

Physiology Lab 1

Red Blood Cell (RBC) Count
White Blood Cell (WBC) Count

Dr. Tamara Alqudah
tamara.alqudah@ju.edu.jo

Hematology lab

- In clinical practice, hematological information accumulated from a series of blood tests conducted on a small volume of blood - even a single drop - can be of great diagnostic and prognostic value.
- In these three lab settings we will learn about some of these tests.

Aim today's experiment

- The purpose of the lab is to determine the count of RBCs and WBCs in a blood sample using a hemocytometer.
- The principle of the procedure is similar in both types of cells with some minor differences.

Red Blood Cells (RBCs)

- Normal RBCs are biconcave discs, they have few organelles and no nuclei.
- A major function of RBCs is to transport hemoglobin, which in turn carries oxygen from the lungs to the tissues.
- The average number of RBCs in healthy men is $5,200,000/\text{mm}^3$ ($\pm 300,000$) and in healthy women $4,700,000/\text{mm}^3$ ($\pm 300,000$)
- The number of RBCs is regulated within narrow limits, so that oxygen is transported adequately to the tissues and at the same time the cells do not become so numerous that they impede blood flow.

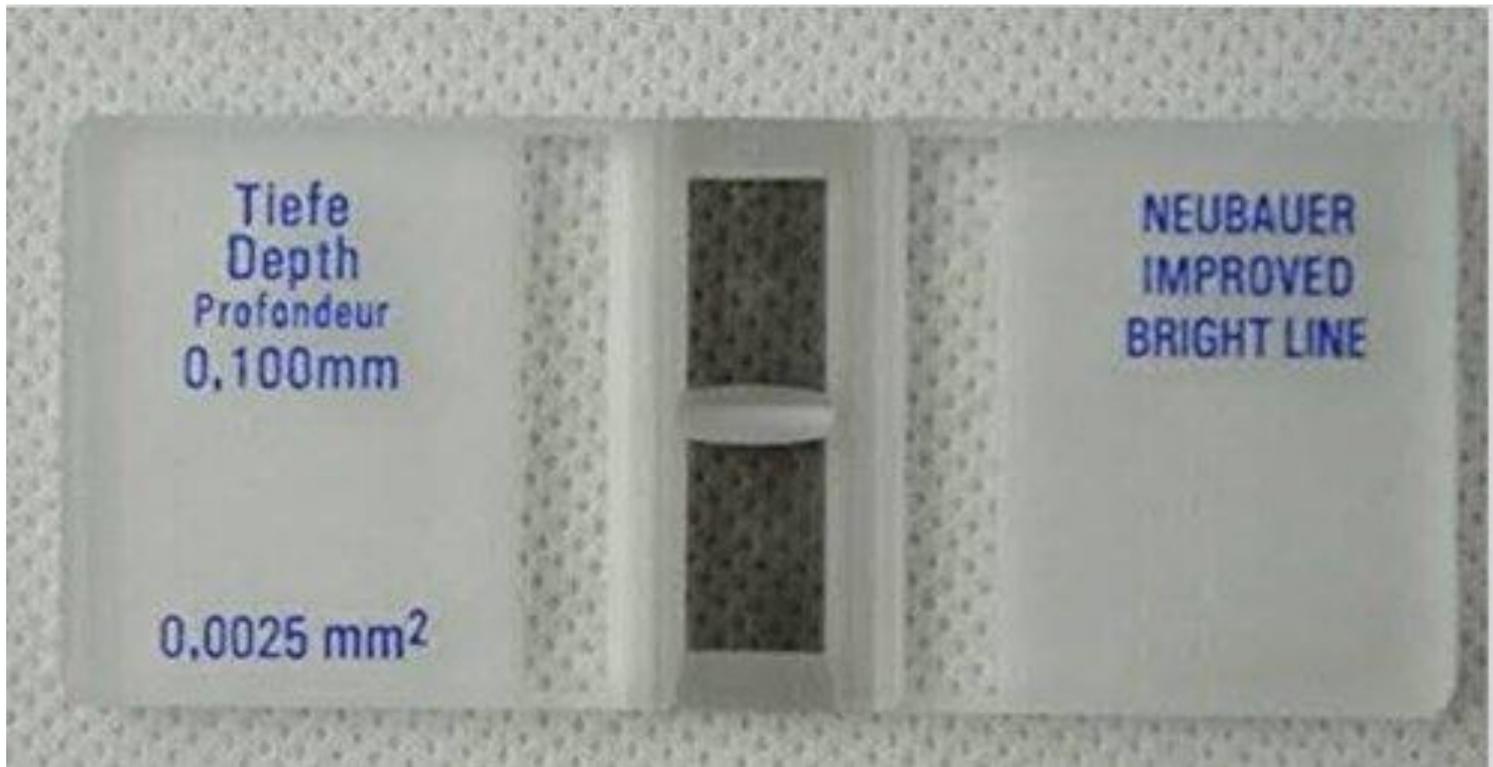
- **Causes of high RBC count (Polycythemia)**

