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# Practical Physiology

## A New Approach



Sameer Mohommed Khan

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Chapter

# 4

# Hemocytometry

## INTRODUCTION

Hemocytometry refers to the process of counting of the blood cells. The apparatus used for this purpose is called hemocytometer.

## PRINCIPLE

Since the number of blood cells is very high it is difficult to count them even under the microscope. To enable easy counting of the cells the process of diluting the blood is carried out with the help of a diluting fluid. The counting chamber is then charged with the mixture of diluting fluid and blood. Finally the cells are counted under the microscope.

## CONSTITUENTS OF HEMOCYTOMETER

1. The Diluting pipettes which are used for the purpose of dilution of blood.
2. The counting chamber using which the cells are counted.
3. Coverslips.
4. RBC and WBC diluting fluids.
5. Watch glasses.

### Diluting Pipettes

The diluting pipettes consists of following parts

1. *The stem*: It is the long and narrow part of the pipette and is divided into 10 equal parts. There are 2 graduations or markings on it. The markings differ in RBC and WBC pipette. In the RBC pipette the markings are 0.5, 1 and 101 whereas in the WBC it is 0.5, 1 and 11.
2. *The bulb*: It is the widened part of the pipette and contains the bead. The color of the bead is different for both the pipettes. In case of RBC pipette the color of the bead is red and in the WBC pipette the color is white thereby making the identification of the pipette possible. The bead has 3 functions:
   - The presence of the bead facilitates the mixing of the blood with its diluent
   - It tells you whether the pipette is dry or wet and
   - It also helps in identifying the pipette.

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3. *Rubber tube and the mouth piece*: The bulb narrows again into a short stem to which a long narrow soft rubber tube bearing a mouthpiece is attached. The color of the mouth piece is also one of the identifying points. It is red in the RBC pipette and white in WBC pipette (Fig. 4.1 and Table 4.1).

RBC Pipette (Thoma glass pipette): It consists of capillary tube, central tube and three graduation marks. Capillary tube is opened at both ends. One end of the tube is narrow while the other end is broad. For sucking blood a rubber tube fixed to the broad end is used. This is a bulb pipette having a long stem with a capillary bore and a pointed tip. The bulb contains a red bead inside. A small rubber tube provided with a mouthpiece is connected to the small narrow portion above the bulb for sucking blood and fluid into the pipette.

The pipette has three markings on it, 0.5 mark in the middle of the stem, 1 mark at the junction between stem and bulb and 101 mark above the bulb. The total volume of the pipette is 101 parts, of which one part is in the stem and blood is drawn to 0.5 mark and then diluting fluid is drawn up to the mark 101. The dilution of blood is 1:200.

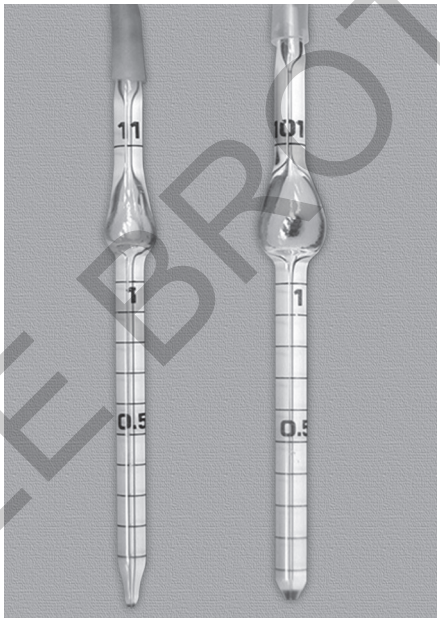


**Figure 4.1:** Figure depicting the RBC and WBC pipette (*For color version, see Plate 1*)

**Table 4.1:** Difference between RBC and WBC pipette (important for OSPE)

| ***Features*** | ***RBC pipette*** | ***WBC pipette*** |
|---|---|---|
| Markings | 0.5,1 and 101 | 0.5,1 and 11 |
| Color of the bead | Red | White |
| Color of the mouth piece | Red | White |
| Capacity of the bulb | 100 | 10 |
| Dilution factor | 1:200 | 1:20 |
| Lumen diameter | Less | More |

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## The Counting Chamber

The counting chamber as the name suggests is used to count the cells. Although there are many counting chambers, many specific as well for certain type of cells, one of the most commonly used is improved Neubauer's counting chamber (Fig. 4.2).

