

HEMATOCRIT (Packed Cell Volume of Whole Blood)

Objectives:

- 1- Determine hematocrit value of blood specimens manually in the lab.
- 2- Understand the principle of hematocrit determination.
- 3- List the mechanical and biological factors that interfere with hematocrit determination.
- 4- State the clinical importance of the test.

Hematocrit:

Hematocrit is defined as percentage of erythrocytes to the whole volume of blood, and is usually expressed as a percentage of the volume of the whole blood sample (expressed as % (vol/vol)). The hematocrit may also be referred to as Packed Cell Volume (PCV).

Principle of the Test:

The hematocrit (PCV) is usually determined by spinning a blood-filled capillary tube in a hematocrit centrifuge.

Hematocrit is a common laboratory test can tell a physician a great deal about the volume of red cells in a blood sample. The volume of RBC's refers to the amount of space that the RBC's occupy within the blood. If whole blood is placed in a special hematocrit tube (a small test tube) and then spun very rapidly in a centrifuge, the heavier components will quickly settle to the bottom of the tube (Figure 1). When the centrifuge spins, the RBCs are forced to the bottom of the tube because they are the heaviest element in the blood. The WBCs and platelets are lighter so, they come to rest on top of the heavier RBCs in a layer called the **buffy coat**. Above the buffy coat rests the plasma.

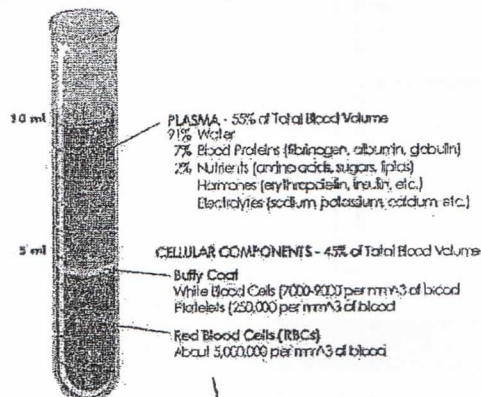


Figure 1: Blood can be separated into its components by putting it into a centrifuge and "spinning it down." The parts separate according to their relative "weights." This test tube shows the components of blood in their relative ratios. It shows a hematocrit of 45 because the RBC layer make up 45% of the total volume of centrifuged blood (4.5 ml. out of 10 ml).

Specimen:

Venous blood anticoagulated with EDTA or capillary blood collected directly into heparinized capillary tubes can be used. Specimens should be centrifuged within 6 hours of collection. Hemolyzed samples cannot be used for testing.

Materials:

1. Capillary tubes, heparinized for fingersticks (red tip) or plain for anticoagulated blood (blue tip) -75 mm long.
2. Clay-type tube sealant.
3. Microhematocrit centrifuge.
4. Microhematocrit reader.
5. Gauze.
6. Alcohol swab.
7. Lancets for capillary puncture.

Procedure:

1. Draw well-mixed anticoagulated blood into two microhematocrit tubes by capillary action avoiding air bubbles. The tubes should be filled about $\frac{3}{4}$ full (**figure 2**).
2. Wipe off excess blood with a Kimwipe or gauze.
3. Seal one end of each tube with a small amount of clay material at a 90° angle. Be sure the seal has a perfectly flat bottom (**figure 3**).

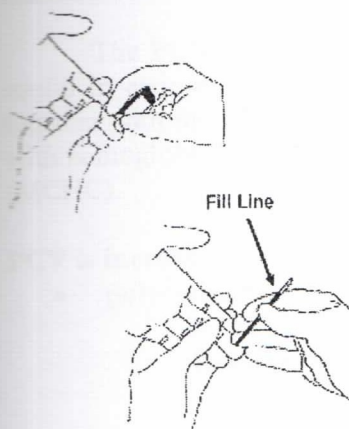


Figure 2: filling the capillary tube.

