Blood & Lymphoreticular System Physiology Lab

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The Experiments

- Packed cell volume (PCV)
- Erythrocyte Sedimentation rate (ESR)
- Osmotic Fragility Test

- Reticulocyte count
- Differential Leukocyte count (DLC)

- Blood Type
- Bleeding Time
- Clotting Time

Blood

- Blood is a body fluid that has two components:

1. Plasma , a watery liquid extracellular matrix that contains dissolved proteins, electrolytes, gases and nutrients

2. Formed elements, which are cells and cell fragments.
   - Red blood cells (RBC)
   - White blood cells(WBC)
   - Platelets.

Red Blood Cells (RBCs)

- Normal RBCs are biconcave discs, they have few organelles and no nuclei.
- A major function of RBCs is to transport hemoglobin, which in turn carries oxygen from the lungs to the tissues.
- The average number of RBCs in healthy males is 5,200,000/mm^3 (±300,000) and in healthy females 4,700,000/mm^3 (±300,000)

- Normal blood hemoglobin content is ~14.0 g/dL in the adult female and ~15.5 g/dL in the adult male.

- The number of RBCs is regulated within narrow limits, so that oxygen is transported adequately to the tissues and at the same time the cells do not become so numerous that they impede blood flow.

Anemia is deficiency of hemoglobin in the blood, which can be caused by either too few RBCs or too little hemoglobin in the cells.

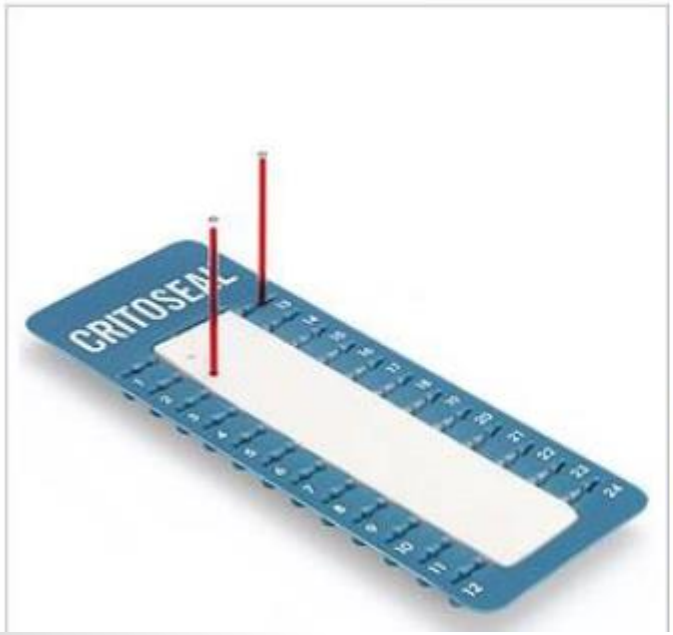
1. Increased RBC loss
   a) Blood loss due to internal or external bleeding
   b) Increased RBC destruction (hemolytic anemia)
2. Decreased RBC production
   - Nutritional deficiencies
   - Bone marrow failure
   - Chronic renal failure

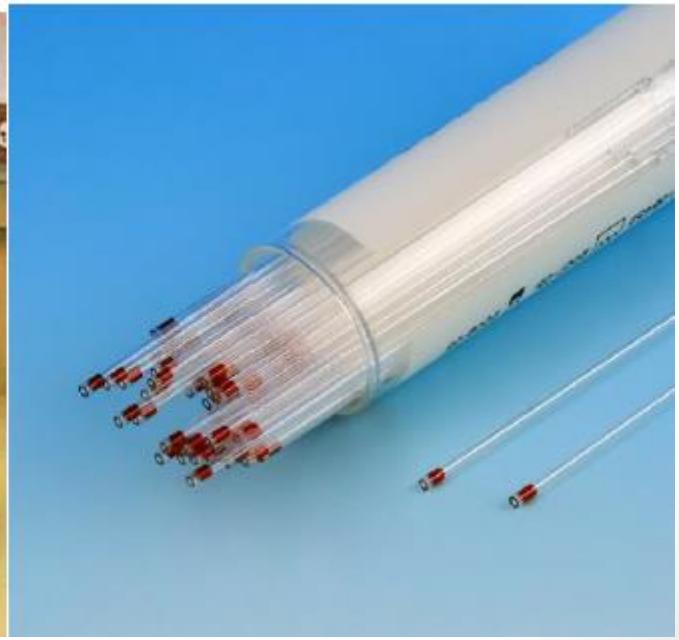
Polycythemia: is increased red blood cell mass

1. Secondary Polycythemia, RBC count commonly rises about 30% above normal, triggered by tissue hypoxia associated with increased erythropoietin
   - Living at high altitudes
   - Cardiac or pulmonary diseases
2. Polycythemia Vera, RBC count commonly rises about 50% above normal. Erythropoietin is normal or low

Packed Cell Volume (PCV)

- PCV is the ratio of the volume of packed red cells to the total blood volume.
  - Adult males: 40–54% (avg = 47%).
  - Adult females: 38–46% (avg = 42%)
- It decreases in cases of anemia and increases in polycythemia and dehydration.



