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Simplified Practical Manual of Biochemistry

THIRD EDITION



Javin Bishnu Gogoi

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CHAPTER 2

Introduction of Basic Instruments Used in a Biochemistry Laboratory

Chapter Outline

- Laboratory test tubes
- Test tube holder
- Test tube stand or rack
- Bunsen burner
- Spirit lamp or alcohol burner
- Beaker
- Volumetric flasks or measuring flask or graduated flask
- Conical flask or titration flask or erlenmeyer flask
- Funnel
- Reagent bottles or media bottles or graduated bottles
- Measuring cylinder or graduated cylinder or mixing cylinder
- Pipettes
- Pipette controllers or fillers
- Urinometer
- Balances
- Centrifuge
- Laboratory water bath
- Magnetic stirrer or magnetic mixer
- Vortex mixer or vortexer
- Glass stirring rod or glass rod or stirring rod or stir rod
- Hot air ovens

LABORATORY TEST TUBES

The various types of laboratory tubes are used for storing, mixing, heating and cooling chemicals, each with specialized designs. The types of laboratory tubes include:

Boiling tube (Fig. 2.1): A boiling test tube is a thick cylindrical alternative test tube primarily used for boiling chemical solutions.

Test tubes (Figs. 2.2A to D): Test tubes are extremely common thin-walled cylindrical tubes that can be used for holding chemical solutions. These are also known as culture tubes or sample tubes. They may be made up of glass or plastic. It varies in sizes from 5 mL to 30 mL capacities. The bottom of a test tube may be round, flat or conical. The test tube may be graduated or non-graduated type with a stopper or without a stopper.

Test tubes used as containers for liquid chemical solutions, mixing, heating or cooling, producing cultures, and other applications.

Uses

- Universally used to hold, mix or heat solid or liquid chemicals, especially for qualitative experiments and assays.
- Used to collect and store samples of blood or other body fluids or biological samples.
- Used to separate the serum.
- A test tube with a stopper is often used for temporary storage of chemical or biological samples.

Screw-cap flat bottom test tube: These multipurpose screw cap test tubes are used for storage or transport of samples.

Test tube conical with graduation: It is used to centrifuge the samples.



Fig. 2.1: Boiling tube.

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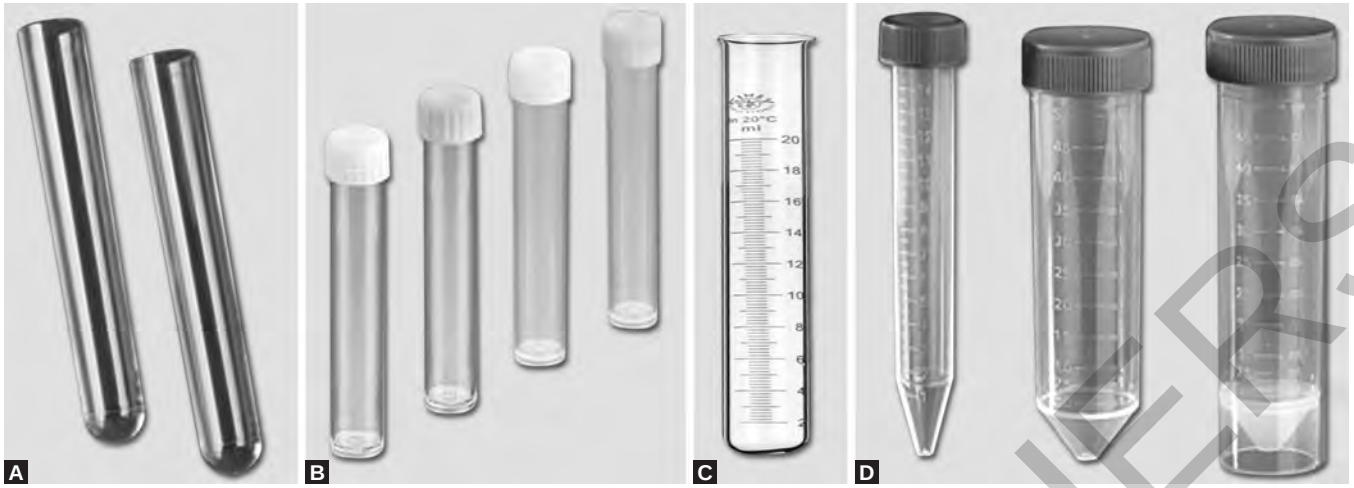
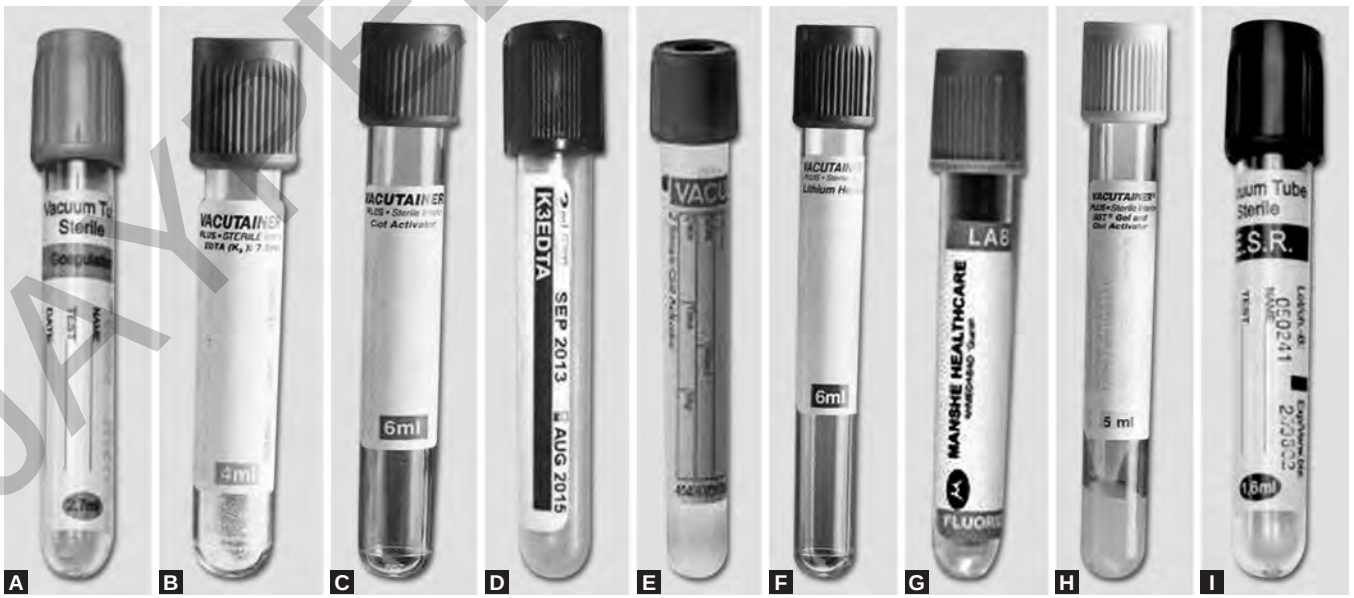


