

اساسيات التعرف على خلايا الدم للتطبيقات المخبرية

Blood is a fluid connective tissue. It circulate continually around the body, allowing constant communication between tissues distant from each other.

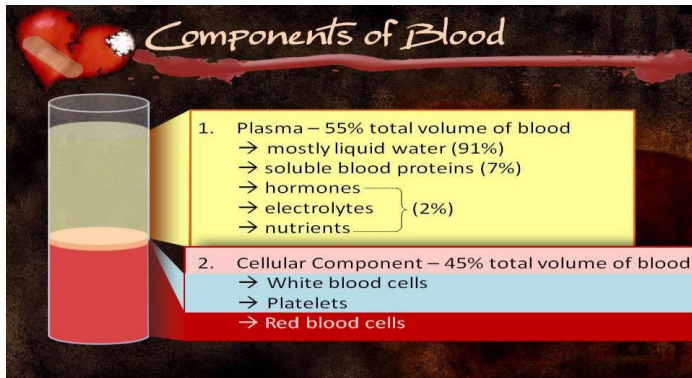
- Blood makes up about 7% of body weight (about 5.6 liters in a 72 Kg man). This proportion is less in women, while in children is greater (gradually decreasing until the adult level is reached).

Functions of the Blood

1. The main function of the blood is to maintain intracellular homeostasis by:
 - a). Carries O₂ and nutrients (glucose, amino acids, lipids, and vitamins) to the cells.
 - b). Carries CO₂ and other wastes (nitrates, creatine, nucleic acid) away from the cell.
2. Providing intercellular communication in the body: carries hormones (secreted by endocrine glands) to the target organs.
3. Protection and defense: it allows cells and immunological proteins to transport from place to place where need them.
4. Self repair mechanism: clotting cascade.

Blood Components

- Blood is composed from 2 fractions:
 1. Plasma: Non living extracellular matrix composes about 55% of total blood volume.
 2. Formed elements (living cells) composes about 45% of total blood volume.
- The two frictions of blood can be separated by spinning.



Plasma

Above the buffy coat is a pale straw-coloured fluid called plasma. Plasma contains hundreds of different substances proteins, vitamins, hormones and minerals.

- The constituents of plasma are:

1. **Water** (90-92%)

2. **Plasma proteins:** make up about 7% of plasma.

- **Albumins**

- **Globins**

- **Clotting factors.**

3. **Inorganic**

4. **Nutrients:** glucose, amino acid, fatty acids and glycerol.

5. **Waste products**

6. **Hormones and gases**

Formed Elements (Cellular Content of Blood)

- There are three types of blood cell:

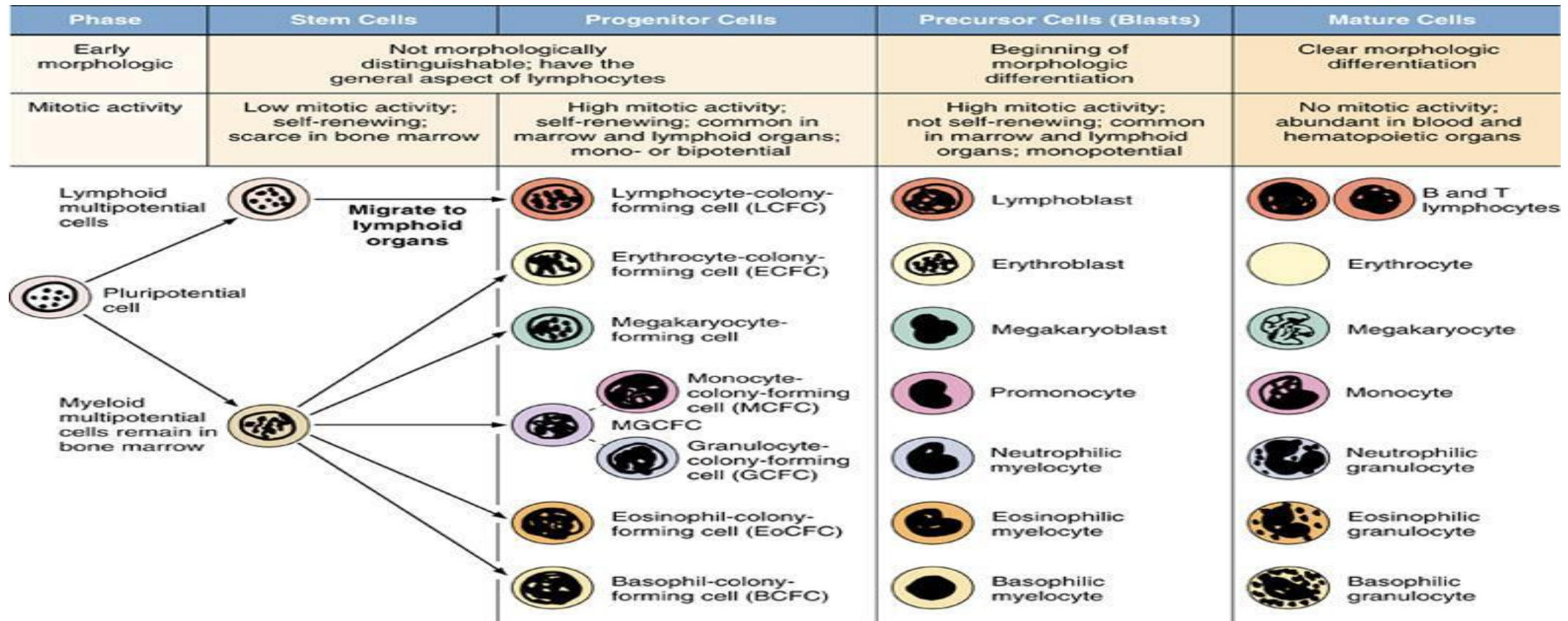
1. **Erythrocytes** (Red Blood Cells =RBC).

