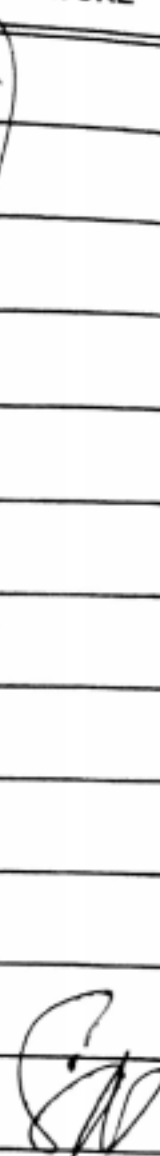
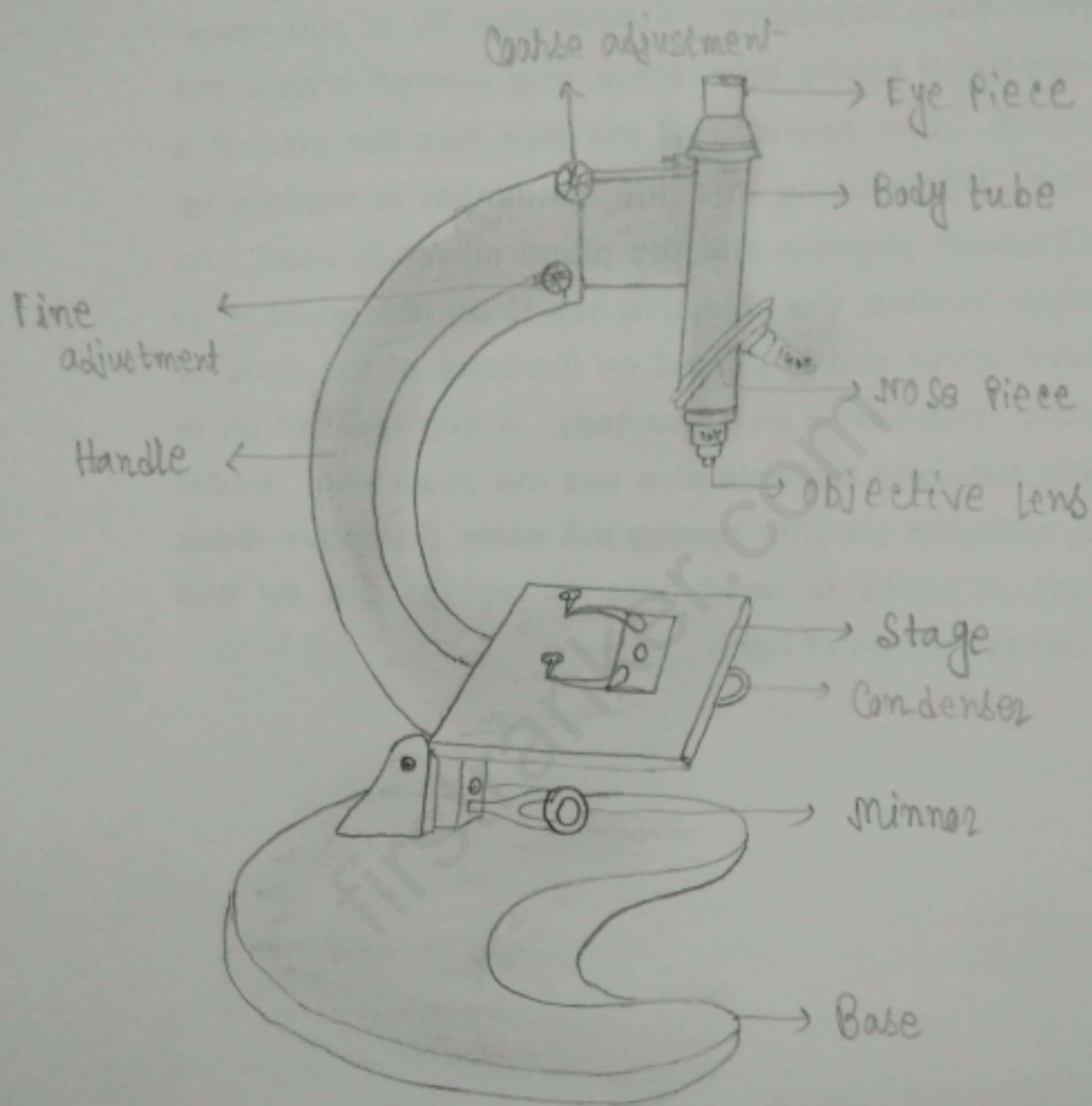


