Ministry of higher education & scientific researches
Foundation of technical education
AL Furat AL Awsat Technical university
Kufa Technical Institute

Hematology

For student Second class

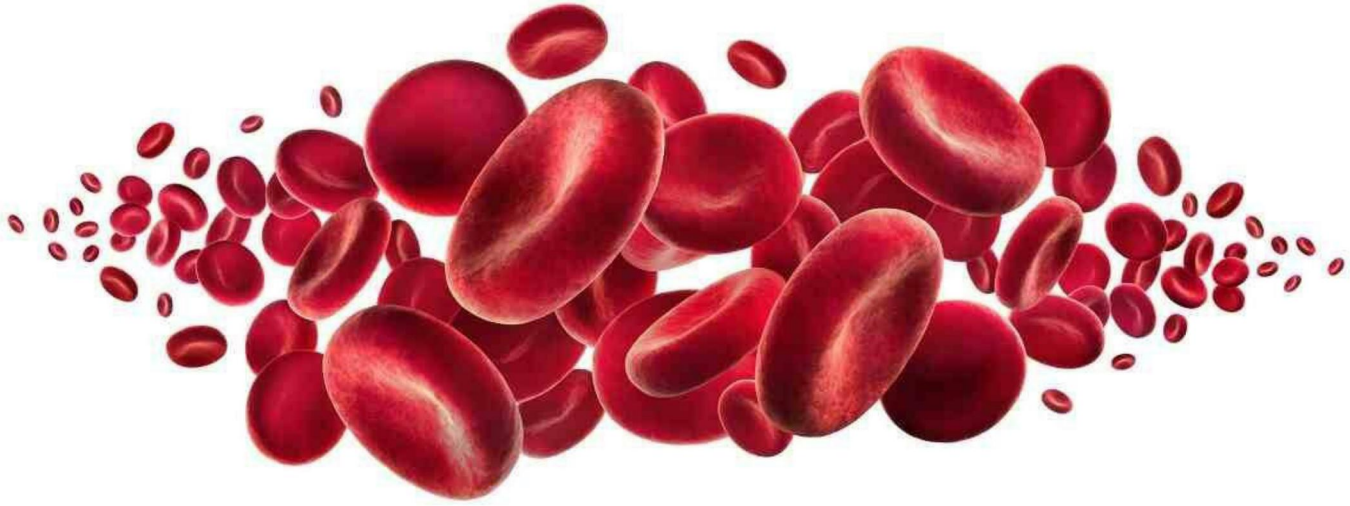
Pathology analysis department

Pathology analysis dept.

Steamer(2024-2025)

The Lecher : Ali K. Metab

The first course



Hematology

Introduction to hematology:

Is the science that study of formation all blood from bone marrow. Through the study compound ,disease, symptom and treatment .

Blood:- is the fluid connective tissue present in the body produce from bone marrow, it is composed of fluid plasma and formed element (RBCs,WBCs,Plateletes).

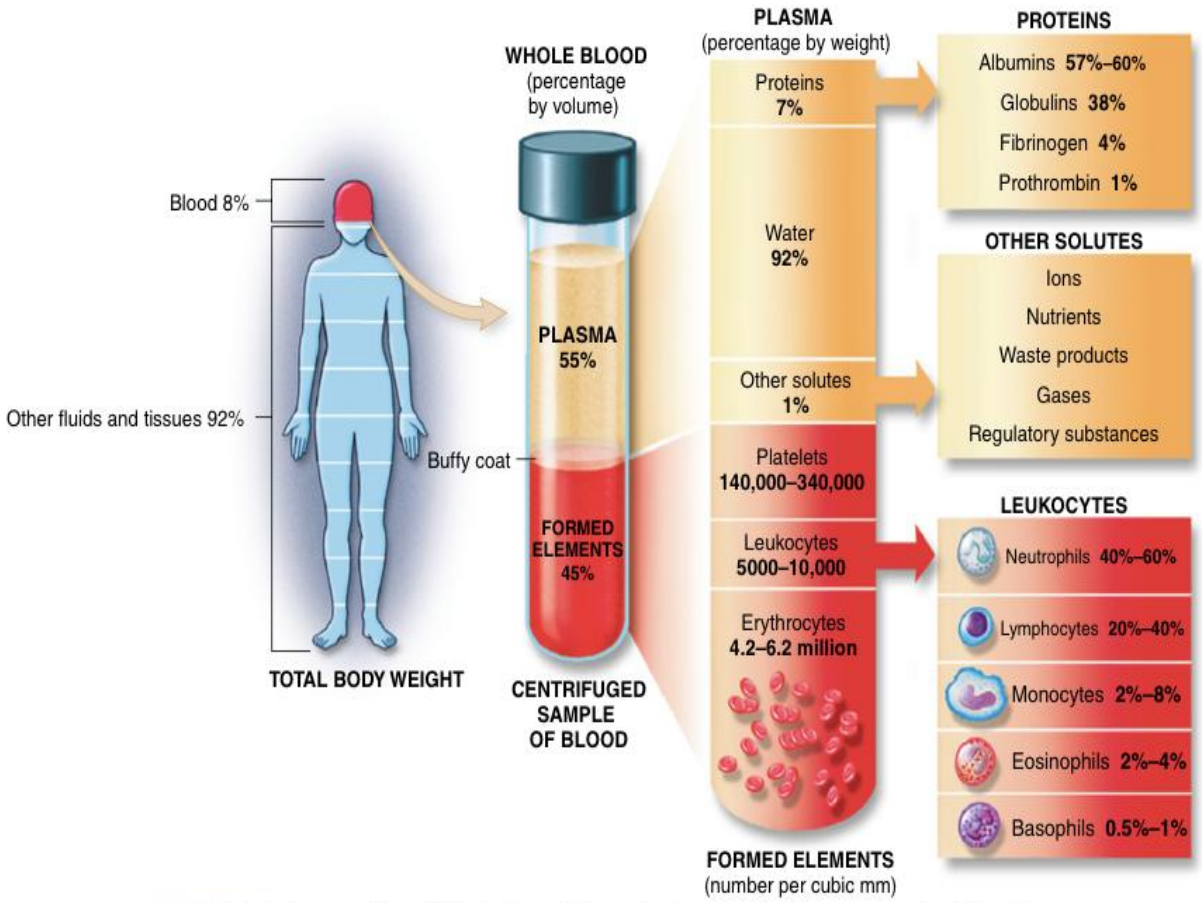
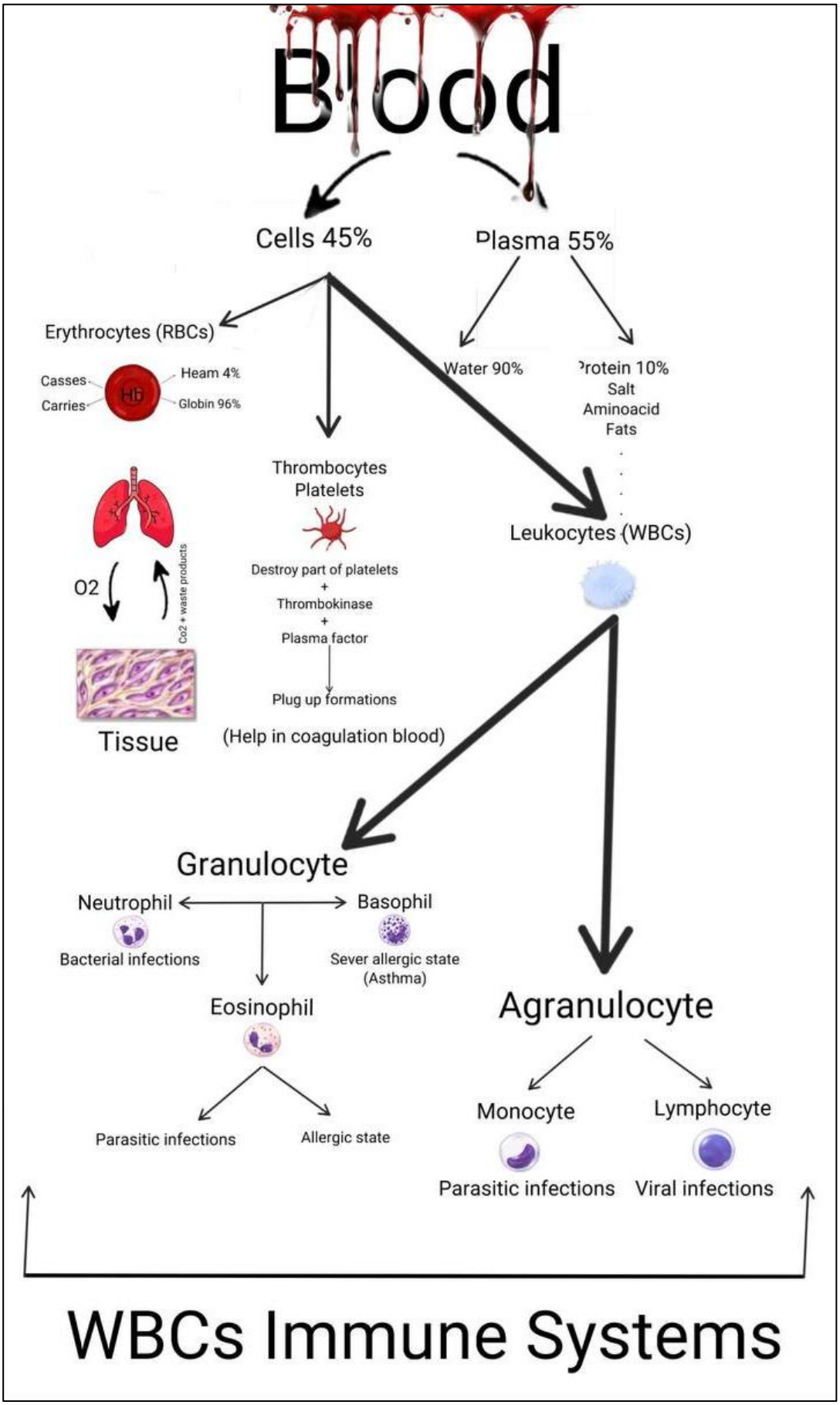


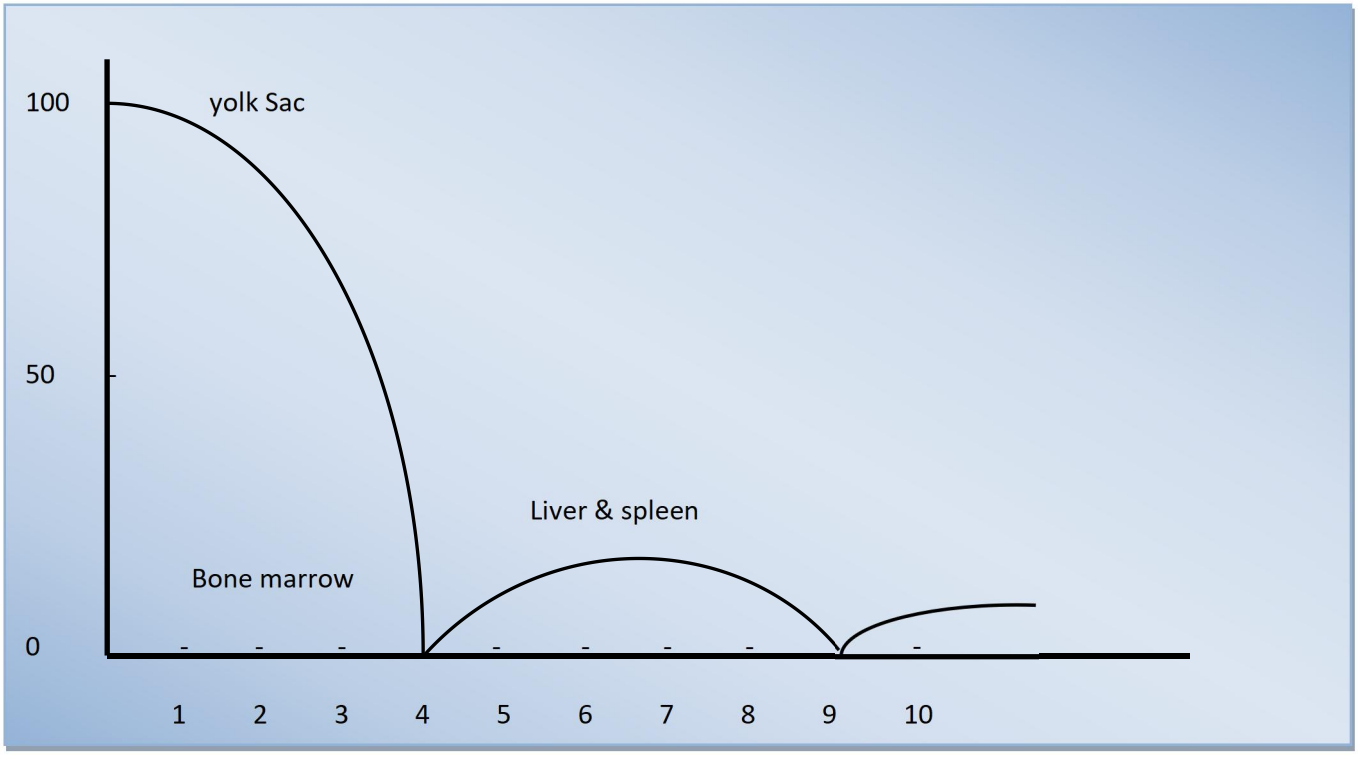
FIGURE 19-1 Composition of Whole Blood. Approximate values for the components of blood in a normal adult. (From Patton KT, Thibodeau GA: *Anatomy & Physiology*, ed 7, St Louis, 2010, Mosby.)


Blood
Cells 45%
Plasma 55%
Erythrocytes (RBCs)
Casses
Carries
Hb
Heam 4%
Globin 96%
Water 90%
Protein 10%
Salt
Aminoacid
Fats
Thrombocytes
Platelets
Destroy part of platelets
+
Thrombokinase
+
Plasma factor
Plug up formations
(Help in coagulation blood)
Leukocytes (WBCs)
O2
Co2 + waste products
Tissue
Granulocyte
Neutrophil
Bacterial infections
Basophil
Sever allergic state
(Asthma)
Eosinophil
Parasitic infections
Allergic state
Agranulocyte
Monocyte
Parasitic infections
Lymphocyte
Viral infections
WBCs Immune Systems

Sites of blood formation

1. Facture (fetus) hemopoiesis in embryo.
   - 2 weeks → 4 months in yolk sac, it called (mesoblast hemopoiesis).

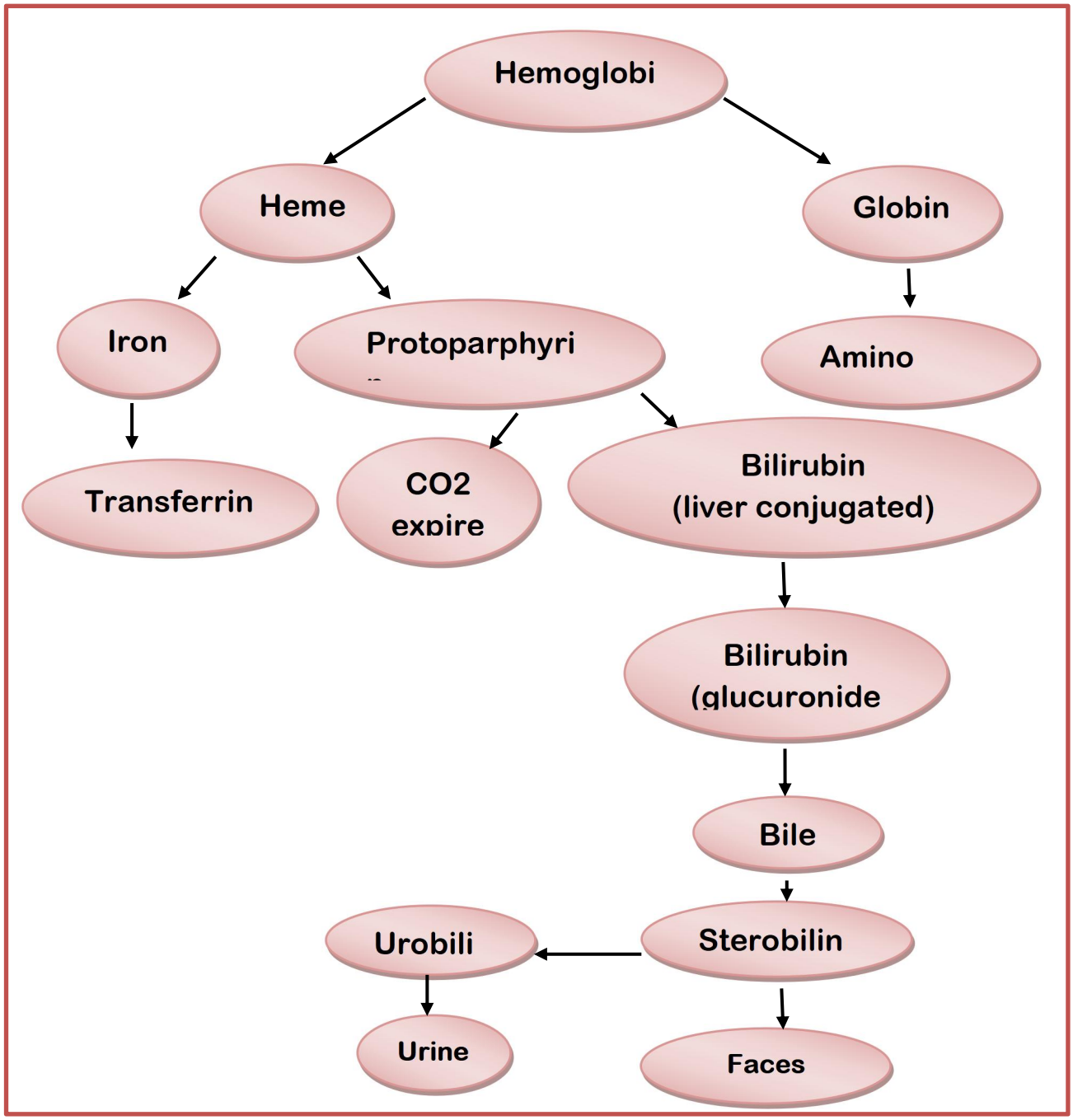
   After fertilization in the mesoderm
   - 4 months → 2 weeks before delivery, liver and spleen (hepatic hemopoiesis).
   - 2 weeks before delivery→ end of life, bone marrow (medullary hemopoiesis).
2. Infant: all bone marrow are active.
3. Adult: (skull, vertebrae, sternum, rib and the end of long bones).



Function of blood

1. Carrier O2 from the lung to other of body.
2. Carrier CO2 and waste products to lung to excrete it out of the body.
3. Carrier nutrients to all parts of body.
4. Regulates body temperature.
5. Defend to body against infection.

Diagram of distraction of RBC



Hemoglobin

It is proteins substance present in side red blood cells it is consist from iron (hem 4%) and protein (globin 96%) soluble in side RBC.

Types of hemoglobin:

1. Normal hemoglobin

A. **Hb-f** **b. Hb-A2** **C. Hb-A**

2. Abnormal hemoglobin

Hb_H , Hb_S , Hb_C ,.....

Formation of hemoglobin need essential material like

1- Iron
2- Protoprophyrine
3- Amion acid
4- R.N.A

Types method

1- Color meter
2- Sahli-method
3- Spector photo meter instrument
4- H.B meter
5- Complete blood count (cell) C.B.C
6- Hemoglobin meter by strep test
7- Used phone

Principle

By this method most of hemoglobin is converted into cyanomet hemoglobin.

Reaction

Hb + potassium ferric cyanide → Met Hemoglobin + potassium cyanid

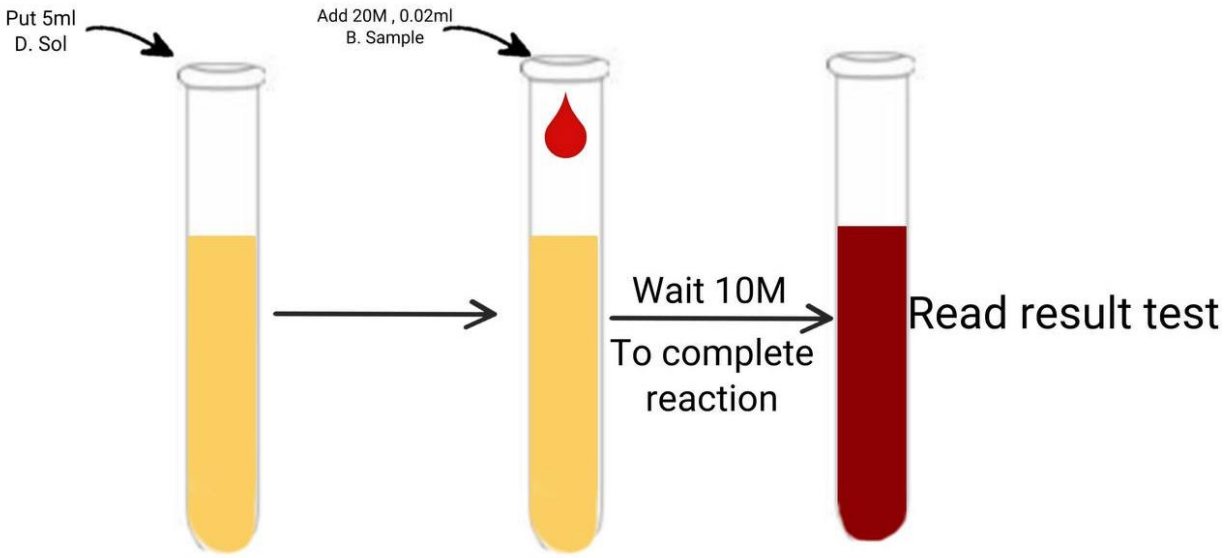
Cyinomethemoglobin

Material required

1. Spectrophotometer or Hb meter.
2. Hb-pipette (Sahli pipette).
3. Blood sample.
4. Test tube.
5. Diluting fluid (Drabkin solution).
6. Standard& blank solution.
7. Graduated pipette & cotton &alcohol.
8. Sterilize lancet.

Procedure

1. Take 5 ml Drabkin solution in test tube.
2. Add 0.02 ml blood sample to test tube.
3. Leave the mixture for 10 minutes.
4. Measure by the spectrophotometer at wave length 540 nm or 250 nm.
5. Write the reading result.



Calculation:

- In spectrophotometer:

$$Hb = \frac{T}{S} \text{ x } 14.3$$

- In Hb-meter:

Hb = ( ) g/ dl

Reagents:

Prepare Drabkin solution as following:

- Potassium cyanide 50 gm. (KCN).
- Potassium ferric cyanide 200 mg $(K3Fe\ CCN)_6$.
- Sodium bicarbonate (NaHCO3) 1 gm.dissolved in one liter distilled water.

The PH of solution is 8.6.

Normal Value:

- Infant = 13.6-19.6 g/dl
- Children 1 year = 11-13 g/dl
- Children 10 years = 11.5 - 14.8 g/dl
- Man = 13.5 - 18 g/dl
- Women = 11.5-16.5 g/dl

Increase Hb Occur in:

1. Dehydration.
2. Burns.
3. Altitude.
4. Smoking.
5. Lung disease.
6. Polycythemia.
7. Kidney disease.
8. Heart Failer.

Decrease Hb Occur in:

1. Anemia.
2. Leukemia.
3. Pregnancy.
4. Bleeding.
5. Panacytopenia (week bone marrow).
6. Decrease Iron , vit B12 , folic acid.
7. Bone marrow disorder (failer).

Packed cell Volume (P.C.V)

Definition of (P.C.V):

It is one blood test to measure concentraction of RBCs spreated from plasma in culum blood it called hematocrit to diagnosis if present Anemia , Normal , Polycythemia.

Types of methods:

1. Macro-method (Wintrob's tube method).
2. Micro-method (capillary tube method).

Material required:

1. Blood sample.
2. Micro hematocritcentrifuge.
3. Micro hematocrit reader.
4. Sealing material & cotton, lancet, alcohol.
5. Capillary tube heparinized 1 cumm, diameter, 2.5 mm and non-heparinized.

Procedure:

1. Allow the blood to enter the capillary tube by capillarity. Leaving at least 15 mm unfilled.
2. Seal the capillary tube by the sealing material.
3. Spin the capillary tube by the micro centrifuge for 5 minutes at 10000 R.P.M.
4. By using the hematocrit reader, read the Value.