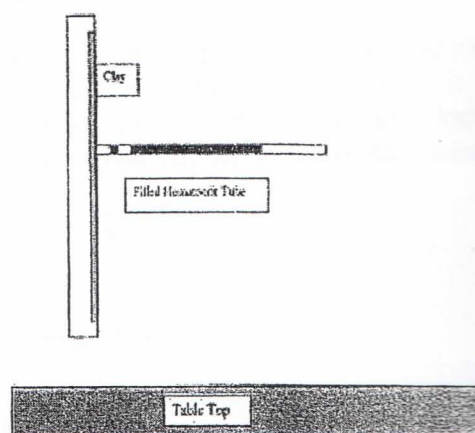


figure 3: sealing of the blood filled capillary tube with the clay.

4. Place the filled and sealed capillary tubes into the centrifuge (**figure 4**). The sealed ends should point toward the outside of the centrifuge. The duplicate samples should be placed opposite each other in order to balance the centrifuge. Record the position

- number of each specimen.
5. Securely fasten the flat lid on top of the capillary tubes.
 6. Centrifuge for 5 minutes at a set speed (11000 rpm). This separates the RBCs from plasma and leaves a band of buffy coat consisting of WBCs and platelets.
 7. Allow the centrifuge to stop on its own. Do not use the hand brake.
 8. After the centrifuge has stopped, open the top and remove the cover plate.
 9. Promptly read the hematocrit on the hematocrit reader (**figure 5**). (Instructor will review directions on using the hematocrit reader.) Do not include the buffy coat layer. (See illustration). If the buffy coat exceeds 2%, it should be recorded and noted as volume of packed WBC/plt.

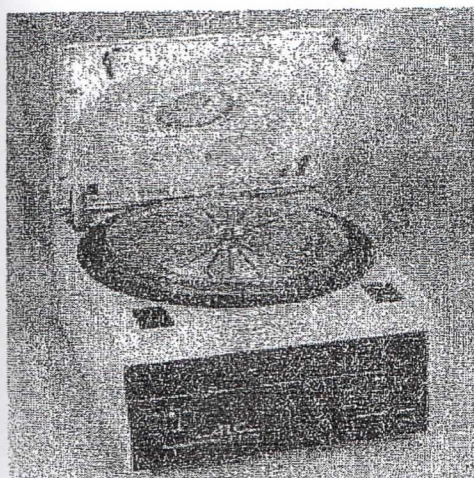


figure 4: microhematocrite centrifuge.

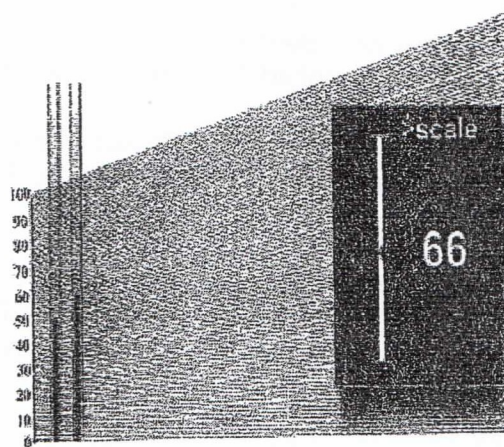


figure 5: reading the hematocrit with hematocrit reader.

Clinical Significance of The Test:

The PCV is an easily measure for detecting anemia or polycythemia and can be useful in estimating changes in hemodilution or hemoconcentration. The PCV is used together with the RBC's count, in calculating the mean cell volume (MCV), and, together with hemoglobin content, in calculating the mean corpuscular hemoglobin concentration (MCHC).

PCV is increased in:

- **polycythemia:** increased RBC's count.
 - A. **Pathological:** bone marrow malignancy.
 - B. **Physiological:**
 - Age: (PCV is higher in infants).
 - Altitudes: (PCV is higher).

PCV is decreased in:

- **Anemia:** RBC's number is decreased.

Reporting Results:

Normal values

Newborn 53-65%

Adult male 42-52%

Adult female 37-47%

Mechanical Sources of Error:

1. Incomplete sealing of the capillary tubes will give falsely low results because, in the process of spinning, RBCs and a small amount of plasma will be forced from the tube.
2. The microhematocrit centrifuge should never be forced to stop by applying pressure to the metal coverplate. This will cause the RBC layer to "sling" forward and results in a falsely elevated value.
3. If the centrifugation time is too short or the speed is too low, an increase in trapped plasma (1-3%) will occur in normal blood. Increased amount of trapped plasma can produce errors in cases where an erythrocyte abnormality exists, such as sickle cell anemia.
4. If too much time elapses between when the centrifuge stops and the capillary tube is removed, the red cells can begin to settle out and cause a false reading of the hematocrit.

