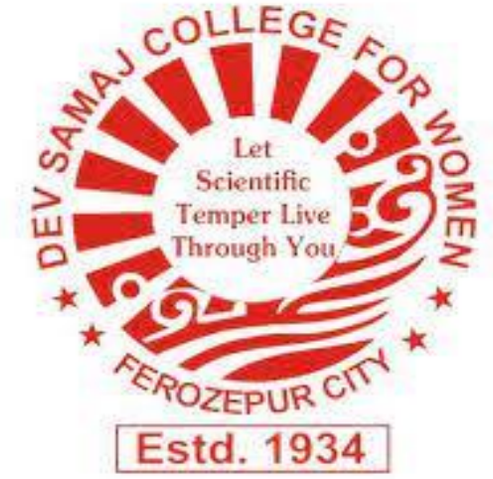
ZOOLOGY

LAB MANUAL

B.Sc. III



Department of Zoology

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EXPERIMENT NO. 1

Studying the development of Chick by preparation of window in the incubated eggs

Lay the eggs sideways in the incubator and mark the uppermost surface of the egg with an X. The yolk will pivot so that its lightest point, the embryonic blastoderm, will rotate from beneath the airspace to the highest point. The X will, therefore, indicate the location of the embryo.

Incubate the eggs at high relative humidity (>50 %) and in the range of 37.5 to 39ºC.

Follow these steps for the windowing:

1. Wipe the egg with 70% ethanol to avoid contaminations.
2. Pierce the blunt end of the egg with the point of a scalpel, thereby opening an air space.
3. Insert a 19 g needle on a 5 mL syringe through the hole, direct the tip of the needle towards the bottom and withdraw 2-4 mL of thin albumen.
4. Cover the top side of the egg with clear sticky tape.
5. With the point of your scissors drill a hole in the middle of the X to admit air.
6. Leave the egg aside for a few moments while the embryo which is immediately beneath the shell subsides into the space created by the withdrawal of albumen.
7. Use the scissors to make a circular cut in the shell about 15 mm in diameter directly over the blastoderm. Do not make a complete cut but leave a strip of the shell and sticky tape so that you can flip back the cut shell.

Now you are ready to observe the embryo under the stereo microscope. Make sure you tape the window before incubation otherwise the egg will dry out.

