

Differential Leukocyte count,
Reticulocyte count, Packed cell
volume, hemoglobin concentration
and Erythrocyte Sedimentation rate

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Differential Leukocyte Count (DLC)

- The 5 main types of WBCs and their percentage:

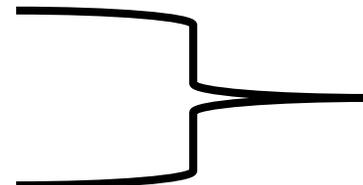
1. Neutrophils: 40-80%

2. Eosinophils: 1-6 %

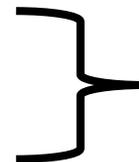
3. Basophils: < 1-2%

4. Lymphocytes: 20-40%

5. Monocytes: 2-10%



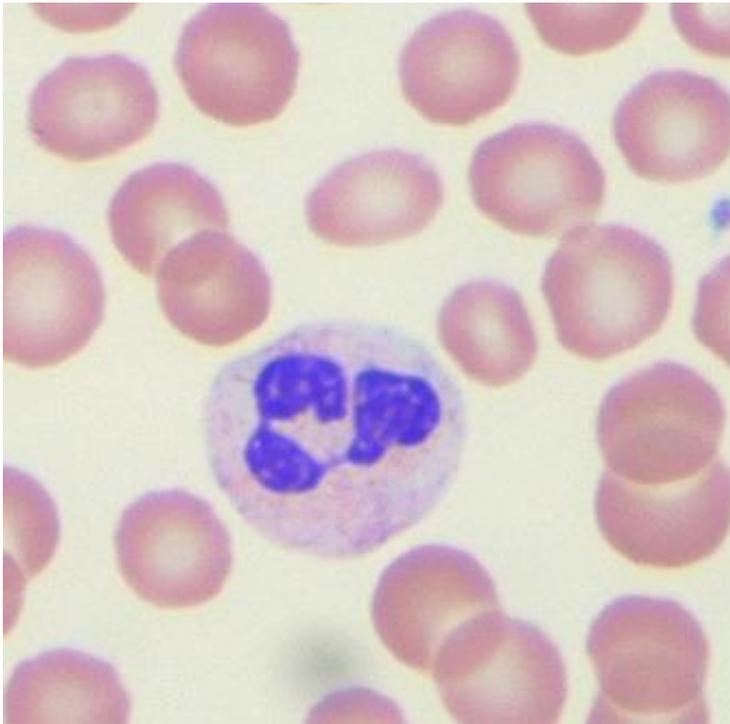
Granulocytes



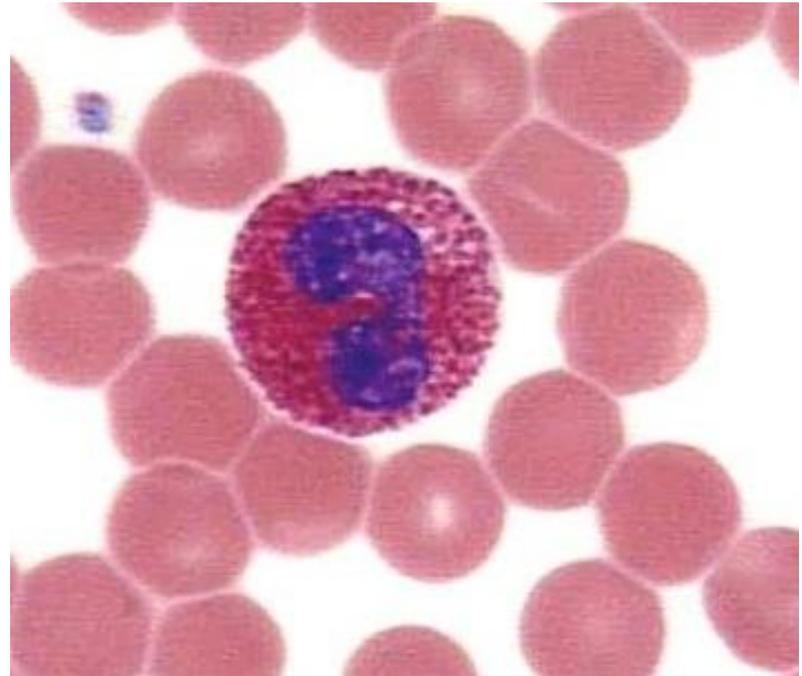
Agranulocytes

- Can be distinguished by staining the blood sample with certain dyes. Then 100-200 WBCs are counted and classified. The number of each type of cells is expressed as a percentage.
- To do this one must be able to distinguish between the 5 types of WBCs.

