



Peripheral Blood Film The Differential Leukocyte Count (DLC)

Physiology Lab-10-

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Definition:

Blood film or peripheral blood smear is a thin layer of blood smeared on a slide and then stained in such a way to allow the various blood cells to be examined microscopically. A blood smear is a blood test that gives information about the number and shape of blood cells. The cell types are examined under a microscope to investigate hematological problems (disorders of the blood)

Goals:

- The student will prepare a blood smear which
- is even, smooth and have an acceptable
- feathered edge.
- To become familiar with the various types of
- white blood cells

Applications of Blood smear:

- to detect infection or inflammation, determine the effects of possible poisoning by chemicals, drugs, chemotherapy, radiation, etc.
- DLC is also done to monitor blood diseases like leukemia, or to detect allergic and parasitic infections.
- The determination of each type of WBC helps in diagnosing the condition because a particular type may show an increase or decrease.

Background

- There are three main cells within the blood that the test focuses on:

red cells: (which carry oxygen throughout the body)

white cells: (which function as part of the body's immune system)

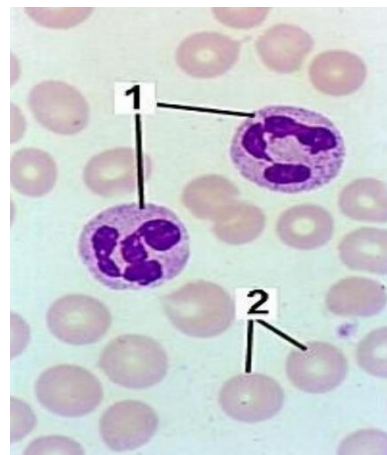
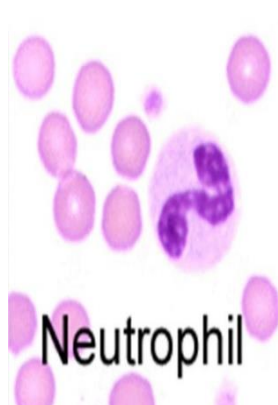
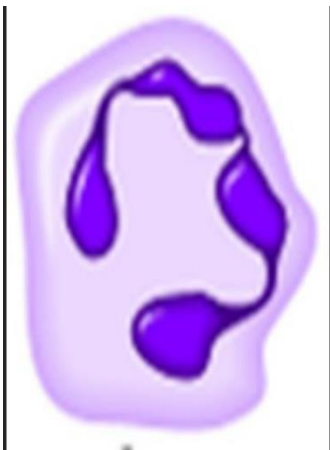
Platelets: (which are important for blood clotting).

Types Of WBCs

- **Granulocytes** are larger than RBCs and have **lobed nuclei** and **granules** in their cytoplasm.
- **Neutrophils**
- **Eosinophils**
- **Basophils**

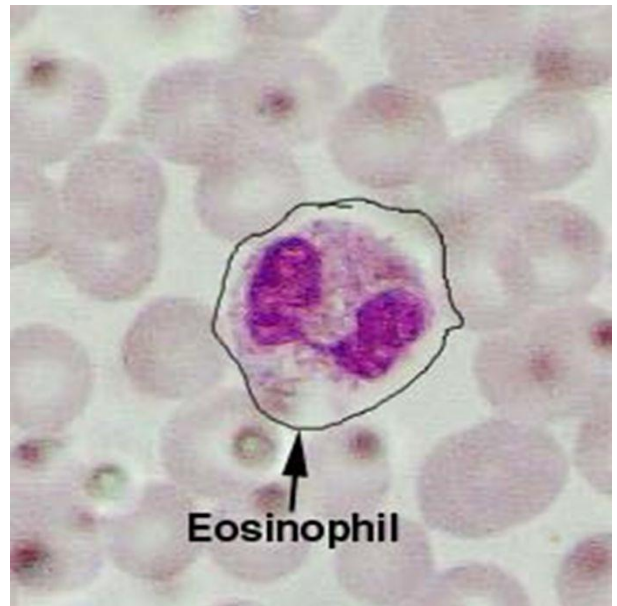
Granulocytes

- 1-**Neutrophils**: 40-70% of WBCs, 3-7 lobed nucleus, pale lilac or pink cytoplasm contains very fine granules which are difficult to see. They are active phagocytes and fight bacterial invasions (important in inflammatory response) as well as cleaning up debris



