

Physiology - HLS

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The Experiments

Today, we'll talk about the first 4 experiments;

- **Red Blood Cell (RBC) Count**
- **White Blood Cell (WBC) Count**
- **Differential Leukocyte count (DLC)**
- **Reticulocyte count**
- Packed cell volume (PCV)
- Hemoglobin concentration
- Erythrocyte Sedimentation rate (ESR)
- Blood Type
- Bleeding Time
- Clotting Time
- Osmotic Fragility Test

RBC and WBC count have the same procedure with minor differences

Red Blood Cells (RBCs)

- Normal RBCs are biconcave discs, they have **a flexible membrane**, few organelles and no nuclei, **they also contain some enzymes in their cytoplasm to help with their function.**
- A major function of RBCs is to transport hemoglobin, which in turn carries oxygen **(by reversibly binding to it)** from the lungs to the tissues.
- **Almost 1/3 of RBCs volume is occupied by hemoglobin.**
- The average number of RBCs in healthy men is 5,200,000/mm³ (±300,000) and in healthy women 4,700,000/mm³ (±300,000)
- **RBCs number in a random blood sample differs depending on many factors, such as age, sex..**
- 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, **so a decrease in RBCs will cause hypoxia, while an increase will lead to an increased blood viscosity.**

- RBC count is typically ordered as a part of complete blood count (CBC) and may be used as a part of health checkup to screen for variety of conditions.

- **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
6. Dehydration (**There's no increase in the actual number but due to decreased water content in RBCs it'll cause a relative increase**)

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

7. Internal or external bleeding
8. Nutritional deficiencies
9. Bone marrow failure
10. Hemolysis of RBCs
11. Chronic renal failure

Complete blood count (CBC) is one of the most important blood tests which gives us an idea about:

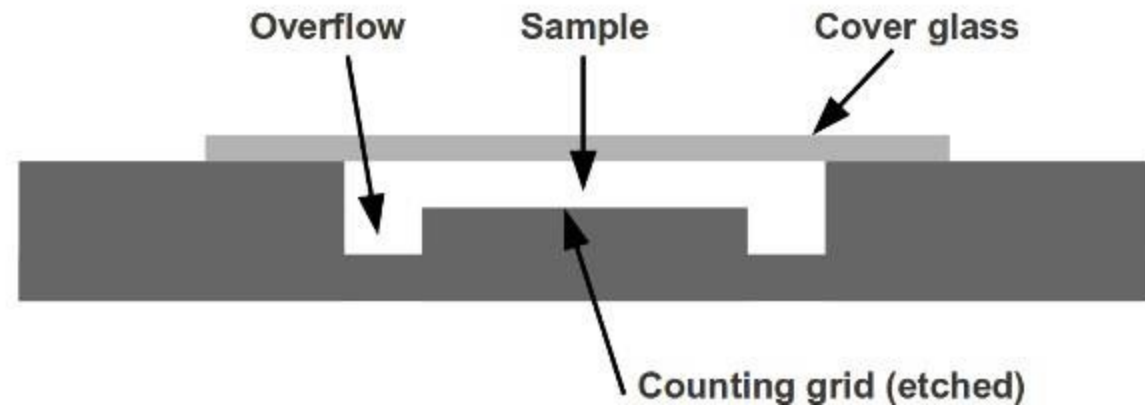
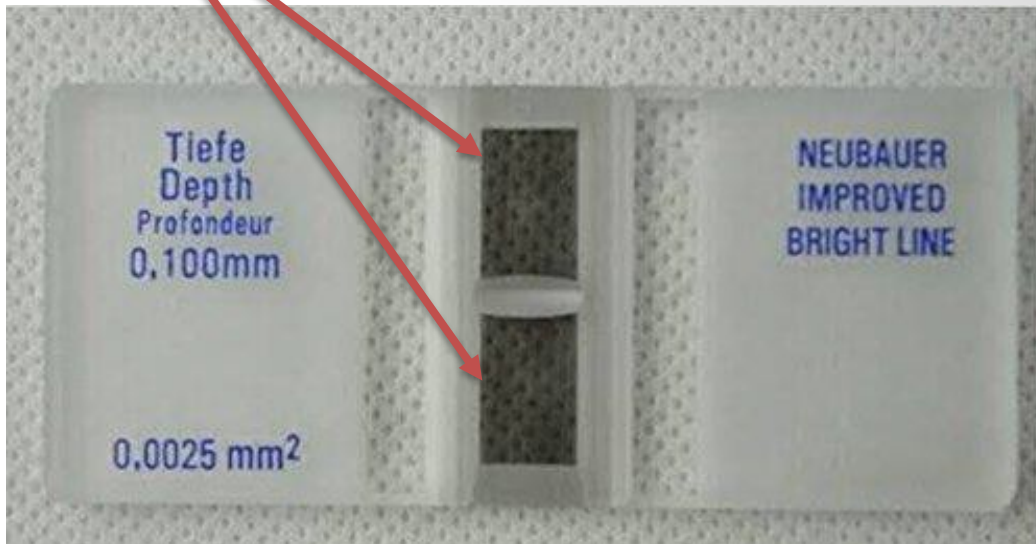
1. RBCs number, size, amount of hemoglobin..
2. WBCs count, types..
3. Platelets number.

A little extra info:

