

## Erythrocyte sedimentation rate. Hemolysis. Blood groups.

### Erythrocyte sedimentation rate (ESR)

Definition:

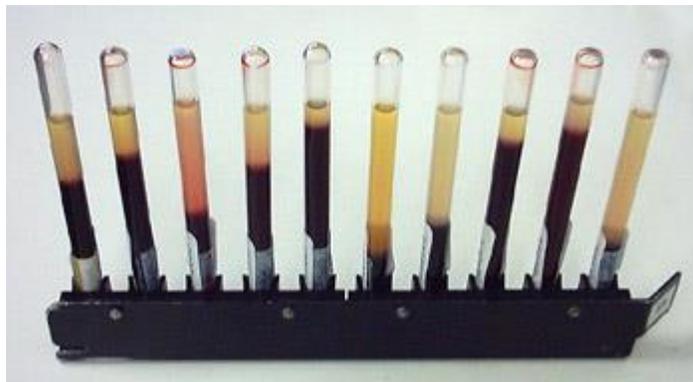
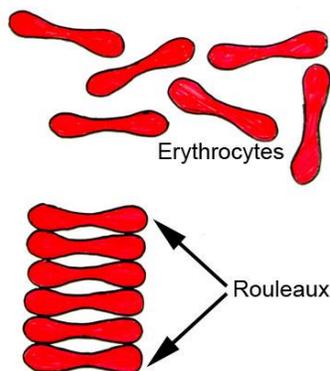
The **erythrocyte sedimentation rate** (ESR), also called a **sedimentation rate**, **sed rate**, or **Biernacki Reaction**, is the rate at which red blood cells precipitate (settle) in a period of 1 hour. It's a common haematology test which is a non-specific measure of inflammation. To perform the test, anticoagulated blood is placed in an upright tube, known as a Westergreen tube and the rate at which the red blood cells fall is measured and reported in mm/h.

For the red blood cells to settle, two processes have to happen:

1 **Biological process**: rouleaux formation, depending on **hematologic factors**, **protein factors** and some other **miscellaneous factors** (see table below). The ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes (zeta potential) generated by the negatively charged proteins (albumins) adsorbed to the surface of the erythrocyte. Globulins are less negative than albumins, and changes in albumin/globulin ratio modify the ESR (e.g., increase of globulins in infectious process or inflammation leads to an increased ESR).

2. **Physical process**: sedimentation of rouleaux

Hematologic Factors	Increases ESR	Decreases ESR
red blood cell count	anemia	polycythemia
red blood cell shape	macrocytosis, cold agglutinins, erythroid aggregates	sickling, microcytosis, spherocytosis
white blood cell count		extremely high WBC
Protein Factors	Increases ESR	Decreases ESR
fibrinogen	hyperfibrinogenemia	hypofibrinogenemia, dysfibrinogenemia
other serum proteins	increased gamma globulins, alpha globulins, beta globulins, paraproteins	hypogammaglobulinemia
dextran	high molecular weight dextran	low molecular weight dextran
coagulation system	heparin	Disseminated Intravascular Coagulation - DIC (low fibrinogen)
Miscellaneous Factors	Increases ESR	Decreases ESR
temperature	fever	hypothermia
nutritional status	extreme obesity	cachexia
serum cholesterol	high serum cholesterol	
organ failures	renal failure	congestive heart failure
gender of the patient	females, especially during pregnancy (not early)	
age of the patient	advanced age	



**Normal values: Men 1 – 15 mm/hr; Women: 5 – 20 mm/hr (<50 years)**

$$\text{ESR (mm/hr)} \leq \frac{\text{Age (in years)} + 10 \text{ (if female)}}{2}$$

### **Hemolysis**

**Definition:** The breakdown or destruction of red blood cells so that the contained hemoglobin is freed into the surrounding medium.

Red blood cells normally live for 110 - 120 days. After that, they naturally break down and are usually removed from the circulation by the spleen.

Some diseases and processes cause red blood cells to break down too soon. This requires the bone marrow to make more red blood cells than normal. The balance between red blood cell breakdown and production determines how low the red blood cell count becomes.

