CHAPTER - 1

Laboratory Safety and Instrumentation

1.1 General Laboratory Rules and Precautions

All Microbiology laboratories maintain live microbial cultures, based on the concept there are certain prescribed rules for personal and environmental safety. The former rules reflect concern for personal safety in terms of avoiding laboratory accidents. The later requires that to maintain a scrupulously clean laboratory setting to prevent contamination of experimental procedures by microorganisms from erogenous sources. All microorganisms should be treated as potential pathogens (Organisms capable of producing disease). Thus, microbiology students must develop aseptic techniques (free of contaminating organisms) in the preparation of pure cultures that are essential in the industrial and clinical market places.

The following:

1. After entering the laboratory, place coats, books and other paraphernalia in specified locations – never on bench tops.
2. Keep doors and windows closed during the laboratory session to prevent contamination from air currents.
3. At the beginning and termination of each laboratory session, wipe bench tops with a disinfectant solution provided by the instructor.

4. Do not place contaminated tools such as inoculating loops, needles, and pipettes, on bench tops. Loops and needles should be sterilized by incineration, and pipettes should be disposed of in designated receptacles.
5. On completion of the laboratory session, place all cultures and materials in the disposal area as designated.
6. Wash your hands with liquid detergent and dry them with paper towels upon entering and prior to leaving the laboratory.
7. Wear a paper cap or tie back long hair to minimize its exposure to open flames.
8. Wear a laboratory coat or apron while working in the laboratory to protect clothing from contamination or accidental discoloration by staining solution.
9. Never apply cosmetics
10. Do not smoke, eat or drink in the laboratory.
11. Never pipette by mouth any broth culture or chemical regents
12. Report accidental cuts or burns to the instructor immediately.
13. Disposable gloves must be worn during the manipulation of these test materials.
14. Masks, safety goggles, and laboratory coats should be worn if an aerosol might be formed or splattering of these fluids is likely to occur.

1.2 Basic Requirements for Microbiology Laboratory Instruments

1. Bunsen burner or spirit lamp
2. Laminar flow safety hood
3. Microscopes with oil immersion lesenes
4. Autoclaves
5. Hot air oven

6. Water bath
7. Hot plate
8. Imcubater
9. Refrigerator
10. Centrifuges
11. Digital balance
12. Colony counter
13. PH meter
14. Distillation unit

Glass Ware

15. Petri dishes
16. Beakers
17. Culture tubes
18. Measuring cylinders
19. Burettes
20. Glass rods
21. Pasteur pipettes
22. Micro pipettes

Miscellaneous

23. Culture media
24. Cotton plugs
25. Test tube racks
26. Stains and staining racks
27. Glass marking pencils
28. Disinfectants

29. Inoculation loop
30. Rubber bands
31. Forceps
32. Syringes and needles
33. Discard container
34. Tape for sealing plates

1.3 Introduction to Equipment and Ware Used in Microbiology Laboratory

Microscope

Microorganisms cannot be seen with naked eye. They can be seen only through a microscope. Antonie Von Leeuwenhock is considered to be the first person who has seen a microorganism through a simple microscope made by him. The microscope that he made was magnified the object to 270-480 times. He describe the size, shape and even the movements of bacteria using such simple microscopes. Apart from bacteria he also observed various free living and parasitic protozoa and algae. His findings were later confirmed after the development of compound microscope by Robert Hooke, which had two lenses, objectives and eye pieces to increase the magnification. By the discovery of powerful microscopes, helped in classifying Microorganisms as algae, fungi, protozoa, bacteria etc., later, discovery of oil immersion lens, which enabled the scientists to study the characteristics more minutely. During this time scientists also introduced various staining techniques to observe the details of the microorganisms seen through the microscopes. Thus, microscopes and staining techniques played an important role in the discovery and identification of many microorganisms. Microscopes are continuously improved to enable us to have higher magnifications and better resolutions.

(a) Microscopy : Microscopes are of two categories light (or optical) and electron, depending upon the principle on which magnification is

based. **Light microscopy**, in which magnification used is light waves that, includes **1. bright field, 2. dark field, 3. fluorescence, and 4. Phase- contrast** microscopy.