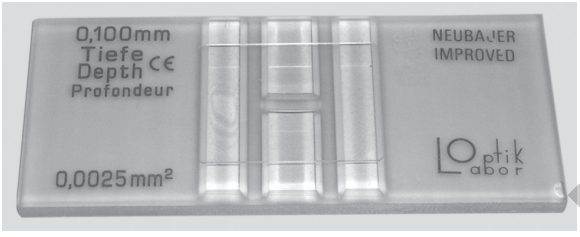


**Figure 4.2:** Improved Neubauer's counting chamber

### *Improved Neubauer's Counting Chamber*

The counting chamber in simple terms is a thick glass slide. The central platform also called as the floor piece is wider and is exactly 0.1 mm lower than 2 lateral pillars. This forms the depth of the counting chamber. The floor piece is divided into 2 equal parts by a short transverse trench in its middle.

On the central platforms are engraved ruled squares which are specific to the RBC and WBC and hence, used for counting.

Each counting chamber is divided into 9 large squares of 1 mm², each has an area of 9 mm² (3 × 3 mm).

Of these 9 squares, 4 corner squares is used for WBC counting and 1 central square is used for RBC counting (Fig. 4.3).

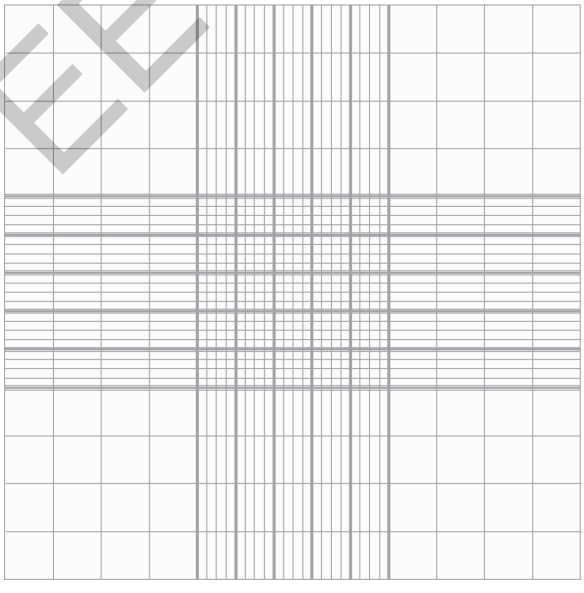

**Figure 4.3:** Counting areas in the Neubauer's chamber

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### The WBC square (Fig. 4.4)

Each of the 4 larger corner squares are further divided into 16 medium-sized squares each of which has a side of 1/4 and an area of 1/16. These 4 larger corner squares are used for WBC counting and hence, known as WBC squares.

WBC square: Let us now calculate the volume of WBC square—

Each WBC square is further divided into 16 small squares

So area of 1 medium square is L × B

That is 1/4 × 1/4 = 1/16

The depth of the chamber is 0.1mm or 1/10 mm

Volume of 1 medium square is L × B × H

That is 1/16 × 1/10 = 1/160 $mm^3$

In total there are 64 squares

So the volume of 64 squares will be 64 × 1/160 = 4/10 $mm^3$

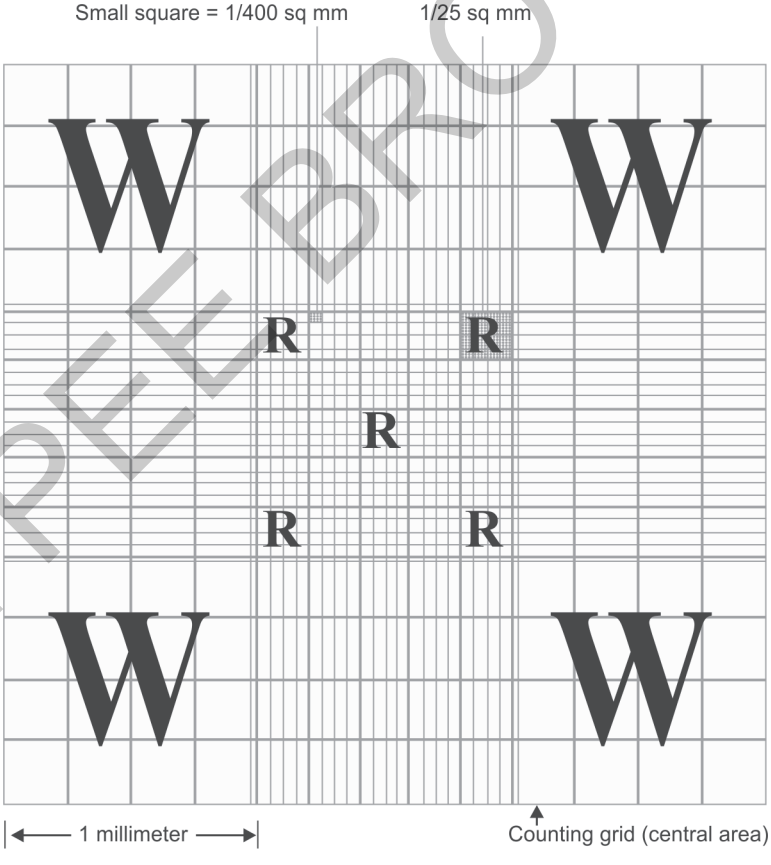


**Figure 4.4:** WBC-counting area and RBC-counting area

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### The RBC square (Fig. 4.4)

