Chapter 1

Laboratory Safety and Instruments Used in Laboratory

Pathology laboratories perform examinations and investigative analyses on patient's specimens and postmortem materials. In the course of such investigations, procedures, which are employed, require the use of chemical reagents and various instruments. Thus, hazards that in pathology laboratories include specific risk from toxic chemicals (including carcinogens), radio-chemicals, pathogenic microorganisms in patient's samples, postmortem specimens and general risk from mechanical, electrical and fire hazards.

To prevent chemical, electrical and biological hazards some precautions should be followed. These precautions are discussed in the forthcoming text.

HAZARDS ASSOCIATED WITH CHEMICAL BURNS AND POISONING

Spattering from acids, caustic materials and strong oxidizing agents is a hazard to clothing and eyes and is a potential source of chemical burns (Table 1.1).

1. All bottles containing reagents must be properly labelled. It is a good practice to label the container before adding the reagent, thus preventing the possibility of having an unlabelled reagent. Expiratory date should be written on the bottle.
2. Proper storage and use of chemicals is necessary to avoid chemical hazards. Thus, knowledge of the properties of chemicals in use and of proper handling procedures greatly reduces dangerous situations.
3. Strong acids, caustic materials and strong oxidizing agents should be dispersed by a commercially available automatic dispensing device.
4. Avoid storing materials and equipment on top of cabinet.
5. Be sure that weight of the chemicals does not exceed the load capacity of the shelf or cabinet.
6. Do not store materials on top of high cabinets where they will be hard to see or reach.
7. Do not store corrosive liquids above eye level.
8. Provide a specific storage location for each type of chemicals, and return the chemicals to those locations after each use.
9. Do not expose chemicals to heat or direct sunlight.
10. Observe all precautions regarding the storage of incompatible chemicals.
11. Use corrosive resistant storage trays or secondary containers to collect materials if the primary container breaks or leaks.

12. Distinguish between refrigerators used for chemical storage and refrigerators used for food storage.
13. A cart should be used to transport heavy or multiple numbers of containers from one area to another.
14. Glass containers with chemicals should be transported in rubber or plastic containers that protect them from breakage and in the event of breakage.
15. Acids must be diluted by slowly adding them to water while mixing; water should

TABLE 1.1 Various chemicals, health hazards and safety precautions

S. No.	*Chemicals*	*Health hazards*	*Safety precautions*
1.	Acetone	Slight eye, nose and throat irritation. Inhalation may cause dizziness, narcosis and coma.	Keep container in well-ventilated area; keep away from sources of ignition. Do not breathe vapour. Use mask
2.	Diethyl ether	Irritation to eyes and respiratory tract. May affect central nervous system causing drowsiness and unconsciousness. Repeated inhalation may cause addiction.	Keep container in well-ventilated area; keep away from sources of ignition. Do not breathe vapour. Use mask.
3.	Absolute alcohol	Harmful if ingested. Irritation of eyes. May affect central nervous system.	Keep container tightly closed. Keep away from ignition sources.
4.	Ehrlich's aldehyde	Severe irritation of eyes and upper respiratory tract; prolonged inhalation exposure or skin contact may cause sensitization.	Work in a well-ventilated area. Use mask and gloves.
5.	Methanol	Effects on central nervous system resulting in unconsciousness; mucous membrane irritation. Chronic exposure can cause damage to retina and optic nerve. Prolonged skin contact may cause dermatitis. May be absorbed through skin.	Keep container in well-ventilated area; keep away from sources of ignition. Do not breathe vapour. Use mask.
6.	Ammonium hydroxide	Corrosive to eyes, skin and respiratory tract. Lung edema at high levels of exposure to gas or vapour.	Prevent skin and eye contact. Avoid inhalation. Use mask.
7.	Xylene	May affect central nervous system resulting headache, dizziness, fatigue, and nausea. Liquid and vapour irritate eyes, skin, mucous membranes and respiratory tract. Harmful if ingested. Prolonged skin contact may remove fat from the skin. Non-specific neurological impairment. Exposure may enhance hearing damage caused by exposure to noise. Animal tests suggest toxicity to human reproduction or development.	Prevent skin and eye contact. Avoid inhalation of fine dust and mist. Use mask.
8.	Sodium hypochlorite	Corrosive to eyes, respiratory system and skin. Inhalation may cause lung edema. Repeated exposure may cause skin sensitization.	In case of contact with eyes, rinse immediately with water and seek medical advice. Do not inhale vapour; use mask. Work in well ventilated area. Wear gloves and eye protection.
9.	Lysol	Substance and vapour are corrosive to eyes, skin and respiratory tract causing severe burns. May be absorbed through skin. Central nervous system disturbances and coma. Kidney and liver damage. Prolonged contact with dilute solutions may cause dermatitis.	Do not breathe vapour; use mask. In case of contact with eyes, rinse immediately and seek medical attention. In case of contact with skin, wash off immediately; remove contaminated clothing. Wear gloves, apron.

never be added to concentrated acid. Always add the sulphuric acid to the water drop by drop, stirring the mixture after each drop.

16. Perchloric acid, because it is potentially explosive in contact with organic materials requires careful handling. Perchloric acid should not be used on wooden bench tops, and bottles of this acid should be stored on a glass tray.
17. Special care is necessary when dealing with mercury. Even small drops of mercury on bench tops and floors may poison the atmosphere in a poorly ventilated room.
18. The alkaline substances, strong acids, dehydrating and oxidizing agents are particularly corrosive or destroy living tissues.
19. Among the more familiar toxic substances are cyanides, and heavy metal salts which cause acute or chronic poisoning. Mercury, lead, arsenic and a number of organic substances are cumulative poisons and are injurious to health on long exposure. There are also compounds like cyanides, sulphides, chlorates and nitrates, which reacts with acid to produce poisonous gases.
20. A number of reagents used in haematological tests contain chemicals which are extremely toxic (e.g. potassium cyanide) or potentially carcinogenic (benzidine).
21. Many chemicals contain protein conjugate and buffer containing sodium azide as a preservative. If these materials are to be disposed off through a sink or other common plumbing systems, flush with generous amount of water to prevent accumulation of potentially explosive compounds.
22. Wash your hands after handling a chemical substance and especially before going on breaks or to lunch.
23. Dispose unused, old or outdated and unlabelled chemical substances, only by proper method.
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ELECTRICAL HAZARDS

1. Ensure that hands and work surfaces are dry before touching electrical equipment.
2. All the pieces of electrical equipment should be grounded using a three-point plugs and use of an extension cord should be prohibited.
3. All wires on all the pieces of electrical equipment should be replaced immediately.
4. Use of extension cord is prohibited. Do not use extension cords through walls, doorways, ceilings and floors, as they are not substitutes for permanent wiring.
5. Electrical equipment and connections should not be handled with wet hands, nor should electrical equipment be used after liquid has been spilled on it.
6. Examine all wirings, plugs and extension cords for any signs of exposed wires, fraying or deteriorating insulation.
7. In the event of a shock (even if minor) or emission of smoke or a burning smell immediately tag the equipment 'out of order' and remove it for servicing.
8. Ensure that the licensed electrician checks all electrical outlets for current grounding and polarity at least annually.

FIRE HAZARDS

Every laboratory should have the necessary equipment to put out a fire in the laboratory, as well as to put out a fire on the clothing of an individual. Easy excess to safety showers should be made.

BIOLOGICAL HAZARDS

Biological hazards can be avoided by following precautions called universal precautions.

- All specimens of human origin, e.g. blood, other body fluids and tissues should be treated if they are potentially infectious.
- Procedures that produce aerosols (such as sonication, mixing, and washing) should be avoided in the open laboratory. Do not mix potentially infectious material by bubbling air through the liquid, which leads to aerosol formation.
- Barrier protection such as gloves, mask, and protective eyewear and gowns must be available and used when drawing blood from a patient. The use of disposable gloves is strongly recommended during the test. This includes removal and handling of all patient specimens. Disposable non-sterile latex or vinyl gloves provide adequate protection.
- Wash hands whenever gloves are changed.
- Facial barrier protection should be used if there is a significant potential for the spattering of the blood or body fluids. Any accidents involving materials of human origin, in particular skin punctures and splashes to the face must be reported immediately. As a rule, affected sites must be cleaned or washed thoroughly with running water.
- Dispose of all sharp objects appropriately.
- Wear protecting clothing, which serves as an effective barrier against potentially infective materials. When leaving the laboratory, protective clothing should be removed.
- Make a habit of keeping your hands away from your mouth, nose, eye and any other mucus membranes. This will reduce possibility of infection.
- Minimize spills and spatters.
- Decontaminate all surfaces and reusable devices after use with appropriate registered hospital disinfectants.
- No warning labels are to be used on patient's specimens.
- Mechanical pipetting, not mouth piptting, must be used for the manipulation of all liquids in the laboratory. Mouth pipettings must be banned at all times. Pipetting devices should be inspected routinely for leakage and services and replaced as necessary. When discharging infectious material from a pipette, it is important to minimize the formation of aerosols; the last drop of pipette should not be formed out but left in the pipette. Care should be taken to avoid bubbling when mixing liquids with pipettes. It may be advisable to discharge the liquid on to the side of the container. Contaminated pipettes should always be discarded into disinfectant fluid, which is prepared daily. Broken and chipped pipettes should be discarded.
- Many needle stick accidents occur when the needle is being disconnected from the syringe so that blood may be discharged into its specimen container gently to avoid haemolysis. Without separating the needle from the syringe, place both, together with the used swab and any other dressings, in a puncture-resistant container for disposal (Table 1.2).
- Before centrifuging tubes, inspect them for cracks; inspect the inside of caps for signs of erosion or adhering matter. Be sure that rubber cushions are free from all bits of glass.
- There should be a jar of disinfectant on each bench at the start of the day's work. The disinfectant must be changed every day.
- Any cuts, insect bites, open sore or wounds should be covered with a waterproof adhesive dressing.
- Periodically clean out freezer, and dry ice chests to remove broken ampoules and tubes of biological samples. Use rubber gloves and respiratory protection during this cleaning.
- Always allow each reagent to fall freely from the dropper tip. Do not touch the dropper tip to any surface as this may contaminate reagents. The reagents may get contaminated, due to interchange of vial caps. Care should be taken while handling the reagent caps to avoid cross contamination of the reagents.

