Experiment → 5

Aim :→ To determine the number of red blood cells in one cubic mm volume of blood.

Requirements :- Haemocytometer, Compound microscope, fresh needle for pricking alcohal, Hayem's solution.

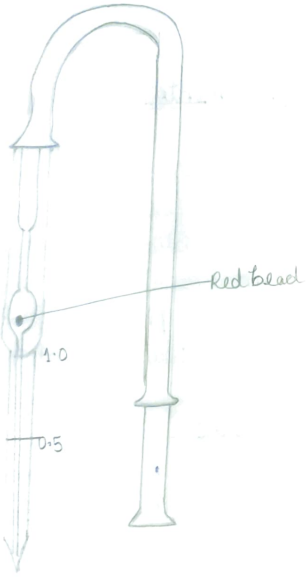
Hayem's solution has the following composition.

i) Mercuric Chloride ($HgCl_2$) : 0.5 g
ii) Sodium Chloride ($NaCl$) : 1.0 g
iii) Sodium Sulphate (Na_2SO_4) : 5.0 g.
iv) Distilled Water (H_2O) : 200 ml.

Procedure

1. Rinse the R.B.C pipette with alcohal and allow it to dry.
2. Wipe a finger with cotton dipped in alcohal or Spirit.
3. prick the finger quickly and gently with a Sterilized needle.
4. With the help of RBC pipette Suck the Oozing blood up to 0.5 mark.
5. Wipe the Outer Surface of the pipette Carefully to remove any traces of blood.

Teacher's Signature: _____



R·B·C pipette

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6. Suck Hayem's solution into the pipette upto 101 mark, causing a dilution of 200 times.
7. Hold the pipette horizontally between the right and left palms and Rotate it so that blood mixes well with Hayem's solution.
8. Place the cover slip on the glass slide allowing it to rest on the lateral platforms.
9. Place the slide carefully on the stage of the microscope and raise the objective lens as high as possible.
10. Place the tip of the pipette between the coverslip and the central platform and allow a few drops of diluted blood to flow on to the counting number.
11. Repeat for the other counting chamber.
12. Care should be taken while filling the counting chambers to avoid any diluted blood from flowing into the grooves.
13. Keep the slide undisturbed for a few minutes to allow the RBCs to settle down on the bottom of the counting chambers.
14. Lower the lens of the microscope and count the cells.

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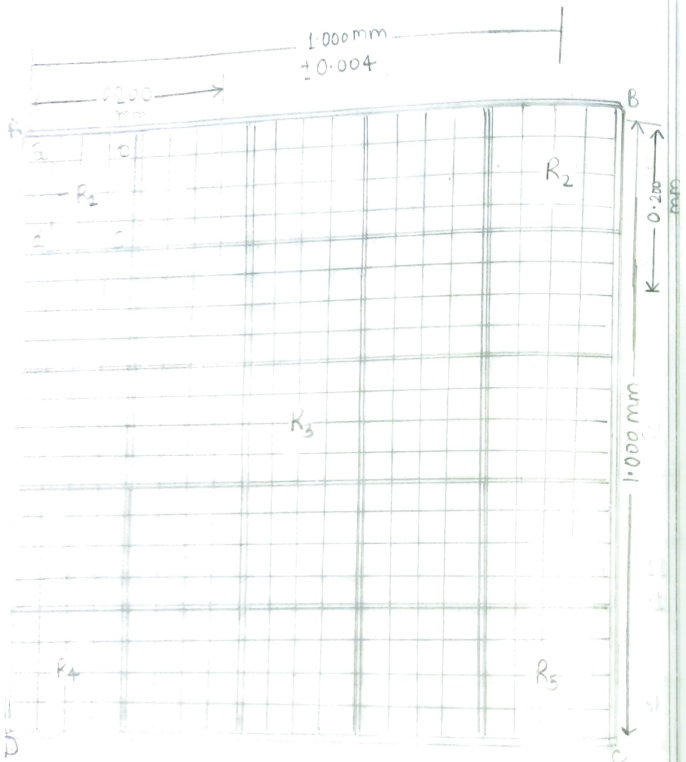

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Counting of RBCs

1. Out of the 9 squares of the counting chamber only the central one is used for counting RBCs.
2. This square is divided into 25 smaller squares. Out of these 1st, 5th, 13th, 21st and
3. The RBCs lying on the lower and right side or edge of a square are added in the total.
4. The RBCs lying on the upper and left side or edge are not included in the total.

Calculation

The central RBC square has a side of 1 mm and a depth of 0.1 mm.

No. of RBCs/mm^3 of blood.

$$= \frac{\text{No. of cells counted} \times \text{Total no. of smallest squares} \times 10 \times \text{dilution}}{\text{No. of small squares counted.}}$$

No. of cells counted

$= R_1 + R_2 + R_3 + R_4 + R_5 = N.$

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Dilution = $\frac{101}{0.5}$ = 200 times.

Total no. of smallest squares = 25 X 16 = 400
No. of smallest Squares Counted = 5 X 16 = 80
Since,
the R.B.C Square is placed lowly by 0.1 mm, We multiply by 10 to make the value 1 mm^3.

No. of RBCs/mm^3 = $\frac{N \times 200 \times 400 \times 10}{80}$

= N X 10000.

