

## Safety Measures in Clinical Biochemistry Lab

Laboratory safety involves *all the measures taken by the laboratory worker, laboratory owner, institution and regulatory agencies to eliminate potential harm to human health and ensure well-being*. It is very important because *it ensures the health and safety of workers and researchers in laboratory* (Fig. 1.1).

### LAB SAFETY DO'S AND DON'TS

#### Do's

1. Before starting any laboratory work, follow all the written and verbal instructions carefully.
2. The work areas or surfaces must be disinfected before and after use.
3. Wear the appropriate personal protective equipment (gloves, mask, etc.).
4. Long hair should be properly tied.
5. Always wear apron/lab coat before performing any experiment. The lab coat should be well buttoned.
6. Waste should be disposed as per BMW (biomedical waste) management criteria.
7. Always turn off the electric instruments after use.

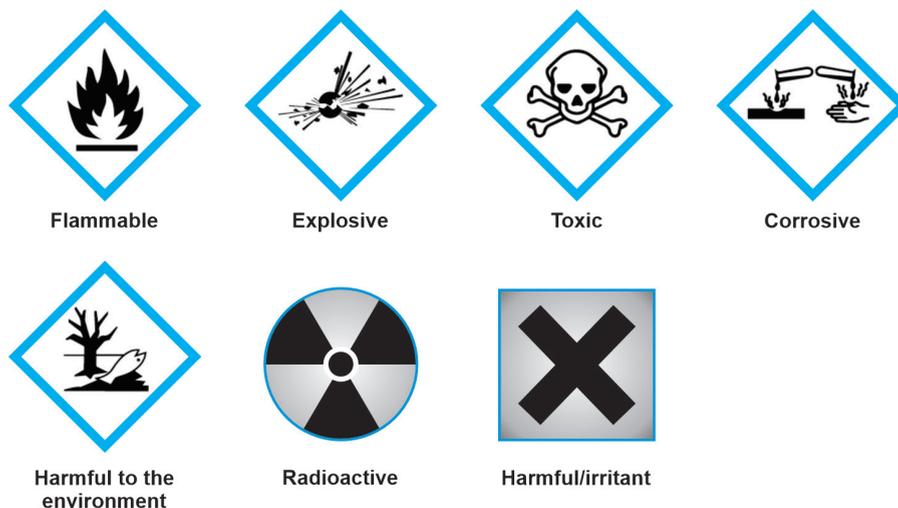


Fig. 1.1: Safety symbols used in the laboratory

8. All the chemicals/reagents should be well-labeled.
9. The first-aid kit should be available in case of any accident and it should also be well-maintained.
10. In case of any accident, report it immediately to the lab incharge or concerned authority.

### Don'ts

1. Smoking, eating and drinking should be absolutely prohibited inside the laboratory.
2. Do not wear open-toed shoes (sandals) in the lab.
3. Do not pour the biohazard fluids in the sink.
4. Do not leave the Bunsen burner/flame open after use.
5. Use of cosmetics in the laboratory should be prohibited.
6. Do not do mouth pipetting.

## LABORATORY HAZARDS

### GENERAL HAZARDS IN THE LABORATORY

#### 1. Chemical Burns

These are caused by accidental exposure to acids, alkaline and other corrosive chemicals. For example: Phenol, HCl, etc.

##### a. Acid Burns

Caused by HCl, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, etc.

##### First Aid

- Wash the inflamed skin with tap water.
- Apply 5% NaHCO<sub>3</sub> and rinse with 5% soap water solution.

##### b. Alkali Burns

More serious than acid burns.

##### First Aid

- Wash affected skin with tap water.
- Use polyethylene glycol mixture with water.
- Contact the physician.

#### 2. Eye Burns

Burns of eye by acids or alkali is a case of ocular emergency. Alkali burns are more dangerous than acid burns.

##### First Aid

- Wash eyes immediately with tap water by holding the eyelids apart.
- Contact ophthalmologist.

### 3. Thermal Burns

Caused by direct contact with open flame or contact with hot objects like hot beaker, hot flask, etc.

#### *First Aid*

- Cool the burnt area with a large amount of cold water.
- Ice should not be used as it may cause loss of body heat.
- Cover burnt area with sterilized dressing and refer patient to burn unit for further medication.

### 4. Injuries by Broken Glass

#### *First Aid*

- Wash the cut wounds with dilute alcohols.
- Apply a tight Band-Aid and call for a doctor.

