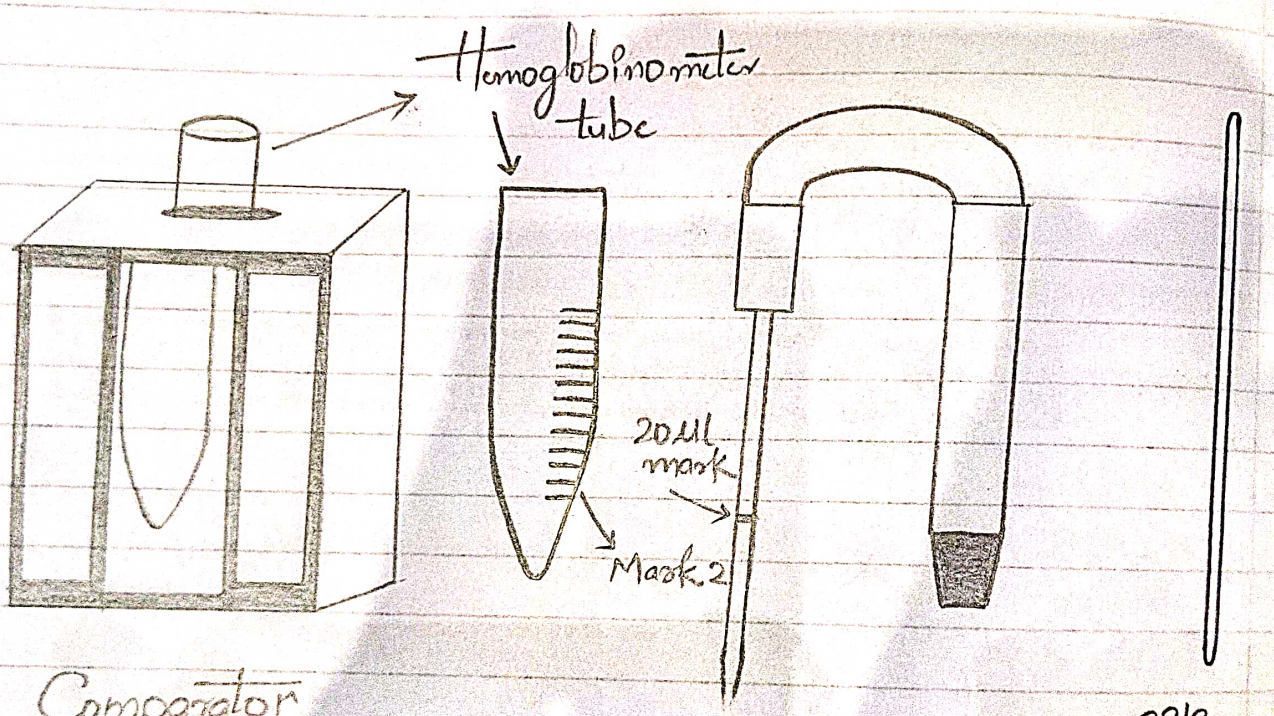
Sahli's Hemoglobinometer.



Date: .....

Expt. No.....01..... Page No. ...01.....

Estimation of human blood Sample for Hemoglobin by Sahli's/acid hematin Method

Principle: Blood is mixed with N/10 HCl resulting in the conversion of Hb to acid hematin which is brown in color. The solution is diluted till it's color matches with the brown colored glass of the comparator box. The concentration of Hb is read directly.

Hemoglobin + (0.1N) HCl → Acid hematin (brown colour)

→ The brown color of compound is matched against a brown glass standard in a comparator.

Equipment required.

→ Hemocytometer which consists of:

1. Comparator box which has brown colored glass on either side
2. Hb pipette which is marked up to 20 mm^3 (0.2ml blood)
3. Tube with markings of Hb on one side
4. glass rod
5. dropper.

Reagents required:

- N/10 HCl
- Distilled water.

Signature .....

Date: .....

Expt. No..... Page No. ...02.....

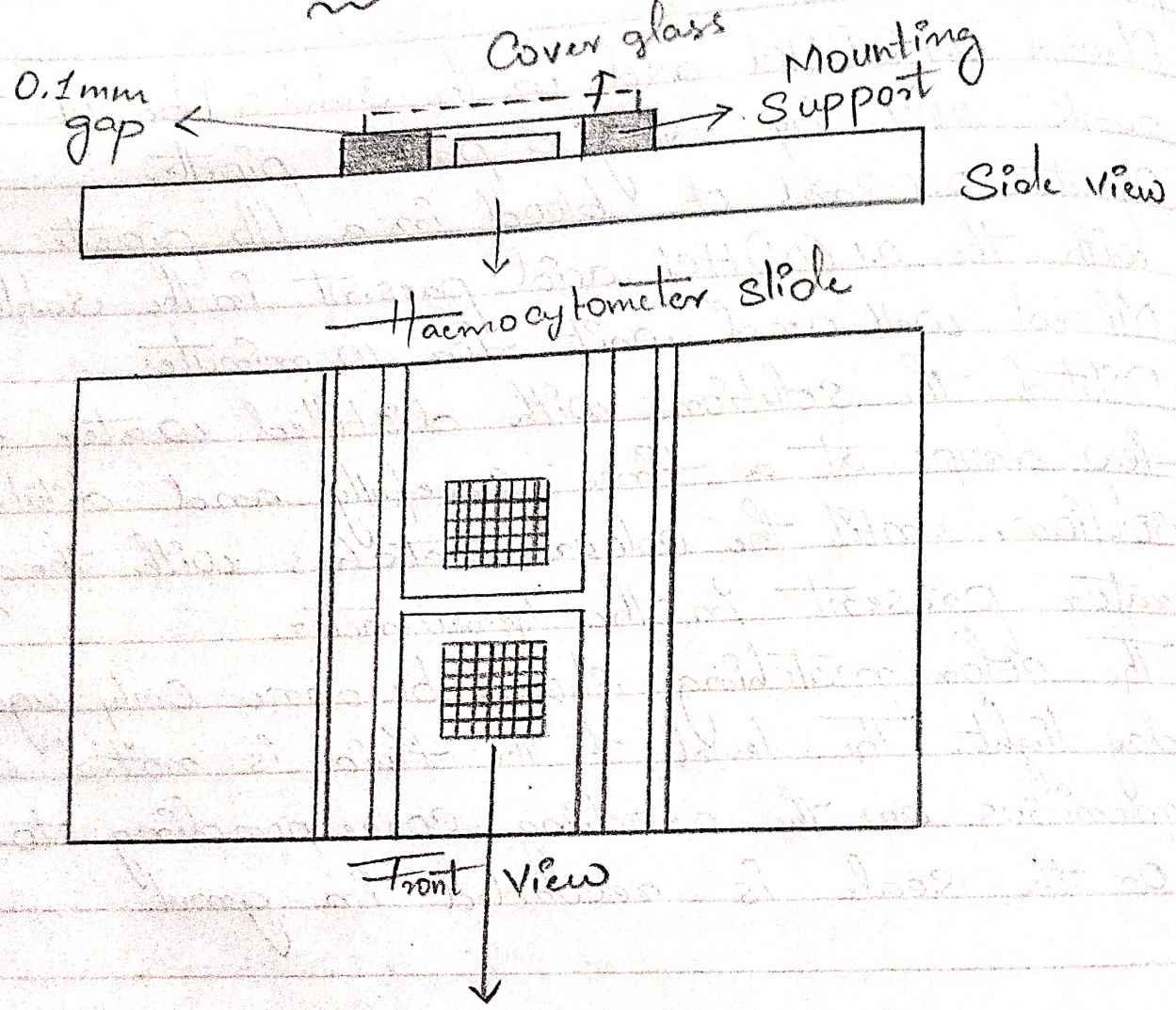
Sample:
Venous blood collected in EDTA as described earlier.