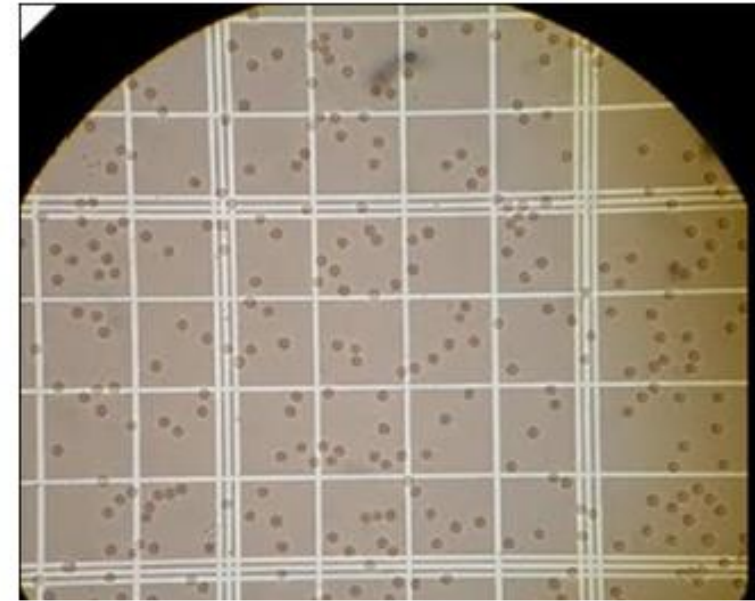
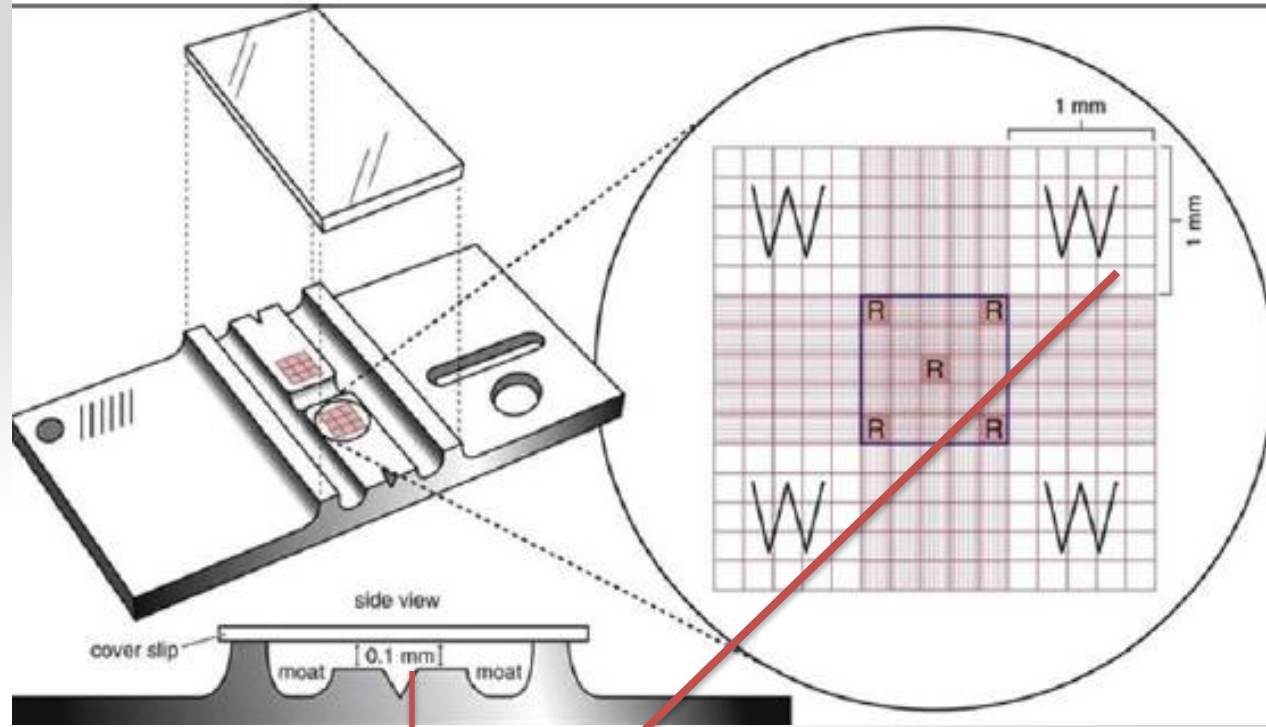
(If the liquid component of the blood (plasma) is decreased, the red blood cell count increases due to the red blood cells becoming more concentrated. The actual number of red blood cells stays the same.)

The method used in our lab for finding RBCs count is manual counting (by counting the number of red blood cells in a blood sample) and is done by a special slide called “Hemocytometer”.

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

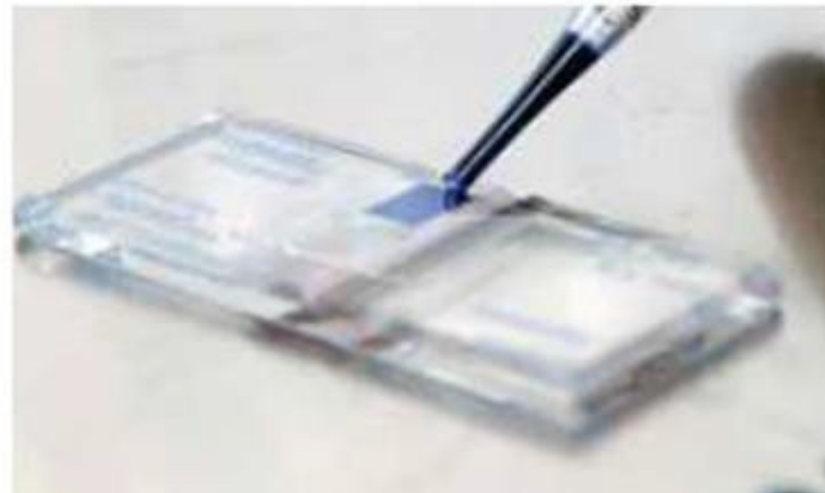


Notice how some areas on the slide are depressed to provide a space “0.1mm” at the bottom when the cover glass is placed.



Notice that the borders of the big square consist of 3 lines, which in turn help in recognizing the boundaries while observing under the microscope.

You can calculate the sample's volume based on the square's dimension, for example if I want to know the volume in the first square, we know that its dimensions are 1×1 , and for the height, it's 0.1 , so the volume will equal $(1 \times 1 \times 0.1 = 0.1 \text{ mm}^3)$



