Physiology Lab 3

Blood type, bleeding time, clotting time and osmotic fragility test

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Blood Groups

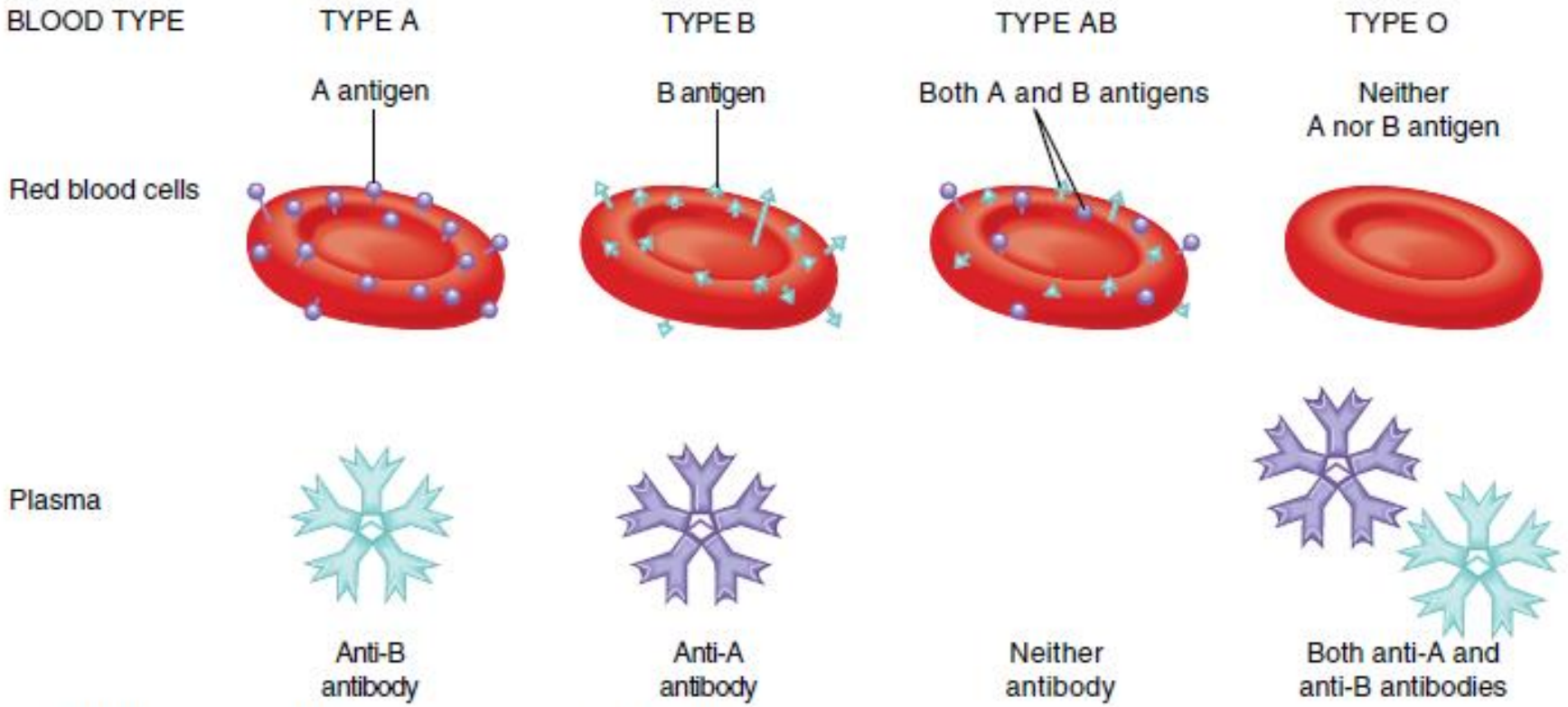
- At least 30 commonly occurring antigens and hundreds of other rare antigens (agglutinogens) composed of glycoproteins and glycolipids are found on the surface of RBCs.
- Each of which can at times cause antigen- antibody reactions leading to immediate or delayed agglutination and hemolysis of RBCs.
- Most of the antigens are weak.
- Two particular types of antigens are likely to cause blood transfusion reactions: the *ABO* system of antigens and the *Rh* system.
- Based on these two systems we have 8 blood groups:
- A +ve, A –ve, B +ve, B –ve, AB +ve, AB –ve, O +ve & O -ve

ABO Blood Group

- The ABO blood group is based on two glycolipid antigens called A and B.

- Blood plasma usually contains antibodies called agglutinins that react with the A or B antigens. These are the anti-A antibody, which reacts with antigen A, and the anti-B antibody, which reacts with antigen B.