Biological Sources of Error:

1. If the buffy coat is included in the RBCs when reading the result, the hematocrit will be falsely elevated.
2. Hemolysis of the specimen can cause a falsely decreased result.
3. When the microhematocrit is spun for the correct time period and at the proper speed, a small amount of plasma still remains in the red blood cell portion. This is termed *trapped plasma*. When comparing spun microhematocrit results with hematocrit results obtained from an electronic cell counter, the spun hematocrit results are generally 1.3 to 3% higher due to this trapped plasma. An increased amount of trapped plasma is found in macrocytic anemias, spherocytosis, thalassemia, hypochromic anemias, and sickle cell anemia.

Differential White Blood Cells count

Objectives:

To:

1. Identify the five different types of WBC's.
2. Make a blood smear to be used for routine differential WBC's count.
3. Calculate the percent of each type of leukocytes in a blood sample.

Introduction:

A differential white cell count: Is a test that consists of an examination of stained blood film under microscope to determine the percentage and the number of different types of white blood cells.

Leukocytes or white blood cells (WBC) are cells in the blood that are involved in defending the body against infective organisms and foreign substances. Like all blood cells, they are produced in the bone marrow. There are 5 main types of white blood cell, subdivided into two main groups (**Figure 1**).

1. **Polymorphonuclear Leukocytes (granulocytes):** The granulocytes account for about 70% of all white blood cells and are characterized by :

- irregular segmented nuclei
- specific granules (*specific size, staining affinities, ultrastructure*)

The granulocytes are subdivided into 3 types:

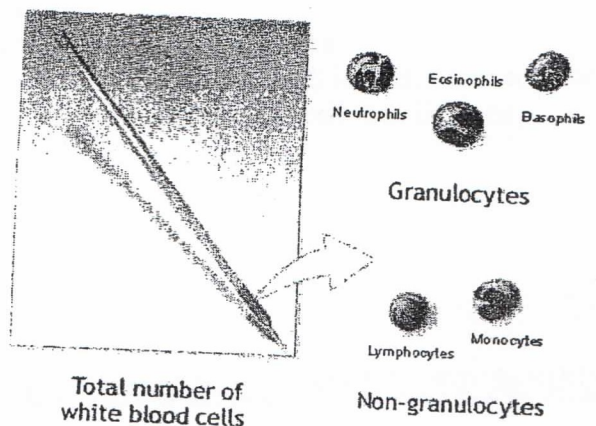
- I.
 - Neutrophils
 - Eosinophils
 - Basophils

2. **Mononuclear Leukocytes(Agranulocytes):** are characterized by :

- regular nuclei (round or kidney-shaped)
- non-specific granules .

- **The Agranulocytes are subdivided into 2 types:**

- Monocytes
- Lymphocytes



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Figure 1: The 5 types of leukocytes

Principle:

A drop of blood is spread over a glass slide, then it is let to dry and stained with special quick stain (Wright –Gimsa stain), and viewed under the microscope.

Materials Required for the Differential Count Procedure:

To perform a differential count, the following materials are required:

1. Plain glass microscope slides clean and dry.
2. Wright-Giemsa stain solution (follow manufacturer's directions for use and storage).
3. Staining containers.
4. Deionized or distilled water.
5. A microscope.
6. Immersion oil.
7. Blood cell counter.

Differential Count Procedure:

The procedure for the differential white cell count is done in 4 steps:

- Step 1: Making the blood smear.
- Step 2: Staining the blood smear .
- Step 3: Counting the cells (cell identification and counting).
- Step 4: Reporting the count.(cell identification)

A- Making Blood Smear: (Wedge Method)

Blood smear: is spreading of the blood on a glass slide to be thin layer of blood..

A good blood smear has a feathered edge that is nearly square and rainbow sheen when reflecting the light. The perfect slide consists of a smear that is exactly *one cell thick in the feathered edge* when viewed microscopically.

