S. S. College, Jehanabad

Department: Zoology

Class: M.Sc. Semester I

Subject: Zoology

Topic: Enumeration of WBC - Differential Count (DC)

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ENUMERATION OF WBC – DIFFERENTIAL COUNT (DC)

White blood cell or leukocytes are heterogeneous group of nucleated cells that are important component of blood like Red Blood Cells or RBCs. However, leukocytes are devoid of hemoglobin, but have prominent subcellular organelle. They are also found in circulation in circulation for at least a period of their life. Their normal concentration in blood varies between 4500 and 11,000 per microliter. They play an important role in defense against the foreign invasion or non-self through phagocytosis and immune response. It is capable of motility and largely involved in ingesting foreign materials and cellular debris by acting as scavenger in the tissue. They are larger than the RBCs in size and also differ in the shape; white blood cells (WBCs) are rounded, amoeboid and irregular in shape while red blood cells (RBCs) are biconcave discoid. The size of WBCs is about 15µm while that of RBCs is 7.5µm.

As leukocytes are heterogeneous group of blood cells, they are classified into various groups depending on their size and cytoplasmic as well as nuclear characteristics. They exist in two forms, such as, granulocytes and agranulocytes. Granulocytes have granular appearance due to the presence of specific granules in their cytoplasm, e.g., eosinophils, basophils and neutrophils, whereas agranulocytes have smooth appearance due to the absence of such granules in the cytoplasm, e.g., lymphocytes and monocytes (see figures below to differentiate the cells). These cells are variedly distributed in terms of numbers as shown in table.

