Differential Count of White Blood Cells

AIM

To enumerate the different types of white blood cells in the given subject.

APPARATUS REQUIRED

Microscope, clean dry grease free glass slides with even edges, a drop bottle containing distilled water, a drop bottle containing Leishman's stain, cedar wood oil, staining rack, lancet, cotton and spirit.

Composition of Leishman's Stain

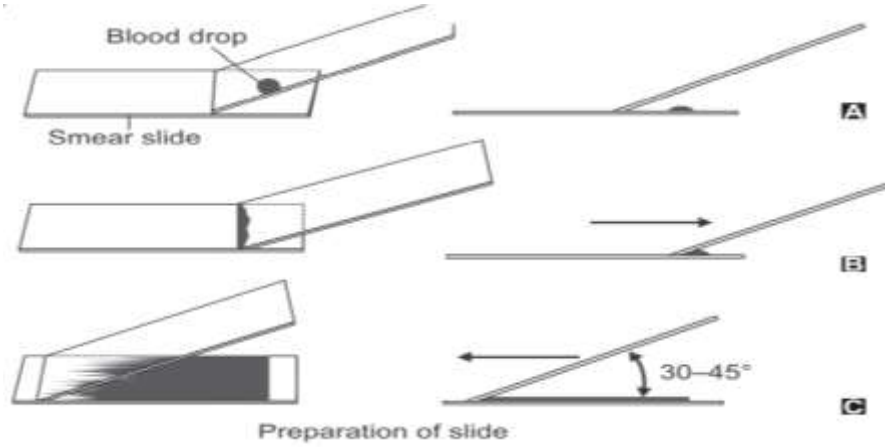
Eosin – An acidic dye, which stains the basic protoplasmic material.

Methylene blue – A basic dye, which stains the acidic nuclear chromatin.

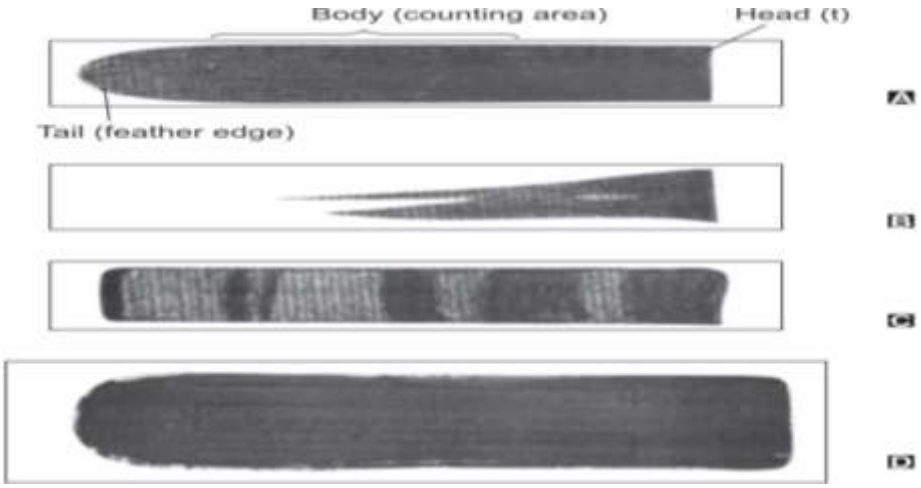
Acetone-free methyl alcohol – To fix the smear to the slide. Acetone being a strong lipid solvent it tends to damage the cell membrane. The stain is prepared by dissolving 750 mg of Leishman's powder in 500 mL of acetone-free methyl alcohol.

PROCEDURE FOR THE PREPARATION OF BLOOD FILM

The finger is cleaned and pricked with a sterile lancet. The first drop of blood is discarded. A clean glass slide is touched to the newly formed drop of blood 1 cm away from the edge. The slide is placed on the table so that the blood drop is on the right side. It is supported with the left hand and the second slide (spreader) is held along its long edges with the right hand at an angle of 45°. The smooth narrow edge of the spreader slide is placed in front of the blood drop and drawn back to touch the drop which then spreads along the edge of the spreader slide evenly. The spreader slide is pushed towards the left by a quick uniform motion with a light but even pressure. The blood follows along the spreader slide to form a blood film. It is dried quickly by waving in the air (Figs 1 and 2).



(Figs 1) Preparation of blood smear



(Figs 2) Diagram show (A) good & (B to D) bad smear.

STAINING THE BLOOD FILM

The blood smear is placed on the staining rack.

Leishman's stain is added drop by drop till the entire film is covered by the stain.

