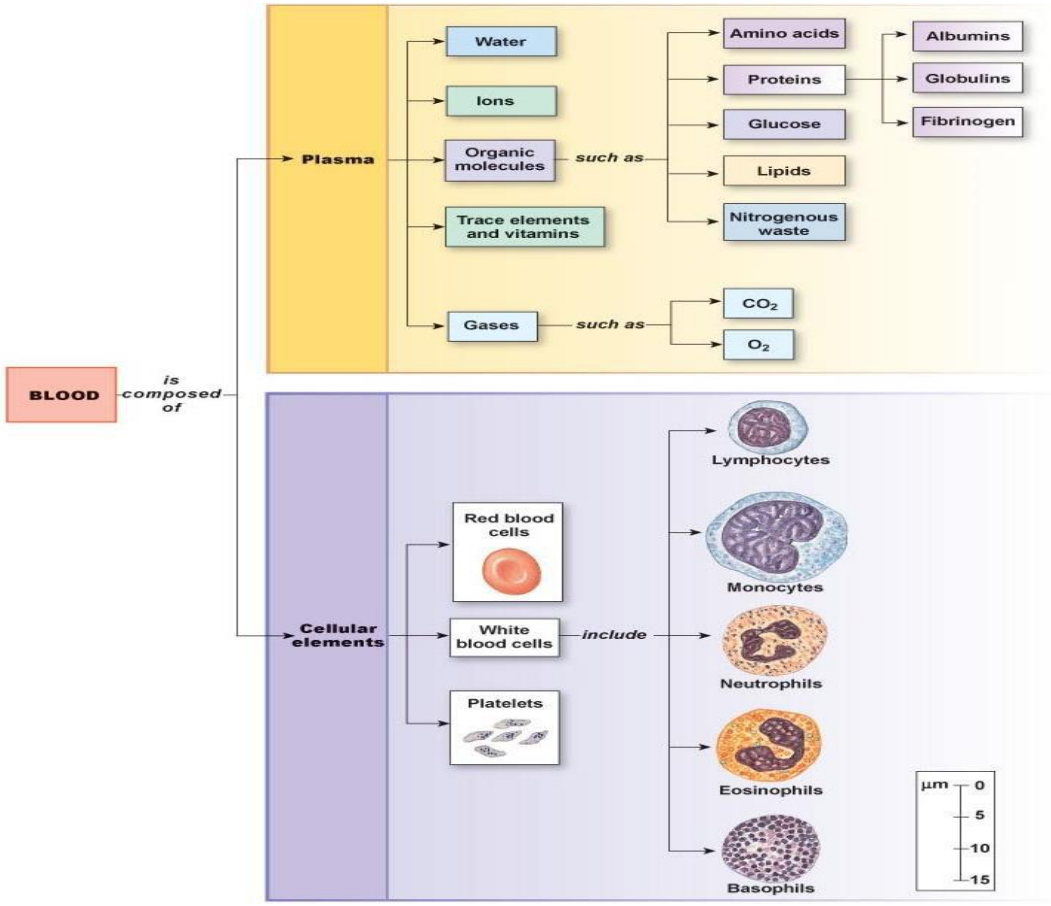
BLOOD EXAMINATION

Blood is made up of two main parts, as shown in the diagram: -



Blood collection:

There are several ways to collect blood samples the most common are the **collection** of the cephalic vein or Basilica vein of the arm. Is possible in other cases we need a few amount of blood so prefers collected sample by finger prick, as well as in children may collect from the jugular vein of the neck.

The most important conditions for the process of blood collection is:-

1. The use of sterilized syringes or sterilized lancet with sterilization of the area to be blood collection
2. Transfer the blood to a sterile tube,
3. Using Anticoagulants, such as heparin or tri sodium citrate or EDTA salts However, if the required tests are to investigate the plasma components do not use Anticoagulants but leave blood sample clotting leaving yellow liquid called serum and which is different from plasma being free of protein (Fibrinogen).