- Agglutinins start to appear in the blood within a few months after birth.
- They are formed naturally. Their production is thought to be stimulated when the immune system encounters the "missing" ABO blood group antigens in food or in micro-organisms.


BLOOD TYPE
TYPE A
TYPE B
TYPE AB
TYPE O
A antigen
B antigen
Both A and B antigens
Neither
A nor B antigen
Red blood cells
Plasma
Anti-B
antibody
Anti-A
antibody
Neither
antibody
Both anti-A and
anti-B antibodies

Rh blood group

- There are six common types of Rh antigens, each of which is called an Rh factor. These types are designated C, D, E, c, d, and e.

- The type D antigen is widely prevalent in the population and considerably more antigenic than the other Rh antigens.
- Anyone who has this type of antigen is said to be Rh positive (85% of population), whereas a person who doesn't have type D antigen is said to be Rh negative.

- In contrast to ABO system there is no preformed Anti-D in the Rh–ve individual

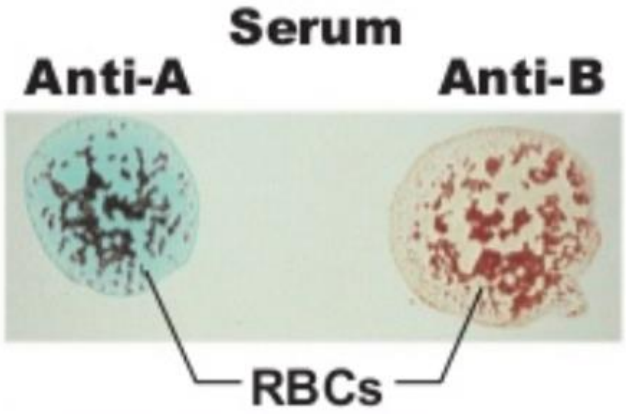
Determination of blood type

1. Prick the tip of a finger with a lancet and put three separate drops of blood on a clean microscopic slide.
2. Add one drop of Anti-A to the first drop, Anti-B to the second drop, and Anti-D to the third drop.
3. Mix well, using separate wooden sticks.
4. The results are read directly from the slide.

- If agglutination occurs in the first drop the blood type is A , if agglutination occur in the second drop the blood type is B, if it occurs in both it is AB and if it doesn't occur in any drop it is type O.
- If agglutination occurs in the Rh drop the blood is considered as Rh+ve. (This reaction might take some time to develop)

- The strength of agglutination reaction is not the same in all people, so in some cases it may be necessary to examine the slide under the microscope to look for agglutination.


Anti-A
MONOCLONAL ANTIBODIES
Store at 2-8°C
DO NOT FREEZE
M L No. KD-522
Anti-B
MONOCLONAL ANTIBODIES
Store at 2-8°C
DO NOT FREEZE
M L No. KD-522
Anti-D
Store at 2-8°C
DO NOT FREEZE
M L No. KD-522

