Chapter

1

Commonly Used Laboratory Apparatus and Equipment

Competencies

BC 14.1: Describe commonly used laboratory apparatus, equipment, good/safe laboratory practice, biomedical hazards and waste management.

BC 14.18: Observe use of commonly used equipment/techniques in biochemistry laboratory including: pH meter, Paper chromatography of amino acid, Protein electrophoresis, TLC, PAGE, Electrolyte analysis by ISE, ABG analyzer, ELISA, Immunodiffusion, Autoanalyser, DNA isolation from blood/tissue.

Various instruments and equipment used in undergraduate biochemistry lab and clinical biochemistry lab are:

- Balances
- Magnetic stirrer
- Centrifuge
- Hot air oven
- Incubator
- Water bath
- Desiccator
- pH meter
- Thermometer
- Various glasswares
- Semiautoanalyzer
- Fully autoanalyzer
- Electrophoresis (described in Chapter 35)
- Chromatography (described in Chapter 36)
- Colorimeter/photometer/spectrophotometer (described in Chapter 5)

There are many instruments which are being used in different biochemistry lab for teaching and diagnostic purposes. Their description is given in this chapter along with the principle of their functioning and the application.

Balances

It is important to prepare reagents and standard solution with accurate measurement of various chemicals. For this purpose, the instrument used is "Balance".

There are various types of balances used in biochemistry lab. They are:

a. Physical/mechanical balance
b. Electronic balance

Physical (Mechanical) Balance

- It is rather a crude equipment which is used for measuring chemicals which are needed for making qualitative reagents and where larger amount of chemical need to be measured.
- They are double-pan balances where on one side known weight is kept and on other side chemical to be measured is equated.
- The weight which can be measured in this instrument vary from **1 mg to 1000 g**.
- Physical balance may be **open** two-pan balance or it may be a balance in **closed** compartment (Fig. 1.1).

Fig. 1.1: Physical balance in closed compartment

Electronic Balances

They are more accurate and are being preferred because of accuracy and ease of operation. They are **single pan balances** which operate on **principle of electromagnetic force** which counterbalance the weighed sample mass. It measures rather smaller amount compared to physical balance. The weight which can be measured ranges from **1 mg to 100 g**.

Facility of **"taring"** in electronic balances makes the measurement easier as because of it the weight of container is adjusted by the machine itself (Fig. 1.2).

Magnetic Stirrer

This is used for thorough mixing of solutes in the solution to make a homogenous mixture. An iron capsule is placed in the vessel containing solution and then this vessel is placed on the pan of magnetic stirrer plate which is built to provide rotating magnetic field.

Movement of iron capsule in the solution on switching on the instrument mixes the solution completely as to provide the homogenous mixture (Fig. 1.3).

Fig. 1.2: Electronic balance

Fig. 1.3: Magnetic stirrer

Centrifuge

This instrument is commonly used in biochemistry lab to separate serum or plasma from whole blood and it also separates sediments in the urine (Fig. 1.4).

Centrifuge is based on the **principle of centrifugal force** which is used to separate solid matters from liquid suspension.

Fig. 1.4: Centrifuge

Centrifuge can be classified as:

a. Bench-top or floor model
b. With refrigeration and without refrigeration
c. Based on rotor design
d. Based on maximum speed which can be attained (normal or ultracentrifuge)

Rotor is the component of the centrifuge machine which is holding the tubes or vacutainers. Based on rotor design, we have two types of centrifuge:

- Horizontal rotor type (swing out head)
- Fixed angle rotor type (angle head)

Fixed angle rotor type of centrifuge can be operated at high speed compare to horizontal rotor type.

This is the reason that lesser air friction is generated in fixed angle type resulting in lesser heat production. In swing out rotor type of centrifuge, more air friction results in more heat production, hence it cannot be operated at very high speed.

Principle of Centrifuge Machine

Key factor in separating the particulate matter from liquid suspension is the **relative centrifugal force (RCF)** which is represented by the following formula

$$RCF = R \times (RPM)^2 \times 118 \times 10^{-7}$$

R is the rotating radius of the rotor which means the radius of rotating path from the central axis.

RPM (revolution per minute): It is the number of revolutions of rotor in a minute. It can be programmed in the centrifuge.

Ultracentrifuge: The centrifuge which can be operated at **very high speed (1,00,000 RPM)** is known as ultracentrifuge.

