

## IDENTIFICATION METHODS AND COMPLICATIONS OF BLOOD GROUPS

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Post-transfusion reactions are observed in 0.002-0.2% of cases where incorrect blood groups are transfused out of 6,000-29,000 blood transfusions. Fatal hemolytic reactions occur in 1 out of 100,000 blood transfusions, with 83% of them being due to incompatible reactions according to the ABO system. In the UK, 1 in 10 transfusions results in a fatal outcome, while in the US it is 1 in 18 and in Russia it is 1 in 3.9.

In 1901, Karl Landsteiner identified blood groups I, II, and III. In 1902, Decastello and Sturli identified group IV. In 1907, Ya. Yanskiy developed a classification for groups I-IV. In 1910, Dungem and Hirszfeld discovered A and B agglutinogens and the ABO system. In 1930, K. Landsteiner received the Nobel Prize for his work on blood groups. In 1940, Karl Landsteiner and his students (Viner and Levin) discovered the Rh factor antigens.

Over 400 antigens are present on erythrocytes, including O, A, B, D, S, s, E, e, M, N, S, Kell(K), Daffy(Fy), Kidd(Jk), Lutheran(Lu), Diego(Di), Lewis(Le), and others. These erythrocyte group and Rh antigens are located on the external membrane and form antigen-antibody complexes with corresponding antigens. Antigens do not change throughout a person's life.

Erythrocytes contain A and B agglutinogens, while  $\alpha$  and  $\beta$  agglutinins are present in plasma. An individual cannot have both A agglutinogen and  $\alpha$  agglutinin or B agglutinogen and  $\beta$  agglutinin, as they would cause agglutination and result in various reactions, including death. Incompatible erythrocyte agglutination and hemolysis occur when blood transfusions are not compatible according to the ABO system and Rh factor.

As a result of the antigen-antibody reaction, large agglutinates form, which block capillaries, damage small blood vessels, and cause various reactions, including death. The antigen-antibody complex activates the complement system, leading to erythrocyte hemolysis. Clinical and laboratory tests are required to identify blood groups before transfusions. Body temperature and blood pressure should be measured, and if there is a history of febrile reactions, premedication should be performed.

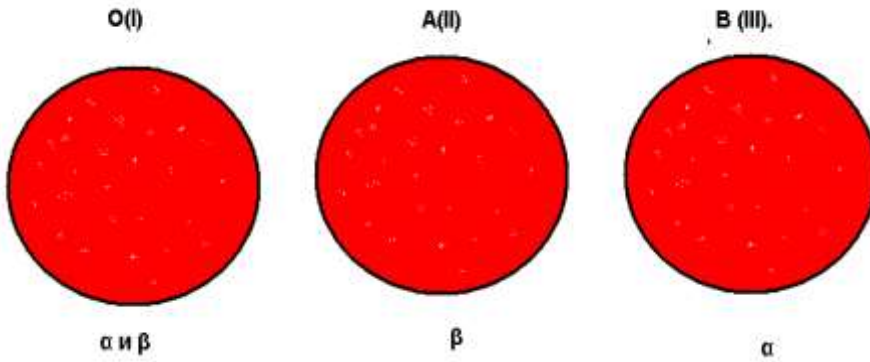
The standard method for blood group identification is based on the agglutination of erythrocytes with corresponding agglutinogens using standard reagents. The following steps are taken:

1. Ten microliters of standard erythrocyte suspensions of groups 1, 2, and 3 are placed on a special slide.
2. One microliter of the patient's blood is added to each suspension.

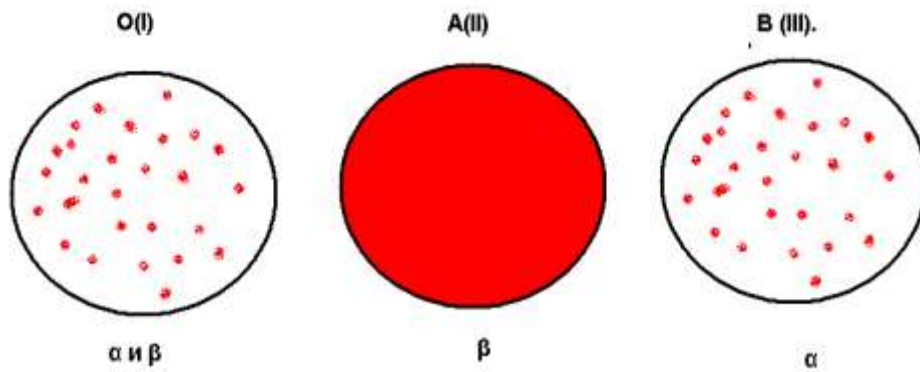
3. The mixture is agitated for five minutes.

