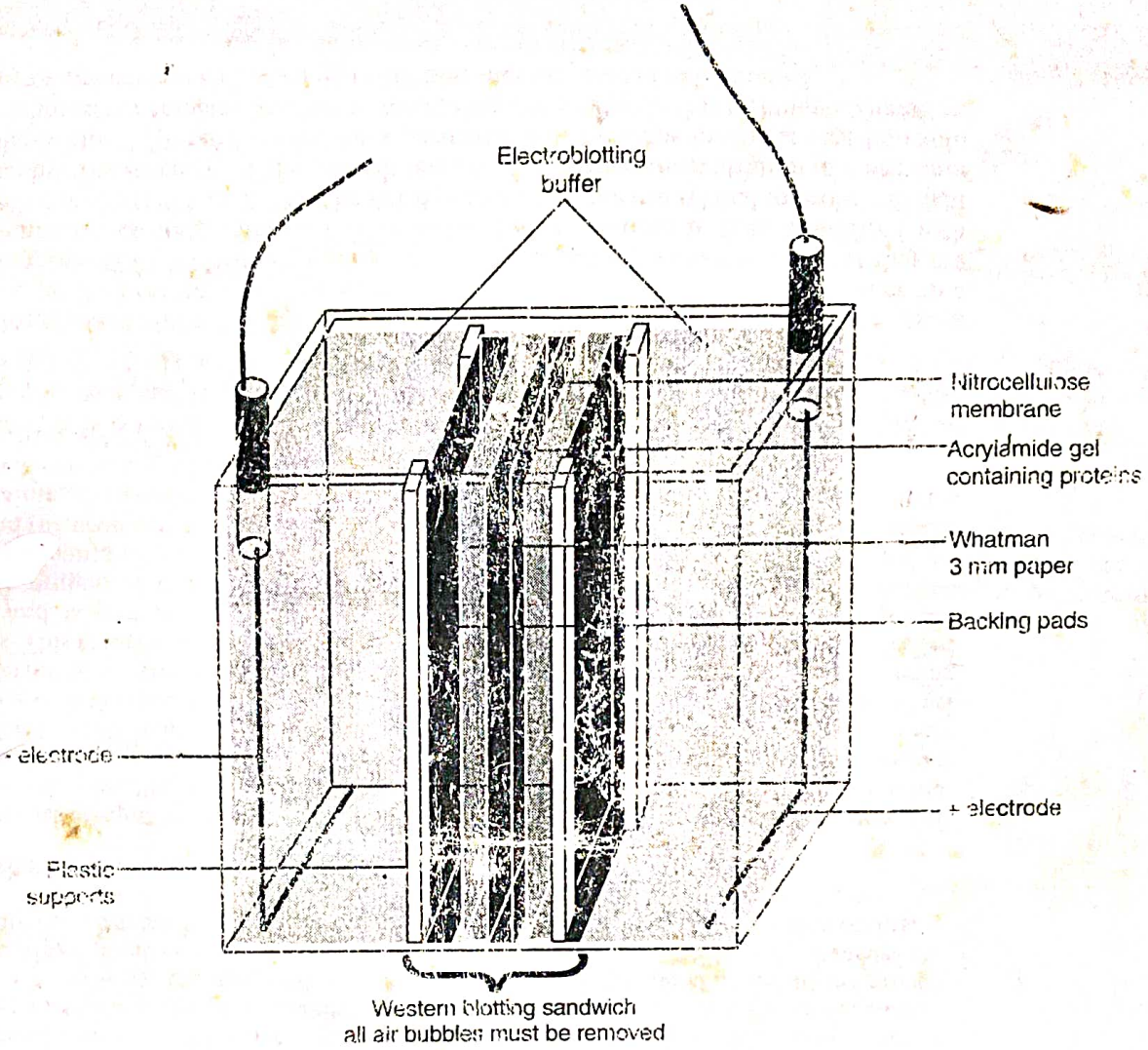



FIG. 22.3: Diagram of a typical Western blotting or electroblotting apparatus. An electric current is used to transfer the proteins from the gel to the nitrocellulose membrane placed next to it in the blotting sandwich. All other components of the sandwich function to provide gentle but firm support; tight contact between the gel and the membrane is essential for good transfer. All bubbles must be carefully removed from the sandwich to assure uniform flow of current and complete transfer of all proteins.