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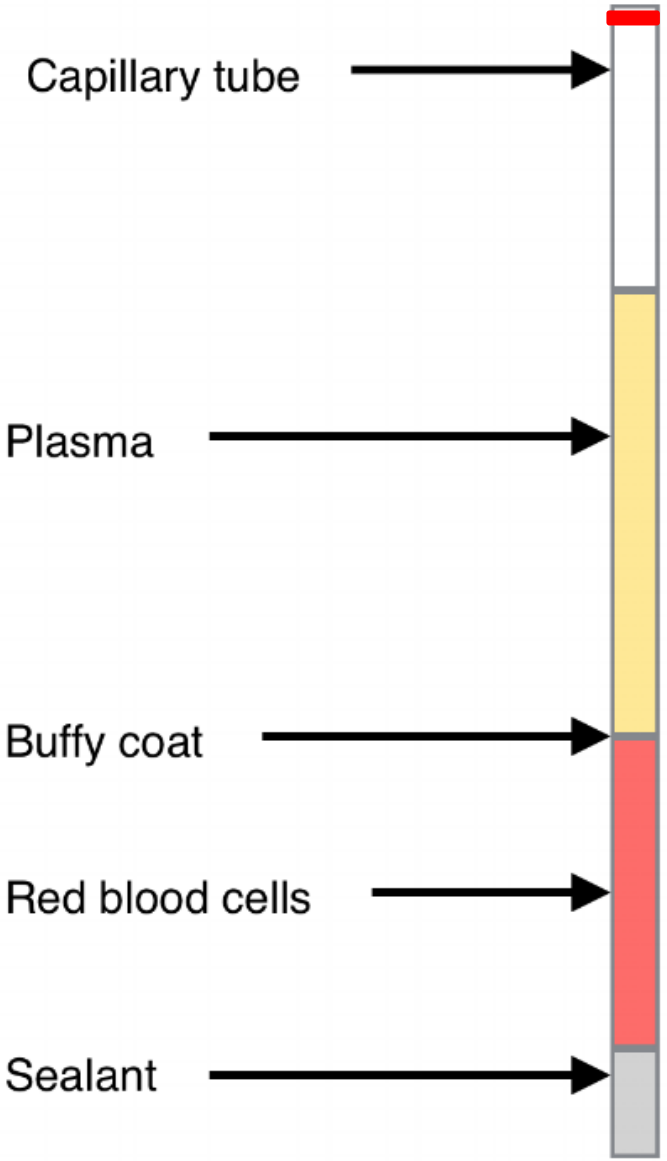
The procedure

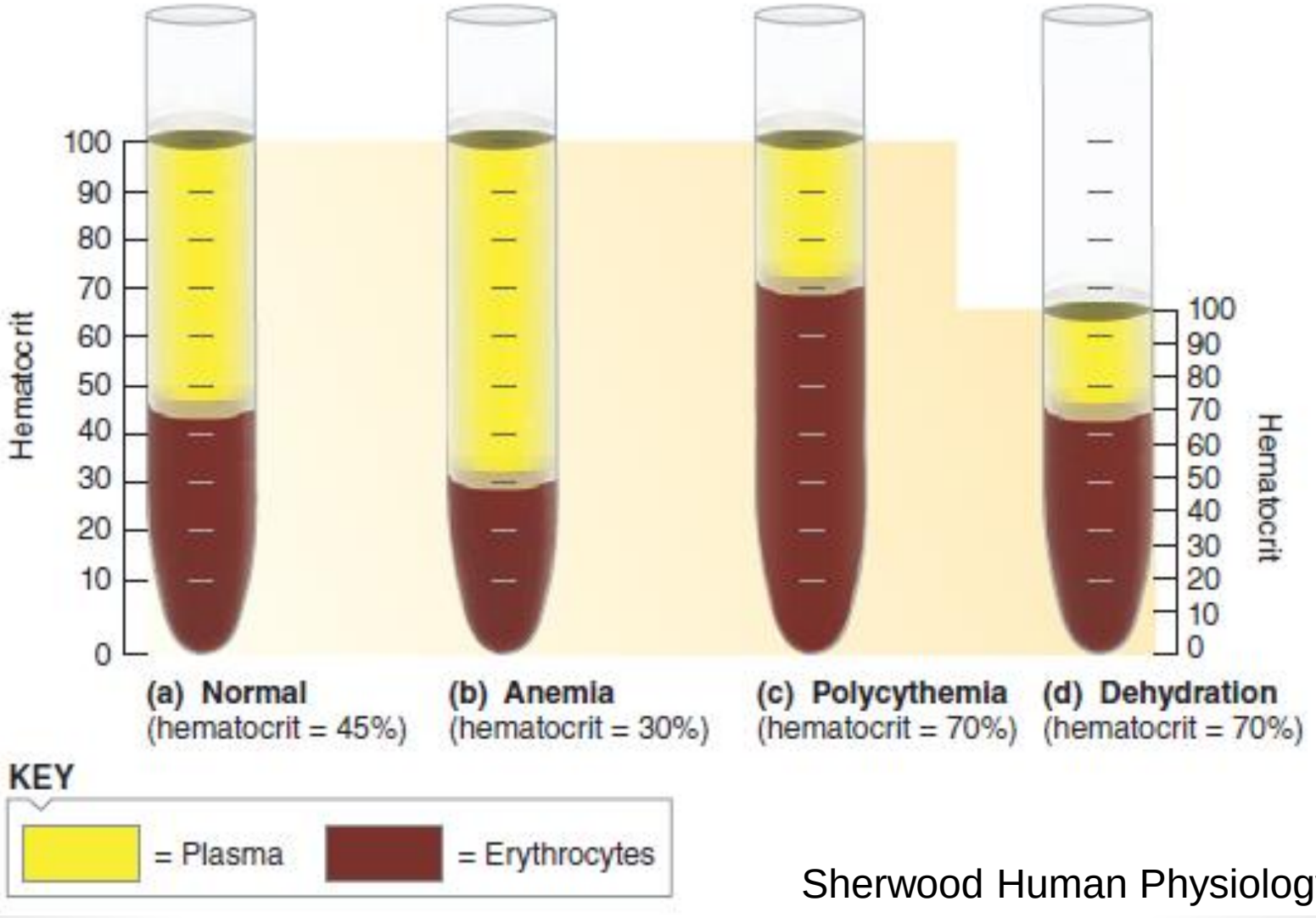
- A heparinized capillary tube (red tip), is filled with blood by capillary action to about ¾ of its capacity.
- The blood-filled end is sealed with clay, and placed in a slot in micro-centrifuge for 5 minutes.

