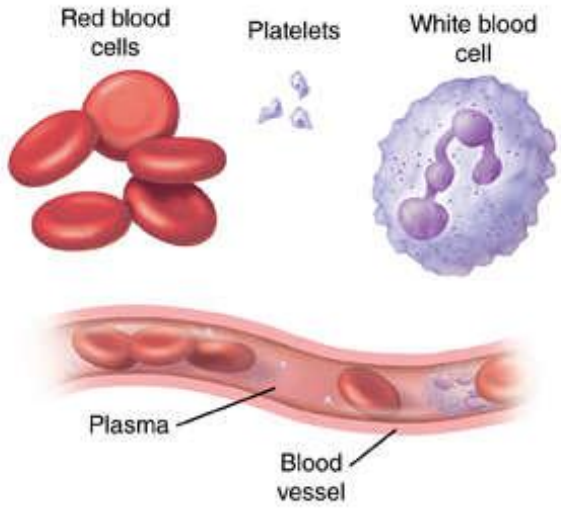
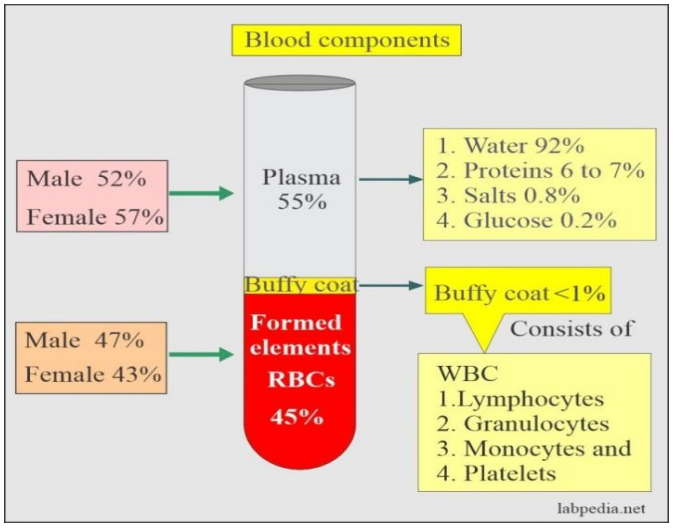
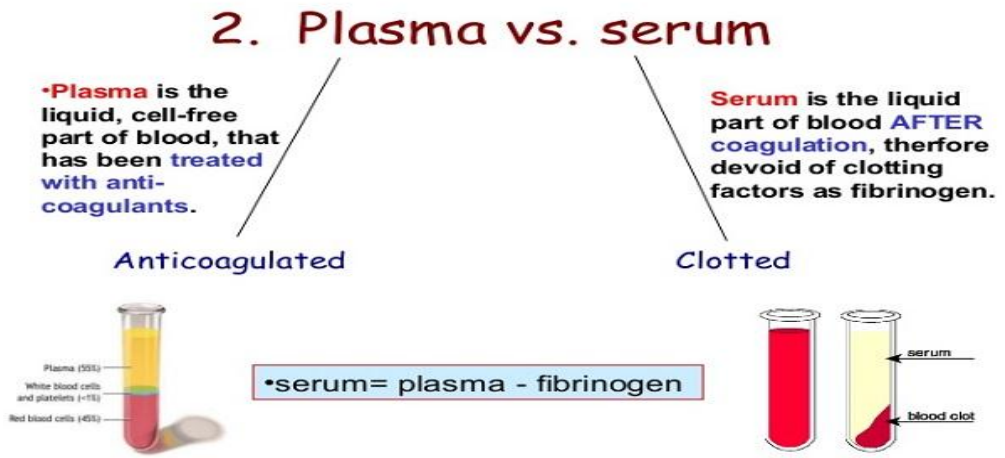
Hematology (Greek Haema = Blood; logy = Study of). Hematology is the branch of medical science that deals with the study of blood.

Blood:- The fluid that circulates through the heart, arteries, capillaries, and veins and is the chief means of transport within the body. It transports oxygen from the lungs to the body tissues, and carbon dioxide from the tissues to the lungs. It transports nutritive substances and metabolites to the tissues and removes waste products to the Kidneys and other organs of excretion.





