Clinical chemistry
Introduction:

- Clinical chemistry (also known as clinical biochemistry or chemical pathology) is used to study the biochemical events and parameter in the body and it is concerned with the biochemical basis of disease.
- It is a type of science that uses the application of biochemical and molecular techniques in the diagnosis of diseases. It searches for biochemical abnormalities or changes and then find interpretation for these changes to see whether they cause effects on certain diseases.
- The clinical chemistry testing system is mainly based on correlating a disease state with the concentration of molecules in body fluids, such as the blood fluid.
- These molecules in the blood are called analytes. The most common tested analytes , include biologically important ions (salts and minerals), small organic molecules such as glucose , cholesterol , urea, creatinine and large macromolecules mainly proteins.

Importance of clinical chemistry testing system

- The most important advantages of clinical chemistry testing is the role played by this system in the management of diseases.
- The management of disease include:

1.Diagnosis: Tests can be used to differentiate between disease and healthy states .The result s of these tests will decide the confirmation or rejection of clinical diagnosis.

2. Screening: Detection of disease before it is clinically diagnosed.

3.Monitoring: Following the progression of disease processes, there will be checking for response to therapy (e.g. measuring glucose levels in diabetes mellitus.

4.Prognosis: providing information on disease outcome in the future, e.g. testing cholesterol level to predict the risk of heart disease in the future.

Variables that may affect the results :Analytical errors

There are two factors can affect the result of a blood test, analytical or physiological. Analytical error is caused by the service, typically the machine or process which produces the result. Physiological error is caused by people, typically how the blood is collected, whether the patient was fasted or taking medication.

Analytical sensitivity and specificity

Each blood test will have an experimental technique to produce the result. Confusingly, two sets of sensitivity and specificity exist in blood sciences; they have totally different meanings and refer to either analytical and physiological for (diagnostic) measures.

Analytical sensitivity refers to the detection limit of the experiment. This is the smallest amount of material of interest that the experiment can detect. As technology has developed it is increasingly easier to measure smaller quantities of material, although at small levels the accuracy and precision may be lower and this can be measured using quality control.

Analytical specificity refers to whether any other similar chemicals interfere with the test.

Example. Insulin levels may be measured in diabetic management, and it is beneficial to have a test that can differentiate between insulin and proinsulin to avoid an artificially elevated result.

Precision, Accuracy, Bias are terms used to describe certain parameters of the test. Precision is how close repeated measures of the same sample lie, accuracy is how close the value reported is to the true value and bias describes variables which may affect precision and accuracy and lead to over and under reporting or large random background changes.