- The RBCs become packed at the bottom of the tube.
- Measure the height of RBC column and the total height of a sample using a ruler.

- The PCV is then calculated according to the following formula:
- $PCV = \frac{\text{RBC height}}{\text{Total height}} x100$



Results



Sherwood Human Physiology

Erythrocyte Sedimentation Rate (ESR)

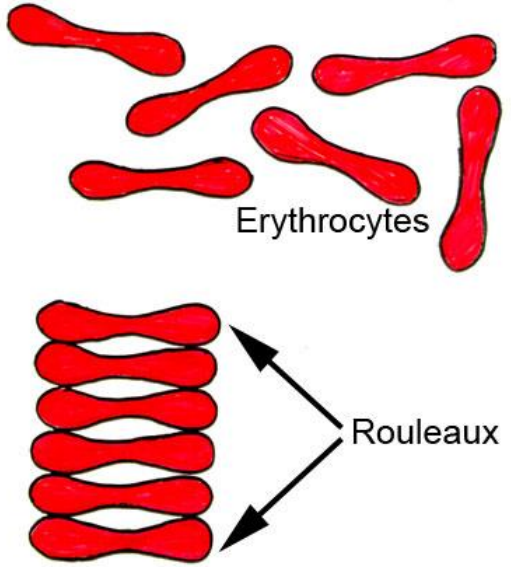
- The rate at which red blood cells settle out when anticoagulated whole blood is allowed to stand for a period of one hour.

- The ESR is a simple, sensitive but **non-specific** screening test that indirectly measures the presence of inflammation in the body.

- It's increase reflects the tendency of red blood cells to settle more rapidly in the presence of inflammatory conditions, usually because of increases in plasma fibrinogen, immunoglobulins, and other acute-phase reaction proteins.
- Changes in red cell shape or numbers may also affect the ESR.

RBCs sedimentation

- The RBCs sediment because their density is greater than that of plasma. The sedimentation increases if stacking of RBCs (rouleaux formation) happens.
  - Rouleaux formation is possible because of the discoid shape of RBCs