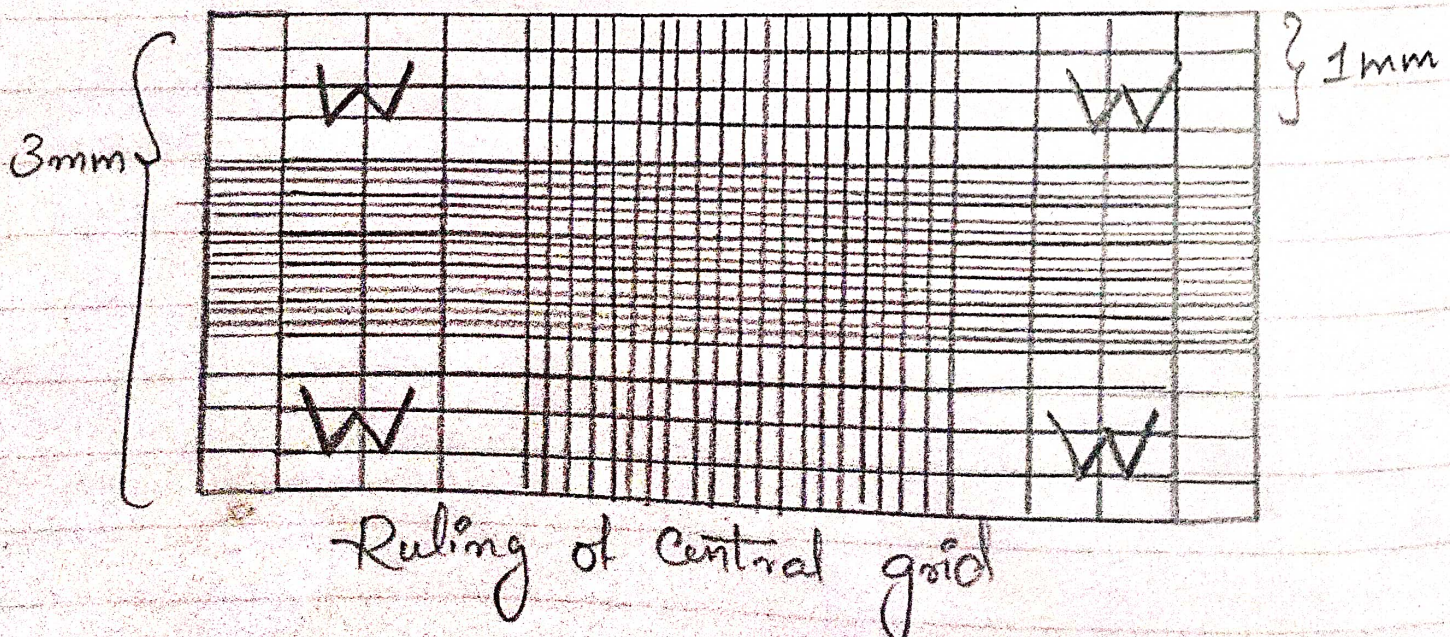
→ Procedure:

1. Placed 0.1 (N) HCl acid in the Sahli's tube up to the lowest mark 20% by using a pasteur pipette.
2. Pipette 0.02ml of blood in a Hb-pipette and added with the 0.1 (N) HCl acid present in the Sahli's tube. Mixed well and wait for 10 minutes.
3. Diluted the solution with distilled water by adding few drops at a time carefully and diluted the solution, until the colour matches with the glass comparator present in the hemometer.
4. The color matching should be done only against natural day light. The level of the fluid is noted at his its lower menisus on the reading corresponding to this level on the scale is recorded in gm/dl.

Signature .....

Neubauer's Chamber





Expt. No. 02 Date:

Page No. 03

Estimation of Total Red blood corpuscles (RBC) Count.

Principle: The basic principle is that the blood specimen is diluted (usually 200 times) with red cell diluting fluid which does not remove the white blood cells but allows the red cells to be counted under magnification in a known volume of fluid. Finally, the number of cells in undiluted blood is calculated and reported as the number of red cells / µl of whole blood.

