PATHOLOGY I PRACTICAL RECORDS

Index

Sr. No	Name of the Experiment	Page No	Date of Experiment	Date of Submission	Remarks
01.	To study the compound microscope & its uses & handling.	01	15.10.2014	~~18~~ 19.11.14	
02.	Anticoagulants	06	29.10.14	26.11.14	
03.	To estimate the amount of Hb in the given blood sample	10	19.11.14	26.11.14	
04.	Estimate total RBC count for given blood sample.	15	25.11.14	14.01.15	
05.	To determine DLC for the given blood sample	18	30.11.14	14.01.16	
06.	Quantitative test for ABO grouping and Rh typing	22	07.12.14	14.01.16	
07.	Peripheral smear Examn	24	23.12.14	14.01.16	
08.	To determine the ESR for the given blood sample	28	03.01.15	14.01.16	
09.	To determine PCV	38	07.02.15	14.01.16	
10.	Determination of Bleeding time	40.	24.02.15	14.01.16	
11.	Determination of Clotting time	42	03.03.15	14.01.16	
12.	Examination of Urine	44	14.04.15	14.01.16	
~~13~~					

Date .2014

Expt. No. 01 Page No. 01

Aim of Experiment :- To study the compound microscope and its uses and handling.

Preface :- Microscope means small and scope means to view.

Microscope magnifies the images of the object visualised through it. Degree of enlargement is known as magnification or magnifying power of microscope.

Light microscope is operated by the white light of the sun or light obtained by tungsten lamp. Laboratory microscope provide a magnification of 40, 100 & 1000 X magnification respectively by scanner, low power, high power or oil immersion.

Total magnification is magnification of objective lens multiplied by magnification of eye piece.

Total magnification = Magnification of objective lens × magnification of eye piece.

Types of microscope :-
1. Simple microscope
2. Compound microscope
3. Electron microscope

Components of microscope :-
It consists of foot limb, mechanical stage and resolving nose piece. The mechanical stage gives the controlled movement to the object on slide.

Teacher's Signature :

Date

Expt. No. Page No. 02

I. MAGNIFICATION SYSTEM :-

It consists of lens bound to each end.

1. Eye piece: Here the observer places his eyes
2. objective lens : These are the lens just above the object to be magnified. Eye piece is made up of two lens upper & lower.

Magnifying power	Magnification
4 X	4 times
6 x	6 times
10 X	10 times

objective lens has a figure engraved on the sleeves of the lens showing magnifying power.

Magnifying power	Magnification
10 X	10 times
40 X	40 times
100 X	100 times

The 100 X lens is also called as oil immersion lens.

Numerical aperture :- It is also engraved on the sleeves of the lens next to magnification.

Teacher's Signature :

Date

Expt. No. Page No. 08

Numerical aperture	Magnification
0.3	10 times
0.6	40 times
1.3	100 times

Greater is the numerical aperture, better is the resolving power. Recommended length between the eye piece & objective lens is 160 mm. Recommended thickness of cover slip is 0.17 mm. Distance between the objective lens and the slide is known as working distance.

Objective	Working Distance
10 x	
40 x	0.5 – 1.5 mm
100 x	0.2 – 1.5 mm

Maximum resolving power of a medical laboratory microscope is 0.25 μ. Oil immersion objective lens increases the resolving power by converging the rays that would have been lost by refraction.

II. ILLUMINATION SYSTEM :-

Nature of light :- For a light microscope broad day light is required. Light can be adjusted by concave or plane mirror and diaphragm. Microscope should not be kept at direct light. In modern microscope, the light is provided by the point in electric lamp beneath the stage.

Teacher's Signature :

Date .....

Expt. No. .....

Page No. 04

Condenser :- It is situated between mirror and stage for maximum illumination is raised and lowered down for minimum illuminated. condenser bring all the rays of light to common focus on the object to be examined.

Diaphragm :- It is within the condenser used to increase or decrease the amount of light by increasing or decreasing the angle.

III. ADJUSTMENT SCREW :-

Consists of five parts :- coarse adjustment screw, fine adjustment screw, condenser, mechanical stage, controller and iris diaphragm.

SETTING A MICROSCOPE :-

Microscope should be placed on a firm level bench. Ideal height of the platform should be 2 ft and 3 inches.

Focussing power :- Adjust a condenser with a lowest level. Lower the objective lens till it is just above the slide to examined. Raise the objective lens slowly by coarse adjustment screw till a clear image is seen.

Using high power light :- Adjust the condenser in b/w the lowest and highest position. Adjust the high power objective lens by coarse

Teacher's Signature : _____

Date

Expt. No. Page No. 07

adjustment screw by lowering untill a clear image is seen. Open the diaphragm for better illumination.

Using oil immersion objective lens :- place the condenser to the highest position. open the diaphragm to it's full position. place a tiny drop of cedar wood oil on the slide to be ~~covered~~. observed. Lower the oil immersion lens till it forms a contact with oil. Slightly raise the oil immersion objective with help of coarse adjustment screw till a clear image is seen.

care and maintenance :-
clean the objective lens with a soft cloth for cleaning oil immersion objective lens absorbant paper is used for removing the oil. for cleaning the eye piece, a soft cloth is used for cleaning a condenser a soft cloth moisten with xylene is used.

cleaning the supporting system :- A soft piece of cloth is used.

other types of compound microscope :-

1. Dark field microscope
2. Contrast microscope
3. Colouring microscope
4. Flourescent microscope

Teacher's Signature :

Date 29-10-14

Expt. No. ---- EXPERIMENT NO.-02 Page No. 06

ANTICOAGULANTS :- These are chemical which prevent the clotting of blood when mixed in proper proportion.

Mechanism of action:
EDTA and sodium citrate removes the calcium as non-ionized form and oxalate salts remove the calcium by precipitating it as insoluble oxalate. Heparin binds to anti-thrombin and prevent coagulation by inhibiting the action of several other factors.

Commonly used anticoagulants are :-

1. EDTA :- The sodium and potassium salts of EDTA are most common anticoagulants suitable for haematological test is mostly available as dipotassium salt and tripotassium salt of EDTA. Potassium salts of EDTA are more preferred for its solubility.

Mechanism :- They remove Ca^{2+} ion from blood by chelation. Thus preventing the coagulation of blood. Recommended conc^n is 1.5 ± 0.25 mg/dl.

Uses :- Used for blood count such as haemoglobin, estimation, RBC count, DLC, platelet count, eosinophill count.
for estimation of erythrocyte estimation rate by wintrobe's method for measuring packed cell volume.

Teacher's Signature : _____

Date .....

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

Advantages :-

i. Best preservation of cellular morphology.
ii. Since platelet clumping is inhibited so it used for platelet count.

Disadvantages :-

i. Excess EDTA cause shrinkage & disintegration of RBC & WBC.
ii. They also disintegrate the platelet giving ~~also~~ false result of increased platelet count, increase MCHC and decreased PCV.

2. TRISODIUM CITRATE :-

Mechanism : It binds loosely with free calcium ions to form calcium citrate complex ~~with~~ thus preventing the coagulation of blood.

Uses :- i) for coagulation studies (for estimating PT and aPTT). for this studies 9 volume of blood is added to 1 vol of 3.8% aq. sodium of trisodium citrate. This ratio of anti-coagulant of blood is critical for maintaining the osmotic effect and the changes in calcium ion conc^{n} affecting the coagulation test.

ii) for estimation of ESR by westergren method. 4 vol. of blood is added to 1 vol, of 3.8% of aq. solution of trisodium citrate.

Teacher's Signature : _____

Date .....

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

3. HEPARIN :-

Lithium or sodium salt of heparin at conc^{n} of 10-20 IU per ml of blood is commonly used as anticoagulant.

Mechanism :- Heparin acts as anti-thrombin thus inhibiting the coagulation of blood.

Uses :- i. commonly used anti-coagulant for gas analysis & emergency gas.

ii. Best anticoagulant for osmotic fragility test for diagnosis of spherocytosis. - suitable for immuno-phenotyping.

iii. for red cell enzyme studies such as G-6PD deficiency & pyruvate kinase deficiency.

Disadvantages:

i. Not suitable for blood count as it cause clumping platelets & leukocytes.

ii. should not be used for peripheral blood cells as it gives a pale blue colouration to background of the peripheral smear when ~~to~~ the blood film is stained by Romanowsky dye in presence of abnormal protein.

4. DOUBLE OXALATE MIXTURE :-

A mixture of potassium and ammonium oxalate salt in ratio of 2:3 was previously used for various haematological test and was replaced by EDTA. Potassium salt causes shrinkage of RBC whereas

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 09

ammonium salt cause swelling of RBC. therefore Double oxalate salt is preferred over single oxalate salt.

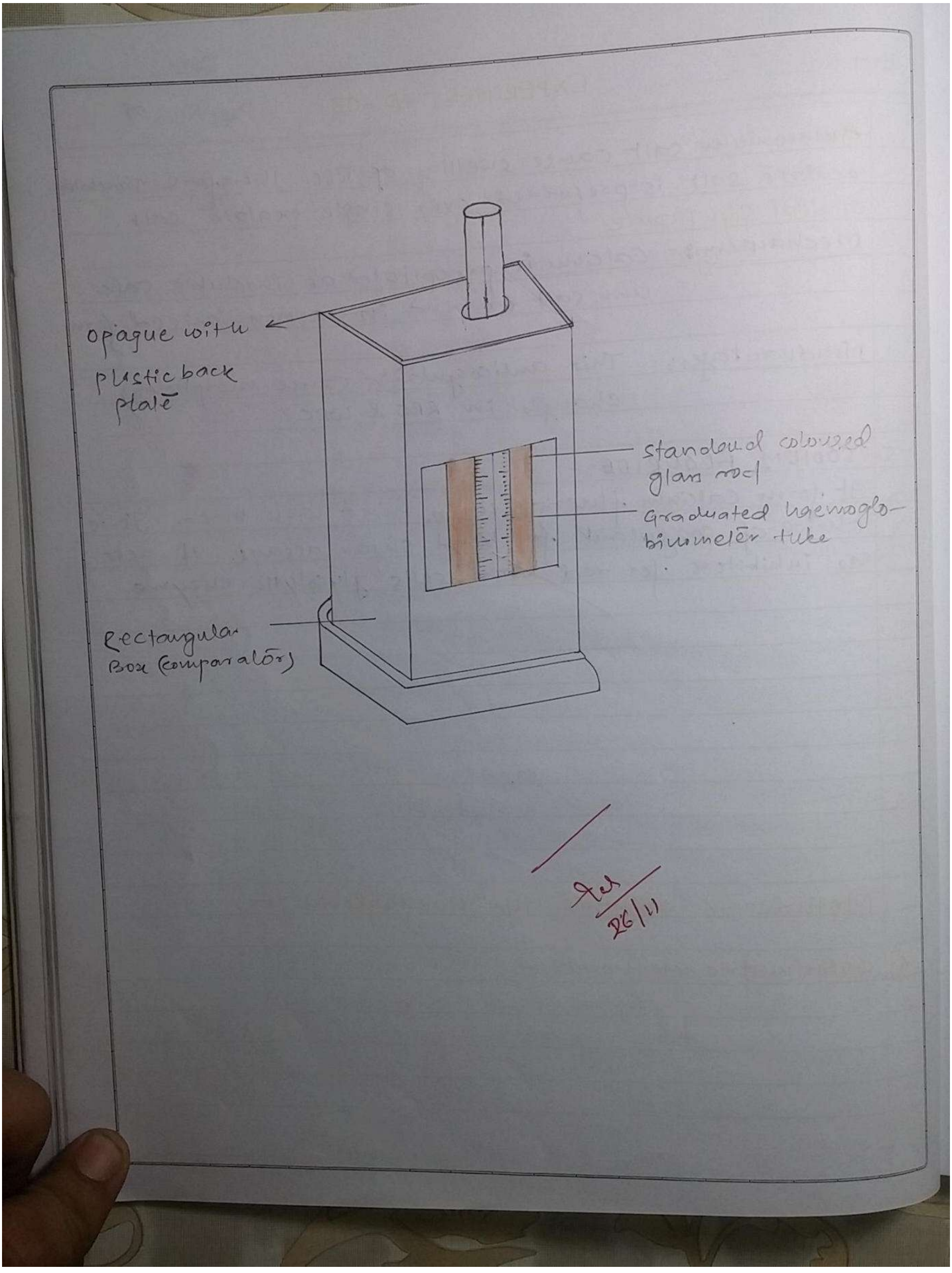
Mechanism:- Calcium is precipitated as insoluble calcium salt or bind in a non-ionised form.

