EXPERIMENT NO.: 7 **DATE:**

AIM: TO STUDY AND INTRODUCTION OF THE HEMOCYTOMETER

REQUIREMENTS: Hemocytometer, cotton swab with spirit, pricking needle.

THEORY:

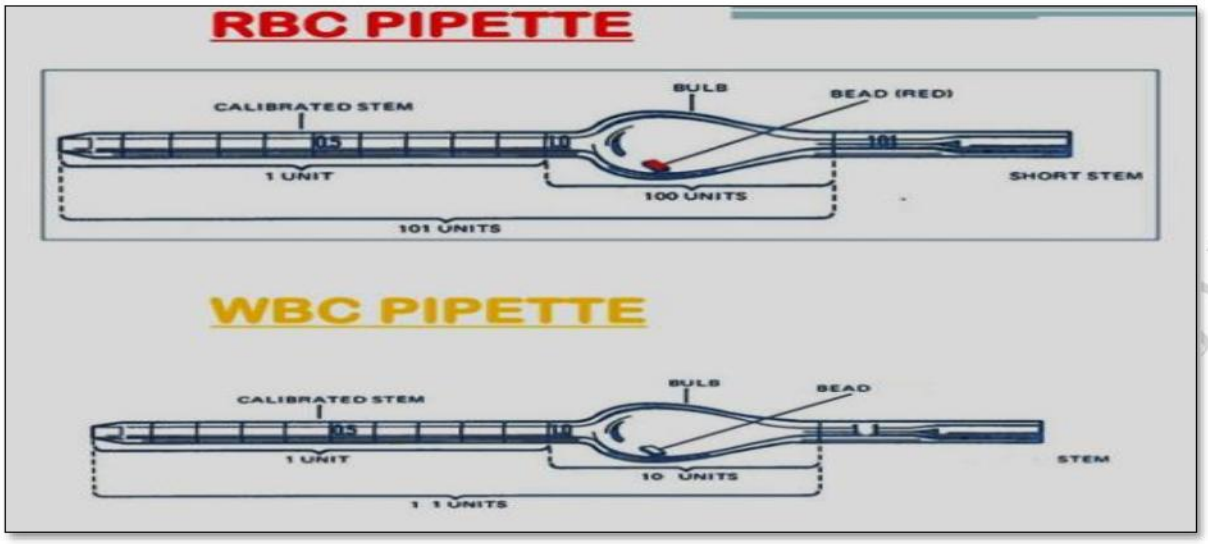
- Hemocytometer, Hemo, for blood; cyto, for cell; meter, for measuring. So altogether: measuring blood cells.
- The counting of cells [RBC and WBC] in blood using hemocytometer set is called hemocytometry.
- It is a device used for determining the number of cells per unit volume of a suspension is called a counting chamber. It is the most widely used type of chamber, since it was mainly designed for performing blood cell counts. It is now used to count other types of cells and other microscopic particles as well.
- The hemocytometer was invented by Louis-Charles Malassez.

Hamocytometer set consisting:

1. Dilution pipette
2. Counting chamber (Thomas or Neubauser's counting chamber) and Special coverslip (Thosmas cover slip)

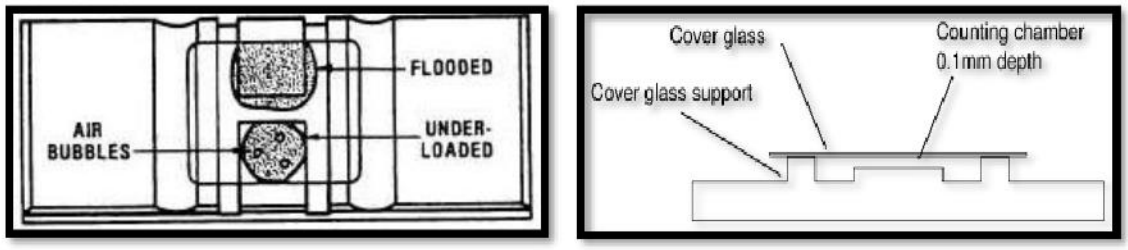
1. Dilution pipette

- It consisting 4 parts- the long stem, the blub, the short stem and the sucker.
- The long stem has a uniform capillary bore extending from a well ground conical tip and merging in the bulb.
- The long stem is divided into 10 equal parts from the tip to the mark '1' just near the bulb.
- The 5^{th} division is heavily marked and labeled as 0.5.
- This part is to measure exact amount of blood taken for counting.
- The fluid in this does not take part in dilution and hence this quantity must must be deducted while calculating the dilution factor.
- The bulb is part where dilution of blood takes place. It consists of while or red bead which helps in uniform mixing of blood with dilution fluid. The bulb ends in a short stem which have the mark '11' [in wbc piptte] or '101'[in RBC pipette]



2. Counting chamber

- The glass microscope slide has a rectangular indentation that creates an 'H' shaped chamber at the centre. Two counting areas with ruled grids are separated by the horizontal groove of the 'H'.
- There is also a very flat, reusable cover slip (Thosmas coverslip). The glass cover slip(Thosmas coverslip) is held at 0.1 mm above the surface of the counting areas by ground glass ridges on either side of the vertical grooves of the H shape.
- The device is carefully crafted so that the area bounded by the depth and lines of the chamber is also known. Because the height is constant, the volume of fluid above each square of the grid is known with precision.
- The hemocytometer is used by putting the cover slip on the device, and filling the space with a liquid containing the cells you want to count.
- There is a "V" or notch at either end which is the place where the cell suspension is loaded into the hemocytometer. The fluid is usually drawn into the space by capillary action.



