Blood smear and differential leukocyte count

by

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Introduction

- DLC: It is relative proportion of different leukocytes expressed as a percentage.

Purpose

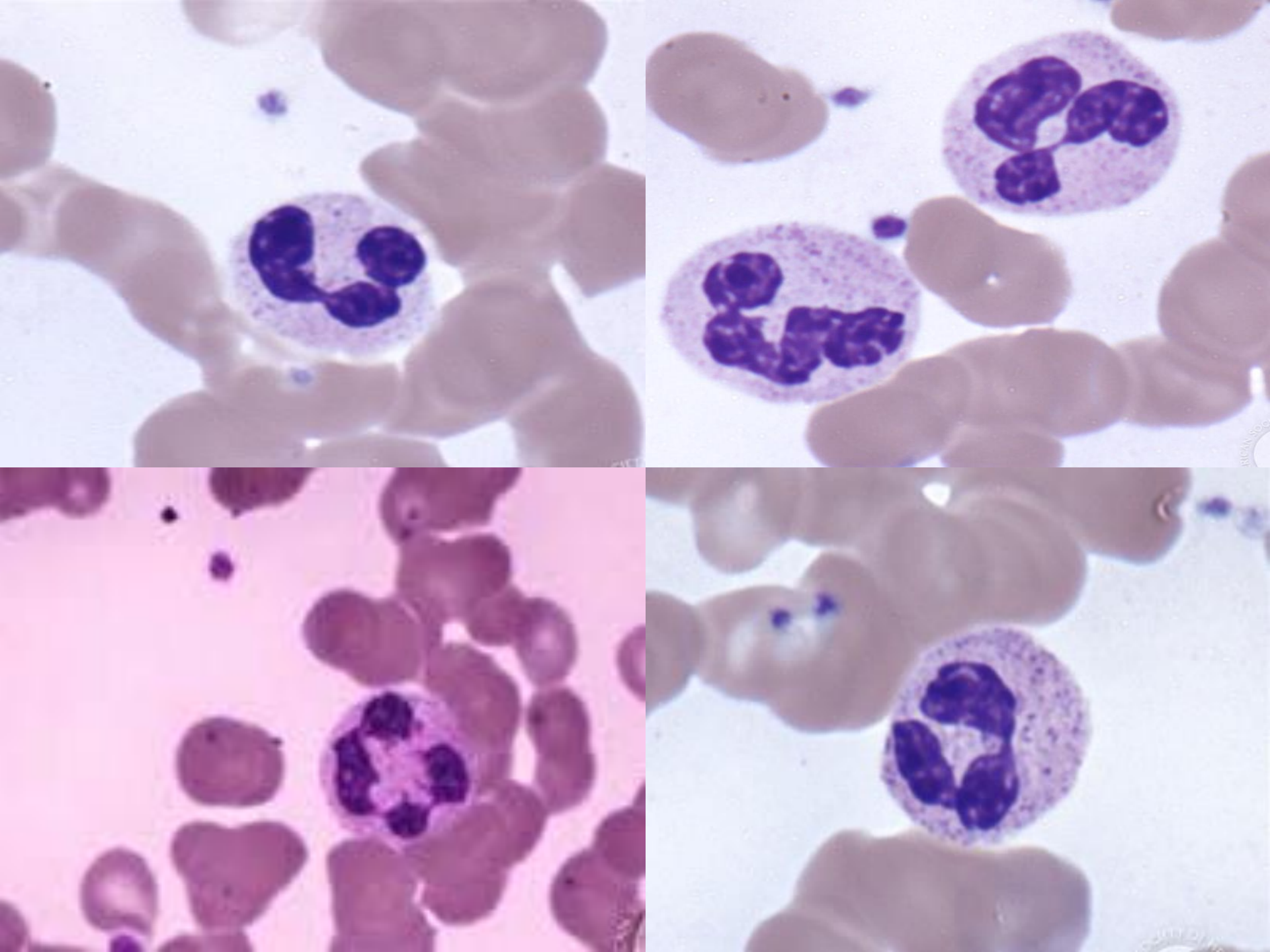
- The differential leukocyte count is performed to determine the relative number of each type of WBC present in the blood. The number of different types may deviate from the normal range, which may indicate certain disease states of the patient such as allergic; parasitic infection and other types of infection. It can also identify various stages of leukemia.

- Leukocytes are divided into two groups according to the type of cytoplasmic granules & the shape of their nuclei:
- Polymorphonuclear granulocytes
- Mononuclear agranulocytes