Normal Value:

Man = 47 ± 7 % (40 % - 54 %).

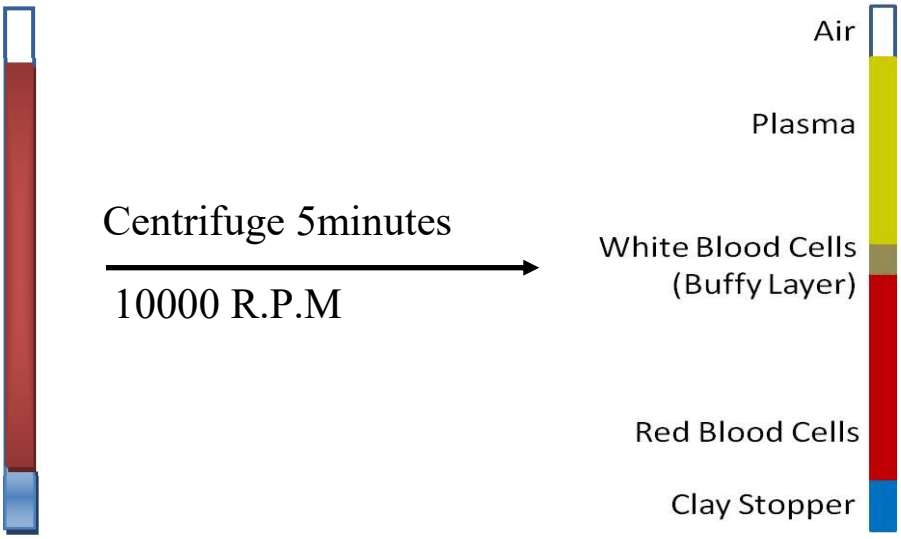
Women = 42 ± 5 %(37 % - 47 %).

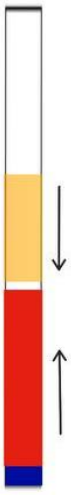
Infant = 54 ± 10 % (54% - 64 %).

Children (3-6 year) = 40 ± 4 % (36% - 44%).

Children (10 -12 year) = 41 ± 4 % (37 % - 45 %).

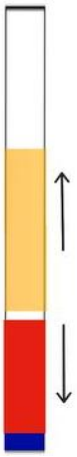
Children (3 month) = 38 ± 6 % (32 % - 44%).





A. Increase packed cell

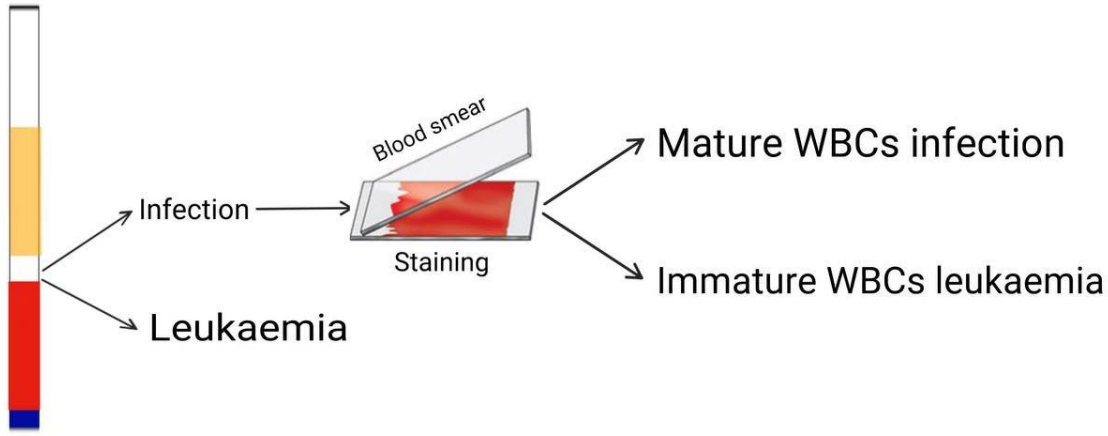
1. Poltcythemia (active bone morrow)
2. Megaloplastic anemia
3. Elliptocytic anemia
4. Spherocytic anemia



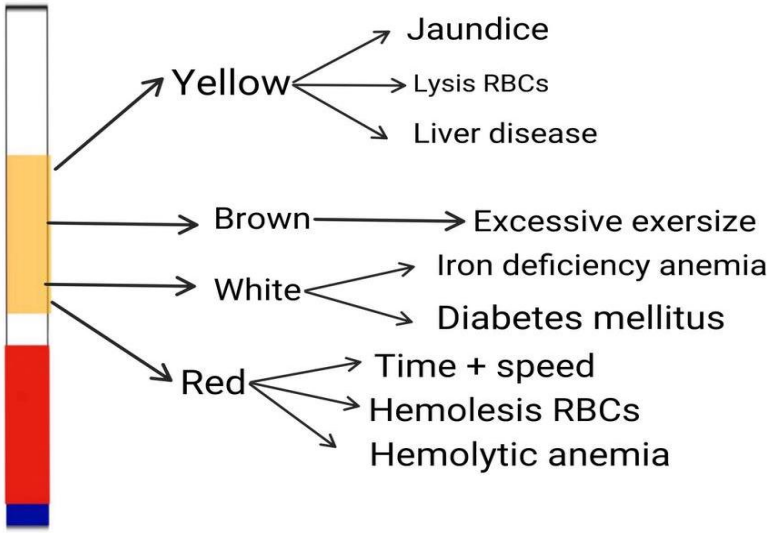
B. Decrease packed cell

1. Bleeding
2. Iron defficiency anemia
3. Thalassemia
4. Sickle cell anemia

C. Increase buffy coat



D.Diagnosis plasma appear these Color



Sources of errors:

1. Bad sealing material & temperature 4℃.
2. Excess anticoagulant.
3. False reading result.
4. Time & speed in centrifuge.
5. Old blood & don't mixed blood before used.
6. Variation in the diameter of the tube.

Notes:

- Reduce plasma occur in (high altitude, burns and dehydration).
- Increase plasma occurs in (pregnancy,severeanemia, liver& spleen disease, kidney disease and extravascular fluid as congestivecardiac failure.
- Yellow colour plasma occurs in jaundice.
- Turbid plasma occurs in haemolytic anemia.
- Light plasma occurs in iron deficiency anemia.

Erythrocyte sedimentation rate (E.S.R.)

It is a blood test to measure the sedimentation of erythrocyte in culum blood and it indirectly measure the level of certain protein in the blood & used to diagnosis any inflammation in the body.

Types of methods of E.S.R.

1. Western green method.
2. Winthrop's method.

Principle:

When the red cells are allowed to settle out from their plasma, the speed of their fall is known as the E.S.R.

E.S.R phase (stage):

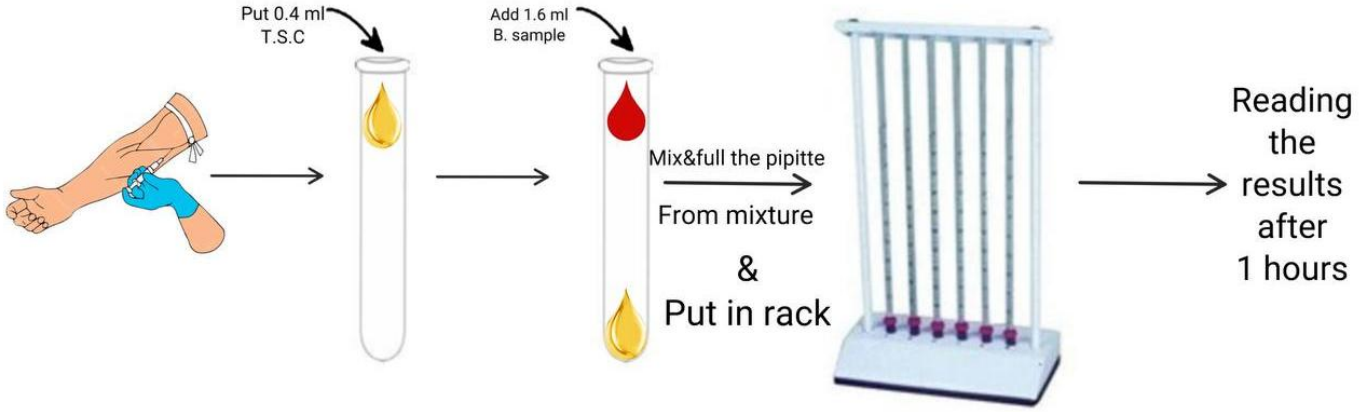
1. First stage **10** minutes (Rolex formation).
2. Second stage **40** minutes (Rapidlysedimentation).
3. Third stage **10** minutes(completedsedimentation).

Material used:

1. Blood sample.
2. Western green rack & pipette.
3. Winthrop's tube + rack.
4. Tri-sodium citrate (1 ml-4 ml blood).
5. Syringe, cotton, alcohol, test tube, watch.

A. Procedure of Western green method :

1. Put 0.4 ml of tri-sodiumcitrate in test tube.
2. Add 2 ml of blood sample & mixed.
3. Full the Western green pipette from the mixture.
4. Let the pipette in Western green rack for 1 hour.



B. Procedure of Winthrop's method:

Normal value:

- Man = 2-15 mm/1h.
- Women = 2 -20 mm/1h.

Notes:

1. Don't use hemolytic blood.
2. Clotted blood must not use.
3. Avoid the form of bubbles in blood column.
4. The tube must be in a vertical position.
5. Temperature 22-25℃.
6. Avoid to direct sunlight.
7. Don't used old blood.

Decrease E.S.R occurs in:

1. Polycythemia.
2. Newborn (birth).
3. Allergic state.
4. Sickle cell anemia.
5. Low plasma fibrinogen.

Increase ESR occurs in:

1. Anemia.
2. Cancer.
3. Nephrosis.
4. Nephritis.
5. Pneumonia.
6. Rheumatic arthritis.
7. Pregnancy test.
8. Liver disease.
9. After large surgery.
10. Appendicitis.
11. Toxemia.
12. Leukemia.

Red blood cell (R.B.C) count

It is blood test to measure the number of red blood cells in cubic millimeter of blood.

- When R.B.Cs increase, called **Erythrocytosis**.
- When R.B.Cs decrease, called **Erythrocytopenia**.

Types of methods:

1. Test tube method.
2. Micro-pipette method.

Test tube method:

Material used:

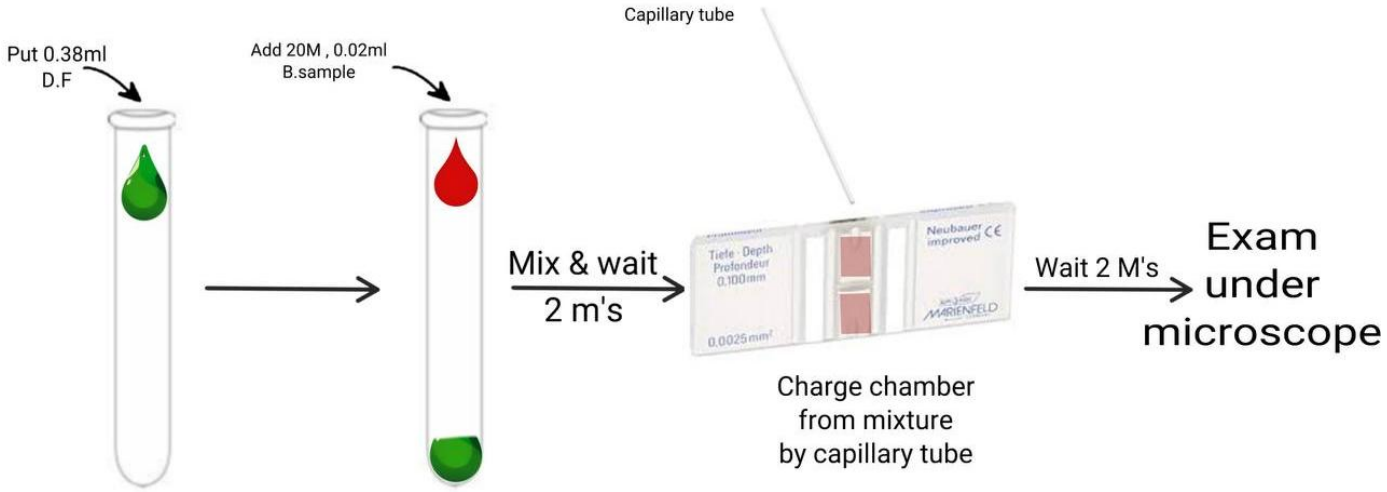
1. Microscope.
2. Diluting fluid (formal citrate).
3. Improved nebulous chamber & cover slide.
4. Test tube & Sahli-pipette.
5. Blood sample, lancet, cotton, alcohol.

Principle:

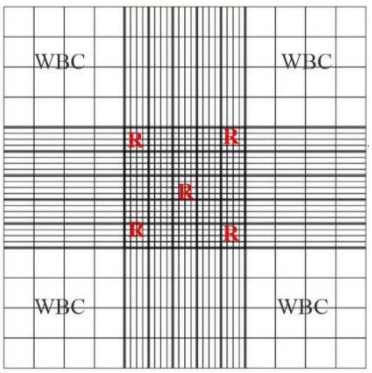
Mix blood sample with diluting fluid & wait 2 Ms & charge chamber & Exam under microscope.

Procedure:

1. Measure 3.98 ml of diluting fluid (formal citrate) and transfer to small test tube.
2. Measure 0.02 ml of blood sample &transfer to the test tube which contains D.F, wash the pipette many times with diluting fluid in test tube.
3. Mix the suspension by tilting & rotating for at least 2 m.
4. Change the chamber by means of capillary tube.
5. Leave the cell to settle down on chamber for 2m.
6. Count the cell in 5 squares of center square.



Calculation:



R.B.Cs count = No.* 10.000

- top left &right, bottom left & right and middle one.

Normal values of R.B.Cs count:

- Man = 4.5-6.5 million c/ cumm.
- Women = 4-5.5 million c/ cumm.
- Infant = 4.5-5.5 million c/ cumm.
- Children (1 year) = 3.5-5.5 million c/ cumm.
- Children (3- 12 years) = 4 -5 million c/ cumm.

Reagents:

R.B.Cs diluting fluid (formal citrate solution), as following.

- Sodium citrate 3 gm.
- Sodium chloride 1.8 gm few.
- Formaldehyde 4ml.
- Safranine few drops.
- D.W 100 ml.

Increase RBCs occur in:

1. Heard muscular failer.
2. Increase in take nutrient like (Iron, B12, B6, C, A, Copper).
3. Alcohol in take.

Decrease RBCs occur in:

1. Pregnancy.
2. Chronic kidney disease.
3. Bariataic surgery.
4. Cancer.
5. Aplastic anemia.
6. Cirrhosis.
7. Hodgkin`s disease.
8. Celiac disease.

Absolute values of Hemoglobin

Use the test to differentiation between two types of anemia through the estimation of Hb, P.C.V, and R.B.Cs count.

1. Mean corpuscular volume (M.C.V).
2. Mean corpuscular Hemoglobin (M.C.H).
3. Mean corpuscular Hemoglobin concentration (M.C.H.C).

1. Mean corpuscular volume (M.C.V):

It's average volume of single cell in cubic micron.

- M.C.V = $\frac{P.C.V\ \%}{R.B.Csmillion} \times 10$ (C.M)

Normal value:

- Adult = 76-96 cm (86 ± 10).
- Infant = 106 cm.
- Children (10-12 year) = 76-93 cm.

Increase M.C.V called **macrocytosis (megaloplastic A, elliptocytic A, sphrocytic A).**

Decrees M.C.V called **microcytosis (I.D.A, S.C.A, Thalassemia).**

Normal M.C.V called **Normocytosis.**

2. Mean Corpuscular Haemoglobin(MCH):

It is amount of Hemoglobin in a single red cell.

Expressed:

- M.C.H = $\frac{Hb}{R.B.Csmillion} \times 10$ mmgm (pigo gram)

Normal value:

- Adult = 27-32 mmgm
- Children = 24-30 mmgm

Increase M.C.H called **Hyperchromic ((megaloplastic A, elliptocytic A, sphrocytic A).**

Decrease M.C.H called **Hypochromic (I.D.A, S.C.A, Thalassemia).**

Normal M.C.H called **Normochromic**

3. Mean corpuscular Haemoglobin conc. (M.C.H.C)

It is a percentage of Hemoglobin in 100 ml of packed red cell but not in whole blood.

- M.C.H.C = $\frac{Hb}{P.C.V}$ × 100 gm/ dl

Normal value:

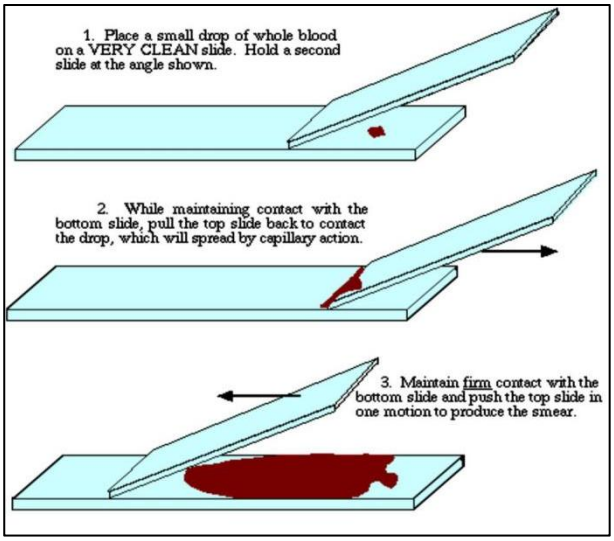
- Adult = 30-35 g/dl
- Infant = 27-34 g/dl

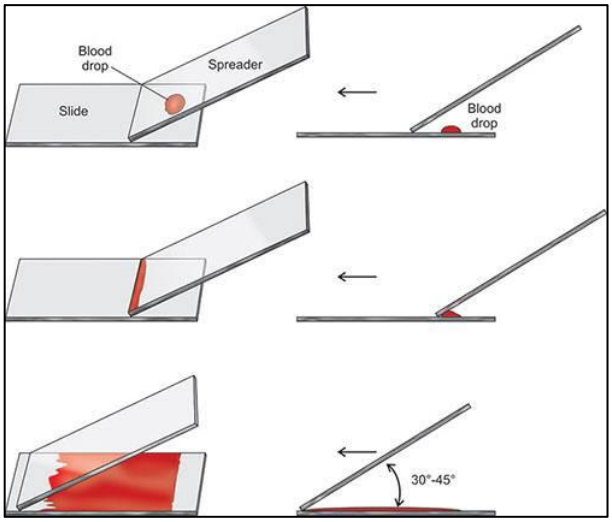
Blood film making

- In making blood film you can use anticoagulant blood or finger puncture.
- Prepare a clean slide, free from grease, dust.
- Select a slide which has smooth edge as a spreader.

Procedure of blood smear:

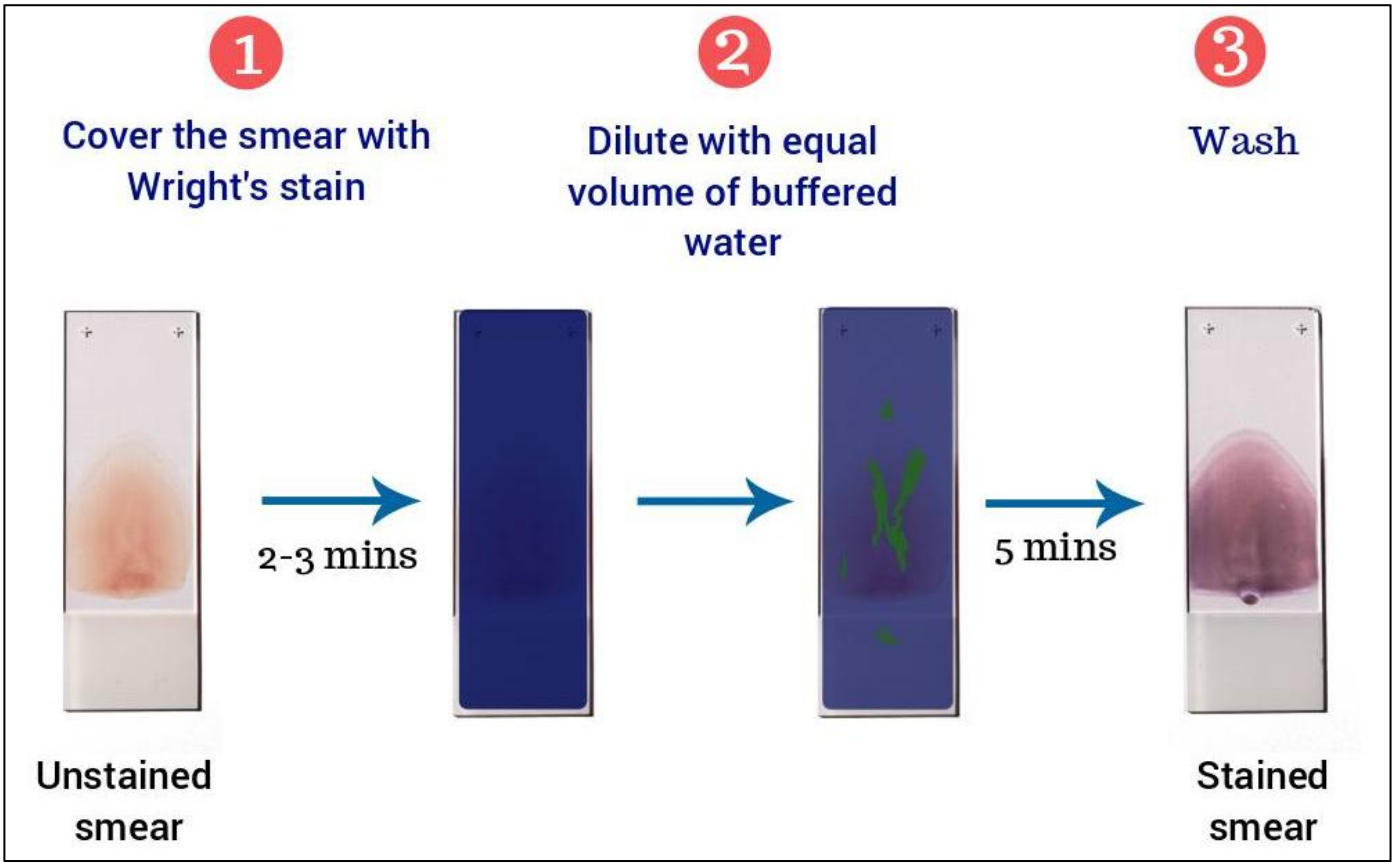
1. Place suitable size drop of blood on a clean slide 1 cm away from one end.
2. Put the spreader on the slide with 45 ℃ angle and draw back.
3. The blood will spread out to the edge of the spreader then push the spreader smoothly, rapidly over the length of the slide.





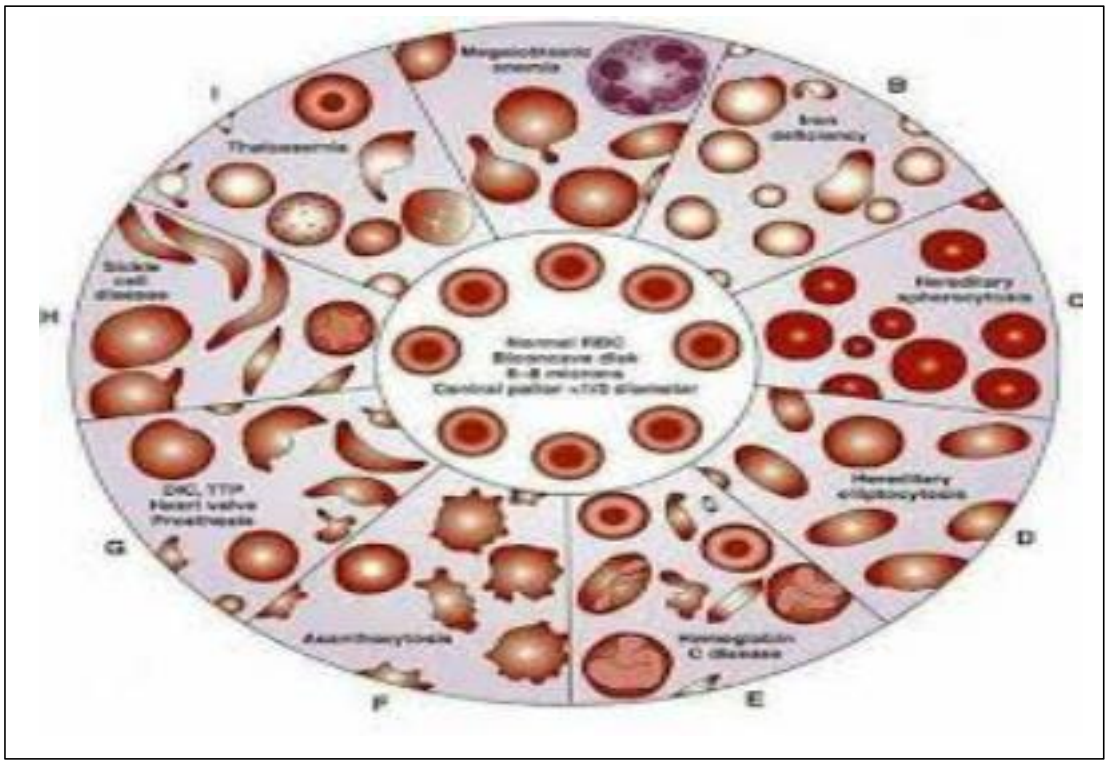
Procedure of staining blood smear:

1. put few drops from leishman stain for 2_3 minutes.
2. Add full double amount of D. W for 5_10 minutes
3. wash the smear by tap. Water.
4. Dry the smear and exam under microscope at oil immersion



Study of variation of red cell

The study variations are signification in distinguishing to diagnosis types of anemia if nutretinal or hereditary anemia.



