Hematopoietic System-2023

Physiology Lab1-2024

Presented by:

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Professor of Medical Physiology

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Important Instructions

1-You must study this lecture and understand what you are going to do in the lab (the lab time is assigned only for rotation on the stations).

2- For anything that is not clear, please Join the office hour of **the next Saturday at 8 PM**.

1-**It is not allowed at all** to change your day, or your session.

3-Please leave the blood group station clean (in the same condition that you will find it when you start the session) and **the used materials (slides, lancets, sticks, cotton) must be placed in the provided yellow safety box.**

2

- Safety Box

3

Physiology Lab 1

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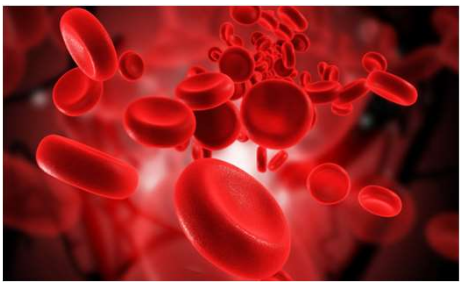
Osmotic Fragility Test

• Fragility ➡ the susceptibility of RBCs to being broken down .



•Hemolysis ➡ breaking down of RBCs and release of Hb into the surrounding fluid.

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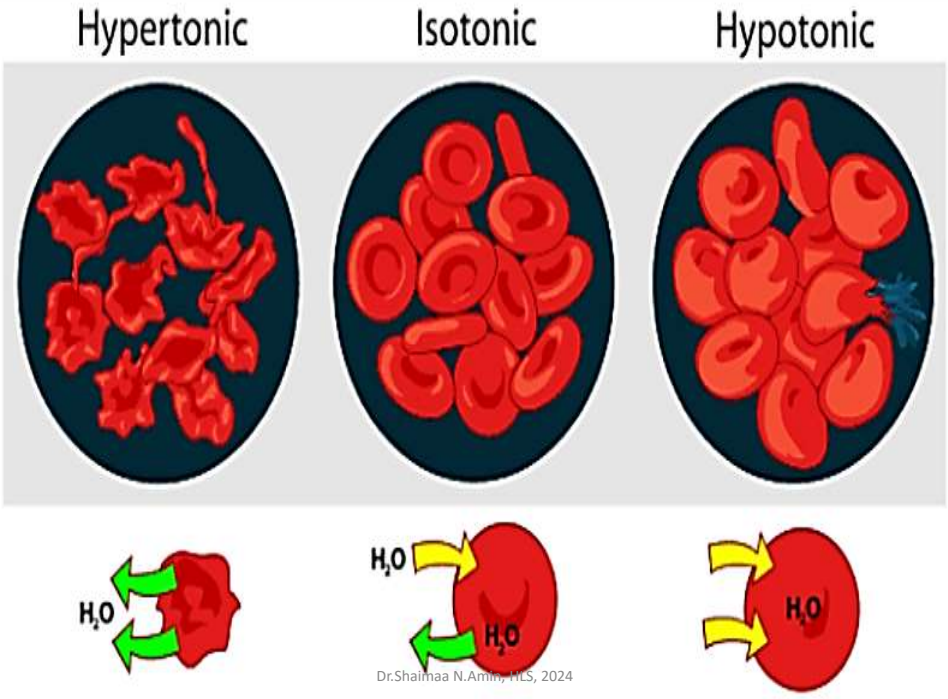

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OSMOTIC FRAGILITY TEST

Importance of test:

In certain types of hemolytic anemia, the fragility of red cells is increased so that they burst and release their Hb into the blood. The osmotic fragility test is used as a screening test for this group of anemia.

Principle:

The normal red cell can remain suspended in normal saline (0.9% NaCl solution) for hours without any change in their size or shape, and without rupturing. But when they are placed in decreasing concentrations of hypotonic saline, their ability to resist hemolysis can be determined quantitatively.

When osmotic fragility is normal, red cells begin to hemolyze when suspended in 0.50% saline. 50% lysis occurs in 0.4% saline and lysis is complete in 0.35% saline.

In hereditary spherocytosis; the red cells are spherocytic in normal plasma (0.9%) and hemolyze more readily in hypotonic NaCl solution. Thus, this test is commonly used to diagnose hereditary spherocytosis, which is one of the most common causes of hereditary hemolytic anemia.

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Materials:

a) Wood or metal rack with 8 clean, dry, glass test tubes

b) 10 ml measuring pipette

c) distilled H_2O

d) NaCl solution in distilled H_2O of the following concentration:

0.25, 0.30, 0.35, 0.40, 0.45, 0.50, and 0.55.

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Method:

1- Number the test tubes from 1-8 and put them in the rack.

2- Using the measuring pipette, place 5ml of the above NaCl solutions in the 1st 7 test tubes in serial order.

3- Place 5 ml distilled H_2O in the 8th tube.

4- Add 1ml of fresh blood to each test tube.

5- Invert gently each test tube to mix the blood and saline (or water). (Don't shake the tubes).

6- Allow the tubes to stand for 30 minutes. Then observe the extent of hemolysis in each tube by holding the rack at eye level, with a white paper sheet held behind it.

Observation and Results:

1- If there is no hemolysis, the red cells will settle down, leaving the saline above clear.

2-If there is some hemolysis, the saline will be tinged red with Hb, with the unruptured cells at the bottom.

3- The color of the saline will be increasingly deeper with decreasing tonicity, i.e, with increasing hemolysis.

4-No hemolysis occurs if the tube contains normal saline, and complete hemolysis occurs in distilled water.

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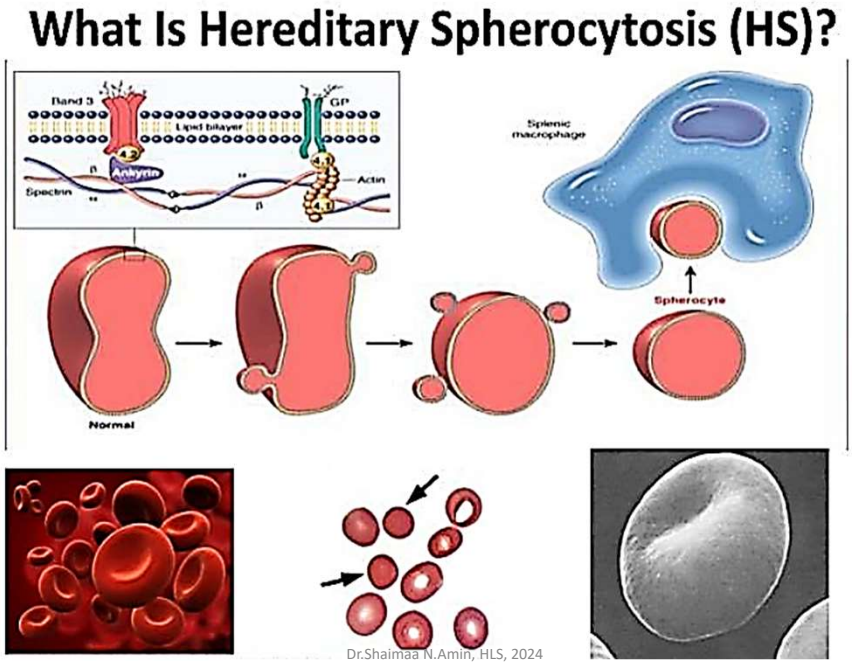