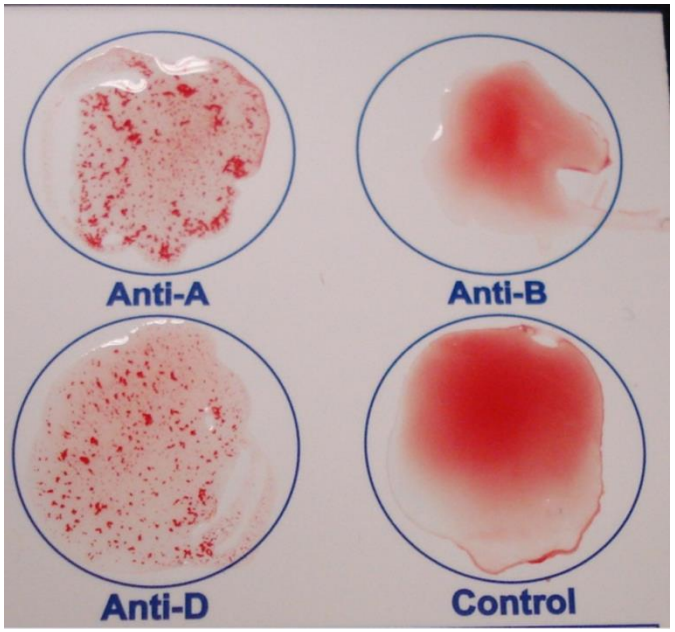


Type AB

Type A

Type B

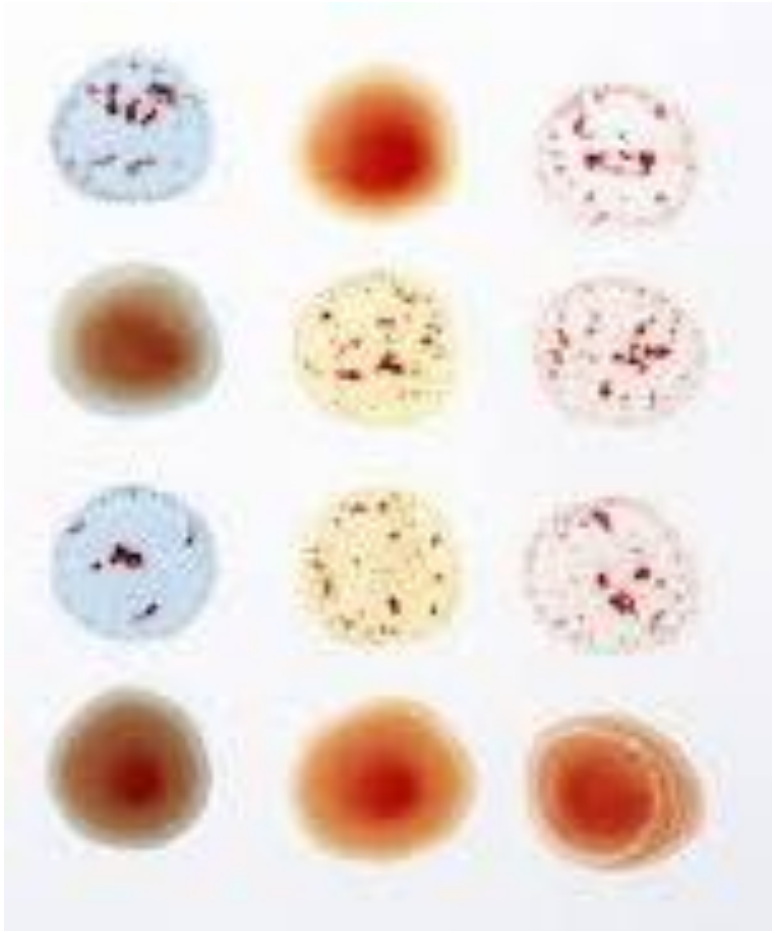
Type O



Type A +ve

Type AB+ve is considered a universal recipient
Type O-ve is considered a universal donor

What is the type of blood in each test presented below?



1.

2.

3.

4.

Bleeding time

- Hemostasis is prevention of blood loss from circulatory system.
- Depends on the integrity of blood vessels, platelets and clotting factors.

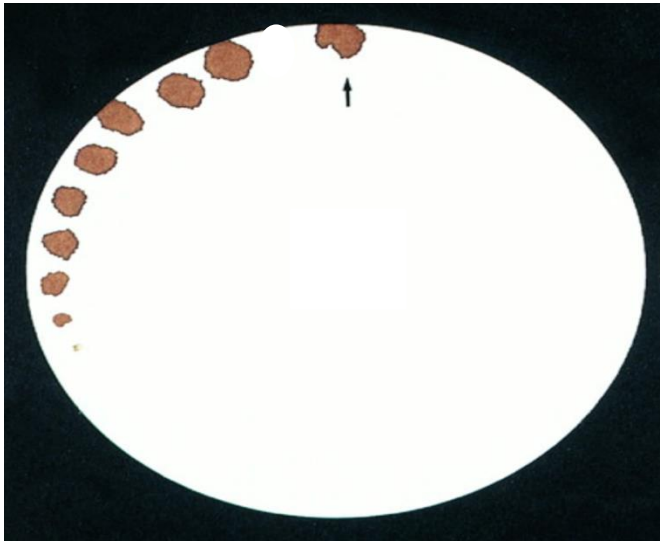
The hemostatic response to vascular injury is achieved by several mechanisms:

1. Vasoconstriction
2. Formation of a platelet plug
3. Formation of a blood clot

- A **bleeding time** is used to evaluate the second phase of hemostasis, which involves adherence of the platelets to the injured vessel, platelet activation and aggregation (formation of a plug).

1. Clean the tip of the finger or the ear lobe with alcohol.
2. Puncture the skin with a special lancet. The wound should be 3–4 mm deep.
3. Wipe the blood drop by a filter paper every 30 seconds
4. Repeat until no more blood is absorbed by the filter paper.
5. Record the time.

- ✓ The time measures how long it takes for a platelet plug to form.
  - ➢ Normal range: 3-5 minutes
- ✓ It increases when the platelets count is low (thrombocytopenia), platelet function is abnormal or with the use of aspirin .
- Disadvantages: Insensitive, Invasive & operator dependent.