Sometimes, an apparatus is used for placing spots on the membrane, through slots made in this equipment. The spots made thus are in the form of oblong slots rather than round blots. These slots are used just like dot blots and are described as slot blots.

## DETECTION OF RFLPS

A RFLP can be demonstrated using the following steps:

1. Extract and purify DNA from several individuals which may differ in some respects among themselves.

## VARIOUS LABORATORY EQUIPMENTS

In Microbiology laboratory certain equipments are basically required to work with microorganisms. It is essential for students to know their working operations / principles. This will facilitate them, while working and obtaining accurate results. (*For details of microscopes, see chapter on Microscopy*).

### Sterilization Equipment

The maintenance of sterilization in lab. is the basic fundamental principle of workign with microbial system. All the lab. Necessities, such as glass wares (Petri dishes, culture tubes, pipettes etc.). Culture media, steel equipments, inoculating needle / loop, cotton and others are required to be sterilized before use. Thus equipment suited for dry / moist heat sterilization is used in the laboratory.

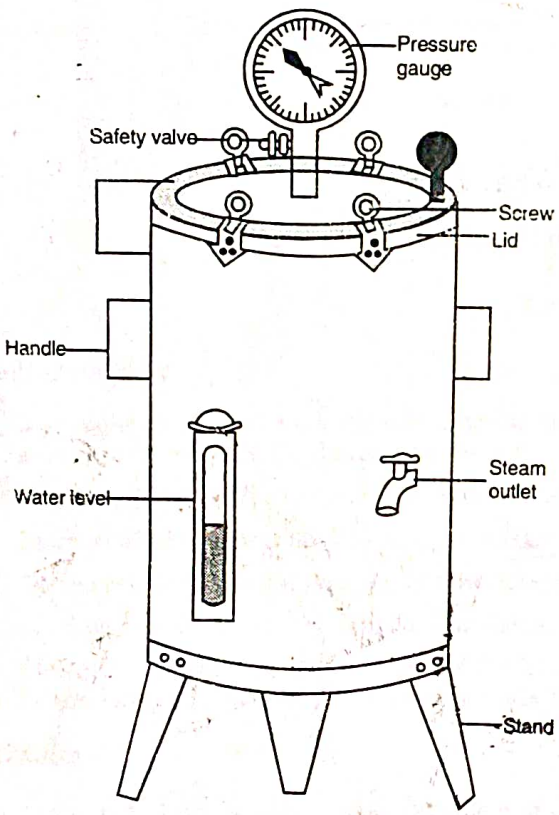



FIG. 47.1: Autoclave.

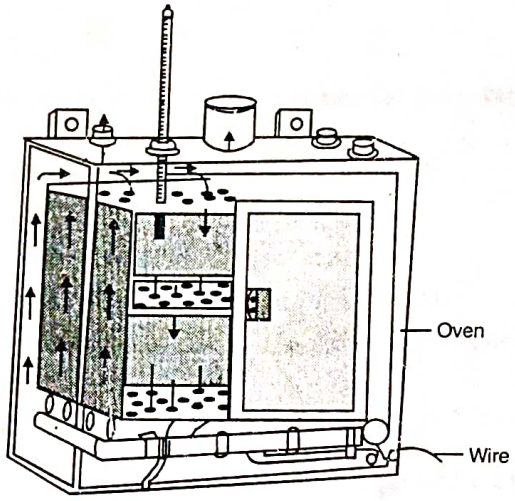



FIG. 47.2: Hot air oven.