Blood tests:-

A sample of blood is one of the more samples performed by the laboratory tests because of their connotations دلالات and indicators مؤشرات for many pathological cases so these tests are classified into several disciplines.

A. Hematology:

1. Complete blood picture (CBP), Blood film.
2. Erythrocyte Sedimentation Rate (ESR)
3. RBC count
4. Packed cell volume (PCV)
5. Hemoglobin content (Hb)
6. WBC count (total & differential)
7. Platelets count
8. Clotting time
9. Bleeding time

B. Biochemistry :

Electrolytes

- Sodium
- Potassium
- Chloride
- Bicarbonate

Liver Function Tests

- Bilirubin; direct; indirect; total
- Aspartate transaminase (AST) (GOT)
- Alanine transaminase (ALT) (GPT).
- Gamma-glutamyl transpeptidase (GGT)
- Alkaline phosphatase (ALP)
- Total protein (serum)
- Albumin
- Globulins
- A/G ratio (albumin/globulin)
- Protein electrophoresis
- Urine protein

Cardiac Markers

- H-FABP
- Troponin
- Myoglobin
- CK-MB
- B-type natriuretic peptide (BNP)

Minerals

- Calcium
- Magnesium
- Phosphate
- Potassium
- Blood Disorders
- Iron
- Transferrin
- TIBC
- Vitamin B12
- Vitamin D
- Folic acid
- Miscellaneous
- Glucose , Suger
- C-reactive protein
- Glycated hemoglobin (HbA1c)
- Uric acid
- Arterial blood gases ([H+], PCO2, PO2)
- Adrenocorticotropic hormone (ACTH)
- Toxicological screening and forensic toxicology (drugs and toxins)
- Neuron-specific enolase (NSE)

Blood Disorders

C. Endocrinology :

- TSH
- Growth hormone GH
- T3 ,T4
- FSH
- Progesterone
- LH
- FSH
- Prolactin
- Testostreone
- Insulun

D. **Serology:**

- Widal test
- Rose Bengal
- ASOT
- Rheumatoid arthritis (RF)
- C-Reactive Protein (CRP)
- VDRL
- TPHA

E. Immunology:

- HLA
- IgG
- IgM
- IgE
- IgD
- IgA

F. Virology :

- HIV
- Hepatitis
- CMV

G. **Bacterial diagnosis:**
Blood culture (Bacteremia).

H. **H)) Cytogenetic :**
Chromosomal Banding
(Hereditary diseases)

- Iron
- Transferrin
- TIBC
- Vitamin B12
- Vitamin D
- Folic acid

Miscellaneous

- Glucose , Sugar
- C-reactive protein
- Glycated hemoglobin (HbA1c)
- Uric acid
- Arterial blood gases ([H+], PCO2, PO2)
- Adrenocorticotropic hormone (ACTH)
- Toxicological screening and forensic toxicology (drugs and toxins)
- Neuron-specific enolase (NSE)
- fecal occult blood test (FOBT)

Renal (Kidney) Function Tests

- Creatinine
- Blood urea nitrogen

These are some laboratory tests that can be performed on a blood sample but the most common and widely used in conventional laboratory tests are Hematology Biochemistry and serology. The rest of it needs to be carefully allocated and high potential, so let's review together some routine tests:

1- Erythrocyte Sedimentation Rate (ESR):-

Speed of descent(going down) of the red cells in a blood sample placed in a tube listed by millimeters and a vertical through the unity of time estimated hourly (1 hour) .Anti coagulated whole blood in tube separates into an upper layer of plasma and lower layer of blood cells because of gravity. **N.V.** in male (1-10 mm. in 1 hr.) and female (2-15 mm in 1 hr.)

