



Clinical Analysis Course
Lecture: 5 - Fourth Stage – Biology Depart.

Dr. Yasir Adil Alabdali

Blood and components and diseases

Blood: fluid viscous complex structure found in entire body through blood vessels and heart, and consists of blood from a liquid plasma, which contains multiple blood cells these include cells: Red Blood cells also called Erythrocytes and white blood cells which called Leukocytes and blood Platelets which called Thrombocytes.

Plasma: Plasma is liquid part of blood plasma and proportion of total blood volume of up to 55%, equivalent to (3.5) liters, can be obtained if liquid plasma added to a sample of blood substance anticoagulant. As if to leave blood to coagulate and then removed from thrombus (clot), the remaining liquid is called serum and clear fluid yellow contains all components of plasma except protein fibrinogen and clotting factors (which will be mentioned later), but rich in serotonin, serotonin resulting from break blood platelets during clotting process.

• **Components of blood plasma**

Blood plasma consists of elements following:

Water is form 95% of plasma volume its importance from its ability to transport organic materials and inorganic as well as maintaining body temperature, and a volume of plasma remaining 15% solid material and material non-solid, which included 9% organic material and 1% inorganic materials.

1-Organic material in plasma:

A- Proteins materials form for 6-8g/100cm³ plasma and include:

1- Albumin: the proportion 3.8-501g/100cm³ of plasma and its importance comes organization osmotic of blood, organization pH of blood, transmission of many hormones and minerals.

2- Globulins: the proportion 3g /100 cm³ of plasma is divided to secondary species of (α 1, α 2, β 1, β 2) in addition to Kama globulines γ or immunoglobulines or antibodies that interact with pathogens or with the antigens (Antigens).

3- Fibrinogen: the proportion 200-400 mg/100cm³ of plasma importance responsible for blood clotting and thus help to stop bleeding.



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The term **Hypoproteinemia** on case that have a low concentration of plasma proteins for normal limit that get as a result lack production or to speed loss in urine which refers to pathogenic case, and a shortage protein plasma to lack of osmotic pressure to blood plasma not back fluid from tissues in to blood and accumulate thus tissue caused **odema**.

B- The material non protein is divided in to:

A-Nutrients are:

1-Glucose rate 80-120 mg/100cm³

2-Lipids proportion 600-800 mg/100 cm³.

Importance of food material is used to produce energy (ATP) necessary for growth and proliferation of cells.

B- Materials excretion such as:

1- Urine rate 11-43 mg / 100 cm³

2- Creatinine rate 0.8 - 1.2 mg / 100 cm³

3- Uric acid and of 0.3 - 0.7 mg / 100 cm³

2- Non organic materials: It includes:

1- Potassium rate 3.5 to 5.5 m Equivalent m Eq / L.

2- Sodium rate 135-153 m Eq / liter.

3- Calcium rate 8.8 to 10.2 mg /100 cm³ of plasma.

4- Manganese rate 1.6 to 2.5 mg/100cm³ of plasma.

5- Iron rate 100-150 mg /100 cm³ of plasma.

6- Chlorine rate 38-110 m Eq / liter.

7- Bicarbonates

8-Phosphate

The importance of inorganic materials in maintaining osmotic of blood and keep on pH blood.

A complete blood count (CBC) is normally include:

1-White blood cells: White blood cells (or leukocytes) are divided into two main groups: granulocytes and agranulocytes. The granulocytes receive their name from the distinctive granules that are present in the cytoplasm of neutrophils, basophils, and eosinophils. The total count of circulating white blood cells is differentiated according to the five types



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of leukocytes, each of which performs a specific function. The differential count is expressed as a percentage of the total number of leukocytes (WBC).

2-Red blood cells: The main function of the red blood cell (RBC or erythrocyte) is to carry oxygen from the lungs to the body tissues and to transfer carbon dioxide from the tissues to the lungs. This process is achieved by means of the Hb in the RBCs, which combines easily with oxygen and carbon dioxide and gives arterial blood a bright red appearance. The RBC test, an important measurement in the evaluation of anemia or polycythemia, determines the total number of erythrocytes in a microliter (cubic millimeter) of blood.

