

### Department of Anesthesia Techniques



# Fragility test (RBC fragility test)

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2<sup>nd</sup> course

Lec.4

- \*The Osmotic Fragility Test: is used to measure erythrocyte resistance to hemolysis while being exposed to varying levels of dilution of a saline solution.
- \* When erythrocytes are exposed to a hypotonic environment, water enters the cell and causes swelling and eventual lysis.
- \* Hemolysis:- breaking down (bursting) of red cells resulting in release of Hb into the surrounding fluid.

- The sooner hemolysis occurs, the greater the osmotic fragility of the cells.
- \* Isotonic Solution: A solution that has the same salt concentration as cells and blood.
- \* Hypertonic Solution: a solution that has a high solute concentration and low water concentration compared to body fluids.
- \* A Hypotonic Tonic Solution is a solution that has a low solute concentration and high water concentration compared to body fluids.

#### **PRINCIPLE**

- Patient sample and normal red cells are placed in a series of graded strength NaCl solution and resulting hemolysis is compared to a 100% standard.
- The normal red cells can remain suspended in normal saline (0.9% NaCl solution) for hours without rupturing or any change in their size or shape.

## **Material & Instruments**

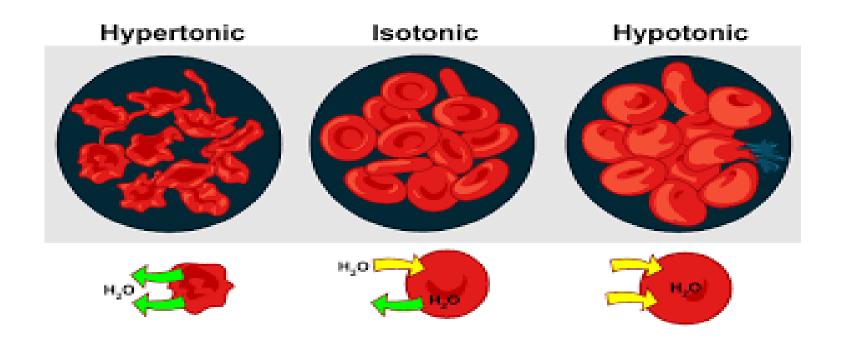
- 1. Test tubes
- 2. NaCl with different concentrations
- 3. Heparinized venous blood
- 4. Distill water

## **Procedure**

- 1- Prepare different concentrations of NaCl start with 0 concentration i.e. put only D.W. then put in the rest of the test tubes the following concentrations of NaCl 0.3, 0.35 0.4, 0.45, 0.5, 0.9 %
- 2- Put a few drops of heparinized blood in each test tube and read the results visually.
- 3-Incubate at room temperature for 30 min. Mix again and centrifuge 1000g for 5 min.
- 4-Remove the supernatants carefully without including cells and estimate the amount of lysis in a photometer at 540 nm. or colorimeter provided with a yellow-green filter.



- \*Red cells in hypertonic saline. In hypertonic solutions, the RBCs, shrink due to movement of water out of the cells.
- \* Red cells in hypotonic saline. In hypotonic saline, water moves into the red cells (endosmosis). They swell up and lose their biconcave shape, becoming smaller and thicker.



- **➤**Normal Range of Fragility
- Normally, hemolysis begins in about (0.45%) saline.
- Hemolysis will be complete at 0.3 % NaCl.
- No cells heamolyze in solutions of 0.5% saline and above.

- ►Increased Red Cell Fragility (increased tendency to hemolysis) caused by:
- 1. Hereditary spherocytosis.
- 2. Autoimmune hemolytic anemia.
- 3. Toxic chemicals, poisons, infections, and some drugs (aspirin).
- 4. Deficiency of glucose 6-phosphate dehydrogenase (G6D).

Decreased red cell fragility (increased resistance to hemolysis) in:

- 1. Thalassemia.
- 2. Iron deficiency anemia.
- 3. Sickle cell anemia