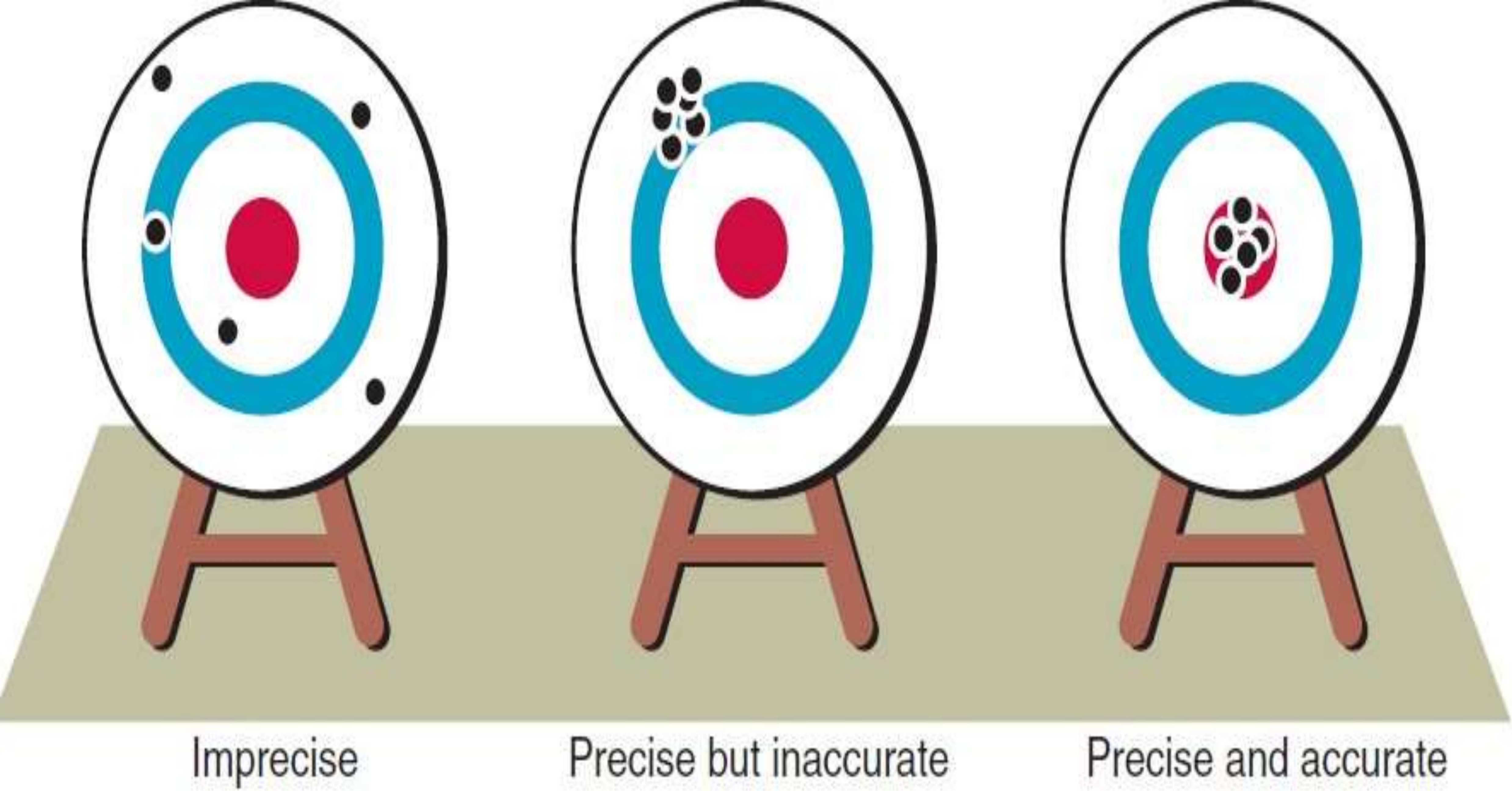