No	DATE	EXPERIMENT	PAGE NO	SIGNATURE	REMARKS
01.	19/8/15	Study of Compound microscope	1		
02.	22/8/15	Drawing of an ideal blood film	8		
03.	25/8/15	Staining with Leishman's stain	12		
04.	9/9/15	Identification of blood cells	18		
05.	3/11/15	Differential count of leucocytes	23		
06.	6/11/15	Estimation of haemoglobin content	29		
07.	17/11/15	Total count of RBC	43		
08.	20/11/15	Total count of WBC	44		
09	24/11/15	Tests for Haemostasis (BT & ET)	45		
10	11/12/15	Determination of PCV	52		
11	11/12/15	Determination of ESR	58		
12	11/12/15	Normal blood standards	69		
13	15/12/15	Total platelet count of blood	73		
14	18/12/15	Determination of blood group	78		
15	22/12/15	Preparation of Haemin crystal	82		
16	11/12/15	Osmotic fragility of Red blood cells	85		



Microscope



Study of Compound Microscope.

Observations :

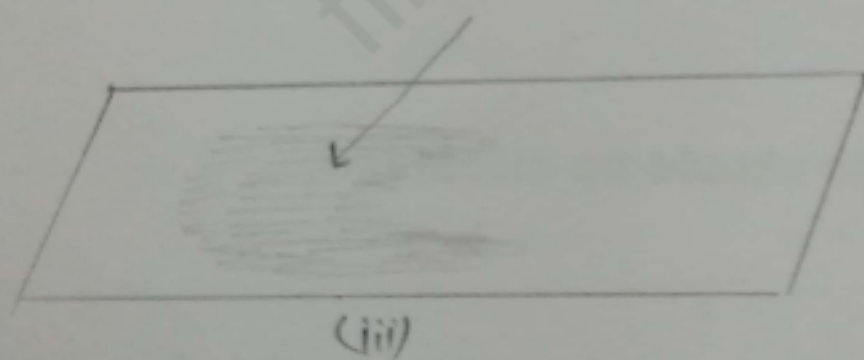
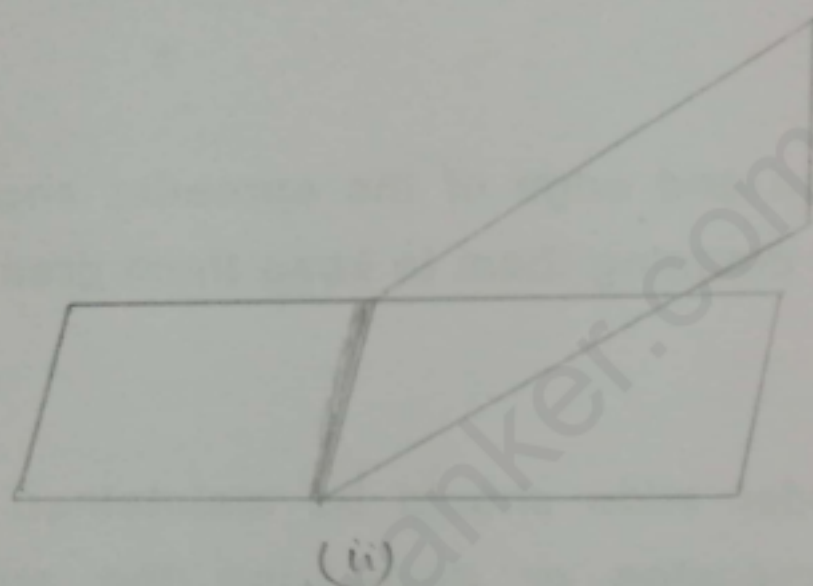
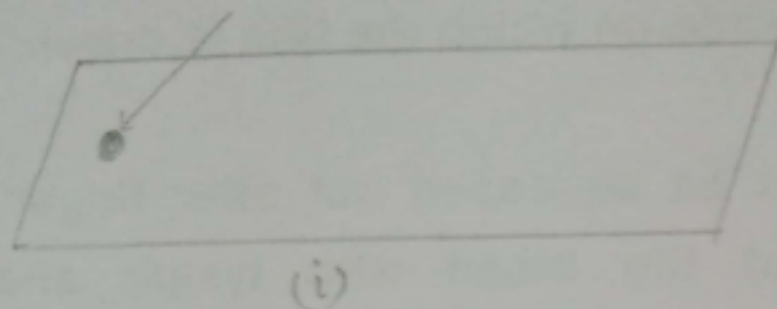
The different characters of different objectives are studied as follows:

	LP	HP	OI
Magnification	- 10x (low)	40-45x (medium)	100x (High)
Length	- Small	Medium	Normal
Aperture	- Maximum	Medium	Minimum
NA	- 0.25	0.66	1.25

The different refractive indices of different media through which light passes before entering the objective:

Air	- 1.00029
Distilled water	- 1.33
Glass	- 1.50
Liquid paraffin	- 1.44
Cedar wood oil	- 1.53

Signature of the teacher with remarks



Stages of blood staining

Drawing of an ideal blood film

Observations :

- The RBCs are seen under the microscope
- (i) The blood film is tongue shaped and small.
 - (ii) It is not too thick or too thin.
 - (iii) The cells are almost uniformly distributed on the slide in a monolayer.
 - (iv) It is covering $\frac{2}{3}$ area of the slide.