**Autoclave (moist heat strategy):** It is an instrument designed on the working principle of steam pressure cooker, the one that is used at home for cooking purposes.

1. It is constructed with that of heavy gauge metallic sheet, so that it can sustain internal steam pressure when in use.
2. Autoclave is normally designed in two shapes, 1. Vertical, 2. Horizontal, and are of verifying capacity suited to one's requirement.
3. In an autoclave saturated moist heat is circulated, hence normally heat labile substances (media, cotton, gauges, liquids and clothing material) are sterilized with it.

5. Incubators can very well called as growth chambers, therefore inside glass d cultures (growth) without disturbing Petri dishes.
6. All the accessories like thermometer, temperature dial, thermostat on / off switch n smooth working with it.

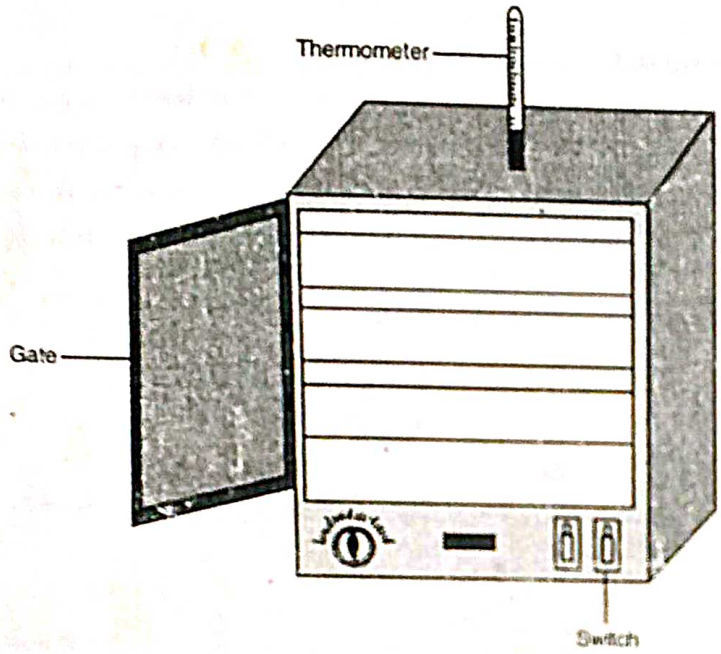



FIG. 47.3: Incubator.

## Laminar Air Flow

1. It is highly improved sophisticated inoculation chamber, used for aseptic transfer for microbial cultures and reducing the danger of infection.
2. It is designed in such a manner that sterile materials remain protected from contamination.
3. Laminar air flow works on the principle of HEPA filters (High Efficiency Particulate Air).
4. On the working bench of laminar air flow, filtered room air is lowed in a single direction.
5. Laminar flow bench are available in two systems, (a) Horizontal, (b) Vertical air flow system.
6. This highly sophisticated instrument of the lab. is equipped with following attachments fiberglass filters, blowers, UV tube light, fluorescent tube for illumination, glass doors.

## pH Meter

Determination of pH (acidity / alkanity) is one of the most important exercise as it is required to set the pH of the media for the cultivation, and optimum biochemical activity of micro organisms.

1. pH meter is an essential instrument which assist in the determination of pH of the solution of unknown pH value.
2. The instrument comprised of electric circuit with one or two electrode system.
3. Out of the two electrodes in a pH meter, one is made up of glass and the other one of calomel 1.
4. pH of the solution is measured on a meter scale ranging from 0 – 14. The number tells concentration of hydrogen ion in the given sample.

5. Glass electrode remains filled with hydrochloric acid (0.1 N), while the calomel electrode contains saturated solution of potassium chloride.
6. The calomel electrode is also known as the reference electrode.
7. The instrument is equipped with adjustment knobs for the standardization of the instrument.
8. Before use, pH meter is standardized with the solutions of the known pH value.

