Diagnostics Methods Used for Analysis of Blood
(Unit-1)
ZOOG-SEC-B-6-4-TH

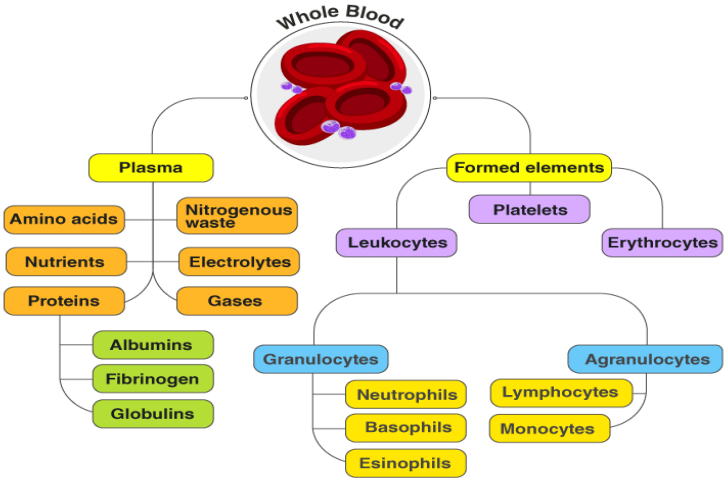
Blood Composition:

Blood is one of the most important components of life. Almost any animal that possesses a circulatory system has blood. From an evolutionary perspective, blood was speculated to have risen from a type of cell that was responsible for phagocytosis and nutrition. Billions of years later, blood and the circulatory system have drastically helped the evolution of more complex life forms. Blood is a specialized body fluid. It has four main components: plasma, red blood cells, white blood cells, and platelets.

Blood has many different functions, including:

- transporting oxygen and nutrients to the lungs and tissues
- forming blood clots to prevent excess blood loss
- carrying cells and antibodies that fight infection
- bringing waste products to the kidneys and liver, which filter and clean the blood
- regulating body temperature

The blood that runs through the veins, arteries, and capillaries is known as whole blood, a mixture of about 55 percent plasma and 45 percent blood cells. About 7 to 8 percent of your total body weight is blood. An average-sized man has about 12 pints of blood in his body, and an average-sized woman has about nine pints.



The Components of Blood and Their Importance:

Many people have undergone blood tests or donated blood, but hematology - the study of blood - encompasses much more than this. People who specialize in hematology (hematologists) are leading the many advances being made in the treatment and prevention of blood diseases. If someone is diagnosed with a blood disorder, the primary care a physician may refer is to a hematologist for further testing and treatment.

Plasma:

The liquid component of blood is called plasma, a mixture of water, sugar, fat, protein, and salts. The main job of the plasma is to transport blood cells throughout your body along with nutrients, waste products, antibodies, clotting proteins, chemical messengers such as hormones, and proteins that help maintain the body's fluid balance.

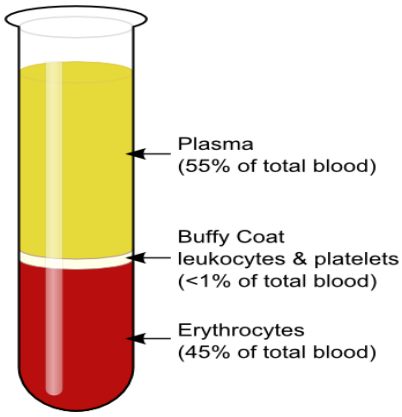
Red Blood Cells (also called erythrocytes or RBCs)

Known for their bright red color, red cells are the most abundant cell in the blood, accounting for about 40 to 45 percent of its volume. The shape of a red blood cell is a biconcave disk with a flattened center - in other words, both faces of the disc have shallow bowl-like indentations (a red blood cell looks like a donut). Production of red blood cells is controlled by erythropoietin, a hormone produced primarily by the kidneys. Red blood cells start as immature cells in the bone marrow and after approximately seven days of maturation are released into the bloodstream. Unlike many other cells, red blood cells have no nucleus and can easily change shape, helping them fit through the various blood vessels in your body. However, while the lack of a nucleus makes a red blood cell more flexible, it also limits the life of the cell as it travels through the smallest blood vessels, damaging the cell's membranes and depleting its energy supplies. The red blood cell survives on average only 120 days. Red cells contain a special protein called hemoglobin, which helps carry oxygen from the lungs to the rest of the body and then returns carbon dioxide from the body to the lungs so it can be exhaled. Blood appears red because of the large number of red blood cells, which get their color from the hemoglobin. The percentage of whole blood volume that is made up of

red blood cells is called the hematocrit and is a common measure of red blood cell levels.