The **electron microscope** use a beam of electrons in place of light waves to produce the image, two types **transmission** or **scanning electron microscopes.**

1. *Bright Field Microscope :* The commonly used microscope is called the bright field microscope which use bright light source to examine microorganisms. Microorganisms can be seen either in an unstained state or in a stained state. In an unstained state they are examined with high power dry objective lens and can be magnified up to 400 times. When they are stained with certain dyes they can be viewed under an oil immersion lens to get a magnification of about 800 to 1200 times. In the bright field microscope image formed is dark against a brighter background.
2. *Dark Field Microscope :* Many transparent and semi-transparent objects are not readily visible in a bright field. Visibility depends upon contrast between the object and its background and can be improved by using dark ground microscopy. The principle involved in the dark ground microscopy is that the object under examination is illuminated not directly but very obliquely. This is done by opening the aperture of the condenser completely and inserting a funnel stop below the condenser. All rays from condenser are made to pass to out side the objective. The rays, which are either reflected or diffracted by the object in the specimen are seen. The field is dark and the object self-luminous. Most of the dark ground condensers have fixed focus and must be used with thin slides and cover slips. By the dark ground microscope bacterial motility can be examined.
3. *Fluorescencence Microscopy :* Certain dyes when exposed to light rays with shorter wave length like blue light and ultra violet light,

they absorb the energy from the light and emit light rays of longer wave length such as yellow rays or green rays. Such dyes are called fluorochromes and the phenomenon called fluorescence. When bacteria are stained with such dyes are called fluorochromes and the phenomenon called fluorescence and seen under fluorescene microscope. For example, *mycobacterium tuberculosis* can be stained with auromine dye which gives of an yellow fluorescene, so they are easily located in a sputum smear.

Electron Microscope

A modern Electron Microscope is complex and sophisticated instrument. It has a resolving power roughly 1,000 times better than light microscope, the points closer than 5^{o}A can be distinguished and the magnification well over 1,00,000 X. The major source of electron microscope is beam of electron, emitted by an electron gun. Magnetic lenses are used rather than glass lenses; a high vacuum column is need for examination of specimens.

Working of Microscopy

A compound microscope has two sets of lenses, one known as the objective and the other as the eyepiece, mounted in a holder is called body tube. The lens system nearest the object called the objective, magnifies the object a definite number of times. The second lens is called eyepiece magnifies the image formed by the objective further. The image seen by the eye has the magnification equal to the product of the magnification of the systems.

A compound microscope consists of three parts. They are :

1. The Stand
2. The Body
3. The series of optical lenses

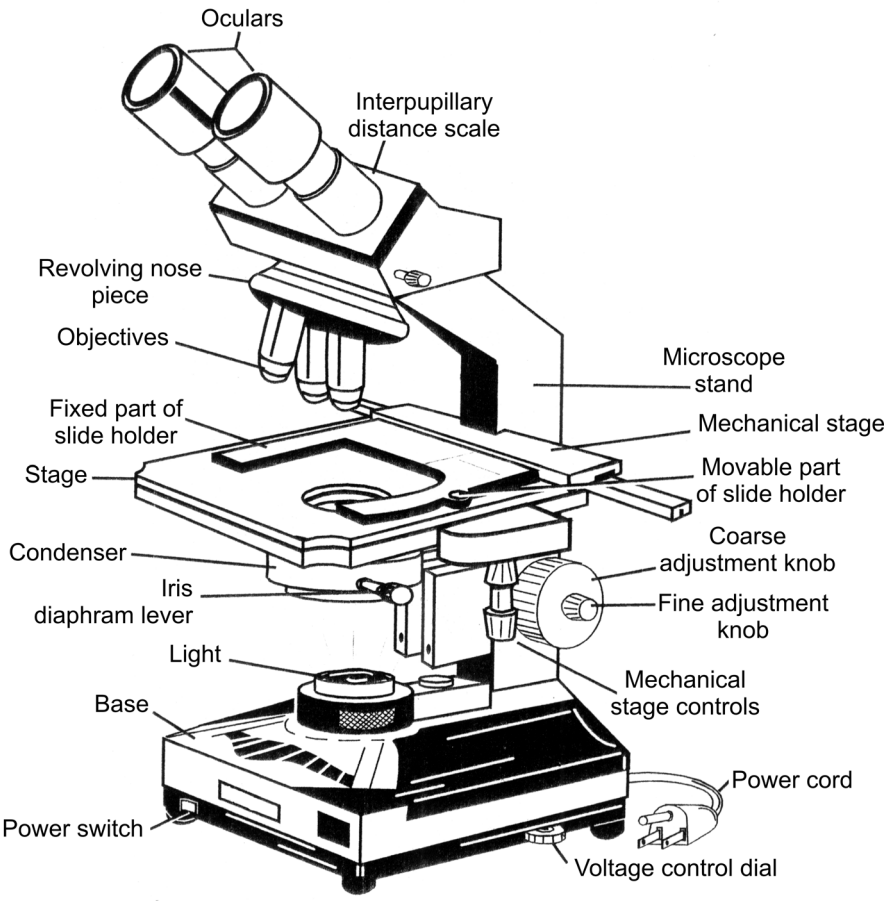

Fig. 1.1 Olympus CHS/CHT binocular microscope (front view).