TABLE 1.2 Containers for disposal of biohazardous materials

S. No.	*Container/ bag type*	*Waste description*
1.	Black bag	General waste: ▸ Office papers ▸ Paper cups ▸ Kitchen waste ▸ Fruit peels
2.	Yellow bag	Infectious waste: ▸ Human tissue organs and body parts ▸ Animal waste ▸ Microbiological and biotechnology waste ▸ Discarded cytotoxic medicines ▸ Items contaminated with blood and body fluids of patients known to be HIV/HBV/HCV positive.
3.	Blue bag	Sharp ▸ Needles ▸ Syringes ▸ Scalpels ▸ Blades ▸ Ampoules ▸ Broken glass ▸ Slides
4.	Red bag	Infectious plastic waste: ▸ Disposable items like infected IV tubes, rubber catheters/ other catheters ▸ Cannula ▸ Cut syringes ▸ Cut gloves ▸ Drains ▸ IV fluid bottles

- Laboratory workers are recommended to have vaccination against hepatitis B.
- Use a separate room if possible or at least a dedicated part of the laboratory and carry out all manual procedures in a microbiological safety cabinet.

Guidelines for Prevention of Needle Stick Injuries

Avoid the use of needles where safe and effective alternatives are available.

Do not recap the needle or bend or break needles with hand.

Used needle should be destroyed properly by needle destroyer (Fig. 1.1) and the mutilated needle and in safe disposal containers (Table 1.2).

Fig. 1.1: Needle destroyer

First Aid for All Exposure

Skin puncture wounds from used and potentially contaminated needles or instrument should be encouraged to bleed and then washed thoroughly (but not scrubbed) with soap and water.

Splashes of blood or body fluid into the mouth should be washed out thoroughly with copious amounts of tap water.

Splashes of blood or fluid into the eyes should be well irrigated with a normal saline wash or running water.

PLANNING FOR SPILLS

A simple spill kit should be readily available and should include the following items:

- Chlorine bleach or some other concentrated disinfectant
- Package or roll of paper towels
- Latex gloves
- Forceps for picking up broken glass
- Report spills to safety officer

Spills Inside a Biological Safety Cabinet

1. Leave the cabinet turned on.
2. Put on gloves and a lab coat.

3. Spray or wipe cabinet walls, work surfaces and equipment with bacillocid solution. If necessary, flood the work surface, as well as drain pans and catch basins below the work surface with disinfectant.
4. Wait at least 10 minutes.
5. Soak up disinfectant and spill with paper towels. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.
6. Autoclave all clean-up materials before throwing them in disposal waste container.
7. Wash hands and any exposed surfaces thoroughly after the clean-up procedure.

Small Spill Outside a Biological Safety Cabinet and Work Bench (Spill that can be Covered by a Few Paper Towels)

1. Put on gloves and a lab coat.
2. Cover spill with paper towels and gently apply bacillocid solution, proceeding from the outer edge of the spill to its center.
3. Leave in place for at least 30 minutes.
4. Pick up the towels and discard into a container (Table 1.2). Use forceps to pick up any broken glass and place them into a sharp container (blue container).
5. Re-wipe the spill area with disinfectant.
6. Remove gloves and thoroughly wash hands.

Large Spill Outside a Biological Safety Cabinet (>10 cm or More)

1. Hold your breath and leave the room immediately.
2. Warn others to stay out of the spill area to prevent spread of contamination.
3. Warn others of the biological materials spill.
4. Remove any contaminated clothing and put it into a plastic bag.
5. Wash hands and exposed skin and inform safety officer or laboratory director about the spill.
6. Put on protective clothing (lab coat, gloves, mask, eye protection, shoe covers) and assemble clean-up materials.
7. Wait 30 minutes before re-entering the contaminated area to allow dissipation of aerosols.
8. Cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its centre.
9. Leave in place for at least 10 minutes.
10. Collect all treated materials and discard in a biohazard container (Table 1.2). Use forceps to pick up any broken glass and place in a sharp container.
11. Re-wipe the spill area with disinfectant.
12. Remove glove and wash hands thoroughly. Disinfect exposed skin (shower if possible).

EXPOSURE TO UV RADIATION

UV radiation is divided into three distinct bands UV A, UV B and UV C.

Each has different penetration properties and potential for damage. The adverse health effects that may occur are erythema (sunburn), photokeratitis in the eyes) retinal burns, cataracts, melanoma and skin cancer (Table 1.3).

TABLE 1.3 Distinct bands of UV radiation and their hazards

Band	*Wavelength*	*Primary visual hazards*	*Other visual hazards*	*Other hazards*
UV A	315–400 nm	Cataracts of lens	–	Skin cancer, retinal burns
UV B	280–315 nm	Corneal injuries	Cataracts of lens, photokeratitis	Erythema, skin cancer
UV C	100–280 nm	Corneal injuries	Photokeratitis	Erythema, skin cancer

COMMON SOURCES OF UV RADIATION IN THE LABORATORY

Germicidal Lamps in Biosafety Cabinets

- **Uses:** The biological safety cabinet is fitted with germicidal ultraviolet (UV) lamps in the work zone. UV can be a useful adjunct to surface cleaning procedures, but should not be seen as a replacement for good cleaning technique.
- **General locations:** These lamps are found within the biosafety cabinet, above the work surface.
- **Signage:** Access to the interior of the biosafety cabinet while the lamp is operating is controlled by closing the glass window pans. Some cabinets are equipped with an interlocking switch which deactivates the UV lamp when the fluorescent lamp is activated; however, personnel must ensure that the UV light is off prior to working at the cabinet. Placing labels that fluoresce when exposed to UV inside the biosafety cabinet should be considered if the lamp is not interlocked with the fluorescent lamp.
- UV lamps should be used for 20–30 minutes at the beginning and end of work. They should not be left on for extended periods.
- Personnel exposed to UV radiation may suffer eye damage and erythema (sunburn). Work opening covers should be in place whenever UV lamps are in use. The personal protective equipment must protect the eyes and skin and include gloves, lab coat with no gap between the cuff and the glove, and mask.
- UV radiation degrades nitrite, plastics, and rubber products and organic coatings.
- UV is ineffective in dynamic airstreams, on dried organic matter, and is not penetrating. Radiation intensity reduces over time due to degradation of the lamps.

Safety Measures Taken Against Radiation

1. Sources of UV lights should be labelled with warning.
2. Warning should indicate that; there is a radiation hazard, shielding should be in place when operating the equipment, and eye/skin protection is needed for operation.
3. All skin should be protected including face, neck, hands, and arms.
4. Face shields should be designed to shield against the UV wavelengths used. Radiation can readily reach the eyes through the open sides of standard eye glasses.

EQUIPMENT SAFETY

The following instructions are to be followed generally for equipment safety.

1. Ensure that all pieces of equipment have appropriate safety features and that such features are properly utilized.
2. Choose the location for an item of equipment considering also its environmental implications (noise, fume/vapour generation, etc.)
3. Monitor the automatic equipment by occasional inspection to determine any significant malfunctions.
4. Review and follow manufacturer's instructions to ensure proper set up.
5. Establish and maintain preventive maintenance schedules as per manufacturer's recommendations.
6. Keep complete and detailed service records for the equipment.
7. Decontaminate all pieces of equipment as per manufacturer's instructions appropriately prior to servicing.

INSTRUMENTS USED IN LABORATORY

Microscope

A microscope allows a sample to be magnified so that it can be examined in more detail.

There are various types of microscopes used in pathological study.

Types of Microscopes

A. **Light microscopes**
   1. Bright field microscope
   2. Dark field microscope
   3. Phase contrast microscope

4. Fluorescence microscope
5. Confocal microscope

B. **Electron microscopes**

1. Transmission electron microscope (TEM)
2. Scanning electron microscope (SEM)
3. Reflection electron microscope (REM)
4. Scanning transmission electron microscope (STEM)
5. Low voltage electron microscope (LVEM)

The light microscope, so-called because it employs visible light to detect small objects, is probably the most well-known and well-used research tool in biology.

Simple microscope: A simple microscope that uses only one lens for magnification and is the original design of light microscope.

