Medical laboratory: **A medical laboratory or clinical laboratory** is a laboratory where tests are done on clinical specimens in order to get information about the health of a patient as pertaining to the diagnosis, treatment, and prevention of disease. Laboratory and diagnostic tests are tools to gain additional information about the patient. By themselves, these tests are not therapeutic; however, when used in conjunction with a thorough history and physical examination, these tests may confirm a diagnosis or provide valuable information about a patient's status and response to therapy that may not be apparent from the history and physical examination alone. Generally, an evidenced-based tiered approach to selecting tests is used:

1. Basic screening (frequently used with wellness groups and case finding)
2. Establishing (initial) diagnoses
3. Differential diagnosis
4. Evaluating current medical case management and outcomes
5. Evaluating disease severity.
6. Detect disease recurrence, and select drugs and adjust therapy.
7. Estimate prognosis.
8. Monitoring course of illness and response to treatment
9. Group and panel testing
10. Regularly scheduled screening tests as part of ongoing care
11. Testing related to specific events, certain signs and symptoms, or other exceptional situations (eg, infection and inflammation [bladder infection or cellulitis], sexual assault, drug screening, pheochromocytoma, postmortem tests, to name a few) .

Departments: Laboratory medicine is generally divided into two sections, each of which being subdivided into multiple units. **These two sections are:**

Section one	Section two
A-Anatomic pathology: units included here are histopathology, cytopathology, and electron microscopy. Academically, each unit is studied alone in one course. Other courses pertaining to this section include anatomy, physiology, histology, pathology, and pathophysiology.	B-Clinical pathology, which includes: 1. Clinical Microbiology: This encompasses five different sciences (units). These include bacteriology, virology, parasitology, immunology, and mycology. 2. Clinical Chemistry: Units under this busy section include instrumental analysis of

	blood components, enzymology, toxicology and endocrinology. 3. Hematology: This section consists of automated and manual analysis of blood cells. It includes two subunits, which are coagulation and blood bank. 4. Genetics is also studied along with a subspecialty known as cytogenetics. 5. Reproductive biology: Semen analysis, Sperm bank and assisted reproductive technology.

Distribution of clinical laboratories in health institutions varies greatly from one place to another. For instance, for microbiology, some health facilities have a single laboratory for microbiology, while others have a separate lab for each unit, with nothing called a "microbiology" lab.

The following is a detailed breakdown of the responsibilities of each unit:

1. **Microbiology** receives almost any clinical specimen, including swabs, feces, urine, blood, sputum, cerebrospinal fluid, synovial fluid, as well as possible infected tissue. The work here is mainly concerned with cultures, to look for suspected pathogens which, if found, are further identified based on biochemical tests. Also, sensitivity testing is carried out to determine whether the pathogen is sensitive or resistant to a suggested medicine. Results are reported with the identified organism(s) and the type and amount of drug(s) that should be prescribed for the patient. 2. **Parasitology** is a microbiology unit that	9. **Immunohaematology, or Blood bank** determines blood groups, and performs compatibility testing on donor blood and recipients. It also prepares blood components, derivatives, and products for transfusion. Regulated by the FDA since giving blood is considered a drug, this unit determines a patient's blood type and Rh status, checks for antibodies to common antigens found on red blood cells, and cross matches units that are negative for the antigen. 10. **Urinalysis tests** urine for many analytes. Some health care providers have a urinalysis laboratory, while others don't. Instead, each component of the urinalysis

investigates parasites. The most frequently encountered specimen here is faeces. However, blood, urine, sputum, and other samples may also contain parasites.

3. **Virology** is concerned with identification of viruses in specimens such as blood, urine, and cerebrospinal fluid.
4. **Hematology** works with whole blood to do full blood counts, and blood films as well as many other specialised tests.
5. **Coagulation** requires citrated blood samples to analyze blood clotting times and coagulation factors.
6. **Clinical Biochemistry** usually receives serum or plasma. They test the serum for chemicals present in blood. These include a wide array of substances, such as lipids, blood sugar, enzymes, and hormones.
7. **Toxicology** mainly tests for pharmaceutical and recreational drugs. Urine and blood samples are submitted to this lab.
8. **Immunology/Serology** uses the concept of antigen-antibody interaction as a diagnostic tool. Compatibility of transplanted organs is also determined.

is performed at the corresponding unit. If measuring urine chemicals is required, the specimen is processed in the clinical biochemistry lab, but if cell studies are indicated, the specimen should be submitted to the cytopathology lab, and so on.

11. **Histopathology processes** solid tissue removed from the body (biopsies) for evaluation at the microscopic level.
12. **Cytopathology** examines smears of cells from all over the body (such as from the cervix) for evidence of inflammation, cancer, and other conditions.
13. **Electron microscopy** prepares specimens and takes micrographs of very fine details.
14. **Genetics** mainly performs DNA analysis.
15. **Cytogenetics** involves using blood and other cells to get a karyotype. This can be helpful in prenatal diagnosis (e.g. Down's syndrome) as well as in cancer (some cancers have abnormal chromosomes).
16. **Surgical pathology** examines organs, limbs, tumors, fetuses, and other tissues biopsied in surgery such as breast mastectomys.

Medical laboratory staff: **The following is the hierarchy of the clinical laboratory staff from highest authority to lowest:**

1. Medical Director
2. Pathologist, Clinical biologist
3. Resident in Pathology, Anatomical

8. Cytotechnologist, Medical Laboratory Scientist, Histotechnologist,
9. Medical Laboratory Technician,

pathology or Clinical biology **4.** Pathologist Assistant, **5.** Laboratory Manager, **6.** Department Supervisor, **7.** Chief/Lead Technologist,	Histotechnician **10.** Medical Laboratory Assistant (Lab Aide), **11.** Phlebotomist, **12.** Transcriptionist, **13.** Specimen processor, Secretary**.**

Terms :

1- Sensitivity: Sensitivity relates to the test's ability to identify a condition correctly. Consider the example of a medical test used to identify a disease. Sensitivity of the test is the proportion of people known to have the disease, who test positive for it. Mathematically, this can be expressed as:

$$\text{sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

$$= \frac{\text{number of true positives}}{\text{total number of sick individuals in population}}$$

$$= \text{probability of a positive test, given that the patient is ill}$$

2- Specificity: Specificity relates to the test's ability to exclude a condition correctly. Consider the example of a medical test for diagnosing a disease. Specificity of a test is the proportion of healthy patients known not to have the disease, who will test negative for it. Mathematically, this can also be written as:

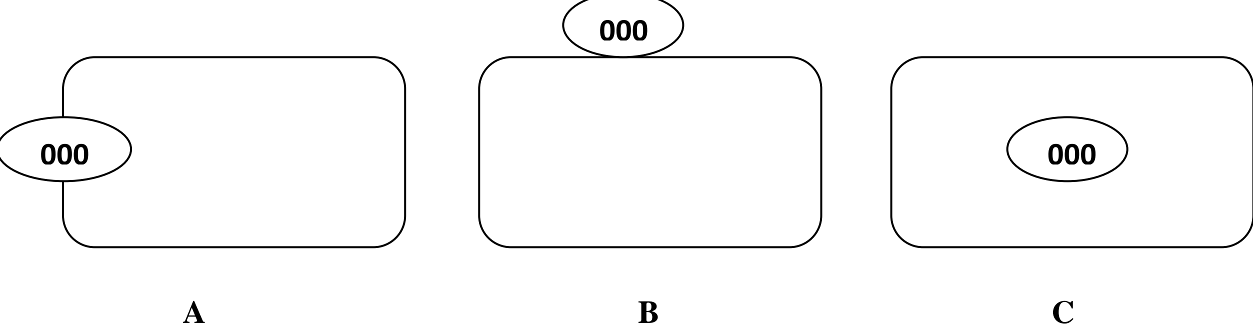
$$\text{specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

$$= \frac{\text{number of true negatives}}{\text{total number of well individuals in population}}$$

$$= \text{probability of a negative test given that the patient is well}$$

3- Accuracy and precision : In the fields of science, engineering, industry, and statistics, the accuracy of a measurement system is the degree of closeness of measurements of a quantity to that

quantity's actual (true) value. The precision of a measurement system, related to reproducibility and repeatability, is the degree to which repeated measurements under unchanged conditions show the same results. Although the two words precision and accuracy can be synonymous in colloquial use, they are deliberately contrasted in the context of the scientific method.



A B C

Figure 1: show a relationship between accuracy and precision in diagnostic tests. The center of the target represents the true value of the substance being tested. Figure **(A)** represents a diagnostic test which is precise but inaccurate; on repeated measurement, the test yields very similar results, but all results are far from the true value. Figure **(B)** shows a test which is imprecise and inaccurate; repeated measurement yields widely different results, and the results are far from the true value. Figure **(C)** shows an ideal test, one that is both precise and accurate**.**

Interfering Factors : The results of diagnostic tests can be altered by factors. Reasons for deviations may include the following: **1-**Incorrect specimen collection, handling, storage, or labeling. **2-**Wrong preservative or lack of preservative .**3-** Delayed specimen delivery. **4-** Incorrect or incomplete patient preparation. **5-**Hemolyzed blood samples. **6-**Incomplete sample collection, especially of timed samples 7-Old or deteriorating specimens.

Patient factors that can alter test results may include the following:

1. Incorrect pretest diet **2.** Current drug therapy **3.** Type of illness **4.** Dehydration **5.** Position or activity at time of specimen collection **6.** Postprandial status (i.e. time patient last ate) **7.** Time of day	**8.** Pregnancy **9.** Level of patient knowledge and understanding of testing process **10.** Stress **11.** Non-adherence or noncompliance with instructions and pretest preparation **12.** Undisclosed drug or alcohol use **13.** Age and gender

General urine examination (GUE)

Urine is a typically sterile liquid by-product of the body secreted by the kidneys through a process called urination and excreted through the urethra. Cellular metabolism generates numerous by-products, many rich in nitrogen, that require elimination from the bloodstream. These by-products are eventually expelled from the body during urination, the primary method for excreting water-soluble chemicals from the body. These chemicals can be detected and analyzed by urinalysis. Certain disease conditions can result in pathogen-contaminated urine. A urinalysis (UA), also known as general urine examination (GUE) is an array of tests performed on urine, and one of the most common methods of medical diagnosis. The target parameters that can be measured or quantified in urinalysis include many substances and cells, as well as other properties, such as specific gravity. A part of a urinalysis can be performed by using urine test strips, in which the test results can be read as color changes. Another method is light microscopy of urine samples. Over 100 different tests can be done on urine. Urinalysis can be part of a routine examination and is frequently performed upon admission to the hospital and before surgery.

Purpose : **1-** General evaluation of health. **2-**Diagnosis of disease or disorders of the kidneys or urinary tract. **3-**Diagnosis of other systemic disease that affect kidney function.**4**-monitoring of patients with diabetes and other conditions .**5-**Screening for drug abuse (eg. Sulfonamide or aminoglycosides). **6-** In general, tests on urine provide information and clues to many diseases, and can also be indications of the condition of a patient's health. A routine urine-screening test may be done to help find the cause for a number of different symptoms.**7-** Liver diseases and haemolytic disorders.

A routine urinalysis or general urine examination usually involves the following tests:

1- Macroscopic urinalysis: includes physical and chemical examination. 2- Microscopic examination

Macroscopic urinalysis: A- Physical examination includes: ❖

Color: Urine varies in appearance, depending principally upon a body's level of hydration, as well as other factors. Normal urine is a transparent solution ranging from colorless to amber but is usually a pale yellow. In the urine of a healthy individual the color comes primarily from the presence of urobilin. Urobilin in turn is a final waste product resulting from the breakdown of heme from hemoglobin during the destruction of aging blood cells..

- Dark yellow urine is often indicative of dehydration.

- Yellowing/light orange may be caused by removal of excess B vitamins from the bloodstream.
- Certain medications such as rifampin can cause orange urine.
- Bloody urine is termed hematuria, a symptom of a wide variety of medical conditions.
- Dark orange to brown urine can be a symptom of jaundice
- Black or dark-colored urine is referred to as melanuria and may be caused by a melanoma.

Odor: Urine does not smell very strong, but it has a slightly "nutty" odor. The odor of normal human urine can reflect what has been consumed or specific diseases. For example, an individual with diabetes mellitus may present a sweetened urine odor. This can be due to kidney diseases as well, such as kidney stones. Eating asparagus can cause a strong odor reminiscent of the vegetable caused by the body's breakdown of asparagusic acid. Likewise consumption of saffron, alcohol, coffee, tuna fish, and onion can result in telltale scents

Turbidity: Turbid (cloudy) urine may be a symptom of a bacterial infection, but can also be caused by crystallization of salts such as calcium phosphate. Urine is normally clear. Bacteria, blood, sperm, crystals, or mucus can make urine look cloudy. **Clarity**: normally clear , **Abnormal color**: cloudy urine Causes: 1. crystals or non-pathologic salts phosphate, carbonate in alkaline urine (dissolve---add acetic acid) uric acid in acid urine (dissolve-warming to 60°C) 2. Various cellular elements: leukocytes, RBCs, epithelial cells.

Volume: Average urine production in adult humans is about 1 – 2 L per day, depending on state of hydration, activity level, environmental factors, weight, and the individual's health. Producing too much or too little urine needs medical attention. Polyuria is a condition of excessive production of urine (> 2.5 L/day), oliguria when < 400 mL are produced, and anuria one of < 100 mL per day.

Density or specific gravity: Reflect the density of the urine (Range of 1.001 to 1.040). This checks the amount of substances in the urine. It also shows how well the kidneys balance the amount of water in urine. The higher the specific gravity, the more solid material is in the urine. When you drink a lot of fluid, your kidneys make urine with a high amount of water in it, which has a low specific gravity.. **Increase**: Dehydration、fever、vomiting, diarrhea、congestive Heart Failure, (urine volume↓ and SG↑); **Decrease:** diabetes mellitus (urine volume↑ and SG ↓)

B - Chemical examination (urine test strip) includes: A chemical examination or urine test strip or dipstick is a basic diagnostic tool used to determine pathological changes in a patient's urine in

standard urinalysis. A standard urine test strip may comprise up to 10 different chemical pads or reagents which react (change color) when immersed in, and then removed from, a urine sample. The test can often be read in as little as 60 to 120 seconds after dipping, although certain tests require longer. Routine testing of the urine with multi-parameter strips is the first step in the diagnosis of a wide range of diseases. The analysis includes testing for the presence of proteins, glucose, ketones, haemoglobin, bilirubin, urobilinogen, acetone, nitrite and leucocytes as well as testing of pH and specific gravity or to test for infection by different pathogens. Semi-quantitative values are usually reported as: trace, 1+, 2+, 3+ and 4+; although tests can also be estimated as milligrams per deciliter. Automated readers of test strips also provide results using units from the International System of Units.

1-pH : The pH of urine can vary between 4.5 and 8 with the first urine produced in the morning generally being more acidic and the urine produced after meals generally more alkaline. The determination of urinary pH has two main objectives, one is diagnostic and the other is therapeutic. On the one hand it provides information regarding the balance between acid and alkali in a patient and allows identification of the substances that are present in the urine in crystalline form. On the other hand, certain illnesses require a patient to keep the pH of their urine within given narrow margins, whether to promote the elimination of chemotherapeutic agents, avoid the precipitation of salts that promote the formation of gallstones, or in order to facilitate the control of a urinary infection.

2- Protein testing: Urine usually contains only low concentrations of proteins, less than 10 mg/dL or 100 mg in 24 hours is excreted, these are mostly low molecular weight proteins, filtered through the kidney's glomerulus, and proteins produced in the genitourinary tract. The main serum protein found in urine is *albumin* as it has a low molecular weight. However, only low concentrations are normally found in urine because it is generally not filtered out in the glomerulus and in addition those molecules that do pass through the glomerulus are reabsorbed in the tubules. Proteinuria can be the first symptom of nephropathy or it can be caused by the accumulation of abnormal proteins due to diseases such as *multiple myeloma*.

3-Glucose test ***:*** Under normal conditions nearly all the glucose removed in the glomerulus is reabsorbed in the proximal convoluted tubule. If the blood glucose level increases, as happens in diabetes mellitus, the capacity of the convoluted tubule to reabsorb glucose is exceeded (an effect know as renal reabsorption threshold) For glucose this threshold is between 160–180 mg/dl. Glucose concentrations vary in an individual, and a healthy person can present with transitory glucosuria after a meal high in sugars, therefore the most representative results come from samples obtained at least two

hours after food is eaten. The detection of glucose by test strips is based on the enzymatic reaction of glucose oxidase.

4-Ketone test : The term ketones or ketone bodies in reality refers to three intermediate products in the metabolism of fatty acids; acetone, acetoacetic acid and beta-hydroxybutyric acid. Elevated concentrations of ketones are not generally found in urine, as all these substances are completely metabolized, producing energy, carbon dioxide and water. However, the disruption of carbohydrate metabolism can lead to metabolic imbalances and the appearance of ketones . The control of urinary ketone is particularly useful in managing and monitoring diabetes mellitus type 1. Ketonuria indicates an insulin deficiency that indicates the need to regulate its dosage. An increase in the blood concentration of ketone produces a water-electrolyte imbalance, dehydration and in the end diabetic coma.

5-Bilirubin test: Bilirubin is a highly pigmented compound that is a by-product of haemoglobin degradation. Conjugated bilirubin appears in urine when the normal degradation cycle is altered due to the obstruction of the biliary ducts or when the kidney's functional integrity is damaged. This allows the escape of conjugated bilirubin into the circulation as occurs in hepatitis and hepatic cirrhosis).The detection of urinary bilirubin is an early indication of liver disease and can be used to determine the causes of clinical jaundice.

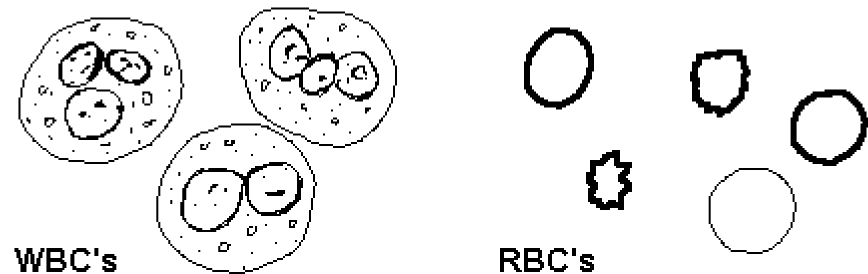
6- Nitrites test: The test for nitrites is a rapid screening method for possible asymptomatic infections caused by nitrate-reducing bacteria. Some of the gram negative bacteria species that most commonly cause urinary tract infections (Escherichia coli, Enterobacter, Klebsiella, Citrobacter and Proteus) have enzymes that reduce the nitrate present in urine to nitrite. The test is a rapid screen for possible infections by enteric bacteria, but it does not replace the urinalysis tests nor microscopic examination as diagnostic tools.