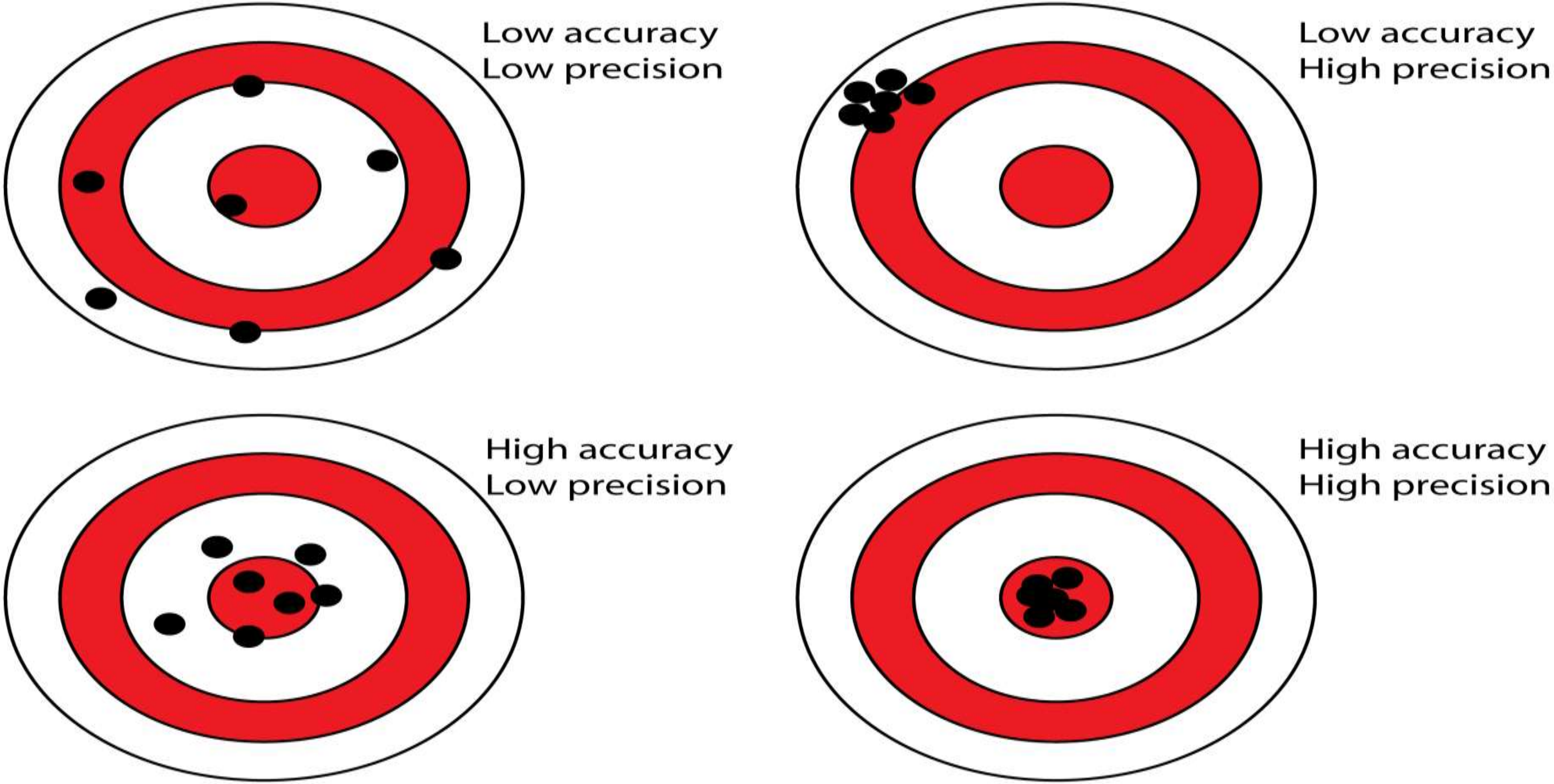