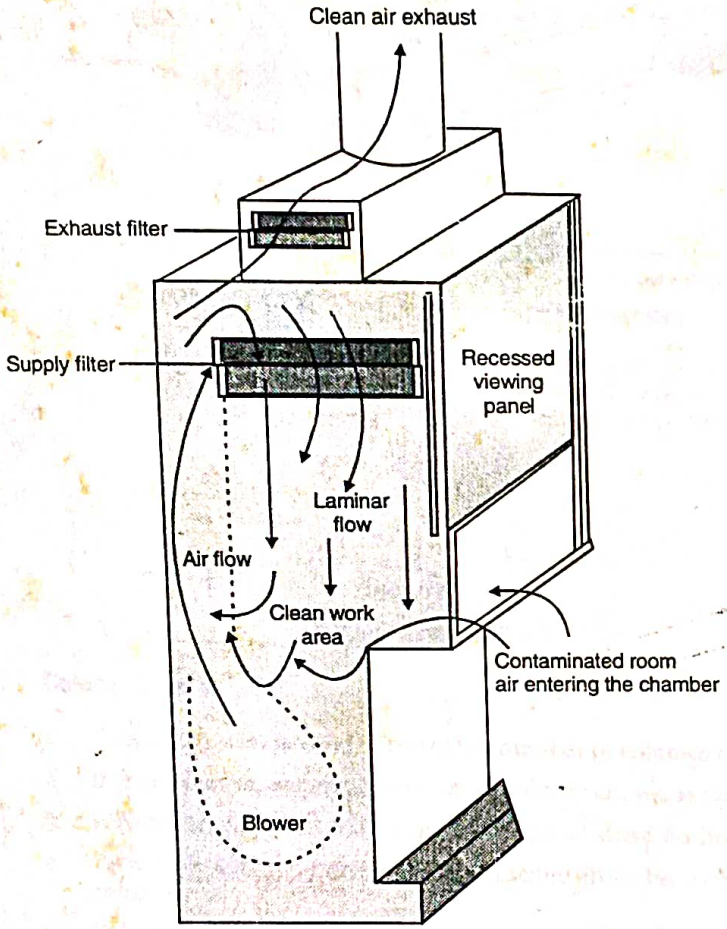



FIG. 47.4: Laminar air flow.

## Centrifuge

1. It is an instrument which works on the principle of centrifugal force.
2. Centrifuge in different shape and models are available with rotation speed capacity, suited to one's requirement.
3. Suspended material in a solution is separated with the help of this instrument.
4. Refrigerated and ultra high speed centrifuge are required in molecular and biochemical studies.
5. The working speed in centrifuge is expressed in terms of revolutiosn per minuted (rpm).
6. During centrifugation, solutions are taken in specially designed glass tube which is then kept in special holding tubes in the machine.

7. The suspended particles in a solution separates and settled at the bottom of the on the basis of mass density.
8. Before putting an instrument in the one position of the care must be taken to check free swinging o fthe hold rings, and the proper closer of the lid.

