

### Hemocytometry (blood cell count)

Hemocytometry is the process the counting formed element of the blood. The elements counted are R.B.Cs, W.B.Cs and platelets. Counting is done either by an electronic device (electronic cell counter) or manually with a special glass slide known as a hemocytometer. Since many element are in the blood in high concentration, blood must be diluted before counting. Diluting fluid is selected for its ability to:

1. Dilute the blood.
2. Lyse cell type not wanted in the count.
3. Stain a particular cell type wanted to count.

Blood cell counts are usually reported in number of cell per cubic millimeter (cells/mm<sup>3</sup>), or per microliter (cell /μL) .

#### Manual white blood cell count

W.B.Cs(leukocytes) are spherical cells that are whitish in color because they lack Hb.they are larger than R.B.Cs about 1.5to 3 times,they have nuclei and move in amoeboid fashion.their function are protect the body against invading microorganisms,and they remove dead cells debris from the tissues by phagocytosis.

Leukocytes are divided into 2 classes based upon the presence or absnce of visible specific granules:

- 1- Granulocytes: they exhibit clearly visible microscopic specific granules in their cytoplasm.there are 3 type of granulocytes that named according to how the granules stain:
  - a- Neutrophils      b. basophils      c. Eosinophils
- 2- Agranulocytes (non-granulocytes): they have small granules in their cytoplasm that cannot be easily seen with the light microscope. There are 2 type of granulocytes:
  - a. Lymphocys      b. Monocytes

\*principle: the blood is diluted with diluting fluid in order to:

- 1- Hemolyse the R.B.Cs & convert hemoglobin into hematin.
- 2- Darken the nuclei of W.B.Cs so that they are easier to see microscopically.
- 3- keep the W.B.Cs in normal stat (isotonic solution).

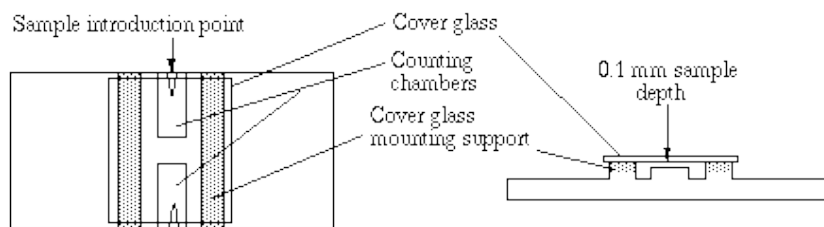
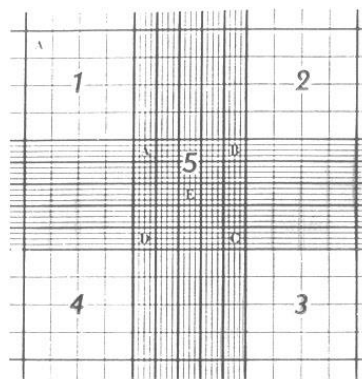
The number of W.B.Cs is counted & the result is expressed as the no. of cells per microliter of blood (cells/\*)

\*Reagents & equipments

1. Anticoagulated blood: the anticoagulant of choice is EDTA as it prevents blood coagulation & preserves the morphology of cellular elements (W.B.Cs)
2. W.B.Cs diluting pipette: it is known as Thoma pipette and allows 1:20 dilution of blood. It does not measure blood in microliters but provides a dilution ratio.
3. W.B.Cs diluting fluid: it is known as Turk's solution, which consists of gentian violet stain. Gentian violet stains the nuclei & making them more identifiable.
4. Hemocytometer & cover slip: it is a specially designed counting chamber. The most commonly used is improved Neubauer. It is a thick glass slide with an H-shaped trough forming 2 counting areas (fig.1). Each of the 2 counting areas is divided into 9 squares (fig.2) the 4 corner squares are used in W.B.Cs counting & are divided into 16 squares by single lines (fig.2).
5. Microscope: the W.B.Cs are counted under low power (10 x) objective lens.

**\*procedure:**

1. pour 0.4 ml of the diluting fluid into a tube
2. The fresh blood is gently inverted to ensure mixing.
3. Insert the stem of pipette into the blood sample & withdraw the blood into the stem to slightly above 0.02 mark.
4. Remove the pipette from the blood & wipe the outside of its stem , adjust the blood level to the 0.02 mark exactly .
5. Pour the blood into the diluting fluid tube & shake it gently for 15-30 sec.
6. The blood is drawn into the hemocytometer chamber by capillary action, make sure no bubbles.
7. Put the chamber on the stage of microscope.

**Fig(1) Hemocytometer counting chamber****Fig(2) Neubaur hemocytometer . The counting area is divided in nine squares**

**Counting the W.B.Cs**

Find the lined square with the objective lens (10 x) . The W.B.Cs in each of 4 corner squares are counted & the total number of these cells should be relatively uniform.

**\*Calculating the W.B.C s**

$$\text{W.B.C. (cell/}\mu\text{l)} = N/4 \times D \times 10$$

**N**=the total W.B.Cs counted in the 4 corner squares.

**D**=the dilution factor of blood sample.

**10**= the volume factor

**\*Reference range**

**\*Male adult :5000-10000 cells/ $\mu$ l**

**\*Female adult : 4500- 9000 cells/ $\mu$ l**

**Leukocytosis** :A total W.B.C.s count above 10000 cell/ $\mu$ l. It is caused by either physiological or pathological condition , like pregnancy , dehydration ,allergy, fever ,sever exercise, injury ,heart attack, kidney failure & cancer.

**Leucopenia**: It is a low W.B.C.s count (below 4000 cells/ $\mu$ l) This condition .may result from viral or bacterial infection (such as ADIS)