Hot Air Oven

It is the equipment which produces dry heat in its internal chamber, the temperature may go up to 250°C. These were originally developed by Pasteur. It has double-walled insulation which conserves the heat (Fig. 1.5).

This is used for:

1. Drying of the glassware
2. Heating the chemicals wherever required
3. Dry sterilization of glassware (in microbiological experiments), metal equipment, swabs, etc.

Fig. 1.5: Hot air oven

Incubator

It is an instrument which is capable of maintaining desired temperature, humidity, oxygen and CO_2 in the atmosphere inside (Fig. 1.6).

It is mainly used in microbiology for culture of organism under specified conditions. In biochemistry, incubator is important to incubate the reaction mixture at specific temperature for set duration which depends upon the method adopted for assessment.

Here the temperature may go up to 60–65°C, never beyond 100°C.

Fig. 1.6: Incubator

Water Bath

This equipment has water filled in it, the temperature of which can be controlled by adjustment (Fig. 1.7).

Fig. 1.7: Water bath

This is used for various experiments where a specified temperature is used for heating the reaction mixture. It is commonly used in medical undergraduate practicals where heating of solution is desired. In this equipment, sample can be heated at constant temperature over a prolonged period of time.

Desiccator and Desiccants

Desiccants are hygroscopic substances which absorb water from atmosphere and from various other hydrated substances. In other words, desiccants are **drying agents**. In biochemical lab, we need to remove moisture from many chemicals before using them for analytical purpose, for that purpose these chemicals are kept in contact with desiccants in a closed, airtight chamber which has a lid with a lubricated seal known as desiccator (Fig. 1.8).

Fig. 1.8: Desiccator

pH Meter

What is pH?

pH of a solution is negative logarithm of the hydrogen ion concentration. It can be expressed as:

$$pH = 1/\log 10\ (H^+)$$

As stated in equation above, pH of a solution is inversely proportional to its hydrogen ion concentration. Normal pH of some important biological fluids in human is enumerated below:

- Plasma = 7.35 to 7.45 [Mean = 7.4]
- Urine = 6.5
- CSF = 7.32
- Gastric juice = 1.0 to 2.0

pH meter: pH meter is a reliable and convenient method of measuring the pH of a solution. The pH meter measures the difference in electric potential between a pH electrode and a reference electrode, and so the pH meter is sometimes referred to as a **"potentiometric pH meter"** (Fig. 1.9).

Fig. 1.9: pH meter

Principle of its Functioning

The electrodes, or probes, are inserted into the solution to be tested. On immersion in the solution to be tested, hydrogen ions in the test solution exchange for other positively charged ions on the glass bulb, creating an electrochemical potential across the bulb. The electronic amplifier detects the difference in electrical potential between the two electrodes generated in the measurement and converts the potential difference to pH units.

This instrument has the following components in it:

- Glass electrode
- Calomel electrode

Glass electrode: It has a thin-walled bulb which is filled with 0.1 M HCl. In this HCl, a silver wire coated with AgCl is dipped (Fig. 1.10).

The hydrogen ion selective glass membrane has the following composition:

- CaO: 6%
- N_2O: 22%
- SiO_2: 72%

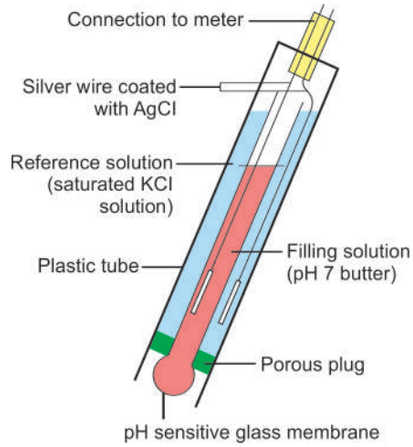


Fig. 1.10: pH meter (glass electrode)

Calomel electrode: It consists of glass tube which is filled with saturated solution of KCl and KCl porous material is plugged at the tip. In this solution, mercuric-mercurous electrode is dipped, the tip of which has porous plug of mercury calomel paste.

Combined electrode: In actual instrument, reference electrode and measuring electrodes are combined.

Thermometer

Many analytical reactions require an optimal temperature at which they occur. Enzymatic reactions specially need precise control of temperature. Many other reactions in biochemical system occur at wide range of temperature. In addition to this, many instruments like laboratory refrigerator, incubators, hot air oven, etc. need to have temperature display to monitor their accurate functioning.