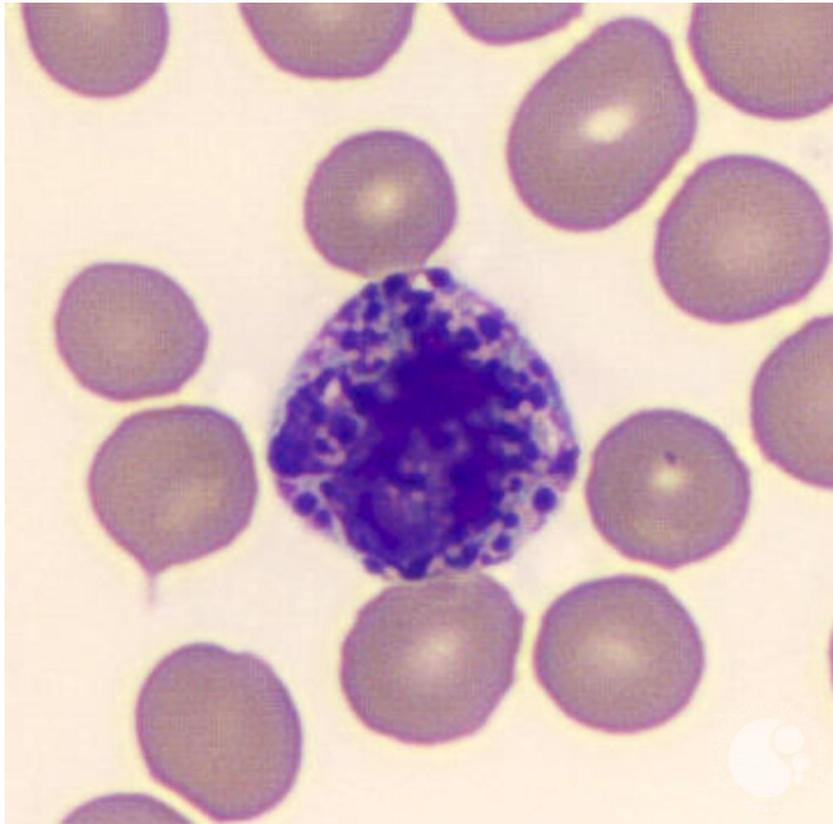
Neutrophils have nuclei with several lobes and fine pink granules in their cytoplasm. They are termed neutrophils, because their granules are not very amenable to staining with either acidic or basic dyes



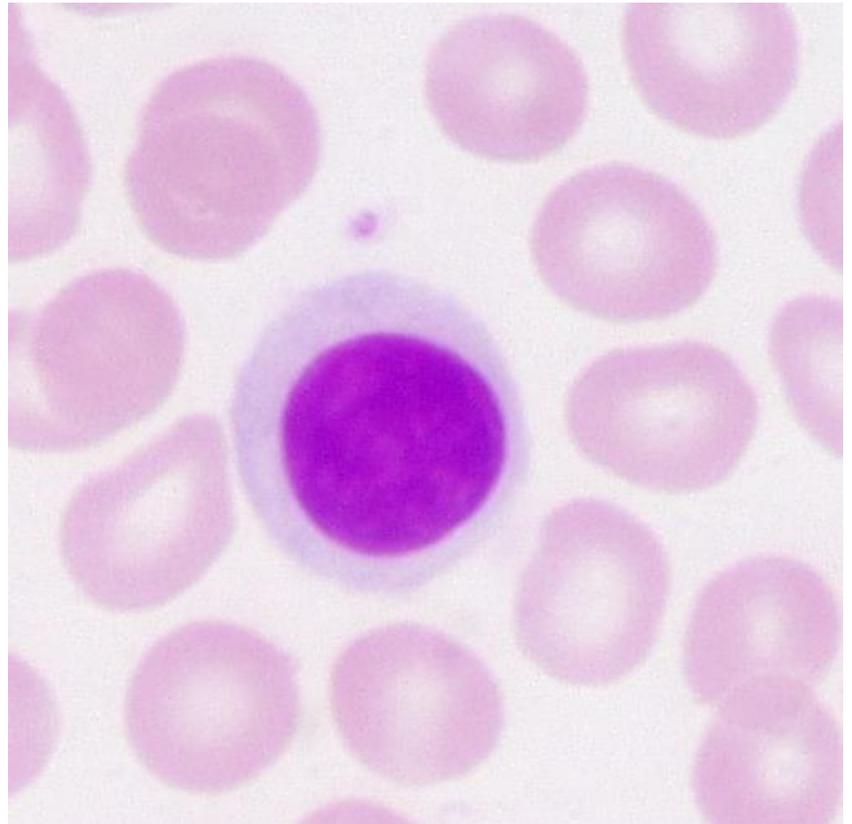
Eosinophils have bi-lobed nuclei and medium-sized granules that can be stained bright red with an acidic dye.