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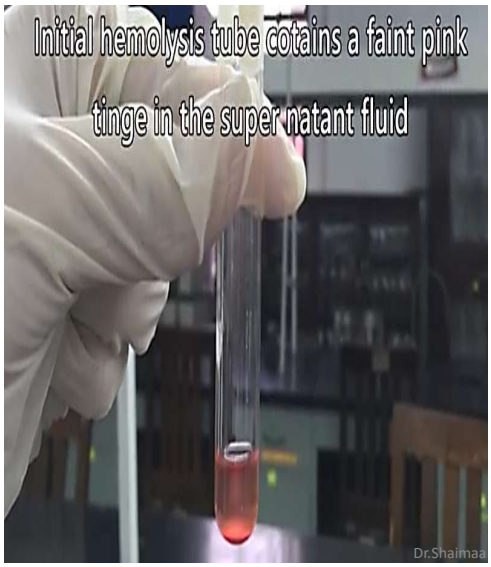
When hemolysis starts and completes?



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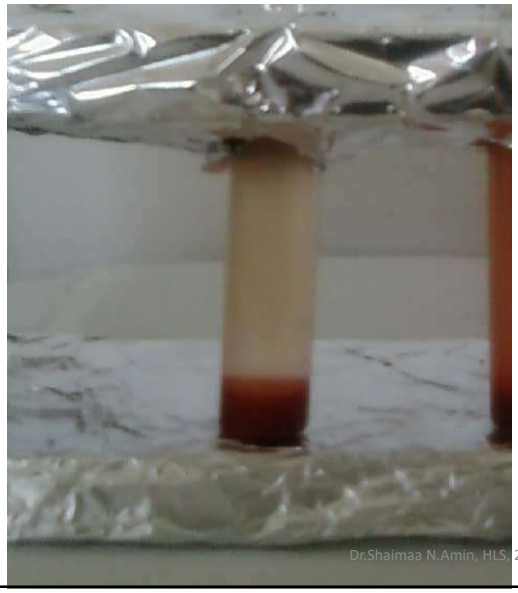
Reading



0.9%

No hemolysis

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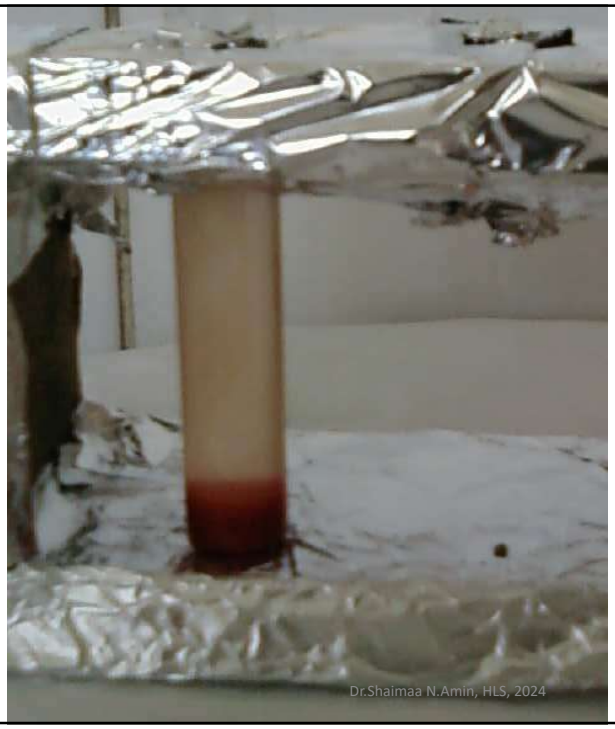


0.7%

No hemolysis

13

0.5%

Partial hemolysis

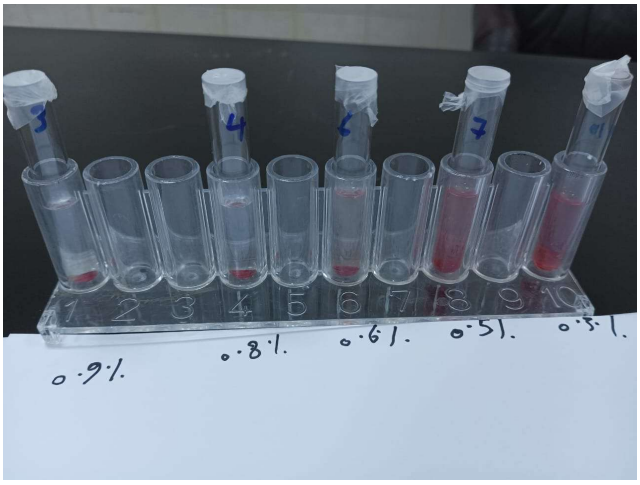
14

0.3%

Complete hemolysis

15

Normal



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- Normally there is a range of hemolysis (from start till it is complete): because of the biconcave shape of RBCs. In addition to the fact that not all RBCs are at the same age(hemolysis of old RBCs starts before young RBCs).

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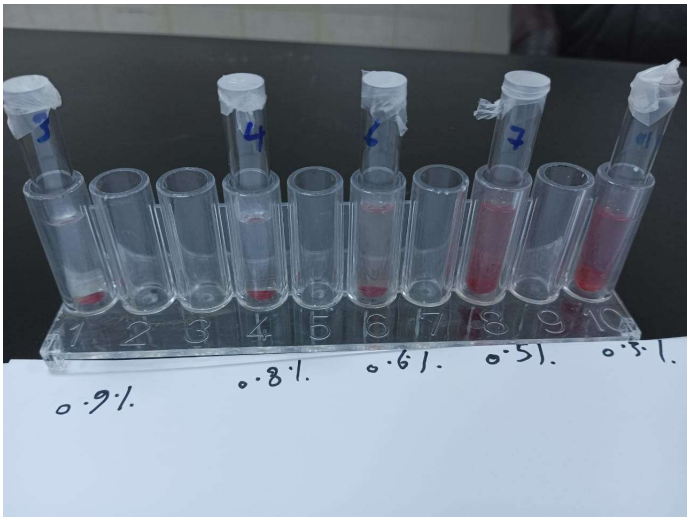
Normal



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Hereditary spherocytosis

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Osmotic Fragility station

- **The station for this experiment contains:**

A rack of test tubes containing Nacl solution with different concentrations.

- **What will you do at the station?**
- Without moving the rack or the tubes check if there is hemolysis or no hemolysis and if there is hemolysis check if it is partial or complete hemolysis (by observing the colour of the solution and the presence or absence of RBCs in the bottom of the tube).

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Packed cell volume (Hematocrit value)

•It's the ratio of red blood cells volume to the whole blood volume.

