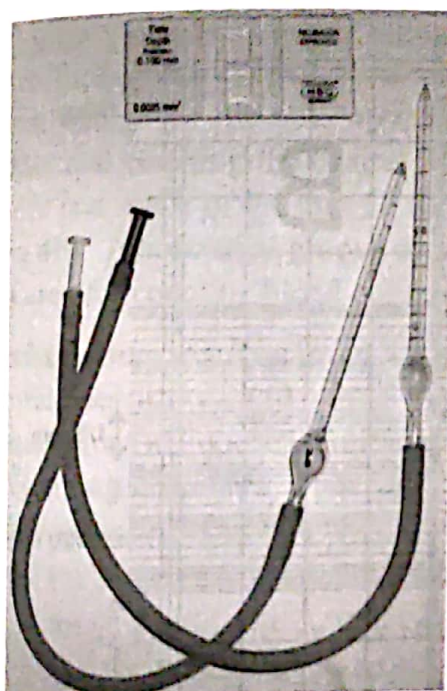


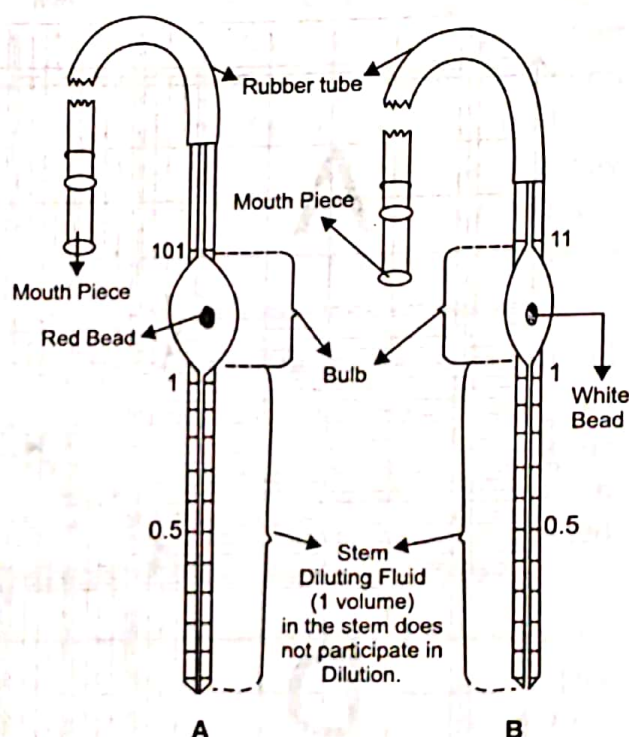
## EXPERIMENT NO. 9

**Aim:** Enumeration of blood cells using haemocytometer.

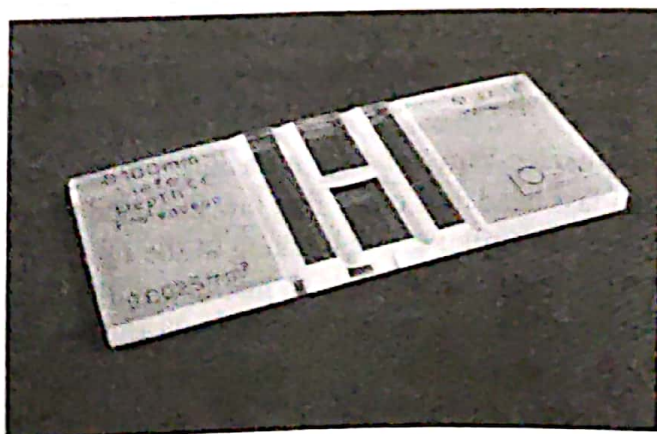
The instrument or device used for counting the number of blood cells is known as **"Thoma Zeiss Haemocytometer"** (Haem = blood, cytometer = cell counting). Though haemocytometer is originally designed for the counting of blood cells, it is now also used to count other type of cells including spermatozoa as well as other microscopic particles. The device is carefully crafted so that the area bounded by the lines along with the depth of the chamber is known. Therefore, it is possible to count the number of cells or particles in a specific volume of fluid and thereby making possible to calculate the concentration of cells in the overall fluid. Haemocytometer apparatus comes in a box. It consists of Haemocytometer slide with cover slip and two Pasteur pipettes as shown in **Figure 1**.



