Differential Leukocyte Count:

The Types of Leukocytes:

1-Granulocyte cell:

a-Neutrophile: is a cell with acidophilic cytoplasm and fine pinkish red granules, the nucleus is usually lobulated (3-5) lobes, connected by thin chromatin filament.

- Percentage 65-70% from blood.
- Number 3000-6000 \( \text{mm}^3 \).
- Disease case: Neutrophilia; increase Neu. In blood ex: inflammation, intoxication.

b-Eosinophile: acidophilic cell, usually larger than the Neu., it is cytoplasm contain bright red granules or orange the nucleus consist usually 2 lobes or (bilobes).

- Per. 2-4% from blood.
- Num. 150-300 \( \text{mm}^3 \).
- Eosinophilia: ex: Allergy, Asthma.

c-Basophile: it is a small cell granules are black or blue in color and over cover most of the cell even the nucleus or (S-shaped).

- Per. 0-1% from blood
- Num. 0-100 \( \text{mm}^3 \)
- Bosphilia: ex: Wounds

2- A granulocyte cell:

a-Lymphocyte: it is also small cell, the nucleus cover most of the cells & it is round dark violet in color, the cytoplasm is usually blue in color with no granules.

- Per. 32-40% from blood.*
- Num. 1500-4000 \( \text{mm}^3 \).
- Lum. is divided into 2 types: T-cell (cytotoxic), B-cell (production of antibodies)
Disease Case:

- Lymphocytosis: Increase lymphocyte in blood ex: Brucellosis and Tuberculosis.
- Monocyte: is the largest mature leukocyte the nucleus is usually kidney shape or horse shape.
  - Per. 5-8% from blood.
  - Num. 300-600 x mm3.
  - Monocytosis: ex: Malaria, Typhus.
  - Function of WBC: it is the first defense line of the body against bacterial infection.
  - The shape of Typical Slide: finger shape, thin smooth, not thick rapid spreader.
  - The evidence of staining: To show the nucleus of the cell.
  - The evidence of diluting: To fixed the stain.

Preparation and Examination of the Peripheral Blood Smear:

The preparation and examination of the peripheral blood smear is one of the most frequently requested tests in the hematology laboratory. Blood Smear must be prepared correctly and examined in such a way as to provide the physician with an accurate interpretation. There are 4 methods used to prepare blood smear:

1- Slide to Slide.
2- Coverslipe to Slide.
3- Coverslipe to Coverslipe.
4- Automated Spinner.

Blood smears are prepared with EDTA anticoagulant blood to minimize degenerative changes in the blood cells. To ensure good preservation of cellular morphology, differential smears should be made as soon as possible and no later than 3 hours after collection.
The procedure of slide to slide:
1- Place a drop of blood from the finger about 2mm in diameter in the central line of a slide about 1-2 cm from one end.
2- The spreader is placed at an angle of 40 degrees to the slide and then moved back to make contact with the drop.
3- The drop should spread out quickly along the line of contact of the spreader with the slide.
4- The moment this occurred the film should be spread by a rapid, smooth, forward, movement of spreader.
5- The drop should be of such size that the film is 3-4 cm in length.
6- The film should be dried rapidly. A good blood film preparation will be thick at the drop end and thin film at the opposite end. The thickness of the spread when pulling the smear is determined by the:
   A- angle of spreader slide (The greater the angle, The thicker and Shorter the smear).
   B- size of the blood drop.
   C- Speed of spreading (Slowly the thicker). While the thin smears are used for describing blood cell, the thick smear are used for detecting malarial parasites.

There are 3 stains that used in differential leukocyte count:*
   1- Wright stain.
   2- Leishman stain.
   3- Giemsas stain.

The composition of Giemsas stain:
Giemsa powder 0.3 gm
Glycerin 25 ml
Methyl alcohol 25 ml this makes stock solution and before use it has to be diluted by adding 1 ml (stain) to 9 ml of buffered water.
Method of staining:
1- The blood film is fixed with methyl alcohol for 2 minutes.
2- Pour Giemsa stain diluted 1:9 with buffer over the smear for 8-10 minutes.
3- Wash off with buffer and dry.

The Count:
The dry and stained film examined without a coverslipe under oil immersion objective. For differential leukocyte counts choose an area where the morphology of the cells is clearly visible. Do differential count by moving the slide in area including the central and peripheral and the smear. A total of 200 cells should be counted in which every white cell seen must be recorded in a table under the following heading: Neutrophile, Eosinophile, Basophile, Lymphocyte, and Monocyte then find the percentage of each type.