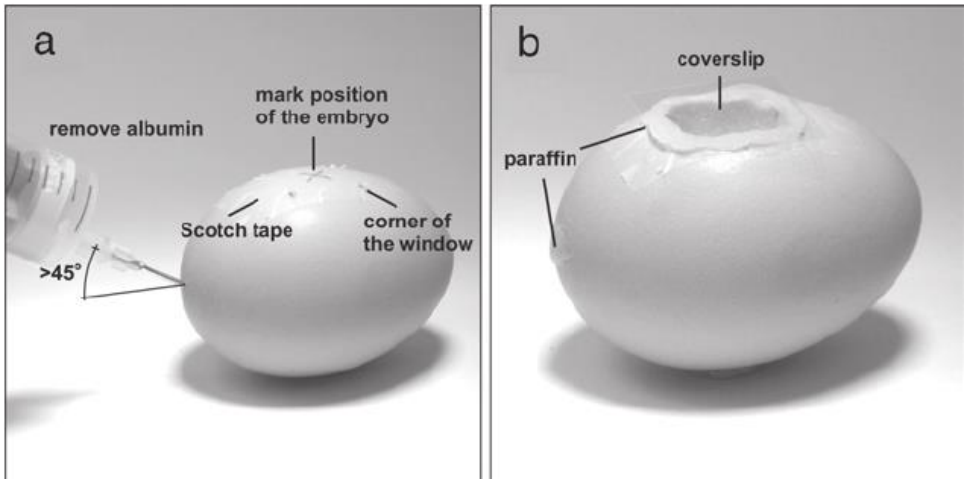


Fig 1: Egg Windowing

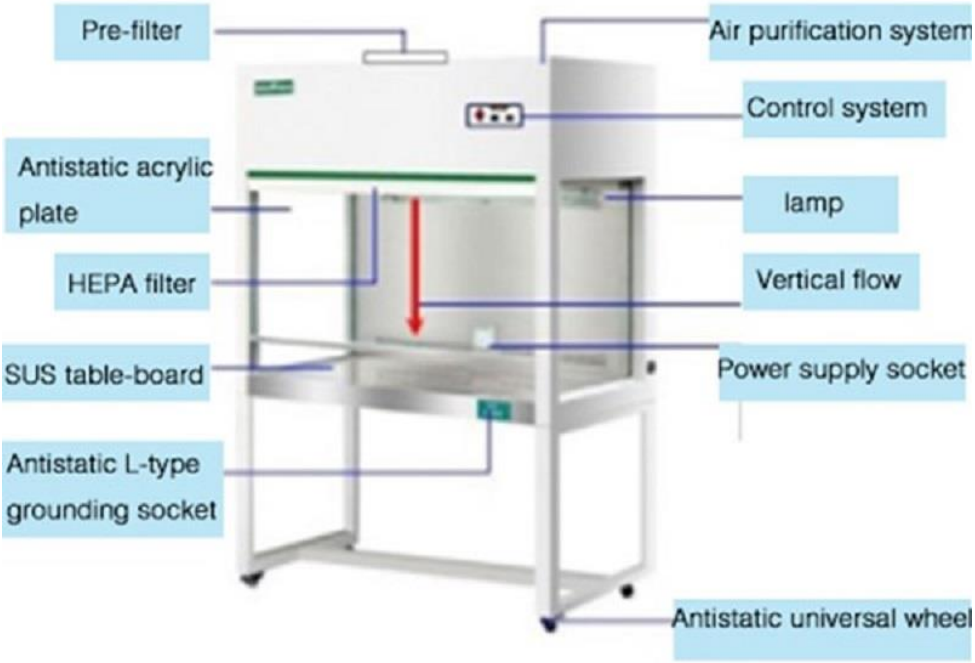
EXPERIMENT NO. 2

1) Cleaning and sterilization of glassware using hot air oven, autoclave etc.,
2) To use the various scientific equipment i.e., autoclave, centrifuge, and spectrophotometer with respect to its operation and function.

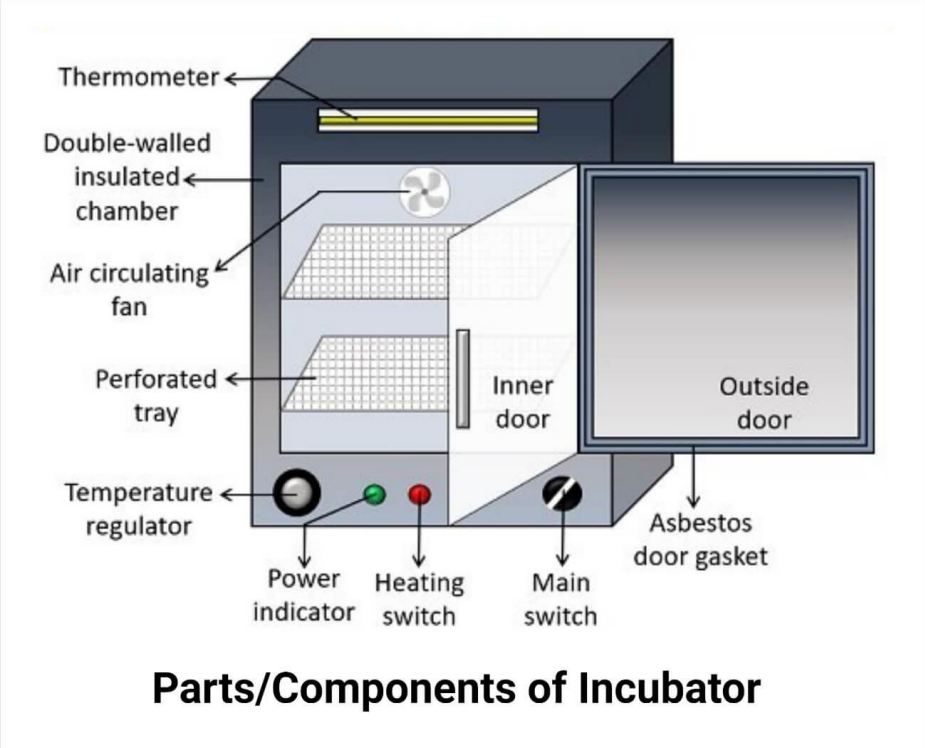
Theory:

1. Autoclave: it is an apparatus in which saturated steam under pressure affects the sterilization. The increased pressure increases the boiling point of water and produces steam due to which microbial cells are destroyed. The temperature, time and pressure conditions are 121°C at 15 psi for 15 minutes. It is the most efficient and the most commonly used instrument for the sterilization of solid state and liquid media, glassware and rubber products. It is double-valued cylindrical vessel made up of steel and the top is opened to receive the materials to be sterilised. Autoclave is provided with pressure gauge for recording pressure and steam cork rises for release of steam. The articles to be sterilized are kept in the basket provided with holes all round for free circulation of steam.