Basophils have bi-lobed nuclei and large granules which stain dark blue with basic dyes and completely obscure the nucleus



Lymphocytes can be large or small. They are spherical and have a very large nucleus taking up most of the cytoplasm. The cytoplasm has no granules



- **Monocytes** are large cells. They have large indented nuclei, often kidney-shaped and very fine cytoplasmic granules



- Differential leukocyte count gives relative percentage of each type of WBC and also helps reveal the presence of abnormal WBCs like blasts or lymphoma cells.
- Differential leukocyte count is also used along with WBC count to generate an absolute value for each type of WBCs.
- Absolute count for any type of cell is the actual number of WBCs that are of that type. It usually gives more meaningful information than the percentage of each, since relative percentages can be misleading. Expressing absolute values are also useful for monitoring certain conditions.

- Absolute neutrophil count (ANC) is the actual number of WBCs that are neutrophils.
 - $ANC = WBC \text{ count} \times (\text{neutrophil}\% + \text{bands}\%) / 100$
 - ✓ **Band cells** are an immature form of neutrophils

- If the WBC count is 6000 cells/mm³ and the lymphocyte make 30% of the DLC, the Absolute lymphocyte count (ALC) will be:

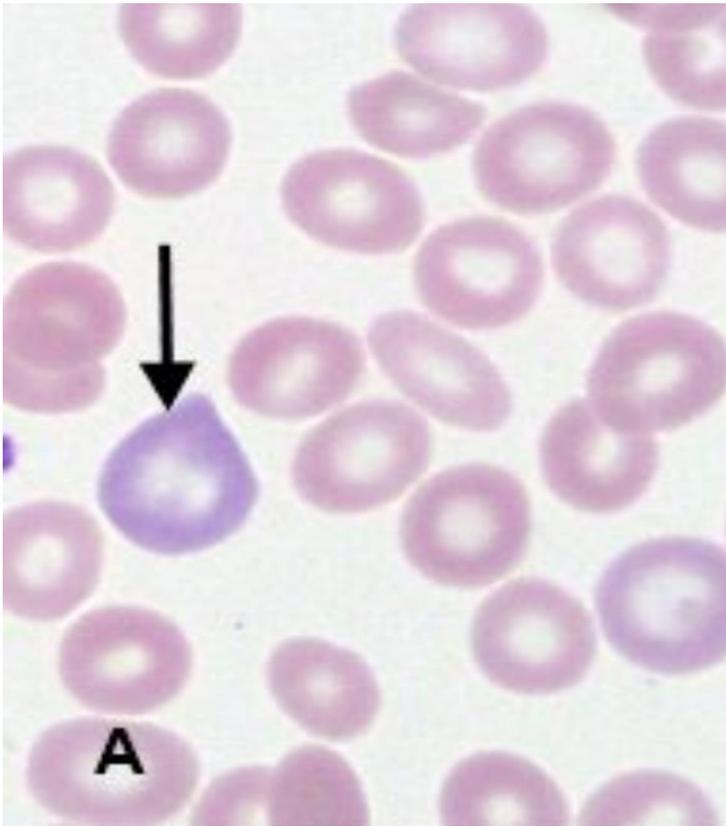
$$WBC \text{ count} \times (\text{Lymphocyte}\%) / 100 =$$
$$(6000 \times 30) / 100 = 1800 \text{ cells/mm}^3$$

- Deviations of different white cell counts from the normal values often indicate a diseased state:
- Neutrophilic leukocytosis: Bacterial infections, inflammatory conditions, CML, stress.
 - Lymphocytic leukocytosis :Viral infections, ALL, CLL.
 - Monocytic leukocytosis: Chronic bacterial infections and chronic inflammation
 - Eosinophilic leukocytosis : Parasitic infections & allergic conditions
 - Basophilic leukocytosis: Allergic conditions
 - Neutropenia :certain infections like TB, typhoid fever, HIV & CMV virus, chemotherapy, radiotherapy, autoimmune diseases
 - Lymphocytopenia: HIV, chemotherapy, radiotherapy.