Polymorphonuclear granulocytes

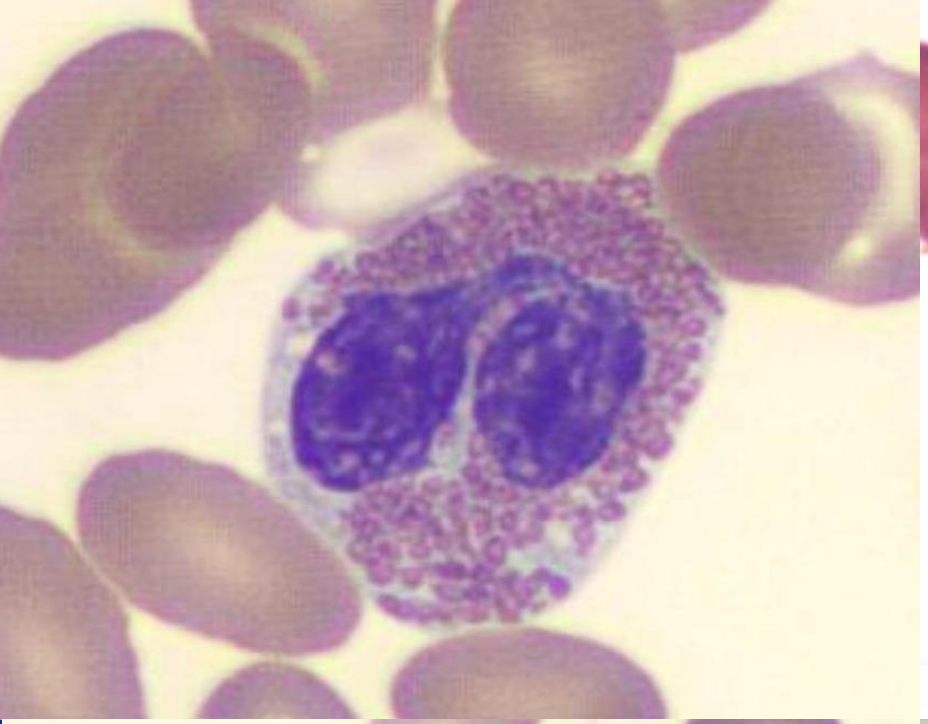
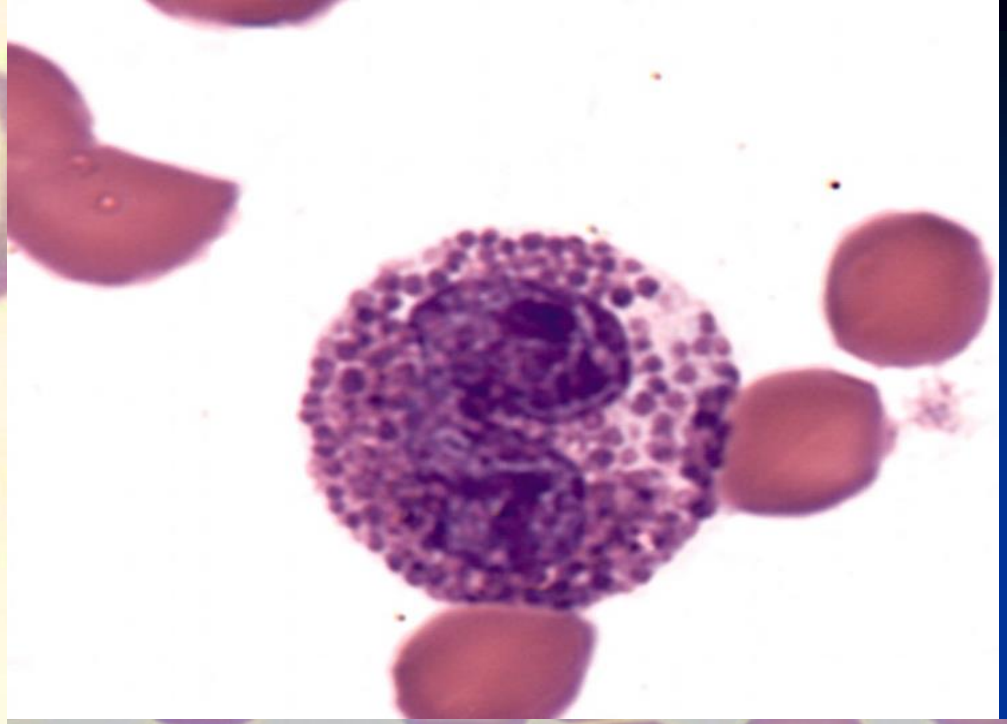
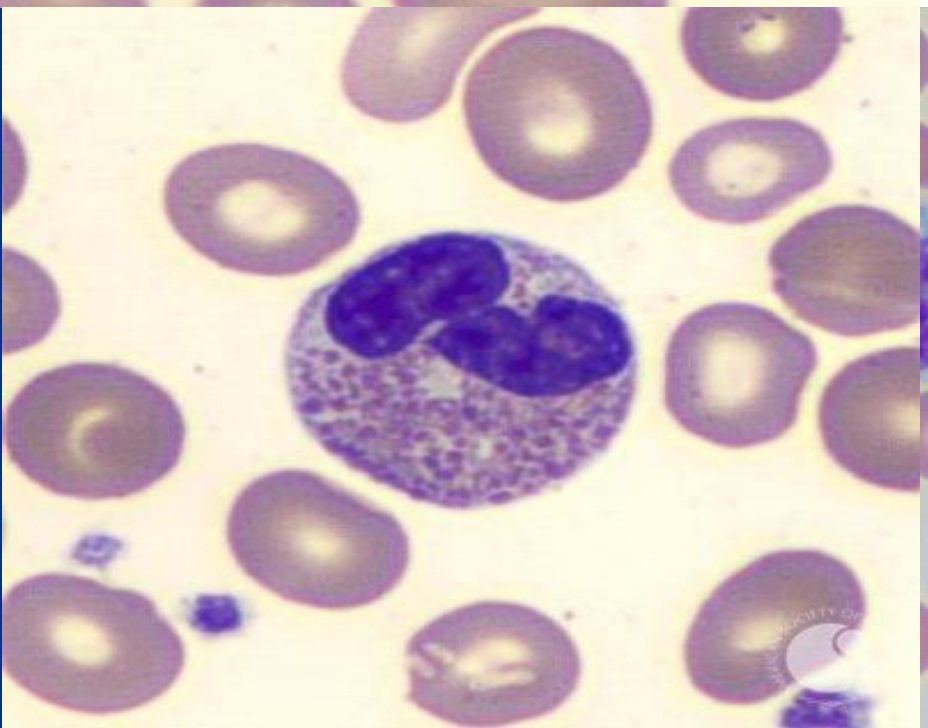
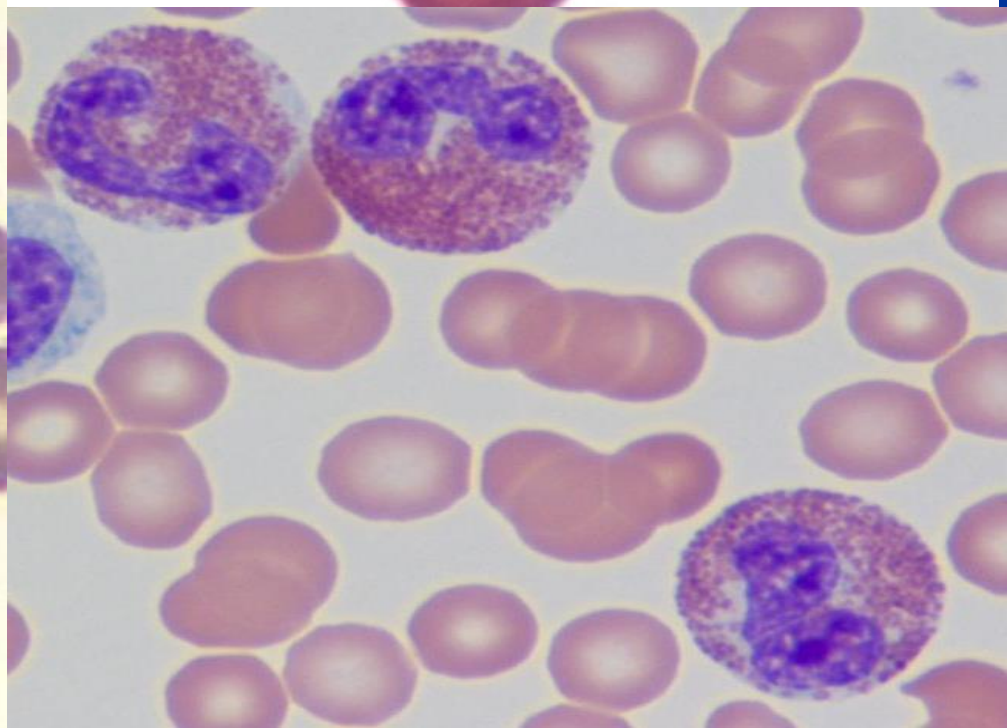
1. Neutrophils