8-Leukocytes esterase: The urine test strip test for white blood cells detects leukocyte esterase, which is present in granules of **monocytes** and **granulocytes** (**neutrophilic**, **eosinophilic** and **basophilic**). Bacteria, lymphocytes and epithelial cells from the genitourinary tract do not contain esterases. Neutrophil granulocytes are the leukocytes most commonly associated with urinary infections. A positive test for leukocyte esterase normally indicates the presence of bacteria and a positive nitrite test

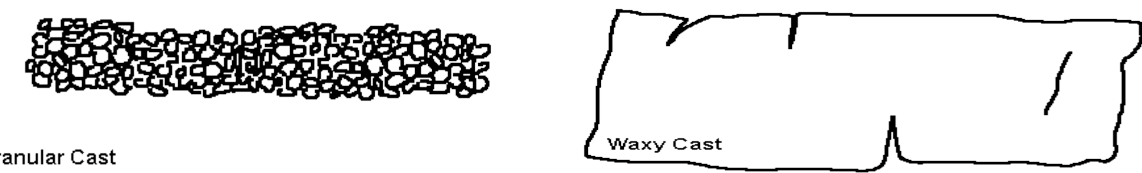
Uses for Urine Test Strips : **A-Screening** : Urine test strips are used for screening both in hospitals and in general practice. The aim of screening is early identification of likely patients by examination of large groups of the population. The importance of screening for diabetes and kidney disease amongst

high-risk populations is becoming very high. **B-Treatment Monitoring:** Treatment monitoring with the aid of urine test strips allows a health professional to check on the results of the prescribed therapy, and if necessary to introduce any changes into the course of therapy. **C-Self-monitoring by Patients :** Self-monitoring with urine test strips under the guidance of a health professional is an effective method for monitoring the disease state. This applies particularly to diabetics, where the idea of self-monitoring of the metabolic status (determinations of glucose and ketones) is self-evident. **D-Veterinary:** In veterinary medicine, especially in cats and dogs, the test strip can be used for urinalysis.

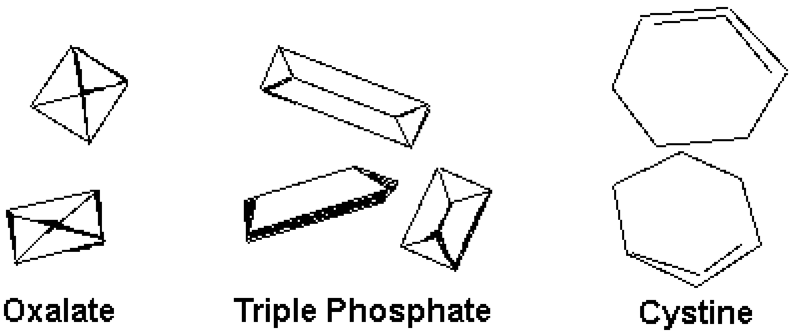
2- Microscopic examination: Examination of urine sediment may reveal the presence of different types of cells such as epithelial cells, leukocytes, erythrocytes, or renal cells. Different types of crystals, yeast, bacteria, or casts may also be present. Casts are cylindrical structures created by protein precipitation in the renal tubules. In this test, urine is spun in a special machine (centrifuge) so the solid materials (sediment) settle at the bottom. The sediment is spread on a slide and looked at under a microscope. Things that may be seen on **the slide include: Red or white blood cells.** Blood cells aren't found in urine normally. Inflammation, disease, or injury to the kidneys, ureters, bladder, or urethra can cause blood in urine. Strenuous exercise, such as running a marathon, can also cause blood in the urine. White blood cells may be a sign of infection or kidney disease.



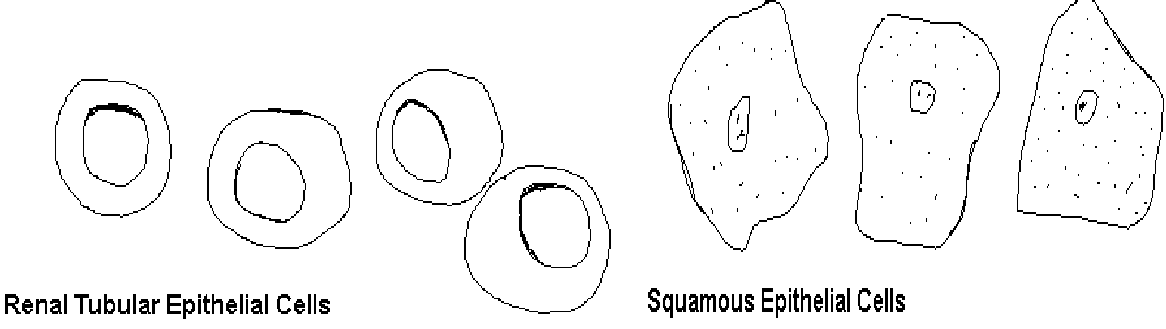
Casts. Some types of kidney disease can cause plugs of material (called casts) to form in tiny tubes in the kidneys. The casts then get flushed out in the urine. Casts can be made of red or white blood cells, waxy or fatty substances, or granular or hyaline . The type of cast in the urine can help show what type of kidney disease may be present.



Crystals. Healthy people often have only a few crystals in their urine. A large number of crystals, or certain types of crystals, may mean kidney stones are present or there is a problem with how the body is using food (metabolism).



Epithelial Cells : Renal tubular epithelial cells, usually larger than granulocytes, contain a large round or oval nucleus and normally slough into the urine in small numbers. However, with nephrotic syndrome and in conditions leading to tubular degeneration, the number sloughed is increased.



Bacteria, yeast cells, or parasites. There are no bacteria, yeast cells, or parasites in urine normally. If these are present, it can mean you have an infection. Spermatozoa can sometimes be seen. Rarely, pinworm ova may contaminate the urine.

General stool examination (GSE) : Overview of Stool Studies

The elimination of digestive waste products from the body is essential to health. These excreted waste products are known as stool or feces. Stool examination is often done for evaluation of gastrointestinal (GI) disorders. These studies are helpful in detecting GI bleeding, GI obstruction, obstructive jaundice, parasitic disease, dysentery, ulcerative colitis, and increased fat excretion.

Feces are composed of the following materials:

- Waste residue of indigestible material (eg, cellulose) from food eaten during the previous 4 days
- Bile (pigments and salts): stool color is normally due to bile pigments that have been altered by bacterial action.
- Intestinal secretions
- Water and electrolytes
- Epithelial cells that have been shed
- Large numbers of bacteria
- Inorganic material (10% - 20%), chiefly calcium and phosphates
- Undigested or unabsorbed food (normally present in very small quantities)

The output of feces depends on a complex series of absorptive, secretory, and fermentative processes. Normal function of the colon involves three physiologic processes: (1) absorption of fluid and electrolytes; (2) contractions that churn and expose the contents to the GI tract mucosa and transport the contents to the rectum; and (3) defecation.

Stool test: A **stool test** involves the collection and analysis of fecal matter to diagnose the presence or absence of a medical condition. A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract . These conditions can include infection (such as from parasites, viruses, or bacteria), poor nutrient absorption, or cancer. For a stool analysis, a stool sample is collected in a clean container and then sent to the laboratory. Laboratory analysis includes microscopic examination, chemical tests, and microbiologic tests. The stool will be checked for color, consistency, amount, shape, odor, and the presence of mucus. The stool may be examined for hidden (occult) blood, fat, meat fibers, bile, white blood cells , and sugars called reducing substances.

The pH of the stool also may be measured. A stool culture is done to find out if bacteria may be causing an infection.

Why It Is Done : Stool analysis is done to: 1-Help identify diseases of the digestive tract, liver and pancreas . Certain enzymes (such as trypsin or elastase) may be evaluated in the stool to help determine how well the pancreas is functioning. **2-**Help find the cause of symptoms affecting the digestive tract, including prolonged diarrhea, bloody diarrhea, an increased amount of gas, nausea, vomiting, loss of appetite, bloating, abdominal pain and cramping, and fever. **3-**Screen for colon cancer by checking for hidden (occult) blood. **4-**Look for parasites, such as pinworms or Giardia . **5-** Look for the cause of an infection, such as bacteria, a fungus, or a virus. **6-** Check for poor absorption of nutrients by the digestive tract (malabsorption syndrome).

Stool Consistency, Shape, Form, Amount, and Odor: Inspection of the feces is an important diagnostic tool. The quantity, form, consistency, and color of the stool should be noted. When diarrhea is present, the stool is watery. Large amounts of mushy (soft), frothy, foul-smelling stool are characteristic of steatorrhea. Constipation is associated with firm, spherical masses of stool. Feces have a characteristic odor that varies with diet and the pH of the stool.

Stool Color : The brown color of normal feces is probably due to stercobilin (urobilin), a bile pigment derivative, which results from the action of reducing bacteria in bilirubin and other undetermined factors. The first indication of GI disturbances is often a change in the normal brown color of the feces. A change in color can provide information about pathologic conditions, organic dysfunction, or intake of drugs. Color abnormalities may aid the clinician in selection of appropriate diagnostic chemical and microbiologic stool tests.

The fecal occult blood test — also known as FOBT — is a lab test used to check stool samples for hidden (occult) blood. Occult blood in the stool may indicate colon cancer or polyps in the colon or rectum — though not all cancers or polyps bleed. Typically, occult blood is passed in such small amounts that it can be detected only through the chemicals used in a fecal occult blood test. If blood is detected through a fecal occult blood test, additional tests may be needed to determine the source of the bleeding. The fecal occult blood test can only detect the presence or absence of blood. The fecal occult blood test doesn't indicate potential sources of bleeding.

Why it's done **1-Screen for colon cancer.** If you're age 50 or older and at average risk of colon cancer, your doctor may recommend a fecal occult blood test every year to screen for colon cancer. **2-Evaluate possible causes of unexplained anemia.** Anemia is a condition in which there aren't enough

healthy red blood cells to carry adequate oxygen to your tissues. Sometimes a fecal occult blood test is used to determine whether gastrointestinal bleeding — such as a bleeding ulcer — is contributing to anemia. **On other hand,** Various foods, dietary supplements and medications can affect the results of some fecal occult blood tests — either indicating that blood is present when it isn't (false-positive) or missing the presence of blood that's actually there (false-negative). To ensure accurate test results, follow your doctor's instructions carefully. For about three days before the test, your doctor may ask you to avoid: **1-Certain fruits and vegetables, including broccoli and turnips 2-Red meat 3- Horseradish 4- Vitamin C supplements 5- Pain relievers, such as aspirin, ibuprofen.**

Results :

- **Negative result.** A fecal occult blood test is considered negative if no blood is detected in your stool samples. If you had the test to screen for colon cancer and you're at average risk — you have no colon cancer risk factors other than age — your doctor may recommend waiting one year and then repeating the test.
- **Positive result.** A fecal occult blood test is considered positive if blood is detected in your stool samples. You may need additional testing — such as a colonoscopy — to locate the source of the bleeding. It's important to remember that false-negative and false-positive results are possible.
- There are four methods in clinical use for testing for occult blood in feces. These look at different properties, such as antibodies, heme, globin, or porphyrins in blood, or at DNA from cellular material such as from lesions of the intestinal mucosa.
- **Fecal Immunochemical Testing (FIT) and immunochemical fecal occult blood test (iFOBT) :** FIT test utilize specific antibodies to detect globin. FIT screening is more effective in terms of health outcomes and cost compared with guaiac FOBT.
- **Stool guaiac test for fecal occult blood (gFOBT):** – The stool guaiac test involves smearing some feces onto some absorbent paper that has been treated with a chemical. Hydrogen peroxide is then dropped onto the paper; if trace amounts of blood are present, the paper will change color in one or two seconds. This method works as the heme component in hemoglobin has a peroxidase-like effect, rapidly breaking down hydrogen peroxide.

- **Fecal porphyrin quantification:** unlike gFOBT and FIT, permits precise quantification of hemoglobin, and is analytically validated with gastric juice and urine, as well as stool samples. Precise quantification measurement has been very useful in many clinical research applications.
- **Fecal DNA test:** test extracts human DNA from the stool sample and tests it for alterations that have been associated with cancer.
- Additional methods of looking for occult blood are being explored, including **transferrin dipstick and stool cytology.**

Stool pH: Stool pH is diet dependent and is based on bacterial fermentation in the small intestine. Carbohydrate fermentation changes the pH to acid; protein breakdown changes the pH to alkaline.

Stool pH testing is done to evaluate carbohydrate and fat malabsorption and assess disaccharidase deficiency. Human feces is normally alkaline. An acidic stool can indicate a digestive problem such as lactose intolerance or a contagion such as E. coli or Rotavirus.

Stool test: bacteria culture: A stool culture helps the doctor determine if there's a bacterial infection in the intestines. A technician places small stool samples in sterile plastic dishes containing nutrients that encourage the growth of certain bacteria. The targeted bacteria will only grow if they're already present in the stool sample. If bacterial colonies form, the technician evaluates them using a microscope and chemical tests to identify the organism. **Why It's Done :** A doctor may request a stool culture to look for illness-causing bacteria such as: (shigella ,salmonella ,yersenia , campylobacter ,Escherichia coli (E. coli) 0157:H7.

Additionally Parasitic diseases such as amoebasis , giardiasis, ascariasis, hookworm, strongyloidiasis and whipworm can be diagnosed by examining stools under a microscope for the presence of cyst or worm larvae or eggs. Toxins from bacteria such as Clostridium difficile ('C. diff.') can also be identified. Viruses such as rotavirus can also be found in stools.

Stool Test: H. Pylori Antigen: *Helicobacter pylori* (*H. pylori*) bacteria are a common cause of peptic ulcers (sores in the lining of the stomach, small intestine, or esophagus). In this test, a stool (feces) sample is used to determine if *H. pylori* antigens are present in your individual's gastrointestinal (GI) system. Antigens are substances that trigger the immune system to fight infection. **Why It's Done** A doctor may request an *H. pylori* antigen stool test if your individual has symptoms that could indicate the presence of a peptic ulcer, such as indigestion, abdominal pain, a full or bloated feeling, nausea, frequent belching , or vomiting. When the sample arrives at the laboratory, a small amount of stool is

placed in tiny vials. Specific chemicals and a color developer are added. At the end of the test, the presence of a blue color indicates the presence of *H. pylori* antigens**.**

Mucus in Stool: The mucosa of the colon secretes mucus in response to parasympathetic stimulation. Recognizable mucus in a stool specimen is abnormal and should be reported and recorded.

Stool Reducing Substances Test: Reducing substances that can be detected in the stool include glucose, fructose, lactose, galactose, and pentose. Carbohydrate malabsorption is a major cause of watery diarrhea and electrolyte imbalance seen in patients with the short bowel syndrome. The finding of elevated levels of reducing substances in the stool is abnormal and suggests carbohydrate malabsorption.

Leukocytes in Stool : Microscopic examination of the feces for the presence of white blood cells (leukocytes) is performed as a preliminary procedure in determining the cause of diarrhea. Leukocytes are normally not present in stools and are a response to infection or inflammation. The greater the number of leukocytes, the greater the likelihood that an invasive pathogen is present.

Fat in Stool; Fecal Fat Stain : Fecal fat is the gold standard test for diagnosing steatorrhea (malabsorption). The three major causes of steatorrhea, which is a pathologic increase in fecal fat, are impairment of intestinal absorption, deficiency of pancreatic digestive enzymes, and deficiency of bile. Specimens from patients suspected of having steatorrhea can be screened microscopically for the presence of excess fecal fat. This procedure can also be used to monitor patients undergoing treatment for malabsorption disorders. In general, there is good correlation between the qualitative and quantitative fecal fat procedures.

Meat Fibers in Stool; Stool Muscle Fiber : The presence of undigested meat fibers (ie, muscle fibers) in stool implies impaired intraluminal digestion. There is positive correlation between the presence of meat or muscle fibers and the presence of fat excreted in the stool.

Urobilinogen in Stool: Increased destruction of red blood cells, as in hemolytic anemia, increases the amount of urobilinogen excreted. Determination of stool urobilinogen is an estimation of the total excretion of bile pigments, which are the breakdown products of hemoglobin.

Trypsin in Stool: Fecal Chymotrypsin: Trypsin is a proteolytic enzyme formed in the small intestine. In older children and adults, trypsin is destroyed by bacteria in the GI tract. Inadequate trypsin secretion can lead to malabsorption and abdominal discomfort. Chymotrypsin, an intestinal

proteolytic enzyme secreted by the pancreas, can be used to assess pancreatic function. Fecal chymotrypsin is a more reliable measurement of pancreatic function than trypsin.

Stool Electrolytes: Sodium, Chloride, Potassium, and Osmolality: Normal colon function involves absorption of fluid and electrolytes. Stool electrolyte tests are used to assess electrolyte imbalance in patients with diarrhea. Stool electrolytes must be evaluated along with the serum and urine electrolytes as well as clinical findings in the patient. Stool osmolality is used in conjunction with blood serum osmolality to calculate the osmotic gap and to diagnose intestinal disaccharide deficiency.

To ensure that good specimens are provided for examination, it is important to note the following points.

1. A clean dry container must be used for the collection of faecal samples. Urine and water will destroy trophozoites, if present, and the presence of dirt also causes identification problems.

2. Ideally the specimen should be brought to the lab as soon as it is passed, to avoid deterioration of protozoa and alterations of the morphology of protozoa and helminths.

3. The specimen container should be clearly labeled with the patients name, date, and time of passage of the specimen.

4. An amount of stool adequate for parasite examination should be collected and a repeat sample requested if too little is supplied.

5. Diarrhoeal specimens, or those containing blood and mucus, should be examined promptly on arrival in the laboratory. The specimens may contain motile amoebic or flagellate trophozoites and may round up and thus be missed if examination is delayed. Where amoebic dysentery is suggested, the laboratory should be informed that a "hot stool" is being supplied so that it can be examined within twenty minutes of being passed.

