Human physiology (II) labaratory

Lab1: The blood

1.1 Introduction

Blood is a type of liquid connective tissue and it consists of two components:

1-**Formed elements**, which include blood cells(RBCs and WBCs) and cell fragments called platelets

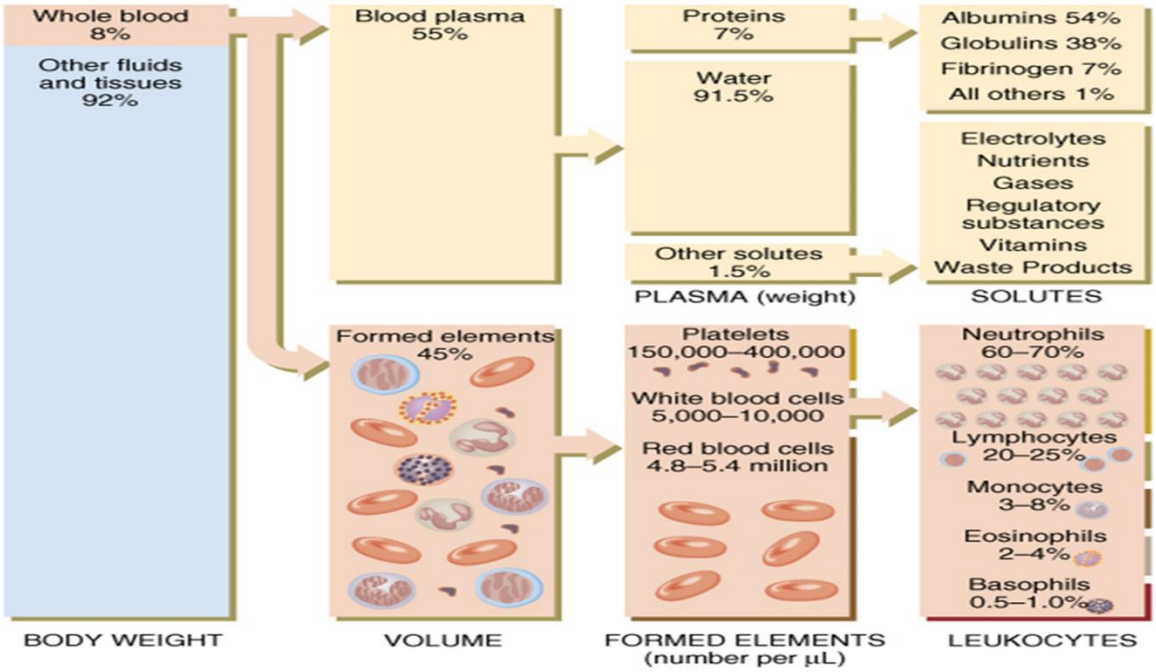
Red blood cells (RBCs) or erythrocytes transport oxygen from the lungs to body cells and deliver carbon dioxide from body cells to the lungs.

White blood cells (WBCs) or leukocytes protect the body from invading pathogens and other foreign substances. There are several types of WBCs: neutrophils, basophils, eosinophils, monocytes, and lymphocytes.

Lymphocytes are further subdivided into B lymphocytes (B cells), T lymphocytes (T cells), and natural killer (NK)cells. Each type of WBC contributes in its own way to the body's defense mechanisms.

Platelets, the final type of formed element, are fragments of cells that do not have a nucleus. Among other actions, they release chemicals that promote blood clotting when blood vessels are damaged.

2-Plasma, Blood plasma is about 91.5% water and 8.5% solutes, most of which (7% by weight) are proteins. which include the albumins (54% of plasma proteins), globulins (38%), and fibrinogen (7%).



Blood's many **important functions** can be grouped into three major types:

1-Delivery: Blood delivers oxygen and nutrients to body cells, carbon dioxide and waste products to their elimination sites (the lungs and kidneys), and hormones to their target organs.

2- Regulatory: such as the regulation of body temperature, pH, and blood pressure.