•According to the specific gravity of blood elements

Blood sample

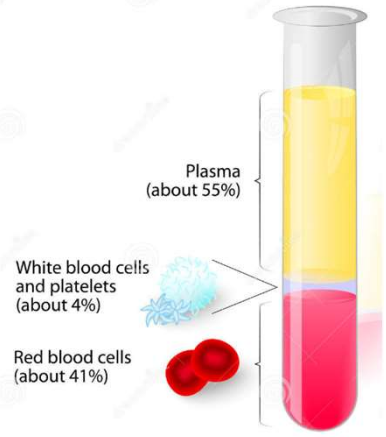
+

Anticoagulant

Centrifuged

↓

RBCs packed at the bottom, then buffy coat, above it plasma



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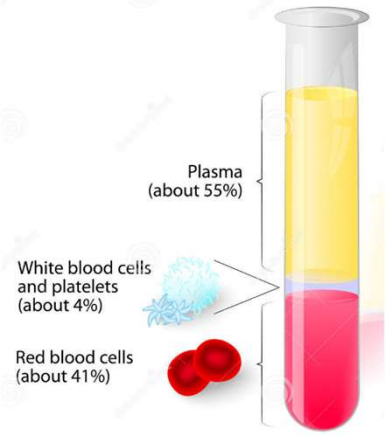
Packed cell volume (Hematocrit value)

$$\text{Hematocrit (Hct)} = \frac{\text{Height of packed red cells (mm)}}{\text{Height of packed RBCs and plasma (i.e., height of blood column)}} \times 100$$

For example, if the height of packed red cells is 45 mm, then

$$= \frac{45}{100} \times 100 = 45 \text{ percent.}$$



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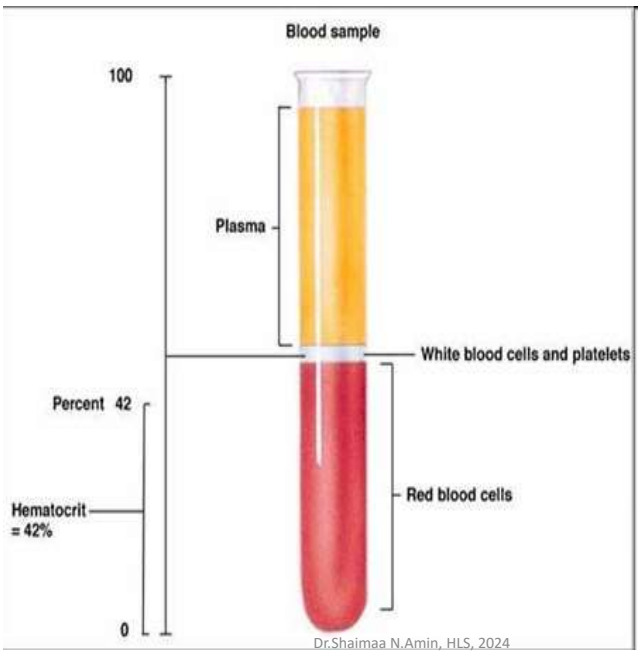

Female sample

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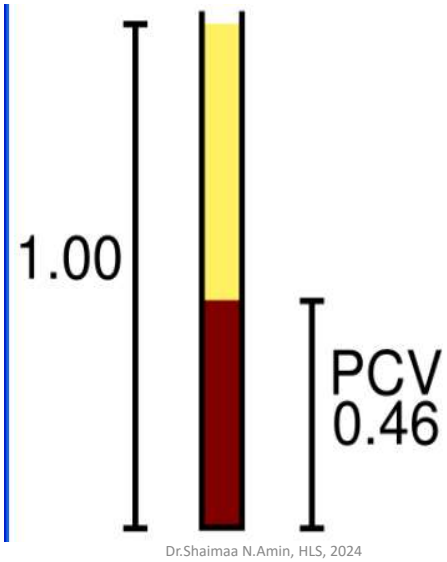

Male sample

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Determination of Packed Cell Volume (PCV)

(Hematocrit Ratio)

Definition

It is the ratio of the RBCs volume to the total blood volume.

Principle:

A sample of blood to which an anticoagulant has been added is centrifuged in a capillary tube. The specific gravity of RBCs is about 1090 and that of plasma is about 1030. Being heavier than plasma, the RBCs get backed towards the bottom of the tube by the centrifugal force and the plasma stays above the RBCs. White cells which have specific gravity intermediate between RBCs and plasma; form a thin layer (buffy coat) above RBCs.

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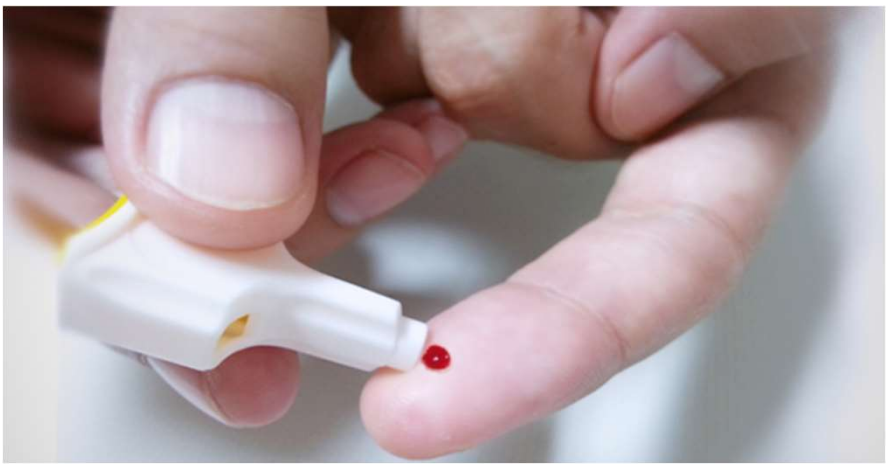
The experiment



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Procedure:

1- Sterile the finger with alcohol and leave it to dry.
2- Prick the fingertip using sterile lancet and allow a large drop of blood to collect.
3- Place the end of the heparinized capillary tube in the drop of blood. Hold the tube in a horizontal position and allow the blood to enter (the blood enters by capillary attraction) until the tube is one-half to three-fourths full.
4- Seal one end of the tube by wax.
5- Place the capillary tube the microhematocrit centrifuge with the sealed end to the outside and centrifuge for 5 minutes. The blood column is separated into plasma above and packed RBCs in the bottom of the tube.
6- After centrifugation, remove the capillary tubes, hematocrit ratio is read with the help of the reading scale.
7- Put the capillary tube on the reading scale, then move the tube on the chart horizontally with the lower end of the packed RBCs on the "O" line and the upper end of the clear plasma intersects the oblique line "100" position.
8- From the upper end of the packed RBCs, hematocrit value is read on the scale on the right side.

