

Experiment #: 3

Determination of red blood cell (RBC) count

APPARATUS

Neubauer's chamber (thick slide), RBC diluting fluid, microscope, coverslip, pricking needle (blood lancet) and spirit swab.

RBC Diluting Fluid (Hayem's Fluid) → *Grower's Solution*

1. Sodium chloride (NaCl): 0.5 gm, to maintain isotonicity of fluid.
2. Sodium sulphate (Na₂SO₄): 2.5 gm, which breaks RBCs and prevents their rouleaux formation.
3. Mercuric chloride (HgCl₂): 0.25 gm act as preservative (antibacterial and antifungal)
4. Distilled water (H₂O): 100 ml. For dilution

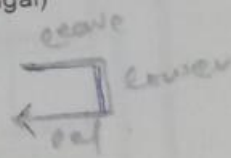
RBC COUNTING METHODS

1. Electronic counting method
2. Visual counting method by Thomas Rule (Leave, Lower, Left) **PRINCIPLE**

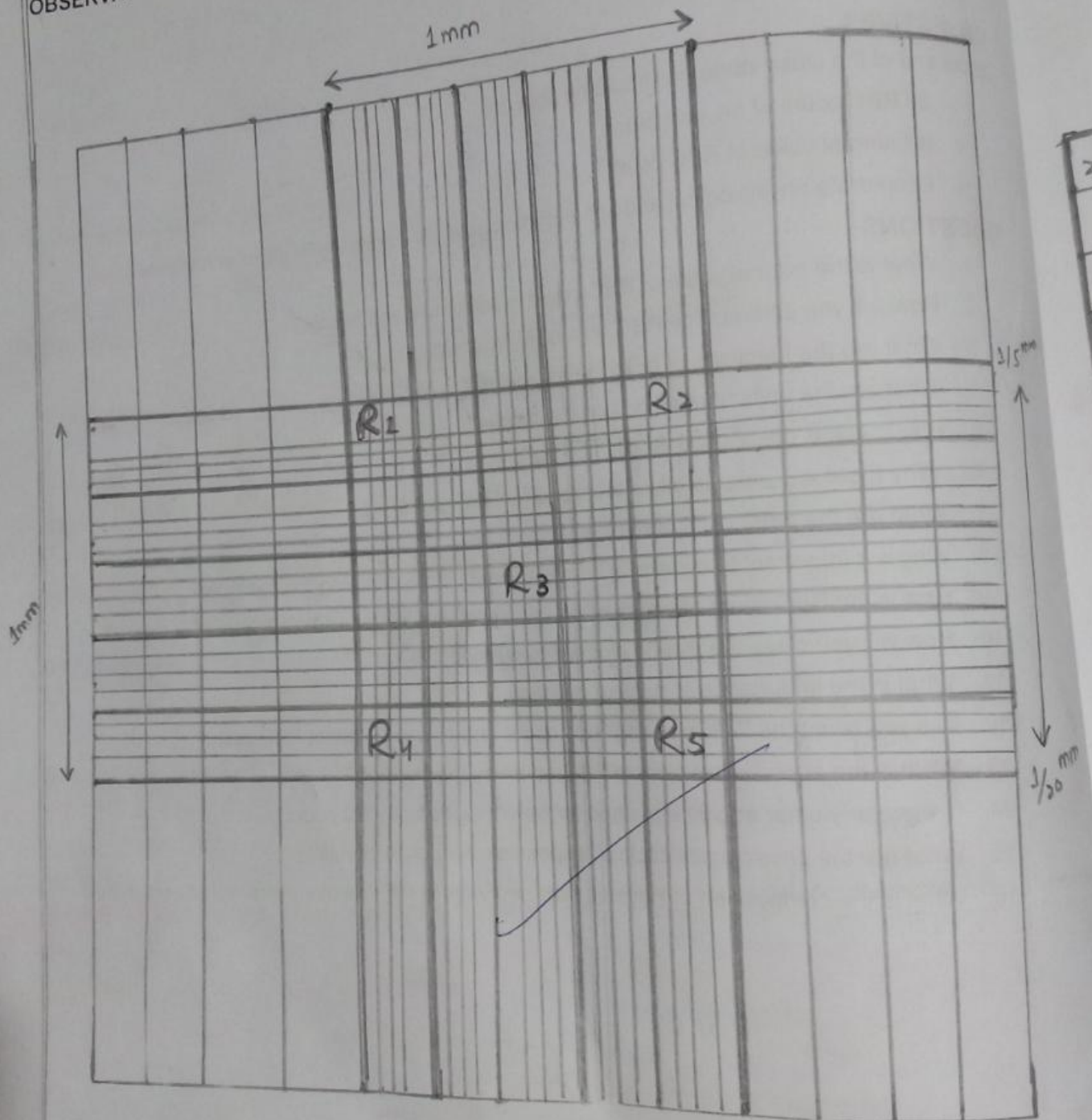
The number of RBC is very high so blood is diluted 200 times with diluents. Red blood cells are counted in diluted blood and actual count is calculated by multiplying by dilution factors. So the product of the number of cells calculated per cu mm (mm^3) diluted blood and the dilution factor gives the number of red blood cells per cu mm in undiluted blood.