Result:

- i) This is an ideal tongue - shaped blood film.
- ii) The best view is from the body region of the tongue - shaped blood film.
- iii) It consists of various types of cells.

Inference:

- i) An ideal blood film is ready and it will be used for the next experiment.
- ii) The tiny particles seen under the microscope are the RBCs. The clear background is the plasma of blood.

Signature of the teacher with remarks

Staining with Leishman's stain.

Observations :

After the film is ready, it is examined first under the low power objective and then the high power objective of the compound microscope. It is finally seen under oil immersion objective.

The stained blood is continuous, bluish violet in colour with a pinkish tint. Some formed are formed under the microscope. The RBCs (erythrocytes); the WBCs (leucocyte) and the platelets (thrombocytes).

Result:

The smaller reddish cells are the RBCs.

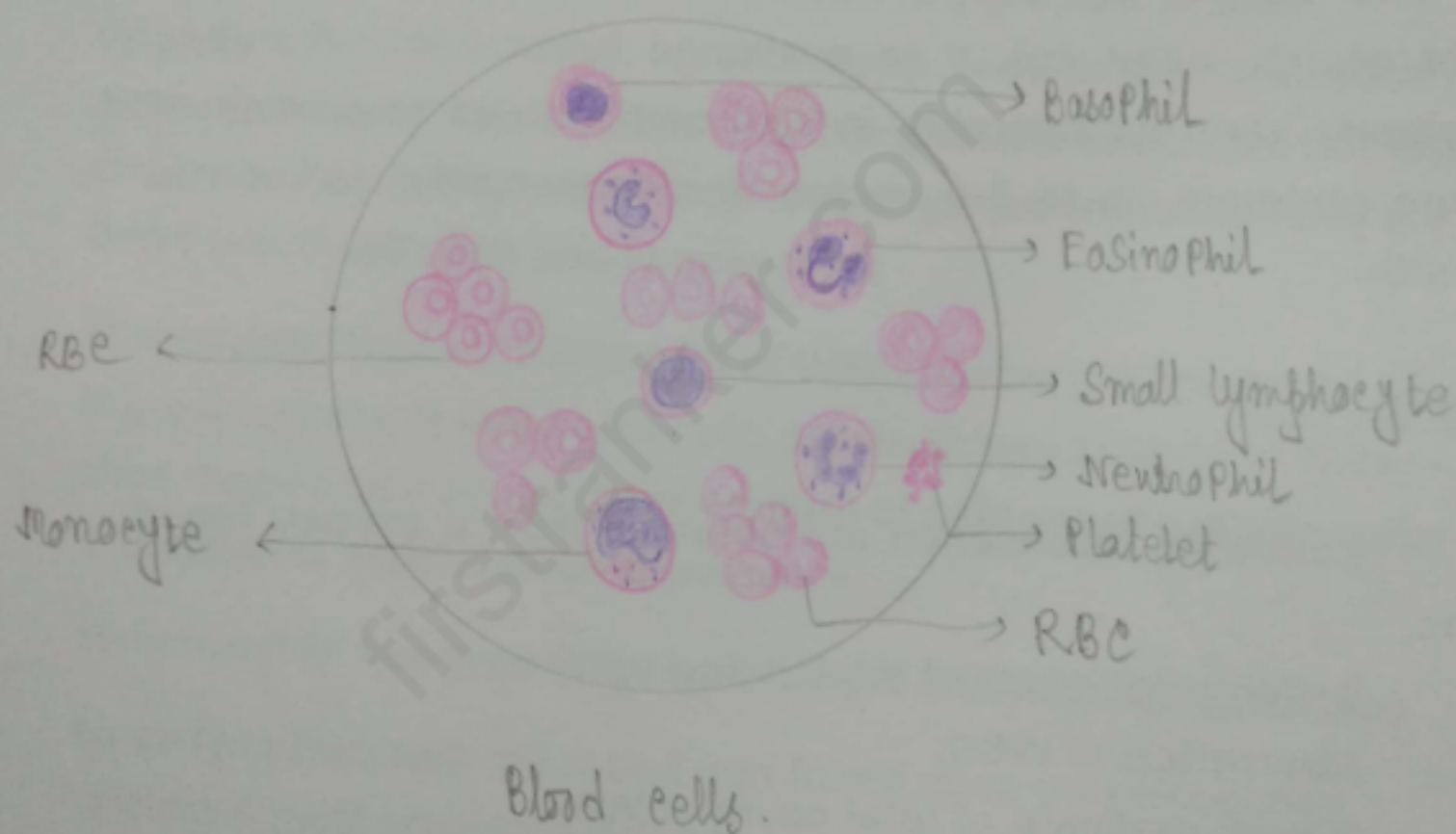
The larger cells are the WBCs.