White Blood Cells (also called leukocytes):
White blood cells protect the body from infection. They are much fewer in number than red blood cells, accounting for about 1 percent of your blood. The most common type of white blood cell is the neutrophil, which is the "immediate response" cell and accounts for 55 to 70 percent of the total white blood cell count. Each neutrophil lives less than a day, so your bone marrow must constantly make new neutrophils to maintain protection against infection. Transfusion of neutrophils is generally not effective since they do not remain in the body for very long. The other major type of white blood cell is a lymphocyte. There are two main populations of these cells. T lymphocytes help regulate the function of other immune cells and directly attack various infected cells and tumors. B lymphocytes make antibodies, which are proteins that specifically target bacteria, viruses, and other foreign materials.

Platelets (also called thrombocytes):
Unlike red and white blood cells, platelets are not actually cells but rather small fragments of cells. Platelets help the blood clotting process (or coagulation) by gathering at the site of an injury, sticking to the lining of the injured blood vessel, and forming a platform on which blood coagulation can occur. This results in the formation of a fibrin clot, which covers the wound and prevents blood from leaking out. Fibrin also forms the initial scaffolding upon which new tissue forms, thus promoting healing. A higher than normal number of platelets can cause unnecessary clotting, which can lead to strokes and heart attacks; however, thanks to advances made in antiplatelet therapies, there are treatments available to help prevent these potentially fatal events. Conversely, lower than normal counts can lead to extensive bleeding.



Types of White Blood Cells:

There are five different types of White blood cells and are classified mainly based on the presence and absence of granules.

- Granulocytes
- Agranulocytes

Granulocytes

They are leukocytes, with the presence of granules in their cytoplasm. The granulated cells include- eosinophil, basophil, and neutrophil.

Eosinophils

- They are the cells of leukocytes, which are present in the immune system.
- These cells are responsible for combating infections in parasites of vertebrates and for controlling mechanisms associated with allergy and asthma.
- Eosinophil cells are small granulocyte, which are produced in the bone marrow and makes 2 to 3 per cent of whole WBCs. These cells are present in high concentrations in the digestive tract.

Basophils

- They are the least common of the granulocytes, ranging from 0.5 to 1 per cent of WBCs.

- They contain large cytoplasmic granules, which plays a vital role in mounting a non-specific immune response to pathogens, allergic reactions by releasing histamine and dilates the blood vessels.
- These white blood cells have the ability to be stained when exposed to basic dyes, hence referred to as basophil.
- These cells are best known for their role in asthma and their result in inflammation and bronchoconstriction in the airways.
- They secrete serotonin, histamine and heparin.

Neutrophils

- They are normally found in the bloodstream.
- They are predominant cells, which are present in pus.
- Around 60 to 65 per cent of WBCs are neutrophils with a diameter of 10 to 12 micrometres.
- The nucleus is 2 to 5 lobed and cytoplasm has very fine granules.
- Neutrophil helps in the destruction of bacteria with lysosomes, and it acts as a strong oxidant.
- Neutrophils are stained only using neutral dyes. Hence, they are called so.
- Neutrophils are also the first cells of the immune system to respond to an invader such as a bacteria or a virus.
- The lifespan of these WBCs extend for up to eight hours and are produced every day in the bone marrow.

Agranulocytes

They are leukocytes, with the absence of granules in their cytoplasm. Agranulocytes are further classified into monocytes and lymphocytes.

Monocytes

- These cells usually have a large bilobed nucleus, with a diameter of 12 to 20 micrometres.
- The nucleus is generally of half-moon shaped or kidney-shaped and it occupies 6 to 8 per cent of WBCs.
- They are the garbage trucks of the immune system.