2. **Platelets** (thrombocytes)

3. **Leukocytes** (white blood cells = WBC) they include monocytes, lymphocytes, neutrophils, eosinophils, and basophils

Source of Blood Cells

- Mature blood cells have a relatively short life span.
- Blood cells are synthesised mainly in the red bone marrow.
- Some lymphocytes, additionally are produced in lymphoid tissue.
- The organ or system responsible for synthesis blood cells are called hematopoietic system and the process of blood cell formation is called hematopoiesis.



Erythrocytes = Red Blood Cells

Erythrocytes are produced in red bone marrow (in the ends of long bones and in flat and irregular bones).

- They pass through several stages of development before entering the blood.
- Their life span in circulation is about 120 days.

- The process of RBC development from stem cells takes about 7 days and is called erythropoiesis.

The count of red blood cells, is the number of red counting: blood cells in mm^3 of whole blood.

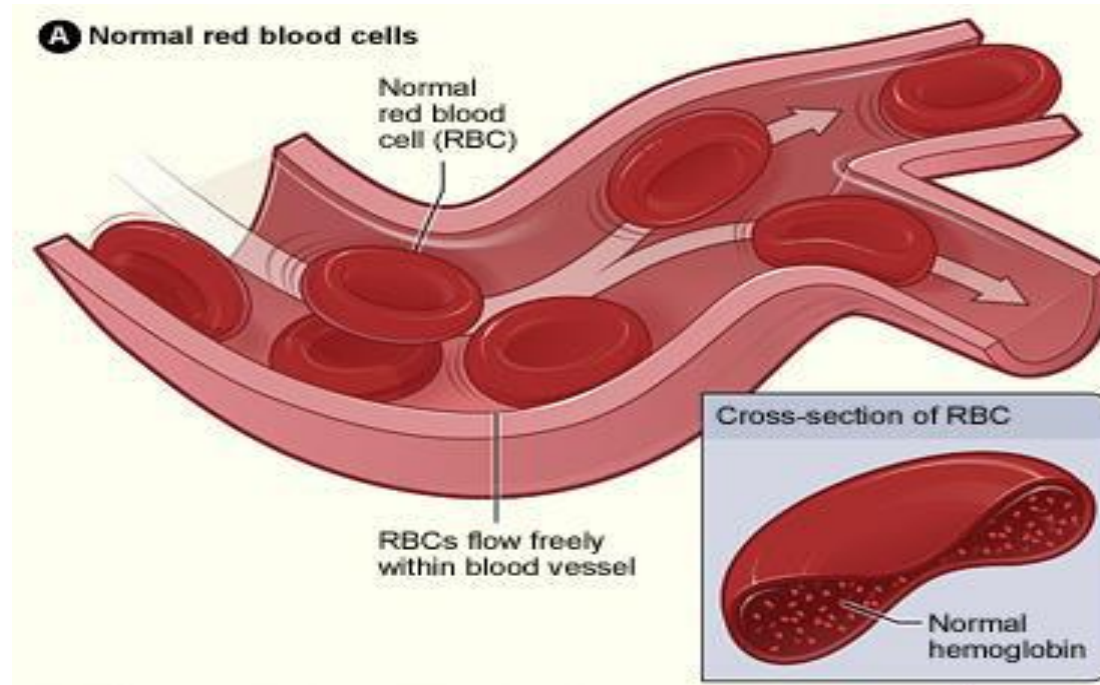
RBC count - Normal Values:

Men: $4.5\text{-}6.2 \times 10^6/\text{mm}^3$

Women: $4.0\text{-}5.5 \times 10^6/\text{mm}^3$

at birth: $4.0\text{-}6.0 \times 10^6/\text{mm}^3$

3 years – 10 years: $4.0\text{-}5.0 \times 10^6/\text{mm}^3$



The RBC counting performed by:

1. Manually by Hemacytometer

2. The blood cells automatic analyzer (Part of CBC; group of tests)

Importance OF RBCS counting:

RBCs countless or more than normal, it is a screen test for anemia or polycythemia

RBC count using Haemocytometer

- Materials:

- Venous blood mixing with EDTA or capillary blood with heparin
- Improved Neubauer's counting chamber with Coverslips
- RBC pipette and RBC Diluting solution (Hayem's Fluid), the Purpose of this fluid: its isotonic solution diluted blood, prevent lysis and blood sedimentation)
- Compound Microscope

Principle: The blood is diluted 200 times with RBCs diluting fluid (1\200) in practice (20ul blood and 4 ml dilution).