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

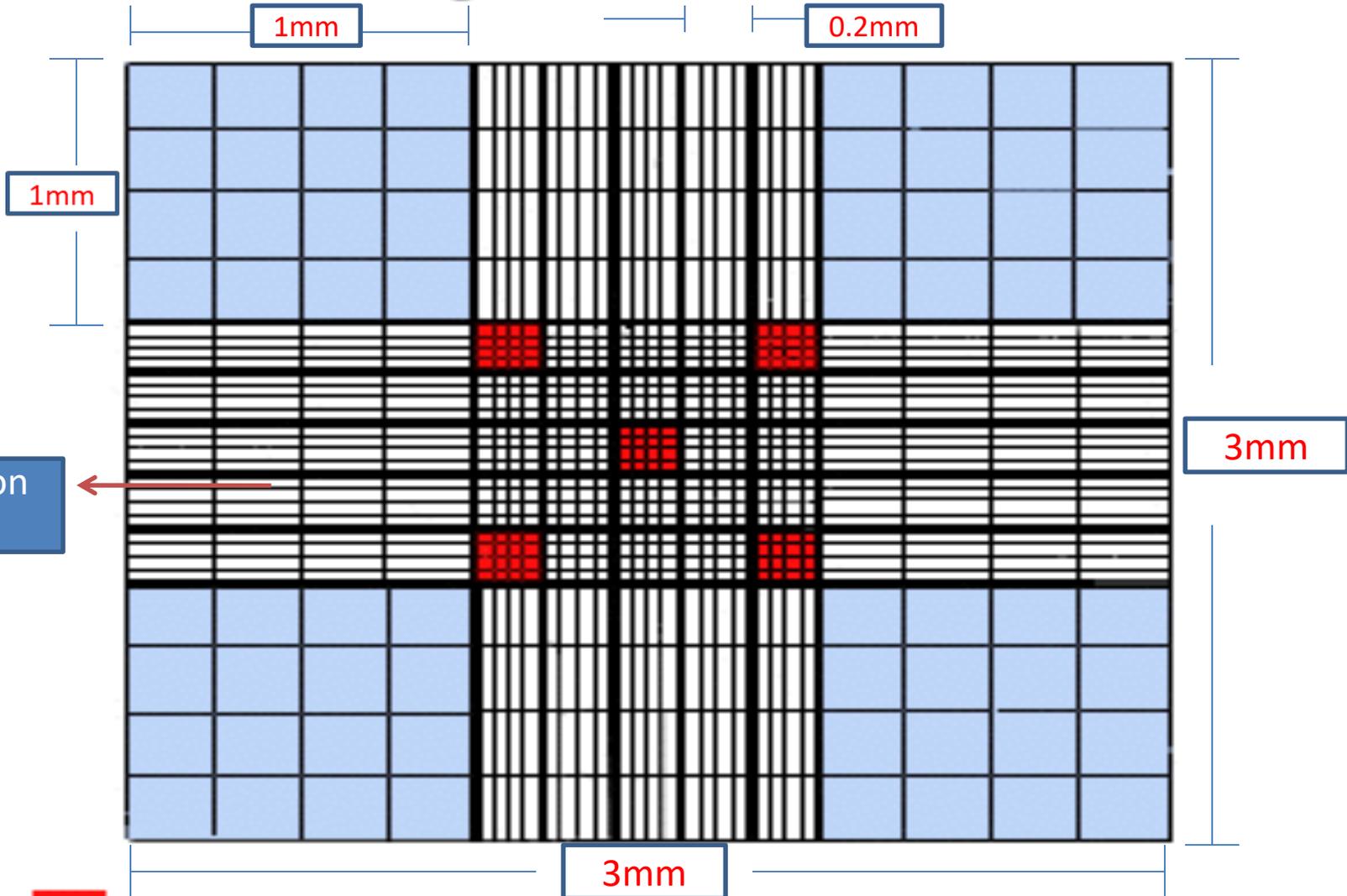
- **Causes of low RBC count (Anemia)**

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

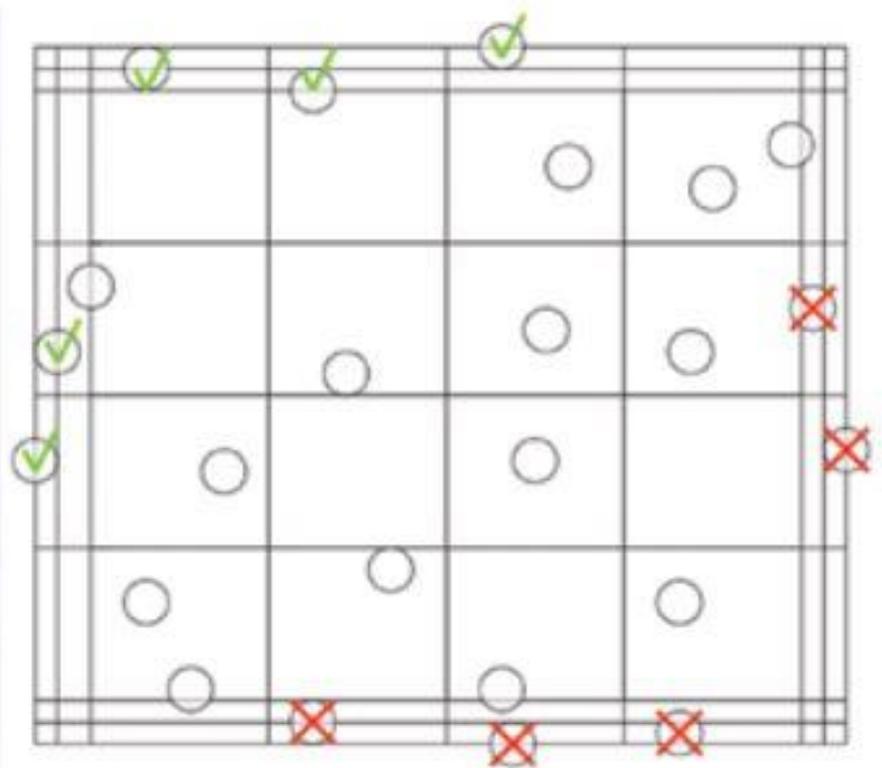
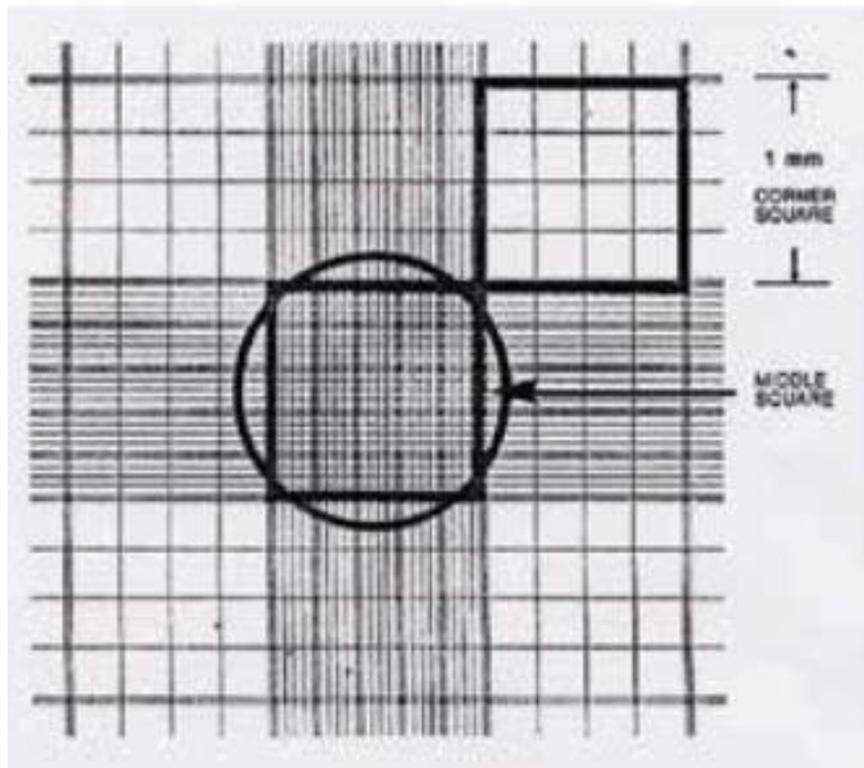
