University of Diyala/ College of Medicine
Department of Physiology
Physiology Lab

Determination of the Differential WBC (Leukocyte) COUNT

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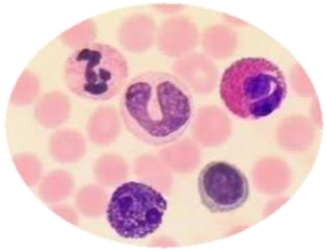


Outlines

- Aims of the Experiment
- Introduction, Principle, and Methods
- Procedure
- Calculations
- Some Medical Considerations

Aims of DWBC Experiment

- Describe the morphological characteristics of each WBC and identify the different blood cells in the blood smear.
- Differentiate between neutrophils, eosinophils, basophils, monocytes, and lymphocytes.
- Describe the functions of each WBC and list the conditions in which their count increases and decreases.
- Learn how to get the % of each WBC type using the manual method.

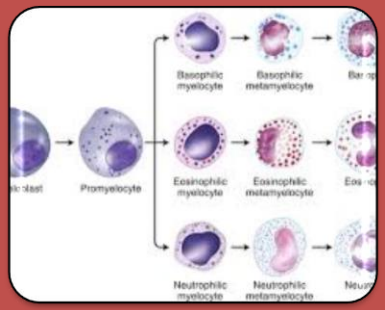
Introduction

- There are two types of leukocytes, **granulocytic** and **agranulocytic**. The granulocytic type is divided into three groups : ***Neutrophils***, ***Eosinophils***, and ***Basophils***. The agranulocytic type is divided into two groups: ***Monocytes*** and ***Lymphocytes***. All WBCs contain nucleus (multilobular in granulocytic and monolobular in agranulocytic).

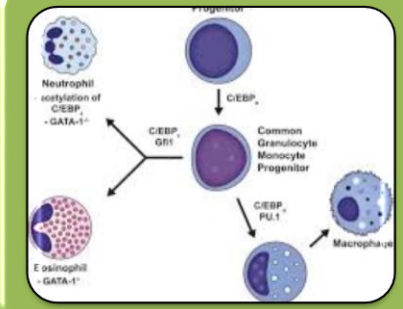
- In the differential leucocyte count the percentage of each type of WBCs in the total leucocyte population is determined. Each type of white cells, performs a different function in the battle against infections and each type of infection yields a different white cell picture in the blood. The morphology and staining characteristics of each type is peculiar and is responsible for the specific typing.

- The ***age of the neutrophils can be determined by the number of lobes of its nucleus***. The cells having less number of lobes are younger whilst those with more lobes are relatively older. The maximum number of lobes is five in general.

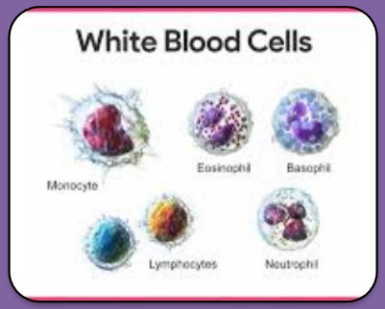
WBCs Maturation



The bone marrow is one of the largest organs in the body and it is also one of the most active. In the bone marrow there are multipotent uncommitted stem cells from which all the cells in the circulating blood are derived.



The uncommitted stem cells have two properties: first; an ability of cell division to give rise to new stem cells (self-renewal). Second; an ability to differentiate into committed stem cells which differentiate into the various differential cell types found in bone marrow and blood.



It appears that the uncommitted stem cells develop into separate pools of committed stem cells for megakaryocytes, erythrocytes, and lymphocytes, whereas the granulocytes (neutrophils, eosinophils, and basophils) in addition to the monocytes arise from the same committed stem cells. The figure below demonstrates the maturation of different types of WBC.