A magnifying glass is, in essence, a basic single lens microscope.

Compound microscope: A compound microscope is microscope, which uses multiple lenses to collect light from sample and then a separate set of lenses to focus the light into the eye or camera.

It is the most widely used instrument in the field of histology. It is a device meant for magnifying biological objects that cannot be seen with the naked eye. A typical compound light microscope is capable of increasing our ability to see detail by 1000 times so that objects as small as 0.1 micrometer (μm) or 100 nanometers (nm) can be seen.

Parts of Microscope

The various components of the microscope can be classified into four systems (Fig. 1.4):

a. Support system
b. Magnifying system
c. Illumination system
d. Adjustment system

A. Support system: This consists of:

1. Foot or base
2. Limb
3. Revolving nosepiece
4. Stage
5. Mechanical slide holder
6. Coarse and fine adjustment knob

1. **Foot or base:** Base of the microscope supports the microscope and houses the illuminator. The base stabilizes the apparatus. When a microscope is being carried, one hand should be under the base and one gripping the arm. It supports the weight of all the microscopic parts.
2. **Arm of limb:** It supports the tube and connects it to the base. It is also known as spine. It keeps the base separate from the viewing portion of microscope, allowing it to be adjusted, even as the stage remains stable. It is used to safely transport microscope.
3. **Nosepiece:** It is the rotating part of the microscope at the bottom of the body tube; it holds the objective.
4. **Stage:** The flat platform where slides are placed. Stage clips hold the slide in place.

B. Magnification system: This consists of a system of lenses.

1. **Body tube:** This part supports the eyepiece and objectives. The tube connected with the arm adjusts up and down using the focus. Tube moves the lenses closer to or farther from the specimen. Most 20th century microscopes with body tubes are designed for a mechanical tube of either 160 mm or 170 mm.
2. **Eyepiece:** Ocular is an alternative name of eyepieces that has widely used in the litreature (Fig. 1.2). The ocular, or eyepiece, is a tubular cylinder containing two or more lenses; its function is to bring the image into focus for the eye. The ocular lens or lenses are closed to the eye when one looks through a microscope. Eyepiece is inserted into the top end of the body tube. This lens can be monocular, with one lens in a tube, or, binocular with two ocular lenses. The eyepieces, like objectives are interchangeable (they are removed and replaced directly). They are usually 4X, 6X, 10X or 15X power. Eyepiece works in combination with microscopic objectives to further magnify the intermediate image so that specimen details can be observed.

Fig. 1.2: Eyepieces

3. **Objective lenses:** Used to magnify the object. Each lens has a different power of magnification, such as 10X, 40X and 100X. The magnifying power is engraved on the side of each objective lens.
   a. *Magnification:* The magnifying power of each objective is shown by a figure engraved on the sleeve of the lens:
      - The 10X objective magnifies 10 times (low power)
      - The 40X objective magnifies 40 times (high power)
      - The 100X objective magnifies 100 times (oil immersion). The 100X objective is usually marked with a red ring to show that it must be used with immersion oil.

   b. *Numerical aperture (NA):* The NA is also engraved on the sleeve, next to the magnification:
      - 0.30 on the 10X objective
      - 0.65 on the 40X objective
      - 0.1.03 on the 100X objective

      Greater the NA, the greater is the resolving power (the ability to reveal closely adjacent details as separate and distinct).

      Moreover, the greater the NA figure, the smaller the front lens mounted at the base of the objective.

      The recommended thickness of the coverslip in mm e.g. 0.17 mm

   c. Other figures may be marked on the sleeve.

   d. *Working distance of an objective:* This is the distance between the front of the objective and the object slide when the image is in focus.

      The greater the magnifying power of an objective, the smaller will be the working distance.
      - 10X objective—working distance 5–6 mm
      - 40X objective—working distance 0.5–1.5 mm
      - 100X objective—working distance 0.15–0.20 mm

   e. *Resolving power:* The greater the resolving power of the objective, clearer the image and greater the ability to reveal closely adjacent details as separate and distinct.

      The maximum resolving power of a good medical laboratory microscope is 0.25 µm (micrometer).

      Immersion oil increases the resolving power by conserving many light rays that would be lost by refraction if a dry objective was used.

4. **Magnification:** Compound microscopes have two magnifying lenses:
   1. Ocular
   2. Objective

Ocular × Objective = Total magnification

Magnification	*Ocular lens*	*Objective lens*	*Total magnification*
Scanning	4X	10X	4 × 10 = 40X
Low power	10X	10X	10 × 10 =100X
High power	40X	40X	40 × 10 = 400X
Oil immersion	100X	10X	100 × 10 =1000X

C. Illumination system

1. **Source of light** (Fig. 1.3)**:** Only the diffused sunlight should be used. Never use direct sunlight as it will produce retinal burns and will damage your eye permanently.

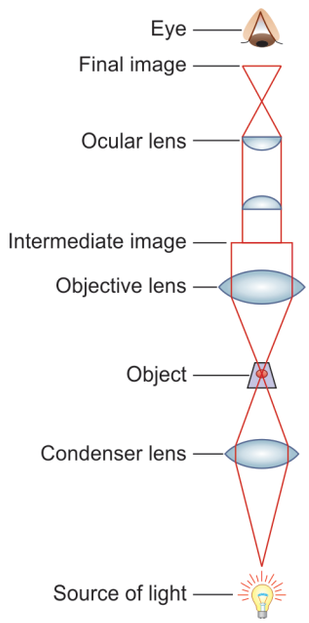

Fig. 1.3: Diagrammatic representation of bright field microscope

- *Light source:* The illumination system begins with a source of light. The light source may be external or internal.
  - *External light source:* It can be a bulb or a mirror, and is usually found near the base of the microscope shining up through the stage. The simplest is the illuminating mirror, which reflects the ambient light source to light the object.
  - *Internal source:* In most modern compound microscope there is built-in light source with an electric lamp, which provides better control of illumination. The lamp, housing has a tungsten lamp, which is placed directly under the stage.

1. **Mirror** (Fig. 1.4)**:** The mirror reflects rays from the light source on to the object. One side has a plane surface, the other concave surface. The concave side forms a lower-power and is not intended to be used if there is already a condenser.
2. **Condenser:** The condenser brings the rays of light to a common focus on the object to be examined. It is situated between the mirror and the stage. It can be raised (maximum illumination). It must center and adjusted correctly.
3. **Diaphragm** (Fig. 1.4)**:** The iris diaphragm is attached below the condenser. A small

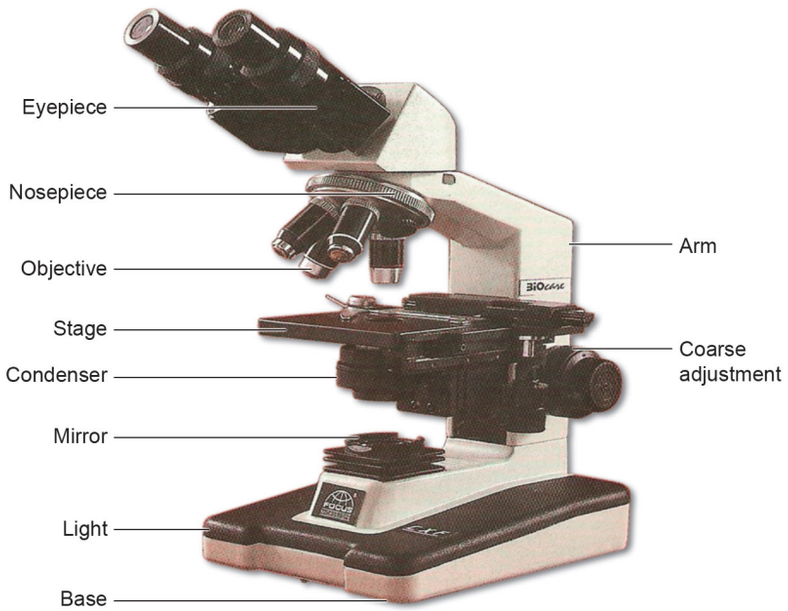

Fig. 1.4: Compound binocular microscope

lever allows controlling the diameter of the iris opening through which the light passes.

When the diameter is decreased to allow only a very narrow cone of light to pass through the specimen, the resolution (sharpness) and depth of the field of the image is increased. Wider the diaphragm there will be wider angle and consequently the greater the NA and the smaller the details seen. But the contrast is correspondingly less.

4. **Filters:** In some microscope coloured filter (particularly blue filters) are fitted below the condenser. These can be left in place or removed according to the type of preparation being examined.

D. Adjustment system

1. **Coarse adjustment knob:** Large round knob on the side of the microscope is used for focusing the specimen. It is used to bring the object into the focal plane of the objective lens.
2. **Fine adjustment knob:** It is a small, round knob on the side of the microscope used to fine-tune the focus of specimen after using the coarse adjustment knob.
3. **The condenser adjustment screw:** This is used to raise the condenser for greater illumination or lower it to reduce the illumination.
4. **Condenser centering screws:** There are three screws placed round the condenser—one in front, one on the left and one on the right. They are used to center the condenser exactly in relation to the object.
5. **The iris diaphragm lever:** This is a small lever fixed on the condenser. It can be moved to close or open the diaphragm, thus reducing or increasing both the angle and the intensity of the light.
6. **Mechanical stage:** Beneath the objective lens is the stage, a flat area where slide/specimen is placed. Some stages are mechanical, meaning that they have a metal apparatus where the slide is inserted, and knobs hanging off the side of the stage (coaxial knobs) for moving the slide. In the center of the stage is a hole through which light passes to illuminate the specimen.