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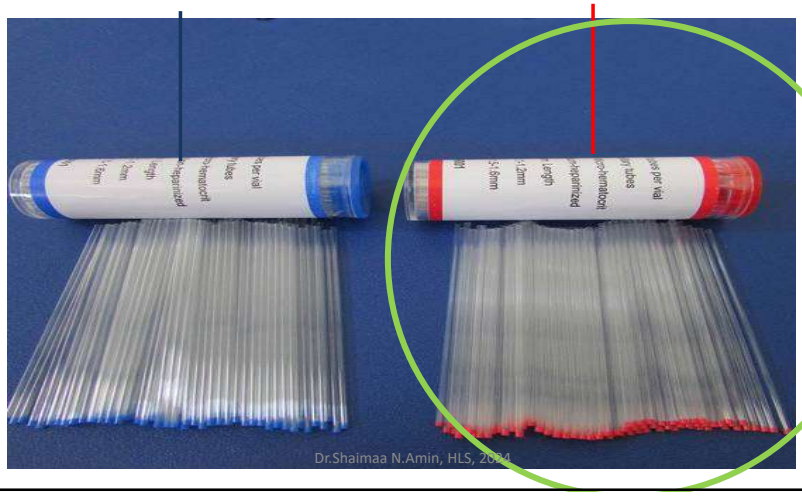
Capillary tubes

- **Non heparinized** **Heparinized**



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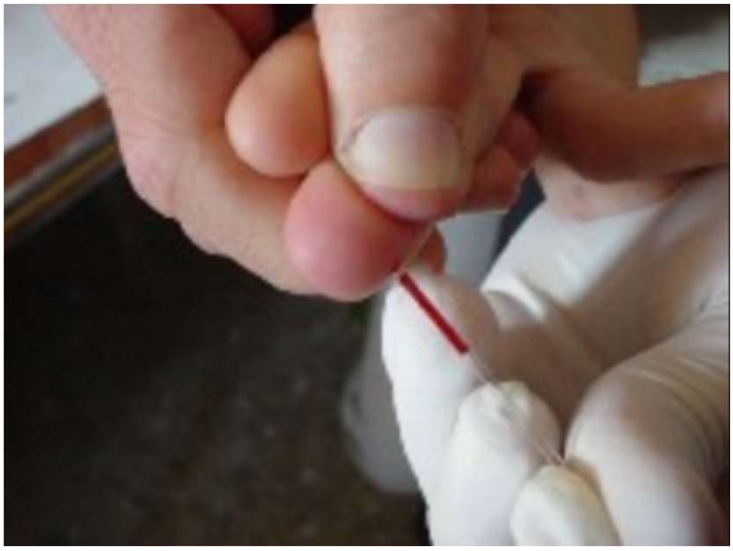

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VITREX
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
MADE IN DENMARK
100
With Heparin
75 mm Length

30

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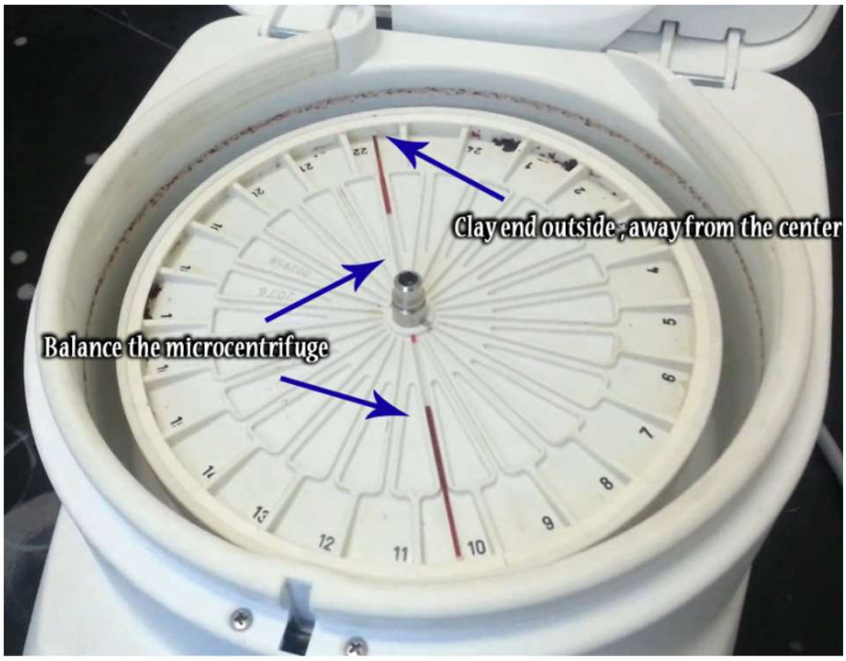

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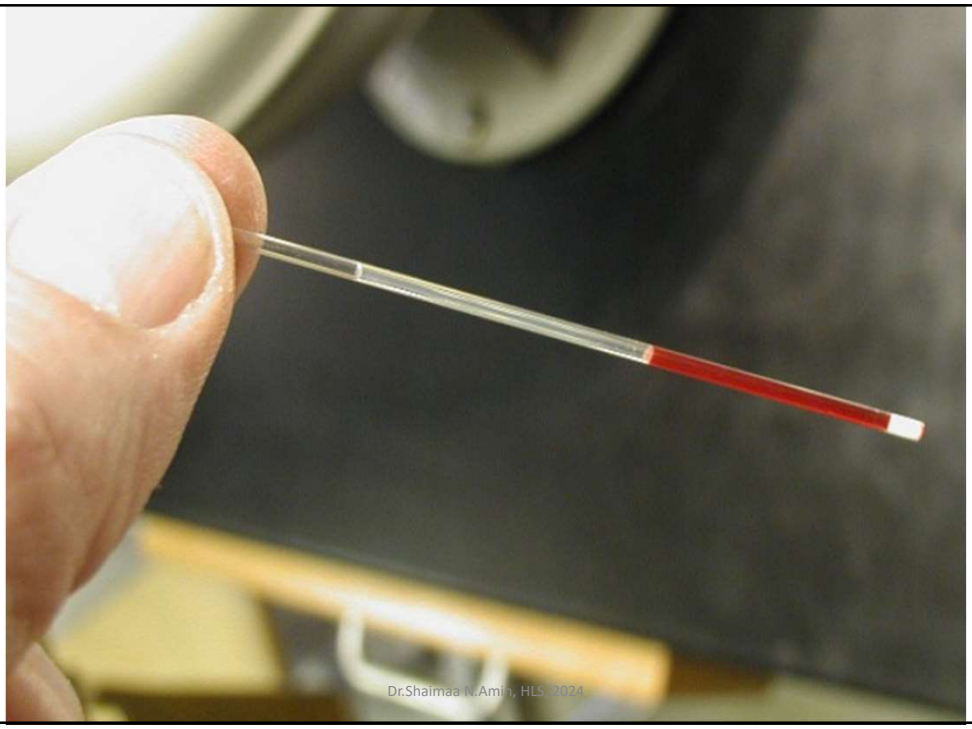