Pathological states that Increase ESR:	**Pathological states that Decrease ESR:**	**Physiological states that Increase ESR:**
1- Anemia's, 2- Chronic and acute diseases of liver, 3-Diseases of connective tissues. 4-Macrocytic RBC 5-Increase in Fibrinogen 6-In pregnancy	1- Increased cases of erythrocytes Polycythemia 2- Red blood cell abnormalities: 3- Microcytic RBC 4- Sickle –cell Anemia 5- Thalassemia	1- After meals, 2- After hot baths, 3- During menstruation, 4- After physical exercises, **5-** Increases with age.

Equipment used in Westergren method:

Westergren tube long straight pipe length is a length of 30 cm and a diameter of 2.5 mm are listed from top to the bottom of zero to 200mm, and using a special tube holder (Westergren stand) placing the pipeline deposition in vertical to the base so as not to spill the blood of the tube, test is performed at room temperature between 18-25°C, If the room temperature is elevated than 25°C, **E.S.R.** will increase and different reference range will acquire. ESR tube must be in strict vertical position. Even a slight tilting will cause elevation in **E.S.R**.

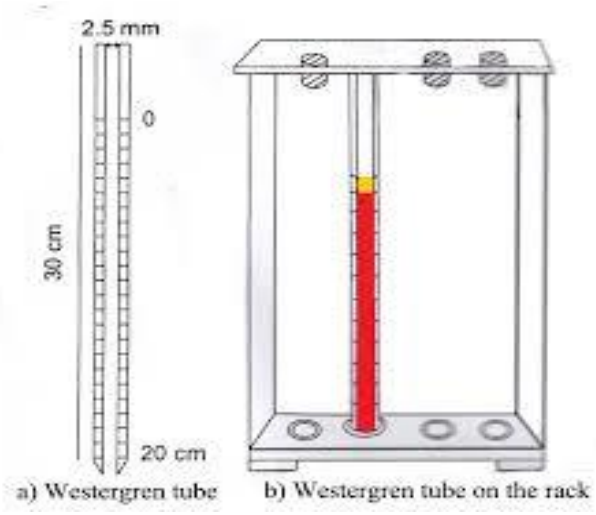
Procedure:

1- Venous blood is collected in trisodium citrate solution (0.321 %) in 4:1 (1.6 ml of blood is placed on a 0.4 ml solution of citrate) proportion. The test should be performed within 4 hours of blood collection.
2- Place the tube in vertical position in the ESR stand and left for an hour.
3- Just exactly after an hour, read the height of the column of plasma above the red cells column in mm.
4- Result is express in the following manner:
Erythrocyte Sedimentation Rate = _____ mm / hour.

Reagent:

- Trisodium citrate dihydrate 32.08 gm
- Distilled water upto 1000 ml

Normal Range: (2-12mm./hr.)



2- (Packed Cell Volume – PCV):

Hematocrit is the proportion of blood volume that is occupied by erythrocytes (the ratio of red blood cells to the whole blood volume). It is usually expressed either as a percentage or as a decimal fraction (e.g. 41% or 0.41).

Procedure:

A) Capillary tubes Method :-

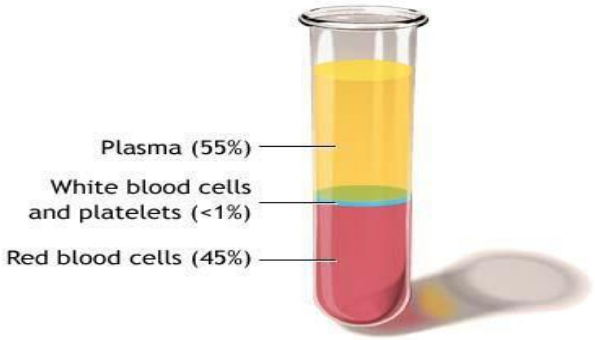
1. Puncture the skin of the finger and collect capillary blood directly into heparinized microhaematocrit tube (Capillary tube), fill 2/3 of the tube.
2. Seal one end of the tube with clay or a sealant. Avoid trapping air between the blood and plug.
3. Place the tube into a calibrated microhaematocrit centrifuge, sealed ends out against a rubber ring. Place firmly the lid over the centrifuge head. Close the cover. Set the timer (most instruments require 3 to 5 minutes centrifugation time). Centrifuge the tube (usually at 10,000 RPM).
4. The tube should be removed and read within a minute or two after the centrifuge has stopped to avoid re-dispersion of cells. Hemolysis should be noted, since this may lower the hematocrit results in relation to the hemoglobin (the hematocrit is 3 times the value of the hemoglobin, if the cells are normocytic).
5. Use a lined card, wheel or other device to determine the hematocrit value. They all work by the same principle, measuring the height of the total blood column and the height of the red cell layer.