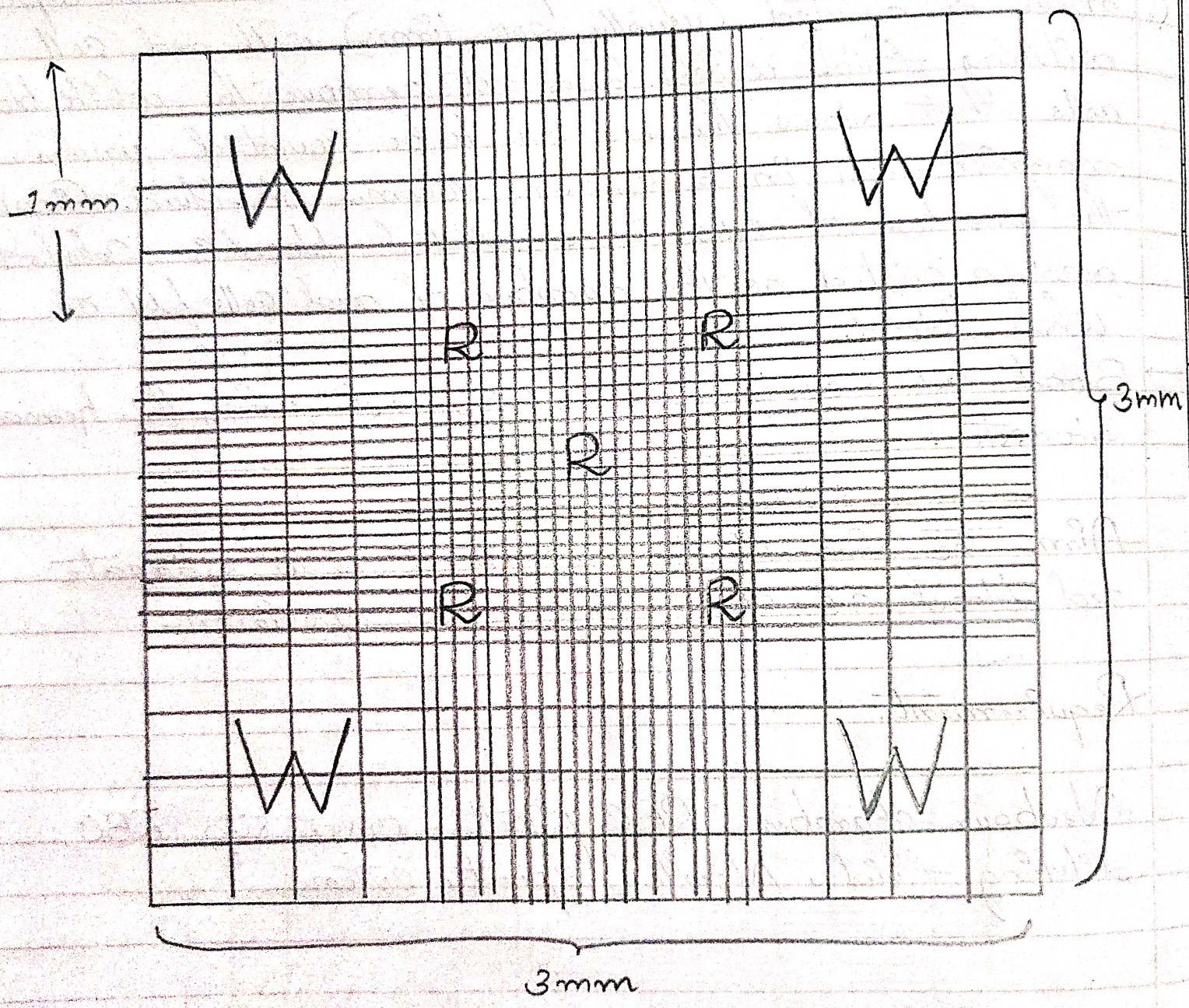
→ Blood cell counts can be performed using the hemacytometer.

Aim: The aim of the experiment is to estimate red blood cell count of a blood specimen.

Requirement:

Neubour chamber, RBC pipette, cover slip, RBC diluting fluid, Needle, spirit, cotton.

Signature .....


1mm
W
W
R
R
R
R
R
W
W
3mm
3mm

Date: .....

Expt. No..... Page No. ...04.....

Procedure:

1. Sterilise the finger tip with cotton plug soaked in spirit and let it dry.
2. Take a bold pick with needle to have free flow of blood and draw the blood in a RBC pipette upto 0.5 mark.
3. Dip the RBC pipette in red blood cell diluting fluid and suck up diluting fluid upto 101 mark
4. Rotate the pipette equally in your hands to mix the solution well by swirling.
5. Take the hemocytometer and place it on the flat surface of the work bench. Place the cover slip on the counting chamber
6. Allow a small drop of diluted blood, hanging from the pipette, to sweep into the counting chamber by capillary action. Make sure that there is no air bubble and the counting chamber must not be flooded
7. Leave the counting chamber on the bench for 3 mins to allow the cells to settle. Observe the cells by placing the counting chamber on the mechanical stage of the microscope.
8. Focus on the centre room of the chamber and start counting the cells from upper left corner of the room. It is advisable to complete all 4 counts of the four squares and then move to the centre square, which is the fifth square to be counted.

Signature .....

Date: .....

Expt. No.....

Page No. ...05.....

Data Analysis:

$$\text{No. of cells} \times \text{Dilution factor} \times \text{Depth factor} \times \frac{\text{Total ruled area}}{\text{Area Count}}$$

where,

Dilution factor = 200 ; Depth factor = 10 ;

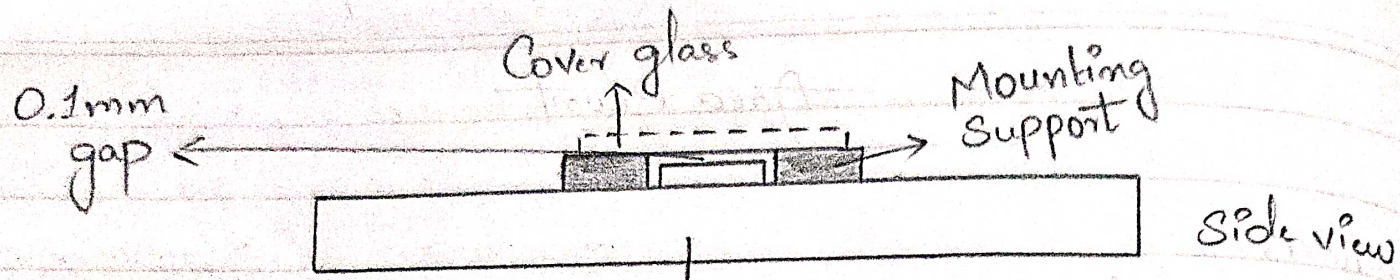
Total ruled area = 25 ; Area Count = 5

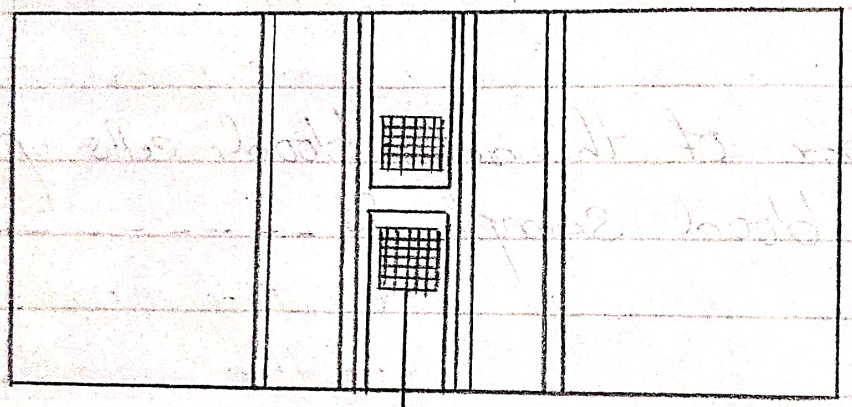
Result:

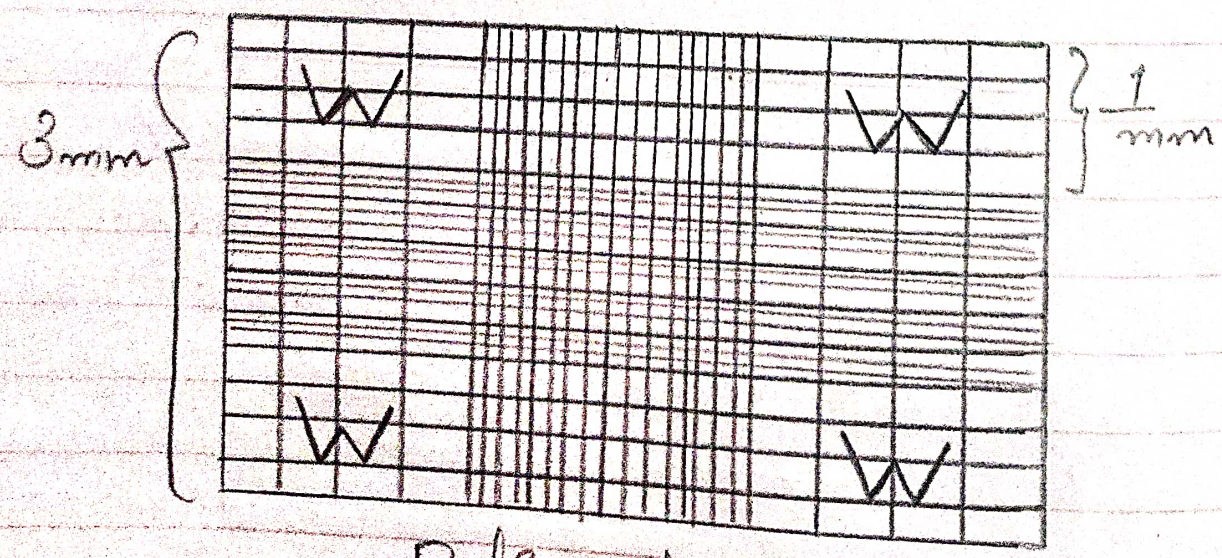
The number of the red blood cells present in One μl of blood sample is _____

Signature .....

Neubauer's Chamber







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Expt. No. 03 Page No. 06

Estimation of White Blood Cell Count

Principle: The basic principle is that the blood is diluted with acid solution which removes the red cells by haemolysis and also accentuates the nuclei of the white cells; thus the counting of the white cells becomes easy → Blood cell counts can be performed using the hemacytometer.

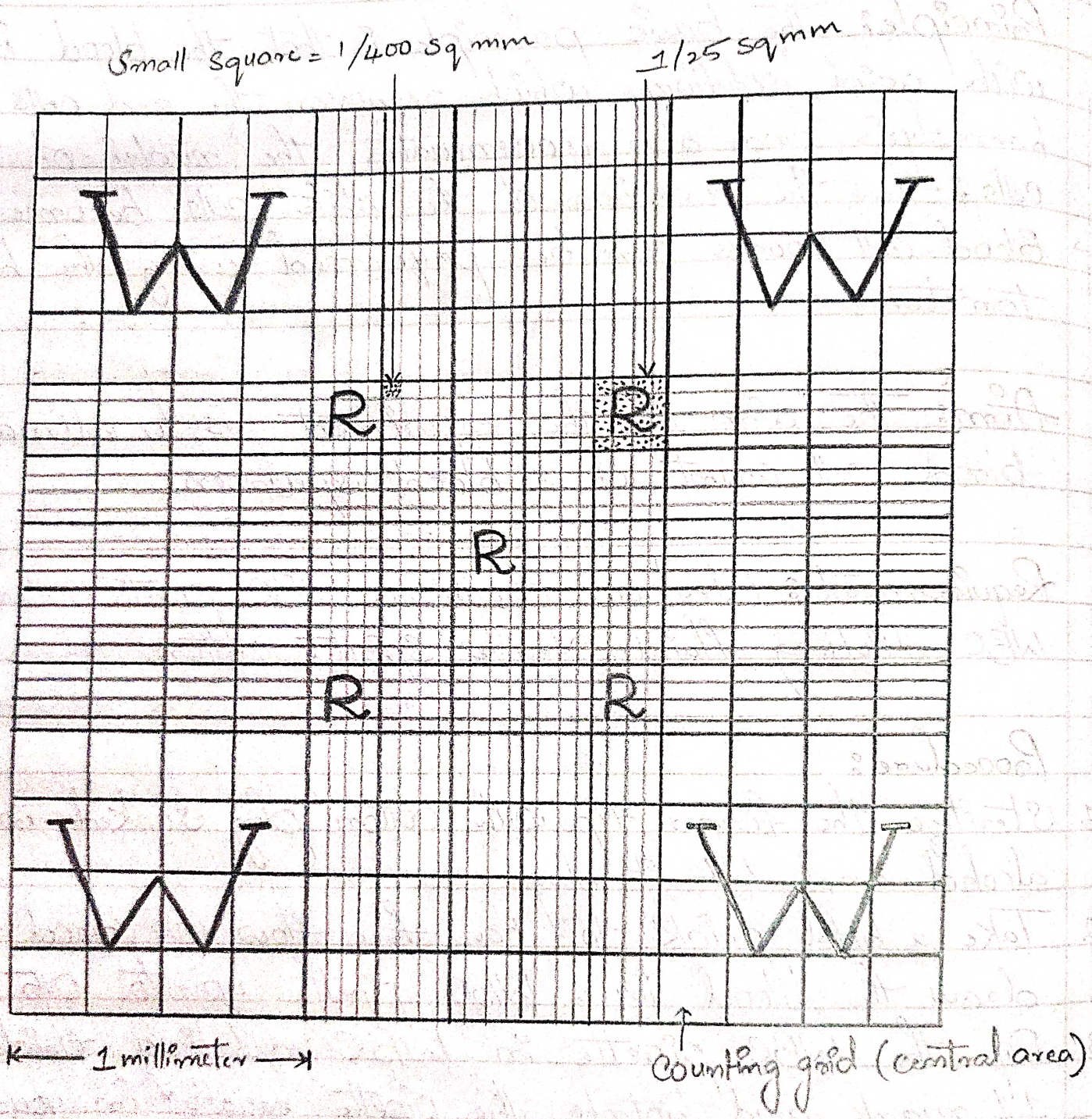
Aim: The aim of the experiment is to estimate white blood cell count of a blood specimen.