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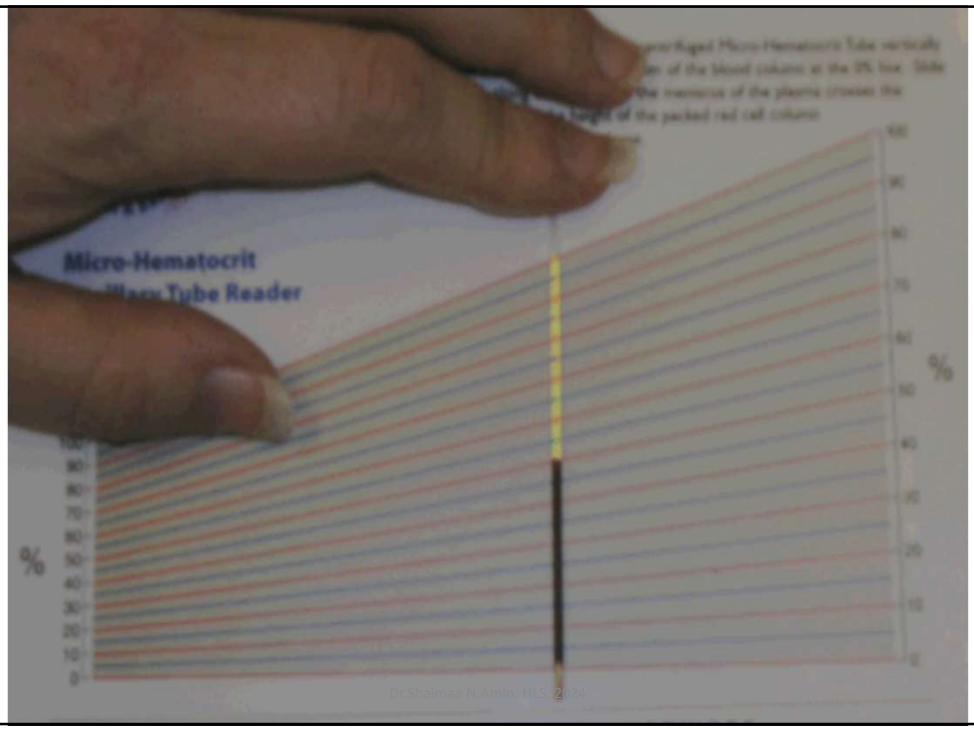

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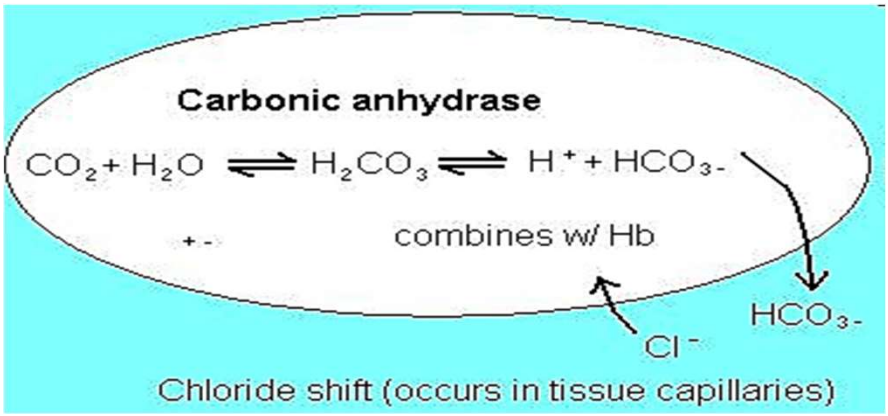
Haematocrite value is higher in venous blood why?

- **Due to Cl^- shift phenomena**



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Clinical significance

- **Diagnosis of anemia, polycythemia.**
- **To determine type of anemia calculate MCV & other blood indices.**

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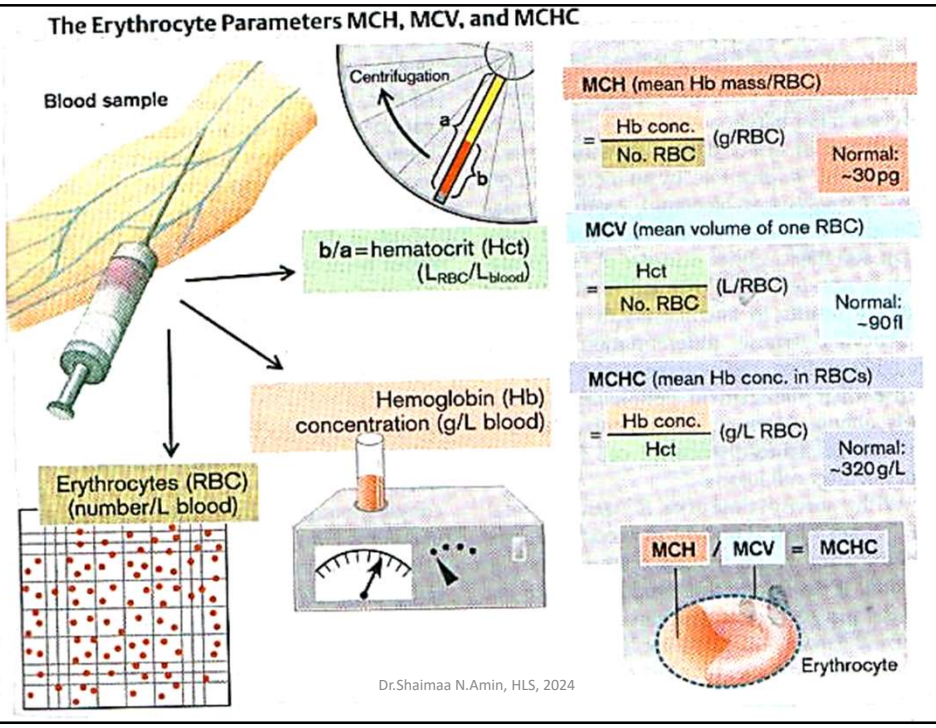

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Conditions in which the PCV varies from normal:

A) The conditions in which PCV is increased:

- Polycythemia: physiological (high altitudes or newly born) or pathological.
- Dehydration (vomiting, diarrhea, profuse sweating), burns , loss of water***(Hemoconcentration).***

B) The conditions in which PCV is decreased:

- Anemia.
- Overhydration, pregnancy ***(Hemodilution)***

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Packed Cell Volume(Hematocrit) station

- **The station for this experiment contains:**

A capillary tube containing blood centrifuged + Reading scale

- **What will you do at the station?**
- You have to read the Hct of the provided sample by adjusting it on the reading scale.