Whole blood: A venous, arterial or capillary blood sample in which the concentrations and properties of cellular and extra-cellular constituents remain unaltered when compared with them invivo state.
Plasma is the part of the blood that contains Serum+ clotting factors.
Serum liquid portion of blood that remains once the clotting factors like fibrin have been removed.



Preparation of serum & plasma

Blood is centrifuged to remove cellular components. Anti-coagulated blood yields plasma containing fibrinogen and clotting factors. Coagulated blood (clotted blood) yields serum without fibrinogen, although some clotting factors remain.

Physical Characteristics of Blood

Thicker than water, 8 % of total body weight, temperature - 100.40F, pH - 7.35 to7.45.

Collection and handling of blood

1- **venous blood:**

It is now common practice for specimen collection. We need: disposable Plastic Syringes and Disposable Needles, Tourniquet, Cotton & Alcohol.

2**-capillary blood.**

- Skin puncture can be used for obtaining a small amount of blood.
- Drawing blood from the finger.

-Disinfect the pad of the middle finger with ethanol. Stop circulation in the middle of the finger with the thumb until the end of the middle finger has deep red color.

-Press the fingerpicker or lancet on the skin of the middle finger.

-Wipe off the first drop of blood as it contains a large amount of other tissue fragments, and start drawing blood. If bleeding subsides, massage the finger.

precautions of blood collection

1. Special care must be taken to avoid risk of infection from various pathogens during all aspects of laboratory practice.
2. The operator should wear disposable plastic or thin rubber gloves
3. Care must be taken to prevent injuries, especially when handling syringes, needles and lancets
4. Disposable sterilized syringes, needles and lancets should be used.
5. Cannot use hemolyzed samples in lab tests (Hemolysis: the breakdown of red blood cells, with the release of hemoglobin into the plasma or serum).

Anticoagulant: Additives that inhibit blood and/or plasma from clotting. The main types:

1. **EDTA**: Ethylene diamine tetra-acetic Acid. The sodium and potassium salts of EDTA are powerful anticoagulants and they are especially suitable for routine hematological work. EDTA acts by its chelating effect on the calcium molecules in blood.

2. **Citrate: Trisodium citrate**: principle: Ca+2 chelating agent forming calcium salts.

A mixture of one-part citrate with nine parts blood to determine the erythrocyte sedimentation rate. It uses in blood transfusion.

3. **Heparin:** for the determination of ionized calcium (inhibit Xa factor).

4. **Sodium Fluoride -Potassium Oxalate**: principle Glycolysis inhibitor AntiCoagulant. used for glucose test.

5. **ACD (Acid-Citrate Dextrose: DNA** Studies.

Types of Plastic/glass vacuum blood test tube:

- SSGT tube (Serum-separating gel tubes), with additive of Gel & clot activator, yellow cap.
- Serum Tube, with additive of Clot activator, red cap.
- Plain Tube, No additives, red cap
- EDTA tube, with additive of EDTA K2/K3, purple cap.
- Heparin Tube, with additive of Sodium Heparin/Lithium Heparin, green cap.
- PT Tube, with additive of Sodium citrate (1: 9), blue cap.
- ESR tube, with additive of Sodium citrate (1: 4), black cap.
- Glucose tube, with additive of Fluoride Oxalate, grey cap.

Complete Blood Count (CBC) What is measure

1-Red blood cell data {Total red blood cell count (RBC), Hemoglobin (Hgb), Hematocrit (HCt), Mean corpuscular volume (MCV), Red blood cell distribution width (RDW)}

2-White blood cell data {Total white blood cell (leukocyte) count (WBC), A white blood cell count differential may also be ordered}.

3-Platelet Count (PLT)

BLOOD FILM

Spreading a drop of blood evenly across a clean grease-free slide using a smooth edged makes a smear or film of blood.

PREPARATION OF BLOOD FILM

1- Select a clean grease-free slide, never put fingers on the surface of the slide.
2- Gently touch a fresh drop of blood onto one end of the slide.
3- Using a beveled piece of glass a little narrower than the slide, allow the drop to spread along it (Fig.1).
4- Holding the slide and the "spreader" at a suitable angle, push the spreader along the slide, drawing the blood behind it, until the whole of the drop has been smeared and allow it to dry.

