

Coombs Test

- ❖ Coombs test is also known as antiglobulin test.
- ❖ The Coombs test tests for antibodies that may stick to the red blood cells and cause red blood cells to die too early.
- ❖ It was discovered by Coombs, Mourant and Race in 1945. Coombs reagent is antihuman globulin.
- ❖ It is made by injecting human globulin into animals, which produce polyclonal antibodies specific for human immunoglobulins and human complement system factors.

Principle:

- Red cells coated with complement or IgG antibodies do not agglutinate directly when centrifuged.
- These cells are said to be sensitized with IgG or complement.
- In order for agglutination to occur an additional antibody, which reacts with the Fc portion of the IgG antibody, or with the C3b or C3d component of complement, must be added to the system.
- This will form a “bridge” between the antibodies or complement coating the red cells, causing agglutination.

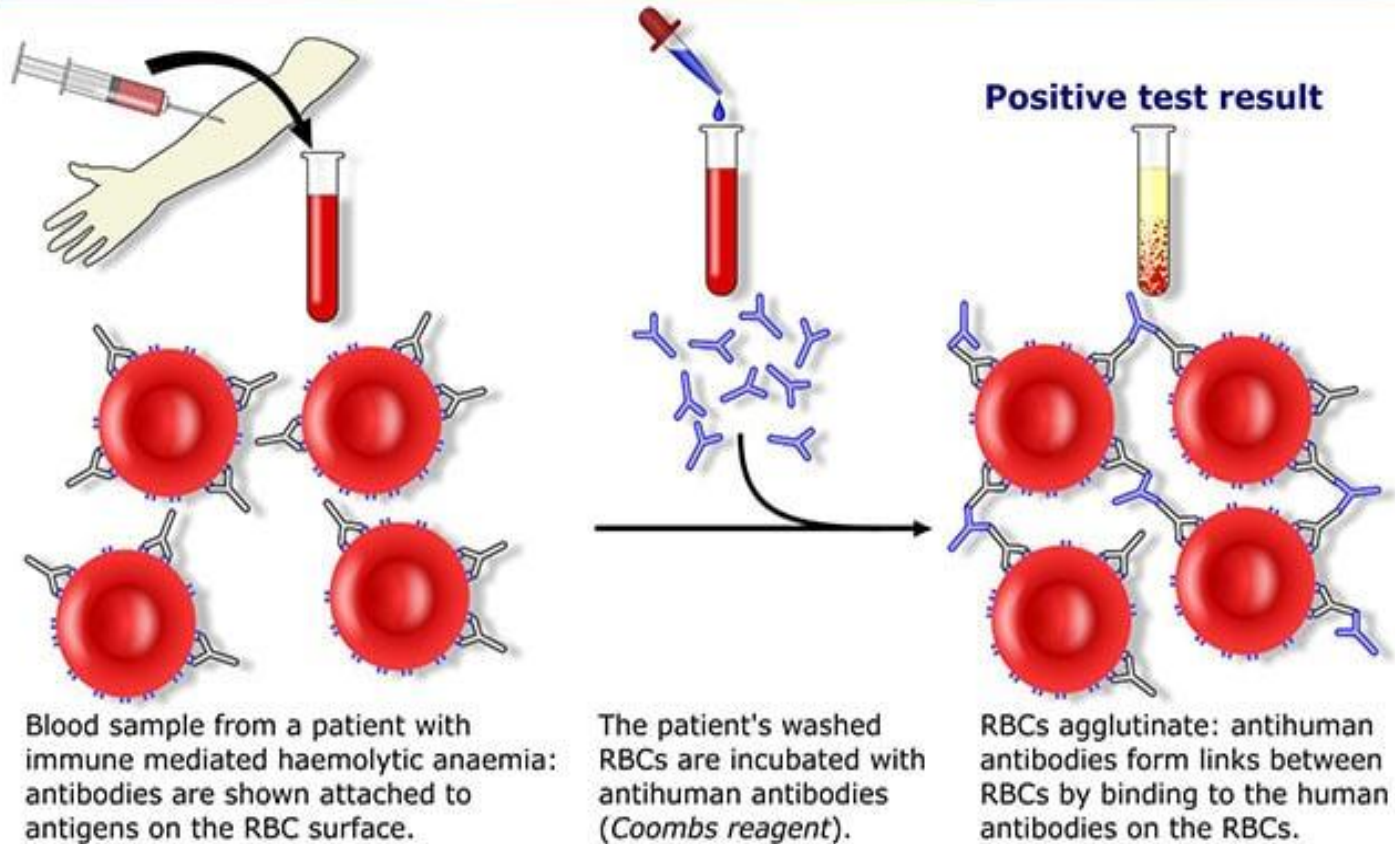
Types:

Direct Coombs Test (Direct Antiglobulin Test- DAT)

The direct Coombs test is used to detect antibodies (IgG or C3) that are stuck to the surface of red blood cells. Many diseases and drugs can cause this. These antibodies sometimes destroy red blood cells and cause anemia.

This is the test that is done on the newborn's blood sample, usually in the setting of a newborn with jaundice. The two most commonly recognized forms of antibody-mediated hemolysis in newborns are Rh incompatibility and ABO incompatibility.

Direct Coombs test / Direct antiglobulin test



Procedure of Direct Coombs Test

1. Prepare a 5 % suspension in isotonic saline of the red blood cells to be tested.
2. With clean pipette add one drop of the prepared cell suspension to a small tube.
3. Wash three times with normal saline to remove all the traces of serum.
4. Decant completely after the last washing.
5. Add two drops of Anti-human serum.
6. Mix well and centrifuge for one minute at 1500 RPM.
7. Resuspend the cells by gentle agitation and examine macroscopically and microscopically for agglutination.

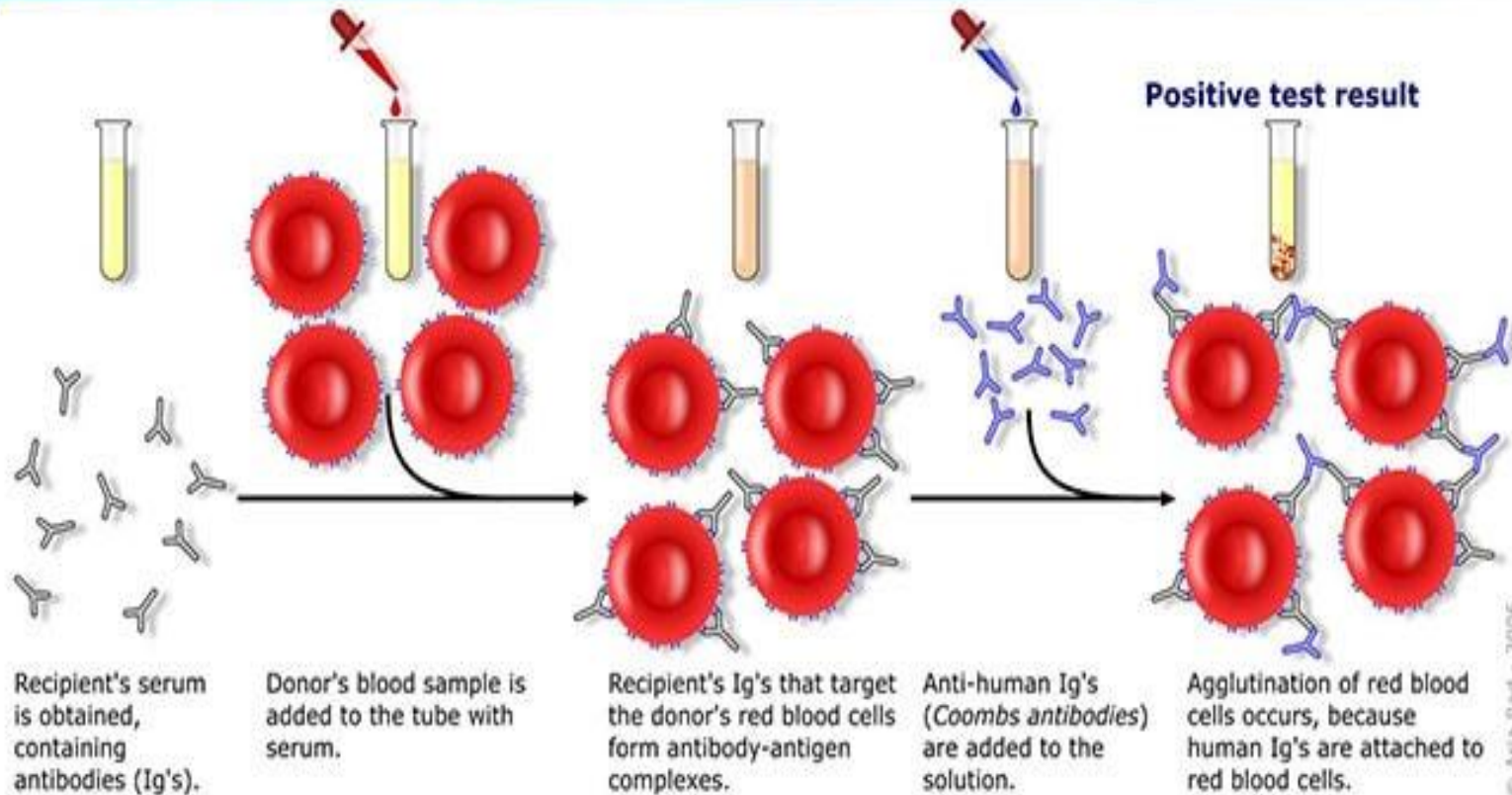
Indirect Coombs Test (Indirect Antiglobulin Test- IAT)

