CHAPTER

1

Basic Laboratory Practice

Competency achievement

The student should be able to:
- **BC 14.1:** Describe commonly used laboratory apparatus, equipments, good/safe laboratory practice, biomedical hazards and waste management.
- **BC 14.18:** Observe use of commonly used equipments/techniques in biochemistry laboratory.

Conducting experiments in a laboratory is an essential part of learning for most science students, especially for students of medical science. Practical work in a biochemistry laboratory helps the student to observe the behaviour and properties of various biochemically important substances and to develop skills which would be of diagnostic importance.

Before undertaking practical laboratory work, a student must be familiar with the instruments, glassware and chemicals used in the laboratory. The student should also be aware of safe working practices.

COMMON INSTRUMENTS AND GLASSWARE IN BIOCHEMISTRY

A number of instruments are used in a biochemistry laboratory out of which some common ones are centrifuge, oven, incubator, burner/spirit lamp, etc. In addition to these, there are some specialized instruments which will be described in later chapters. The common glassware includes test tubes, pipettes, burettes, reagent bottles, flasks, beakers, etc.

Centrifuge

A centrifuge is used for centrifugation. Centrifugation is a separation technique by which particles of different shapes, sizes and density are separated from each other on the basis of their sedimentation rate. The separation is done by spinning the material under the influence of centrifugal force. Centrifuge is the instrument used to hold the material and generate the centrifugal force (Fig. 1.1A). The centrifuge has a powerful motor that creates the spin. A rotor is attached to this motor. The rotor has got the containers (test tube holders) in which the tubes containing the material to be centrifuged are placed. The tubes used in the centrifuge are generally 12 × 100 mm in size and are known as centrifuge tubes. The rotor spins the tubes along its axis at different speeds to generate centrifugal force. There are different types of centrifuges, e.g. benchtop centrifuge, floor-standing centrifuge, refrigerated centrifuge, ultracentrifuge, etc.

A common use of a centrifuge machine in a biochemistry laboratory is to separate plasma/serum from blood cells. To use a centrifuge machine: (i) put the tube containing the sample in one of the holders, (ii) put another tube filled with sample/water in the opposite holder for maintaining balance, (iii) close the lid and select the desired speed, (iv) start the centrifuge and wait for completion of the cycle, (vi) when the centrifuge stops spinning, open the lid and take out the tubes.

Oven

Ovens (or hot air ovens) are used in biochemistry laboratories to provide uniform temperature for heating, drying, and sterilizing glassware (Fig. 1.1B). The oven consists of a double-walled aluminium or stainless-steel cabinet filled with glass wool, a rectangular chamber of aluminum or stainless steel inside the cabinet in which aluminium shelves can be put at different heights, a heating element to generate heat, a thermostat to regulate temperature, a fan to circulate air inside the chamber and a door on one side to close the chamber.

The temperature inside the oven can range from ambient to above 300°C. The glassware (or other material) to be dried or sterilized is put inside the oven. The door is closed. The desired temperature is selected, and the instrument is switched on. When done, the oven should be switched off, and the material inside should be removed after the interior has cooled down.

Incubator

An incubator or laboratory incubator is an insulated cabinet used to maintain a precise temperature in the enclosed chamber (Fig. 1.1C). The construction of an incubator is similar to that of an oven, but the temperature inside is lower and more precise. The temperature is maintained by water or air jackets. Experiments involving the use of enzymes are generally conducted inside an incubator at a constant temperature because the enzymes work best at their optimum temperature. Assay of human enzymes is usually done in incubators at a temperature of 37°C.

Burner

Bunsen burners (sometimes spirit lamps) are commonly used as a heat source in laboratory experiments. A Bunsen burner is a type of gas burner which consists of a flat base with a straight tube extending vertically, known as the barrel or chimney. Natural gas or liquefied petroleum gas is supplied at the bottom of the chimney. Bunsen burners are normally equipped with a hose barb at the base of the chimney to allow rubber tubing to supply the gas from a gas nozzle on the laboratory bench.

