAKAGES, or injuries to the instructor.
6. Any sharp objects like Scalpels and Razors should be used only after getting proper handling instructions and authorization from the instructor.
7. Do not keep unnecessary books, backpacks and other personal items on laboratory benches.
8. Avoid open long hair, flowing clothing, and open-toed shoes in the laboratory.
9. Pregnant or immuno compromised students must inform the instructor. Pregnant students will not be allowed to do dissections or work with any body fluids without having a doctor's note for permission.
10. Before the laboratory, wash hands thoroughly and line the work area with clean paper towels. After the laboratory, wipe down the work area with disinfectant and wash hands thoroughly. Dispose of used slides, chemicals, any tissue wastes or hazardous wastes in the proper disposal container. Follow the instructor's instruction before disposal of anything in the laboratory.

Leave the laboratory in better condition than you entered. Put all microscopes, glass and plastic materials or others back in place properly. Clean laboratory benches, wash glass wares, slides, trays or any reusable things of such kind. Dispose of specimens and other things properly.

SOME COMMONLY USED INSTRUMENTS IN ZOOLOGY LABORATORY

Compound Microscope

This is a high power light microscope with multiple lenses i.e. the objective lens (typically 4x, 10x, 40x or 100x) is compounded (multiplied) by the eyepiece lens (typically 10x) to obtain a high magnification of 40x, 100x, 400x and 1000x. This microscope has multiple uses in biological laboratories. Learners can observe biological slides or thinly cut sections of any object by this tool. Histological slides, bacteria, protozoa etc. can be studied under this microscope.

The up-right microscope is basically used for research purposes. Here, the source of transmitted light and the condenser are located below the stage, pointing up and the objective is placed on the top of the stage pointing down. The specimen is observed from the top. This is used for observing the living cells or samples that are squeezed between a slide and coverslip.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

Compound Microscope
Photo Credit: IndiMART

Up-right Microscope (Light Compound)
Photo Credit: Microscopy Land

Microtome

Microtome (Greek *mikros*, meaning "small", and *temnein*, meaning "to cut") is a tool which is used to cut several materials into extremely thin slices. Based on the mechanism, microtomes are of different types i.e. Rocking, Rotary, Base-sledge, Sliding, Freezing, Vibrating, Saw, Cryostat.

Generally, in biological laboratories Rotary Microtomes are commonly used. Different sized Knife blades are used to cut any desired item and microtome sections with thickness between 50 nm and 100 µm, can be produced. In biological science this tool is used to study the histology of organisms i.e., tissue sections from different organs of the animals. Tissues to be studied are cut into thin sections by the microtome, processed, stained and observed under microscope.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

Rotary Microtome

Photo Credit:

Brand: Safire Scientific Company

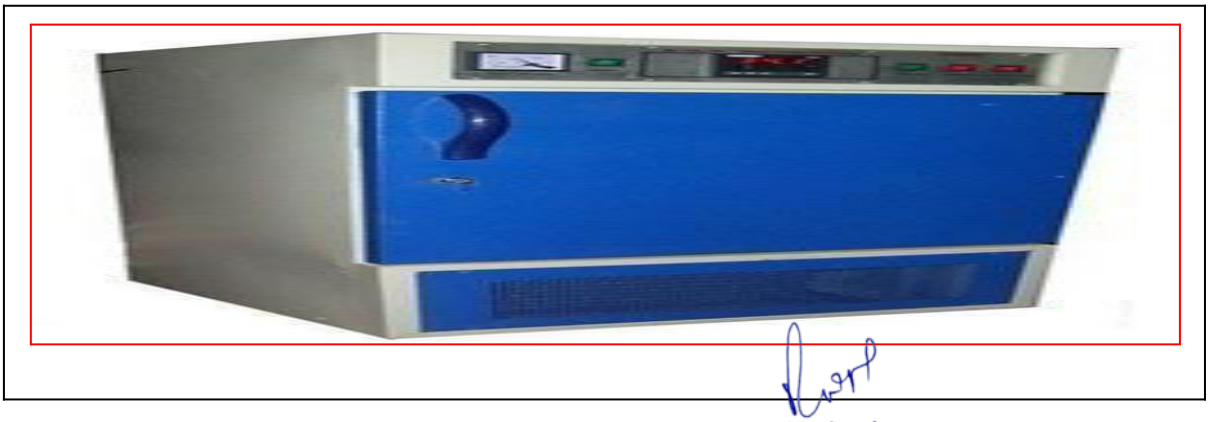
Hot Plate

These are electrically controlled plates which are used to dry slides or straighten the paraffinized tissue sections. The metalized surface of the tool gets heated by the electric coils underneath. Also such instruments are used in heating solutions and preparing reagents etc.