Care of Microscope

A microscope is usually a fairly expensive piece of equipment and contains a number of different components, which require proper care and maintenance.

1. When not in use, keep the microscope protected with a plastic cover.
2. While picking up the microscope and walking with it grab the arm with one hand and place other hand on the bottom of the base. Carry microscope close to your body.
3. Do not touch any of the lenses with your fingers. Eyepiece should not be removed from the microscope for a long duration; otherwise dust will enter the body tube and will be deposited on or near lens of the objective.
4. Do not remove the lenses from nosepiece.
5. Do not interfere with any mechanical part of microscope.
6. When using the low-power objective lens, be very careful when focusing not to drive the head of the lens into the cover slip or slide.
7. Always visually adjust the head of the lens about 1 mm or so above the coverslip, then looking through the eyepiece, use the fine focus control to bring the sample into sharp focus.
8. Do not leave slide, sample or coverslip on the microscope stage for extended periods of time, and when through viewing, raise the objective lens away from the cover slip and turn the objective lens setting. Remove the slide and put the microscope away.
9. Always turn off the light when not using microscope.
10. Use lens paper, alcohol-ether, alcohol, and lens cleaner to clean all lenses before each lab session and after using oil immersion lens. The lens paper is designed for this purpose and will not scratch the lenses.

Never use xylene in any part of the microscope specially to clean the lenses, unless the microscope company directs it. The cementing materials of the lenses are dissolved in xylene.

11. Clean all slides, materials and work area when work is done.
12. Unplug microscope. Coil cord and tuck under band on arm. Return to appropriate space on shelf.
13. The user must be seated away from direct sunlight.

Focusing procedure for monocular compound microscope

- Turn on the light source.
- Switch to the 10X objective.
- Back off on the coarse focus to raise the nosepiece.
- Place the specimen slide on the stage and secure in the proper position. Look at the slide and place it so the specimen is over light aperture in the stage.
- Lower objective lens to lower limit (close to slide).
- Raise the lens using the coarse focus knob until you see the image come into focus and then go out again, then focus back until you find center focus. Adjust fine focus similarly.
- Centre the image and adjust the light using the diaphragm.
- Recenter and adjust focus, first coarse, then fine focus.
- Readjust diaphragm as needed.
- Now switch objectives to the 40X, if a higher magnification is needed.
- Readjust fine focus and light (diaphragm) as needed.

Focusing procedure for oil immersion lens

1. Place slide on stage.
2. Rotate the nosepiece to the 10X objective (low power lens).
3. Adjust the nosepiece to its lowest limit.
4. Adjust the fine adjustment control knob to bring the image of the slide into focus.
5. Swing the low power lens out of position, place a drop of immersion oil on the slide, swing the oil immersion lens (100X objective) into position.
6. Focus with fine adjustment knob.
7. Raise the condenser as high as it can go to improve the image contrast.

Light Microscope

Bright field microscopes.

Polarising Microscope

- Polarizing microscopes employ two polarizing filters in the light path called polarizer and an analyzer.
- By 'crossing' the filters one can examine birefringence specimens such as calcite or quartz.
- A compound to light microscopes may be converted easily to a polarizing microscope by placing one piece of polarizing film on top of the light source (polarizer) and another on top of the microscope slide analyzer.
- They are used in a variety of scientific studies. These include geology, petrology, mineralogy, toxicology, chemistry, pharmaceutics, medicine, forensics, the pulp and paper industry, studying atmospheric pollution, and evaluating ceramics. It is also used to make the identification of amyloid stained with Congo red more specific.
- Because these microscopes work with polarized light, all lenses in the microscope must be 'stain free'.

Dark-field Microscope

- In dark ground microscope the background is dark and object is highly illuminated.
- The microscope is fitted with a special type of condenser lens that contains an opaque disc in the center.
- The disk blocks the light from entering the objective lens directly.
- Certain very slender bacteria, such as the spirochetes are so freely refractile that they cannot be seen by ordinary microscopic method; and dark ground illumination is necessary for their demonstration.

- The scattered light falls on the object from the side. The light that becomes off the object lens and the object appears bright.

Phase Contrast Microscope

- It widely used for examining such specimens of unstained biological tissues.
- Phase contrast microscope is fitted with a special phase contrast objective and a phase contrast condenser with a ring-shaped diaphragm.
- The microscope works on the principle that light passing through one material into another material of a slightly different refractive index undergoes a change in phase.
- The difference in phase is converted into brightness giving rise to contrasting light and dark regions.
- Through this system details of internal structures in the living organisms can be clearly studied.
- It is a type of microscopy that enhances contrasts of transparent and colourless objects by influencing the optical path of light.
- It is able to make the components in a cell or bacteria visible which would be difficult to see in an ordinary light microscope.
- This type of microscope is generally used in microbiology laboratory.

Fluorescence Microscopy

The technique involves conversion of invisible ultraviolet light into visible light by the use of appropriate filters to visualize the reaction of antigen and antibody complex in a histological section.

- Certain organisms are capable of absorbing fluorescent dye such as auromine and rhodamine.
- When exposed to ultraviolet (UV) rays, these specimens absorb the short wavelength of UV rays and emit rays of longer wavelength (visible region) and become highly luminous.
- Under a fluorescence microscope, microorganisms appear bright yellow against a black background when potassium permanganate is used as a counterstain.

Fluorescence microscopy can be utilized:

i. For detection of *tubercle bacilli*, if a smear is stained with auromine rhodamine.
ii. For diagnosis of all types of glomerular diseases to detect the deposits of immune complexes and antibodies or antigens, complements and fibrin.
iii. In the diagnosis of skin diseases, e.g. pemphigus.
iv. For demonstration of antinuclear antibody and other tissue antibodies in SLE and other autoimmune diseases.
v. It is used for detection of viral antigens in frozen tissue sections, acetone fixed cell smears, and cells from virus infected cultures or vesicles fluid by direct or indirect fluorescent antibody technique.
vi. Fluorescence microscopy of brain biopsy can be used for the diagnosis of *herpes simplex* encephalitis and subacute sclerosing pancephalitis.
vii. It is used for verification of rabies in the brain of animals suspected to be rabid.

Confocal Microscopy

Confocal microscopy is a powerful tool in which we are able to obtain high resolution images of real life objects as they were meant to be seen in three dimensions, rather than two dimensions.

It is an optical technique used to increase optical resolution and contrast of a micrograph by using point illumination and a special pinhole to eliminate out-of-focus light in specimens that are thicker than the focal plane.

Like fluorescent microscope, the specimens are stained with fluorochrome dyes.

In confocal microscope laser light is used to illuminate the specimen.

The microscope is mainly used in biochemical research.

Electron Microscopy

Uses of Electron Microscope

1. **Renal pathology:** Electron microscope (EM) aid is used for classification of kidney

diseases; reveals all structural details of glomerulus. In renal pathology EM is used in conjunction with immunofluorescence.
2. It is used to study pathology of liver diseases.
3. To study ultrastructure of tumours of uncertain histogenesis. EM plays an important but selective role in diagnostic study of tumours.
4. **Storage diseases:** EM study of skin, brain, rectum, muscle and nerves.
5. Other tissues and cells studied by EM are liver, heart, nerves, macrophages and respiratory epithelium.

Types of Electron Microscope

1. Transmission electron microscope: It is used to study structural details within a cell which are too small to be seen by light microscope.

This type of microscope is provided with a tall column; a beam of electrons is fired from the top of column, which passes through electromagnetic lenses.

An extremely thin section of the object is placed in the path of the electron beam.

The image of the object is produced on a phosphor-coated screen at the bottom of the column.

The magnification obtained is about 1000 times greater than that in the case of light microscope.

2. Scanning electron microscope: It scans the surface of the objects. Advantages of SEM are:

i. Provides three-dimensional image
ii. Specimens of large size or thickness can be studied.
iii. Differentiate B and T lymphocytes on the basis of their surface morphology.
iv. For viewing of podocytes in renal epithelium.

Advantages of Electron Microscope

Minute structures, not visible by light microscope, are visible by electron microscope, e.g. intracellular organelle, viruses and structure of amyloid fibres.

Disadvantages of Electron Microscope

1. Only very small pieces of tissue/material (1–2 mm) can be used in one block; so chance of missing the exact site of lesion is there.
2. The tissue must be processed in vacuum, thus living cells cannot be examined and such some of the functional properties of living organisms cannot be studied, e.g. locomotion of any living organism.
3. Tissue must be fixed immediately if artifacts are to be removed.

WEIGHT SCALES OR ANALYTICAL BALANCE

There are three types of balances, which are very useful in the laboratory. They are:

1. Analytical balance
2. Electronic balance
3. Direct read out and top loading balance.

Weighing pieces of equipment are essential in a medical laboratory. They are used to weigh substances for the preparation of reagents.