Disadvantages:- This anticoagulant cause morphology changes in RBC & WBC.

5. SODIUM FLOURIDE:-

It form calcium fluoride when mixed with blood. It is choice of coagulant for blood sugar assays. It acts as inhibitors for red blood cells glycolytic enzyme.

Teacher's Signature : _____



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Date 19.11.2014

Expt. No. 03 EXPERIMENT NO.-03 Page No. 10

Aim:- To estimate the amount of Hb in the given blood sample.

Clinical Significance:

Normal Range: 15 ± 2 gm% for male 13.5 ± 1.5 gm% ♀
13.5 ± 1.5 gm% for female 15 ± 2 ♂
16.5 ± 3 gm% for infant 16.5 ± 3 infant

- Decrease in Hb below the normal range is indication of anaemia.
- Increase in Hb conc^{n}. is known as Polycythemia.

a) Primary Polycythemia : Increase in total Red cell mass with low erythropoetin.

b) Secondary Polycythemia : Increase in total Red cell mass with increase in erythropoetin.

c) Relative :- Decrease in plasma vol. with Red cell mass retaining.

ex:- for primary - polycythemia vera
for secondary - hypoxia
for Relative - Dehydration

Methods to estimate the Haemoglobin :-

A] Calorimetric methods :-

i) calorimetric :- In this method colour composition is made b/w the standard & the test sample either usually by calorimetric method

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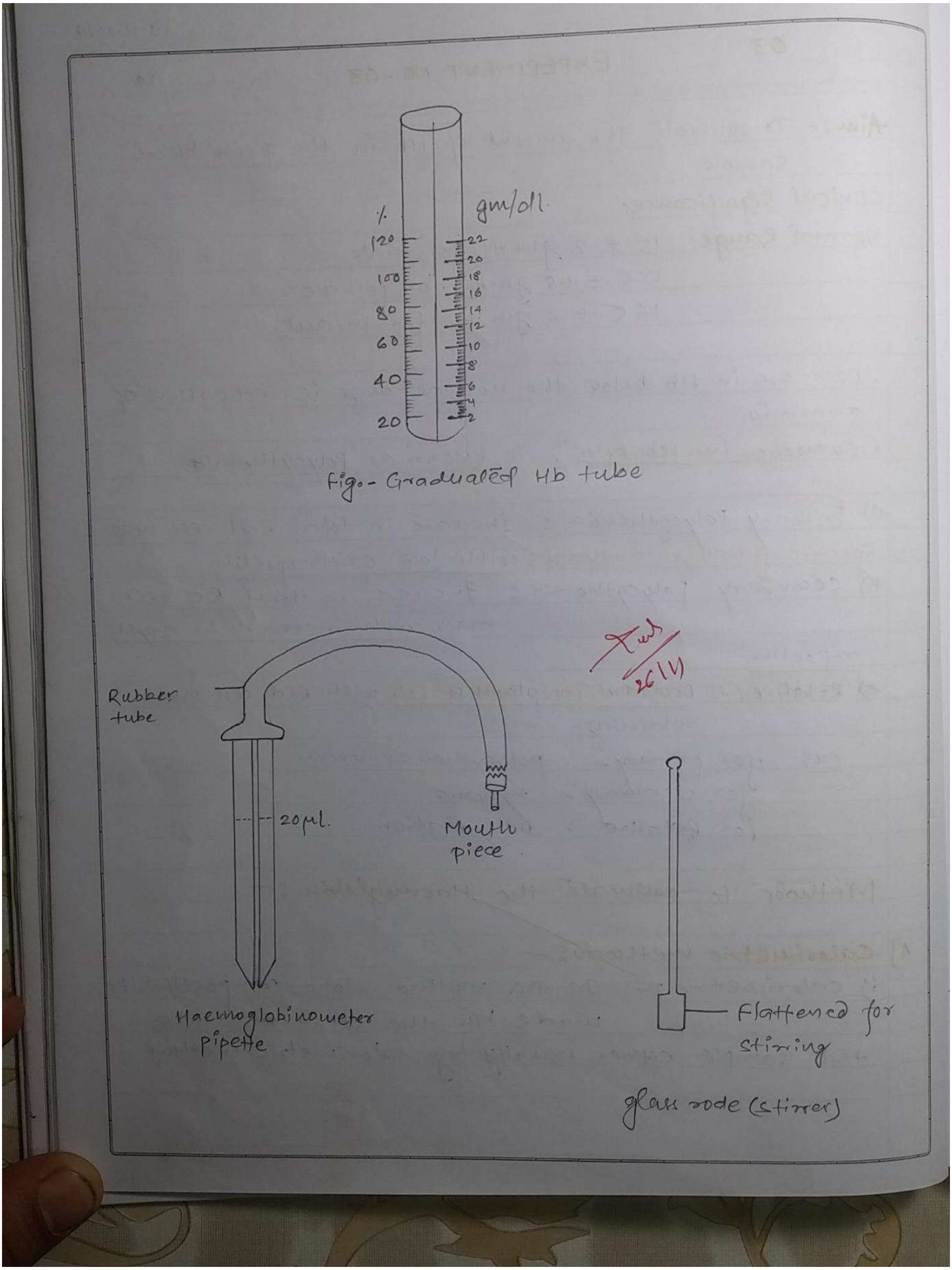

Fig.- Graduated Hb tube

Date .....

Expt. No. ..... Page No. 11

iii) Visual method:- Tall equest-
- Sahlis and Haematic method
- WHO Hb colour scale.

iiii) Photoelectric method:-
- cyn. method Hb method Cinabkin's method.
- oxy-Hb method.
- Alkaline Haematin method.

B) Gasometric method:- carrying capacity of blood is measured by non-styke method.

c) Specific gravity method:- This method is useful in man training like selection of blood doners.

SAHLI'S ACID HAEMATIN METHOD:-

The blood is mixed with acid sol" so that Hb is converted to brown coloured acid haematin. This acid haematin is then diluted with distilled water. Till brown colour method with brown glass ~~star~~ std. of comparative box. The Haemoglobin value is read directly from the scale.

Apparatus:-
- Add N/10 HCl in the Hb tube by dropping pipette up to the mark ~~of~~ 2 gm i.e. of tube.
- add 20 µl of blood at mark in Hb pipette below the blood from the pipette into the tube

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 12

and rinse the pipette by and discharging blood solution at least.

- withdraw the pipette and also the acid to act with the RBC for 10mm. this will lies the RBC and convert the Haemoglobin into acid Haematin.

- Mix the acid Hematin with glass rode provided and match it with the colour of brown glass std. of comparator box if the acid Haematin colour dark then add a few drops of distilled water to Haemoglobinometer tube.

- Stir the solution and match the colour with standard provided pipette in the process. till the solution colour match with the standard.

- Take out the stirrer from the solution but it should be drawn fully out of tube.

- The stirrer should be upper part of the tube. The reading is taken from the upper meniscus and in gm%.

Advantages:-
- simple bed site test
- Reagents & apparatus are cheap.

Disadvantages:-
i) There can be visual error.
ii) Cyn. method surface Hb cannot be converted

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 12

into acid Haematin.

iii) Brown glass standard of comparator may fell.

iv) Colour of acid haematin faids quickly.

Cyn. meth Haemoglobin method :-

This is the method for estimation of Hb.

Procedure :-

- The blood is mixed tropkins fluid.

Tropkins fluid contain potassium ferricyanide and potassium cyanide.

Tropkins fluid contain oxy-Hb, Co-Hb & meth-Hb. into acid Haematin with the conversion of acid haematin. There will be development of pink colour. The intensity of pink colour is measured by spectrophotometer or photo-electric colorimeter at 540 nm.

This is compared with standard cyn-meth Hb.

Advantages :- i) Their is no change of visual error in all form of Hb except sulpho-Hb

ii) The standard comparison is very stable.

Disadvantages :- i) Reagiy cannot be taken immediately.

ii) If the blood is turbid due to plasma protein the absorbance is more hence incorrect value may be obtained.

iii) Result may be affected by Hyperbilirubinimia.

Teacher's Signature : _____

Date .....

Expt. No. .....

Page No. 14

Result:- The amount of Haemoglobin in the given blood sample is 13.2 gm/dl.

Teacher's Signature :

Date .....

Expt. No. ..... Exp. No. - 04 Page No. 15

Aim :- To estimate total RBC count for given blood sample.

PRINCIPLE :- The blood sample is diluted 200 times with the diluting fluid & does not remove RBC but allow RBC to settle down & count in known vol. of fluid. The final result is calculated as no. of RBC in diluted blood and reported as cells / mm^3 of blood.

APPARATUS :- Microscope, Improved Neubaur's chamber, cover slip, Counting chamber, Haem's fluid, RBC pipette.

Constituent of Haem's fluid :-

Mercuric chloride	:	0.25 gm
NaCl	:	0.5 gm
Na_2SO_4	:	2.5 gm
Distilled water	:	

- NaCl provides isotonicity.
- Na_2SO_4 acts as anticoagulant & fixation and also prevents Roulear formation.
- $HgCl_2$ acts as antifungal & antibacterial agent.

Dacie's fluid -

40 % formaldehyde	=	5 ml
30 % Sodium Citrate	=	495 ml.

METHOD :- Blood sample is mixed properly & sucked upto mark of .5 Draw the diluting

Teacher's Signature : _____

Date .....

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

fluid till mark of 1 0 1. Mix the blood & diluting fluid in the bulb ~~c~~ the help of rubbing in the palm.