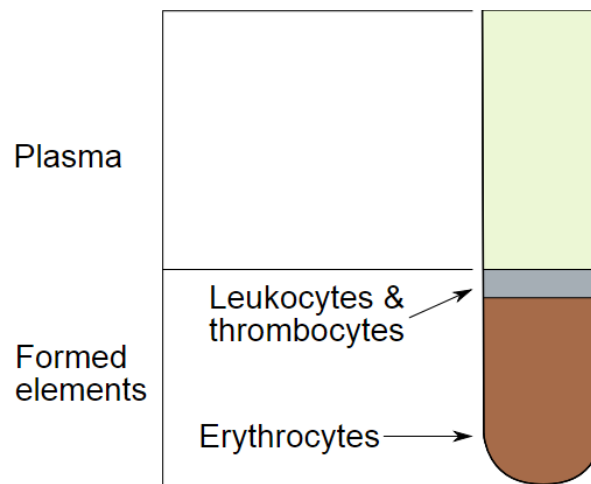
3-Hemoglobin: Hb, the main component of erythrocytes, serves as the vehicle for the transportation of oxygen and carbon dioxide. The oxygen-combining capacity of the blood is directly proportional to the Hb concentration rather than to the RBC because some RBCs contain more Hb than others. This is why Hb determinations are important in the evaluation of anemia. The Hb determination is part of a CBC. It is used to screen for disease associated with anemia, to determine the severity of anemia, to monitor the response to treatment for anemia, and to evaluate polycythemia.

4-Hematocrit: The Hct test is part of the CBC. This test indirectly measures the RBC mass. The results are expressed as the percentage by volume of packed RBCs in whole blood (PCV). It is an important measurement in the determination of anemia or polycythemia. The hematocrit also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF), is the volume percentage (%) of red blood cells in blood. It is normally about 45% for men and 40% for women.

5-Red Blood Cell Indices: The red cell indices define the size and Hb content of the RBC and consist of the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), and the mean corpuscular hemoglobin (MCH). The RBC indices are used in differentiating anemia. When they are used together with an examination of the erythrocytes on the stained smear, a clear picture of RBC morphology may be confirmed. On the basis of the RBC indices, the erythrocytes can be characterized as normal in every respect or as

abnormal in volume or Hb content. In deficient states, the anemia can be classified by cell size as macrocytic, normocytic, or microcytic, or by cell size and color as microcytic hypochromic.

6-Stained Red Cell Examination (blood film; Stained Erythrocyte Examination): The stained film examination determines variations and abnormalities in erythrocyte size, shape, structure, Hb content, and staining properties. It is useful in diagnosing blood disorders such as anemia, thalassemia, and other hemoglobinopathies. This examination also serves as a guide to therapy and as an indicator of harmful effects of chemotherapy and radiation therapy. The leukocytes are also examined at this time.



7-Platelet Count; Mean Platelet Volume (MPV): Blood normally contains 150,000 to 400,000 per microliter (μl). If this value should drop much below 20,000/ μl , there is a danger of uncontrolled bleeding. This is because of the essential role of platelets

Platelets (thrombocytes) are the smallest of the formed elements in the blood. Platelet activity is necessary for:

in maintaining the integrity of the adherens junctions that provide a tight seal between the endothelial cells that line the blood vessels; in forming a clot where blood vessels have been broken.

The platelet count is of value for assessing bleeding plugs that occlude disorders that occur with thrombocytopenia, uremia, liver disease, or malignancies and for monitoring the course of disease associated with bone marrow failure. It is also part of a coagulation profile or workup. The mean platelet volume (MPV) is sometimes ordered in conjunction



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with a platelet count. The MPV indicates the uniformity of size of the platelet population. It is used for the differential diagnosis of thrombocytopenia.

Reticulocyte Count: A Reticulocyte young, immature, nonnucleated RBC contains reticular material (RNA) that stains gray-blue. Reticulum is present in newly released blood cells for 1 to 2 days before the cell reaches its full mature state. Normally, a small number of these cells are found in circulating blood. The reticulocyte count is used to differentiate anemia caused by bone marrow failure from those caused by hemorrhage or hemolysis (destruction of RBCs), to check the effectiveness of treatment in **pernicious anemia** and **folate** and **iron deficiency**, to assess the recovery of bone marrow function in **aplastic anemia**, and to determine the effects of radioactive substances on exposed workers.