A. Variation in size.

B. Variation in color.

C. Variation in shape.

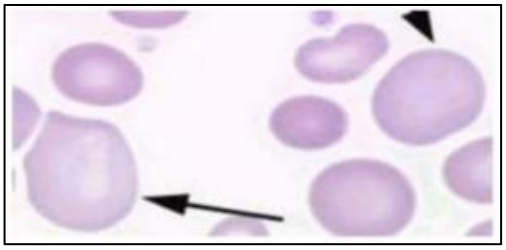
D. Variation in content (inclusion body).

A. Variation in size (Anisocytosis):

1. **Macrocytosis**:

It is increase in size of erythrocyte than the normal cell.

may be found in:



1. Healthy-new born infant.
2. chronic liver disease.
3. pernicious anemia.

Caused by decrease in folic acid & vitamin B_{12} in food.

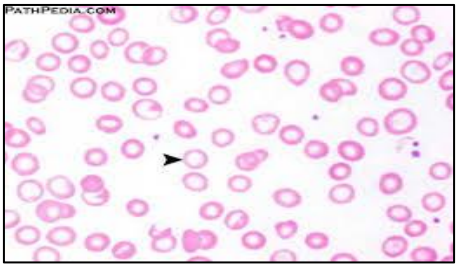
2. **Microcytosis :**

It is decrease in size of erythrocyte (smaller) than the normal red cell.

cause by deficiency (Iron deficiency)

in food occur in (Iron deficiency anemia)

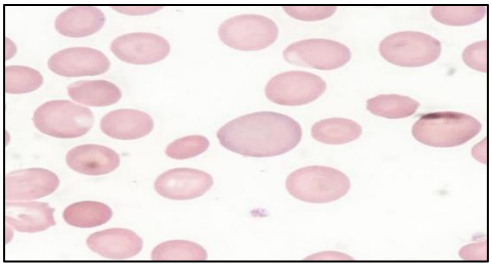
and thalassemia, S.C.A, I.D.A



3. **Anisocytosis**:

It may be present of larger& smaller cells than normal erythrocyte

both are microcytosis& macrocytosis with normocytosis in slide caused by abnormal development of red cell may be occur in anemia and leukemia.



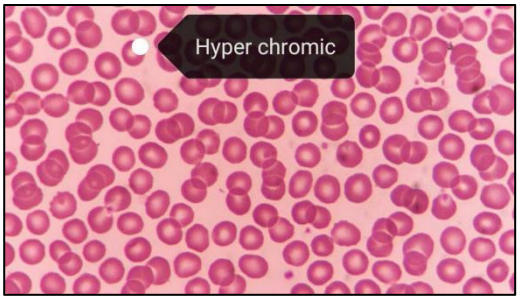
B. Variation in color :

1. **Hyperchromic** :

   The red cells appear dark red color than normal

   Because high concentration Hb in red cell seen in:

   1. Megaloplastic anemia.
   2. Elliptocytic anemia.
   3. Spherocytic anemia.

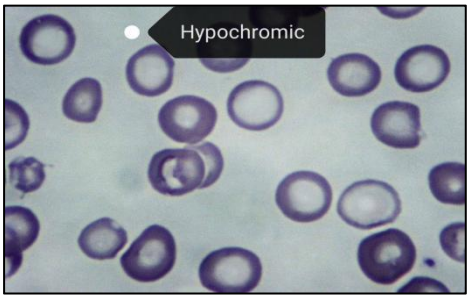


2. **Hypochromic**:

   The red cell appears pale red colour than normal.

   Because low concentration Hb in red cell seen in:

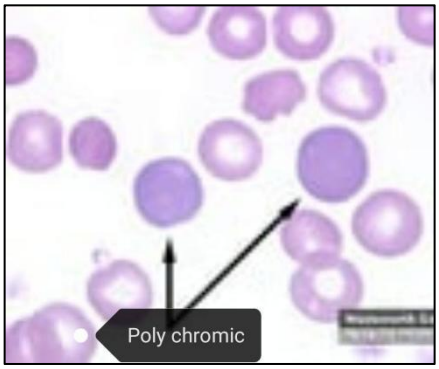
   1. Scikle cell anemia.
   2. Iron deficiency anemia.
   3. Thalassemia.



3. **Polychromic :**

   red cell appear violet-colour by staining Leishman's stain

   (brilliant stain) appear in reticulocyte cell caused by bleeding loss.



Properties of mature erythrocyte:

1. Didn`t contain nucleus.
2. Biconcave.
3. Diameter 7.2 micron.
4. Red colour.
5. Contain hemoglobin.
6. Function : cases & carrier.
7. Circular.
8. Smooth.
9. Age 120 days.
10. Didn`t contain cytoplasm.

Reading result of anemia:

- Macrocytic _ hyperchromic, N.
- Microcytic _ hypochromic.
- Normocytic _ noromchromic.

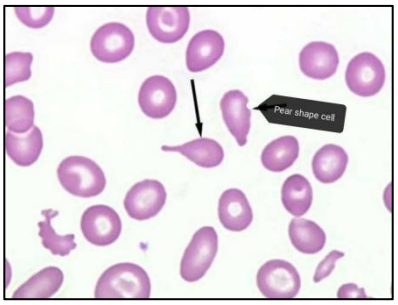
C. Variation in shape (poikilocytosis)

1. **Pear shape:**

   ar this cell in blood smear caused defect in bone marrow

   Seen in:

   - Hemolytic anemia.



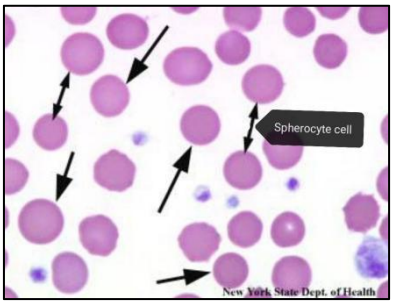
2. **Spherocyte (spherocytosis):**

   phere in shape which is thicken than normal

   because take much dark stain.

   Seen in:

   - Hemolytic anemia.
   - Spherocytic anemia.

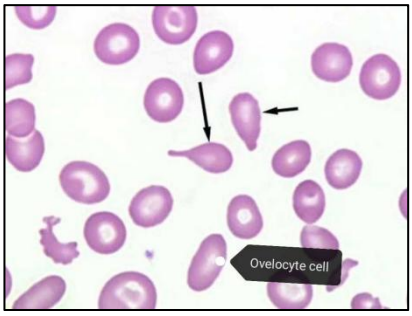


3. **Ovalocyte (ovalocytosis):**

   The cell appear oval shape.

   seen in:

   - Thalassemia.
   - Sickle cell anemia.
   - Macro ovalocyte (megaloplastic anemia).

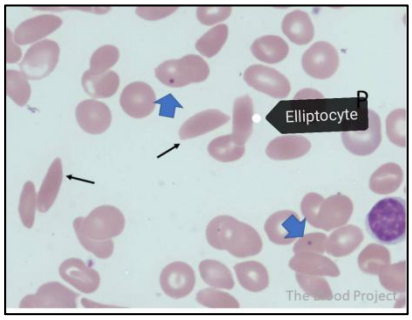


4. **Elliptocyte (Elliptocytosis):**

   cell appear elliptical in shap.

   seen in:

   - hereditary elliptocytic anemia.

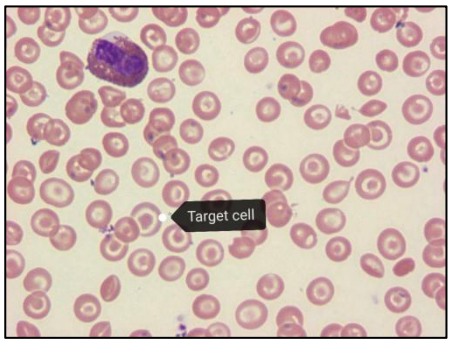


5. Target cell (codocyte):

This cell appears in airing of hemoglobin and found presentation of Hb in center cell.

Seen in:

- Thalassemia.
- Sickle cell anemia.
- Liver disease.
- Iron deficiency anemia.

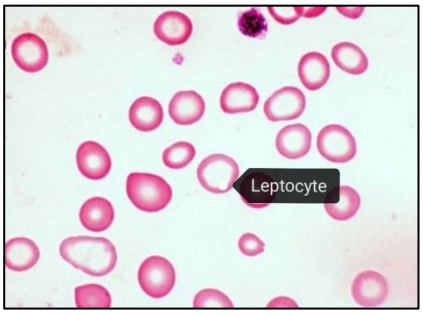


6. Leptocyte (leptocytosis)

This cell appear ring of hemoglobin in cell wall and it have large center didn`t stain.

Seen in:

- Thalassemia.
- Sickle cell anemia.
- Iron deficiency anemia.

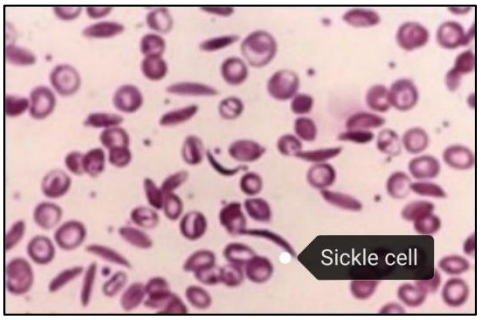


7. Sickle cell:

Sickle or cresent shape .

Seen in :

- sickle cell anemia.

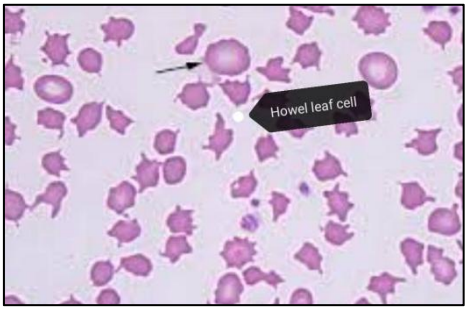


8. Howell leaf:

It is leaf in shape .

Seen in:

- Hereditary sickle cell anemia.

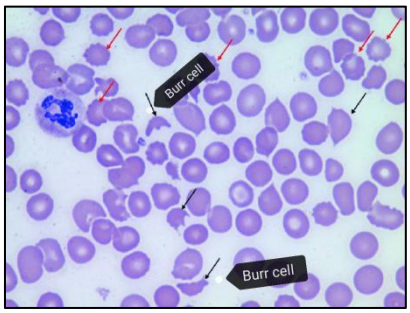


9. Burr cell:

It is a particular red cell has blind projections in cell wall

seen in:

- ❖ Anemia.
- ❖ Carcinoma of stomach.

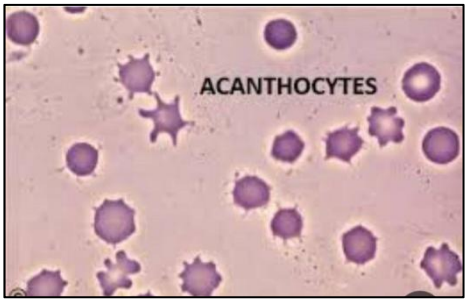


10. Acanthocyte (spur):

The cell is star shape has numbers of sharp projectionsin cell wall.

Seen in:

- ❖ Chronic liver disease.
- ❖ Hereditary haemolytic anemia.

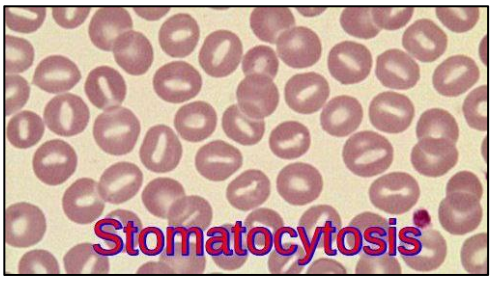


11. Stomatocyte cell:

The cell appear mouth shape and it have center biconcave not stained in blood film.

seen in:

- ❖ hereditary haemolytic anemia.

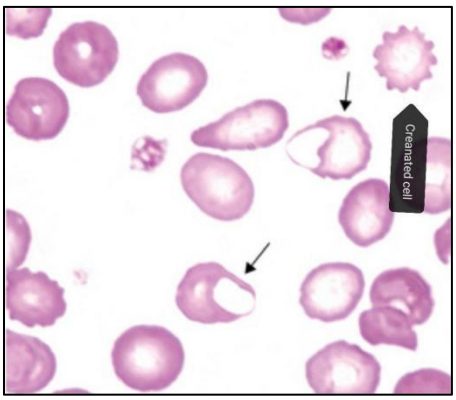


12. Creanated cell:

The cell has numbers of projections in extra-cell wall

Seen in:

- ❖ Normal.
- ❖ Hemolytic anemia.
- ❖ Age.
- ❖ Working.

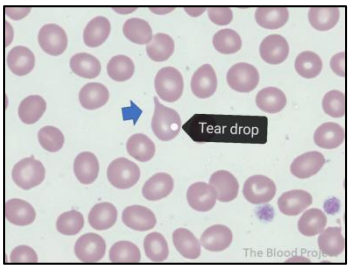


13. **Tear drop:**

Tear-like shape and has a small sharp project in the other side of external cell wall.

seen in:

- bone marrow.

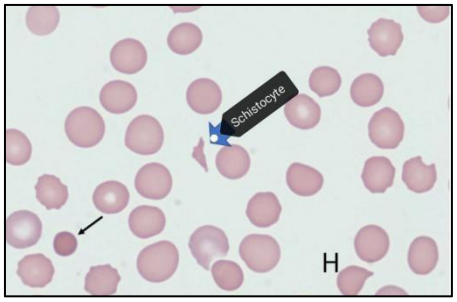


14. **Schistocyte:**

It is a part of destroyed red cell.

Seen in:

- Hemolytic anemia.
- Severe burns.
- Thalassemia & megaloplastic anemia.
- Iron deficiency anemia.

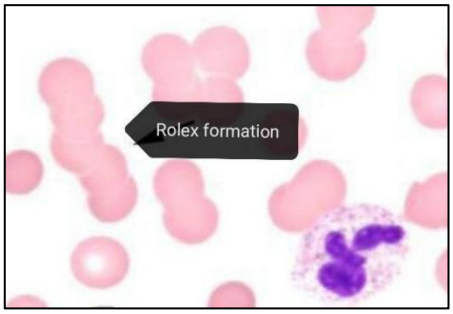


15. **Rolex formation :**

It is a chain of a number of red cells.

It is non-pathogenic, caused by:

- Defect in plasma factor.

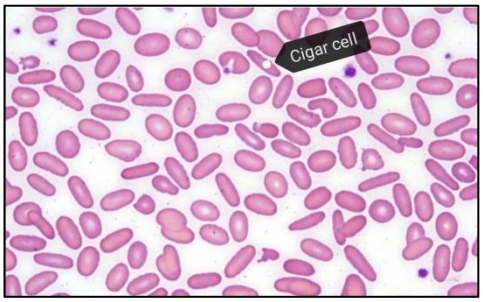


16. **Cigar cell (non-pathogenic):**

The cell appear pencil shape.

Seen in:

- iron deficiency anemia.

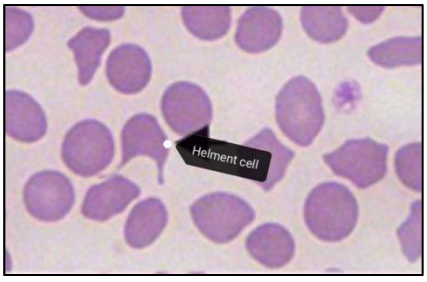


17. **Helminth cell:**

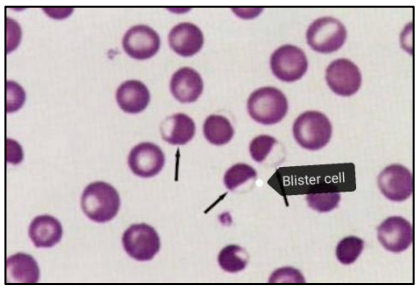
The cell appear drafts shape.

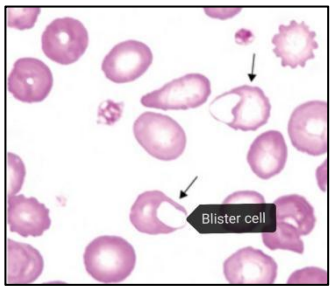
seen in:

- nutritional anemia.

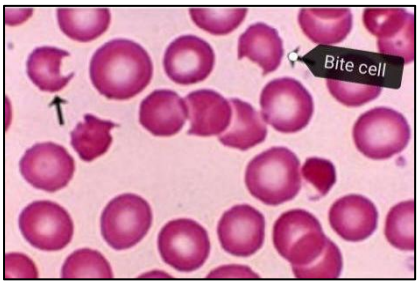


18. Blister cell(Eccentrocyte)

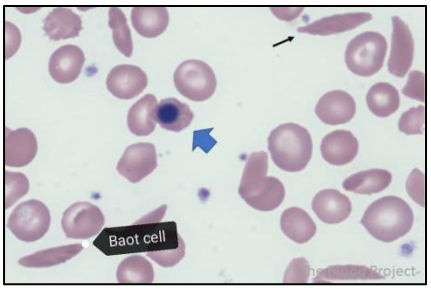




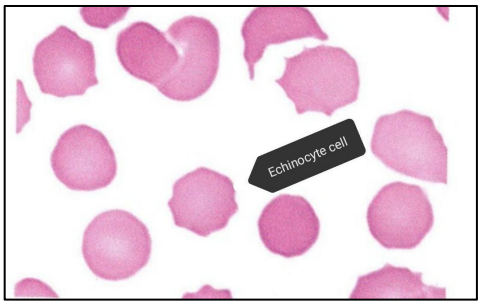
19. Bite cell



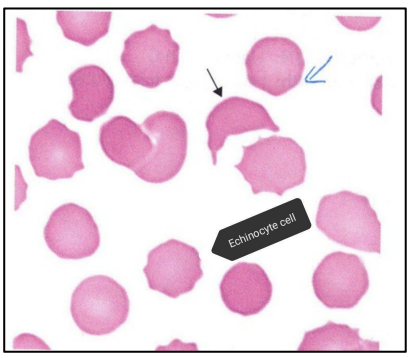
20. Boat-shape cell(Limocyte)



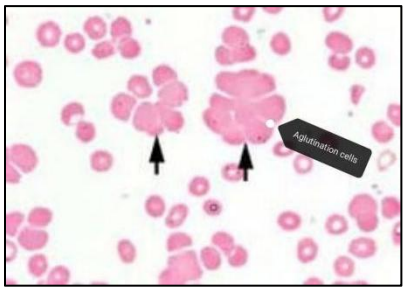
21. Echinocyte (regular progection)



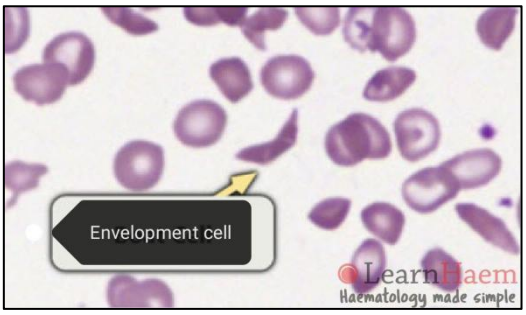
22. Echinocyte



23. Agglutination erythrocyte



24. Envelopment cell

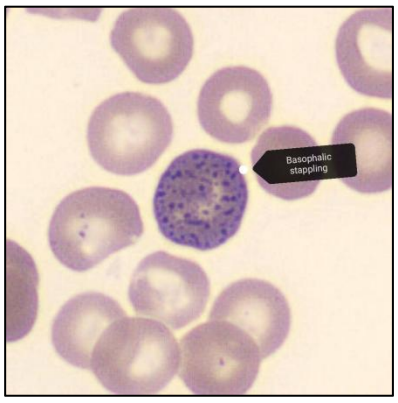


D. Variation in content:

1. Basophilic stippling:

The cell appear as Blue-brown inside red cell.

It indicates the presence of development R.B.Cs.

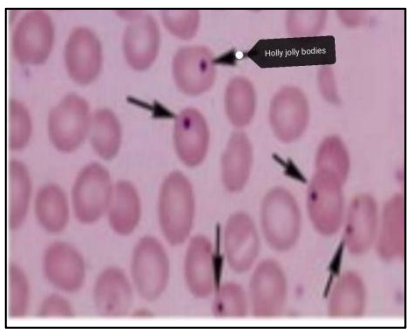


(it is denatured R.N.A) seen in:

- ❖ Lead-poisoning.
- ❖ Anemia.
- ❖ Leukemia.

2. Howell-jolly bodies:

The cell appear blue-purple-brown-reddish in color.



(it is D.N.A remained) Seen in:

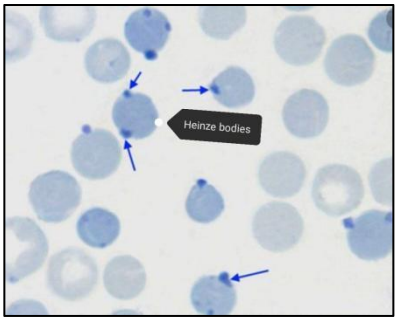
- ❖ Severe anemia, thalassemia, M.A, H.A, S.C.A.
- ❖ Erythroblast fetalies.
- ❖ Splenectomy.

3. **Heinz bodies:**

It is round or irregular particles, deep-purple color, usually lies near the edge of RBC.

(it is denatured hemoglobin) Seen in:

- ❖ hemolytic anemia.

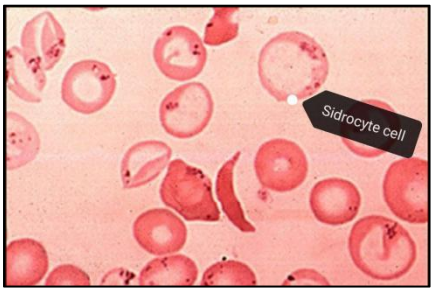


4. **Siderocyte (Pappenheimer bodies):**

It is small blue-gray granules side erythrocyte is free iron.

seen in :

- ❖ Sideroblastic anemia.
- ❖ Hemolytic anemia.

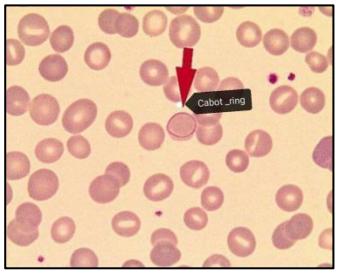


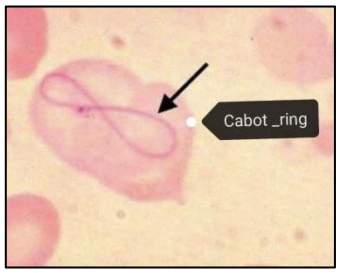
5. **Cabot-ring :**

It is blue-purple, brown or reddish color. It thread-like a ring or 8 number.

(it is denatured protein). Seen in:

- ❖ Polychromic.
- ❖ Lead posining.

