# Coomb's Test (Antiglobulin Test)

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- **There are Two types of Coomb's Test**

- ✓ **Direct Coomb‘s Test**
- ✓ **Indirect Coomb's Test**

# Mechanism

- The two Coombs tests are based on :
- anti-human antibodies binding to human antibodies, commonly IgG or IgM.
- These anti-human antibodies are produced by plasma cell of non-human animals after immunizing them with human serum.
- Additionally, these anti-human antibodies will also bind to human antibodies that may be fixed onto antigens on the surface of red blood cell (RBCs).
- In the appropriate test tube conditions, this can lead to agglutination of RBCs and allowing for visualization of the resulting clumps of RBCs.
- If clumping is seen, the Coombs test is positive;
- if not, the Coombs test is negative.

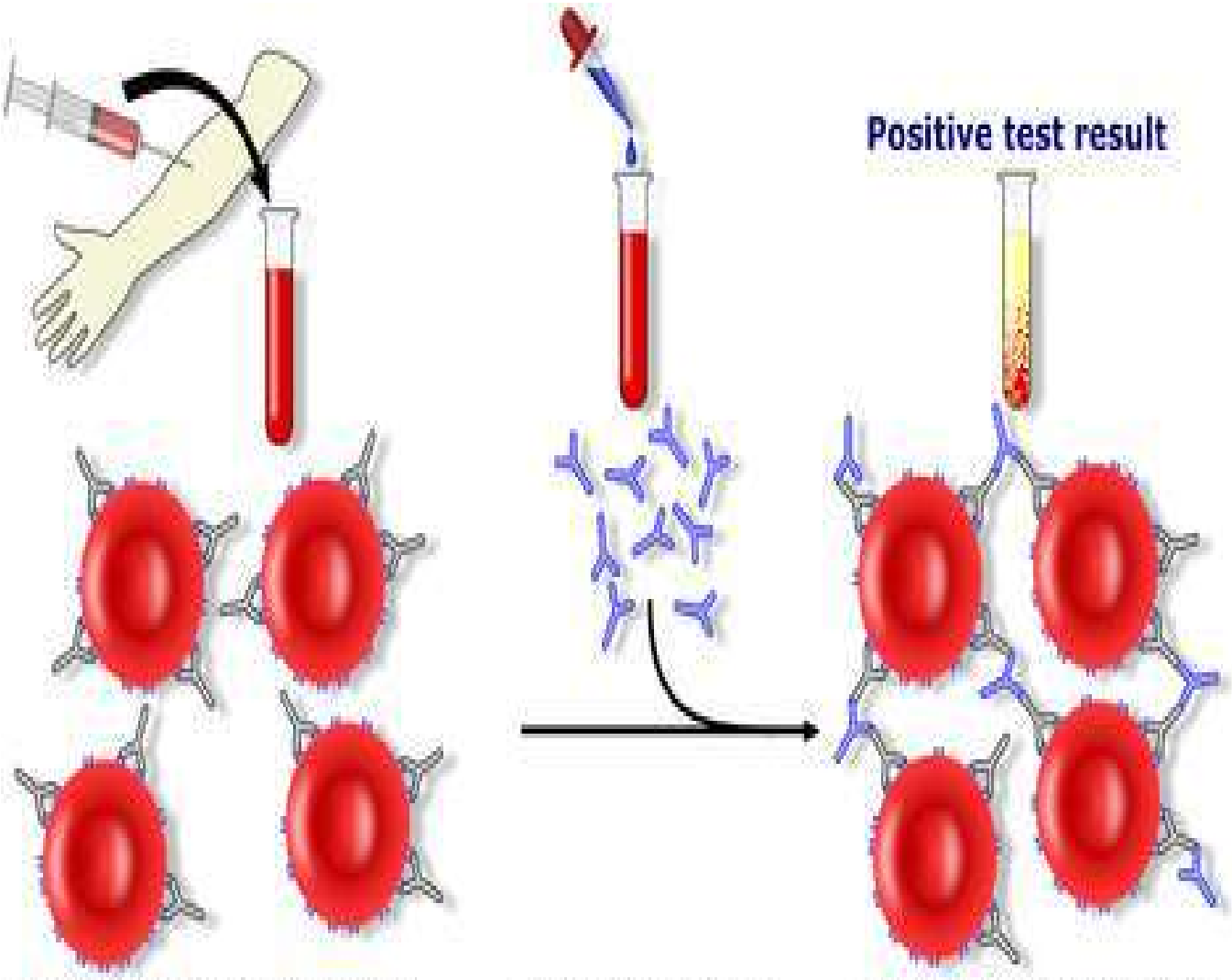
# THE DIRECT COOMBS TEST (DIRECT ANTIGLOBULIN TEST ( DAT).

- The Direct Coombs test is used to test for :
- autoimmune hemolytic anemia i.e., a condition of a low count of(RBCs) caused by immune system lysis or breaking of RBC membranes causing RBC destruction.
- In certain diseases or conditions an individual's blood may contain IgG antibodies that can specifically bind to antigens on the RBC surface membrane, and their circulating RBCs can become coated with IgG autoantibodies.
- Complement proteins may subsequently bind to the bound antibodies and cause RBC destruction.