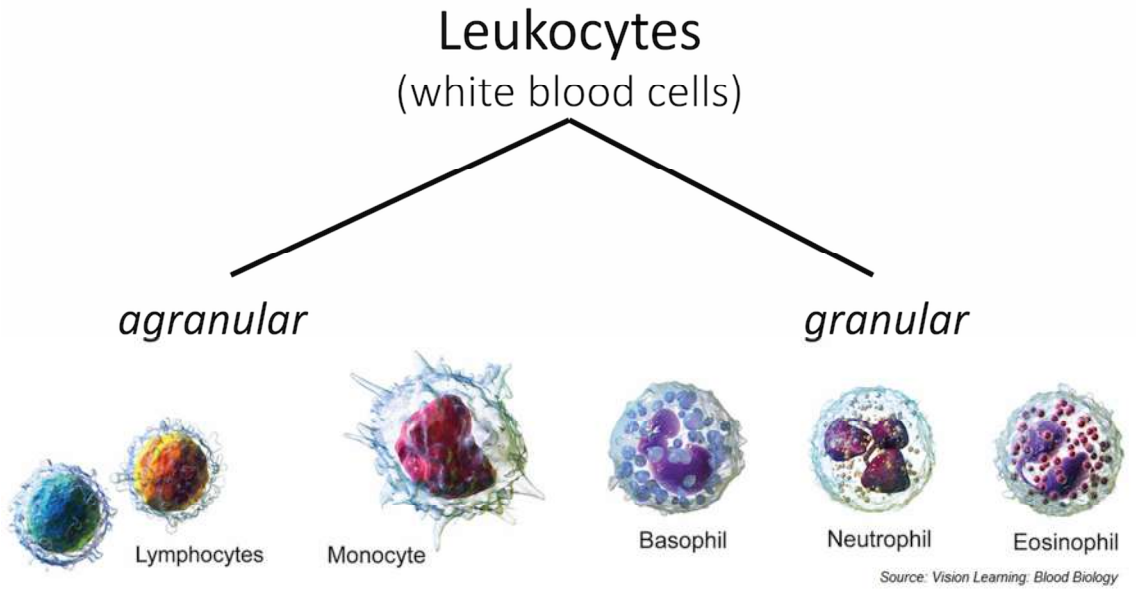


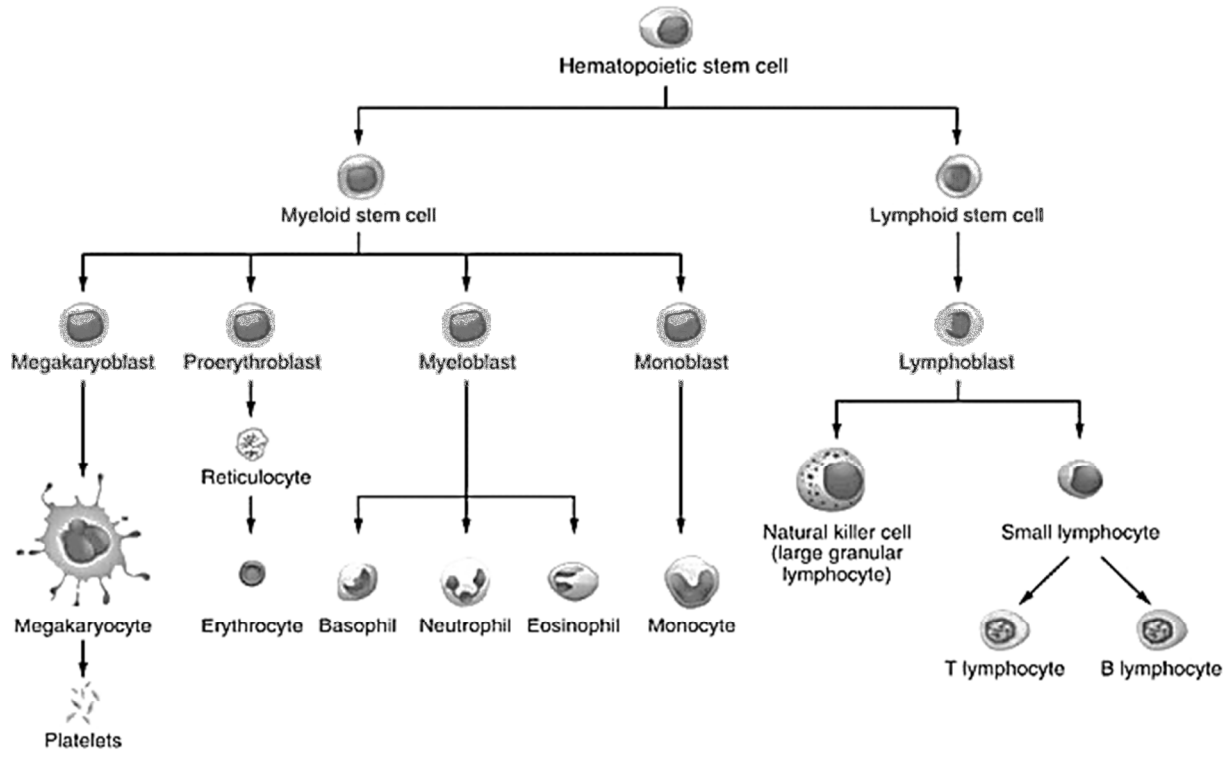
Table: Percentage of different cell types of white blood cells

Name of leukocyte	Male (% of total leukocytes)	Female (% of total leukocytes)
Neutrophils	40 – 75%	30 – 75%

Lymphocytes	20 – 50%	20 – 50%
Monocytes	2 – 10%	2 – 10%
Eosinophils	1 – 6%	1 – 6%
Basophils	0.3 – 1%	0.3 – 1%

Genesis of white blood cells (WBCs)

White blood cells develop from the hematopoietic stem cells (HSCs) in the bone marrow. The HSCs differentiate into two intermediate precursors namely myeloid stem cells and lymphoid stem cells. Lymphoid stem cell leads the formation of lymphoblast cells which later on differentiate into large granular natural killer cells or NK cells and small lymphocytes. Myeloid lymphoid cells are differentiated into four types of intermediate cells namely megakaryoblast, proerythroblast, myeloblast and monoblast. Megakaryoblast differentiate into platelets with megakaryocyte is a, intermediate stage, proerythroblasts are differentiated into erythrocytes with reticulocyte as an intermediate stage, myeloblasts are differentiated into eosinophils, basophils, and neutrophils, while monoblasts are differentiated into monocytes.



Differential count of WBCs

The process of evaluation of relative percentage of each type of wile blood cell is known as differential blood count or differential count of WBC (DC). It also helps to reveal abnormal

white blood cell populations (e.g., blasts, immature granulocytes, and circulating lymphoma cells in the peripheral blood.

Aim of the differential count

Aim of the blood differential leukocyte count (DLC) is to make a blood smear and to count the different types of leukocytes present in a stained blood smear and express their relative value in percentage.

Principle

The differential blood count is based on the staining of nucleus and cytoplasm of the white blood cells. Staining of both nucleus as well as cytoplasm enables us to determine the morphology and other properties of cells. For differential count, generally the combination of polychrome methylene blue and eosin stains are used because of their selective staining properties; methylene blue stains nucleus while eosin stains cytoplasm. Staining is followed by quantitating the different types of cells

Equipment

1. Micropipette
2. Glass slides with cover slips
3. Microscope
4. Clean gauge or cotton

Sample

1. Generally blood sample is collected in EDTA (Ethylene-Diamine-Tetraacetic acid).
2. Freshly prepared peripheral blood smear can also be used.