Analytical balance: Analytical balances are mainly of two types:

I. Single pan balance
II. Double pan balance

Single pan balance: The single pan balance is a commonly used balance in the clinical laboratory. It is most often electronically operated and self balancing (Fig. 1.5).

Double pan balance: Analytical balance is basically a two-pan balance. It has a simple operation by which a set of known weights is added in one pan while the substance being weighed balances the other pan.

Electronic balance: The advent of microprocessor in the field of electronics has brought about the introduction of extremely sensitive electronic balance (Fig. 1.6). This type of balance correlates weight of a substance with electromagnetic force. It is a monopan balance, supported by an electric coil, which is suspended in an electromagnetic field. Adding weight to the pan causes the coil to move.

Fig. 1.5: Single pan analytical balance

Fig. 1.6: Electronic balance

The simple rules for the care and maintenance of the balances are:

1. Position the balance on a vibration-free surface, preferably in a draught-free area.
2. Level the balance with the help of a spirit level by adjusting the screws on the stand.
3. Zero the balance before weighing.
4. Always use forceps to add or remove weights when using a pan balance.
5. Never weigh any substance directly on pan of a balance. Use a weighing container (Fig. 1.6).

GLASSWARES

Glass slides: A microscope slide is a thin piece of glass, typically 75 mm × 25 mm (3 by 1 inch) and about 1 mm thick, used to hold objects for examination under a microscope. The white area can be written on to label the slide.

A range of other sizes is available for various special purposes such as 75 × 50 mm for geological use, 46 × 27 mm for petrography and 48 × 28 mm for thin sections.

Slides are usually made of common glass and their edges are often finely ground or polished. Microscopic slides are usually made of optical quality glass, such as soda lime glass or borosilicate glass (Fig. 1.7).

Fused quartz slides are often used when ultraviolet transparency is important, e.g. in fluorescence microscopy.

White plain slides are most common, there are several specialized types. A concavity slide or cavity slide has one or more shallow depressions (wells) designed to hold slightly thicker objects, and certain sample such as liquids and tissue cultures.

Coverslips: A coverslip or coverglass is a thin flat piece of transparent material usually square or rectangular, about 20 mm (4/5 in) wide and

Fig. 1.7: Glass slides

a fraction of a millimeter thick, that is placed over objects for viewing with a microscope (Fig. 1.8).

Fig. 1.8: Coverslips

The object is usually held between the coverslip and a somewhat thicker microscope slide, which rests on the microscope's stage or slide holder and provides the physical support for the object and coverslip.

Functions

- The main function of the coverslip is to keep solid specimens pressed flat, and liquid samples shaped into a flat layer of even thickness. This is necessary because high-resolution microscopes have a very narrow region within which they focus.
- It holds the specimen in place (either by weight of coverslip or in the case of a wet mount, by surface tension) and protects the specimen from dust and accidental contact.
- It protects the microscope's objective lens from contacting the specimen and vice versa; in oil immersion microscopy or water immersion microscopy the coverslip prevents contact between the immersion liquid and the specimen.
- The coverslips are usually made of silicate or borosilicate glass, but especially plastics are also used. Fused quartz coverslips may be used where ultraviolet trans-parency is required, e.g. for fluorescence microscopy.
- Coverslips are available in a variety of widths, lengths and thickness. They are usually sized so as to fit well inside the boundaries of the microscopic slide, which typically measures 25 mm by 75 mm.
- Rectangular slips measuring up to 24 × 60 mm are commercially available.
- Coverslips for haematocytometer are rectangular. Size is 20 × 26 mm and thickness is 0.4–0.6 mm.

Test tube: Test tubes are round bottom cylinders made of borosilicate glass so that they can withstand temperature changes and resists reaction with chemicals. In some cases test tubes are made from plastic (Figs 1.9 and 1.10).

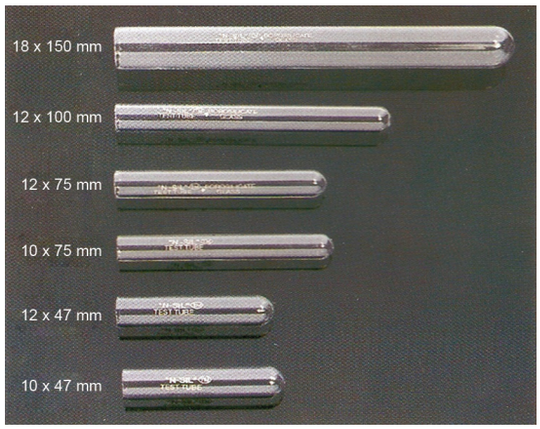

Fig. 1.9: Different sizes of test tubes

Fig. 1.10: Test tubes

The test tube in laboratories is used by chemist to hold, mix or heat small quantities of solids or liquid chemicals, especially for qualitative, experimental and assay.

Centrifuge tubes: Centrifuge tubes or centrifuge tips are tapered tubes of various sizes made of glass or plastic (Fig. 1.11). They may vary in capacity from tens of millimeters, to much smaller capacities used in microcentrifuges and used extensively in molecular biology laboratories. The most commonly encountered tubes are of about the size and shape of a normal test tube (10 cm long).

Fig. 1.11: Centrifuge tube

15 ml centrifuge tubes are suitable for general centrifugation, urine analysis procedure and serum separation. These conical bottom tubes are chemically clean and metal free, ready to use and uniform in size and shape measuring 17 × 120 mm. Graduations are at 0.25, 1.0, 2.5, 5, 10 and 15 ml.

Microcentrifuges typically accommodate microcentrifuge tubes with capacities from 250 µl. These are exclusively made of plastics (Fig. 1.12).

Pasteur pipettes: Pasteur pipettes, also known as teat pipettes or droppers, are plastic or glass pipettes used to transfer small amounts of liquids, but are not graduated or calibrated for any particular volume (Fig. 1.13).

They are usually glass tubes tapered to a narrow point, and fitted with a rubber bulb

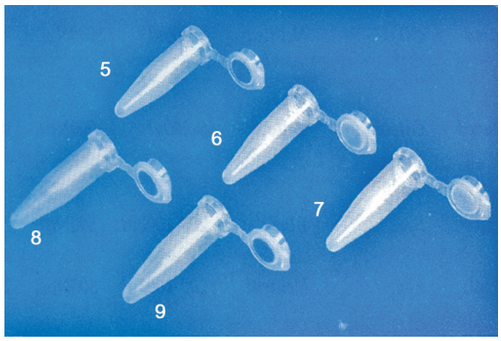

Fig. 1.12: Microcentrifuge tubes

Fig. 1.13: Pasteur pipettes

at the tip. Pasteur pipettes come in various lengths and are sold in boxes of hundreds.

Volumetric pipettes: These are highly accurate adjustable pipettes which come in a variety of different volume ranges and are fitted with disposable tips in which the liquid is actually held. This means a new tip can be used for each new sample and avoids the problem with contamination of a sample with anything that has previously been used in the pipette (Fig. 1.14).

Method of Using Pipette

1. First of all choose the appropriate range of pipette. Pipettes are most accurate when

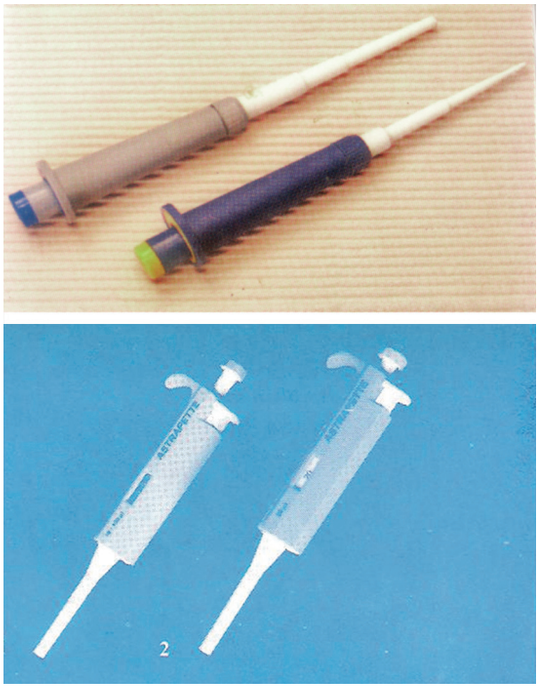

Fig. 1.14: Volumetric pipettes

they are not very close to their maximum or minimum volume.

2. Set the volume which you want to dispense on the pipette. For most types of automatic pipette this involves revolving a wheel on the top of the pipette until the correct numbers appear in the number below. The numbers shown usually only represent the first three significant figures.

   This means if you were using a pipette with a maximum volume of 1000 µl.

   1 0 0 0—this would represent 1000 µl.

   However, if you were using a pipette with a maximum volume of 200 µl.

   1 0 0—would represent a volume of 100 µl.
3. Fit a new disposable tip of the appropriate size to the end of the pipette. Different volume range pipettes need different size disposable tips—these are usually colour coded so you can quickly select the correct tip.
4. Hold the pipette vertically. Carefully draw up the liquid into the pipette tip. This is done by depressing the dispenser button on the top of the pipette to position 1. Place the tip carefully in the liquid and then gently release the dispenser button and observe the liquid being drawn up.
5. You will notice as you press the dispenser button that there are two positions—position 2 where the dispenser button is fully depressed. However, there is a position just above this where you will feel some resistance although the dispenser button is not yet fully depressed—this is position 1.