The stand comprises a heavy foot to give stability and a limb, which bears the optical system. The limb is attached to the foot by hinge joints or fused directly.

The optical system is mounted in the tube which is usually in two parts an external tube bears at its lower and a revolving nose piece, in which inter changeable objective lenses of various magnifications are fitted and the inner draw tube which carries eye piece at its upper end. The coarse and fine adjustments are fixed by adjustments, where by the height of tube can be adjusted to focus the object by the lens systems.

Principle

In the compound microscope, the object is placed between the F and 2F of the objective lens. The objective produces the primary image, the primary image is real, inverted and magnified. The eye piece consists of two lenses; a field lens and an eye lens which is near the eye, and a diaphragm between the two lenses. The field lens of the eye piece brings the real image to focus at the plane of the diaphram. This is with the focal length (f) of the eye lens. The eye lens produces the virtual magnified image that is seen by the eye.

Magnification of the object depends on the eye piece and objective. It is equal to objective magnification and eye piece magnification.

(b) Bunsen Burner : It is a type of gas burner. This burner is commonly used in chemistry, biochemistry and microbiological laboratories. On illumination, the burner provides a very hot blue coloured non-illuminous flame. The temperature of the hottest point reaches to 187 ^{o}C when optimum amount of air is allowed to enter in to the tube at the base.

In microbiology, the Bunsen burner or spirit lamp is used to sterilize the inoculating needle or loop before they are used for transfering the cultures. It is also used for flaming the mouth of cultures tubes, flasks or petri dishes before their opening or closing to prevent the contamination through air and the microbial transfer operations are done in the vicinity of Bunsen burner or spirit lamp flame.

Sterilization of inoculating needles, loops, is done by inceration i.e., by placing the needle or loop vertically on the flame until they become hot red so that any particle attached to them is burnt to ashes. Flame sterilization of needle or loop is the most rapid and reliable method of all heat treatments for sterilization.

(c) Autoclave : Autoclave is an essential instrument in every microbiology laboratory. It is used for sterilization of media, heat stable liquids, heat resistant instruments, glassware and rubber products.

The principle of the autoclave is that when saturated steam comes in contact with a cooler surface, it condenses to water and gives up its latent heat to the surface. Thus, the object becomes sterilized. When water inside a closed vessel boils, then vapours are formed which in turn increases pressure inside a closed vessel. This increased pressure results in the elevation of boiling point of water and produces steam with high temperature. This high temperature of the steam kills the organisms but not the pressure. The boiling point of water at 15 lbs pressure is 121 ^{o}C. Most of the organism are killed at 121 ^{o}C in 15 minutes.

Autoclave consists of a vertical or horizontal cylinder of stain less steel, in a supporting sheet of iron case. The lid is tightened by screw clamps and made air tight by a suitable washer. The upper side of the lid of the autoclave has a discharge tap for air and steam, a pressure gauge (for recording pressure) and a safety valve which can be set to blow off at any desired pressure. The autoclave can be heated through electricity.

Working of Apparatus

Sufficient water is put in the cylinder, the material to be sterilized is placed on the tray and the auto clave is heated.

The lid is screwed tight with the discharge tap open.

The safety valve is adjusted to the required pressure.

The steam-air mixture is allowed to escape freely till all the air has been displayed.

The discharge tap is closed.

The steam pressure rises inside and reaches the desired set level – the safety valve opens and the excess steam escapes.

The holding period is calculated from this point.

When the holding period is over, the heater is turned off and the autoclave is allowed to cool till the pressure gauge indicates that the pressure inside is equal the atmospheric pressure.

The discharge tap is opened slowly and air is let into the autoclave.

Precautions

Water level inside the autoclave should be well above the heating mantle.

The air in the chamber of autoclave must be completely replaced by pure steam. The steam outlet must be kept open for some time so that the air is let out.

The required pressure must be maintained constantly for the required period of time.

Do not open the lid until the pressure gauge show zero.