Reagents and solution

1. Well mixed whole or anticoagulant blood
2. Stains – Commonly used satins are Leishman's stain, Wright stain, Giemsa stain, and Filed stain. Out of these stains, only one stain needs to be used while doing differential count (DLC) test.
3. 70% Ethanol
4. Distilled water

Preparation of stains

Leishman's stain: It is constituted by mixing 0.150gm of Leishman stain in 100 ml of absolute methanol. Leishman's staining results in following colour formation:

Nuclear staining	Staining of granules

Chromatin	Chromatin	Basophils	Purple black
Nucleoli	Nucleoli	Eosinophils	Red orange
Cytoplasm staining		Neutrophils	Purple
Erythrocytes	Erythrocytes	Platelets	Purple
Reticulocytes	Reticulocytes		
Lymphocytes	Lymphocytes		
Monocytes	Monocytes		
Neutrophils	Neutrophils		
Basophils	Basophils		

Wright's stain: It is a histologic stain that facilitates the differentiation of blood cell types. It is classically mixture of eosin (red) and methylene blue dyes. It is used primarily to stain peripheral blood smears, urine samples, and bone marrow aspirates and examined under light microscope. It is prepared by mixing 1.0gm of Wright's stain powder in 400ml absolute methanol. Thereafter 100ml phosphate buffered saline (Potassium dihydrogen phosphate 0.663gm and disodium hydrogen phosphate 0.256gm added in 100ml distilled water; 0.15M, pH 6.5/6.8) is added to the mixture. Staining with Writght's stain results in following colour formation:

WBC types	**Colour**	**WBC types**	**Colour**
Erythrocytes	Yellowish – red	Eosinophils: Granules	Red or Orange – red
Neutrophils: Nucleus	Dark purple	Basophils: Nucleus	Purple to dark blule
Neutrophils: Cytoplasm	Pale – pink	Basophils: Granules	Very dark purple
Neutrophils: Granules	Reddish – lilac	Lymphocytes: Nuclei	Dark purple
Eosinophils: Nulclei	Blue	Lymphocytes: Cytoplasm	Sky blue
Eosinophils: cytoplasm	Blue	Platelets	Violet to purple granules

Giemsa stain: It is a type of Romanowsky stain (neutral stains composed of a mixture of oxidized methylene blue (azure) dyes and Eosin Y). It was named after Gustav Giemsa, a German chemist who created a dye solution for the demonstration of malarial parasites in blood smears, but now it is applied in routine examination of blood smear because it is

specific for the phosphate groups of DNA and attaches itself to where there are high amounts of adenine-thymine bonding. It is prepared by adding 7.6gm of Giemsa powder to the 500ml of glycerol and finally 500ml of absolute methanol is added to the mixture. *For thin blood smears, 1:20 dilution and for thick blood smear, 1:50 dilution of this preparation is used.* Staining with Giemsa stain results in following colour formation:

Blood cell types	Colour	Blood cell types	Colour
Red Blood cells	Mauve – pink	Lymphocytes	Nuclei: Dark blue Cytoplasm: Light blue
Neutrophils	Nuclei: Reddish purple Cytoplasm: Pink	Monocytes	Nuclei: Purple Cytoplasm: Pink
Eosinophils	Nuclei: Purple Cytoplasm: Faintly pink Granules: Red to orange	Platelets	Violet to purple colour granules
Basophils	Nuclei: Purple Granules: Blue coarse		

Field stain: It is also a type Romanowsky stain developed in order to discover malarial parasites in thick blood smears and now used for histological staining of blood smears. It enables worker for rapid processing of the specimens. It contains methylene blue (basic dyes) and eosin (acidic dyes). It is prepared in two parts; Field's stain A and Field's stain B.

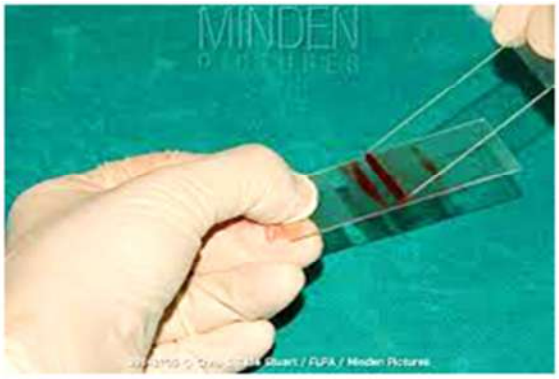
Field's Stain A (Methylene Blue Solution)		Field's Stain B (Eosin Blue Solution)	
Methylene blue	1.300gm	Eosin	1.300gm
Potassium dihydrogen phosphate	6.250gm	Potassium dihydrogen phosphate	6.250gm
Disodium hydrogen phosphate	5.000gm	Disodium hydrogen phosphate	5.000gm
Distilled water	550.000ml	Distilled water	500.000ml