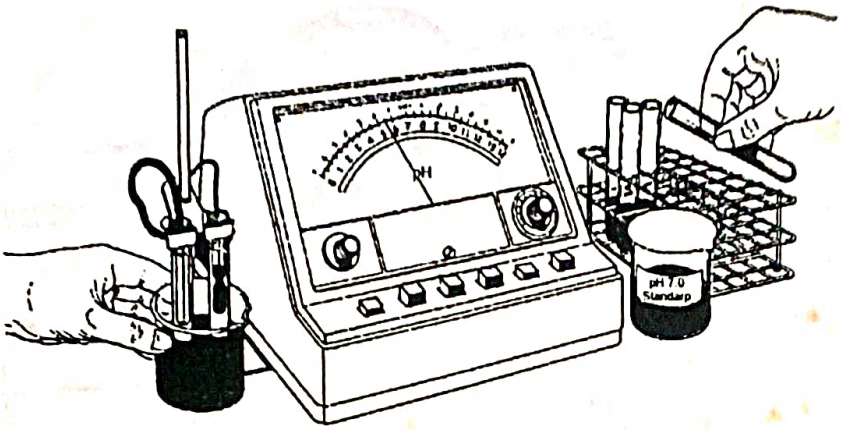
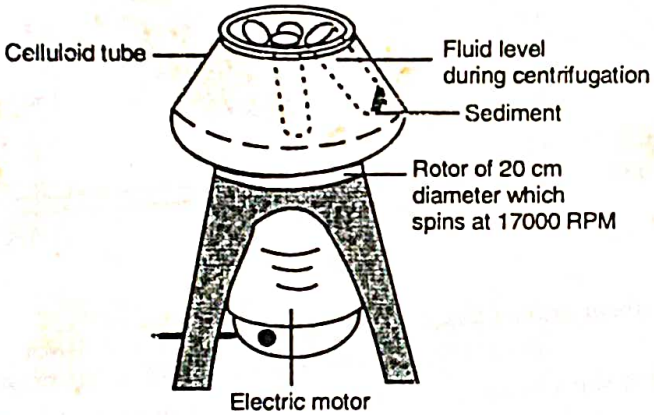



FIG. 47.5: pH meter.




A superspeed centrifuge

FIG. 47.6: Centrifuge.

## Colony Counter

1. This instrument is used to count the number of colonies on a solid medium in a Petri dish.
2. It is an electronic instrument and commonly known as Quebec Colony Counter.
3. It consists of Petri dish holding platform and stand for holding the magnifying lens.
4. Petri dish holding platform is illuminated from beneath, which assist in clear visibility of small colonies.
5. Colony counters with automatic counting systems are also available, and they remain equipped with sensitive electrode system.

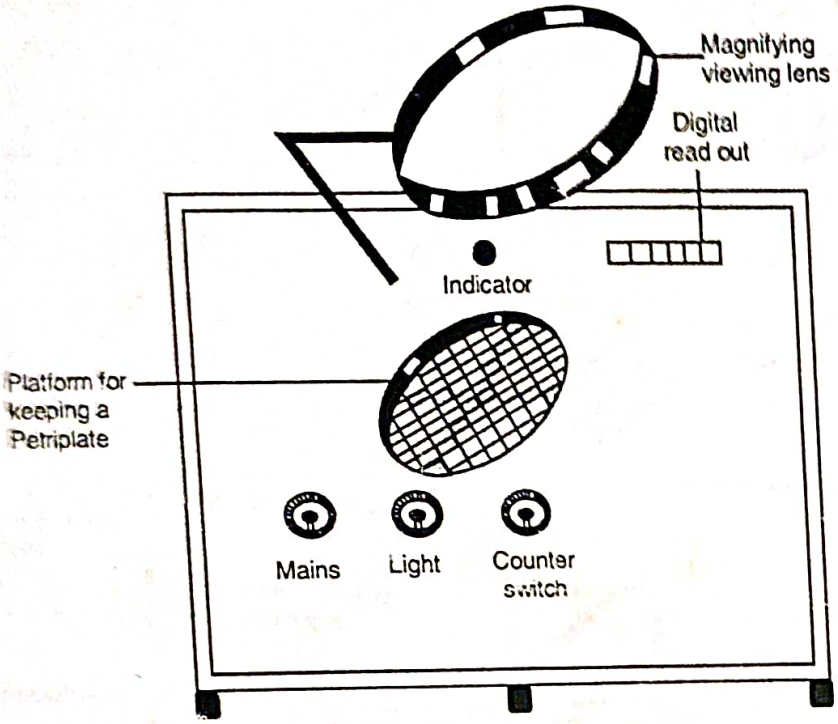



FIG. 47.7: Colony counter.

