EXPERIMENT (1)

1. Separation of Plasma and Serum from Whole Blood

1.1 Objectives:

1- To know how to separate plasma and serum from whole blood.
2- To know what is haemolysis and the nature of haemolysing agents.
3- To detect the presence of Blood in a sample.

1.2 Introduction

The average person circulates about 5L of blood (1/13 of body weight), of which 3L is plasma and 2L is cells. Plasma fluid derives from the intestines and organs, and provides a vehicle for cell measurement. The cells are produced primarily by bone marrow and account for blood "solids". Blood cells are classified as red blood cells (erythrocytes), white cells (leukocytes) and platelets. The size of cells differs: white cells are the largest, red cells fall into the middle, and platelets are the smallest.

1.2.1 The General Functions of Blood

The general functions of blood are in metabolism and its regulation, transport, osmotic balance and defense. The metabolic and transport roles of blood overlap to some extent, for instance in the carriage of oxygen and carbon dioxide. Blood plays an important part in the body‟s defense mechanism. The immune response system is able to recognize foreign material within the body and a sequence of events is triggered that neutralizes and destroys the foreign material. The complex composition of blood is not constant, but changes during stress, starvation, exercise and as the result of injury or disease.

1.2.2 Whole Blood

It is living tissue that circulates through the heart, arteries, veins, and capillaries carrying nourishment, electrolytes, hormones, vitamins, antibodies, heat, and oxygen to the body‟s tissues. Whole blood contains

red blood cells, white blood cells, and platelets suspended in fluid called plasma. If blood is treated to prevent clotting and permitted to stand in a container, the red blood cells, which weigh more than the other components, will settle to the bottom; the plasma will stay on top; and the white blood cells and platelets will remain suspended between the plasma and the red blood cells. A centrifuge may be used to fasten this separation process. The platelet-rich plasma is then removed and placed into a sterile bag, and it can be used to prepare platelets and plasma.

1.2.3 Blood Cells

1.2.4 Red Blood Cells

They are perhaps the most recognizable component of whole blood. Red blood cells contain hemoglobin, a complex iron-containing protein that carries oxygen throughout the body and gives blood its red color. The percentage of blood volume composed of red blood cells is called the "hematocrit". The average hematocrit in an adult male is 47 percent. There are about one billion red blood cells in two to three drops of blood, and, for every 600 red blood cells, there are about 40 platelets and one white cell. Manufactured in the bone marrow, red blood cells are continuously being produced and broken down. They live for approximately 120 days in the circulatory system and are eventually removed by the spleen.

1.2.5 White Blood Cells

They are responsible for protecting the body from invasion by foreign substances such as bacteria, fungi, and viruses. The majority of white blood cells are produced in the bone marrow, where they outnumber red blood cells by two to one. However, in the blood stream, there are about 600 red blood cells for every white blood cell. There are several types of white blood cells; Granulocyes and macrophages protect against infection by surrounding and destroying invading bacteria and viruses, and lymphocytes aid in immune defense.

1.2.6 Platelets (or thrombocytes)

They are very small cellular components of blood that help the clotting process by sticking to the lining of blood vessels. Platelets are made in the bone marrow and survive in the circulatory system for an average of 9-10 days before being removed from the body by the spleen. The platelet is vital to life, because it helps prevent massive blood loss resulting from trauma, as well as blood vessel leakage that would otherwise occur in the course of normal, day-to-day activity.

1.2.7 B. The Liquid Phase of Blood

1.2.7.1 Plasma

It is the liquid portion of the blood in which red and white blood cells and platelets are suspended. Plasma, which is 90 percent water, constitutes about 55 percent of blood volume. Plasma contains albumin (the chief protein constituent), fibrinogen (responsible, in part, for the clotting of blood), globulins (including antibodies), and other clotting proteins.

1.2.7.2 Serum

Serum resembles plasma in composition but lacks the coagulation factors. It is obtained by letting a blood specimen clot prior to centrifugation or by centrifugation of plasma to precipitate Fibrinogen and the liquid phase will be the serum. Serum is preferred for many tests as the anticoagulants in plasma can sometimes interfere with the results.

1.2.8 Collection of Blood Specimens

1.2.8.1 Capillary Blood

It is most frequently obtained from a finger or thumb. The most convenient place is one the thumb about 5 mm from the side of the nail. The tip of a finger is also used.