The broken fragments are the platelets.

Inference:

The stained blood film is ready to observe all the types of blood corpuscles (RBCs, WBCs and platelets) and these cells can be obtained easily under the microscope.

Signature of the teacher with remarks



Identification of blood cells.

Observations :

	Blood cells	Nucleus	Cytoplasm	granules	Size (μm)
1	Neutrophil	2-5 lobes	granular	light pink	10-14
2	Eosinophil	2 lobes	granular	brick red	10-15
3	Basophil	multilobe	granular	bluish black	10-15
4	large lymphocyte	round, no lobed	agranular	—	12-15
5	Small lymphocyte	round, ink spot	agranular	—	7-10
6	monocyte	large, kidney shape	agranular	—	15-20
7	RBCs	absent	agranular	—	7-10
8	Platelets	anucleated	—	—	

Result:

The Various types of blood cells are discussed to identify them by comparing the size, staining and the number of lobes of nucleus etc - Among WBCs, we find that-

(i) Neutrophil (50-60%), (ii) Eosinophil (1-4%), (iii) Basophil (0-1%)
(iv) (large & small) lymphocytes (20-40%), (v) monocyte (2-8%) &
(vi) RBCs occupy major volume of the blood film.

Inference:

- After identification of different kinds of blood cells is done, their total count and differential count can be measured.
- RBCs are easily identified, because it is the most numerous cells found.
- WBCs have different types on the basis of granules in cytoplasm and size and shape of their nucleus.

Signature of the teacher with remarks

N	N	N	N	L	L	L	N	N	N
L	N	L	N	N	E	N	M	M	L
N	L	L	N	N	N	N	L	L	N
N	L	L	N	N	N	L	N	N	L
L	N	N	N	N	N	E	E	L	L
N	N	M	L	N	N	N	N	L	L
L	N	N	N	M	N	L	N	N	N
N	L	N	N	E	N	N	L	L	L
L	N	N	N	N	N	N	L	L	N
N	N	L	L	N	N	N	N	L	E

N → Neutrophil - 59.1%

L → Lymphocyte - 32.1%

E → Eosinophil - 0.5%

M → Monocyte - 0.4%

Differential Count of leucocytes of blood

Observations :

While performing this experiment with Compound light microscope under oil immersion objective, mainly Neutrophils are seen. There are lymphocytes having small and large category. There can also be found very little amount of eosinophil. Little amount of monocytes are also found.

Result:

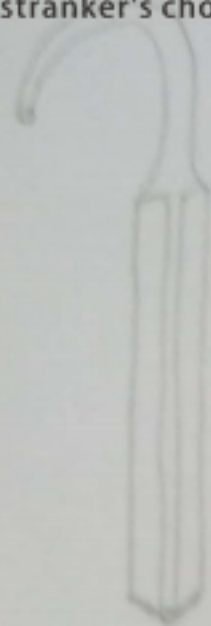
Cells observed are identified as follows -

Neutrophil	Basophil	eosinophil	Lymphocyte	Monocyte
Range - 50-60%.	0-1%.	1-5%.	20-40%.	2-8%.
Found - 59%.	0%.	5%.	32%.	4%.

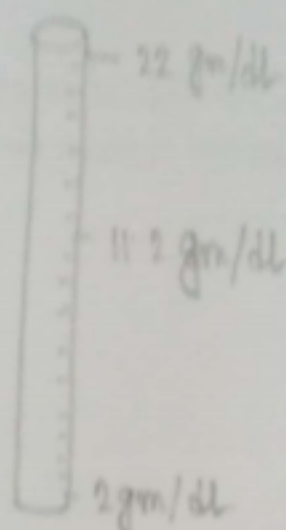
Inference:

After Comparing the result with the normal differential count of the human blood, we find that the differential count of leucocyte is normal.

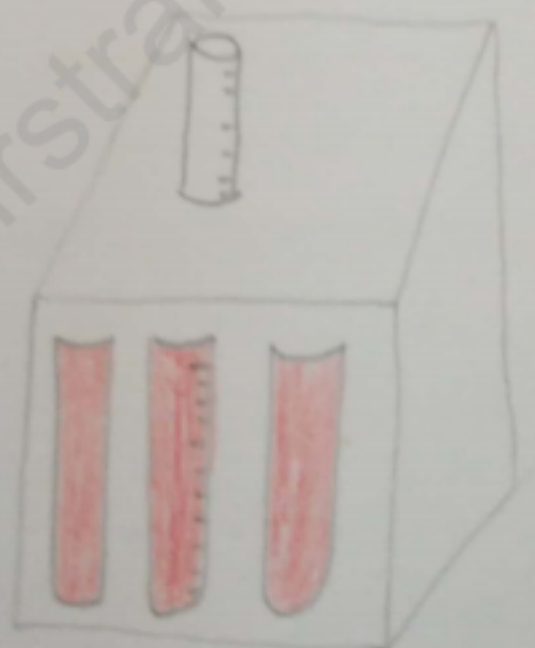
Signature of the teacher with remarks



Pipette



Comparator tube



Comparator box

NAME OF THE EXPERIMENT:

DATE:

Estimation of Haemoglobin Content of blood

Observations :

After proper mixing of blood and 2 ml N/10 HCl, the haemoglobinometer tube is kept in the comparator box for 10 minutes.

