

BLOOD GROUPING

ABO SYSTEM

INTRODUCTION

Erythrocytes contain several types of antigens (at least 600) belonging to membrane grouped in several systems. Since the antibodies formed against antigens produce erythrocytes agglutination they are called agglutinogens. The erythrocyte membrane contains A, B antigens and plasma antibodies called agglutinins: alpha (against A) and beta (against B). The corresponding/homologous agglutinogen and agglutinin do not normally coexist in the blood of one and the same person (rule of Landsteiner). Depending on their presence in the OAB system there are four blood groups. The name of the blood type is given by the agglutinogen.

Table no. OAB blood group system

Blood group	Genotype	Agglutinogen	Agglutinins	Frequency in population*
O I	OO	----	alpha, beta	45 %
A II	OA or AA	A	beta	40 %
B III	OB or BB	B	alpha	11 %
AB IV	AB	A,B	----	4 %

* According to <http://www.bloodcenters.org/blood-donation/blood-types-and-the-population>

Agglutinogens are glycolipids located on the erythrocyte surface. They appear in the third month of fetal life and found in all tissues except the lens, vitreous; less in the nervous tissue, cornea, cartilage, compact bone, but there are in some secretions: saliva, gastric and intestinal juice, milk, semen, amniotic fluid. Agglutinins occur between 3-6 months of intrauterine life and reach their highest level in 10 years. Some samples of erythrocytes from group A, B or AB give weak reactions with specific antiserum, characterizing the presence of antigens which are variations or subgroups of ABO system. Subgroups A: A1 (A loud) and A2 (A weak), the latter characterized by weak agglutination reactions to anti-A serum. Because of these subgroups, groups A and AB are divided into A1, A2, A1B and A2B. Other subgroups A and B subgroups are exceptionally rare and not important for hemolytic disease of the newborn. Blood groups are transmitted hereditary; each person has two genes (one from each parent). In this sense they are useful in establishing paternity.

When there is incompatibility between several types of blood (agglutinins come into contact with homologous agglutinogens) red blood cells agglutinate due to their surface attachment agglutinins, so that cells are gathered into a conglomerate in the process of agglutination. These conglomerates will cause the occlusion of small blood vessels throughout the circulatory system. In the next hours / days those will be phagocytosed resulting the destruction of erythrocyte membranes that hemolysis (plasma hemoglobin release).

PRINCIPLE: The determination is based on the agglutination reaction, antigen-antibody reaction between erythrocyte antigens A, B and serum agglutinins alpha, beta in the tube or on the slide. You can use the Beth-Vincent test that identifies agglutinogens when we know agglutinins or using the Simonin method which identifies agglutinins knowing agglutinogens.

WORK MATERIALS: glass slides (possibly with well), Anti-A, Anti-B, Anti-A,B(hemotests), erythrocytes test 0, A, B; collection blood from fingertip required needle, wool and alcohol for Beth

Vincent method, blood collected by venopunction for serum separation for Simonin method. Hemotests are blood sera from persons with known blood group; we use anti-A, anti-B, anti-AB; hemotest anti-A contains agglutinins alpha, hemotest anti-B agglutinins beta and hemotest anti-A B agglutinins alpha and beta. Erythrocytes test are group O, A and B.

TECHNIQUE



Figure

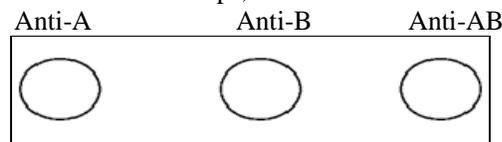
1. Beth-Vincent method

Successively put on a slide with different pipettes cleaned with alcohol, one drop of hemotest in the following order: AB, B and A. The pulp of the finger is disinfected with alcohol and is pricked with the sterile needle. With upper blades put a small amount of blood in each drop of hemotest using each time another corner and mixing it. Drop of blood should be 10/20 times lower than the hemotest. Slowly move the slide circularly and watch for the agglutination appearance in the first 2-3 minutes.

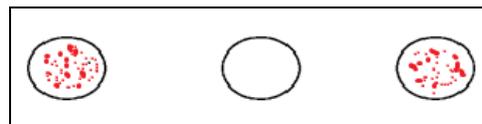
Interpretation of results

These situations are possible:

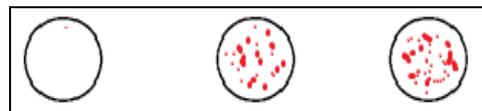
1. Agglutination is absent in all three drops, researched blood belongs to group O;



2. Agglutination occurs only with sera anti-AB and anti- A, then we have group A;



3. Agglutination occurs only with sera anti-AB and anti-B, then we have group B;



4. Agglutination in all three drops, the blood group is AB.

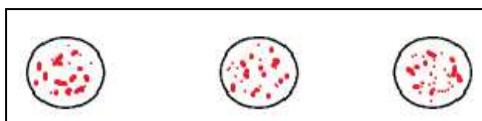


Figure no. Interpretation of of blood groups using Beth-Vincent method

4. Simonin method

On a glass slide put three drops of serum to research; the first drop of serum is added to an erythrocytes O test, in the second A and the last B. Addition and mixing is performed for each corner of a drop slide, changing at every drop. Erythrocytes and serum ratio should be about 1 to 10. Reading is done by the slow rotation of the blade in the first 2 to 3 minutes.