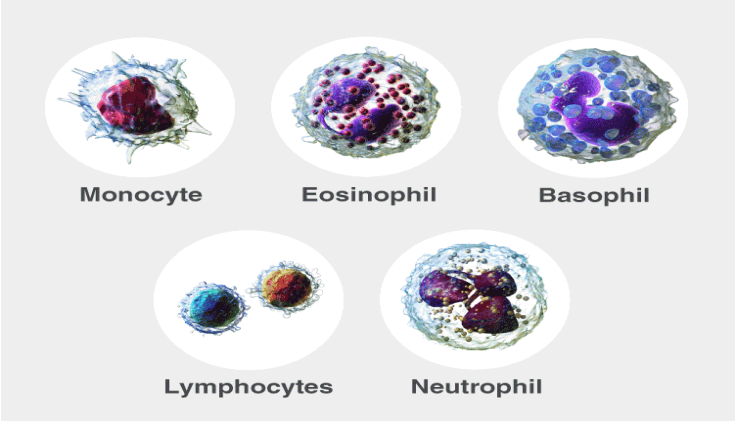
- The most important functions of monocytes are to migrate into tissues and clean up dead cells, protect against the bloodborne pathogens and they move very quickly to the sites of infections in the tissues.
- These white blood cells have a single bean-shaped nucleus, hence referred to as Monocytes.

Lymphocytes

- They play a vital role in producing antibodies.
- Their size ranges from 8 to 10 micrometres.
- They are commonly known as natural killer cells.
- They play an important role in body defence.
- These white blood cells are colourless cells formed in lymphoid tissue, hence referred to as lymphocytes.
- There are two main types of lymphocytes – B lymphocytes and T lymphocytes.
- These cells are very important in the immune systems and are responsible for humoral and cell-mediated immunity.

Platelets (Thrombocytes)

- Thrombocytes are specialized blood cells produced from bone marrow.
- Platelets come into play when there is bleeding or haemorrhage.
- They help in clotting and coagulation of blood. Platelets help in coagulation during a cut or wound.



Preparation of Blood Smear:

Blood collection for thick or thin blood smears -

Capillary blood obtained by finger stick:

1. Pre-cleaned slides (preferably frosted-end) are labeled with patient's name, date and time of collection.

2. Gloves are to be used.

3. Slides are cleaned with 70 to 90% alcohol and allowed to dry. The surface of the slide should not be touched where the blood smear will be made.

4. The finger is selected to puncture, usually the middle or ring finger.

5. The area is cleaned to be punctured with 70% alcohol and allowed to dry.

6. The ball of the finger is punctured.

7. The first drop of blood is wiped away with clean gauze.

8. The next drop of blood is touched with a clean slide. It is repeated with several slides (at least two thick and two thin smears should be made). If blood does not well up, the finger is squeezed gently.

For venous blood obtained by venipuncture:

1. Collection tubes and pre-cleaned slides (preferably frosted-end) are labeled with the patient's name (or other identifier), date and time of collection.

2. The site for blood collection is cleaned well using 70% alcohol and allowed to dry.

3. The venous blood is collected in a vacuum tube containing anticoagulant (preferably EDTA); alternatively, the blood is collected in a syringe and transferred it to a tube with anticoagulant after that it is mixed well.

4. At least two thick smears and two thin smears are prepared as soon as possible after collection.

Making thick and thin blood smears

Separate slides are used for thick and thin smears.

Thin film:

(a) A clean spreader slide, held at a 45° angle, is brought toward the drop of blood on the specimen slide.

(b) The slide is left until the blood spreads along the entire width of the spreader slide.

(c) While holding the spreader slide at the same angle, it is pushed forward rapidly and smoothly.

Thick film:

(a) Using the corner of a clean slide, the drop of blood is spread in a circle of diameter 1-2 cm.

(b) The slide is kept until the thin and thick films are completely dry before staining. The thin film is then fixed with methanol (100% or absolute) and it is left to dry completely before staining.

(c) If both thin and thick films need to be made on the same slide, only the thin film is fixed with methanol.

Differential Leukocyte Count (DLC):

DLC is important for the diagnosis of various blood related disorders, involving white cell or red cell. Generally, it is performed to check the normal number or distribution of different leucocytes. It also gives the morphology of different cells.