Hot Plate Photo Credit: Leica Biosystems

BOD Incubator

Generally, any incubator is used to maintain optimal temperature, humidity and other conditions such as the CO (CO2) and oxygen content of the atmosphere inside a device, which is used to grow and maintain microbiological cultures or cell cultures. BOD incubators are used in determining the Biological Oxygen Demand (BOD) as this tool helps to maintain a favourable temperature (20^0 C) inside. BOD incubator is also helpful to measure molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron.



Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

Centrifuge

This tool is useful to separate different components of any solution based on their molecular size, weight and concentration. This machine puts an object in rotation around a fixed axis (spins it in a circle), applying a force perpendicular to the axis of spin (outward) that can be very strong. This equipment works on the basis of sedimentation principle. Putting the object in centrifuge tubes, the machine can be set to centrifuge at required rate. This tool has a wide application in biological laboratories to differentiate different components of the tissue, cell etc. biochemical components with different molecular weights can also be differentiated via this machine.

Centrifuge

Photo Credit: Moglix.com

pH meter

This instrument is used to measure the hydrogen-ion activity in water-based solutions, indicating its acidity or alkalinity expressed as pH. Electric potential between a pH electrode and a reference electrode is measured to detect the pH of any solution and so the pH meter is sometimes referred to as potential pH meter. The voltage between the two electrodes is converted into pH values and displayed. This instrument is very common in use in the biological and chemical laboratories to prepare different chemicals, reagents, buffers with specific pH.

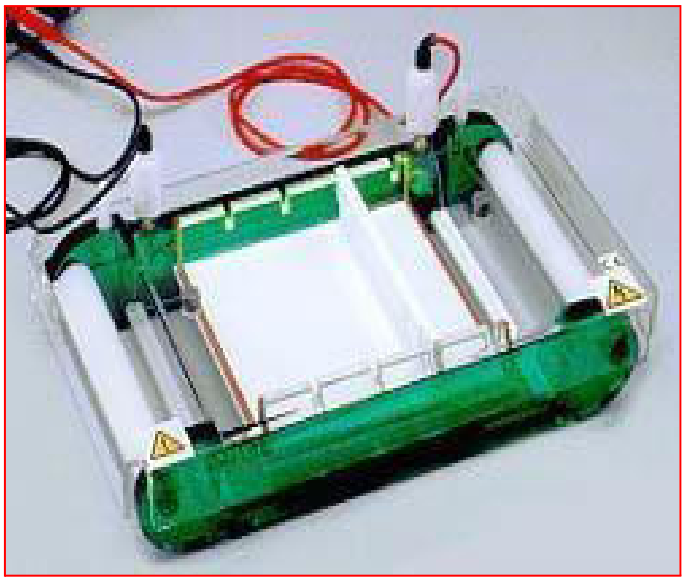
Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

Gel Electrophoresis System

Gel electrophoresis is used in separation of nucleic acids and proteins based on their size. This apparatus is composed of a porous gel matrix. Nucleic acids or proteins pass through these porous structures and form bands at different parts forming gradients according to their comparative size. The gel box features a cathode at one end and an anode at the other. An Ionic buffer fills the box and creates an electric field when charge is applied. Electrophoresis is used by laboratories studying vaccines, medications, forensics, DNA profiling or other life science applications. Gel electrophoresis can be done in two ways i.e. horizontal or vertical orientation.

a. **Horizontal Gel Electrophoresis**: In this case the gel is casted in horizontal orientation and submerged by a running buffer in the gel box. The gel box is divided into two compartments, with agarose gel separating the two.

b. **Vertical Gel Electrophoresis**: This apparatus utilizes a discontinuous buffer system with two chambers in the gel box. The bottom of the gel is submerged in a buffer in one chamber and the top is submerged in a buffer in another chamber. A small amount of buffer comes through the gel from the top to the bottom chamber. Unlike the Horizontal system acrylamide can be used in vertical gel electrophoresis.



Horizontal Gel Electrophoresis System

Vertical Gel Electrophoresis System

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

There are several other instruments which are in use in a students' laboratory in the Zoology Department along with a considerable number of preserved specimens of different taxa of animal kingdom. A student or learner may receive the exposure of these with the PCP and LCES programmes of the University.