The number of drops added is counted (usually 8–10 drops).

The undiluted stain is allowed to act for 2 minutes. It should not be allowed to dry up. The cells are fixed in these 2 minutes.

Then, double the number of distilled water drops is added to dilute the stain. It is mixed by gently blowing through a pipette. The actual staining occurs now only. The stain should not be allowed to dry. After 7 minutes the stain is drained off.

The slide is washed in a gentle stream of running tap water until the film turns pink. The slide is kept in a vertical position to drain and dry. Thick uneven smears should be discarded. It is preferable to make 3–4 smears.

The best stained film should be selected for microscopic examination.

A good smear should:

1. Be buff colored.

2. Be uniform.

3. Be broader at the head and taper off into a tail.

4. Occupy the middle-third of the slide leaving a margin of about 5 mm along the edges.

5. Have no longitudinal or transverse striations or windows.

6. Have no stained granules or precipitates. 7. Have discrete red cells without overlapping each other.

MICROSCOPIC EXAMINATION

The microscope is adjusted for oil immersion lens. The condenser is raised, the diaphragm is completely opened and the plane mirror is used.

Two drops of cedar wood oil are placed near the head end. The oil immersion objective lens is made to touch the oil by viewing from the side. The fine adjustment screw is adjusted till the cells are brought into focus.

Hundred squares are drawn for recording the cell count.

The white blood cells are identified and entered using the letter N for neutrophil, E for eosinophil, B for basophil, L for lymphocyte and M for monocyte.

The slide is slowly moved towards the tail end and the cells are counted. The slide is then shifted up and moved in the opposite direction. This pattern of movement of the slide (zigzag pattern) takes into consideration all the parts of the film and ensures that a cell is not counted more than once.

Hundred white cells are identified and entered. The number of each type of white blood cell is counted and expressed in percentage.

IDENTIFICATION OF THE CELLS

A leukocyte is identified from its size, the nucleus and the cytoplasm. Size The size of the white cell is assessed by comparing it to that of the red cells if the white cell is as big as the red cell, it probably is a small lymphocyte. If the cell is twice as big as the red cell, it may be a granulocyte or a large lymphocyte. If the cell is about 2.1/2 to 3 times, then it is possibly a monocyte.

Nucleus

The following characters of the nucleus must be observed: 1. Whether it appears as a single mass or lobed 2. If single, whether the shape is round, oval or kidney shaped 3. Whether partially or completely fills the cell A small cell with a single round or oval nucleus may be a small lymphocyte. A bigger cell with distinct nuclear lobes joined by chromatin strands should be a granulocyte. A big cell with a kidney shaped nucleus is a monocyte. The nucleus in a lymphocyte completely fills the cell.

Cytoplasm

1. The amount of cytoplasm in relation to the size of the nucleus is noted.

2. The presence of visible granules and their nature is observed.

A small cell with a thin crescent of cytoplasm is a small lymphocyte.

A bigger cell with a rim of cytoplasm all around is a large lymphocyte.

A big cell with large amount of clear cytoplasm in relation to the nucleus is a monocyte (Fig. 3).

If a granulocyte has fine neutral granules of light violet color, the cell is a neutrophil.

If the granules are coarse and orange or red colored, the cell is an eosinophil.

In a basophil, the granules are large, coarse and deep blue in color. It is the smallest of granulocyte.