Thermometer is the device which is used to monitor the temperature of the environment. It may be an integral part of the instrument or can be placed in the device for this purpose. Three major types of thermometers are:

1. Liquid in glass thermometer
2. Electronic thermometer (thermistor)
3. Digital thermometer

Liquid in glass thermometer has coloured liquid or mercury which is filled in glass or transparent plastic stem with bulb at one of its ends. Stem is graduated to record the temperature (Fig. 1.11).

In era of automation, electronic thermometer (thermistor) is incorporated in many devices which give accurate and fast reading.

Fig. 1.11: Thermometer

Glasswares

A number of glasswares are used in biochemistry laboratories. The glassware which is used to transfer the liquid may be the one which is to contain (TC) or to deliver (TD) type based on how accurately the specified volume of liquid is transferred.

Various glasswares are described below:

- Flask
- Griffin beaker

- Graduated cylinder
- Pipettes
- Burets

Flask

Volumetric flask: This flask has round lower portion with flat bottom, along with a long narrow neck where calibrated line is marked. This kind of flask is used to prepare a specific volume of reagent (Fig. 1.12).

Erlenmeyer flask: This flask has wide bottom with narrow neck and is designed to hold different volumes of liquid. For this purpose, there is marking of various volumes on its wall (Fig. 1.13).

Fig. 1.12: Volumetric flask

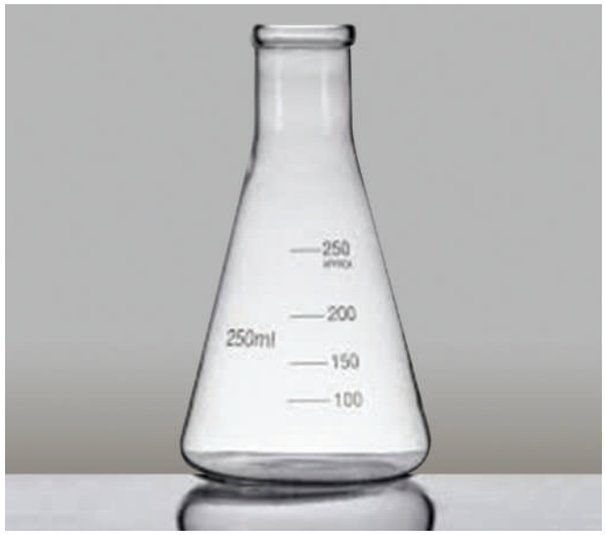


Fig. 1.13: Erlenmeyer flask

Griffin Beaker

It has flat bottom and straight wall with wide opening. It has marking on its side wall and can be used to store various volumes of solution (Fig. 1.14).

Fig. 1.14: Griffin beaker

Graduated Cylinder

These are cylinders having long stem and round or octagonal base. The side wall has markings of various volumes and this is used to measure volume of the reagent. It is available in various capacities from 10 to 2000 mL (Fig. 1.15).

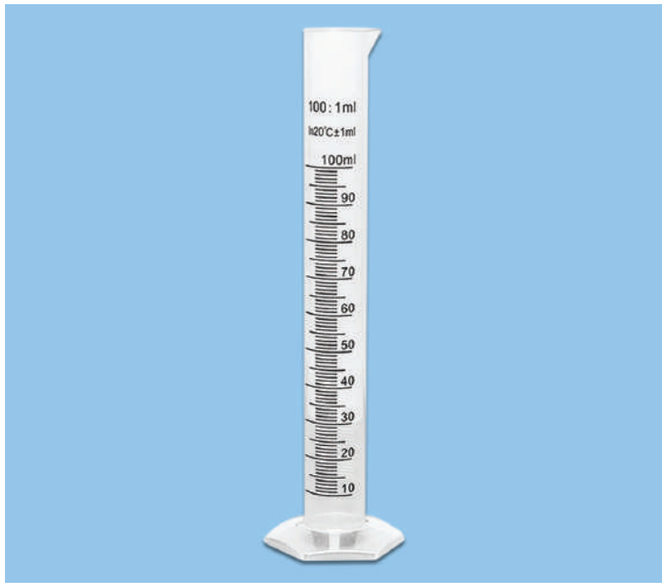


Fig. 1.15: Graduated cylinder

Pipette

Pipettes may be **TC (to contain) or TD (to deliver)** type depending upon whether it is delivering the exact specified volume or not as explained above.