To make a good blood smear, follow these steps:

1. Select two glass slides that are clean and free of chipped edges.
2. Make a capillary puncture (**Figure 2, A, B**) and fill a hematocrit tube with blood, hold the filled hematocrit tube over the slide, tilt the tube and place a drop of blood 1 to 2 mm in diameter on one of the slides. The drop should be in the center line approximately 1/4 inch from the frosted edge of the slide. ***Make the smear immediately after you have applied the drop of blood.***
3. Hold the slide with the drop of blood at the opposite end with the thumb and Fore finger of your non-dominant hand. Grasp the spreader slide similarly with your dominant hand.
4. Rest the left end of the spreader slide at a 45-degree angle just in front of the drop of blood (wedge smear technique) . Draw the spreader slide backward until it just touches the drop of blood. Allow the drop of blood to spread in the angle between the slide and the spreader.
Not spreading the blood evenly will cause a rounded feathered edge.
5. Keep the spreader slide at the 45-degree angle. Push the spreader slide rapidly across the stationary slide with one even stroke and pressure. Avoid any jerky movements. (**Steps 1-4, Figure3**).

Note: Any pressure exerted on the spreader slide should be directed across the slide in the direction that the film is made rather than down on the stationary slide. The faster the spreader slide is moved, the longer and thinner the film will be. The slower the slide is moved, the shorter and thicker the slide will be. The angle will also vary the results. An angle greater than 45 degrees makes the smear thicker; less than 45 degrees, the smear is thinner. *Speed, angle and drop size can be varied slightly to produce a good smear*

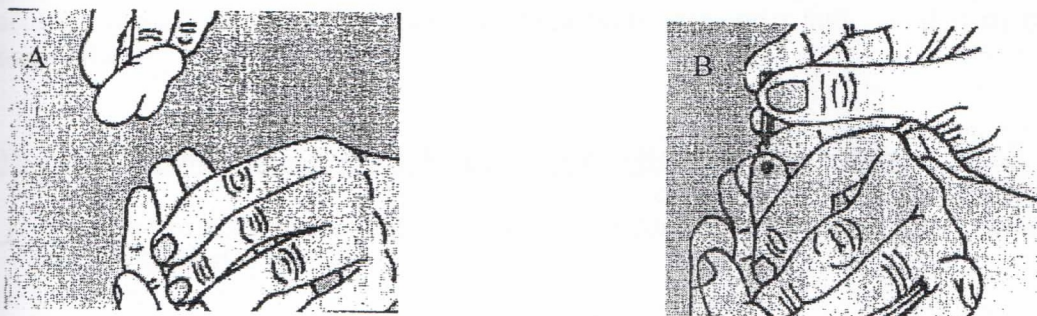


Figure 2: Making a capillary puncture

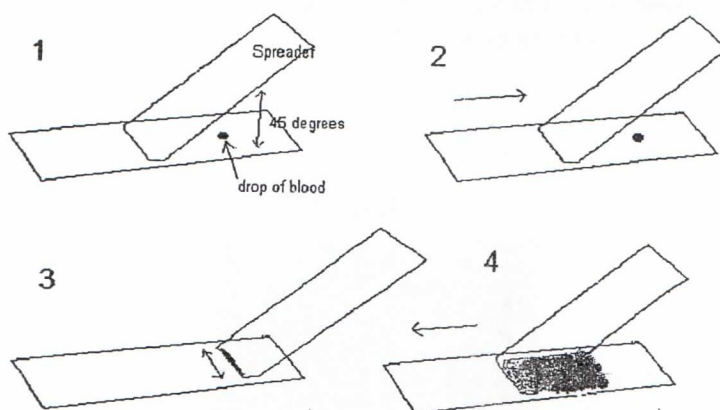


Figure3: The main steps in making a blood smear

Allow the slide to air dry.
Fix the slide by methanol.

B- Staining the Cells:

Once a blood smear is made, it should be stained. Staining the blood smear highlights the differences among the different types of leukocytes for easier recognition during the counting process. The most popular stain used for this purpose is Wright's stain.

Wright's stain is a methyl alcohol (methanol) solution of an acid dye and a basic dye. The acid dye in Wright's stain is known as Eosin and it is red in color. The basic dye in Wright's stain is known as Methylene blue and is blue in color. Generally, white blood cells are identified by their affinity to the dye. For example, cells that prefer the acid dye (eosin) are called eosinophils. Other cells that prefer the basic dye are called basophils.