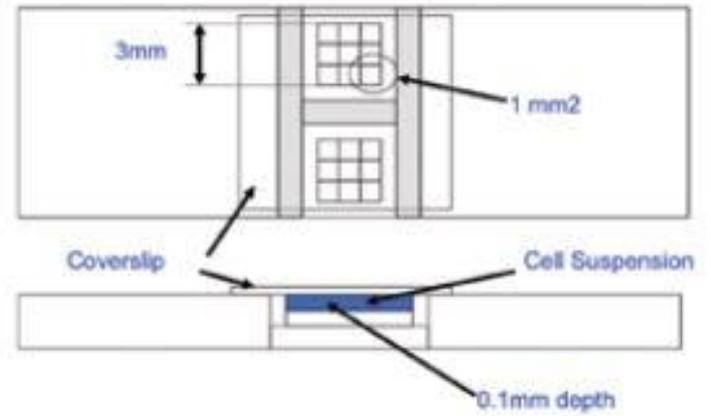
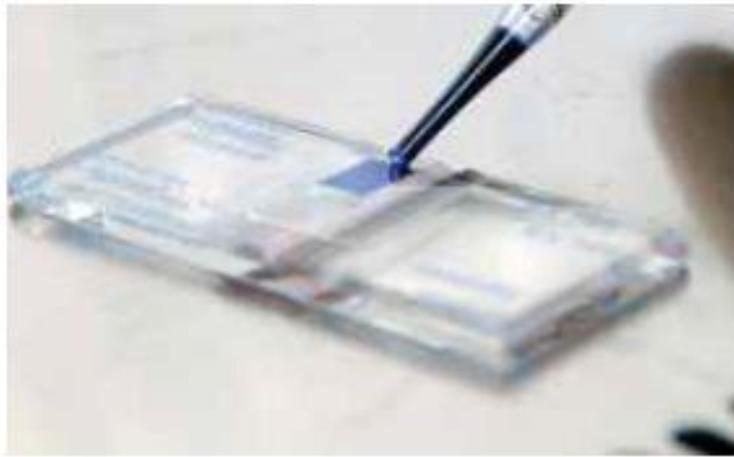
- Hemocytometer is a special microscopic slide that has specific grids engraved on it's counting chamber and is designed to hold a specific volume of fluid.