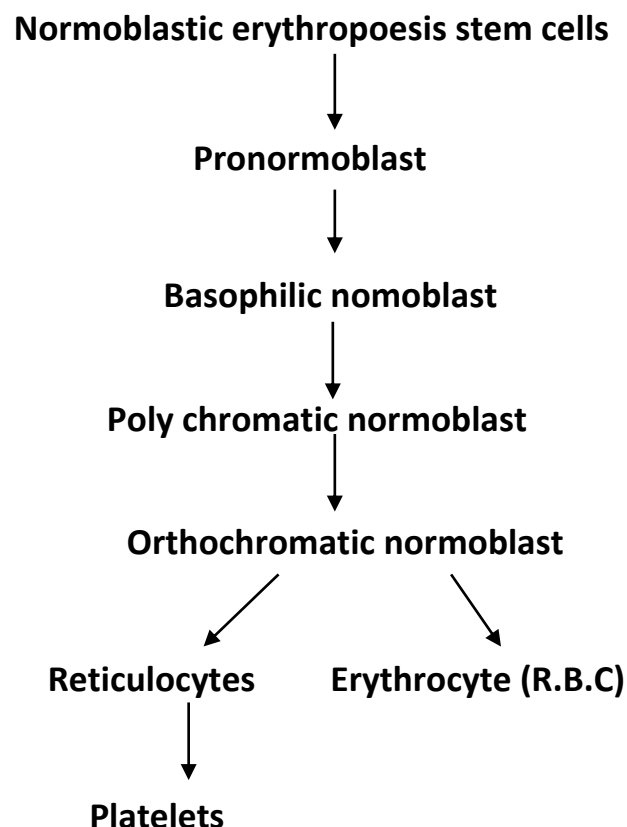


Figure formation erythropoiesis



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- **Blood chemical tests**

Specializes chemicals testing for blood assess level of certain chemicals in blood plasma and also called Basic metabolic panel (BMP) allows these tests to doctor identify the work of some tissues and organs of body different muscles (including the heart muscle), bone, liver, kidney, and require most of these withdrawn blood venous blood with exception of tests for assessing level of oxygen and carbon dioxide and pH blood, it requires arterial blood, and tests commonly used in laboratories include:

- **Glucose**

Represents glucose that sugar used by cells of body to release energy required for effectiveness and both pancreas and liver, organization level in the blood to remain within its normal limits and that amount (70-110 mg / dl), the increased concentration on normal limit the pancreatic secretion hormone **insulin**, insulin which encourages cells on capture from blood stream also encourages liver cells to store sugar more than enough in form **glycogen**, if either lack of concentration glucose on minimum natural the pancreatic secretion hormone **glucagon**, Which encourages liver to break down glycogen to glucose and pumped to circulatory system of blood to keep level available to some of sensitive cells as cells RBCs and brain cells, but that these mechanisms fail to maintain level of glucose in blood will show one of these cases:

- 1- Hypoglycemia: in this case the blood glucose least level at about 54 mg / dl due to several reasons including:

That person has already diabetic and take insulin injection but he did not eat food, leading to a rapid fall and a sudden level of sugar blood, whatever the reason for decline in blood sugar, it is a serious condition requiring transfer patient to the emergency directly regulation level of blood sugar immediately before it gets damaged **brain cells** and thus a coma, which ends with death.

- 2- Hyperglycemia: is known *diabetes mellitus* D.M, which is caused by either a decrease or absence of insulin is called Diabetes type I (D.M-type-I) or non-effectiveness of insulin hormone receptors found on liver



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cells and muscle (insulin resistance), which called Diabetes Type II (D.M type II) and in both cases the will increase glucose level in blood more 126 mg/dl for purpose of determining level of blood sugar. **There are two test basic:**

1- Fasting test

This test work after a break person from eating for 8-12 hours If glucose level of less than 54 mg/dl **Hypolycemia** case and if its level of more than mg /dl 126 case **Hyperglycemia**.

2- Glucose tolerance test (GTT)

The test work to confirm injury by diabetes (DM), which stops a person from eating at night, in early morning is measuring the level of glucose to him and prove its value then gives solution sugary consists of 75g glucose dissolved in 300 mL water to drink and then wait two hours then makes sugar measerment again **if the result of test more than 200 mg/dl this person is infect with diabetic**. While, if it is more than 140 and less than 200 mg/dl person has a **weakness in mechanism for taking of sugar from blood and has a susceptibility to infect with diabetes later**. It is worth mentioning that these two tests (1 and 2) to be through them to determine **type of diabetes**, but it needs to be examined other, more specialized.

● Blood diseases

1-Anemia

Anemia is disease due to the reduction in the hemoglobin concentration of blood, resulting poor oxygen carrying capacity.

