HEMACYTOMETER

It is a technique for enumerating the total cell count in Blood or other Biological body fluids. This can be done either manually using a Haemocytometer or automatically using an Electronic cell counter.

Hemo: blood

Cyto: cell

Meter: measurement/counter

Thus, it is an instrument used to count the blood cells.

The hemacytometer chamber (Figure 1) counts white and red blood cells. The large square areas indicated by the numbers **1, 2, 3**, and **4** in the figure are used for counting **white blood cells**. The area indicated by the number **5** is used for counting **red blood cells**. (In hemacytometers that are 0.1 mm deep). **Errors can be introduced in several ways:**

1. Dilution errors
2. Loss of cells during pipetting
3. Uneven suspension of cells
4. Overfilling or under filling of the chamber, and the counting of cells before.

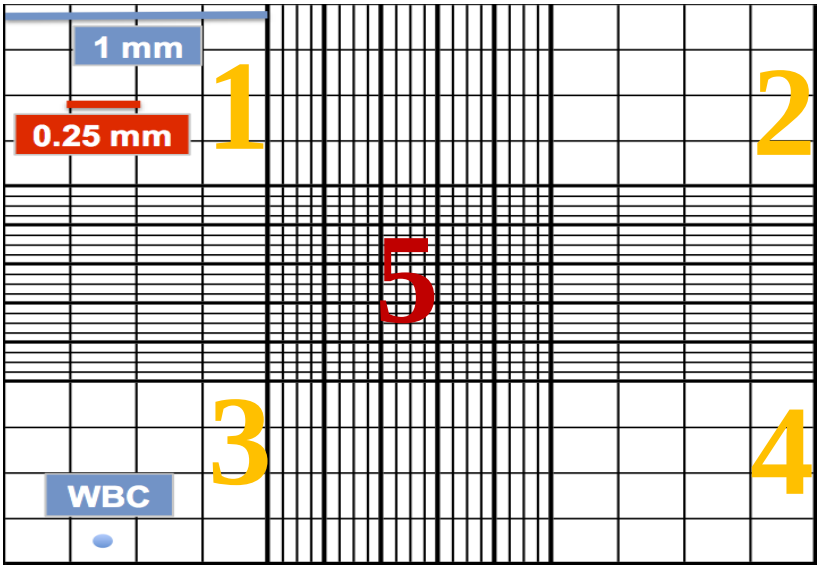


Figure 1: Hemocytometer

✓ Principle of cell counting:-

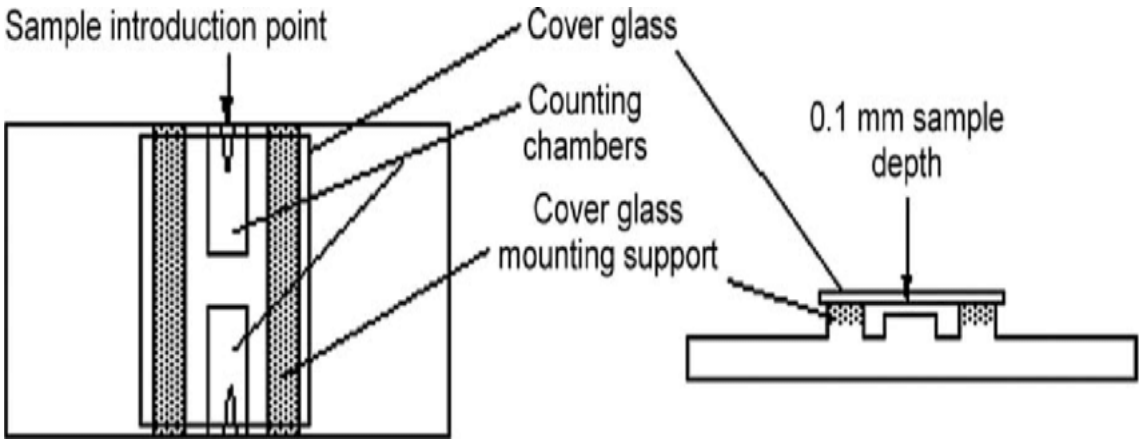
The Number of blood cells is very high, so it is difficult to count them even under microscope. This difficulty is partly overcome by diluting the blood to a known degree with suitable diluting fluids and then counting them.