■ areas of the grid where WBC are counted

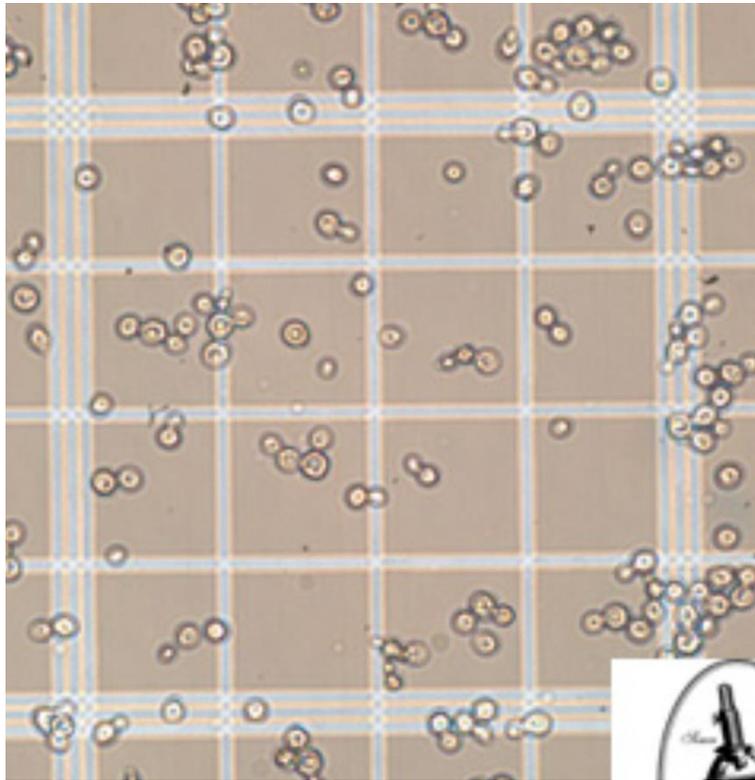


■ areas of the grid where RBC are counted



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 experiment.
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.