Figs. 2.2A to D: (A) Glass test tube, round bottom; (B) Screw-cap flat bottom test tube; (C) Graduated test tube with round bottom; (D) Test tube conical with graduation *(For color version, see Plate 1)*.

Different types of clinical test tubes (Figs. 2.3A to I):

- *Blue-top/cap test tube-sodium citrate (NaCitrate) tube:* This tube is primarily used for coagulation studies (PT and PTT). Complete filling of this tube is essential to obtain accurate results.
- *Lavender-top/cap test tube–EDTA tube:* EDTA is the anticoagulant used for most hematology procedures. Its primary use is for the CBC and individual components of the CBC. The larger (6 mL) tube is used for blood bank procedures.
- *Red-top/cap test tube:* This tube has no anticoagulant and is used for many chemistry tests, drug levels, and blood bank procedures.
- *Navy blue-top/cap test tube:* There are two general types—one with K2 EDTA and one with no anticoagulant. This tube is used primarily for trace metal analysis.
- Serum separator test tube (SST): This tube contains a clot activator and serum gel separator. It has no anticoagulant and is used for many chemistry tests. It cannot be used for certain drug levels or any blood bank procedures.
- Green-top/cap test tube: Sodium heparin or lithium heparin
- Gray-top/cap test tube: Potassium oxalate. This tube is used primarily for blood glucose testing.
- Yellow-top/cap test tube: ACD (Sol. A or Sol. B)
- Black-top/cap test tube: Contains 3.8% buffered sodium citrate (4NC) for Westergren erythrocyte sedimentation rate determination.



Figs. 2.3A to I: (A) Blue-top/cap test tube; (B) Lavender-top/cap test tube; (C) Red-top/cap test tube; (D) Navy blue-top/cap test tube, (E) Serum separator test tube; (F) Green-top/cap test tube, (G) Gray-top/cap test tube; (H) Yellow-top/cap test tube; (I) Black top/cap test tube *(For color version, see Plate 1)*.

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Centrifuge tube or centrifuge tips (Fig. 2.4): Centrifuge tubes are used in laboratory centrifuges to separate solids out of liquid chemical solutions.

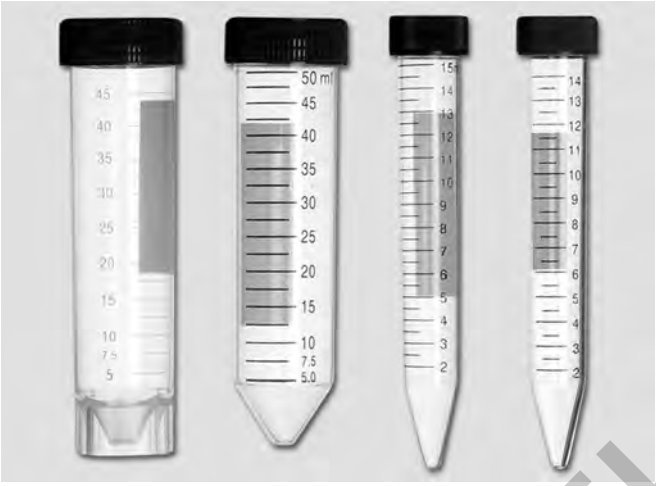
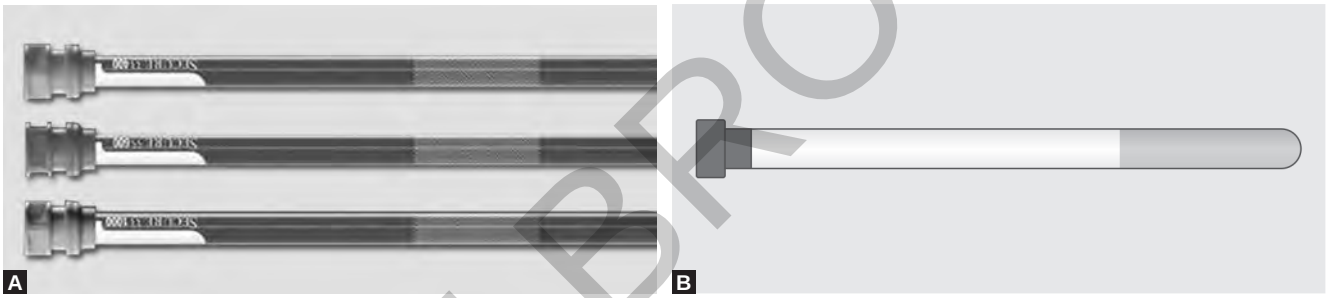


Fig. 2.4: Centrifuge tube.

