Netaji Subhas Open University
School of Sciences
DEPARTMENT OF ZOOLOGY

MANUAL FOR ZOOLOGY LABORATORY

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 nearest sink, flush and wash 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 instructor.
7. Do not keep unnecessary books, backpacks and other personal items on laboratory benches.
8. Avoid open long hair, flowing clothing, open-toed shoes in laboratory.
9. Pregnant or immunocompromised student 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 laboratory, wash hands thoroughly and line the work area with clean paper towels. After laboratory, wipe down work area with disinfectant and wash hands thoroughly.

11. Dispose of used slides, chemicals, any tissue wastes or hazardous wastes in 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 specimen and others 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 use 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.

Up-right microscope is basically used in research purpose. 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.

Compound Microscope
Photo Credit: IndiMART

Up-right Microscope
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.

Rotary Microtome
Photo Credit: IndiaMART
Brand: Safire Scientific Company

Hot Plate

These are electrically controlled plate which are used to dry slides or straighten the paraffinized tissue sections. Metalized surface of the tool gets heated by the electric coils underneath. Also such instrument is 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 (CO_2) 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.

BOD Incubator
Photo Credit: IndiaMART,
Brand: Adarsh International

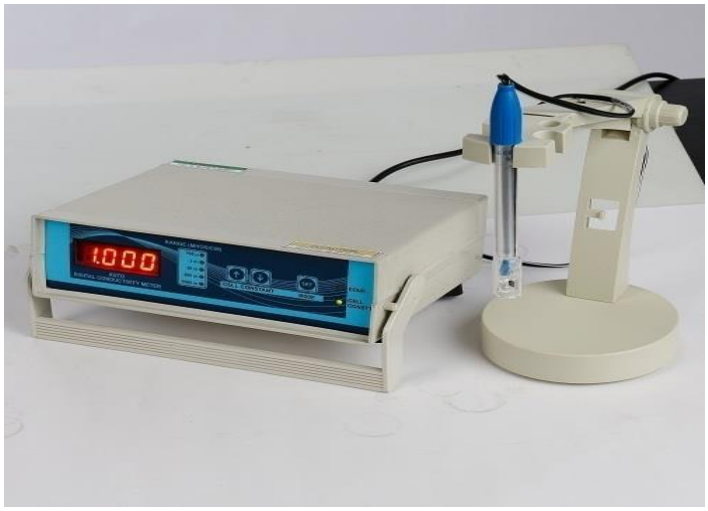
Centrifuge

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 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 differentiae 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 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.



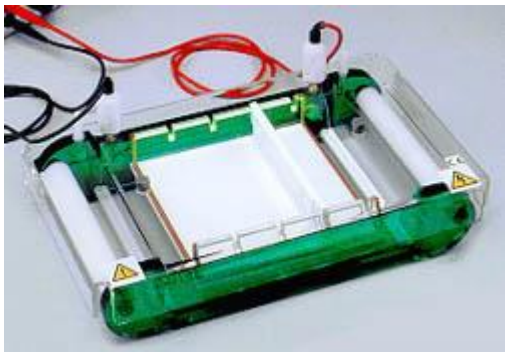
pH meter
Photo Credit: IndiaMART
Brand: Sigma Scientific Glass Company

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 structure and forms bands at different parts forming gradient according to their comparative size. The gel box features a cathode at one end and an anode at the other. Ionic buffer fills the box and creates 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 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 buffer in one chamber and the top is submerged in buffer in another chamber. A small amount of buffer comes through the gel from the top to the bottom chamber. Unlike Horizontal system acrylamide can be used in vertical gel electrophoresis.



Horizontal Gel Electrophoresis System
Photo Credit: Topac Inc.

Vertical Gel Electrophoresis System
Photo Credit: Topac Inc.

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.

SOME LABORATORY PROTOCOLS in ZOOLOGY UG and PG COURSES

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 albumen **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 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 stair the saline for few minutes. This will produce as suspension of gut content in the saline of the watch glass.
- Take a clean glass slide which is layered by Mayer's albumen.
- Put few drops of the suspension of the gut content from the watch glass.
- Spread the solution uniformly over the glass slide. Wire loop or another slide may be used for this purpose.
- Now transfer the slide to fixative and start downstream process. Then the slides are stained for observation under microscope. This transfer process should be done before the smear gets air dried.