Procedure:

1. Draw blood by micropipette micropipette 20 ul.
2. Mix the blood with diluting fluid 4ml .
3. Mix the contents in glass tube for 2min.
4. Introducing the sample into the Neubauer chamber (10 ul from from mixture) mixture).
5. To count the RBCs RBCs the microscope Microscope must be switched to 40 X objective
6. Count RBCs from from R marked 5squares squares
7. RBCs count in* 10 .000 cells cells of blood

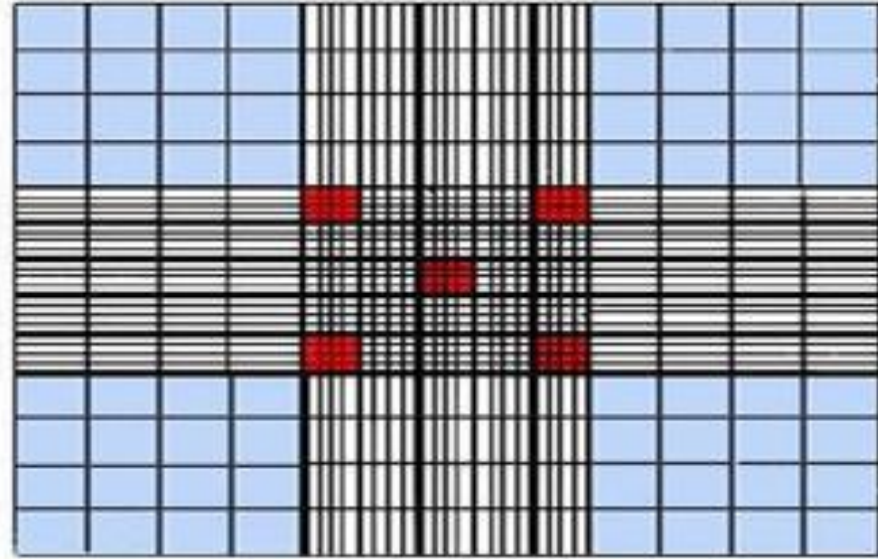
Calaulation:

$$\text{Count (/l)} = \frac{\text{No. of the counted cells.}}{\text{Volume of the blood (}\mu\text{l)}} \text{ dilution} \times 10^6.$$

$$\text{Count (/l)} = \frac{N}{0.02} \times 200 \times 10^6 / l.$$

$$\text{Number of red cells count / l} = N \times 10,000$$

■ areas of the grid where WBC are counted



■ areas of the grid where RBC are counted

Causes of decreased RBC count:

- Impaired red blood cell (RBC) production
- Increased RBC destruction (hemolytic anemia's)
- Blood loss
- Fluid overload (hemodilution)

Causes of high RBC count:

- High altitudes , Hypoxia, Smoking, Dehydration
- Polycythemia vera (often a hereditary problem)
- Congestive heart disease, Certain lung diseases
- Erythropoietin doping by athletes to boost their performance
- Some types of anemia has high RBC count such as β -thalassemia

Physiological effect on RBC count:

1-age 2-sex 3-Activity 4-nutrition 5-pregnancy 6-breast feeding 7 psychological Emotions

Red cells indices:

They are calculated from total red cell count, hematocrit (pcv) and hemoglobin.

It can be determined by automated hematology analyzer in CBC test or manual

Usefulness of RBCs Indices

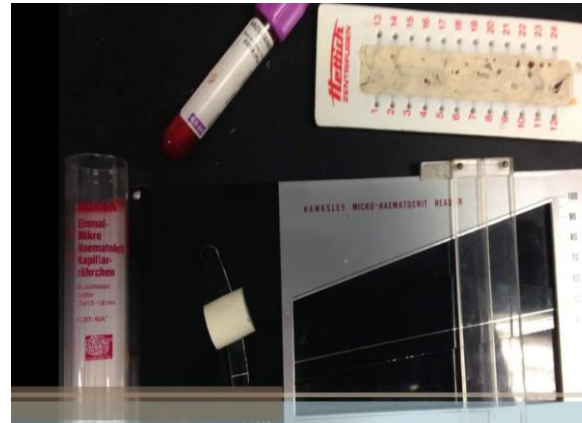
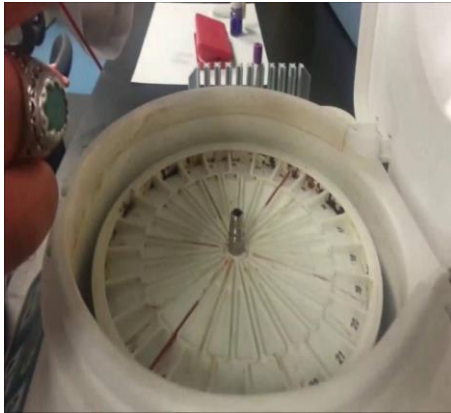
These parameters are useful in classifying anemia's into microcytic, normocytic, or macrocytic; and hypochromic or normochromic

Three most used RBCs indices are the MCV, MCH and MCHC

They include:

1. Total red blood cells (RBC): The number of red cells is given as an absolute number per litre.
2. Hemoglobin (Hb): The amount of hemoglobin in the blood, expressed in (grams per decilitre) (g/dl). (Low hemoglobin is called anemia.)