Place the Neubaur's chamber over stage of microscope. Place the coverslip over the counting area of Neubauer's chambers. Focus the counting chamber under low power microscope. Discard the first few drop from RBC pipette and carefully charge the counting chamber. Do not overcharge or undercharge the chamber. Allow the cells to settle down. First observe it under low power & ~~the~~ count the cells under high power.

Alternative method :-
Take 4 ml of RBC diluting fluid in test tube & add .02 ml of blood by sahli's pipette.

CALCULATION :-
Area of 1 small square = $\frac{1}{5}$ mm x $\frac{1}{5}$ mm

= $\frac{1}{25}$ mm^2

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

& Vol. of single counting chamber

= $\frac{1}{25}$ mm^2 x $\frac{1}{10}$ mm

= $\frac{1}{250}$ mm^3

Each big counting chamber consists of 5 small counting chamber = $5 \times \frac{1}{250}$ mm^3 = $\frac{1}{50}$ mm^3.

Teacher's Signature : _____

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9	9	5	9
8	9	10	4
7	8	4	16
10	11	6	7

RU

8	6	9	10
8	9	4	11
4	7	7	8
7	5	8	9

M

9	9	8	11
7	12	7	5
9	8	5	14
12	12	8	5

LL

8	5	3	15
8	7	10	14
4	10	7	8
9	11	8	6

RL

5	12	5	9
9	6	5	9
6	12	8	10
14	7	6	13

$N = 132 + 118 + 135 + 133 + 134$

$= 652$

TOTAL no of ROBOCS $= 652 \times 10^4$

$= 6.52$ million.

Date .....

Expt. No. ..... Page No. 17

No. of cells in 1/50 mm^3 = n

So, 1 mm^3 of diluted blood = n x 50 cells.

Dilution factor = 200

So, 1 mm^3 of undiluted blood has = n x 50 x 200 cells.
= n x 10000 cells.

PRECAUTIONS :-

- Both haemocytometer & cover slip should be dried.
- pipette should be dried.
- should not be undercharging or overcharging of counting chamber.

Clinical significance :

Normal RBC Count -

At birth = 6.5 - 7.25 million/mm^3.
Adult male = 5 - 6 million/mm^3
Adult ♀ = 4.5 - 5.5 million/mm^3.

↓se in RBC count - Anaemia
physiological - pregnancy.
pathological - Fe deficiency, megaloblastic, hemolytic.

↑se in RBC count - polycythemia.
physiological - High altitude, new born.
pathological - 1° polycythemia, 2° polycythemia due to chronic lung disease, hypoxia.

Teacher's Signature : _____

Date .....

Expt. No. 05 Page No. 18

AIM :- To determine the DLC for the given blood sample.

(Tally bar - method 100 - Box.)

PRINCIPLE :-

It is based on Romanowsky technique. The polychromatic staining solution like Leishman's stain etc. contain and acidic and basic dye.

Acidic is eosin & basic is methylene. The acidic & basic dyes include multiple colours when applied on cells. Fixation does not allow any further changes in cells. & make them adhere to glass slide.

Basic component are stained by acidic dye i.e. eosinophilic particles.

Acidic component take blue to purple stain by basic dye i.e. basophils.

APPARATUS :-

Two slides, blood sample, Leishman's stain, distilled water, glass rod, blowing pipette, cedar wood oil, microscope.

Composition of Leishman stain :-

- .15 mg of powdered stain in 100 ml of acetone free methanol.

PROCEDORE :-

Take a clean glass slide & place a drop of blood at edge of slide. Make a thin film of blood. Dry the film and cover it Leishman stain it & drops. wait for a min. Add 2 times the amount of stain

Teacher's Signature : _____

Leucopenia with relative lymphocytosis - Enteric fever
malaria

Leucopenia & relative monocytosis - Kala-azar

Arneth count -

① Shift to left → band forms & stab forms (Immature cells)
few myelocytes.
eg:- severe bac inf, septicemia.

② Shift to Right → Org older leucocytes
hypersegmented nuclei.
Ex:- megaloblastic anemia, thalassemia.

Schilling count -

The 4 groups of leucocytes are expressed as a % age of WBCs.
groups are -
myelocytes, metamyelocytes, Band forms, segmented cells.

Reticulocytosis	Reticulopenia (cyto)	Normal value
- Acute blood loss	cytotoxic drugs	Adult 0.2 - 2%
- post splenectomy	Radiation	infant 2 - 6%
- B_{12} - Cy	myelofibrosis	Absolute count 2400 - 8000.
- hemolytic anemia		
- Regen phase of anemia		

Date .....

Expt. No. ..... Page No. 19

DW. Mix the stain & DW under blowing pipette.
wait for 10 min.
wash the slide under tap water. Dry the slide in room temp. observe the slide under low & then high power & at last oil immersion.

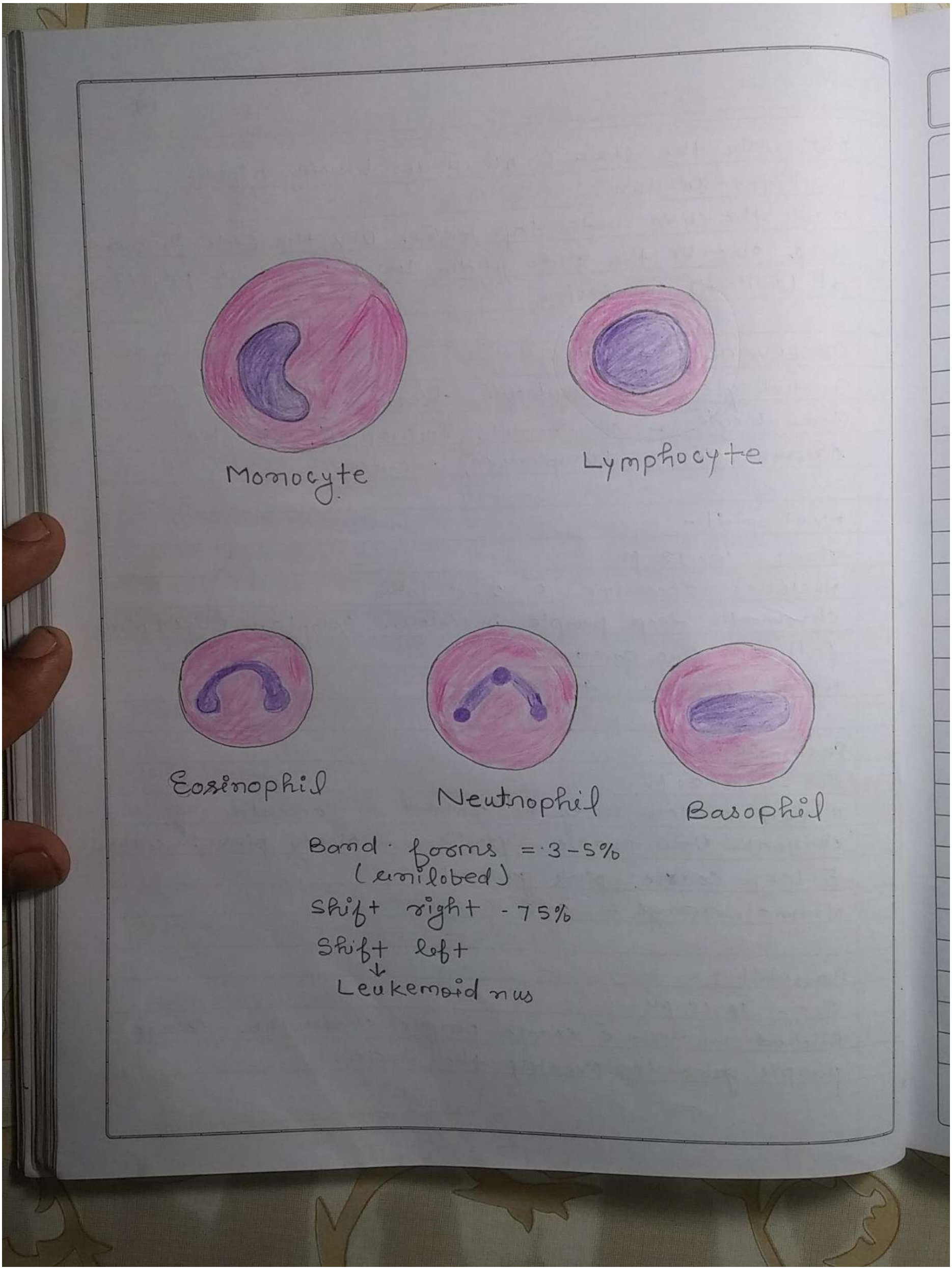
OBSERVATION :-
Granulocyte & agranulocyte observed.
Granulocyte - Neutrophil, Eosinophill, Basophill.
Agranulocyte - Lymphocyte, monocyte.

Neutrophil -
size: 10-12 μ
Nucleus: eccentric & 2-5 lobes.
chromatin deep purple in colour. cytoplasm light pink. full of fine granules.
Normal range: 40-70 %

Eosinophill :-
size - 10-20 μ
Bilobed nucleus may be central or eccentric
chromatin light purple. cytoplasm - plenty, pink in colour & large coarse, pink granules.
Normal range - 2-6 %.

Basophil :-
size - 10-15 μ
Bilobed nucleus & coarse purple chromatin. coarse purple granule overlap the nucleus.

Teacher's Signature : _____



Date .....

Expt. No. ..... Page No. 20

Normal range - 0-1 %.

Monocyte -
size: 12-20 μ.
central or oval or horse shoe shaped.
pale blue in colour. cytoplasm is plenty & light.
pale blue ~~in~~ in colour.
Range - 2-8 %.

Lymphocyte :-
size: 7-8 μ
smaller than neutrophil, single nucleus placed eccentrically. Coarse purple in colour. cytoplasm is scanty blue in colour without granules.
cytoplasm seen as thin rim around nucleus.
Range - 30-40 %.

Precautions :-
Never understain or overstain the slide.

Neutrophilia :
Acute bact. infection, ARF, MI, CML,
Polycythemia Vera., Suppurative, Haemorrhage, Intoxication.

Neutropenia : steroid therapy, HIV → lymphocytopenia
Typhoid fever, Influenza, chem. like benzene, Antimetabolites drugs,

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 21

Aplastic anemia, megaloblastic anemia, bone marrow suppression due to infiltration of BM by leukemic cells, radiations, & chemo.