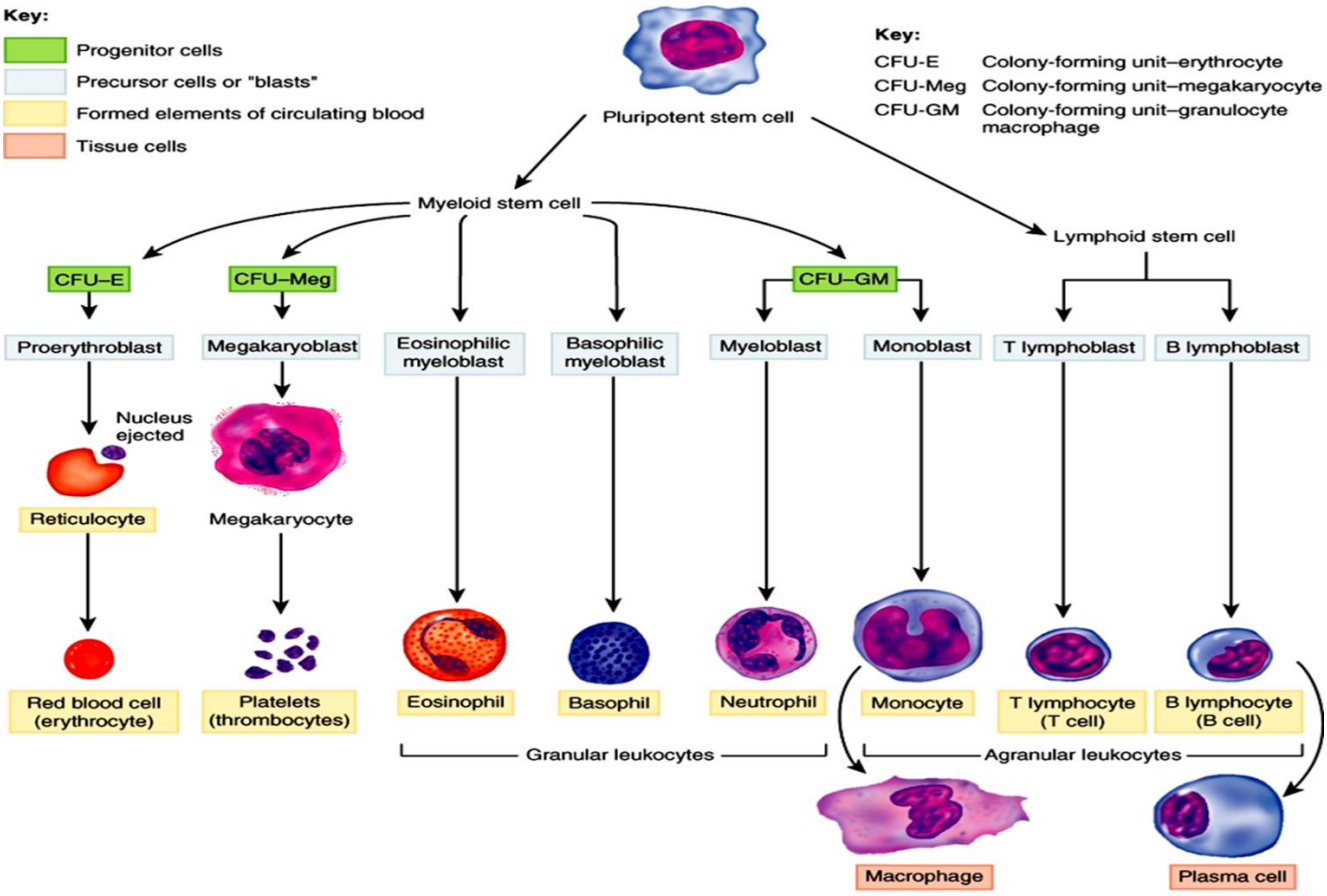
3-Protective functions: blood's protective functions include immunity and blood clotting.

1.2 Hemopoiesis

The process by which the formed elements of blood develop is called hemopoiesis or hematopoiesis. Before birth, hemopoiesis first occurs in the yolk sac of an embryo and later in the liver, spleen, thymus, and lymph nodes of a fetus.

Red bone marrow becomes the primary site of hemopoiesis in the last3 months before birth, and continues as the source of blood cells aft erbirth and throughout life.

About 0.05–0.1% of red bone marrow cells are called pluripotent stem cells or hemocytoblasts and These cells have the capacity to develop into many diff erent types of cells.