Interpretation:

1. Agglutination with erythrocytes group A and B corresponds to group O (serum containing both agglutinins type);
2. agglutination with erythrocytes B and without agglutination with erythrocytes A (serum contains agglutinins beta) corresponds to group A;
3. Agglutination RBC A and lack of agglutination with RBC B corresponds to the group B, serum researched containing agglutinins alpha;
4. if no agglutination occurs in any drops, agglutinins are missing corresponding serum group AB.

Sources of error in determination

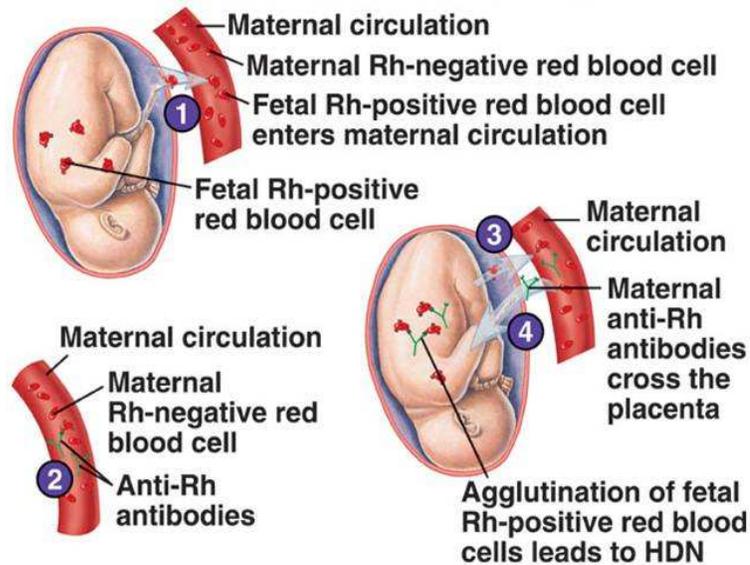
1. Use of infected/ old hemotests may cause unspecific reactions or lack of the agglutination
2. Delay of reading may occur false agglutination;
3. Use of outdated test hemotests or erythrocytes;
4. Abnormal proportion between blood and hemotest, serum and erythrocytes test.

Rh SYSTEM

The Rh is another group of erythrocyte membrane antigens. There are six antigens (C, D, E, c, d, e) in the Rh system. The most important antigen is antigen D (or Rh antigen from the monkey *Macacus Rhesus*), which can be found on the RBC membrane of 85% of the population. Caucasian people who have this antigen are called Rh positive (85%) and those that don't have it are called Rh negative. The presence of antigen c excludes antigen C, as well as for other antigens. Between ABO and Rh system are the following major differences:

-the Rh-antigen is present only in erythrocyte membranes (not in other cells)
-in the Rh system there aren't spontaneously Rh antibodies in plasma. These antibodies appear in the following circumstances:

- after transfusion of Rh negative blood to a Rh positive person (high concentrations occurring after 2-4 months);
- Rh negative mother and the fetus Rh positive (Rh positive dominant character). An Rh negative mother with Rh positive fetus develops in the first pregnancy usually not a lot of anti Rh agglutinins to produce adverse effects; 3% of Rh-positive children born to the second pregnancy erythroblastosis signs of fetal (or hemolytic disease of the newborn) and 10% of children in the third pregnancy develops the disease. The severity of this condition can vary widely. In some instances, the baby has no symptoms of the disease. In other cases, it can lead to the death of the baby before or shortly after birth. It can be treated before birth by intrauterine transfusion.



Hemolytic disease of the newborn, also known as **hemolytic disease of the fetus and newborn** or **erythroblastosis fetalis**, is an alloimmune condition that develops in a fetus, when the IgG molecules (one of the five main types of antibodies) produced by the mother pass through the placenta. Among these antibodies there are some which attack the red blood cells in the fetal circulation; the red cells are broken down and the fetus can develop anemia and reticulocytosis. This fetal disease ranges from mild to very severe, and fetal death from heart failure (hydrops fetalis) can occur. When the disease is moderate or severe, many erythroblasts are present in the fetal blood and so these forms of the disease can be called erythroblastosis fetalis (or erythroblastosis foetalis).

Solutions:

- exchange transfusion with a blood type compatible with both the infant and the mother (complete replacement of newborn blood with Rh-negative blood);
- administration to Rh-negative mother during the first 36-72 hours after birth intravenous anti D serum (anti-D IG) (destroy Rh positive erythrocytes reached maternal circulation).

Principle: agglutination reaction between serum anti Rh (D) and blood research.

Materials required: anti Rh serum, slides, glass pipettes, needle wool, alcohol.

Technique: put on a glass slide a drop of anti Rh serum than, with the corner of a slide, a drop of blood (from fingertip). Mix gently and prints blade circular motion. Read the first 2-3 minutes.

Interpretation

If the blood examined is Rh positive agglutination will be noticed, if agglutination occurs it is Rh negative.