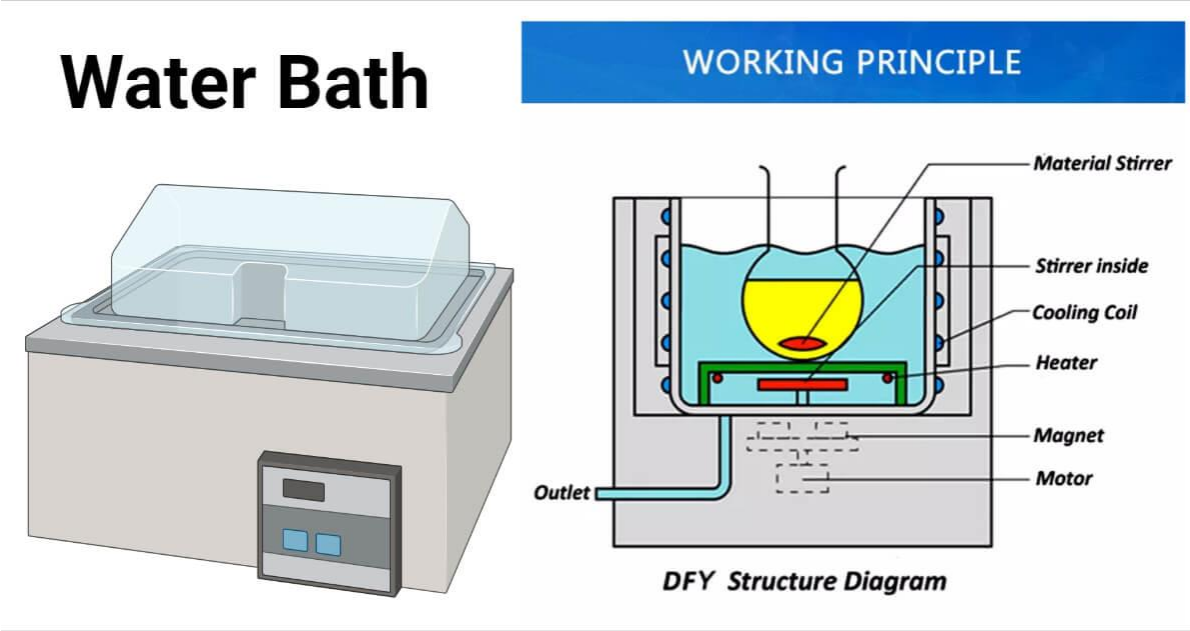
2. Laminar flow: it is used for reducing danger of contamination while working with the infectious microbes. It works on the principle of application of fibrous filters usually HEP filter i.e. high efficiency particulate air filters. Room air is filtered before entering the working chambers and it moves in a single direction. UV light is also provided to reduce the risk of spores if present.



3. Incubator: it consists of an insulated, fitted with the heating element at the bottom. The temp of the incubator can be maintained at a desired level by an aromatic device called thermostat which cuts the connection when the temp falls below that point. They are provided with double doors, the inner one is made up of glass so that the contents maybe viewed from outside. It helps in culturing of the microbes at a constant temp and helps in growth and development of various organisms. it uses dry heat of maintaining temperature.