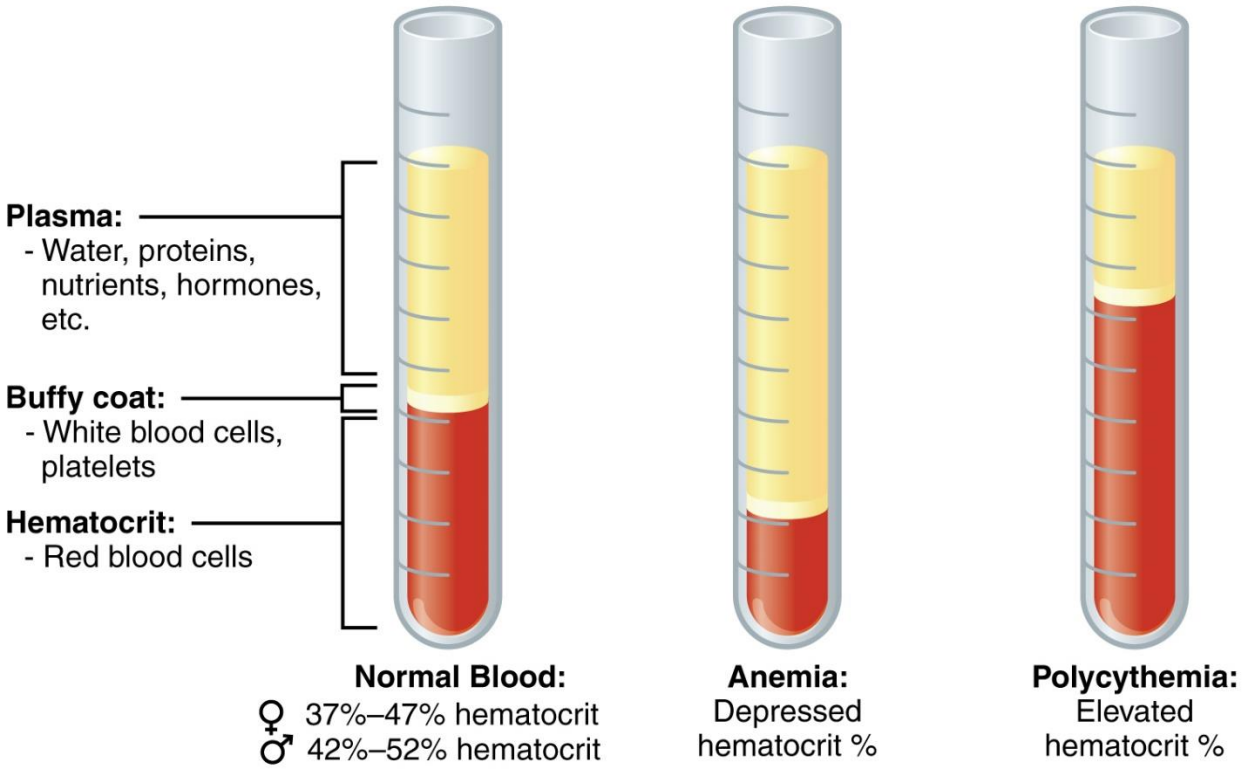


1.3 Selected hematological blood tests

1- The hematocrit (HCT) or Packed cell volume (PCV).

The percentage of total blood volume occupied by RBCs is called the hematocrit (; a hematocrit of 40 indicates that 40% of the volume of blood is composed of RBCs. **The normal range of hematocritfor adult females is 37–47%; for adult males, it is 42–52%.** The hormone testosterone, present in much higher concentration in males than in females, stimulates synthesis of erythropoietin (EPO), the hormone that in turn stimulates production of RBCs. Thus, testosterone contributes to higher hematocrits in males.

Hemoglobin concentration can be estemated from HCT by dividing Hct level by 3 (Hb = Hct / 3).

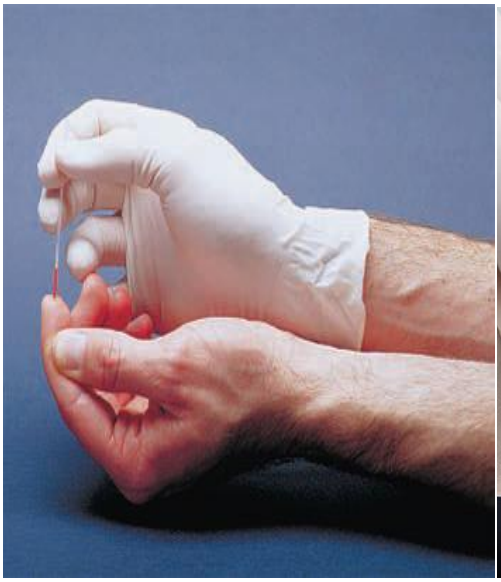
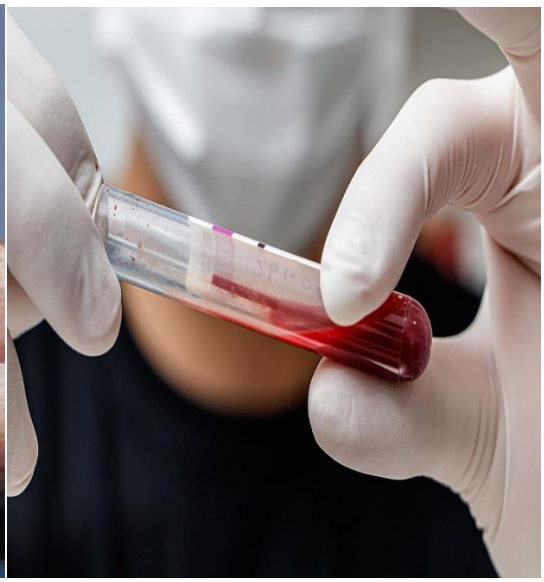


Procedures

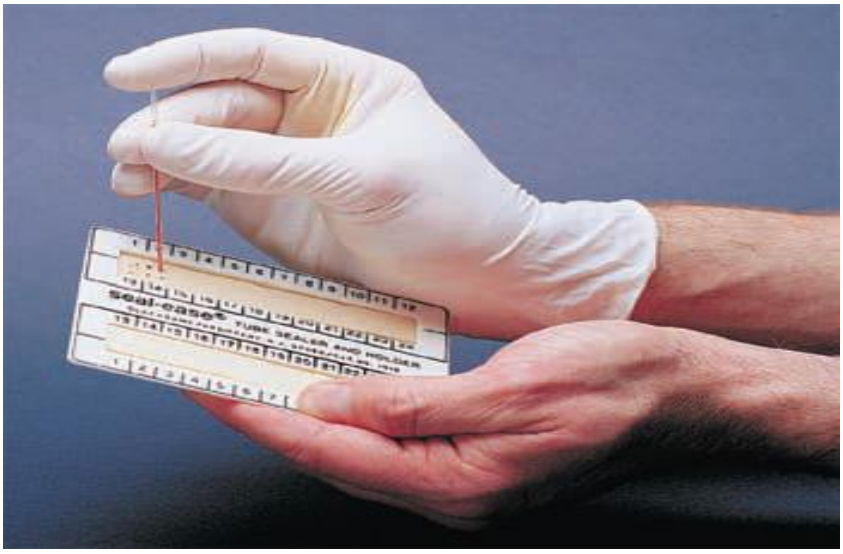
Determine the hematocrit of a blood sample by performing the following steps:

1. Put on safety goggles and disposable gloves (PPE).
2. 2. Obtain a blood sample. clean the fingertip of the third or fourth finger with an alcohol wipe, and prick the finger with a sterile lancet. Dispose of the lancet in the sharps biohazard disposal container.
3. Use a small square of sterile gauze to wipe away the first drop of blood.
4. place one end of a sterile, heparinized capillary tube (contains heparin, which prevents the blood from coagulating) just into the drop of blood, holding the tube at an angle so that blood will enter the tube by capillary action