WBCs Maturation

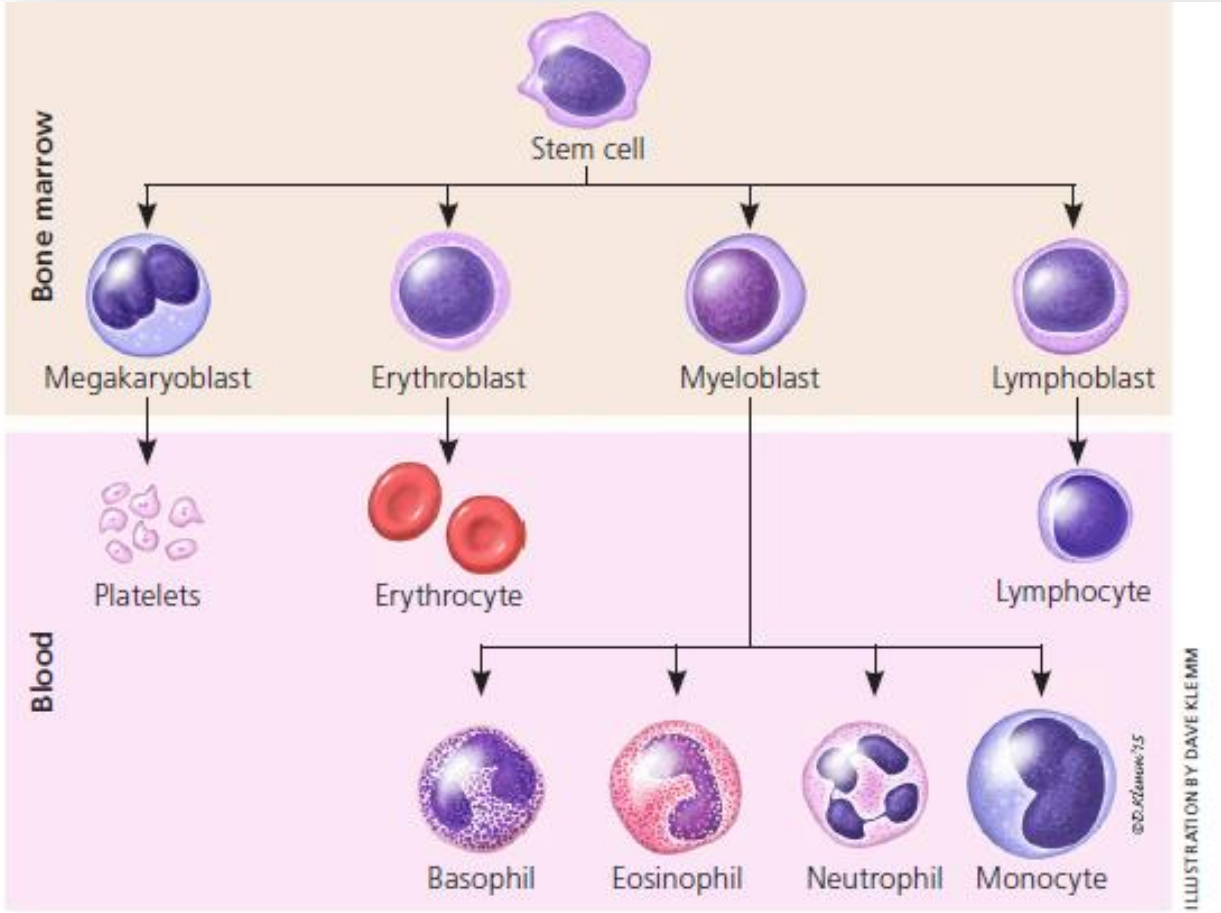
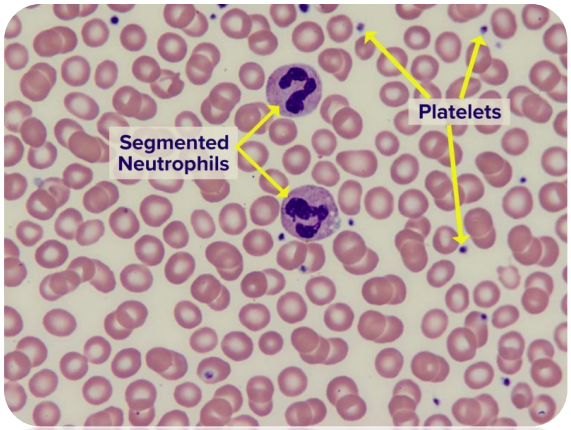