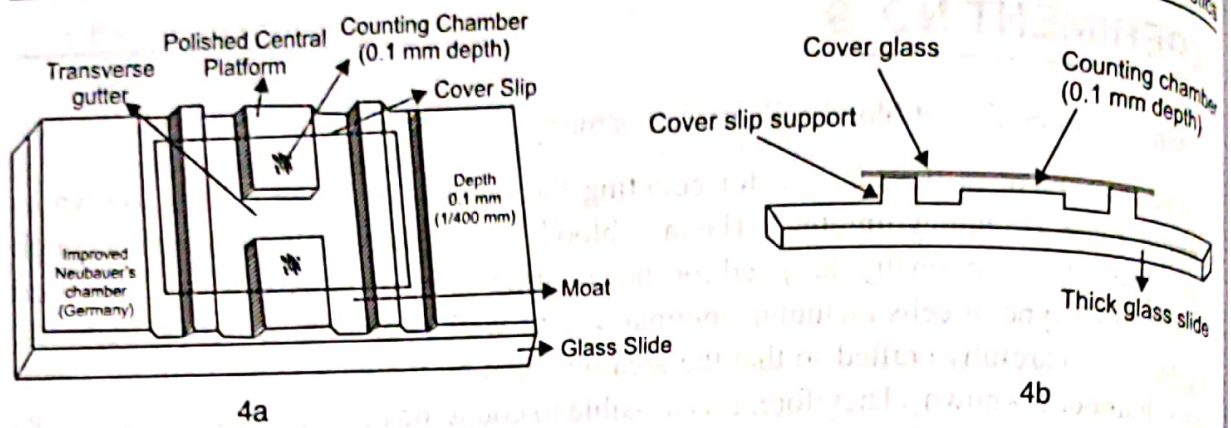
**Figure 1:** Photograph of Haemocytometer Apparatus-A Slide and Two Pasteur Pipettes.



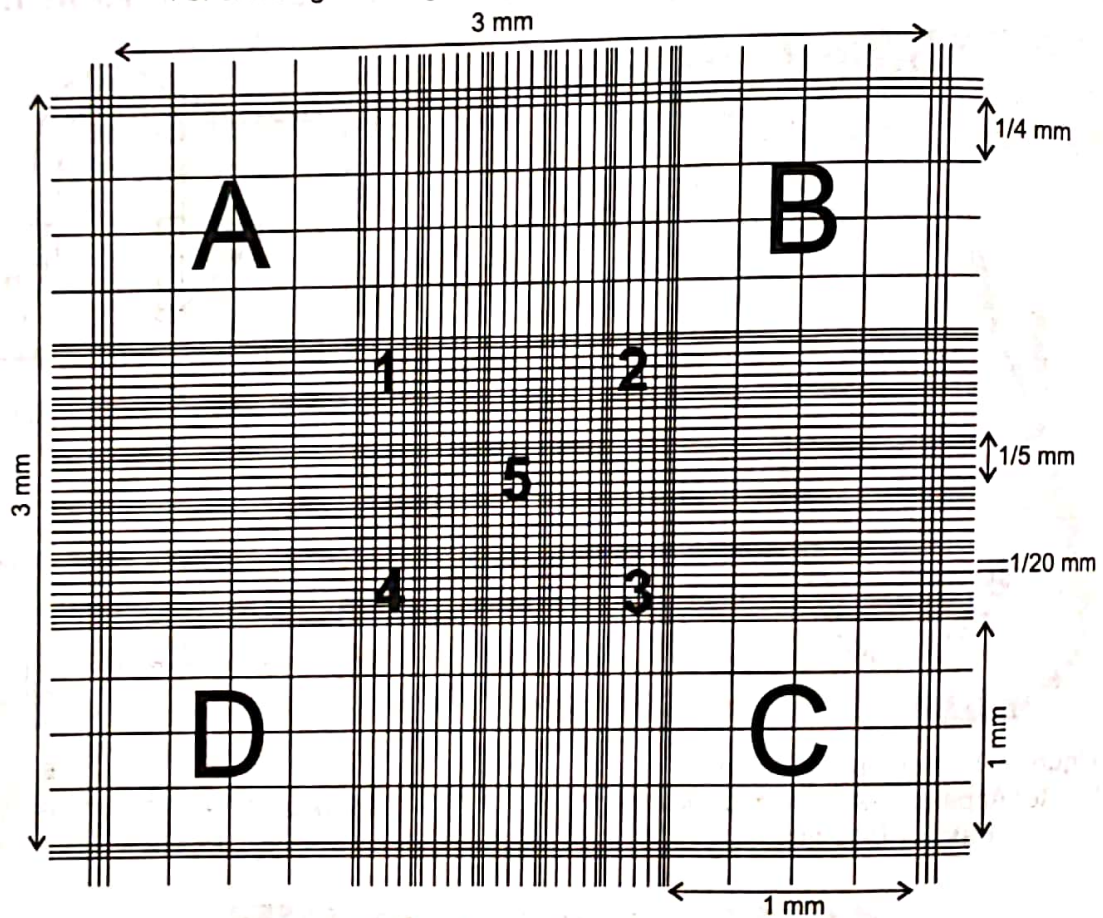
**Figure 2:** A- RBC Pipette; B- WBC Pipette.