3. Hematocrit or packed cell volume (PCV): This is the fraction of whole blood volume that consists of red blood cells.
4. Mean corpuscular volume (MCV): The average volume of the single red cell.
5. Mean corpuscular hemoglobin (MCH): The average amount (or weight) of hemoglobin per single red blood cell.
6. Mean corpuscular hemoglobin concentration (MCHC): The average concentration (or weight) of hemoglobin inside all the red blood cells. It correlates with the degree of hemoglobinization of the red cells on the peripheral blood film.
7. Red blood cell distribution width (RDW): A measure of the variation of the RBC population.



RBC indices

RBCs indices		Unit	Normal range	usefulness
MCV	$Mcv = \frac{hct}{rbc} \times 10$	Femtoliter fl	80-100 fl	MCV increased in macrocytic anemia and decreased in microcytic anemia like IDA and thalassaemia
MCH	$MCH = \frac{Hb}{rbc} \times 10$	Picogram pg	27_32 pg	MCH increased in macrocytic anemia and decreased in microcytic anemia like IDA and thalassaemia
MCHC	$MCH = \frac{Hb}{pcv} \times 100$ <i>Hb g/dl</i> OR $MCH = \frac{MCH\ pg}{MCV\ fl} \times 100$	Percentage Or g/dl or g/l	32_36 g/dl or percentage	MCHC is useful guide to measure the degree of hypochromasia present in IDA

Leucocytes = White blood cells (WBC)

- Leucocytes are the largest blood cells.
- They account for only about 1% of the blood volume.

Leucocytes are different from erythrocytes in several ways:

1. They are true cells, each leucocyte having a nucleus, mitochondria, and other organelles.
2. They do not contain Hb.
3. Leucocytes can actively move while erythrocytes do not have mobility of their own.
4. Normally erythrocytes do not leave the vascular system but leucocytes can leave vessels and enter the surrounding tissue.
5. Most leucocytes have a relatively short life span.

White blood cells count is important in the diagnosis of disease

Normal Value

Infants 15,000-20,000 cell/mm³

Children 4,500-13,500 cell/mm³

Adult 4,000-11,000 cell/mm³

Types of WBC

- There are two main types:
 1. **Granulocytes** which contain granules in their cytoplasm and they *Neutrophils*, *easinophils* and *basophils*.
 - **Neutrophils (polymorph):**
 - ☐ Cell diameter: 12-15 µm
 - ☐ Nucleus: 2-5 lobes , clumped

- Cytoplasm: pink\white granules

- Normal value : 40 – 80%

- Absolute count per μl : 2000- 7500

- ❖ **increase in neutrophil count above 7500\ μl (neutrophilia):**

- 1-acute infections (by bacteria , fungi,..)

- 2-inflammation from damage tissue

- ❖ **Decrease in neutrophil count below 2000\ μl (neutropenia):** ex: Typhoid , malaria , aplastic anemia

- **Eosinophila :**

- Cell diameter : 12-15 μm

- Nucleus : Bilobed

- Cytoplasm : coarse red granules

- Normal value :1 - 6 %

- Absolute count per μl :40 – 400

- ❖ **Increase in eosinophil count above 400 \ μl (Eosinophilia):** ex : Allergy, Asthma

- ❖ **Decrease in eosinophil count below 40 \ μl (Eosinopenia) :** ex : steroid administration

- **Basophil :**

- Cell diameter : 12-15 μm

- Nucleus : Bilobed

- Cytoplasm : Large , coarse purplish granules

- Normal value :0 - 1 %

- Absolute count per μl :10 – 100

- ❖ **Increase in Basophil count above 100\ μl (Basophilia) :** ex: chronic myeloid leukemia

2. Agranulocytes

- *Monocytes* and *lymphocytes*.

- **Lymphocytes:**

- ☐ Cell diameter :

- Small lymphocyte : 9-12 μm

- Large lymphocyte : 12-16 μm

- ☐ Nucleus : large nucleus round to indented fills the cell

- ☐ Cytoplasm : no granules

- ☐ Normal value : 20 – 40 %

- ☐ Absolute count per μl : 1500 – 4000

- ❖ **Increase in Lymphocyte count above 4000\ μl (Lymphocytosis) :** ex : viral hepatitis and Tuberculosis