NMR tubes (Figs. 2.5A to B): NMR tubes are used exclusively in nuclear magnetic resonance (NMR) spectroscopy, a type of imaging that can be performed on solid-state molecules.



Figs. 2.5A and B: NMR tubes *(For color version, see Plate 1—Fig. A)*.

Thiele tubes (Fig. 2.6): Thiele tubes are hand-blown tubes shaped in a triangular fashion, resembling a test tube that then loops back upon itself. They are typically used to determine the boiling points or melting points of different chemicals.



Fig. 2.6: Thiele tubes *(For color version, see Plate 2)*.

Thistle tubes (Fig. 2.7): Thistle tubes are tubes designed to allow chemists to add material through a semi-permeable membrane or especially designed stopper. They have long stems that end in a reservoir bulb with a flared rim. Thistle tubes are specifically designed to fit in complementary stoppers on Erlenmeyer and other flasks, so that new materials can be introduced to other compositions.

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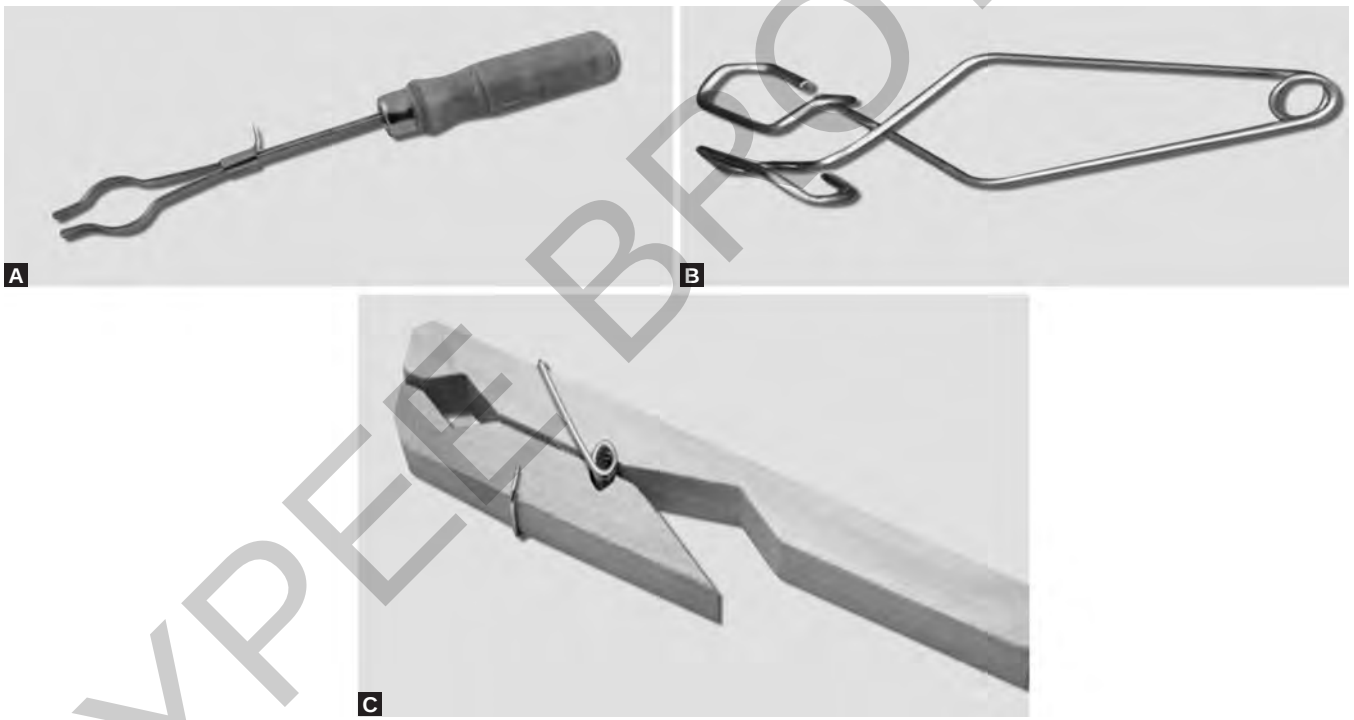
Fig. 2.7: Thistle tubes.

TEST TUBE HOLDER (FIGS. 2.8A TO C)

Uses

It is used to hold or support test tubes containing chemicals to perform tests.



Figs. 2.8A to C: (A) Common test tube holder; (B) Stoddard test tube holder; (C) Wooden test tube holder.

TEST TUBE STAND OR RACK (FIGS. 2.9 TO 2.12)

Uses

It is used to keep multiple test tubes in an upright position to keep chemicals or samples from spilling.

Main purpose served by these stands is safety, in situations where the tests calls for acids or other hazardous reagents; for safe storage of test tubes, to ease the transport of multiple tubes and for efficient organization of test tubes.

Types

Test tube racks come in a variety of size, composition, material, and color. The racks are made of metal wires, stainless steel, plastic, polystyrene, foam, fiberglass, and polypropylene.

Test tube racks may be a classic rack, an interlocking cube form, a stack-able form, test tube drying rack, slant rack, 1-well rack, vial racks, 96 hole test rack for ELISA, etc.