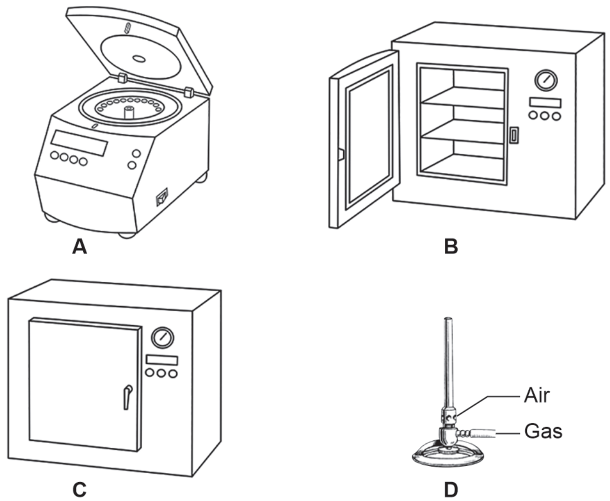

Fig. 1.1A–D: (A) Centrifuge; (B) Oven; (C) Incubator; (D) Burner

There is an air hole near the bottom just above the gas inlet, which allows pre-mixing of air and gas before combustion occurs at the top of the chimney. A collar around the base of the chimney, with a hole, acts as an air regulator, allowing the air in the mixture to be adjusted (Fig. 1.1D).

Before lighting the burner, check connections to burner and bench outlet valve. Close needle valve and collar. Open the bench outlet valve fully. Open the needle valve half turn. Use a spark lighter to light the flame. Adjust the collar and needle valve till a blue flame is seen.

Test Tubes

Test tubes are the most common glassware used in a biochemistry laboratory. Test tubes used for general biochemical work are usually made of glass for their relative resistance to heat. These are available in a variety of lengths and diameters. The top usually has a flared lip to help in pouring out the contents (Fig. 1.2A). Most biochemical experiments are done in test tubes. Most of the tests are done in test tubes of 18 × 150 mm. Centrifugation is usually done in test tubes of 12 × 100 mm. Test tubes made of synthetic materials are used for specialized work.

Burettes

Burettes are also used to dispense measured quantity of liquids but are less accurate than pipettes. They are used mainly for titration. Use of burettes has decreased now. A burette can be made of glass or plastic, and is a straight tube with a graduation scale. At the tip of burette, there is a stopcock and a valve to control the flow of the solution (Fig. 1.2B). The barrel of the stopcock can be made of glass or plastic. Stopcocks with glass barrels need to be lubricated with vaseline or a specialized grease. The solution to be dispensed is filled into the burette from the top end. The burette is fixed to its stand with a clamp.

The burette must be observed with the eye level at the bottom of the meniscus. The solution is dispensed by opening the valve at the bottom. The liquid levels before and after dispensing are noted. The difference between the two equals the volume dispensed.

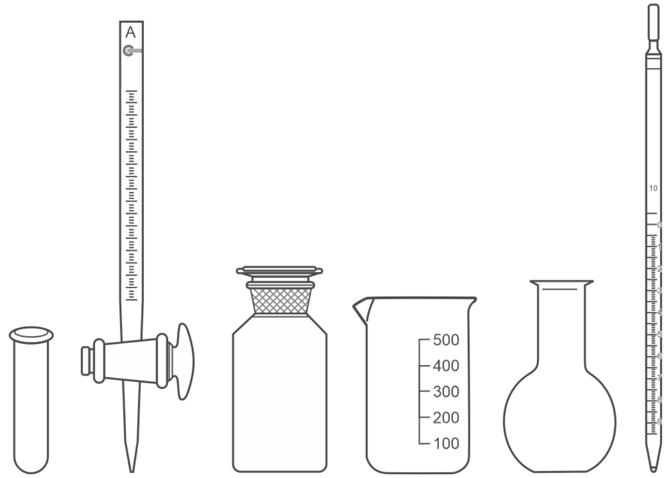

Fig. 1.2A–F: (A) Test tube; (B) Burette; (C) Reagent bottle; (D) Beaker; (E) Flask; (F) Pipette

Reagent Bottles

Reagent bottles are containers made of glass or plastic. They are used to store chemicals in liquid or powder form. They are stored in cabinets or on shelves. Most of the reagent bottles are made of colourless material, but some are tinted amber or brown to protect light-sensitive chemicals from visible light. Reagent bottles are topped by special caps or stoppers (Fig. 1.2C). A label is put on the bottle to show the identity of the chemical. For accurate dispensing, the solution present in the bottle is aspirated into a pipette. For approximate dispensing, the solution can be poured into a test tube, flask or beaker.