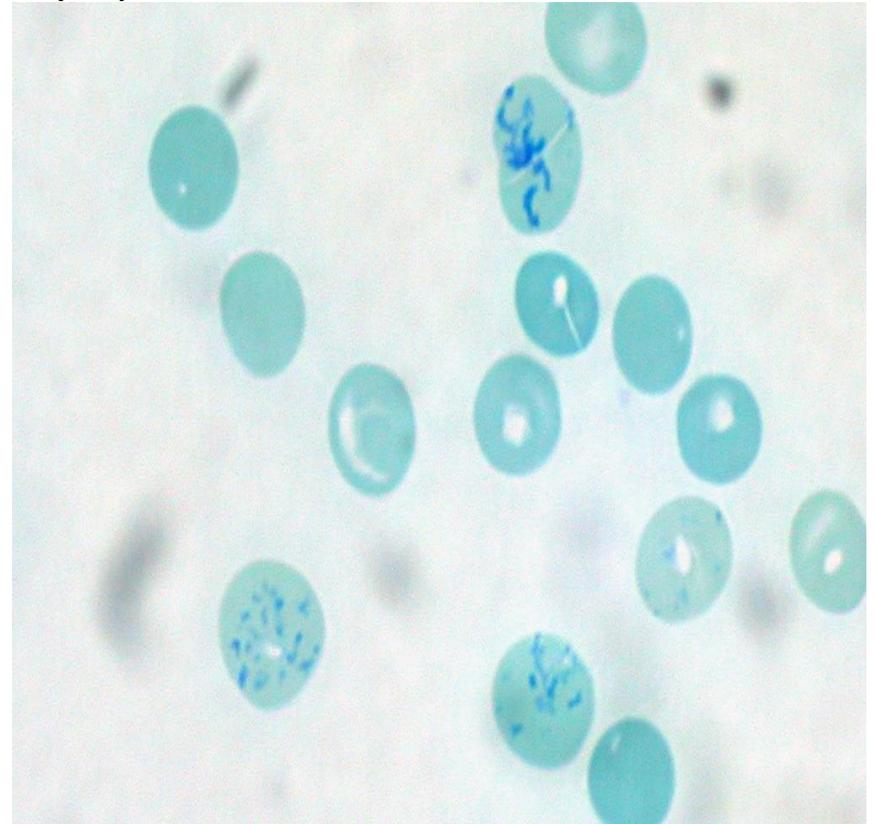
Reticulocyte Count

- Reticulocytes are the immediate precursor of RBCs, following their release to the blood stream they mature within 1-2 days into RBCs.
- Contain a small amount of basophilic material, mainly remnants of the Golgi apparatus & mitochondria
- They make less than 1-2% of all RBCs
- Used to estimate the degree of effective erythropoiesis
- Their number increases in cases of bleeding and RBC hemolysis and decreases in cases of bone marrow failure

On a blood smear stained with Wright-Giemsa, reticulocytes appear larger than mature RBCs with irregular borders and are blue-grey in color. They often lack the central pallor.



If supravital staining (new methelene blue) is performed on a blood smear, the reticulocytes appear larger than RBCs and contain dark blue dots and curved linear structures in their cytoplasm.



How it's performed

- 500-1000 RBCs should be counted and the number of reticulocytes noted. The count is expressed as a percentage which can be used to calculate the absolute reticulocyte count (ARC) .
- ARC accurately reflects the degree of reticulocytosis regardless of the degree of anemia. The normal absolute reticulocyte count is between 25,000 to 75,000/mm³
- $ARC = (RBC \text{ count} \times \text{reticulocyte}\%) / 100$

Packed Cell Volume (PCV)

- PCV or Hematocrit HCT is the percentage of blood that is in RBCs
 - Males: 40%- 54%
 - Females: 36% - 46%
- It decreases in cases of anemia and increases in polycythemia.