✓ Purpose:-

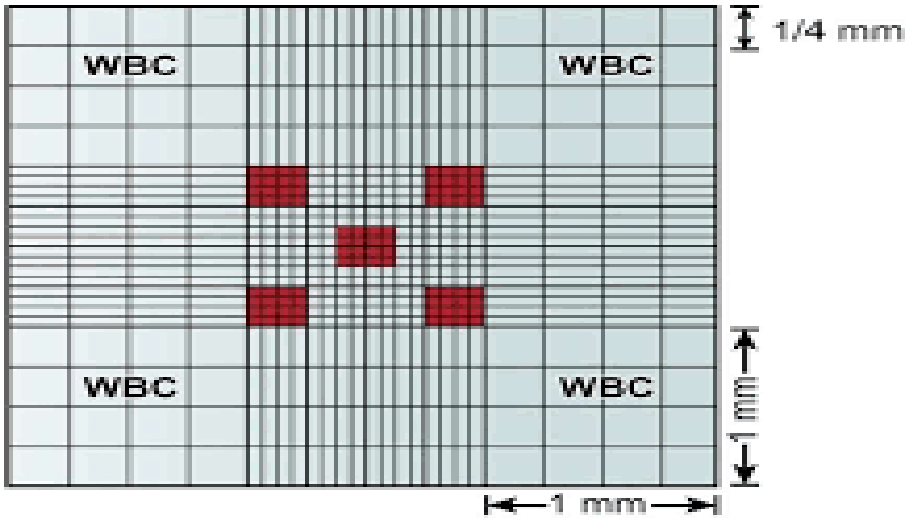
In certain pathological conditions, the value of different types of cells may vary. Thus, counting the cells in the blood or body fluids can determine whether an individual is normal or abnormal. Broadly, the cell count is done:

1) To determine the **normal** and **abnormal** cell counts,
2) To support and confirm the **clinical diagnosis** of the patient,
3) To find out the **response** of the patient to the treatment

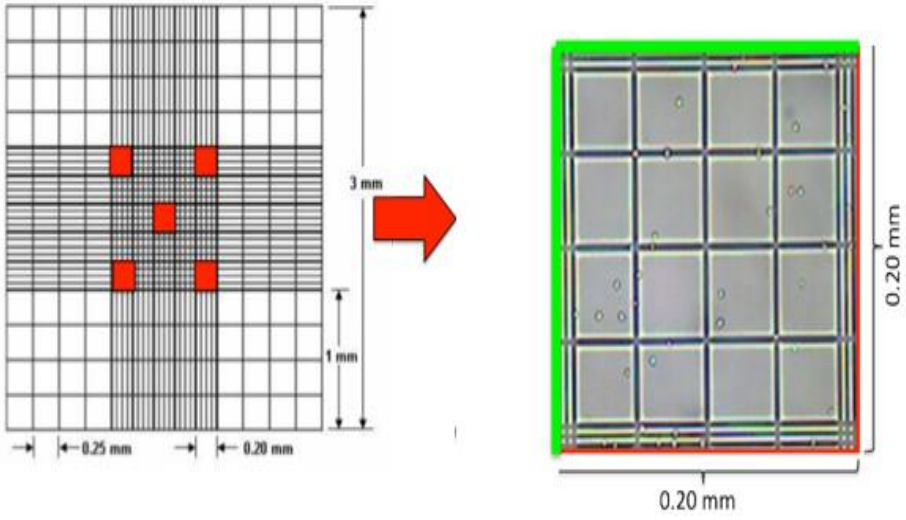




- The four corner squares are divided into sixteen (16) smaller squares, which are used for WBC counting. The total number of small squares is 64.

