

Practical immunity

Leucocytes

White Blood Cells

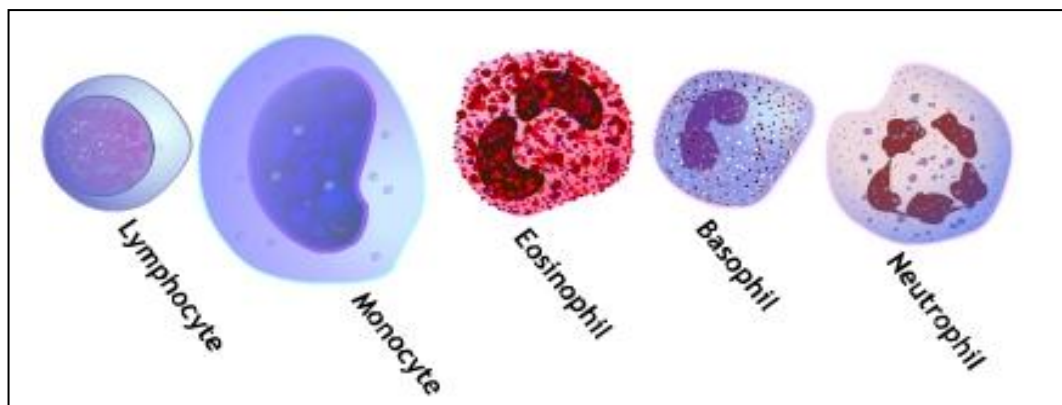
White blood cells or leukocytes are cells of the immune system which defend the body against both infectious disease and foreign material.

White Blood cells are also known as Leucocytes as they are colorless due to lack of Hemoglobin .

Types OF White Blood Cells

Classified according to the **presence or absence of granules** and the **staining characteristics of their cytoplasm** .

Leucocytes appear brightly colored in stained preparations , they have a **nuclei** and are **generally larger in size than RBC's**.



Type of WBC's

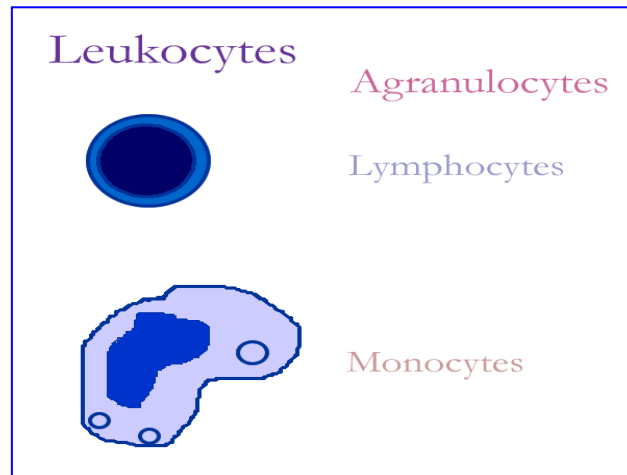
A / Granulocytes - have large granules in their cytoplasm

- 1- Neutrophils
- 2- Eosinophils
- 3- Basophils

B / Agranulocytes - do not have granules in their cytoplasm

1- Lymphocytes

2- Monocytes



Granulocytes

1-Neutrophils

Stain light purple with neutral dyes .

Granules are small and numerous

Several lobes in nucleus

Highly mobile / very active

#Can leave blood vessels and enter tissue space

Phagocytosis (eater) , contain several lysosomes .

2- Eosinophils:

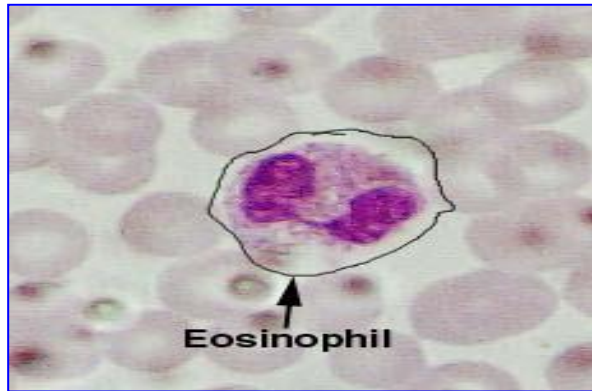
Large , numerous granules .

Nuclei with two lobes .

Found in lining of respiratory and digestive tracts

Important functions involve protections against infections caused by parasitic worms and involvement in allergic reactions.