(d) Hot Air Oven : Hot air oven is a common instrument of microbiological laboratories. It involves the method of sterilization by dry heat. The killing effect of dry heat is due to protein denaturation, oxidative damage and the toxic effect of elevated levels of electrolytes. A holding period of 160 ^{o}C for 1 hour is used to sterilize glassware, forceps, scissors, scalpels, all glass – syringes, swabs, some pharmaceutical products such as liquid paraffin, dusting powder, fats and grease.

Discription of Apparatus

An oven consists of an insulated heat proof cabinet which maintains a desired constant temperature with the help of electric heating mechanism and thermostat. As hot air is a bad conductor of heat and its penetrating power is low, the instrument is therefore provided with fan. The fan ensure even distribution of air at constant temperature and a thermometer is provided for recording the temperature at which the oven is maintained. The shelves inside the oven are perforated to fecilitate the free circulation of air.

Workings of Apparatus

The apparatus should not be overloaded. The material should be arranged so as to allow free circulation of air in between the objects. The oven is heated by electricity. The oven must be allowed to cool slowly for about two hours before the door is opened.

Precautions

Glassware should be perfectly dry before being placed in the oven.

Rubber material will not withstand the temperature.

Test tubes and flasks should be wrapped in paper.

Cotton plugs get charred at high temperature.

The oven must be allowed to cool slowly for about two hours before the door is opened, otherwise there are chances for the glassware to crack.

(e) Water Bath : This is a common instrument of microbiology laboratories. The principle involved in this instrument is boiling. Boiling is not an example of sterilization. It is a means of disinfection. Boiling is not recommended for sterilizing of instruments used for surgical procedures. Hard water should not be used. The lid of the sterilizer should not be opened during the period of boiling.

This method is used during the preparation of media, where the ingredients of the media can be quickly dissolved and then substituted to autoclaving.

(f) Incubator : The working principle of incubator is similar to oven. It works at low thermostat i.e., designed in such a way to maintain low temperature (below 80 ^{o}C).

Incubator plays an important role in maintaining the culture at constant desired temperatures at or above the ambient temperature.

Description

It is similar to oven. It consists of a heating element at the bottom, a Thermostat, temperature probe and devices for regulating the temperature. The shelves are perforated for proper ventilation. The incubator have double doors the inner one made of glass so that the contents of the incubator may be viewed without disturbing

temperature condition of the cabinet. Some incubators are provided with light arrangements to provide light to micro organisms which require light for their growth and sporulation.

Precautions

The uses of dry heat leads to dehydration and show evaporation of culture medium. This can be prevented by keeping a beaker containing sterile water inside the incubator.

(g) Centrifuge : Centrifugation is the most widely used technique for studying cellular and sub cellular structures of a cell. The process is carried out by a centrifuge. It rotates at high speed and thereby separates the cellular contexts. The separation is based on the property of mass and density.

The principle of centrifugation is that, an object moving in a circular motion at an angular velocity w is subjected to an outward force F through a radius of rotation 'r' (in cm) is expressed as $F = w^2 r$.

Sometimes the velocity of a moving particle is expressed in the form of sedimentation coefficient

$$n = S(w^2r)$$

Sedimentation coefficient is a characteristic for a particle. It is a function of the size, shape and density. It is equivalent to the average velocity per unit of acceleration. The unit Sved berg (s) is often used for centrifugation.

Centrifuges can be broadly categorized into three types:-

(a) Low speed — Maximum speed 500 rpm

(b) High speed — Maximum speed 1800 rpm.

(c) Ultracentrifuge — Maximum speed 20,000 rpm to 60,000 (rpm-revolution per minute)

Description of apparatus

A typical centrifuge consist of a head which rapidly revolves by an up right motor. Metal caps or containers are attached to the head for holding tubes or container having material which is to be separated. During centrifugation, the liquid containing the particulate matter is taken in the tubes, run at particular speed and when the centrifugation is complete, the particular matter gets settled at the bottom of the tubes.

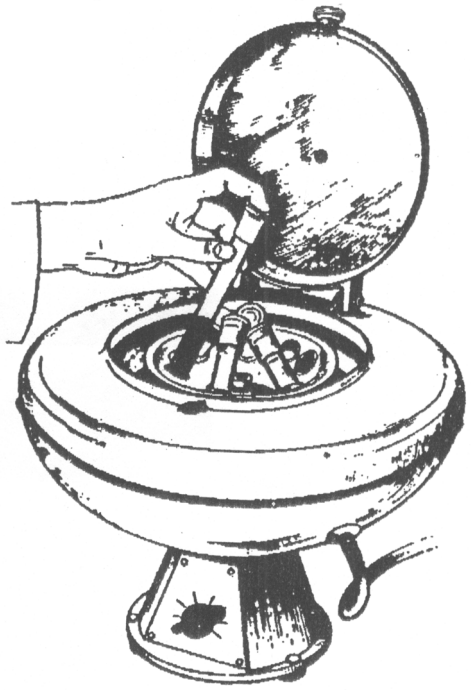

Fig. 1.2 Low speed or clinical centrifuge.