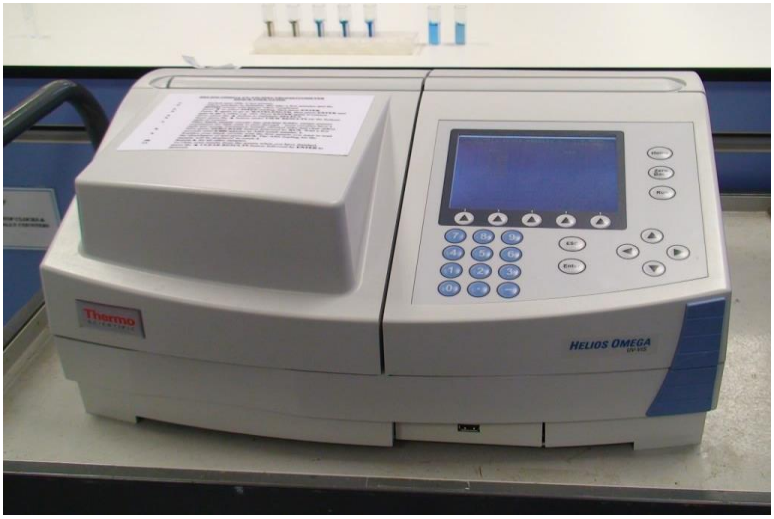


4. Water bath: a water bath is an insulating metal box filled with an electric heating mechanism and thermostat which maintains the temperature at a desired level. The bath is filled with water until it reaches almost to the top of the brim, as it contains racks for holding test tubes and flasks. Water bath is mainly used for melting up of various microbes in Wassermann's test.

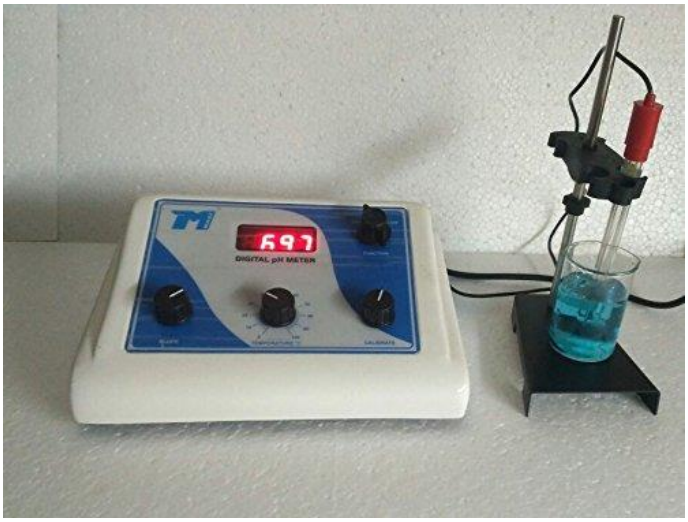


5. Magnetic hot plate stirrer: it is an extremely useful in making media as well as to stir to make a suspension. It is fitted with stirrer and heat. A control stirring is done by creation of magnet kept in the beaker to spin resulting in stirring of the medium.

6. Spectrophotometer: it is used for counting population of bacteria based upon the principle of turbidity or optical density. Turbidity is the cloudiness of the suspension. The more turbid suspension is, less light will be absorbed and is proportional to the mass of cells in the light part. Bacteria may grow in broth, clean broth may become turbid. Since, the turbidity increases, the indicator is used for determination of bacterial density in the broth. Turbidity is also used for standardising of bbacteria structures of chemical significance.



7. pH meter: it is used to determine pH that maybe acidic or alkaline of the solution of unknown pH as well as setting of pH. Various media used for cultivation and testing of biochemical activities of microbes. The measurement of ph and ph meter is done electrometrically and depends upon development of membrane potential by a glass electrode. A pH meter consists of another electrode known as reference electrode. This consists of metallic internal element typically of mercury i.e. HgCl2 or AgCl2 immersed in an electrolyte. Usually, a saturated solution of KCl is used. The function of electrolyte is to form a conductive salt bridge between the metallic element and the sample solution in which electrodes are placed to keep stable electrically communication between the internal metallic element and sample solution. A liquid is present in the tip of the outer body of reference electrode.



8. Oven: an oven is based on the principle of sterilization which is accomplished by dry heat or hot air. Hot air oven are mostly used for sterilisation of glassware like petridishes, pipette and metallic instruments. An oven consists of an insulated cabinet which is held at a constant temperature by means of thermostat. It is fitted with fan to keep hot air circulation. The shelves are perforated for normal sterilisation, the oven will be operated at 160°C will require the period of two hours for total sterilisation.

EXPERIMENT NO. 3

To prepare the permanent slides with the help of Fixation, Embedding, Cutting of tissue section and their staining

Histopathology- Definition it is a branch of pathology which deals with the study of disease in a tissue section. The tissue undergoes a series of steps before it reaches the examiners desk to be thoroughly examined microscopically to arrive at a particular diagnosis. To achieve this it is important that the tissue must be prepared in such a manner that it is sufficiently thick or thin to be examined microscopically and all the structures in a tissue may be differentiated. The objective of the subsequent discussions will be to acquaint the staff with their responsibility; the basic details of tissue handling, processing and staining. The term histochemistry means study of chemical nature of the tissue components by histological methods.