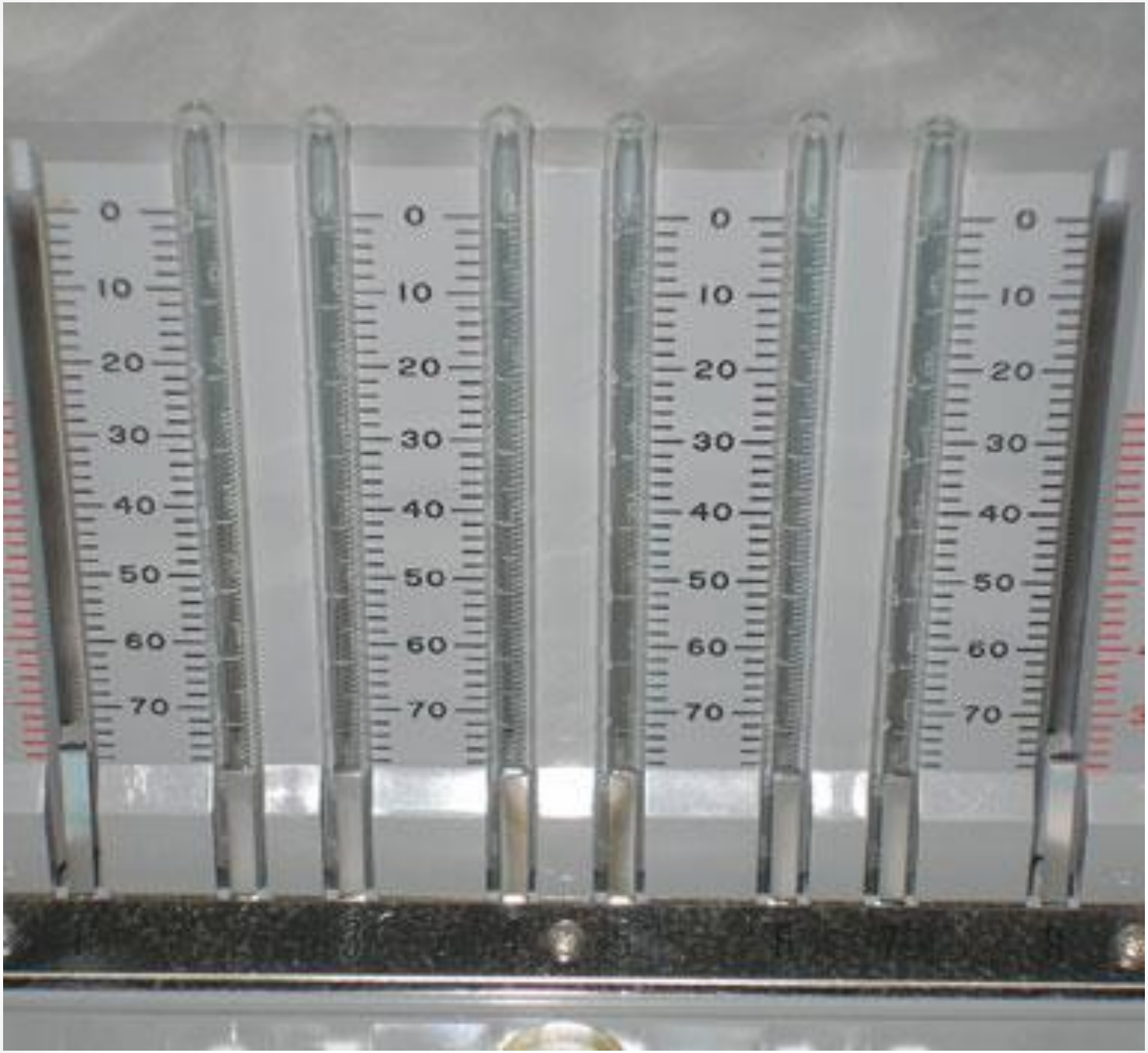


- Normally, RBCs have negative charges on the outside of the cells, which cause them to repel each other and decreases or prevents rouleaux formation.

- Many plasma proteins have positive charges and can neutralize the negative charges of the RBCs, which allows for the formation of the rouleaux.
- Therefore, an increase in plasma proteins (present in inflammatory conditions) will increase the rouleaux formations, which settle more readily than single red blood cells leading to increased ESR during inflammation

The procedure

- ESR tubes have the zero mark on top
- EDTA anticoagulated blood is drawn into the ESR tube till the zero mark
- The tube is placed in its rack in a strictly vertical position for **1 hour** at room temperature
- the RBCs – under the influence of gravity - settle out from the plasma.
- This will leave a clear, straw-colored fluid (plasma) at the top of the tube.
- The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the RBC column after one hour **(mm/hr**).


0
10
20
30
40
50
60
70
0
10
20
30
40
50
60
70
0
10
20
30
40
50
60
70
0
10
20
30
40
50
60
70

At the beginning of the experiment

After one hour

- # Normal ESR values
  - Adult males < 15mm/hr
  - Adult females < 20mm/hr

- High ESR
  - Inflammation
  - Anemia
  - Old age
  - Pregnancy
  - Technical factors: tilted ESR tube, high room temperature.