To stain a blood smear with Wright's stain, follow the steps below.

1. Prepare two staining containers by filling one with the stain solution and the other with deionized water.
 2. Immerse the slide or slides (blood smear) in the stain for 15 to 30 seconds (Figure 4).
 3. Remove the slide and allow excess stain to drain from the edge of the slide.
 4. Immerse the slide in the deionized or distilled water for 5 to 15 seconds. (Change the water when it becomes dark blue or when film forms on the surface).
 5. Drain excess water and wipe the back of the slide to reduce background color.
 6. Place slide in horizontal position on table and allow to air dry.
7. Once the slide is dry, proceed counting the cells.



Figure 4: immersing slides in the stain

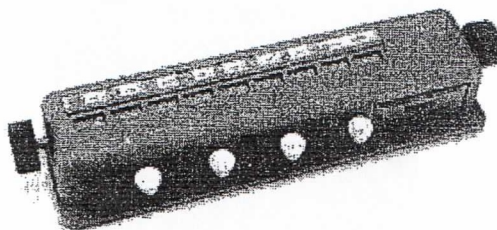
C- Counting the Cells

Once the blood smear has been stained, it is placed under a microscope, and the differential count is conducted. To perform a differential white cell count, you should follow the steps listed below:

1. Place the slide under the microscope and focus the thin area under the low power lens.
2. Place a large drop of immersion oil on the thin area of the blood smear
3. Switch the oil immersion objective (100X) into position above thin area covered with the oil drop.
4. Now, while continually looking through the eye piece. A very slow rotate the coarse adjustment toward you until you see some cells. After you have brought the cells into view with the coarse adjustment, bring the cells into perfect focus by rotating the fine adjustment.

NOTE: Always rotate the fine adjustment back and forth when identifying cells. This step will help you see the various layers of the cell and thereby help you to identify the different types of white cells.

5. Count 100 consecutive white cells, pressing the correct key on the cell counter for each type of white cell identified. (If the cell counters is not available, record cell type and number of cells encountered on a piece of paper.) Follow path similar to one illustrated in **figure 5** to count cells. Then calculate the percent of each type by using the relation:



Relative count of certain cell % = number of cells counted / 100 × 100%



Figure 5: Counting path for differential count

D- Cell Identification

To perform a differential white cell count, you must be able to identify the different types of white blood cells. The ability to properly identify the different types of white blood cells is not difficult to develop, but it does require a thorough knowledge of staining characteristics and morphology. This knowledge can be gained only by extensive, supervised practice.

To further assist you, identifying characteristics of each type of leukocyte as they appear on a stained blood smear will be covered in the following sections:

1. Erythrocyte and a Platelets (figure 6).

Erythrocytes are biconcave discs without nuclei. There are 5,000,000 RBC/cubic ml of blood. About one third of the volume of each cell is hemoglobin.

2. Platelets

Platelets are small fragments of cells. There are approximately 200,000 to 400,000 platelets per cubic milliliter of blood. (figure 2).

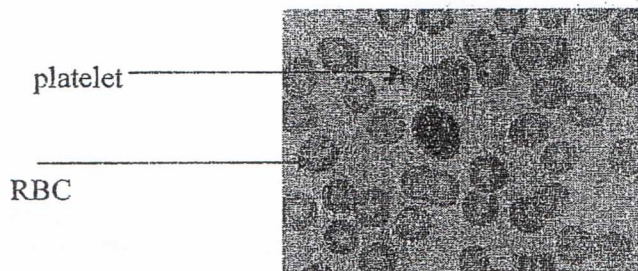


Figure 6: Erythrocyte and Platelets

3. Neutrophils

Neutrophils constitute the vast majority of granulocytes 40 to 60%. It is the most common granular leukocytes. They are somewhat larger than RBC and are

distinguished by having a polymorphic nuclear structure (**3 to 5 lobes**). What appears to be several nuclei are in reality one joined by narrow stands of nuclear material. These cells are very activity phagocytotically. They are called neutrophils because their cytoplasmic granules stain with neutral dyes (**figure 7**). Increased numbers usually indicates an infection.