Cerebrospinal Fluid (CSF) Analysis

Cerebrospinal fluid (**CSF**) is a clear colorless bodily fluid found in the brain and spine. It is produced in the choroid plexus of the brain (a network of blood vessels in each ventricle of the brain). It acts as a cushion or buffer for the brain's cortex, providing a basic mechanical and immunological protection to the brain inside the skull, and it serves a vital function in cerebral autoregulation of cerebral blood flow..Analysis of cerebrospinal fluid is used in the diagnosis of a wide variety of diseases and conditions affecting the central nervous system. CSF analysis includes measurement of normal CSF

components (eg, proteins, glucose), as well as examining for abnormal elements (eg, cells, pathogens, abnormal proteins, serology). CSF samples are usually collected through lumbar puncture.

Indications/Applications

CSF analysis may be indicated in patients whose history or examination suggests a CNS process. Such symptoms and signs may include the following:

1. Changes in mental status and consciousness	7. Sensitivity to light
2. Sudden, severe, or persistent headache or a stiff neck Confusion, hallucinations, or seizures	8. Numbness or tremor
3. Muscle weakness or lethargy, fatigue	9. Dizziness
4. Nausea (severe or prolonged)	10. Difficulties with speech
5. Flu-like symptoms that intensify over a few hours to a few days	11. Difficulty walking, lack of coordination
6. Fever or rash	12. Mood swings, depression
	13. Infants: Persistent irritability, body stiffness, poor feeding, or bulging fontanel

Functions: CSF serves four primary purposes:

1-Buoyancy: The actual mass of the human brain is about 1400 grams; however the net weight of the brain suspended in the CSF is equivalent to a mass of 25 grams. The brain therefore exists in neutral buoyancy, which allows the brain to maintain its density without being impaired by its own weight, which would cut off blood supply and kill neurons in the lower sections without CSF.

2-Protection: CSF protects the brain tissue from injury when jolted or hit. In certain situations such as auto accidents or sports injuries, the CSF cannot protect the brain from forced contact with the skull case, causing hemorrhaging, brain damage, and sometimes death. **3-Chemical stability**: CSF flows throughout the inner ventricular system in the brain and is absorbed back into the bloodstream, rinsing the metabolic waste from the central nervous system through the blood-brain barrier. This allows for homeostatic regulation of the distribution of neuroendocrine factors, to which slight changes can cause problems or damage to the nervous system. For example, high glycine concentration disrupts temperature and blood pressure control, and high CSF pH causes dizziness and syncope. **4-Prevention**

of brain ischemia: The prevention of brain ischemia is made by decreasing the amount of CSF in the limited space inside the skull. This decreases total intracranial pressure and facilitates blood perfusion.

Examination of CSF includes the following :

1. Glucose	12. Fungal tests
2. Total protein	13. Beta-2 microglobulin
3. CSF electrophoresis	14. Antibodies and DNA of common viruses
4. AFB smear and culture	15. Bacteria (culture)
5. Herpes	16. Cell count (WBC,RBC)
6. Lyme disease.	17. Chloride
7. Rubella	18. Cryptococcal antigen
8. Syphilis	19. Glutamine
9. West Nile virus	20. Lactate dehydrogenase
10. Toxoplasmosis	21. Oligoclonal banding to look for specific proteins
11. EBV antibodies	**22.** CSF cytology (whether there are cancerous cells present).

CSF Pressure : Pressure measurement is done to detect impairment of CSF flow or to lower the CSF pressure by removing a small volume of CSF fluid. Elevation of the opening CSF pressure may be the only abnormality found in patients with cryptococcal meningitis and pseudo-tumor cerebral.

CSF Color and Appearance: Normal CSF is crystal clear, with the appearance and viscosity of water. Abnormal CSF may appear hazy, cloudy, smoky, or bloody. The initial appearance of CSF can provide various types of diagnostic information. Inflammatory diseases, hemorrhage, tumors, and trauma produce elevated cell counts and corresponding changes in appearance.

CSF Microscopic Examination of Cells; Total Cell Count; Differential Cell Count:

Normal CSF contains a small number of lymphocytes and monocytes in adults. A higher proportion of monocytes is present in young children. An increase in the number of white blood cells (WBCs) in CSF is termed pleocytosis. In general, inflammatory disease, hemorrhage, neoplasms, and trauma cause an elevated WBC count.

CSF Glucose : The CSF glucose level varies with the blood glucose levels. It is usually about 60% of the blood glucose level. This measurement is helpful in determining impaired transport of glucose from plasma to CSF, increased use of glucose in the CNS, and glucose utilization by leukocytes and microorganisms. The finding of a markedly decreased CSF glucose level accompanied by an increased WBC count with a large percentage of neutrophils is indicative of bacterial meningitis.

CSF Glutamine: Glutamine, an amino acid, is synthesized in brain tissue from ammonia and ketoglutarate. Production of glutamine, the most prominent amino acid in CSF. The determination of CSF glutamine level provides an indirect test for the presence of excess ammonia in the CSF. A CSF glutamine test is therefore frequently requested for patients with coma of unknown origin.

CSF Lactic Acid, L-Lactate : The source of CSF lactic acid (L-lactate) is CNS anaerobic metabolism. Lactic acid in CSF varies independently with the level of lactic acid in the blood. Destruction of tissue causes the production of increased CSF lactic acid within the CNS because of oxygen deprivation levels. Thus, elevated CSF lactic acid levels can result from any condition that decreases the flow of oxygen to brain tissues. The CSF lactic acid test is used to differentiate between bacterial and nonbacterial meningitis. CSF lactate levels are frequently used to monitor severe head injuries.

CSF Lactate Dehydrogenase (LD/LDH); CSF Lactate Dehydrogenase (LDH) Isoenzymes:

Although many different enzymes have been measured in CSF, only lactate dehydrogenase (LDH) appears useful clinically. Sources of LDH in normal CSF include diffusion across the blood-CSF barrier, diffusion across the brain-CSF barrier, and LDH activity in cellular elements of the CSF, such as leukocytes, bacteria, and tumor cells. Because brain tissue is rich in LDH, damaged CNS tissue can cause increased levels of LDH in the CSF.High levels of LDH occur in about 90% of cases of bacterial meningitis and in only 10% of cases of viral meningitis.

CSF Total Protein: The CSF normally contains very little protein because the protein in the blood plasma does not cross the blood-brain barrier easily. The CSF protein is a nonspecific but reliable indication of CNS pathology such as meningitis, brain abscess, MS, and other degenerative processes

causing neoplastic disease. Elevated CSF protein levels may be caused by increased permeability of the blood-brain barrier, decreased resorption of the arachnoid villi, mechanical obstruction of the CSF flow, or increased intrathecal immunologic synthesis.

CSF Albumin and Immunoglobulin G (IgG) : Albumin composes most (50% -75%) of the proteins in CSF. The albumin and IgG that are present in normal CSF are derived from the serum. Increased levels of either or both are indicative of damage to the blood-CNS barrier. The combined measurement of albumin and IgG is used to evaluate the integrity and permeability of the blood-CSF barrier and to measure the synthesis of IgG within the CNS. The IgG index is the most sensitive method to determine local CNS synthesis of IgG and to detect increased permeability of the blood-CNS barrier.

CSF Protein Electrophoresis; Oligoclonal Bands; Multiple Sclerosis Panel: Fractionation (i.e., electrophoresis) of CSF is used to evaluate bacterial and viral infections and tumors of the CNS. However, the most important application of CSF protein electrophoresis is the detection and diagnosis of multiple sclerosis (MS) .

CSF Syphilis Serology: Negative (ie, nonreactive) for syphilis. Neurosyphilis is characterized by an increase in protein, an increase in the number of lymphocytes, and a positive test for syphilis.

Normal Results of CSF analysis

1. Antibodies and DNA of common viruses: None
2. Bacteria: No bacteria grows in a lab culture
3. Cancerous cells: No cancerous cells present
4. Cell count: less than 5 white blood cells (all mononuclear) and 0 red blood cells
5. Chloride: 110 to 125 mEq/L
6. Fungus: None
7. Glucose: 40to 80 mg/dL(or greater than

10. Oligoclonal bands: 0 or 1 bands that are not present in a matched serum sample
11. Gross appearance: Normal CSF is clear and colorless.
12. CSF opening pressure: 50–175 mm H_2O.
13. Specific gravity: 1.006–1.009.
14. Total protein: 15–45 mg/dL.
15. LD: 1/10 of serum level.
16. Lactate: less than 35 mg/dL.
17. Red blood cell count: Normally, there are no red blood cells in the CSF unless the

two-thirds of blood sugar level) 8. Glutamine: 6 to 15 mg/dL 9. Lactate dehydrogenase: less than 2.0 to 7.2 U/mL 10-Gram stain: negative.	needle passes through a blood vessel on route to the CSF.

An abnormal CSF analysis result may be due to many different causes, including:

1-viral and bacterial infections, such as meningitis and encephalitis. **2-**tumors or cancers of the nervous system.**3-** syphilis, a sexually transmitted disease. **4-** bleeding (hemorrhaging) around the brain and spinal cord.**5-**multiple sclerosis, a disease that affects the myelin coating of the nerve fibers of the brain and spinal cord. **6-** Guillain-Barré syndrome, an inflammation of the nerves.7- Alzheimer's disease. 8-Autoimmune disease

Factors that affect results:

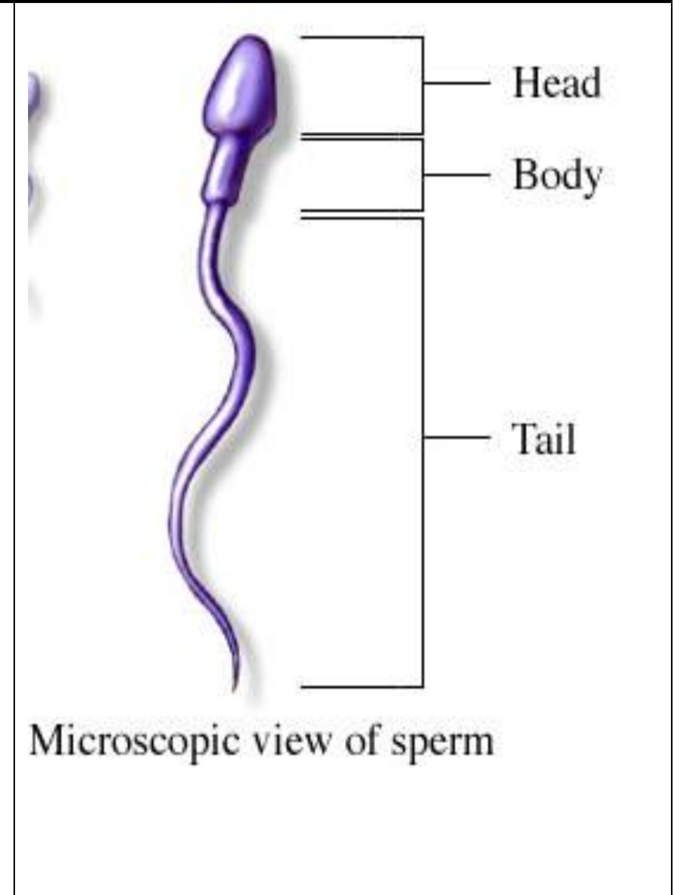
1. The results are invalid when the specimen stands over 1 hour at room temperature.
2. Previous radiation, intrathecal therapy, myelogram, or pneumoencephalogram may cause cytologic changes that produce false results.
3. Microbiologic studies should be performed on tube 3 or higher to lessen the chance of skin contamination.
4. At least 5 mL is necessary to detect fungal and mycobacterial infections.
5. If CNS infection is strongly suspected but initial cell counts are normal,the test should be repeated a few hours later to detect rising white cell counts.
6. Store CSF for culture in a bacteriologic incubator when not tested promptly.
7. A portion of the sample should be frozen at -20 degrees C when viral meningitis is suspected.

Seminal fluid analysis : Semen analysis has long represented the standard test for evaluating male fertility. A semen analysis measures the amount of semen a man produces and determines the number and quality of sperm in the semen sample. A semen analysis is usually one of the first tests done to help determine whether a man has a problem fathering a child (infertility).A problem with the semen or sperm affects more than one-third of the couples who are unable to have children (infertile).A semen analysis evaluates certain characteristics of a male's semen and the sperm contained therein. It is done to help evaluate male fertility, whether for those seeking pregnancy or verifying the success of vasectomy. Collection techniques and precise measurement method may influence results.

What is the spermatozoon : **1-** The human sperm cell is about 70 µm long. **2-**The nucleus is in the head – contain the 23 chromosomes. **3-**It is the head which binds to the egg at fertilization. **4-**Mid-piece: the energy for motility is generated. **5-**Tail: (motility the beat is initiated just behind the mid-piece, and then propagated along the tail).

Sperm function

1. The ejaculated sperm pass through the cervix, then the uterus, and enter the oviduct. The fertilizing sperm swims through the layers of cells around the egg (cumulus and corona), and reaches the zona pellucida.
2. The sperm then loses the front membranes of its head (the “acrosome reaction”), binds to the zona, then forces its way through the zona to the egg membrane.
3. When the sperm head binds to the egg membrane, its tail stops beating and the egg incorporates the whole sperm cell.
4. The egg unpacks the sperm, then the male and female pronuclei form.



What the purpose of the test : **1-**Investigation of fertility. **2-** Identify treatment options (surgical treatment, medical treatment, assisted conception treatment, Determine the suitability of semen for IVF **In vitro fertilisation** (artificial insemination). **3-**Occasionally a man will have a semen analysis done as part of routine pre-pregnancy testing.**4-** Animals semen analysis is commonly used in farm animal breeding. **5-**Evaluate the function of the accessory glands.

Methods of seminal fluid collection : Different methods used for semen collection are masturbation, coitus interruptus, condom collection, epididymal extraction, etc.

How To Prepare : You may be asked to avoid any sexual activity that results in ejaculation for 2 to 5 days before a semen analysis. This helps ensure that your sperm count will be at its highest, and it improves the reliability of the test. If possible, do not avoid sexual activity for more than 1 to 2 weeks before this test, because a long period of sexual inactivity can result in less active sperm. You may be asked to avoid drinking alcohol for a few days before the test. Be sure to tell your doctor about any medicines or herbal supplements you are taking.

Seminal Fluid Fractions::

1. Urethral glands (2-5%) are very small mucus secreting glands.
2. Prostate: (produce about 13-33 % of the fluid volume of semen). Prostate glands secretion is a milky, acidic fluid that plays a role in activating sperm, the secretion contains acid phosphatase and proteolytic enzymes that act on the fluid from the seminal vesicles, resulting in the coagulation and liquefaction of the semen.
3. Seminal vesicles (produce about 46-80 % of the fluid volume of semen" alkaline ") Viscous, yellowish secretion is rich in fructose, vitamin C, prostaglandin, and other substances, which nourish and activate the sperm passing through the tract.
4. Testis & Epididymis: (5%) Spermatozoa are produced in the testis under the influence of testosterone, and then the epididymis (is the first part of the duct system) provides a temporary storage site for the immature sperm that enter it from testis. This fraction still in the inactive form until ejaculation.

Tests that may be done during a semen analysis include:

1- Volume. This is a measure of how much semen is present in one ejaculation. ***Volume***: normal is (2-5 milliliters). Using disposable volumetric pipette .WHO criteria specify that any volume

greater than 2.0 mL is normal. Low volume may indicate partial or complete blockage of the seminal vesicles, or that the man was born without seminal vesicles.

2- **Liquefaction time.** Semen is a thick gel at the time of ejaculation and normally becomes liquid within 20 minutes after ejaculation. Liquefaction time is a measure of the time it takes for the semen to liquefy, as well as the liquefaction is the process when the gel formed by proteins from the seminal vesicles is broken up and the semen becomes more liquid.

3- **Sperm count.** This is a count of the number of sperm present per milliliter (mL) of semen in one ejaculation.

4- **Sperm morphology.** This is a measure of the percentage of sperm that have a normal shape.

5- **Sperm motility.** This is a measure of the percentage of sperm that can move forward normally. The number of sperm that show normal forward movement in a certain amount of semen can also be measured (motile density).

A more specified measure is *motility grade*, where the motility of sperm is divided into four different grades:

- **Grade 4**: Sperm with progressive motility. These are the strongest and swim fast in a straight line. Sometimes it is also denoted motility **a**.
- **Grade 3**: (non-linear motility): These also move forward but tend to travel in a curved or crooked motion. Sometimes also denoted motility **b**.
- **Grade 2**: These have non-progressive motility because they do not move forward despite the fact that they move their tails. Sometimes also denoted motility **c**.
- **Grade 1**: These are immotile and fail to move at all. Sometimes also denoted motility **d**.

6- **pH.** This is a measure of the acidity (low pH) or alkalinity (high pH) of the semen. The pH of the sample is measured. WHO criteria specify normal as 7.2-7.8. Acidic ejaculate (lower pH value) may indicate one or both of the seminal vesicles are blocked. A basic ejaculate (higher pH value) may indicate an infection. A pH value outside of the normal range is harmful to sperm.

7- **White blood cell count.** White blood cells are not normally present in semen.

8- **Fructose level.** This is a measure of the amount of a sugar called fructose in the semen. The fructose provides energy for the sperm. Absence of fructose may indicate a problem with the seminal vesicles.

9- **The liquefaction** is the process when the gel formed by proteins from the seminal vesicles is broken up and the semen becomes more liquid. It normally takes less than 20 minutes for the sample to change from a thick gel into a liquid. An abnormally long liquefaction time (more than 40 - 45 minutes at 37 °C) may indicate an infection.

Semen analysis		
Semen volume	Normal:	2–5 milliliters (mL) per ejaculation
	Abnormal:	An abnormally low or high semen volume is present, which may sometimes cause fertility problems.
Liquefaction time	Normal:	20–30 minutes after collection
	Abnormal:	An abnormally long liquefaction time is present, which may indicate an infection.
Sperm count	Normal:	20 million spermatozoa per milliliter (mL) or 0 sperm per milliliter if the man has had a vasectomy
	Abnormal:	A very low sperm count is present, which may mean infertility. But a low sperm count does not always mean that a man cannot father a child. Men with sperm counts below 1 million have fathered children.
Sperm shape (morphology)	Normal:	More than 30% of the sperm have normal shape. Kruger criteria: More than 14% of the sperm have a normal shape.
	Abnormal:	Sperm can be abnormal in several ways, such as having two heads or

		two tails, a short tail, a tiny head (pinhead), or a round (rather than oval) head.
Sperm movement (motility)	Normal:	More than 50% of the sperm show normal forward movement after 1 hour.
	Abnormal:	Sperm must be able to move forward (or "swim") through cervical mucus to reach an egg. A high percentage of sperm that cannot swim properly may impair a man's ability to father a child.
Semen pH	Normal:	Semen pH of 7.1–8.0
	Abnormal:	An abnormally high or low semen pH can kill sperm or affect their ability to move or to penetrate an egg.
White blood cells	Normal:	No white blood cells or bacteria are detected.
	Abnormal:	Bacteria or a large number of white blood cells are present, which may indicate an infection.