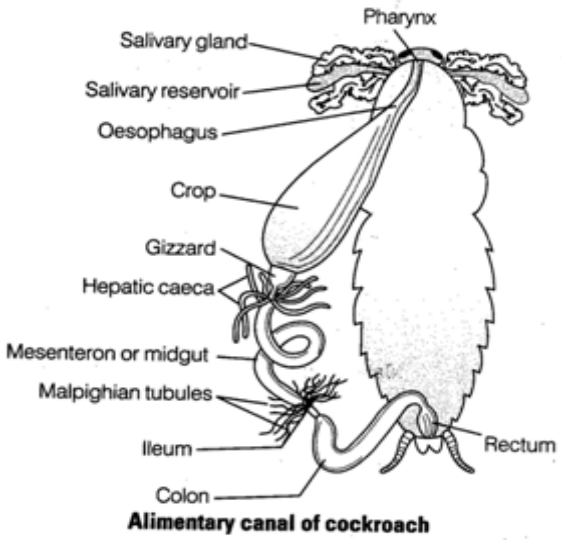


Fig 1: Alimentary canal of cockroach
(**Image credit- www.studyadda.com)**

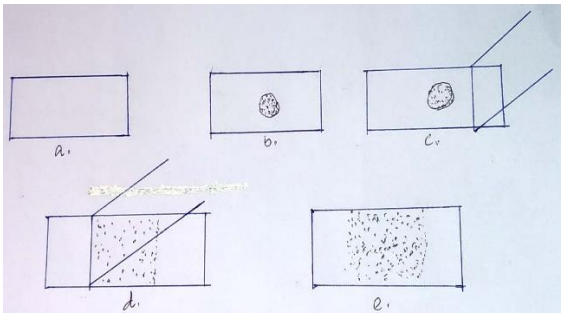


Fig 2: a. clean glass slide, layered by Mayer's albumen. b. drop of the suspension of gut content on glass slide. c. another slide is taken at 45^0 to spread the suspension d. smear is spread over the slide e. prepared slide.

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.

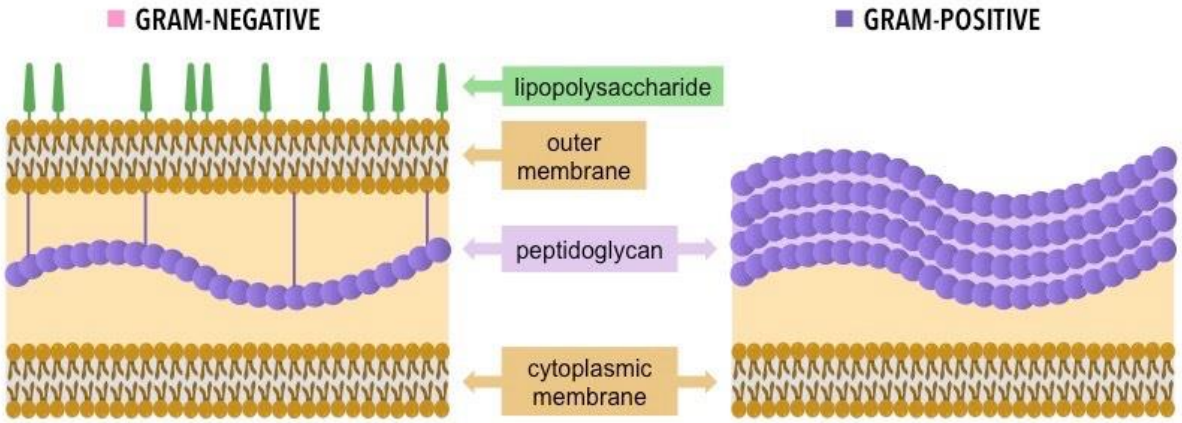


Fig. 1: chemical components of cell wall in Bacteria (Image Credit: BioNinja; Link- https://ib.bioninja.com.au/options/untitled/b1-microbiology-organisms/gram-staining.html)

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

- At first clean the glass slide and take a wire loop. Then allow the wire loop to cool after faming it on 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.

- Then pour the iodine solution over the bacterial smear and keep it for another 30 seconds. All the cell still appears 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.