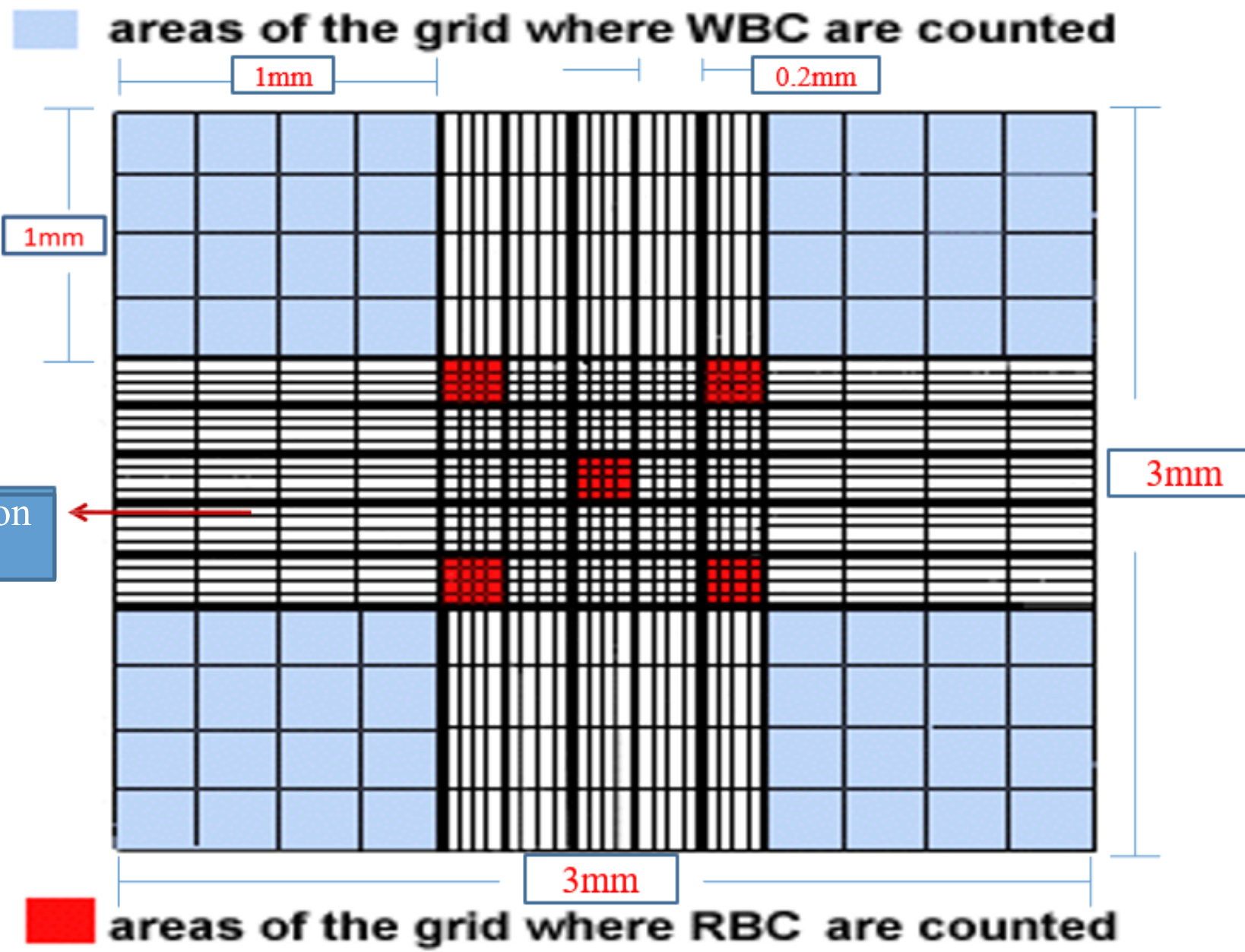
Notice that the counting chamber is shaped like a square which is also divided into 9 smaller squares.

The 4 blue corner squares are for WBCs count and are further divided into 16 smaller squares to make counting easier. while the center big square is where RBCs are counted, though only the 5 red squares are the ones used for counting.

Orientation lines

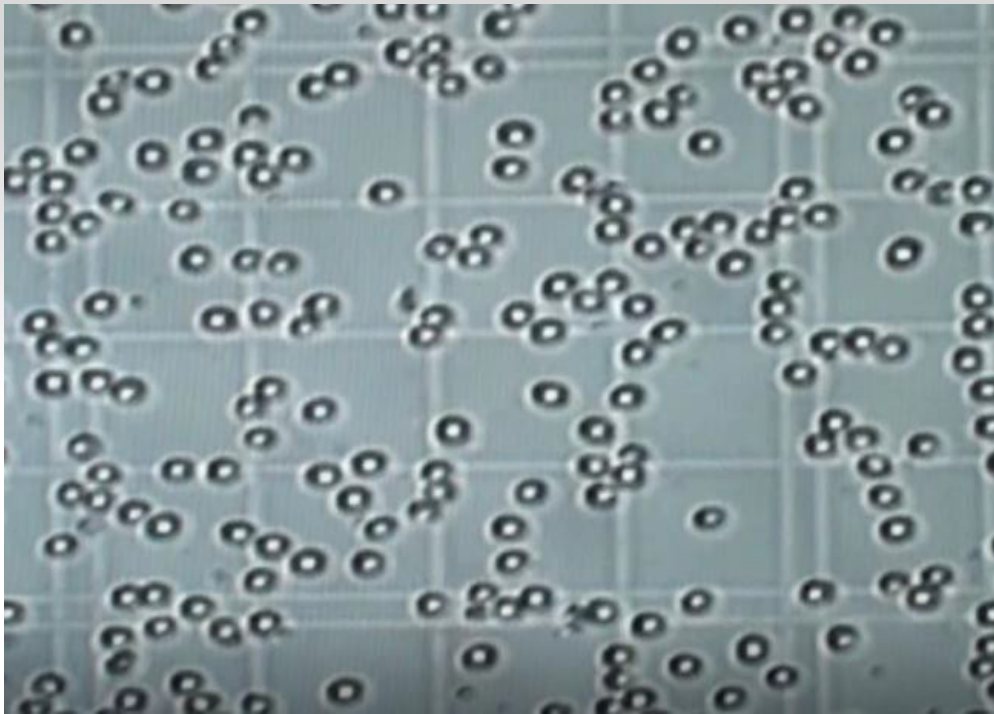
The orientation lines serve as a guide to tell which area of the hemacytometer we're observing under the microscope to avoid mistakes.

*square dimensions are important.

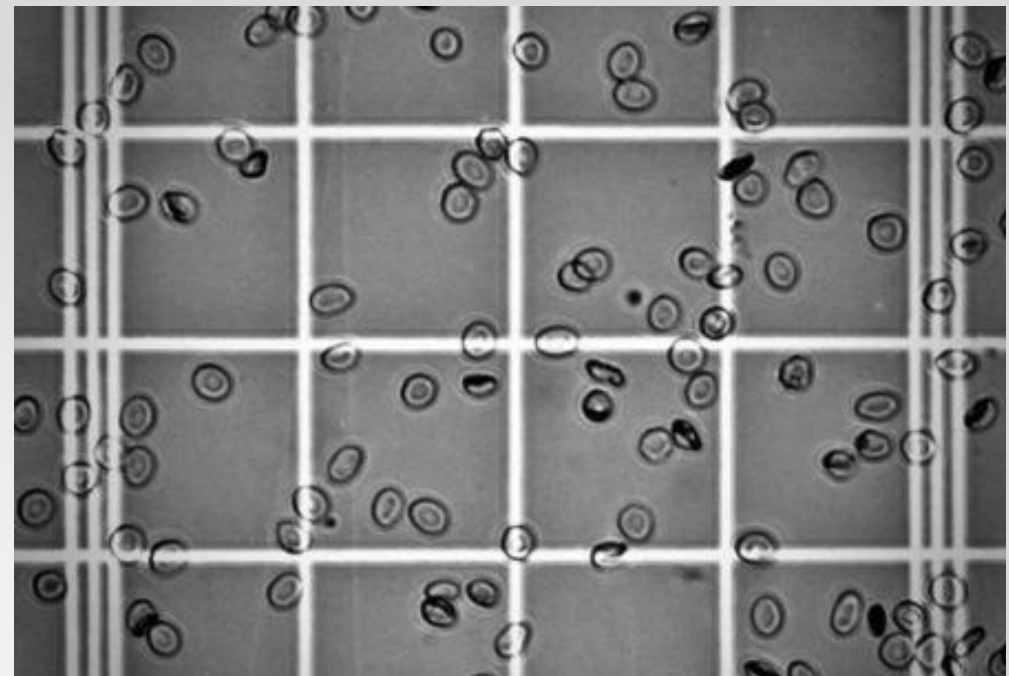


The procedure

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 (**so number of cells would be manageable**) by adding 1 unit of blood to 199 units of an isotonic solvent (**to preserve the RBCs**) 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 start counting the cells.
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 with a light center)
6. Count the number of cells in the five small squares and obtain an average number.