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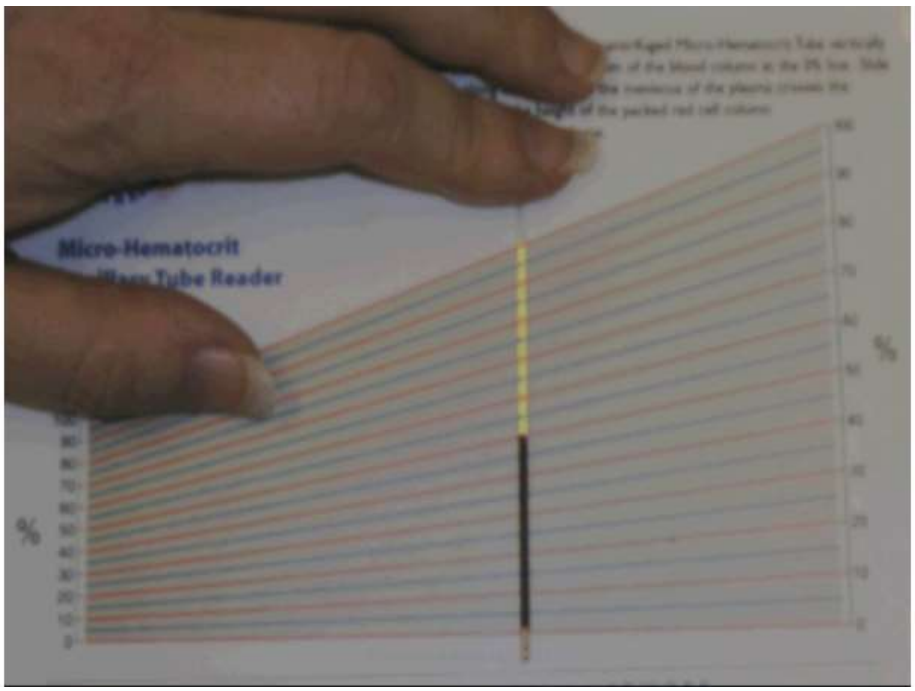
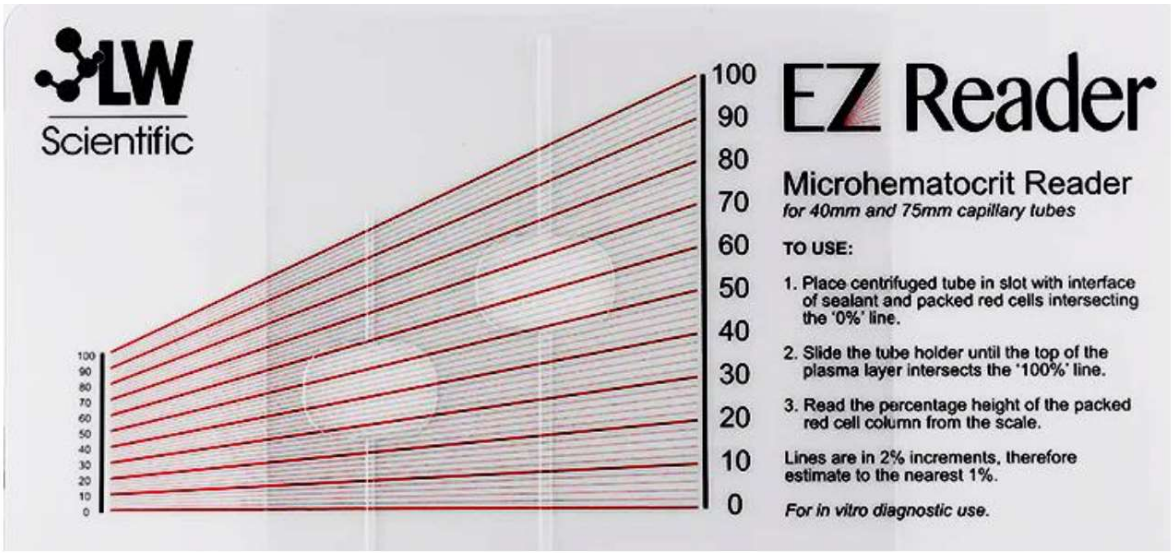

Micro-Hematocrit
Tube Reader
%

41


LW
Scientific
100
90
80
70
60
50
40
30
20
10
0
EZ Reader
Microhematocrit Reader
for 40mm and 75mm capillary tubes
TO USE:
1. Place centrifuged tube in slot with interface of sealant and packed red cells intersecting the '0%' line.
2. Slide the tube holder until the top of the plasma layer intersects the '100%' line.
3. Read the percentage height of the packed red cell column from the scale.
Lines are in 2% increments, therefore estimate to the nearest 1%.
For in vitro diagnostic use.

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Erythrocyte Sedimentation Rate (ESR)

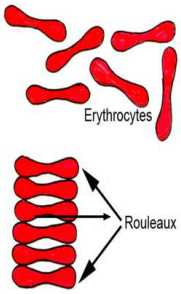
- **Definition:** distance sedimented in a vertical column of blood after 1 & 2 hours.

Westergren tube

Principle

- if anticoagulant is added to blood and blood is left to stand for 2 hours → RBCS sediment
- specific gravity

RBCS	1090
plasma	1030



- Plasma proteins neutralize charges of RBCS→
- ↓ repulsion force between RBCS →rouleaux formation → RBCS sedimentation

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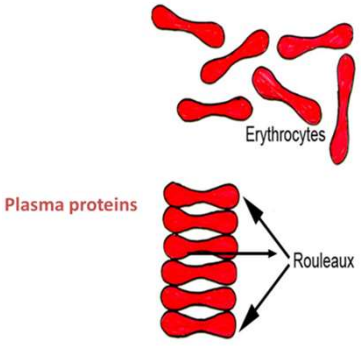
What is Rouleaux formation? Describe its possible mechanism.

- Globullins & fibrinogen→
- Neutralize charges of RBCs → Rouleaux formation.
- ++ ratio of mass to surface area of RBCs ++ sedimentation



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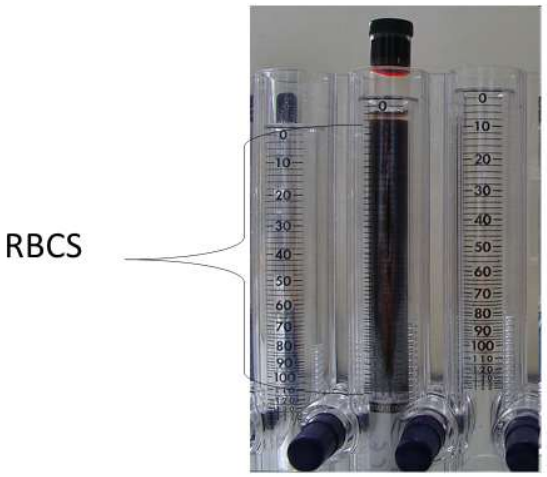
steps

1. In a test tube 2 ml blood +0.5 ml na^+ citrate
2. Fill **Westergren tube** to the mark 0 and put the tube in the stand vertically
3. Read the height of plasma column on top of RBCS after 1 and 2 hours



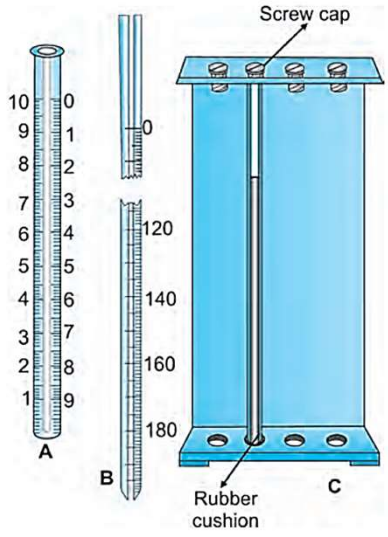
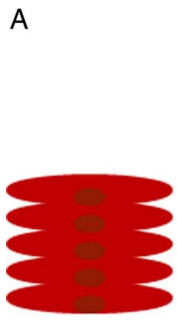


Figure 1-17: Determination of ESR. (A) Wintrobe tube; (B) Westergren tube; (C) Westergren stand with the Westergren tube in position



Normal value

mm	1st hour	2nd hour
male	6	8
female	10	16

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A. *Physiological Variations in ESR.*

Sex. The ESR is somewhat higher in females, probably due to lower hematocrit (PCV).