- Constitute (50%-70%) of circulating leukocytes.
- The nuclei having 3-5 lobes linked by thin nuclear extension.
- The pink cytoplasm contains very fine granules which are difficult to see.
- Function they are active in phagocytosis and killing bacteria and are usually the first leukocytes to arrive at sites of infection



2. Eosinophils

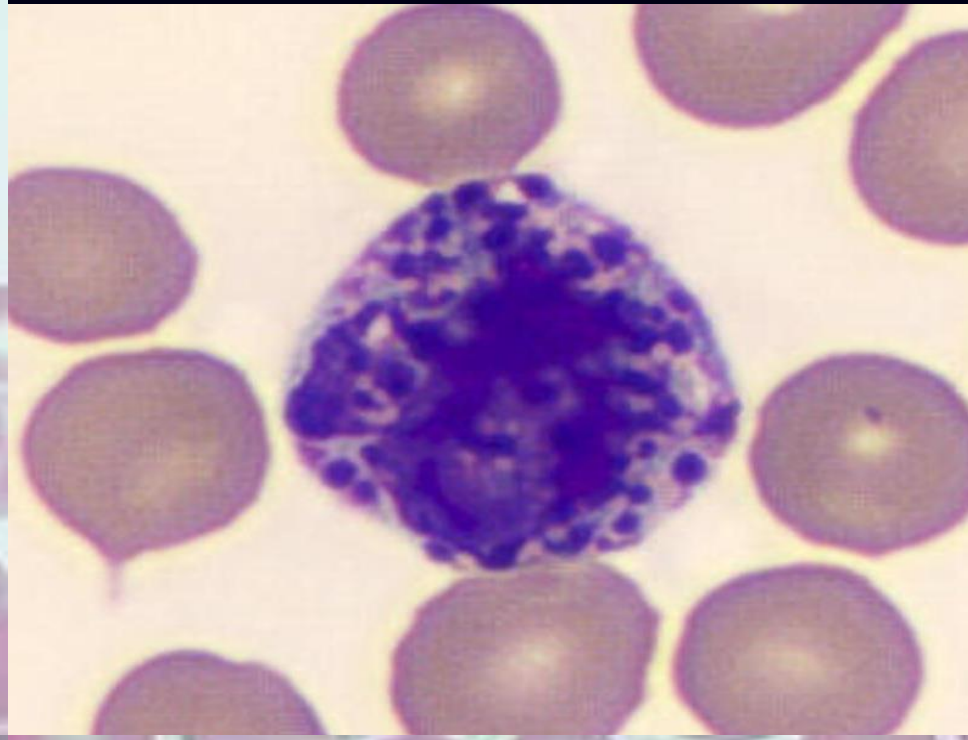
- Constituting only **(1%-3%)** of leukocytes
- The nucleus has bi-lobed.
- The main identifying characteristic is the abundance of large, acidophilic specific granules typically staining red.
- **Function** eosinophils play an important role in the inflammatory response triggered by allergies. Also, they act to kill parasitic worms.