There are three major steps involved in differential cell count:
1. Preparation of blood smears
2. Staining of blood
3. Staining of smear

Preparation of Blood Smear:
A clean grease free slidkene is taken.Capillary blood is obtained directly from the finger or EDTA anti-coagulated blood. It is placed on a corner of a slide and a spreader (Spreader is a slide with sharp edges) is taken.

The edge of the spreader is touched to drop the blood on the slide. It is pushed slightly backward so that, the drop is spread evenly to the edge of the spreader. Now, the blood is spread with the help of a spreader across clean grease free slide. The angle between spreader and slide is kept at about 45°. With a quick movement, the spreader is pushed towards the other end of the slide. Blood film should not be too thick. It should be 1 cm. from the edge of slide and 5 mm. in width.

Staining of Blood:

Blood cells have different structures, which take different stains. Some are basophilic; others are acidophilic while some cells accept neutral stain.

Staining of Smear:

Leishman Stain:

Leishman stain is the most common and cheapest of all stain.

Composition:

i. Leishman stains powder – 0.15 gm.
ii. Methyl alcohol – 100 ml.

Leishman stain crystals are grounded in a glass mortar. This powder is first dissolved in few ml of methyl alcohol, and then the remaining quantity of alcohol is added, so that the entire volume becomes 100 ml. The stain is poured in a clean dry bottle and closed well. After 3 weeks it is ready for use.

Blood films are placed in a staining tray. The dry blood film is then covered with stain. The stain should be evenly distributed over the entire slide. After 1 min distilled water or buffer solution (Sodium- potassium phosphate buffer at pH 6.8) is added to the slide. The distilled water / buffer solution is carefully mixed with the stain. It is kept as it is for about 7 to 8 min. Then the slide is washed with distilled water to remove excess of stain. The film is air dried and observed under oil immersion objective of microscope.

Microscopic Examination:

First stained blood smear is examined under low power objective. The background colour and distribution of cells is noted.

In an ideal staining smear, three zones can be identified:
1. Thick area or head of smear
2. The central area is body
3. At the end of smear is tail region

The portion of the smear in the body region is chosen, slightly before the tail end. The slide is observed under oil immersion objective by putting a drop of oil over the slide.

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The portion of the smear in the body region is chosen, slightly before the tail end. The slide under oil immersion objective is observed by putting a drop of oil over the slide. Each type of white cell observed and counted. The observations are recorded either on a piece of paper in a tabular form or on a cell counter. The cell counter has different keys for different types of WBC. The counting is continued till 100 cells are counted. This will give the average number of white cells.

Observation:

With Leishman stain, the cells are observed as:

1. Neutrophil:

Purple coloured nuclei with pink cytoplasm.

2. Eosinophil:

Cytoplasm is faint pink, nucleus is purple and granules are orange red.

3. Basophil:

Granules stain dark blue with purple nucleus.

4. Monocytes:

Pink cytoplasm with purple colour nucleus.

5. Lymphocyte:

Dark blue nucleus with light blue cytoplasm.

Significance:

The blood differential test is used to diagnose a variety of medical conditions. These may include infections, autoimmune diseases, anemia, inflammatory diseases, and leukemia and other types of cancer. It is a common test that is frequently used as part of a general physical exam.

A blood differential test is used for many reason :

- Monitor your overall health or as part of a routine checkup
- Diagnose a medical condition. If you are feeling unusually tired or weak, or have unexplained bruising or other symptoms, this test may help uncover the cause.
- Keep track of an existing blood disorder or related condition

Platelet count using Hemocytometer:

The purpose of performing Total Platelets count is to know whether or not a person is suffering from Thrombocytosis, Thrombocythemia (i.e. the increase in the no. of Platelets or Thrombocytes to more than 700,000/mm^3) or Thrombocytopenia , Thrombopenia (i.e. the Decrease in the no. of Platelets / Thrombocytes to less than 50,000/mm^3).

Very large numbers of Platelets/ Thrombocytes are present in the Blood Specimen. Practically, counting this amount of Platelets directly under the microscope is highly impossible. So, the Platelets / Thrombocytes are counted by using a special type of chamber, designed for the counting of blood cells in the specimen, known as Hemocytometer or Neubauer's chamber.