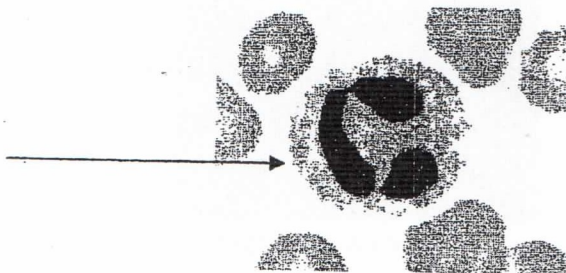


Figure 7: A Neutrophil

4. Eosinophils:

An **Eosinophil** is a granular leukocyte which stains with acidic dyes (**figure 8**). They represent 5% of the leukocytes with 200/cc of blood. They are about the same size as neutrophils but have only 2 or 3 lobes in their nuclei. The granules stain red to orange and are much larger than in neutrophils. Increased eosinophil counts are found in response to allergies and to parasitic infections.

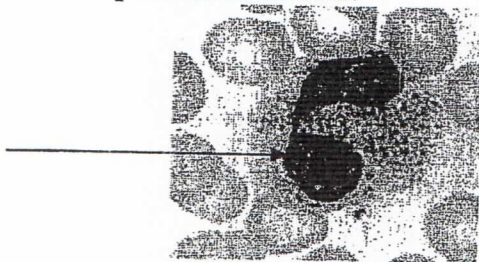


Figure 8: A Eosinophil

5. Basophils:

A **Basophil** is the rarest form of leukocyte and found at the rate of 1%. They are about the same size as neutrophils and eosinophils but stain selectively with basic dyes and have small, numerous, blue to black granules which often obscure the nucleus (**figure 9**)

The granules contain **Histamine**, a potent vasodilator, **Heparin**, an anticoagulant.

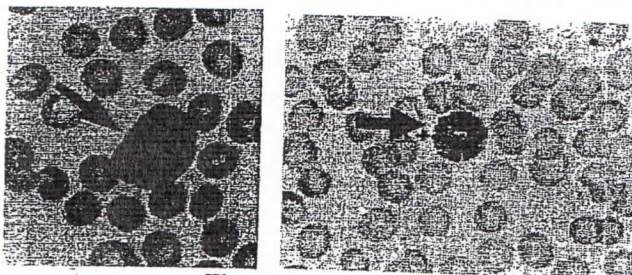


Figure 9: A Basophil

6. Lymphocytes:

Lymphocytes are the most abundant of the agranulocytes. Approximately 2500/cc and they represent about 20-40% of leukocytes. They have a round, densely stained nucleus which occupies most of the volume of the cell (**figure10**). They are variable in size and can be smaller than RBC or larger than granulocytes. They are the key cells of the immune system. They are subdivided in B and T lymphocytes reflecting their location and/or function.

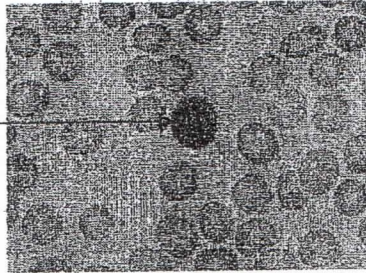


Figure 10: A Lymphocyte

7. Monocytes:

Monocytes represent about 7% of the leukocytes or 300/cc. They are the largest of the leukocytes with a more or less round nucleus but often with depressions giving it a kidney-bean shape (**figure 11**). The monocytes often move into the tissue spaces where they become **Macrophages** and **Histiocytes**. The macrophages and histiocytes show significant phagocytotic activity.

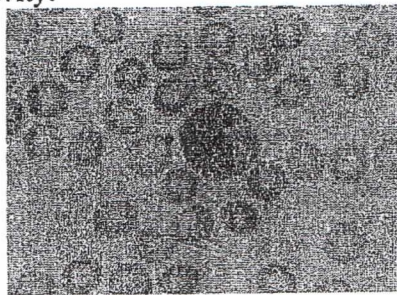


Figure 11: A Monocyte

Significance of the test:

Differential leukocytes count is useful in:

- general health examinations

- Help investigate a variety of illnesses, including infection, allergy, and leukemia.
 - Treatment monitoring
1. An increase in number (**leucocytosis**) may result from such conditions as bacterial or viral infection, metabolic disorders, chemical and drug poisoning, and acute hemorrhage.
 2. A decrease in number (**leucopenia**) may result from typhoid infection, measles, infectious hepatitis, tuberculosis, or cirrhosis of the liver.