Requirement: Neubaur chamber, WBC pipette, cover slip, WBC diluting fluid, Needle, Spirit, cotton.

Procedure:

1. Sterilize the finger tip with cotton plug soaked in 70% alcohol and let it dry.
2. Take a bold prick to have free flow of blood and draw the blood in a WBC pipette up to 0.5 mark.
3. Dip the WBC pipette in WBC diluting fluid up to 11 mark and rotate the pipette equally in your hands to mix the solution well by swirling.
4. Take the haemacytometer and place it on the flat surface of the work bench. Place the cover slip on the counting chamber.

Signature .....


Small square = 1/400 sq mm
1/25 sq mm
W
W
R
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R
R
W
W
1 millimeter
Counting grid (central area)

Date: .....

Expt. No..... Page No. ...07.....

5. Allow a small drop of diluted blood, hanging from the pipette, to sweep into the counting chamber by capillary action. Make sure that there is no air bubble and there is no overfilling beyond the ruled area.

6. Leave the counting chamber on the bench for 3 mins to allow the cells to settle. Observe the cells by placing the counting chamber on the mechanical stage of the microscope.

Focus on one of the corner squares of the counting chamber and count the white cells schematically, starting from the upper left small square of each square. Repeat the count in all the four corners of the chamber. Apply the margin rules i.e count the cells lying on two adjacent margins, and discard those on the other two margins.

Data Analysis:

$$\frac{\text{No. of cells} \times \text{Dilution factor} \times \text{Depth factor}}{\text{Area count}}$$

Where: Dilution factor = 20, Depth factor = 10, Area Count = 4

Result: The number of white blood cells present in one ml of bloodspecimen is - - - - - - - -

Signature .....

Date: .....

Expt. No. 04 Page No. 08

Estimation of Packed Cell Volume [Hematocrit]

Basic: Hematocrit literally means "blood Separation" Packed cell volume is the percentage of volume of blood occupied by the red cells.

Aim: To estimate packed cell volume using Macro-hematocrit method (Wintrobes method)

Apparatus required:

→ Wintrobes tube:

- It is 110mm long, narrow, thick walled tube with 3mm internal bore.
- Graduated from 0 to 10 cm with graduation on both sides in ascending and descending order on 2 sides of tube.
- Scale with the markings from 0 to 10 from above downwards is used in ESR determination and from below upwards is used for PCV determination.
- Pasteur pipette
- Centrifuge.

Procedure:

- 2ml of venous blood is collected and mixed with double oxalate (ammonium oxalate and potassium oxalate) or EDTA powder in the proportion of 1.5mg/ml.

Signature .....

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- Blood is drawn into Pasteur pipette and introduced in the wintrobes tube from the bottom to 0 or 10 mark above.
- Place the wintrobes tube in the centrifuge machine and other wintrobes tube filled with water on the opposite side so as to balance it.
- Centrifuge the tube at the speed of 3000 rpm for 30 minutes.
- After 30 minutes stop the centrifuge, take out the tube and note the readings
- Calculation -

Hematocrit = [Height of RBC's in mm/Height of RBC and plasma] x 100

Result:

Zones Separated after centrifugation.

- Top layer - Plasma (48-52%)
- Normally amber or pale yellow colour
- Yellow - jaundice
- pink or red colour indicates - hemolysis
- Creamy white - hyperlipidemia.
- Brown coloured - meth hemoglobinemia.
- Cloudy (increased viscosity) - Multiple myeloma.
- Intermediate zone - Buffy coat - Zone of platelets and leukocytes (2-3% or 1mm thick)

Signature .....

- Greyish - white tan layer
- Smears prepared from buffy coat can be used to diagnose
  - Sub leukemic leukemia
  - LE cells
  - Detection of plasma cells
  - Hemoparasites.

- lower most zone or bottom layer - Zone of packed RBC's (45% - 50%)
  - Normal PCV

    Males :- 40 - 50%

    Females:- 37 - 47%

    New born:- 55 - 60%

Anti-A	Anti-B	Anti-D	Blood type
			O-positive
			O-negative
			A-Positive
			A-negative
			B-positive
			B-negative
			AB-positive
			AB-negative

Date: .....

Expt. No. 05 Page No. 11

Identification of blood group

Aim: Blood group testing.

Materials Required:
Toothpicks, Blood sample, Alcohol swabs, Lancet, clean glass slide, sterile cotton balls, Biohazard disposal container, monoclonal Antibodies (Anti-A, B and D)

Procedure:

- Take a clean glass slide and draw three circles on it.
- Unpack the monoclonal Antibodies kit. In the first circle add Anti-A, to the second circle add Anti-B and to the third circle add Anti-D with the help of a dropper.
- Keep the slide aside safely without disturbing.
- Now wipe the ring finger with the alcohol swabs and rub gently near the fingertip, where the blood sample will be collected.
- Prick the ring fingertip with the lancet and wipe off the first drop of the blood.
- As blood starts oozing out, allow it to fall on the three circles of the glass slide by gently pressing the fingertip.

Signature .....

Date: .....

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- Apply pressure on the site where it was pricked and to stop blood flow, use cotton ball if required.
- Mix the blood sample gently with the help of a toothpick and wait for a minute to observe the result.

Conclusion:

Here is the chart which predicts the different types of blood groups along with it Rh-factor.