- ❖ **Decreases in lymphocyte count below 1500\ μl (Lymphopenia) :** ex : AIDS

- **Monocytes:**

- ☐ Cell diameter : 12 -20 μm

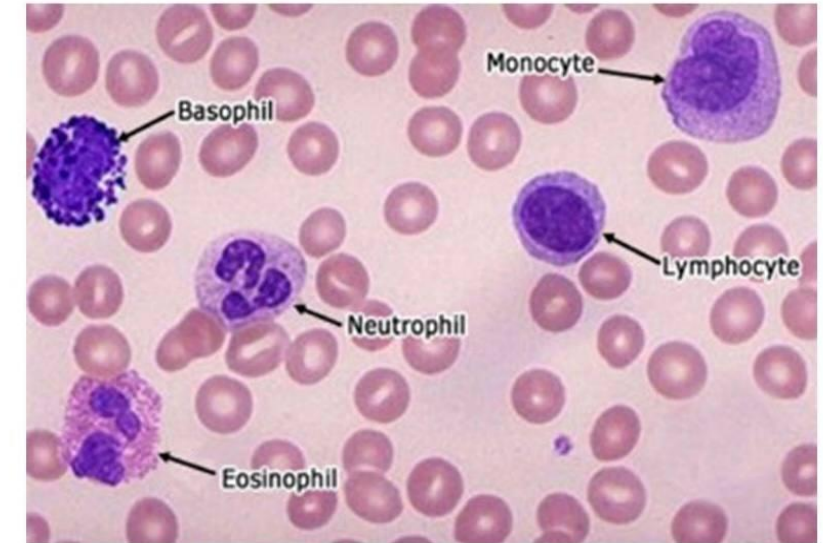
- ☐ Nucleus : large lobulated

- ☐ Cytoplasm : no granules

- ☐ Normal value : 2-10 %

- ☐ Absolute count per μl : 200 -800

- ❖ **Increase in monocyte count above 800\ μl (Monocytosis) :** ex: Malaria, syphilis.



Experiment of white blood cell count:

The Principle

- The blood is diluted **20 times** with the WBCs diluting fluid .
- The leukocytes are then counted in **4 corners squares** of the **improved Neubauer chamber**.

Purpose

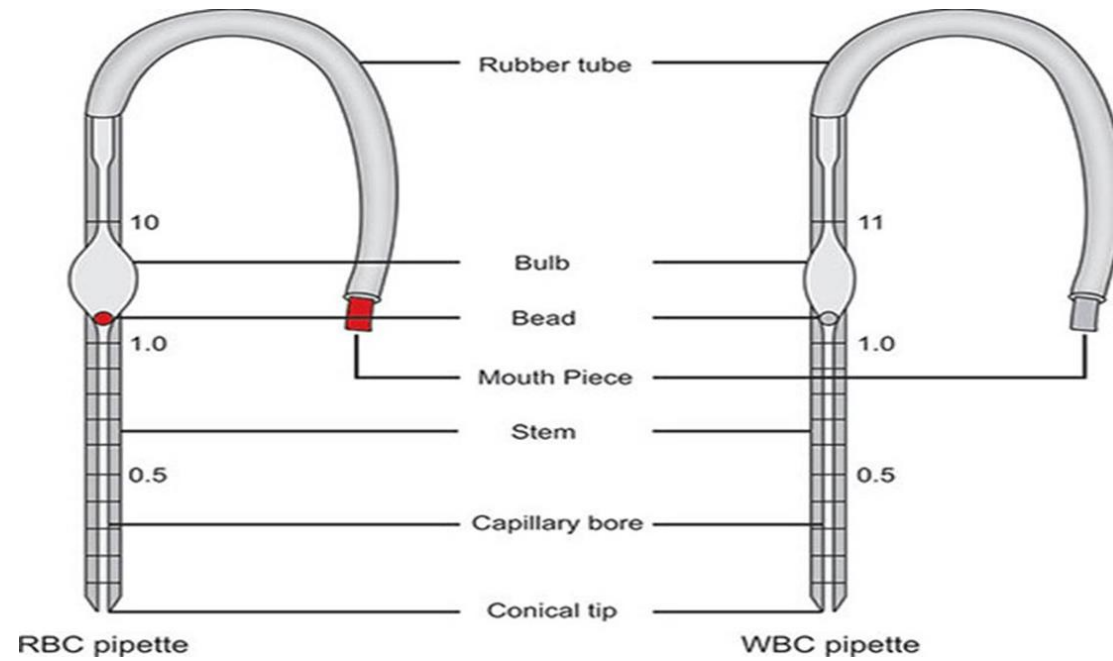
The WBCs count is **to determine the number of circulating WBCs in the body**

Apparatus and Reagents

- Hemocytometer counting chamber (Neubauer chamber with Coverslip).
- WBCs counting pipette.
- Rubber tube with a plastic mouth piece for drawing the fluid into a pipette.
 - Microscope
 - Diluting fluid (Gentian violet (1% solution) (1.5 ml) (stains the nuclei of WBC).
- Disposable blood lancet, sterile cotton, & 70% alcohol

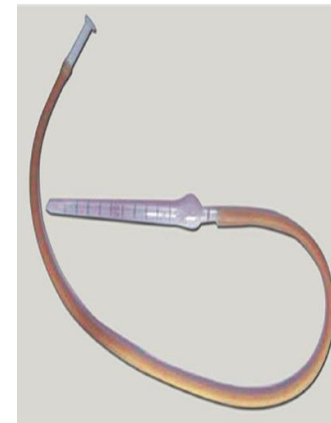
pipette

- **WBC pipette** have white bead in the bulb and its mark **(0.5, 1, 11)**
- **While RBC pipette** have red bead in the bulb and its mark **(0.5, 1, 101)**