The distilled water is then added to the test tube drop by drop to match the colour with that of the glass rods of the comparator box.

With further addition of drops of distilled water, colour of the solution is almost same in comparison to standard comparator tube.

Result:

Haemoglobin Content of blood sample -

1st reading $\rightarrow$ 14.2 gm/dl

2nd reading $\rightarrow$ 14.8 gm/dl

3rd reading $\rightarrow$ 15.2 gm/dl

$\therefore$ Mean reading $\rightarrow (14.2 + 14.8 + 15.2)/3 = 14.8$ gm/dl

Inference:

The haemoglobin content in the donor's blood is 14.8 gm/dl. It is within the normal range.

Signature of the teacher with remarks

WBL																WBL			

STUDY OF NEUBAUER CHAMBER

Date:

The Neubauer's Chamber has two chambers forming two counting areas. The raised support helps to hold the cover glass. Each counting area is divided by triple lines into nine large equal areas of 1mm width. Out of these nine areas, 4 corner areas (squares) are meant for counting WBCs and each square is further divided into 16 small squares of the big 4 squares. The central square is meant for RBC counting and is divided by 3 lines into 25 medium sized squares i.e. there are in all 400 smaller squares; the red cells are counted in 4 corners and central medium squares. The depth of the chamber is $\frac{1}{10}$ mm.

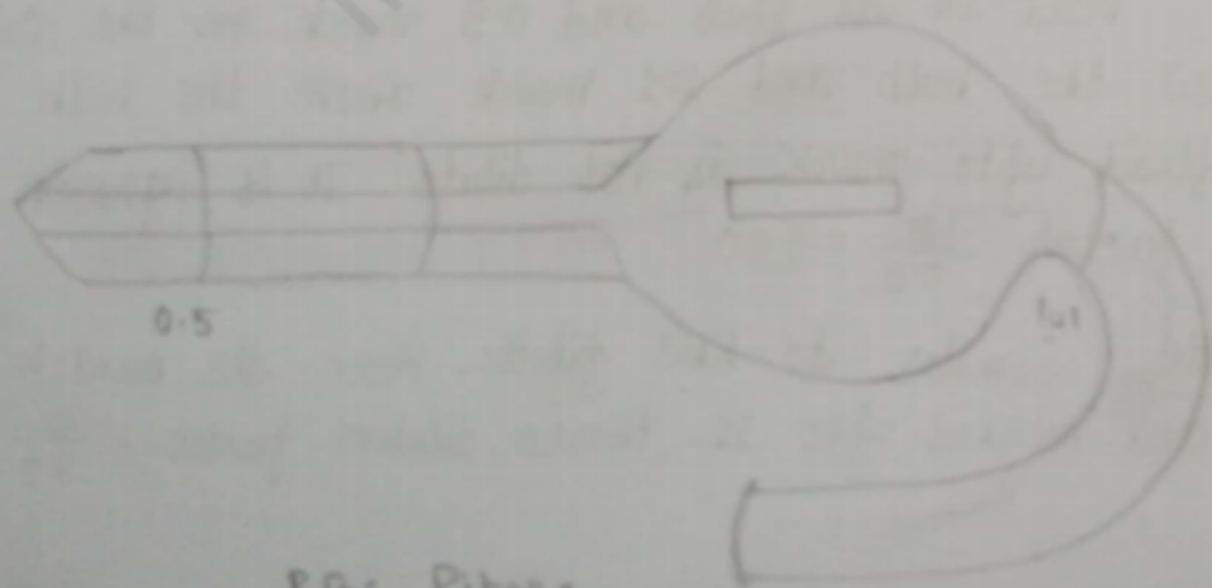
RBC Pipette - It can be recognised by presence of a red bead in its bulb and 0.5 mark on the capillary tube below the bulb and 101 mark above the bulb.

Since fluid upto mark is not dilute. It is ignored.