Clotting time

- It measures the time required for a blood sample to coagulate in vitro. Clotting time depends on the availability of coagulation factors.

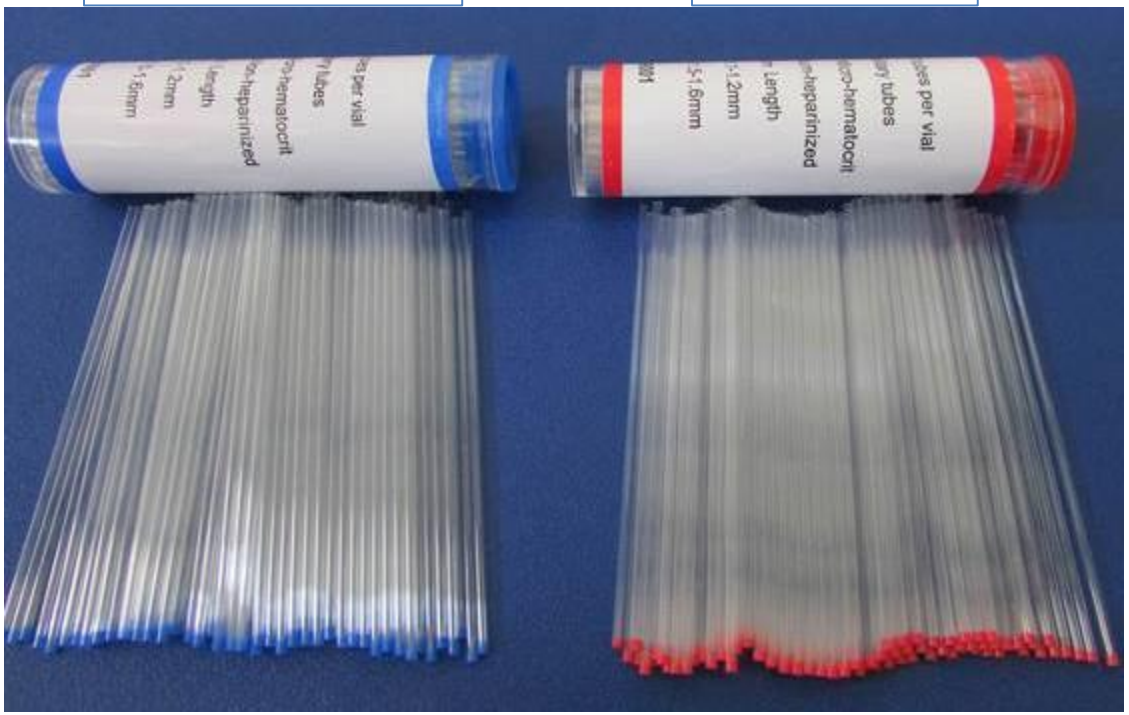
1. Clean the tip of the finger with alcohol then prick it with a lancet.
2. Draw blood into non-heparinized capillary tubes.
3. After 2 minutes, start breaking the capillary tubes to see whether a thread of coagulated blood is formed between the two broken ends.
4. It is preferred to calculate the clotting time from the average of two capillary tubes.

- Normal value is 6-10 minutes.

- It is prolonged in conditions like hemophilia, vitamin K deficiency, liver diseases, and warfarin overdose.

Non-heparinized

Heparinized



Prothrombin Time & Activated Partial Thromboplastin Time

- Two laboratory tests are used commonly to evaluate coagulation disorders and to monitor patients taking certain medications :

1. Prothrombin Time (PT) which measures the integrity of the extrinsic & common pathways
2. Activated Partial Thromboplastin Time (aPTT), which measures the integrity of the intrinsic & common pathways

Prothrombin Time

- The PT test is performed by adding Tissue Factor and calcium ions to the patient's plasma, the time required for coagulation to happen is then measured.
- It evaluates the presence of factors VII, V, X, prothrombin, and fibrinogen.
- A PT within 11 -15 second is considered normal.
- A prolonged prothrombin time indicates:

1. Deficiency in factors VII, X, V, prothrombin, or fibrinogen.
2. Vitamin K deficiency
3. Liver disease
4. Warfarin overdose

Activated Partial Thromboplastin Time

- Activating substances (kaolin and cephalin) are added to the plasma to start the intrinsic pathway of the coagulation cascade.
- An aPTT within 33-35 second is considered normal.
- A prolonged aPTT indicates:

1. Deficiency in factors XII, XI, VIII, IX, prothrombin, or fibrinogen.
2. Heparin overdose
3. Vitamin K deficiency
4. Liver disease

Osmotic fragility

- when RBCs reside in an isotonic medium, the intracellular and extracellular fluids are in osmotic equilibrium across the cell membrane, and there is no net influx or efflux of water.