B) Wintrob's Method:-

Less common need is greater than the amount of blood where the blood is placed in a tube listed from zero to 100 Tube Top is open while the closed bottom centrifugation at 3500 rpm/min for 5 minutes and then read the result.

Normal values:

Newborn at one week of age	7 D	0.54 - 0.68
Infant at three month of age	13W	0.31 - 0.43
Infant at one year of age	52 W	0.31 - 0.46
Child at ten years of age	10 Y	0.36 - 0.45
Adult male M	Adult	0.4 - 0.54
Adult female F	Adult	0.35 - 0.47



3- Estimation of hemoglobin (Hb. Estimation):

It is a respiratory pigment that responsible of the red color of blood and found inside red blood cells and is made up of the two major parts of the synthetic protein and the second part is the non-protein, there are several forms of blood Hemoglobin:

1. 1-Reduced Hemoglobin Hb-Fe^{++}
2. Oxygenated Hemoglobin Oxy –Hb
3. Met Hemoglobin (Oxidized –Hb)
4. Carbiaminohemoglobin

And it can be estimated in several ways

A- SAHLI'S ACID HEMATIN METHOD:

AIM: To determine the hemoglobin content in 20µl of blood sample.

PRINCIPLE: The working principle of this method depends on the conversion of hemoglobin to acid hematin compound using HCL (10 N) which turns to light brown color intensity is compared with the standard color chromaticity and calculate the amounts of hemoglobin.

MATERIALS:

1. Sahli's hemoglobinometer: This equipment consists of a comparator with a brown glass standard and Sahli's graduated hemoglobin tube which is marked in percent and gram.
2. Hemoglobin pipette or Sahli's pipette (marked at 0.02 ml or 20 µl).
3. Stirrer (a small glass rod).
4. Dropping pipette (dropper).
5. Distilled water.
6. HCl (10 N).

PROCEDURE:

1. Take 1/10 HCl in the Hb tube up to the lowest mark (20%).
2. Prick the finger with needle and collect 20µl of blood sample with single mark pipette.

3. Place the Hb tube on working table for five minutes for the formation of Hematin acid compound
4. Place the Hb tube in the hemoglobinometer and add drop by drop of distilled water into it until the colour of the solution similar with color tubes, double standard at that level solution includes the amount of hemoglobin.

Note \\ Despite the ease of this method, but it is not accurate because it does not include all kinds of hemoglobin in blood and also all the other substances found in the blood, such as proteins may give false read.

B- DRABKIN'S METHOD:

1. Put in a test tube x ml of t Drabkin's reagent then add a 20 microliter of blood and mixed well for 5 minutes
2. Read color intensity by chromatography spectrometer at a wave length of 540 nm.
3. The result compared with the standard values

Drabkin's reagent consists of

Sodium bicarbonate	19 gm.
Potassium cyanide	0.05 mg
Potassium Ferricyanide	0.2 mg.

Dissolved in one liter of distilled water and kept in sterile bottles in the fridge and is fit for use for one month from the date of preparation.