### 5. Needle Stick Injury

Needle stick injury means the accidental puncture of skin by a used needle.

#### *First Aid*

- As soon as possible, wash the area around the puncture for at least 30 seconds, using soap and warm water.
- Do not squeeze or rub the area around the puncture.
- Cover the site with a Band-Aid or similar dressing.
- Seek medical advice immediately.

## IMPORTANT EXAMINATION QUESTIONS

### VIVA VOCE QUESTIONS

#### 1. What is laboratory safety?

Laboratory safety involves all the measures taken by the laboratory worker, laboratory owner, institution and regulatory agencies to eliminate potential harm to human health and ensure well-being.

#### 2. Why is laboratory safety important?

Laboratory safety is important because it ensures the health and safety of workers and researchers in laboratory.

#### 3. What are 4 hazards in a laboratory?

Chemical, biological, physical and radioactive hazards.

#### 4. Enlist some of the safety measures that need to be followed in clinical biochemistry lab.

Some of the safety measures that need to be followed in clinical biochemistry lab are as follows:

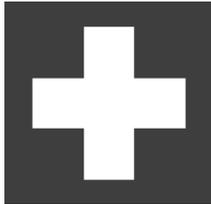
- Wear safety gear.
- Maintain personal hygiene.
- Take responsibility for your personal safety.
- Maintain a clean workspace.
- Follow work procedures.

- Learn emergency procedures.
- Report accidents if they occur.

**5. What are the aims of first aid?**

The aims of first aid include preserving life, preventing injury from getting worse, aiding recovery, relieving pain, and protecting the unconscious.

**6. What is the symbol of first aid?**



**7. Who is the father of first aid?**

Friedrich Esmarch.

**8. What are the most common acid burns?**

Common causes of chemical burns include the following acids: Sulfuric, nitric, hydrofluoric, hydrochloric, acetic acid, formic, phosphoric, phenols, and chloroacetic acid.

**9. What first aid can be given if acid/alkali splashes on the skin?**

Wash thoroughly; bath the affected skin with cotton wool soaked in 5% aqueous sodium carbonate if acid and 5% acetic acid or undiluted vinegar, if alkali.

**10. What first aid can be given if acid/alkali splashes in the eye?**

Spray water immediately from a wash bottle or rubber bulb into the medial corner of the eye. Put four drops of 2% aqueous sodium bicarbonate into the eye, if acid, and saturated solution of boric acid, if alkali.

**11. What first aid can be given in case of needle stick injury?**

- As soon as possible, wash the area around the puncture for at least 30 seconds, using soap and warm water. Bottled water can also be used if no hand washing facilities are available.
- Do not squeeze or rub the area around the puncture.
- Cover the site with a Band-Aid or similar dressing.
- Seek medical advice immediately.

# Introduction to Laboratory Glasswares

Laboratory glasswares are made of complex silicate containing boron dioxide/borosilica, therefore, they are also known as borosilicate glassware.

## PROPERTIES OF BOROSILICATE GLASS

The properties of borosilicate glassware are listed as follows:

- They are usually resistant to acid, alkali, corrosion, temperature, radiation, etc.
- They also have a low coefficient of expansion.

Table 2.1: Composition of borosilicate glass

Material	Percentage
SiO <sub>2</sub>	80.6%
B <sub>2</sub> O <sub>3</sub>	13.0%
Na <sub>2</sub> O	4.0%
Al <sub>2</sub> O <sub>3</sub>	2.3%

## GLASSWARES WHICH ARE COMMONLY USED IN LABORATORY

### BEAKER

- Beakers are used as containers.
- They are available in a variety of sizes.
- Although they often possess volume markings, but are only rough estimates of the volume. These markings are not necessarily accurate.

### MEASURING CYLINDER

- It is available in different volume ranging from 10 mL to 2 L.
- They are used to measure volume of liquids.
- They are also commonly used for dilutions and reagent preparation.

### PIPETTES

- Pipettes are the most important glassware and are available from 0.1 mL to 25 mL.
- Pipettes are used to deliver a measured volume of the liquid.



Fig. 2.1: Beaker

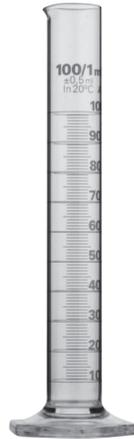


Fig. 2.2: Measuring cylinder



Fig. 2.3: Pipette

- Pipettes can be either:
  - Blowout variety
  - Non-blowout variety
  - Micropipette/autopipette

### Blowout Pipette

It has graduations right up to the tip of the lower end and the contents have to be blown out till the last drop.