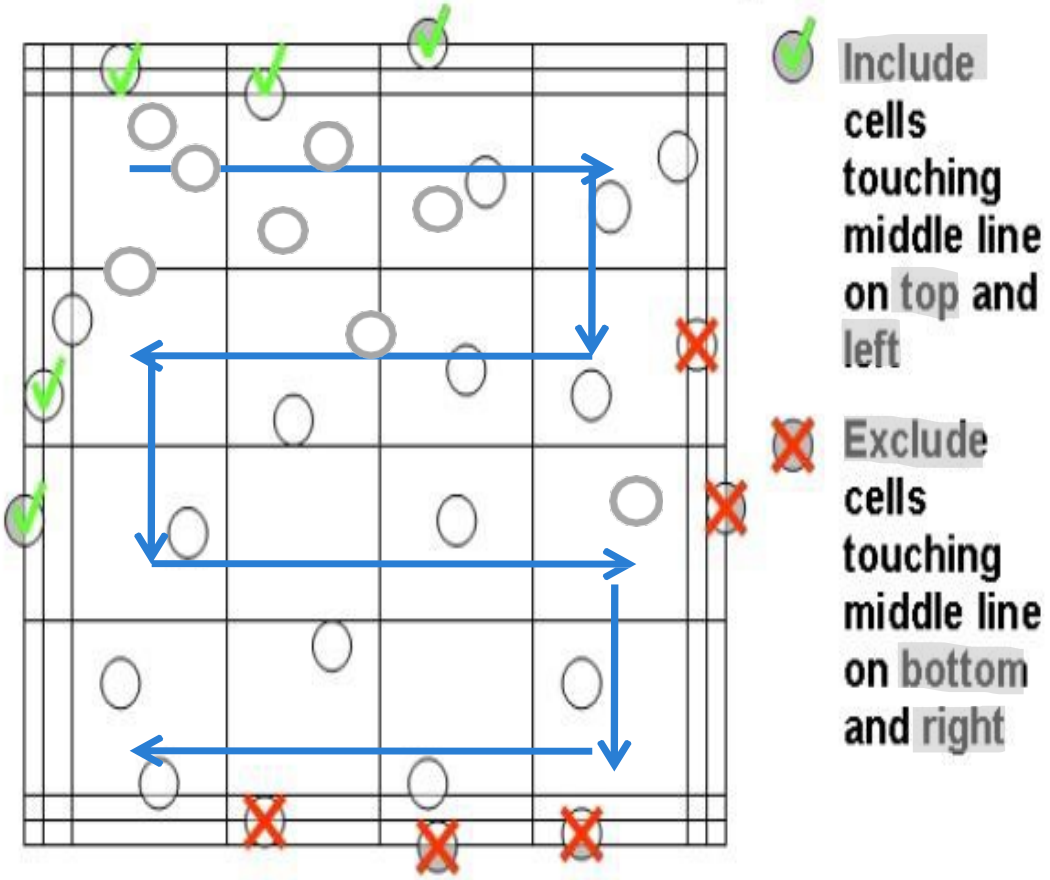
Count all the cells contained within the square and those touching the **upper** and **left** outer lines. The cells that touch the **right** and **bottom** outer lines are not to be counted. In each of the four areas, conduct the count in a zig-zag line.



- 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.

Counting system to ensure accuracy and consistency



- If the RBC count was (100, 90, 94, 96, 95). What is the RBC count in the blood sample?
 1. In this case the average RBC count is 95
 2. Dilution factor (DF) = Final volume/ volume of blood
 - Blood is diluted at (1:199) so DF= 200
 3. The volume of fluid contained in one small square = $(0.2 \times 0.2 \times 0.1) = 0.004 \text{ mm}^3$
 - Volume Correction Factor (VCF)= Desired Volume/ Actual volume
Desired volume = 1 mm^3
 - $VCF = 1/0.004 = 250$
 - The number of RBCs in blood sample= The average number of RBCs X DF X VCF = $95 \times 200 \times 250 = 4,750,000$ cells/ mm^3

FURTHER EXPLANATION

For Example; if I have an **average number of 100 RBCs**, and I wanted to calculate the total count; I need to have two things; a correction of the dilution and a correction of the volume

Dilution factor (DF) = Final volume/ volume of blood

So if I diluted my blood at 1:199, the DF would equal final volume (which is $1+199=200$) divided by the volume of blood which is (1), so **DF=200**.

The volume of fluid contained in one small square as we said before is the dimensions*height, so it's $(0.2 \times 0.2 \times 0.1) = 0.004 \text{ mm}^3$

Volume Correction Factor (VCF)= Desired Volume/ Actual volume

Desired volume = 1 mm^3 (the volume I want to find the count for) , Actual volume = 0.004 mm^3 (which I used in the sample).

So **VCF = $1/0.004 = 250$** .

So now to calculate the number of RBCs in blood sample = The **average number of RBCs X DF X VCF**
= **$100 \times 200 \times 250$**
= **$5,000,000 \text{ cells/mm}^3$**

Important Points

- 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.
- **DF can change** based on the dilution performed during the experiment.
(we'll consider it 200 in our calculations unless mentioned otherwise)
- **VCF is always the same** **(cause the squares have fixed dimensions)**

WBC count

- White Blood Cells are part of the immune system
- Move 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
- This test is often included in the complete blood count (CBC), it is done to get an impression about the immune system since the Leukocytes (WBC) play an integral role, to get more informative results it is often combined with the differential count

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

1. Active inflammation or infection.
2. Certain malignancies
3. Recent vigorous exercise, thermal burn, electric shock, surgery, or trauma.
4. Certain medications e.g. glucocorticoids (neutrophilia)
5. Dehydration.