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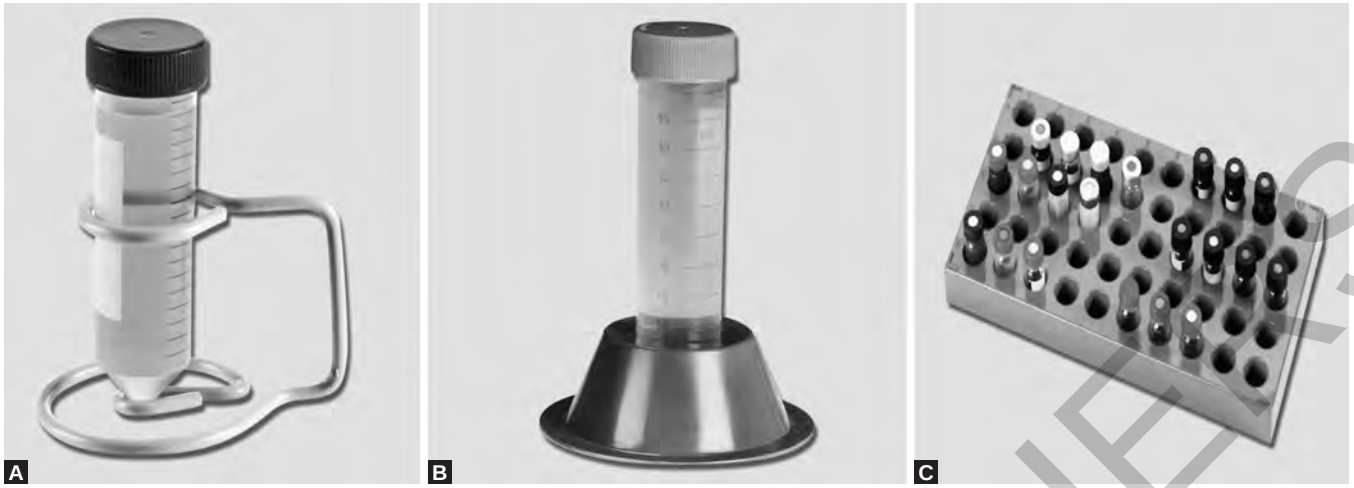


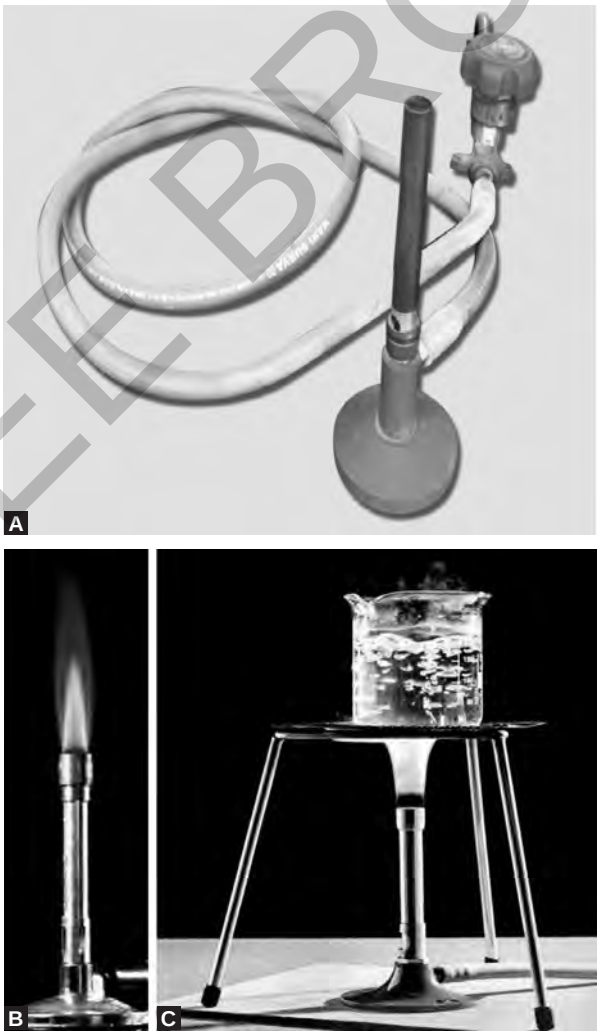
Figs. 2.12A to C: (A) and (B) 1-Well rack; (C) Vial rack *(For color version, see Plate 3)*.

BUNSEN BURNER (FIGS. 2.13A TO C)

Named after Robert Bunsen.

Uses

It produces a single open gas flame, which is used for heating, sterilization and combustion **(Figs. 2.14 and 2.15)**.



Figs. 2.13A to C: (A) Bunsen burner; (B) Flame; (C) Use of Bunsen burner for boiling/heating water/solution in a beaker *(For color version, see Plate 3)*.