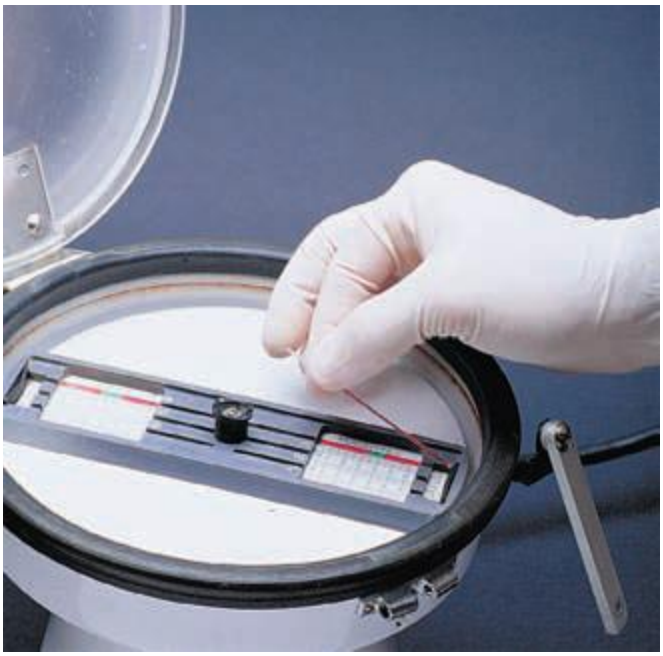
5. Fill the tube at least two-thirdsfull. (If using a blood sample provided by your instructor, simply immerse the capillary tube in the blood sample and fill it two-thirds full.)

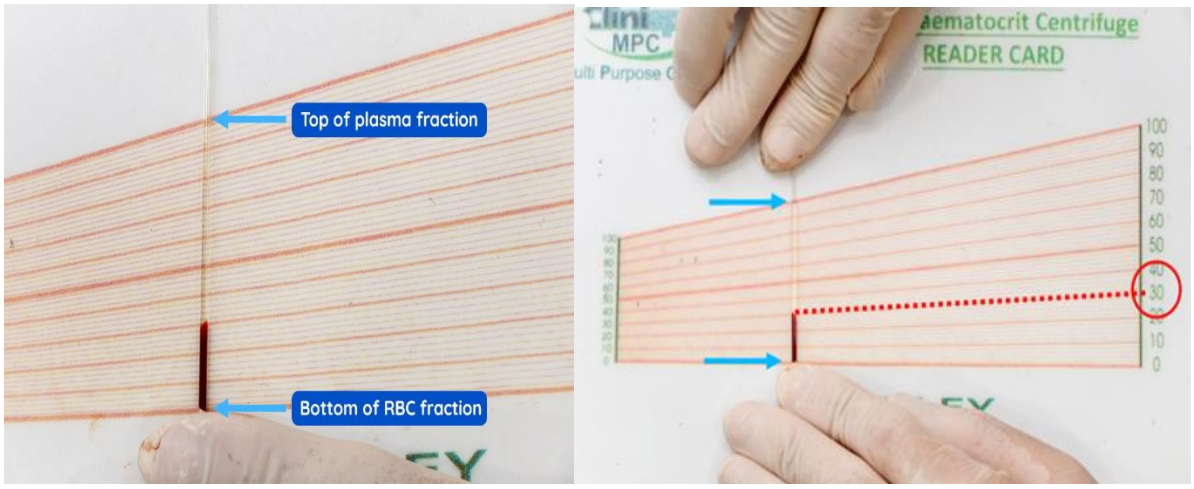
6. Stop the flow of blood by holding a folded square of sterile gauze over the prick site for a minute
7. While holding your index finger over the dry end of the capillary tube, seal the blood-containing end of the tube by gently pushing it into the sealing clay.



8. Place the tube into of the microcentrifuge, with the sealed end of the tube against the outer lining on the rim.(Note that the centrifuge must be balanced by having another student place a capillary tube directly opposite yours).



9. Close the cover of the centrifuge and spin the capillary tubes for 2-4 minutes.
10. When the centrifuge stops, carefully remove your capillary tube. The erythrocytes are now packed into the bottom of the tube, and the clear liquid on top is the plasma. Between the erythrocytes and the plasma is the buffy coat, which contains leukocytes and platelets.
11. Determine the hematocrit using a microhematocrit reader.



2-Red Blood Cells (RBCs) count.

Normal RBCs are biconcave discs, they have few organelles and no nuclei.

