

Differential WBC Count= Differential Leukocyte Count (DLC)

Aim:-

1. To check general health condition.
2. To help diagnose the diseases that affect one or more type of WBCs.
3. It is useful in confirming the diagnosis of specific neoplastic disorders (lymphoma and leukemia).

Principle

1. The blood contains various types of white blood cells. The number of different types may deviate from normal acceptable range. The percentage deviation from normal may indicate certain disease state of the patient such as allergic and drug reactions; parasitic infection and other types of infection. It can also identify various stages of leukemia.
2. Cell morphology and number will also differ among species.

Requirements

1. Glass slides
2. Giemsa's stain
3. Microscopes
4. Disposable needles
5. Vials containing anticoagulants (blood sample)
6. immersion oil
7. Staining rack

Procedure

A. Blood Smear Preparation

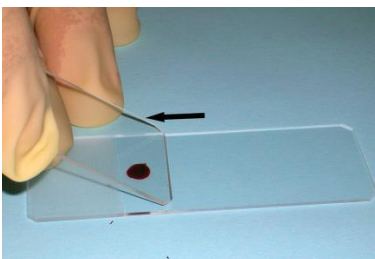
1. Put a drop of blood on the end of a clean glass slide use hematocrit tube.
2. Hold another slide (or cover slip) firmly in a horizontal position (an angle of about $30-45^{\circ}$) just in front of a drop of blood.
3. Pull the horizontal slide somewhat back ward in order to make the drop of blood to run along the line of contact between the 2 slides.

4. Push the horizontal slide gently but firmly along the surface of the first slide to the far end.
5. Dry rapidly by waving in the air or using a blower

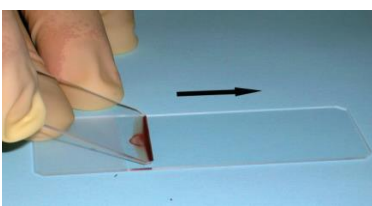
B- Procedure of Giemsa Stain

Giemsa's stain:- is a mixture of methylene blue, eosin, and Azure B.

1. Immerse the blood film in absolute methanol (99%) for 5 minutes to fix the film.
2. Drain the methanol and stand the slide upright to dry.
3. Immerse the slide in for 15 minutes
4. Wash the slide with D.W. to remove excess stain.
5. Place it in an upright position to dry completely.
6. Mount with DPX, and put cover slip.
7. Examine the slide under oil immersion lens (100X)



1. Place a drop of blood towards the end of a slide (on a flat surface). Then reverse the spreader slide into the drop of blood.



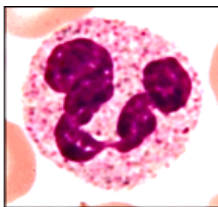
2. Once the slide is in contact with the blood, pause movement of the slide to allow the blood to spread laterally. Then propel the spreader slide forward with a gentle, smooth motion to spread the blood along the slide.

Counting

1. Place the slide on the microscope stage and observe under low power. If the smear is not good enough for counting, repeat the whole process.
2. Focus the smear under oil immersion lens(100x).
 - (a) In the first phase, looking from the side raise the stage until the oil immersion lens touches the oil drop placed on the slide.

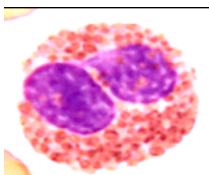
- (b) In the second phase, looking from the eye-piece raise the stage slowly using coarse knob till the cells appear vaguely.
 - (c) In the third phase, focus the cells clearly by using fine knob,
3. Identify and count different types of the cells. Use its characteristics.
 4. Count the cells from the body areas of the smear in a zig-zag manner up to 100 cells.
 - (a) Identified cells as "N" for neutrophil, "B" for basophil, so on
 - (b) Count each type of cells separately and express it as percentage

A- Granular WBCs



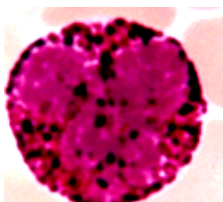
Neutrophils 60-70%

1. 10-12 μ in size; 2-5 lobed nucleus; Older the cell more the lobes- (polymorphonuclear leukocytes);
2. Small colored granules which contain enzymes, Defensins, oxidants
3. Action: by Phagocytosis
4. Life span: a few hrs: 4-8 hrs in blood; 4-5 days in tissues
5. Fast response; neutrophilia in bacterial diseases; neutropenia in drug toxicity



Eosinophils 0-4%; 10-12 μ

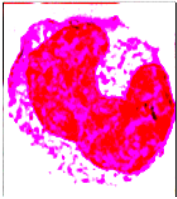
1. Bi-lobed nucleus; coarse acidophilic granules
2. Phagocytose antigen-antibody complexes; destroy parasites
3. Granules contain various enzymes : MBP
4. Increase in allergic conditions; decrease during stress-corticoids



Basophils 0-1% 8-10 μ

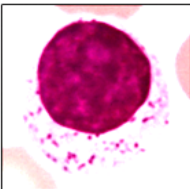
1. Nucleus usually bi-lobed;
2. Large deep purple colored granules which often cover the nucleus
3. Secrete heparin, serotonin, histamine: help in inflammation

B-Agranular WBCs



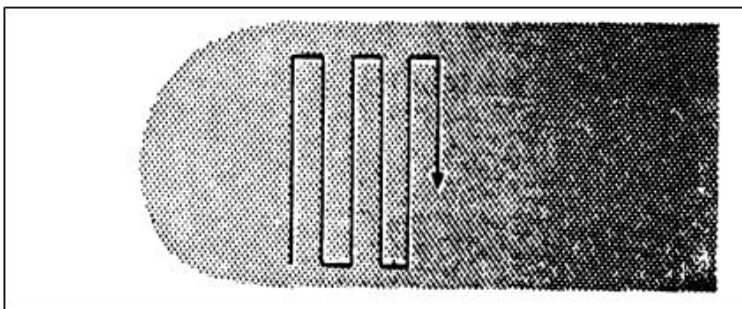
Monocyte 12-20 μ ;3-8%;

1. circulate in blood for about 20-30 hrs.
2. long life span (many weeks to months) after change into macrophages in tissues
3. Single kidney shaped nucleus
4. Phagocytosis after converting in to macrophages



Lymphocyte 6-9 μ

1. (small); 10-14 μ (large); 20-25%; mostly formed in lymphoid tissue; some in bone marrow.
2. Nucleus surrounded by thin ring of cytoplasm; long life span inside tissues, but only a few hours in blood
3. B and T lymphocytes: Immune mechanisms
4. B lymphos form plasma cells
5. Natural Killer (NK) cells against viruses, cancer



- Pathway for differential cell count.