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

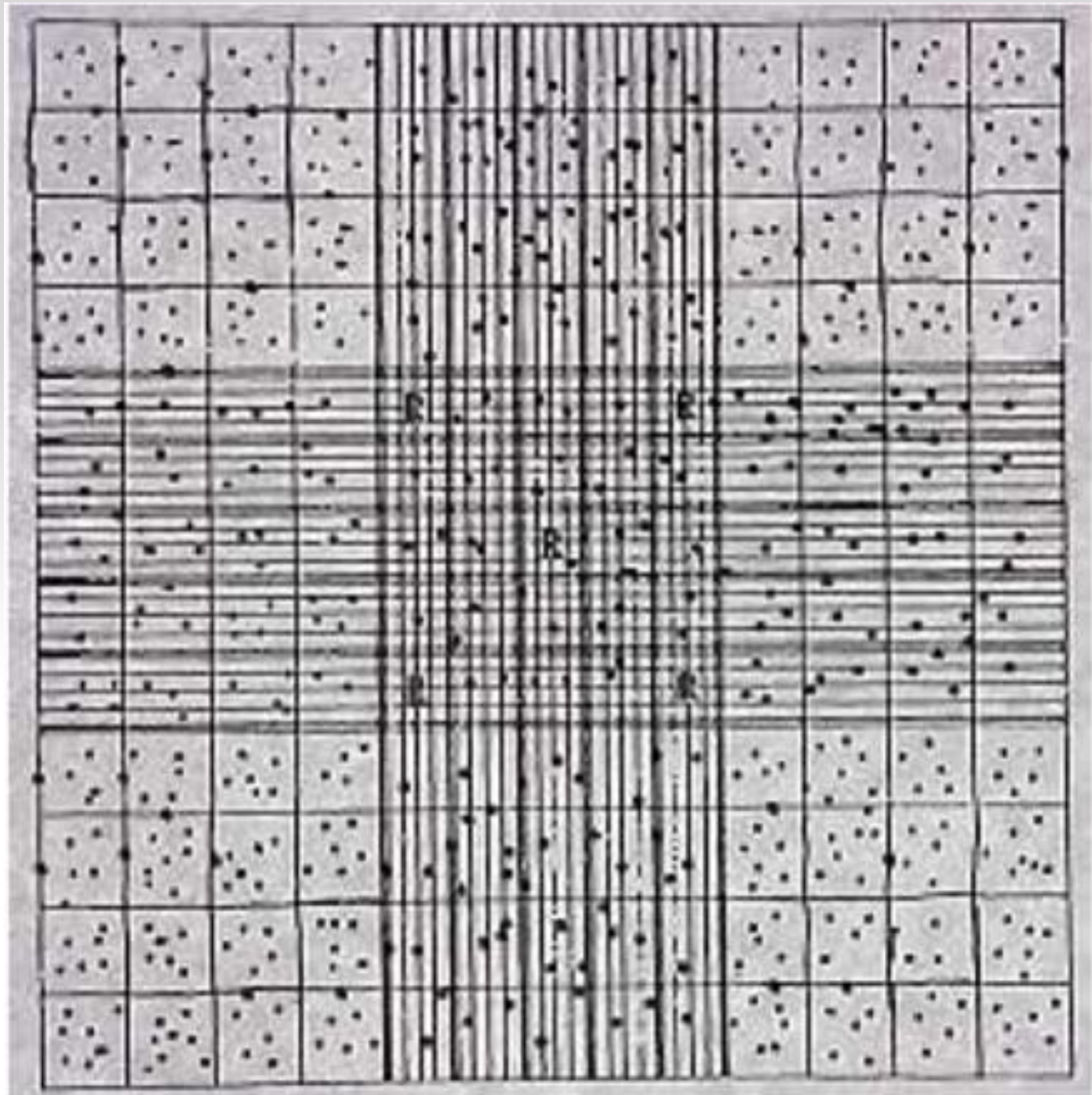
1. Bone marrow failure due to aplastic anemia, fibrosis, metastatic cancer, radiotherapy or chemotherapy
2. Autoimmune diseases.
3. Infections like HIV & tuberculosis.

The procedure

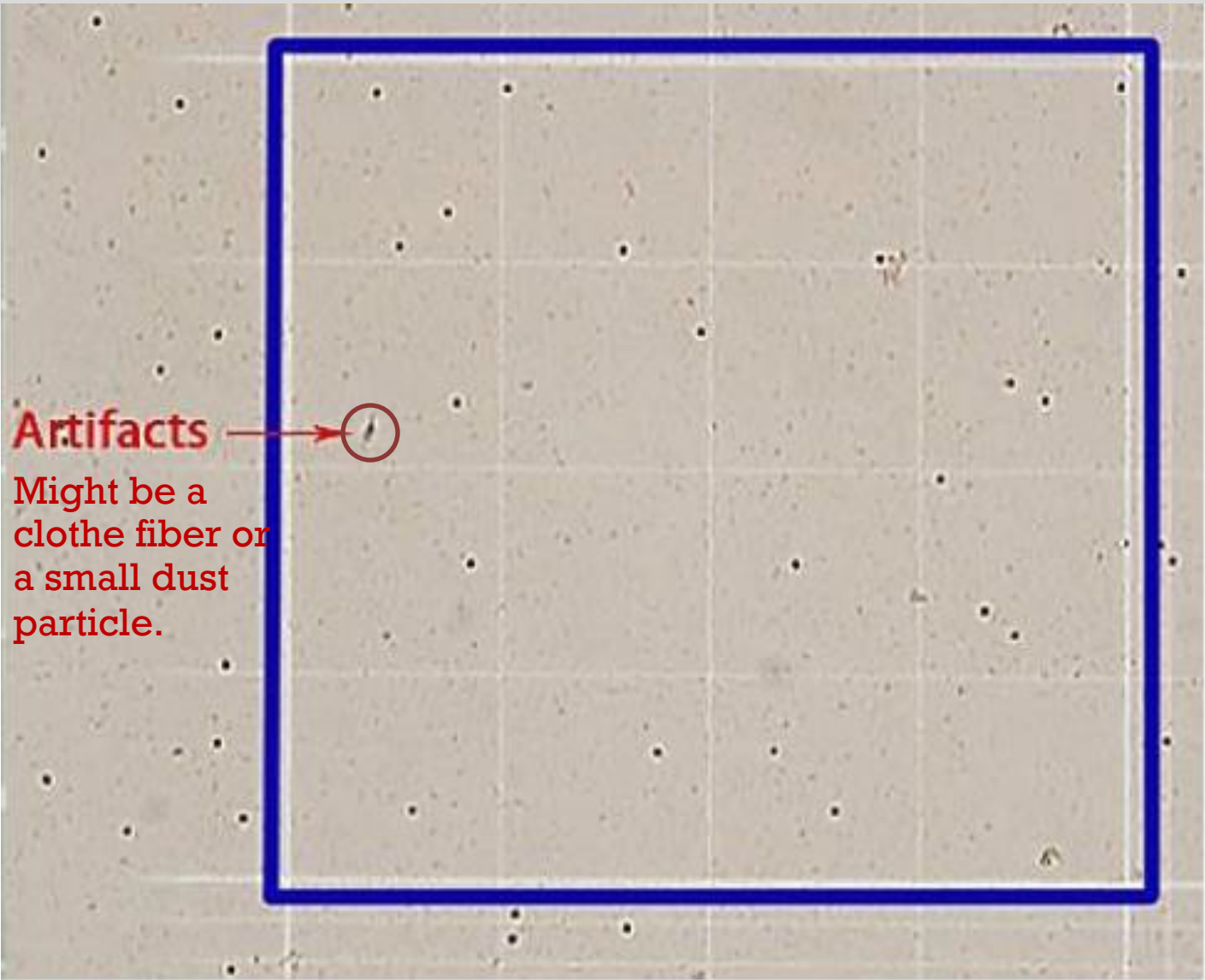
Same procedure as RBCs count, with minor differences only.