Fig 3.2 **Precision and accuracy**.


Low accuracy
Low precision
Low accuracy
High precision
High accuracy
Low precision
High accuracy
High precision

Blood composition

- Blood consists of two main parts – a fluid portion (called plasma, which contains the dissolved ions and molecules) and a cellular portion (the red blood cells, white blood cells and platelets).
- Most clinical chemistry analytes are found in the plasma. Part of the preparation of blood for testing these analytes involves removing the cells and collection of the liquid part.

HEMOLOYSIS

The rupture or lysis of RBC, releasing the cellular constituents .The presence of these components may interfere with the laboratory tests carried out on serum or plasma samples.

Therefore, care should be taken to avoid hemolysis when these liquid samples are used for biochemical tests.

Collection of blood sample:

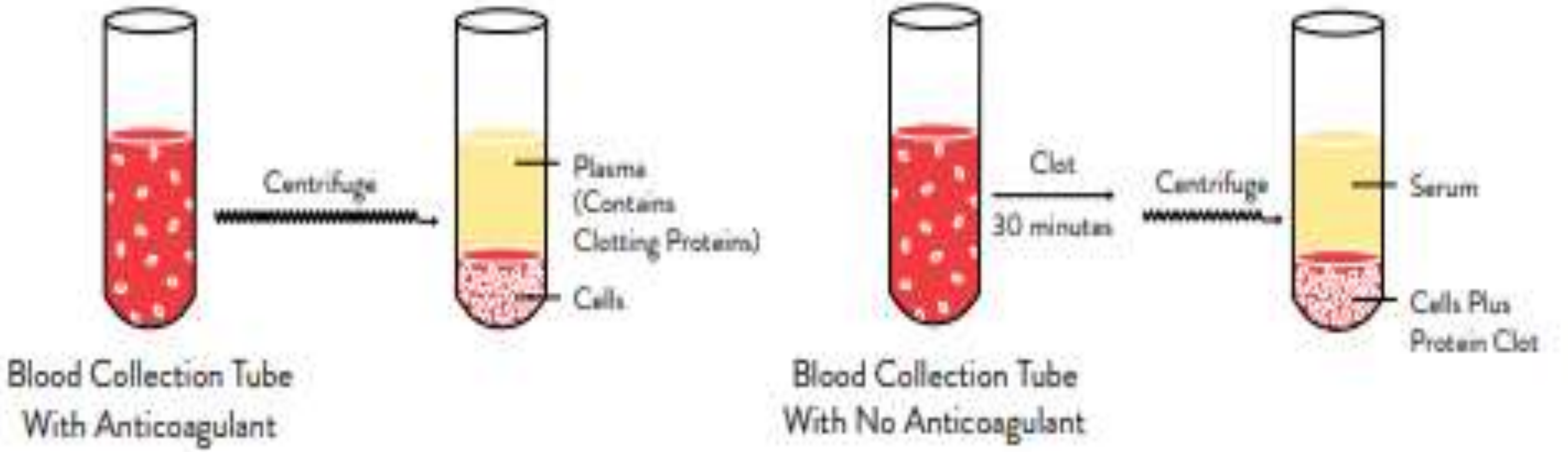
- Biochemical investigations can be performed on 4 types of blood specimens – whole blood, plasma, serum and red blood cells. The selection of the specimen depends on the analyte to be estimated.

1. **Whole blood**: When the blood is mixed with an anticoagulant to prevent clotting. It will contain all blood components.

2. **Plasma**: If the blood is mixed with an anticoagulant and centrifuged. The cell components (RBC and WBC) are precipitated. The separated supernatant is called plasma , which make up about 55-60 % of the total blood volume.

3. **Serum** :If the blood is withdrawn without anticoagulant and allowed to clot. After about two hours, the liquid portion will be separated from the clot. This defibrinated liquid is called serum, which lacks coagulation factors

4. **Red blood cells.** The non-nucleated cells isolated from whole blood . It is used for the determination of few blood components such hemoglobin.



Blood Composition

Plasma

Plasma is fluid component of blood. Comprises ~55% of total volume of whole blood. Contains proteins, sugars, vitamins,minerals, lipids, lipoproteins and clotting factors.
95% of plasma is water

White Blood cells (WBC) & Platelets

Red Blood cells (RBC)

Cellular Components

Whole Blood

Whole Blood after centrifugation
Note: clotting has been prevented

Blood Composition

If blood is collected and allowed to stand it will clot. Formation of an insoluble fibrin clot. If blood is then centrifuged the fluid portion is known as SERUM

Serum

Plasma is fluid component of blood. Comprises ~55% of total volume of whole blood. Contains proteins, sugars, vitamins,minerals, lipids, lipoproteins
No clotting factors
95% of plasma is water

Blood Clot
-comprised of clotting factors (Fibrin,platets etc)
-RBCs

Whole Blood

Whole Blood after clotting and centrifugation

- Serum contains all the components of plasma except the clotting proteins, which are consumed in the cascade of reactions that form the blood clot.
- Some clinical chemistry tests are best performed using plasma, others are best performed using serum, and still others can be performed using either plasma or serum.