### Non-blowout Pipette

It does not have graduations till the last and the final drop is not blown out.

### Micropipette

Micropipettes or autopipettes are one of the most widely used equipment for scientific research and experimentation. Their function is to dispense and/or transfer very small volumes of liquid. By definition, the principle remains the same as that of any other pipette, however, the measure of volume can range from 0.1 to 5000 units of volume.

It has both a volume adjuster and a volume indicator. Micropipettes require micropipette tips or microtips which are available in various sizes and colors (for easy identification) depending upon the volume. A plastic shaft at the lower end of the micropipette is where the tip is to be attached and ejected after it is used by pressing the tip ejector.

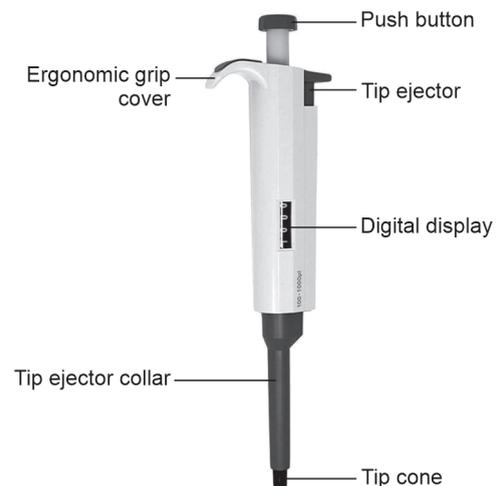


Fig. 2.4: Micropipette



Fig. 2.5: Reagent bottle



Fig. 2.6: Test tube



Fig. 2.7: Conical flask

### Reagent Bottle

Reagent bottles, also known as media bottles or graduated bottles, are containers made of glass, plastic, borosilicate or related substances and topped by special caps or stoppers. They are used to store the reagents.

### Test Tube

A test tube, also known as a sample tube, is a common piece of laboratory glassware consisting of a finger-like length of glass or clear plastic tubing, open at the top and closed at the bottom.

The purpose of a test tube is to hold, heat, or mix substances used in experimentation.

### Conical Flask

Conical flasks (Erlenmeyer type) are used for performing titrations and for boiling purpose.

### Volumetric Flask

Volumetric flasks are used to make standard solutions and other reagents. They are usually round bottomed with a long neck and stopper.

### Flat Bottom Round Flask

Round bottom flask can withstand higher temperature. Thus, they are used for heating purpose.

### Desiccator

Desiccators are sealable enclosures containing desiccants used for preserving moisture-sensitive chemicals. A common use for desiccators is to protect chemicals which are hygroscopic or tend to absorb moisture from air.



Fig. 2.8: Volumetric flask

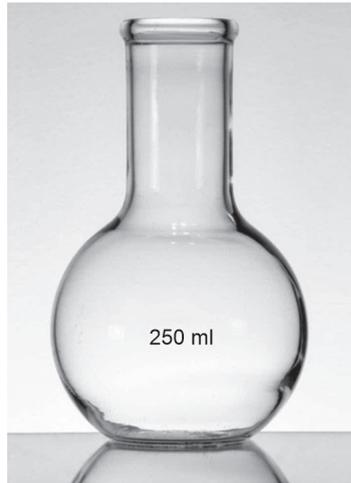


Fig. 2.9: Flat bottom round flask

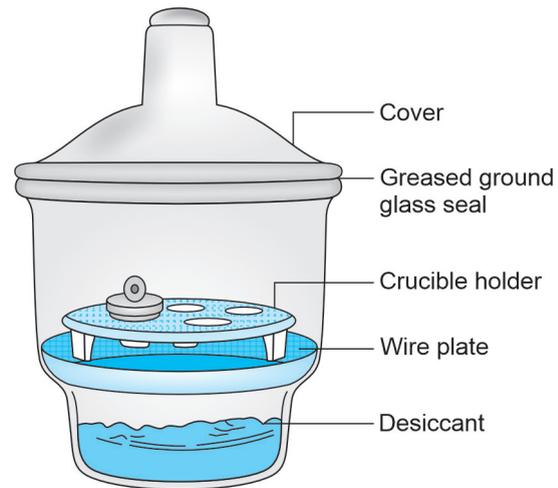


Fig. 2.10: Desiccator

### Funnel

A tube or pipe that is wide at the top and narrow at the bottom, used for guiding liquid or powder into a small opening.

