

White blood cell count = Total Leukocyte Counting

Aims :- To enumerate the total number of leukocyte (White Blood Cell: WBC) of a given blood sample.

Significance

The normal WBC performs some important physiological functions. The chief function of it is imparting immunity to the body.

Therefore, the decrease or increase in circulating WBC indicates physiological condition i.e., increase in WBC indicates inflammation, whereas decrease in WBC may indicate AIDS.

Leukocyte Disorders :- Normal WBC count: 4000-11000

1. Leucytosis >11000

Neutrophils:- Infections, Burns, inflammation

Lymphocytes:- Viral infections

Monocytes:-Viral, fungal infections, TB, Chronic disease

2. Leuopenia < 4000

Some viral infections, Bacterial infections (typhoid)

Bone marrow suppression

Drugs: antibiotics, corticosteroids

Material and method :-

- 1- The special pipette, consisting of a capillary tube marked with figures 0.5,11 with a bulb between the marks 0.5 and 11.The bulb contain small white glass bead.
- 2- Blood cell counting chambers are called (**haemocytometers**); the most commonly used type is the **Improved Neubauer chamber**. The Improved Neubauer chamber is a thick glass slide with two recessed central areas each having a finely ruled grid. The grid consists of 9 squares, each with an area of 1mm^2 . The central square is divided into 25 smaller squares, and each of these is further divided into 16 squares, giving a total of 400 squares. This central portion is used for red cell counts. The four corner squares are divided into 16 squares only are used for white cell counts. When the special (heavy) cover glass is placed over the recessed central area, the depth of the counting chamber is 0.1 mm.

- 3- Special thick cover slide of standard weight and thickness
- 4- Fluid dilution to the White blood cells count may be one of the following :-

1. Acetic acid 2% (v/v) in distilled water.
2. HCL 1% (v/v) in distilled water.
3. Turks' solution which is formed of:
 - A. Glacial acetic acid 3 ml
 - B. Crystal violet 1 ml
 - C. 100 ml distilled water

Procedure

1. The counting chamber and the cover slip are cleaned and the cover slip placed on the lateral bars across the middle of the counting chamber.
2. Clean the figure and puncturing it gently by lancet.
3. Using the special pipette and quickly draw the blood up to the mark 0.5 and then immediately draw Turkey's solution up to the mark 11
4. Mix well for 1 – 2 min.
5. Discard the first three drops of mixture from the capillary prior to loading the hemocytometer
6. Introduce a little of the diluted blood into the chamber with the cover slid from the pipette. No pressure is required to fill the chamber, capillary action is quite sufficient.
7. Leave the cells to settle for 2 min.
8. Focus on the ruled area of the chamber using the **x10 microscope objective**.
9. . Under 10x magnification, scan to ensure even distribution. Leukocytes are counted in **THE FOUR OUTSIDE large squares** of counting chamber . Avoid counting the same cell twice.

Calculation:-

- ❖ Depth= 0.1
- ❖ Correction for dilution:
- ❖ The pipette is 0.5-11 which= 1:20
- ❖ Dilution factor 20
- ❖ Correction of volume:
 - Volume of 1small 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 (0.4mm^3), found the count in 1mm^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 =
- **No. of cells x 50 =**