4. The results are observed.

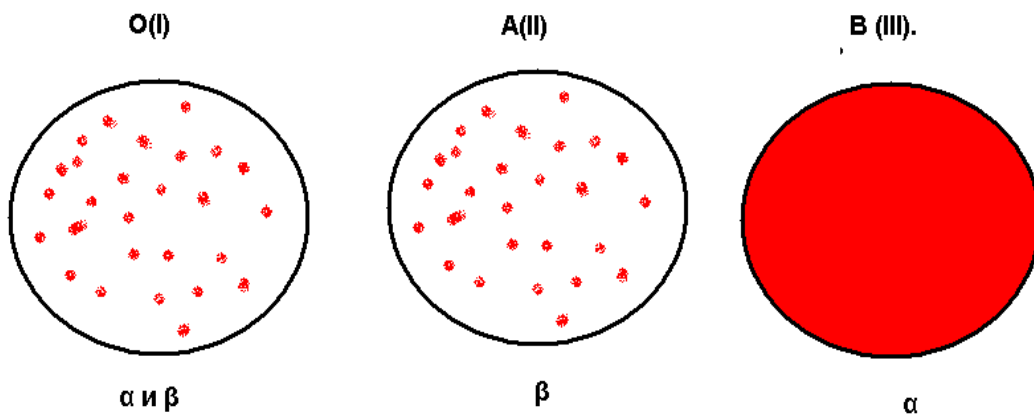
If there is no agglutination in any sample, it is group O (I)  $\alpha\beta.\alpha\beta$ .

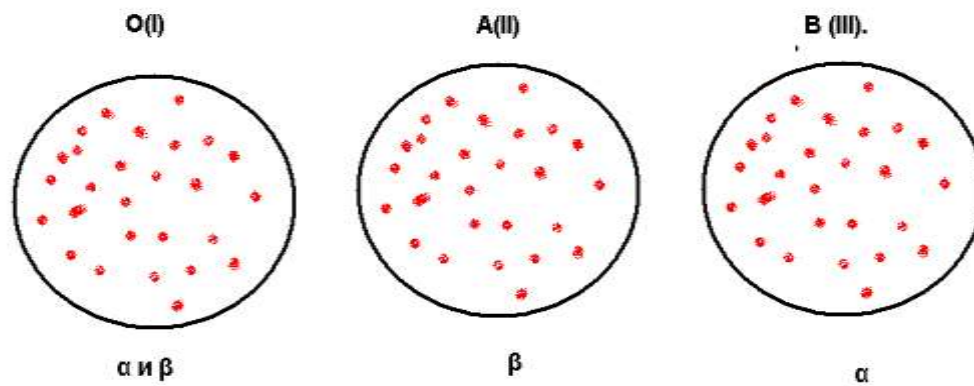


If there is agglutination in groups 1 and 3, but not in group 2, it is group A (II)  $\beta$



If there is agglutination in groups 1 and 2, but not in group 3, it is group B(III) $\alpha$ .





If positive results are obtained for groups I (O), II (A), and III (B) in two series of standard reagents, a cross-matching test is performed using the standard reagent for group IV (AB). No agglutination should occur with the IV (AB) standard reagent. The presence of agglutination indicates a para-agglutination or false reaction.

#### **Identifying blood groups using standard red blood cells.**

1. The standard red blood cells are diluted with 1 drop per milliliter in 1st, 2nd, and 3rd groups in the special Petri dishes.
2. Blood serum or plasma, which can be detected within 10 seconds, is diluted with 10 drops each.
3. Mixed with a glass stirrer.
4. The dishes are shaken for 5 minutes and then the results are observed.

If there is no agglutination in the 1st group, and agglutination is observed in groups 2 and 3, it belongs to O(I)  $\alpha\beta$ , which is related to group 1. If agglutination is absent in groups 1 and 3 and present in group 2, it belongs to A(II) $\beta$ , which is related to group 3. If there is no agglutination in all samples, then the tested blood group is AB (IV).