1. Clean the hemocytometer well
2. Place a coverslip over the counting area.
3. Dilute the blood sample by adding 1 unit of blood to 19 units of solvent and thoroughly mix the mixture.
 - ✓ The dilution fluid contains an agent (**glacial acetic acid**) which lyses the red blood cells (**because of their big number, we worry it'll obscure the WBCs**). It also contains a dye that stains the nuclei of WBCs. This allows a proper count of WBCs.
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 count the cells.
5. Use the **10X** lens to count the WBC in the four large corner squares. (WBCs appear as dark dots)

This is how the WBCs appear on hemacytometer (black dots), remember we only count the WBCs in the large four squares, same counting technique as RBCs, and we start from the upper right big square then upper left and so on.



The black dots are the WBCs



Artifacts →

Might be a
clothe fiber or
a small dust
particle.

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....
 $40 \times 20 \times 10 = 8000 \text{ cells/mm}^3$ 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

Differential Leukocyte Count (DLC)

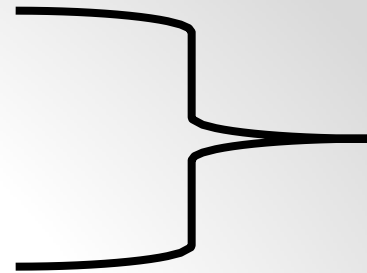
Its not enough to only know the WBCs count,
that's why we do this test, for clearer details.

- The blood contains 5 different types of white blood cells which are classified into:
 1. Granulocytes: have cytoplasmic granules which contain enzymes or chemicals, and have a single multi lobed nucleus (segmented)
 2. Agranulocytes: have a single non lobulated nucleus, their cytoplasmic granules are too small to be seen under the light microscope.

1. Neutrophils: 40-80 %

2. Eosinophils: 1-4 %

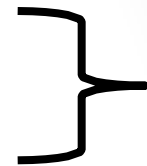
3. Basophils: < 1%



Granulocytes

4. Lymphocytes: 20-40%

5. Monocytes: 2-8%



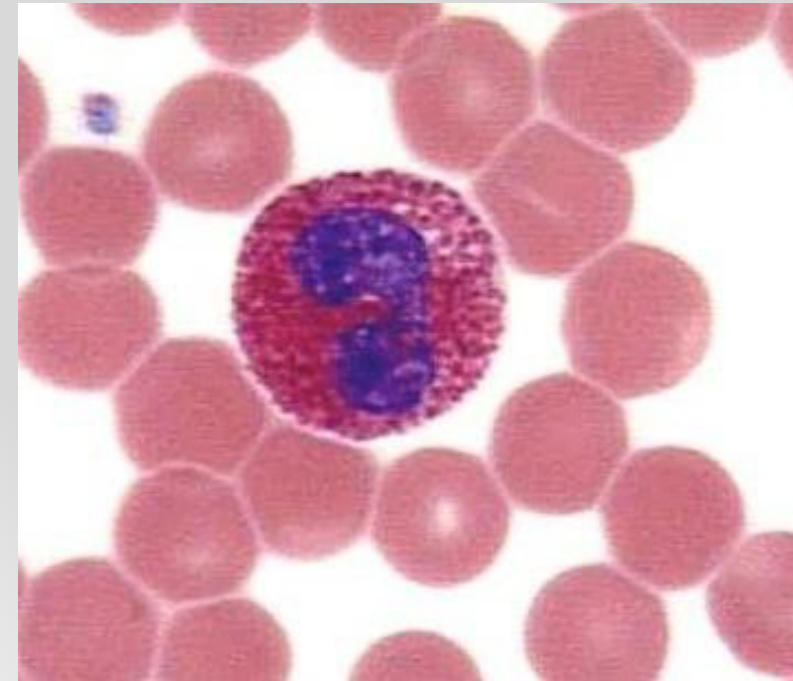
Agranulocytes

Neutrophils have nuclei with several lobes and fine pink granules in their cytoplasm.

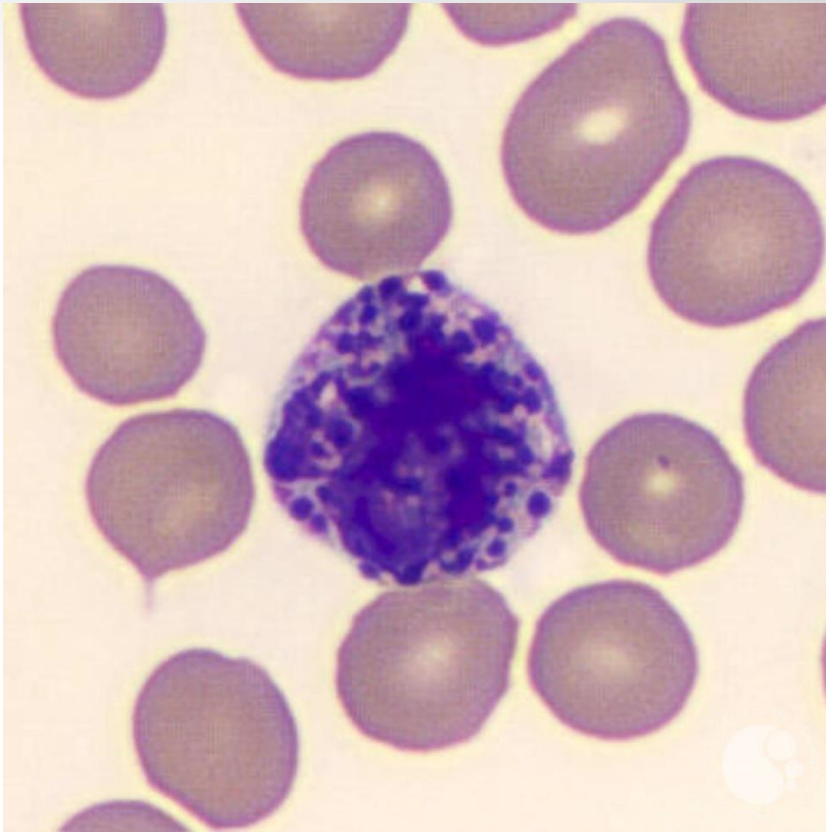
They are called neutrophils, because their granules are not very amenable to staining with either acidic or basic dyes