Lymphocytosis :- TB, Syphilis, Brucellosis, measles, chicken pox, CLL (chronic infections)

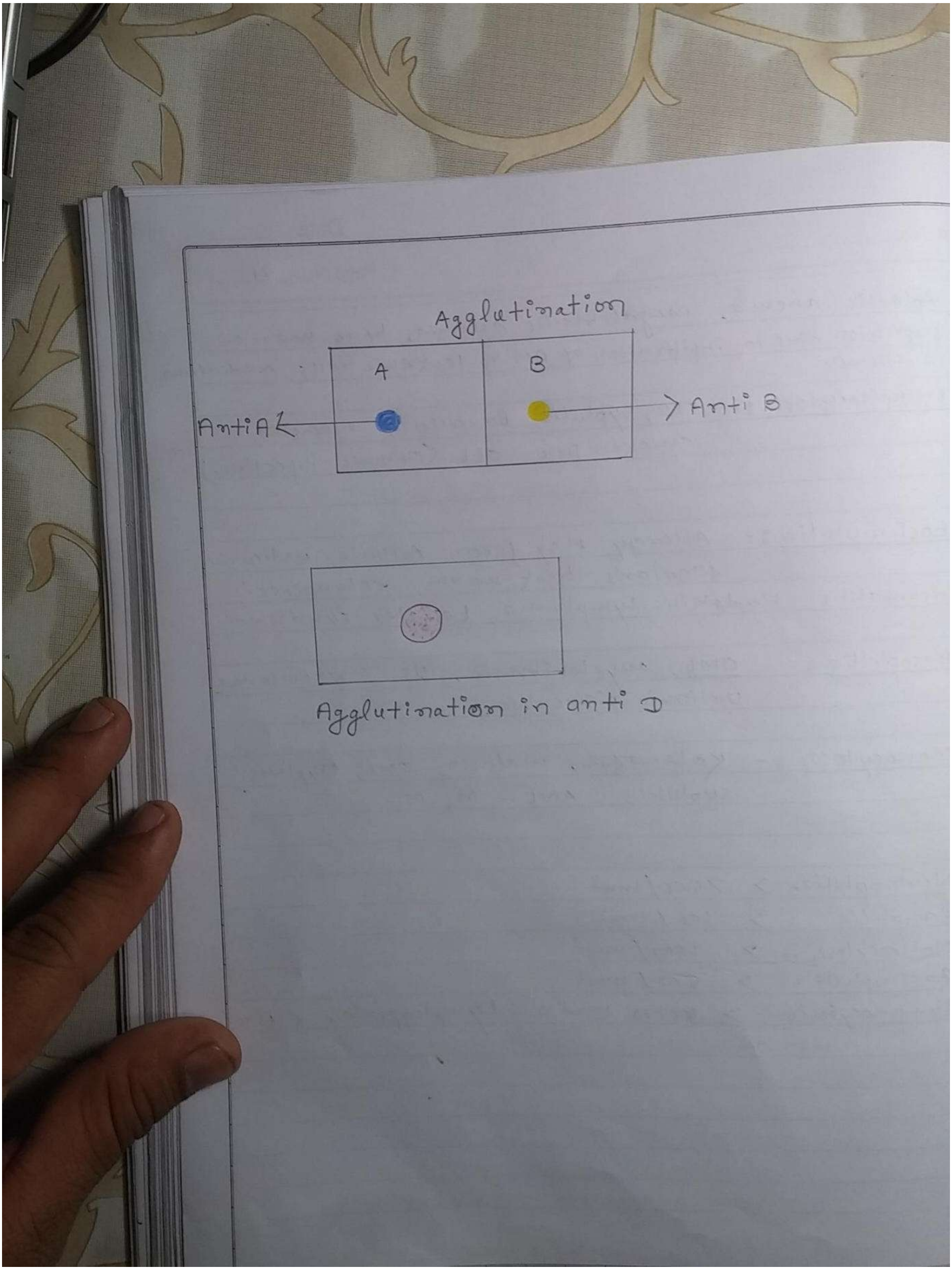
Eosinophilia :- Allergy, Hay fever, Asthma, urticaria, filariasis, hook worm, echinococci, dermatitis, Hodgkin's lymphoma, Loeffler syndrome.

Basophilia :- CML, myelofibrosis, UC, myxoedema, urticaria pigmentosa.

Monocytosis :- Kala-azar, malaria, CML, Typhus, syphilis, AML - M_4, M_5.

Neutrophilia - > 7500/mm^3
Basophilia - > 100/mm^3.
Monocytosis - > 1000/mm^3.
Eosinophilia - > 500/mm^3
Lymphocytosis - > 5000 mm^3 Lymphopenia - < 1500/mm^3.

Teacher's Signature : _____



Agglutination in anti D

Date .....

Expt. No. ..... Page No. 22

AIM :- Quantitative test for ABO grouping & Rh typing.

PRINCIPLE :
The procedure of blood grouping & Rh typing is based on principle of agglutination.
Normal human RBC parses the Ag & chem in presence of corresponding antibody in the serum.

Method - slide method.

APPARATUS -
Glass slide, blood sample, antisera A, Antisera B, Rh, glass marking pencil.

Antisera A - (Blue in colour). can be human polyclonal or murine monoclonal antibody of IgG type.
Antisera B - (Yellow). can be human polyclonal or murine monoclonal antibody of IgG.

Antisera D - Rh colourless. Can be human polyclonal & murine monoclonal antibody of IgM type.

PROCEDURE :- Take a glass slide and divide into 3 compartment by glass marking pencil.
Take a drop of blood in each compartment & add antisera A to compartment having mark A.
Antisera B to mark B. & antisera Rh to mark D.

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 23

Mix blood and antisera $\bar{e}$ 3 diff applicator stick. settle it down for 2-3 min.

Interpretation :-

Rxn $\bar{e}$ antisera A	Rxn $\bar{e}$ antisera B	Interpretation of Blood group.
+	−	A
−	+	B
+	+	AB
−	−	O

Note: The test does not ~~down the~~ slow the agglutination within 2 min. considered as −ve.

Precautions :

- Never add a drop of antisera on drop.
- Never interchange the applicator stick.

Significance:

Blood grouping is done for knowing the blood group of patient during transfusion in clinical field and in forensic to know blood group of unknown person to identify the person.

Result:- Blood group of given sample is A^+.

Teacher's Signature : _____

Date .....

Expt. No. 07. Page No. 24

Peripheral smear Examination

It is the single most important investigation which gives a lot of information about RBC, WBC, platelets & abnormal cells along with hemoparasites.

- To access the red cell morphology, a well made & stained PS is essential.
- The smear gives information about size, shape & the variation of RBCs and the extent of hemoglobination.
- This features help in characterisiziny diff. kinds of anemia.
- Preparation of P.S. :- clean glass
  1. A drop of venous blood is taken on a slide, 1 cm. from end of slide.
  2. Spread the drop in the forward movement ē the help of spreader slide making an angle of 30° - 45°.
  3. The smear should be 2.5 - 3.5 cm. in length.
  4. Smear is dried in air.

Staining of smear :-
Stained by Romanovsky stain. which stains red cells, white cells, platelets & parasites.
Mostly the smear is stained by Leishman's stain.

Evaculation of Peripheral Smear :-
Examine the smear under low power objectives to look for quality of P.S. & uniformity of staining.

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 25

A thin layer of oil is spread over smear & observed under the microscope.

Area for Red cell morphology - Area is marked in smear where RBC are evenly distributed and there is slight overlap of RBC.
Head, tail, & edges of smear should be avoided, Look for red cell morphology, platelet clumps & diff. type of WBCs.

Terminology used for describe red cells feature -

i) Normal → RBC - Biconcave disc ē diameter 7.2 - 7.7 μ (normocytic) ē a central pallor of 1/3rd area (normochromic).

ii) Macrocytes :- large RBCs ē diameter > 8 μ.
MCV > 100 fm litres.
seen in vit. B_{12} & folic acid deficiency.
usually oval in shaped & called as macro-ovalocytes.

iii) Microcyte - RBC diameter < 7.2 μ & MCV < 80 fm litres associated ē hypochromia (↑ed as. of central pallor) observed in Fe deficiency anemia & Thalassemia major & minor.

iv) Anisocytosis :- variation in size of cell population.

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 26

Clinically significant :-

v) Poikilocytosis - variation in shape of RBC population, Tear drop cells, helmet cells, oval cells. sickle cells - SCA.

vi) Spherocytes :- small deeply stained cells, central pallor is not observed. seen in HS & auto-immune hemolytic anemia.

vii) Target cells :- This cell possess redistributed Hb so that periphery & central region of cell appear hemoglobinised. found in SCA, Thalassemia major, Hemolytic anemia, post splenectomy & Liver disease.

viii) Sickle cell :- Thin elongated slightly curved RBC with irregular outlines & seen in sickle cell disease.
Induced by low O_2 states.

ix) Schistocytes :- (fragmented cells) irregular shaped triangular & speculated cells. mostly these cells are on process of fragmentation.
characteristic feature for microangiopathic hemolytic anemia & DIC.

x) Acanthocytes - RBC posses large, coarse spicules having sharp end & irregular features mostly

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 27

seen in β-lipoproteinemia & liver ~~cells~~ disease. (associated) & ↑sed chl & chl/pl ratio).

xi) ~~Bush~~ Bush cells - RBCs & numerous uniform projection, incised & regularly spaced seen in uremia & drug induced hemolytic anemia in infants.

xii) Tear drop cells - RBC has tear drop shape. seen in myelofibrosis & MD Anemia.

xiii) Polychromatin red cells :- slightly ~~change~~ large RBC & faint blue grey tint due to r-RNA. Hemolysis & Blood loss.

xiv) Basophilic stippling / punctate basophilia - presence of fine / coarse purple blue granules (ribosomal aggregates) in RBCs. Lead poisoning, thalassemia (coarse) & megaloblastic anemia (fine)

Howell jolley bodie - round purple nucleus remnant in RBC in megaloblastic anemia, post spleenectomy, thalesemia.

xv) Rouleux formation - arrangement of RBC like a stack of coins seen in hyper- gamma globulinemia.

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 28

xvi) Dimorphic RBC : Presence of 2 diff. population of RBC, macrocytic hypochromic, & normocytic. Sideroblastic Anemia, post blood transfusion, partially treated anemia.

ANEMIA :-
Decrease in no. of circ RBC ↓ dec in Hb. ↓ compared to normal in that group.

♂ - Hb < 12 gm/dL } Anemia
♀ - Hb < 10.5 gm/dL }

Based on morphology Anemia divided as -
i) Macrocytic
ii) Microcytic hypochromic
iii) Normocytic macrochromic

i) ↓ sed Hb, TLC ↓ or ↔, platelets ↓ or ↔
MCV - >100 fl, MCHC - ↔ MCH - > 35
(PS) - mild to moderate degree to anisopoikilocytosis.
RBC - macroovalocytic, elongated, tear drop cells, normal RBCs. ↓ inclusion like cabots ~~sign~~ rings, punctate basophilia, howell jolley bodies, few large hypersegmented neutrophils are seen.
Platelets are normal or ↓ sed.
smear may show occassional megaloblasts.
serum Bb - 1.5 to 3 gm %.

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 29

Hemolysis resulting from intramarrow death of megaloblast (Dyserythropoetic anemia).

~~Polyc~~

ii) Hb ↓, TLC ↔, platelet ↔ or ↑sed,
MCV < 80 fl., MCHC < 31 , MCH < 30 pg.

(PS) -
RBC smaller than normal, central pallor 1/2 or more than 1/2 of RBC diameter.
smear - mild - moderate Anisopoikilocytosis & microcytic RBC.
mild to severe degree of hypochromia.
Severe hypochromia - RBC shows a rim of Hb at periphery - Rim of pessary cell.