To avoid lengthy calculations simply add 4 across to the Value of N.

Result

The number of RBCs, depending on the source of blood is approximately as follows.

Source	Approximate no. of RBCs/mm^3 of blood
frog	1.0 to 1.5 million.
Male humans	4.2 to 5.4 million.
female humans	3.6 to 5.0 million.
New borns	5.0 to 6.5 million.

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In the Example shown

$N = (R_1 + R_2 + R_3 + R_4 + R_5)$

$N = 81 + 80 + 85 + 87 + 87.$

$N = 420$

No. of RBC /mm^3 = 42,00,000

= 4.2 million / mm^3.

Precautions

1. Clean and dry the slide and pipette before and after use.
2. Hayem's solution should not be sucked beyond 101 mark.
3. Care should be taken to prevent Overflowing of the Counting Chamber.

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Pratical-6

Estimation of WBC Count in blood by Haemocytometer.

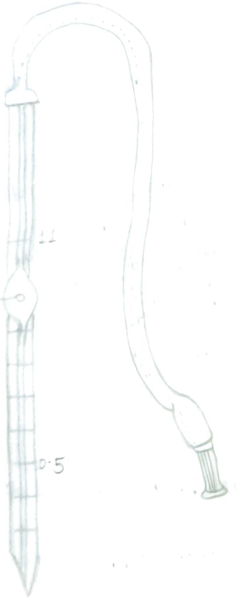
Aim:→ To Estime total WBCs in blood by haemocytometer.

Requirements:→ Haemocytometer, Compound microscope, spirit, Cotton, Sterilized needle, diluting-fluid (Turk's fluid). prepare diluting fluid by adding 3 ml glacial acetic acid and 1% (W/V) gentian Violet to 96 ml distilled Water.

procedure

1. Rinse the WBC pipette With alcohol and allow it to dry.
2. Wipe a finger With cotton dipped in alcohol or spirit.
3. prick the finger Quickly and gently With a sterilized needle.
4. Using the WBC pipette Suck the Oozing blood up to 0.5 mark.

Teacher's Signature: _____



W B C pipette

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5. Wipe the outer surface of the pipette carefully to remove any traces of blood.
6. Immediately suck the diluting fluid into the WBC pipette upto the 11 mark.
7. Hold the pipette between the palms and rotate it gently so that blood mixes well with the diluting fluid.
8. Place the cover slip on the glass slide allowing it to rest on the lateral platforms.
9. Place the slide carefully on the stage of the microscope and raise the objective lens as high as possible.
10. place the tip of the pipette between the coverslip and the central platform and allow a few drops of diluted blood to flow on the counting Chamber.
11. Repeat for the other counting Chamber.
12. Care should be taken to fill the counting Chambers without any diluted blood flowing into the grooves.
13. Keep the slide undisturbed for a few minute to allow the WBCs to settle down on the bottom of the counting Chambers. Lower the lens and count the cells.

Teacher's Signature:

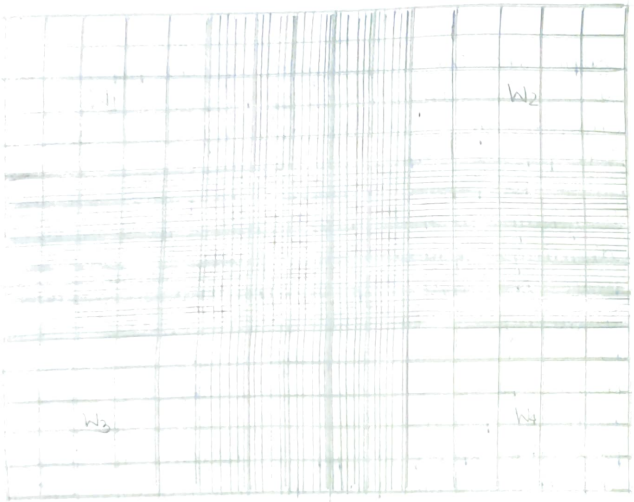

Fig: The counting area used for WBCs

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Counting the WBCs

- The WBCs are counted in the 4 corner squares.
- They are recognized by the slight colour given to them by gentain violet and by their refractile appearance.
- The WBCs that touch the boundary lines of the square are not counted.

Calculations:-

WBCs /mm^3 of blood

$$= \frac{\text{No. of WBCs counted} \times \text{dilution} \times 10}{\text{No. of } 1\,mm^2 \text{ squares counted.}}$$

$$\text{Dilution} = \frac{11}{0.5} = 20 \text{ times.}$$

One square has a side of 1mm and a depth of 0.1mm ∴ the number of WBCs is multiplied by a factor of 10 to make the volume $1\,mm^3$.

Eg. Let us suppose that the number of WBCs in the 4 squares are 20, 23, 27, 25=

$$\text{WBCs}/mm^3 \text{ of blood} = \frac{95 \times 20 \times 10}{4} = 4750.$$

Teacher's Signature

Result

The Normal WBCs number varies from 4000 - 6000 / mm^3. In a healthy person.

precautions

1. Clean the slide and pipette before and after use.
2. the diluting fluid should be sucked up to the 11 mark.
3. Care should be taken to prevent Overflowing of the counting Chamber.