FIG. 47.8:

Racks for test t

## Colorimeter

1. To calculate the growth index of bacteria in broth (liquid culture medium) spectrophotometer or photoelectric colorimeter is used in the microbiology laboratory.
2. The growth of bacteria is measured in this instrument on the principle of turbidity determination.
3. Bacterial growth in liquid medium in indicated by the cloudiness (optical density) of the solution which interferes with the light rays passing through it.
4. In this instrument light rays of known wavelength (passing through colored filters) allows to travel through the solution.
5. The cell mass present in the medium intervenes light path via light absorption / reflection.
6. This optical density is measured with this instrument for quantitative estimation of microbial growth.

## Water Bath

1. In microbiology laboratory water baths are used for melting solid media and other biochemical activity purpose.
2. It is an electronic instrument made up of thick stainless steel and having space for holding water, and is of box type.
3. The upper side of the box is holed so that test tubes or conical flasks can be put into it dipping in underneath.
4. The basal part of the box is fitted with hot plate for heating mechanism.
5. Thermostat attached with the circuit in the box helps in maintains temperature at desired level.
6. In water bath uniform heating is provided to growing cultures.

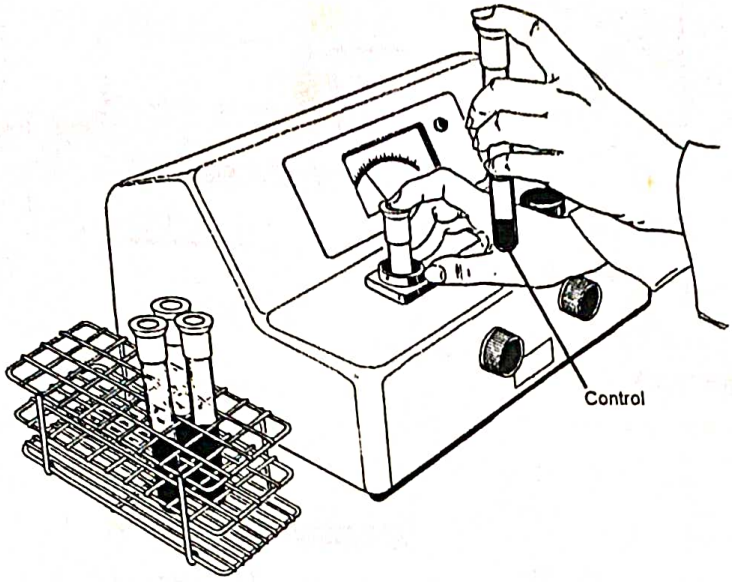



FIG. 47.8: Colorimeter.

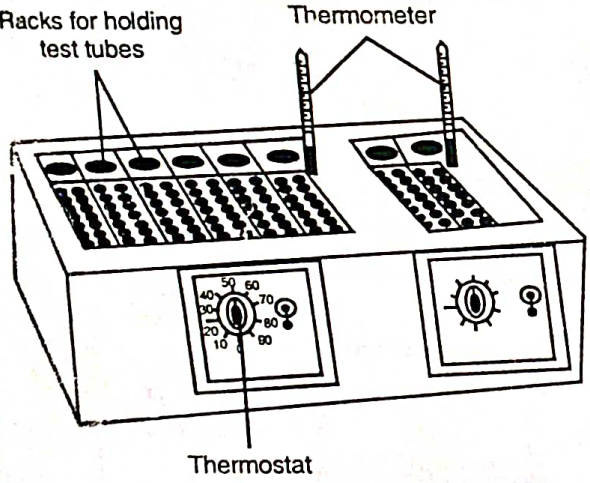



FIG. 47.9: Water bath.

## EXPERIMENT NO. 1

### Temporary Wet Mount (TWM) Technique for Microscopic Observation of Living Microorganisms.

**Requirements**

(1) 18-18 hour-old broth culture of *Bacillus cereus*, (2) *Spirogyra* sp. (culture or in pond water), (3) *Paramecium* sp. (in pond water), (4) Glass slides (Three in number), (5) Cover slips (Three in number), and (6) Dropping pipettes (Three in number).