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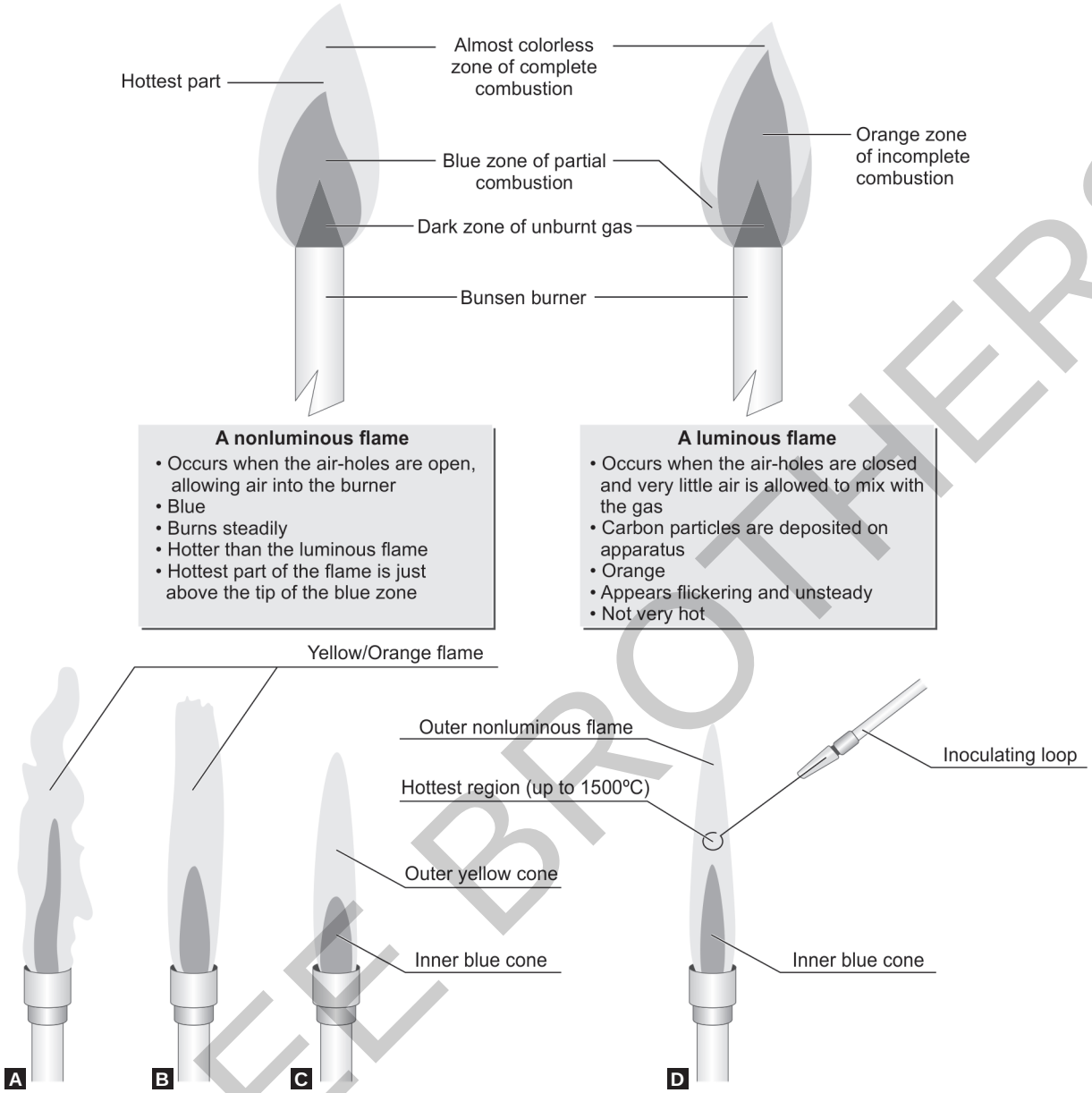
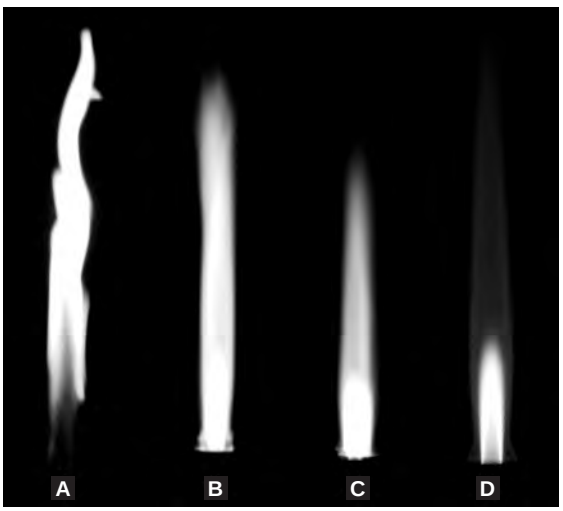


Figs. 2.14A to D: Different types of flame produced by a Bunsen burner.



Figs. 2.15A to D: Different types of Bunsen burner flames depend on air flow in the throat holes: (A) Air hole closed (safety flame used for lighting or default); (B) Air hole slightly open; (C) Air hole half-open; (D) Air hole fully open (roaring blue flame) *(For color version, see Plate 3)*.

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SPIRIT LAMP OR ALCOHOL BURNER (FIG. 2.16)

A spirit lamp is like an ordinary lamp, but it uses ethanol, i.e., ethyl alcohol, methanol or isopropanol as a fuel. It is used to produce an open flame in the laboratory. It can be made from brass, glass, stainless steel or aluminum.

Uses

It is used for heating, sterilization and combustion in laboratories.

Their flame is limited to approximately 5 centimeters in height, with a comparatively lower temperature than the gas flame of the Bunsen burner. A cap is used as a snuffer for extinguishing the flame.



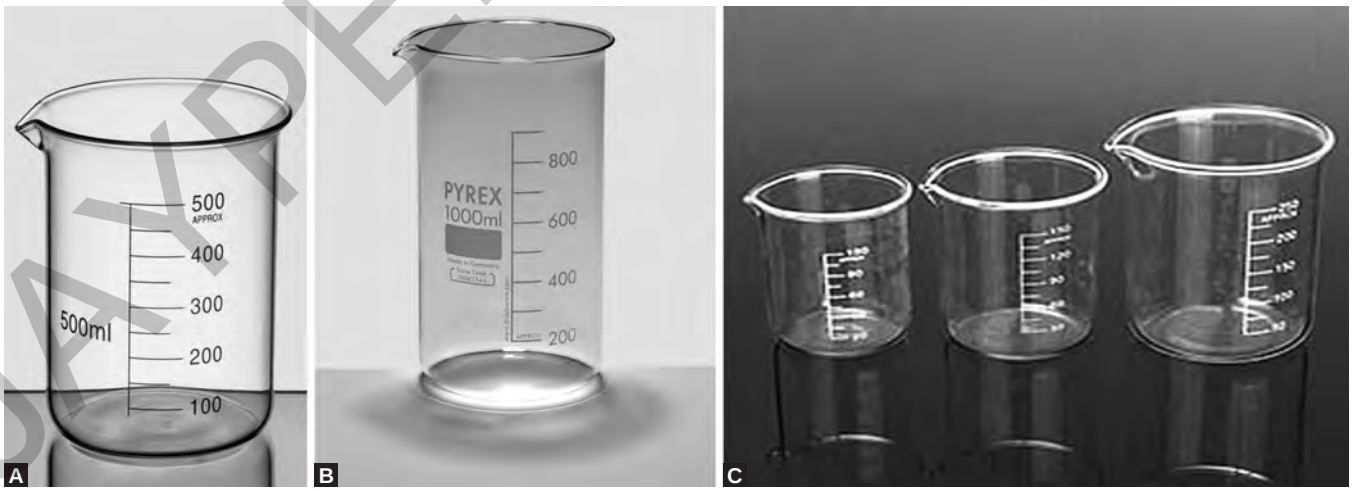
Fig. 2.16: Spirit lamp *(For color version, see Plate 3)*.