- **Some interferences which decrease ESR:**
- Abnormally shaped RBC (sickle cells and spherocytosis)
- Polycythemia
- Technical factors: low room temperature, delay in test performance (>2 hours), clotted blood sample

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

- 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**

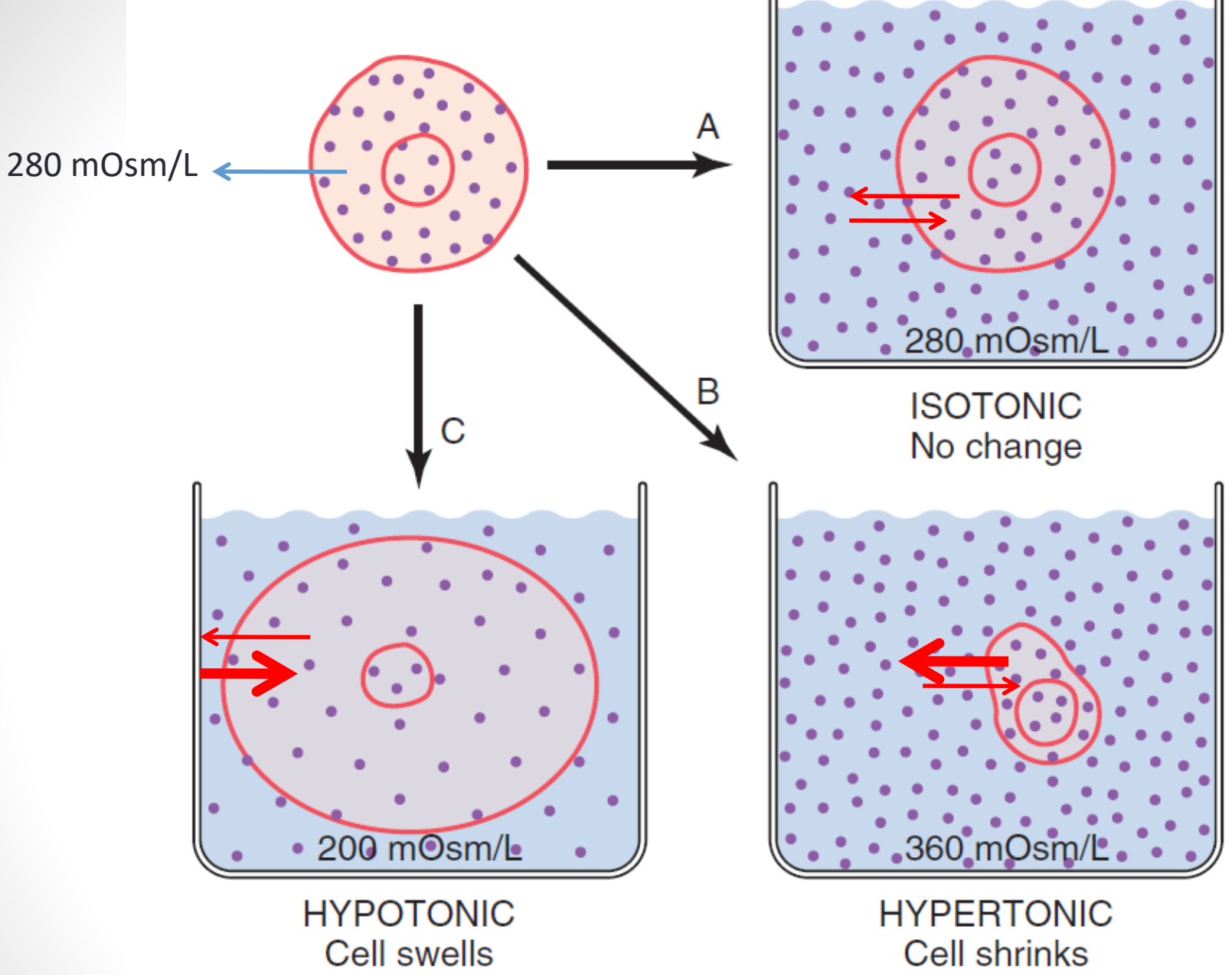


Figure 25-5. Effects of isotonic (*A*), hypertonic (*B*), and hypotonic (*C*) solutions on cell volume.

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.

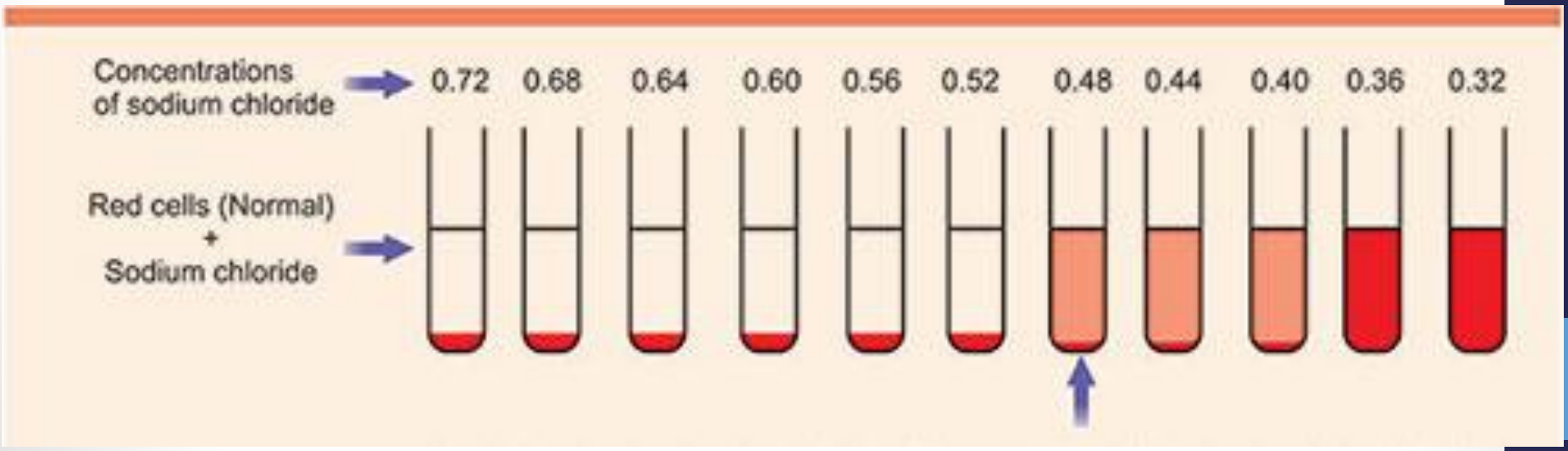
- The susceptibility of RBCs to hemolysis is determined by:
  - **Surface area to volume ratio.**
  - Cell membrane composition and integrity