Blood Type	A	B	O	AB
Rh-positive	A+	B+	O+	AB+
Rh-negative	A-	B-	O-	AB-

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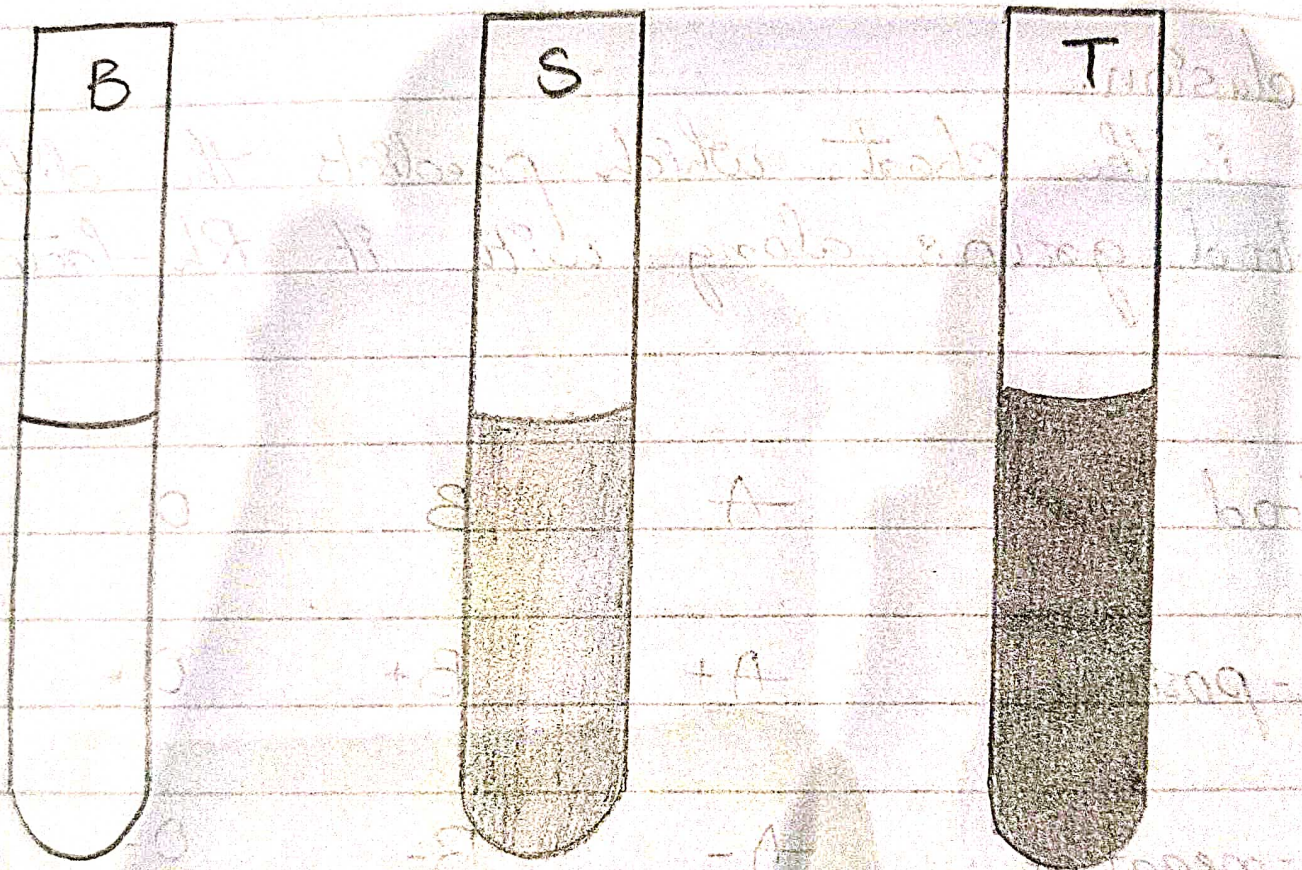

BLOOD GLUCOSE

Date: .....

Expt. No....06.....

Page No. ...13.....

- Estimation of the amount of glucose present in the given blood sample by glucose Oxidase-peroxidase method [GOD POD Method]

Principle:

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$\text{4 Amino Phenazone} + \text{Phenol} + H_2O_2 \xrightarrow{\text{POD}} \text{Quinonimine [pink colour Compound]}$$

- Intensity is determined at on 505 nm filter

Requirements:

- Specimen

Serum or plasma free of hemolysis. Sodium fluoride is preferred as an anticoagulant due to its antiglycolytic activity.

- Reagents:

1. Glucose standard [100 mg/dl]
2. GOD-POD reagent: Enzyme reagent mixture Containing glucose Oxidase (GOD), peroxidase (POD), 4-amino-antipyrine, phenol and phosphate buffer (PH ≈ 7.0) Some stabilizers and activators.

Signature .....

	Blank	Standard	Test
GOD-POD Reagent	1ml	1ml	1ml
Distilled water	10 µl	-	-
Glucose Standard	-	10 µl	-
Sample	-	-	10 µl

Date: .....

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Instruments:

1. Test tubes
2. Pipettes, disposable tips, rack
3. Water bath.
4. Colorimeter.

Procedure:

1. Label three clean, dry test tubes as Blank (B), standard (S), and Test (T).
2. Pipette as given in the table.
3. Mix well and incubate at 37°C for 10 minutes. Or at room temperature (25°C) for 30 minutes.
4. Measure the absorbance of the standard and test sample at 540 nm (green filter) against blank within 60 minutes.

Calculation:

Calculate the concentration of blood glucose in the specimen using the following formula:

$$\text{Conc. of Glucose in the Specimen (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100$$

Signature .....

Date: .....

Expt. No. 07 Page No. 15

- Estimation of the amount of glucose in the given urine sample by Benedict's test Method.

Principle:

Glucose $(R\text{-}CHO) + 2Cu^{+2} + 2H_2O \longrightarrow$ Glucomic acid $(R\text{-}COOH) + Cu_2O + 4H^+$

Procedure:

5ml of Benedict's reagent + 8 to 10 drops of urine Boiling the mixture & cool it down, Observe the change in colour.

Result & Interpretation on Benedicts Test

- Blue - Sugar absent;
- Green - 0.5 gm% Sugar = +1
- Yellow - 1.0 gm% Sugar = +2
- Orange - 1.5 gm % Sugar = +3
- Brick red - 2.0 % or more Sugar = +4

Signature .....

Date: .....

Expt. No....08..... Page No. ..16.....

Estimation of the Serum Albumin in the given Urine sample by Bromocresol Green Method.

Principle: The method is based on the protein error of indicators. Binding of a protein to an indicator changes its colour. Among serum protein, only albumin binds to BCG this binding produces a change in the colour of BCG which is measured colorimetrically. The pH is maintained during the reaction by a buffer.

Reagents:

(i) Succinate buffer
(ii) BCG solution
(iii) Buffered BCG solution
(iv) Standard albumin solution.

