

INTRODUCTION TO HAEMOCYTOMETRY

HAEMOCYTOMETRY

It is a technique used to enumerate the total cell count in the blood or other biological body fluids. This can be done either by using haemocytometer or by electronic cell counter.

PURPOSE

In certain pathological conditions the value of different type of cells may have the variation. Thus, by counting the cells in the blood or body fluids, it can be found out if an individual is normal or not

Broadly, the cell count is done mainly:

- To find out normal and abnormal count of the cells
- To support and confirm clinical diagnosis of the patient
- To find out the response of the patient to the treatment

PRINCIPLE OF CELL COUNTING

The blood is diluted with appropriate known volume of diluting fluid and then counting is done by using haemocytometer.

HAEMOCYTOMETER

- This is an instrument used for counting the cells in blood or fluid.
- It consists of a special instrument called counting chamber, cover glass, pipette for diluting the blood rubber tube with plastic mouth piece for drawing blood or fluid in pipette.



FIGURE: Showing a haemocytometer consisting of red blood cell pipette, white blood cell pipette and improved Neubauer chamber

1. Counting Chamber: It is a thick glass slide with two identical ruled areas separated by empty space and having two elevated ridges on their both sides. Either of the ruled areas is used for counting the cells. There are different types of counting chamber viz. old Neubauer counting chamber, improved Neubauer counting chamber, Burker counting chamber and Fuch's Rosenthal counting chamber.
 - **OLD NEUBAUER COUNTING CHAMBER:** In this, the central platform is set 0.1 mm below the level of the two sides, which gives the chamber a depth of 0.1 mm. The ruling covers an area of 9 sq. mm divided into 9 squares of 1 sq. mm. each. The four corner squares are subdivided into 16 squares, each with an area of 1/16 of a sq. mm. the central ruled area of 1 sq. mm. is divided into 16 large squares by set of triple lines. These large squares are further subdivided into 16 small squares by single lines.
 - **IMPROVED NEUBAUER COUNTING CHAMBER:** Here, the counting square is of 9 sq. mm area (3 mm × 3 mm). The four corner squares of area 1 sq. mm. each (1 mm × 1 mm) are used for white blood cell count. In this the triple lines which divide the central large square are very much closer to each other. The central ruled area is divided into 25 large squares. These squares are subdivided to form 16 smaller squares each with an area of 1/400 of 1 sq. mm. The four corners and one middle subsquare are used for red blood cell count. The depth of improved Neubauer chamber is same that is 0.1 mm.

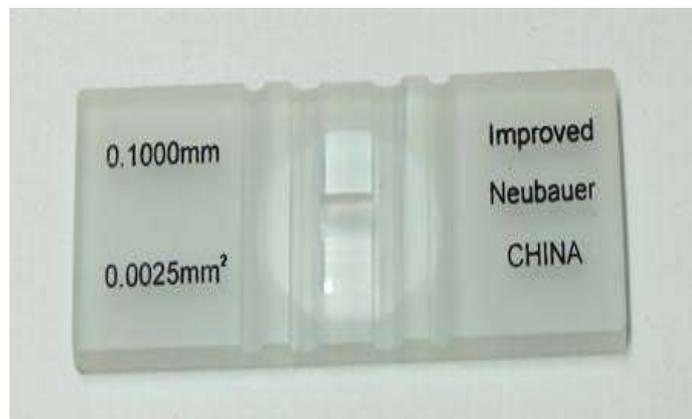


FIGURE: Showing an improved Neubauer chamber

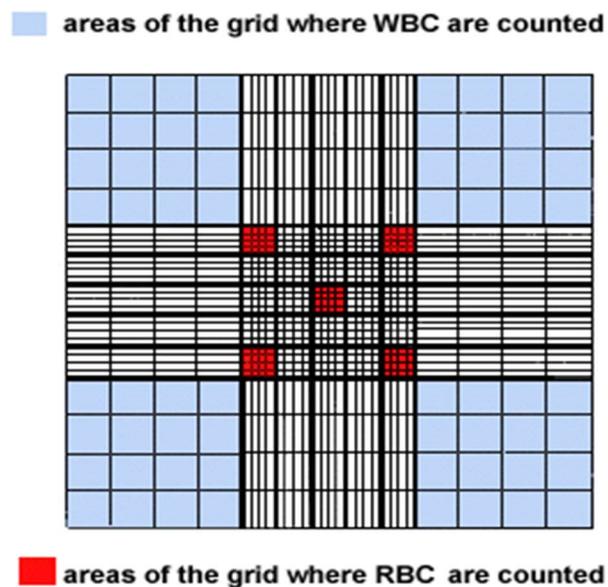


FIGURE: Showing the cell counting area of Neubauer chamber

Old V/S Improved Neubauer Chamber

- The space occupied by the triple lines in old Neubauer chamber being used to produce extra large squares.
- In old Neubauer chamber the gap b/w triple lines was very wide and the rectangular space between them look as similar as the squares in which cells are to be counted. This makes the count very difficult and chances of error was very high.
- In old Neubauer chamber the lines were very dull and sometimes it was very difficult to recognize them
- But in improved Neubauer chamber these all faults are removed.
- By dividing central square in 25 squares the RBC and platelet count has become easy to do.

2. Cover Glass: A special cover glass is used which has a very smooth, flattened surface and even thickness. Different thickness are: 0.3mm, 0.4mm (most common), 0.5mm. two sizes are common: 16×22 sq.mm and 22×23 sq.mm.
3. Diluting Pipette: It is a glass tube pipette with rubber sucking arrangement. The tubular part of the pipette is graduated from 0 to 1 with the division of 0.1 units. The bulb portion can accommodate 100 units of volume graduated from 1 to 101 on both sides of the bulb. Bulb serves as diluting and mixing chamber for blood. The red bead in it, aids in mixing and for identification of red cell pipette from white cell pipette.

REFERENCE:

1. Haematology, Practical Human Anatomy And Physiology, S.R. Kale et al., Nirali Prakashan, Eight Edition, 2002, pp. 11-12