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



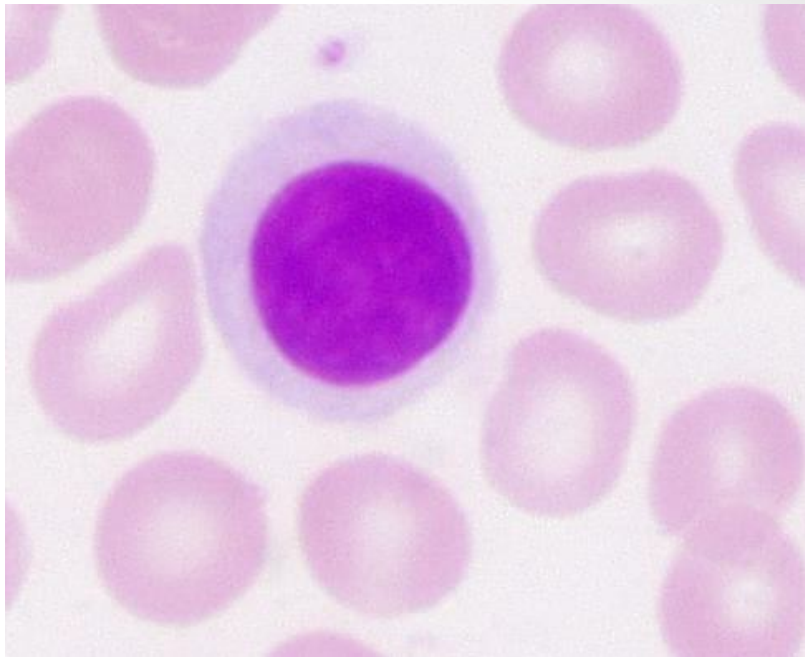
Basophils have bi-lobed or S shaped nuclei and large granules which stain dark blue with basic dyes and completely obscure the nucleus



Neutrophilic Band cells are immature neutrophils, usually make less than 5% of the total WBC count, their nucleus isn't segmented



- **Lymphocytes** have a very large nucleus taking up most of the cytoplasm. The cytoplasm has no granules. Most cells are small in size.
- We can't differentiate between the B and T lymphocytes under the light microscope.



- **Monocytes** are large cells. They have large indented nuclei, often kidney-shaped. Their cytoplasm has fine purple granules which give it a "ground glass" appearance.

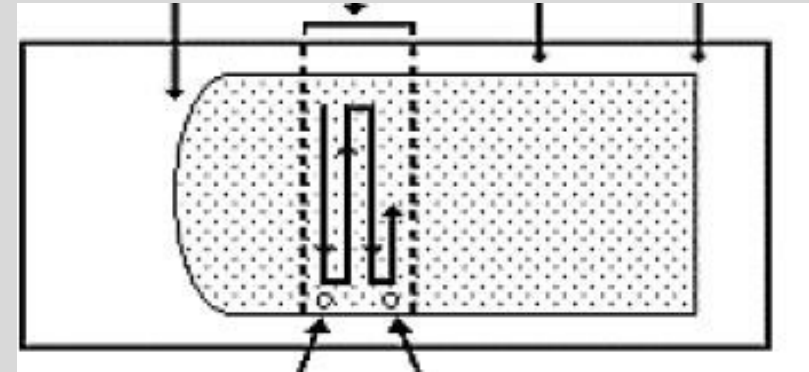




The procedure

1. A drop of blood is thinly spread over a glass slide, air dried, and stained with an **acidic dye (red)** and a **basic dye (blue- purple)**.
2. The slide is examined under a microscope using an oil immersion lens.
3. Two hundred white cells are then counted and classified.
4. 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

We basically count and record the number of different cells as we go down the slide, for example if we counted 100 WBCs and it had (60 Neutrophils , 30 Lymphocytes , 5 Monocytes , 3 Eosinophils and 2 Basophils) their % out of 100 would be (60% , 30% , 5% , 3% and 2%)



Importance of DLC

- Gives relative percentage of each type of WBC
- Helps reveal the presence of abnormal WBCs like blasts or lymphoma cells.
- Used along with WBC count to generate an **absolute value** (as a number not a percentage) for each type of WBCs.
 - Relative percentages can be misleading
 - Absolute values are also useful for monitoring certain conditions.
 - **Absolute count = WBC (cells/ μ L) x percent of the specific WBC type \div 100**

Absolute count calculation

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

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

- Absolute neutrophil count (ANC) = WBC (cells/ μL) x percent (neutrophils + neutrophilic band cells) ÷ 100

1. **Neutrophilic leukocytosis**: is defined as a total WBC above 11,000/ μ L along with an absolute neutrophil count (ANC) greater than **7700/ μ L**
 - Bacterial infections, inflammatory conditions, stress.

2. **Lymphocytic leukocytosis** : is defined as a total WBC above 11,000/ μ L along with an absolute lymphocyte count greater than **4500/ μ L**
 - Viral infections as infectious mononucleosis, mumps, rubella and pertussis or in acute and chronic lymphocytic leukemias.

3. Monocytic leukocytosis:
 - Acute or chronic bacterial infection and chronic inflammation

4. Eosinophilic leukocytosis :
 - Parasitic infections & allergic conditions

6. Basophilic leukocytosis:

- Allergic conditions

7. Neutropenia : absolute neutrophil count is less than 1,500 cells/ mm³

- Certain infections like typhoid fever, HIV & CMV, chemotherapy, radiotherapy, and autoimmune diseases.