Dilution factor $\frac{100}{0.5} = 200$

WBC Pipette - Similar to RBC pipette. Here the bead is white and marked upto 11. Hence dilution factor $= \frac{10}{0.5} = 20$

Signature of the teacher with remarks



0.5

1.0

RBC Pipe

Total Count of RBC

Observations :

Four corners and central chamber of the RBC chamber, the RBCs are found in every chamber almost -

10	8	4	8	9	5	7	10	5	9	7	4	9	6	7	5	7	4	6	9
8	5	7	6	5	4	6	9	6	8	9	4	5	9	7	5	9	3	6	7
5	7	7	8	8	4	6	6	5	5	7	8	5	7	5	6	7	4	4	5
8	5	4	9	8	8	8	6	9	5	6	8	8	8	5	9	7	6	5	8
↓				↓				↓				↓				↓			
Total: 109				+ 109				+ 105				+ 106				+ 97			

Result:

Total five small RBC chambers contain $(\frac{1}{5} \times \frac{1}{5} \times \frac{1}{10} \times 5) = \frac{1}{50} \text{ mm}^3$
diluted blood which presents (n) = 526 RBCs

Dilution factor = 200

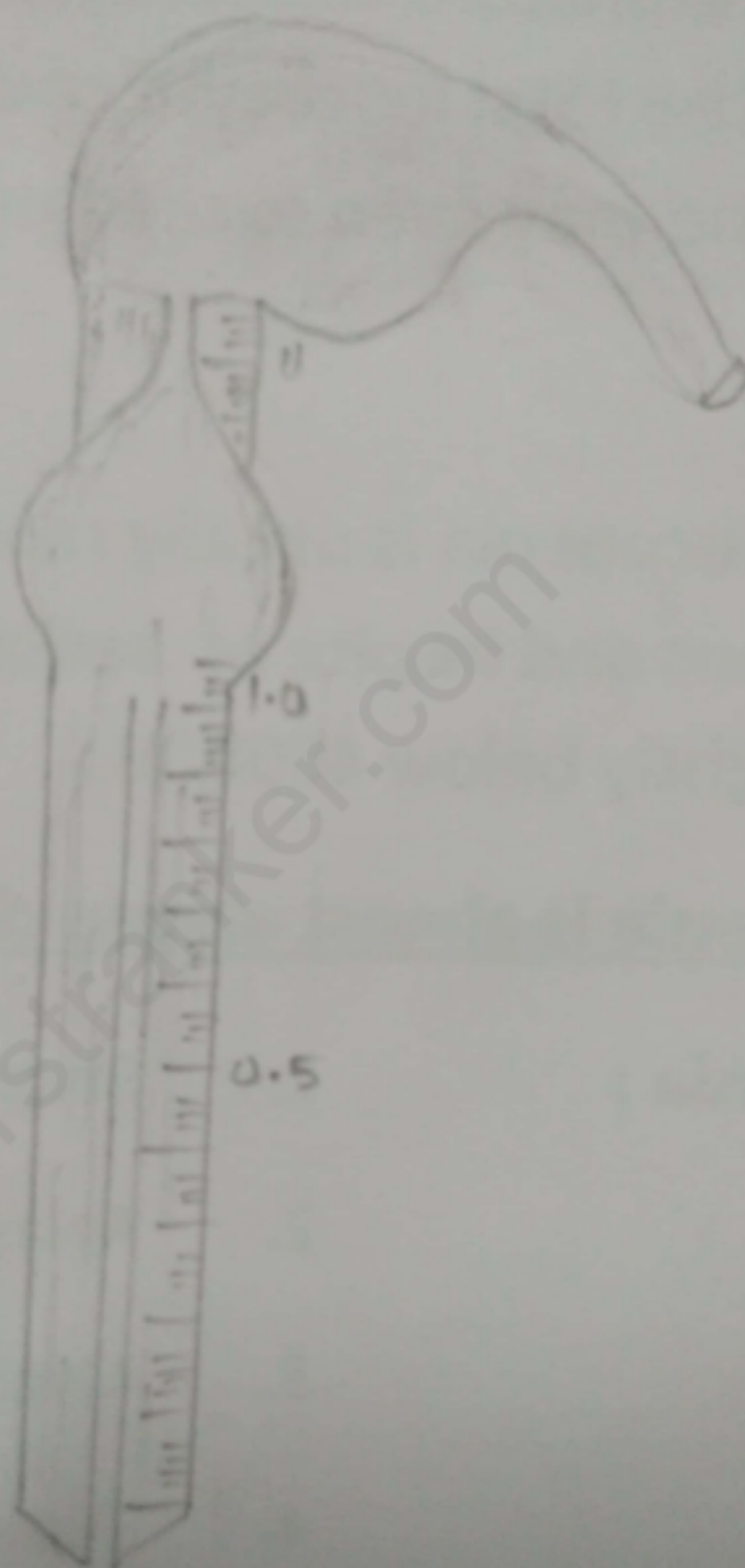
$\therefore$ Total number of RBCs in 1 mm^3 blood = $50 \times n \times 200$
= $n \times 10000$
= 5260000

Inference:

Total number of RBCs in 1 mm^3 blood $\rightarrow 5260000$

Therefore, RBC count of the given blood sample is within the normal limit.

Signature of the teacher with remarks



WBC Pipette.

