

Basic Laboratory Practice

Competency achievement: The student should be able to:

BI 11.1: Describe commonly used laboratory apparatus and equipment, good safe laboratory practice and waste disposal

BI 11.16: Observe use of commonly used equipment/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 are 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

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

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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 got a rotor. The rotor holds the material in tubes, and spins the tubes along its axis at different speeds to generate centrifugal force. There are different types of centrifuges. Their common use in biochemistry laboratory is to separate plasma/serum from blood cells.

Oven

Laboratory ovens are used in biochemistry laboratories to provide uniform temperature for heating, drying and sterilizing glassware (Fig. 1.1B). The temperature inside the oven can range from ambient to above 300°C. The glassware 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 apparatus used to maintain a precise temperature inside the apparatus (Fig. 1.1C). The temperature is maintained by water or air jackets. The operation of incubator is similar to that of an oven but the temperatures are lower and more precise. Experiments involving the use of enzymes are generally conducted inside an incubator to maintain a constant temperature throughout the experiment.

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 liquified 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

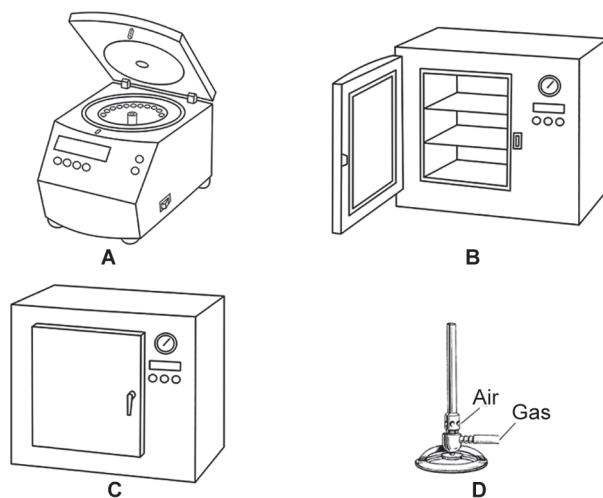


Fig. 1.1A to D: A. Centrifuge; B. Oven; C. Incubator; D. Burner

laboratory bench. 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 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 generally made of glass for its 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. Test tubes made of synthetic materials are used for specialized work.

Pipettes

Pipettes are used very commonly in biochemistry laboratories for quantitative work. A pipette is used to transfer/dispense a measured volume of liquid. Accuracy of quantitative experiments depends to a large extent on accurate dispensing of liquids by means of pipettes. Manual pipettes are now going out of use but are not fully abandoned (Fig. 1.2B). The stem of the pipette has one or more graduation marks. The tip of the pipette is dipped in the liquid to be dispensed but should not touch the bottom of the container. The liquid is aspirated into the pipette up to the desired mark by sucking from the upper end. It is dispensed by allowing it to flow out of the pipette.

Autopipettes have to a large extent replaced the manual pipettes. Autopipettes can be broadly divided into: (i) air displacement pipettes and (ii) positive displacement pipettes. The latter are more accurate. The autopipettes can be single volume, multiple volume or continuously variable volume pipettes. To transfer a liquid using an autopipette: (i) set the volume on the pipette, (ii) depress the plunger, (iii) immerse the tip to the correct depth and smoothly 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°, 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.

Burettes

Burettes are also used to dispense measured quantity of liquids but are less accurate than pipettes. 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 are a stopcock and valve to control the flow of the solution (Fig. 1.2C). 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

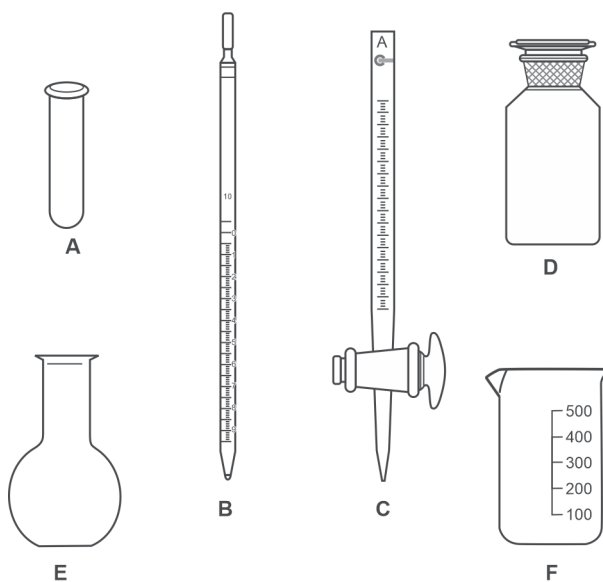


Fig. 1.2A to F: A. Test tube; B. Pipette; C. Burette; D. Reagent bottle; E. Flask; F. Beaker

the top end. The burette is fixed to its stand with a clamp. The burette must be observed at eye level straight to the bottom of the meniscus. The solution is dispensed by opening the valve at the bottom. The levels before and after dispensing are noted. The difference between the two equals the volume dispensed.

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. 1.2D). 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 and a narrower tubular section at the top called neck which has an opening at the top (Fig. 1.2E). They are of several types, e.g. flat-bottom flask, round-bottom flask, conical flask, etc. Some of them have graduation scales. Flasks are used as vessels for making solutions or for holding, containing or collecting solutions for chemical reactions or for other purposes, e.g. mixing, heating, cooling, etc. Volumetric flasks are a type of flasks that have a single graduation mark on their neck. They are used to make solutions having an accurate volume.

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. 1.2F). 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.

SAFE PRACTICES IN THE LABORATORY

All laboratory work requires safety measures to safeguard the health of persons working in the laboratory, and 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.

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.

Waste Disposal

A biochemistry laboratory usually generates chemical waste and infectious (biohazard) waste. Laboratory waste from analyzers, calibrators, cleaners, reagents, and test kits must be evaluated to determine whether they are hazardous. Due to the large number of chemicals used in a laboratory, one will need an expert to evaluate and advise about waste disposal in accordance with the regulations. Biohazard infectious waste needs primary treatment by autoclaving and/or incineration at a licensed disposal facility. Bags of appropriate colors should be placed in bins for collections of different types of waste.

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 bandage, non-adhesive pads, scissors, tweezers, anaesthetic spray or lotion, disinfectant, e.g. betadine, antibiotic cream, gloves, etc.

In case of 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.