Al Muthanna University College of Pharmacy

Differential W.B.C count

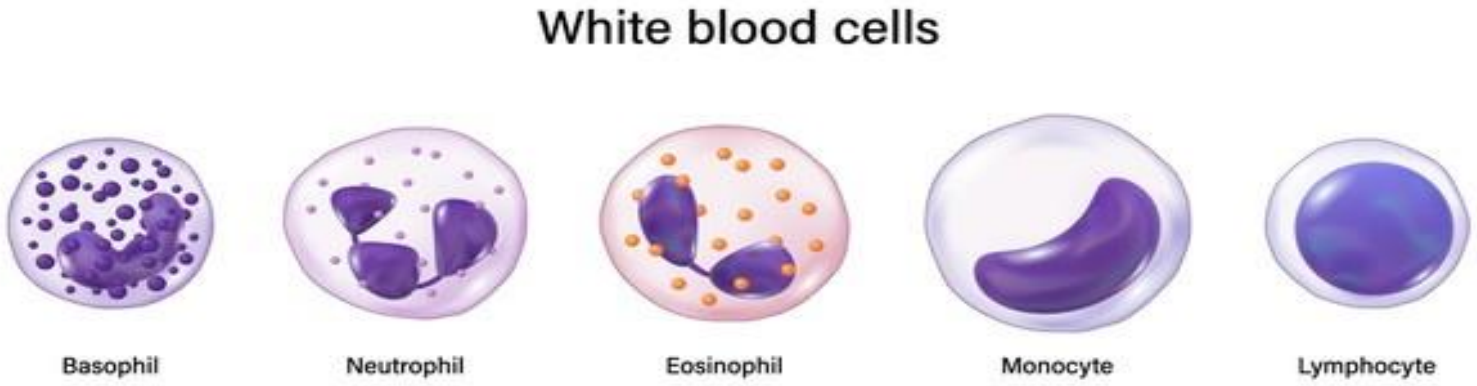
By
Dr. Sarah Zghair Hussein
Second Stage

Leucocytes

They are commonly known as white blood cells. They are larger than erythrocytes, contain a nucleus and do not contain haemoglobin. Normal leucocytes count various from 4000 to 11000 cells / C. mm of blood.

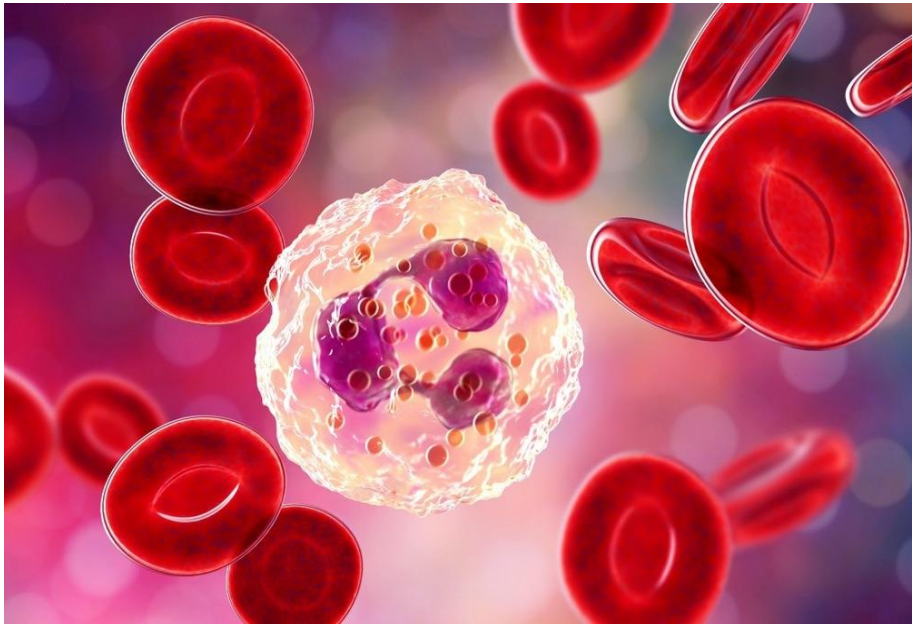
The leucocytes are classified into:

1. Granulocytes and
2. Agranulocytes.



Granulocytes:

1. Neutrophil: They constitute 60 – 70% of the total leucocyte count. They have a multilobed nucleus and the number of lobes usually range from 3 to 5, cytoplasm contains fine granules, which take neutral stain. Neutrophils exhibit phagocytosis and their count increases in acute infections. They form the first line of defense in the body. Increase neutrophil count during menstruation, pregnancy and muscular exercise, in acute infections like pneumonia, appendicitis, tonsillitis. Neutrophil count decreases in typhoid, malaria, aplastic anemia and under the influence of various drugs.



Neutrophilia: is an increase in neutrophils.

Common Causes of Neutrophilic Leukocytosis:

1. Acute pyogenic infection such as tonsillitis, appendicitis, pneumonia.
2. Tissue necrosis as in myocardial infarction .
3. Following hemorrhage.
4. Trauma, postoperative burns
5. Hemolysis
6. Malignant neoplasm, Myeloproliferative disorders (bone marrow hyperactivity diseases).
7. Metabolic disorders like gout, diabetic acidosis, uremia.
8. Drugs such as glucocorticoids, adrenaline, digitalis, phenacetin
9. Poisoning with lead, mercury, insect venom.
10. Physiological: Exercise, stress, after meals, pregnancy and parturition, nervousness.
11. Inflammatory disorders (non-infectious) such as rheumatic fever, rheumatoid arthritis.

Q/ What are the following:

1.Glucocorticoids

2.Adrenaline

3.Digitalis

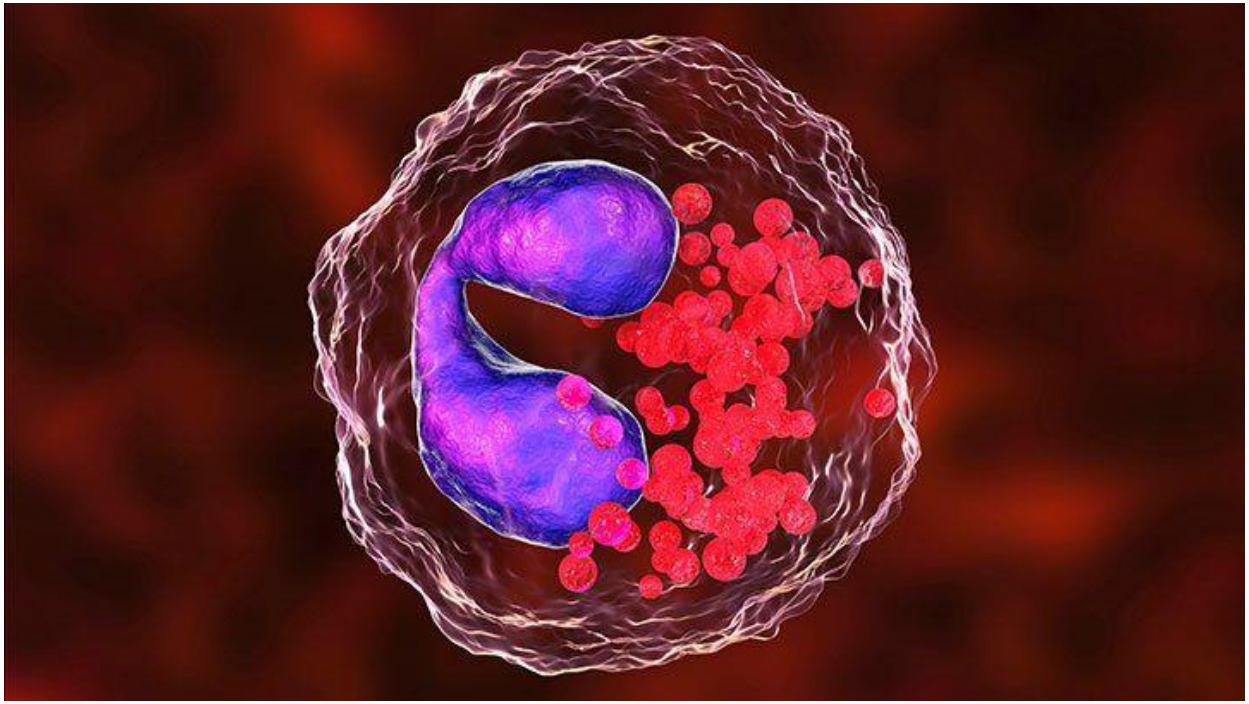
4.Phenacetin

Neutropenia: is decrease in the number of neutrophils.

Causes:

1. Typhoid and paratyphoid fever.
2. Viral infection as measles, influenza.
3. Depression of bone marrow due to irradiation, bone marrow diseases and chemotherapy.
4. Drugs such as chloramphenicol.
5. Autoimmune diseases. Increased destruction of neutrophils can be due to the body's immune system targeting neutrophils for destruction. This may be related to having autoimmune diseases, such as systemic lupus erythematous. It can be also due to large spleen (hypersplenism).
6. Nutritional such as anorexia nervosa and in starvation.

2. Eosinophil: It has usually a bilobed nucleus and shows the presence of relatively large granules, which take an acidophilic or orange stain. Normal eosinophil count ranges from 2 to 5%. It has been observed that in allergic conditions eosinophil count increases (eosinophilia), the decrease in eosinophil count constitutes eosinopenia and can be seen in acute pyogenic infections or steroid therapy.



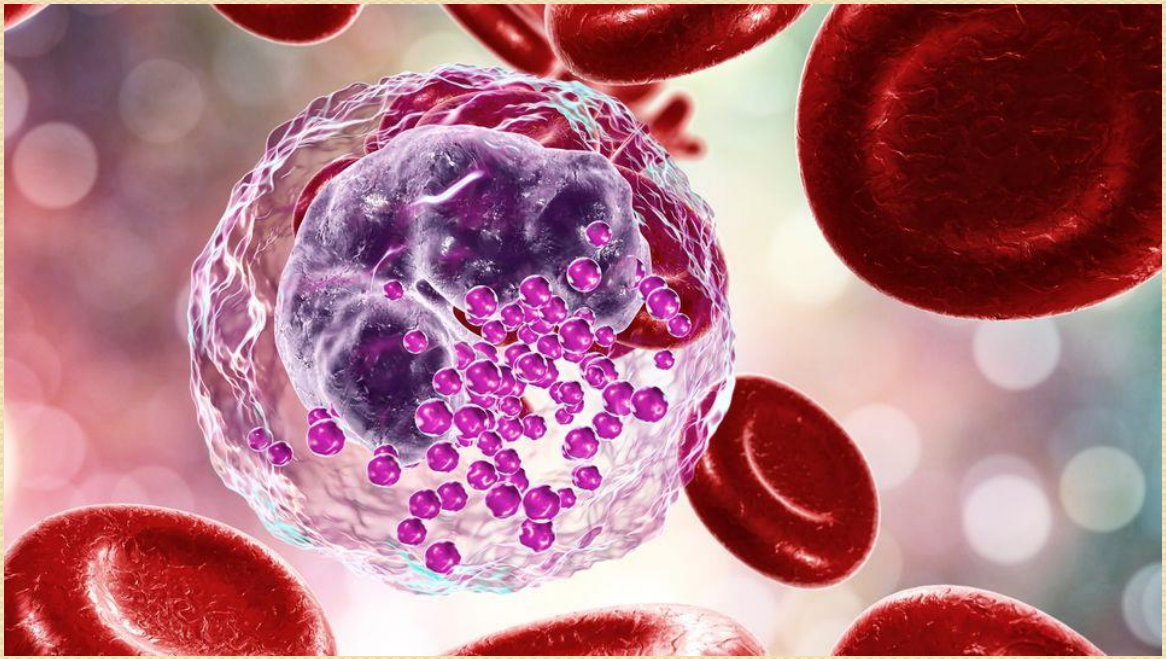
Causes of Eosinophilic Leukocytosis (increase eosinophil):