Secrete anti-inflammatory substances in allergic reactions



3- Basophils

production histamine

A granulocytes

Lymphocytes

Smallest W.B.C

Large nuclei / small amount of cytoplasm

types of lymphocytes

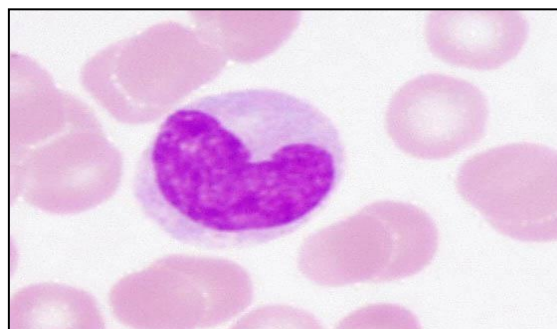
1- T lymphocytes - attack an infect or cancerous cell .

2- B lymphocytes - produce antibodies against specific antigens (foreign body)

Monocytes

Largest of WBCs # Highly phagocytic

Dark kidney bean shaped nuclei



WBC Numbers

If number goes up there is some kind of infection and surgery might be needed.

Clinics will count the number of WBC's in a blood sample, this is called differential count.

Leukocytosis is a condition characterized by an elevated number of white cells in the blood, which is usually due to:

- Bacterial infection such as appendicitis, tonsillitis , ulcers and urinary tract infection
- Leukemia.
- Pregnancy.
- Hemolytic disease of new born.

Leukopenia is a condition characterized by a decreased number of white cells in the blood , which is usually due to:

- Viral disease such as **measles** and **infectious hepatitis**.
- Some bacterial infections such as **typhoid fever, brucellosis** .
- Rheumatoid arthritis.
- Certain drugs such as radio therapy and chemotherapy.

W.B.C COUNT

- Specimen:

EDTA- anticoagulant blood tube .

- Reagents, supplies and equipment:

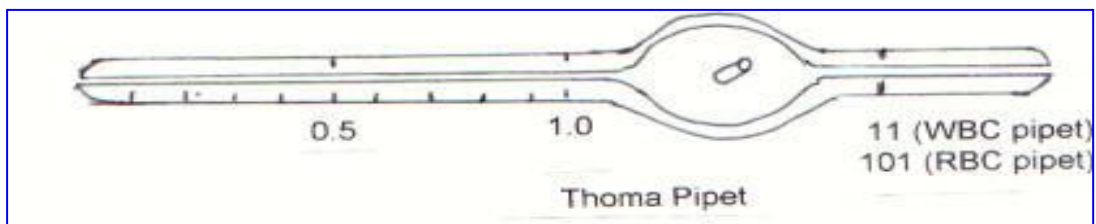
White blood cells count diluting fluid which may be one of the following: W.B.C solution

Turks' solution which is formed of : 1.5 Glacial acetic acid 3%

- Crystal violet 1 ml
- 100 ml distilled water.

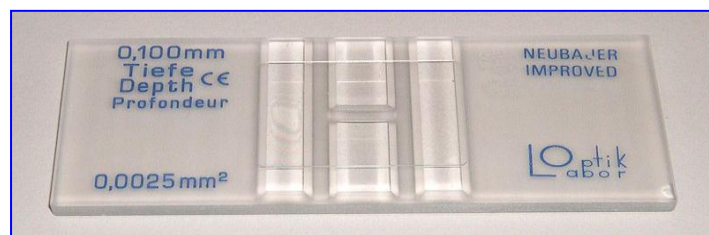
- Equipment & instrument

- 1-White blood cells count diluting fluid
- 2-Thoma white pipette
- 3-Hemocytometer and cover slip
- 4-Microscope
- 5-Lint-free wipe
- 6-Alcohol pads



Hemocytometer

- * The hemocytometer **counting chamber** is used for cell counting.
- * It is constructed so that the distance between the bottom of the cover slip and the surface of the counting area of the chamber is **0.1 mm**.
- *The surface of the chamber contains two square ruled areas separated by an H-shaped moat