Causes of high RBC count (Polycythemia)

1. Living at high altitudes 2. Cardiac or pulmonary diseases 3. Erythropoietin secreting tumors 4. Smoking. 5. Polycythemia Vera

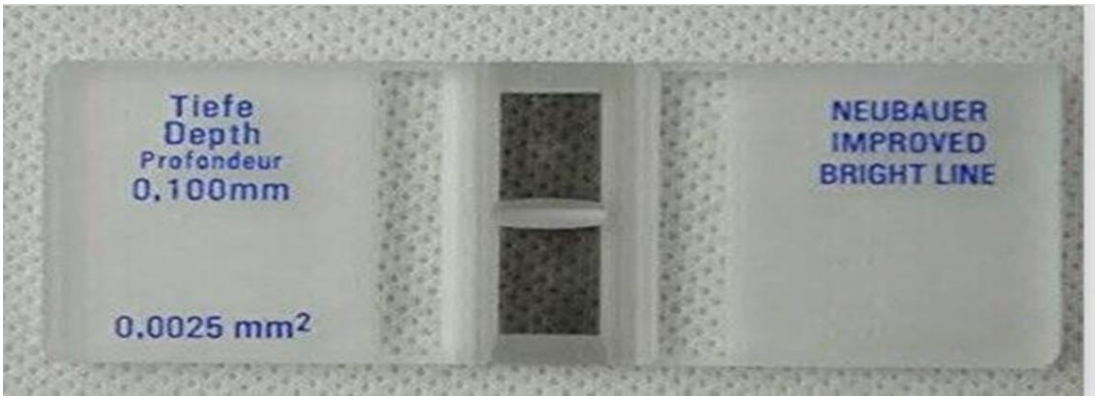
Causes of low RBC count (Anemia)

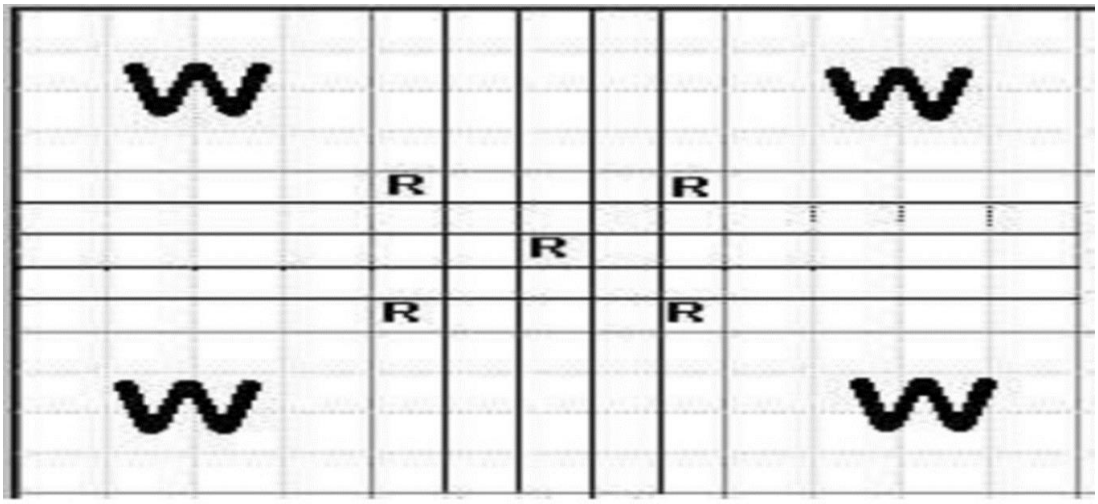
1. Internal or external bleeding 2. Nutritional deficiencies 3. Bone marrow failure 4. Hemolysis of RBCs 5. Chronic Renal failure

Hemocytometer (The Neubauer's Chamber)is a special microscopic slide that has specific grids engraved on it's counting chamber and is designed to hold specific volume of fluid.

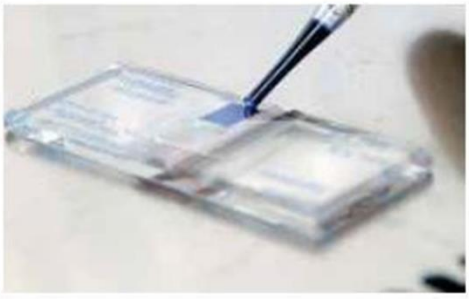
The Hemocytometer has ruled the area of total 9 square mm and the depth is 0.1 mm as when the coverslip is placed on the surface of the counting chamber.

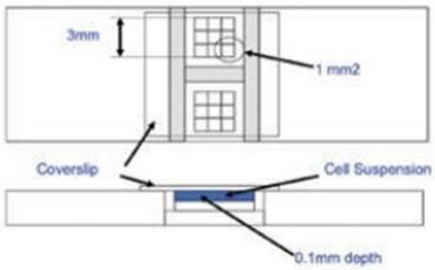
Each square of the Central Square (divided into 25 squares) contains 16 small squares so the total no. of the area to be counted for RBC Count 16 × 5 = 80 small squares

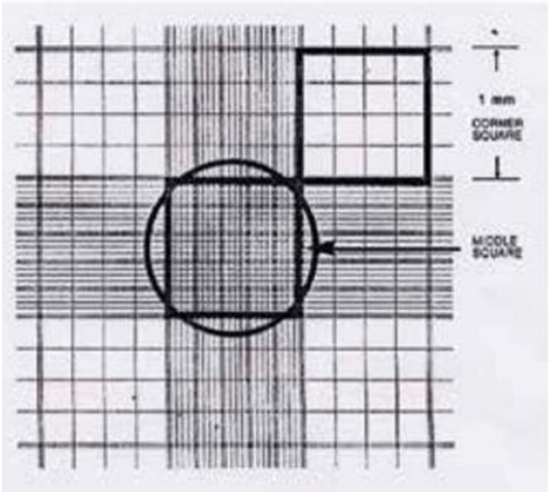


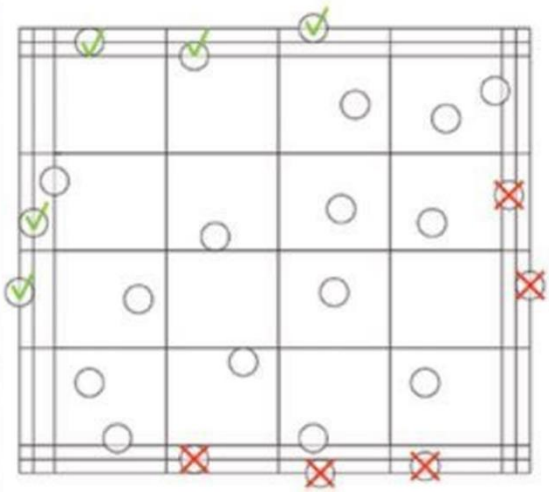


❖RBC use 5 small squares in the center large square
❖WBC , sperm cells, culture cells use 4 corner large squares