1. Allergic conditions like asthma, hay fever, urticarial, eczema.
2. Parasitic infestations—such as trichinosis, schistosomiasis, hookworm infestation.
3. Tropical eosinophilia.
4. Chronic myeloid leukemia.
5. Hodgkin's disease.
6. Other disease states: Such as scarlet fever, acute rheumatic fever.

3. Basophil: The nucleus in these cells is usually bilobed and cytoplasm contains blue granules. Their count ranges from 0 1o 1%. Basophil releases heparin, which is an 10 anticoagulant and it also releases histamine and this occurs specially in hypersensitivity reactions such as anaphylactic shock, the basophil count increases in polycythemia and chronic myeloid leukemia.

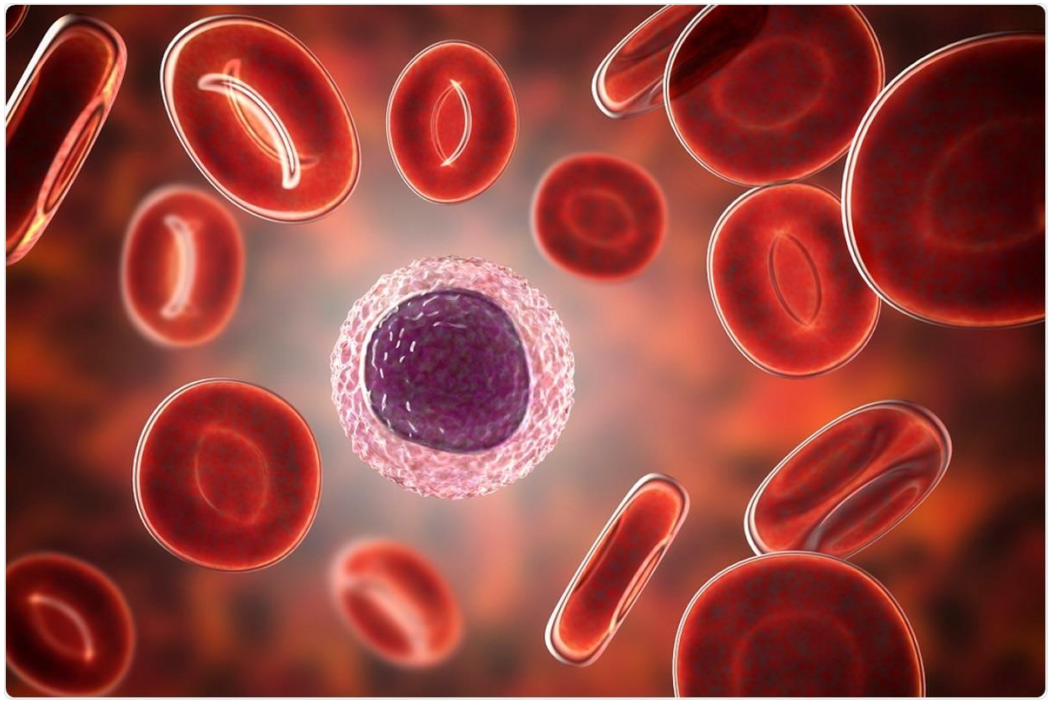
Causes of basophilia :

1. Myeloproliferative disease and hemolytic anemia.
2. Allergic reactions.
3. Smallpox.



Agranulocytes:

1. **Lymphocyte:** The cell has a large oval or round nucleus and there is a thin strip of clear nongranular cytoplasm between the nucleus and cell membrane. The lymphocytes are responsible for providing immunity to the body. There are two functionally distinct types namely T lymphocytes and B lymphocytes, they are involved in immune and defense mechanisms.