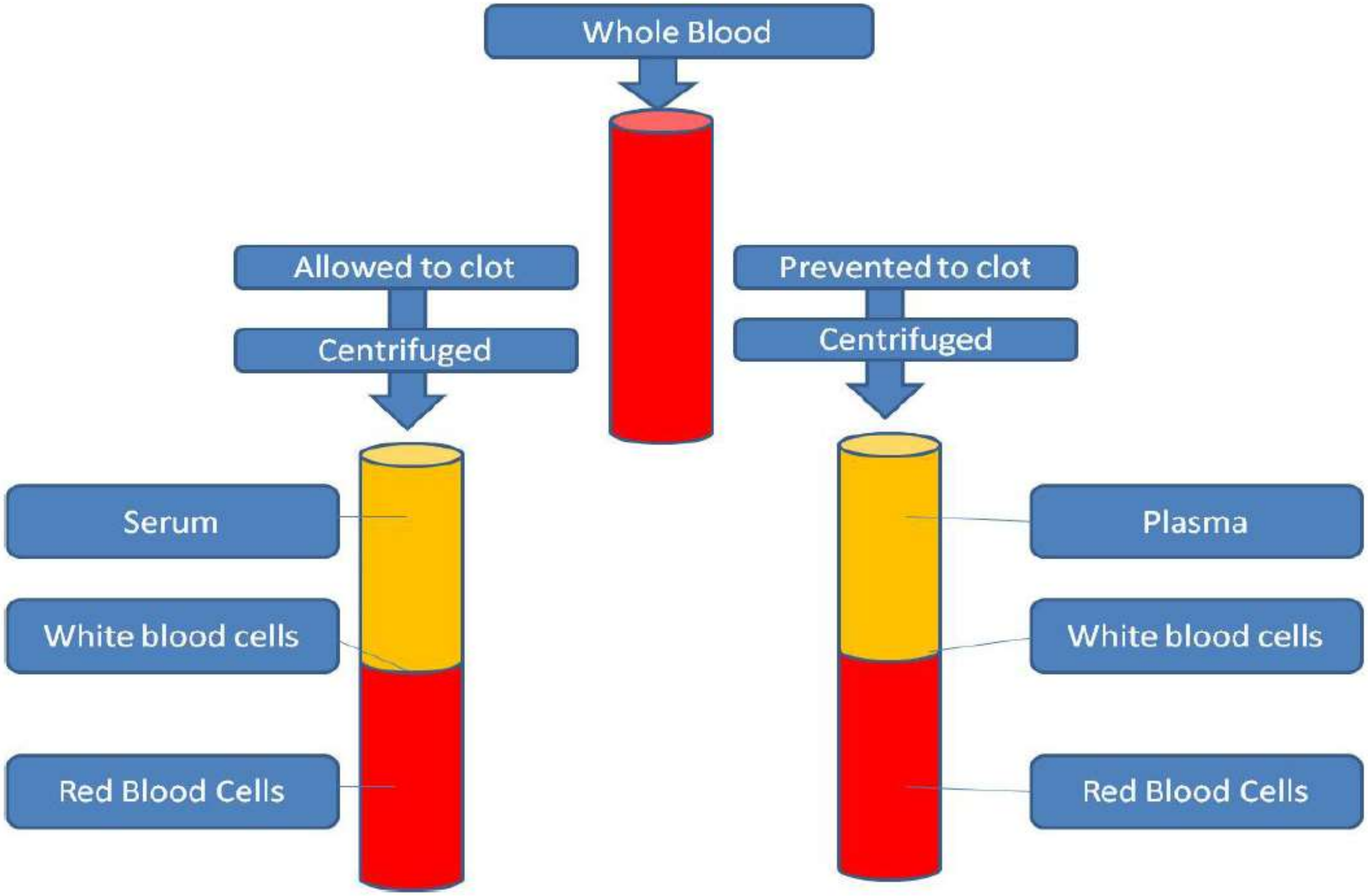


Figure 1.6: The difference between plasma and serum

ANTICOAGULANTS

- Certain biochemical tests require unclotted blood, which can be obtained by adding certain chemicals called anticoagulants.
- **Heparin** (inhibits the conversion of prothrmobin to thrombin) is the most widely used anticoagulant for clinical chemical analysis. Heparin is an ideal anticoagulant, since it does not cause any change in blood composition. However, other anticoagulants are preferred to heparin, due to the cost factor.
- **Ethylene diamine tetra acetic acid (EDTA)** is a chelating agent, and is particularly useful for hematological examination because it preserves cellular components of the blood. It chelates with calcium and stops coagulation.
- **Sodium fluoride** is an anticoagulant and also used as antiglycolytic agent to preservative blood glucose by inhibiting the enzyme systems involved in the glycolysis. Without sodium fluoride , the blood glucose concentration decreases about 10 mg/dl per hour and false results may be obtained.
- **Oxalate** inhibits blood coagulation by forming insoluble complexes with calcium ions.

Tube cap color	Additive	Function of Additive	Common laboratory tests
Light-blue 	3.2% Sodium citrate	Prevents blood from clotting by binding calcium	Coagulation
Red or gold (mottled or "tiger" top used with some tubes is not shown) 	Serum tube with or without clot activator or gel	Clot activator promotes blood clotting with glass or silica particles. Gel separates serum from cells.	Chemistry, serology, immunology
Green 	Sodium or lithium heparin with or without gel	Prevents clotting by inhibiting thrombin and thromboplastin	Stat and routine chemistry
Lavender or pink 	Potassium EDTA	Prevents clotting by binding calcium	Hematology and blood bank
Gray 	Sodium fluoride, and sodium or potassium oxalate	Fluoride inhibits glycolysis, and oxalate prevents clotting by precipitating calcium.	Glucose (especially when testing will be delayed), blood alcohol, lactic acid

EDTA

Sodium fluoride

Heparin

Other biologic fluids