Hemolysis may be:

1. Physiological –within the reticulo- endothelial system, mainly in the spleen.
2. Pathological intravascular lysis of red blood cells. Conditions that can cause pathological hemolysis include:
  - Transfusion of the wrong blood type, or Rh incompatibility of fetal and maternal blood, a condition called erythroblastosis fetalis
  - Inherited defects in the blood cells (e.g., hereditary spherocytosis, thalassemia)
  - Infections
  - Medications
  - Toxins and poisons
  - G6PD deficiency and other enzyme deficiencies
  - Mechanical (heart valves, microvascular disease, hemodialysis, heart-lung bypass machine)
3. Experimental -hemolysis may be produced in the laboratory by various physical agents (heat, freezing, flooding with water, sound)

Mechanisms that produce hemolysis:

1. colloid –osmotic : hemoglobin leaves the RBC through a rise in permeability, not by rupture of the membrane. i.e: hypotonic medium
2. non-osmotic/stromatolysis: membrane is torn (destruction of the cell membrane),

### **Osmotic fragility test**

Cell membranes are semipermeable barriers, and osmotic gradients are established between intracellular and extracellular fluids which can cause water to flow into and out of the cells. The amount of osmotic pressure depends upon the difference between the concentrations of non-diffusible ions on each side of the membrane

The intracellular fluid of erythrocytes is a solution of salts, glucose, protein and hemoglobin. A 0.9% NaCl solution is said to be **isotonic**: when blood cells reside in such a 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 subjected to **hypertonic** media (e.g. 1.8% NaCl), the cells lose their normal biconcave shape, undergoing collapse (leading to **crenation**) due to the rapid osmotic efflux of water.

On the other hand, in a **hypotonic** environment (e.g. 0.4% NaCl or distilled water), an influx of water occurs: the cells swell, the integrity of their membranes is disrupted, allowing the escape of their hemoglobin (**hemolysis**) which dissolves in the external medium.

In this experiment, we make use of the property that the osmotic fragility (or susceptibility to hemolysis) of erythrocytes is **not uniform**, and the number of cells undergoing hemolysis depends on the degree of hypotonicity of the extracellular medium. The concentration of liberated hemoglobin in each test medium is an index of the extent of osmotic hemolysis. Your task is to examine the relationship between extent of hemolysis and osmolarity of the medium in which the erythrocytes are suspended, in other words to determine the globular resistance.

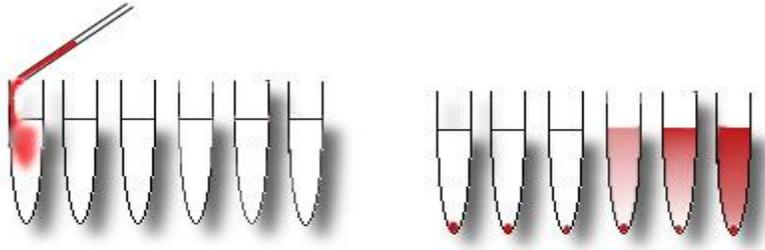
Minimal globular resistance relates to the concentration of the least hypotonic solution that causes the hemoglobin to leave the cell.

Maximal resistance is represented by the concentration that caused complete hemolysis.

Technique:

- 18 tubes for hemolysis are prepared containing 1,8ml, 1,7ml, 1,6ml.....0,2ml, 0,1ml of NaCl solutions.
- Volume of each tube is completed up to 1,8 ml with distilled water, so that the concentrations of NaCl decrease from 0,9% in the first one to 0.01% in the last one.

- 1 drop of blood is delivered to each tube and left for one hour.



#### Results:

- You need to observe the difference between the tubes: some of them have sedimented RBC on the bottom of the tube, supernatant is clear - no hemolysis
- Following tubes have reduced sediment and the supernatant is pink – partial hemolysis; concentration in the first tube with pink supernatant determines the minimal resistance. i.e: minimal resistance = 0,45% corresponding to the tenth tube where the concentration was 0,45%.
- Last tubes have no sediment and reddish supernatant – total hemolysis; concentration in the last tube with sediment (last tube with partial hemolysis) determines the maximal globular resistance. i.e. maximal resistance=0.3%, corresponding to the thirteenth tube where the concentration was 0,3%.