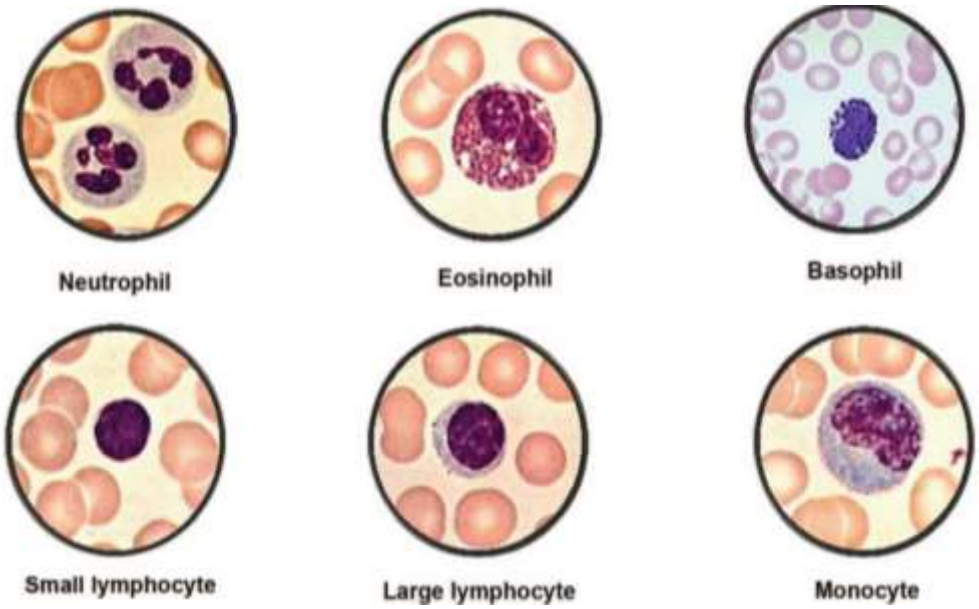


Figure (2) White Blood Cells .

Cell type		Size	Nucleus	Cytoplasm
Granulocytes				
1. Neutrophil		10–14 μm	2-6 lobes connected by chromatin.	Fine light violet colored granules.
2. Eosinophil		10–14 μm	Bilobed connected by a chromatin strand. (Spectacle shaped)	Coarse orange to brick red granules.
3. Basophil		10–14 μm	Irregular may be bilobed or S shaped. Not seen clearly as it is obscured by the granules.	Coarse deep blue granules completely filling the cell.
Agranulocytes				
1. Lymphocyte	a. Small	8–10 μm	Large-round nucleus completely, filling the cell, stains deep blue	Thin crescent of pale blue cytoplasm. No granules
	b. Large	10–15 μm	Large round or indented nucleus, stains deep blue	Thin rim of pale blue cytoplasm all around. No granules seen
2. Monocyte		12–24 μm	Large, placed centrally, kidney-shaped nucleus	Large amount of pale greayish blue cytoplasm. No granules seen

RESULTS

Neutrophils – %

Eosinophils – %

Basophils – %

Lymphocytes – %

Monocytes – %

DISCUSSION

The percentage of the different types of white blood cells is called the differential count. It is done to find out if there is an increase or decrease in a particular type of leukocyte. But it shows only a relative increase or decrease of the cell type with a corresponding change in the other cell types. Absolute values are more significant than relative values.

NORMAL COUNT

- Neutrophils – 50–70%
- Eosinophils – 1–4%
- Basophils – 0–1%
- Lymphocytes – 20–40%
- Monocytes – 2–8%

VARIATIONS

Neutrophilia: Is an increase in the number of neutrophils , some examples ;

1. Acute pyogenic infection such as tonsillitis, appendicitis, pneumonia

2. Malignant neoplasm

3. Drugs such as glucocorticoids, adrenaline, digitalis, phenacetin

4. Poisoning with lead, mercury, insect venom.

5. Physiological: Exercise, stress, after meals, pregnancy and parturition.

Neutropenia: Is decrease in the number of neutrophils , some examples ;

1. Typhoid and paratyphoid fever, kala-azar

2. Viral infection

3. Depression of bone marrow due to irradiation

Eosinophilia: Increase in the number of eosinophils.

1. Allergic conditions like asthma, hay fever, urticaria

2. Parasitic infestations—such as trichinosis, schistosomiasis, hookworm infestation

3. Chronic myeloid leukemia

Eosinopenia: Decrease in the number of eosinophils.

1. Administration of ACTH and glucocorticoids

2. Stress

3. Cushing's syndrome

Basophilia: Increase in the number of basophils.

1. Viral infections such as small pox and chickenpox

2. Allergic diseases

3. Chronic myeloid leukemia and polycythemia vera.

Basopenia: Decrease in the number of basophils.

1. Acute pyogenic infection

2. Glucocorticoid treatment.

Monocytosis: Increase in the number of monocytes , some examples ;

1. Chronic infection like tuberculosis, syphilis, subacute bacterial endocarditis, brucellosis

2. Protozoal infections like malaria, kala-azar

Monocytopenia: Decrease in the number of monocytes occurs rarely in:

1. Bone marrow failure

2. Aplastic anemia

3. Septicemia.

Lymphocytosis: Increase in the number of lymphocytes.

1. Infants and young children (relative lymphocytosis)
2. Whooping cough, diphtheria
3. Chronic infections like TB, syphilis and malaria
4. Viral infection like chicken pox
5. Lymphocytic leukemia

Lymphocytopenia: Decrease in the number of lymphocytes.

1. Steroid therapy
2. Acute infections and illnesses.
3. Hodgkin's disease
4. Bone marrow failure