STAINING OF BLOOD FILM

Before staining, the blood films need to be fixed with acetone free methyl alcohol for ¼ to 1 minute in order to prevent hemolysis when they come in contact with water while staining them.

White blood cells have structures that are acidophilic and basophilic structures, so they vary in their reaction pH. The nuclei are basophilic and stain blue. The highly basophilic granules also stain blue. Haemoglobin stains acidophilic or red.

STAIN PREPARATION & STAINING

1- Leishman's stain:
Powdered Leishman's stain 0.15 g
Aceton free methyl alcohol 137 ml
Mix and warm for 15 min. with shaking

2- Wright's stain:
Wright's stain powder 0.2 g
Aceton free methyl alcohol 100 ml
Let stand this solution for a few days.

Procedure:

1- Cover the blood film with 8-10 drops of Leishman's stain for 1-2 min.
2- Dilute the stain with an equal volume of distilled water (DW) and mix by gentle rocking then leave it for 10 min.
3- Wash with (DW).
4- Drain and dry in the air at room temperature.
5- Clean the back of the slide and examine it microscopically.

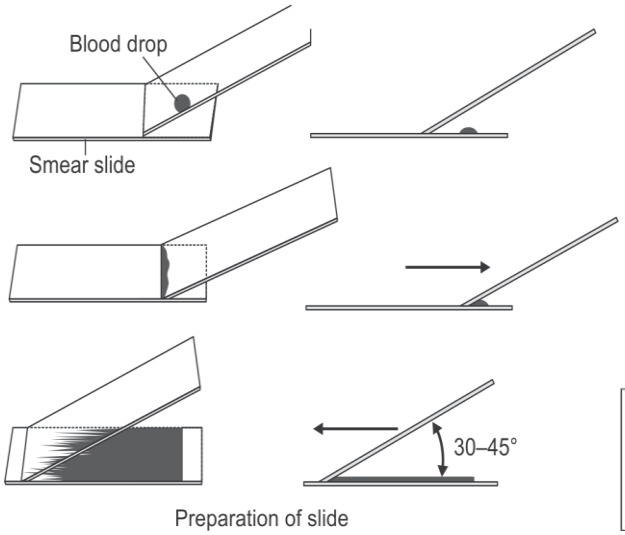


Fig. (1): Making blood smear

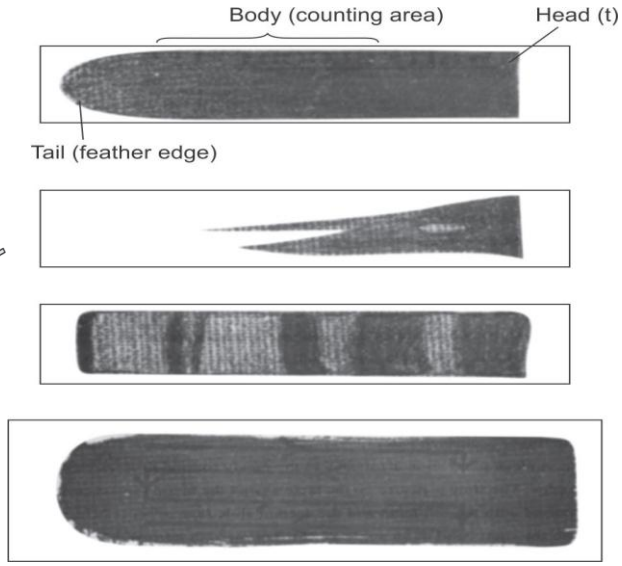


Diagram showing (A) Good and (B to D) Bad smears

Notes on technique

The slide should be very clean and free from any greasy materials to get a good spreading. The film should be such that there is some overlap of the red blood cells, diminishing the separation near the tail of film, but it should not be so thick that the leukocytes in the body of the film are badly distributed, if films are made too thinly, or if a rough edged spreader is used. Many of the leukocytes perhaps 50% of them accumulate at the edges in tail.