The procedure

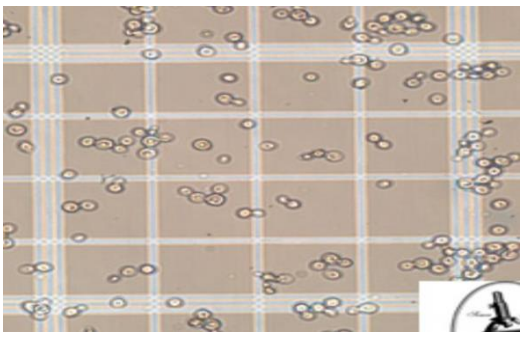
1.Clean the hemocytometer well

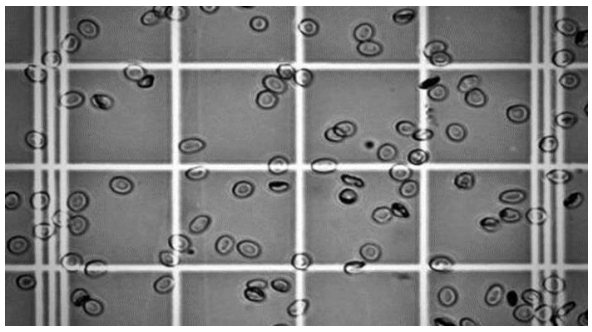
2.Place a coverslip over the counting area. Now the distance between the bottom of the coverslip and the surface of the counting area is 0.1 mm

3.Dilute the blood sample by adding 1 unit of blood to 199 units of an isotonic solvent and thoroughly mix the mixture

4.Draw a sample using a pipette and gently touch the junction of the coverslip and hemocytometer . The diluted blood will flow by capillary attraction to fill the chamber. Let it stand for 3 min before you complete the expirement.

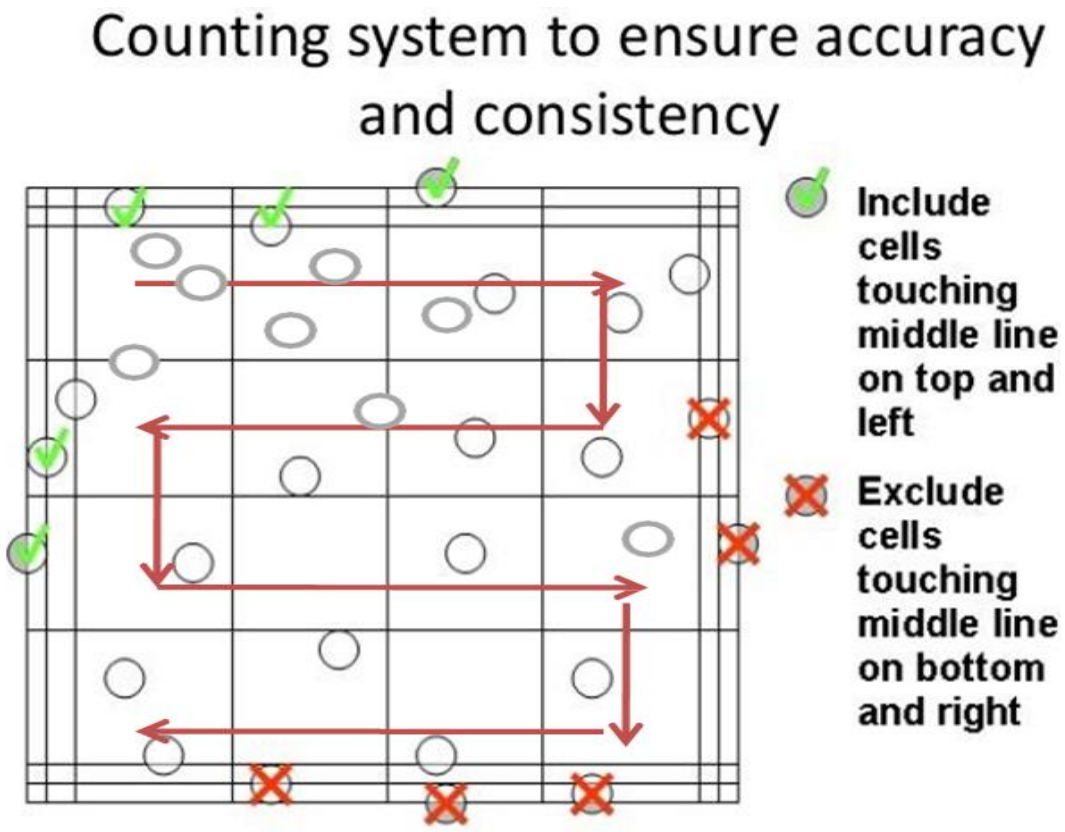
5.Use the 10X lens to identify the center square , then use 40X lens to focus on the smaller squares and count the RBCs (RBCs appear circular in shape)





6.Count the number of cells in the five small squares and obtain an average number.