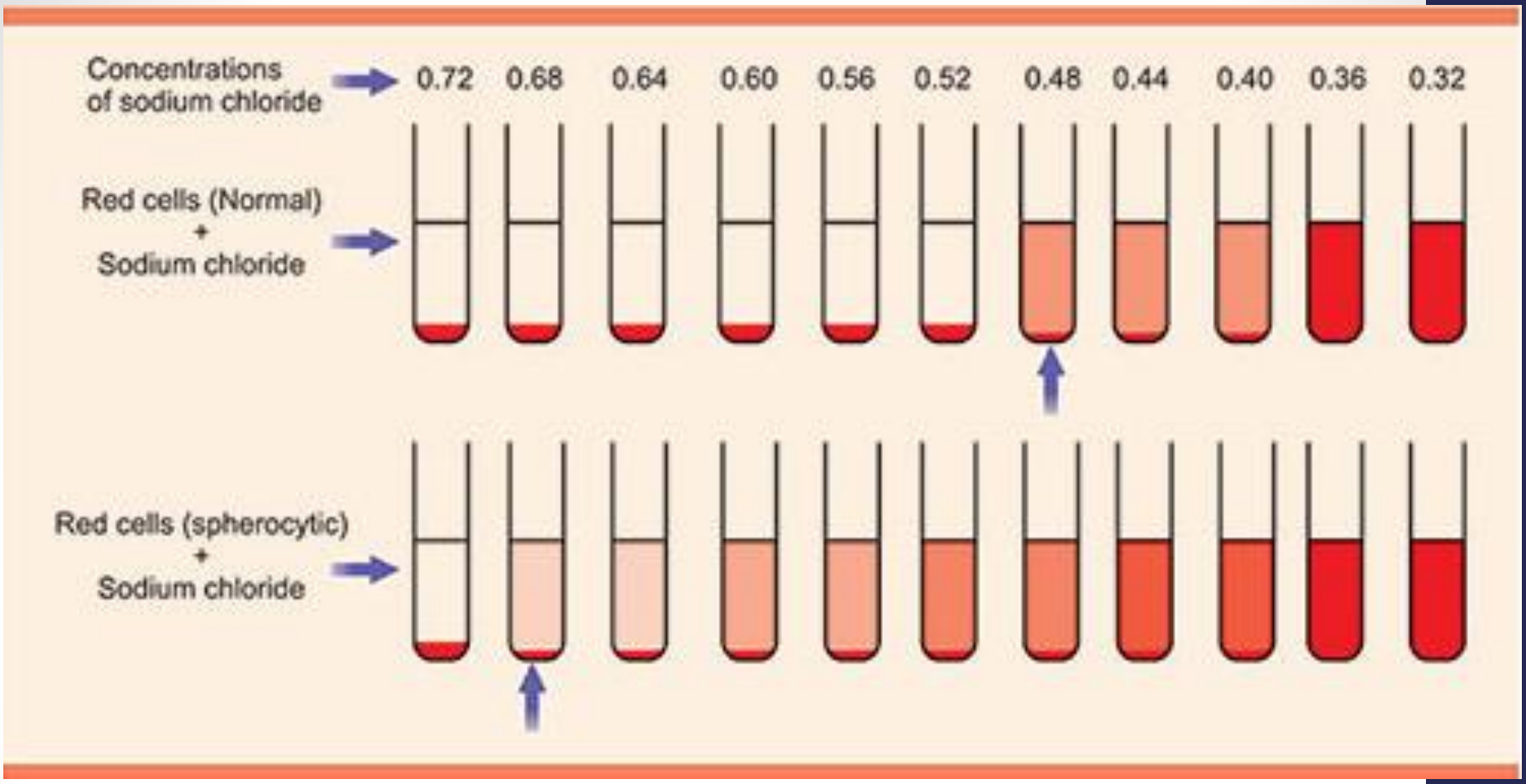
- This test is mainly used to diagnose hereditary spherocytosis.