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#### Blood groups. ABO blood groups

In 1901, Karl Landsteiner, an Austrian pathologist, randomly combined the serum and red blood cells of his colleague and following the reactions he observed, he reported that blood could be classified into blood "groups". By matching these blood groups, a successful blood transfusion could be made between a healthy donor and a patient in need of blood replacement due to an injury, disease or surgery. A blood transfusion can either be whole blood, or a blood component, such as red cells or platelets or plasma. This discovery earned him the 1930 Nobel Prize in Medicine.

The antibody reaction that occurs when two different blood groups are mixed, causes the foreign red cells to be destroyed (hemolysis). This can lead to kidney damage and death. That is why matching blood groups between donor and patient is so important before a transfusion is given.

A person's ABO blood type—A, B, AB, or O—is based on the presence or absence of the A and B antigens on his red blood cells.

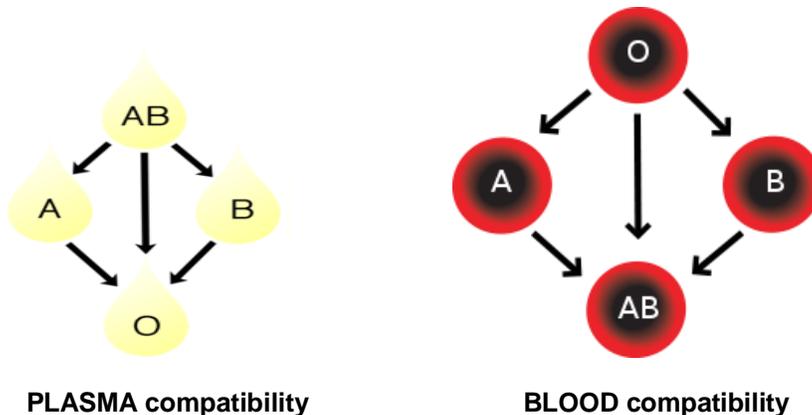
#### There are four basic blood groups:

1. **Group A** with A antigen on the red cells and anti-B antibodies in the plasma.
2. **Group B** with B antigen on the red cells and anti-A antibodies in the plasma.
3. **Group AB** with both A and B antigens on the red cells and neither anti-A nor anti-B in the plasma.
4. **Group O** with no A or B antigens on the RBC and both anti-A and anti-B antibodies in the plasma.

# The ABO Blood System

Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	 A agglutinogens only	 B agglutinogens only	 A and B agglutinogens	 No agglutinogens
Plasma Antibodies (phenotype)	 b agglutinin only	 a agglutinin only	NONE. No agglutinin	 a and b agglutinin

Although the distribution of each of the four ABO blood types varies among racial groups, O is the most common and AB is the least common in all groups.



## Blood group testing - Beth Vincent method (most used)

**Principle:** contact between the erythrocytes to be tested and three types of hemotest serum (that contain known antibodies) will produce agglutination or not, depending on the type of agglutigen on the cell.

## THE RH BLOOD GROUP SYSTEM.

The Rh, or Rhesus, system was first detected in 1940 by Landsteiner and Wiener when they injected blood from rhesus monkeys into guinea pigs and rabbits. More than 50 antigens have since been discovered that belong to this system, making it the most complex red blood cell antigen system.

In routine blood typing and cross-matching tests, only one of these 50 antigens, the D antigen, also known as the Rh factor or Rh<sub>0</sub>[D], is tested for. If the D antigen is present, that person is Rh-positive; if the D antigen is absent, that person is Rh-negative. Unlike the ABO system, antibodies to Rh antigens don't develop naturally. They develop only as an immune response after a transfusion or during pregnancy. The same principle is used for typing the blood as for ABO groups. About 15% of the human population is Rh negative.

Rh system becomes important when one considers the eventuality of Rh incompatibility between mother and fetus; in such a case, the antibody-mediated cytotoxicity mechanism involved threatens the fetus. During birth, a leakage of the baby's red blood cells often occurs into the mother's circulation. If the baby is Rh positive (inheriting the trait from its father) and the mother is Rh negative, these red cells

will cause the mother to manufacture antibodies against the Rh antigen. The antibodies (IgG class) do not cause problems for that first born, but can cross the placenta and attack the red cells of a subsequent Rh+ fetus. The red cells are destroyed, leading to anemia and jaundice. The disease - erythroblastosis fetalis or hemolytic disease of the newborn- may result in fetal death.