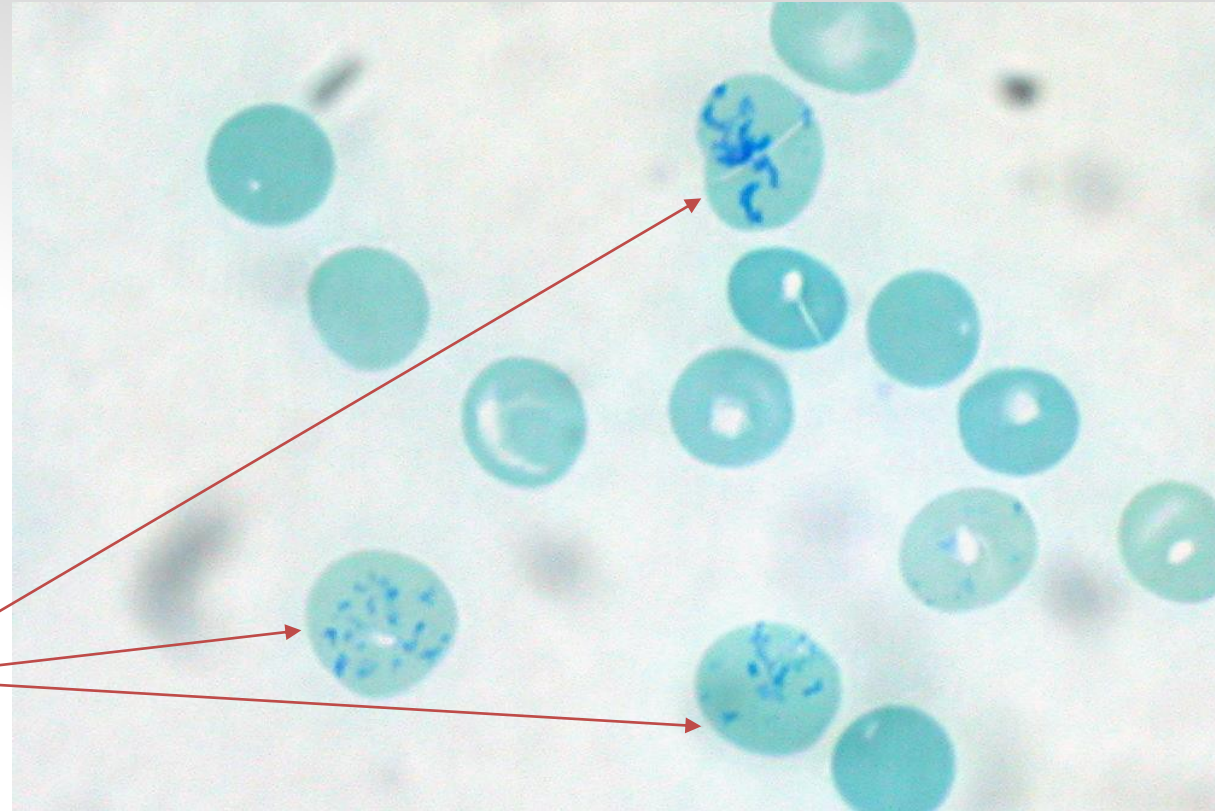
8. Lymphocytopenia:

- May occur in the normal elderly or be associated with chronic infection or malignancy.

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 normally 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

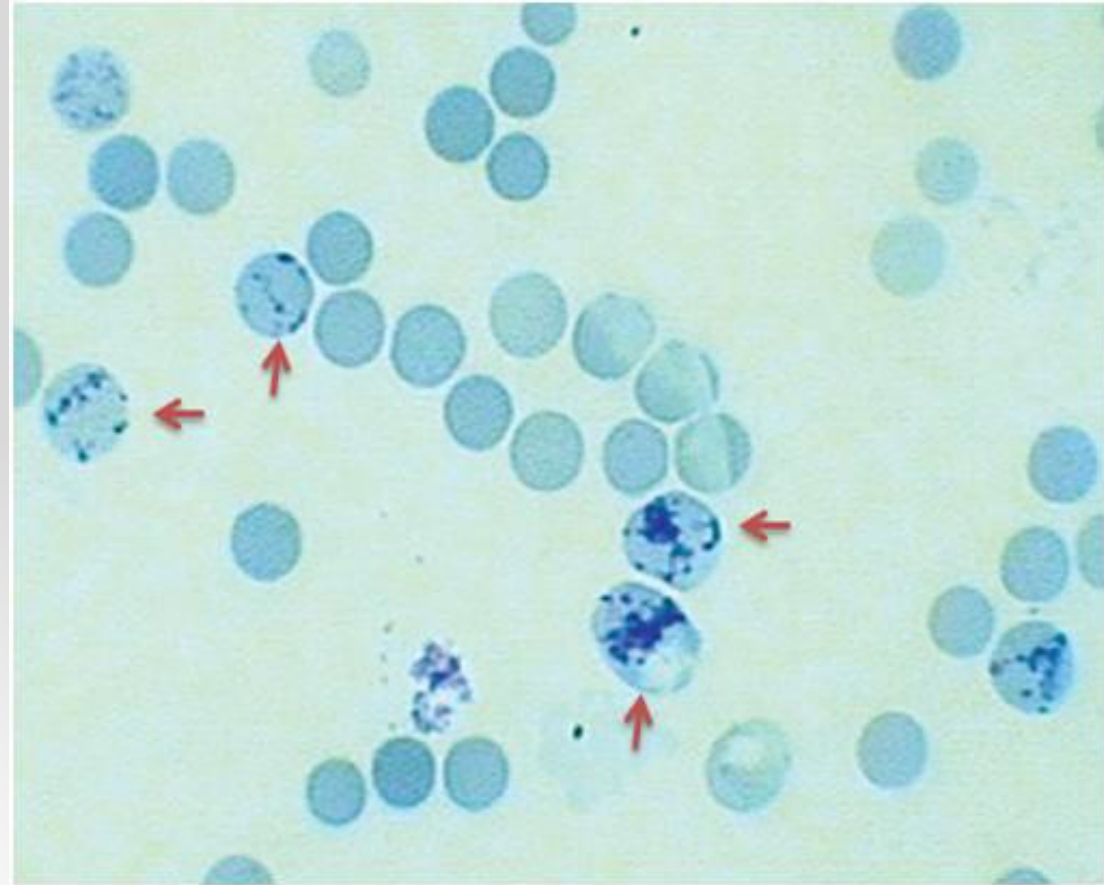
If supravital staining (new methelene blue) is performed on a blood smear, the reticulocytes appear larger than RBCs and contain dark blue dots and curved linear structures in their cytoplasm (remnants of ribosomes).



Reticulocytes.

The procedure

- 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 count X reticulocyte%)/100**



Reticulocytosis and Reticulocytopenia

- Condition associated with an increase in reticulocytes:
 - Hemolytic anemias: Immune hemolytic anemia, RBC membrane defects, Sickle cell diseases,
 - Following hemorrhage
 - Following treatment of anemias
- Condition associated with a decrease in reticulocytes:
 - Iron deficiency anemia
 - Aplastic anemia
 - Radiation therapy
 - Tumor in bone marrow