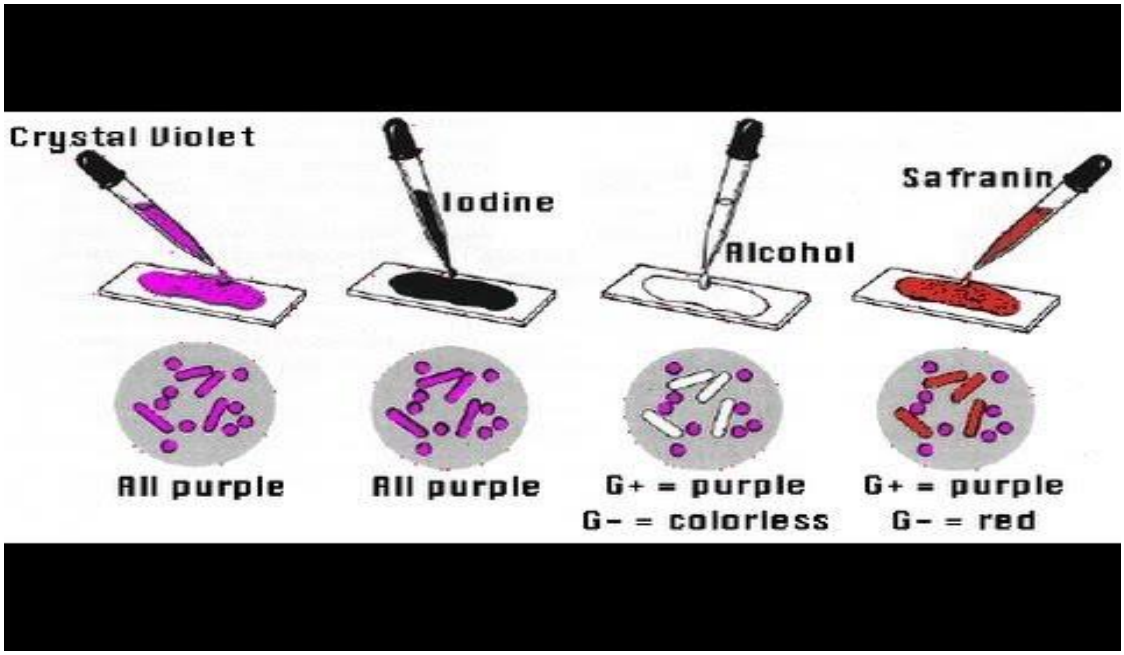


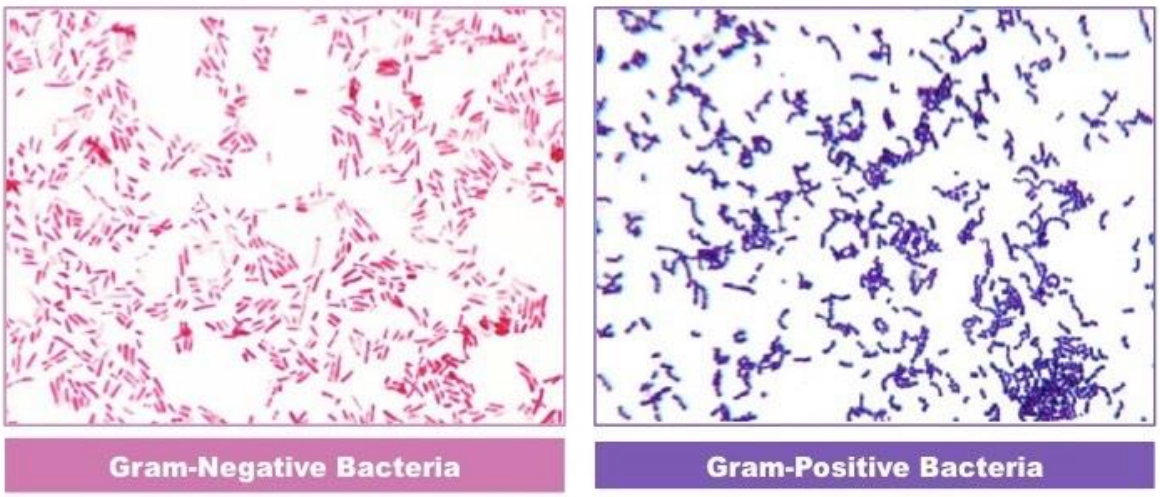
Fig 2: use of different chemicals (photo courtesy- Uploaded on YouTube by: Amrita Vlaboratory; Link- http://www.amrita.edu/create)

Observations

Under 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 glass slide from the bacterial colony.
- Glass slide needs to be properly cleaned.
- Correct thickness of bacterial smear is very important.
- Don't wash the slide by acetone or alcohol for longer time unnecessarily.



(Image Credit: BioNinja; Link- https://ib.bioninja.com.au/options/untitled/b1-microbiology-organisms/gram-staining.html)

Qualitative Estimation of Protein

Proteins are composed of one or more than one numbers of amino acids. Proteins, which are composed of only one amino acid, is called as mono-peptide. Poly-peptides contains 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 following.

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 cupper 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-napththol 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-napththol 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 boil gradually.

Observation:

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

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.

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 are 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 TE buffer for further analysis and experiments.

Details of such laboratory experiments and protocols are available in the Self Learning Materials (SLM) of the University and a learner may receive exposure of these laboratory procedures in PCP/ LCES programmes of the University.