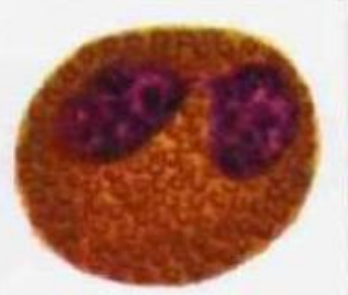
Figure 1. White blood cell maturation. The life cycle of leukocytes includes development and differentiation, storage in bone marrow, margination within vascular spaces, and migration to tissues.

Recognition of WBC Type: Neutrophils (Microphages)

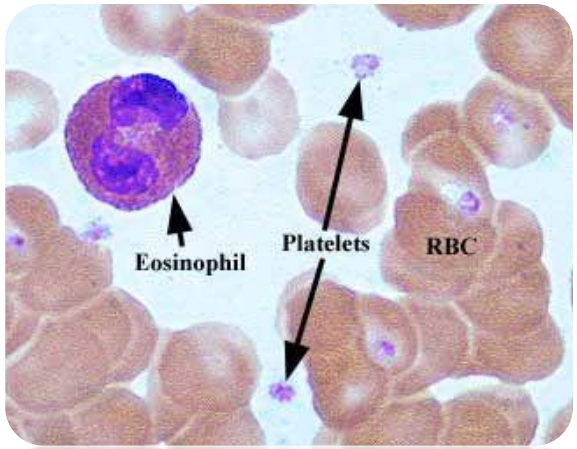
- ✓ They are average in size and the first cells to migrate to the site of infection. They can engulf 5-25 bacteria.
- ✓ Multilobulated nucleus (3-5lobes).
- ✓ Small granules (usually pinkish red granules) in the cytoplasm that respond to both acid and basic stains.
- ✓ Abundant cytoplasm.
- ✓ Their plasma half-life time is around 6 hr after which they can migrate to tissue.
- ✓ Normal %: 50-70 of total WBCs.
- ✓ Absolute count per µl : 2000- 7500.



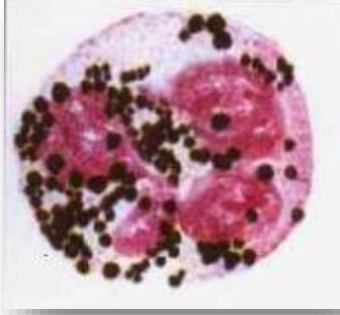
Recognition of WBC Type: Eosinophils



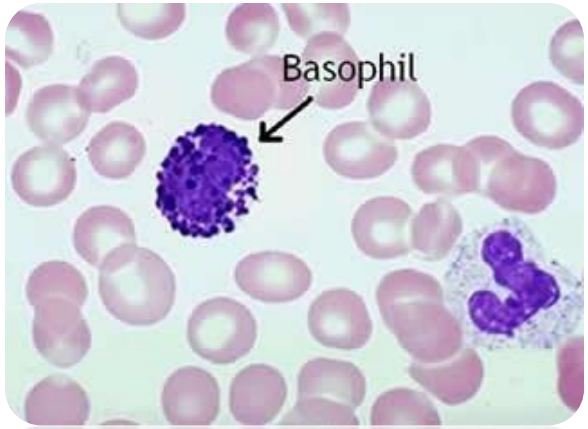
- ✓ They are large in size .
- ✓ Abundant cytoplasm that contains large red granules.
- ✓ Multilobulated nucleus.
- ✓ Remain in the blood for 8-12 hr period after which they migrate to tissue, they predominate in the tissue (one eosinophil in blood for 100 in tissue).
- ✓ The main function is detoxification of foreign proteins as in allergic conditions.
- ✓ Normal % : 1-4 of total WBCs.
- ✓ Absolute count per µl : 40-400.