Fe↓ tear drop, elliptical, pencil cells +nt.
DLC normal.
Normally seen in Fe deficiency anemia. if it is caused due to hookworm → ↑sed eosinophilia is seen.

Thalasemia major -
smear shows mod-severe anisopoikilocytosis, & target cells, microcytic hypochromic RBC.
presence of stippled RBC fragment cells & nucleated RBC. seen.
smear shows polychromasia due to ↑ Reticulocyte count. serum Bb ↑sed, HbF - 20-70 %. (raised, marrow Fe stores increased.

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		Fe deficiency Anemia	Thalassemia
1.	Cause	deficiency of Fe.	Hereditary hemolytic anemia
2.	PS.		
	Target cell	occassional	many
	Anisopoikilocytosis	mild	mod- severe
	Nucleated RBC	rare	+ve
	Reticulocyte count	normal	high
	BM - Fe Stores	nil	markedly ↑ed.
	B		
3.	Biochem finding		
	serum Fe	↓se markedly	↑se markedly
	% of Transferrin saturation	< 16 %	> 70 %
	serum ferritin	< 15 mg/ml.	300 - 3000 mg/ml
	S. Bb	Normal	↑ed.
	Urobilinogen	Normal	↑ed.
	AbF	Nil.	20 - 70 %.
4.	Bone changes	Nil.	+ve of hair on end. appearance on skull.
5.	Clinical features	Pallor	Hepatosplenomegaly & pallor.

Date .....

Expt. No. ..... Page No. 30

Sickle cell Anemia :-

PS - severe anisopoikilocytosis ~ large no. of target cells. fragment RBCs, nucleated RBCs & sickle cells.
RBC - normochromic to mildly hypochromic.
Appears as long elliptical to oval or narrow curve cells ~ pointed ends.

iii) **Normochromic Normocytic :-**

present ~ normal Red cell indices in post hemorrhagic anemia & bone marrow suppression.

Leukaemia -

Malignant neoplasm of blood forming cells which undergo uncontrolled proliferation of BM & replaces erythroid & megakaryocytic series of cell resulting in anemia & thrombocytopenia.

Acute & chronic leukemia

① ALL & AML

PS findings -
TLC ↑↑ (14000 - 100000)
characterised by ↑ce of blast cells (>30% in BM).
and in PS of leukaemia Leukamia.
peripheral picture of subleukamic leukamia shows <30% of blast cells.

Blast cells are large ~ ↑ N/C ratio.
N. chromatic is opaque ~ 1-2 nucleoli in ALL & 3-5 in AML.

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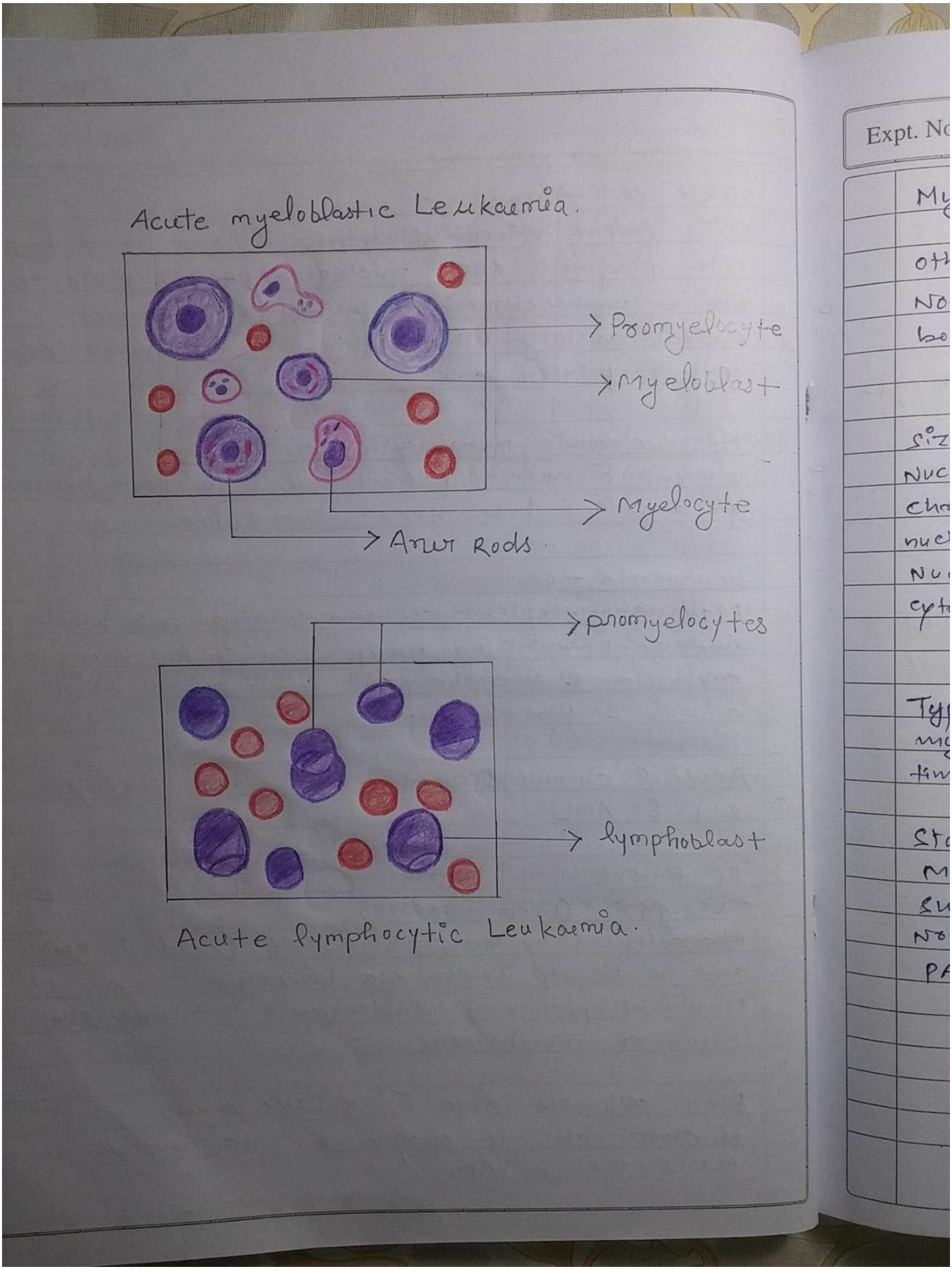

Date .....

Expt. No. ..... Page No. 31

Myeloblast demonstrates Auer rods.

other findings –
Normocytic normochromatin anemia & severe thrombocytopenia.

	Lymphoblast	Myeloblast
size	10-18 μ	10-18 μ
Nucleus	Round to oval	Round to oval
Chromatin	slight clumped	fine meshwork
nuclear memb.	fairly dense	very fine.
Nucleoli	1-2	3-5
cytoplasm	Scanty clear blue agranular	Scanty blue & ↑ce of auer rods.

Typing of blast cells are essential for chemotherapy. myeloid & lymphoid cells are extremely diff. & at times it is difficult to demonstrate by morphology.

Stain	AML	ALL
MPO	+ve	-ve
Sudan Black	+ve	-ve
Non-specific esterase	M_4 M_5 +ve	-ve
PAS	-ve mostly (M_6) +ve	+ve.

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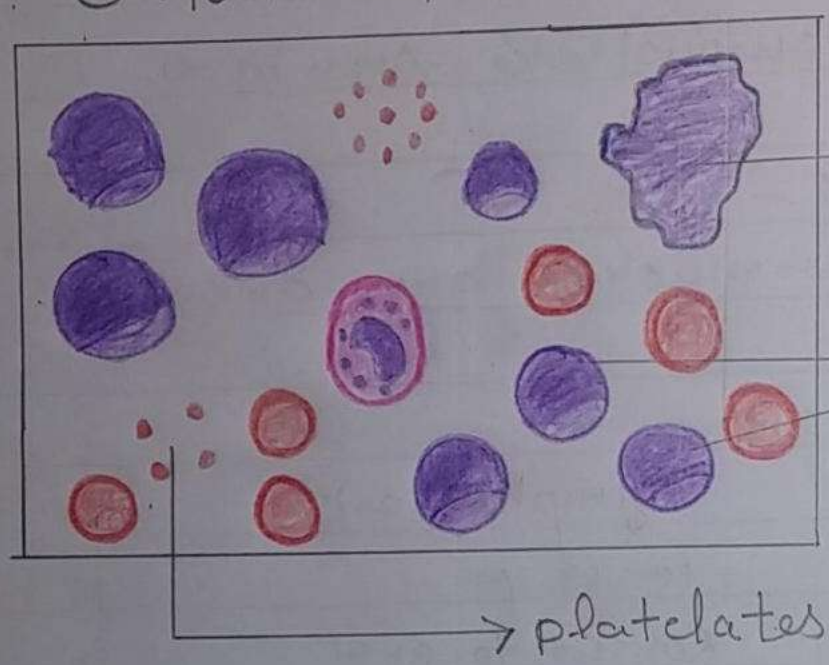
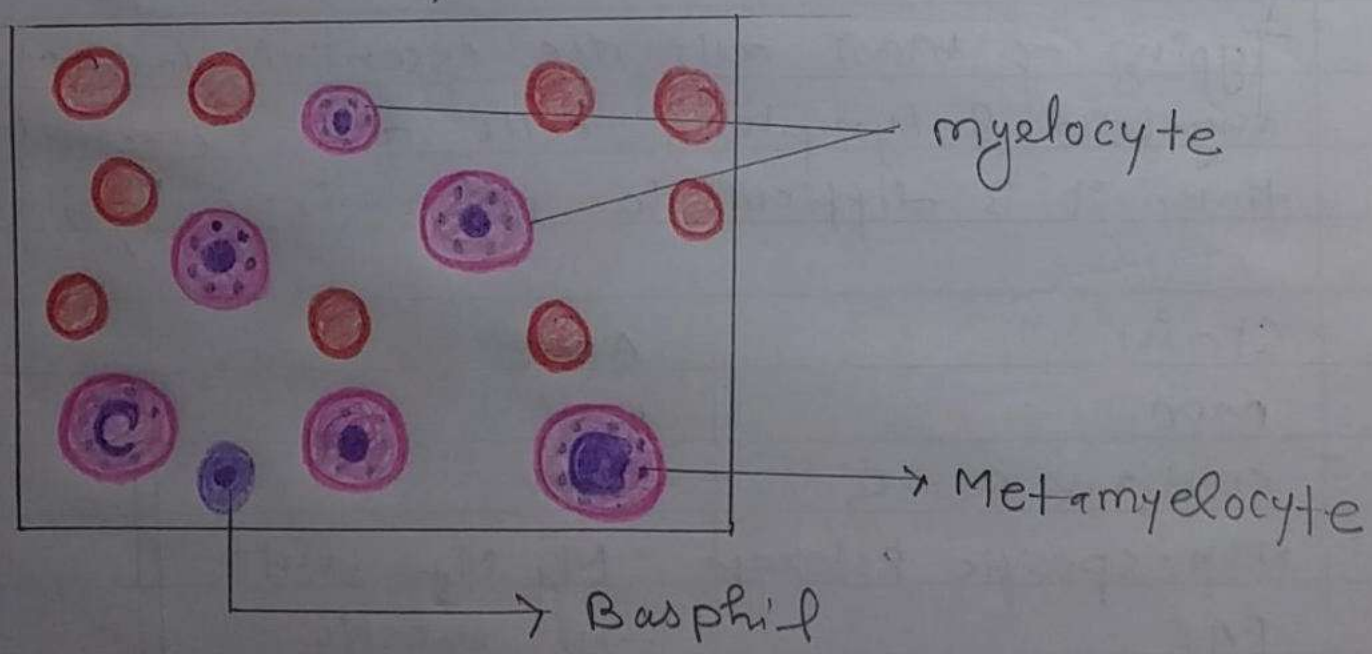
Chronic lymphoblast Leukaemia.