   This is something you will have to try 'hands on' with a real pipette before moving to the next step.
6. Place the tip against the side of the destination tube and slowly press the dispenser button to the position 1. Most of the liquid will be ejected from the tip. Wait a couple of seconds.
7. Then, press the dispenser button all the way down to position 2. This will eject any remaining liquid which has remained in the pipette tip.
8. Finally eject the used tip. This can usually be done by pressing an ejector button on the side of the pipette.

These tips for micropipettes are made from very special quality raw material, which ensure high quality at its user ends.

Micropipettes use a disposable plastic tip (Fig. 1.15). This is the only part of the micropipette that actually comes in contact with the solution. The tips come in various sizes and colour. A colour coding system between the micropipette and the tips is used. Each size of micropipette uses a specific size of tip.

Drop bottles: Bottle top dispenser is a valuable tool to aliquot reagents quickly, reliability and safety (Fig. 1.16).

Built-in safety features protect the user from accidental spills and ensure smooth, trouble free dispenser operation for use with wide range of compatible liquids including corrosive and inflammable liquids. Bottle

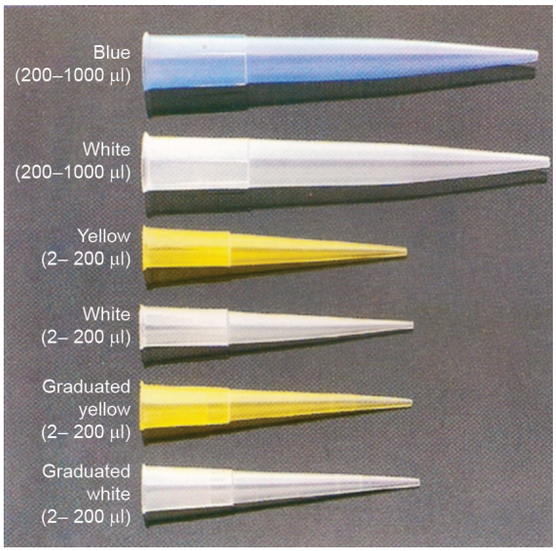

Fig. 1.15: Various sizes and colours of pipette tips

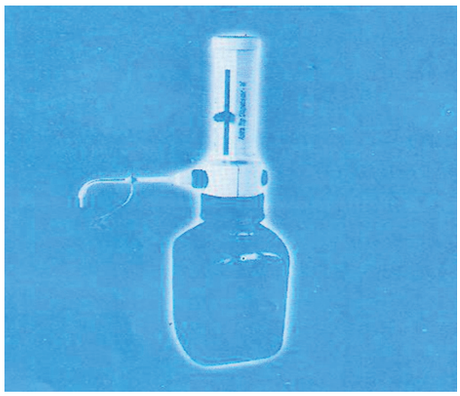

Fig. 1.16: Drop bottle

drop dispensers are used in biological, pharmaceutical, chemical and forensic laboratories. They are available in four different volume ranges 0.5–5 ml, 1–10 ml, 2.5–25 ml and 5–50 ml.

Funnel: A funnel is a conical piece of glass- ware that terminates in a narrow tube. It is used to transfer substances into containers that have narrow mouths. Funnels may be made of any material. A graduated funnel may be called a conical measure (Fig. 1.17).

Some funnels act as filters, either because of their design filter paper or a sieve is placed on a funnel.

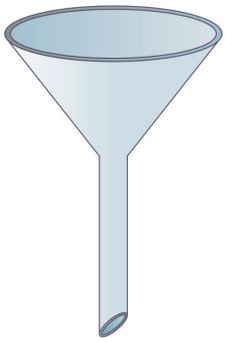

Fig. 1.17: Funnel

Measuring cylinders: A graduated cylinder, measuring cylinder or mixing cylinder is a piece of laboratory equipment used to measure the volume of a liquid (Fig. 1.18). It is used to accurately measure the volume of chemicals for use in reactions. Graduated cylinders are generally more accurate and precise than laboratory flasks and beakers. However, they are less accurate and precise than volumetric glassware such as a volumetric flask or volumetric pipette. Often the largest graduated cylinders are made of polypropylene for its excellent chemical resistance or promethylpentene for its transparency, making them lighter and less fragile than glass (Fig. 1.18).

Fig. 1.18: Measuring cylinders

Types of graduated cylinders:

1. Narrow and high
2. Wide and low

Flask

Erlenmeyer flask (Volumetric flasks): An Erlenmeyer flask is a cone-shaped container with neck, so we can hold the flask or attach a clamp or use stopper (Fig. 1.19). Erlenmeyer flasks are used to measure, mix and store liquids.

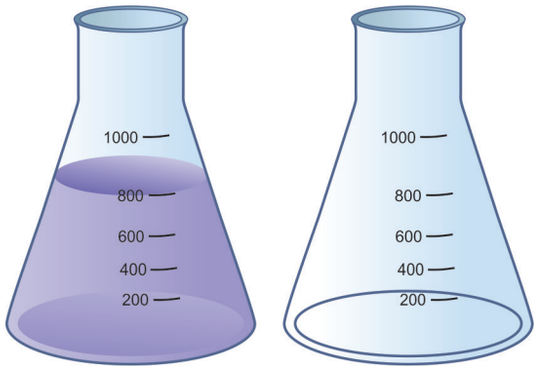

Fig. 1.19: Erlenmeyer flask

The shape makes this flask very stable. They are one of most common and useful pieces of chemistry laboratory glasswares. Most Erlenmeyer flasks are made of borosilicate so that they can be heated over a flame or autoclaved.

The most common sizes of Erlenmeyer flask probably are 250 ml and 500 ml. They can be found in 50, 125, 250, 500 and 1000 ml. We can seal them with a cork or stopper or place plastic or paraffin film or a watch glass on the top of them.

Volumetric flasks are used to measure exact volumes; they are commonly found in sizes varying from 1 to 4000 ml. In practice, they are primarily used in preparing solutions of known concentration, and they are available in various grades.

Conical flask (Erlenmeyer flask) is shaped like a cone, usually completed by the ground joint. The conical flasks are very popular because of their low prices.

They are used for performing titrations and for boiling solutions, since evaporation is minimum.

Round bottom flask: Round bottom flasks are shaped like a tube emerging from the top of the sphere. The flasks are often long neck; sometimes they have the incision on the neck, which precisely defines the volume of flask (Fig. 1.20).

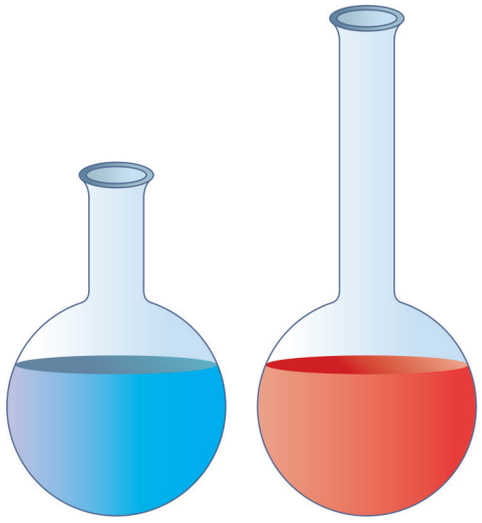

Fig. 1.20: Round bottom flask

They can be used in distillations, or in the heating a product. These can withstand higher temperature. They may be heated in a naked flame, or in an electrothermal mantle.

Beaker: A beaker is a simple container for stirring, mixing and heating liquids commonly used in many laboratories. Beakers are generally cylindrical in shape, with a flat bottom. Most also have a small spout (or beak) to fluid pouring (Fig. 1.21).

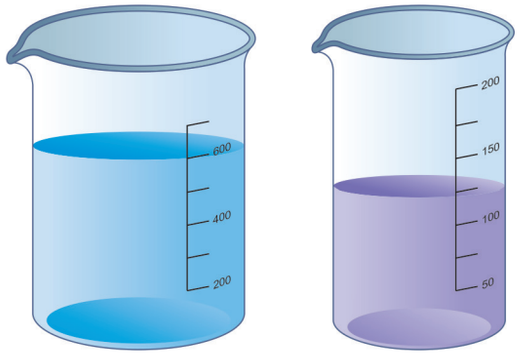

Fig. 1.21: Beaker

Beakers are commonly made of glass (borosilicate glass), but can also be in metal (such as stainless steel or aluminium) or certain plastics (polythene and polypropylene).

Beakers are often graduated, that is, marked on the side with lines indicating the volume contained. For instance, a 250 ml beaker might be marked with lines to indicate 50, 100, 150, 200, and 250 ml.

Watch glass: Watch glasses are concave dishes that have a variety of uses.

A watch glass is circular, slightly concave piece of glass is used in biochemistry as a surface to evaporate a liquid, to hold solids while being weighed or as a cover for a beaker (Fig. 1.22). Watch glasses can even be gently heated in a burner flame to dry precipitates or evaporate solutions.