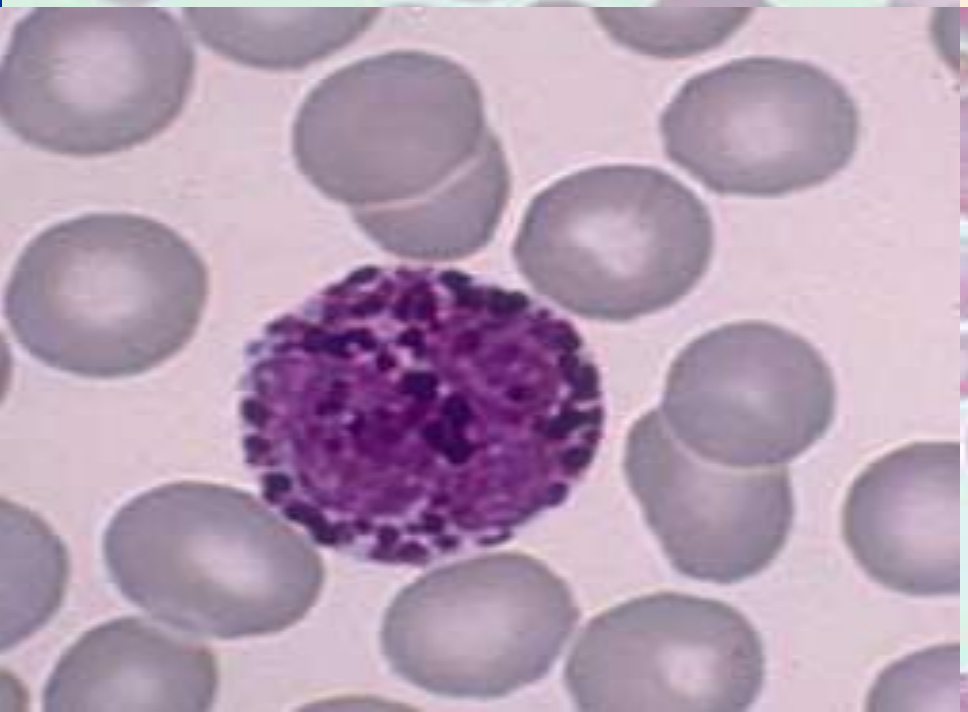





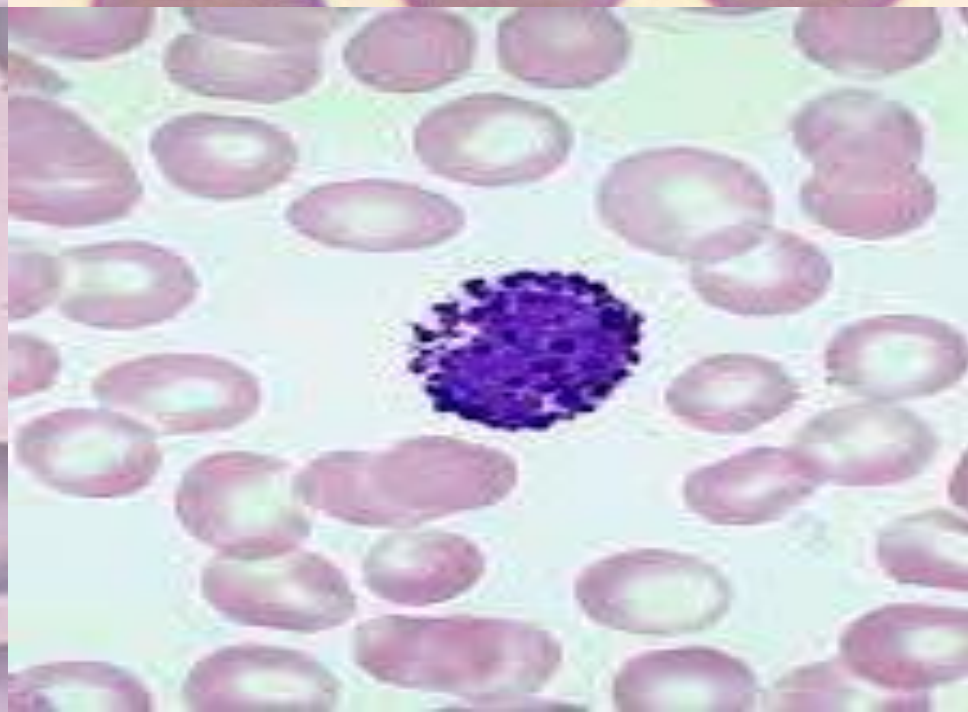
3. Basophils

- Constituting (0-1%) of circulating leukocytes.
- The nucleus is divided into two irregular lobes (U - shaped).
- The cytoplasmic granules are coarse & stained dark blue.
- Function they are also involved in the hypersensitivity response (allergy) and chronic inflammatory conditions.


Basophil







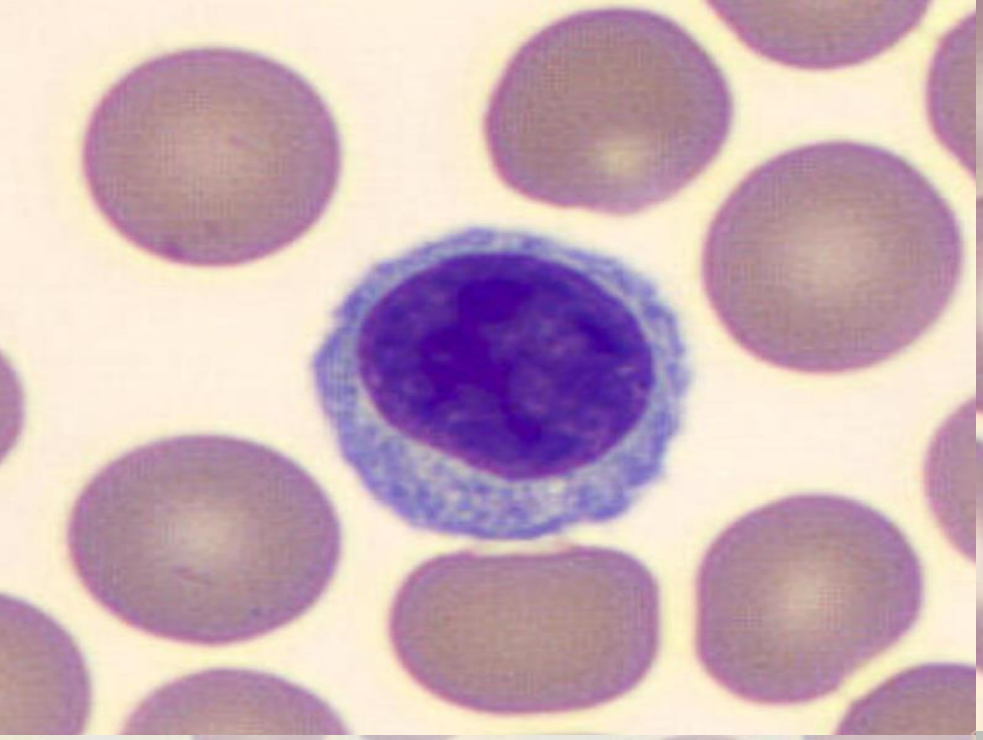
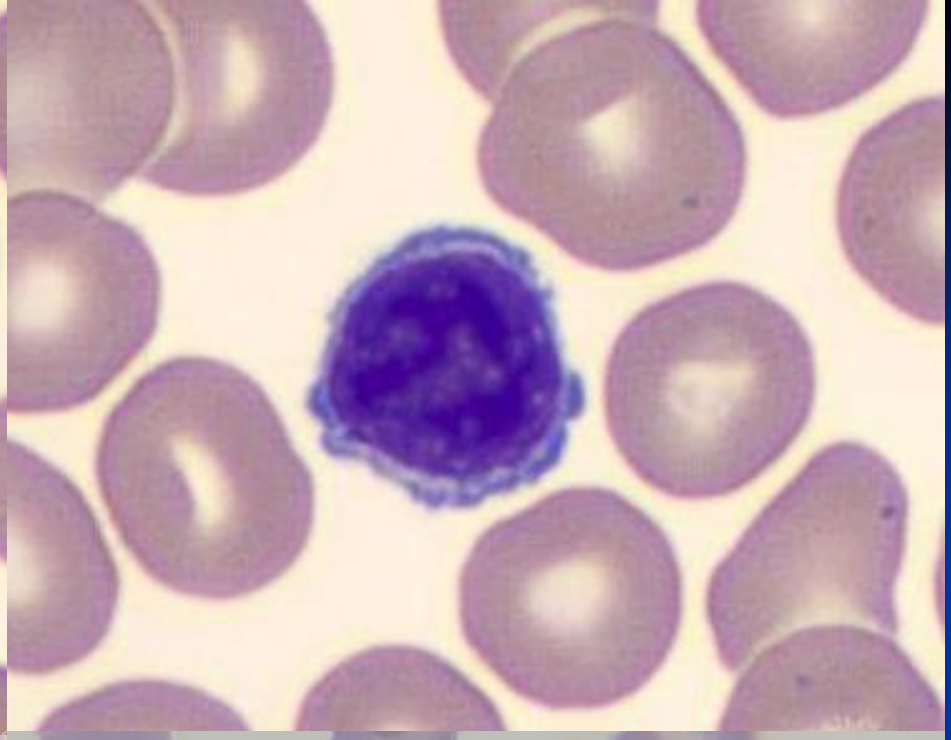
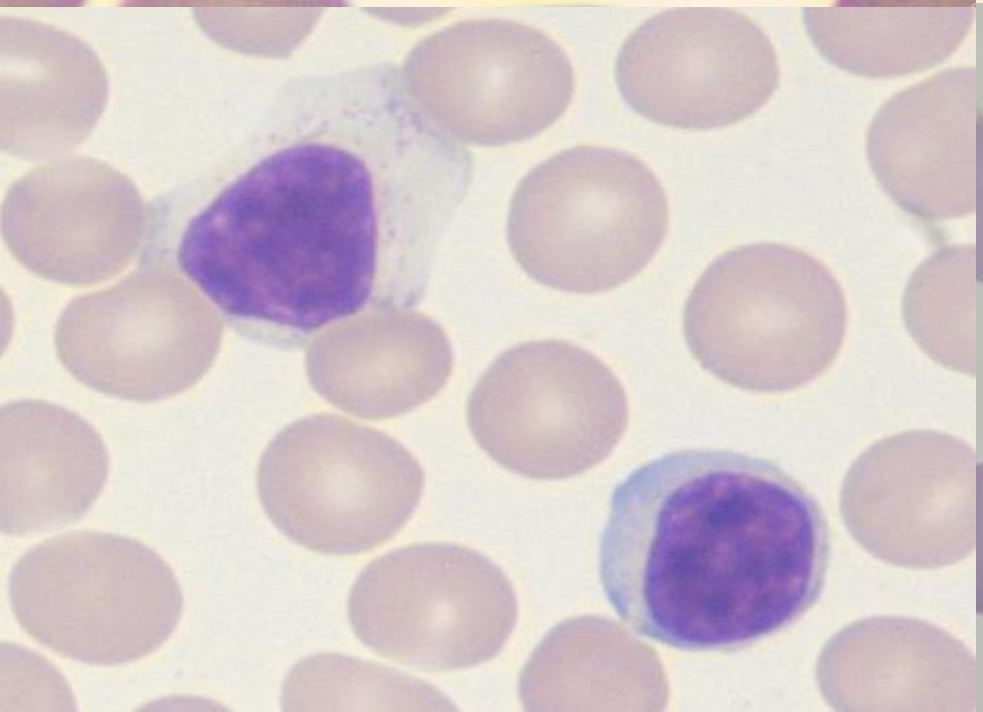
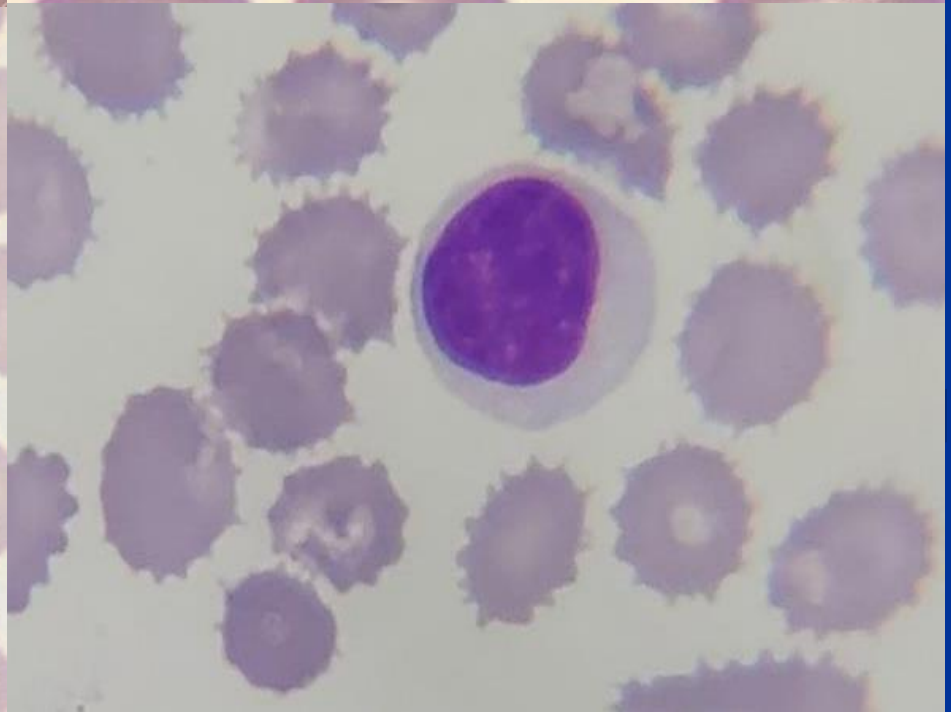
Mononuclear agranulocytes