Total count of WBC

Observations :

The count of leucocytes in 4 corner squares are as following

2	0	3	2
0	3	2	3
1	2	1	4
2	3	0	3

3	2	2	0
1	0	3	2
1	3	1	4
2	0	2	1

2	2	0	3
2	2	4	0
0	1	5	2
1	2	0	1

3	0	2	2
2	0	1	3
2	2	0	1
2	0	2	0

↓ ↓ ↓ ↓
 Total: 31 + 27 + 27 + 22

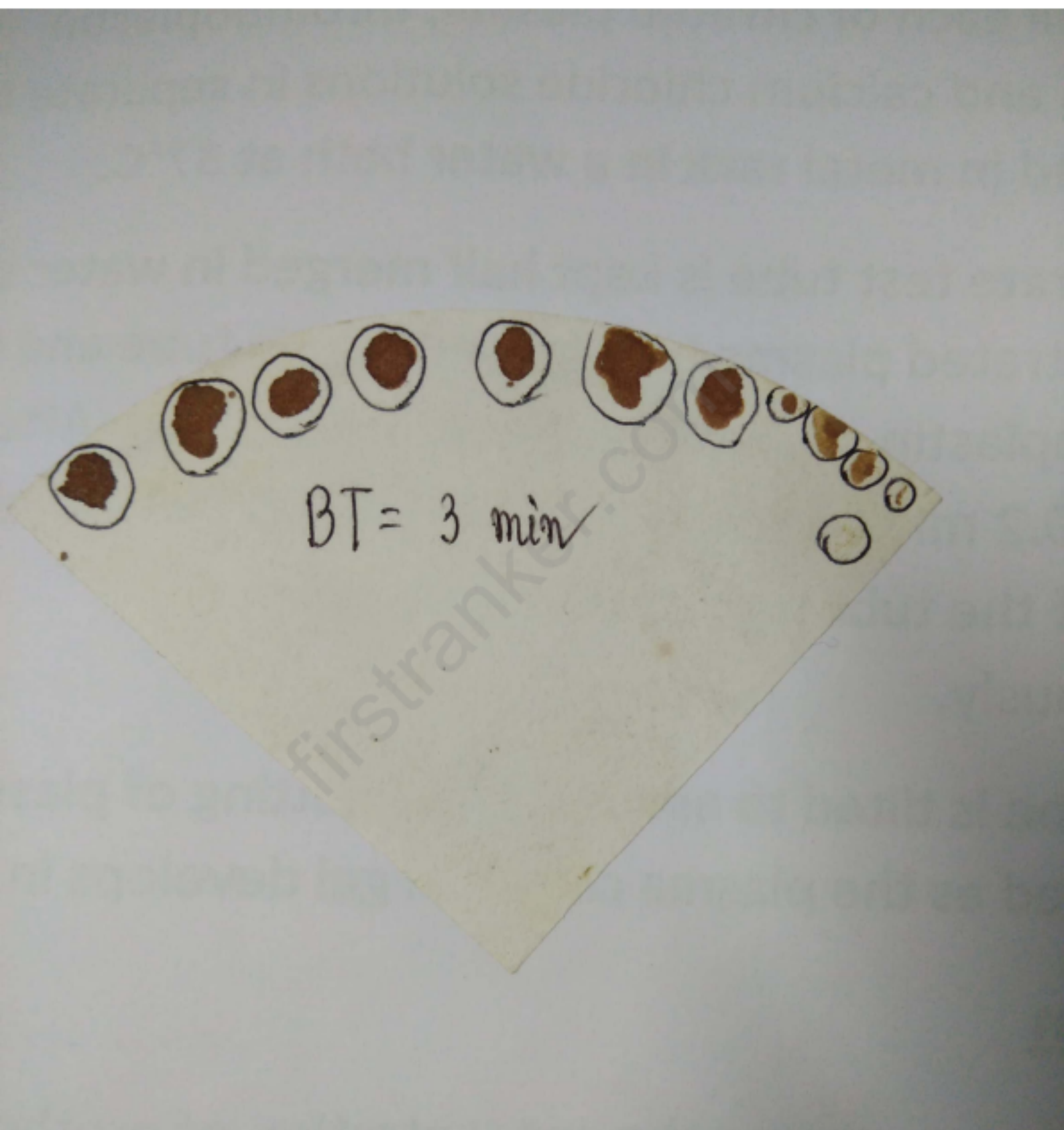
Result:

Number of WBCs counted in 4 corner square (n) = 107
 Dilution factor = 20

$\therefore$ Total number of WBCs in 1 mm^3 blood = $(20 \times \frac{5}{2} \times n)$
 $= 5350$

Inference:

The total count of WBC in 1 mm^3 of blood is 5350
 $\therefore$ WBC count of given blood sample is within the normal limit.