H. pH - Meter

The pH meter is used to determine pH of an unknown solution. It is also used for adjusting the pH of various media used in the cultivation of and testing of biochemical activities of micro-organisms.

The measurement of pH with pH meter is carried out electrometrically and the measurement of pH depends upon the development of membrane potential by a glass electrode. The glass electrode consists of an internal sealed tube with a metallic tip and an external tube that contains a standard solution. A pH sensitive glass bulb forms the immersion tip of the electrode. The potential of glass electrode is proportional to the pH of the solution in which it is immersed. In addition to the glass electrode, a pH meter consists of another electrode, the reference electrode.

The only purpose of this electrode is to complete the measuring circuit with a device that is not sensitive to any of the ions in the solution. The reference electrode consists of a metallic internal element typically of mercury immersed in an electrolyte, usually a saturated solution of potassium chloride. The function of electrolyte is to form a conductive salt bridge between the metallic element and the sample solution in which the two electrodes are placed. To keep stable electrical communication between the internals metallic element and the sample solution, a liquid junction is present at the tip of the outer body of the reference electrode. This junction consists of an extremely small hole through which electrolyte solution streams continuously in the solution to be measured.

The pH meter is also equipped with a temperature compensation circuit for introducing a known potential to balance out the potential caused by different sample temperatures. The instrument is also provided with a standardizing potential which is used to balance the circuit to indicate the correct pH of the standard used as a reference to measure the pH of a sample solution.

Procedure

The procedure for the determination of pH with a simple laboratory pH meter is as follows:-

1. Set the temperature compensation dial to room temperature or temperature of the solution.

2. Immerse the two electrods in standard buffer solution of known pH (say pH 4.0)
3. Using the standardization knob, set the meter reading to the proper pH i.e., 4.0.
4. After these calibration steps, replace the buffer with the sample solution of unknown pH.
5. Read the pH directly from the scale of the pH meter in millivolts in pH units.
6. The pH is expressed in numbers (0 to14). The number is an expression of – log [H]

(i) Laminar Air Flow : All the microbiological operations are to be carried out under aseptic conditions to prevent contamination. Similarly when we handle pathogenic organisms then contaminant precautious should be taken. Laminar air flow cabinets provide aseptic working environment and also prevent spill over of pathogenic organism

Description of apparatus

1. Laminar air flow cabin consists of an air blower in the rear side of the chamber which can produce air flow with uniform velocity among parallel flow lines. There is a special filter (HEPA) which can remove particles as small as 0.3 mm.
2. In front of the blower, there lies a mechanism through which air blow from the blower produce air velocity along parallel flow lines.
3. The laminar flow is based on flow of air current of uniform velocity along parallel flow lines, which help in transferring microbial cultures in aseptic condition. Air is passed through the filters in to the enclosure and the filters do not allow any kind of microbe to enter into the system.

4. The chamber is filled with a fluorescent tube and a UV tube. In the presence of these parallel flow of air current having uniform velocity, all microbiological operation like transfer of cultures, pouring of media plating, perforation etc. are carried out without any contamination.

Working Apparatus

(a) At first the front door of the laminar air flow cabin is opened, the platform of the cabin is cleaned with a disinfectant (preferably alcohol) using a smooth cloth.

(b) The chamber is decontaminated by using UV light, the UV chamber for 30 minutes before using.

(c) The air blower has to be set at desired degree so that the air inside the chamber is expelled.

(d) The UV light is switched off and the fluorescent light is switched on to start work on the platform

(e) The cabinet should be provided with a Bunsen burner or spirit lamp. All operations inside the cabinet should be carried out in the flame zone of the burner.

Precautions

(a) Don't get exposed to UV light, wear black goggle.

(b) Leave the shoes before entering to operate the apparatus.

(c) Wash hands with detergent or soap before starting the work.

(d) After finishing work, clean the platform with surgical spirit. Switch on the UV light and keep for 15 minutes before, finally switch off the cabinet.