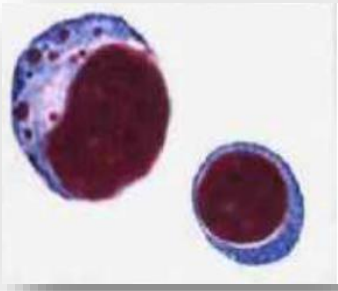
Recognition of WBC Type: Basophils



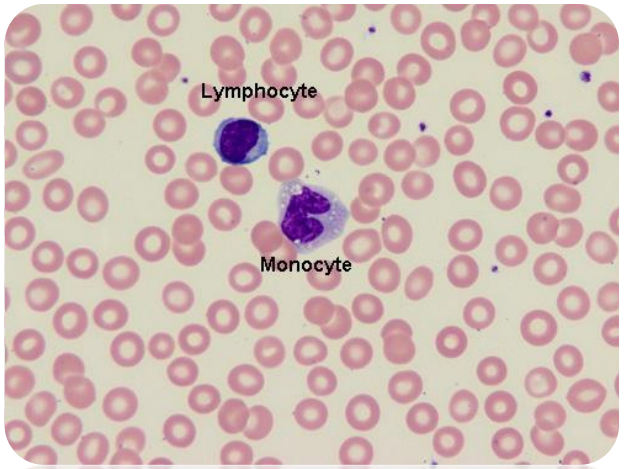
- ✓ They are average in size .
- ✓ Scanty cytoplasm that contains large deep blue granules.
- ✓ Lobulated nucleus.
- ✓ Remain in the blood few hours then leave to the tissue.
- ✓ They contain histamine and contain receptors for immunoglobulin E IgE: Basophils+IgE → histamine release → hypersensitivity. They also contain heparin (anticoagulant), thus they increase in the healing process to maintain open vessels promoting healing.
- ✓ Normal % : 0.4.
- ✓ Absolute count per µl : 10-100.



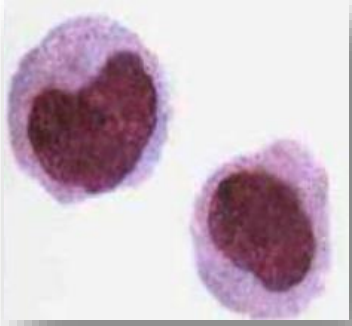
Recognition of WBC Type: Lymphocytes



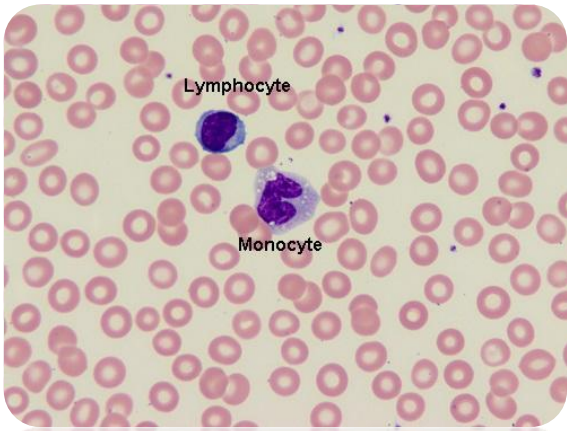
- ✓ They are variable in size (small or large) .
- ✓ Scanty cytoplasm pushed to the periphery by a large round nucleus.
- ✓ Absence of granules.
- ✓ Play a major role in chronic infections as in TB.
- ✓ Normal % : 20-40.
- ✓ Absolute count per µl : 1500-4000.



Recognition of WBC Type: Monocytes(Macrophages)



- ✓ Irregular large size.
- ✓ Abundant cytoplasm.
- ✓ Absence of granules.
- ✓ Kidney or round shape nucleus.
- ✓ As neutrophils, they are phagocytic and also contain peroxidase enzymes.
- ✓ They remain in the blood for 24 hr after which they migrate to the tissue (tissue macrophages).
- ✓ They form the second line of defense against infection(after neutrophils).
- ✓ Each monocyte can engulf 100 bacteria , that's why we call them macrophages.
- ✓ Normal % : 2-10
- ✓ Absolute count per µl : 200-800.