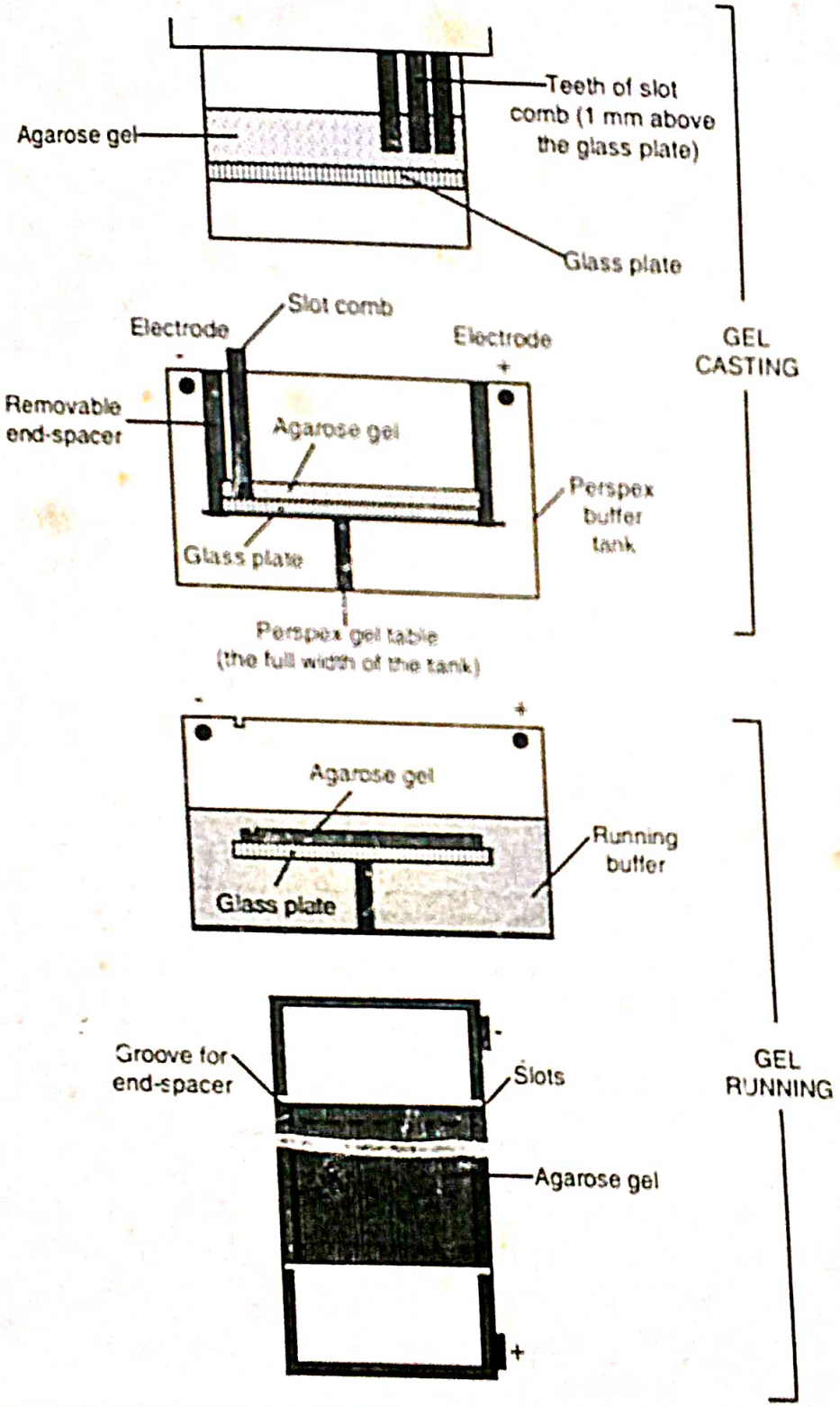



FIG. 21.1: Agarose gel electrophoresis.