Chronic Myeloid Leukaemia



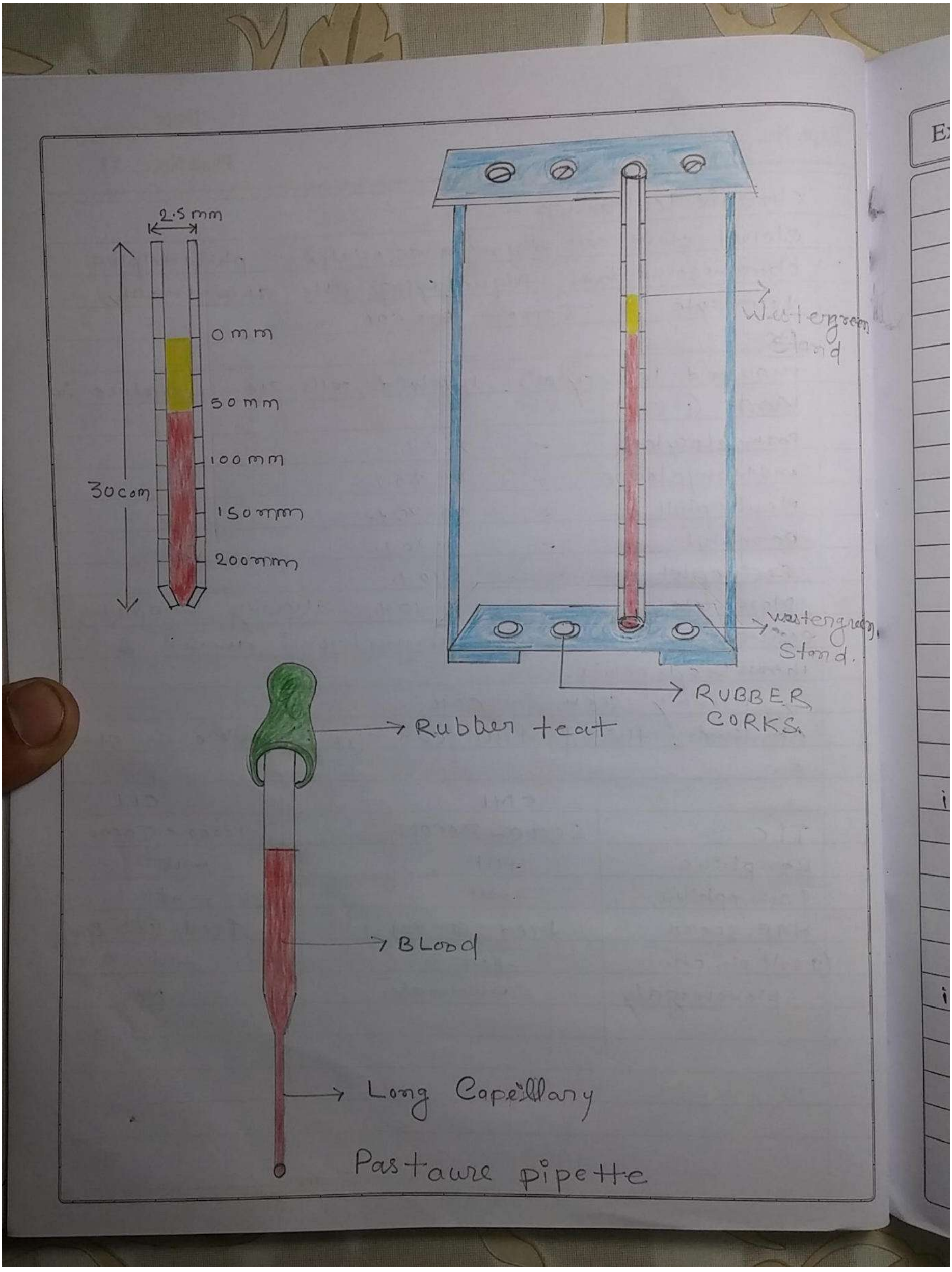
Date .....

Expt. No. ..... Page No. 32

Chronic leukaemia -
clonal stem cell disorder associated ē philadelphia chromosome has unique cytogenetic abnormality.
leucocyte - 50000 - 300,000 mm³.
PS -
marked leucocytosis, myeloid cells are immature in blood (1-5 %)
promyelocyte - 2-5 %.
metamyelocyte - 15-20 %.
Neutrophill - 40-70 %.
Basophil - 1-10 %.
Eosinophil - 10 %.
Monocyte - 2-10 %. showing basophilia, eosinophilic normocytic normochromic anemia & thrombocytopenia.
→ mainly seen in CML.
At times, this feature can also resemble as CLL.

	CML	CLL
TLC	50000 - 300000	10000 - 50000
Basophilia	+ve	-ve
Eosinophilia	+ve	-ve
NAP score	↓sed (0-40)	↑sed (150-200)
(9:22)t ph. ch.	+ve	-ve
Splenomegaly	moderate	No.

Teacher's Signature : _____



Date .....

Expt. No. ..... Page No. 33

Aim – To determine the ESR for the given blood sample.

Principle :- When well mixed anticoagulated blood is placed in a vt. tube the erythrocyte tends to fall towards the bottom of tube or pipette till they form a packed column in the lower part of tube in a given specific time.

Mechanism of ESR :- Fall of RBC depends on following factors.

i) Rouleau formation - The RBC sediments in the tube because density is greater than that of plasma. When a no. of erythrocyte aggregate, in form a rouleau & settle down, this area is much less than that of some of the area of constituents, RBC.

Rouleau formation is a very imp. factor which ↑ses the ESR.

ii) Concentration of fibrinogen - leads to colloidal changes in plasma which causes ↑sed viscocity in of plasma. If the concentration of fibrinogen is ↑sed, the ESR is also ↑sed. In defibrillated blood, ESR is very low.

iii) Concentration of α & β globulins - these protein molecules have a greater impact than other proteins in decreasing the -ve charge of the RBC. This tends to keep them together thus promoting the rouleau formation.

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 34

iv) Albumin retards the ESR rate ~~to~~ so in conditions where albumin is lysed the ESR is ↑ed.

Sickeling retards ESR

polycythemia

diameter.

Methods of ESR -

* Westergreen's method - This the most commonly employed standard method for detecting ESR prior to the AIDS era.

westergreen pipette is a straight 30 cms. tube open at both ends & an internal diameter of 2.5 mm & calibrated from 0 to 200 mm from top to bottom.

Anticoagulant - Trisodium Citrate 3.8 gm/dl liq. is used in conc. of 1:4 (4 part of blood & 1 part of anticoagulant.

Procedure :-

- The patient is advised for moving faster before the collection of blood.
- With 1.6 ml of pt's blood, 0.4 ml of tri-sodium citrate is mixed in a test tube.
- This test has to be conducted or performed within 2 hrs of collection.
- Fill the pipette upto mark 0 with the help of a rubber teat & fix it in the rack vertically away from sunlight & vibrations.

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 35

- wait for 1 hr. after which the reading is taken at upper meniscus of RBC.

Normal values -
for ♂ - 3-5 mm at the end of 1 hr.
♀ - 4-7 mm at the end of 1 hr.

Advantages -
i) more sensitive method.
ii) easy to fill & clean the westergreen's pepette.

Disadvantages :
i) require more amount of blood.
ii) if dilution ratio differs, esr is affected.

* Wintrobe's Method -
Wintrobe's tube is a glass tube closed at one end. The tube is 110 mm long has internal bore diameter - 2.5 mm. The tube graduated on both sides as 0-10 on one side 10-0 on other side.
Anticoagulant - EDTA of conc. 1-2 mg/mL & double oxalate 2-3 mg/mL.
former causes swelling & the latter causes shrinkage of RBC hence RBC shape is maintained.

Procedure -
- The patient is asked for morning fasting before the collection of blood.

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Clinical Significance of ESR :-
non-specific test to evaluate disease
seldom used for diagnostic purpose but its use
is limited to the progresis of disease:-

Dignostic ulcer :-
i) Rheumatoid Arthritis
ii) Collegen Ds.
iii) Multiple Myeloma
iv) chromic Infection
v) Macroglobulinemia.

Monitering the progresis of disease :-
i) TB (ii) temporal arthiritis iii) Polymyalgia Rheumatein iv) Hodgkins disease :-
ESR < 10mm good progresis
ESR > 16mm poor progresis

Diseases Causing raised E.S.R :-
i) TB
2) acute bacterial endocardilis
(3) Acute MI
(4) Rheumatoid Arthiritis
(5) shock
(6) Anemia
(7) Liver ds.
(8) Multiple Myeloma
(9) Pregnancy
(10) Ankylosing spondylitis.

Date .....

Page No. 36

Expt. No. .....

- 1 ml of blood + anticoagulant
- The wintrobe tube is filled ε anticoagulated blood ε pasteur pipette upto the mark 0 so as the tube should be free from any air bubble.

The tube is placed vertically and the ESR is noted after 1 hrs.

Normal value - for ♂ - 0-7 mm after 1 hrs.
♀ - 0-15 mm after 1 hrs.

Advantage -
simple method and requires small amount of blood. PLV can be done by same pipette.

Disvantage :-
because of short and choice of antivagulant, this method is not sensitive for ESR.
Addition of more antivoagulant can level the ESR

Micro ESR method :—

Automated mathod :—

Diseases cause for ESR —

① Polycythemia:

Teacher's Signature :

Date .....

Expt. No. .....

Page No. 37

② Spherocytosis
③ S C anemia
④ Congestive HF (CHF)
⑤ Hypofibronemia.