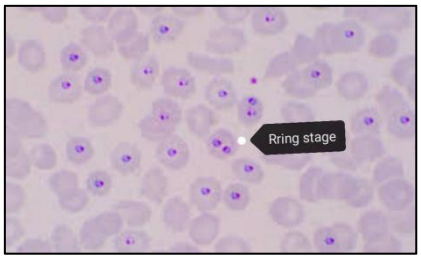


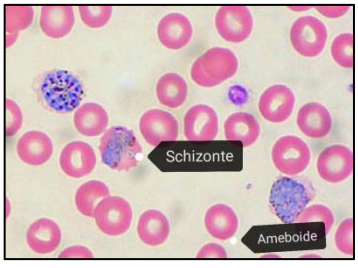


6. **Malaria parasites:**

It is spend part of life inside R.B.Cs appear as:

- ❖ Ring stage.
- ❖ Ameloaid stage.
- ❖ Schizonte stage.





Anemia

It is reduction in health R.B.Cs or reduction in Hb or both of them in peripheral blood than normal.

Anemia classified into:

- Nutritional Anemia.
- Hereditary Anemia.

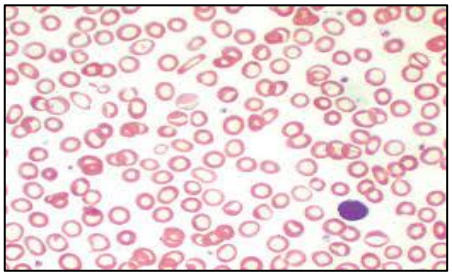
Types of anemia:

1. Iron deficiency anemia (I.D.A).
2. Pernicious anemia (P.A).
3. Sickle cell anemia (S.C.A).
4. Thalassemia anemia (T.A).
5. Elliptocytic anemia. (E.A).
6. Spherocytic anemia (S.A).
7. Hemolytic anemia (H.A)
8. Panacytopenia

1. Iron deficiency anemia:

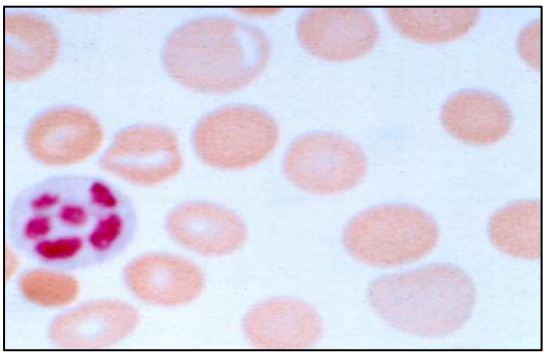
This type of anemia occurs due to lack of supply or absorption of iron or due to continue blood loss. See in blood picture:

- Microcyte cell.
- Hypochromic.
- Leptocyte cell.
- Anisocytosis & Poikilocytosis.
- M.C.V, M.C.H, M.C.H.C, Hb, R.B.Cs decrease.



2. Pernicious anemia

This type of anemia is caused by lack of vitamin B12 or folic acid or lack of absorption in the body.

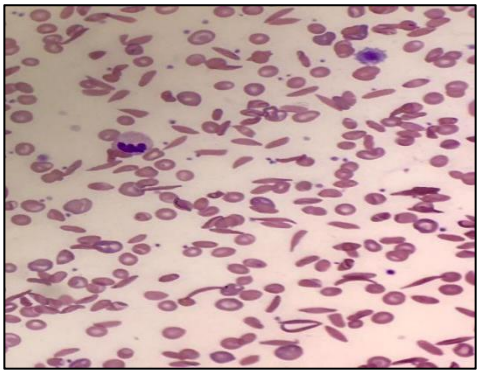


See in stained blood film:

- Macrocytic cell.
- Ovalocytic cell.
- Anisocytosis & poikilocytosis.
- Hyper segment neutrophil.
- M.C.V > 95 , M.C.H, M.C.H.C decrease.

3. Sickle cell anemia:

In this type of anemia the Hb.s is insoluble and form crystals when exposed to low oxygen tension. It is fromhereditary disease.



See in blood picture:

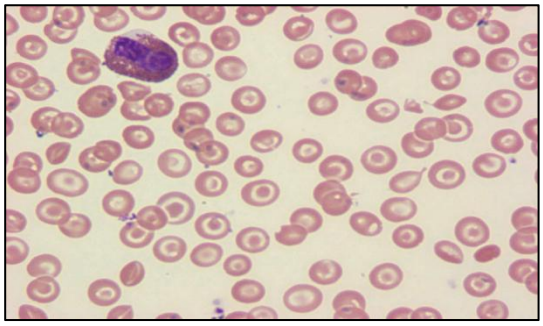
- Sickle cell.
- Target cell.
- Hypochromic.
- Anisocytosis & poikilocytosis.
- Hb < 9 g/d
- M.C.V, M.C.H, H.C.H.C, Hb, RBCs decrease

4. Thalassemia:

This type of anemia is hereditary disease which contains hemoglobin called Hb.h abnormal and it divided into:

a. Major B. Minor.

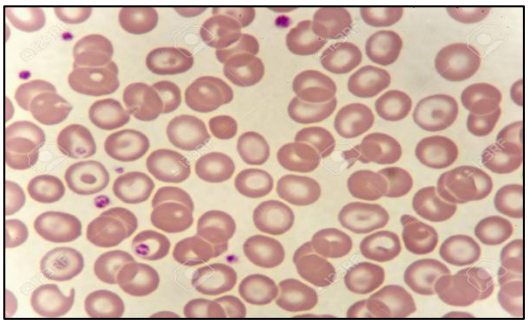
a- Thalassemia major:



See in blood film:

- Target cell (more).
- Leptocyte cell (less).
- Anisocytosis& poikilocytosis.
- M.C.V(N), M.C.H, M.C.H.C, Hb decrease.
- Basophil cell.

b. Thalassemia minor:

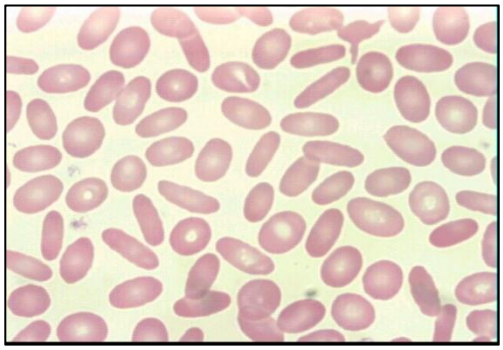


See in blood film:

- Leptocyte cell (more).
- Target cell (less).
- Anisocytosis & poikilocytosis.
- M.C.V(N), M.C.H, M.C.H.C, Hb decrease.

5. Elliptocytic anemia

This type of anemia is hereditary disease.



See in stained blood film:

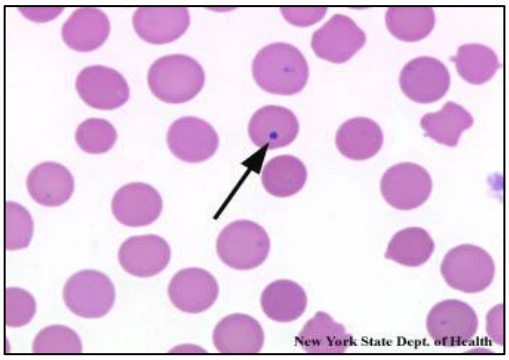
- Elliptocyte cell about 90%.
- Anisocytosis & poikilocytosis.
- M.C.V, M.C.H, M.C.H.C, Hb, RBCs (N).

6. Spherocytic anemia

This type of anemia is hereditary disease.

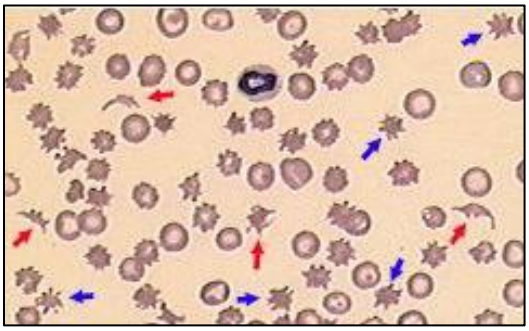
See in blood picture:

- Spherocyte about 95%.
- Anisocytosis & poikilocytosis.
- M.C.V, RBCs (N).
- M.C.H, M.C.H.C, Hb increase.



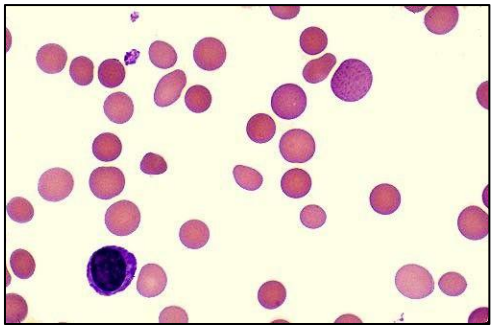
7. Hemolytic anemia

- **Acanthocyte cell**
- **Creanated cell.**
- **Schistocytr cell.**
- **Bite cell.**
- **M.C.V (N), M.C.H, M.C.H.C, Hb decrease.**



8. Panacytopenia

- **Decrease number of erythrocyte in blood film.**

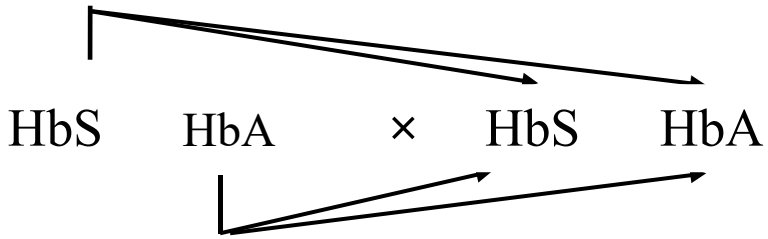


Sickle cell anemia by slide test

Principle:

Hb.S has low solubility when we reduce the oxygen tension.

- Normal haemoglobin : Hb.A
- Abnormal haemoglobin: Hb. S



Hb .ss + Hb.SA + Hb.AS + Hb. AA

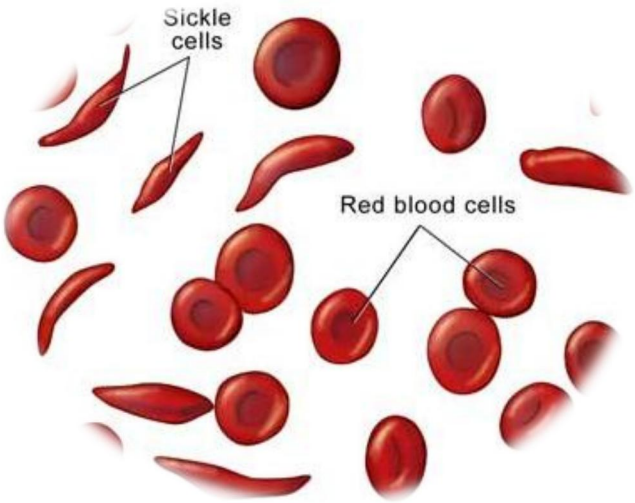
25% 50 % 25%

Homozygous Heterozygous Homozygous Abnormal Hb.SS

Abnormal Hb. SA Normal Hb. A Sickle cell anemia Sickle cell trait

Material used:

1. Blood sample.
2. Slide & cover slide.
3. Reducing agent.
4. Petroleum jelly.
5. Incubator37℃.
6. Microscope.



Reducing agent:

A. Sodium dithionite ($Na_2S_2O_4$) 0.114 mol/L prepare freshly just before use. 19.55 g/L.

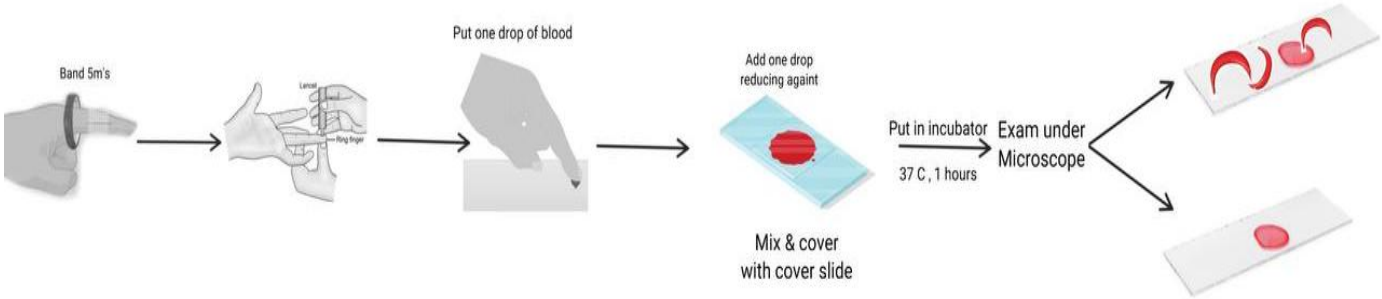
B. Disodium hydrogen phosphate (Na_2HPO_4) 0.114 mol/L, 16.29 g/L.

- Mix 2 volumes of A with 3volumes of B, the PH of reducing agent should be 6.8 the solution should prepare before the use.

❖ Hb.S has low solution when reducing oxygen tension.

Procedure:

1. Apply a band on finger and after 5 minutes make finger puncture and take one drop of blood on the slide clean & dry.
2. Add one drop of reducing agent & mixed.
3. Covered by cover slide & seal the preparation with petroleum jelly.
4. Keep it for 1 hrs.At 37 ℃ in incubator.
5. Examine under high power for sickling.



Results:

1. Crescent shape → Hb.S disease after 30 minutes

2. Howellleaf shape → Hb.sA trait after 12 -24 hrs

Sickle cell anemia by screening test for HbS

Principle:

Mix the whole blood with sodium dithionate and phosphate buffer, and then examine the solubility of the solution.

Material required:

1. Blood sample (whole blood).
2. Sodium dithionate.
3. Potassium diphosphate.
4. Positive sickle cell blood.
5. Negative sickle cell blood.
6. 3test tubes& test tube rack.
7. Wax pencil.

Preparation of potassium diphosphate buffer:

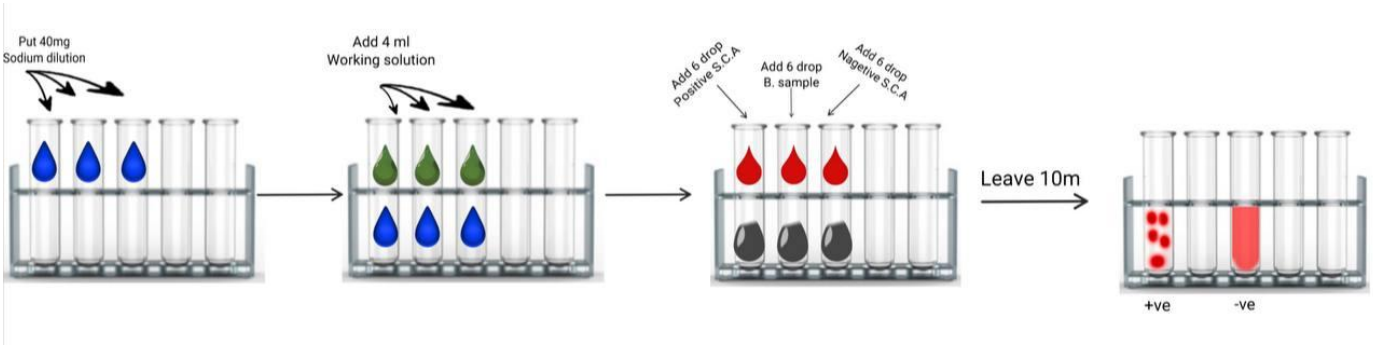
1. Potassium dihydrogen phosphate (KH2Po4) 33.78 g.
2. Dipotassium hydrogenphosphate (K2HPO4) 59.33g
3. White saponin 2.5 g.
4. Distilled water 250 ml.

Working solution:

Dissolve 0.1 g of sodium dithionate in ml of buffer just prior to use.

Procedure:

1. Arrange 3 test tubes in the test tube rack.
2. Mark the test tube by wax pencil positive, negative, and the sample.
3. In to 3 test tubes place 40 g sodium dithionate.
4. Add to each tube 4 ml of working solution.
5. Mix by tilting.
6. To the first tube add 6 drops of sickle cell positive blood.
7. To the second tube add 6 drops of sickle cell Negative blood.
8. To the third tube add 6 drops of blood sample.
9. Leave for 10 minutes.
10. Examine by eye above the light.
11. If the number of tubes could be recognize from the other side of tube, the test is Negative.
12. If not recognized from the other side of tube, the test is positive.
13. Compare with first and second tubes.



Result:

1. If solution is soluble, the sickle is negative
2. If solution is insoluble, the sickle is positive.

Acid – Elution for Hb-F

It is normal Hb present in fetal.

Principle:

- Hb-F resistance of PH acidic but other cell sensitive of PH acidic.

Material used:

1. Blood sample.
2. Slide & spreader.
3. Ethanol 80 %.
4. Eosin stain.
5. Microscope.
6. Elution solution.

Preparing Elution solution:

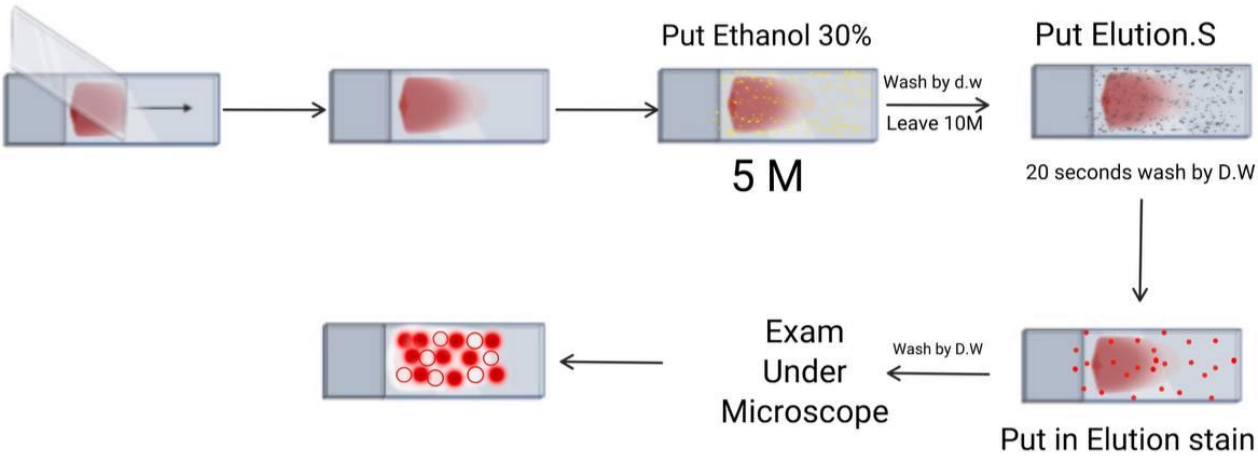
- Solution A 7.5 g/l haematoxylin in 95% ethanol.
- SolutionB 249 Fecl3
- 20 ml Hel 25%
- 1 L D.W

- For use: 5 volume of sol. A and 1 volume of sol. B are well mixed. The PH is 1.5 approximately.

Procedure:

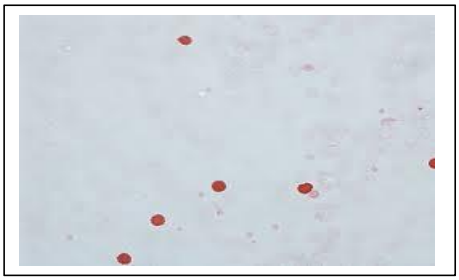
1. Prepare fresh blood film.
2. Fix the blood film in 80 %. Ethanol for 5 minutes.
3. Wash the slide in D.W & leave to dry 10 minutes.
4. Pleas slide in elution solution for 20 seconds.

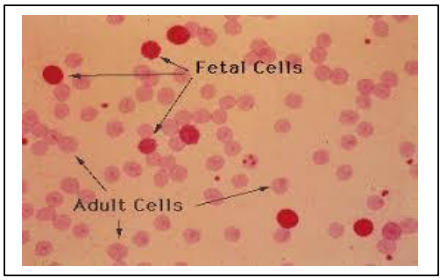
5. Wash the slide in D.W.
6. Pleas slide in eosin solution stain for 2 minutes.
7. Wash in D.W & leave to dry in air.
8. Examine microscopically under oil-immersion.



Results:

- Fetal cells stain … red.
- Adult cell, stain ….. pale pink (ghost cells).
- Lymphocyte, stain.. grey





Demonstration of HbH

It is abnormal Hb present in thalassemia.

Principle:

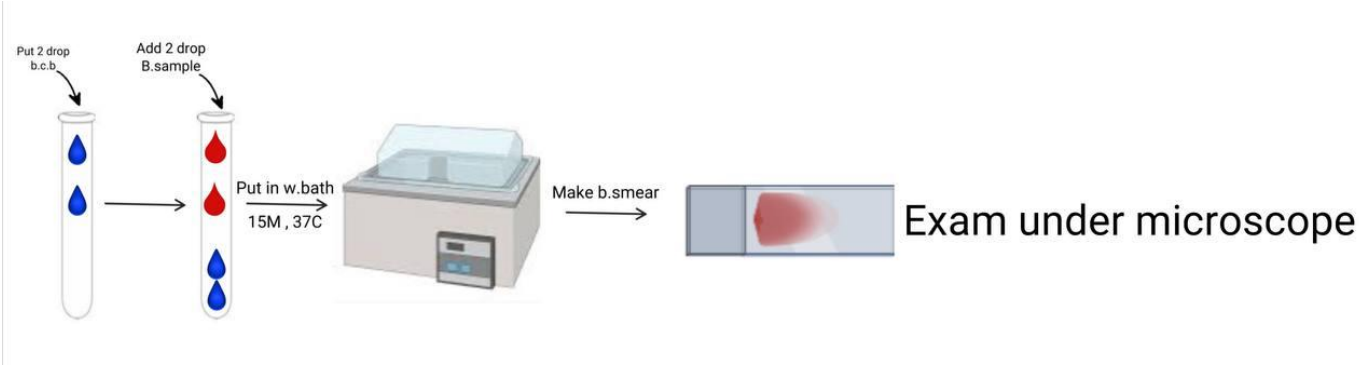
- Hb.H undergoes denaturation in the presence of brilliant crystal blue.
- Hb.H is resist haemolysis by found this stain.

Material required:

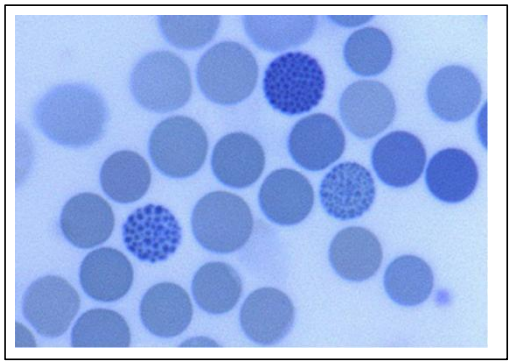
1. Blood sample.
2. Brilliant crystal blue stain.
3. Slide & spreader.
4. Incubator (water-bath).
5. Microscope.

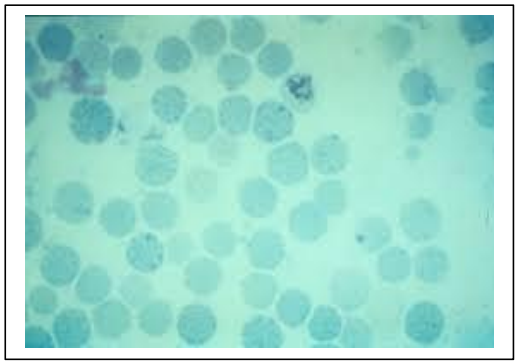
Procedure:

1. Take 2 drops of brilliant crystal blue in test tube.
2. Add 2 drops of fresh blood sample to test tube.
3. Put the tube in water-bath at 37 ℃ for 1-3 hrs.
4. Making blood film & leave to dry in air.
5. Examine under oil-immersion of microscope.



Result:





Reticulocyte count

It is a flat disc shape, non-nucleated, slightly large volume & diameter than mature erythrocyte.