Fig. 1.22: Watch glass

Petri dishes: Petri dishes come as a set, with a flat bottom dish and a flat lid that rests loosely over the bottom. The contents of the dish are exposed to air and light but air is exchanged by diffusion, preventing contamination of microorganisms. Petri dishes are made of borosilicate glass, such as Pyrex® or Kimax (Fig. 1.23). Single use sterile plastic Petri dishes are also available. Petri dishes are commonly used for culturing bacteria in a microbiology laboratory containing small living specimens, and holding chemical samples.

Fig. 1.23: Petri dish with lid

Koplin staining jars: Koplin staining jars have a rectangular body with a round base and the opening. Both the cover and the body are made of heavy-duty molded glass. Coplin jars are wide-mouth glass jars, usually with vertically grooved interior walls, used for the storage or staining of slides containing blood smears or tissue sections (Fig. 1.24).

It is used for staining slides or use as a developing chambers in the layer chromatography. Unit holds 5 single (3″ × 1″ or 75 × 25 mm) slides vertically or 10 slides back-to-back. Screw cap is white linerless polypropylene which reduces solvent evaporation.

Fig. 1.24: Coplin staining jar

VACUTAINER SYSTEM

Vacutainer system consists of a double pointed needle, a plastic holder or adapter and a series of vacuum tubes with rubber stoppers of various colours. The colour indicates the type

of additive or no additives. Blood collection using the evacuated tube collective system produces the best samples for analysis in laboratory. They also are safe and easy to use because the patient's blood flows directly into the appropriate test tube.

Vacutainer needle: The vacutainer needle is a sharp point at both ends, and usually is covered by a rubber sheath, with one end being shorter than the other. The long end of needle is used for penetrating the vein, the shorter end is used to pierce the rubber stopper of the vacuum tube.

The sheath makes it possible to draw several tubes of blood by preventing leakage of blood as tubes are changed, this is called multidraw. If the short end is not covered with a rubber sheath, it is single sample needle and only one tube of blood can be collected.

There are several sizes of vacutainer needles available, the size depends on the length and gauge of the needle. Vacutainer needle lengths range from 1 to 1½ inches. The most frequently used gauges for phlebotomy are 20, 21, and 22 (Fig. 1.25).

Vacutainer sample collection tubes: Vacutainer tubes are glass tubes sealed with a partial vacuum inside the rubber stoppers (Table 1.4). The air pressure inside the tube is negative, less than normal environment. After inserting the longer needle into the vein, the phlebotomist pushes the tube into holder

TABLE 1.4 Types of specimen collection containers

Additive	*Cap colour*	*Blood volume*	*Suitable for*
Clot activator	Red	6 ml	Most chemistry, including drug levels and serological tests that require serum. Also for immunohaematological test that requires clotted blood such as abnormal blood group antibody tests and crossmatching.
Dipotassium ethylenediamine tetra-acetic acid (K2-EDTA)	Pink	6 ml	For crossmatching requests only. After the tube has been filled with blood drawn by vacuum, it should be gently inverted at least 6 times to prevent clotting.
K2-EDTA	Purple	3 ml	Most haematological tests,cyclosporine, homocysteine and molecular genetics (when using blood DNA). After the tube has been filled with blood drawn by vacuum, it should be gently inverted 6 to 10 times to prevent clotting.
Lithium heparin	Green	4 ml	Tests requiring plasma or whole blood (such as cytogenetics, when using blood DNA) and STAT biochemistry test such as electrolytes, renal screen and ammonia test. After the tube has been filled with blood drawn by vacuum, it should be gently inverted 6 to 10 times to prevent clotting.
Sodium citrate	Blue	2.7 ml	Coagulation studies. The ratio of blood to anticoagulants is very critical for valid prothrombin time and activated partial thromboplastin results. Allow 2.7 ml blood to be drawn by vacuum. This tube should be inverted gently at least 3–4 times to prevent clotting.
Sodium fluoride and potassium oxalate	Grey	6 ml	Glucose tests. After the tube has been filled with blood drawn by vacuum, it should be inverted gently at least 6 times to prevent clotting.
SST II	Yellow	5 ml	All tests requiring serum except those few need red cells as well (such as abnormal blood group antibody screen, cold agglutinins). After centrifugation, the gel forms an effective barrier between the blood clot and the serum.

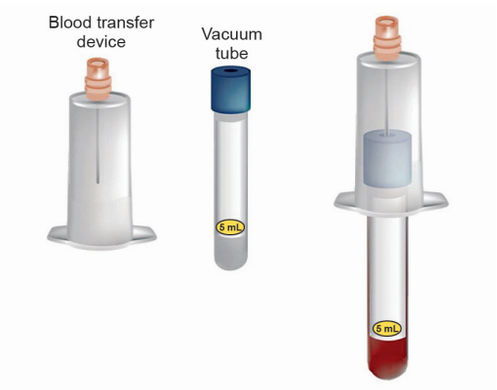

Fig. 1.25: Vacutainer holder, needle and tube

so that the shorter needle pieces the stopper. The difference in pressure between the inside of the tube and the vein causes blood to fill the tube. The tubes are available in various sizes for adult and pediatric phlebotomist. Adult tubes have volumes of 5, 7, 10 and 4 ml (Fig. 1.26).

Vacutainer holder: The vacutainer holder is a plastic sleeve into which the phlebotomist screws.

The double pointed vacutainer needle holders are available in different colours and in two sizes, one for adults and one for pediatric use (Fig. 1.25).

Fig. 1.26: Different coloured top vacutainer tubes

CENTRIFUGE

A laboratory centrifuge is a piece of laboratory equipment, driven by a motor, which spins liquid samples at high speed. Increasing the gravitational force more rapidly and completely cause precipitate together on the bottom of the tube as pellet. The remaining solution is called supernatant (Fig. 1.27).

Centrifugation

The process of separating molecules by size or density using centrifugal forces generated by a spinning rotor. Gravitational forces of several hundred thousand times of gravity are generated in ultra-centrifugation.

Fig. 1.27: Centrifuge

Principle: A body is rotated in a circular movement at speed. This creates a force that drives the body away from the center of the circular movement. To calculate the relative centrifugal force (RCF) for an individual centrifuge, measure the radius (R) of the rotor arm (in cm) and the number of resolutions per minute (RPM) and use the formula shown below:

$$RCF = 1.118 \times 10^{6} \times r \times (rpm)^{2}$$

For example, if the radius is 25 cm and rpm is 1300 rev/minute the RCF is about 50 g.

There are various types of centrifugation:

1. *Differential centrifugation:* Based on the differences in the sedimentation rate of the biological particles different size, shape, and density.
2. *Isopycnic centrifugation:* It is often used to isolate nucleic acid such as DNA.

3. *Density gradient centrifugation:* It is an important technique for purifying proteins and particularly nucleic acids.

Two different types of density gradient centrifugation, for two different purposes are:

1. Zonal or rate zonal centrifugation (Sucrose density gradient centrifugation)
2. Iso-density (isopycnic centrifugation) (Caesium chloride density gradient centrifugation)

Three general types of centrifuges are available:

1. Swinging bucket or horizontal-head centrifuge
2. Fixed angle or angle head centrifuges
3. Ultracentrifuges

1. *Swing out head or horizontal-head:* It is designed to swing the tubes out to a horizontal position during centrifugation. This is the type most frequently needed.
2. *Angle head or fixed head:* The angle head holds the tubes at an angle of about 45° during centrifuging. It is useful for certain techniques, e.g. agglutination tests in blood grouping by the test tube method.

Following centrifuges are available as floor or table models, allowing the laboratory to purchase the instrument that best suits its needs.

1. Hand centrifuge
2. Battery operated bench centrifuge
3. Electric bench centrifuge
4. Microhaematocrit centrifuge
5. Ultracentrifuge
6. Cytocentrifuge or cytospin

1. *Hand centrifuge:* Hand centrifuge is ideal for rapid settling of suspended solids from small quantities of liquid in analytical or clinical procedures at speed up to 2000 RPM.
2. *Battery operated bench centrifuge:* It is battery powered/cordless portable bench top mini-centrifuge. Excellent for use in the field of where electricity is not available or other applications such as running inside an incubator or refrigerated environment.
3. *Electric bench centrifuge:* Electric centrifuges are more accurate than hand operated centrifuges and should be used whenever possible.
4. *Microhaematocrit centrifuge:* Microhaematocrit centrifuges are intended to aid in the diagnosis of blood disorders and diseases. By applying centrifugal force, it is possible to separate suspended particles in a fluid or to separate liquids that have different densities.
5. *Ultracentrifuge:* The ultracentrifuge is a centrifuge optimized for spinning a rotor at very high speeds, capable of generating acceleration as high as 1000000 g (approximately 9800/ km/S^2). There are two kinds of ultracentrifuges, the preparative and analytical ultracentrifuge. Both classes of instruments find important uses in molecular biology, biochemistry and polymer science.
6. *Cytocentrifuge or cytospin:* Cytocentrifuge or cytospin is a special centrifuge, which is used for transferring cells in suspension to a circumscribed area on a glass slide. This instrument concentrates the cell suspension and minimizes cell damage.