**Figure 3:** A photograph of Haemocytometer Slides.



**Figure 4:** Diagrammatic representation of Haemocytometer slide-  
 4 a. Showing counting chambers and moats,  
 4 b. Showing counting chamber having a depth of 0.1 mm.



**Figure 5:** Neubauer Counting Chamber as seen under low power of microscope. Squares marked as A, B, C and D are for WBCs counting and 1, 2, 3, 4 and 5 are for RBCs counting.

- 1. Red Blood Corpuscles Pipette:** It is a pasture pipette with a bulb or reservoir containing a red glass bead thus indicating that it is used for counting of RBCs. The bulb leads into a fine capillary tube opening at the tip. The capillary is graduated lengthwise as "0.5" near the tip, as "1" just below the bulb and as "101" at the top end of the bulb as seen in the **Figure 2**. The capillary towards the bulb end is connected with a rubber tube by which the blood is sucked.
- 2. White Blood Corpuscles Pipette:** It is also a pasture pipette and has similar structure as that of RBCs pipette except a few points. 1- It has a white glass bead



in the bulb or reservoir indicating that it is used for counting WBC's. 2- Its bulb is little smaller in size. And 3- The capillary is graduated lengthwise as "0.5" near the tip, as "1" just below the bulb and as "11" at the top end of the bulb as shown in the **Figure 2**. Like RBC pipette, the capillary towards the bulb end is connected with a rubber tube by which the blood is sucked.

3. **Haemocytometer Chamber:** The haemocytometer was invented by Louis Charles Malassez. The counting chamber was originally invented by Crammer in 1885 and was later modified by Neubauer. It is used to get a very thin film of fluid of known volume for counting of cells.

Haemocytometer consists of a thick glass slide (**Figure 3**) and a perfectly ground cover slip (thicker than the ordinary cover slip to avoid floating). This slide is 3 inches long and 1.5 inches wide with a rectangular indentation that creates a chamber. One side of the haemocytometer slide bears polished central platform bordered on either side by an edge-shaped groove called as moat (**Figure 4a**). On either side of this moat, there is another platform further demarcated by a lateral groove and is slightly higher than the central platform. The cover slip rests upon the two lateral platforms (**Figure 4b**), thus bridging the moats and covering the central platform. The chamber is constructed in such a way that there is a space of 0.1 mm ( $1/10$  mm) between the ruled platform and the cover slip (**Figure 4b**). A horizontal groove divides the central platform into two parts, each having a ruled area for counting blood corpuscles as shown in **Figure 4a**.