PROCEDURE

1. Take about 3-5 ml Hayem's fluid in a watch glass.
2. Prick the ring finger after cleaning it with spirit swab.
3. Wipe off the first drop of blood. Suck the next drop in RBC pipette exactly up to 0.5 mark, taking care that there should be no air bubble. If excess blood has been drawn, remove it by touching the pipette on the cotton swab very carefully.
4. Wipe off the blood sticking around the tip of the pipette with cotton swab.
5. Now suck the haymes fluid in the pipette up to mark 101.
6. The pipette is then kept horizontally between palms and rolled gently for a minute to mix the blood with diluting fluid.
7. Focus Neubauer's chamber under low power (10 x) objective of microscope.
8. remove the chamber form microscope and place a coverslip on it.
9. Discard first-2 drops of fluid from the pipette which is unmixed fluid present in the stem of the pipette.
10. Charge Neubauer's chamber:
 - i. Small drop of fluid is allowed to form at the tip of pipette.
 - ii. Bring the top of the pipette near the edge of the cover slip on central platform in such a way that it will make an angle of about 45° with central platform



OBSERVATIONS AND CALCULATIONS



EXPER
OBSER

2	6
6	11
3	5
3	6

OBSERVATIONS AND CALCULATIONS

Dilution :-
$$\frac{\text{Total Volume of bulb (100 parts)}}{\text{Volume of blood taken (0.5 parts)}} = \frac{100}{0.5} = 200$$

→ Area of medium size square (R)
$$\Rightarrow \frac{1}{5} \times \frac{1}{5} = \frac{1}{25} \text{ mm}^2$$

→ Depth of chamber = $\frac{1}{10} \text{ mm}$

→ Volume of each medium size square (R)
$$\Rightarrow \frac{1}{25} \times \frac{1}{10} = \frac{1}{250} \text{ mm}^3$$

→ Total volume of 5 square = $(R_1 + R_2 + R_3 + R_4 + R_5)$

$$= \frac{1}{250} \times 5 = \frac{1}{50} \text{ mm}^3$$

→ Suppose total no. of cells counted in five $(R_1 + R_2 + R_3 + R_4 + R_5)$ squares = 510

→ No. of cells counted in $\frac{1}{50} \text{ mm}^3$ in diluted blood = 510

No. of cells counted in 1 mm^3 diluted blood.

$$510 \times 50 = 25,500$$

→ No. of cell in 1 mm^3 undiluted blood

$$= 25,500 \times 200$$

$$= 5,100,000$$

RBC count = 5,100,000

EXPE
OBS

Ans 1 :-

Ans 2 :-

Ans 3

Ans 4

Ans 5

Ans 6

OBSERVATIONS AND CALCULATIONS

Ans 1 :- In adult male = 5.5 million/mm³ (5-6 million)
 In adult female = 4.8 million/mm³ (4.5-5.5 million)

Ans 2 :- (i) In RBC pipette, there will be a red bead while in the WBC pipette the bead will be white.
 (ii) In the RBC pipette the graduation are upto 101 and in the WBC the graduations are upto 11.
 (iii) The size of bulb is larger in RBC pipette when compared to the size of WBC pipette.

Ans 3 :- The beads help in mixing the contents of bulb thoroughly. It help in identifying the pipette at the distance. And thirdly it tells whether the bulb is dry or not (if it is not the bulb will not roll freely).

Ans 4 :- The markings don't represents any unit but relative volumes relation to each other.

Ans 5 :- Blood is diluted 200 times dilutant in order to make the RBC countable (in diluted blood).

Ans 6 :- Dilution is needed b/c the no. of RBC's are very high.

STUDENT'S NOTES

12-1

In case of high leucocyte count as in leukemia, dilution may have to be increased to 100 times or more, so RBC pipette is used for this purpose.

13-00

The first few drops of the fluid are discarded before charging bcz they don't contain RBCs.

13-01

When the chamber is overcharged, diluted blood flows into the chamber, where the red cells being heavier sink down giving a low count. Under charging due to less blood in the chamber will also give low count.

13-00 Ans

To remove particles of coagulated blood or dirt, a cleaning soln should be used. It is best to fill the pipette with cleaning soln and allow it to stand overnight. NaCl or detergent should be used. H_2O_2 is also useful in difficult cases. Use HNO_3 .