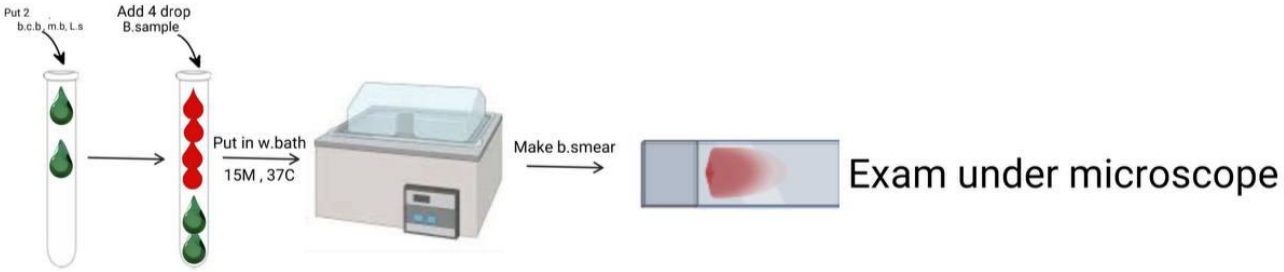
- The retic. count indicator in the bone marrow when the red cell production decrease.
- The retic. count is useful in diagnosis several types of anemia.

Material required:

1. Blood sample.
2. Microscope.
3. Special stain (brilliant crystal blue).
4. Water-bath.
5. Slide & spreader.

Procedure:

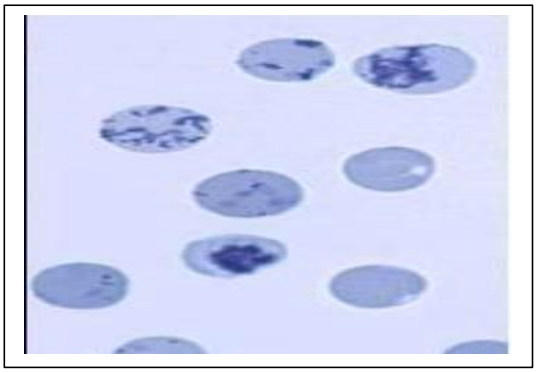
1. Put 2 drops of stain in a test tube.
2. Add 4 drops of blood to the stain.
3. Shake and put in water-bath at 37 ℃ for 15 minutes.
4. Leave to dry in air.
5. Examine under the microscope oil-immersion.



The counting:

In sets paper diaphragm with 2 mm × 2 mm hole in the eye piece of the microscope. This will make counting easier in a smaller area.

Calculation:



Retic. count = $\frac{Noofretic}{NoofR,B.Cs}$ × 100%

Ex.: retic= 13, R.B.Cs = 1000 cell

Retic. count = $\frac{13}{1000}$× 100%

= 1.3% cell

Normal value = 0.2-2 % cell

Increase count of reticulocyte called reticulocytic, seen in:

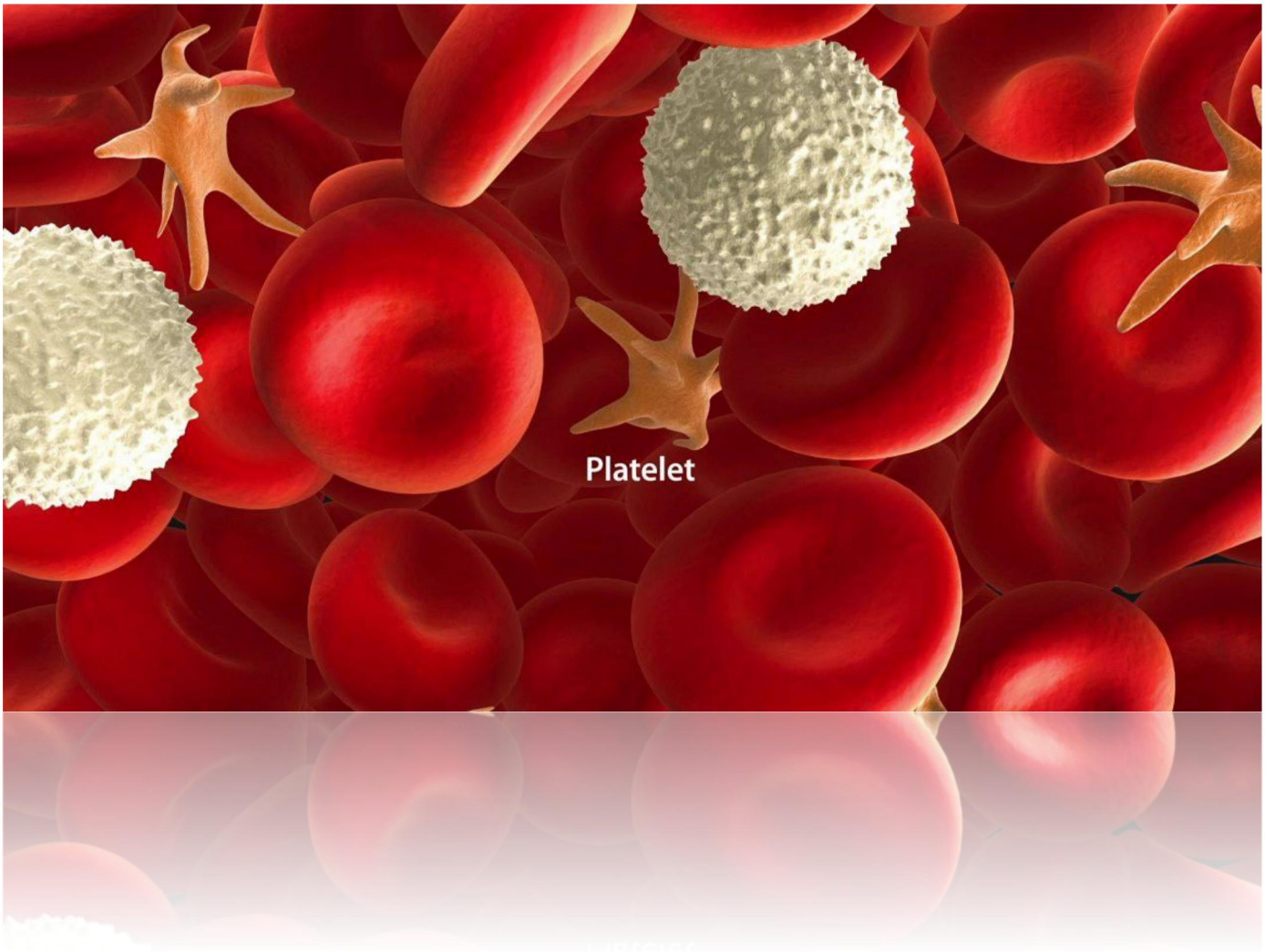
1. Megalobastic anemia.
2. Plastic anemia.

Decrease count of reticulocyte called reticulocytosis, seen in:

1. Thalassemia.
2. Sickle cell anemia.
3. Hereditary spherocytic anemia.

Reticulocyte cell contain ribonucleated protein of the nucleus (RNA).

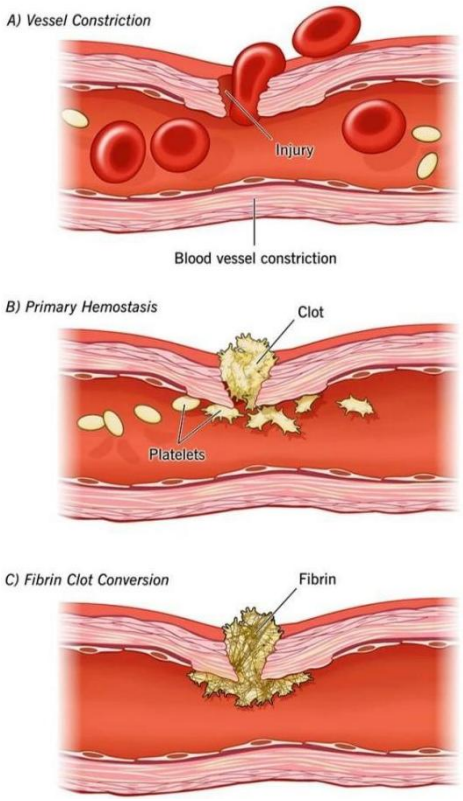
The second course



Investigation on hemorrhage disorder

Haemostasis:

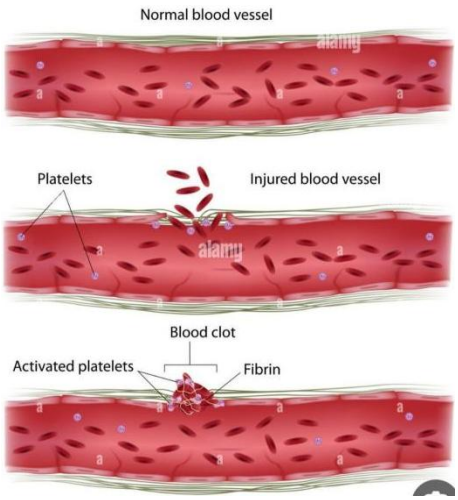
Mean the process which stop the bleeding and prevent excess blood loss.



- Used 3 tests to diagnosis haemorrhage disorder.
  1. Platelets count.
  2. Hess's test.
  3. Bleeding time.

Platelets count

It is number of thrombocyte per one cm of blood.



Platelets:

Are small refraction, non-nucleated bodies

and they have an important role in hemostasis

and in plug formation after injury.

Types of methods used:

1. **Basic method.**
2. **Modified method.**

Basic method:

- ***Use blood sample only.***

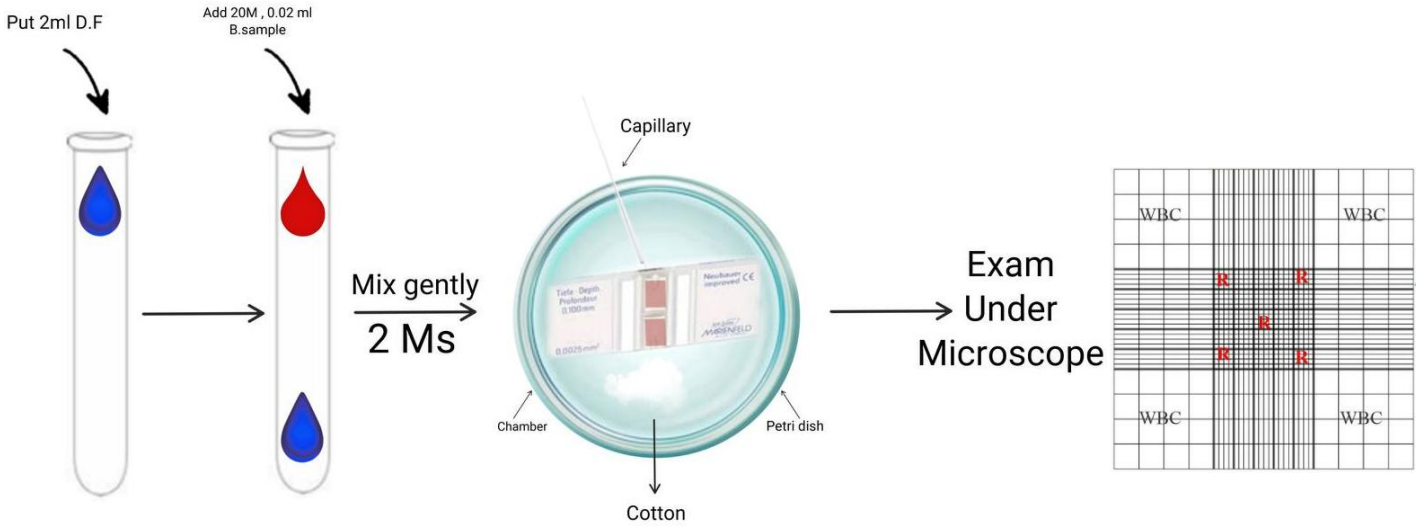
Material Required:

1. Blood sample.
2. Improved Neuber chamber.
3. Petri-dish & test tube & pipette.
4. Sahli-pipette, W.B.Cs & R.B.Cs pipette.
5. Microscope.
6. Diluting fluid (ammonium oxalate).
7. Vaseline& piece of cotton.
8. Lancet, cotton, alcohol.

- Ammonium oxalate consists of:
- 1. Ammonium oxalate 10 gm
- D.W 1 liter

Procedure:

1. Draw 2 ml of dilution fluid in test tube.
2. Add 0.02 ml of blood by Sahli-pipette.
3. Mix gently for 2 minutes.
4. By capillary tube charge the chamber.
5. Leave the chamber in wet environment (20-30) M.
6. Count the platelets in one W.B.Cs square.

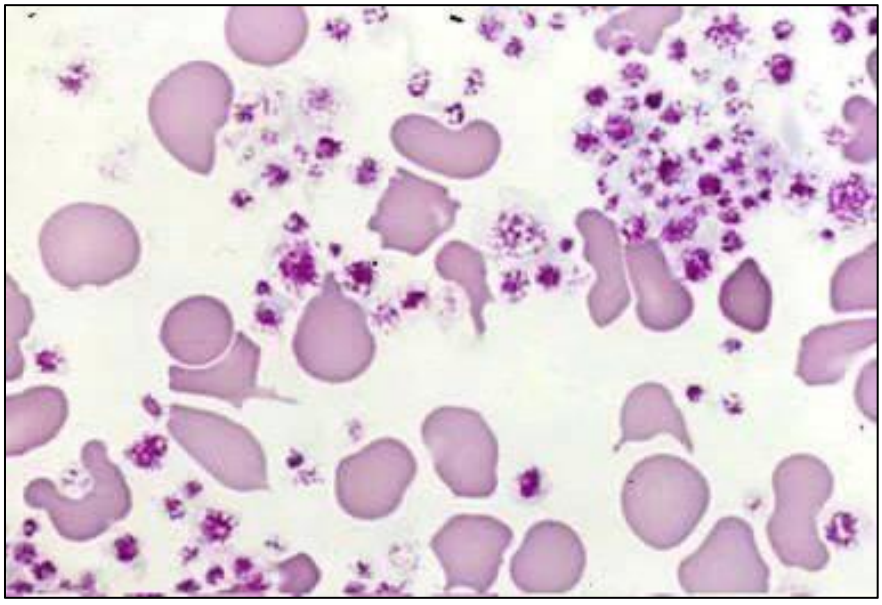


Calculation:

- Platelets count = N × 1000 p/cumm If W.B.Cs pipette & R.B.Cs square.
- Platelets count = N × 500 p/cumm If R.B.Cs pipette & W.B.Cs square.

Normal value:

150.000 - 400.000 p/ cumm



Modified method:

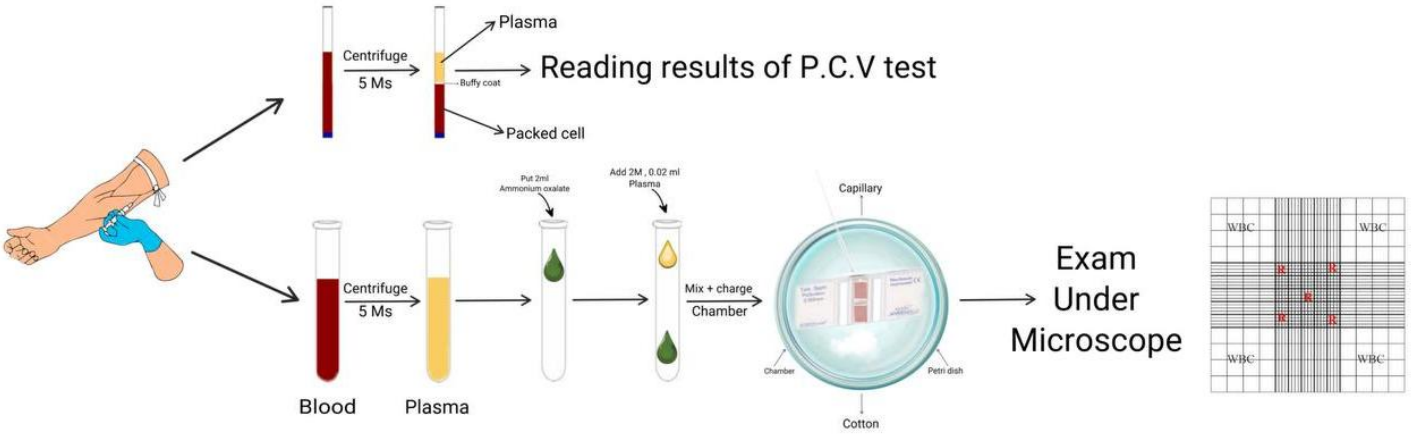
- **Use blood sample & plasma sample.**

Material required:

1. Blood & plasma sample.
2. Diluting fluid (Ammonium oxalate).
3. Micro-centrifuge& reader.
4. Capillary tube & sealing material.
5. Test tube & pipette.
6. Microscope &centrifuge.
7. Petri-dish & cotton, alcohol.
8. Improved chamber.

Procedure:

1. Draw vein blood & put in tube gently.
2. Take part one of blood to do P.C.V. test.
3. Take part two of plasma after separated.
4. Put 0.02 ml of plasma in small test tube.
5. Add 2 ml of Ammonium oxalate to the test tube.
6. By capillary tube charge the chamber.
7. Leave for 30 minutes in petri-dish (in wet environment)
8. Count the platelets in one square.

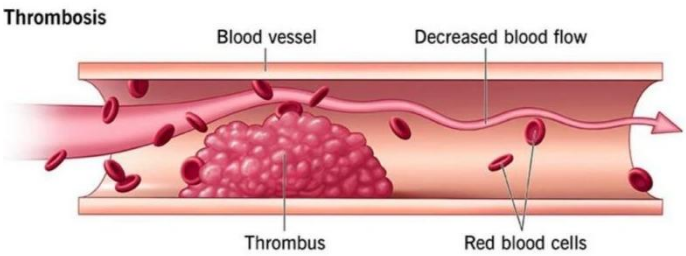


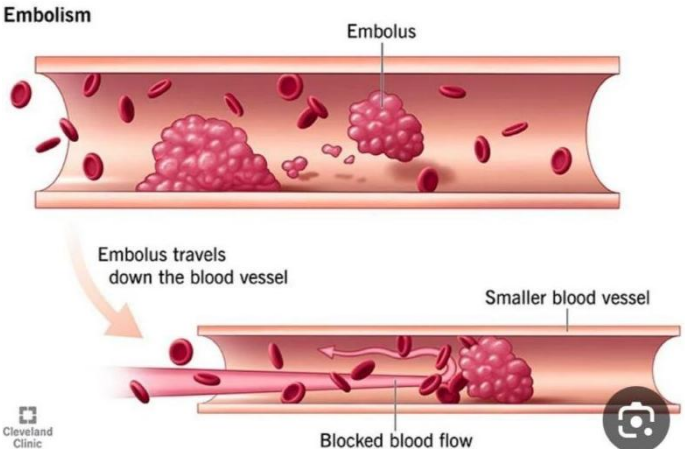
Calculation:

Platelets count = N × $\frac{100 - PCV}{100}$ × 1000 p/cumm

❖ Increase platelets count called **thrombocytosis** occurs in:

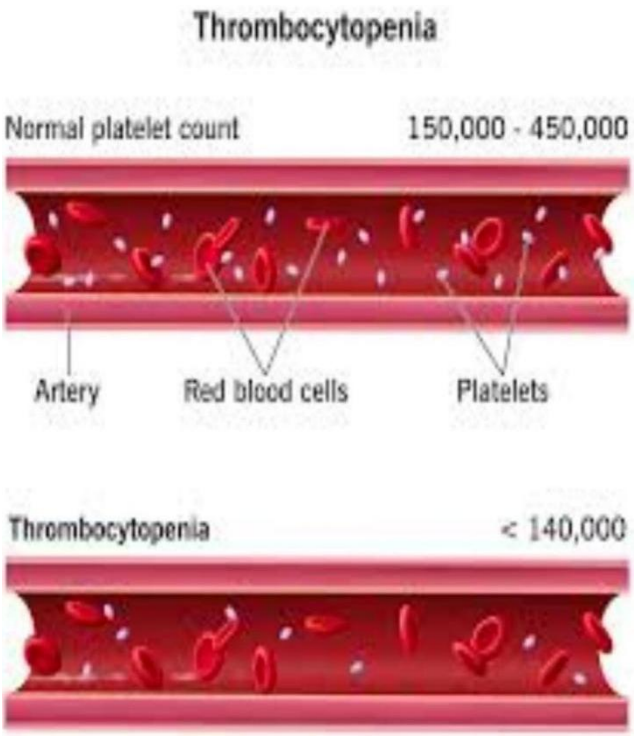
1. Chronic myelocytic leukemia.
2. After acute bleeding.
3. I.D.A.
4. Hemalytic anemia.
5. Inflammatory disease.





❖ Decrease platelets count called **Thrombocytopenia** occurs in:

1. Weak megakaryocyte:
   A. Drugs (sulfate).
   B. Chemical material (Arsenic).
   C. Fever infections
      (viral hepatitis &influenza).
   D. Auto immune disease.



2. Weak bone marrowplasia:
   A. Aplastic A, megaloplstic A.
   B. Chronic lymphatic leukemia.
   C. Myelosclerosis.
   D. Lymphoma.

Bleeding time

Definition:

It is the time required for a small cut to stop bleeding after occur injury.

Used bleeding time test to detect:

1. Haemorrhage disorders.
2. Capillary response to injury.
3. Platelets function test by:
   A. Adhesive the platelets to plug formation.
   B. Destroy platelets to release thromboplastin.

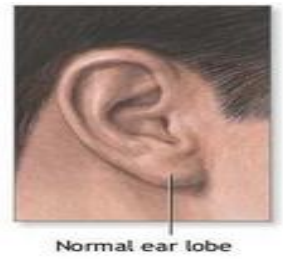
Types of methods used:

1. Duke's method.
2. Ivy's method.

Duke's method:

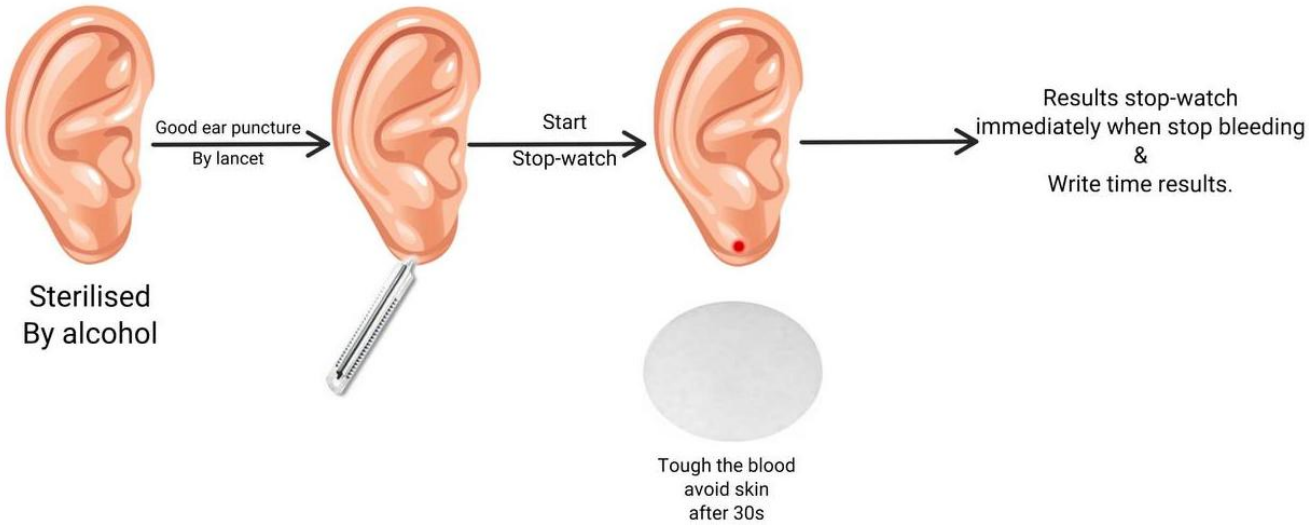
Material required:

1. Filter paper.
2. Stop-watch.
3. Lancet (sterile)&alcohol& cotton.


Normal ear lobe

Procedure:

1. Make the ear-lab warm by hand.
2. Make good puncture & start watch as soon as blood appear.
3. Tough the blood by filter paper every 15 second till blood stop.
4. Stop the watch immediately, this is bleeding time and read the result.