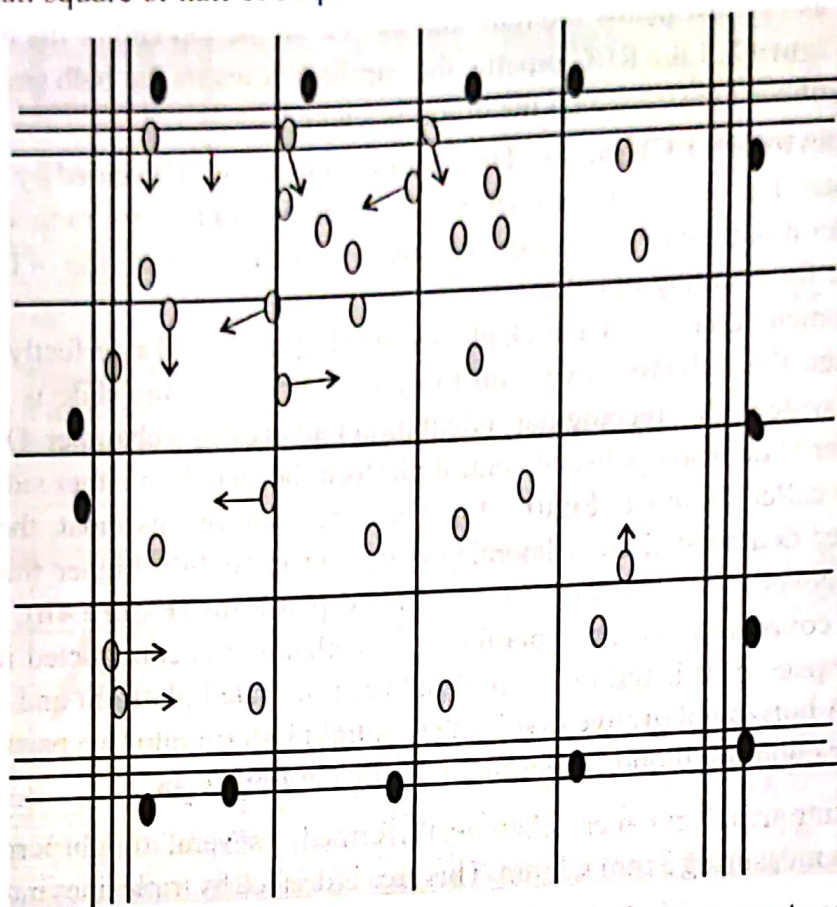
Each counting area (Neubauer's chamber) is formed by several straight perpendicular and horizontal lines measuring 3 mm x 3 mm. This area is divided by triple lines into 9 large equal squares as shown in **Figure 5**. Four squares at the four corners marked as A, B, C and D form the WBC counting chambers, while the central chamber is meant for counting RBCs. Each of these 9 squares is 1x1 mm having a depth of 0.1 mm. This gives each square a defined volume.

Each WBC square with an area (Neubauer's chamber) of 1 square mm (1 mm x 1 mm) at the corners of the ruled area is further divided into 16 small squares (**Figure 5**). The central large square with an area of 1 square mm (1 mm x 1 mm) is further divided by means of triple lines into 25 squares, each with an area of  $1/25$  square mm ( $1/5$  mm x  $1/5$  mm). Each of these 25 squares is again divided into 16 small squares each with an area of  $1/400$  square mm ( $1/20$  mm x  $1/20$  mm). Thus, 1 square mm RBC counting area is divided into 400 small squares; hence one small square has  $1/400$  square mm area. For counting RBCs, only five squares of RBC counting area marked as 1, 2, 3, 4 and 5, four corner ones and a central of five diagonal squares are used (**Figure 5**).

There is a universal rule for counting of cells with haemocytometer in order to avoid counting of same cell twice. According to this rule, any cell which is lying on the left or upper border of a square is counted in that particular square while cells lying on the right or lower border of a square are to be omitted from counting. In addition, the middle of the triple lines is assumed to be the boundary of the square bordered by triple lines. In other words, the cell-sized structures to be counted are those which lie between the middle of the three lines on the top and left of the square and the inner of the three lines on the bottom



and right of the square as shown in Figure 6. A RBC is counted as one only if it is fully entering a small square or half of its part otherwise not.



**Figure 6:** Rule for counting of cells with haemocytometer- The dark cells are not counted while light colour cells are counted as per the arrow in the respective square.

### RELEVANT QUESTIONS

**Q.1. Which cells can be counted in counting chambers of the haemocytometer?.**

**Ans.** The cells which can be counted in the counting chambers of the haemocytometer are all types of blood cells, reticulocytes, bacteria and sperms.

**Q.2. Why is a thick cover slip used in counting the cells in haemocytometer?**

**Ans.** The thick special type of cover slip is used in counting the cells in haemocytometer to avoid any increase in depth due to floating of the cover slip which would otherwise occur if it is thin.

**Q.3. Enumerate the functions of the bead of RBC or WBC pipette.**

**Ans.** The bead in RBC or WBC Pasteur pipette is to identify and differentiate between RBC and WBC Pasteur pipettes. It helps in mixing the blood with the RBCs or WBCs diluting fluid. In addition, the bead also checks the dryness of the interior of the bulb i.e. if the bead sticks to the wall of the bulb, this indicates that the interior of the pipette is not dry.