- When RBCs reside in a hypotonic medium, a net influx of water occurs so the cells swell and the integrity of their membranes is disrupted resulting in **hemolysis**

- When RBCs reside in a hypertonic media , a net efflux of water occurs so the cells lose their normal biconcave shape, undergoing collapse.

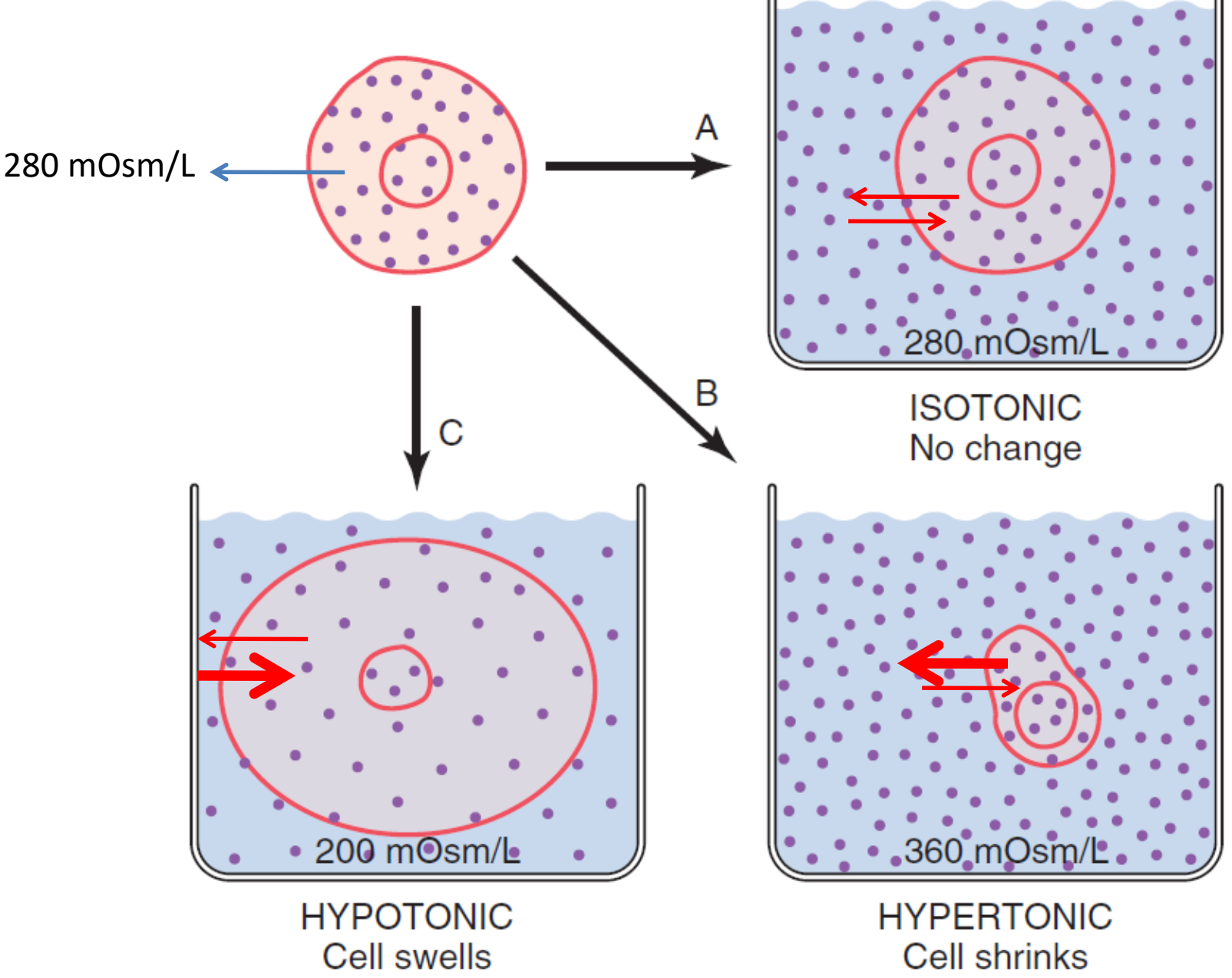


Figure 25-5. Effects of isotonic (*A*), hypertonic (*B*), and hypotonic (*C*) solutions on cell volume.