For this, the blood specimen is diluted (usually in 1:20 ratio) with the help of Platelet diluting fluid (commonly the Rees – Ecker Fluid) which preserve and fix the Platelets / Thrombocytes and stains it. The Rees – Ecker fluid is isotonic to the Platelets / Thrombocytes and does not cause any damage to it whereas causes the lysis of red blood cells. After diluting the specimen in appropriate dilutions, the content is charged on Hemocytometer / Neubauer's chamber and the cells are counted in the areas specific for Platelets count.

The composition of Platelet diluting Fluid (Rees – Ecker Fluid)

COMPONENTS	QUANTITY
Trisodium Citrate	3.8 gm
Brilliant Cresyl Blue	0.1 gm
40% Formalin	0.2 ml

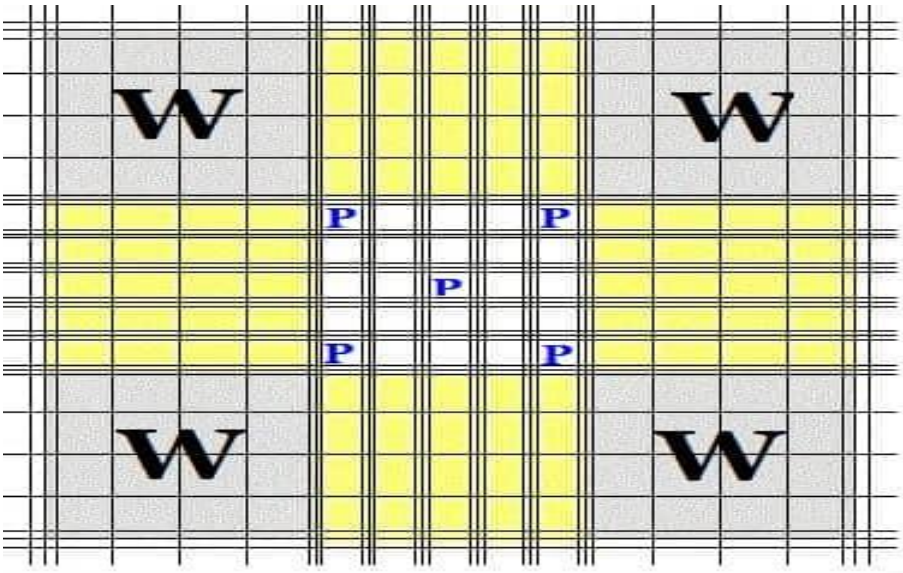
COMPONENTS	QUANTITY
Distilled Water	100 ml

The Final pH of the solution (at 25°C) varies from 6.8 – 7.0 which depends on the composition and companies who manufacture it.

Hemocytometer / Neubauer's Chamber –

This is a special type of glass chamber that is used for the cell counting, especially for Blood cells. Nowadays, most commonly Improved Neubauer's Chamber is used and in some laboratories, other types of chambers are also employed like Burkers chamber, Levy's chamber and Fusch – Rosenthal chamber etc.

The Neubauer's Chamber has ruled the area of total 9 square mm and the depth is 0.1 mm as when the coverslip is placed on the surface of the counting chamber, the space between the bottom of the cover glass and the base of grooved area measures 0.1 mm in depth. The central 1 square is highly ruled which is divided into 25 squares. Each square of the Central Square is further subdivided into 16 small squares. For Platelets Count, the cells are counted in the 5 squares of the Central square as 4 Corner squares of the Central square (divided into 25 squares) and 1 central square of the Larger Central Square (divided into 25 squares) which is same as for Red Blood Cells.



Each square of the Central Square (divided into 25 squares) contains 16 small squares so the total no. of the area to be counted for Total Platelet Count –

16 × 5 = 80 small squares

Two Method has been developed for the Manual Estimation of Total Platelets / Thrombocytes Count Using Hemocytometer / Neubauer's chamber –

1. **Microdilution Method**
2. **Macrodilution Method**

MICRODILUTION METHOD FOR THE ESTIMATION OF PLATELETS USING HEMOCYTOMETER

Materials Required for the Total Platelet Count by Microdilution Method

- Blood sample (Capillary blood or EDTA anticoagulated specimen)
- Platelet diluting fluid (preferably Rees – Ecker fluid)
- Gauze piece or Cotton
- WBC pipette
- Hemocytometer a.k.a. Neubauer's Chamber
- Coverslip
- Microscope