Uses of centrifuge

1. Centrifuge is used in clinical laboratory to separate substances of significantly different masses or densities.
2. The two substances to be separated can be a solid (particles) and a liquid or two liquids of different densities.
3. Centrifuges are used in the chemical laboratory primarily to separate clotted blood or cells from serum or plasma and body fluids.
4. Remove cellular elements from blood to provide cell-free plasma or serum for analysis.
5. Separate protein-bound or antibody-bound ligand from ligand in immunochemical and other assays.
6. A small air driven ultracentrifuge has been used to separate chylomicron from serum,

allowing accurate analysis to be performed on clear infranatant. It has also been used to fractionate lipoproteins, perform drug-binding assays, and prepare tissue for hormone-receptor assay.

7. Analytical ultracentrifuges are used to determine sediment coefficients of proteins, allowing assessment of molecular weights.

ROTORS

There are mainly three types of rotors:

1. Angle head rotor
2. Fixed angle rotor
3. Swing out rotor

Angle Head Rotors

- Angle head holds the tubes at a specified angle, 25–52° to the vertical axis of the rotation.
- It is useful for certain techniques, e.g. agglutination tests in blood grouping by the test tube methods.
- During centrifugation particles move along the side of the tube to form sediment that packs against the side and bottom of the tube.
- The surface of the sediment in this case is parallel to the shaft of the centrifuge. As the rotor slows and then stops, gravity may cause the sediment to slide down the tube, forming a poorly packed pellet.

Fixed Angle Rotors

- Fixed angle rotors are used when rapid sedimentation of small particles is required.
- The design of these rotors is more aerodynamic, allowing operation at speeds higher than those achievable with a swinging bucket rotor.
- The capability allows microhaematocrit centrifuges to operate at 11,000–15,000 resolutions per minute (rpm) with an RCF as high as 14,000 xg.

Swinging Bucket or Horizontal-head Rotors

- Swinging bucket or horizontal-head rotor holds the tubes in a vertical position when the centrifuge is at rest, the tubes move to and remain in a horizontal position when the rotor is in motion.
- During centrifugation, particles constantly move along the tube while it is in the horizontal position, distributing the sediment uniformly against the bottom of the tube.
- After centrifugation is complete the rotor has ceased turning, the surface of the sediment is flat with a column of liquid above it. This is the type most frequently needed.
- Ultracentrifuges are high-speed centrifuges that use fixed angle or swinging bucket motors.
- They are often refrigerated to counter the heat generated as a result of friction.
- Small air-driven ultracentrifuges, the airfuge is a miniature air turbine with a small rotor operating at 9,000–10, 0000 rpm.

Bucket Tube Holders

There are several types of buckets for use in electric centrifuge. The choice depends on the model of centrifuge:

- Buckets designed to hold one round-bottomed or conical tube.
- Buckets that hold nine small precipitin tubes.

Some models are also fitted with:

- A timer that stops the centrifuge automatically when the time is up (e.g. after 5 or 10 minutes).
- A cooling chamber that prevents heating of the specimen during centrifuging.
- A resolution counter, e.g. a dial with a needle that indicated the speed of the machine during centrifuging.
- Some centrifuges are equipped with alarm that sounds when the malfunction such as a tube imbalance occurs.
- Some centrifuges automatically shut down under these conditions, preventing tube breakage and the potential for exposure to biohazardous agents.
- All modern centrifuges have a required safety latch that prevents the operator from opening the instrument before the rotor has stopped.

Rotor Lid

The rotor is closed by a rotor lid.

The rotor is located in a rotor chamber, which is covered by a metal centrifuge lid.

CENTRIFUGE TUBES

1. The material used for the tube must withstand the RCF to which the tube is likely to be subjected.
2. Polypropylene tubes are generally capable of withstanding RCFs of up to 5000 xg.
3. The tubes should have tapered bottom, particularly if a supernatant is to be removed, and should be of a size to fit securely into the rack to be centrifuged.
4. The top of the tube should not protrude so far above the bucket that the swing into a horizontal position is impeded by the rotor.

Procedure of Use

1. Carefully transfer required amount of material to be centrifuged to the tubes.
2. Place the centrifuged tubes in the rotor head.
3. The materials (liquid) should be filled into centrifuge tubes only up to a specific level.
4. Centrifuged tubes placed diagonally opposite must be perfectly balanced. The weight of racks, tubes, and their contents on opposite sides of a rotor should not differ by more than 1% or by an acceptable limit established by the manufacturer.
5. In case only one centrifuge tube or odd numbers of tubes are to be used for spinning distilled water should be fitted in another centrifuge tube and placed diagonally opposite the sample tube to achieve balancing.
6. After placing the centrifuge tubes close the lid and perform any other operations such as adjusting the temperature, speed, etc.
7. Switch on the power and spin at the required speed and time.
8. At the end of the spinning switch off the apparatus, open the lid, and take out the material.

Care of Centrifuge

1. Balance the containers with sample before placing them in the opposite cavities of the motor.
2. The sample should be checked for any damage before spinning.
3. The rotor should be checked for any damage before spinning.
4. All the speeds at which the centrifuge is commonly operated should be checked. The speed given for rotor should not exceed. The speed of a centrifuge should be checked at least once every 3 months. The measured speed should not differ by more than 5% from the rated speed under specified conditions.
5. The unit should be switched off (power) if any irregularity noticed during operation.
6. Rotors and chambers must be absolutely clean.
7. Rotors should be checked for any metal fatigue. After a certain amount of use, the rotors should be used at less than the allowed speed.
8. Centrifuge timer should be checked weekly against a reference timer (such as stopwatch) and should not be more than 10% in error.
9. Brushes should be checked at least every 3 months. Brushes should be replaced when they show considerable wear.

Cleaning and Maintenance of Centrifuge

- Cleanliness of a centrifuge is important in minimizing the possible spread of infectious agents, such as hepatitis viruses.
- Clean the bowl of the centrifuge daily or after any spillage occurs. Use 70% ethanol for metal bowls and 1% bleach for plastic ones.
- Ringed the centrifuge buckets after use and remove any traces of blood.
- Check the wiring for fraying and loose connections at regular intervals. If the centrifuge is sparking or running irregularly, the carbon brushes may need replacing.
- Lubrication of centrifuge should be carried out by a specialist.

Precautions

- Infectious material may be dispersed by a centrifuge either through broken tubes or other means such as through the threads of the tubes and caps. In case of breakage, the racks and chamber of the centrifuge must be carefully cleaned. Any spillage should be considered a possible blood borne pathogen hazard.
- Gray dust arising from the sandblasting of the chamber by fragments of glass indicates tube breakdown and possible contamination, necessitating cleaning of the chamber.
- Broken glass embedded in cushions of tube holders may be a continuing cause of breakage if cushions are not inspected and replaced in the cleanup procedure.
- Open centrifuge in biological safety cabinet.
- Wearing gloves remove unbroken tubes and wipe exterior with 1.0% sodium hypochlorite. Remove broken glass with forceps and discard into sharps containers.
- Sealed centrifuge buckets should be used when centrifuging infectious material.
- Care should be taken to ensure that centrifuge tubes are not cracked or flawed.
- Tubes should not be more than three-quarters full, especially if angle centrifuges are used.
- Tubes should be capped and the buckets should be balanced carefully to avoid vibration, which may lead to breakage.
- Material containing agents, which are particularly likely to cause laboratory infections should be centrifuged in sealed centrifuge buckets which are subsequently opened in a safety cabinet.
- Pouring of the supernatant after centrifuging cultures or viruses, and diarrhoeal specimen is a common practice. These fluids are usually poured into disinfectant.
- Two hazards may arise—production of aerosols, and contamination of the outside of the tubes from which the material is poured. Pouring infected fluids into a funnel may minimize these hazards and that small strips of blotting paper may be used to wipe open the rim of tubes after pouring.
- Use centrifuges only with covered centrifuge rotors.
- Do not operate centrifuges in a biological safety cabinet because the motor may produce strong air currents and turbulence, which may disrupt the laminar air flow.

SEROLOGICAL WATER BATH

The water bath, like the incubator, is required for controlled temperature incubation of cultures and liquids and many other laboratory tests. The temperature of water bath is thermostatically controlled and can be set at any desired level ranging usually from 20° to 100°C (Fig. 1.28).

Use and Care of Water Bath (Fig. 1.28)

1. Maintain the minimum level in the water bath with chemically pure water.
2. Avoid use of tap water as salts from tap water may be deposited on the coil and so after its function.
3. Always use a thermometer to check that the temperature is stable at the desired level.
4. Make sure that the substance being incubated is below the surface of water in the bath.

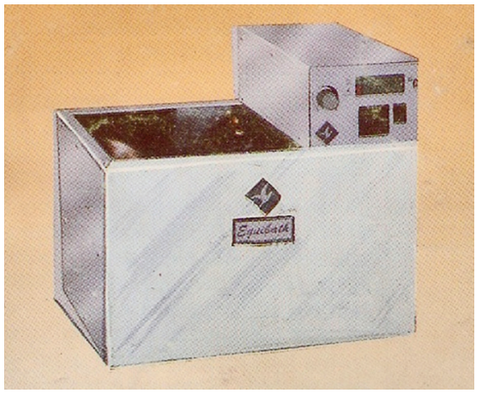

Fig. 1.28: Water bath used in laboratory