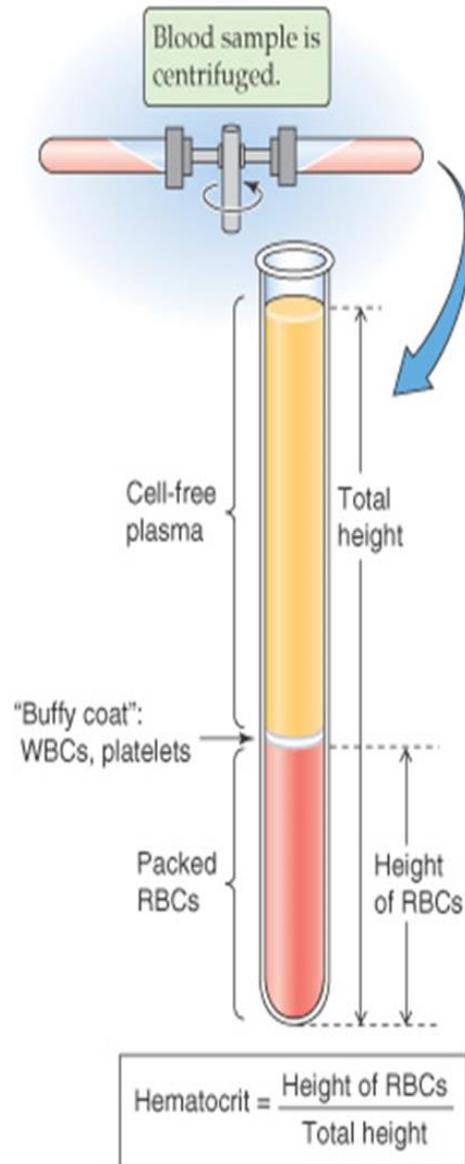


Figure 18-1 Determination of the hematocrit.

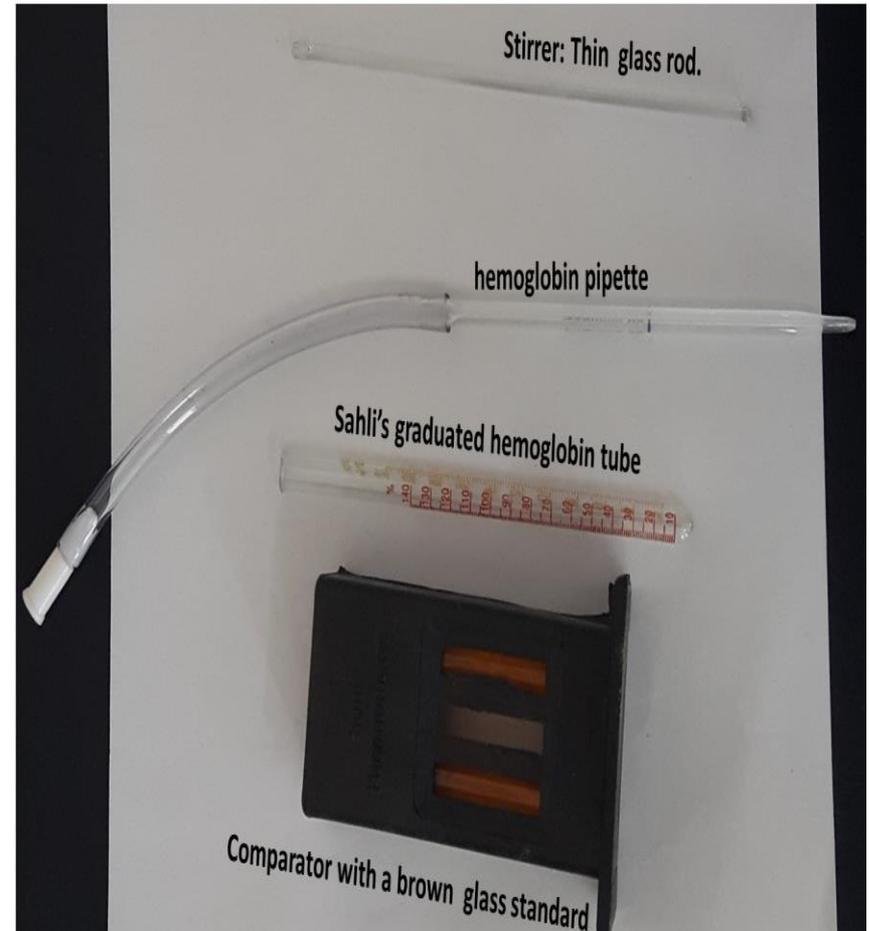
- When centrifuging a blood sample in a heparinized capillary tube, the RBCs become packed at the bottom of the tube. The PCV is then calculated according to the following formula:
- $PCV = \frac{\text{RBC height}}{\text{Total height}} \times 100$
- Beware not to include the buffy coat