Procedure

1. Ensure that Hemocytometer chamber, coverslips and lenses of the microscope are clean, WBC pipette is dry.



2. Sterilize the tip of the middle finger of hand

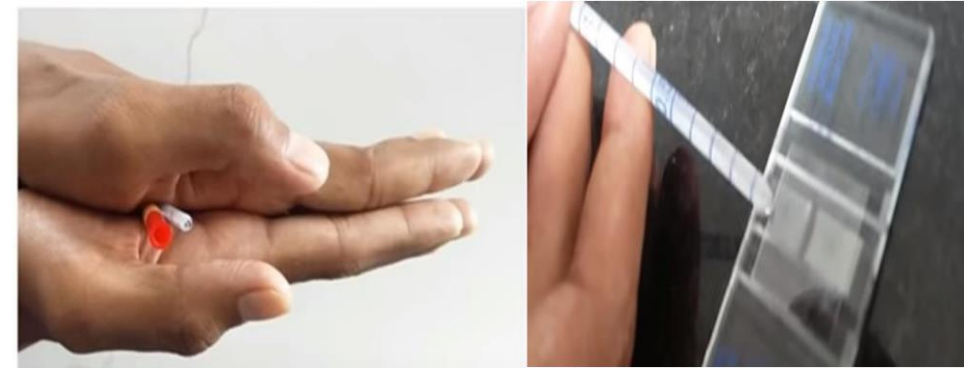


3. Suck the blood up to 0.5 marking of WBC pipette followed by the diluting fluid (Thomas solution) up to 11 marking without any air bubble.

4. Mixing is done by rotating the pipette between the palms

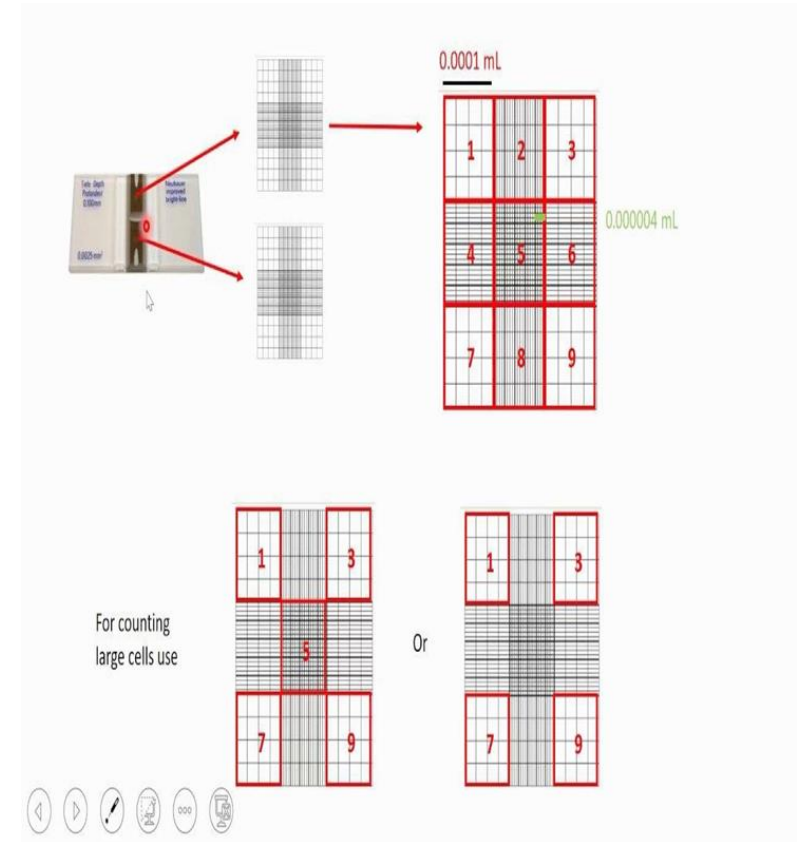
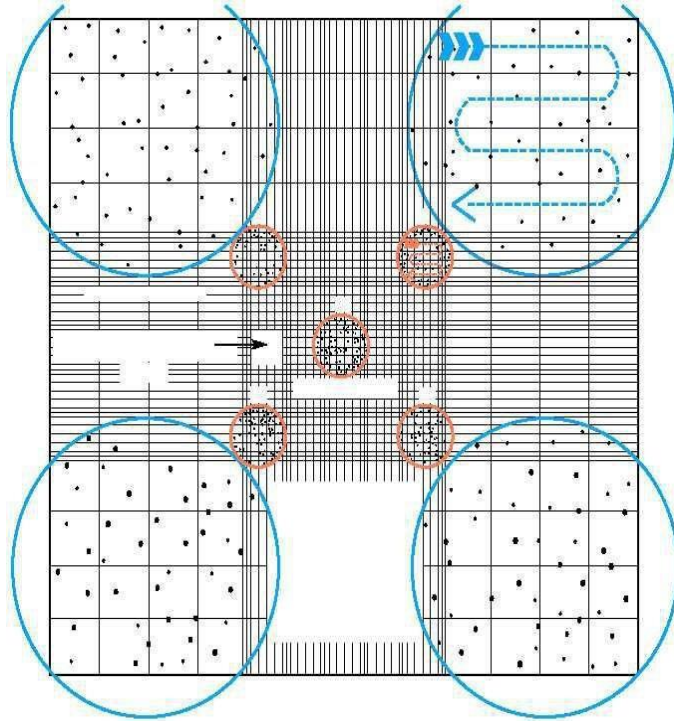
5. After mixing the blood with diluting fluid, keep it aside for 3 minutes