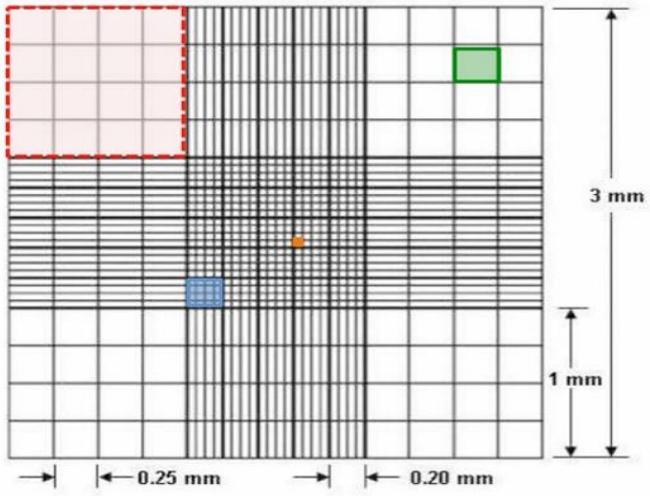


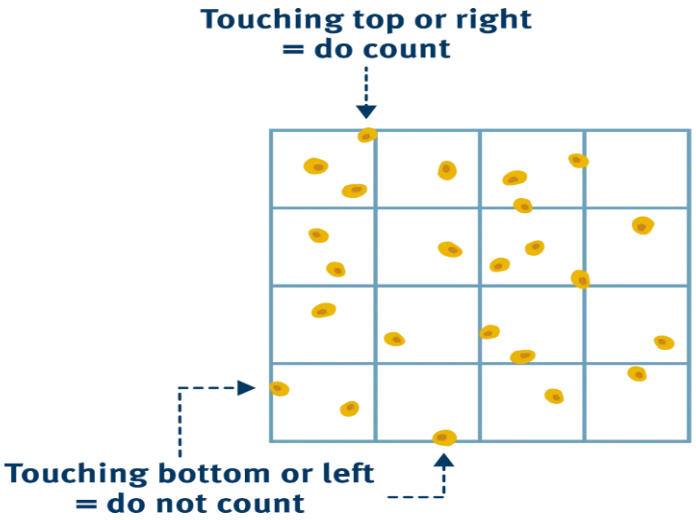
- The central square is subdivided into twenty-five (25) smaller squares, and each of these smaller squares is further subdivided into sixteen (16) smallest squares.



Each smaller square is (0.2 mm) wide and (0.2 mm) long. These squares are used for **platelet** and **RBC** counting. The platelets are counted in all the small squares of the central square, while the RBCs are counted in five small squares, **four corners**, and **one in the center** (a total of **80** smallest squares).

Unit	Width	Volume (mm3)	#
chamber	3 mm	0.9 mm3	2
Square (red)	1 mm	0.1 mm3	9
Small square (green)	0.25 mm	0.00625 mm3	16
Smaller square (blue)	0.2 mm	0.004 mm3	25
Smallest square (orange)	0.05 mm	0.00025 mm3	16





Total RBC counting

- The methodology involves accurately diluting the blood specimen **(1:200)** with the RBC diluting fluid, preferably **Hayem's fluid**.
- Count the cells in five medium-sized corner RBC squares (four corners and one central medium-sized RBC square) (16x5 = 80).
- **RBC count** = $\frac{\text{Number of cells counted (N)} \times \text{Dilution factor}}{\text{Number of squares counted x Volume of area } (mm^3)}$

- Where is the number of red blood cells (N) =?
- Dilution factor = 200
- The volume of each 5 small squares = 0.004
- Total RBCs/mm^3 = N X 200 / (5 X 0.004) = N X 10,000 million/mm^3

For example, suppose N or the number of RBCs in the five squares is 500, then the total RBCs will be: = 500 x 10, 000 = 5,000,000 (5.0 million)

Total WBC counting

- The methodology involves accurately diluting the blood specimen **(1:20)**. The dilution factor is less than RBC's. We used the WBC diluting fluid **Turk's fluid**.
- Count the cells in the WBC squares at four corners of the chamber.
- **WBC count** = $\frac{\text{Number of cells counted (N)} \times \text{Dilution factor}}{\text{Number of squares counted x Volume of area } (mm^3)}$

- Where is the number of red blood cells (N) =?
- Dilution factor = 20
- The volume of each 4 small squares = 0.1
- Total WBC mm^3 = N X 20 / (4 X 0.1) = N X 50 mm^3