The central RBC square is divided further into 25 medium-sized squares each of which has a side of 1/5 and an area of 1/25. Each of these are further divided into 16 small squares by triple lines. Thus, each smallest square has a side of 1/5×1/4 that is 1/20 mm². The red cell are counted in 4 corner groups and one central group of medium squares each of which has 16 smallest squares that is in total 80 smallest squares.

Calculation of the volume of RBC square:

Each medium square is divided into 25 small squares

Each of the medium squares has a side of 1/5 × 1/4 = 1/20

Area of smallest square is L × B

That is 1/20 × 1/20 = 1/400 mm²

Volume of the smallest square will be L × B × H

That is 1/400 × 1/10 = 1/4000 mm³

Volume of 80 small-sized squares will be 80 × 1/4000 = 1/50 mm³.

The process of counting the cells in the Neubauer's chamber includes two important preliminary steps which are as follows:

### A. Pricking the finger

1. Clean the fingers with the antiseptic spirit provided. Do not use excess of spirit. Allow the spirit to dry for more efficient action.
2. It is common to use any of the 3 fingers for the pricking namely index, ring or the forefinger. The thumb and little finger are preferably avoided.
3. Use a sterile lancet for pricking.
4. With the help of the lancet prick the finger deeply so as to ensure free flow of blood.
5. Remember the prick should be deep otherwise you will be just pricking the tissue.
6. Allow the blood to flow freely.
7. Wipe off the first drop of blood and allow the other drop of blood to form.
8. Insert a clean pipette entirely into the blood drop to prevent entry of air bubbles.
9. This step should be done quickly otherwise the blood drop will clot in which case you might have to prick again.
10. Suck the blood up to the mark of 0.5 or 1 in the pipette as the case may be for RBC and WBC pipette.
11. If you have sucked in excess from the mark tap the pipette against your palm to bring it to the level of 0.5. Do not use cotton or filter paper as it might suck of excess of blood.
12. Immediately take in the diluting fluid into the pipette to avoid clotting of the blood inside the pipette.
13. Mix both the blood and the fluid gently by keeping the pipette horizontally in between the palms of both the hands. Do not mix vigorously as it might lead to mechanical hemolysis of the cells.
14. Keep the pipette aside for 1–2 mins.

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### B. Charging of the Neubauer's chamber

1. Clean the Neubauer's chamber and the coverslip.
2. Place the coverslip on the central platform of the chamber.
3. Mix the contents of the bulb thoroughly.
4. Discard the first 2 drops of fluid from the pipette as it contains only the diluting fluid.
5. Place the tip of the pipette on the surface of the chamber touching the edge of the coverslip at an angle of 45°. Be aware that the angle slightly varies when you use a WBC pipette.
6. Allow the diluted blood to flow under the coverslip by capillary action.
7. Remove the pipette quickly from the edge of the coverslip as soon as the counting chamber is filled with blood.
8. The fluid should cover the entire counting area and not flow into the gutters.
9. Any excess of fluid in the counting area will enter the gutters and it is called overcharging.
10. Any decrease in the amount of fluid in the counting area so that it doesn't fill the counting area completely is called undercharging.
11. Both overcharging and undercharging should be avoided.
12. Ideally both the sides of the counting area are charged.

## IMPORTANT POINTS FOR EXAMINATION

1. Read about the differences between the RBC and WBC pipette.
2. Read about the counting areas for RBC and WBC count.
3. Read about the difference between the RBC and WBC squares.

## QUESTIONS

1. What are the uses of the hemocytometry in hematology?
2. Enumerate the differences between RBC and WBC pipette.
3. What are the precautions to be taken while pipetting?
4. How do you ensure that the blood sucked is exactly up to the 0.5 mark in the pipette?
5. How do you prevent entry of air bubbles into the pipette?
6. How do you calculate the area and volumes of RBC and WBC squares?
7. What are the steps to be followed while charging the Neubauer's chamber?
8. What are the precautions to be followed while charging the chamber?
9. Why are 2 drops of fluid discarded from the pipette before charging?
10. What do the terms overcharging and undercharging mean?

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