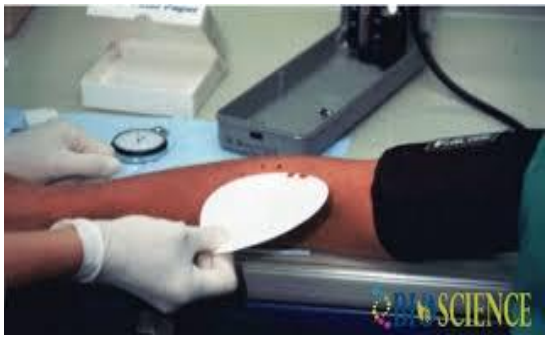
Ivy is method:

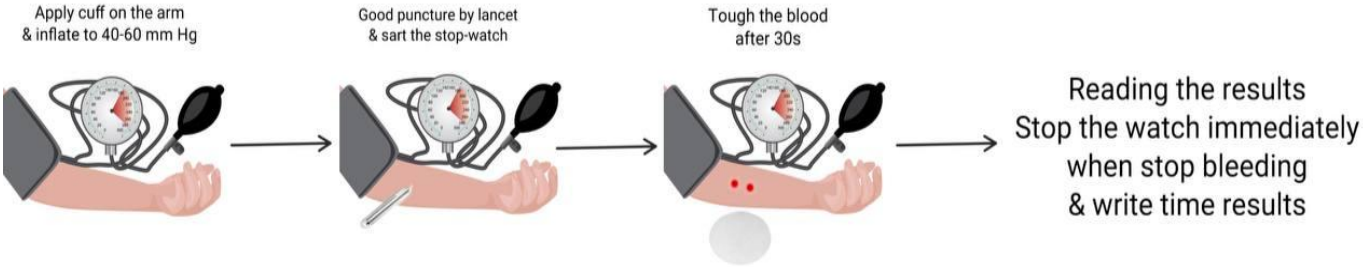
Material used:

1. Sphygmomanometer.
2. Filter paper & lancet & cotton &alcohol.
3. Stop-watches.

Procedure:

1. Apply sphygmomanometer cuff on the arm.
2. Inflate cuff to 40-60 mm/Hg.
3. Clean the arm, avoiding visible vein, make two puncture 3 cumm apart.
4. Start stop-watch for each puncture assoon as blood appears.
5. Tough the blood every 15 second and avoid touch the skin by filter paper.
6. Stop the watch for each puncture immediately at bleeding stop.
7. Take the average time for each puncture.



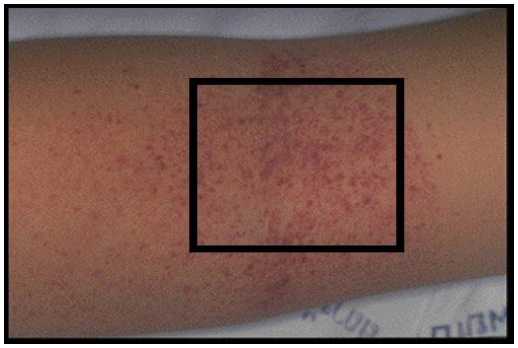


Normal value: 2 -4 minutes.

The reasons lead to long haemorrhage disorder.

1. Severe leukemia.
2. Decrease number of platelets.
3. Severe allergic.
4. Decrease plasma factor (fibrinogen).
5. Drugs (Aspirin).

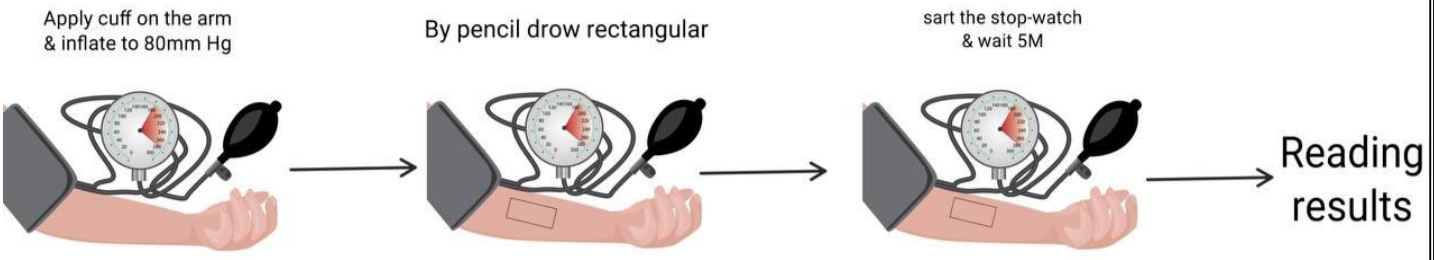
Hess's test



- It is measure the ability of the capillary to resist pressure under 80 mm/ Hg for 5 minutes.
- In healthy conditions, the capillaries in the arm will resist pressure under 100 mm/Hg.

Procedure:

1. Apply sphygmomanometer cuff on the arm.
2. 2 inches below the joint, make an area 3 cm × 3 cm, on the fore arm.
3. If there is any black spot is present, cancel it with pen.
4. Inflate sphygmomanometer cuff to80 mm/Hg and keep it for 5 minutes, after count the number of blue spots.



Results:

1. If you see (1-5) spots as normal is negative.
2. If you see (10-20) spots report as double full.
3. If you see more than 20 spots is positive report. It occurs in:
   A. Vit. K deficiency.
   B. Aplastic anemia.
   C. Influenza.
   D. Haemorrhage disorder.

Mechanism of clotting Factors

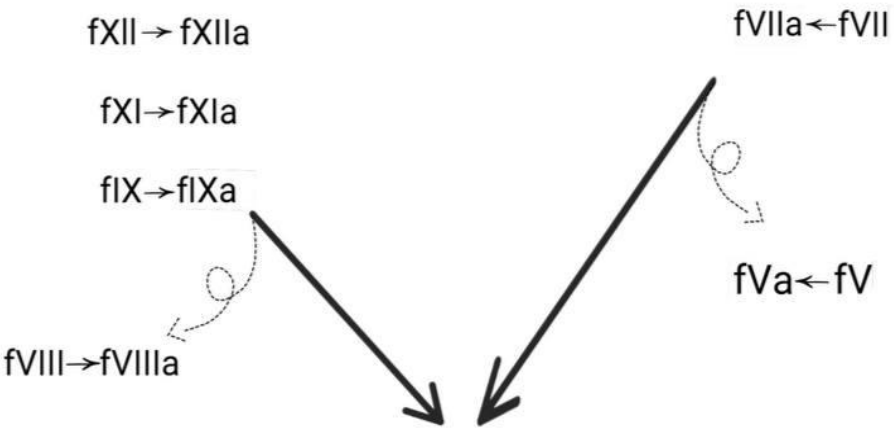
Intrinsic activation
(In vessels)

Destroy platelets ⟶ Thromboplastin

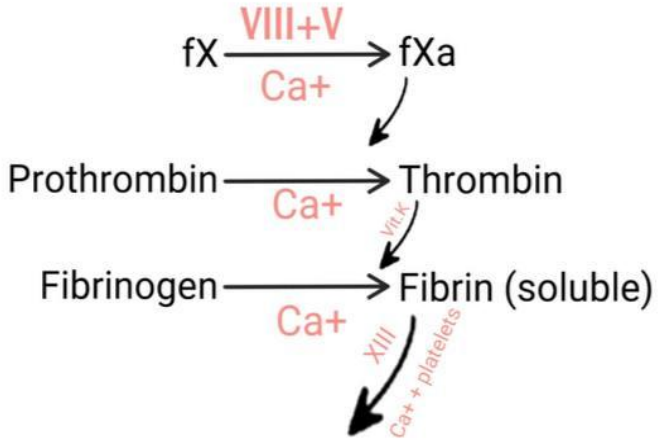
(Collagen, Kallikrein, HMWK)

Extrinsic activation
(In vitro)

Tissue injury



Common pathway



Fibrin (insoluble)

Clotting time

It is the time required to clotting the blood without adding any substance to it.

- Use this test to detect any disturbance in intrinsic factor (I, II, III, VIII, X, XI, XII).

Types of methods:

1. Dale & Laid method (capillary tube method & slide method).
2. Lee & White's method (test tube method).

Dale & Laid method:

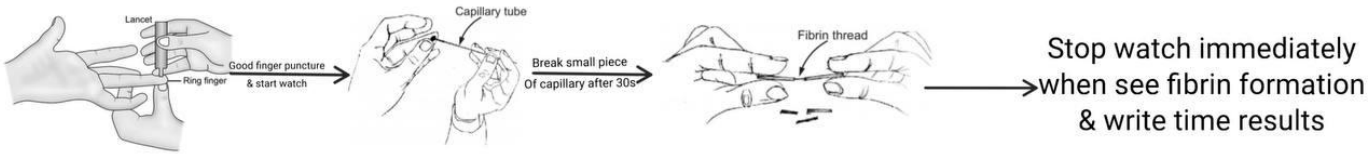
1. Capillary method:

Material required:

1. Capillary tube (non-heparinized).
2. Stop-watch.
3. Lancet & cotton &alcohol.

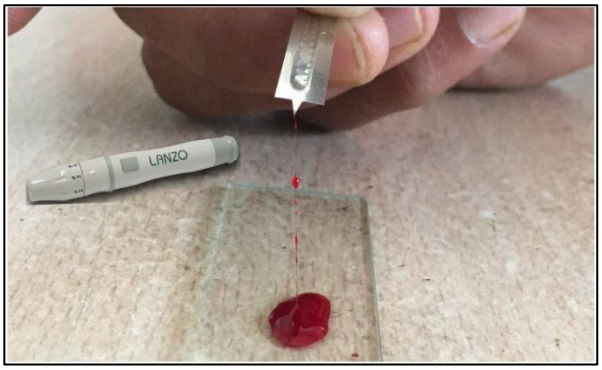
Procedure:

1. Moisten apiece of cotton with Alcohol& sterilize the finger & do good finger puncture.
2. Start stop-watch as soon as blood appears.
3. Fill the capillary tube from blood puncture.
4. Leave the capillary in your hand to keep 37 ℃ for 1 minute.
5. Break small piece of capillary and see fibrin formation. If didn't see fibrin, repeat the steps all 30 second.
6. Stop the watch if you see fibrin immediately.
7. Write the long time between seeing blood to fibrin formation.



2. Slide method:

Material required:



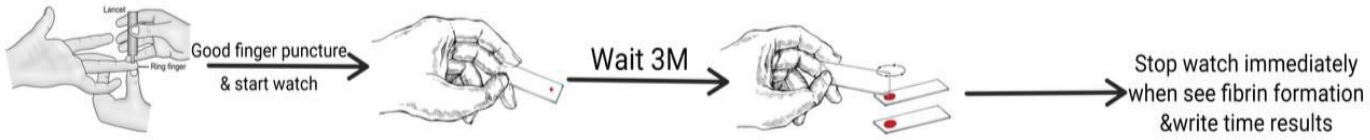
1. Slide**.**

2. Stop-watch.

3. Lancet & cotton &alcohol.

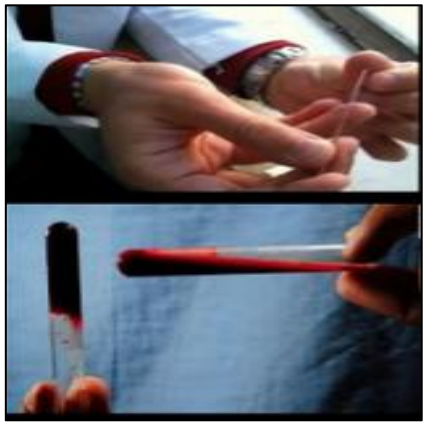
Procedure:

1. making Good finger puncture and start stop watch.
2. put few drops of blood patient on the slide and wait 2 mints.
3. Raise the blood by lancet to see clotted.
4. when occur fibrin stop the stop watch and write time result.



Lee & white method

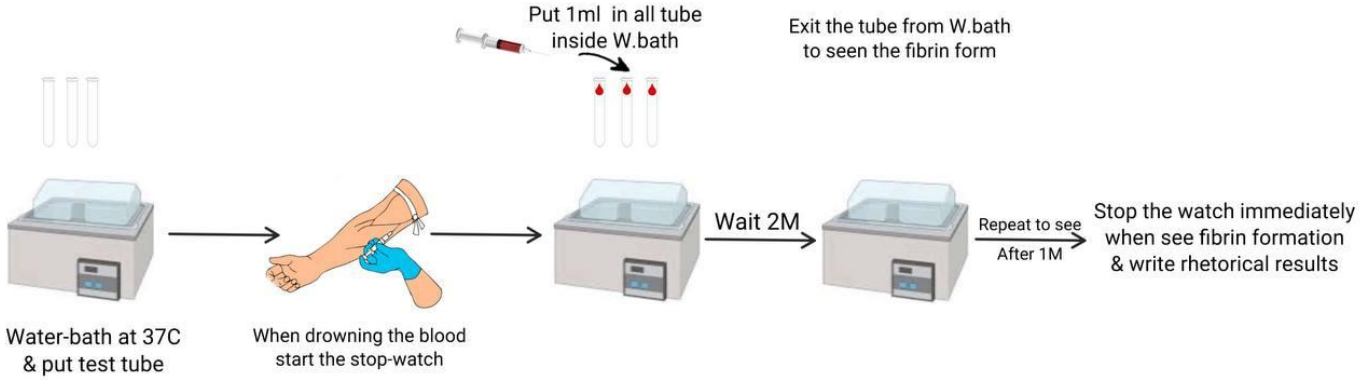
Material used:



1. Four test tubes & rack.
2. Water-bath.
3. Stop-watch four.
4. Blood sample.
5. Plastic syringe& cotton &alcohol.

Procedure:

1. Fill the water-bath with D.W at 37 ℃ .
2. Let the rack with four test tubes in water-bath.
3. Draw 4 ml of blood by plastic syringe from vein.
4. Start the watch after blood appear in the syringe.
5. Transfer 1 ml of blood to all test tubes.
6. After one minutes exit the test tubes from water bath in step & see the fibrin formation. If youdidn't see fibrin, leave all tubes in water-bath 30 second and the long time.
7. Stop the watch immediately if you see the fibrin.



Normal value = 5-11 minutes.

Use clotting time to detect any disturbance in the intrinsic factors: I,II,X,VIII,X, XI, XII.

Prothrombin time

Prothrombin:

It is protein substance which produce by the liver and it have important role in haemostasis after occur injury.

Prothrombin time:

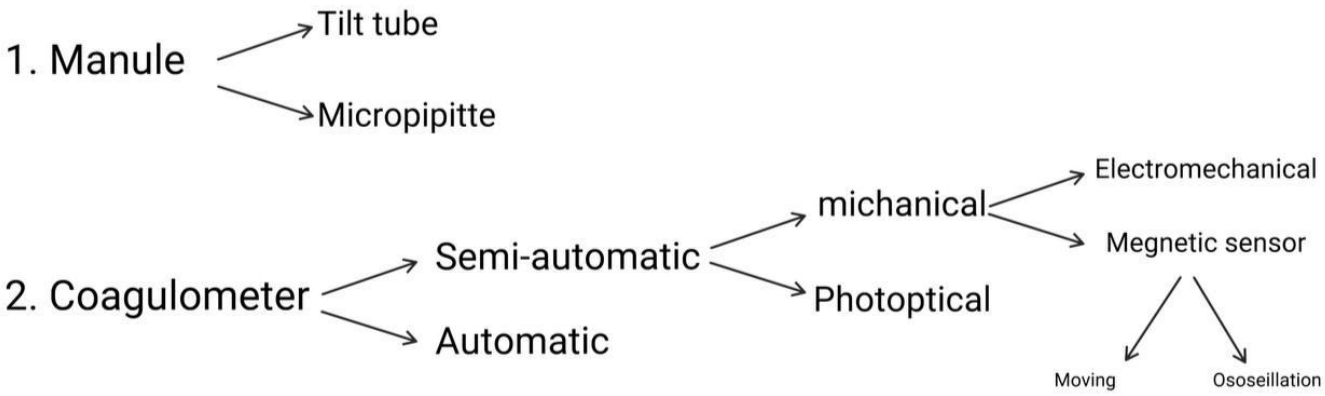
It is the time required for clotting of citrate plasma after addition of tissues thromboplastin and calcium chloride.

- Use this test to detect the deficiency in external factor (I, II, III, V, VII, X).

Principle:

Used equal volume of reagent 0.1 ml citrate plasma + 0.1 ml thromboplastin $\xrightarrow{waterbath\ 1min}$ 0.1 ml CaCl2 → clotted formation

Type method:

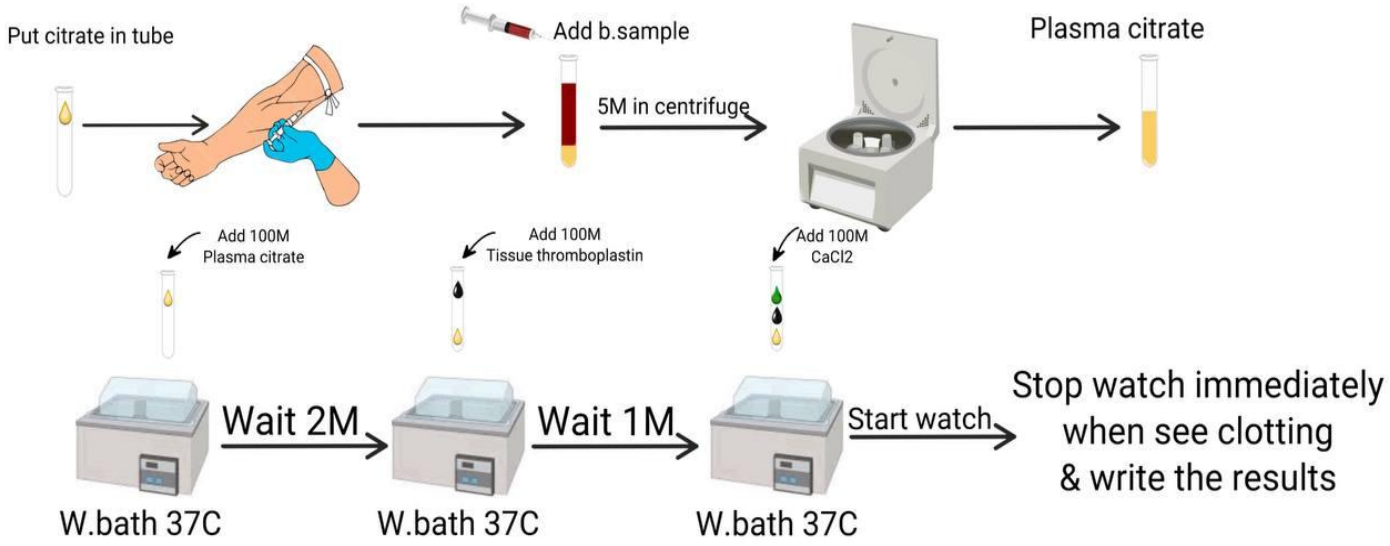


Material used:

1. Plasma citrate.
2. Water-bath, stop-watch.
3. Thromboplastin and CaCl2.
4. Small test tube &pipette.

Procedure:

1. Put 0.1 ml plasma citrate in small test tube and leave 1 minutest in water-bath.
2. Add 0.1 ml thromboplastin preparation with Ca^{+2} to the test tube & leave 1 minute in water-bath.
3. Add 0.1 ml of $CaCl_2$ to it & startstop watch.
4. Lift the tube out the water-bath until fibrin appears.
5. Stop the watch immediately& write the time result.



Normal Value = 10-14 Seconds

Thrombin Time

It is the time required for clotting of plasma after addition thrombin substance, prepared without addition calcium Ca^{+2}.

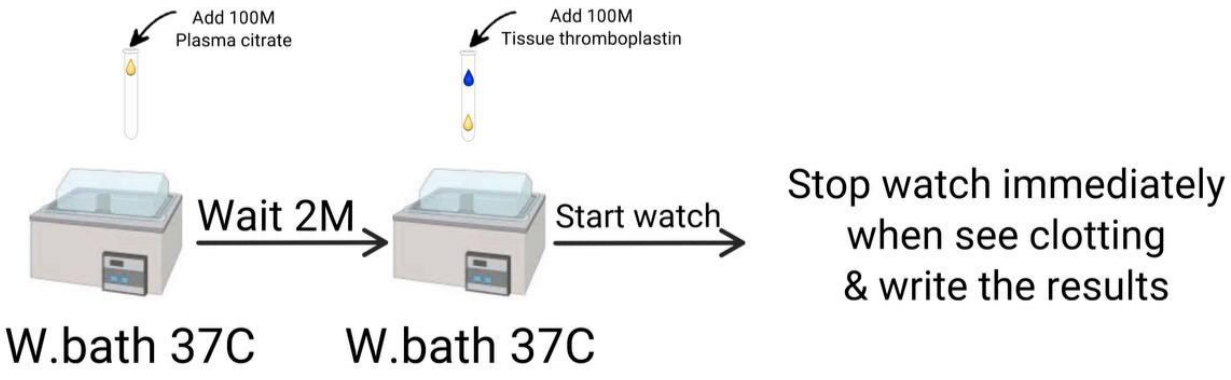
- It depends on:
  1. Strength of thrombin.
  2. Concentration of fibrinogen.
  3. Presence of anti-Thrombin (heparin).
  4. Temperature 37°C.

Material required:

1. Plasma sample.
2. Water-bath & stop-watch.
3. Thrombin substance.
4. Test tube, micropipitt, syringe, cotton, alcohol.

Procedure:

1. Take 0.2 ml plasma in clean & dry test tube.
2. And leave in water bath at 37 °C for 2 minutes.
3. Add rapidly 0.2 ml thrombin & start the watch.
4. Mix, then leave 5-10 second & see the clot, if it appear, stop the watch immediately and write the long time result.



Increase prothrombin & thrombin time occurs in these conditions.

1. Heparin therapy 2. Dicumarin therapy. 3. Fibrinogen deficiency.
4. Prothrombin deficiency. 5. Vit. K deficiency (Malabsorption states).
6. Deficiency factors (II, V, VII, X) (one of them) 7. Some liver diseases.
8. Haemorrhage disorder disease of new born.

Partial thromboplastin time

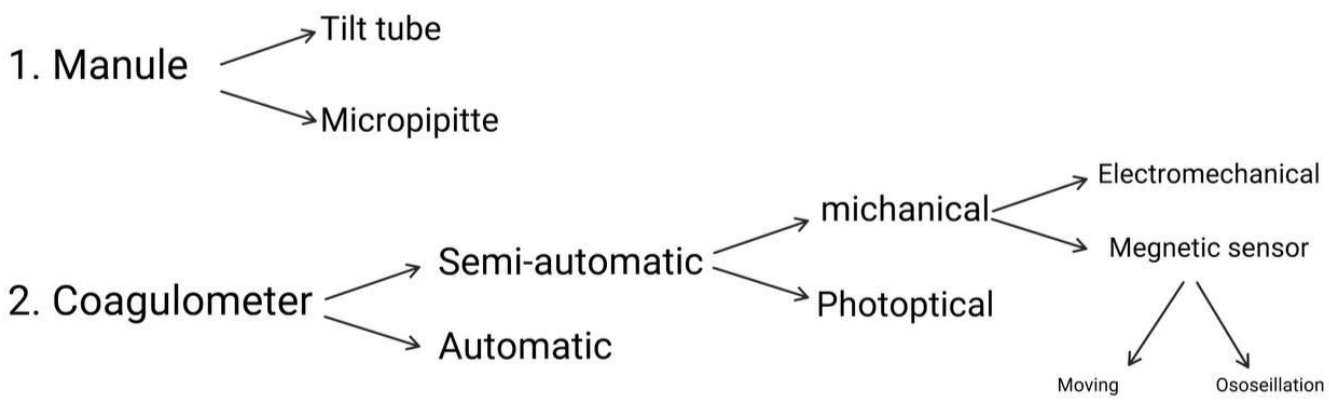
It is the time required to plasma clotting after addition calcium with found Kaolin phospholipids.

- Used the P.T.T widely to:
  1. Detect of Christmas disease& haemophilia.
  2. Detect of intrinsic factor (II, V, VIII, IX, X, XI, and XII).

Principle:

Each equal volume of reagent activator + plasma + CaCl2.