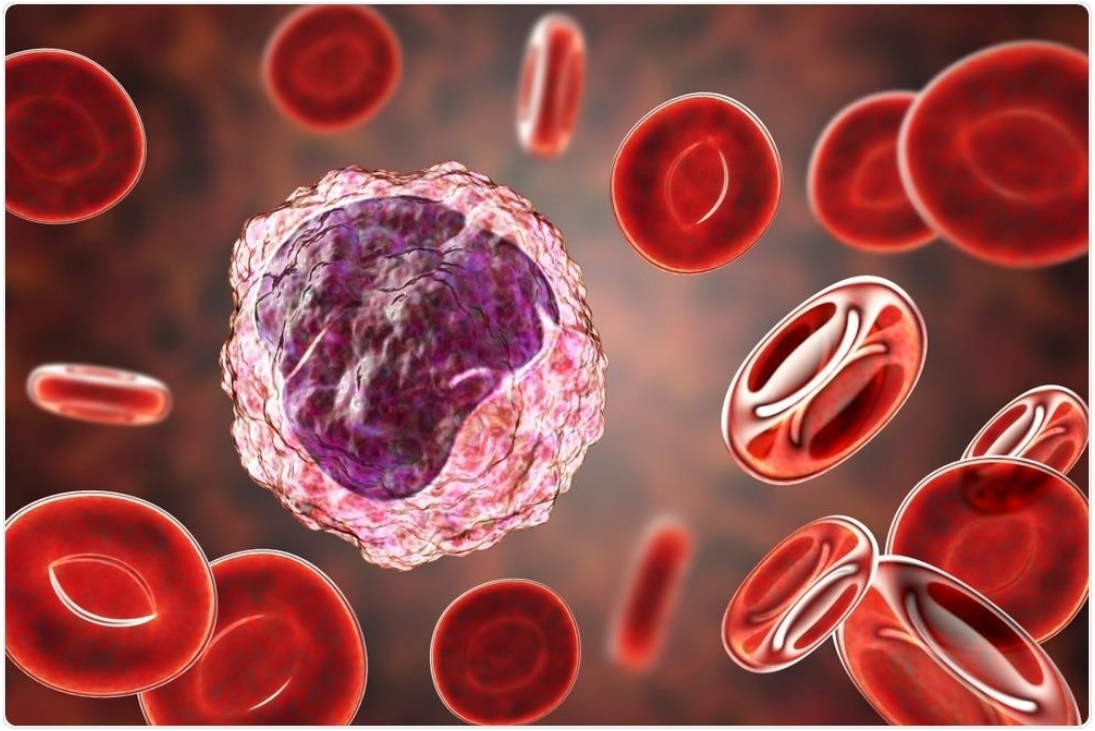
Causes of Lymphocytosis (increased number/percentage of lymphocytes):

a. Specific acute bacterial infection such as Whooping cough.
b. Specific chronic infections like TB, syphilis and malaria.
c. Specific protozoal infections, such as toxoplasmosis.
d. Viral infection like chicken pox.
e. Lymphocytic leukemia.

Causes of lymphopenia (decreased number of lymphocytes):

1. Steroid therapy.
2. Radiation (lymphocytes are most sensitive to whole body irradiation).
3. Characteristic of AIDS.
4. Recent temporary infection such as common cold.

2. **Monocyte:** They are the largest white cell (15 microns) and have a kidney shaped nucleus. The normal monocyte count is 3 to 8%. Monocytes are phagocytic in function and they are the second line of defense in the body.



The life span of white cells is very short and ranges from a few hours to 2 -3 days.