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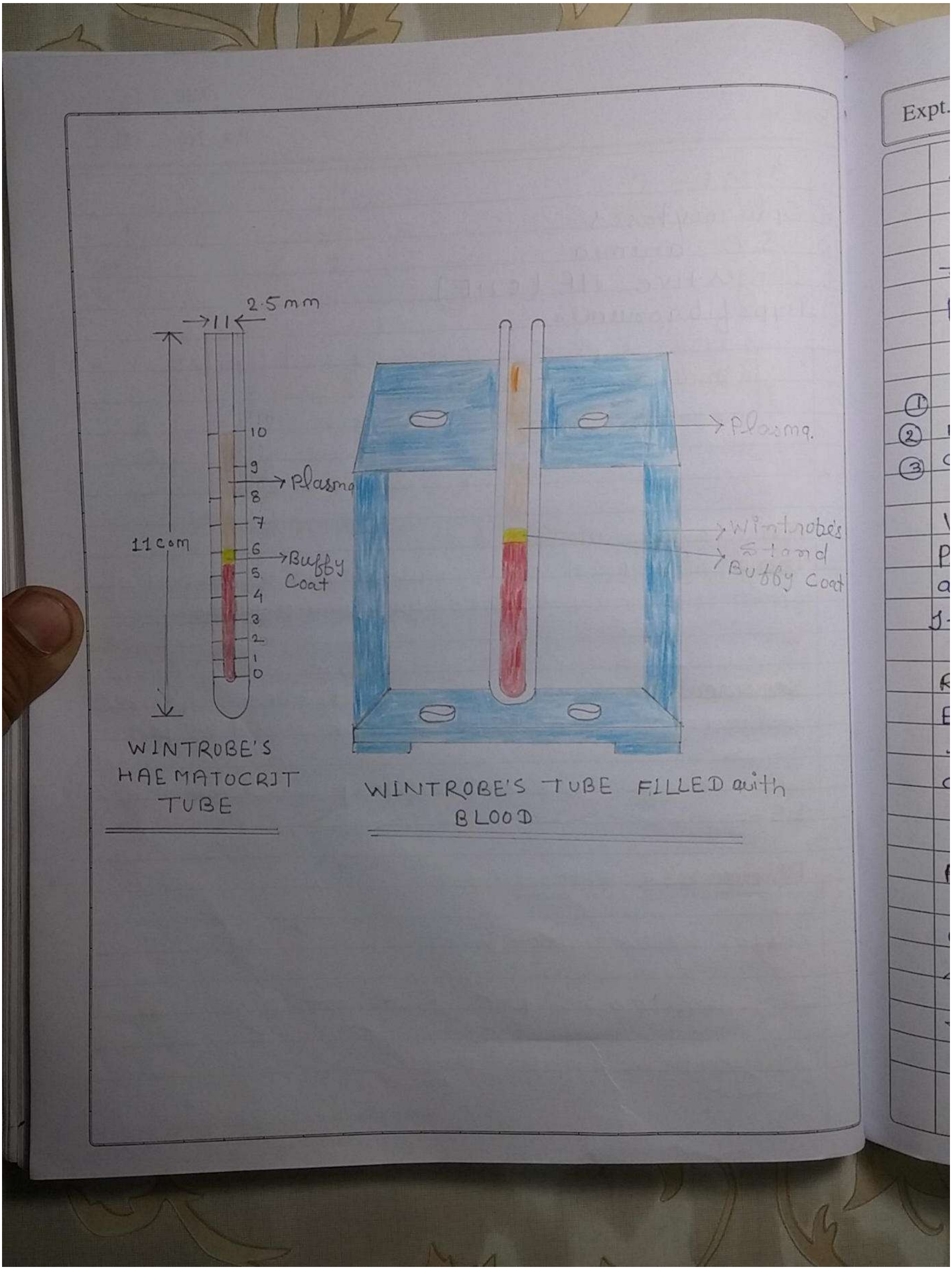

WINTROBE'S HAEMATOCRIT TUBE

WINTROBE'S TUBE FILLED with BLOOD

Date .....

Expt. No. ..... Page No. 38

AIM :- To determine the PCV

PCV is difined as ratio of volume of R.B.C to that of whole blood and is expressed as percentage.

Mathod of estimation of PCV :-
1. Macro (Wintrelus method)
2. Micro hematocrit method
3. Electronic mathod.

Wintrolles mathod :-
Principal :- Antiwagulent whole blood is centrifugel and the volume of Red cell mass denotes the hematocrit. It is expressed as persentage.

Requirment :-
EDTA or double oxalate blood, wintrolu tube of length 11 c.m (110 mm) and internal base of 2.5 c.m diameter and graduated from 0-10 both direction. Paeustem pipatte, needle, syringe.

Produce :-
Collect 2ml Blood of in EDTA vial. shake the Blood sample so that plasma cell and cells are mixed separately. Take blood in pipette from vial and place the end of the wosel to mark 0 or 100

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 39

Place tube in Centrifuge mc. Centrifuge it for 30 min. at the rate of 3500 Note the reading and evaluate it in % age

↑ in PCV –	↓ in PCV
polycythemia	Anemia
Emphysema	Excess fluid in blood in
Dehydration.	case of pregnancy.

NORMAL RANGE :-
♂ - 40 - 52%
♀ - 36 - 48%
Late pregnancy - 22-37%

Colour and ofacity of plasma :-

Yellow jaundice
Milky lipemia
Cleudy multiple myeloma
Reddish Heamoglobelimia.

Buffy Coat :- Normally it is of .5 - 1 mm and .1 mm buffy coat contains 1000 cells/mm^3

Precautions :-
Buffy Coat
should not be included while calculating PCV

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 40

AIM :- Determination of bleading

PRINCIPAL :- A small cut of std. size and depth is made on the skin and oozing blood is swiped off with filter paper. Bleeding stop's when capillaries contract and platelet seal the vessels. Therefore bleeding time is a measure of platelet, and fuction, capillary function and platlet plug formation.

Normal Range - 1-5 min.

PROCEDURE :-

1. clean the finger with spirit cotton swab.
2. Allow it to dry
3. Puncture the finger tip about 1 cm by using steride needle.
4. Start the stop watch, Blood should flow freely without squeezing finger.
5. After every 30 Sec. collect the drop of blood at one of filter paper.
6. When bleeding ceases, stop the watch.
7. Note the time on stop watch if bleeding is >10min
8. Discontinue the test and apply pressure on bleeding stop.

Causes of prolong BT :-

① Thrombocytopenia
② Functional platelet defect eg - aspirin

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 41

* Von Willebrand Ds.
* Anemia, Leukaemia, Cirrhosis.

Vessel wall disease like prolonged T/t o Corticosteroids. Vascular Abnormality like Pseudo

Deficiency of Vit. C
Infection
Allergic purpura. Senile purpura

Methods used for determining BT :-

* Dake's method
* Ivy's method
* Template method.
* Hess ~~method~~ Test
* Simplate Test

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 42

AIM :- To determine clotting Time.

CLINICAL SIGNIFICANCE :-

clotting time is a rough Indication of intrinsic pathway of coagulation. It is prolonged when there is seven dificiency factor VIII, IX and fibrinogen.
Most sensitive test is @PTT [Activated partial thromboplastin time]

PROCEDURE :-
Blood is collected in a capillary tube after a finger and stop watch is started. The formation of fibrin streak is noted by b the capillary tube at repeated intervals. Time is noted at 1st appearance of fibrin streaks
Normal Range - 4-9 min.

Apparatus Required :-
sterile , sterile needle, capillary tube of dimension 1.5 cm diameter is of 10-15 cm length, cotton swale, spirit, stopwatch.

PROCEDURE :-
clean the finger tip I cotton spirit and dry the area. Make a deep insion of about sterile needle and start the stop watch. Wipe off the 1st blood drop and collect in a capillary

Teacher's Signature : _____

Date .....

Expt. No. ..... Page No. 42

tube about 2/3rd of it's length.
After every 30 sec, break off about tern of capillary tube to find out if the fibrin steaks have formed or not.
when it appears, skip the stop watch and note down the time:

9ther methods :-
① Drop Method
② Lee white mathod / whole blood clotting time more stab reliable than Capillary Tube.

Causes of prolonged CT :-

Heriditary Coagulation disorders :-
Hemophilia A, Hemophilia B, Dysfibrogenea, Afibrogenea, Von Willebrand Ds. ; - Cy of factor VIII

Cross linkage ex. DIC, liver ds, anticoagulant tharapy.

Teacher's Signature : _____

Date

Expt. No. 12 Page No. 44

EXAMINATION of URINE

AIM :- Examination of given sample of urine.

It is imp. for diagnosis and assistance of various ds. Routine examination is divided in 4 parts :-

1) Adequacy of specimen.
2) physical and gross examination.
3) chemical and microscopic examination.

Adequacy of urine :- Sp. should be properly Collected in a clear containes & should be properly labelled with the age, name and name and sex of pt. It shld not shad sign of contamination. clean glass tube used for bacteriological examination. Sterilised tube or ~~bottle~~ bottle used.

Method of preservation of urine :-
Urine should be examined fresh with 1 hrs of voiding. beat it is delayed, the following preservation in added, to prevent decomposition.

Refrigeration at 4°C - add preservation like touline, formalin, thymol etc.

Physical examination of urine :-
Volume : 700 - 2500 ml of urin normally voided

Teacher's Signature :

polyuria :- Excess of urine voided in 24 hrs. > 2500ml
It can be physiologically due to excess H_2O intake
seasonal in winter.
Pathological in DI and DM
Oliguria :- when uria voided < 500 ml in 24 hrs.
may be due to.
* Less H_2O intake
* Dehydration
* Renal Ischemia.
Anuria :- Complete cessation of urine < 250ml of urine in 24 hrs may be due to renal stone (tumour) and ischemia.

Colour :-
Normal appearance :- Pale straw coloured due to pg. urochrome.
Colourless in Condition -
- DM, DI, excess water in take.
Deep amber colour -
• muscular excessive and High grade fever.
Orange
↑ red urobilinogen. Drugs :- INH
Smoky Red :-
Small amt. of Blood and aniline dye.
Red Colour
hematuria, hemoglobinuria.
Milky colour -
mixt of pus and fat
Green Colour -
phenol poisoning

Date .....

Expt. No. ..... Page No. 45

in 24 hrs.

Nocturia :- Urine passed in exess 500 ml during night is an early sign of renal failure.

odous :- normal faint ammonia odour.
pungent odours due to ↑sed production of NH_3 by bac. contamination.

Bac. Contamination of urine
Putrid - due to UTI
fruity odour - ketoacidosis
odem like mouse :- PKU.

PH :- Reflect ability of kidney to maintain conc. of H^+ ion in ECF and plasma. It can be measured by PH paper as electronic PH meter.
freshly voided urine :- slightly acidic PH: 4.6 - 7.0

Acidic Urine :-
High protein diet
Respiration and metabolise acidosis

Alkaline Urine :-
Resp. and Metabolic alkalosis
UTI by protein and pseudomonas.

Specific Gravity :-
Ratio of gravity of 1 ml urine to that of 1 ml of distil water. It depends on conc. of various particles or solute in urine. Sp. Gravity is used to measure the conc and diluting power of kidney.

Teacher's Signature : _____

Urinometer procedure :-
Fill the urinometer containes 3/4 with urine. Insect urinometer in it so that thoats in urine count touching the wall and bottom of container. Read the graduation on the rim of urinometer at lower urinary miniscus. Add or ~~solve~~ subtract .001 from the final reading for each 3°C above or bellow the calibration temp. moved on urinometer

Normal sp. Gravity - 1.003 to 1.030
Low sp. Gravity - High water intake
Excess water intake Dehydration
Albuminoruria
DI Glycomia.