SPECTROPHOTOMETER

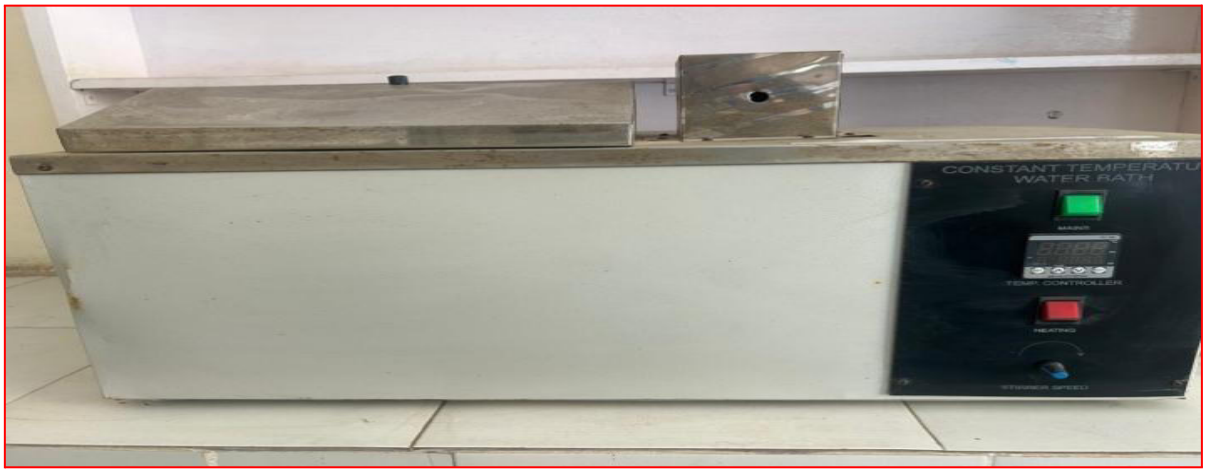
1. **Switch on the instrument** and allow it to warm up (usually 10–15 minutes).
2. **Select the desired mode** (e.g., Absorbance or Transmittance).
3. **Set the wavelength** required for the analysis.
4. **Fill a cuvette with a blank solution**, wipe it clean, and place it in the sample holder.
5. **Zero or calibrate the instrument** using the blank.
6. **Replace the blank with the sample cuvette** (filled and wiped clean).
7. **Close the lid and record the absorbance or transmittance** reading.
8. **Repeat for all samples**, cleaning the cuvette between measurements.
9. **After use**, remove cuvettes, turn off the instrument, and clean the sample area.
10. **Document the readings** and store data as per lab protocol.

HEATING MANTLE

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

1. Adjust the three-pin plug and supply the electricity to the instrument.
2. Start the instrument by switching on the black switch.
3. Adjust the temperature by thermo regulator.
4. Keep the solution being heated in the netted wool.
5. Heat the sample at desired time.
6. Remove the sample assembly being used for heating.
7. Cut off the power supply.

WATER BATH



1. **Check water level** and fill if needed.
2. **Turn on** the water bath.
3. **Set the desired temperature** using the control panel.
4. **Wait until temperature stabilizes** (monitor display).
5. **Place samples** in the bath securely.
6. **Cover lid** to maintain temperature (if applicable).
7. **Monitor regularly** during use.
8. **Turn off**, **remove samples**, and **drain water** after use.
9. **Clean and dry** the unit properly.

COMPOUND MICROSCOPE

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

1. **Clean lenses** with lens paper.
2. **Turn on** the light source.
3. **Place the slide** on the stage and secure it with clips.
4. **Start with a low power** objective lens.
5. **Use a coarse focus** knob to bring the image into view.
6. **Use fine focus** for clarity.
7. **Switch to higher power** if needed and refocus.
8. **After use**, lower stage, turn off light, and **cover** the microscope.
9. **Store safely** in a dust-free place.

PROJECTION MICROSCOPE

1. **Clean lenses** and projection screen.
2. **Place the slide** on the stage and secure it.
3. **Switch on** the light source.
4. **Adjust mirror/illumination** for clear lighting.
5. **Select a low power** objective lens first.
6. **Focus** using coarse, then fine adjustment.
7. **Project image** onto screen.
8. **Adjust clarity and magnification** as needed.
9. **After use**, turn off light, remove slide, and **cover the microscope**.
10. **Store properly** to prevent dust or damage.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

DIGITAL BALANCE

1. Adjust the three-pin plug and supply the power to the instrument.
2. Switch on the instrument by pressing the blue indicator button.
3. Adjust the mode by pressing the mode indicator button.
4. Tare the container being used to weigh the sample and it is indicated by zero reading.
5. For reweighing of successive components of the sample, the zero reading is put by pressing zero indicator buttons.
6. Weigh the sample being weight.
7. Remove the sample.
8. Switch off the instrument by pressing the blue indicator button.
9. Cut off the power supply.
10. Instruments should be cleaned properly.