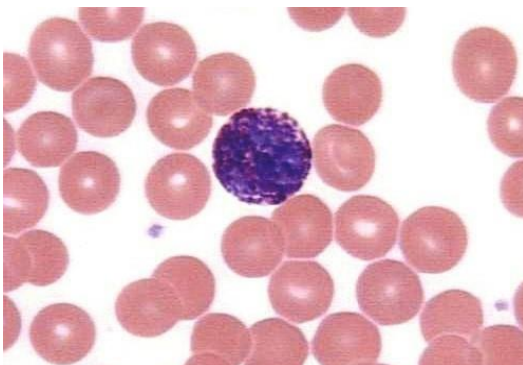
Granulocytes

2-**Eosinophils**: 1-4% of WBCs, bilobed nucleus, large red/orange cytoplasmic granules. Important in ending allergic reactions (phagocytize antibody-bound allergens) and fighting parasitic worms.



Granulocytes

- 3- **Basophils**: less than 1% of WBCs, nucleus often U or S shaped with indentations, large dark purple cytoplasmic granules. Mediate inflammatory response (release histamine and other molecules) during allergic responses and parasitic infections.

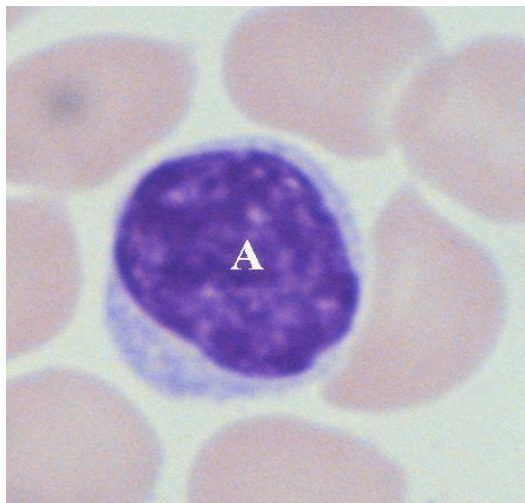


Types Of WBCs

- **Agranulocytes** have no observable granules and nuclei are usually roughly spherical.
- **Lymphocytes**
- **Monocytes**

Agranulocytes

- 1-**Lymphocytes**: 20-40% of WBCs, about the size of a RBC, dark blue or purple nucleus, sparse gray/blue cytoplasm. Important role in immune system.



Agranulocytes

- 2- **Monocytes**: 4-8% of WBCs, largest of the WBCs, dark blue nucleus, abundant gray/blue cytoplasm. They are active phagocytes and considered important in long-term clean up



Materials 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

Blood film is a blood test that gives information about the number and shape of blood cells

Procedure

MAKING A SMEAR

- One drop of blood on one end of slide
- Spreader slide is placed at 45° on the drop and moved along the slide
- It is moved smoothly and once such that the blood film is thin
- Care should be taken to prevent the formation of air bubbles
- Air dry the smear
- Make identification mark on one edge

How to stain a blood film ?

1- Staining with Leishman's (neutral) stain:

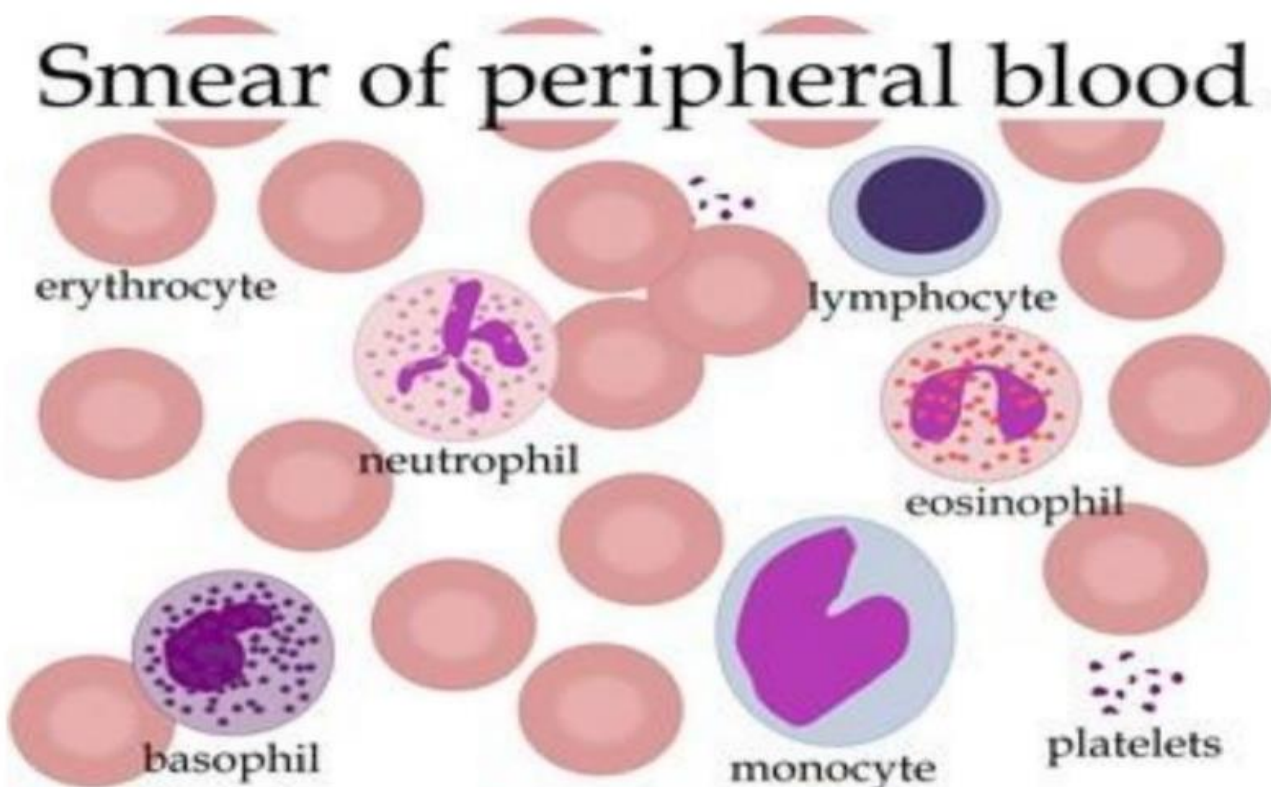
- It is formed of eosin & methylene blue dissolved in methyl alcohol as a fixative.

- The blood film is covered with drops of Leishman's stain for 5 minutes.

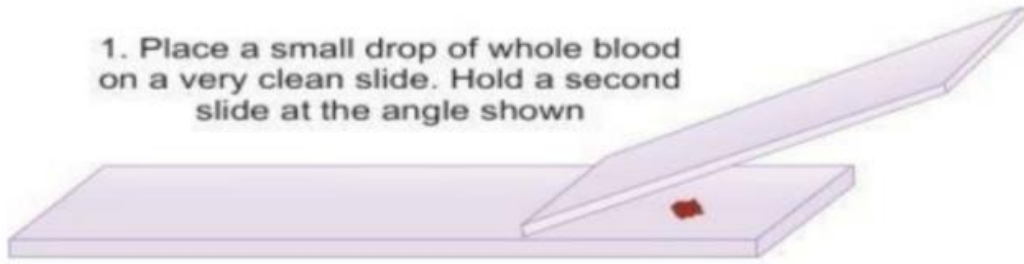
2- Then dilution of the stain with distilled water for 10 minutes.

3- Washing of the slide in tap water then left to dry in air.