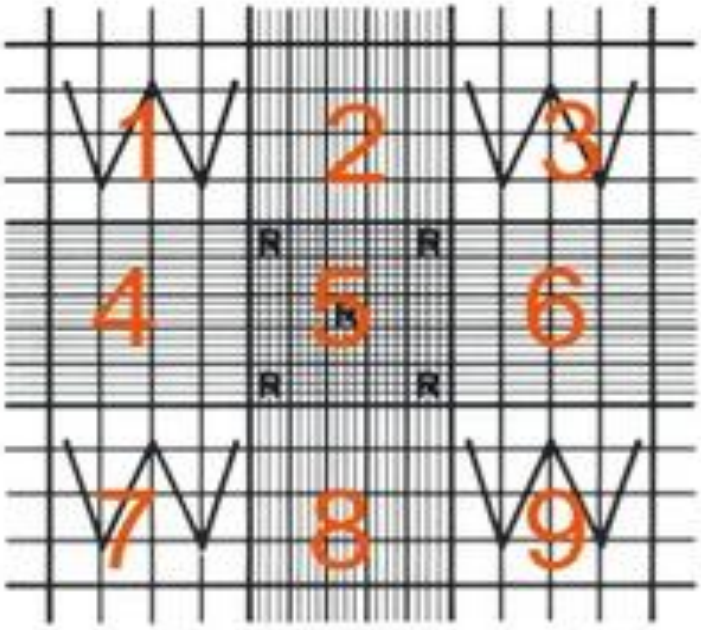
NORMAL VALUES:

Adult Males; --------13.0 – 18.0 grams/dl.
Females; ----------11.5 – 16.5 grams/dl.
Children; ----------12.0 – 14.0 grams/dl.
Newborn infants; ---15.5 – 21.0 grams/dl.

4-Total Count of Red Blood Cells:

Blood cell counter device consists of a glass slide is very much like ordinary microscopic slide but it is much thicker ones. This segment contains in the middle of the square equals one space each equal to 9 mm 2 and a depth of 0.1 mm and each divided square to a huge number of the straight lines carved to be an enormous number of very

small squares and one consists of nine equal squares along one side of which 1 mm. This little box is also divided into smaller boxes totaling 25 square means that each square of the nine squares consists of 25 square side length of 0.2 mm square. Smaller squares divided are the other into tiny squares so that each smaller box contains 16 square very small length of the rib 0.05 mm, has characterized the ribs of some of the smaller squares (0.2 mm) three lines in the sense that every 16 square too small to be limited to three lines and so it seems the basic box (9 mm left) and with the plus sign (+).



Chamber haemocytometer

AIM: To determine total number of red blood cells present in one µl of blood specimen.

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

Materials: Haemocytometer chamber, RBC pipette, Cover slip, **Hayem's** diluting solution, Needle, spirit, cotton.

Procedure:

1. Prick the finger with needle and draw the blood in a RBC pipette up to 0.5 marks.
2. Take RBC diluting fluid up to 101 marks in single mark pipette or RBC pipette.
3. Rotate the pipette equally in your hands to mix the solution well by swirling.
4. Take the haemocytometer and place it on the flat surface of the work bench. Place the cover slip on the counting chamber.
5. Allow a small drop of diluted blood, hanging from the pipette, to sweep into the counting chamber by capillary action. Make sure that there is no air bubble and there is no overfilling beyond the ruled area.
6. Leave the counting chamber on the bench for 3 minutes to allow the cells to settle. Observe the cells by placing the counting chamber on the mechanical stage of the microscope.
7. Focus on the center room of the chamber and start counting the cells from upper left corner of the room. It is advisable to complete all counts of the four squares and then move to the center square, which is the fifth square to be counted.