MAGNETIC STIRRER WITH HOT PLATE

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

1. Adjust the three-pin plug and supply the electricity.
2. Switch ON to start the instrument.
3. Adjust the temperature by regulator.
4. Adjust the R.P.M. by the regulator.
5. Keep the sample on the heating plate for desired time. Sample containers should contain magnetic beads.
6. Remove the sample from the metal plate.
7. Switch OFF the power supply.

LABORATORY HEATING PLATE

1. **Place the hot plate on a stable, heat-resistant surface** in a well-ventilated area.
2. **Ensure the surface of the plate is clean and dry** before use.
3. **Place the sample container (e.g., beaker, flask)** centrally on the plate.
4. **Set the desired temperature** using the control knob or digital panel.
5. **Switch on the hot plate** and monitor the heating process.
6. **Use appropriate tools (e.g., tongs, gloves)** when handling hot containers.
7. **Do not leave the hot plate unattended** while in use.
8. **After use**, turn off the heating and allow the plate to cool completely.
9. **Unplug the hot plate** and clean the surface if necessary.
10. **Store the equipment properly** once cooled and cleaned.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

BACTERIOLOGICAL INCUBATOR

1. **Check cleanliness** and ensure no leftover samples.
2. **Set the desired temperature** using the control panel.
3. **Wait for temperature** to stabilize.
4. **Label and place samples** inside evenly spaced.
5. **Close the door** firmly to maintain temperature.
6. **Monitor temperature** regularly.
7. **After incubation**, remove samples carefully.
8. **Turn off** the incubator.
9. **Clean and disinfect** the interior.

CENTRIFUGE

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

- Ensure that the instrument is clean & free from dust.
- Open the upper lid by lifting it up.
- Place the centrifuge tubes in the compartments provided for it.
- The compartment is designed in such a way to place six centrifuge tubes at a time.
- Switch “ON” the mains.
- Adjust the RPM speed by the knob provided on the front panel of the instrument.
- Switch “OFF” the mains when not required.

HOT AIR OVEN

1. Ensure that the instrument is clean & free from dust.
2. Switch ‘ON’ the mains.
3. The green indicator light will be ‘ON’.
4. There is a three- way switch for the selection of temperature range.
5. A thermostatically controlled knob can set the required temperature 105, by rotating it in clockwise or anti-clockwise direction.
6. Inside the oven, there are two trays. Put any object that is drying, on this tray and close the door of the oven.
7. A small hole is provided on the right corner of the instrument, to place the thermometer or any other temperature measuring device.
8. A vapor or fumes releasing value is also provided on the top middle part of the instrument.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

- First of all the balance is arrested & the sides doors are carefully opened.
- Check the state of the balance before each weighing.
- Remove dust from the pans with a soft brush.
- Before weighing is started the zero point is first determined.

With the balance case closed the arrest knob is carefully turned fully to the left & the pointer swings are observed. The balance is so adjusted as to be in equilibrium in the unloaded state, when the rider is on the zero division. After that the object to be weighted is put in the middle of the "left hand pan" & the door is closed; weights are placed on the "right hand pan" by means of the forceps.When the pan with weights begins to swing over in addition to one hundred grams. Thousand & ten thousand fractions

RULES FOR HANDLING THE ANALYTICAL BALANCE

1. Do not move the balance from its place.
2. Do not put the object to be weighted directly on the balance pan.
3. Don't weigh hot or very cold objects.
4. Always use only the side doors of the balance case when weighing.
5. Do not touch the balance, weights or rider with the fingers
6. Never overload the balance above the permitted load (usually 100 gm) as this causes damages.
7. Check the states of the balance before each weighing

RECTANGULAR WATER BATH

1. **Check the water level** and ensure it is above the heating element.
2. **Connect the power supply** and switch ON the water bath.
3. **Set the desired temperature** using the temperature controller.
4. **Wait for stabilization** and verify temperature with a thermometer (if needed).
5. **Place samples** in test tubes, flasks, or beakers inside the bath.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

6. **Cover the bath** with a lid to minimize evaporation (if applicable).
7. **After use, switch OFF**, remove samples carefully.
8. **Drain and clean** the tank regularly to avoid contamination or scale buildup.
9. **Dry and store** with lid open when not in use.