Procedure:

Level 3 test tubes 'Unknown', 'Standard' and 'Blank'
Measure 4 ml of buffered BCG solution into each
Wash 0.02 ml of serum into 'Unknown', 0.02ml of Standard albumin solution into 'Standard' and 0.02 ml of water into 'Blank'. Mix and allow the tube to stand for 5 minutes.
Read 'Unknown' and 'Standard' against 'Blank' at 630 nm or using a red filter.

Signature .....

Date: .....

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Calculations:

$$\text{Serum albumin (gm/100 ml)} = \frac{A_4}{A_5} \times 4$$

Results Interpretation:

The normal range of serum albumin is 3.7 - 5.3 gm/100 ml. Serum globulin ranges from 1.8 - 3.6 gm/100 ml. The ratio of A:G is roughly 2:1 though it may range from 1.2:1 to 2.5:1.

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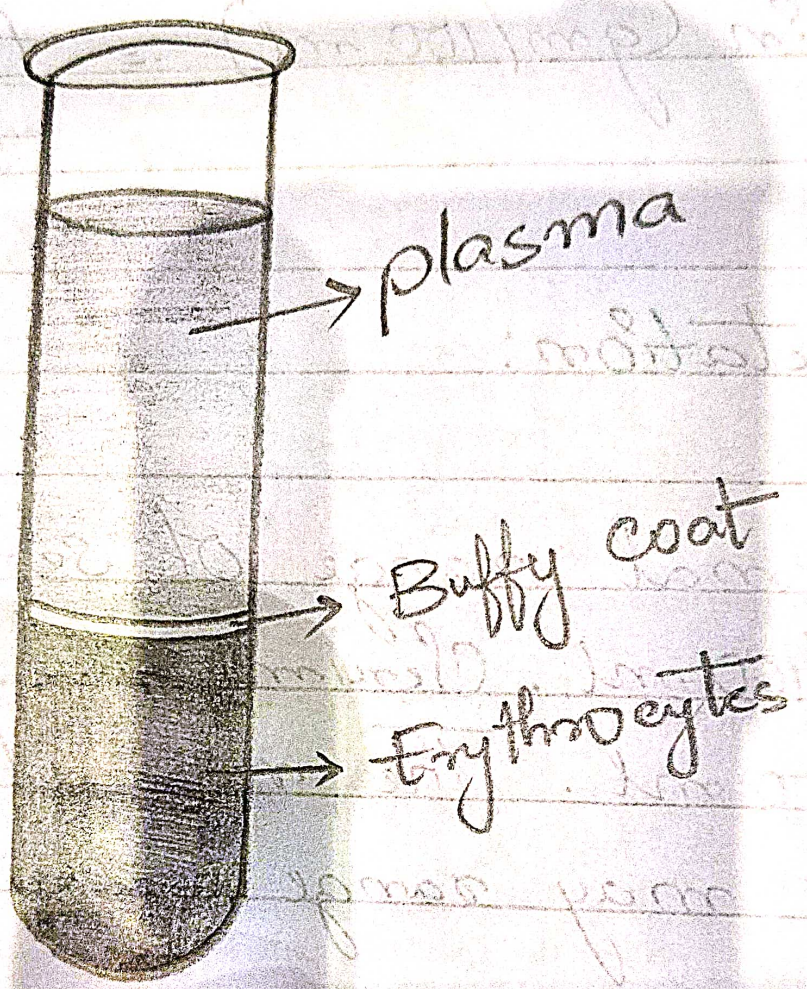

plasma
Buffy coat
Erythrocytes

Date .....

Expt. No..... Page No. ..18.....

Spotters:

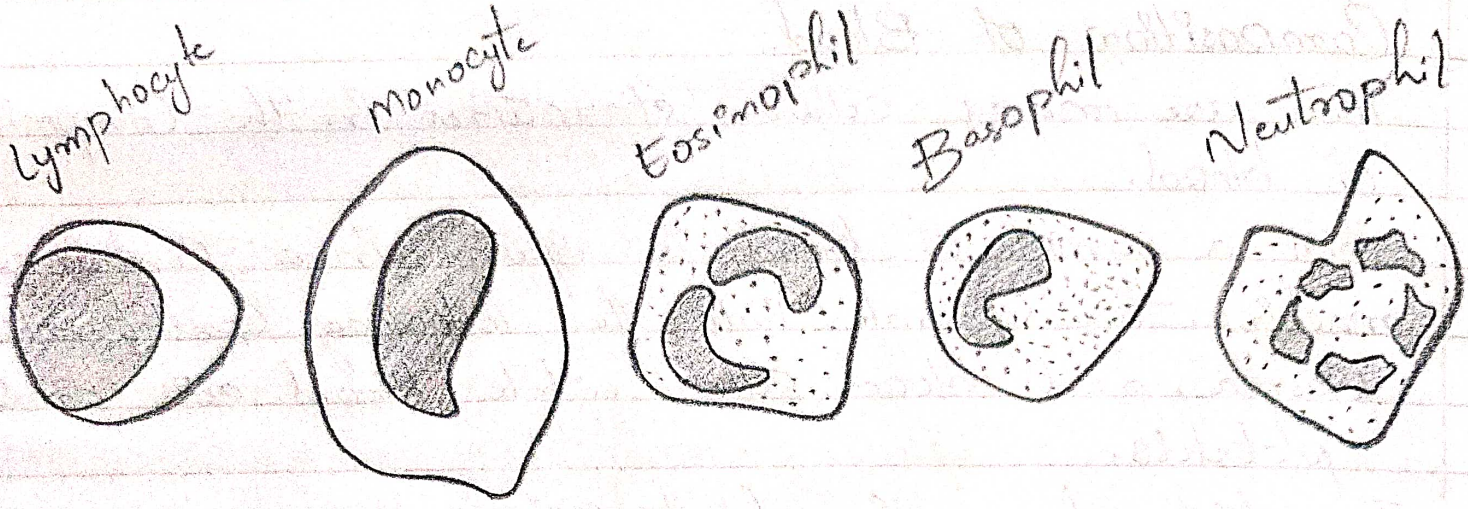
1. Composition of Blood.