The indirect Coombs test looks for free-flowing antibodies against certain red blood cells. It is most often done to determine if you may have a reaction to a blood transfusion.

This is the test that is done on the mother's blood sample as part of her prenatal labs. Frequently referred to as the "antibody screen", this test identifies a long list of minor antigens that could either cause problems in the newborns or cause problems in the mother if transfusion is necessary.

Approximately 5% of patients have a positive IAT due to IgG antibodies, IgM antibodies, or both.

Indirect Coombs test / Indirect antiglobulin test



Procedure of Indirect Coombs Test

1. Label three test tubes as T (test serum) PC (Positive control) and NC (negative control).
2. In the tube labeled as T (Test), take 2 drops of test serum.
3. In the test tube labeled as PC (Positive control), take 1 drop of anti D serum.
4. In the test tube labeled as NC (Negative control), take 1 drop of normal saline.
5. Add one drop of 5 % saline suspension of the pooled 'O' Rho (D) positive cells in each tube.
6. Incubate all the three tubes for one hour at 37°C.
7. Wash the cells three times in normal saline to remove excess serum with no free antibodies, (in the case of inadequate washings of the red cells, negative results may be obtained).
8. Add two drops of Coombs serum (anti human serum) to each tube.
9. Keep for 5 minutes and then centrifuge at 1,500 RPM for one minute.
10. Resuspend the cells and examine macroscopically as well as microscopically.

Result Interpretation of Coombs Test

Negative Result:

No clumping of cells (no agglutination). This means you have no antibodies to red blood cells.

Positive Result:

Clumping (agglutination) of the blood cells during a direct Coombs test means that you have antibodies on the red blood cells and that you may have a condition that causes the destruction of red blood cells by your immune system (hemolysis). This may be due to

- ✓ Hemolytic anemia,
- ✓ Chronic lymphocytic leukemia or similar disorder,
- ✓ Infectious mononucleosis,
- ✓ Mycoplasmal infection,
- ✓ Syphilis,
- ✓ Transfusion reaction, such as one due to improperly matched units of blood.