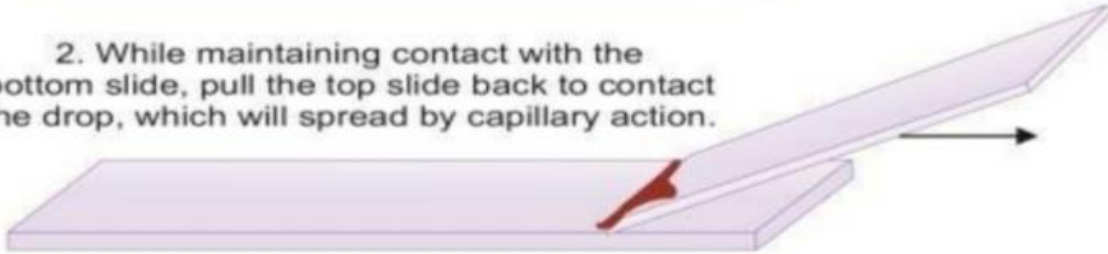
4- Then the film is examined by oil immersion lens.



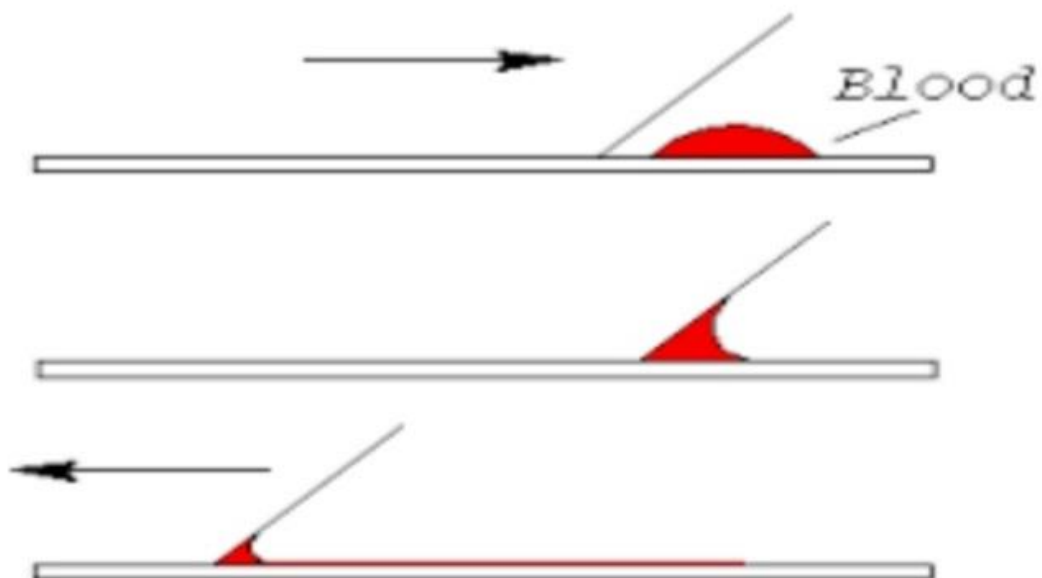
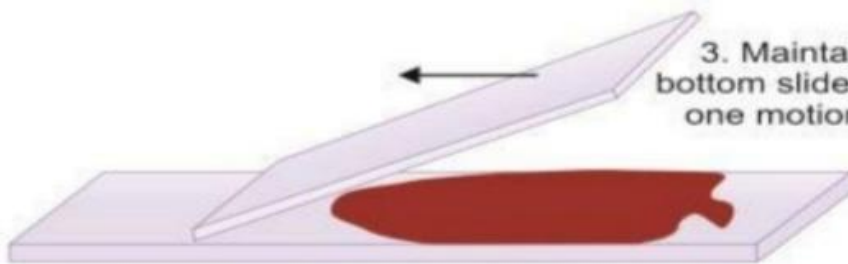
1. Place a small drop of whole blood on a very clean slide. Hold a second slide at the angle shown



2. While maintaining contact with the bottom slide, pull the top slide back to contact the drop, which will spread by capillary action.