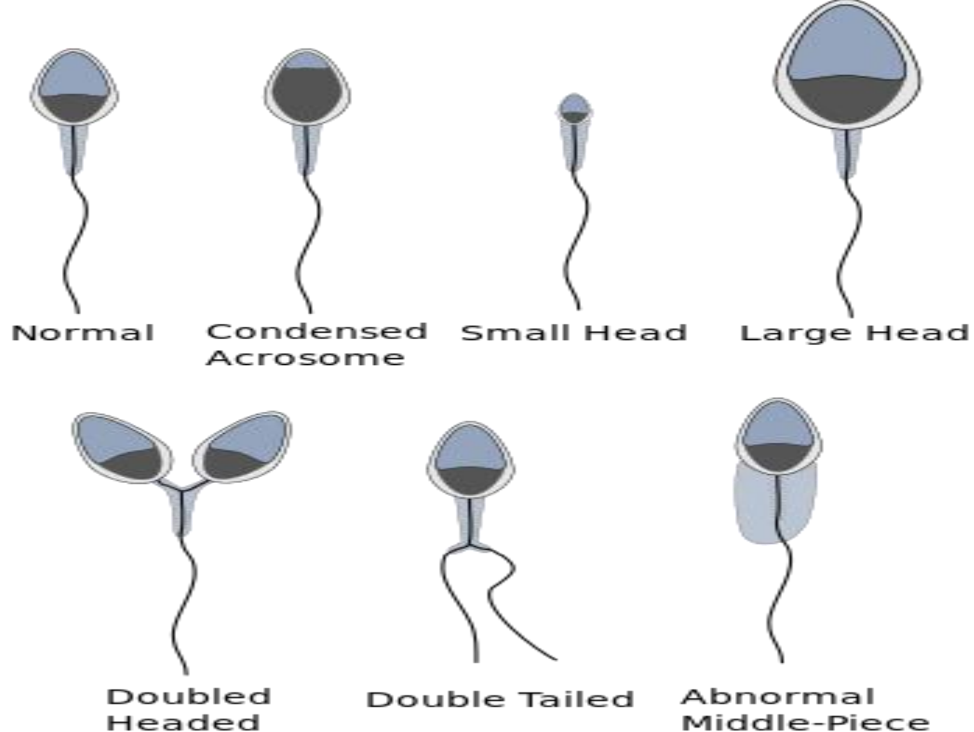
Terms (Abnormalities)

- Aspermia: absence of semen
- Azoospermia: describe a total absence of spermatozoa in semen. (After centrifuge sperm count is zero/HPF).
- Oligozoospermia: refers to a reduced number of spermatozoa in semen and is usually used to describe a sperm concentration of less than 20 million/ml. Sperm count 5-10 sperm/HPF.
- Severe oligospermia: sperm count 1-2 sperm/HPF.
- Polyzoospermia: denotes an increased number of spermatozoa in semen and is usually refers to a sperm concentration in excess of 350 million/ml.
- Teratozoospermia: sperm carry more morphological defects than usual .

- Hypospermia: low semen volume
- Asthenozoospermia: poor sperm motility

Factors That Affect Results : 1- Document specimen collection time. Specimens must be received within 1 hour **. 2-** Reject semen specimens more than 1 hour old..**3-** Heavy tobacco smoking and heavy coffee consumption may decrease the number of motile spermatozoa**.4-** Coital lubricants reduce sperm motility.**5-** The presence of antisperm antibodies has been shown to affect sperm linearity, but not sperm motility.**6-** Do not have intercourse or masturbate for 48 hours before specimen collection.**7-** For home collection: After collecting the specimen, keep the container of semen warm by putting it in a pocket next to the body.**8-** If the specimen is collected at home, it must be delivered to the laboratory within 1 hour.**9-** Consult with your physician before using natural or herbal remedies or medicines because some have been shown to impair the activity of or damage sperm.**10-** Temperature extremes decrease the sperm count.11- Repeat testing may be necessary because results vary with samples.

Other cells in semen includes : **1-**Leukocytes: normally (1-3/HPF), increase number (leukocytospermia) indicates reproductive tract infection.**2-**Epithelial cells: normally (1-2/HPF) . **3-** Spermatocytes: (Immature germ cells) 1-2/HPF. **4-**Erythrocytes: (1-2/HPF). Increased number may indicate a reproductive tract infection or damage to a small capillary during sample production. **5-** bacteria and protozoan such as Trichomonas vaginalis are uncommon in human semen but their presence is indicative of possible male reproductive tract infection and should be reported to the referring doctor for further evaluation.



<u>Complete Blood Count (CBC)</u>

A **complete blood count** (**CBC**), also known as **full blood count** (**FBC**) or **full blood exam** (**FBE**) or **complete blood picture (CBP)** or **blood panel**, is a test panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood. The cells that circulate in the bloodstream are generally divided into three types: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes). Abnormally high or low counts may indicate the presence of many forms of disease, and hence blood counts are amongst the most commonly performed blood tests in medicine, as they can provide an overview of a patient's general health status. A CBC is routinely performed during annual physical examinations in some jurisdictions .The CBC is a basic screening test and is one of the most frequently ordered laboratory procedures. The findings in the CBC give valuable diagnostic information about the hematologic and other body systems, prognosis, response to treatment, and recovery. The CBC consists of a series of tests that determine number, variety, percentage, concentrations, and quality of blood cells:

1. White blood cell count (WBC): leukocytes fight infection 2. Differential white blood cell count	7. Mean corpuscular volume (MCV) shows the size of the red blood cells. 8. Mean corpuscular hemoglobin concentration (MCHC) measures the

(Diff): specific patterns of WBC

3. Red blood cell count (RBC): red blood cells carry O_2 from lungs to blood tissues and CO_2 from tissue to lungs
4. Hematocrit (Hct): measures RBC mass
5. Hemoglobin (Hb): main component of RBCs and transports O_2 and CO_2
6. Red blood cell indices: calculated values of size and Hb content of RBCs; important in anemia evaluations

concentration of hemoglobin in an average red blood cell.

9. Mean corpuscular hemoglobin (MCH) is the amount of hemoglobin in an average red blood cell.
10. Stained red cell examination (film or peripheral blood smear)
11. Platelet count (often included in CBC): thrombocytes are necessary for clotting and control of bleeding
12. Red blood cell distribution width (RDW): indicates degree variability and abnormal cell size
13. Mean platelet volume (MPV): index of platelet production

Interfering Factors on CBC results

RBCs

Many physiologic variants affect outcomes: posture, exercise, age, altitude, pregnancy, and many drugs.

Hematocrit

- Physiologic variants affect Hct outcomes: age, sex, and physiologic hydremia of pregnancy.

MCH

- Hyperlipidemia and high heparin concentrations falsely elevate MCH values.

WBC Count

- Hourly variation, age, exercise, pain, temperature, and anesthesia affect test results.

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Hemoglobin

- Physiologic variations affect test outcomes: high altitude, excessive fluid intake, age, pregnancy, and many drugs.

MCHC

- High values may occur in newborns and infants.

Platelets

- Physiologic factors include high altitudes, strenuous exercise, excitement, and premenstrual and postpartum effects.

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Neutrophils and Eosinophils

- Physiologic conditions such as stress, excitement, exercise, and Steroid administration affects levels for up to 24 hours.
- The eosinophil count is lowest in the morning and then rises from noon until after midnight. Stressful states such as burns, postoperative states, and drugs such as steroids, epinephrine, and thyroxine affect eosinophil levels.

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Blood Specimen Collection Procedures

Proper specimen collection presumes correct technique and accurate timing when necessary. Most hematology tests use liquid ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Tubes with anticoagulants should be gently but completely inverted end over end 7 to 10 times after collection. This action ensures complete mixing of anticoagulants with blood to prevent clot formation. Even slightly clotted blood invalidates the test, and the sample must be redrawn. For plasma coagulator studies, such as prothrombin time (PT) and partial thromboplastin time (PTT), the tube must be allowed to fill to its capacity or an improper blood-to-anticoagulant ratio will invalidate coagulator results. Invert 7 to 10 times to prevent clotting. A phlebotomist collects the sample, drawing the blood into a test tube containing an anticoagulant (EDTA, sometimes citrate) to stop it from clotting. The sample is then transported to a laboratory. Sometimes the sample is drawn off a finger prick using a Pasteur pipette for immediate processing by an automated counter. In the past, counting the cells in a patient's blood was performed manually, by viewing a slide prepared with a sample of the patient's blood under a microscope (a blood film, or peripheral smear). Presently, this process is generally

automated by use of an automated analyzer, with only approximately 10–20% of samples now being examined manually.

Why It Is Done : A complete blood count may be done to:

1. Find the cause of symptoms such as fatigue, weakness, fever, bruising, or weight loss. 2. Check for anemia. 3. See how much blood has been lost if there is bleeding. 4. Diagnose polycythemia. 5. Check for an infection. 6. Diagnose diseases of the blood, such as leukemia. 7. Check how the body is dealing with some types of drug or radiation treatment.	8. Check how abnormal bleeding is affecting the blood cells and counts. 9. Screen for high and low values before a surgery. 10. See if there are too many or too few of certain types of cells. This may help find other conditions, such as too many eosinophils may mean an allergy or asthma is present. 11. A complete blood count may be done as part of a regular physical examination. A blood count can give valuable information about the general state of your health.

Manual blood count: Counting chambers that hold a specified volume of diluted blood (as there are far too many cells if it is not diluted) are used to calculate the number of red and white cells per litre of blood. To identify the numbers of different white cells, a blood film is made, and a large number of white blood cells (at least 100) are counted. This gives the percentage of cells that are of each type. By multiplying the percentage with the total number of white blood cells, the absolute number of each type of white cell can be obtained. Manual counting is useful in cases where automated analyzers cannot reliably count abnormal cells, such as those cells that are not present in normal patients and are only seen in peripheral blood with certain haematological conditions. Manual counting is subject to sampling error because so few cells are counted compared with automated analysis. Medical technologists examine blood film via a microscope for some CBCs, not only to find abnormal white cells, but also because variation in the shape of red cells is an important diagnostic tool. Although automated analyzers give fast, reliable results regarding the number, average size, and variation in size

of red blood cells, they do not detect cells' shapes. Also, some normal patients' platelets will clump in EDTA anticoagulated blood, which causes automatic analyses to give a falsely low platelet count. The person viewing the slide in these cases will see clumps of platelets and can estimate if there are low, normal, or high numbers of platelets.

A complete blood count will normally include:

White blood cells : White blood cells (or leukocytes) are divided into two main groups: granulocytes and agranulocytes. The granulocytes receive their name from the distinctive granules that are present in the cytoplasm of neutrophils, basophils, and eosinophils. The total count of circulating white blood cells is differentiated according to the five types of leukocytes, each of which performs a specific function. The differential count is expressed as a percentage of the total number of leukocytes (WBC). The distribution (number and type) of cells and the degree of increase or decrease are diagnostically significant. The percentages indicate the relative number of each type of leukocyte in the blood. The absolute count of each type of leukocyte is obtained mathematically by multiplying its relative percentage by the total leukocyte count.

Red blood cells: The main function of the red blood cell (RBC or erythrocyte) is to carry oxygen from the lungs to the body tissues and to transfer carbon dioxide from the tissues to the lungs. This process is achieved by means of the Hb in the RBCs, which combines easily with oxygen and carbon dioxide and gives arterial blood a bright red appearance. The RBC test, an important measurement in the evaluation of anemia or polycythemia, determines the total number of erythrocytes in a microliter (cubic millimeter) of blood.

Hemoglobin: Hb, the main component of erythrocytes, serves as the vehicle for the transportation of oxygen and carbon dioxide. The oxygen-combining capacity of the blood is directly proportional to the Hb concentration rather than to the RBC because some RBCs contain more Hb than others. This is why Hb determinations are important in the evaluation of anemia.The Hb determination is part of a CBC. It is used to screen for disease associated with anemia, to determine the severity of anemia, to monitor the response to treatment for anemia, and to evaluate polycythemia.

Hematocrit : The Hct test is part of the CBC. This test indirectly measures the RBC mass. The results are expressed as the percentage by volume of packed RBCs in whole blood (PCV). It is an important measurement in the determination of anemia or polycythemia.The hematocrit also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF), is the volume percentage (%) of red blood cells in blood. It is normally about 45% for men and 40% for women. It is considered an integral part

of a person's complete blood count results, along with hemoglobin concentration, white blood cell count, and platelet count.

Red Blood Cell Indices: The red cell indices define the size and Hb content of the RBC and consist of the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), and the mean corpuscular hemoglobin (MCH).The RBC indices are used in differentiating anemia. When they are used together with an examination of the erythrocytes on the stained smear, a clear picture of RBC morphology may be ascertained. On the basis of the RBC indices, the erythrocytes can be characterized as normal in every respect or as abnormal in volume or Hb content. In deficient states, the anemia can be classified by cell size as macrocytic, normocytic, or microcytic, or by cell size and color as microcytic hypochromic.

Red Cell Size Distribution Width (RDW): This automated method of measurement is helpful in the investigation of some hematologic disorders and in monitoring response to therapy. The RDW is essentially an indication of the degree of anisocytosis (abnormal variation in size of RBCs). Normal RBCs have a slight degree of variation.

Stained Red Cell Examination (blood film; Stained Erythrocyte Examination): The stained film examination determines variations and abnormalities in erythrocyte size, shape, structure, Hb content, and staining properties. It is useful in diagnosing blood disorders such as anemia, thalassemia, and other hemoglobinopathies. This examination also serves as a guide to therapy and as an indicator of harmful effects of chemotherapy and radiation therapy. The leukocytes are also examined at this time.

Platelet Count; Mean Platelet Volume (MPV): Platelets (thrombocytes) are the smallest of the formed elements in the blood. Platelet activity is necessary for blood clotting, vascular integrity and vasoconstriction, and the adhesion and aggregation activity that occurs during the formation of platelet (plug) breaks in small vessels. The platelet count is of value for assessing bleeding plugs that occlude disorders that occur with thrombocytopenia, uremia, liver disease, or malignancies and for monitoring the course of disease associated with bone marrow failure. It is also part of a coagulation profile or workup. The mean platelet volume (MPV) is sometimes ordered in conjunction with a platelet count. The MPV indicates the uniformity of size of the platelet population. It is used for the differential diagnosis of thrombocytopenia.

Reticulocyte Count: A Reticulocyte young, immature, nonnucleated RBC contains reticular material (RNA) that stains gray-blue. Reticulum is present in newly released blood cells for 1 to 2 days before the cell reaches its full mature state. Normally, a small number of these cells are found in circulating

blood. The reticulocyte count is used to differentiate anemia caused by bone marrow failure from those caused by hemorrhage or hemolysis (destruction of RBCs), to check the effectiveness of treatment in pernicious anemia and folate and iron deficiency, to assess the recovery of bone marrow function in aplastic anemia, and to determine the effects of radioactive substances on exposed workers.

Sedimentation Rate (Sed Rate); Erythrocyte Sedimentation Rate (ESR): Sedimentation occurs when the erythrocytes clump or aggregate together in a column-like manner (rouleaux formation). These changes are related to alterations in the plasma proteins. Normally, erythrocytes settle slowly because normal RBCs do not form rouleaux. The ESR is the rate at which erythrocytes settle out of anticoagulated blood in 1 hour. This test is based on the fact that inflammatory and necrotic processes cause an alteration in blood proteins, resulting in aggregation of RBCs, which makes them heavier and more likely to fall rapidly when placed in a special vertical test tube. The faster the settling of cells, the higher the ESR. The sedimentation rate is not diagnostic of any particular disease but rather is an indication that a disease process is ongoing and must be investigated. It is also useful in monitoring the progression of inflammatory diseases.

Red blood cell (RBC) count	**Men:**	4.7-6.1 million RBCs per microliter (mcL)
	Women:	4.2-5.4 million RBCs per mcL
Hematocrit (HCT)	**Men:**	42%-52% or
	Women:	37%-47%
Hemoglobin (Hgb) : In general, a normal hemoglobin level is about one-third the value of the hematocrit.	**Men:**	13-16 grams per deciliter (g/dL)
	Women:	11-15 g/dL
Platelet (thrombocyte) count	Adults: 150,000-400,000 platelets per mm^3	
White blood cell (WBC, leukocyte) count	5,000–10,000 WBCs per cubic millimeter (mm^3)	
	Neutrophils:	50%–62%

White blood cell types (WBC differential)	**Band neutrophils:**	3%–6%
	Lymphocytes:	25%–40%
	Monocytes:	3%–7%
	Eosinophils:	0%–3%
	Basophils:	0%–1%
Blood smear	Blood cells are normal in shape, size, color, and number.	

Immunodiagnostic methods: A laboratory techniques that makes use of the binding between an antigen and its homologous antibody in order to identify and quantify the specific antigen or antibody in a sample .