### Cleaning of Glassware

- Soak new glassware for several hours in 1% hydrochloric acid since new glassware is slightly alkaline in reaction which could affect test results.
- Soak glassware in tap water or detergent immediately after use. Brushes which are used for cleaning glasswares should be in good condition.
- When glassware is contaminated with material that is very hard to remove, treat it with nitric acid, fuming sulfuric acid or chromic acid reagent.



Fig. 2.11: Funnel

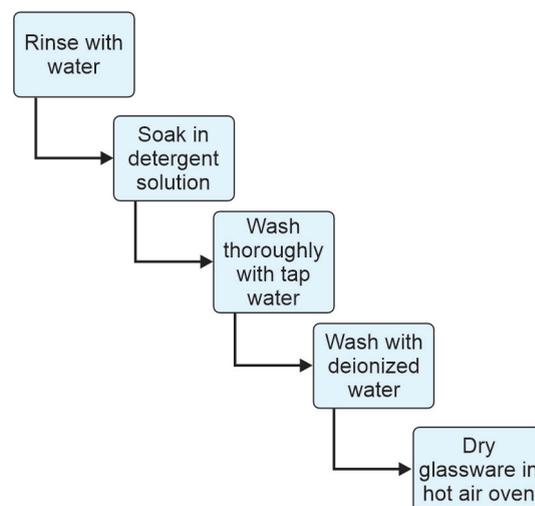


Fig. 2.12: Steps of cleaning glassware

**IMPORTANT EXAMINATION QUESTIONS****LONG QUESTIONS**

1. Discuss in brief about the uses of various laboratory glasswares.
2. Write down the steps of cleaning of laboratory glasswares.

**SHORT AND VIVA VOCE QUESTIONS**

- 1. What are laboratory glasswares made of?**  
Borosilicate glass or fused quartz.
- 2. What are the properties of borosilicate glass?**
  - It is usually resistant to acid, alkali, corrosion, temperature, radiation, etc.
  - It also has a low coefficient of expansion.
- 3. What is 100% borosilicate glass?**  
Borosilicate glass is a type of glass that contains boron trioxide which allows for a very low coefficient of thermal expansion.
- 4. What is the composition of borosilicate glass?**  
 $\text{SiO}_2$ ,  $\text{B}_2\text{O}_3$ ,  $\text{Na}_2\text{O}$  or  $\text{K}_2\text{O}$  and  $\text{Al}_2\text{O}_3$ .
- 5. What is the use of pipette?**  
Glass pipettes are used to transfer precise quantities of fluids.
- 6. What is the use of reagent bottle?**  
To store reagents or samples.
- 7. What is the use of flask?**  
Flasks are narrow-necked glass containers, typically conical or spherical, used in a laboratory to hold reagents or samples.
- 8. What is the use of test tube?**  
Test tubes are used to hold, mix or heat small quantities of chemicals, especially for qualitative experiments and assays.

## Chapter

# 3

## Blood Collection

Laboratory test results are dependent on proper collection and handling of the specimen. Specific instructions for the proper collection and handling of specimens must be made available to laboratory personnel.

The most common types of specimen collected in the clinical laboratory include:

1. Blood
2. Urine
3. Sputum
4. Throat swab
5. Nasal swab
6. Body fluids
7. Biopsy

### BLOOD

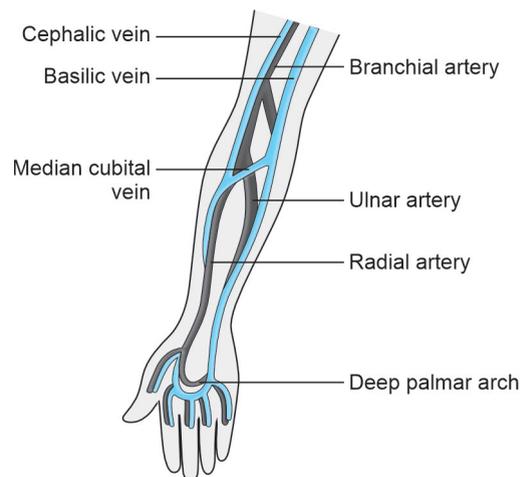
Blood is made up of cellular components (RBCs, WBCs and platelets) and fluid component. The pH of blood is 7.4 and its specific gravity is 1.054–1.060.