Determining group compatibility. The group compatibility test is performed before blood transfusion. This is because AB antigens are present along with S, D, E, F, G, K antigens. Even though the blood groups are the same, the post-transfusion reaction may be different.

Group compatibility testing is performed using the Petri dish. 1 drop of blood is taken from the patient's blood and 10 drops from the donor's blood and mixed. It is then subjected to a 5-minute shake and observed. If agglutination occurs, the recipient and donor blood have different groups. If there is no agglutination, then they are compatible.

Determining the Rh factor. Anti-Rh antibodies are used to determine the Rh factor. 10 drops of standard anti-Rh blood serum are mixed with each sample in the Petri dishes. It is then subjected to a 5-minute shake. If agglutination is observed, the patient has Rh-positive blood, otherwise, they have Rh-negative blood.



### **Blood transfusion rules:**

1. Before each blood transfusion, consent is obtained from the patient and an expert group reviews the recommendations and contra-indications.
2. The same group of donors and recipients should be used for blood transfusion.
3. Before each blood transfusion, both donor and recipient blood groups are checked and compatibility tests are performed.
4. The red blood cells (1 unit) of the patient and plasma (10 units) of the recipient are tested for compatibility.
5. The reverse group compatibility test is also performed by matching the plasma (10 units) of the patient and the red blood cells (1 unit) of the donor (using the centrifuge).
6. Rh compatibility test. A tube containing 2 units of patient plasma, 1 unit of donor blood, and 2 units of 30% Polyglucin is placed in a water bath at 37 ° C for 5 minutes. After adding 5 ml of saline, it is checked. If the tube turns blue, it indicates the presence of the Rh factor, otherwise it becomes red, indicating that the blood is Rh negative.
7. With the Bezredko method, 10 ml of donor red blood cells are administered and monitored for 5 minutes. This procedure is repeated three times; if the patient's condition is good, the blood is administered at a rate of 1 unit per second. The patient is monitored for the first 2 hours after blood transfusion and monitored for 24 hours after blood transfusion.
8. Tested blood groups and compatibility tests are not stored and sent for transfusion within 2 hours.
9. After blood transfusion, general blood tests and total bilirubin tests are performed.

**Testing blood group using dry monoclonal reagents.** Monoclonal reagents are contained in Eldoncards and are an express method for determining blood group according to the ABO system and rhesus factor. Eldoncards were first produced in Denmark in 1955. The principle of the reaction is agglutination (antigen-antibody).

### **Eldoncard advantages and superiority:**

1. No need for specialized personnel, fasting, or laboratory conditions (except for physiological stress or in case of a patient with a blood disorder)

2. No need for the employee to have special skills to perform the test
3. The results are obtained in a few seconds
4. Minimal contact between the personal and the blood sample
5. "Dry" reagent is used, there is a control group
6. The obtained results are accurate and highly sensitive
7. Stored for up to 3 years
8. Storage temperature ranges from 5-65 °C

**Eldoncard disadvantages: the reagent is relatively expensive.**

**Application areas:**

1. For initial blood grouping and rhesus testing.
2. In blood services, especially when providing services in remote areas.
3. In emergency situations, intensive care units, and laboratories, and other places where medical treatment is provided.
4. In educational institutions.
5. Suitable for home use, especially in remote areas.
6. Useful in remote locations, oil fields, and other remote locations.
7. Convenient for military operations and emergency situations.

ELDONCARD







### Technical errors in determining blood groups and Rh factor:

1. Incorrect marking of collected blood, mixing of samples.
2. Testing of clotted blood.
3. Lack of blood group marking on the testing tablet or plate.
4. Non-standard solutions, incorrect dilution of red blood cells, reagents with a ratio of 10:1.
5. Using expired reagents.
6. Failure to observe the reaction time (5 minutes or overnight).
7. Room temperature should be between 15°C and 25°C.
8. Mixing different reagents with one pipette or transferring all groups with one dropper.
9. Poor quality of reagents, low activity (avidity) of reagents.
10. Failure to follow storage rules (temperature, air tightness, contamination).
11. Lack of specificity of anti-A antibodies prepared by a specialist in reagent preparation.
12. Reagents without quality control should not be used.
13. Standard solutions and red blood cells should be tested alongside the blood group.
14. Standard sera prepared from individuals with known blood groups should not be used, monoclonal reagents are used instead.
15. Blood group and Rh factor are tested before collecting blood and plasma.

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