1-Enzyme-Linked Immunosorbent Assay: ELISA – primary immunological test, using an enzyme as a label to determine presence of target protein, is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality control check in various industries.The enzyme linkage or labeling allows you to follow your target protein and if present (qualify) and at what amounts (quantify).An enzyme conjugate is an enzyme bound or joined with an antibody which binds with your target protein. This enzyme labeling is a safe and effective way to track your antibody. Literally hundreds of ELISA kits are manufactured for: 1- research 2- human and veterinary diagnosis

Types : **1- Indirect ELISA 2- sandwich ELISA 3- competitive ELISA**

Indirect ELISA

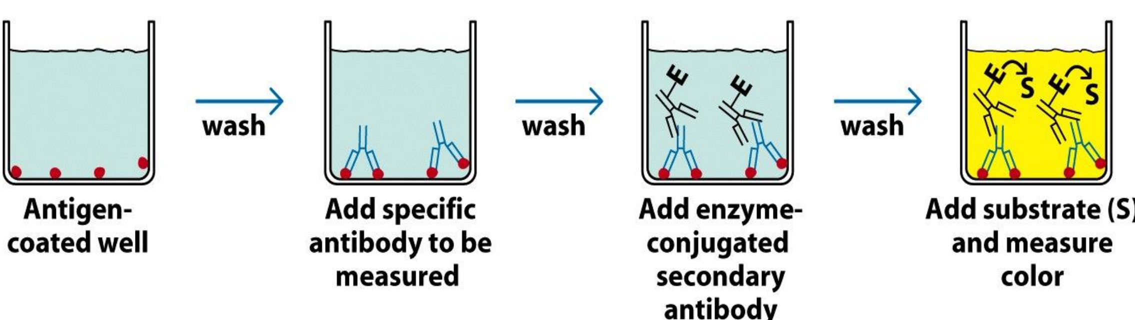

Figure 6-10a
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Sandwich ELISA

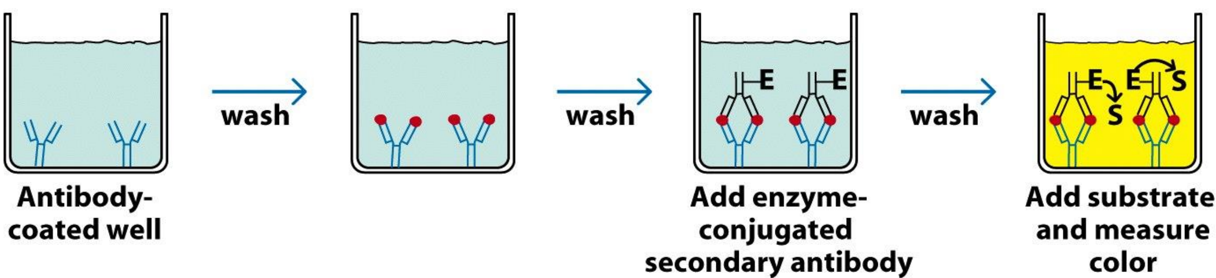

Figure 6-10b
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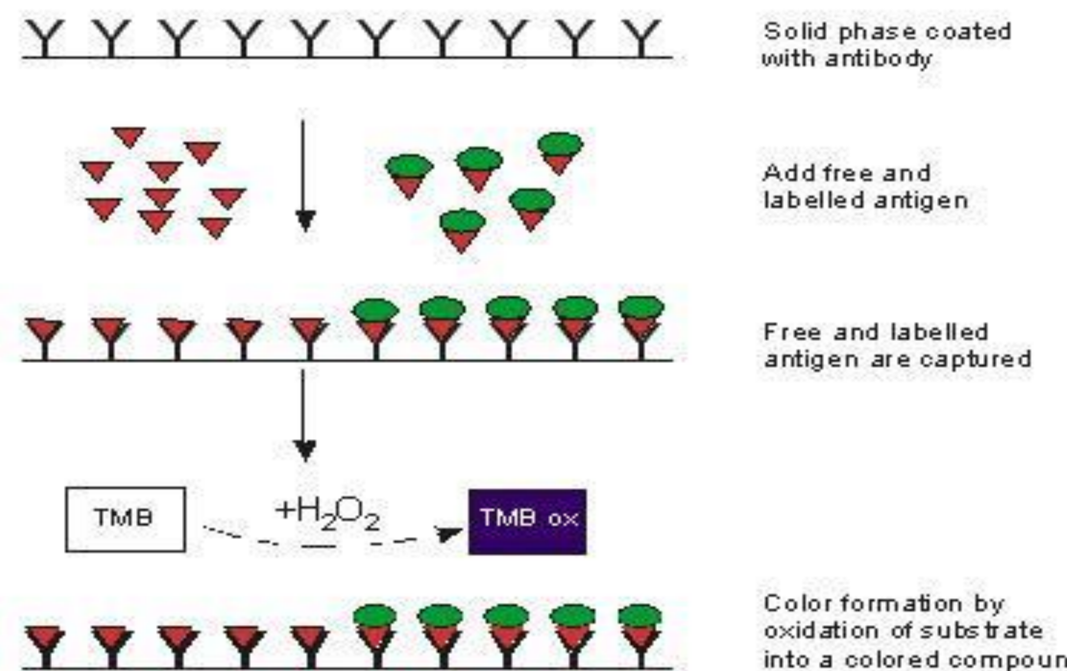
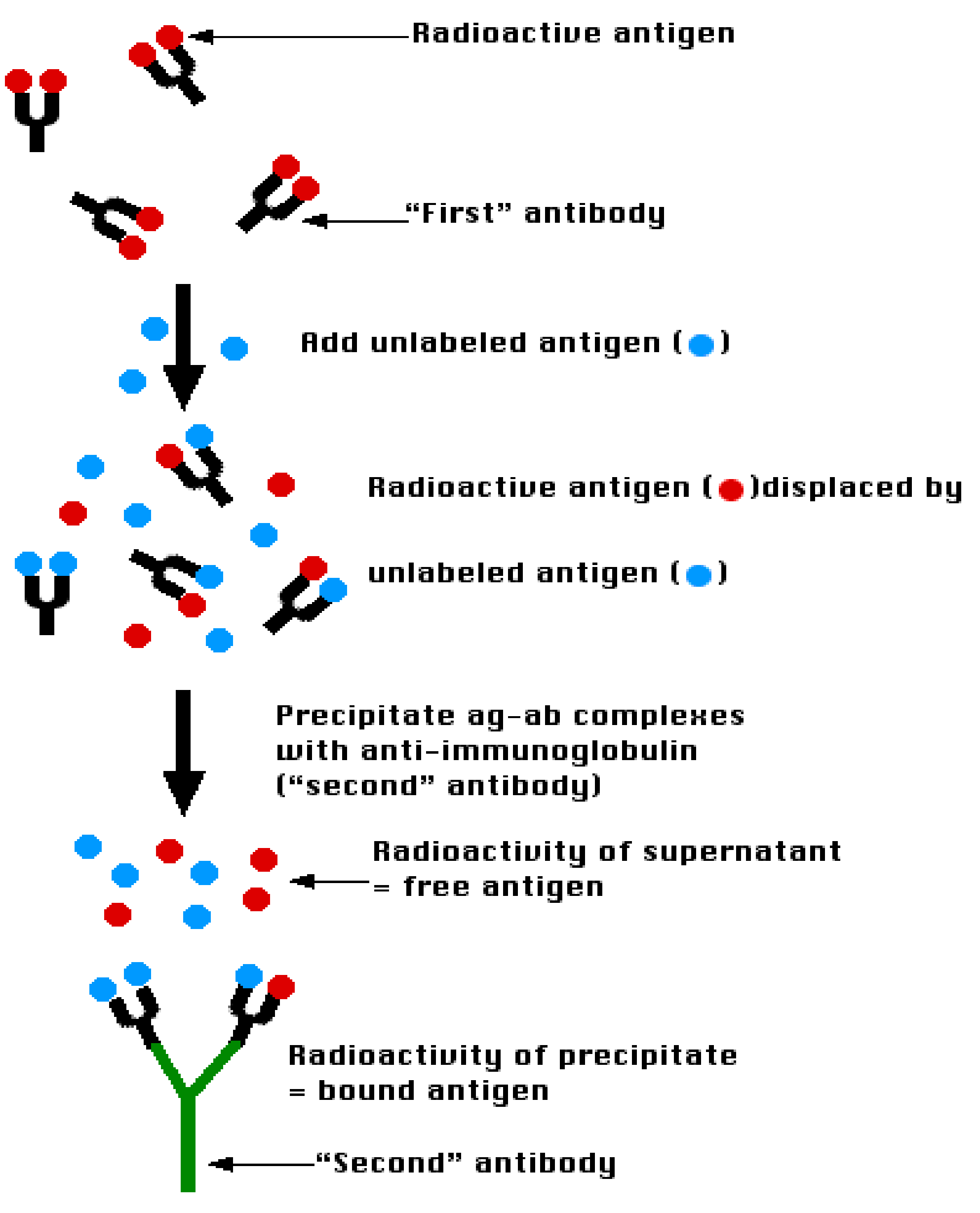


A very wide variety of substances can be detected: **1. Proteins:** Plant and animal proteins (hormones, enzymes, cytokines) ; Microbial proteins (viral, bacterial, fungal, bacterial). **2. Other Bio-molecules- small to large** : *Peptides, Polysaccharides, Gycolipids, Steroids, Organic Toxins ; *Drugs (aminoglycosides, cocaine)

Components of reaction : **1-Antigen**: The antigen is your target protein which comes from your sample extract. **2-Antibody**: binds only to the specific wanted antigen. **3- Enzyme Conjugate** horseradish peroixdase or alkaline phosphatase : An enzyme conjugate (EC) is an antibody joined with an enzyme. Enzyme labeling allows the researcher to follow the antibody.This joining of the enzyme to antibody is often called conjugation.**4-Substrate**: ELISAs traditionally utilize chromogenic substrates, though newer assays employ fluorogenic and electrochemiluminescent substrates enabling much higher sensitivity.**5-Solution of Stop reaction** (HCL , H_2SO4).
Advantages of the ELISA: 1-Can detect either antibody or antigen.2-Can quantify amounts of antigen or antibody. 3-Easy to perform, inexpensive, and can test many samples quickly.4-Plates coated with antigen and gelatin can be stored for later testing

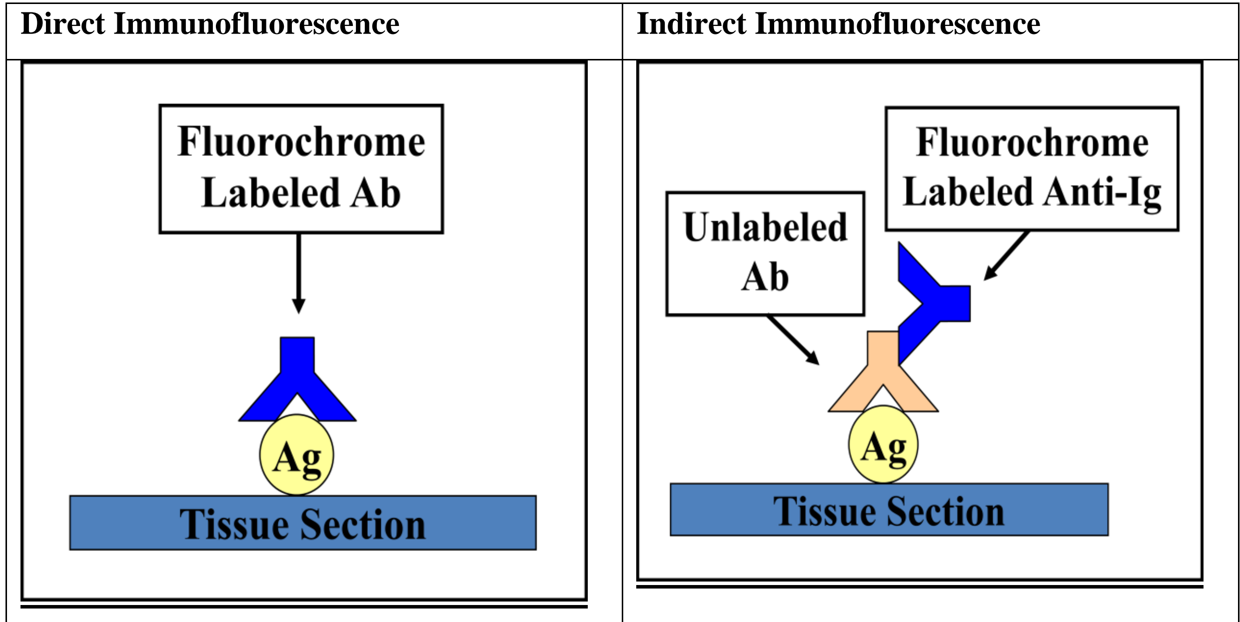
Radioimmunoassay : A highly sensitive and specific assay method that uses the competition between radiolabeled and unlabeled substances in an antigen-antibody reaction to determine the

concentration of the unlabeled substance; it can be used to determine antibody concentrations or to determine the concentration of any substance against which specific antibody can be produced. Radioimmunoassay (RIA) is a very sensitive technique used to measure concentrations of antigens (for example, hormone levels in the blood) without the need to use a bioassay. Although the RIA technique is extremely sensitive and extremely specific, it requires specialized equipment and is costly. It also requires special precautions, since radioactive substances are used. Therefore, today it has been largely supplanted by the ELISA method, where the antigen-antibody reaction is measured using colorimetric signals instead of a radioactive signal. The RAST test (radioallergosorbent test) is an example of radioimmunoassay. It is used to detect the causative allergen for an allergy.



Immunofluorescence (IF): Immunofluorescence is a powerful technique that utilizes fluorescent-labeled antibodies to detect specific target antigens. It is used widely in both scientific research and clinical laboratories, this technique used fluorescence microscope and is used primarily on microbiological samples. This technique uses the specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell, and therefore allows visualization of the distribution of the target molecule through the sample. Immunofluorescence is a widely used example of immunostaining and is a specific example of immunohistochemistry that makes use of fluorophores to visualize the location of the antibodies. Immunofluorescence can be used on tissue sections, cultured cell lines, or individual cells, and may be used to analyze the distribution of proteins, glycans, and small biological and non-biological molecules. Several microscope designs can be used for analysis of immunofluorescence samples; the simplest is the epifluorescence microscope, and the confocal microscope is also widely used. Various super-resolution microscope designs that are capable of much higher resolution can also be used.

As with most fluorescence techniques, a significant problem with immunofluorescence is photo-bleaching. Loss of activity caused by photobleaching can be controlled by reducing the intensity or time-span of light exposure, by increasing the concentration of fluorophores, or by employing more robust fluorophores that are less prone to bleaching (e.g. Alexa Fluors or DyLight Fluors).Many uses of immunofluorescence have been outmoded by the development of recombinant proteins containing fluorescent protein domains, e.g. green fluorescent protein (GFP). Use of such "tagged" proteins allows much better localization and less disruption of protein function. There are two classes of immunofluorescence techniques, primary (or direct) and secondary (or indirect). DIF uses fluorescent-tagged antibodies to bind directly to the target antigen. Indirect immunofluorescence utilizes a two-step technique, in which a primary, unlabeled antibody binds to the target, after which a fluorophore-labeled second antibody (directed against the Fc portion of the primary antibody) is used to detect the first antibody. This technique is more complicated and time consuming than direct immunofluorescence (because it requires a second incubation period); however, it is more sensitive because more than one secondary antibody can bind to each primary antibody, which amplifies the fluorescence signal.



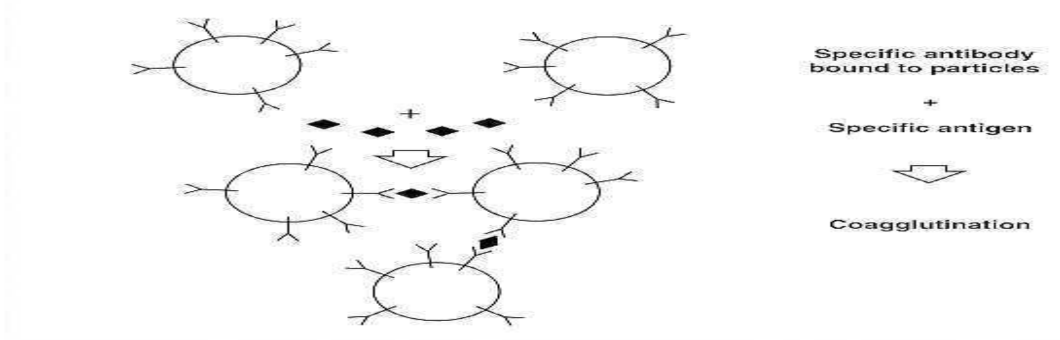
Agglutination: It is one of important laboratory method to detect antigen antibody reaction. It provides flexible and useful method for semi quantitating of either antigen or antibody concentration. The reaction occurs between insoluble antigen and appropriate antibody. The reaction will results in forming aggregate or agglutinate. **Stages of agglutination reaction: 1- one Phase :** Antibody reacts with single antigenic determinants on or close to particle surface. It is a rapid reaction.

IgG and IgM in serum

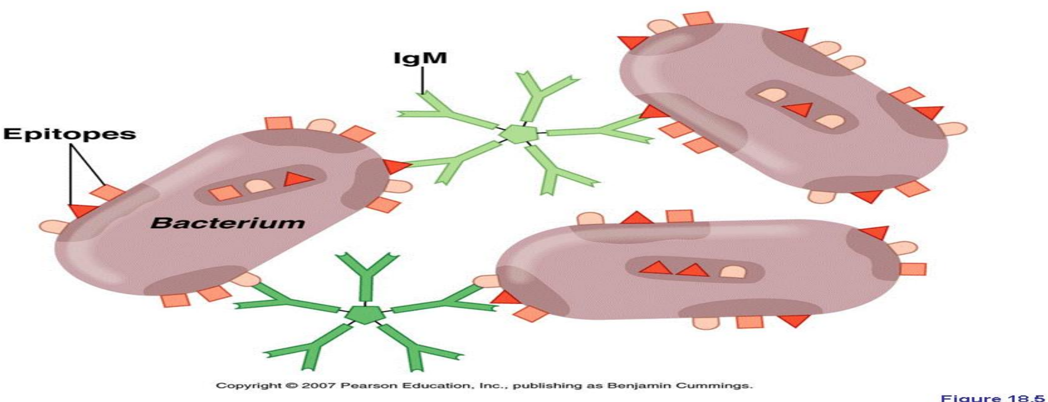
IgG binds to antigenic sites and blocks the ability of IgM molecules to cross-link antigens

Legend: Antigen
IgM Antibody molecules
IgG Antibody molecules

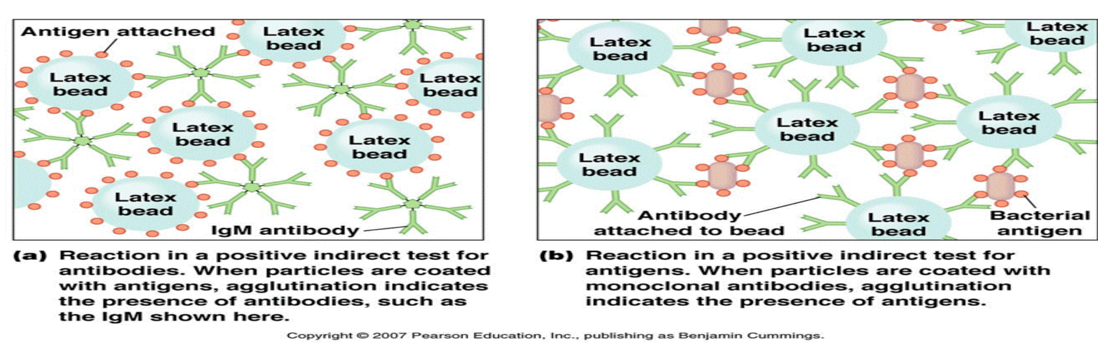
2- Two phases: A single antibody molecule binds to antigenic determinants on adjacent particles. The visible reaction occur under appropriate conditions and over time, particles remain connected and interconnected by antibody bridge.