• Start counting from the left to the right and proceed in a zig-zag.–To avoid counting the same cells twice, cells that are touching the lines at the tops and left sides of the squares are counted, but cells that are touching the bottoms and right sides of the squares are not counted.



•Regarding cells that touch the outer boundaries, count the cells that touch the left and upper boundaries and ignore the cells touching the other two boundaries.

The calculation

- If we counted an average of 95 cells in the five squares what is the number of RBCs in the sample?

- RBCs/mm^3 = average number of counted cells **X** dilution factor (DF) **X** volume correction factor(VCF)
- DF = $\frac{Final\ volume}{Volume\ of\ blood} = 200$
- The volume of fluid in one small square is (0.2 **X** 0.2 **X** 0.1 = 0.004 mm^3)
- VCF = Desired Volume/ Counted Volume = 1/.004 = 250
- RBCs/mm^3 = 95 **X** 200 **X** 250 = 4,750, 000 cells /mm^3

- Before you obtain the average number of RBCS make sure the count in the five squares doesn't vary by more than 20 cells.
- 1 mm^3 = 1 ul
- If there is a big variation discard the sample from the slide and repeat the experiment

3-White Blood Cells (WBCs) count.

White Blood Cells are the mobile units of the body's protective system. Specifically transported to areas of severe infection or inflammation to provide a rapid and potent defense for the body

Normal WBC count is 4000 - 11,000 cells/mm3

Medical applications

Increased number of WBCs indicates that there is leukocytosis which could be

physiological or pathological such as infection.

Physiological Conditions

1. Age:

a. Newborn will always have an increased count. a newborn has a high white

blood cell count, ranging from 9,000 to 30,000 leukocytes / mm3 . This number falls to adult levels within two weeks.

b. Childhood, pregnancy and delivery shows increased count.

c. There is no significant change in old age compared to adult values.

2. Females during pregnancy and parturition.

3. Stress like severe exercise, severe pain, and excitation.

4. Diurnal variation: WBC count may vary from hour-to-hour (highest count in evening and lowest count in morning).

5. Digestive leukocytosis (after digestion).

6. Injection of adrenaline.

7. After removal of spleen (spleenectomy)

Pathological Conditions

1. Acute pyogenic infections (e.g. pneumonia, appendicitis and tonsillitis).

2. Leukemia (abnormal increase with immature cells) count may go up to 1,00,000 to 3,00,000 per cu mm.

3. Acute hemorrhage.

4. Tissue damage resulting from burns, operations, myocardial infarction

Causes of Low WBC count (Leukopenia)

1. Bone marrow failure due to radiation or malignancy
2. 2. Autoimmune diseases.
3. 3. Infections like HIV & tuberculosis.

The procedure

1. Clean the hemocytometer well

2. Place a coverslip over the counting area. Now the distance between the bottom of the coverslip and the surface of the counting area is 0.1 mm

3. Dilute the blood sample by adding 1 unit of blood to 19 units of Turk's diluting fluid and thoroughly mix the mixture.

Turk's diluting fluid composed of :

 Glacial acetic acid, 3 ml to haemolyse RBCs.

 Aqueous gention violet (1% w/v) 1 ml to color the nuclei of WBC

 Distilled water up to 100 ml

4. Draw a sample using a pipette and gently touch the junction of the coverslip and hemocytometer . The diluted blood will flow by capillary attraction to fill the chamber. Let it stand for 3 min before you complete the expirement.

5. Use the 10X lens to count the WBC in the four large corner squares .(WBCs appear as dark dots)

***The dilution fluid contains an agent (glacial acetic acid) which lyses the red cells. It also contains a dye that stains the nuclei of WBCs. This allows a proper count of WBCs.**

The calculation

1. Blood is diluted at (1:19) so DF = 20

2. The volume of fluid in the corner square is (1X 1 X 0.1= 0.1 mm3) SO the VCF is 10

If we counted an average of 40 cells in the 4 squares the count of WBCs is.... 40 X 20 X 10 = 8000 cells/mm3 which is a normal value

• Before you obtain the average number of WBCS make sure the count in the four squares doesn't vary by more than 10 cells.

4-Differential White Blood Cell Count

A differential white blood cell count is performed to determine the percentages of each white blood cell type in a blood sample.

WBC is devided into

1-Granulocytes: 3 types

A-Neutrophile B-Eosinophile C-Basophile

2- A granulocyte: 2 types

A-Lymphocytes B-Monocyte

The procedure

A-Spreding the blood smear (blood film).

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 of slide.
2-slide spreader is placed at an angle of 40 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- spread by a rapid, smooth, forward, movement of spreader for thin film preparation.
5-The film should be dried rapidly

B- The stain.

By using on of the follwoing stains 1-Wright stain or Leishman stain or 3-Giemsas stain.

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

2-Pour Giemsa stain diluted 1:9 with buffer over the smear for 8-10 minutes.

3-Wash off with buffer and dry

C- The Count.

The dry and stained film examined under oil immersion objective .