1.2.8.2 Venous Blood

It is most often used, while the blood may be taken from any prominent vein, a vein on the front of the elbow or forearm is almost universally employed.

1.2.9 Anticoagulants

If whole blood or plasma is desired, an anticoagulant must be added to the specimen immediately after it is drawn or placed into the tube into which the blood is collected.

1.2.10 Types of Anticoagulants

1.2.10.1 Heparin (20 mg / 10ml of blood)

It is the most satisfactory anticoagulant since it does not produce a change in red cell volume or interfere with subsequent determinations. It inhibits the formation of thrombin from prothrombin and thus preventing the formation of fibrin from fibrinogen.

1.2.10.2 EDTA. disod.salt (20 mg / 10ml of blood)

It is a chelating agent, drives its anticoagulant activity from the fact that it binds calcium, which is essential for the clotting mechanism.

1.2.10.3 Potassium Oxalate (20 mg / 10ml of blood)

Oxalates act by precipitating the calcium, and we use potassium oxalate since it is the most soluble. It inhibits blood coagulation by forming rather insoluble complexes with calcium ions, which is necessary for coagulation.

1.2.10.4 Sodium Citrate (30 mg / 10ml of blood)

It does not precipitate the calcium, but converts it into a non-ionized form, and hence prevent clotting of blood.

1.2.10.5 Sodium Fluoride (10 mg / 10ml of blood)

It acts as a weak anticoagulant, therefore larger amounts are required than of either oxalates or citrates. It has been used chiefly as a preservative since it inhibits red cell metabolism and bacterial action.

1.2.10.6 Laboratory Use of Plasma and Serum

Different anticoagulants interfere with different tests; using serum means the same sample can be used for many tests. Some tests require serum, others plasma, while some can be carried out on either or whole blood.