- Other biologic fluids (matrices) often used for testing include urine, saliva, cerebrospinal fluid (CSF), amniotic fluid, synovial fluid, pleural fluid, peritoneal fluid and pericardial fluid.
- These fluids often contain the same biologic analytes of interest – such as glucose and protein – but differ greatly from each other in physical and chemical properties. These differences in fluid characteristics are termed matrix differences. Test methods that are designed for determination of an analyte in blood plasma may not be suitable for determination of that same analyte in other fluids (other matrices).
- When using a test method for analysis of a fluid other than blood plasma or serum, it is important to validate that the method is acceptable for the type of fluid sample being used.

FLUIDS TYPICALLY USED FOR CLINICAL CHEMISTRY TESTS
Blood (whole blood, serum or plasma)
Urine
Cerebrospinal fluid (CSF)
Amniotic fluid
Saliva
Synovial fluid (fluid that is found in joint cavities)
Pleural fluid (from the sac surrounding the lungs)
Pericardial fluid (from the sac surrounding the heart)
Peritoneal fluid (also called ascitic fluid; from the abdomen)

URINE

- Urine, containing the metabolic waste products of the body in water and considered the most important excretory fluid.
- It is especially suitable sample for tests that evaluate kidney functions, by using tests that look at waste products that are excreted by the kidneys, and for metabolites that are cleared quickly from the bloodstream and accumulate in the urine, such as drugs of abuse.
- Sometimes both serum and urine concentrations of a substance are analyzed to know if its expected excretion is taking place correctly or to determine if its leakage is unexpectedly occurring.
- Urine samples can be concentrated or dilute depending on the hydration status and kidney function of the patient. These differences in urine can affect the amount of a substance found in a sample at different times.
- Since creatinine is excreted at fairly constant rates over time, urine analytes are sometimes normalized to the amount of creatinine in the sample in order to correct for the differences in the hydration state of the patient and concentrated versus dilute samples.