Flasks

A flask is a vessel having a wider body below and a narrower tubular section above called neck which has an opening at the top (Fig. 1.2D). The flasks are of several types, e.g. flat-bottom flask, round-bottom flask, Erlenmeyer flask, etc. Among flasks, Erlenmeyer flasks, also known as conical flasks, are used very commonly. An Erlenmeyer flask has a flat bottom to give it stability. It has a conical body with sloping sides and a cylindrical neck. The conical shape allows swirling of liquids for mixing without the risk of the liquid spilling out. Many Erlenmeyer flasks have graduations on their side, but these are not precise. Erlenmeyer flasks are used for a wide range of applications, e.g. mixing solutions, heating liquids, titration, etc.

A volumetric flask is calibrated to contain a precise volume of liquid. It has a bulbous body below and a long, narrow neck above. There is a single calibration mark on the neck. Volumetric flasks are used to prepare solutions of known concentrations with high accuracy. To prepare a solution of a desired and accurate concentration, weigh the solute accurately. Transfer the weighed solute into the volumetric flask. Add a portion of the solvent to the flask, stopper the flask and swirl it gently to dissolve the solute. When the solute is fully dissolved, add more solvent to the flask carefully until the meniscus of the liquid is at the level of the calibration mark. Stopper the flask again and invert it several times to ensure homogeneity of the solution.

Beakers

A beaker is usually a cylindrical vessel with a flat bottom. Most of the beakers have a small spout on one side at the top to enable pouring out the solution (Fig. 1.2E). Beakers come in a wide range of sizes. A beaker differs from a flask in having straight rather than sloping sides. Beakers are commonly made of glass, but some are made of plastic nowadays. Some beakers may have graduation scales, but the graduations are not precise. Beakers are used for preparing solutions and for decanting supernatant liquids.

Pipettes

Pipettes are used very commonly in biochemistry laboratories for quantitative work. A pipette is a long tube, open at both ends, used to transfer/dispense a measured volume of a liquid. Accuracy of quantitative experiments depends to a large extent on accurate dispensing of liquids by means of pipettes. Pipettes can be manual or automatic. Manual pipettes (Fig. 1.2F) are now being replaced by automatic pipettes (autopipettes), but are still used, especially in undergraduate laboratories.

Manual pipettes are generally of two types—volumetric pipettes and graduated pipettes. Volumetric pipettes are designed to dispense a fixed volume of liquid. They are also known as transfer pipettes. They are more precise than graduated pipettes.

Graduated pipettes are less accurate than volumetric pipettes and can be divided into Mohr pipettes and serological pipettes. Mohr pipettes have the last graduation mark at their lower end just above the tip. While dispensing from Mohr pipettes, any liquid remaining in the tip is not to be blown out.

Serological pipettes do not have the last graduation mark at the lower end. While dispensing from serological pipettes, the last drop of liquid in the tip must be blown out so as to deliver the full volume (Fig. 1.3).

For dispensing by manual pipettes, the tip of the pipette should be dipped in the liquid well below the surface but not touching the bottom of the container. The liquid should be aspirated into the pipette by sucking from the upper end until the liquid is slightly above the desired mark. The pipette should be held vertically with the calibration mark at eye level. The liquid should be allowed to flow out slowly until the meniscus of the liquid is at the desired calibration mark. Any excess liquid on the outer side of the pipette should be wiped off. To dispense, the tip of the pipette should be placed inside the receiving vessel, touching against the inner wall of the vessel at an angle. The liquid should be allowed to flow out freely by gravity. If it is a Mohr-type pipette, the liquid below the last graduation mark

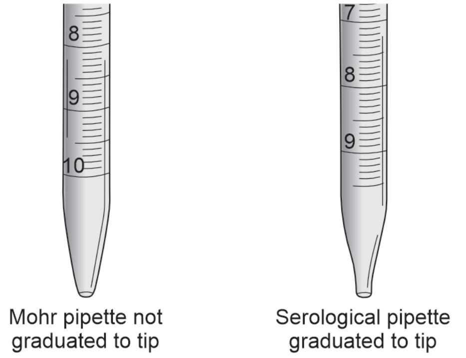

Fig. 1.3: Mohr pipette and serological pipette

should not be blown out. If it is a serological pipette, the last drop should be blown out.

