

Blood Tests

- **Collection of whole blood:**

Whole blood usually collected by venipuncture mainly from the vein in the arm using a needle or syringes, if only drops of blood are needed, then blood sample can be collected from finger. Then blood should be transformed into tubes with anticoagulant (EDTA, heparin) and mixed gently.

Tests that use whole Blood:

1. Total WBCs Count:

This test can be done manually using whole blood and special slide called Hemocytometer. The whole blood should be diluted by a special diluting fluid made from mixing D.W. with a dye usually crystal violet and glacial acetic acid, the dye is for WBCs nucleolus staining and glacial acetic acid is for destroying RBCs. Counting chambers hold a specified volume of diluted blood, by multiplying the dilution factor by the total number of white blood cells then divided on volume, the total number of white blood cells can be obtained. Normal range for adult: 5,000-10,000.

Total WBCs (cell/ml)= cell count (mean of 4 squares)x dilution factor

0.1

= cell count (mean of 4 squares) x dilution factor x10

= No.x 20 x10

2. Differential WBCs count:

Also called complete blood picture or blood film. This test can be done using whole blood, a small drop of blood is placed on a clean slide and by another slide called spreader, spreading that drop making fingerprint like

shape, blood should be left to dry at room temperature for the next step of staining. Leishman's or Gemsa stains are commonly used for staining, then after slides dry they must be examined by 40 X then oil lenses. Counting 100 WBCs and determining each type percentage, platelets are also counted and if there is any abnormalities must be mentioned.

3. Blood Type Test

This test mainly performed for patients receiving blood transfusion, pregnant women, tests before marriage and pregnant women. This test includes ABO system and Rh test. These tests depend on antigens (proteins) on the surface of red cells, A, B, Rh.

4. Erythrocyte Sedimentation Rate(ESR)

The erythrocyte sedimentation rate (ESR) is the rate of the red blood cells sediment in a period of one hour. It is a common hematology test, and is a non-specific measure of inflammation. To perform the test, anticoagulated blood is placed in an upright tube, known as a Westergren tube, and the rate at which the red blood cells fall was measured and reported in mm/h.

The ESR depends on the balance between sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation,). When an inflammatory process starts, the high levels of fibrinogen in the blood causes red blood cells to stick to each other. The red cells form stacks called 'rouleaux,' which settle faster. The ESR is increased in pregnancy, inflammation, anemia or rheumatoid arthritis.

5. Hemoglobin % (Hb %)

This test represents the amount of hemoglobin in the blood, expressed in grams per deciliter (dl). The low level of Hemoglobin is a sign of anemia. Normal range: Male: 13.5 - 17.5 g/dl and Female: 12.0 - 16.0 g/dl.

6. Hematocrit value or Packed cell volume (PCV) or erythrocyte volume fraction (EVF):

The hematocrit (Ht or HCT), 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.

It is considered as a part of a person's complete blood count results, with hemoglobin concentration, white blood cell count, and platelet count. Usually gives an indicator for hemoglobin concentration and mainly ordered for anemia cases.

This test can be done using fresh heparinized blood loaded in capillary tube (also known as a microhematocrit tube or pcv tube) and special instrument called microcentrifuge at 10,000 rpm for five minutes. This instrument will separate the blood into layers RBCs layer and plasma layer. The volume of packed red blood cells divided by the total volume of the blood sample gives the PCV. Because a tube is used, this can be calculated by measuring the lengths of the layers.

7. Erythrocytes count and Erythrocytes indices (MCV, MCH and MCHC):

These indices with erythrocytes number are used as clues for the classification of anemia types as rotten laboratory tests

MCV or Mean cell volume, the average volume of the red cells, detects conditions that can affect MCV include thalassemia, reticulocytosis, alcoholism, chemotherapy, vitamin B12, and/or Folic acid deficiency.

MCH or Mean cell hemoglobin, the average amount of hemoglobin per red blood cell, in picograms.

MCHC or Mean cell hemoglobin concentration, the average concentration of hemoglobin in the cells.

8. Prothrombin Time or clotting time:

Prothrombin time (PT) is a blood test that measures how long it takes blood to clot. A prothrombin time test can be used to check for bleeding problems liver function because the liver is responsible for the production of coagulation factors (prothrombin). This test is usually ordered for patients before operations as a checkup step. Normal range for adult: 9.9-13 seconds. This test can be done using capillary tube(or pcv tube) loaded with fresh blood and breaking one centimeter every couple of seconds while recording time the time when the clott appears it is the PT.

9. Blood culture

Blood culture usually ordered to detect infections that are spreading through the bloodstream (such as bacteremia, septicemia). No microorganisms should be found in blood because bloodstream is usually a sterile environment.

When a patient shows signs or symptoms of a systemic infection, results from a blood culture can confirm that an infection is present, and they can identify the type (or types) of microorganism that is responsible for the infection to give inappropriate treatment. For example, blood tests can identify the causative organisms in severe pneumonia, puerperal fever, pelvic inflammatory disease, sepsis, and fever of unknown origin. Also, negative growths do not mean that there is no infection.

Method of blood culture involves 5-10 ml of blood is taken through venipuncture and injected into two or more "blood bottles" with specific media for aerobic and anaerobic organisms. A common media used for anaerobes is thioglycollate broth. The blood is collected using aseptic technique. This requires that both the tops of the culture bottles and the venipuncture site of the patient are cleaned prior to collection with cotton and 70% isopropyl alcohol. Ordering multiple sets of cultures increases the probability of discovering a pathogenic organism in the blood and reduces the probability of having a positive culture due to the flora of skin contaminants.

After inoculating the culture vials, they are entered into incubator which incubates the specimens at body temperature (37° C). The blood culture instrument reports positive blood cultures (cultures with bacteria present, thus indicating the patient has "bacteremia"). Most cultures are kept for 5 days for negative vials removing.

If a vial is positive, a microbiologist will perform a Gram Stain on the blood for a rapid, general identification of the bacteria. The blood is also subcultured or "subbed" onto agar plates to isolate the pathogenic organism for culture and susceptibility testing, which takes up to 3 days. This culture & sensitivity (C&S) process identifies the species of bacteria. Antibiotic

sensitivities are then assessed on the bacterial isolate to inform physician about the appropriate antibiotics for treatment.

Cases that request blood culture:

1. Septicemia or bacterimia cases: when infection with any bacteria succeeded to inter blood stream and circulating system causing spreading of pathogen to all body organs and tissues. Examples: *Pseudomonas aeroginosa* or *Escherichia coli*.
2. Meningitis: suspecting for example with bacteria e.g. *Hemophilus influenza*, *Neisseria meningitides*, *Streptococcus pneumoniae*, *Listeria monocytogens* and fungi e.g. *Cryptococcus neoformans*.
3. Endocaditis: suspecting for example with *Staphylococcus aureus* and *Streptococcus pyogens*.
4. Recurrent cases of typhoid fever (*Salmonella typhi* and *Salmonella paratyphi*) and Malta fever (*Brucella melatanses*).
5. Unknown causative pathogen for fever cases: for example post delivery infections when pregnant women getting birth and suffer from fever due to spreading of pathogens to maternal blood during delivery process e.g. *Streptococcus pyogens* and *Mycoplasma hominis*