A Brief Introduction to WBC Pipette

WBC pipette is a graduated pipette that gives the dilution of 1:20. It has two markings at the bottom as 0.5 and 1 and the top of the pipette is marked 11. It has a round shape bulb which contains the White bead to mix the blood specimen and the diluting fluid. On the top, a rubber tube is attached to the pipette for sucking the blood specimen and diluting fluid. When blood is sucked up to 0.5 mark and the diluting fluid up to 11 marks, gives the 1:20 dilution of Blood: Diluting fluid and When the Blood is sucked up to 1 mark and the diluting fluid up to 11, gives the 1:10 dilution of Blood: Diluting fluid which less commonly used. After sucking the Specimen & Diluting fluid, the content is gently mixed by rotating the pipette on its long axis to ensure thorough mixing of blood and diluting fluid.

Note: *Nowadays Mouth pipetting is banned in most of the laboratories due to the high risk of getting infected with highly contaminated specimens of the patients. So instead of Microdilution method, the Macrodilution methods are employed in Laboratories…..*

Procedure of the Total Platelet Count by Microdilution Method

⇒ The WBC pipette is filled up to the 0.5 mark with the blood specimen and the pipette is wiped out externally to avoid false high results.

⇒ The same pipette is filled with the Platelet diluting fluid (i.e. the Rees – Ecker Fluid) up to the mark 11.

⇒ Caution has to be taken that there should be no air bubble in the pipette bulb.

⇒ The Blood and Diluting fluid is mixed in the pipette by rotating the pipette (horizontally) between your palms.

⇒ The Neubauer's chamber / Hemocytometer is taken out from its case and cleaned using a swab or gauze piece. Similarly, the cover glass is cleaned and placed it over the grooved area of Hemocytometer.

Note: *Here a special type of cover glass is used which is 0.4 mm thick with very smooth surface and even thickness so that the space between the grooved area of the chamber and cover glass is exactly 0.1 mm.*

⇒ Now, the WBC pipette is put, the solution present in it is mixed again and then discarded 1-2 drops from the pipette before charging the chamber.

⇒ The rubber tube is pressed gently of the WBC pipette, so that the next drop of fluid is in hanging position.

⇒ The Tip of the pipette is touched with the hanging drop against the edge of the coverslip making an angle of 45° approximately.

⇒ A small amount of fluid from the pipette is allowed to fill into the chamber which occurs by the Capillary action. Care is taken that the chamber it is not overcharge and there should be no air bubble in the Chamber.

⇒ After charging, the charged chamber is put in a moist condition for 10-15 min so that the cells settle down in the chamber without getting dried & then the chamber is focused under the microscope to calculate Platelets.

MACRODILUTION METHOD FOR THE ESTIMATION OF PLATELETS USING HEMOCYTOMETER

Materials Required for the Total Platelet Count by Macrodilution Method

- Blood sample (Capillary blood or EDTA anticoagulated specimen)
- Platelet diluting fluid (preferably Rees – Ecker fluid)
- Hb pipette or Micropipette (0.02 ml or 20 µl)
- Hemocytometer / Neubauer's Chamber
- Gauze piece or Cotton swab
- Graduated Pipette (5 ml)
- Test tubes
- Cover Slip

COUNTING THE PLATELETS / THROMBOCYTES UNDER THE MICROSCOPE

⇒ The ruling is focused using the 10x Objective lens and then the Platelets are ncounted in 5 small squares of the central square as described above, using the 40x Objective lens.

⇒ The cells are counted which are lying on the right and lower lines of the 5 small squares but not the opposite line. In case of marginal cells, the cells are counted on 'L' line that is either on Right and Lower lines or Left and Upper lines.

CALCULATIONS FOR THE TOTAL PLATELET COUNT USING HEMOCYTOMETER

⇒ After counting the cells under the microscope, the No. of Platelets in 5 squares of the central square are known. It is considered it as 'N' no. of cells.

⇒ Now, the volume of the fluid inside the chamber is the product of Area and depth of the Hemocytometer / Neubauer's chamber.