6. Touch the tip of the pipette on the surface of the counting chamber in angle 45 degree.

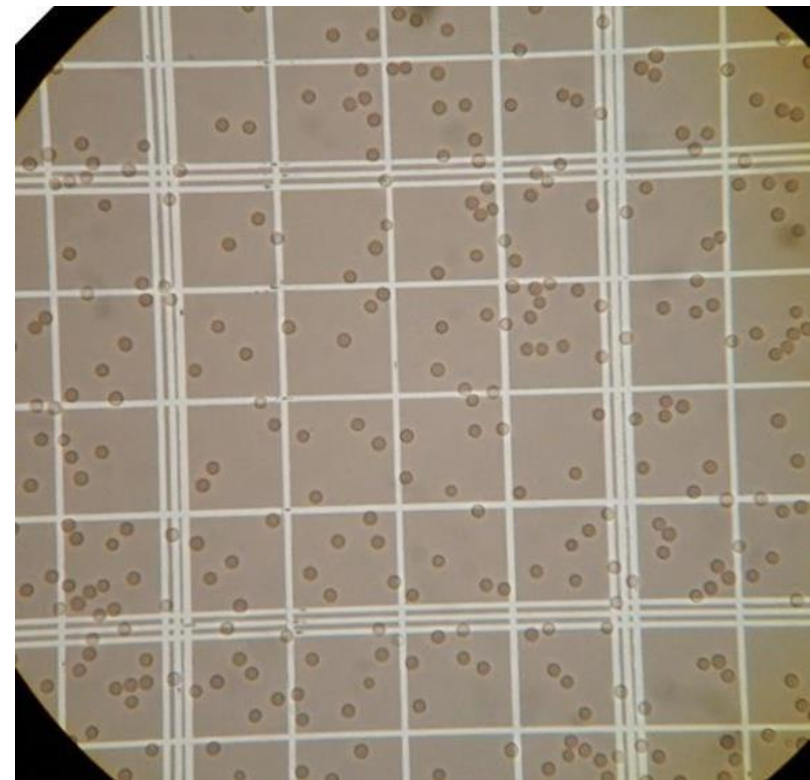
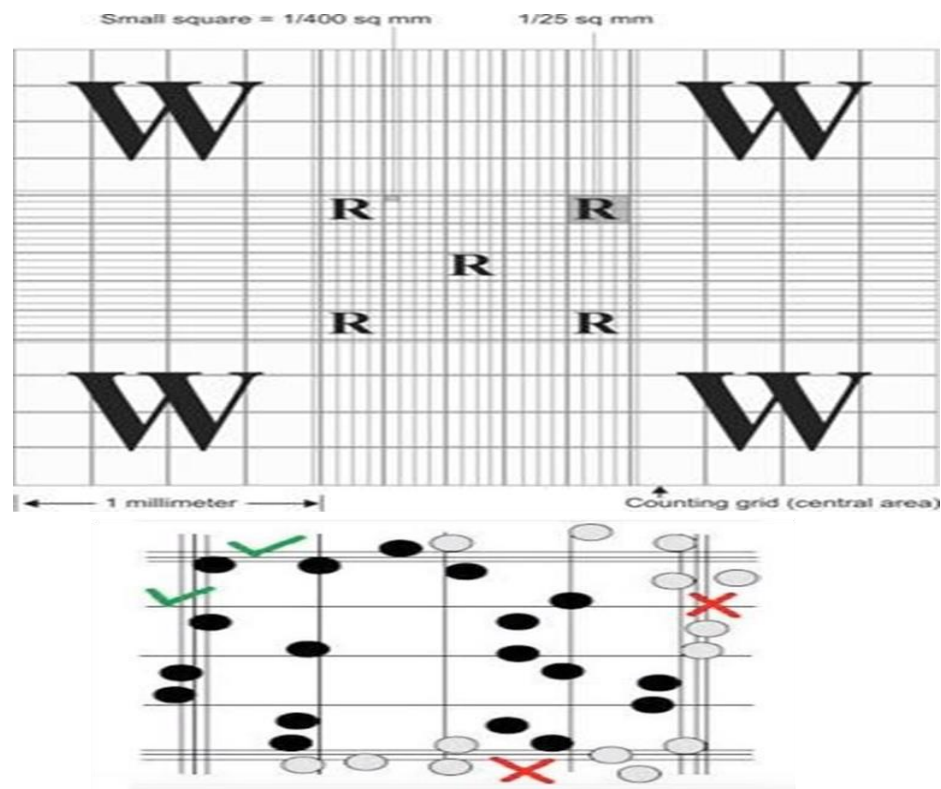


7. Place the chamber on the stage of the microscope and allow 2 minutes for the cells to settle.

8. Scan the counting area with 10x objective lens then 40x objective



Calculation number of WBCs



Calculation

**The total number of WBCs/mm³ of
4 squares = $N \times 50$.**

**The total number of WBCs/mm³ of
2 squares = $N \times 100$.**

Differential Leukocytes Count (DLC)

The Leukocyte Differential Count is the determination of the proportion of, or absolute count per unit volume of defined classes or subsets of leukocytes in a blood sample.