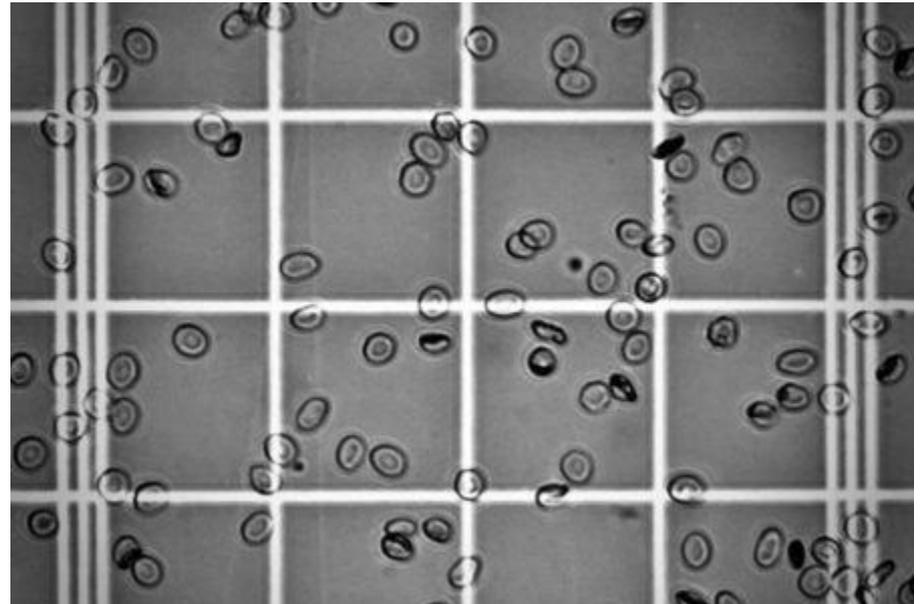
8 μm



Surface view



Sectioned view

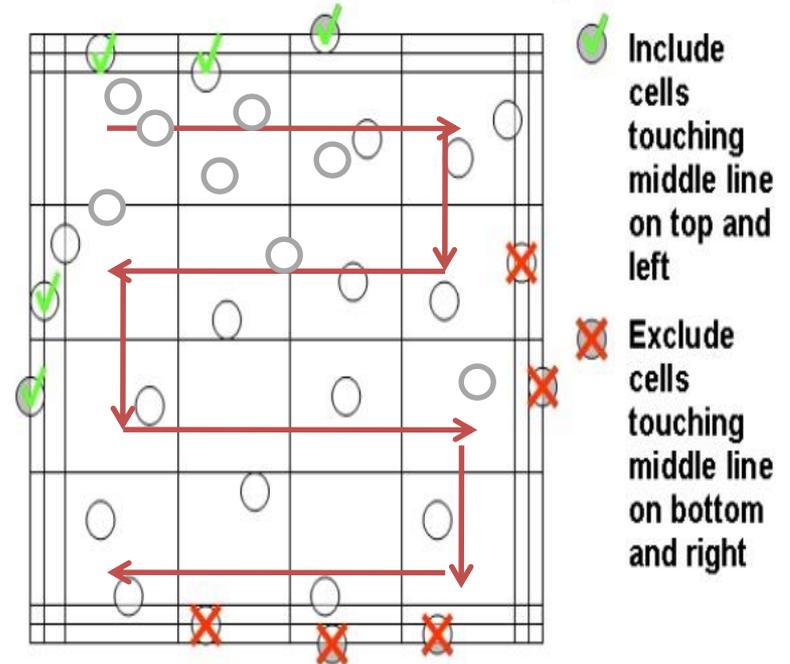


- Start counting from the left to the right and proceed in a zig-zag.

–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

Counting system to ensure accuracy and consistency



The calculation

- If we counted an average of 95 cells in the five squares what is the number of RBCs in the sample?
- $\text{RBCs/mm}^3 = \text{average number of counted cells} \times \text{dilution factor (DF)} \times \text{volume correction factor (VCF)}$
- $\text{DF} = \frac{\text{Final volume}}{\text{Volume of blood}} = 200$
- The volume of fluid in one small square is $(0.2 \times 0.2 \times 0.1 = 0.004 \text{ mm}^3)$