Depending upon the draining characteristic, pipette may be **self-draining type or blowout type. Blowout pipette has got two continuous rings at the top.** Pipettes without this marking are self-draining type.

There are two major classes of pipettes: Measuring (graduated) and transfer types.

Measuring (graduated) pipettes: They are designed to dispense different volumes for which they have markings on it. They are of two types:

1. *Mohr type*: Mohr pipette does not have graduations till tip and it is self-draining type.
2. *Serological type*: Serological pipette has graduation till tip and is blowout type (Fig. 1.16).

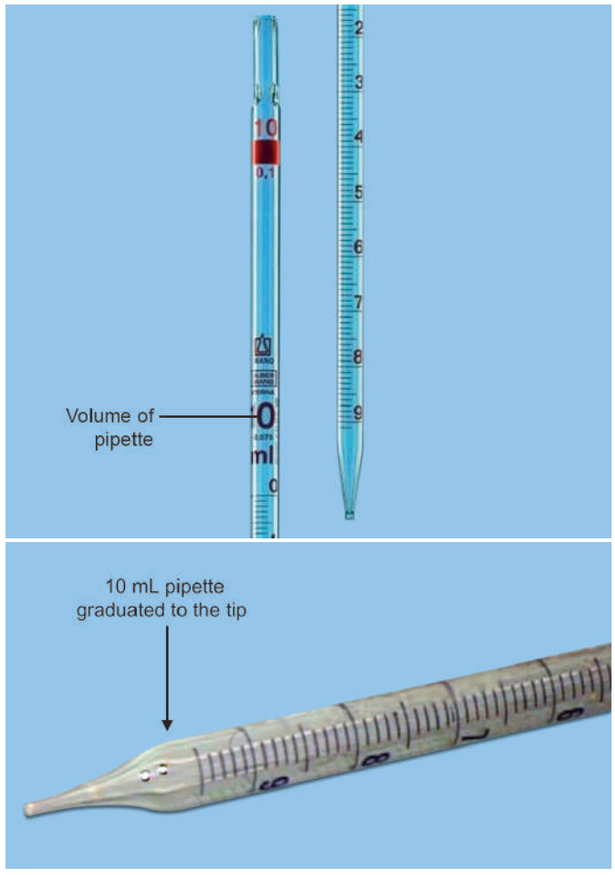


Fig. 1.16: Serological blowout pipette. Notice the marking till tip

Transfer type of pipettes: They are designed to deliver a specific volume alone. They are of following types (Fig. 1.17):

1. *Volumetric*: This type of pipette has bulb in it and it is self-draining type.
2. *Ostwald-Folin*: This type of pipette has bulb in it and is blowout type.
3. *Pasteur*: No calibration mark, not used for quantitative test (Fig. 1.18).