Performing the test

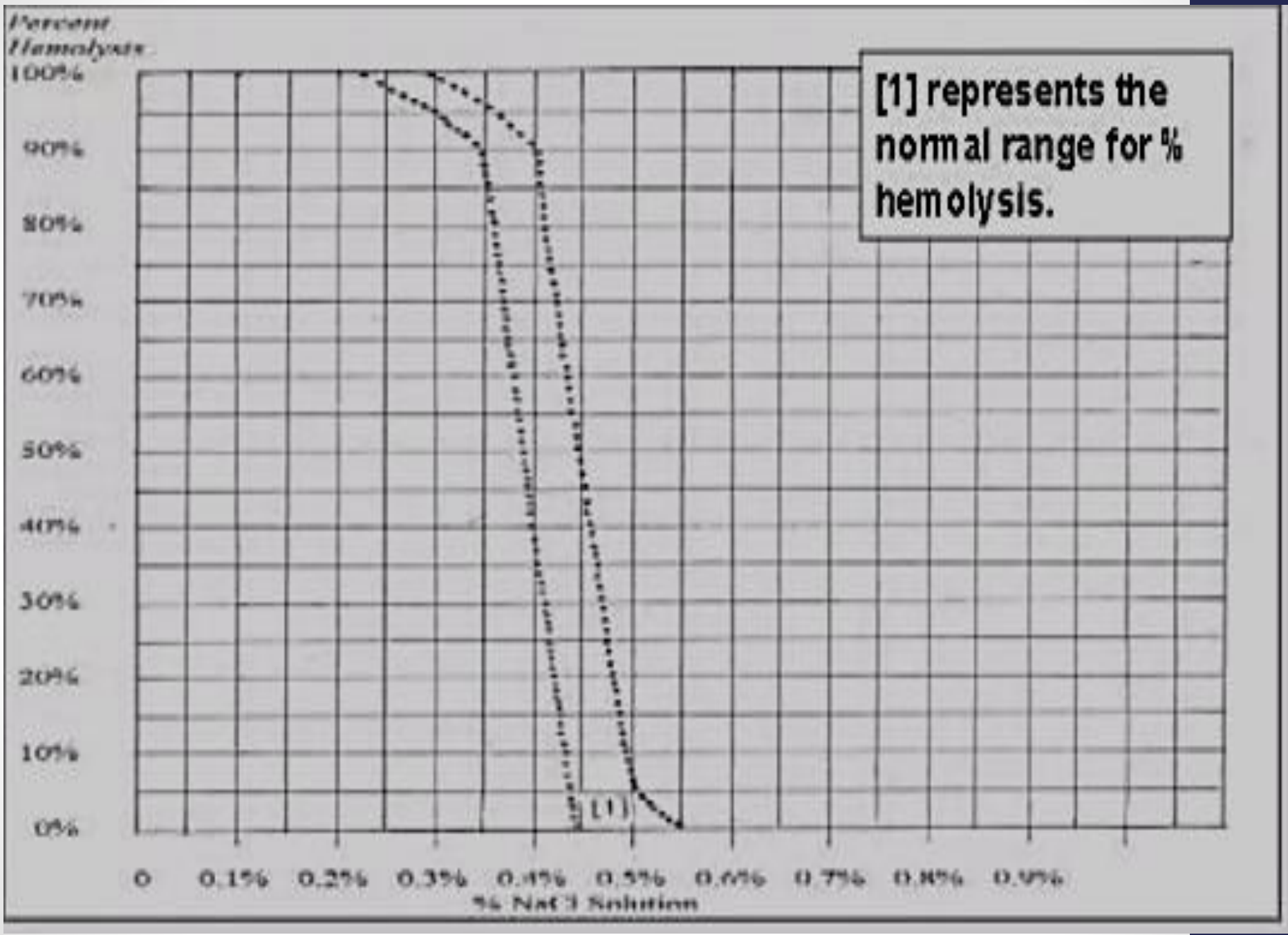
1. Put labeled centrifuge tubes in a rack.
2. Prepare NaCl solutions of different concentrations starting from 0.9% NaCl till 0.2% NaCl.
3. Add 10 ml of each solution to a different tube then add one drop of blood to each tube.
4. Shake each tube well and allow them to stand for 20 minutes. After 20 minutes, the tubes are centrifuged for 10 minutes
   - Normal findings :
     - Hemolysis begins ~ 0.5% NaCl
     - Hemolysis complete ~ 0.3% NaCl
5. Transfer supernatant fluid from each tube into spectrophotometer cuvettes
6. The absorbance is then measured at 540 nm and used to calculate the percentage of hemolysis for each solution.
7. The results are plotted against the NaCl concentrations, this yields an osmotic fragility curve which is then compared to a standard curve.