- $\text{VCF} = \text{Desired Volume} / \text{Small square Volume} = 1 / .004 = 250$
- $\text{RBCs/mm}^3 = 95 \times 200 \times 250 = 4,750,000 \text{ cells /mm}^3$

Knowing that the blood sample was taken from a female, the number of RBCs is normal

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

WBC 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

➤ **Causes of High WBC count (Leukocytosis)**

1. Active inflammation or infection.
2. Certain malignancies
3. Physiological processes (stress, exercise)

➤ **Causes of Low WBC count (Leukopenia)**

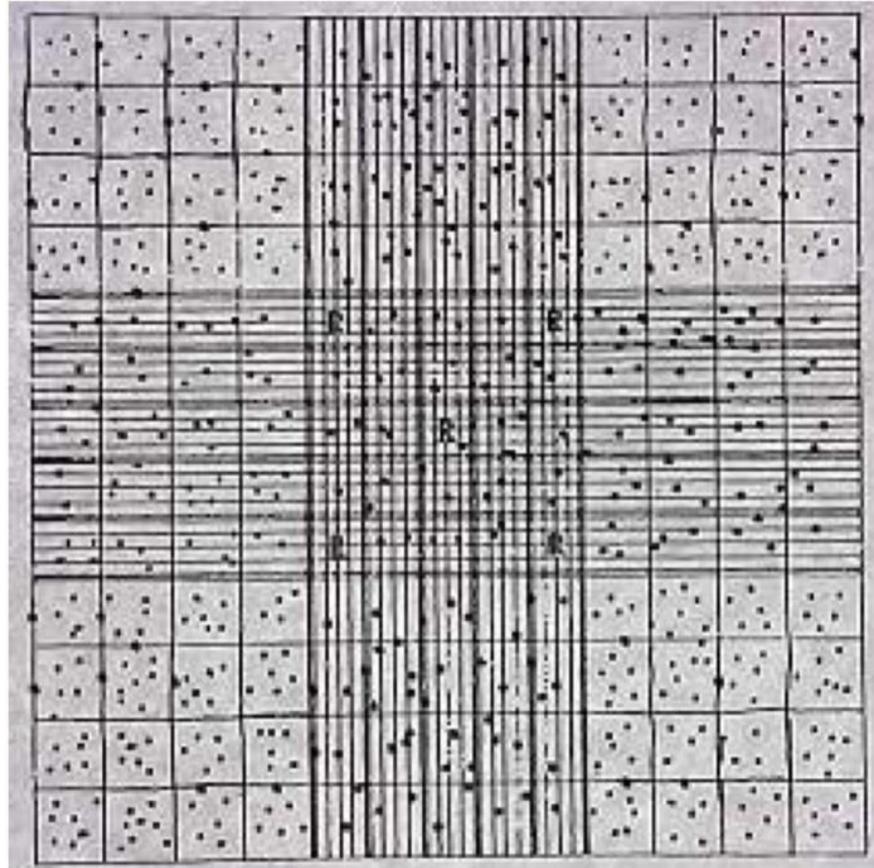
1. Bone marrow failure due to radiation or malignancy
2. Autoimmune diseases.
3. Infections like HIV & tuberculosis.