Type method:

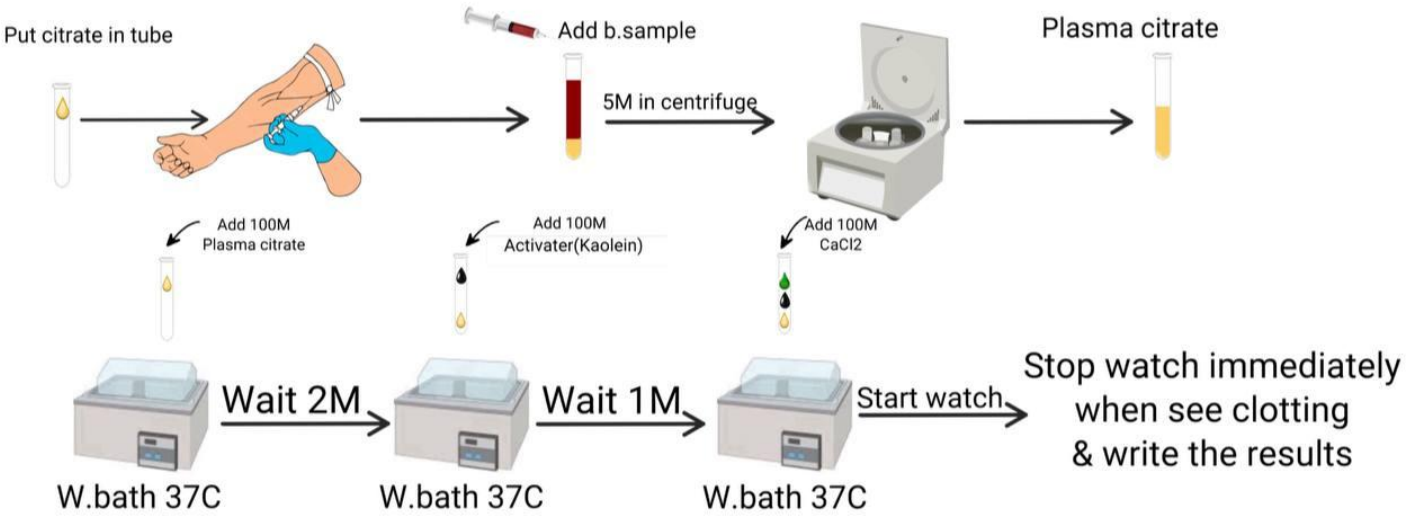


Material required:

1. Plasma citrate.
2. Activator reagent (kaolein, Phospholipids, cephalin, silica plastin)
3. Water-bath & test tube & pipette.
4. Stop watch & syringe & cotton & alcohol.

Procedure:

1. Keep equal volume of $CaCl_2$ + Phospholipids in water-bath.
2. Put 0.1 ml plasma citrate in small test tube.
3. Add 0.1 ml of Kaolin& shake & mix.
4. Leave for 10 minutes with occasional shaking.
5. Add 0.2 ml of $CaCl_2$ + phospholipid & start the watch immediately.
6. Stop the watch when clot forms.



Normal value = 40-45 seconds.

Fibrinogen deficiency test F.D.T

It is measure amount of fibrinogen in 100ml plasma.

- Use to detect any deficiency in fibrinogen in the body.

Fibrinogen:

It is least soluble plasma protein synthesis in the liver. It is important factor in stage 3 of clotting in which it is converted to fibrin by thrombin.

Principle:

Fibrinogen is precipitated from plasma at 56 ℃ for 3 minutes.

Type method:

1. Manule → Capillary tube method
   Manule → Strep method
2. Photoptical clot detection.
3. Immuno assay methods (ELIAS)

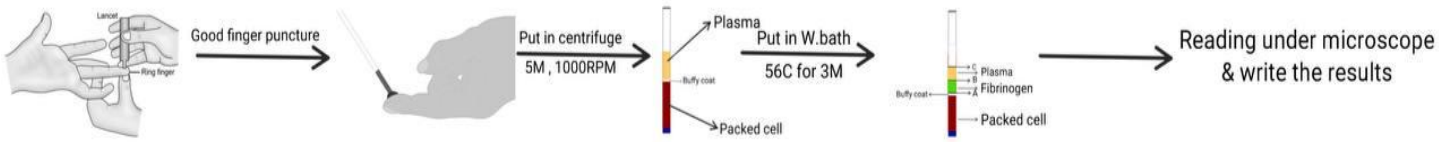
Material required:

1. Plasma.
2. Thrombin substance.
3. capillary tube & strep.
4. Micro-haematochrite centrifuge.
5. Test tube, micropipitte, syringe, cotton, alcohol.
6. Water-bath at 56℃.

Procedure:

1. Full the capillary tube with blood & seal one end of capillary by sealing material & put in micro-centrifuge for 5 minutes at 10.000 RPM.
2. Place the capillary tube in water bath at 56 ℃ for 3 minutes.
3. Put the capillary in centrifuge 5 minutes.
4. Place& fix the capillary tube on the slide.
5. Examine the component of the capillary tube under the microscope & give reading result.

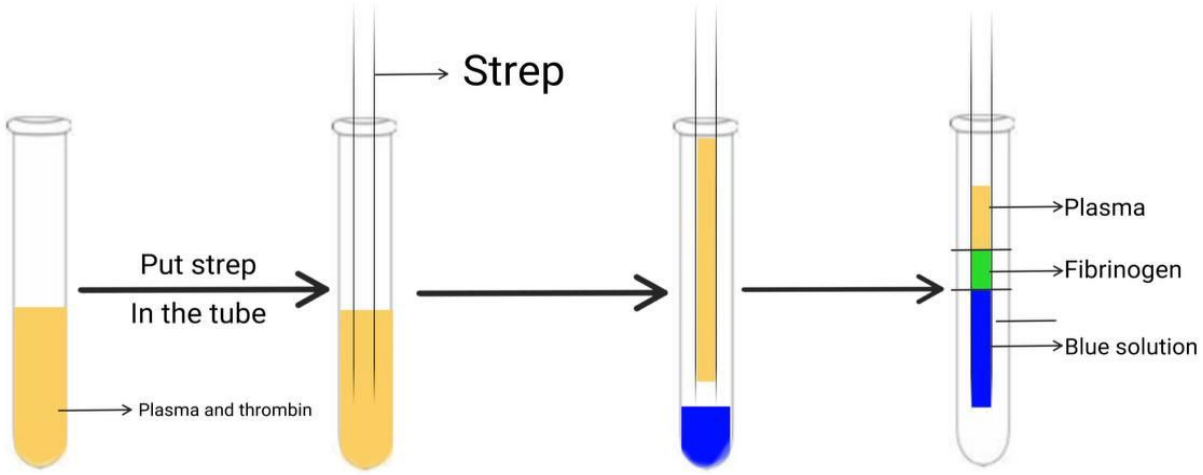
1. Capillary method:



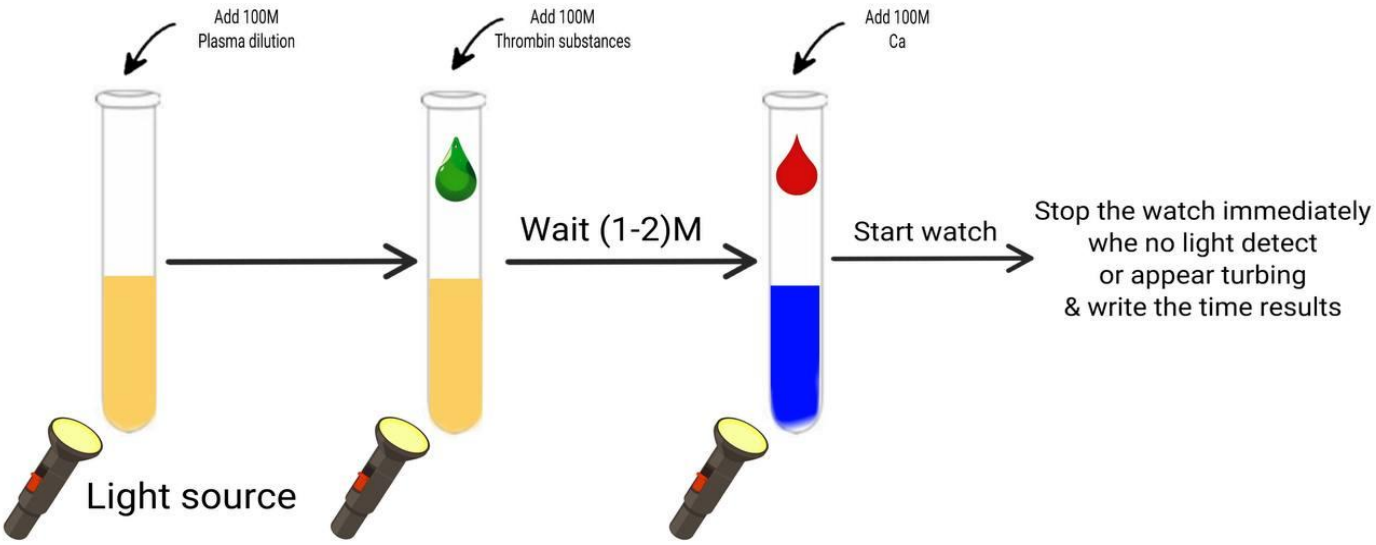
Calculation:

Fibrinogen = $\frac{A-B}{A-C}$× 10.000

2. Strep method:



3. Photo optical:



Normal value = 150-400 mg/ 100 ml plasma

The value below the normal in these conditions:

1. Congenital hypo-fibrinogemia.
2. Severe liver disease.
3. During or follow surgery.
4. Complication of pregnancy.
5. Abortion.
6. Acute leukemia.
7. Metastatic carcinoma.
8. Severe burns.

Total leukocyte count

It is measure the number of the different type of leucocyte in one cumm of circulating blood.

- Increase leukocyte than normal called **Leukocytosis** occur in:

1. Response to infection (Bacterial, virus).
2. Bone morrow disease (disorder).
3. Leukemia, cancer.
4. Allergic state (Asthma).

- Decrease leukocyte than normal called **leukocytopenia** occur in:

1. Cancer treatment like (Chemotherapy).
2. Infection such as HIV or Hepatitis.
3. Autoimmune condition like rheumatic arthritis.

Principle:

Diluting blood 20 times with diluting fluid causing the lysis of the R.B.Cs and leave W.B.Cs cells indicate with deep violet-black color of the nuclei.

Type method:

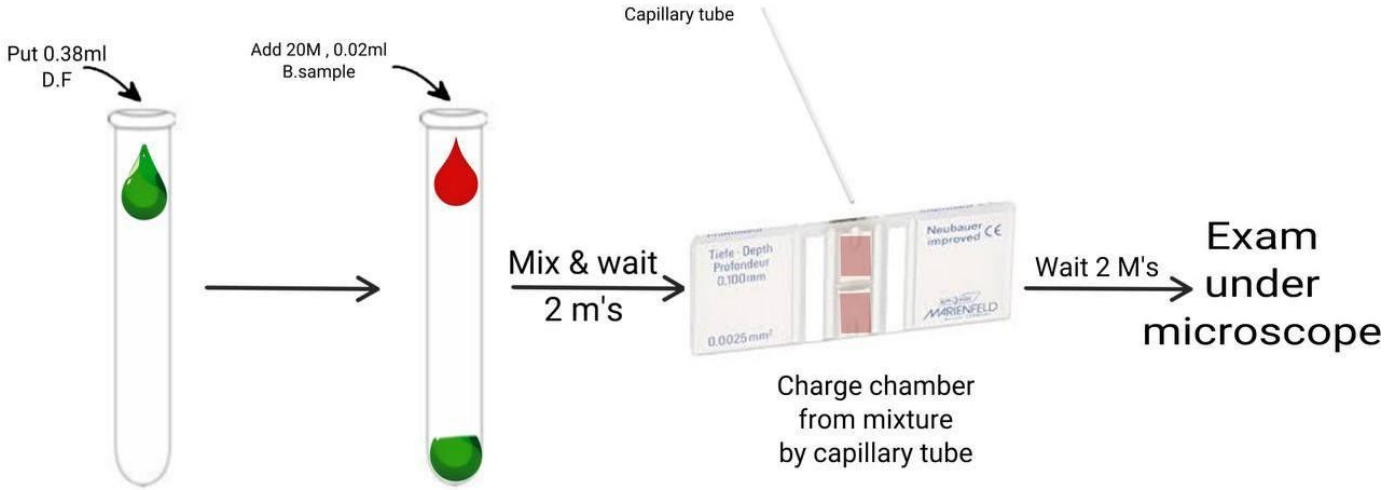
1. Manual methods → Test tube
   → Hemometer (thomapipitte)
2. CBC (complete blood cell).

Material required:

1. Blood sample (vain, finger).
2. Micro-pipette with 0.02 ml.
3. Microscope.
4. Test tubes & pipette.
5. Diluting fluid (glacial acetic acid 1%).
6. Improved number chamber & cover slide & capillary.
7. Syringe & lancet & cotton & alcohol.

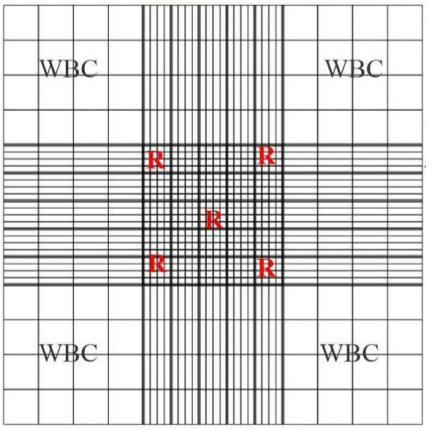
Procedure:

1. Measure 0.38 ml of diluting fluid in test tube.
2. Measure 0.02 ml of blood and put in test tube by micro-pipette & mix, the suspension by tilting and rotating for at least 2 minutes.
3. Charge the chamber by capillary tube and leave 2 minutes.
4. Count the cells in the 4 corner squares.



Calculation:

W.B.Cs count = N × 500 c/cumm



Normal value:

- Adult = 4000-11000 c/mm.
- Children = 4500-13000 c/mm.
- Infant = 10000-25000 c/mm

Notes:

- Don’t use heparin anticoagulant because it may cause the clumping the cells.
- Don’t use bad (old) blood sample.
- Not filtering the diluting fluid from time to time.
- Bad charging not using a clean and dry. Apparatus (chamber).
- Not mixing the blood & diluting fluid.

Differential count of Leucocytes

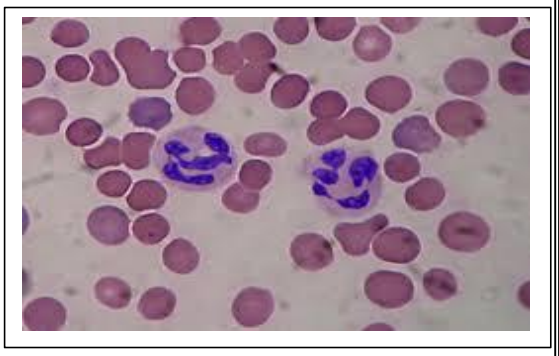
It is the percentage of each type of leukocytes.

Type of leucocyte:

1. **Granulocyte cells:**
   A. Neutrophil.
   B. Eosinophil.
   C. Basophil.
2. **Agranulocyte cells:**
   A. Lymphocyte.
   B. Monocyte.

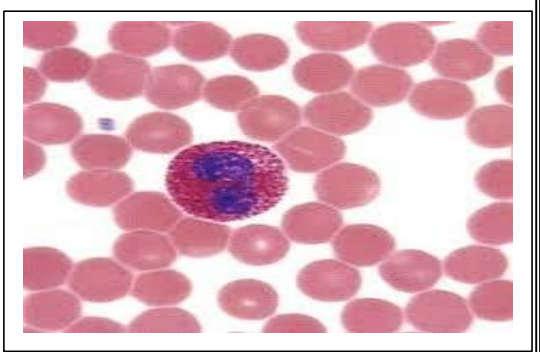
Granulocyte cells:

A. Neutrophil cell:



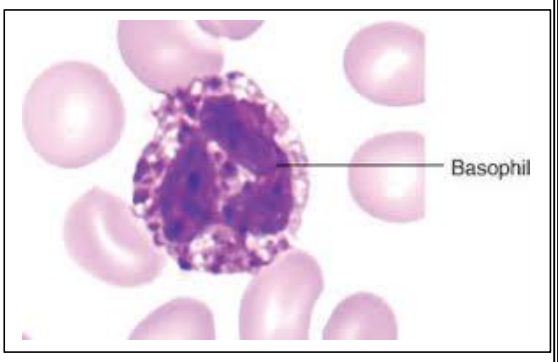
- Pink cytoplasm
- Pink,fine granules.
- (2-5) lobes nucleus.
- 40-70 %.
- Diameter (12-14) micron.
- Bacterial infection.

B. Eosinophil cell:



- Pink coarse granules.
- Pink cytoplasm.
- (1- 3) lobes nucleus.
- (1-6) %.
- Diameter 16 micron.
- Allergic state & parasitic infection.

C. Basophil cell:



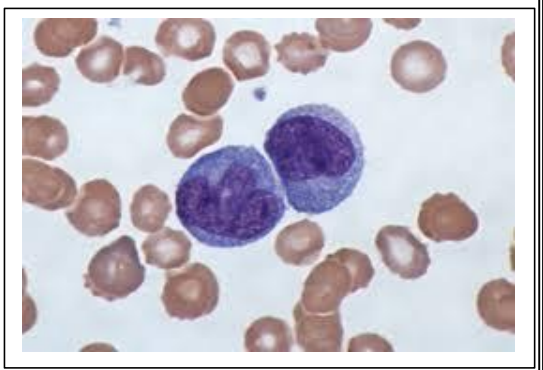
- Blue coarse granules
- Blue cytoplasm
- 2 lobes nucleus.
- 0-1 %.
- Diameter (14-16) micron.
- Sever allergic state (Asthma).

Agranulocyte cells:

A. Lymphocyte cell:

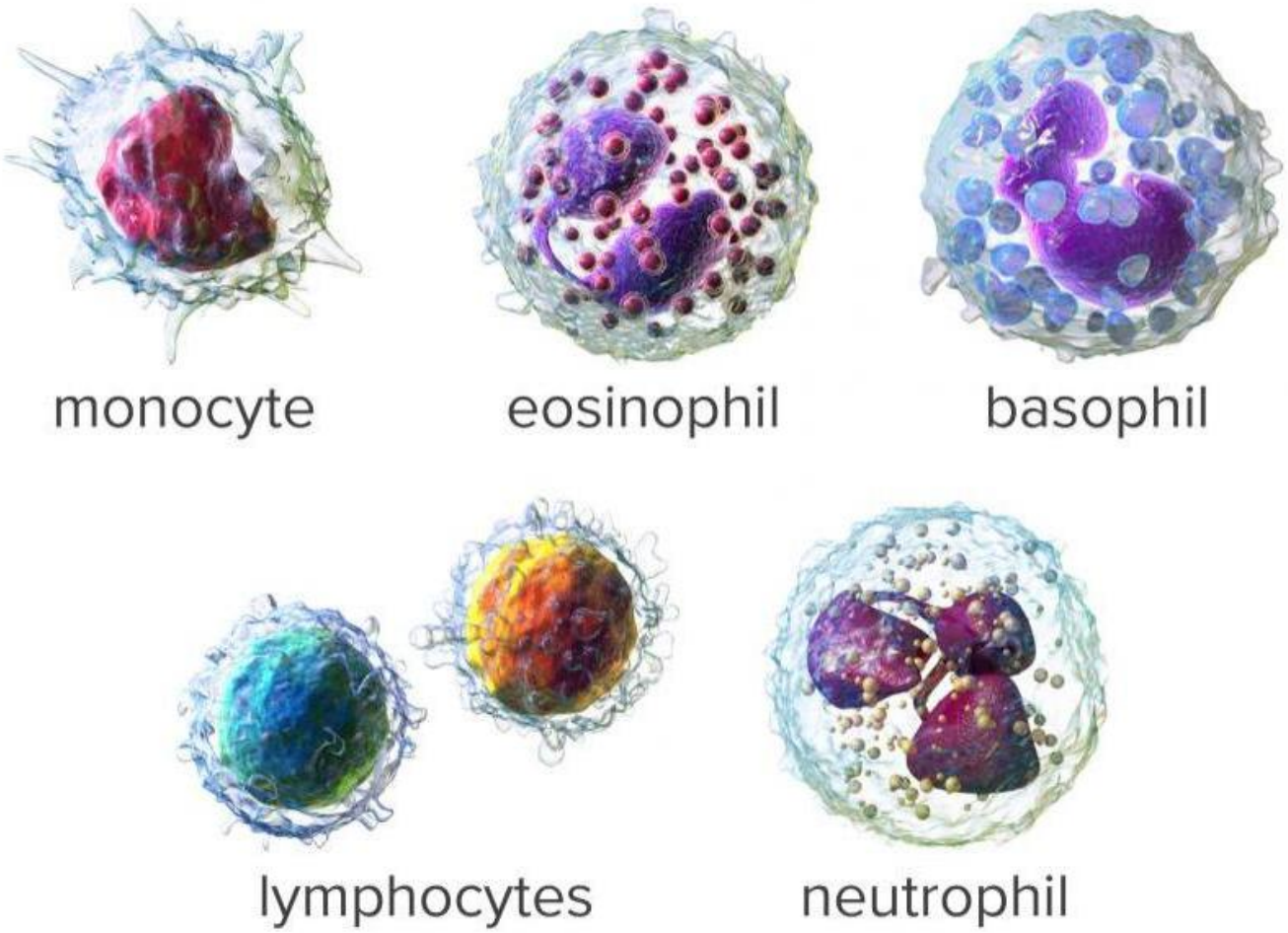
- Sky blue cytoplasm.
- No granules.
- One lobe nucleus.
- 20-40 %.
- Diameter (12-16)(9-12).
- Viral infection.

B. Monocyte cell:



- Greenish-blue cytoplasm.
- No granules.
- Kidney or spongy nucleus.
- 2 -8 %.
- Diameter (15-18) Micron.
- Parasitic infection.

White blood cells



Relative (Absolute count of W.B.Cs)

Absolute count:

It is the number of each type of leucocyte per one cu.mm of blood.

Relative count:

It is percentage of each type of leucocyte per one hundred of leucocyte.

Absolute count = $\frac{\%\ count of cell}{100}$ × Total WBCs count

Normal value:

WBCs type	Absolute	Relative	Increase	Decrease
Neutrophil	2500-2700	45 - 75 %	Neutrophilia	Neutropenia
Eosinophil	50-300	1-6 %	Eosinophilia	Eosinopenia
Basophil	0-100	0-1 %	Basophilia	Basopenia
Lymphocyte	1000-3500	20-40 %	Lymphocytosis	Lymphocytopenia
Monocyte	100 - 600	2-8 %	Monocytosis	Monocytopenia

Total count of Eosinophil cell

It is the number of eosinophil cell per one cumm of blood.

❖ Increase eosinophil cell than normal called **Eosinophilia** occur in:

1. Asthma. 2. Allergic food sensitive. 3. Allergic drugs sensitive.

4.High fever. 5. parasitic infection. 6. Malignant disease.

7.Pulmonary disorder.

❖ Decrease eosinophil cell than normal called **Eosiopenia** occur in:

1. Burns & tumor. 2. After large surgery. 3. Acute sever infection.

4.Shock. 5. Post operation. 6. After excessive exercise.

Type method:

1. Manual methods → Test tube
                  → Hemometer (thomapipitte)

2. CBC (complete blood cell).

Material required:

1. Blood sample(vein, finger).
2. Funchs-Rosenthal chamber.
3. Diluting fluid (Dugar's fluid).
4. Microscope.
5. Sahli-pipette or WBCs pipette.
6. Small test tube& capillary tube.
7. Petri-dish and piece of cotton.

Preparation of Eosinophil cell diluting fluid:

- Eosin (29/ 100 ml) 10 ml.

  It istostains the eosinophil granules by red color.

- Acetone 10 ml.