DIGITAL pH METER

1. Adjust the pH electrode at the stand & attach the backside of the instrument.
2. Adjust the three-pin plug and supply the power to the instrument. Adjust the required temperature-by-temperature regulator.
3. Calibrate the pH electrode by dipping in the standard buffer solution.
4. Keep the electrode in standard buffer solution for 3 to 5 minutes to stabilize the reading.
5. pH electrode wash 3 times with distilled water and place in test solution being examined.
6. Measure the pH of the solution.
7. Cut off the power supply of the instrument.
8. Remove the electrode from test solution and wash with distill Water and again keep in fresh distill water

MICROPIPETTE

1. **Select appropriate micropipette** based on desired volume range.
2. **Attach a sterile tip** firmly to the micropipette.
3. **Set desired volume** using the volume adjustment dial.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

4. **Press plunger to first stop**, insert tip into liquid.
5. **Slowly release plunger** to aspirate liquid.
6. **Dispense liquid** by pressing plunger to the **second stop** into the desired container.
7. **Eject tip** using the tip ejector button into a waste container.
8. **Store micropipette vertically** on a stand after use.
9. **Calibrate and clean** regularly for accurate performance.

STAGE MICROMETER

1. **Clean the slide** gently before use.
2. **Place the stage micrometer** on the microscope stage and secure it.
3. **Focus the microscope** using low power, then switch to desired magnification.
4. **Align the scale** of the stage micrometer with the eyepiece reticle.
5. **Count how many divisions** on the eyepiece scale correspond to a known length on the micrometer.
6. **Calculate calibration factor** (e.g., 1 division = X µm).
7. **Record the calibration** for future measurements.
8. **Remove the micrometer** and clean if necessary.
9. **Store in dust-free case** to prevent

STOP WATCH

1. **Ensure the stopwatch is functional** (battery charged or wound up).
2. **Press the start button** to begin timing at the start of the event.
3. **Press the stop button** when the event ends.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

4. **Record the time displayed** accurately.
5. If required, **use the lap/reset button** for multiple readings.
6. **Reset the stopwatch** after recording.
7. **Clean the surface** if necessary and **store in a safe place**.

HEMOGLOBINOMETER

1. **Clean the apparatus** before use (especially the comparator tubes and pipette).
2. **Fill the hemoglobin pipette** with fresh blood up to the mark (usually 20 µL).
3. **Transfer the blood** into the hemoglobin tube containing **N/10 HCl** up to the fixed mark.
4. **Mix well** and allow the mixture to stand for 5–10 minutes to convert hemoglobin into acid hematin.
5. **Add distilled water** drop by drop, mixing after each drop.
6. **Compare the color** of the solution against the standard comparator scale.
7. **Record the hemoglobin value** in g/dL when the color matches.
8. **Clean all parts thoroughly** with water and dry before storing.

STETHOSCOPES

1. **Ensure the stethoscope is clean** and disinfected before use.
2. **Place earpieces** comfortably and correctly in your ears.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

3. **Select the appropriate side** of the chest piece (diaphragm or bell).
4. **Place the chest piece** firmly on the patient's body (over clothing should be avoided).
5. **Listen carefully** to the body sounds (heartbeats, breath sounds, etc.).
6. **Adjust position** as needed for better clarity.
7. **After use**, clean earpieces and diaphragm/bell with alcohol swabs.
8. **Store the stethoscope** in a dry, dust-free place.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

SOME LABORATORY PROTOCOLS IN ZOOLOGY UG LAB

Experiment no.1

Smear Preparation of Gut Content of Cockroach

Smear preparation of the gut content of different animals is done to examine the endo-parasites living inside the organism. After smear preparation, we watch the prepared slides under the microscope and can identify different living organisms residing there. We need the following materials for smear preparation of a cockroach.

1. Living cockroach **2**. dissecting instruments and tray **3**. pipette **4**. 0.7% saline **5**. Watch glass **6**. Glass slide **7.** Mayer's album **8**. Fixatives **9.** Stains **10.** A wire loop **11**. Cover glass

Protocol

- At first the gut of the cockroach needs to be dissected out without using water.
- Find the mid-gut and hindgut of the gut. Then cut them free.
- Take a watch glass containing a few drops of 0.7% saline. Put the hindgut and mid-gut portion into the saline.
- Cut the gut portions longitudinally in the watch glass. Then gently stir the saline for a few minutes. This will produce a suspension of gut content in the saline of the watch glass.
- Take a clean glass slide which is layered by Mayer's albumen.
- Put a few drops of the suspension of the gut content from the watch glass.
- Spread the solution uniformly over the glass slide. A wire loop or another slide may be used for this purpose.
- Now transfer the slide to fixative and start the downstream process. Then the slides are stained for observation under a microscope. This transfer process should be done before the smear gets air dried.