Principle and Methods

Principle:

The test depends on making a blood film from peripheral blood and staining it with a Lishman stain. Different types of WBCs are differentiated by their morphological and staining characteristics.

Methods:

1.Manual method (***the one we are going to do in our lab***).

2.Electronic cell counter (automated method).

Materials and Instruments

1.Whole blood using EDTA as anticoagulant or capillary blood drawn from a finger or toe puncture.

2.Glass slides

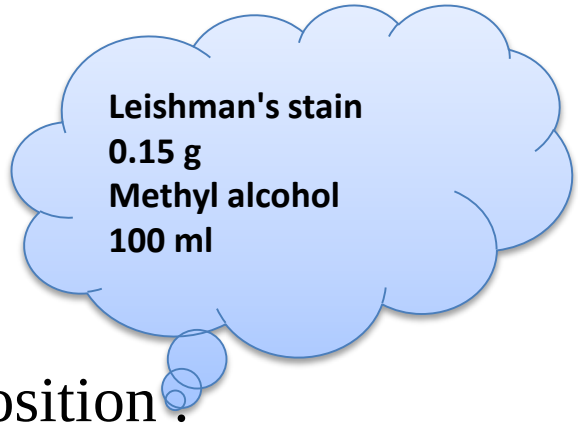
3.Microscope

4.Alcohol 70%

5.Lancet

6.Leishman's stain – it's composition :

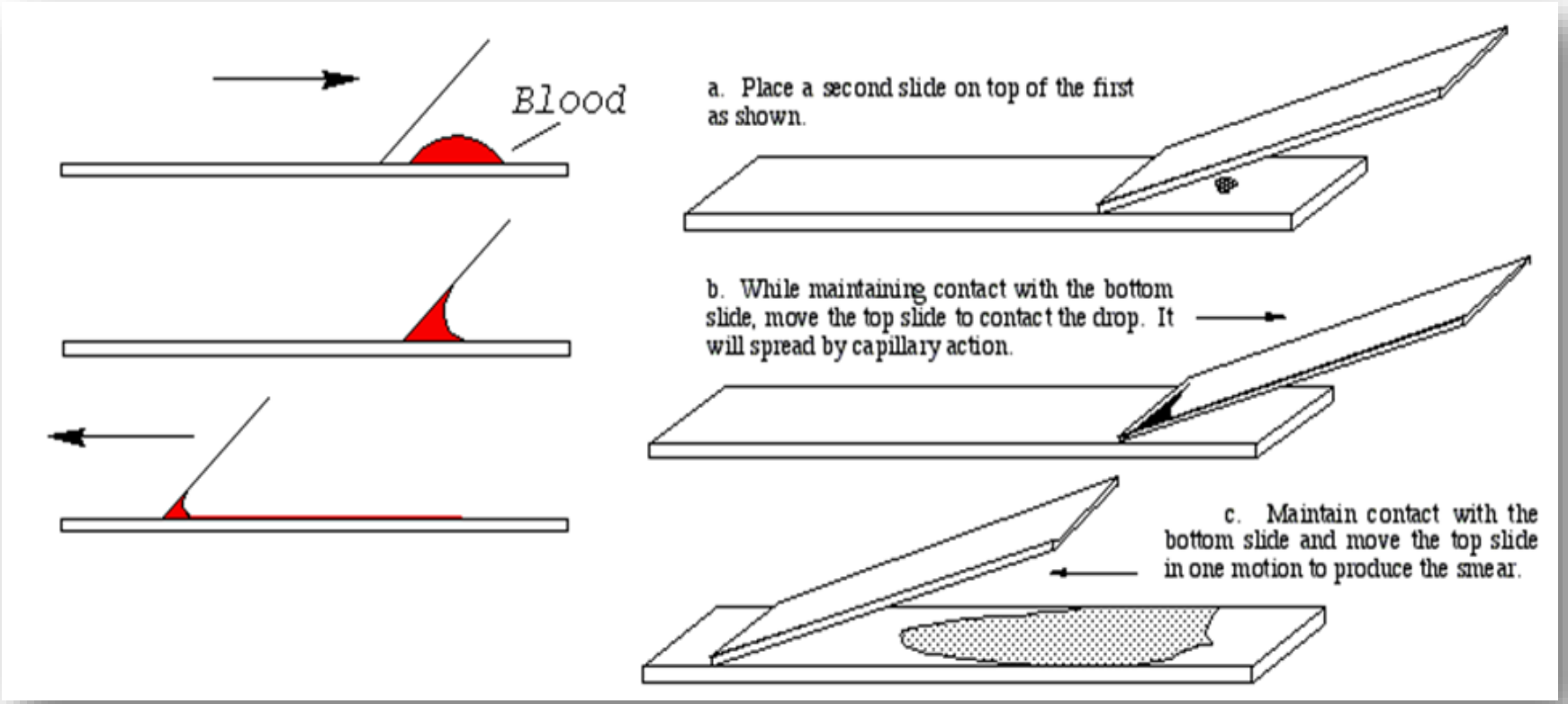
7.Immersion oil.