1. Lymphocytes:

- Lymphocytes are typically the smallest leukocytes and constitute (20% to 35%) of the WBCs.
- Spherical cell with a dark purple round nucleus in sparse blue cytoplasm.
- Function play important role in the immune defense against invading microorganism.

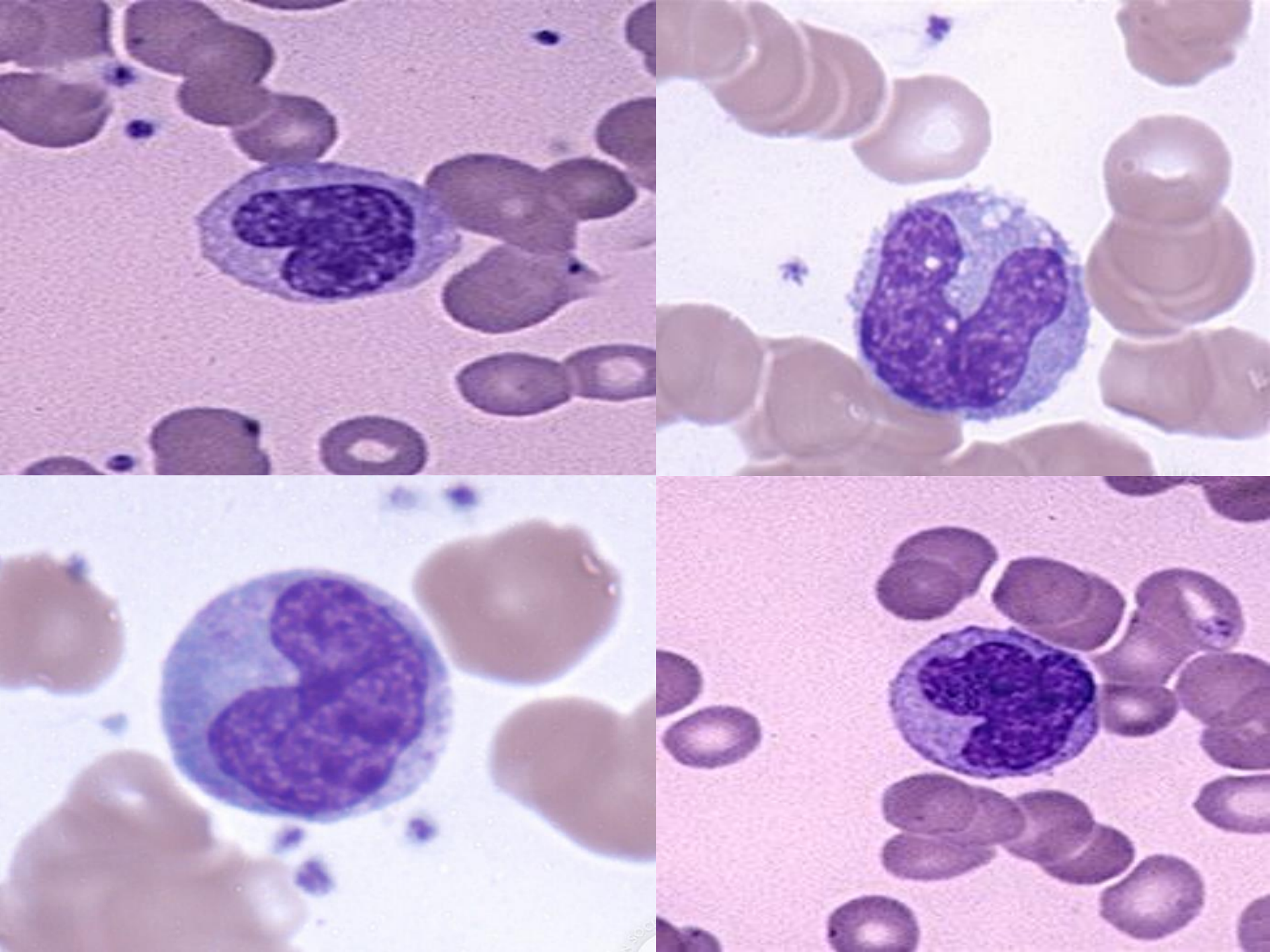
There are two types of lymphocytes:

- B cell: produce antibodies in response to antigens
- T cell: attack cancer cells.

2. Monocytes

- Constitute only (1% to 6%) of the WBC count.
- The nucleus have kidney-shape in abundant cytoplasm stains blue.
- Function: circulating monocytes are precursor cells of the mononuclear phagocyte system, function in phagocytosis and antigen presentation.



Methods

- Manual method (blood film)
- Electronic cell counter

Materials and instruments

- Glass slide
- Microscope
- Alcohol 70%
- Lancet
- Immersion oil.
- Differential cell counter
- Leishman's stain

Leishman stain composition:

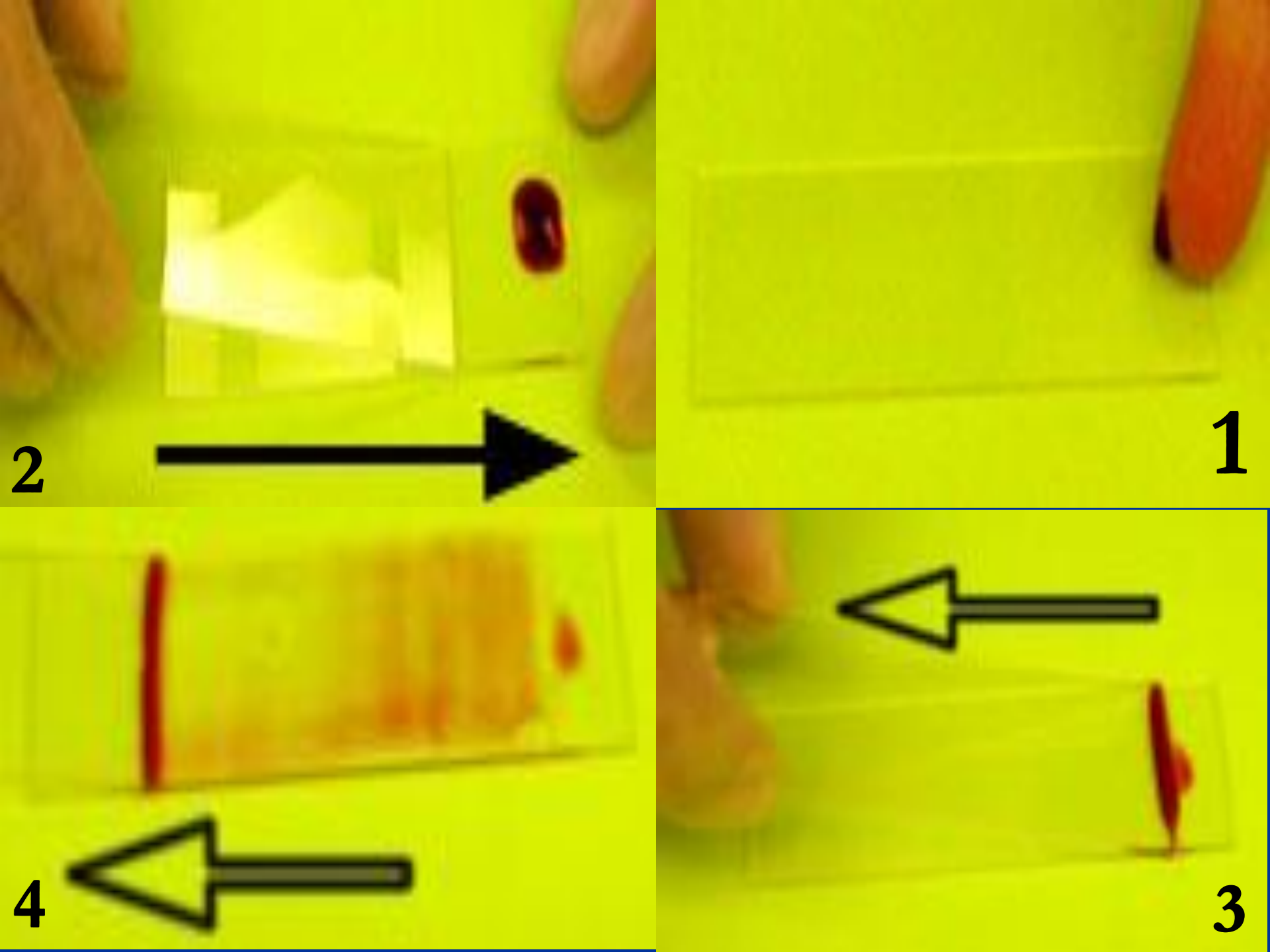
- **Methylene blue:** basic dye, positively charged and stains negatively charged particles (cytoplasmic granules of basophils).
- **Eosin:** acidic dye, negatively charged and stains positively charged particles (cytoplasmic granules of esinophils)
- **acetone free methyl alcohol:** to fix the smear to the slide and preserves the morphology and chemical status of the cells. The alcohol must be free from acetone due to being a strong lipid solvent which tends to damage the cell membrane

Procedure

Prepare the blood smear

- Clean the finger with alcohol, allow it to dry and then prick it with a disposable lancet to obtain a drop of blood.
- Make a fine touch of one end of a slide with the drop of blood (only a small amount is required).
- Place the edge of the other slide on the surface of the first slide just in front of the drop of blood and at an angle of 45˚.