Test for Haemostasis: Determination of Bleeding time (BT) and Clotting time (CT)

Observations :

Bleeding time (BT) - The interval between onset of oozing of blood from a puncture site and its complete stoppage.

Clotting time (CT) - The time interval taken by the blood to clot in formation of fibrin by capillary tube.

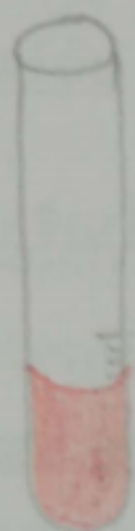
Result:

Bleeding time is counted by summing all the 30 sec between the first and last drop of blood which is equal to 3 min.

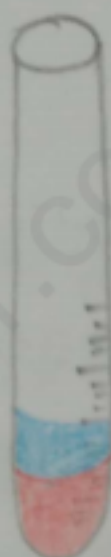
Clotting time is counted by the total 30 sec intervals taken at broken pieces. The clotting time is equal to 3 min 20 sec.

Inference:

So, the bleeding time (BT) is within normal range (2-4 min)
The clotting time (CT) is also within normal range (3-5 min)



Filled with
blood



RBC Volume after
centrifuge

Determination of Packed Cell Volume (PCV)

Observations :

After centrifugation, the blood is separated into 3 layers of packed cells (red). Thin layer of white cells and platelets are formed at the top layer of clear plasma.

Result:

The PCV is 45%. After centrifugation, red, white and clear plasma levels are separated. They demarcate RBC, WBC and plasma respectively. Packed Cell Volume (PCV) in this case is 45 ml

Inference:

The PCV is within the normal range.

Signature of the teacher with remarks



Determination of erythrocyte sedimentation Rate (ESR)

Observations :

After one hour, a clear column of plasma layer is separated, about the blood cell column in the westergren's tube.

Result:

The ESR is 12 mm after one hour.

Inference:

The Value of ESR (12 mm after one hour) thus obtained with female blood is within the normal range.

Signature of the teacher with remarks



Determination of Normal Blood Standards.

Observations :

The Values -

Total count of RBC;

PCV and

Net haemoglobin content of blood are observed and obtained from the respective experiment.

Result:

$$MCV = 70.37 \mu^3$$

$$MCH = 19.5 \text{ pg}$$

$$MCHC = 27.7\%$$

$$CI = 0.69$$

$$MCV = \frac{PCV(\text{ml}) \times 10}{RBC(\text{in ml/mm}^3)}$$

$$MCH = \frac{Hb\%(\text{gm/dl}) \times 10}{RBC(\text{in ml/mm}^3)}$$

$$MCHC = \frac{Hb(\text{g/l}) \times 100}{PCV}$$

Inference:

The Values are slightly below the normal range.

Signature of the teacher with remarks



Total platelet Count of blood

Observations :

Number of fields	Number of RBCs	Number of Platelets
01	15	1
02	20	2
03	18	1
04	17	2
05	19	0
06	11	1
Total	100	7

Result:

Total Count of RBC = $5260000 / \text{mm}^3$ of blood

$$\therefore \text{total platelet count} = \frac{7 \times 5260000}{100}$$

$$= 368200 / \text{dl of blood}$$

Inference:

The platelet count is within the normal range.

Signature of the teacher with remarks

Determination of Blood group

Observations :

4 spaces in 2 glass slides (2 spaces each) are marked as A, B, C & D.

A for anti serum A.

B for anti serum B.

C for Control and D for anti - Rh Serum.

The blood drops are mixed with 0.9% NaCl solution. Now anti A, Anti-B, Anti-Rh are taken in A, B, D respectively. Then the slides isolated to mix blood and anti-serum without mixing contents of different halves of slides.

Result:

Reactions with -

[Slide A]	[Slide B]	[Slide C]	[Slide D]
Anti A Serum	Anti B Serum	Controller Slide	Anti Rh Serum
(+) Occur	(-) not occur	(-) not occur	(+) Occur

Inference:

The blood group of the sample taken is B⁺

Signature of the teacher with remarks



Haemin crystal

(Under microscope)

NAME OF THE EXPERIMENT:

DATE:

Preparation of Haemin crystals

Observations :

Shining rhombic or tricyclic structures were found under low power objective of the compound light microscope.

Result:

The Haemin crystals are formed from acid haemin.

Inference:

We can determine that the sample was of human.

Signature of the teacher with remarks

Osmotic fragility of Red blood cells

Observations :

Red blood cells are allowed to rest on various suspended osmotic saline solution and in gradually diversing tonicity. The sample in the test tube is kept for many hours under careful observation. Thus the shape of RBC is factor for its fragility.

Result:

The red cells starts partial haemolysis at 0.5% saline solution which gradually increases. Ultimately haemolysis is completed at about 0.25% of saline solution.

Inference:

The fragility of the red cells of the subject under clinical check up was normal.

Signature of the teacher with remarks