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| Aims:- to determine the percentage of each type of leukocytes in peripheral blood film. Certain types of leukocytes may increase or decrease during certain diseases, so that test is valuable for the diagnosis of these diseases. |

**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 away as to provide the physician with an accurate interpretation. There are four methods used to prepare blood smears:-

1) Slide-to-Slide

2) Coverslip- to- Slide

3) Coverslip-to-Coverslip

4) Automated Spinner

Blood smears are prepared with EDTA anticoagulated 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.

**Slide-to-Slide Method**

**Procedure**

1) place a drop of blood from the finger .about 2 mm 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 45 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 occurs the film should be spread by a rapid, smooth, forward movement of the 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 the 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 smears are used for detecting malarial parasites.

**Fixing of Blood Films**

Before staining, the blood films need to be fixed with acetone free methyl alcohol for up to 1 minute in order to prevent hemolysis when they come in contact with water when water has to be added subsequently. Alcohol denatures the proteins and hardens the cell contents.

**Stain preparation and staining**

**I. Wright's stain**

**Method of staining**

1- Cover the slide with stain for 1-2 minutes taking care that it do not dry on the slide.

2- Now dilute this with equal amount of buffer water.

3-Diluted stain is allowed to act for 3-5 minutes and then flooded off with buffer (or top water)

4- Shake excess buffer away and dry in the air

**II. Giemsa's stain**

**Composition**

Gimsa powder 0.3 gm

Glycerine 25 ml

Methyl alcohol 25 ml

This make stoke solution and befor use it will 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 minute
2. pour Gimsa stain diluited 1:9 with buffer over the smear for 8-10 minutes.
3. Wash off with buffer and dry.

**III. Leishman's stain**

**Composition**

Powdered Leishman's stain 0.15 gm

Methyl alcohol 133ml

**Method of staining**

Like that for Wright's stain but with double dilution of buffer.

1-pour few drops ( 8 drop) on the slide , wait for two minutes.

2-Add double amount (16 drops) of buffered water .mix by rocking and not by blowing and wait for 7-10 minutes.

3-The stain is floored off with distilled water and this should be complete in 2-3 seconds and allow it to dry.

**Examination of blood Film**

There are several necessary steps in the examination of a peripheral blood smear.

**Low -power (x10) Scan**

1) Determine the overall staining quality of the blood smear.

2) Determine if there is a "good distribution" of cells on the smear.

a) Scan the edges and center of the slide to be sure there are no clumps of red cells, white cells or platelets.

b) Scan the edges for abnormal cells

3) Find an optimal area for the detailed examination and enumeration of cells.

a) The red cells should not quite touch each other

b) There should not be areas containing large amounts of broken cells or precipitated stain.

c) The red cells should have a graduated central pallor.

**High - Power (x 40) Examination**

1) Determine the white cell estimate

2) The morphology of the WBCS should be evaluated and any abnormalities.

**Oil immersion (x100) Examination**

1-Perform white cell differential count, platelet estimate and evaluate platelet morphology.

2-Evaluate red cell anisocytosis, poikilocytosis, hypochromasia,

polychromasia and inclusions.

**The count**

The dry and stained film is examined without a cover slip 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 of 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 Neutrophil, Eosinophil, Basophil, Monocyte and Lymphocyte. Then find the percentage of each type.

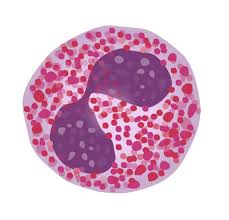
**RECOGNITION:-**

The following parameters can be used for identification of WBC:- 1- **Neutrophil**:- has following features:-



* Average size
* Abundant cytoplasm
* Small granules in the cytoplasm
* Multilobulated nucleus (3-5 lobes).
* Increase in number (neutrophilia) in acute pyogenic (bacterial) infection.
* It constitutes the largest proportion of leukocytes 55-75%.

1. **Eosinophil** have following characteristic:-

* Large size cell. 
* Abundant cytoplasm
* Large red granules in the cytoplasm
* Multilobulated nucleus (2 lobes)
* Increase in number (eosinophilia)

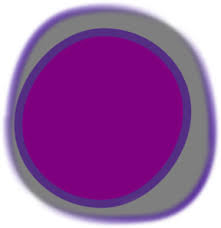
during allergic conditions and parasitic infestation.

* Its percentage for total WBC is 1-4%.

1. **Basophil**:-

* Averaged cell size. 
* Abundant cytoplasm
* Large deep blue granules in the cytoplasm
* Lobulated nucleus (S shaped nucleus)
* They increase in number (basophilia) produce heparin into the blood and take part in allergic reaction.
* Its percentage is 0-0.5%

1. **Lymphocyte**:-

* Variable size 
* Scanty cytoplasm pushed to the

periphery of the cell by large nucleus

* Absence of granules in the cytoplasm
* Large round nucleus
* It perform important role in immune system and increase in number (lymphocytosis) in chronic bacterial and viral infection.
* Its percentage is 20-40%

1. **Monocytes**:-

* Large cell size 
* Large irregular nucleus
* Abundant cytoplasm
* Absence of cytoplasmic granules
* Irregular kidney shaped nucleus
* They are scavenger cells and increase in number (monocytosis) to be transferred into tissues to be macrophages during final stages of inflammation to engulf dead tissue and bacteria.
* increase in number in chronic bacterial infection.
* Its percentage is 2-8%.