Pregnancy. The ESR begins to rise after about 3rd month of pregnancy and returns to normal a few weeks after delivery. Hemodilution during pregnancy and increased fibrinogen: albumin ratio are probably the cause of increased rouleaux formation.

B. Pathological *Increase in ESR.*

infections, tissue destruction (myocardial infarction) & malignancy, anemia, TB & fractures.

ESR is increased in inflammatory conditions by 2 mechanisms:

1. Tissue destruction.
2. Increasing antibodies which are plasma proteins.

Since ESR increases with age, the upper limit of normal can be calculated as:

Males = Age ÷ 2,
Females = Age + 10 ÷ 2.

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What is the clinical significance of this test?

- ESR is not a diagnostic test (not a specific test).
- It is a prognostic test i.e. indicates progress of the disease & effect of treatment.
- Increased ESR?
- Decreased ESR → polycythemia, afibrinogenemia.

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Erythrocytes Sedimentation Rate (ESR)Station

- **The station for this experiment contains:**
- A vertically held tube (for 2 hours) containing anticoagulated blood.
- **What will you do at the station?**
- Without moving the rack or the tubes check how many mm is the height of the clear plasma column (cleared from RBCs that were sedimented).

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Erythrocytes Sedimentation Rate (ESR)Station

- **The station for this experiment contains:**
- A vertically held tube (for 2 hours) containing anticoagulated blood.
- **What will you do at the station?**
- Without moving the rack or the tubes check how many mm is the height of the clear plasma column (cleared from RBCs that were sedimented).

For example: in the given photo ESR =40 mm

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DETERMINATION OF BLOOD GROUPS

Importance of test:

Blood grouping and cross matching is an essential requirement before blood can be transfused into any individual. Blood grouping is also done to settle paternity disputes and other medico-legal problems.

Principle:

The red cells contain a series of antigens (agglutinogens) on their surface, while the plasma contains antibodies (agglutinins). To determine the blood group, the red cells are made to react with sera containing known agglutinins. The slide is then examined under the microscope to detect the presence or absence of agglutination and hemolysis of RBCs. In actual practice most agglutination in transfusion is caused by two antigen-antibody systems: the ABO and Rh system.

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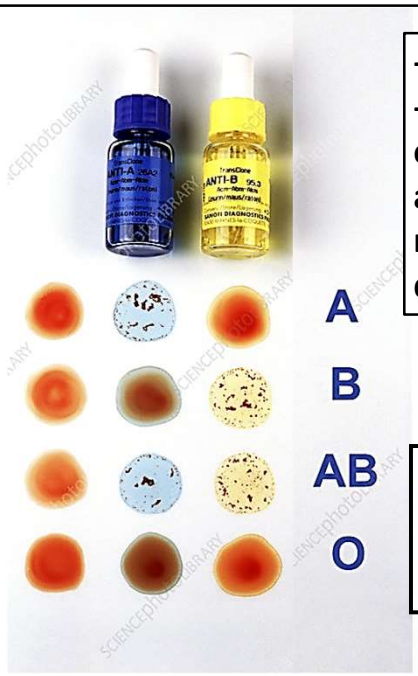

-Wait for 8–10 minutes
-For agglutination to occur, the concentration of agglutinogens and agglutinins has to be about the same. If the difference is too much, there may be a doubtful reaction

Why should you wait for 8–10 minutes before checking for agglutination?
This much time is required for antigen-antibody reaction to occur.

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Materials:

1-Sterile disposable lancet
2- Alcohol and cotton swabs
3- Anti-A , Anti-B and anti-D Sera
4- Clean glass slides
5- Glass marking pencil
1- Mixing sticks

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Procedure:

1. Divide the slide by the marker to 2 parts A and B.
2. Sterile your finger with alcohol and leave to dry.
3. Prick your finger to obtain blood. Place 1 drop of blood on each part of the marked slide.
4. Add1drop of anti-A serum (blue) to the A-side. And1drop of anti-B serum (yellow) to the side B.
5. Mix the blood and serum in each compartment by tilting the slide gently to and fro for 1-2 minutes or by using a mixing stick (one for each side).
6. Observe the slide for any agglutination of red cells. Agglutination is usually visible by the naked eye as dark red clumps of different sizes.
7. If there is no agglutination, keep the slide tilting occasionally and look for agglutination from time to time

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Results:

1. If agglutination occurs on side A only: you have the blood type A.
2. If it occurs on side B only: you have type B.
3. If a reaction occurs on both sides: you have type AB.
4. If no reaction occurs on both sides: you have type O.

Agglutination is observed with Anti: ……

Your blood group is: ……

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Rh Factor:

Procedure:

1- Place 2 drops of your blood with one drop of anti-D on a dry clean microscopic slide.
2- Mix the anti-serum and blood by racking the slide gently to and fro for 2-3 minutes.
3- If agglutination occurs, the blood is Rh positive.

Result:

Agglutination present or absent: ……

You are Rh: ……

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Blood groups Station

- **The station for this experiment contains:**
- Sterile lancet, Alcohol, cotton, glass slides, Antibodies (Anti A, Anti B, Anti-D), A blood-containing tube and pipette to use if you don't want to prick your finger (please keep the safety precautions).
- **What will you do at the station?**
- Add blood drops over 3 sites on the slide, add a drop of each antibody to each drop separately, and check for agglutination (clumping of cells), what is the blood group?