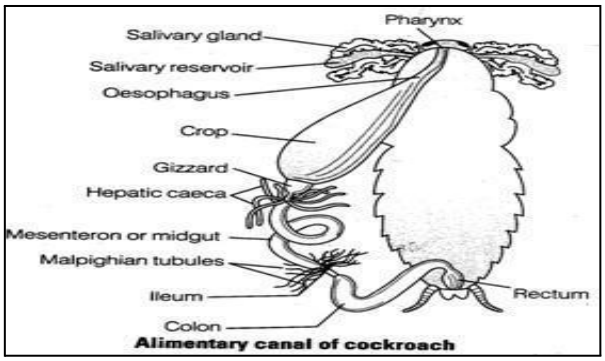


Fig 1: Alimentary canal of cockroach (**Image credit-www.studyadda.com)**

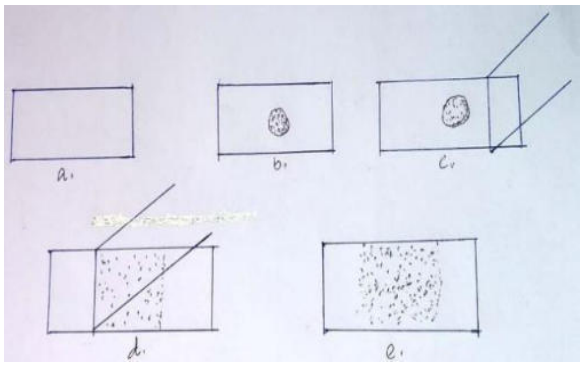


Fig 2: a. clean glass slide, layered by Mayer's albumen. b. drop of the suspension of gut content on the glass slide. c. another slide is taken at 45^0 to spread the suspension d. smear is spread over the slide e. prepared slide.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

Experiment no.2
Gram staining of Bacteria

Danish microbiologist and physician Hans Christian Gram in 1884, developed a staining procedure by which bacteria can be classified in two groups as Gram positive and Gram negative bacteria. Most of the bacteria is encased by a strong cell wall in which a carbohydrate matrix is cross-linked by short polypeptide chains. Gram positive bacteria (encased by thick layer of peptidoglycan- 90% of cell wall) retain the color of the stain and the Gram-negative bacteria (thin layer of peptidoglycan- 10% of cell wall and high lipid content) don't retain the color of the stain, when the bacteria is subjected to it. After the staining, Gram-positive bacteria will appear as violet or blue-black and the Gram-negative bacteria will appear as red/pink.

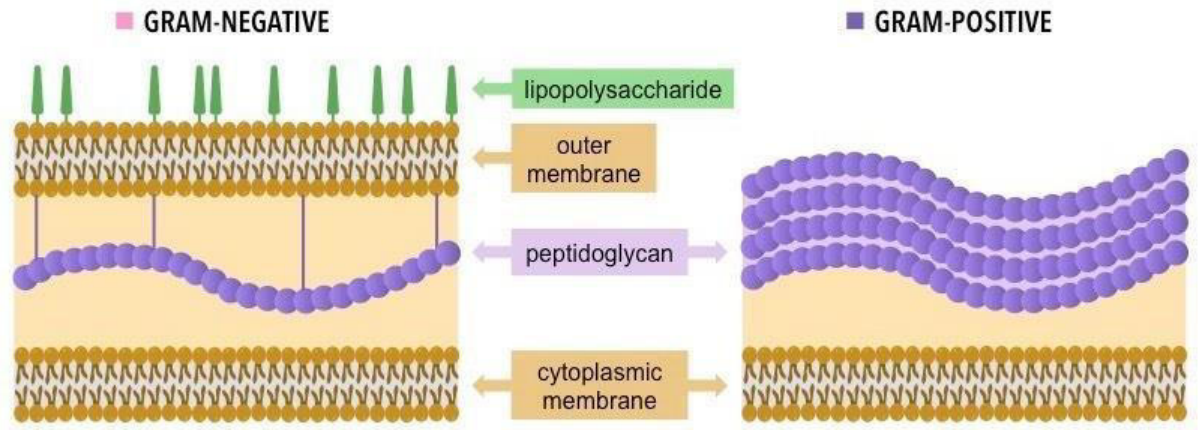


Fig. 1: chemical components of cell wall in Bacteria (Image Credit: BioNinja; Link- https://ib.bioninja.com.au/options/untitled/b1-microbiology-organisms/gram-staining.html)

Materials required for the experiment

Methanol, aqueous solution (0.5%) of crystal violet, Gram's iodine, acetone alcohol (1:1), safranin (1% aqueous solution), glass slide, Bunsen burner, wire loop, forceps, distilled water in wash bottle, blotting paper, bacterial culture on plate and compound microscope.