Microtomes are special instruments which have automatic mechanism for cutting very thin sections. To cut the sections on the microtome; the tissue must be made hard enough to not get crushed. There are 2 methods of hardening the tissues. One is by freezing them and the other is by embedding them in a hard material such at paraffin wax or gelatin.

Types of Histological preparation

The histological specimen can be prepared as

1. Whole mount 2. Sections

3. Smears.

1. Whole mounts- These are preparation entire animal eg. fungus, parasite. These preparations should be no more than 0.2-0.5 mm in thickness.
2. Sections- The majority of the preparations in histology are sections.

   The tissue is cut in about 3-5 mm thick pieces processed and 5 microns thick sections are cut on a microtome. These are then stained and permanently mounted.
3. Smears- Smears are made from blood, bone marrow or any fluid such as pleural or ascitic fluid. These are immediately fixed in alcohol to presence the cellular structures are then stained. Smears are also made by crushing soft tissue between two slides or an impression smear in made by pressing a clean slide in contact with the moist surface of a tissue. By doing this, the cells are imprinted on the slide and these may be stained for cytological examination.

FIXATION

Definition It is a complex series of chemical events which brings about changes in the various chemical constituents of cell like hardening, however the cell morphology and structural detail is preserved.

Unless a tissue is fixed soon after the removal from the body it will undergo degenerative changes due to autolysis and putrefaction so that the morphology of the individual cell will be lost.

Mode of teaching - Overhead projector and practical demonstration.

Principle of fixation- The fixative brings about crosslinking of proteins which produces denaturation or coagulation of proteins so that the semifluid state is converted into semisolid state; so that it maintains everything in vivo in relation to each other. Thus semisolid state facilitate easy manipulation of tissue.

Aims and Effects of fixation

If a fresh tissue in kept as such at room, temperature it will become liquefied with a foul odour mainly due to action of bacteria i.e. putrefaction and autolysis so the first and fore most aim of fixation is

1. To preserve the tissue in as lf like manner as possible.

2. To prevent postmortem changes like autolysis and putrefaction.

   Autolysis is the lysis or dissolution of cells by enzymatic action probably as a result of rupture of lysosomes.
   Putrefaction The breakdown of tissue by bacterial action often with formation of gas.

3. Preservation of chemical compounds and microanatomic constituents so that further histochemistry is possible.
4. *Hardening* : the hardening effect of fixatives allows easy manipulation of soft tissue like brain, intestines etc.
5. *Solidification*: Converts the normal semifluid consistency of cells (gel) to an irreversible semisolid consistency (solid).
6. *Optical differentiation* - it alters to varying degrees the refractive indices of the various components of cells and tissues so that unstained components are more easily visualized than when unfixed.
7. *Effects of staining* - certain fixatives like formaldehyde intensifies the staining character of tissue especially with haematoxylin.

Properties of fixatives

1. Coagulation and precipitation as described above.

2. Penetration Fixation is done by immersing the tissue in fluid containing the fixative. Faster a fixative can penetrate the tissue better it is penetration power depends upon the molecular weight e.g. formalin fixes faster than osimic acid.
3. Solubility of fixatives - All fixatives should be soluble in a suitable solvent, preferably in water so that adequate concentrations can be prepared.
4. Concentration - It is important that the concentration of fixative is isotonic or hypotonic

5. Reaction - Most fixatives are acidic. It may help in fixation but can affect staining so has to be neutralized e.g. formalin is neutralized by adding of calcium carbonate.

Amount of fixative

The fixative should be atleast 15-20 times the bulk of tissue. For museum specimens the volume of fixative is > 50 times.

Note : If the specimen is large then see that the sections are made to make slices which have a thickness of 1.5 cm so that fixative can penetrate the tissue easily

Preparation of the specimen for fixation

1. For achieving good fixation it is important that the fixative penetrates the tissue well hence the tissue section should be ≥ 4mm thick, so that fixation fluid penetrates from the periphery to the centre of the tissue. For fixation of large organs perfusion method is used i.e. fixative is injected through the blood vessels into the organ. For hollow viscera fixative is injected into the cavity e.g. urinary bladder, eyeball etc.
2. Ratio of volume of fixative to the specimen should be 1:20.
3. Time necessary for fixation is important routinely 10% aqueous formalin at room temperature takes 12 hours to fix the tissue. At higher temperature i.e. 60-65°C the time for fixation is reduced to 2 hours.