Autopipettes have largely replaced manual pipettes, especially in diagnostic and research laboratories. Autopipettes can be broadly divided into: (i) air displacement pipettes and (ii) positive displacement pipettes (Fig. 1.4). The latter are more accurate. The autopipettes can be single volume, multiple volume or continuously variable volume pipettes. Single-volume autopipettes are designed to deliver a single, fixed volume of liquid. Multiple volume autopipettes can dispense two or three different volumes by adjusting the volume adjustment knob. Continuously variable volume pipettes can be used to deliver any particular volume in the given range.

To dispense a liquid using an autopipette: (i) set the volume on the pipette, (ii) depress the plunger, (iii) immerse the tip to the correct depth in the liquid and let the plunger go to its resting position, (iv) wait a few seconds for the liquid to flow into the tip, (v) put the pipette, held at 30–45 degrees, against the wall of the receiving chamber, and smoothly depress the plunger to the first stop, (vi) wait a second and then depress the plunger to the second stop and (vii) move the tip up the vessel wall to remove the pipette.

Air displacement autopipettes

Positive displacement autopipettes

Fig. 1.4: Autopipettes

SAFE PRACTICES IN THE LABORATORY

All laboratory work requires safety measures to safeguard the health of persons working in the laboratory, and the biochemistry laboratory is no exception. A worker in a biochemistry laboratory is exposed to certain health risks. These risks arise due to mechanical, chemical and microbial hazards.

Certain hazards, viz., mechanical and electrical, are common to most laboratories. Majority of the instruments used in biochemistry laboratories are electrically operated. Operation of such instruments requires the usual safety measures.

Chemical and biological hazards need specific safety precautions in biochemistry laboratories. The disposal of waste material should also be safe. In addition, such laboratories should be equipped with first aid material and should have personnel trained to use first aid measures.

Chemical Hazards

A major hazard in a biochemistry laboratory is the risk posed by the dangerous properties of hazardous chemicals. When dangerous chemicals are not handled in a safe manner, they can cause a number of health problems. These problems can vary in severity from mild to life-threatening. Many chemicals used in the laboratory, e.g. concentrated acids and alkalis, are corrosive substances, which pose a serious risk to health on contact in the form of chemical burns. Immediate washing with water is the primary safety measure. Even if there is no exposure to dangerous chemicals, hands should be washed thoroughly with soap and water after working with chemicals. Bunsen burners and other heating devices are routinely used in the laboratory. Just like with any activity involving fire, accidents can occur and can result in heat burns. One must keep a safe distance from any open flames or heating devices to minimize the risk of thermal injury. Keep skin, clothing and any other flammable material away from flames and sources of heat. It is also imperative not to leave a Bunsen burner on or unattended after use. If a heat burn occurs while working in the laboratory, put the affected area immediately under cold running water and hold it there until the burning sensation goes away.

Glassware can break if handled roughly or improperly. Broken glassware can have sharp edges, which can cause cuts. The best preventive measure to avoid cuts from broken glassware is to handle it with care. Glassware should be held firmly and never with wet or slippery hands. When not being used, glassware should be kept in a secure location where there is no risk of it falling and shattering. In case a cut occurs from broken glassware, the first aid personnel should be contacted immediately.

Biological Hazards

Persons working in a biochemistry laboratory come in contact with biological material of patients, e.g. blood, urine, cerebrospinal fluid, etc. in which pathogenic microorganisms may be present. The pathogens can enter the body of laboratory personnel through cuts in skin or breach in mucosa. Needle stick injuries can also be a source of infection. Gloves should be used when working with potentially infected material. Disinfection should be done regularly where necessary. If exposure to pathogens is suspected, medical help should be sought without delay.

First Aid

Even after taking all safety measures, accidents cannot be avoided altogether. Therefore, first aid measures must be available in the laboratory. The laboratory should have a first aid kit with common emergency aid items, e.g. sterile gauze pads, cotton wool, adhesive tape, bandages of different width, crepe bandages, non-adhesive pads, scissors, tweezers, anaesthetic spray or lotion, disinfectant, e.g. betadine, antibiotic cream, gloves, etc.

In case of an accidental chemical spill/splash on the body, the affected area should be washed with plenty of cold water. In case a corrosive liquid has accidentally entered the mouth, mouth should be rinsed with plenty of water. In case of bleeding from a cut, pressure should be applied at the site of injury until the bleeding stops. If the injury seems to be serious, medical help should be sought.