⇒ The central area is the 1 sq. mm which is divided into 25 parts so the area is 25 squares = 1 sq. mm

⇒ Out of these 25 squares, the Platelets are counted in 5 squares. So the Area of 5 small squares is 5/25 i.e. 1/5

⇒ The depth of the Hemocytometer is 0.1 mm as described above in a short description of Hemocytometer.

Now the Following formula is applied to get the Total Platelets Count –

Total Platelets Count = N × Dilution / Area × Depth

$$N \times 20 / (1/5 \times 0.1)$$

Total Platelet count = $N \times 1000 / mm^3$

Using the Above formula the Total No. of Platelets / Thrombocytes can be calculated present in the Blood Specimen.

NORMAL VALUES OF PLATELETS / THROMBOCYTES

Normally about 150,000 to 450,000 platelets are present in Healthy individuals. These Values show variations in various physiological and Pathological Conditions.

Erythrocyte Sedimentation Rate (ESR):

An erythrocyte sedimentation rate (ESR) is a type of blood test that measures how quickly erythrocytes (red blood cells) settle at the bottom of a test tube that contains a blood sample. Normally, red blood cells settle relatively slowly. A faster-than-normal rate may indicate inflammation in the body. Inflammation is part of your immune response system. It can be a reaction to an infection or injury.

Inflammation may also be a sign of a chronic disease, an immune disorder, or other medical condition. An ESR test can help determine if you have a condition that causes inflammation. These include arthritis, vasculitis, or inflammatory bowel disease. An ESR may also be used to monitor an existing condition.

A sedimentation rate is performed by measuring the rate at which red blood cells (RBCs) settle in a test tube. The RBCs become sediment in the bottom of the test tube over time, leaving the blood serum visible above. The classic sedimentation rate is simply how far the top of the RBC layer has fallen (in millimeters) in one hour. The sedimentation rate will be higher in the presence of increased inflammation. The normal sedimentation rate (Westergren method) for males is 0-15 millimeters per hour, females are 0-20 millimeters per hour. The sedimentation rate may normally be slightly higher in the elderly. Here is the normal range for the sedimentation rate chart.

The normal range for the sedimentation rate chart

Gender and Age	**Sed Rate (mm/hour)**
Males younger than 50	0 to 15
Males older than 50	0 to 20
Females younger than 50	0 to 20
Females older than 50	0 to 30

Theoretical considerations:

The RBCs sediment because their density is greater than that of plasma; this is particularly so, when there is an alteration in the distribution of charges on the surface of the RBC (which normally keeps them separate form each other) resulting in their coming together to form large aggregates known as rouleaux. Rouleaux formation is determined largely by increased levels of plasma fibrinogen and globulins, and so the ESR reflects mainly changes in the plasma proteins that accompany acute and chronic infections, some tumors and degenerative diseases. In such situations, the ESR values are much greater than 20mm/hr. Note that the

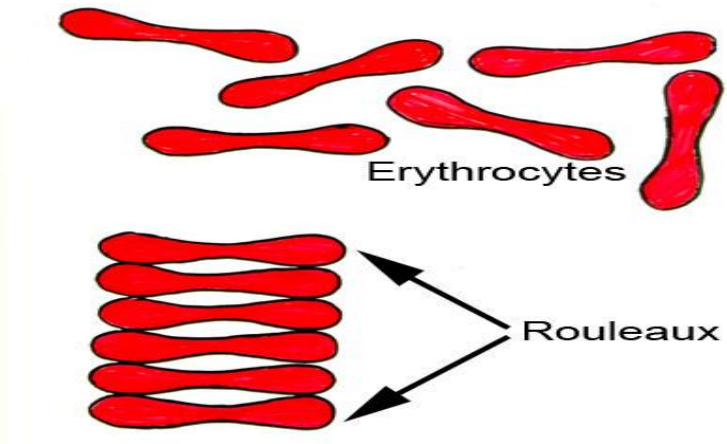
ESR denotes merely the presence of tissue damage or disease, but not its severity; it may be used to follow the progress of the diseased state, or monitor the effectiveness of treatment.

Some interferences which increase ESR:

- increased level of fibrinogen, gamma globulins.
- technical factors: tilted ESR tube, high room temperature.