The Neubauer's counting chamber

*Ruling area on The Neubauer's counting chamber

The total ruing area of each slide is 3mm in length and 3mm in breadth. It is divided into nine equal squares os 1sq. mm area. The boundary lines of these squares are triple linings. Four squares of the corners are used for WBC counting while the central square is for RBC counting. Each WBC square is divide into 16 equal squares by single lining. The area of each square is

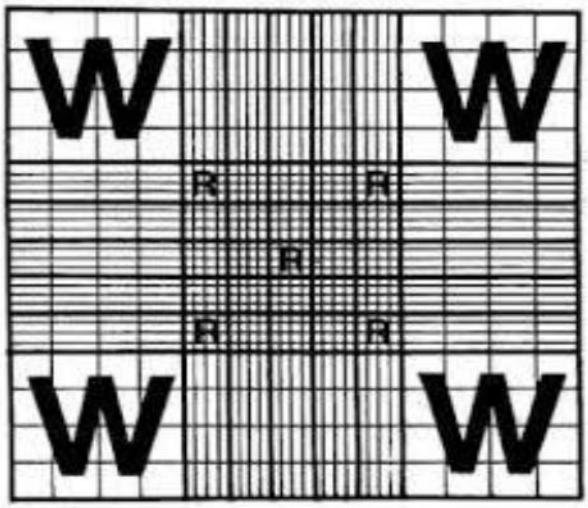
$$\frac{1}{4} \times \frac{1}{4} = \frac{1}{16} \text{ sq.mm.}$$

Each RBC square is divide by triple lines into 25 equal small RBC squares, and each of these 25 small RBC squares are further divided into 16 smallest squares by single lining. Thus whole Central Square is divided into 400 smallest squares the area of each is

$$\frac{1}{20} \times \frac{1}{20} = \frac{1}{400} \text{ sq.mm.}$$

Summary of Neubaur's counting chamber

	AREA	VOLUME OF FLUID
One small WBC square	1 sq.mm	0.1 cmm
One smallest WBC square	1/16 sq.mm	1/600 cmm
One small RBC square(16 smallest RBC squares)	1/25 sq.mm	1/250 cmm
One smallest RBC square	1/400 sq.mm	1/4000 cmm



The dilution fluids

Various dilution fluids are used in haemocytometry but the basic criteria for preparing the dilution fluid is that it should be isotonic to blood plasma. The composition of the dilution fluid depends on other requirements such as staining, fixation etc.

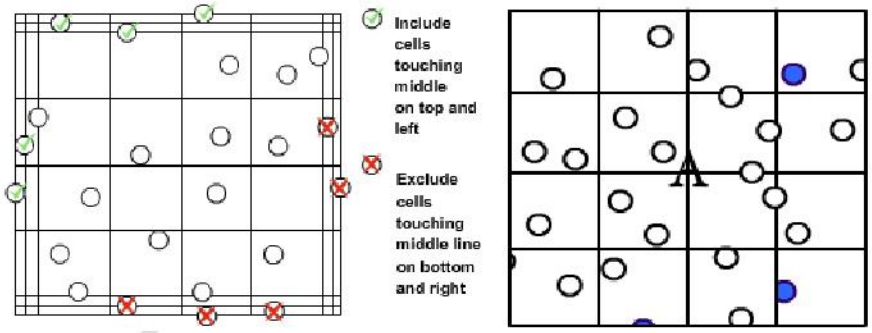
1. Haymen's RBC dilution fluid

SUBSTANCE	AMOUNT	PURPOSE
Sodium Chloride	1.0gm	Provides isotonicity Prevents hemolysis.
Sodium-sulphate	5.5 gm	Provides isotonicity
Mercuric – sulphate	0.5 gm	Causes fixation of the cells, Prevents bacterial growth.
Water	Up to 100 ml	Diluent

2. WBC dilution fluid

SUBSTANCE	AMOUNT	PURPOSE
Glacial acetic	2.0 ml	Destroys RBCs
Gentian or Methyl violet	1.0 ml	Stains nuclei of WBCs
Water	Upto 100 ml	Diluent

NOTE: DO NOT COUNT CELLS ON LININGS.



SIGNATURE OF TEACHER