A well spread blood film should have the following characteristics (Fig.2):

1. Lateral edges.
2. An adequate zone of morphology.
3. Straight feature-edge.
4. Adequate length.

The zone of morphology is the area of the film where the RBCs barely touch each other (Fig.3). This is appropriate area for carrying out blood film examination.

Preparing a good quality smear depends on three main factors:

1. The size of the drop of blood.
2. The angle applied to spreader.
3. The speed and steadiness in pushing the spreader.

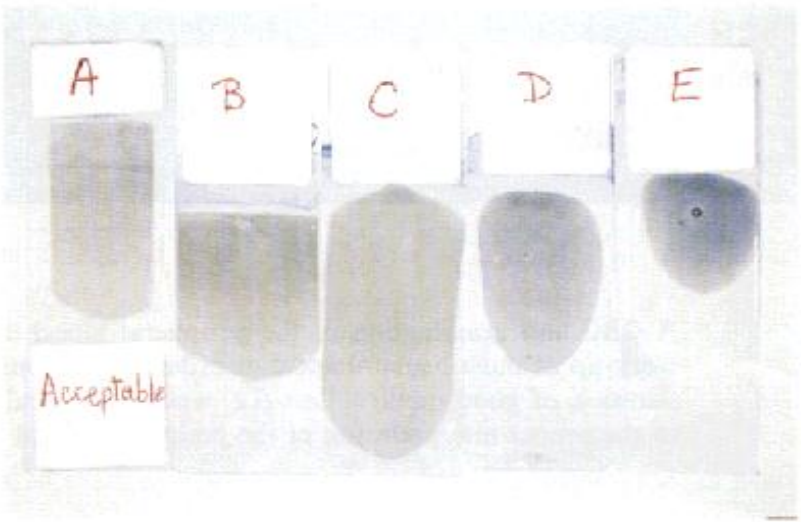


Fig.(3): Examples of blood films. The optimal blood film **(A**) is thin and of medium length. The others are suboptimal because of the lack of lateral edges **(B,C)**. A curved zone of morphology **(D)**. Or excessive thickness **(E)**.

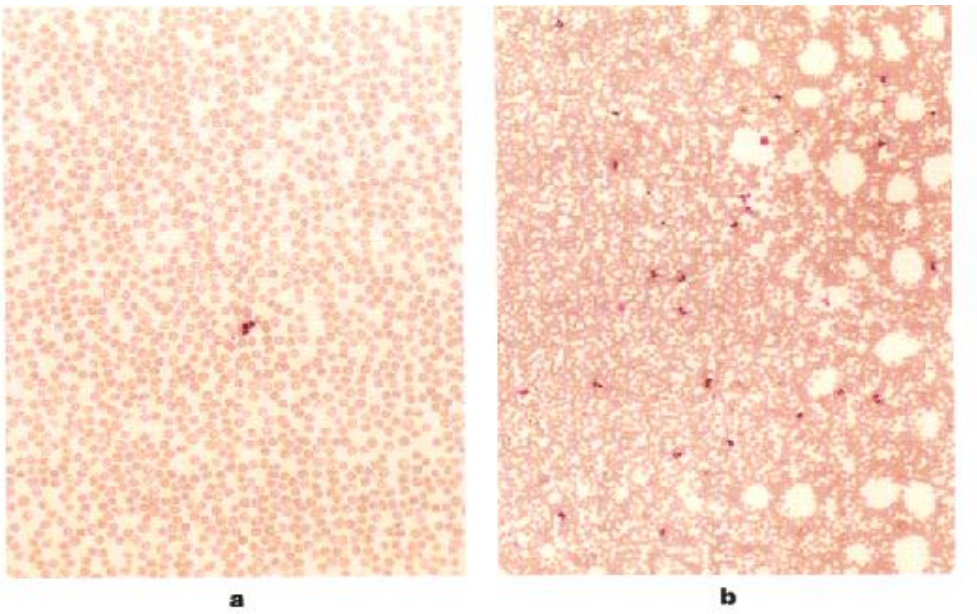


Fig.(3): a:Zone of morphology. The RBCs are well separated from each other . **b:** Thick film with no zone of morphology. The RBCs are crowded