BEAKER (FIGS. 2.17A TO C)

A beaker is a simple container for stirring, mixing and heating liquids commonly used in laboratories.

Beakers are generally cylindrical in shape, with a flat bottom. Most also have a small spout (or "beak") to aid pouring. Beakers are available in a wide range of sizes, from one milliliter up to several liters. Beakers are often graduated. Beakers are commonly made of borosilicate glass, but can also be in other non-corrosive materials (such as stainless steel or aluminum) or certain plastics (e.g., polythene, polypropylene, etc.). Beakers may be:

- Standard or "low-form" beakers (Griffin beaker)
- "Tall-form" beakers (Berzelius beakers) and
- Flat beakers (often called as crystallizers)



Figs. 2.17A to C: (A) Standard or "low-form" beakers or griffin beaker; (B) "Tall-form" beakers or Berzelius beakers; (C) Flat beakers or crystallizers.

VOLUMETRIC FLASKS OR MEASURING FLASK OR GRADUATED FLASK (FIG. 2.18)

It is a type of laboratory flask, calibrated to contain a precise volume at a particular temperature. These flasks are usually pear-shaped, with a flat bottom, and made of glass or plastic. The neck of the volumetric flasks is elongated and narrow with

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an etched ring graduation marking. The marking indicates the volume of liquid contained when filled up to that point. The flask's mouth is furnished with a plastic snap/screw cap or glass stopper.

Uses: Volumetric flasks are used for precise dilutions and preparation of standard solutions.

Sizes: Volumetric flasks are of various sizes, containing from 1 milliliter to 20 liters of liquid.



Fig. 2.18: Volumetric flasks.

CONICAL FLASK OR TITRATION FLASK OR ERLENMEYER FLASK (FIG. 2.19)

It is a laboratory flask with flat bottom, a conical body and a cylindrical neck. The German chemist Emil Erlenmeyer created it in 1860. It differs from the beaker in its tapered body and narrow neck. Like beakers, Erlenmeyer flasks are not normally suitable for accurate volumetric measurements. They can be made up of glass or plastic with a wide range of volumes depending upon their applications.

Uses

They are widely used in chemistry laboratories for holding liquids and mixing them by swirling. It is used to manipulate solutions or to carry out titrations.

They are also used in microbiology for the preparation of microbial cultures.



Fig. 2.19: Conical flasks.

FUNNEL

A funnel is a pipe with a wide mouth and a narrow stem. Funnels are usually made of stainless steel, aluminum, glass, or plastic. There are different kinds of funnels used in laboratory, such as filter funnels, thistle funnels, dropping funnels, separatory funnels, powder funnel, etc.

The Tullgren funnel is used to collect arthropods from plant litter or similar material.

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Uses

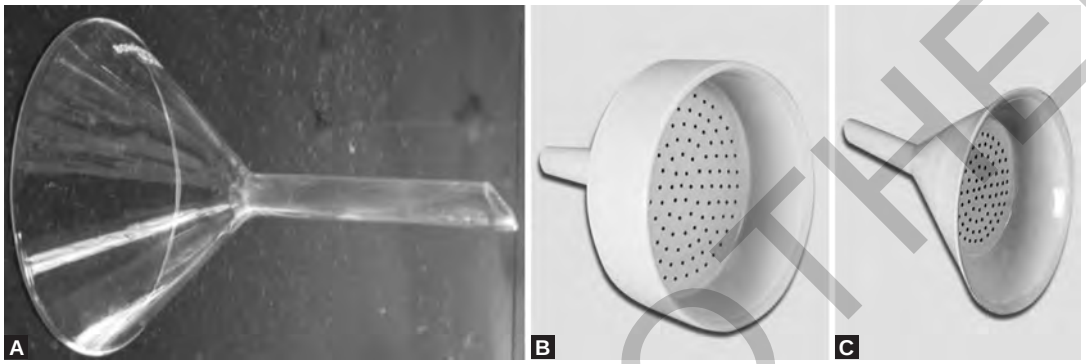
Funnel is used to channel liquid or fine-grained substances into containers with a small opening.

A **filter funnel (Fig. 2.20A)** is used for separating solids from liquids via the laboratory process of filtering.

Büchner and Hirsch funnels

A **Büchner funnel (Fig. 2.20B)** is used in filtration. It is traditionally made of porcelain, but glass and plastic funnels are also available. On top of the funnel-shaped part, there is a cylinder with a fritted glass disc/perforated plate separating it from the funnel.

The **Hirsch funnel ((Fig. 2.20C)** has a similar design; it is used similarly, but for smaller quantities of material. The main difference is that the plate of a Hirsch funnel is much smaller, and the walls of the funnel angle outward instead of being vertical.



Figs. 2.20A to C: (A) Filter funnel; (B) Büchner funnel; (C) Hirsch funnels.