Types-of-agglutination-reaction: 1. Direct agglutination: To test patient's sera (contain antibody) against large antigen. Direct agglutination can be used to determine antibody titer.



2. Indirect agglutination: serum is mixed with latex spheres (inert substance) with the soluble antigens attached. Antibodies will then cause visible agglutination of the latex spheres with the soluble-antigens-attached.

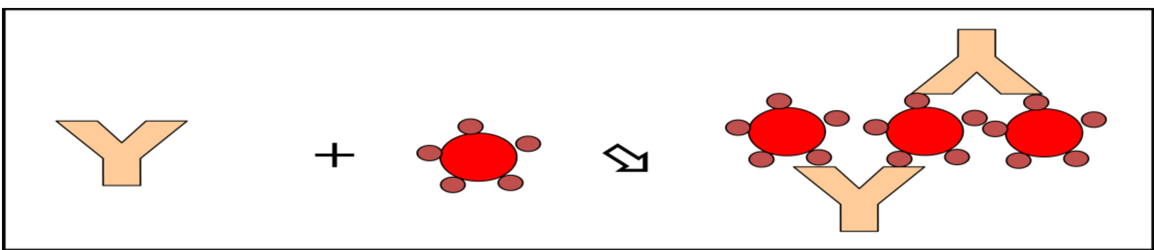


(a) Reaction in a positive indirect test for antibodies. When particles are coated with antigens, agglutination indicates the presence of antibodies, such as the IgM shown here.

(b) Reaction in a positive indirect test for antigens. When particles are coated with monoclonal antibodies, agglutination indicates the presence of antigens.

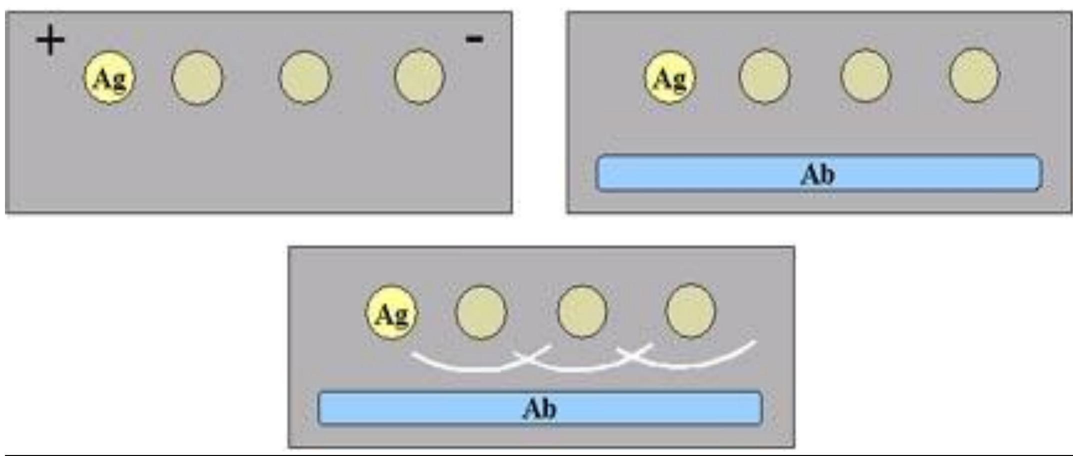
Figure 18.7 - Overview

3-Indirect hemagglutination: The red blood cell are coated with soluble, then incubated with patient serum (contain Ab against Ag). The interaction between AB in the patient's sera and antigen on the surface of red blood cell resulting on agglutination of the red blood cell

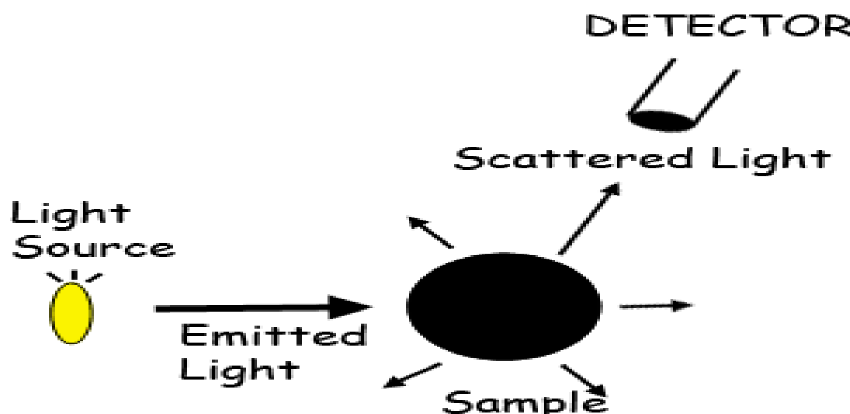


Advantages of agglutination methods: ease of performance. speed of performance, usually requiring few minutes. high degree of sensitivity. **Disadvantages of agglutination methods** : the reaction are only semi-quantitative. The occurrence of the prozone phenomenon, in which agglutination is inhibited by extreme antibody excess as a result of poor lattice formation.

Precipitation: When a specific antibody - **precipitin** combines with a colloidal antigen - **precipitinogen** in solution or in gel the antigen - antibody complex is thrown out of solution - **precipitate**. The precipitate is most heavy in the **equivalence zone**, when antigen and antibody are fully combined. In some tests optimum relation between antigen and antibody must be kept up to carry out the reaction - the so called **flocculation.** Flocculation test is used for the quantitative measurement of toxin, toxoid or antitoxin. Precipitation reactions may be carried out in various ways: **1- Capillary tube precipitation (Ring Test)**: layer Ag over Ab , Simplest test ,qualitative ,pecipitate occurs at the interface of the two reagents, forming a ring. **2- Ouchterlony Double Diffusion (Immunodiffusion) :** As the materials diffuse toward one another, ppt. lines form resulting from the Ag-Ab interactions.**3- Radialimmunodiffusion (RID):** Antibody mixed with agar poured into plate, holes punched , add standards, controls and patients to wells , antigen will diffuse out and form precipitin ring , the diameter of the ring directly proportional to concentration ,Create standard curve and read results. **4- Immunoelectrophoresis (IEP)**: serum sample is electrophoresed through an agar medium, trough is cut in the agar and filled with Ab , precipitin arc is then formed ,because Ag diffuses radially and Ab from a trough diffuses, the reactants meet in optimal proportions for precipitation. **5- Rocket Electroimmunodiffusion (EID).6- Counterimmunoelectrophoresis (CIEP). Factors affecting rate of diffusion:** Size of the particles, temperature, gel viscosity and hydration, interaction of reactants with gel



Nephelometry : Widely used in clinical laboratories because it is relatively easily automated. Based on the principle that a dilute suspension of small particles will scatter light passed through it rather than simply absorbing it. The amount of scatter is determined by collecting the light at an angle (usually about 70 or 75 degrees).



<u>Comparison of the most frequently used assays</u> : Comparison of the RID-, nephelometry and ELISA assays for determination of human IgG subclass levels .

Assay	Detection Range	Inter assay variation	Automation	Assay time	Work load
RID	ug/ml	small	no	long (>48 hours)	moderate
Nephelometry	ug/ml	very small	complete	very short (few minutes)	minimal
ELISA	ng/ml	medium	partly	Short (few hours)	high

Polymerase chain reaction: polymerase chain reaction, (PCR), a technique used to make numerous copies of a specific segment of DNA quickly and accurately. The polymerase chain reaction enables investigators to obtain the large quantities of DNA that are required for various experiments and procedures in molecular biology, forensic analysis, evolutionary biology, and medical diagnostics.

PCR (polymerase chain reaction) is a method to analyze a short sequence of DNA (or RNA) even in samples containing only minute quantities of DNA or RNA. PCR is used to reproduce (amplify) selected sections of DNA or RNA. Previously, amplification of DNA involved cloning the segments of interest into vectors for expression in bacteria, and took weeks. But now, with PCR done in test tubes, it takes only a few hours. PCR is highly efficient in that untold numbers of copies can be made of the DNA. Moreover, PCR uses the same molecules that nature uses for copying DNA.PCR specifically targets and amplifies a single sequence from within a complex mixture of. Most PCR methods typically amplify DNA fragments of between 0.1 and 10 kilo base pairs (kb), although some techniques allow for amplification of fragments up to 40 kb in size.

History: PCR was first conceived in April, **1983** by Kary Mullis. Mullis and Faloona, 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. In **1989** PCR was selected as the major scientific development and Taq DNA polymerase as molecule of the year by the science magazine. In **1993** Kary Mullis was awarded the Nobel Prize in chemistry for this achievement.

A basic PCR set up requires several components and reagents. These components include:

1. DNA template that contains the DNA region (target) to be amplified. **2.** Two primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strand of the DNA target. **3.** Taq polymerase or another DNA polymerase with a temperature optimum at around 70 °C. **4.** Deoxynucleoside triphosphates (dNTPs, sometimes called	**5.** Buffer solution, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase. **6.** Divalent cations, magnesium or manganese ions; generally Mg2+ is used, but Mn2+ can be utilized for PCR-mediated DNA mutagenesis, as higher Mn2+ concentration increases the error rate during DNA synthesis **7.** Monovalent cation potassium ions. **8.** The PCR is commonly carried out

"deoxynucleotide triphosphates"; nucleotides containing triphosphate groups), the building-blocks from which the DNA polymerase synthesizes a new DNA strand.	in a reaction volume of 10–200 µl in small reaction tubes (0.2–0.5 ml volumes) in a **thermal cycler**. **The thermal cycler** heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction. (**Machine** that automatically changes the temperature at the correct time for each of the stages and can be programmed to carry out a set number of cycles) is used for a PCR reaction.

PCR stages **1- Thermal denaturation:** Initial denaturation temperature of 94ºC for 8 min.For subsequent cycles, 94ºC for 1-2 min is usually adequate. **2- Primer annealing:** The temperature and length of time required for primer annealing depends on the base composition and the length and concentration of the primers. 3- **Primer Extension**: Primer Extension is typically carried out at 72ºC ,which is close to the temperature optimum of the Taq polymerase.

Notes: 1- Most thermal cyclers have heated lids to prevent condensation at the top of the reaction tube. Older thermocyclers lacking a heated lid require a layer of oil on top of the reaction mixture or a ball of wax inside the tube. **2-** The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters. These include the enzyme used for DNA synthesis, the concentration of divalent ions and dNTPs in the reaction, and the melting temperature (Tm) of the primers. **3-** Typically the annealing temperature is about 3–5 °C below the Tm of the primers used. Stable DNA–DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence.**4-** The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified. **5-** The amplified product can be detected using gel electrophoresis to view the band containing DNA fragments of a particular size containing the gene of interest in the original starter DNA sample. **6-**DNA fragments separate according to size , and the dye ethidium bromide forms a brightly fluorescent adduct as it binds to DNA ,Standard low-molecular weight marker is used (DNA ladder).

Applications of PCR:

1. Genetic fingerprinting (A- Forensic analysis at scene of crime to identify a person who suspected of committing a crime by comparing his or her DNA with a given sample (blood, hair, semen….etc) obtained from a crime scene. B- Paternity testing). **2.** Analysis of ancient DNA. **3.** Cloning genes. **4.** Genetic diagnosis - Mutation detection (PCR facilitates the advancement of prenatal diagnosis of genetic defects such as: Cystic fibrosis , Duchenne muscular dystrophy ,Haemoglobinopathies).	**5.** Mutagenesis to investigate protein function. **6.** Quantitative differences in gene expression by Reverse Transcription (RT)-PCR. **7.** Detection of pathogens especially when applied to those which are: (difficult or costly to culture, slow growing , present in low concentration , hazardous to propagate in the lab.

Real-time PCR: is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR), which is used to amplify and simultaneously quantify a targeted DNA molecule. For one or more specific sequences in a DNA sample, quantitative PCR enables both detection and quantification. The quantity can be either an absolute number of copies or a relative amount when normalized to DNA input or additional normalizing genes. The procedure follows the general principle of polymerase chain reaction; its key feature is that the amplified DNA is detected as the reaction progresses in "real time". This is a new approach compared to standard PCR, where the product of the reaction is detected at its end. Two common methods for the detection of products in quantitative PCR are: (1) non-specific fluorescent dyes that intercalate with any double-stranded DNA, and (2) sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence to quantify messenger RNA (mRNA) and non-coding RNA in cells or tissues. There are numerous applications for quantitative polymerase chain reaction in the laboratory. It is commonly used for both diagnostic and basic research. Uses of the technique in industry include the

quantification of microbial load in foods or on vegetable matter, the detection of GMOs (Genetically modified organisms) and the quantification and genotyping of human viral pathogens.

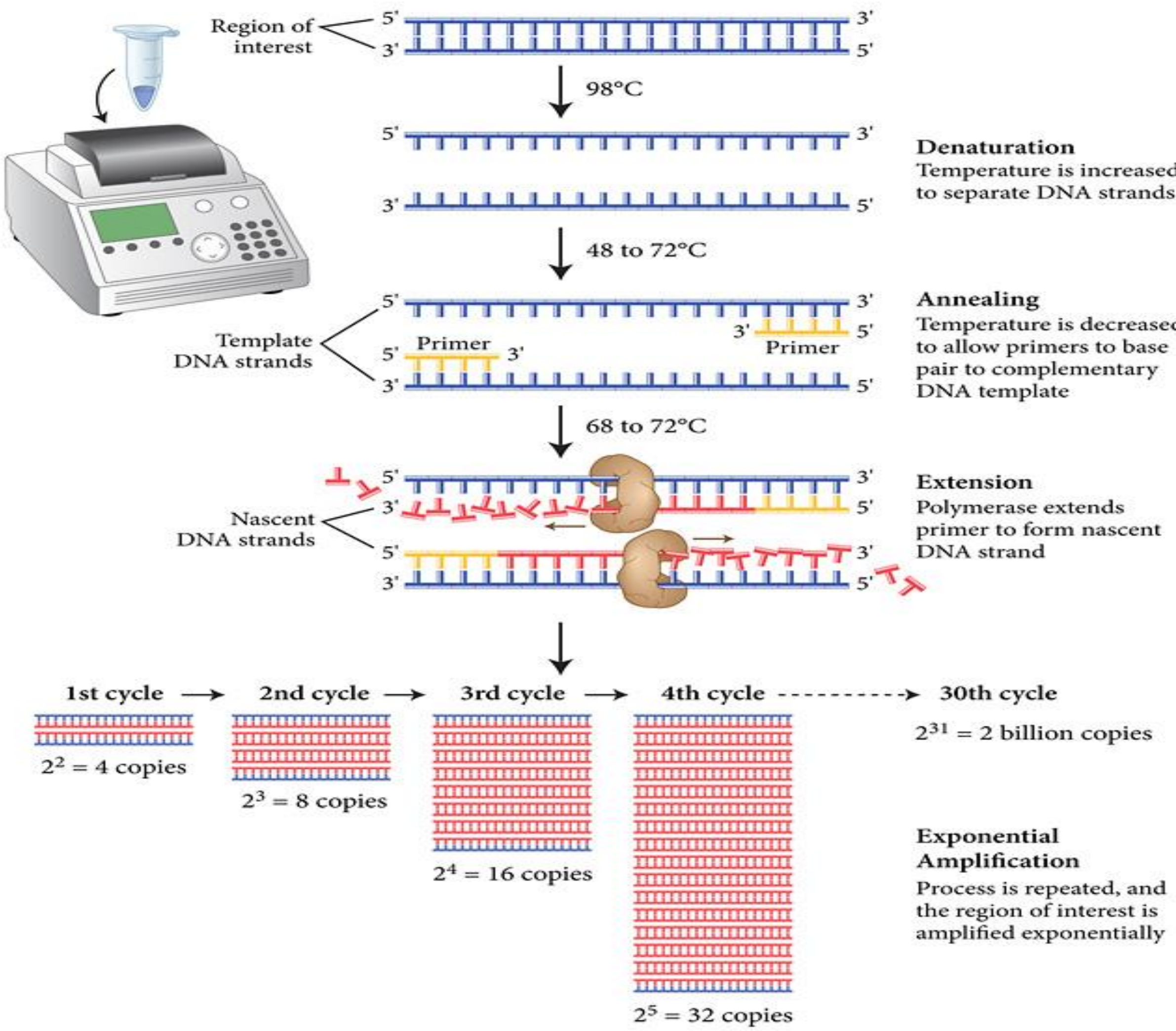


Figure 1: principle of polymerase chain reaction.

<u>**Cytogenetic :**</u> **Cytogenetic** is a branch of genetics that is concerned with the study of the structure and function of the cell, especially the chromosomes. It includes routine analysis of G-banded chromosomes, other cytogenetic banding techniques, as well as molecular cytogenetics such as fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH).

G-banding or Giemsa banding is a technique used in cytogenetics to produce a visible karyotype by staining condensed chromosomes. It is useful for identifying genetic diseases through the photographic representation of the entire chromosome complement. The metaphase chromosomes are treated with

trypsin (to partially digest the chromosome) and stained with Giemsa. Dark bands that take up the stain are strongly A,T rich . Banding can be used to identify chromosomal abnormalities, such as translocations, because there is a unique pattern of light and dark bands for each chromosome.

Comparative genomic hybridization is a molecular cytogenetic method for analyzing copy number variations (CNVs) relative to ploidy level in the DNA of a test sample compared to a reference sample, without the need for culturing cells. The aim of this technique is to quickly and efficiently compare two genomic DNA samples arising from two sources, which are most often closely related, because it is suspected that they contain differences in terms of either gains or losses of either whole chromosomes or sub-chromosomal regions (a portion of a whole chromosome). Ploidy is the number of sets of chromosomes in the nucleus of a cell

Fluorescence in situ hybridization (FISH): is a cytogenetic technique that is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence complementarity. Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes. FISH is often used for finding specific features in DNA for use in genetic counselling, medicine, and species identification. FISH can also be used to detect and localize specific RNA targets (mRNA) in cells, circulating tumor cells, and tissue samples. In this context, it can help define the spatial-temporal patterns of gene expression within cells and tissues.