Protocol

- First, clean the glass slide and take a wire loop. Then allow the wire loop to cool after farming it on a Bunsen burner.
- Dip the wire loop very quickly into the bacterial colony and transfer the bacteria in the loop of the glass slide.
- Then spread the bacteria gently on the glass slide after mixing it properly.
- After that the bacterial source needs to be fixed on the glass slide by using methanol. The fixation can also be done by holding the slide horizontally with forceps and passing it quickly over the flame of the Bunsen burner.
- Flood the slide with crystal violet stain and leave it for about 30 seconds. All the cells turn into blue or violet.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

- Then pour the iodine solution over the bacterial smear and keep it for another 30 seconds. All the cells still appear blue.
- Decolorize the cells by using organic solvents such as acetone or alcohol by keeping it on slide for not more than 2- 5 seconds. Decolorization step is helpful to distinguish the Gram-positive bacteria from Gram-negative bacteria.
- Then counter stain the slide by using red dye safranin and keep it for 1-2 minutes.
- Wash with water and blot dry.

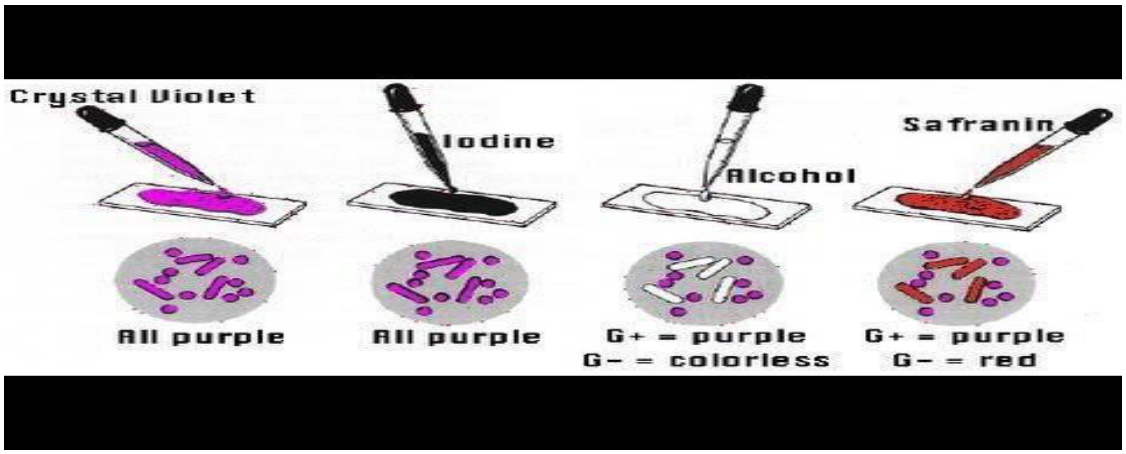


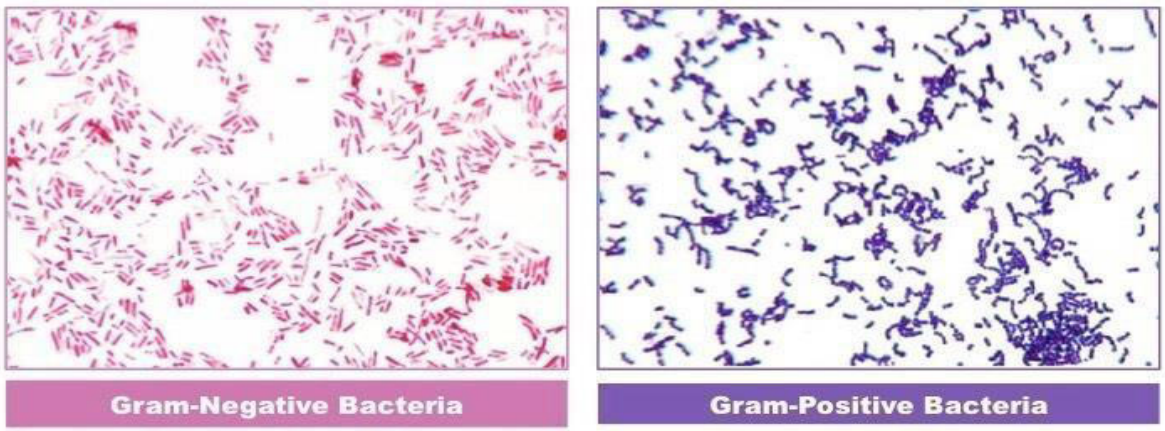
Fig 2: use of different chemicals (photo courtesy- Uploaded on YouTube by: Amrita Vlaboratory; Link- http://www.amrita.edu/create)

Observations

Under a compound microscope the Gram-positive bacteria will appear in violet or blue-black and the Gram-negative bacteria will appear in red or pinkish color.