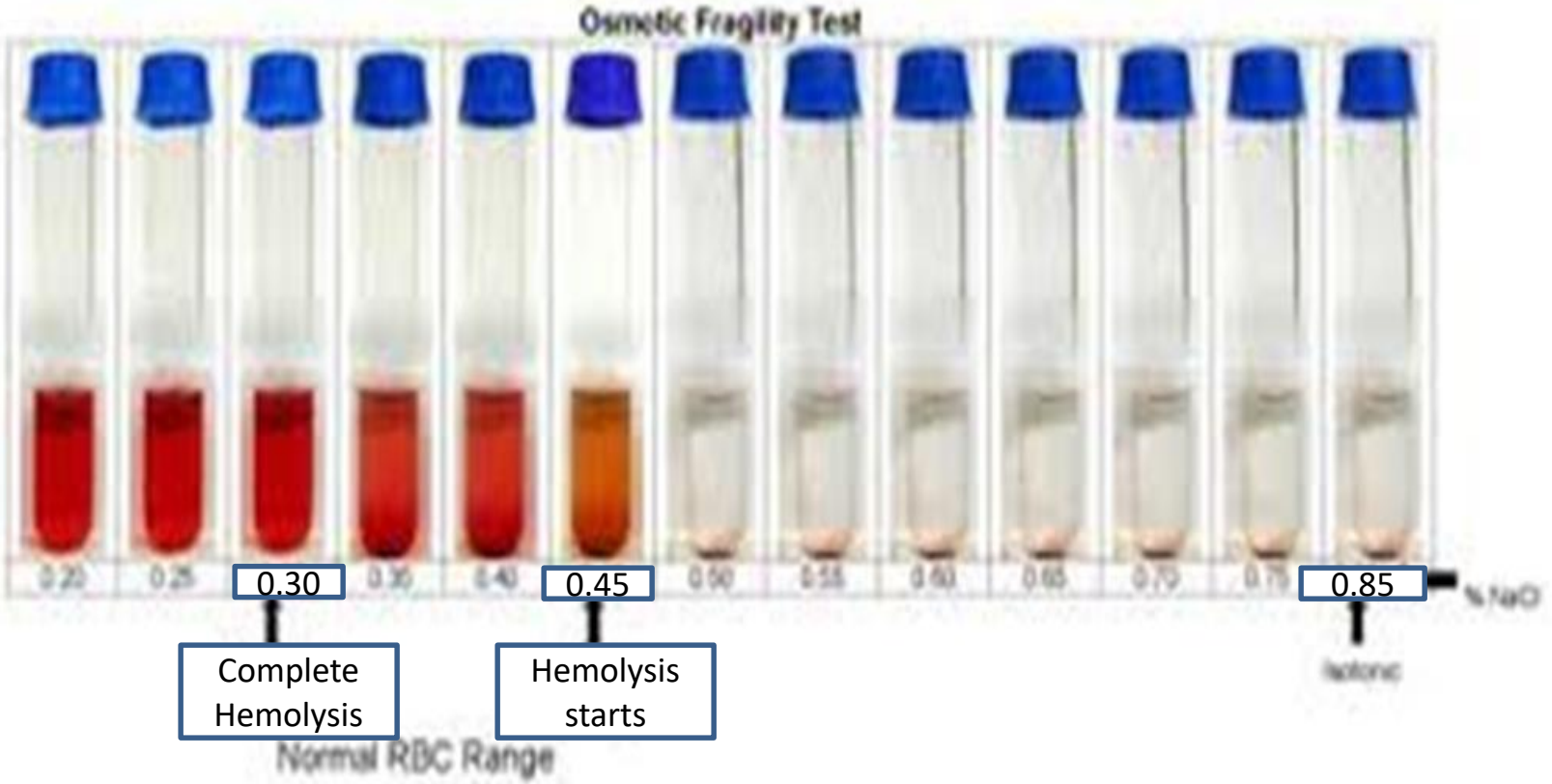
Osmotic fragility test

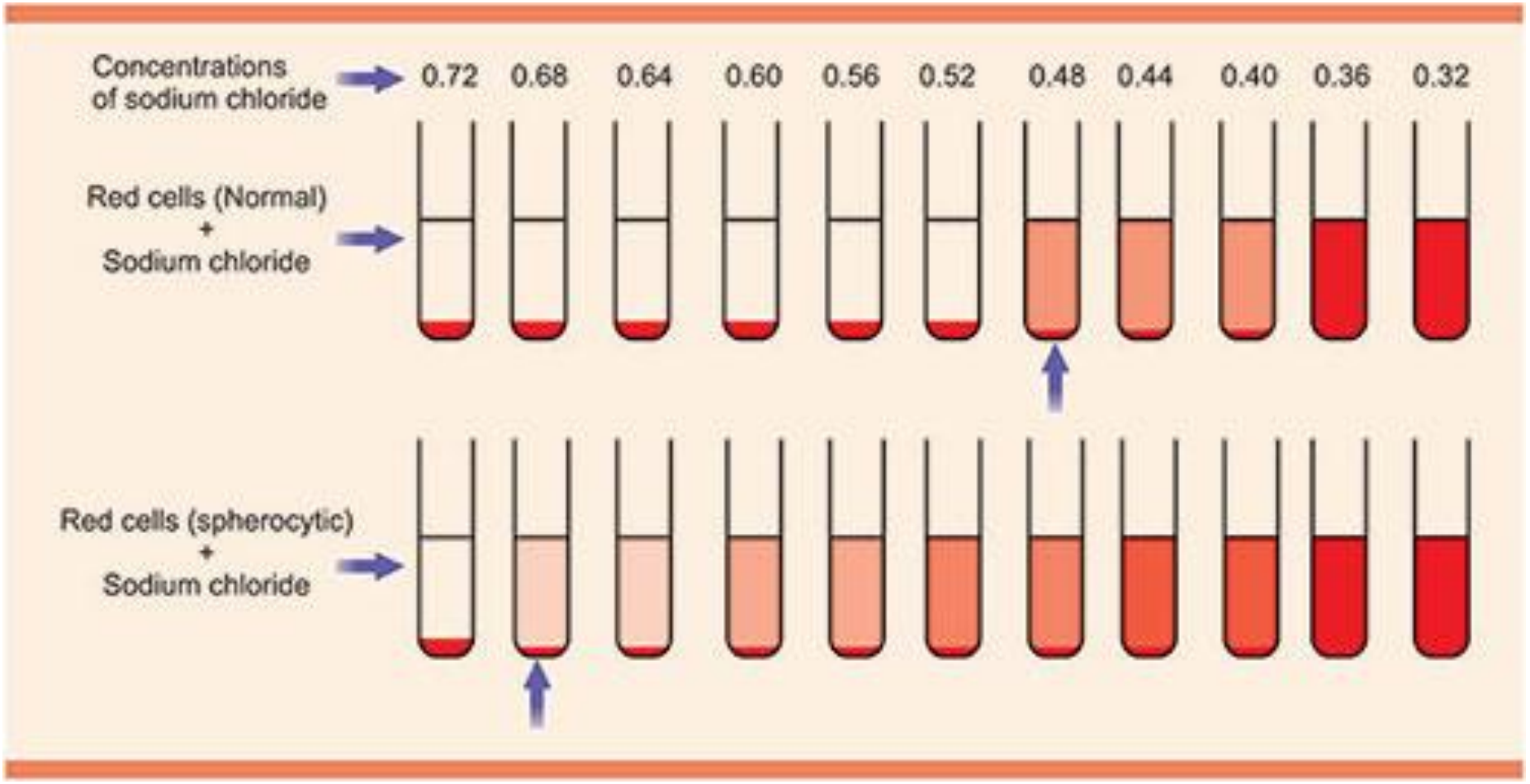
- A test designed to measures red blood cell's resistance to hemolysis when exposed to a series of increasingly dilute saline solutions.
- The susceptibility of RBCs to hemolysis is a function of:
  - Surface area to volume ratio.
  - Cell membrane composition and integrity
- This test is performed to diagnose certain medical conditions like thalassemia and hereditary spherocytosis .