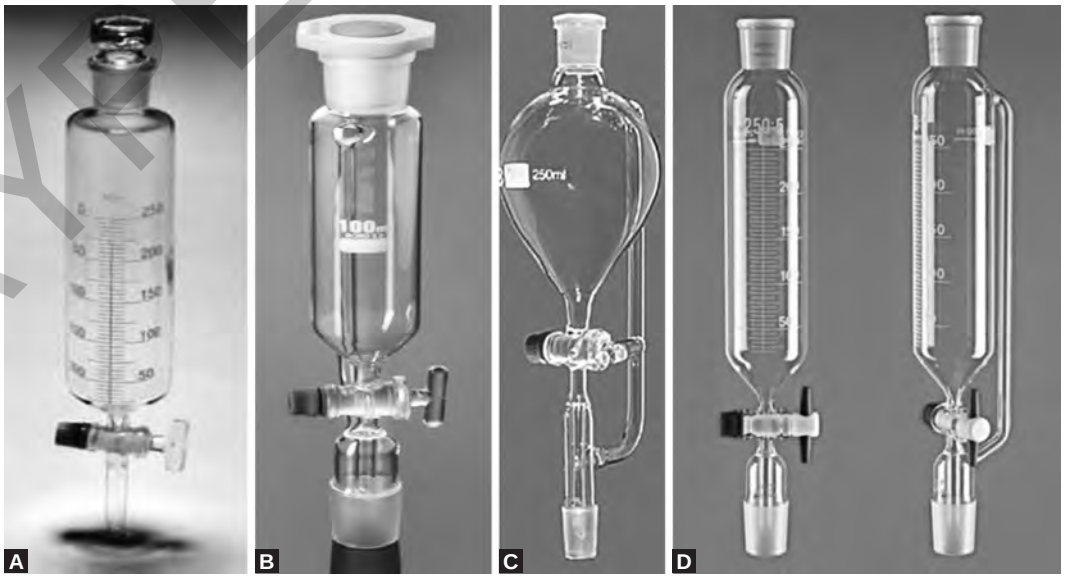
A **thistle tube/funnel (Fig. 2.21)** has a shaft of tube, with a reservoir and funnel-like section at the top. They are typically used to add small volumes of liquids to an exact position.



Fig. 2.21: Thistle funnel.

A **dropping funnel (Figs. 2.22A to D)** is used to transfer fluids. They are fitted with a stopcock which allows the flow of fluid to be controlled. Dropping funnels are useful for adding reagents slowly, i.e., drop-wise. Types of dropping funnel

a. Cylindrical type graduated and ungraduated with cone and socket
b. With pressure equalizing tube
c. Pear-shaped, graduated and ungraduated



Figs. 2.22A to D: (A) Dropping funnels-cylindrical, graduated; (B) Dropping funnels-cylindrical, ungraduated; (C) Dropping funnel-pear shaped, ungraduated; (D) Dropping funnels with pressure equalizing tube.

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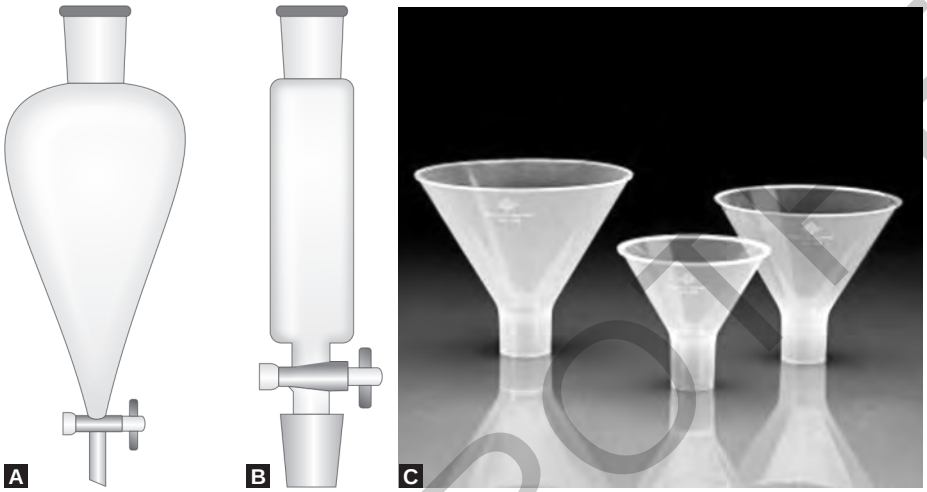
Separatory Funnel

A **separatory funnel or separation funnel** or **separating funnel**, or **sep funnel (Figs. 2.23A and B)** is a piece of laboratory glassware used in liquid-liquid extractions to separate the components of a mixture into two immiscible solvent phases of different densities**.** Typically, one of the phases will be aqueous, and the other a lipophilic organic solvent, such as ether, chloroform or ethyl acetate. All of these solvents form a clear delineation between the two liquids. The more dense liquid, typically the aqueous phase unless the organic phase is halogenated, sinks and can be drained out through a valve away from the less dense liquid, which remains in the separatory funnel.

Powder funnels (Fig. 2.23C) feature a large bore, making them ideal for use with powders, larger particles and viscous liquids. For solids, a powder funnel with a wide and short stem is used.



Figs. 2.23A to C: (A) and (B) Separatory funnel (A: Cone or pear shaped; B: Cylindrical); (C) Powder funnel.

REAGENT BOTTLES OR MEDIA BOTTLES OR GRADUATED BOTTLES (FIGS. 2.24 AND 2.25)

These containers are made of glass, plastic, borosilicate glass, soda-lime glass or related substances. They are topped by special caps or stoppers which protect the contents from spilling or contamination with outside environment. Some reagent bottles are tinted amber (actinic), brown or red to protect light-sensitive chemical compounds from visible light, ultraviolet and infrared radiation which may alter them. Reagent bottles are available with narrow mouths for better control while pouring, or wide mouths for easy filling or content retrieval. The common bottle sizes include 100 mL, 250 mL, 500 mL, 1000 mL (1 liter) and 2000 mL (2 liter).

Uses

Reagent bottles are excellent for storing powders and liquids. They are used to keep chemicals in liquid or powder form for laboratories and stored.



Fig. 2.24: Different sizes of reagent bottles *(For color version, see Plate 4)*.