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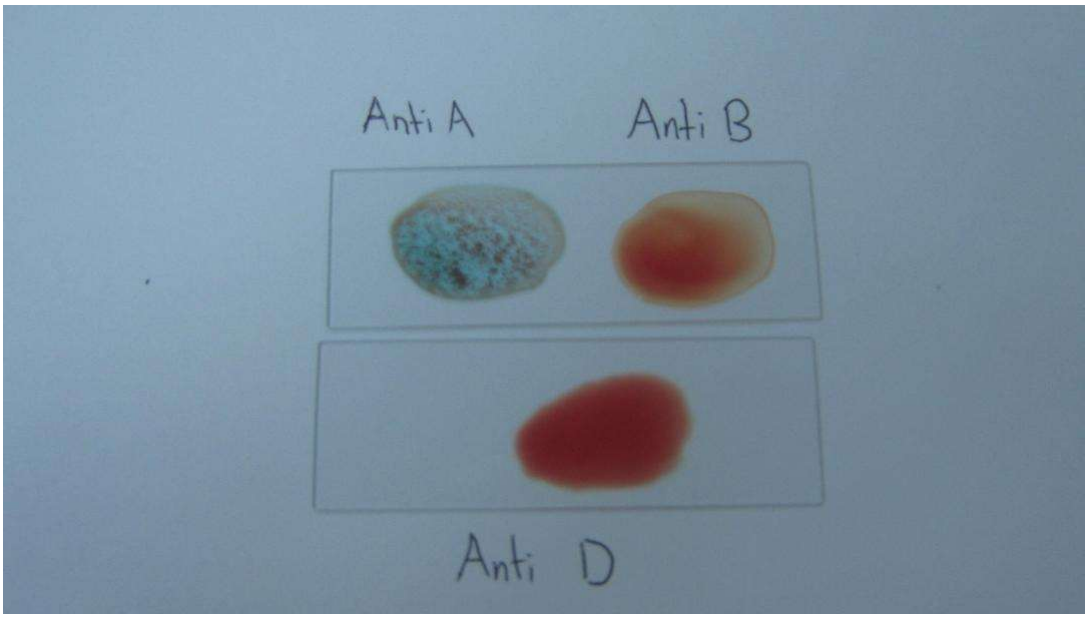

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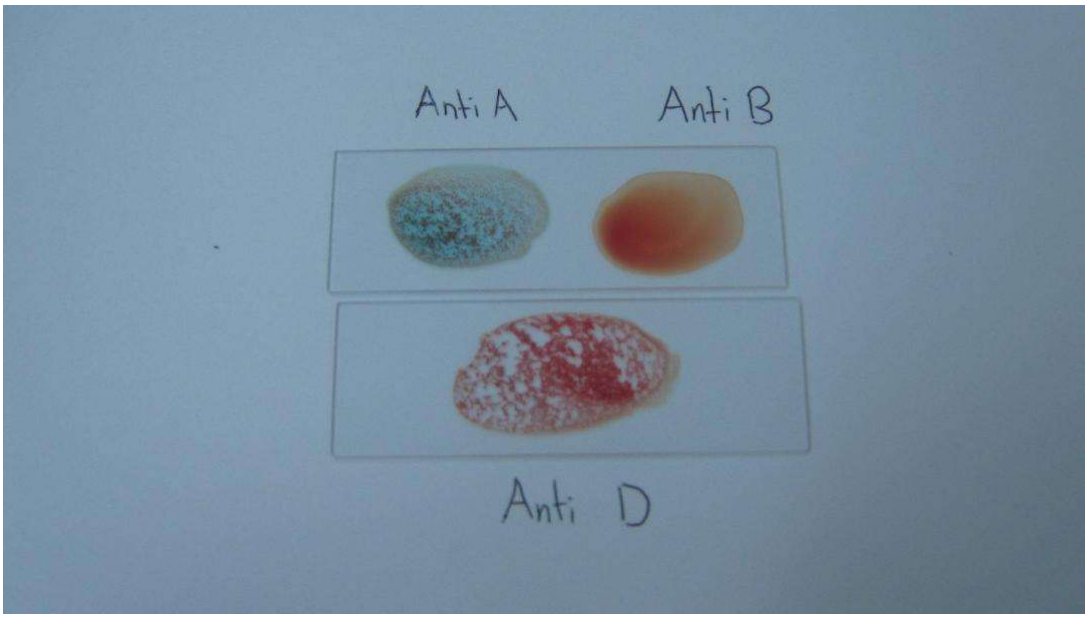

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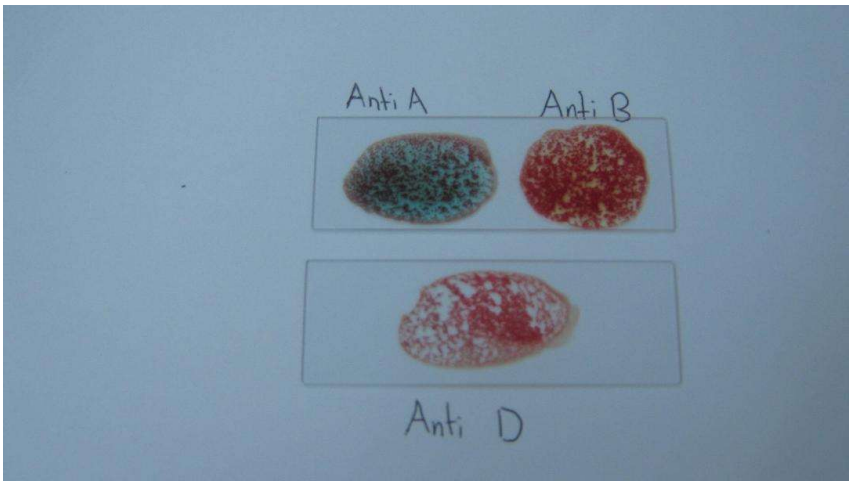

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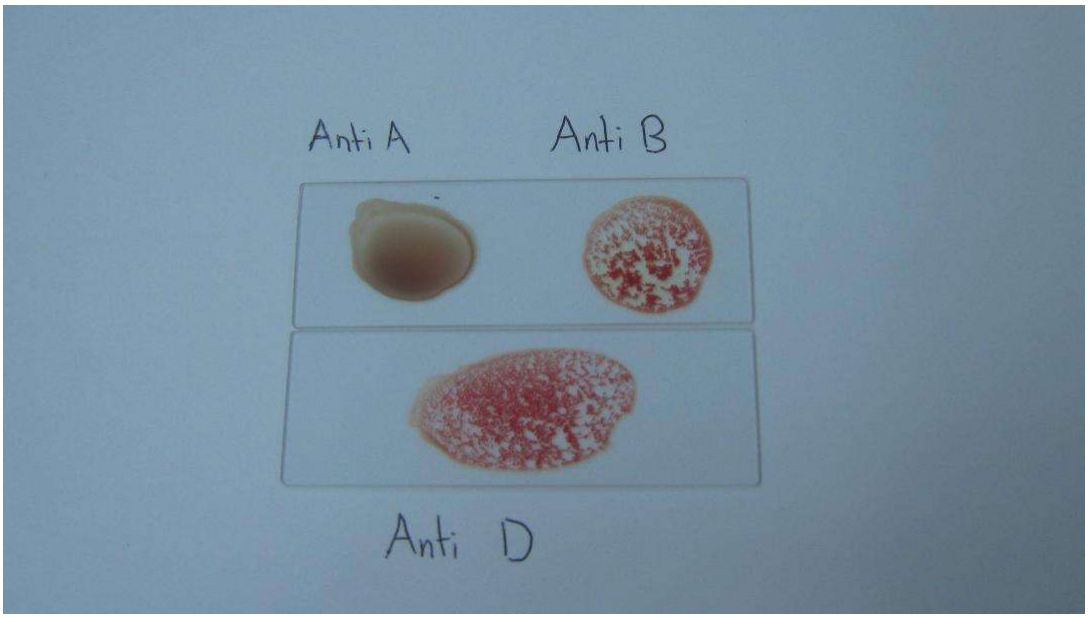

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