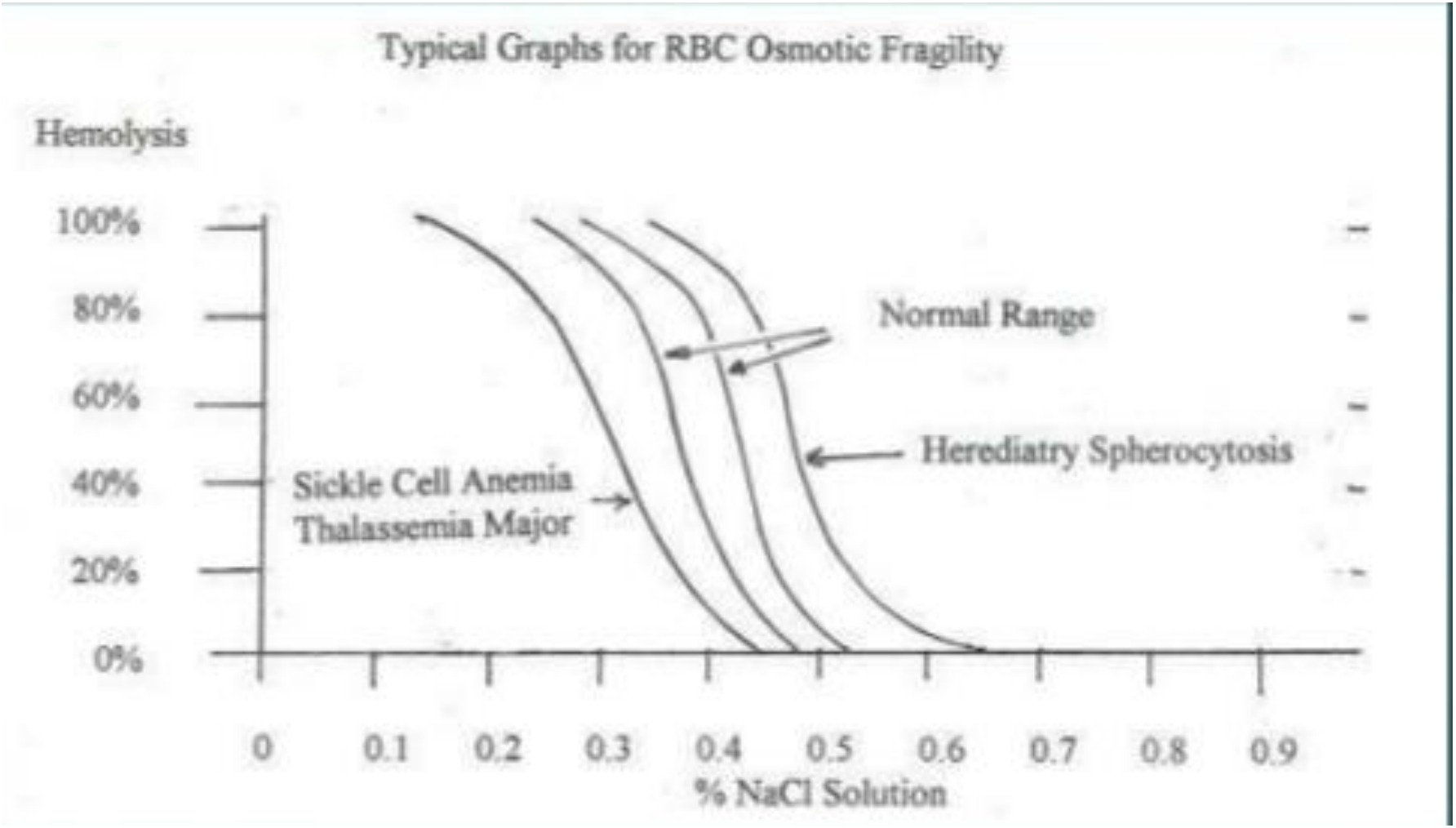
- The procedure:

1. Put labeled centrifuge tubes in a rack.
2. Prepare NaCl solutions of different concentrations starting from 0.9% NaCl till 0.2% NaCl.
3. Add 10 ml of each solution to a different tube then add one drop of blood to each tube.
4. Shake each tube well and allow them to stand for 20 minutes. After 20 minutes, the tubes are centrifuged for 10 minutes at maximal speed.
5. Transfer supernatant fluid from each tube into spectrophotometer cuvettes.
6. The percentage of hemolysis for each solution is calculated. Then the results are plotted against the NaCl concentrations, this yields an osmotic fragility curve which is then compared to a standard curve.

- In this example
- From 0.85% to 0.45% there is no hemolysis.
- At the concentration of 0.45% hemolysis starts and the solution becomes red in color, but there are some settled RBCs in the tube.
- At the concentration of 0.30%, the solution is clear red and there are no settled RBCs (complete hemolysis).




Concentrations of sodium chloride
0.72
0.68
0.64
0.60
0.56
0.52
0.48
0.44
0.40
0.36
0.32
Red cells (Normal) + Sodium chloride
Red cells (spherocytic) + Sodium chloride


Typical Graphs for RBC Osmotic Fragility
Hemolysis
100%
80%
60%
40%
20%
0%
Normal Range
Herediatry Spherocytosis
Sickle Cell Anemia
Thalassemia Major
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
% NaCl Solution

- Decreased red cell fragility (increased resistance to hemolysis) is seen with the following conditions:
  - ➢Thalassemia.
  - ➢Iron deficiency anemia.
  - ➢Sickle cell anemia

  ✓These cells have a high surface area: volume ratio

- Increased red cell fragility (increased susceptibility to hemolysis) is seen in the following conditions:
  - ➢Hereditary spherocytosis
  - ➢Autoimmune hemolytic anemia

  ✓These cells have a low surface area: volume ratio