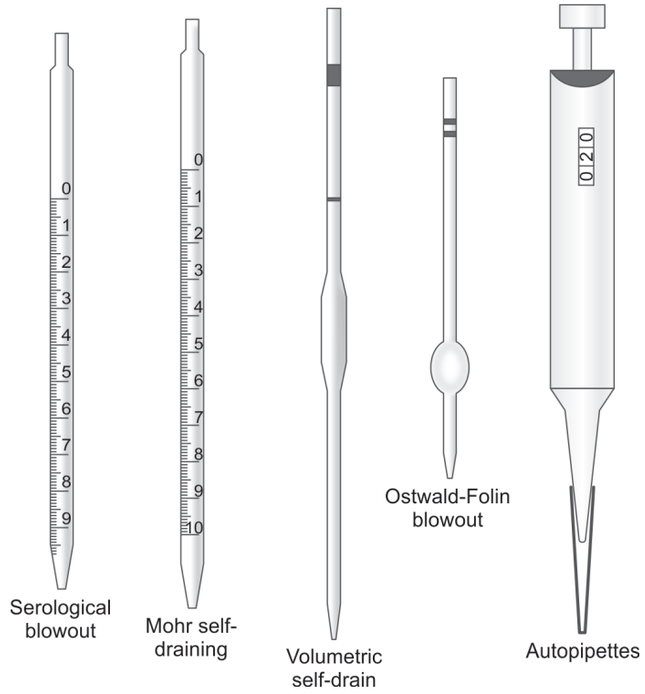


Fig. 1.17: Various types of pipettes

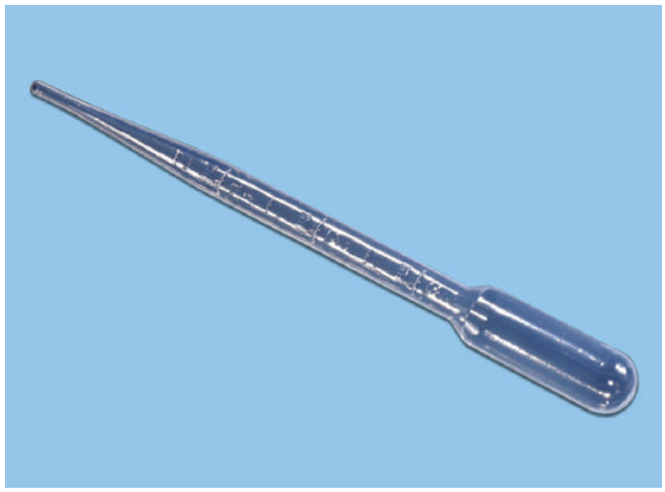


Fig. 1.18: Pasteur pipette

Nowadays **autopipettes** are used commonly to transfer volume less than 1 mL. They are easy to use and accuracy is high. They are of mainly two varieties: **Fixed volume and variable type.** Those which are designed to transfer volume of fluid less than 1 mL are called micropipettes and those which transfer larger volume of liquid are called **automatic macropipettes** (Fig. 1.19).

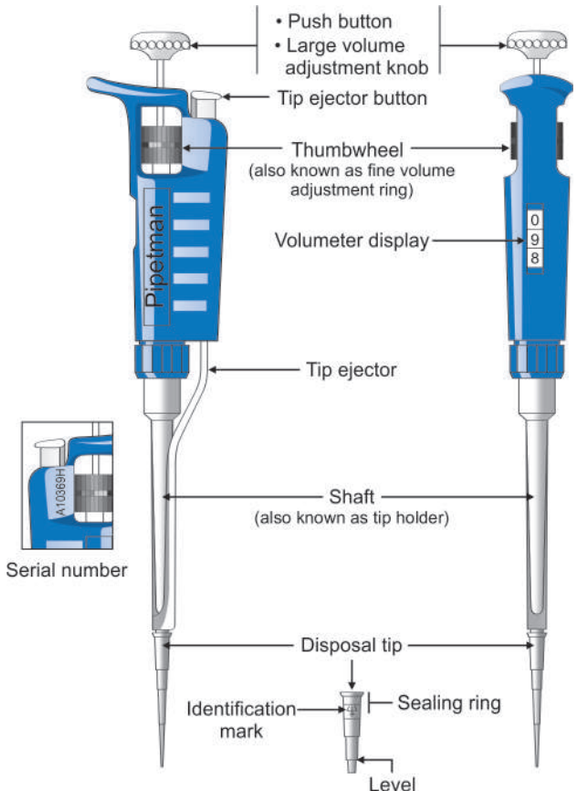


Fig. 1.19: Autopipette

Burette

It is long, graduated big pipette-like structure with a stopcock at the lower end. Its volume ranges from 25 to 100 mL. It is used to dispense desired volume of liquid drop by drop (Fig. 1.20).

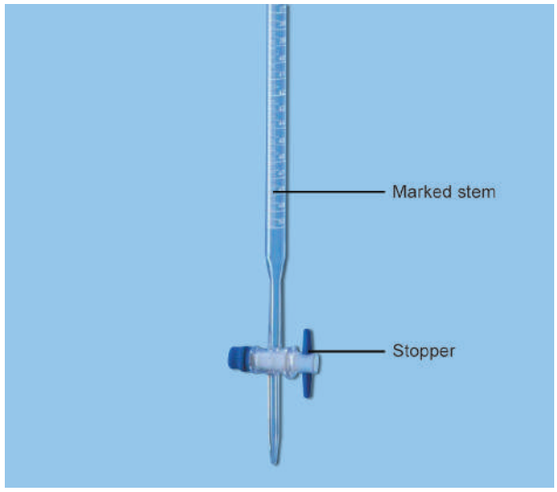


Fig. 1.20: Burette

Semiautoanalyzer

Principle and Functioning

Semiautomatic biochemistry analyzer is capable of performing routine biochemistry, hormonal assay, electrolytes, therapeutic drugs and drug-enzyme investigations. This instrument is based on principle of photometry described under colorimeter description in Chapter 5.