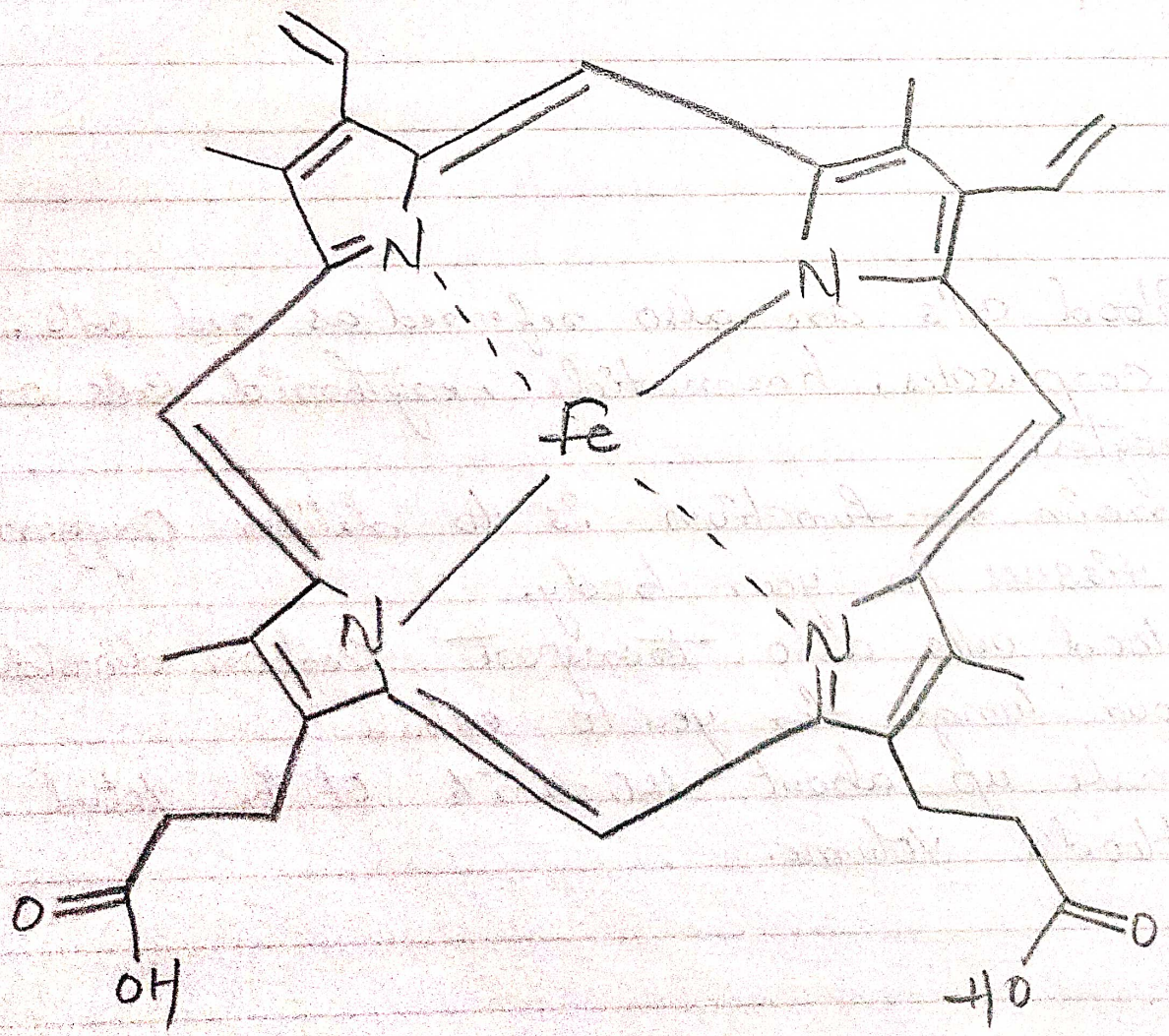
- There are many cellular structures in the Composition of blood.
- When a sample of blood is spun in a Centrifuge machine, they separate into the following Constituents: plasma, red blood cells, white blood cells and platelets.
- In which plasma is about 55%.
- leukocytes & platelets about <1%.
- Erythrocytes / RBC about 45%.

2. RBC.

- Red Blood cells are also referred as red cells, red blood corpuscles, haematids, erythroid cells or erythrocytes.
- Their main function is to deliver Oxygen to the tissues in your body.
- Red blood cells also transport Carbon dioxide to your lungs for you to exhale
- They make up about 40-45% of the total blood's volume.

Signature .....


Lymphocyte
Monocyte
Eosinophil
Basophil
Neutrophil


N
N
Fe
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N
O
OH
HO
O

Date .....

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3. White blood cells

- White blood cells are also known as leukocytes.
- They are responsible for protecting your body from infections.
- As part of your immune system, white blood cells circulate in your blood and respond to injury or illness.
- White blood cells a broadly of five types:
  i) monocyte ii) eosinophil iii) basophil
  iv) lymphocytes v) neutrophil.

4. Hemoglobin

- Hemoglobin is a protein.
- It is present in red blood cells that carries Oxygen to your body's organs and tissues and transports Carbon dioxide from your organs and tissues back to your lungs.
- The normal Hb level for males is 14 to 18 g/dl; that for female is 12 to 16 g/dl.
- If hemoglobin level is lower than normal it means you have a low red blood cell count (anemia).

Signature .....

5. Platelets

- platelets or thrombocytes are small colourless cell fragments in blood.
- They form clots and stop or prevent bleeding.
- platelets are made in our bone marrow
- A normal platelet count in adults ranges from 15,000 to 4,50,000 platelets per microliter of blood.
- low platelet levels causes thrombocytopenia that may increase your risk of issues like excessive bleeding and bruising.

6. Serum protein

- Serum proteins are classified as albumin and globulins.
- Albumin is the most abundant protein in the Serum.
- It carries many small molecules.
- It is also important for keeping fluid from leaking out from the blood vessels into the tissues.
- Serum protein test is often performed to diagnose nutritional problems, kidney disease or liver disease.

Signature .....

Date .....

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7. Antibodies in blood group - A

- Blood group A has anti-B antibodies in the plasma and A antigens on the red blood cells.

8. Antigens present in blood group - B

- Blood group - B has B antigens with anti - A antibodies in the plasma.

9. Composition of urine.

- Human urine is composed primarily of water [95%]
- The rest is:
  urea (2%), Creatinine (0.1%), uric acid (0.03%), chloride, sodium, potassium, sulphate, ammonium, phosphate and other ions and molecules in lesser amounts.
- Protein is only found in trace amounts compared to their value in blood plasma.

Signature .....

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10. CBP test.

- Complete blood picture test or CBP test is done as a routine screening test.
- It gives the count of different blood cells count (like RBC, WBC, platelets, Hb levels, ESR etc).
- The test is recommended when there are signs and symptoms related to the conditions that effect the different blood cells, when suspecting infections like malaria, microfilaria etc.
- Also helps to diagnose various conditions like anemia etc....