Hemoglobin Concentration

- Hemoglobin is a globular protein made up of four subunits. Each subunit contains a **heme** group conjugated to a polypeptide. Heme is an iron-containing porphyrin derivative. The polypeptides are referred to collectively as the **globin** portion of the hemoglobin molecule.
- Heme has the ability to bind oxygen reversibly and carry it to tissues.
- Normal values of hemoglobin
 - 14-17.5 g/ 100 ml in males
 - 12-15 g/ 100 ml of in females
- Different methods can be used to find the hemoglobin concentration one of them is Sahli's method.

Sahli's Method

Sahli's haemoglobinometer



When blood is mixed with HCl, hemoglobin is converted to acid hematin which is brown in color

- The procedure:
 1. Add HCl into the tube up to 2g% mark
 2. Mix the EDTA sample gently and fill the pipette with 0.02ml blood.
 3. Wipe the external surface of the pipette to remove any excess blood.
 4. Add the blood into the tube containing HCl.
 5. Mix the blood with the acid thoroughly. Allow the mixture to stand undisturbed for 10min.
 6. Place the hemoglobinometer tube in the comparator and add distilled water to the solution drop by drop stirring with the glass rod until it's colour matches that of the comparator glass.
 7. Remove the stirrer and take the reading directly
- Hemoglobin concentration is read directly from the graduated scale on the dilution tube.

Erythrocyte Sedimentation Rate (ESR)

- The rate at which RBCs sediment in a period of one hour.
 - Men < 15mm/hr
 - Women < 20mm/hr
- It is a common & simple test
- Non-specific measure of inflammation.
- Governed by the size & shape of RBCs & the presence of plasma proteins associated with inflammation and tissue damage e.g. fibrinogen and immunoglobulins

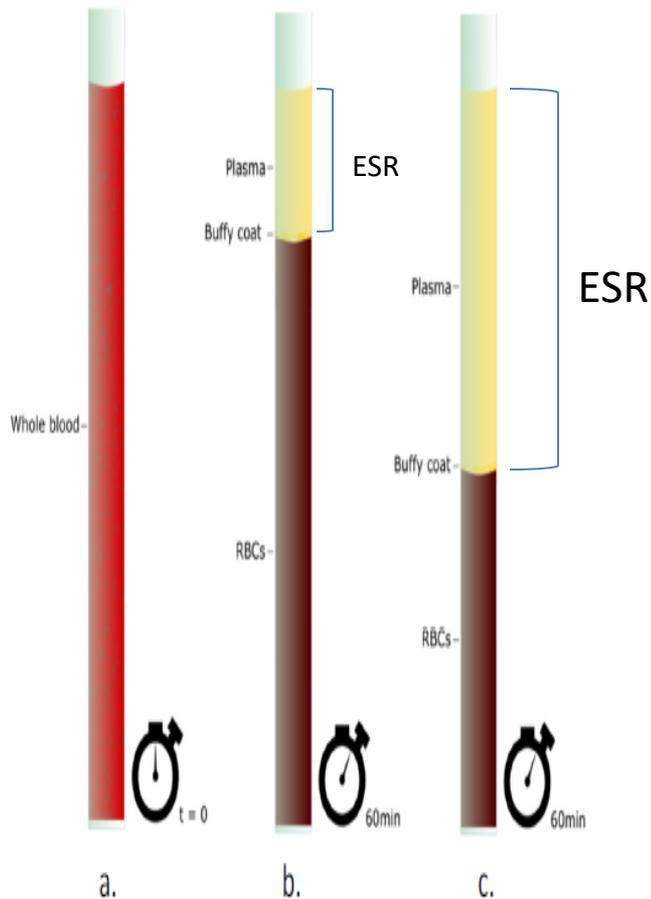


Fig 3: a. The diluted sample is aspirated and transferred to the Westergren tube.
 b. A normal ESR after 60 minutes; <20 mm plasma.
 c. An elevated ESR after 60 minutes.

- Anticoagulated whole blood is allowed to stand in a narrow vertical tube for an hour.
- The ESR is measured as the distance from the bottom of the plasma meniscus to the top of the descended erythrocytes and recorded in mm/hr.

- High ESR

- Inflammation

- Anemia

- Old age

- Low ESR

- Polycythemia

- Sickle-cell disease

- Spherocytosis