BLOOD TYPING.

Objectives:-

At the end of this session the students are expected to:

- 1:-Acquired the skill of determining blood group(ABO,Rh system)
- 2:-Describe the importance of blood groups determination in the process of blood transfusion.

The History behind the Blood Group of Humans

In 1901 Karl Landsteiner demonstrated the existence of blood group antigens on human red blood cells as well as antibodies directed against those antigens in human sera. Blood was collected from members of his laboratory staff. He then separated the red blood cells from the serum, and then studied the results of mixing serum and red blood cells from different individuals. He discovered that some sera could agglutinate the red blood cells of some individuals but not others. He realized that individuals could be grouped. Group A individuals had an antigen, called A, on their red blood cells and antibodies to another antigen, called B, in their serum. Group B individuals had antigen B on their red blood cells and antibodies to antigen A in their sera. A third group, called group O, had neither A nor B on their red blood cells but had both anti-A and anti-B in their sera. Some time later, individuals were described who had both A and B antigens on their red blood cells but no antibodies to A or B in their sera. This group was called AB.

The ABO blood groups are defined by specific inherited molecules, or antigens, that are present on the surface of red blood cells. Thus, one inherits either A or B antigens (group A or B), both A and B antigens (group AB), or neither antigen (group O). Conversely, a person develops a natural immunity, or antibody, in their serum to the ABO antigens that are absent on their own red cells. Thus, a group A person has anti-B antibodies, and group O person has both anti-A and anti-B antibodies.

If group A red cells are mistakenly transfused to a group O recipient, for example, the anti-A antibody in the recipient's plasma destroys the transfused group A cells and a serious transfusion reaction occurs. Because group O has anti-A as well as anti-B antibodies, group O recipients can only accept blood from group O donors. Conversely, group AB recipients can receive blood from all groups.

Principle:

The test procedure based upon the agglutination (clumping) of red blood cell which carries a specific antigen in the presence of a corresponding specific antibody.

Table 1. ABO blood group. ABO genotypes and corresponding phenotypes, agglutinations, and isohemagglutinins.

Genotype	Blood group phenotype	Antigens on erythrocytes	Serum antibodies
AA or AO	A	A	Anti-B
BB or BO	B	B	Anti-A
AB	AB	A and B	None
OO	O	None	Anti-A and Anti-B

TO WHO CAN I DONATE BLOOD, AND FROM WHOM CAN I RECEIVE BLOOD?

	If you are:	You can donate to:	You can receive from:
Group	O	A, B, AB, O	O
	A	A, AB	O, A
	B	B, AB	O, B
	AB	AB	A, B, O, AB
Rh status	Rh +	Rh +	Rh +, Rh -
	Rh -	Rh +, Rh -	Rh -

Materials

Blood group Sera (Anti A,B,and D), Disposable blood group slide, lancets, Alcohol swap, Viewing box, glass rod and Microscope.

Method

- 1:-Obtain a drop of blood from a finger prick in each of the circles of the disposable blood group slide.
- 2:-Add one drop of Anti A (blue) and a drop of Anti B (yellow) and a drop of Anti D sera in the proper circles on the disposable blood group slide.
- 3:-Using clean (uncontaminated) glass rod to mix the blood with antiserum.

4:- Tilt the slide from side to side occasionally, and after 2 minute read macroscopically for agglutination.

5:-Any apparently negative tests should be read microscopically after 5 minute.

Report the result as follow.

-Agglutination (clumping) take place in the circle which has Anti A, but not with that of Anti B then Blood Group for this sample is A.

-Agglutination (clumping) take place in the circle which has Anti B, but not with that of Anti A then Blood Group for this sample is B.

-Agglutination (clumping) takes place in the circle which has Anti A and Anti B then The Blood Group for this sample is AB.

-No agglutination take place in both Anti A and Anti B circles the the Blood Group for this sample is O.

For Rh if Agglutination (clumping) take place in the Anti D circle then this mean Rh positive while if not it mean Rh negative.