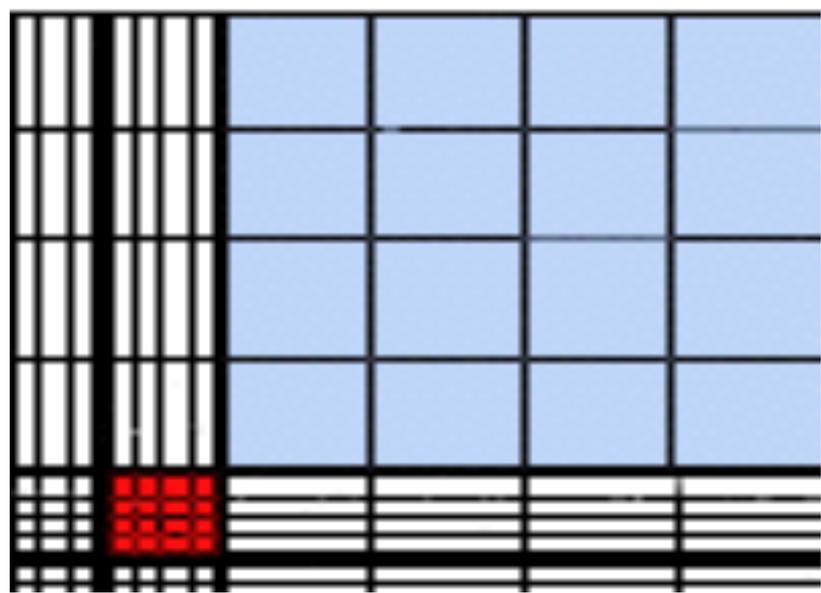
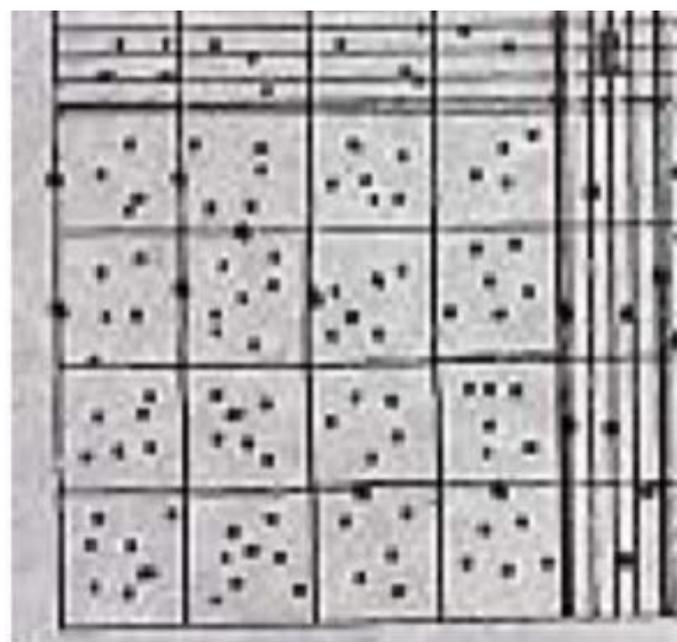
The procedure

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 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 experiment.
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.

WBCs





The calculation

1. Blood is diluted at (1:19) so DF = 20
2. The volume of fluid in the corner square is (1 X 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