Some interferences which decrease ESR:

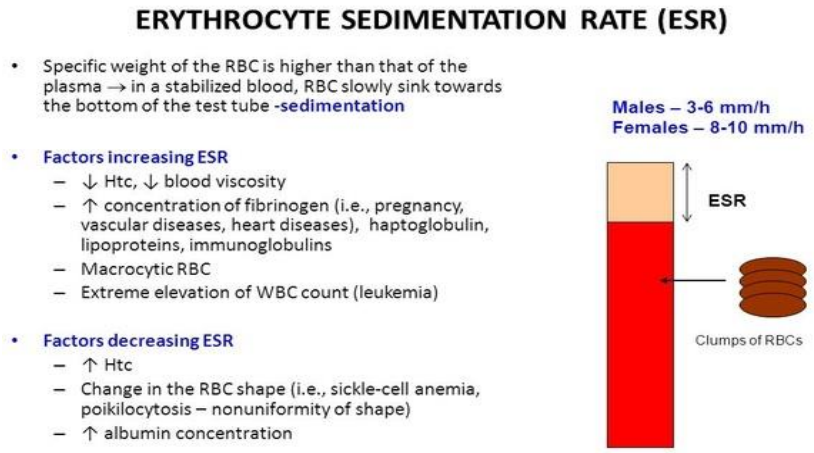
- abnormally shaped RBC (sickle cells, spherocytosis).
- technical factors: short ESR tubes, low room temperature, delay in test performance (>2 hours), clotted blood sample, excess anticoagulant, bubbles in tube.



Procedure:

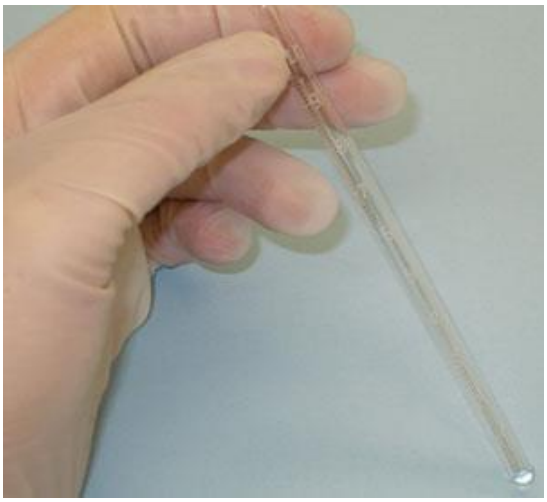
The ESR test measures the rate at which the red blood cells, or erythrocytes, in a sample of blood settle at the bottom. This process of settling is called sedimentation. A small amount of blood from the individual's vein and send it to a laboratory. The blood is transferred to a vertical test tube in which the red blood

cells will slowly settle at the bottom. This will leave a clear, yellowish fluid at the top, which is blood plasma. The result of the test will depend on the amount of plasma at the top of the tube after 1 hour. The measurement will be in millimeters per hour (mm/hr). Red blood cells settle at a faster rate in people with inflammatory conditions. These conditions trigger an inflammatory process in the body, which leads to an increase in the number of proteins in the blood. This increase causes red blood cells to clump together and settle more quickly. People whose red blood cells settle faster will have elevated ESR values, indicating to doctors that a medical condition may be present.



When anticoagulated whole blood is allowed to stand in a narrow vertical tube for a period of time, the RBCs – under the influence of gravity - settle out from the plasma. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour (mm/hr).

Westergren method: The Westergren method requires collecting 2 ml of venous blood into a tube containing 0 .5 ml of sodium citrate. It should be stored no longer than 2 hours at room temperature or 6 hours at 4 °C. The blood is drawn into a Westergren-Katz tube to the 200 mm mark. The tube is placed in a rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment is measured. The distance of fall of erythrocytes, expressed as millimeters in 1 hour, is the ESR.



Wintrobe method: The Wintrobe method is performed similarly except that the Wintrobe tube is smaller in diameter than the Westergren tube and only 100 mm long. EDTA anticoagulated blood without extra diluent is drawn into the tube, and the rate of fall of red blood cells is measured in millimeters after 1 hour. The shorter column makes this method less sensitive than the Westergren method because the maximal possible abnormal value is lower. However, this method is more practical for demonstration purposes.