A **karyotype** (Greek karyon = kernel, seed or nucleus): is the number and appearance of chromosomes in the nucleus of a eukaryotic cell. The term is also used for the complete set of chromosomes in a species, or an individual organism. Karyotypes describe the number of chromosomes, and what they look like under a light microscope. Attention is paid to their length, the position of the centromeres, banding pattern, any differences between the sex chromosomes, and any other physical characteristics. The preparation and study of karyotypes is part of cytogenetics.

The basic number of chromosomes in the somatic cells of an individual or a species is called the somatic number and is designated 2n. Thus, in humans 2n = 46. In the germ-line (the sex cells) the chromosome number is n (humans: n = 23). So, in normal diploid organisms, autosomal chromosomes (not a sex chromosome) are present in two copies. Polyploid cells have multiple copies of chromosomes and haploid cells have single copies. The study of karyotypes is important for cell biology and genetics, and the results may be used in evolutionary biology (karyosystematics) and medicine. Karyotypes can be used for many purposes; such as to study chromosomal aberrations, cellular function, taxonomic relationships, and to gather information about past evolutionary events.

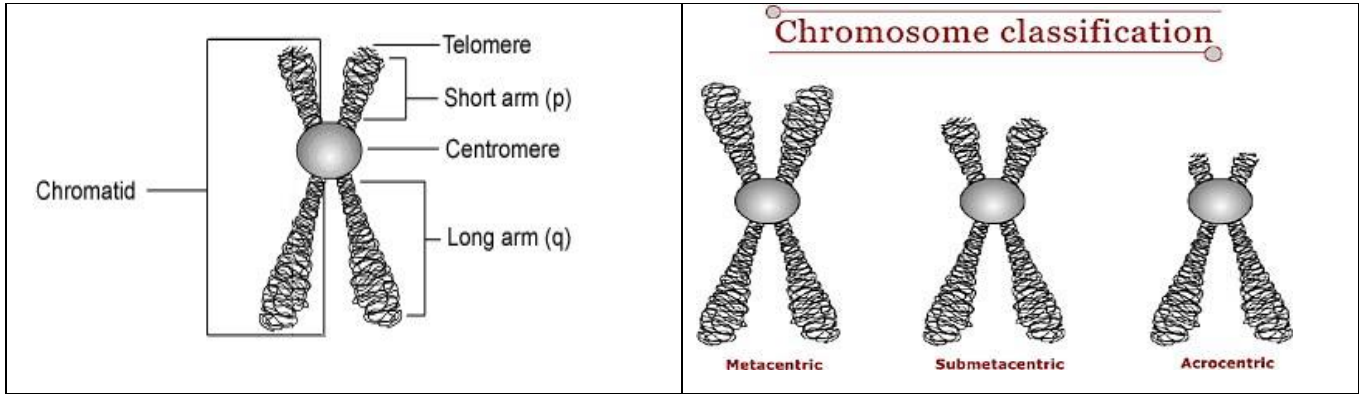
Chromosome Preparation includes :

1. Culture blood in media and bovine calf serum 2. PHA - a mitogen is added to stimulate white blood cells to divide in culture 3. Centrifuge cells - white blood cells go to the bottom of the tube (red cells on top) 4. Hypotonic solution - causes white blood cells to swell and ruptures the red cells	5. Fixation of cells - stabilizes cells and chromosomes 6. Drop cells onto slides - cells burst open and chromosomes fall out 7. Band chromosomes by staining with Giemsa 8. Analyze under the microscope

How are chromosomes classified? By size and position of centromere:

1- Metacentric – centromere in the middle of the chromosome , **2-** Submetacentric – centromere divides the chromosome into 1/3 and 2/3 **3-**Acrocentric – centromere near the end of the chromosome

Cytogeneticists use three things to tell chromosomes apart: 1- chromosome size , **2-** the position of the centromere , **3-** characteristic banding patterns of alternating light and dark bands (caused by staining the chromosomes with dyes).



Blood typing (ABO blood group system): The classification of human blood based on the inherited properties of red blood cells (erythrocytes) as determined by the presence or absence of the antigens A and B, which are carried on the surface of the red cells. Persons may thus have type A, type B, type O, or type AB blood. The A, B, and O blood groups were first identified by Austrian immunologist Karl Landsteiner in 1901. ABO blood types are also present in some other animals, for example rodents, apes such as chimpanzees, bonobos, and gorillas. Human blood is grouped according to the presence or absence of specific blood group antigens (ABO). These antigens, found on the surface of red blood cells, can induce the body to produce antibodies. More than 300 distinct antigens

have been identified. Compatibility of the ABO group is the foundation for all other pre-transfusion testing. All blood donors and potential blood recipients must be tested for blood type to prevent transfusion with incompatible blood products. Specifically linked sugars determine the antigenic activities named A and B. One sugar, N-acetylgalactosamine, gives the molecule A activity; another sugar, galactose, determines B activity. The backbone molecule, without galactose or N-acetylgalactosamine, has antigenic activity termed H. This H substance, as well as H gene activity, is essential for the function of the ABO antigens.

Table 1: shows the relationship between blood antigens and antibodies.

Antigen Present on Red Blood Cell	**Antibodies Present in Serum**	**Major Blood Group Designation**	**Distribution in the world**
None	Anti-A, anti-B	O (universal donor for red blood cells)	O (46%)
A	Anti-B	A	A (41%)
B	Anti-A	B	B (9%)
AB	None	AB (universal recipient for red blood cells) (universal donor for fresh frozen plasma)	AB (4%)
Called universal donor because no antigens are present on red blood cells; therefore, the person is able to donate to all blood groups. Called universal recipient because no serum antibodies are present; therefore, the person is able to receive blood from all blood groups. Called universal donor for plasma because no serum antibodies are present; therefore, the plasma can be given to all blood groups. The associated anti-A and anti-B antibodies are usually IgM antibodies			

Rh Typing : The Rh (Rhesus) blood group system (including the Rh factor) is one of thirty-three current human blood group systems. It is the most important blood group system after ABO. At present, the Rh blood group system consists of 50 defined blood-group antigens, among which the five antigens D, C, c, E, and e are the most important. The commonly used terms Rh factor, Rh positive and Rh negative refer to the D antigen only. Besides its role in blood transfusion, the Rh blood group system—specifically, the D antigen—is used to determine the risk of hemolytic disease of the newborn as prevention is the best approach to the management of this condition. As part of prenatal care, a blood test may be used to find out the blood type of a fetus. If the Rh antigen is lacking, the blood is called Rh-negative. If the antigen is present, it is called Rh-positive. When the mother is Rh-negative and the father is Rh-positive, the fetus can inherit the Rh factor from the father. This makes the fetus

Rh-positive too. Problems can arise when the fetus's blood has the Rh factor and the mother's blood does not.A mother who is Rh-negative may develop antibodies to an Rh-positive baby. If a small amount of the baby's blood mixes with the mother's blood, which often happens in such situations, the mother's body may respond as if it were allergic to the baby. The mother's body may make antibodies to the Rh antigens in the baby's blood. This means the mother has become sensitized and her antibodies may cross the placenta and attack the baby's blood. Such an attack breaks down the fetus's red blood cells, creating anemia (a low number of red blood cells). This condition is called hemolytic disease or hemolytic anemia. It can become severe enough to cause serious illness, brain damage, or even death in the fetus or newborn. Sensitization can occur any time the fetus's blood mixes with the mother's blood. It can occur if an Rh-negative woman has had a spontaneous or undetected miscarriage of a Rhesus positive fetus.

	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in Red Blood Cell	A antigen	B antigen	A and B antigens	None

Rh Antibody Titer Test: This antibody study determines the Rh antibody level in an Rh-negative or pregnant woman whose partner is Rh positive. If the Rh-negative woman is carrying an Rh-positive fetus, the antigen from the fetal blood cells causes antibody production in the mother's serum. The firstborn child usually shows no ill effects; however, with subsequent pregnancies, the mother's serum antibodies increase and eventually destroy the fetal red blood cells, causing hemolytic disease of the newborn.

Cross-matching: Refers to the testing that is performed prior to a blood transfusion in order to determine if the donor's blood is compatible with the blood of an intended recipient, or to identify matches for organ transplants .The primary purpose of the cross-match, or compatibility test, is to prevent a possible transfusion reaction. There are two types of cross-matches: **1-Major cross-match:** This is the most important cross-match, comparing donor erythrocytes to recipient serum (i.e. you are checking for preformed (acquired or naturally occurring) antibodies in recipient serum against donor erythrocytes. For the major cross-match, you need red blood cells from the donor (this can be whole blood from a donor animal or packed red blood cells) in EDTA or citrate and serum from the recipient (non-anticoagulant tube). **2-Minor cross-match:** This compares donor serum to recipient erythrocytes and checks for preformed antibodies in donor serum that could hemolyse recipient red cells. This cross-match is less important as usually the donor serum is markedly diluted after transfusion and is unlikely to produce a significant transfusion reaction. This type of cross-match could be important if transfusing small patients, in which hemodilution is less likely to occur.

Coombs test: A Coombs test is either of two clinical blood tests used in immunohematology The two Coombs tests are the direct Coombs test, and the indirect Coombs test. The Direct Coombs test is used to test for autoimmune hemolytic anemia; i.e., a condition of a low count of red blood cells (aka RBCs) caused by immune system lysis or breaking of RBC membranes causing RBC destruction.In certain diseases or conditions an individual's blood may contain IgG antibodies that can specifically bind to antigens on the RBC surface membrane, and their circulating RBCs can become coated with IgG alloantibodies and/or IgG autoantibodies. Complement proteins may subsequently bind to the bound antibodies and cause RBC destruction.

The direct Coombs test is used to detect these antibodies or complement proteins that are bound to the surface of red blood cells; a blood sample is taken and the RBCs are washed (removing the patient's own plasma) and then incubated with antihuman globulin (also known as "Coombs reagent"). If this produces agglutination of RBCs, the direct Coombs test is positive, a visual indication that antibodies (and/or complement proteins) are bound to the surface of red blood cells.

The indirect Coombs test is used in prenatal testing of pregnant women, and in testing blood prior to a blood transfusion. It detects antibodies against RBCs that are present unbound in the patient's serum. In this case, serum is extracted from the blood sample taken from the patient. Then, the serum is incubated with RBCs of known antigenicity; that is, RBCs with known reference values from other patient blood samples. If agglutination occurs, the indirect Coombs test is positive. Common clinical

uses of the Coombs test include the preparation of blood for transfusion in cross-matching, screening for atypical antibodies in the blood plasma of pregnant women as part of antenatal care, and detection of antibodies for the diagnosis of immune-mediated haemolytic anemia.

Laboratory method: The IAT is a two-stage test. **First stage:** washed test red blood cells (RBCs) are incubated with a test serum. If the serum contains antibodies to antigens on the RBC surface, the antibodies will bind onto the surface of the RBCs. **Second stage:** The RBCs are washed three or four times with isotonic saline and then incubated with antihuman globulin. If antibodies have bound to RBC surface antigens in the first stage, RBCs will agglutinate when incubated with the antihuman globulin (also known Coombs reagent) in this stage, and the indirect Coombs test will be positive.

Blood Banking : The process of collecting (donation), testing, processing, and storing blood for later use (transfusion), is a cornerstone of emergency and surgical medicine and is dependent on the clinical laboratory for ensuring the safe use of blood and its components. blood bank A blood bank is a cache or bank of blood or blood components, gathered as a result of blood donation, stored and preserved for later use in blood transfusions.

Table 2: shows pre-transfusion testing of blood recipient and donor blood All donated blood, as it is processed, must undergo several measurements. These include tests for the following factors**.**

1. ABO groups **2.** Rh type **3.** Antibody screen **4.** Hepatitis B surface antigen (HBsAg) **5.** Hepatitis B core antigen (HBcAg) **6.** Hepatitis C virus (anti-HCV)	**7.** Syphilis (VDRL) **8.** HIV-1 and HIV-2 **9.** HTLV-I and HTLV-II **10.** HIV antigen (HIV-1-Ag) **11.** Nucleic acid tests (NAT) (narrow window of infection for HIV and HCV) **12.** Chagas' disease caused by Trypanosoma cruzi

Types of Transfusion Reactions: **1-Acute Hemolytic Transfusion Reaction (HTR)**

HTR is triggered by an antigen-antibody reaction and activates the complement and coagulation systems. These are most always due to ABO incompatibility because of misidentification resulting in the patient receiving incompatible blood. Symptoms include fever, chills, backache, vague uneasiness, and red urine. HTR is potentially fatal.

2-Bacterial Contamination : Bacteria may enter the blood during phlebotomy. These microbes will multiply faster in components stored at room temperature than in refrigerated components. Although rare, bacteria in blood or its components can cause a septic transfusion reaction. Symptoms include high fever, shock, hemoglobinuria, disseminated intravascular coagulation, and renal failure. Such reactions can be fatal.

3-Cutaneous Hypersensitivity Reactions: Urticarial reactions are very common, second in frequency only to febrile nonhemolytic reactions, and are usually characterized by erythema, hives, and itching. Allergy to some soluble substance in donor plasma is suspected.

4-Non-cardiogenic Pulmonary Reactions (NPR): Transfusion-related acute lung injury (TRALI) should be considered whenever a transfusion recipient experiences acute respiratory insufficiency or x-ray films show findings consistent with pulmonary edema without evidence of cardiac failure. These are possibly reactions between the donor's leukocyte antibodies and the recipient's leukocytes.

5-Febrile Non-hemolytic (FNH) Reactions: FNH reactions are defined as a temperature increase of >1-2 °C. They are seldom dangerous and may be caused by an antibody-antigen reaction.

6-Anaphylactic Reactions : Anaphylactic reactions occur after infusion of as little as a few milliliters of blood or plasma. Anaphylaxis is characterized by coughing, bronchospasm, respiratory distress, vascular instability, nausea, abdominal cramps, vomiting, diarrhea, shock, and loss of consciousness. Some reactions occur in IgA-deficient patients who have developed anti-IgA antibodies after immunization through previous transfusion or pregnancy.

7-Circulatory Overload : Rapid increases in blood volume are not tolerated well by patients with compromised cardiac or pulmonary function. Symptoms of circulatory overload include coughing, cyanosis, orthopnea, difficulty breathing, and a rapid increase in systolic blood pressure.

Apheresis : is a medical technology in which the blood of a donor or patient is passed through an apparatus that separates out one particular constituent and returns the remainder to the circulation. It is thus an extracorporeal therapy. An extracorporeal medical procedure is a medical procedure which is performed outside the body. **Plasmapheresis** is the removal, treatment, and return of (components of) blood plasma from blood circulation. It is thus an extracorporeal therapy. The method can also be used to collect plasma for further manufacturing into a variety of medications. The procedure is used to treat a variety of disorders, including those of the immune system, such as Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura.

Leukoagglutinin Test: Leukoagglutinins are antibodies that react with white blood cells and sometimes cause febrile, nonhemolytic transfusion reactions. Patients who exhibit this type of

transfusion reaction should receive leukocyte-poor blood for any subsequent transfusions.This test is done when a blood reaction occurs even though compatible blood has been given. The donor plasma contains an antibody that reacts with recipient white cells to produce an acute clinical syndrome of fever, dyspnea, cough, pulmonary infiltrates, and, in more severe cases, cyanosis and hypertension.

Role of ABO antigens: The carbohydrate molecules on the surfaces of red blood cells have roles in cell membrane integrity, cell adhesion, membrane transportation of molecules, and acting as receptors for extracellular ligands, and enzymes. ABO antigens are found having similar roles on epithelial cells as well as red blood cells. The ABO antigen is also expressed on the von Willebrand factor (vWF) glycoprotein, which participates in hemostasis (control of bleeding).

Tests of Hemostasis and Coagulation

The prime function of the coagulation mechanism is to protect the integrity of the blood vessels while maintaining the fluid state of blood. Serious medical problems or even death may occur with the inability to stem the loss of blood or with the inability for a normal clot to form. Hemostasis and coagulation tests are generally done for patients with bleeding disorders, vascular injury or trauma, or coagulopathies. **coagulation (clotting)** is the process by which blood changes from a liquid to a gel. It potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair. The mechanism of coagulation involves activation, adhesion, and aggregation of platelets along with deposition and maturation of fibrin. Disorders of coagulation are disease states which can result in bleeding (hemorrhage or bruising) or obstructive clotting (thrombosis).

Hypercoagulability States : Two general forms of hypercoagulability exist: hyperreactivity of the platelet system, which results in arterial thrombosis, and accelerated activity of the clotting system, which results in venous thrombosis. Hypercoagulability refers to an unnatural tendency toward thrombosis. The thrombus is the actual insoluble mass (fibrin or platelets) present in the bloodstream or chambers of the heart.

Conditions and classifications associated with hypercoagulability include the following:

1. **Platelet Abnormalities.** These conditions are associated with arteriosclerosis, diabetes mellitus, increased blood lipids or cholesterol levels and smoking. 2. **Clotting System Abnormalities.**	3. **Venous Thrombosis.** This can be related to stasis of blood flow, to coagulation alterations, or to increases in pro-coagulation factors or decreases in

These are associated with congestive heart failure, immobility, artificial surfaces (eg, artificial heart valves), use of oral contraceptives or estrogen, pregnancy and the postpartum state, and the postsurgical state.	anticoagulation factors

Normally, haemostastis is maintained by three key events:1- Primary haemostasis involves platelets and the blood vessels themselves and is triggered by injury to a vessel - platelets become activated, adhere to endothelial connective tissue and aggregate with other platelets. **2- Secondary haemostasis** occurs when proteinaceous clotting factors interact in a cascade to produce fibrin to reinforce the clot. Two arms of the cascade are activated simultaneously to achieve coagulation: the intrinsic and extrinsic pathways. The intrinsic pathway is activated by contact with collagen due to vessel injury and involves the clotting factors XII, XI, IX and VIII. The extrinsic pathway is triggered by tissue injury and is affected via factor VII. **3-Fibrinolysis** is the final stage of restoring haemostasis - it prevents uncontrolled, widespread clot formation and breaks down the fibrin within blood clots. The two most important anticoagulants involved in fibrinolysis are antithrombin III (ATIII) and Protein C. The end products of fibinolysis are fibrin degratation products (FDPs).