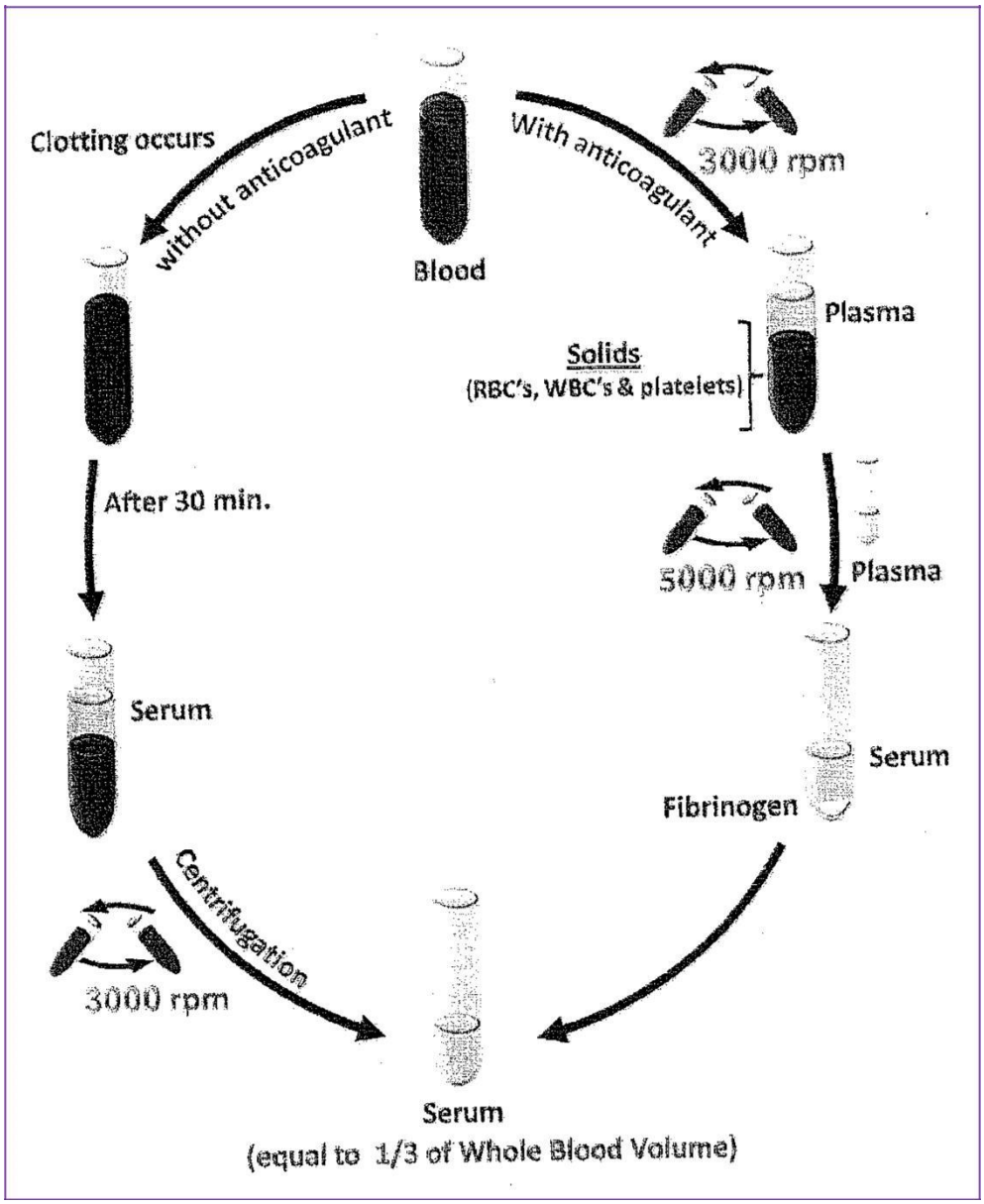
1.2.11 Changes in Blood on Keeping

1- Loss of carbon dioxide.
2- Conversion of glucose to lactic acid (glycolysis).
3- Increase in plasma inorganic phosphate.
4- Formation of ammonia from nitrogenous substances.
5- Passage of substances through the red cell envelope.
6- Conversion of pyruvate into lactate.

1.2.12 Normal Values

Total blood volume	55 – 80 ml / kg
RBC‟s volume	20 – 35 ml / kg
Plasma volume	30 – 45 ml / kg
Serum volume	18 – 27 ml / kg

Separate Blood Components



Part 1

Method of Separation of Plasma and Serum from Whole Blood

1.3 Materials

1- Whole blood
2- Centrifuge (up to 5000 rpm)
3- Centrifuge tubes suitable for the rotor of the centrifuge (preferably plastic and capped).
4- Disposable gloves
5- Disposable Pasteur pipette.
6- Measuring cylinder 10 ml.

1.4 Principle

1- If the blood is placed in a plain container and allowed to clot, the clot shrinks and expresses serum which can be obtained by centrifugation at 5000 rpm.

(Volume of serum obtained = 1/3 of the volume of whole blood)

2- Clotting can be prevented by placing the blood into a container containing an anticoagulant.

3- For hematological, biochemical, blood bank-related, immunological and other kinds of tests, plasma is obtained from whole blood. To prevent clotting, an anticoagulant is added to the blood specimen immediately after it is obtained and then we get (**Whole Blood**). The specimen is then centrifuged to separate plasma from blood cells.

Further centrifugation of plasma at 5000 rpm will precipitate the fibrinogen and we get SERUM (Plasma – Fibrinogen = Serum).

4- To obtain platelets, units of platelets are prepared by using a centrifuge to separate the platelet-rich plasma from the donated unit of whole blood. The platelet-rich plasma is then centrifuged again to concentrate the platelets further.

1.5 Procedure

1- Into dry clean Centrifuge tube, pipette 15 ml of whole blood (V_1).

2- Place the centrifuge tube in the centrifuge machine and run it at 3000 rpm for 10 minutes. Centrifugation of whole blood separates the solid from the supernatant plasma.

3- Remove the tube, withdraw the liquid layer (plasma) by pasture pipette and measure its volume using small measuring cylinder (V_2). Determine the volume of blood cells too V_3 (equal to $V_1 - V_2$). Red blood cells which prepared from whole blood by removing the plasma, are kept to be used in part 2.

4- **Transfer** the supernatant (plasma) in another centrifuge tube and make further centrifugation at 3000 rpm. This will precipitate fibrinogen and the supernatant will be **SERUM**. Measure its Volume (V_4).

1.6.**RESULTS**

-Record your results in the following table:

	Component	**Total Volume**	**Percentage**	
1	Whole Blood	V_1 =		
2	RBC"s	V_3 =		
3	Plasma	V_2 =		
4	Serum	V_4 =		

1.7.DISCUSSION

1.8.REFERANCES

Dacie.J.V. and Lewis. S.M .2001.Practical hematology .Longman group UK Limited. Ninth edition