Titrate Leishman's stain with 100 ml methyl alcohol (acetone free)

Procedure: Blood Smear

- Made on an ordinary thin slide. Two clean slides are used , one to be covered with the blood film and one to be used as spreader.



Procedure: Blood Smear

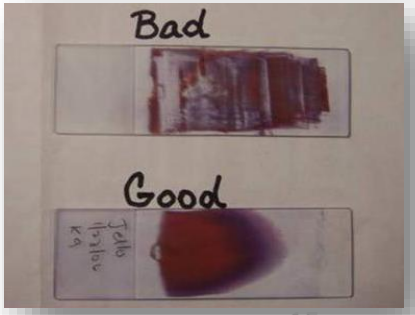
• Slides must be spotlessly clean. Hold them from the edges and never put your fingers on the surface of the slide. Clean the finger with alcohol, allow it to dry and then prick it with a disposable lancet to obtain a drop of blood.

• 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 slowly and smoothly to the other end of the slide in one motion.

• Allow the film to dry at room temperature i.e. the blood smears should be air-dried

• They should be also labeled immediately with the student's name and the date at the end of slide. ***A satisfactory film is very evenly distributed. The red cells must be close together not overlapped (you can check this under the microscope).***



Procedure: Staining the Blood Smear

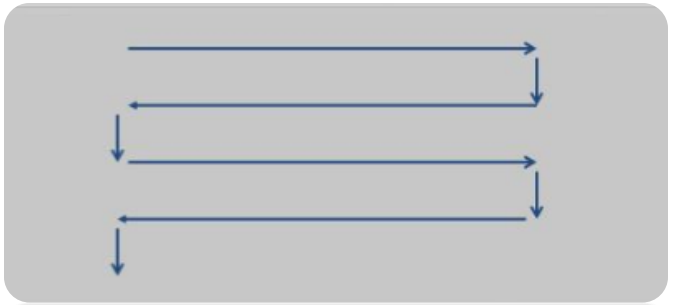
- Do not stain a film until you have a satisfactory one. Put the dried slide on a staining rack.
- The blood smear should be stained as soon as possible certainly within 1 to 2 hours.
- Carefully drop Leishman's stain on the blood film until the film is covered.
- Allow the stain to act for one to two minutes.
- Add distilled water to the stain, this gives dilution of 1:1 or 1:2. Water should added carefully to prevent the stain from overflowing.
- The diluted stain should act for 15-30 minutes.
- Then wash it off with distilled water, continue washing until the film has a pink color.
- Shake off excess water and allow it to dry at room temperature.