Bleeding Time : Bleeding time is the best single screening test for platelet function disorders and is one of the primary screening tests for coagulation disorders. This test is of value in detecting vascular abnormalities and platelet abnormalities or deficiencies.

Platelet Count; Mean Platelet Volume (MPV): Platelets (thrombocytes) are the smallest of the formed elements in the blood. Platelet activity is necessary for blood clotting, vascular integrity and vasoconstriction, and the adhesion and aggregation activity that occurs during the formation of platelet plugs that occlude (plug) breaks in small vessels. The platelet count is of value for assessing bleeding disorders that occur with thrombocytopenia, uremia, liver disease, or malignancies and for monitoring the course of disease associated with bone marrow failure. This test is indicated when the estimated platelet count (on a blood smear) appears abnormal. It is also part of a coagulation profile or workup. **The mean platelet volume (MPV)** is sometimes ordered in conjunction with a platelet count. The MPV indicates the uniformity of size of the platelet population. It is used for the differential diagnosis of thrombocytopenia.

Platelet Aggregation: Platelet aggregation is used to evaluate congenital qualitative functional disorders of adhesion, release, or aggregation. It is rarely used to evaluate acquired bleeding disorders.

Thrombin Time (TT); Thrombin Clotting Time (TCT) : Stage III fibrinogen defects can be detected by the TT test. It can detect DIC and hypofibrinogenemia and may also be used for monitoring streptokinase therapy. A TT test is often included as part of a panel for coagulation defects.

Partial Thromboplastin Time (PTT); Activated Partial Thromboplastin Time (APTT):

The PTT, a one-stage clotting test, screens for coagulation disorders. Specifically, it can detect deficiencies of the intrinsic thromboplastin system and also reveals defects in the extrinsic coagulation mechanism pathway. The APTT is used to detect deficiencies in the intrinsic coagulation system, to detect incubating anticoagulants, and to monitor heparin therapy. It is part of a coagulation panel workup.

Prothrombin Time (Pro Time; PT) : Prothrombin is a protein produced by the liver for clotting of blood. Prothrombin production depends on adequate vitamin K intake and absorption. During the clotting process, prothrombin is converted to thrombin. The prothrombin content of the blood is reduced in patients with liver disease.The PT is one of the four most important screening tests used in diagnostic coagulation studies. It directly measures a potential defect in stage II of the clotting mechanism (extrinsic coagulation system) through analysis of the clotting ability of five plasma coagulation factors (prothrombin, fibrinogen, factor V, factor VII, and factor X). In addition to screening for deficiency of prothrombin, the PT is used to evaluate dysfibrinogenemia, the heparin effect and coumarin effect, liver failure, and vitamin K deficiency.

Plasminogen (Plasmin; Fibrinolysin) : Plasminogen is a glycoprotein, synthesized in the liver, present in plasma. This test is done to determine plasminogen activity in persons with thrombosis or DIC.

Fibrin Split Products (FSPs); Fibrin Degradation Products (FDPs) : When fibrin is split by plasmin, positive tests for fibrin degradation (split) products, identified by the letters X, Y, D, and E, are produced. These products have an anticoagulant action and inhibit clotting when they are present in excess in the circulation. Increased levels of FDPs may occur with a variety of pathologic processes in which clot formation and lysis occur.This test is done to establish the diagnosis of DIC and other thromboembolic disorders.

D-Dimer : is a fibrin degradation product (or FDP), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It is so named because it contains two crosslinked D fragments of the fibrinogen protein. D-dimer concentration may be determined by a blood test to help diagnose thrombosis,it has become an important test performed in patients suspected of thrombotic disorders. D-

dimer testing is of clinical use when there is a suspicion of deep venous thrombosis (DVT) or pulmonary embolism (PE). In patients suspected of disseminated intravascular coagulation (DIC), D-dimers may aid in the diagnosis.Various kits have a 93-95% sensitivity and about 50% specificity in the diagnosis of thrombotic disease.

Fibrinopeptide A (FPA): Fibrinopeptides A and B are formed by the action of thrombin on fibrinogen; therefore, the presence of FPA indicates that thrombin has acted on fibrinogen. The measurement is the most sensitive assay done to determine thrombin action.

Prothrombin Fragment (F1 + 2): Prothrombin F1 + 2 is used to detect activation of the coagulation system before actual thrombosis occurs. It is used to identify patients with low-grade intravascular coagulation (DIC) and to judge the effectiveness of oral anticoagulant therapy. F1 + 2 levels may assist in the study of the hypercoagulable states and in the assessment of thrombotic risk.

Fibrinogen : Fibrinogen is a complex protein (polypeptide) that, with enzyme action, is converted to fibrin. The fibrin, along with platelets, forms the network for the common blood clot. Although it is of primary importance as a coagulation protein, fibrinogen is also an acute-phase protein reactant. It is increased in diseases involving tissue damage or inflammation.This test is done to investigate abnormal PT, APTT, and TT and to screen for DIC and fibrin-fibrinogenolysis. It is part of a coagulation panel.

Protein C (PC Antigen) : Protein C, a vitamin K “dependent protein that prevents thrombosis, is produced in the liver and circulates in the plasma. This test evaluates patients with severe thrombosis and those with an increased risk for or predisposition to thrombosis. Patients with partial protein C or partial protein S deficiency (heterozygotes) may experience venous thrombotic episodes, usually in early adult years.

Protein S : Both protein S and protein C are dependent on vitamin K for their production and function. A deficiency of either one is associated with a tendency toward thrombosis. Protein S serves as a cofactor to enhance the anticoagulant effects of activated protein C. This test is done to differentiate acquired from congenital protein S deficiency. Congenital deficiency of protein S is associated with a high risk for thromboembolism.

Antithrombin III (AT-III; Heparin Cofactor Activity): This test detects a decreased level of antithrombin that is indicative of thrombotic tendency. In some families, several members may have a combination of recurrent thromboembolism and reduced plasma antithrombin (30%- 60%). A significant number of patients with mesenteric venous thrombosis have AT-III deficiency. It has been

recommended that patients with such thrombotic disease be screened for AT-III levels to identify those patients who may benefit from coumarin anticoagulant prophylaxis rather than heparin therapy.

Biochemical Test: A biochemical test refers to the chemical identification of unknown substances within a living thing. The test quantitatively and qualitatively determines a particular substance like an enzyme within the blood. A biochemical test can be used to diagnose a given disease. Clinical chemistry (also known as chemical pathology, clinical biochemistry or medical biochemistry) is the area of clinical pathology that is generally concerned with analysis of bodily fluids. Most current laboratories are now highly automated to accommodate the high workload typical of a hospital laboratory. All biochemical tests come under chemical pathology. These are performed on any kind of body fluid, but mostly on serum or plasma. A large medical laboratory will accept samples for up to about 700 different kinds of tests. Even the largest of laboratories rarely do all these tests themselves, and some must be referred to other labs.

This large array of tests can be further sub-categorized into sub-specialties' of:

1. General or routine chemistry - commonly ordered blood chemistries (e.g., liver and kidney function tests). **2.** Special chemistry - elaborate techniques such as electrophoresis, and manual testing methods. **3.** Clinical endocrinology - the study of hormones, and diagnosis of endocrine disorders. **4.** Toxicology - the study of drugs of abuse and other chemicals.	**5.** Therapeutic Drug Monitoring - measurement of therapeutic medication levels to optimize dosage. **6.** Urinalysis - chemical analysis of urine for a wide array of diseases, along with other fluids such as CSF and effusions **7.** Fecal analysis - mostly for detection of gastrointestinal disorders.

Common clinical chemistry tests include:

Liver Function Tests	**Renal (Kidney) Function Tests** 1-Creatinine

1. Total protein (serum)
    - Albumin
    - Globulins
    - A/G ratio (albumin-globulin)
    - Protein electrophoresis
    - Urine protein
2. Bilirubin; direct; indirect; total
3. Aspartate transaminase (AST)
4. Alanine transaminase (ALT)
5. Gamma-glutamyl transpeptidase (GGT)
6. Alkaline phosphatase (ALP)

Miscellaneous

1. Glucose
2. C-reactive protein
3. Glycated hemoglobin (HbA1c)
4. Uric acid
5. Arterial blood gases ($[H^+]$, P_{CO2}, P_{O2})
6. Adrenocorticotropic hormone (ACTH)
7. Toxicological screening and forensic toxicology (drugs and toxins)
8. Neuron-specific enolase (NSE)
9. fecal occult blood test (FOBT)

2-Blood urea nitrogen

Cardiac Markers

1. Troponin
2. Myoglobin
3. CK-MB
4. B-type natriuretic peptide (BNP)

Minerals

- Calcium
- Magnesium
- Phosphate
- Potassium

Blood Disorders

- Iron
- Transferrin
- TIBC
- Vitamin B12
- Folic acid

Electrolytes

1. Sodium
2. Potassium
3. Chloride
4. Bicarbonate

Panel tests: A set of commonly ordered tests are combined into a panel:

- Basic metabolic panel (BMP) - 8 tests - sodium, potassium, chloride, bicarbonate, blood urea, creatinine, glucose, calcium
- Comprehensive metabolic panel (CMP) - 14 tests - above BMP plus total protein, albumin, alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), bilirubin.

Glucose test: A glucose test is a type of blood test used to determine the amount of glucose in the blood. It is mainly used in screening for any pre-diabetes or diabetes. Patients are

instructed not to consume anything but water during the fasting period. Caffeine will also distort the results.

The **blood sugar concentration** or **blood glucose level** is the amount of glucose (sugar) present in the blood of a human or animal. The body naturally tightly regulates blood glucose levels as a part of metabolic homeostasis. With some exceptions glucose is the primary source of energy for the body's cells, and blood lipids (in the form of fats and oils) are primarily a compact energy store. Blood sugar levels outside the normal range may be an indicator of a medical condition. A persistently high level is referred to as hyperglycemia; low levels are referred to as hypoglycemia. Diabetes mellitus is characterized by persistent hyperglycemia from any of several causes, and is the most prominent disease related to failure of blood sugar regulation. Intake of alcohol causes an initial surge in blood sugar, and later tends to cause levels to fall. Also, certain drugs can increase or decrease glucose levels.

Blood glucose laboratory tests:

1. fasting blood sugar (i.e., glucose) test (FBS) **2.** two-hr postprandial blood sugar test (2-h PPBS) **3.** oral glucose tolerance test (OGTT) **4.** intravenous glucose tolerance test IVGT(T)	**5.** glycosylated hemoglobin (HbA_{1C}) **6.** self-monitoring of glucose level via patient testing **7.** Random blood sugar (RBS) **8.** Average blood glucose may be estimated by measuring glycated hemoglobin (HbA1c)

Routine blood test of kidney function : The usual blood test which checks that the kidneys are working properly measures the level of urea, creatinine, and certain dissolved salts.

Urea : is a waste product formed from the breakdown of proteins. Urea is usually passed out in the urine. A high blood level of urea ('uraemia') indicates that the kidneys may not be working properly, or that you are dehydrated (have a low body water content). **Creatinine** is a waste product made by the muscles. Creatinine passes into the bloodstream, and is usually passed out in urine. A high blood level of creatinine indicates that the kidneys may not be working properly. Creatinine is usually a more accurate marker of kidney function than urea.

Estimated glomerular filtration rate (eGFR) : provides a guide to kidney function. Although the level of creatinine in the blood is a useful guide to kidney function, the eGFR is a more accurate measure. Blood creatinine can be used to estimate the eGFR using age, sex, and race. This is often calculated by either manual or computer and reported with the creatinine blood test. The normal value for eGFR is 90-120 ml/min. An eGFR below 60 ml/min suggests that some kidney damage has occurred. The value becomes lower with increasing severity of kidney damage. **Dissolved salts** that are routinely measured are sodium, potassium, chloride and bicarbonate. They are sometimes referred to as 'electrolytes'. Abnormal blood levels of any of these may be due to a kidney problem. (Some other conditions may also alter the salt balance in the blood.)

Routine kidney function is one of the most commonly performed blood tests. It may be done:

1. As part of a general health assessment. **2.** Before and after starting treatment with certain medicines. Some medicines occasionally cause kidney damage as a side-effect. Therefore, kidney function is often checked before and after starting treatment with certain medicines. **3.** If you have suspected dehydration (when the urea level increases).	**4.** If you have suspected kidney failure. The higher the blood levels of urea and creatinine, the less well the kidneys are working. The level of creatinine is usually used as a marker as to the severity of kidney failure. (Creatinine in itself is not harmful, but a high level indicates that the kidneys are not working properly. So, many other waste products will not be cleared out of the bloodstream.) You normally need treatment with dialysis if the level of creatinine goes higher than a certain value.

Aspartate Transaminase (Aminotransferase, AST); Serum Glutamic-Oxaloacetic Transaminase (SGOT) : Aspartate transaminase (AST) is an enzyme present in tissues of high metabolic activity; decreasing concentrations of AST are found in the heart, liver, skeletal muscle, kidney, brain,

pancreas, spleen, and lungs. The enzyme is released into the circulation following the injury or death of cells. Any disease that causes change in these highly metabolic tissues will result in a rise in AST levels. The amount of AST in the blood is directly related to the number of damaged cells and the amount of time that passes between injury to the tissue and the test. Following severe cell damage, the blood AST level will rise in 12 hours and remain elevated for about 5 days.This test is used to evaluate liver and heart disease. The ALT is usually ordered along with the AST.

Alanine Aminotransferase (Aminotransferase, ALT); Serum Glutamic-Pyruvic Transaminase (SGPT) : ALT is an enzyme. High concentrations occur in the liver, and relatively low concentrations are found in the heart, muscle, and kidney. This test is primarily used to diagnose liver disease and to monitor the course of treatment for hepatitis, active post-necrotic cirrhosis, and the effects of later drug therapy. ALT is more sensitive in the detection of liver disease than in biliary obstruction. ALT also differentiates between hemolytic jaundice and jaundice due to liver disease.

Alkaline phosphatase (ALP) is an enzyme originating mainly in the bone, liver, and placenta, with some activity in the kidney and intestines. It is called alkaline because it functions best at a pH of 9. ALP levels are age and gender dependent. Post puberty ALP is mainly of liver origin. Alkaline phosphatase is used as an index of liver and bone disease when correlated with other clinical findings. In bone disease, the enzyme level rises in proportion to new bone cell production resulting from osteoblastic activity and the deposit of calcium in the bones. In liver disease, the blood level rises when excretion of this enzyme is impaired as a result of obstruction in the biliary tract. Used alone, alkaline phosphatase may be misleading.

Bilirubin : Bilirubin results from the breakdown of hemoglobin in the RBCs and is a byproduct of hemolysis (ie, RBC destruction). It is produced by the reticuloendothelial system. Removed from the body by the liver, which excretes it into the bile, bilirubin gives the bile its major pigmentation. Usually, a small amount of bilirubin is found in the serum. A rise in serum bilirubin levels occurs when there is excessive destruction of RBCs or when the liver is unable to excrete the normal amounts of bilirubin produced. There are two forms of bilirubin in the body: indirect or unconjugated bilirubin, which is protein bound; and direct or conjugated bilirubin, which circulates freely in the blood until it reaches the liver, where it is conjugated with glucuronide transferase and then excreted into the bile. An increase in protein-bound bilirubin (unconjugated bilirubin) is more frequently associated with increased destruction of RBCs (hemolysis); an increase in free-flowing bilirubin is more likely seen in dysfunction or blockage of the liver. A routine examination measures only the total bilirubin. A normal level of total bilirubin rules out any significant impairment of the excretory function of the liver or excessive hemolysis of red cells. Only when total bilirubin levels are elevated will there be a call for

differentiation of the bilirubin levels by conjugated and unconjugated types.The measurement of bilirubin allows evaluation of liver function and hemolytic anemias. For infants younger than 15 days, a neonatal bilirubin measurement may be necessary.

Albumin: Albumin (along with total protein) is a part of a diverse microenvironment. Its primary function is the maintenance of colloidal osmotic pressure (COP) in the vascular and extravascular spaces (eg, urine, cerebrospinal fluid, and amniotic fluid). Albumin is a source of nutrition and also a part of a complex buffer system. It is a negative acute-phase reactant. It decreases in response to acute inflammatory infectious processes. Albumin is used to evaluate nutritional status, albumin loss in acute illness, liver disease and renal disease with proteinuria, hemorrhage, burns, exudates or leaks in the GI tract, and other chronic diseases. Hypoalbuminuria is an independent risk factor for older adults for mortality, admission serum albumin in geriatric patients is a predictor of outcome.

Uric Acid : Uric acid is formed from the breakdown of nucleonic acids and is an end product of purine metabolism. The basis for this test is that an overproduction of uric acids occurs when there is excessive cell breakdown and catabolism of nucleonic acids (as in gout), excessive production and destruction of cells (as in leukemia), or an inability to excrete the substance produced (as in renal failure). Measurement of uric acid is used most commonly in the evaluation of renal failure, gout, and leukemia. In hospitalized patients, renal failure is the most common cause of elevated uric acid levels, and gout is the least common cause.

Cholesterol : Cholesterol testing evaluates the risk for arthrosclerosis, myocardial occlusion, and coronary arterial occlusion. Cholesterol relates to coronary heart disease (CHD) and is an important screening test for heart disease. It is part of the lipid profiles. Elevated cholesterol levels are a major component in the hereditary hyperlipoproteinemia. Cholesterol determinations are also frequently a part of thyroid function, liver function, renal function, and diabetes mellitus studies. It is also used to monitor effectiveness of diet, medications, lifestyle changes (eg, exercise), and stress management.

Triglycerides : Triglycerides account for >90% of dietary fat intake and comprise 95% of fat stored in tissues. Because they are insoluble in water, they are the main plasma glycerol ester. Normally stored in adipose tissue as glycerol, fatty acids, and monoglycerides, the liver reconverts these to triglycerides. Of the total, 80% of triglycerides are in VLDL, and 15% are in LDL. This test evaluates suspected atherosclerosis and measures the body's ability to metabolize fat. Elevated triglycerides, together with elevated cholesterol, are atherosclerotic disease risk factors. Because cholesterol and triglycerides can vary independent of each other, measurement of both values is more meaningful. Triglyceride level is needed to calculate the LDL-C and is also used to evaluate turbid samples of blood and plasma.