Disposal of Biomedical Waste

A biochemistry laboratory usually generates chemical waste and infectious (biohazardous) waste. Laboratory waste from analyzers, calibrators, cleaners, reagents, and test kits must be evaluated to determine whether it is biomedical waste. Biomedical waste (BMW) includes any waste generated during diagnosis, treatment, immunization or research involving human beings or animals. Unsafe disposal of such waste can pose a serious risk to health in the form of spread of infectious diseases, injuries from sharps, and contamination of the environment. Proper biomedical waste management (BMWM) is imperative for the protection of health and the environment.

Government Regulations

The management and disposal of biomedical waste are governed in India by the Biomedical Waste Management Rules, 2016. Key features of these rules are:

1. Categorization of biomedical waste into four simplified groups.
2. Mandatory pre-treatment of laboratory and microbiological waste.
3. Barcoding and tracking of waste from source to disposal.
4. Responsibilities assigned to healthcare facilities (HCFs) and common biomedical waste treatment facilities (CBWTFs).

HCFs must follow the rules with respect to segregation, collection, and disposal protocols for BMW. CBWTFs play a vital role by servicing multiple healthcare units.

Segregation and Collection

The first and most critical step in BMWM is segregation of BMW at the point of generation. Biomedical waste must be segregated into colour-coded bins (Fig. 1.5):

- Yellow—Human/animal anatomical waste, soiled waste, expired medicines.
- Red—Contaminated waste (recyclable) like tubing, bottles.
- White—Sharps like needles and scalpels in puncture-proof containers.
- Blue—Broken glassware and metallic body implants.

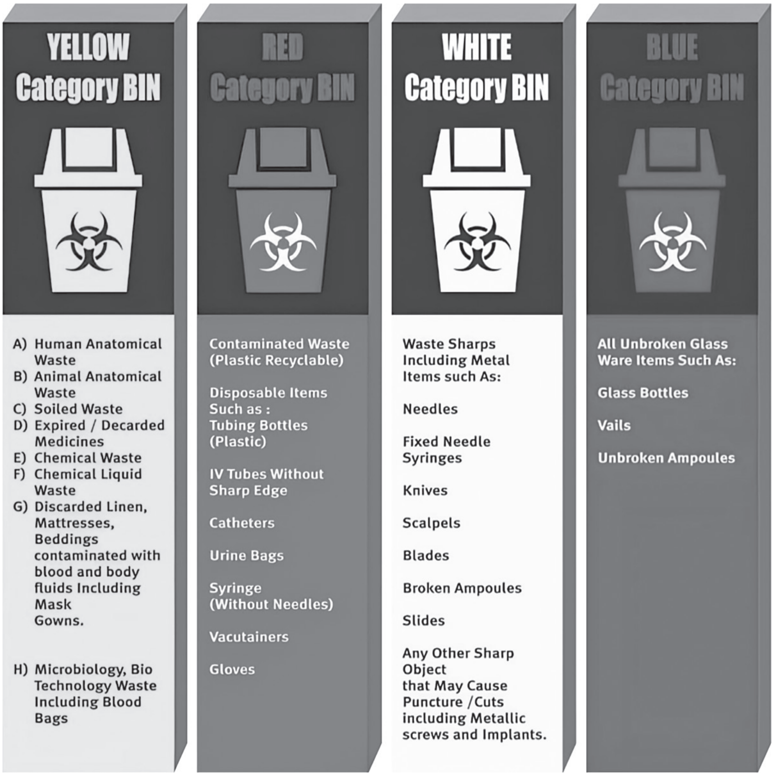

Fig. 1.5: Colour codes for segregation of biomedical waste
(For Colour Figure see also Colour Plate 1)

All healthcare workers must be familiar with the rules for segregation and disposal of biomedical waste. They are required to segregate waste promptly and properly at their place of work. Large healthcare facilities must have their own arrangements for the disposal of BMW. Small healthcare facilities can use common biomedical waste treatment facilities.

Treatment and Disposal

Different methods have to be used to treat and dispose of different types of biomedical waste:

1. Incineration: Yellow-category waste should be burned in incinerators at high temperatures so as to destroy the

pathogens. However, incinerators may emit harmful pollutants into the atmosphere if not operated properly.

2. Autoclaving: Steam sterilization should be done for red and white category waste. It destroys the pathogens in an eco-friendly way.
3. Microwaving: This is a cost-effective alternative to incineration. It is a non-burning technology for waste disinfection.
4. Deep burial: Deep burial of BMW is permitted in rural or remote areas where there are no CBWTFs.