3. Maintain firm contact with the bottom slide and push the top slide in one motion to produce the smear.



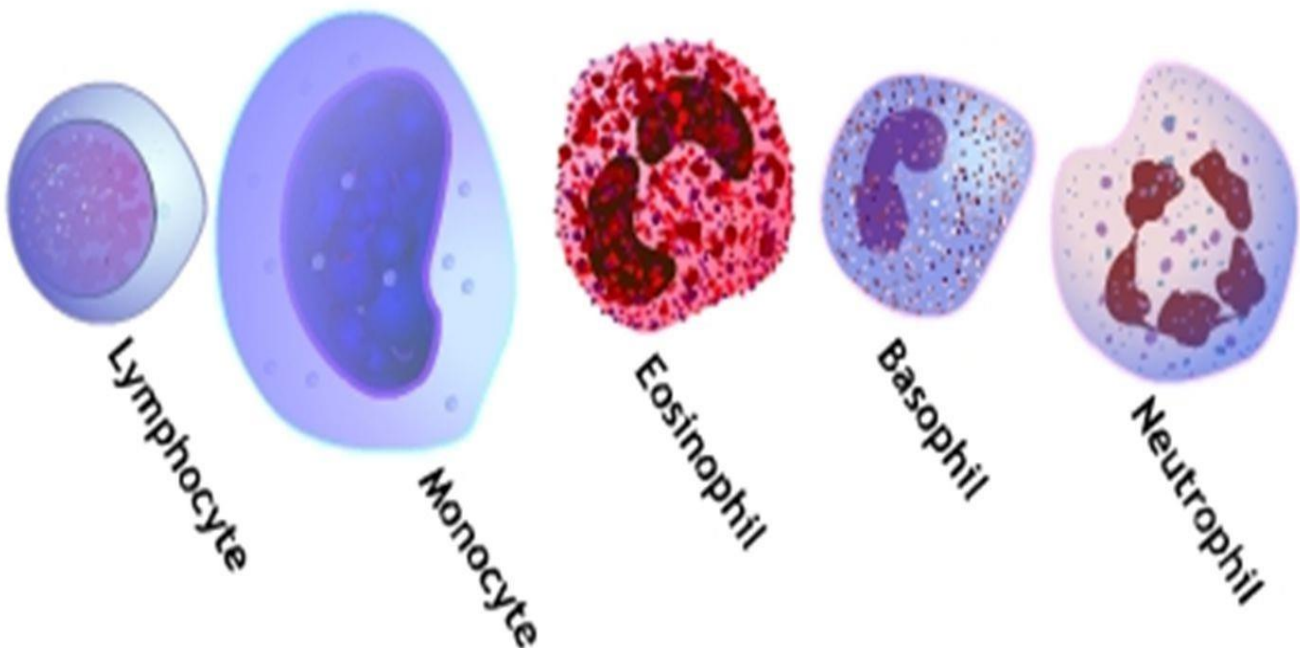
Identifying a Leukocyte

- . A leukocyte is identified from its size, its nucleus, and the cytoplasm—its color, whether vesicles (granules) are visible or not, their color and size if visible, and the cytoplasm/nucleus ratio.

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 were 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	1-4%
Basophils	0.4%
Monocytes	2-8%
Lymphocytes	20-40%



	High indicate	Low indicate
Neutrophils	bacterial infection, burns, stress, inflammation	radiation exposure, drug toxicity, vitamin B12 deficiency, systemic lupus erythematosus (SLE)
Eosinophils	allergic reactions, parasitic infections, autoimmune diseases	drug toxicity, stress
Basophils	allergic reactions, leukemias, cancers, hypothyroidism	pregnancy, ovulation, stress, hyperthyroidism
Lymphocytes	viral infections, some leukemias, Bacterial infections.	prolonged illness, radiation. AIDS, immunosuppression, treatment with cortisol
Monocytes	viral or fungal infections, tuberculosis, some leukemias, other chronic diseases	bone marrow suppression, treatment with cortisol

Thank you