Procedure

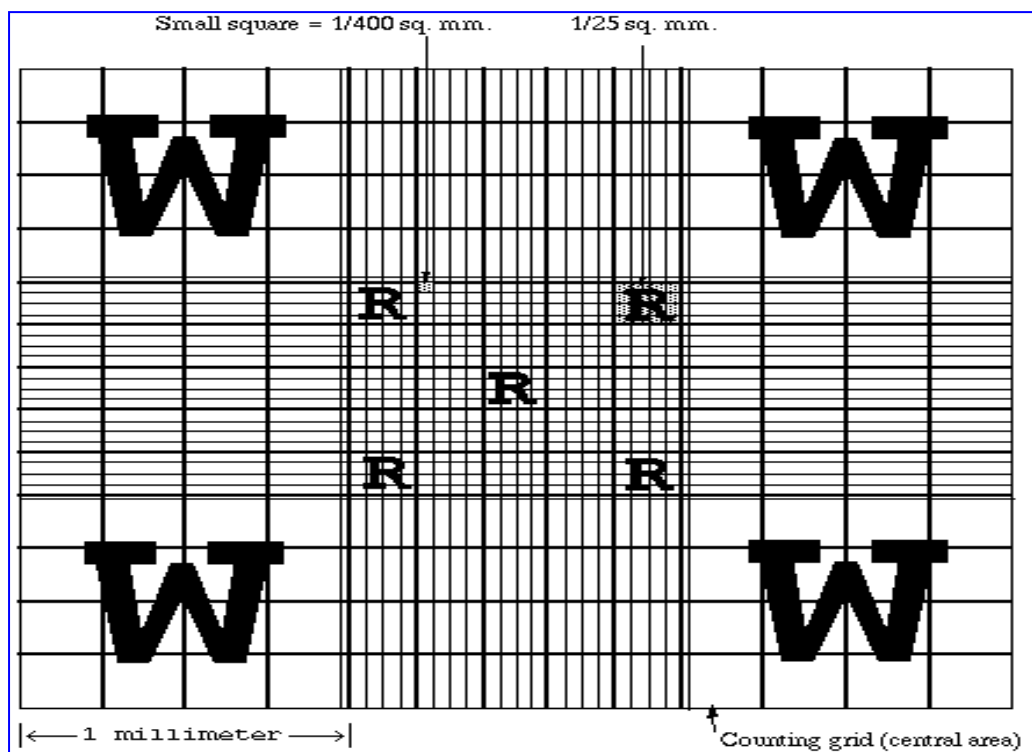
- 1-Draw the blood up to 0.5 mark in the thoma pipette .
- 2-Wipe the outside of the capillary pipette to remove excess blood that would interfere with the dilution factor.
- 3-Holding the pipette almost vertical place into the fluid , Draw the diluting fluid into the pipette slowly until the mixture reaches the 11 mark, while gently rotating the pipette to ensure a proper amount of mixing.
- 4-Place the pipette in a horizontal position and firmly hold the index finger of either hand over the opening in the tip of the pipette, detach the aspirator from the other end of the pipette now the dilution of the blood is completed
- 5-Mix the sample for at least 3 minutes to facilitate hemolysis of RBCs.
- 6-Clean the hemacytometer and its cover slip with an alcohol pad and then dry with a wipe.
- 7-Before filling the chamber, discard the first four to five drops of the mixture on a piece of gauze to expel the diluents from the stem.
- 8-Carefully charge hemacytometer with diluted blood by gently squeezing sides of reservoir to expel contents until chamber is properly filled.

Procedure for counting WBC's

1-Under **40 x** magnifications, scan to ensure even distribution. Leukocytes are counted in all nine large squares of counting chamber.

2- Count cells starting in the upper left large corner square. Move to the upper right corner square , bottom right corner square , bottom left corner square and end in the middle square.

3- Count all cells that touch any of the upper and left lines , do not count any cell that touches a lower or right line.



Depth= 0.1

Correction for dilution:

The thoma pipette is 1:20

Dilution factor 20

Correction of volume:

Volume of 1 small square = $1 \times 1 \times 0.1 = 0.1 \text{ mm}^3$

Volume of 4 large squares = $4 \times 0.1 = 0.4 \text{ mm}^3$ or μL

Suppose that you count 50 cells in 4 squares (0.4 mm^3), found the count in 1 mm^3 ?

50 0.4 mm^3

X 1 mm^3

X = $50 \times 1 \div 0.4$

Volume correction = $1 \div 0.4$

Total count \ 1mm³ =

No. of cells x **volume correction** x dilution =

no. of cells x $(1 \div 0.4)$ x 20 =

A/granulocytes (5000-10000) per microliter or cubic mm(mm^3)

1-Neutrophils ----- 60-70% == phagocytosis

2-Basophils----- 0.5-1% == production of histamine

3-Eosinophils ----- 2-4% == production toxin proteins against
certain parasites ;some phagocytosis

B/Agranulocytes

1- Monocytes----- 3-8% ===== phagocytosis (when they mature in to
macrophages

2- Lymphocytes--- 20-25%== Ab production (B) lymphocytes ; cell
mediated immunity (T) lymphocytes