Fixed sp. Gravity - 1. Chronic Nephritis
2. ADH deficiency.

chemical constitments frequently observed :-
Protein, ketose bodies, Glucose, bile derivatives.

Test for proteinouria :-
If urine is not clear, it shald be filtered or Centrifugal before tested. Urine may be tested for proteinouria.
by qualitative and quantitative method.

Qualitative :- * Heat and acidic acid test
* Sulphosalicylic acid
* Hales test and reagent strip test

① Heat and Acedic Acid test
Cause coagulation of protein.

Date .....

Expt. No. ..... Page No. 46.

Procedure :- Take 5ml of test-tube filled 2/3 of the tube with urine. Acidity it with adding 40% Glacial acetic acid if urine is alk. boil upper portion for 2 min (Lower part act as control). if PPt or turbility appear add few drops of 10% acetic acid.

Interpretation :- If ppt disaffear on adding citric acid it is alt. phasphate. If turbidity or ppt persist after addn of acetic it is due to proteins
No cloudiness - -ve
cloudiness algst dark background < 0.1 g/dl
cloudiness con't granulasily — 0.1 gm/dl
Granular cloudiness - 2+ (0.1 - 0.2 gm/dl)
(ppt and floculation) - 3+ (0.2 - 0.4 gm/dl)

thick solid PPt - 4+ .5 g/dL
Quantitative examination of protein in urine
Esbachs albuminometer method
Imbidity metric method.

Esbachs mathod :- Fill the take with urine upto mark 'U' add Eshbach reagent (Picric acid + Citric acid) upto R. close the tube with stopper and mix it and let it stand for 24 hrs. take the reading from the level of P.Pt in albenometer and divide it by 10 to get % of protein
Cause of peoteinuria - Normally there is very

Teacher's Signature : _____

Heavy proteinuria :- > 3 gm/day is seen in
* Nephrotic Syndrome
* DM
* SLE
* Renal ~~Vessel~~ Vein thrombosis.

Mederate proteinuria :- 1-3 gm/day seen in
* ch. glomerulonephritis.
* Multiple myeloma
* Selerosis
* Pyelonephritis.

Mid. proteinuria :- < 1 gm/day seen it
→ HTN
→ polycystic kidney
→ ch. pyelonephritis
→ UTI
→ fever

Microalbuminuria :- it excretion of 20-200 mg/day of albumin and it indicates early and passibly. reversible glomerular damage.

Test for Benes Jones proteins :- they are light chain of γ-globulin. These are execretion in multiple myeloma and other proteinuria

These protein are ppt at lower temp. and disappear on further heating above 90°C but reappeares on ~~cooling~~ Cooling to low tempreture again It In both Keres joins and albumin not in urine.

Date .....

Expt. No. ..... Page No. 47

scanty amount of protein in urine < 150mg/dl
sample of urine heated to boiling ppt
so formed dlt albumin are filtered.
out and test for bence jones protein
and repeated under temp. Constant.

Test for Glycosuria :-

Glucose is most imp. sugar with may be ~~not~~ in
urine normally 0-15 mg/dl of glucose 24 hrs. is
passed in urine with is ~~not~~ undetectable by
qualitative test.
May be qualitative as quantitative.

① Benedict Test :-
In this test, cupric ion is reduced to glucose by
Cuprous oxide and a Colourless ppt is formed.

Procedure :- Take 5ml of Benedict reagent in
a TT Add 8 drops of urine. Heat to mixture to
boiled for 2 min. cool in water both or under
running tap water.

:: Benedicts test for reducing substance. excreted
in urin the test is +ve for all reducing
sugar eg :- Glucose, fructose, maltose, Lactose
but not Sucrose
other reducing substance are ascorbic acid
antibiotic t dopa.

Teacher's Signature : _____

Interpretation - No change of balue Colour -ve
greenish colour traces - <0.5 gm/dl
cloudy green PPt + gm/al
yellow —11— —— ++ (1-1.5 gm/dl)
Orange PPT +++ (1.5 -2 gm/al)
Bride red —11— ++++ (>2g/al)

Qualitative test for Glucose :-
Benedicts test :- cause of Glycosuria - DM
~~-Renal~~ Glycosuria
Severe burns, severe sepsis, pregnancy, Administration of Corticosteroids.

Test for Ketonuria :- These are the product of fat metabolism the 3 ketone bodies encreated in urine are aceto-acetic acid (20%) Acetone (2%) and β hydroxy butyric acid (18%)

1) Rothra's test
2) Gerhadhetis test
3) Reagent Strip test

Rothra's test

Principal :- ketone bodies combined with alkaline solution of Sodium nitroprusside forming.

Date .....

Expt. No. .....

Page No. 48

Thymol - I ½ hrs at 8°C
purple Complex :-

Procedure :- Take 5ml of urine in test tube saturate it with solid $(NH_4)_2SO_4$ and a few cystal of Sodium nitroprusside and shake add liq. NH_3 from side of test tube.

Interpretation :- Appearance of purple coloured ring at function indicate the +ve of ketone bodies.

Cause of ketonuria - Diabetic ketoacidosis, Dehydration, Hyperemesis graviderum fever (acheria) after general anesthesia.

Test for Bile derivatives :-
The Bile derivatives excreted in urine are urobilinogen bile salt and bile pigments while urobilinogen is excreted normally in urine and small amount of bile salt and bile pigment appear in urine only in liver disease.

Test for Bile salt :- Bile salts excreted in urine are cholic acid and chenodeoxycholic acid.
test are - ① Hays test
② Strip method

Teacher's Signature : _____

Hays test :- Bile salt if +ve in urine lower the Surface tension of urine.

Procedure :- Fill a 50 or 100 ml of beaker upto 2/3 or 3/4 with urine. sprinkle finely powdered Sulphur power over it.

Interpretation :- If Bile Salt are ~~not~~ +ve in urine 4mg/day the sample should be always Collected in a dark Coloured bottle as urobilinogen get oxidised on exposure to light.

Test are :- Ehrlich test
Reagent stip test

Ehrlich test :- Urobilinogen in urine combines with ehrlich reagent to give a red purple Coloured compound.

Procedure :- Take 2ml of urine combines with ehrlich reagent or Ehrlich aldehyde wait for 3-5 minutes.

Interpretation :- Dev. of red purple Colour indicater urobilinogen is +ve and +ive test is Subsequently in dilution normally it is -ve in dilⁿ upto 1:20

Expt.

Date .....

Expt. No. ..... Page No. 49

Significance :- Cause of ↑d urobilinogen in urine hemolytic anemia and hemolytic jaundice causes of -nt urobilinogen in urine.

obstructive Jaundice

Test for bilirubin - Bilirubin is breakdown product of Hb normally no urobillinogen in urine.

Test for Bilirubin in urine
- Fouchet's test
- Foam test
- Reagent stip test.

Fouchet's test :-

Principal :- $FeCl_3$ oxide bilirubin to green biliverdin.

Procedure :- Take 10 ml of urine in a test tube add 3-5 ml of 10% barium chloride filtered through filter paper to the ppt on filter add a few drop of fouchet's reagent ($FeCl_3$ + trichloro acetic Acid)

Interpretation :- Development of green colour indicate bilirubin.

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Cause of bilirubin :- obstructive and Hepatocellular jaundice.

Test for Blood in urine :- (a) Benzidine test
(b) Arthotoulidine test
(c) Reagent strip test.

Benzidine test :- Take 2 ml of urine in a test tube. Add 2ml of Saturated Solution of benzidine with glacial acetic Acid.
Add 1 ml of H_2O_2 to it.

Interpretation :- Appearance of blue Colour indicate the presence of Blood.

Cause of Blood in urine :- Renal stone
Renal tumour.
Bleeding disorder, polycystic kidney trauma.

Microscopic examination of urine

Principal :- Microscopic element present in urine Supervision collected in the form of deposited by Centrifugation on as well a small drop of Sediment is examined by a Coverslip preparation under Microscope.

Date .....

Expt. No. ..... Page No. 50

Requirement :- Centrifuge tube glass slide coverslip Pasteur pipetted centrifuge machine and microscope.

Specimen :- Freshly voided, mid stream, catch early morning Sample.

Procedure :- Fill the centrifuge tube up to 3/4 make it balance in centrifuge machine with another tube with 5ml. If water 2500 rpm. pour of supernatant quickly into another test the tube resuspend the deposite on glass side cover with coverslip and make it to identification no. observe it under low power In sub due light can be obtain by partially closing the axis and diaphragm and then adjusting the condesar until satisfication contract is obtain

Observation :- Following are observed

① Pus cell enter the urine from glomerulan to urethra - normal urine contain 2-3 pus cells/ high power field most of the pus cell are neurtrophils.

② Epithelial cell :- 3-5 cell/high power field

3 types are :-

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Polygonal Small round

i) Tubular epithelial cell :- Slightly larger than WBCs and contain larger round nucleous may be cuboidal flat / Columnar in shape.

ii) Transitional epithelial cell 2-4 times larger than WBCs may be pear shape or round occassionally may contain 2 nuclei.

iii) Sq. epithelial cell :- larger flat Irregular shape (Urethra and vagina) contain abundant cytoplasm and small centeral nuclei.

(3) Erythrocyte :- They appear smooth biconcave and about 7µ in diameter and 2µ in thickness. They don't contain nuclei In less urin the red blood cells swell up and large. these lyse cell appear as Colouress sample called as ghost cell. In hypertonic urin red cell appear as created.

Casts :- The renal tubular secrete a mucoprotein it is believed to form basic protein Called Cast.

(i) Granular Cast :- Always indicate renal d/s due to ge degeneration of cellular cast or due to direct aggregation of serum protein -

Date .....

Expt. No. ..... Page No. 51

② Hyaline test :- These are colourless homogenous transpart ē rounded field, these cast can be seen in ↑d ⁱno. even in mild. kidney ds. fvo hyaline cast may be tnt in normal urine.

3. Red all cast :- Always pathogenic usually and glomerular ds found in acute glomerular nephritis SLL Renal infraath and Renal Pyelonephritis.

4. White cell cast :- tnt in renal infection can be seen in accute pyelonephritis glomerulo nephritis.

5. Epithelial cell Cast :- Rare Rarely seen in urin of these cast indicate tubular degeneration and necrosis may be due to nepthotic agent ab virus also tnt in severe chronic renal d/s.

⑥ Waxy Cast :- Yellow grey or colourless cast homogenous in appearance occure due to glomerular cast.

⑦ Fatty Cast :- After occurence of fatty degenerate of tubular epithelium mostly seen in nephrotic syndrome glomerulonephric toxic renal poisoning.

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Smooth muscle colourless avoid in shape vary in size have double refractile wall may be found in UTI mainly in diabetic pt.
Bacteria +nce of larger no. of bacteria with pus cells may indicate UTI.