The purpose of the differential Leukocytes count: is to obtain a picture of the true distribution of the leukocytes in peripheral blood, to support the diagnosis of infectious, inflammatory or allergic disorders and diagnosis of malignant blood disorders .The leukocytes present in the peripheral blood are composed of five types of mature cells:

Neutrophils, Eosinophils, Basophils, Lymphocytes, Monocytes.

Manual differential leukocytes count

Materials and instruments :

- ☐ whole blood drawn from finger or collected in EDTA.
- ☐ Stain
- ☐ Microscope
- ☐ Slides and coverslips
- ☐ Immersion oil
- ☐ Lancet
- ☐ Alcohol 70%

Preparation and Examination of the Peripheral Blood Smear :

☐ The preparation and examination of the peripheral blood smear is one of the most frequently requested tests in the hematology laboratory. blood Smear must be prepared correctly and examined in such a way as to provide the physician with an accurate interpretation. there are 4 methods used to prepare blood smear :

1-Slide to Slide.

2-Coverslip to Slide.

3-Coverslip to Coverslip.

4-Automated Spinner.

☐ Blood smears are prepared with EDTA anticoagulant blood to minimize degenerative changes in the blood cells. to ensure good preservation of cellular morphology ,differential smears should be made as soon as possible and no later than 3 hours after collection .

The procedure of slide to slide:

1-Place a drop of blood from the finger about 2mm in diameter in the central line of a slide about 1-2 cm from one end.

a. If the drop is too small, the smear will be too short.

b. If the drop is too thick, the smear will extend to the end of the slide and be too thick.

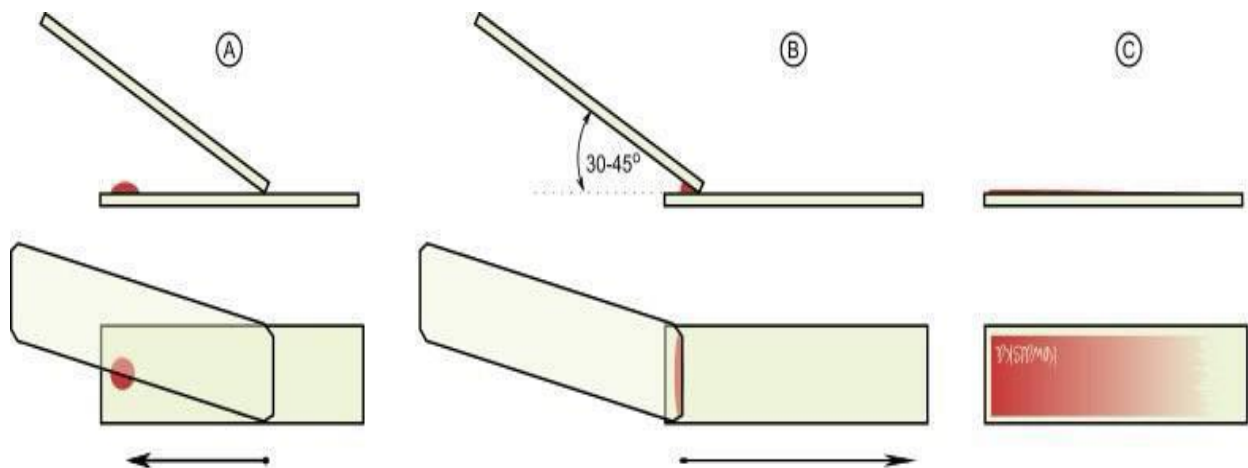
2-The spreader is placed at an angle of 30-45 degrees to the slide and then moved back to make contact with the drop.

3-The drop should spread out quickly along the line of contact of the spreader with the slide.

4-The moment this occurred the film should be spread by a rapid, smooth, forward, movement of spreader.

5-The drop should be of such size that the film is 3-4 cm in length.

6-The film should be dried rapidly.



Preparation of blood smear

There are 3 stains that used in differential leukocytes count:

1-Wright stain.

2-Leishman stain.

3-Giemsas stain

Method of staining:

1-The blood film is fixed with methyl alcohol for 2 minutes.

2- Cover the slide with stain for 5 minutes taking care that it do not dry on the slide

3-Wash off with buffer and dry.

The Count:

The dry and stained film examined without a coverslip under oil immersion objective .for differential leukocyte counts choose an area where the morphology of the cells is clearly visible .do differential count by moving the slide in area including the central and peripheral and the smear .a total of 200 cells should be counted in which every white cell seen must be recorded in a table under the following heading: Neutrophile, Eosinophile, Basophile, Lymphocyte, and Monocyte then find the percentage of each type.