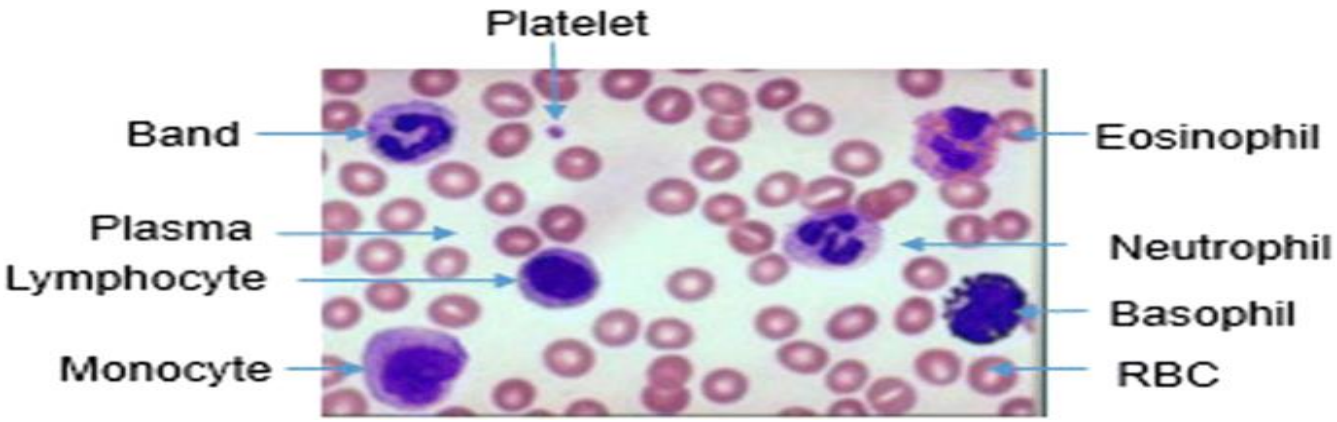
for differential leukocyte counts:

1- choose an area where the morphology of the cells is clearly visible .

2- do differential count by moving the slide in area including the central and peripheral and the smear.

3- A minimum of of 100 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.

Formed element	Major subtypes	Numbers present per microliter (μL) and mean (range)	Appearance in a standard blood smear	Summary of functions	Comments
Erythrocytes (red blood cells)		5.2 million (4.4–6.0 million)	Flattened biconcave disk; no nucleus; pale red color	Transport oxygen and some carbon dioxide between tissues and lungs	Lifespan of approximately 120 days
Leukocytes (white blood cells)		7000 (5000–10,000)	Obvious dark-staining nucleus	All function in body defenses	Exit capillaries and move into tissues; lifespan of usually a few hours or days
	Granulocytes including neutrophils, eosinophils, and basophils	4360 (1800–9950)	Abundant granules in cytoplasm; nucleus normally lobed	Nonspecific (innate) resistance to disease	Classified according to membrane-bound granules in cytoplasm
	Neutrophils	4150 (1800–7300)	Nuclear lobes increase with age; pale lilac granules	Phagocytic; particularly effective against bacteria. Release cytotoxic chemicals from granules	Most common leukocyte; lifespan of minutes to days
	Eosinophils	165 (0–700)	Nucleus generally two-lobed; bright red-orange granules	Phagocytic cells; particularly effective with antigen- antibody complexes. Release antihistamines. Increase in allergies and parasitic infections	Lifespan of minutes to days
	Basophils	44 (0–150)	Nucleus generally two-lobed but difficult to see due to presence of heavy, dense, dark purple granules	Promotes inflammation	Least common leukocyte; lifespan unknown
	Agranulocytes including lymphocytes and monocytes	2640 (1700–4950)	Lack abundant granules in cytoplasm; have a simple-shaped nucleus that may be indented	Body defenses	Group consists of two major cell types from different lineages
	Lymphocytes	2185 (1500–4000)	Spherical cells with a single often large nucleus occupying much of the cell's volume; stains purple; seen in large (natural killer cells) and small (B and T cells) variants	Primarily specific (adaptive) immunity: T cells directly attack other cells (cellular immunity); B cells release antibodies (humoral immunity); natural killer cells are similar to T cells but nonspecific	Initial cells originate in bone marrow, but secondary production occurs in lymphatic tissue; several distinct subtypes; memory cells form after exposure to a pathogen and rapidly increase responses to subsequent exposure; lifespan of many years
	Monocytes	455 (200–950)	Largest leukocyte with an indented or horseshoe-shaped nucleus	Very effective phagocytic cells engulfing pathogens or worn out cells; also serve as antigen-presenting cells (APCs) for other components of the immune system	Produced in red bone marrow; referred to as macrophages after leaving circulation
Platelets		350,000 (150,000–500,000)	Cellular fragments surrounded by a plasma membrane and containing granules; purple stain	Hemostasis plus release growth factors for repair and healing of tissue	Formed from megakaryocytes that remain in the red bone marrow and shed platelets into circulation



Lab 1 report.

Q1. From a blood sample 10ul were diluted with isotonic solution to final volume of 1000 ul.

A total of 4 RBC squares were counted as follows : 85, 91, 99 and 81.

A-Calcualte the RBC count in the sample:

B-Indicate if the results is normal and if not what is the condiction called.

Q2 From a blood sample 10ul were diluted with isotonic solution to final volume of 100 ul.

A total of 3 WBC squares were counted as follows : 159, 173 and 169.

A-Calcualte the WBC count in the sample:

B-Indicate if the results is normal and if not what is the condiction called.