Semiautomated biochemistry analyzer is capable to perform tests on whole blood, serum, plasma, cerebrospinal fluid and urine as sample and it is suitable for small-sized laboratory (Fig. 1.21).

Fig. 1.21: Semiautoanalyzer

Fully Autoanalyzer

Principle and Functioning

The automatic biochemical analyzer has automated the sequence of operation processes which used to be conducted manually in past (Fig. 1.22).

It is based on colorimetric principle.

Important components of fully autoanalyzer are given in Fig. 1.23.

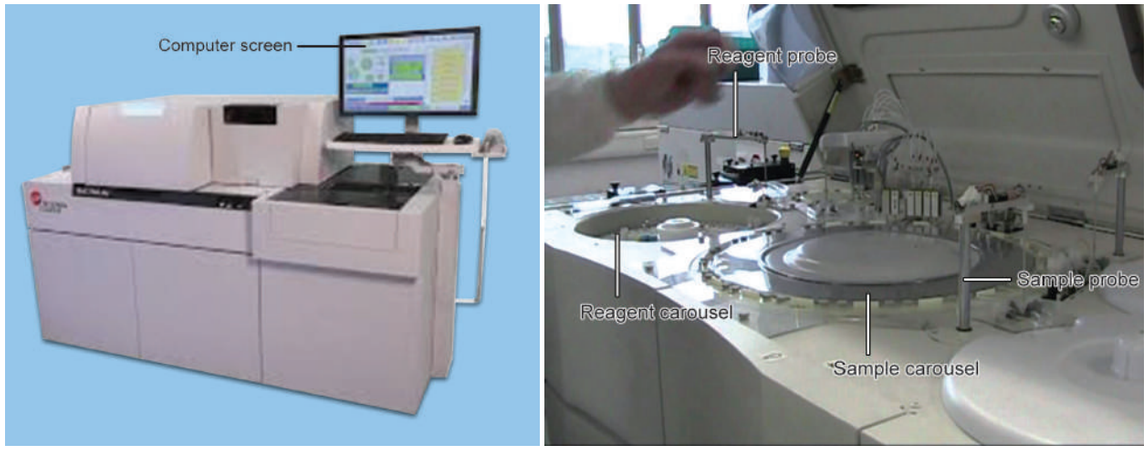


Fig. 1.22: Fully autoanalyzer

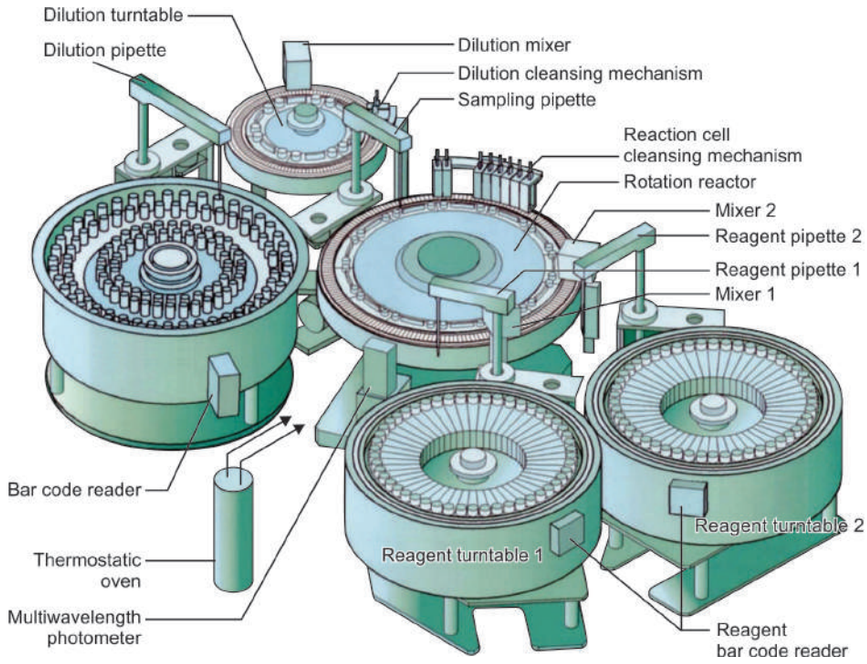


Fig. 1.23: Core components of fully automated biochemistry analyzer

Following are important parts in a fully autoanalyzer:

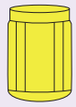
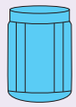
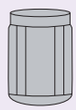
1. Reagent carousel
2. Sample compartment
3. Reaction cell
4. Sample probe (pipette)
5. Reagent probe (pipette)
6. Dilution pipette
7. Mixer

There are varieties of blood collection tube (vacutainers) available for collection of blood for specific purposes (Fig. 1.24).