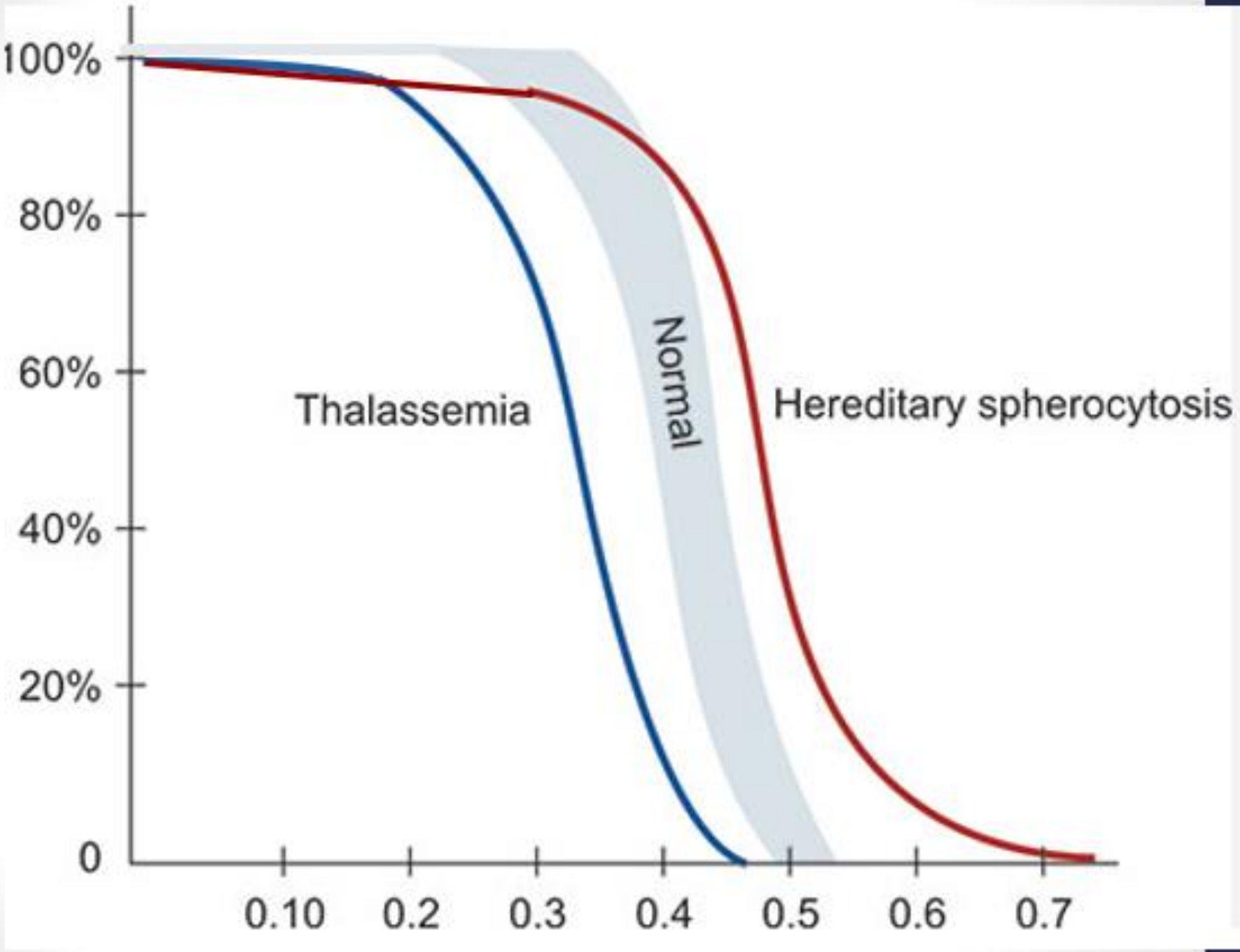
- From 0.7% to 0.5% there is no hemolysis.
- At the concentration of 0.48% hemolysis starts and the solution becomes red in color, but there are some settled RBCs in the tube.
- At the concentration of 0.36%, the solution is bright red and there are no settled RBCs (complete hemolysis).





- With spherocytosis hemolysis starts at the concentration of 0.68% which means RBCs can't resist hemolysis as they normally do (they are more fragile)


Percent Hemolysis
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
[1] represents the normal range for % hemolysis.
[1]
0
0.1%
0.2%
0.3%
0.4%
0.5%
0.6%
0.7%
0.8%
0.9%
% NaCl Solution


100%
80%
60%
40%
20%
0
0.10
0.2
0.3
0.4
0.5
0.6
0.7
Thalassemia
Normal
Hereditary spherocytosis

- Increased red cell fragility (increased susceptibility to hemolysis) is seen in the following conditions:
  - Hereditary spherocytosis
  - Autoimmune hemolytic anemia
  - Toxic chemicals, poisons, infections, and some drugs.
  - Severe burns.

✓ These cells have a low surface area: volume ratio

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

✓ These cells have a high surface area: volume ratio