DATA ANALYSIS:

No. of cells * Dilution factor * Depth factor * 5
Dilution factor = 200
Depth factor = 10
Total ruled area = 25 because we count the RBC in five squares only we multiply by 5
Area count = 5
$RBC/mm^3 = N * 5 * 200 * 10$
$= N * 10000 = (N * 10^4)/ mm^3$

Normal range:

Adult Male; ---------- 450 – 650 $*10^4$ cells/mm^3 Or (4.5 – 6.5) millions/mm^3
Adult Female; ------- 380 – 580 $*10^4$ cells/mm^3 Or (3.8 – 5.8) millions/mm^3
Children 5-15 y; ---- 350 – 550 $*10^4$ cells/mm^3 Or (3.5 – 5.5) millions/mm^3
Newborn infants; --- 410 – 700 $*10^4$ cells/mm^3 Or (4.1 – 7.0) millions/mm^3

Increased in numbers of RBC called **polycythemia**.
Decreased in numbers of RBC is due to
• Anemia
• Bone marrow failure
• Hemorrhage
• Leukemia
• Nutritional deficiencies of (Iron, Copper, Folate, Vit B12, B6)

5-Total Count of WBC: White blood cells It also derived from the bone marrow , its play important role in immune system of the body and is a natural number up to 4- 11 × 103/ mm 3 in males and females, and can be an indicator of many disease states Excellency gives the number increased from the normal limit is called leukocytosis as is the case in some cancers break blood and some infections such as chronic appendicitis Appetites, while the number of low natural rate is called leukopenia and gets in some bacterial diseases like Typhoid, as well as viral diseases.

PRINCIPLE

Free-flowing capillary or well-mixed anti coagulated venous blood is added to a diluent (ammonium oxalate) at a specific volume in the unspotted reservoir. The diluent lyses the erythrocytes but preserves leukocytes and platelets. The diluted blood is added to the haemocytometer chamber. Cells are allowed to settle for 10 minutes before leukocytes and platelets are counted.

SPECIMEN

EDTA-anti coagulated blood or capillary blood is preferred.

Procedure:

By using Haemocytometer, following steps:-

1. Clean and dry sorbent white beads
2. Drag the amount of blood until the 0.5 mark and then drag the amount of this liquid diluted up to the mark of 11.
3. Use a dilute solution of the dye Gention violet in **1% acetic acid**, this solution is analyzed red blood cells and dying all at the same time the nuclei of the white blood cells and thus easily distinguish them.
4. Disconnect the rubber tube and then sucking party grabbed by finger forefinger and thumb and mix the solution well diluted blood sucking move between the fingers for about a minute.
5. Calculates the number of white blood cells in 4 large boxes (Squares) on the slide.
6. By the equation:

   WBC /mm^3 =N/4 *10* 20

   N= Total No of WBC in 4 boxes

   10 =Volume factor

   20= diluted factor

   WBC / mm^3 = N *50

Normal range:

Adults; ---------- 4000 – 11000 cells/mm^3

Children 10 y; ------- 5000 – 14000 cells/mm^3

Children 1 y; -------- 5000 – 17000 cells/mm^3

Newborn infants; --- 5000 – 21000 cells/mm^3

Note:

Increased in number of WBCs over normal called **Leukocytosis**.

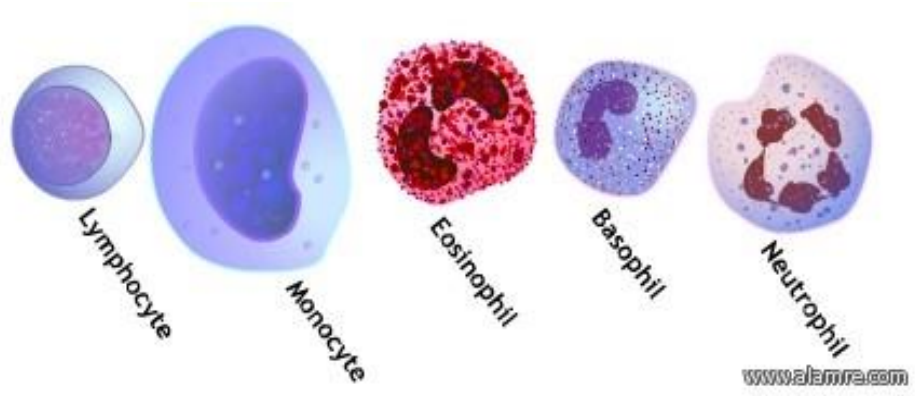
Decreased in Number of WBCs under normal called **Leukopenia**.