● Symptoms of anemia

Weakness, pallor of skin and mucous membrane, dyspnea, headache, fatigue.

● Morphology of classification of anemia

- 1- Iron deficiency anemia (hypochromic, microcytic anemia).
- 2- Megaloblastic anemia (macrocytic anemia).
- 3- Pernicious anemia (normochromic, macrocytic anemia).
- 4- Aplastic anemia (normochromic).



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- **Iron deficiency anemia:**

Body iron content divided:

- 1- 70% of iron in the hemoglobin
- 2- 25% as a storage ferritin enzyme & hemosidren
- 3- 5% myoglobin

- **Absorption of Iron**

Iron absorbed in the duodenum and jejunum. Iron is present in the food, both in organic form (hem) and inorganic form (Fe^{++} and Fe^{+++}).

Fe^{+++} iron and hem iron are easily adsorbed.

- **Iron transport:**

Iron enters plasma in ferric form, bound to transferrin (B. globulin). The molecule of transferrin can combined two atoms of iron. Transferring transport iron to erythroblast and in the bone marrow for synthesis of hemoglobin. In each day approximately 6g of Hb synthesised which require 20mg of iron.

- **Cause of iron deficiency anemia**

- 1- Chronic blood loss hemorrhage – excessive menstruation.
- 2- Deficient iron in the food (children taking only milk and adult with poor diet).
- 3- Deficient absorption of iron (surgery in G.I.T) chronic malabsorption.
- 4- Deficient transport of iron, due to decrease in transferrin (Rheumatoid arthritis).
- 5- Increased physiological requirement (children during active growth & in pregnancy).

- Source of iron: meat, liver, eggs, green vegetable.

- **Laboratory finding in iron deficiency anemia**

- 1- Hb decreased (6-10g/dl).
 - 2- P.c.v. decreased (20-30%).
 - 3- R.B.Cs decreased (2-3.5 million).
 - 4- M.C.V, M.C.H, M.C.H.C (increase).
- **M.C.V:** mean cell volume.
 - **M.C.H:** mean cell Hb.
 - **M.C.H.C:** mean cell Hb concentration.
 - **Poikilocytosis:** that means change in shape R.B.Cs.

- **Polychromasia:** that means change in the size R.B.Cs.
- **Hypochromic:** that means decreased in the dye R.B.Cs.
- **Morphology of red cells**

Hypochromic, microcytic with anisocytosis and poikilocytosis.
Leukocyte-normal. Platelets normal or slightly increased.

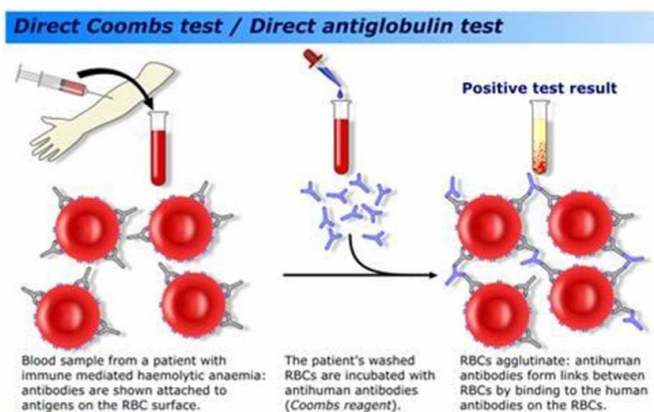
Serum iron decreased

Total iron binding capacity (T.I.B.C) increased. N.R: 250-400mg/dl.

Coombs test: A Coombs test is either of two clinical blood tests used in immunohematology. The two Coombs tests are the direct Coombs test, and the indirect Coombs test. The Direct Coombs test is used to test for **autoimmune hemolytic anemia**; i.e., a condition of a low count of red blood cells (aka RBCs) caused by immune system lysis or breaking of RBC membranes causing RBC destruction. In certain diseases or conditions an individual's blood may contain IgG antibodies that can specifically bind to antigens on the RBC surface membrane, and their circulating RBCs can become coated with IgG alloantibodies and/or IgG autoantibodies. Complement proteins may subsequently bind to the bound antibodies and cause RBC destruction.

The direct Coombs test is used to detect these antibodies or complement proteins that are bound to the surface of red blood cells; a blood sample is taken and the RBCs are washed (removing the patient's own plasma) and then incubated with antihuman globulin (also known as "Coombs reagent"). If this produces agglutination of RBCs, the direct Coombs test is positive, a visual indication that antibodies (and/or complement proteins) are bound to the surface of red blood cells.