Precautions to be taken:

- Don't over-stain the bacterial smear either by crystal-violet or safranin stain.
- Don't delay in collecting the sample on a glass slide from the bacterial colony.
- The glass slide needs to be properly cleaned.
- Correct thickness of bacterial smear is very important.
- Don't wash the slide with acetone or alcohol for a longer time unnecessarily.



Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

Experiment no.3

Qualitative Estimation of Protein

Proteins are composed of one or more than one number of amino acids. Proteins, which are composed of only one amino acid, are called mono-peptides. Poly-peptides contain many amino acids that are linked to each other by forming peptide bonds. Proteins (by using Bovine Serum Albumin or white part of the egg as protein sample) can be identified by the use of different chemical reactions in different experiments as follows.

1. Biuret test

Chemical reagents required: 2 ml protein solution, 2 ml 10% NaOH solution, 1% copper sulphate solution.

Procedure

- Add 2 ml protein solution and 2 ml 10% NaOH solution. Mix it thoroughly.
- Then add 2 drops of copper sulphate solution. Mix the whole solution properly.

Observation

A purple color develops slowly.

Chemical basis of the reaction- In basic medium Cu^{+2} gets attached with the peptide bonds and forms a purple complex.

2. Sakaguchi reaction

Chemical reagents required:

3 ml protein solution, 10% NaOH solution, 1% α-naphthol in 70% alcohol, 2% sodium hypochlorite (or 2 drops of bromine water).

Procedure

Add 5 drops of 10% NaOH solution, 3 drops of 1% α-naphthol in 70% alcohol and 2% sodium hypochlorite (or 2 drops of bromine water) in 3 ml protein solution and mix.

Observation

Appearance of deep red color.

Chemical basis of the reaction:

This reaction takes place due to presence of guanidyl group present in arginine. This amino acid is present in all the proteins.

3. Millon's Test:

Chemical reagents required:

2 ml protein solution and 1 ml Millon's reagent.

Procedure:

Take 2 ml of protein solution in the test tube and then add 1 ml of Millon's reagent. Mix thoroughly and bring to a boil gradually.

Observation:

Appearance of white precipitate, which will turn into red as it will coagulate by absorbing heat. In the presence of peptone, there will be less precipitation. Then the whole solution becomes red as the precipitate gets mixed in the solution.

Principal
Deepshikha College of Technical Education
Varun Path, Mansarovar

Chemical basis of the reaction:

Amino acid, tyrosine is present in almost all the proteins. In presence of Millon's reagent, tyrosine reacts with the mercuric and mercuric nitrate and due to the presence of phenol group in tyrosine, red colored mercuric (II) compound is produced.

Experiment no.4
Isolation of Genomic DNA

The genomic DNA can be isolated from the cells or tissue portions by different methods. Presently different nucleic acid or DNA extraction kits and protocols have developed. However, one of the commonly used and basic methods is the phenolic extraction method of genomic DNA. Basic steps of such method is described below –

1) Tiny amount of tissue (e.g. goat liver) of about 10 mg taken into 1.5-2 ml of micro- centrifuge tube,
2) Lysis buffer (composed of Tris-HCl, EDTA, NaCl and SDS) added and tissue homogenized,
3) Tissue lysate centrifuged at 13,000 rpm for 15 mins,
4) Supernatant discarded, pellet taken, resuspended into lysis buffer,
5) RNaseA is added and incubated at 37^0C for an hour,
6) ProteinaseK is added and incubated at 50^0C overnight,
7) Phenol/Chloroform/Isoamylalcohol (25:24:1) was added and mixed well,
8) Centrifuged at 13,000 rpm for 10 mins,
9) Aqueous phase is pipetted into other tube,
10) Repetition of steps 7-9 is done twice,
11) Chloroform added, mixed well,
12) Mixture centrifuged at 13,000 rpm for 10 mins,
13) Aqueous phase is pipetted into other tube,
14) Repetition of steps 11 and 12,
15) 5M NaCl of one tenth of the volume of aqueous phase is added with twice the volume of 100% chilled ethanol and mixed,
16) Whitish thread like DNA starts to precipitate,
17) Kept at – 20^0C for 30 mins,
18) Centrifugation at 13,000 rpm for 15 mins,
19) DNA pellet found and air dried removing excess ethanol,
20) DNA dissolved in the TE buffer for further analysis and experiments.