Procedure: Examination of the Stained Smear

- For examining the blood smear, a microscope with a low-power objective (10x) and an oil immersion objective (100x) is necessary.
- Place the slide (smear side up) on the microscope stage.
- Examine the blood smear using the low power (10x) objective.
- Choose an area where there are plenty of WBCs.
- 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 are lymphocytes, then the percentage of lymphocytes is 25% & so on.
- ✓ The direction of DWBCs calculation is as in this figure:



- ✓ This sheet can be used to help you getting the number of each WBC type.

N	N	M	N	N	N	N	E	L	N
N	N	N	L	N	N				

Neutrophil = %
Eosinophil = %
Basophil= %
lymphocyte = %
Monocyte = %

Some Medical Implications

Neutrophilia

Neutrophilia ***is an increase in neutrophils count. This is seen in any acute insult to the body (both infectious and non-infectious), the common causes are:***

1. Infections (especially) bacterial.
2. Inflammatory disorders (non-infectious) such as rheumatic fever, rheumatoid arthritis.
3. Pregnancy, exercise and nervousness due to movement of marginated neutrophils to enter the blood stream.
4. Myeloproliferative disorders.

Neutropenia

Neutropenia ***is a fall in neutrophils count , the common causes are:***

1. Problem in the production of neutrophils in the bone marrow which may be congenital, bone marrow failure, radiation, and chemotherapy.
2. Increased destruction of neutrophils which 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 erythmatosus It can be also due to large spleen (hypersplenism).
3. Nutritional such as anorexia nervosa (starvation).
4. Infections as in bacterial infection such as typhoid fever, or viral infections such as measles, influenza or infectious hepatitis.

Eosinophilia and Eosinopenia

Eosinophilia ***is an increase in the absolute eosinophils count. Causes of Eosinophilic Leukocytosis include:***

1. Allergies (most common).
2. parasites (second most common cause).
3. Other disease states: Such as scarlet fever, acute rheumatic fever, irradiation, polyarteritis nodosa, RA, sarcoidosis, and tuberculosis.

Eosinopenia (*low eosinophils count*) may be produced by an elevated secretion of corticosteroids (in states of sever stress), shock, trauma, surgery, and major pyogenic infection.

Basophilia and Basopenia

Basophilia ***is an increase in the absolute basophils count. Causes of basophilia include:***

1. Myeloproliferative disease and hemolytic anemia.
2. Allergic reactions
3. Chicken pox
4. Ulcerative colitis
5. Myxedema
6. Chronic hemolytic anemia
7. Hodgkin s disease
8. Post splenectomy
9. Drugs :estrogens ,antithyroid medication

Basopenia *:A rapid decrease in basophils is associated with an anaphylactic reaction.*

Monocytosis

Monocytosis ***is an increase in absolute monocytes count. Causes of monocytosis include:***

1. Recovery phase of acute infection

2. Chronic inflammatory disorders such as tuberculosis, malaria, syphilis, brucellosis, and Chrohn's disease .

3. Hematologic neoplasms.

4.drugs:carbon,disulfide poisoning &phosphorus.

Lymphocyte

Lymphoctosis *is an increase in absolute lymphocytes count. It is of two types:*

Relative

- Total number of circulating lymphocytes unchanged but WBC count low due to neutropenia. Relative lymphocytosis is normal in infants and children (aged 4 months - 4 years).
- Causes include: age less than 2 years; acute viral infection, and splenomegaly with splenic sequestration of granulocytes.

Absolute

- Number of lymphocytes increases.
- Causes include: Lymphocytic leukemia (most common) , acute viral infections such as measles, rubella, and chicken pox, ***Lymphocytosis is a feature of infection, particularly in children,*** Some Bacterial infection such as Pertussis (whooping cough), and some protozoal infections, such as toxoplasmosis .

Lymphopenia

Causes of Lymphopenia (*decreased number of lymphocytes*) include:

1. Characteristic of AIDS.
2. Recent temporary infection, such as the common cold.
3. Radiation (lymphocytes most sensitive to whole-body irradiation).
4. Corticosteroid use.

Thank you