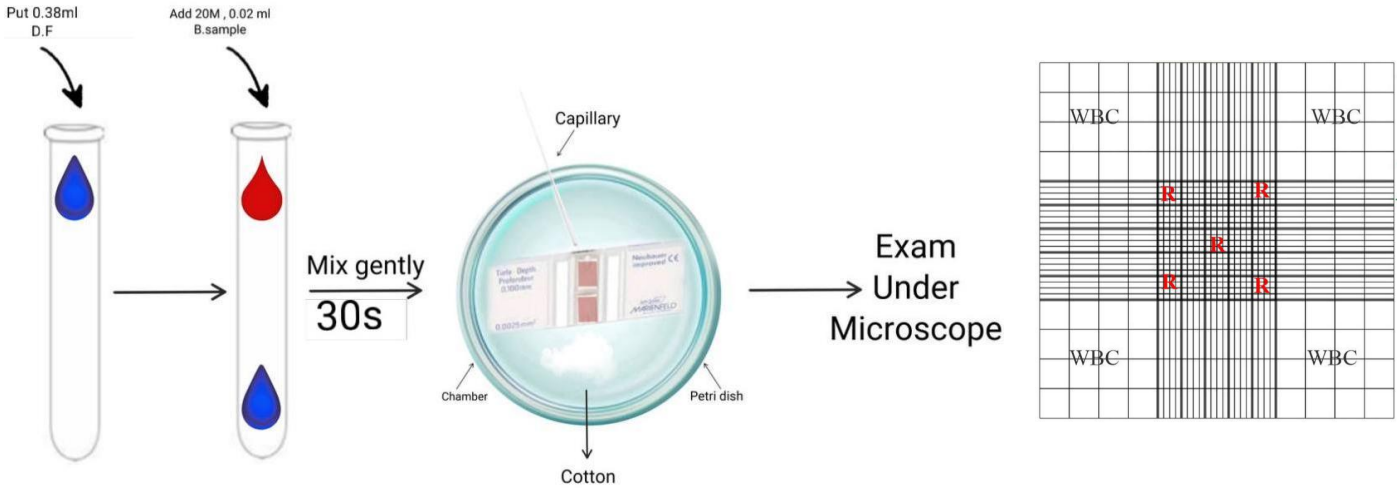
  The action of acetone is to inhibit the lytic action of water on the leucocyte, or to increase the resistance toward the water.

- D.W 80 ml.

  The action of wateris destroying all cells except eosinophil cell.

Procedure:

1. Measure 0.38 ml of Dugar's fluid in test tube.
2. Measure 0.02 ml of blood by micro-pipette and transfer to test tube & mix for 30 seconds.
3. Charge the chamber by capillary tube & leave it 30 minutes, in a damp atmosphere.
4. Count Eosinophil cell in the chamber under the low power in microscope.

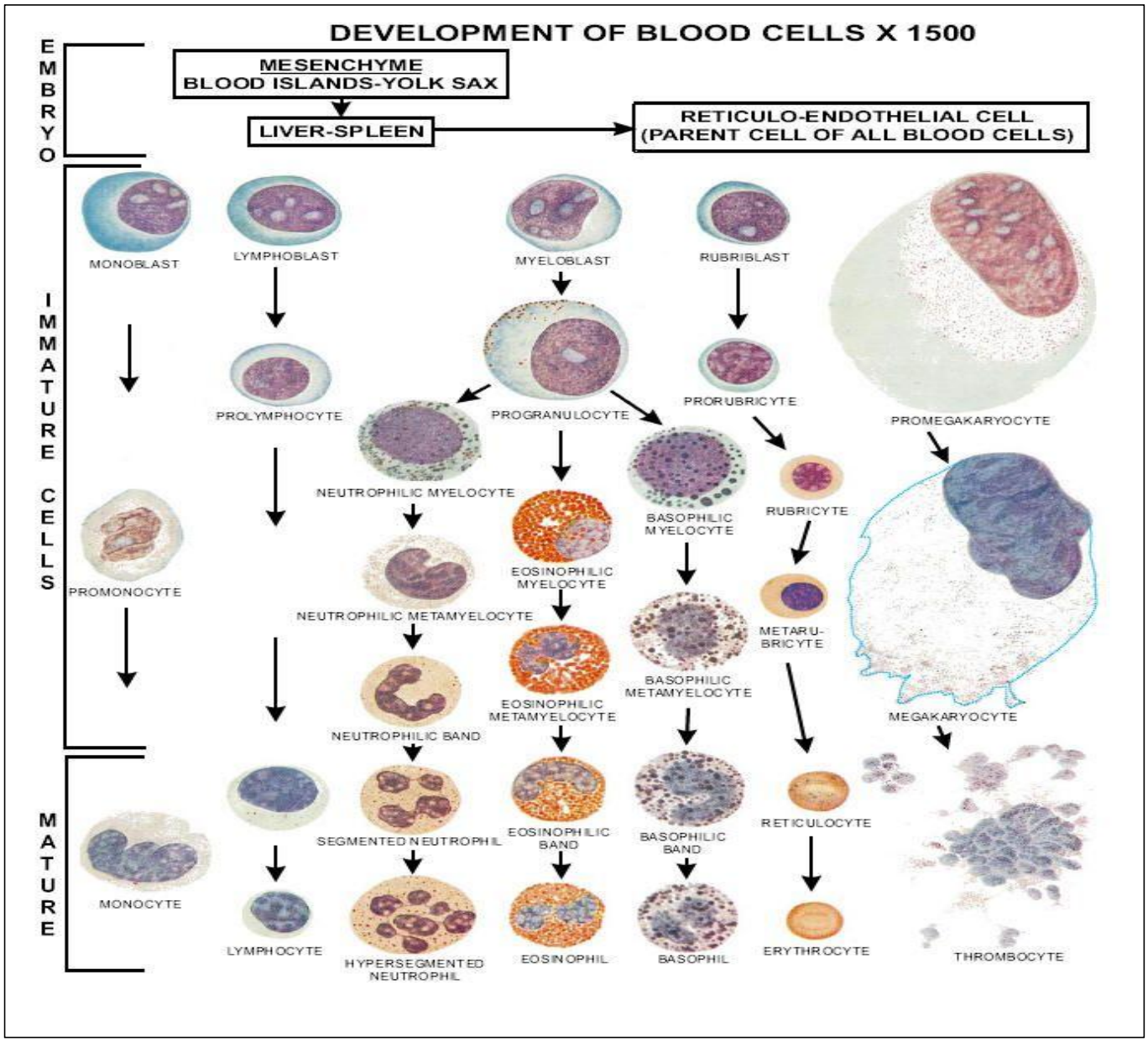


Calculation:

Eosinophil count = N × 22.2 c/cumm

Normal value = 40 - 440 c/cumm

Study of primitive leucocytes

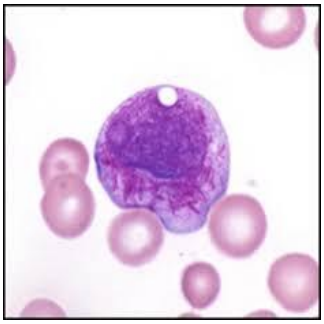


Study of primative leukocyte (immature)

- Granulocyte cells development in bone morrow before release to peripheral blood.
- Spreated immature leukocyte in blood stream indicator to stimulation immature system in early stage of **infection** or **disorder in bone morrow (Leukemia)**.

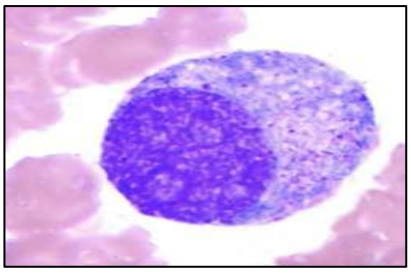
1. Myeloid series: produce from bone morrow.

A. Myeloblast:



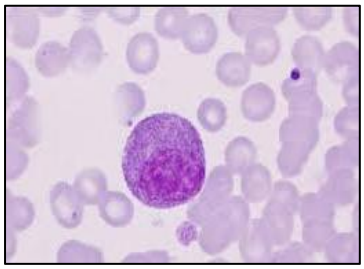
- Large size.
- Basophilic cytoplasm.
- Large Nucleus contains (2-5) nucleolus.
- Cytoplasm contains **Auer's bodies** red color bodies.
- Diameter 15-20 micron.

B. Promyelocyte:



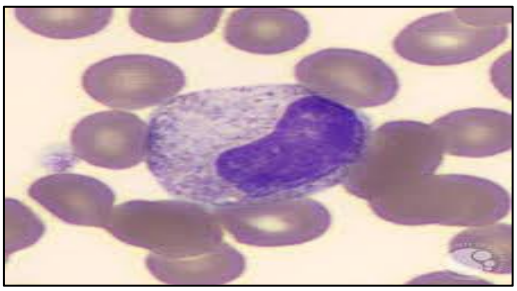
- Larger in size than myeloblast.
- Basophilic cytoplasm light-blue cytoplasm.
- Nucleus oval or round shape.
- Cytoplasm contain red **azurophilic** granules
- Diameter 22-25 micron.

C. Myelocyte (0-1%):



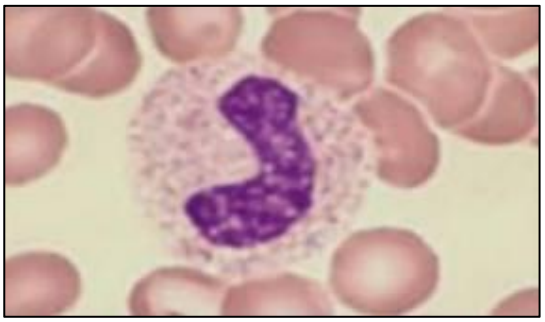
- Smaller size than promyelocyte.
- Nucleus round or oval.
- Acidophilic cytoplasm which contain granules.
- Diameter (20-22) micron.

D. Metamyelocyte (1-3 %):



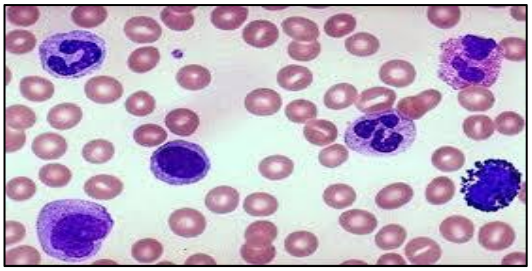
- Small size than myelocyte.
- Kidney shape nucleus.
- Acidophilic cytoplasm pink.
- Found pink granules in cytoplasm.

E. Band cell:



- Small size than metamyelocyte.
- Band like shape W, N, S, C, Y.
- Acidophilic cytoplasm pink color.
- Pink fine granules in cytoplasm.

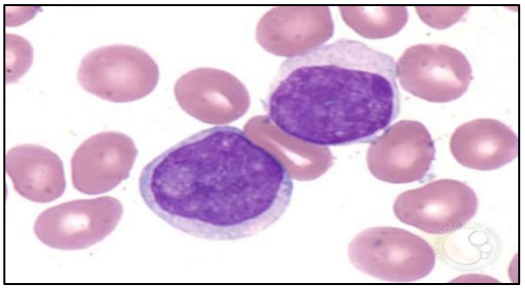
F. Segment mature leucocyte:



- Neutrophil.
- Eosinophil.
- Basophil.

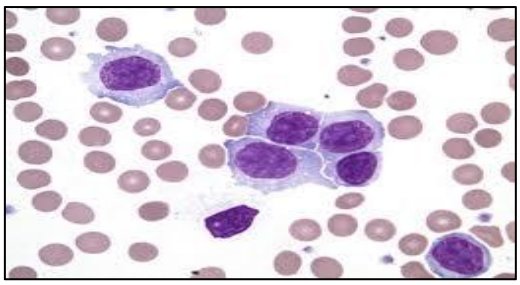
2. Lymphocytic series : Produce from lymphatic system.

A. Lymphoblast:



- Large size.
- Basophilic cytoplasm.
- Large nucleus contains2 nucleoli.
- Diameter 15-20 micron.

B. Prolymphocyte:



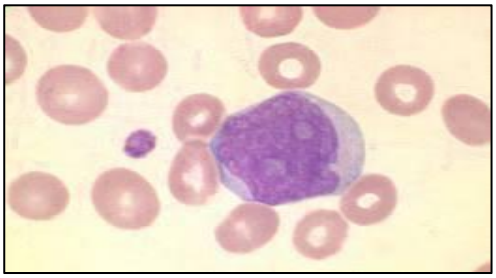
- Large size than lymphoblast.
- Large nucleus with out nucleolus.
- Basophilic cytoplasm.
- Diameter 15-20 micron

C. Mature lymphocyte large & small.

- Sky blue cytoplasm.
- Large nucleus.
- Diameter (12-16)(9-12).

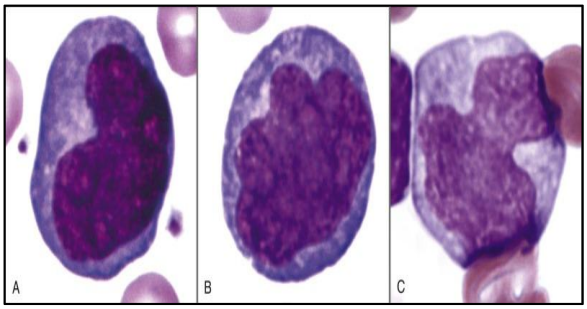
3. Monocytic series: Produce from bone morrow & Lymphatic system

A. Monoblast:



- Large size.
- Acidophilic & basophilic cytoplasm.
- Large nucleus irregular.
- Diameter 18-22 micron.

B. Promonocyte:



- Small size than monoblast.
- Band nucleus shape.
- Basophilic & Acidophilic cytoplasm.
- Diameter 15 -18 microns.

C. Mature monocyte.

- Basophilic cytoplasm (blue).
- Kidney shape nucleus.
- Diameter 18 micron.

Leukemia

Are groups of disorder in bone morrow , white blood.

❖ It is white of blood formation has two characteristics:

1. The total WBCs increasing above 10-20 times than normal in blood stream & bone morrow.
2. The differential WBCs count show immature white cell such as

*Myeloblast. *Lymphoblast. *Monoblast.

☒ The cause of leukemia is unknown; it is total and classified according to

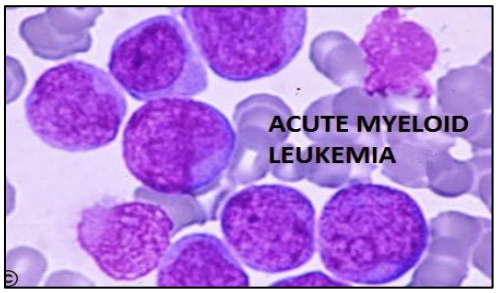
1. The type of white cell, series which predominant in blood or bone morrow (myelocytic, lymphocytic, monocytic).
2. The intensity and duration of disease, so it's either
   - acute : A.ML, A.L.L, C.M.L
   - Chronic : C.M.L, C.L.L

Classification of leukemia:

1. **Acute myelocytic leukemia.**
2. **Chronic myelocytic leukemia.**
3. **Acute lymphocytic leukemia.**
4. **Chronic lymphocytic leukemia**.
5. **Acute monocytic leukemia.**
6. **Acute myelomonocytic leukemia.**

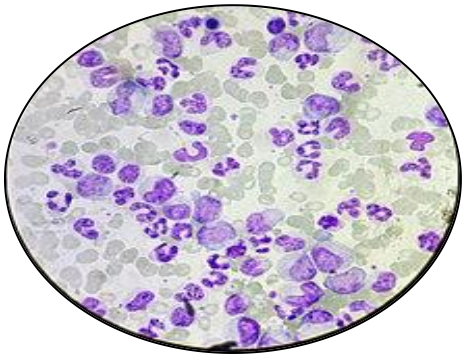
1. Acute Myelocytic leukemia (A.M.L)

- Myeloblast cell increase.
- Promyelocyte.
- Myelocyte.
- Metamyelocyte cell.
- Segment cell decrease.
- Anisocytosis & pokilocytosis.



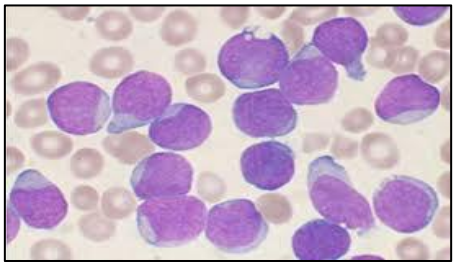
2. Chronic myelocytic leukemia (C.M.L)

- Metamyelocyte cell.
- Myelocyte cell.
- Band cell.
- Segment cell increase.
- Anisocytosis & pokilocytosis.



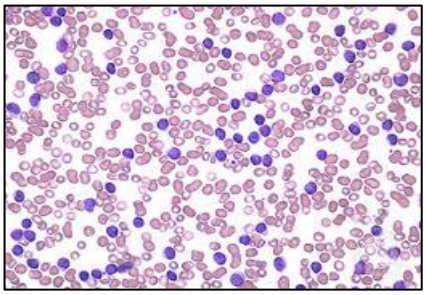
3. Acute lymphocytic leukemia (A.L.L)

- Lymphoblast.
- Prolymphocyte.
- Mature lymphocyte decrease.
- Anisocytosis & pokilocytosis.

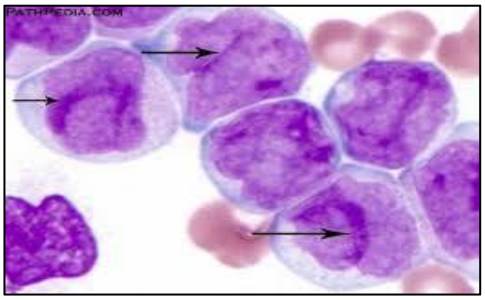


4. Chronic lymphatic leukemia (C.L.L)

- Lymphoblast cell decrease.
- Prolymphocyte cell.
- Mature lymphocyte.
- Anisocytosis & pokilocytosis.

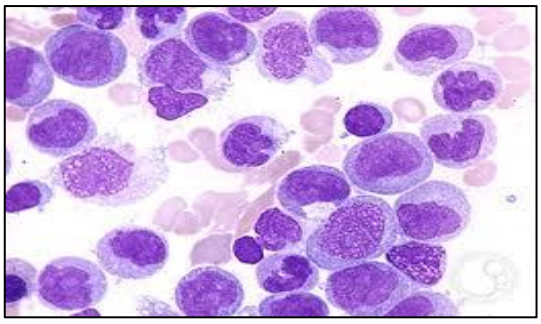


5.Acute Monocytic leukemia (A.M.L)



- Monoblast.
- Promonocyte.
- Mature monocyte.
- Anisocytosis & pokilocytosis.

6.Acute myelo-monocytic leukemia (A.M.M.L)



- Myeloblast.
- Monoblast.
- Metamyelocyte.
- Promonocyte.

Cytochromistar test

Peroxidase test:

It is specific test to diagnosis & differential between mylocytic leukemia & lymphatic leukemia.

Principle:

Mylocytic series is contain specific granules called **azurophilic granules.**

This granules produce **peroxidase enzyme** which oxidase chemical agent such as benzidine in the presence of **H_2O_2** (catalase) as PPT. which then take **C4SO4** staining greenish blue color.

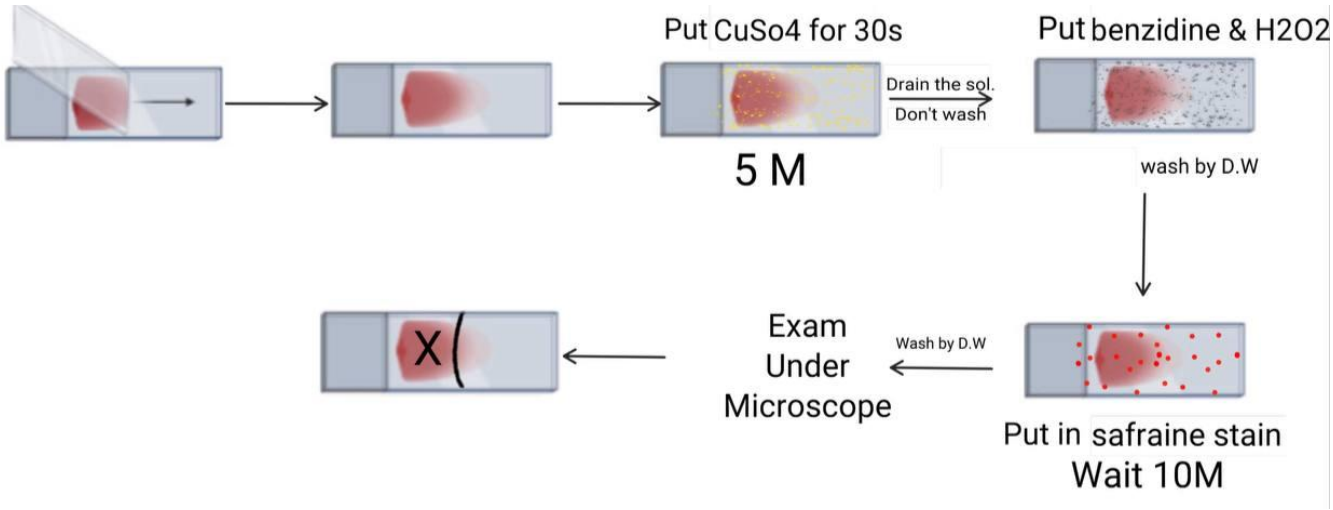
Materials required:

1. Spreader the blood & slide.
2. Solution $CuSO_4$, Benzidine with H_2O_4

3. Counter stain safranine.
4. Microscope
5. Blood sample.

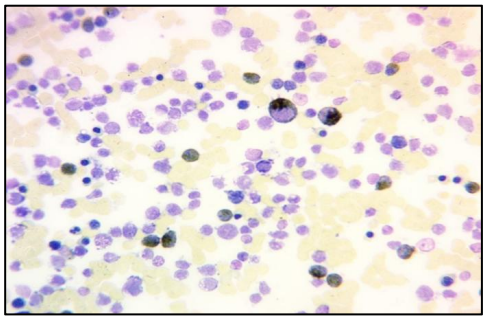
Procedure:

1. Spread the blood on the clean & dry slide.
2. Leave the blood film to dry in air.
3. Cover the blood film with solution $CuSO_4$ for 30 seconds.
4. Drain off the solution and do not wash.
5. Cover the slide with solution NO.2 Benzidine +few drop H_2O_2 for 15 seconds & wash with water.
6. Cover the slide with solution No.3 (safranine stain) for 10 minutes. & wash with water.
7. Leave to dry in air.
8. Examine under the microscope using oil-immersion.



Results:

- If present greenish granules in all cell is **Myelocytic** or **Monocytic leukemia.**
- If absent granules in all cell is Lymphatic **leukemia.**



Sudan black test

It is specific test to diagnosis & different between Myelocytic leukemia & Monocytic leukemia.

Principle:

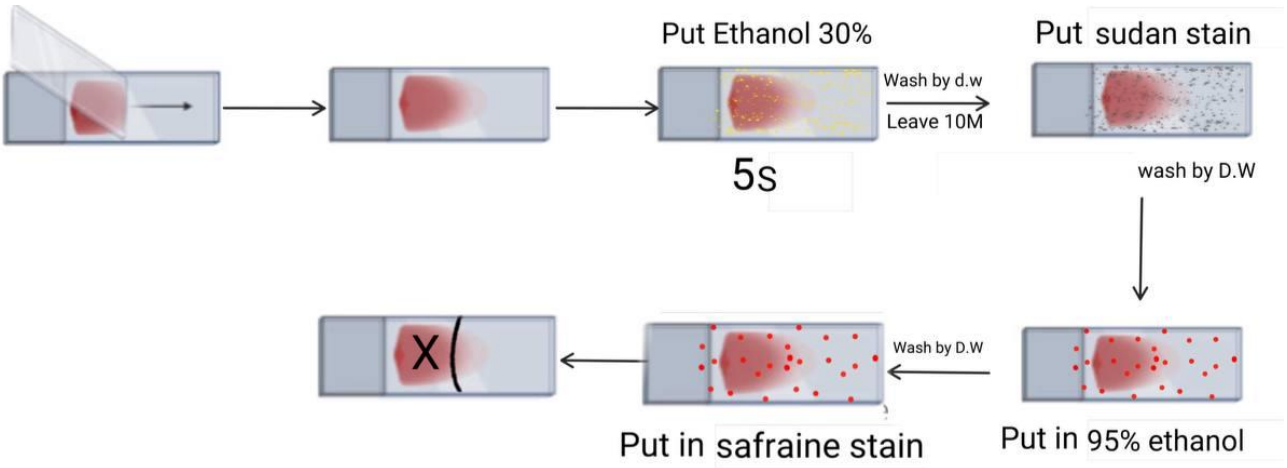
Sudan black stain, stain granules of myeloid series with black color, many of which appears to contain phospholipid.

Material used:

1. Blood sample.
2. Spreader & clean slide.
3. Sudan black stain.
4. Microscope.

Procedure:

1. Fix air dried film for 5 second in ethanolfixative.
2. Wash in D.W 1-minute allow to dry.
3. Filter stain on to slide & leave for 1 hour.
4. Wash off excess stain with D.W.
5. Dipin 95% ethanol for 3 minutes.
6. Wash immediately with D.W.
7. Counter stain with safranine for 3 minutes.
8. Wash with water & dry &examine under microscope.


Put Ethanol 30%
Put sudan stain
Wash by d.w
Leave 10M
5s
wash by D.W
X
Wash by D.W
Put in safraine stain
Put in 95% ethanol

The end