- Cerebrospinal Fluid (CSF): CSF is a fluid of the nervous system , formed by a process of selective dialysis of plasma from the interspace ventricles of the brain.
- Spinal fluid is used primarily for assessment of patients with symptoms of diseases such as meningitis or multiple sclerosis or patients who may have suffered a cerebrovascular accident.
- Saliva is rarely used in clinical laboratory testing, but is recognized as a specimen whose composition reflects the blood plasma levels of many low molecular weight substances such as drugs or alcohol. Saliva also has an advantage for hormones like cortisol for pediatric patients, when blood collection is too painful or stressful
- Amniotic fluid is typically used for tests of fetal health.

Diagnostic tests and objective of their request

- Clinical biochemistry is one of the most important parts of laboratory diagnostics together with laboratory haematology, immunology, clinical serology and microbiology, clinical toxicology.
- It possesses the largest number of diagnostic tests that help understand different pathological diseases.
- There are over 400 different tests which may be carried out in clinical biochemistry laboratories.
- Clinical biochemical tests represent over ⅓ of all hospital laboratory investigations.

Types of Diagnostic tests

1.Core biochemical tests: (Routine tests)

- They are the core analyses or commonly requested tests carried out by most biochemistry laboratories

2. Specialized tests:

- Not every laboratory is equipped to carry out all possible biochemistry requests. Those less commonly asked tests are performed by special labs called reference centers.
- Example of specialized tests:

  Hormones, Specific proteins, Vitamins, Drugs, DNA analyses

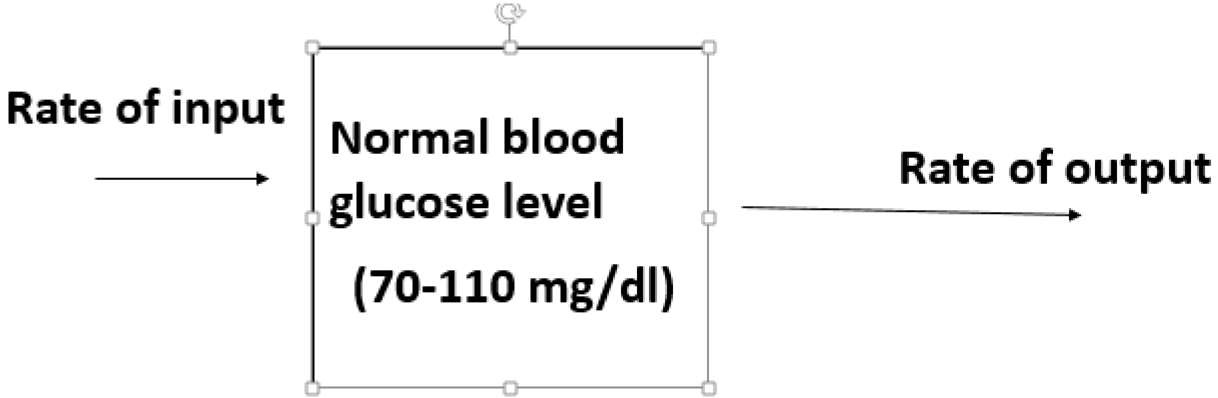
3. Emergency tests:

These urgent test specially used in special care unites to obtain very quick results that can be used by the clinicians to suggest an immediate treatment.

- Emergency tests are mainly :Urea , electrolytes and glucose

Principle of clinical chemistry testing

- Under normal conditions (healthy), the analytes almost maintain constant levels in circulations due to a balance between rate of input and rate of output.
- Example is the homeostasis of glucose to maintain a constant level in blood.



- Under disease conditions this balance is disturbed causing either increase or decrease in concentration of blood analyte.
- This change in concentration of the analyte can be measured to diagnose the type of disease that caused the imbalance in analyte concentration.