The process of collecting blood sample is known as phlebotomy and the technician who performs it known as phlebotomist. There are 3 types of blood sample. They are:

- Capillary blood
- Venous blood
- Arterial blood

### Steps of Blood Collection

- Site for venipuncture is cubital fossa. Most preferred vein is median cubital vein (Fig. 3.1).
- The patient should sit in a chair, lie down or sit up in bed. Hyperextend the patient's arm.
- Apply tourniquet 3–4 inches above the selected puncture site to make vein prominent.
- Clean the site with 70% alcohol.
- Insert the syringe and collect blood in one prick.

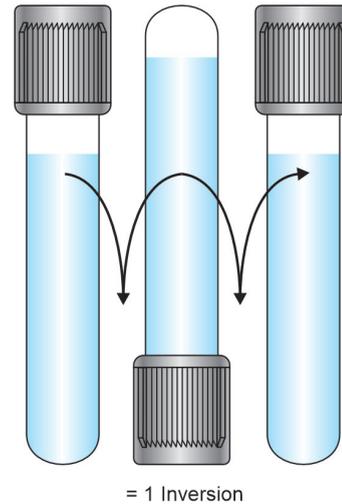


**Fig. 3.1:** Site for venipuncture

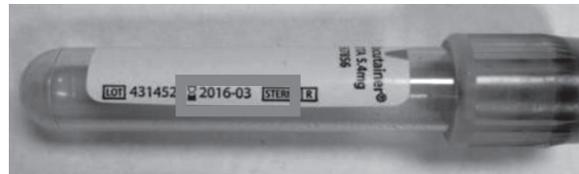
- Release the tourniquet and remove the syringe.
- Apply dry cotton over the site of venipuncture.
- Transfer the blood into the blood vial and destroy the needle.

#### Points to be Considered

- All tubes must be collected in the correct order of draw and inverted gently to ensure proper mixing of additive or anticoagulant (Fig. 3.2).
- All collection tubes must be filled with the required volume. Do not use expired tubes. Expiration dates can be found on each paper label on the tube.
- Incorrect order of draw will introduce contamination with anticoagulants and often produce inaccurate results.
- If testing cannot be completed within that 8-hour timeframe, the sample is typically placed in cold storage at 2°C to 8°C for no longer than seven days.
- If testing assays are not completed within seven days, or if the sample is to be stored for longer than seven days, it should be frozen at -15°C to -20°C.



**Fig. 3.2:** Correct way of mixing anticoagulant



**Fig. 3.3:** EDTA vial along with its expiry date

#### Routinely Used Anticoagulants

##### 1. Ethylenediaminetetraacetic acid (EDTA):

- EDTA has chelating action on calcium ion and prevents coagulation.
- The quantity of EDTA to be used is 20 mg/10 mL blood.

##### 2. Oxalates (potassium oxalate):

- It acts by precipitating calcium ion.
- The quantity of potassium oxalate to be used is 30 mg/10 mL.

##### 3. Heparin:

- It inhibits the formation of thrombin from prothrombin.
- The quantity of heparin to be used is 2 mg/10 mL of blood.

##### 4. Sodium citrate:

- This does not precipitate the calcium, but converts it into a non-ionized form.
- The quantity of sodium citrate to be used is 30 mg/10 mL blood.

##### 5. Sodium fluoride:

- It is usually used as a preservative in blood glucose estimation as it inhibits glycolysis. Sodium fluoride also acts as an anticoagulant, but larger amounts are required (10 mg/1 mL blood).

## Separation of Serum and Plasma

### *Separation of Serum*

1. The whole blood is collected following standard procedures in a serum separator tube (SST) or red-top vial.
2. Allow samples to clot for half an hour at room temperature.
3. Centrifuge for 10 minutes at approximately 3500 rpm.
4. The clear yellow supernatant is serum.
5. Using a clean pipette, the supernatant is collected.

### *Separation of Plasma*

1. Blood is collected in purple top EDTA tube and any other tube containing anticoagulant.
2. It is centrifuged at 2000 rpm for 20 minutes.
3. The supernatant is called “plasma”.

## Precautions

### *To Prevent a Hematoma*

- i. Puncture only the uppermost wall of the vein.
- ii. Remove the tourniquet before removing the needle.
- iii. Use the major superficial veins.
- iv. Make sure the needle fully penetrates the upper most wall of the vein. (Partial penetration may allow blood to leak into the soft tissue surrounding the vein by way of the needle bevel.)
- v. Apply pressure to the venipuncture site.