The age of a neutrophil can be determined by the number of lobes present in its nucleus.

The normal range percentage of the different types of WBCs is as follows:

Neutrophils	50-70%
Eosinophils	1-4%
Basophils	0.4%
Monocytes	2-8%
Lymphocytes	20-40%

Functions of Leucocytes:

1. **Phagocytosis**: It is a process by which leucocytes engulf bacteria and foreign material.
2. **Antiallergic effect**: histamine release during allergic conditions is inhibited by eosinophil, their count increases in allergy.
3. **Heparin production**: Basophils produce heparin, which prevents intravascular clotting.

What is the difference between Leucocytosis and Leukemia?

Differential WBC count

Introduction and principle:

The differential WBC count is performed to determine the relative number of each type of WBC in 1 liter of whole blood. **In a normal healthy individual** WBC range 4.5 - 11.0 x 10^3/ c. mm this count varies with age. **In disease states** a particular white blood cell type shows increase in number in the blood.

Objectives: To do total WBC count of a provided sample.

Methods:

1. Manual method.
2. Electronic cell counting (coulter counter)

Manual differential white cell count material and instruments:

1. Whole blood using EDTA as anticoagulant or capillary blood drawn from a finger or toe puncture.
2. Glass slide
3. Microscope
4. Alcohol 70%
5. Lancet
6. Leishman's stain
7. Immersion oil

Leishman's stain composition:

Leishman's stain: It is probably one of the simplest and most precise methods of staining blood for diagnostic purposes.

It is consist of:

a. **Eosin**: an acidic dye (negatively charged) and stains basic (positive) particles—granules of eosinophils, and RBCs a pink color.
b. **Methylene blue**: a basic dye (positively charged) and stains acidic (negatively charged) granules in the cytoplasm, nuclei of leukocytes, especially the granules of basophils, a blue-violet color.
c. **Acetone-free methyl alcohol**: preserves the morphology and chemical status of the cells and to fix the smear to the slide. Acetone being a strong lipid solvent it tends to damage the cell membrane.

- The stain is prepared by dissolving 750 mg of Leishman's powder in 500 mL of acetone-free methyl alcohol.

We add oil immersion for blood smear's slide that contain when examine under microscope, why?

Mechanism of Leishman's stain?

Prepare the blood smear:

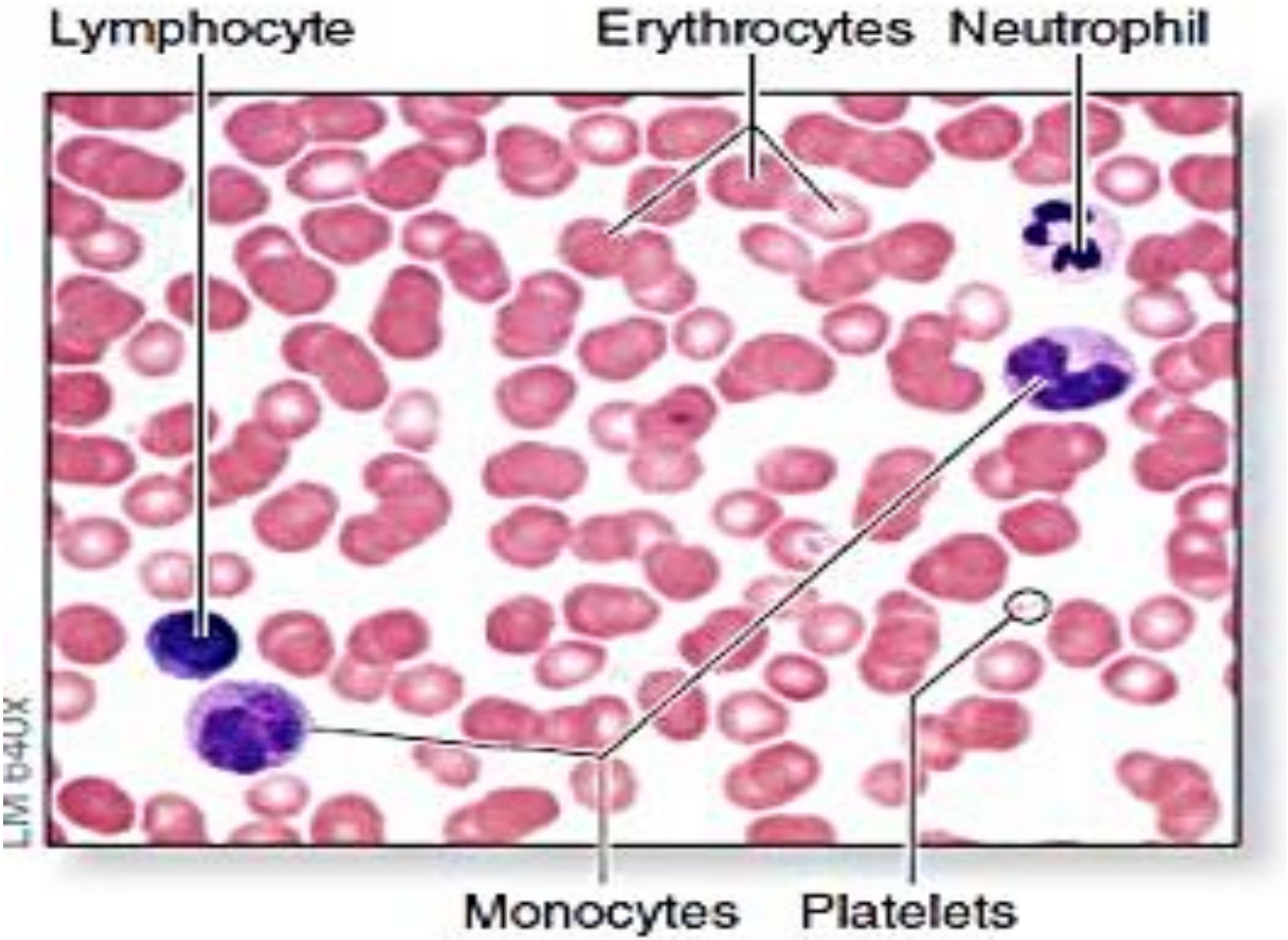
a. Clean two slide, one to be covered with the blood film and one to be used as spreader.
b. Clean the finger with alcohol, allow it to dry and then prick it with a disposable lancet to obtain a drop of blood.
c. Make a fine touch of one end of a slide with the drop of blood (only a small amount is required).
d. 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˚.
e. Draw the spreader back until it makes contact with the drop of blood.
f. Push the spreader slowly and smoothly to the other end of the slide in one motion.
g. Allow the film to dry at room temperature i.e. the blood smears should be air-dried.
h. They should be also labeled immediately with the student's name and the date at the end of slide.

Staining the blood smear by:

a. Put the dried slide on a staining rack.
b. The blood smear should be stained as soon as possible certainly within 1 to 2 hours.
c. Carefully drop Leishman's stain onto the blood film until the film is covered.
d. Allow the stain to act for one to two minutes.
e. Add distilled water to the stain.
f. The diluted stain should act for 15-30 minutes.
g. Then wash it off with distilled water, continue washing until the film has a pink color.
h. Shake off excess water and allow it to dry at room temperature.

Examination of the stained smear:

- For examining the blood smear a microscope with a low-power objective (10 x) and an oil immersion objective (100 x) is necessary.
- Place the slide (smear side up) on the microscope stage.
- Examine the blood swear 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 blood smear.
- Place a drop of immersion oil on the selected site and carefully change to the oil immersion objective (100 x).
- Perform the differential cell count and, at the same time examine the morphology of the WBCs.


Lymphocyte
Erythrocytes
Neutrophil
LM b4UX
Monocytes
Platelets


Thank you!