Their types, color of cap, additives (if any), purpose and mixing instruction is given in Table 1.1.

Fig. 1.24: Actual picture of various types of vacutainers

Table 1.1: Various types of vacutainers as coded by colour of its cap and their purpose

Type of vacutainer/ Colour of the cap	*Representation*	*Additive*	*Purpose*	*Mixing instruction*
Red [glass] [clotting time: 60 min]		Nil	Serum biochemistry	Do not invert
Red [plastic] [clotting time: 60 min]		Silicone coating	Serum biochemistry	Invert 4 times
Yellow [clotting time: 30 min]		Clot activator	Serum biochemistry	Invert 4 times
Orange [clotting time: 5 min]		Rapid serum tube [thrombin based clot activator]	Serum biochemistry	Invert 4 times
Light blue		Sodium citrate [1:9]	Coagulation studies	Invert 4 times
Black		Sodium citrate [1:4]	Coagulation study	
Green		Heparin	ABG analysis	Invert 8 times
Lavender/purple		EDTA	Hemogram, ESR, HbA1c	Invert 8 times
Grey		Potassium oxalate and sodium fluoride	Blood glucose, blood alcohol	Invert 8 times

VIVA VOCE

Q1. How many types of balances are being used in biochemistry lab?
Ans. There are various types of balances used in biochemistry lab. They are:
a. Physical/mechanical balance
b. Electronic balance

Q2. What do you understand by term "Taring" in electronic balance?
Ans. Facility of "taring" in electronic balances makes the measurement easier as the weight of container is adjusted by the machine itself.

Q3. What is magnetic stirrer and why it is used?
Ans. This is used for thorough mixing of solutes in the solution to make a homogenous mixture

Q4. How many variations of centrifuge you know?
Ans. Centrifuge can be classified as
a. Bench-top or floor model
b. With refrigeration and without refrigeration
c. Based on rotor design
d. Based on maximum speed which can be attained (normal or ultracentrifuge)

Q5. What is the rotor in a centrifuge?
Ans. Rotor is the component of the centrifuge machine which is holding the tubes or vacutainers. Based on rotor design, we have two types of centrifuge:
- Horizontal rotor type (swing out head)
- Fixed angle rotor type (angle head)

Q6. What is the principle of centrifuge?
Ans. Principle of centrifuge machine

Key factor in separating the particulate matter from liquid suspension is the relative centrifugal force (RCF) which is represented by the following formula:

$$RCF = R \times (RPM)^2 \times 118 \times 10^{-7}$$

R is the rotating radius of the rotor which means the radius of rotating path from the central axis.

RPM (revolution per minute): It is the number of revolution of rotor in a minute. It can be programmed in the centrifuge.

Q7. What is ultracentrifuge? Mention its use.
Ans. The centrifuge which can be operated at very high speed (1,00,000 RPM) is known as ultracentrifuge.

This is used for:
1. Drying of the glassware
2. Heating the chemicals wherever required
3. Dry sterilization of glassware (in microbiological experiments mainly), metal equipment, swabs, etc. (in surgical units).

Q8. What is desiccator and what is desiccant?
Ans. Desiccator and desiccants

Desiccants are hygroscopic substances which absorb water from atmosphere and from various other hydrated substances. In other words, desiccants are drying agents.

In biochemical lab, we need to remove moisture from many chemicals before using them for analytical purpose, for that these chemicals are kept in contact with desiccants in a closed, airtight chamber with a lid with a lubricated seal is known as desiccator.

Q9. What is pH?

Ans. pH of a solution is negative logarithm of the hydrogen ion concentration. It can be expressed as

$$pH = 1/\log 10\ (H^+)$$

As stated in equation above, pH of a solution is inversely proportional to its hydrogen ion concentration.

Plasma = 7.35 to 7.45 [Mean = 7.4]
Urine = 6.5
CSF = 7.32
Gastric juice = 1.0 to 2.0

Q10. Mention the difference between Mohr pipette and serological pipette.

Ans.

Mohr type: Mohr pipette does not have graduations till tip and it is self-draining type.

Serological type: Serological pipette has graduation till tip and is blowout type.

Notes