- Draw the spreader back until it makes contact with the drop of blood.
- Push the spreader smoothly and tightly to the other end of the slide.
- Allow the film to dry at room temperature i.e. the blood smears should be air-dried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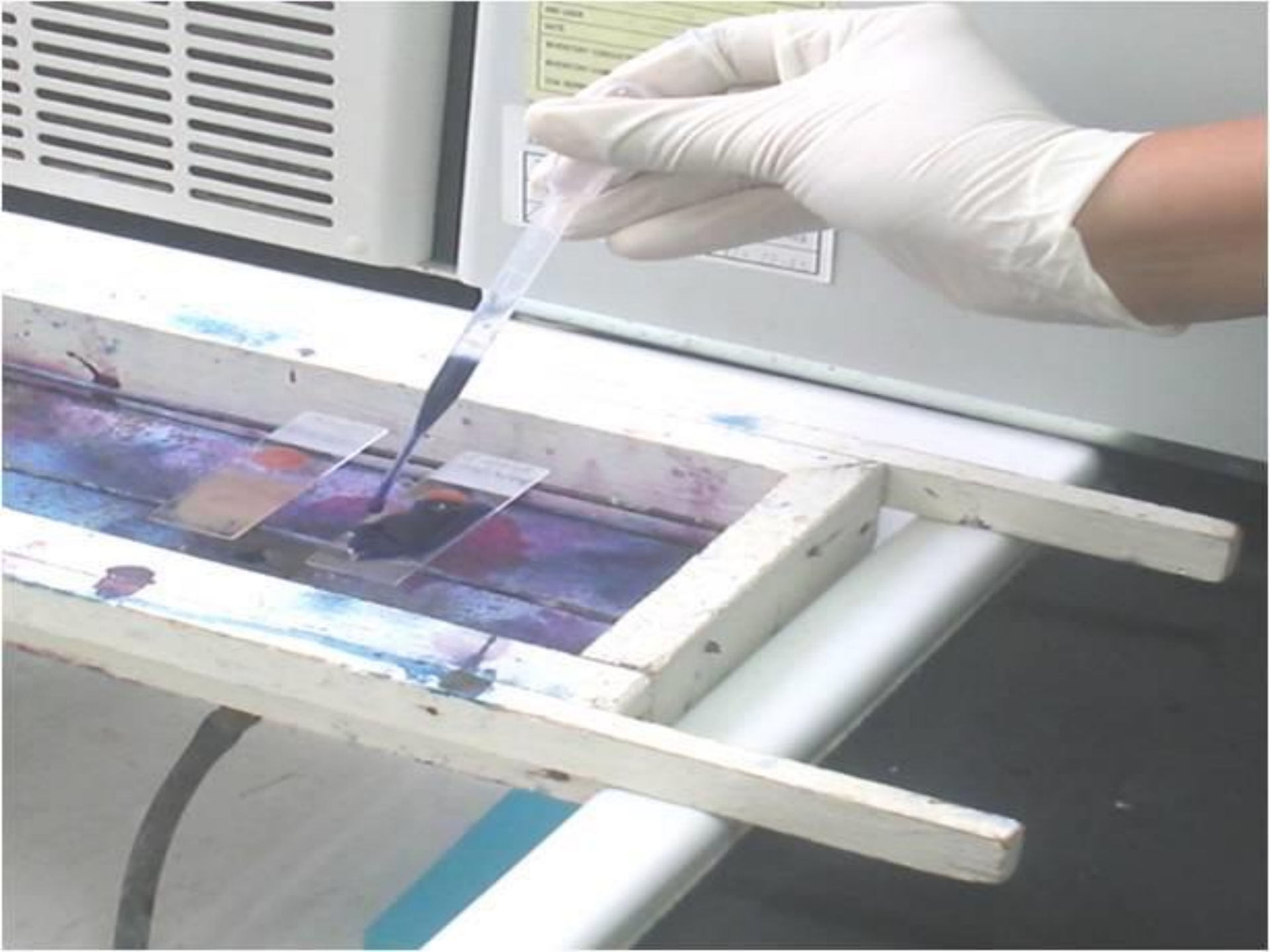
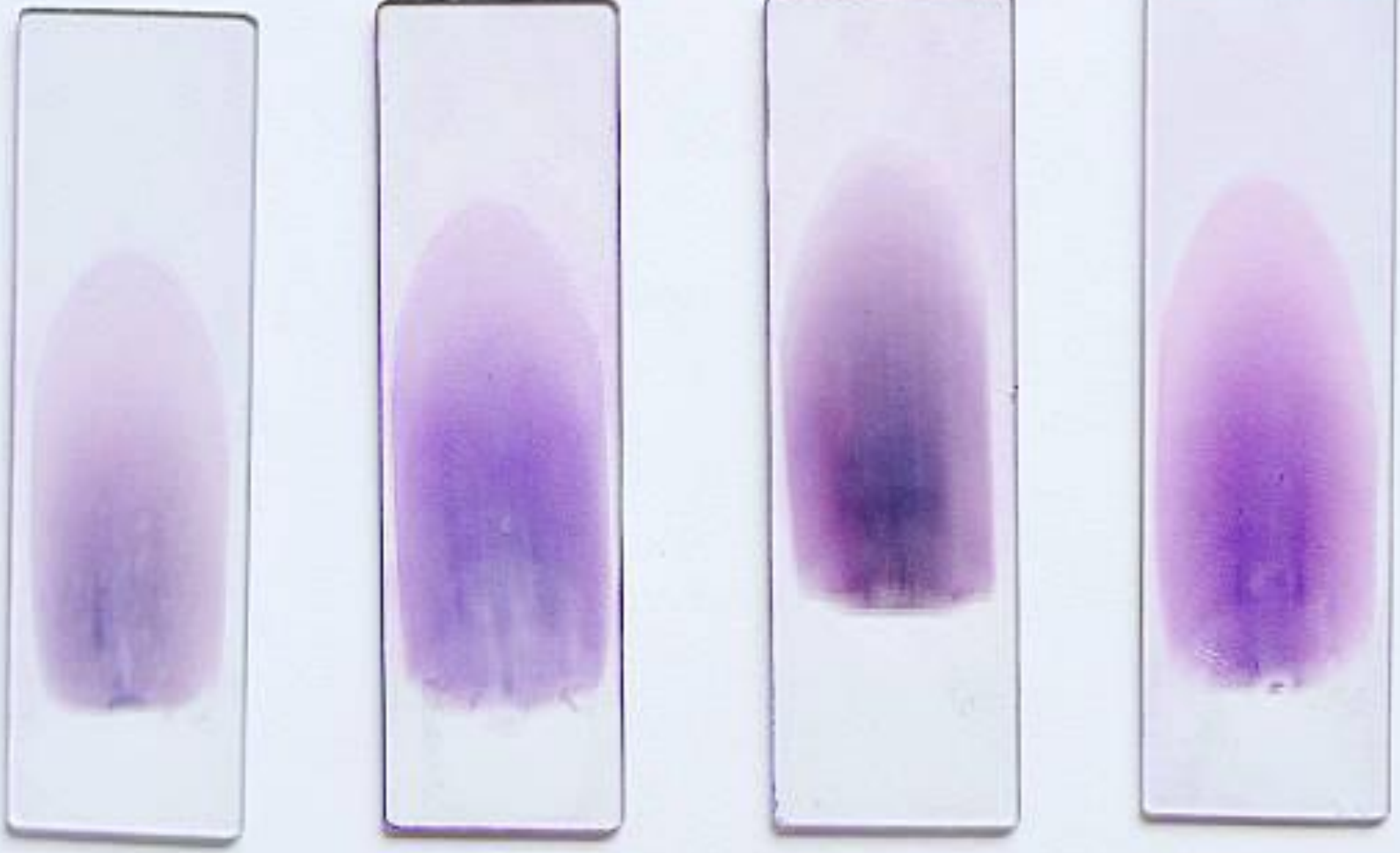
Staining the blood smear by:-