### *To Prevent Haemolysis (Which Can Interfere with Many Tests)*

- Mix tubes with anticoagulants gently (5–10 times).
- Avoid drawing blood from a hematoma.
- Avoid drawing the plunger back too forcefully.
- Make sure the venipuncture site is dry.
- Avoid a probing, traumatic venipuncture.
- Avoid prolonged tourniquet application or fist clenching.

### *Effects of Prolonged Tourniquet Application*

- Primary effect is hemoconcentration of non-filterable elements (i.e. proteins). The hydrostatic pressure causes some water and filterable elements to leave the extracellular space.
- Significant increases can be found in total protein, aspartate aminotransferase (AST), total lipids, cholesterol, iron.
- Affects packed cell volume and other cellular elements.
- Hemolysis may occur with pseudohyperkalemia.

### *Changes in Blood on Keeping*

The following changes may take place in blood when it is kept:

1. **Loss of carbon dioxide:** In order to prevent loss of  $\text{CO}_2$ , the blood is collected under liquid paraffin and the plasma is separated immediately.
2. **Glycolysis:** Conversion of glucose to lactic acid by the action of enzymes present in RBCs. In order to prevent this blood is collected in grey-top tube.
3. Increase in plasma inorganic phosphate due to formation from ester phosphate present in the cells. To avoid this, serum or plasma is separated within a short time from taking the blood.
4. Formation of ammonia from nitrogenous substances may occur in blood which has been contaminated with bacteria. The blood should be kept sterile or in refrigerator.

### REASONS FOR CANCELLING A LABORATORY TEST

The most common causes for cancellations include:

- Duplicate test request
- Incorrect test ordered
- Test no longer needed

A test may be cancelled due to a technical problem in the specimen collection process:

- Hemolysis of the specimen
- Clotted specimen
- Quantity of specimen not sufficient
- Collection of specimen in incorrect tube
- Contaminated specimen
- Identification of the specimen is suspicious
- Delay in transport—specimen too old

### IMPORTANT EXAMINATION QUESTIONS

#### LONG QUESTIONS

1. Explain in brief about the process of venipuncture.
2. What are the precautions that must be followed while blood collection?

#### SHORT QUESTIONS

1. What are the effects of prolonged tourniquet application?
2. What precautions should be taken to avoid the haemolysis of the blood sample?
3. What precautions should be taken to avoid the hematoma?
4. Discuss in brief about the anticoagulants.

#### VIVA VOCE QUESTIONS

1. **What is the composition of blood?**
  - Cellular components (This fraction includes erythrocytes, leukocytes, and platelets).
  - Plasma.
2. **What is the pH of blood?**

7.4.
3. **What is the specific gravity of blood?**

1.054–1.060.

**4. What is the difference between serum and plasma?**

Plasma contains clotting factors and blood cells, and serum does not. Basically, serum is plasma minus the clotting factors.

**5. How should the needle, syringe and blood sample be disposed?**

The needle and syringe must be disposed of in approved sharps disposal containers. Other contaminated waste must be discarded in an appropriate bio-hazard bag.

**6. What should be the angle of syringe for venipuncture?**

45°.

## Normal Urine Analysis

Urine is the ultra-filtrate of plasma which is formed by nephrons (functional unit of kidney). The daily output of urine on an average diet and normal fluid intake is between 1,200 and 1,500 mL but may vary widely.

The various types of urine sample include:

- Mid-stream urine (MSU) sample
- 24-hour urine sample
- Random urine sample

### PROCEDURE OF URINE COLLECTION

- i. The patient is given a urine container which should be labelled with patient's name, age, sex and date (Fig. 4.1).
- ii. Prior to the urine collection, following instructions are given to the patient:
  - Proper hand wash should be done prior to sample collection.
  - Wash the external genitals.
  - Initial few drops of urine are to be discarded.
  - The midstream urine is to be collected in the container.
  - The remaining urine is to be discarded.
  - The container is closed tightly and immediately transferred to the laboratory for the analysis.



Fig. 4.1: Urine container

### PROCEDURE OF 24-HOUR URINE COLLECTION

- Instructions for collecting a 24-hour urine sample vary from laboratory to laboratory.
- Typically, the patient's first voided morning urine is discarded.
- Collect all voided urine during the 24-hour in the container that is provided by the laboratory.
- A preservative solution is added to preserve the urine sample.

### Physical Characteristics of Urine

1. **Color of urine:** Normally urine is pale yellow or straw colored. This is due to presence of urobilinogen and urobilin.