It can also be prepared by adding 1.3gm methylene blue in 550ml phosphate buffer saline (PBS) for Field Stain A and 1.3g, eosin in 500ml PBS for Field stain B. Filed stain A has a dark violet coloured solution, while Field stain B has a orange coloured solution. Staining is performed by flooding or dipping slide in Field's stain A for 2 – 3 seconds and in Field's stains B for 2 – 3 seconds after washing which leads to following colour formation:

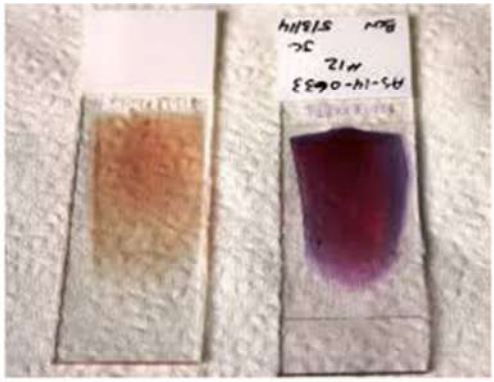
Cell types	Colour	Cell types	Colour
Neutrophils granules	Lilac	Leukocytes Nuclei	Purple
Eosinophils granules	Orange	Leukocytes cytoplasm	Pale blue
Nuclei	Blue	Malarial parasite	Deep red chromatin & pale blue cytoplasm
Red Blood cells	Red Blood cells are lysed & only background stroma remains		

Preparation of slides and staining

- Collect drops of blood on the end side of a glass slide.
- Spread the blood drop with another glass slide by placing it at an angle of 45 degree and move sidewise.
- Hold the spreader firmly and move it on the previous slide to the other end in a straight line with same force and pressure.
- Allow the glass slide to dry after formation of the smear.
- Air dry the slide or use any fixative to fix the smear.
- Stain the slides with a stain stated above.