- Put the dried slide on a staining rack.
- The blood smear should be stained as soon as possible certainly within 1 to 2 hours.
- Carefully drop Leishman's stain onto the blood smear until the smear is covered.
- Allow the stain to act for one to two minutes.
- Add distilled water to the stain and mix by blowing
- The diluted stain should act for 10-15 minutes
- Then wash it off with distilled water, continue washing until the smear has a pink color.
- Shake off excess water and allow it to dry at room temperature.





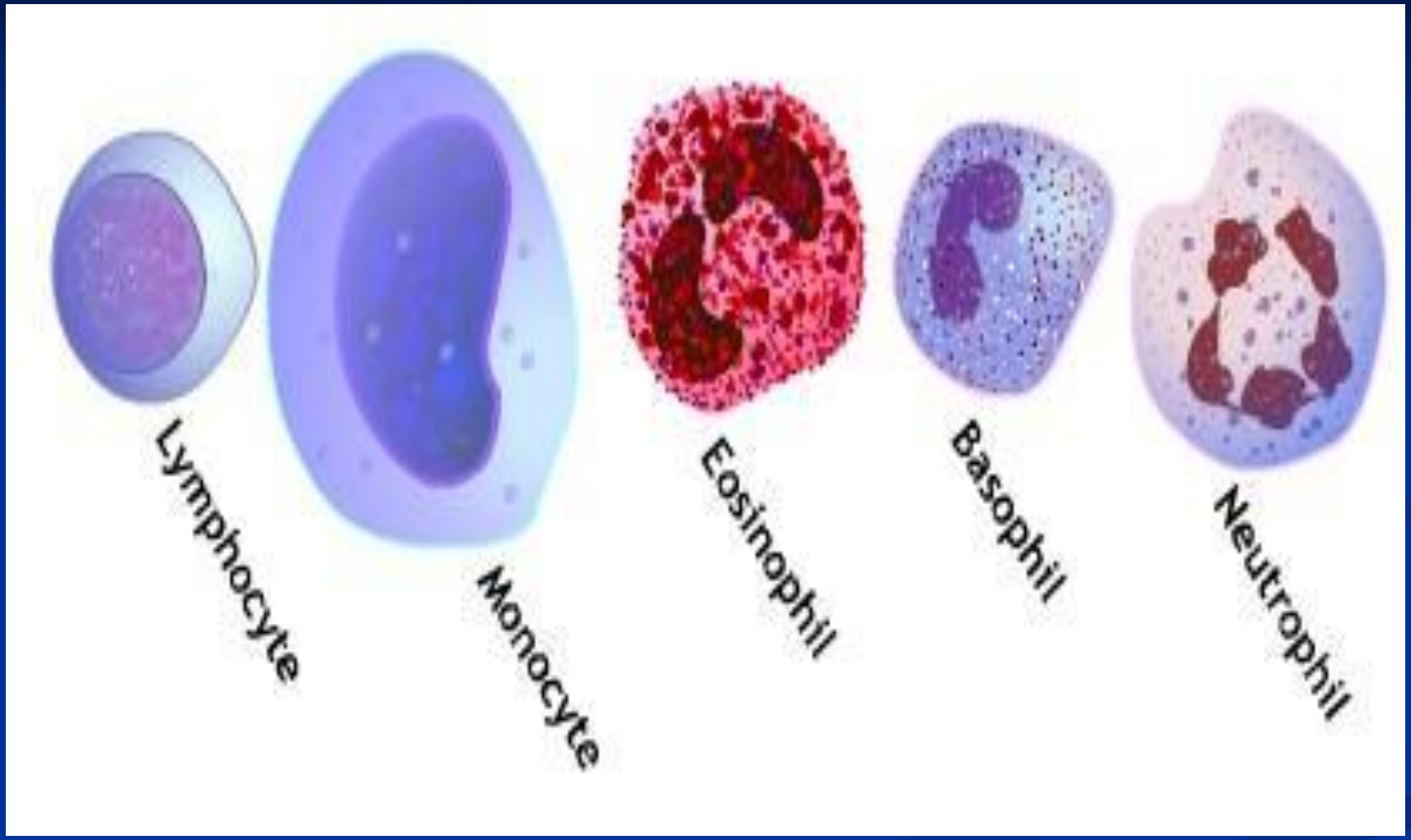
Examination of the stained smear

- Place the slide (smear side up) on the microscope stage.
- Examine the blood smear using the low power (10 x) objective. Choose an area where there are plenty of WBCs. This area is usually located near the wedge-shaped end of the blood smear.
- Place a drop of immersion oil on the selected site and carefully change to the **oil immersion objective (100x)**
- Perform the differential cell count and at the same time examine the morphology of the WBCs.

Calculation

- Count each WBC seen and record on a differential cell counter until 100 WBCs have been counted. For instance if 25 of the 100 WBCs where lymphocytes, then the percentage of lymphocytes is 25%.
- **The normal range percentage of the different types of WBCs is as follows:**

- **Neutrophils 50-70%**
- **Eosinophils 1-3%**
- **Basophils 0.1%**
- **Monocytes 1-6%**
- **Lymphocytes 20-35%**


Lymphocyte
Monocyte
Eosinophil
Basophil
Neutrophil