Direct Antiglobulin Test





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2- Megaloblastic anemia:

Is due to the deficiency of vitamin B12 and folic acid both are required for DNA synthesis.

Lack of these vitamins leads to delayed maturation of nucleus. Cytoplasm is normal. Due to the delayed maturation of nucleus, macrocytes are released in to the circulation instead of normocytes.

• Vitamin B12 absorption

Vit. B12 is cobalt containing terapyrol reddish colour. It is synthesised in nature by microorganism and found in food of animal orgin (liver, fish & meat).

Absorption: B12 (extrinsic factor) must combine with internsic factor (glycoprotein secreted by gastric cells) & then complex is absorded by illem.

Transport of vit.B12: vit.B12 from the illem is taken to portal blood to plasma protein (transcobalamin I, transcobalamin II bind the vit.B12) transcobalamin I transfer vit. B12 to the bone marrow and other tissues.

• Folic acid (pteroglutonic acid)

Is a yellow, water soluble, stable found in green vegetable, liver, kidney and yeastes. Absorded in from duodenum and jejunum. It acts as a coenzyme in the synthesis of DNA and RNA.

• Cause of megaloblastic anemia

- 1-Deficiency of vit.12 and folic acid.
- 2-Abnormal vit. B12 and folic acid metabolism.
- 3-Defective absorbtion of vit. B12 and folic acid due to G.I.T. disease.
- 4-Fish tape worm infestation (*Diphyllobothrium lutum*).
- 5-Congenital defet in DNA synthesis.
- 6-Lake of intrinsic factor. Secreted by stomach.

3- Pernicious anemia

It is an autoimmune disease due to gastric atrophy the wall stomach is thin the secretion of intrinsic factor is absent.

• Laboratory findings in pernicious anemia

- 1- Hb low (3-4g/dl).
- 2- P.C.V. low (10-15g/dl).



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- 3- R.B.C. low (1 million-2 million).
- 4- M.C.V. & M.C.H (increase).
- 5- M.C.H.C. (normal).
- 6- Leucocytes & platelets also reduced.

• Morphology of R.B.C. in blood smear reduced

R.B.C. is macrocytic, normochromic, macro ovalocyte, anisocytic, poikilocytosis, cobots ring, howelly jolly bodies and hyper segmented neutrophils seen (neutrophils having more than 5 lobes).

4- Aplastic anemia

It is due to failure of bone marrow to produce cell.

• Cause of plastic anemia

- 1- Due to chemicals (benzene, carbone tetra chloride) and drugs (nitrogen mustard, chloramphenicol).
- 2- Ionizing radiation (gamma rays, neutrons).
- 3- Genetic factors
- 4- Idiopathic (without known causes).
- 5- Associated with viral hepatitis.

• Laboratory findings

Blood smear shows pancytopenia (pancytopenia is a disease due to the reduction of all three formed elements, erythrocyte, leukocyte and thrombocyte).

• Morphology of R.B.C.

Normochromic, normocytic with slight anisocytosis and poikilocytosis, polychromasia.

• Blood count

- 1-Leucocyte low (2000-3000/ml).
- 2-R.B.C. low (50.000-3.5 million).
- 3-Platelets low (25000-7500/ml).
- 4-Differential count shows 90% lymphocytes.

• Leucocyte maturation function and abnormalities

Mainly two types of leucocytes. Seen in normal blood:

- 1- Granulocytes
- 2- AGranulocytes



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Granulocytes are those leucocytes having specific granules. Granulocytes are also known as polymorphnuclear leucocyte. They are:

- A- Polymorphnuclear neutrophil.
- B- Polymorphnuclear esonophil.
- C- Polymorphnuclear basophil.

• Function of granulocytes:

A- Polymorphnuclear neutrophil, actively motile, eliminates microorganism and other foreign particles. There are three phase in this process.

- 1- Chemotaxis (cell mobilization & migrate to the affected).
- 2- Phagocytosis (foreign particles, bacteria, fungi).
- 3- Killing and digestion (occur in two pathways).

A- Oxidative pathway. **B-** Non Oxidative pathway.

Neutrophil leucocytosis

The team for an increase in the total number of neutrophil above 7.5×10^3 /ml of blood seen in pyogenic bacteria infection, inflammation, uremia, gout, and acidosis.