Smearing of glass slide

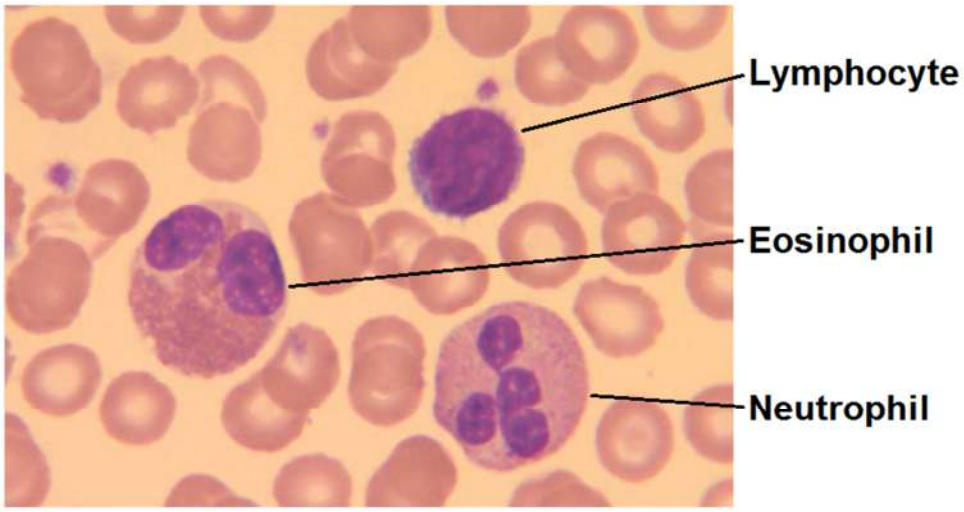


Smears on glass slides

Observation and counting

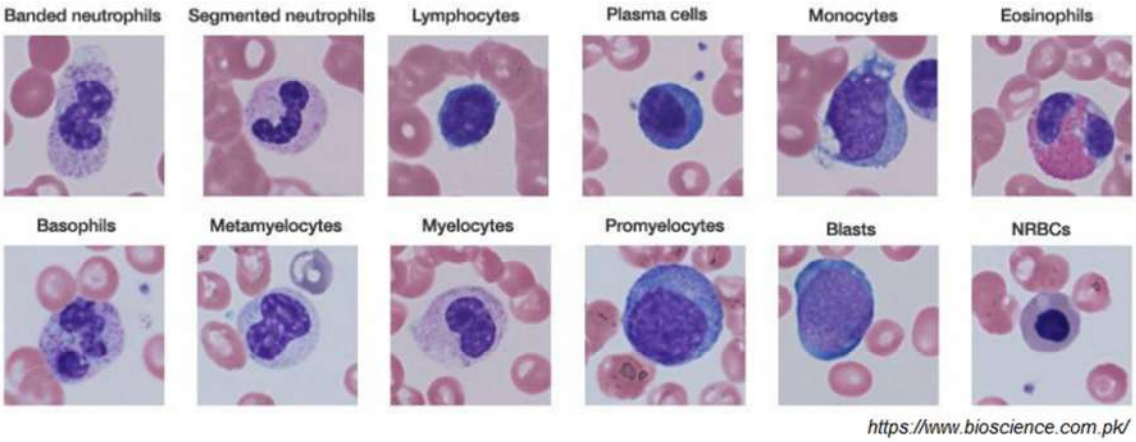
- Put the slides on the stage of light microscope and focus appropriately.
- Identify different types of WBC under medium magnification on the basic nuclear morphology and granules.
- Count all cells without differentiating cell types present on the slide.
- Move downwards and in chain like manner till 100 cells are observed.

- Count the different types of WBC one by one observed on the equivalent area of the slide and calculate percentage out of total leukocytes.



Significance of differential count

- It gives the relative percentage of each type of white blood cell.



- It helps in analyzing for any abnormal white blood cell population, e.g. blasts, immature granulocytes and circulating lymphoid cell, etc., that may be a sign of potential health issue.
- It helps us diagnosing an infection or inflammation in the body.
- It helps in detecting any disorder of the immune system.

Interpretation of test results

- An **increased percentage of neutrophils** in your blood can mean that you have:
  - Neutrophilia, a white blood cell disorder that can be caused by an infection, steroids, smoking, or rigorous exercise
  - an acute infection, especially a bacterial infection

- acute stress, tissue injury due to trauma
- pregnancy
- inflammation, such as inflammatory bowel disease or rheumatoid arthritis
- chronic leukemia

Condition associated with increased or decreased number of WBC types		
Type of WBC	**Increased number**	**Decreased number**
Neutrophils	Neutrophilia	Neutropenia
Lymphocytes	Lymphocytosis	Lymphocytopenia
Monocytes	Monocytosis	Monocytopenia
Eosinophils	Eosinophilia	Eosinopenia
Basophils	Basophilia	Basopenia

- A **decreased percentage of neutrophils** in your blood can indicate:
  - Neutropenia, a white blood cell disorder that can be caused by a lack of neutrophil production in the bone marrow
  - aplastic anemia, a decrease in the number of blood cells produced by your bone marrow
  - a severe or widespread bacterial or viral infection
  - recent chemotherapy or radiation therapy treatments
- An **increased percentage of lymphocytes** in your blood may be due to:
  - lymphoma, a white blood cell cancer that starts in your lymph nodes
  - a chronic bacterial infection
  - hepatitis
  - multiple myeloma, a cancer of the cells in your bone marrow
  - a viral infection, such as mononucleosis, mumps, or measles
  - lymphocytic leukemia
- A **decreased percentage of lymphocytes** in your blood can be a result of:
  - bone marrow damage due to chemotherapy or radiation treatments
  - HIV, tuberculosis, or hepatitis infection
  - leukemia
  - a severe infection, such as sepsis
  - an autoimmune disorder, such as lupus or rheumatoid arthritis
- A **heightened percentage of monocytes** in your blood can be caused by:
  - chronic inflammatory disease, such as inflammatory bowel disease

- a parasitic or viral infection
- a bacterial infection in your heart
- a collagen vascular disease, such as lupus, vasculitis, or rheumatoid arthritis
- certain types of leukemia

– An **increased percentage of eosinophils** in your blood can indicate:
  - eosinophilia, which can be caused by allergic disorders, parasites, tumors, or gastrointestinal (GI) disorders
  - an allergic reaction
  - skin inflammation, such as eczema or dermatitis
  - a parasitic infection
  - an inflammatory disorder, such as inflammatory bowel disease or celiac disease
  - certain cancers
– An **increased percentage of basophils** in your blood might be caused by:
  - a serious food allergy
  - inflammation
  - leukemia

Precautions

– Always wear protective gloves/protective clothing/eye protection/face protection before handling the dilution fluid.

– Follow good microbiological lab practices while handling specimens and culture.

– Standard precautions as per established guidelines should be followed while handling clinical specimens.

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