6– Differential count of WBC:

This test assesses the body's ability to respond to infection and disposal, also reveals the severity of allergic reactions and pharmaceutical, as well as responding to the parasite infection and other types of infections. It is necessary to assess the reaction to viral infection and respond to chemical processing. It can also calculate the various stages of leukemia.

Procedure is made by preparing blood smear stained with (**Leishman`s Stain,** composed of 2gm Leishman`s dye dissolved with 99.9% Methanol), surveyed in microscope at 40x power lens counting a minimum of 100 WBCs of all types.

Natural values	Characteristics and diseases
Neutrophils **54-62 %**	Large lobed nucleus, coarse purple granule. Increase in bacterial infection or inflammatory diseases.
Lymphocytes **28-33 %**	The smallest one, about the size of RBC, blue large nucleus fills most of the space cell, violet color. Increase in cases of viral infection and leukemia and cancer of the bone
Eosinophilia **1-6 %.**	Large nucleus with two lobes connecting chromatid thread, red senior granule. Increase in allergic and skin inflammation and infection parasites.
Basophiles **less than 1 %**	Large nucleus resembling the letter S, soft blue Granules. Increase in leukemia and chronic inflammation and severe allergic reaction to food or radiotherapy
Monocyte **2-10 %**	The largest type of nucleus, resembles a grain of beans, cytoplasm-free grained, gray or light blue. Increase in malignant diseases, including leukemia.



7- Bleeding time:

Is defined as the time taken for a standard skin wound to stop bleeding .upon vessel injury , platelets adhere and form a hemostatic platelet plug by effect of some chemical media as prostaglandin. Bleeding time measures the ability of these platelets to arrest bleeding and therefore measures platelet number and function.

It increases in the case of low platelets count or a defect in the synthesis of chemical mediators substances like prostaglandin.

Procedure:

1. Clean the lobe of the ear or a fingertip with alcohol and let dry.
2. For ear-glass slide is placed behind the ear lobe and held firmly in place this provided a firm site for incision.
3. Discard the glass slide if ear lobe has been incised.
4. Pierce the ear lobe (or tip of a finger) with the lancet .making the incision 3mm deep start the stopwatch.
5. Blot the blood with the filter paper at regular 30 second intervals. Move the filter paper so that each drop touches a clean area .do not touch the incision with the filter paper.
6. When the filter paper no longer shows singe of blood stop the stopwatch and record the time, normal values of 2 to 7 minutes.

Bleeding time = interval time * No. of blood drops

8- Coagulation time or (Clotting Time):

It is the time between wound appeared and the result of clot turning prothrombin to thrombin, which converts to Fibrinogen to fibrin which in turn are fibrous filaments limit between them red blood cells component network closes the aperture.

Procedure:

1. Clean your finger with spirit and allow the spirit to dry.
2. Pricked the finger by lancet .remove the first drop of blood.
3. Squeeze the finger to obtain a larger drop of blood and fill the capillary tube with blood.
4. The capillary tubes are sealed plasticize and immersed in water bath at 37centegrate.
5. After one minute start breaking small pieces of the capillary tube every 30 second until a fibrin thread is seen between the two broken ends.
6. By these methods the normal clotting time is 5 to 11 minutes at 37^{o} centigrade.

Coagulation time = (No. of pieces *30 sec.) + Additional time
For example; = (6*30) + 90 sec.
= 180 + 90 = 270/60
= 4.5 min =

The result must be written as: Clotting time = 4 minutes: 30 seconds or/ (4': 30")

(Normal range: 5 min – 11 min.)

** The most important factors that increase the clotting time is the case of shortages occurring in clotting factors, which include more than 13 factors, including

- Vitamin K,
- Calcium ions,
- Thrombin
- And Fibrinogen