Neutropenia: is the term for the decrease in the total number of neutrophil below 2.5×10^3 /ml of blood seen in viral infection (hepatitis, influenza) bacteria infection (tuberculosis, typhoid fever) and after treatment with antibacterial and anti-inflammatory drugs.

B- Bacteremia

Is presence of bacteria in the blood. The blood is normally a sterile environment, so the detection of bacteria in the blood (most commonly with blood cultures) is always abnormal.

Bacteria can enter the bloodstream as a severe complication of infections (like pneumonia or meningitis), during surgery (especially when involving mucous membranes such as the gastrointestinal tract), or due to catheters and other foreign bodies entering the arteries or veins (including intravenous drug abuse). Either in case of bacteria with secreted toxins are called case of Septicemia (so-called blood poisoning or toxemia), either presence of fungi in the blood are called Fungaemia. The most types of bacteria that exist in the blood which because infections are:



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- 1- G+Ve cocci for example: *Staph* , *Strep*
- 2- G- Ve cocci for example: *Neisseria meningitidis*
- 3- G-Ve bacilli for example: *E.coli*, *Proteus*, *Ps. aeuginosa*, *Brucella*
- 4- Fungi for example: *Candida alicans* , *Cryptococcus neoformans*.

● Blood collection

- 1- Collections samples before taking any treatment to reduce difficulty of isolating pathogenic bacteria.
- 2- Collected 1-3 samples in one day from same patient and preferred withdraw one specimen went high temperature of patient.
- 3- Sterilized place withdraw with iodine or alcohol 70% to reduce contamination by microorganisms pathogens.
- 4- Withdraw blood with amount of 10 ml of adults, from 2 to 5 ml of the children and 1-2 ml of infants.
- 5- If not available blood culture bottles mixed blood with heparin to prevent clotting.

● Blood culturing

Through this test investigation for infections different spread through bloodstream, including bacteremia and septicemia, is supposed to be blood stream a sterile environment has adopted this test as a means of investigation for disease in humans since beginning of the last century, when showing patient's symptoms or signs of systemic infections, the results of blood cultures determine possible presence of inflammation and type of microorganisms pathogens that causes it, and this test diagnoses organisms causing for following cases:

- 1- Sever pneumonia: this causes bacteria inflammation *Streptococcus pneumonia* or *Mycoplasma*, or *Actinomyces* or *Nocardia* or *Mycobacterium tuberculosis*).
- 2- Puerperal fever: or childbed fever is a bacterial infection contracted by women during childbirth or abortion. It can develop in to puerperal sepsis, which is a serious form of septicemia. If untreated, it is often fatal.
- 3- Inflammatory diseases variety.
- 4- Fever of unknown origin (FUO).



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However, lack of growth in the culture of blood discharge found infection because bacteria may be unable to grow on a culture in blood but present in the sample of blood.

• The process of blood culture in two ways:

1-The test of culture blood by withdraw at least 10 cc of venous blood and injected in to two or multiples tubes of blood bottles aerobic and non-aerobic containing among culture media especially bacteria aerobic and non-aerobic, and are cultured by repeat to increase probability detection of pathogen found in the blood and causes disease, the blood is collected using sterile techniques, as sterilizes both surface of bottles used to cultured blood and surface skin of person that meaning place of withdrawal blood by using alcohol.

Incubating tubes after cultured blood sample in incubator at 37C indicates appearance is positive for bacteremia, then examines staining with Gram stain to take idea of diagnostic principle type bacteria and then work subculture on culture media solid for purpose of isolating pathogen bacteria followed bt examined sensitivity test to determine antibiotic appropriate for treatment.

2- Mixed blood with culture media by a size equal to 10 times the volume of blood (5 ml of blood +50 ml culture media), and used often media **Tryptic soy broth** or **Brain heart broth** which is benefit to increase to ensure isolation of pathogens and minimize impact of antibiotics found in the patient's blood which is reduce the bacteria growth, as well as have blood (serum) of antibody or antiviral agents for growth of pathogenic microorganisms.

A- Examine bottles at 37 C for 7 days and observe growth or lacking the growth any pathogen by RBC sediment layer in bottom and see bottle topped with a transparent layer. If microbial growth is observed through turbid media with presence degradation red blood cell and with production of gas and formation layer of thick dark in the bottom bottle.

B- Starts from day diagnosis showing turbidity, by culturing sample of bottle on media MacConkey agar and blood agar and then complete diagnostic tests such as biochemical tests and serological test and after sensitivity test to examine determine antibiotic correct for treatment.