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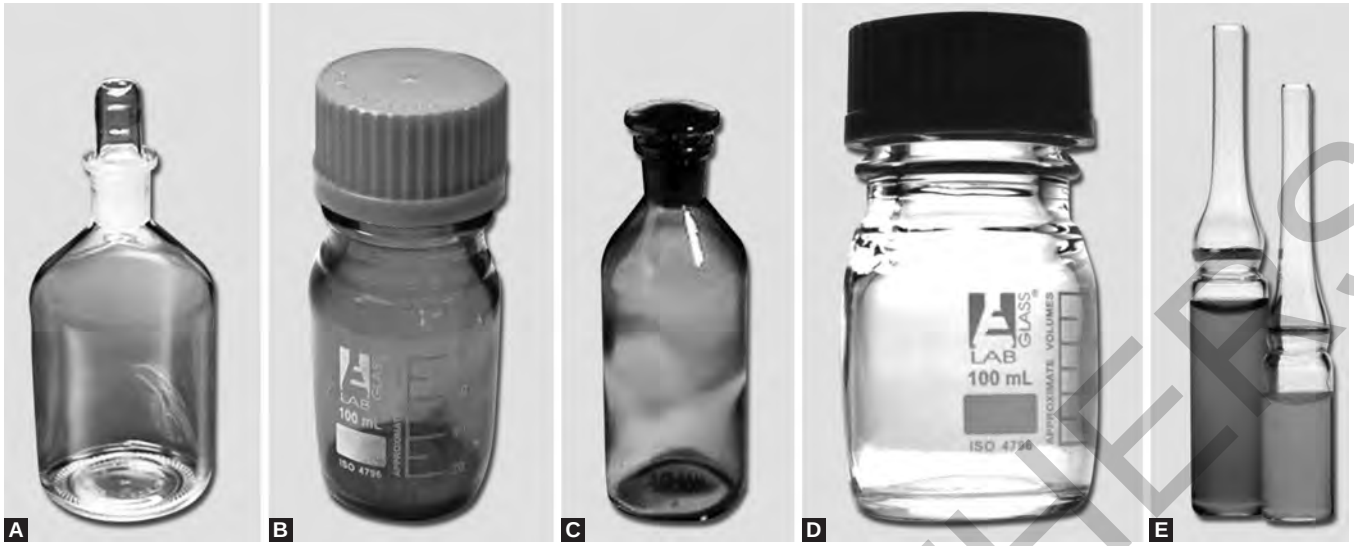

Figs. 2.25A to E: (A) Reagent bottles with hollow standard taper stoppers; (B) Amber reagent bottle with screw cap; (C) Amber glass reagent bottle with ground glass stopper; (D) Clear reagent bottle with screw cap and graduations; (E) Safety-coated reagent bottles *(For color version, see Plate 4)*.

MEASURING CYLINDER OR GRADUATED CYLINDER OR MIXING CYLINDER (FIGS. 2.26A TO C)

Measuring cylinders are used to measure the volume of liquids. Typical capacities of graduated cylinders are between 5 mL and 2000 mL. A traditional graduated cylinder is usually narrow and tall so as to increase the accuracy and precision of volume measurement.

Mixing cylinders have ground glass joints instead of a spout, so they can be closed with a stopper or connect directly with other elements of a manifold.

Advantage: Graduated cylinders are generally more accurate and precise than laboratory flasks and beakers.

Disadvantage: They are less accurate and precise than volumetric glassware, such as a volumetric flask or volumetric pipette. They should not be used to perform volumetric analysis.



Figs. 2.26A to C: (A) and (B) Graduated measuring cylinders; (C) Mixing cylinder *(For color version, see Plate 4)*.

PIPETTES

A pipette is a laboratory tool commonly used in chemistry, biology and medicine to transport a measured volume of liquid. They are available in several designs for various purposes with differing levels of accuracy and precision, from single piece glass pipettes to more complex adjustable or electronic auto-pipettes or micropipettes.

Graduated pipettes (Fig. 2.27): These are a type of macropipette consisting of a long tube with a series of graduations to indicate different calibrated volumes. Graduated pipettes commonly come in 5, 10, 25 and 50 mL volumes. Originally pipettes were made of soda-lime glass, but now many are made of borosilicate glass; disposable graduated pipettes are often made of polystyrene.

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Simplified Practical Manual of Biochemistry

The third edition of *Simplified Practical Manual of Biochemistry* is extensively revised as per the Competency Based Medical Education (CBME) guideline of National Medical Commission (NMC), New Delhi, India. This book includes a brief description of hazards and precautions related to laboratory, laboratory waste management, pH and buffers, analysis of normal and abnormal urine, urine screening for inborn error of metabolism, automation, quality control, description (principle, procedure and application) of basic techniques used in biochemistry laboratory, such as enzyme-linked immunosorbent assay (ELISA), chromatography, electrophoresis, ion-selective electrode (ISE), arterial blood gas (ABG), DNA isolation; qualitative and quantitative analysis of some important biochemical parameters with clinical significance and reference values of few bio-metabolites, which are a must-know to the undergraduate students. The tests procedures are represented in tabular form wherever possible has made it unique in itself. The book also contains a section describing various organ function tests, such as liver function test (LFT), kidney function test (KFT), gastric function test (GFT), pancreatic function test (PFT) and thyroid function test (TFT) with their clinical significance. It also includes chapters on analysis of milk and cerebrospinal fluid (CSF). It has separate sections on clinical case study, point-of-care testing (POCT), spotters and reference values of biochemical parameters. The relevant diagrams and graphs are included in the book along with color plates to make it easier for the reader to understand the text matter. The whole book has been divided into ten sections to categorize the content. Also, blank pages (worksheets) are left after the chapters so that it can be utilized to do calculations, draw diagrams, and write text matters if required in the practical class room. All the comprehensible measures are taken to make this book a reader-friendly.

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