# TO PERFORM THE TEST:

- The **direct Coombs test** is used to detect these antibodies or complement proteins that are bound to the surface of red blood cells.
- a blood sample is taken and the RBCs are washed **(removing the patient's own plasma)**
- then incubated with anti-human globulin (also known as "Coombs reagent").
- If this produces agglutinate of RBCs, the direct Coombs test is positive

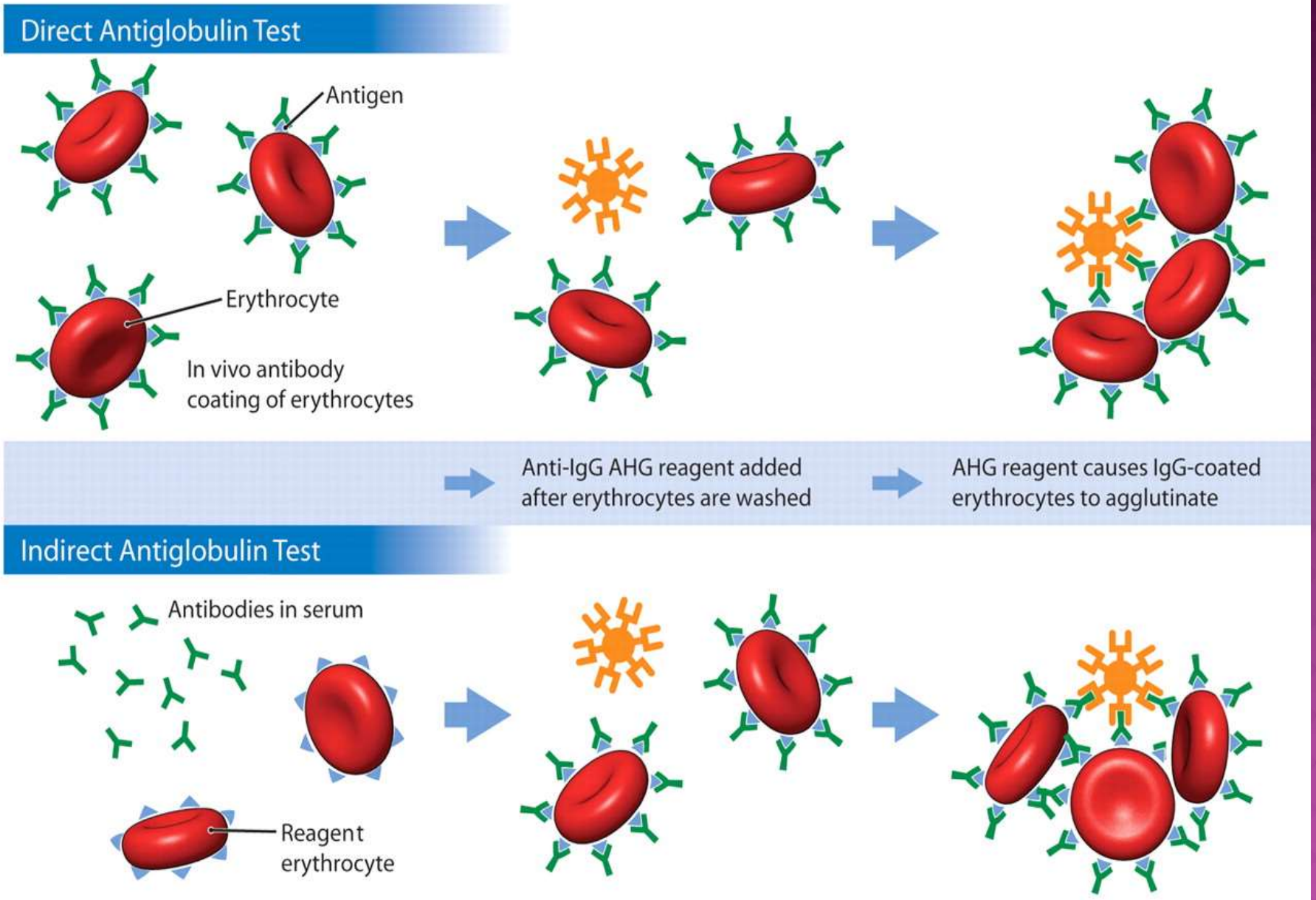
- **a. Direct Coomb's Test**
- When antibodies bind to erythrocytes, they do not always result in agglutination.
- **This can result from the :**
- antigen/antibody ratio being in antigen excess or antibody excess .
- some cases electrical charges on the red blood cells preventing the effective cross linking of the cells.
- These antibodies that bind to but do not cause agglutination of red blood cells are sometimes referred to as **incomplete antibodies.**
- In order to detect the presence of non-agglutinating antibodies on red blood cells, one simply adds **a second antibody directed against the immunoglobulin (antibody) coating the red cells.**
- This anti-immunoglobulin can now cross link the red blood cells and result in agglutination




Blood sample from a patient with immune mediated haemolytic anaemia: antibodies are shown attached to antigens on the RBC surface.

The patient's washed RBCs are incubated with antihuman antibodies (*Coombs reagent*).

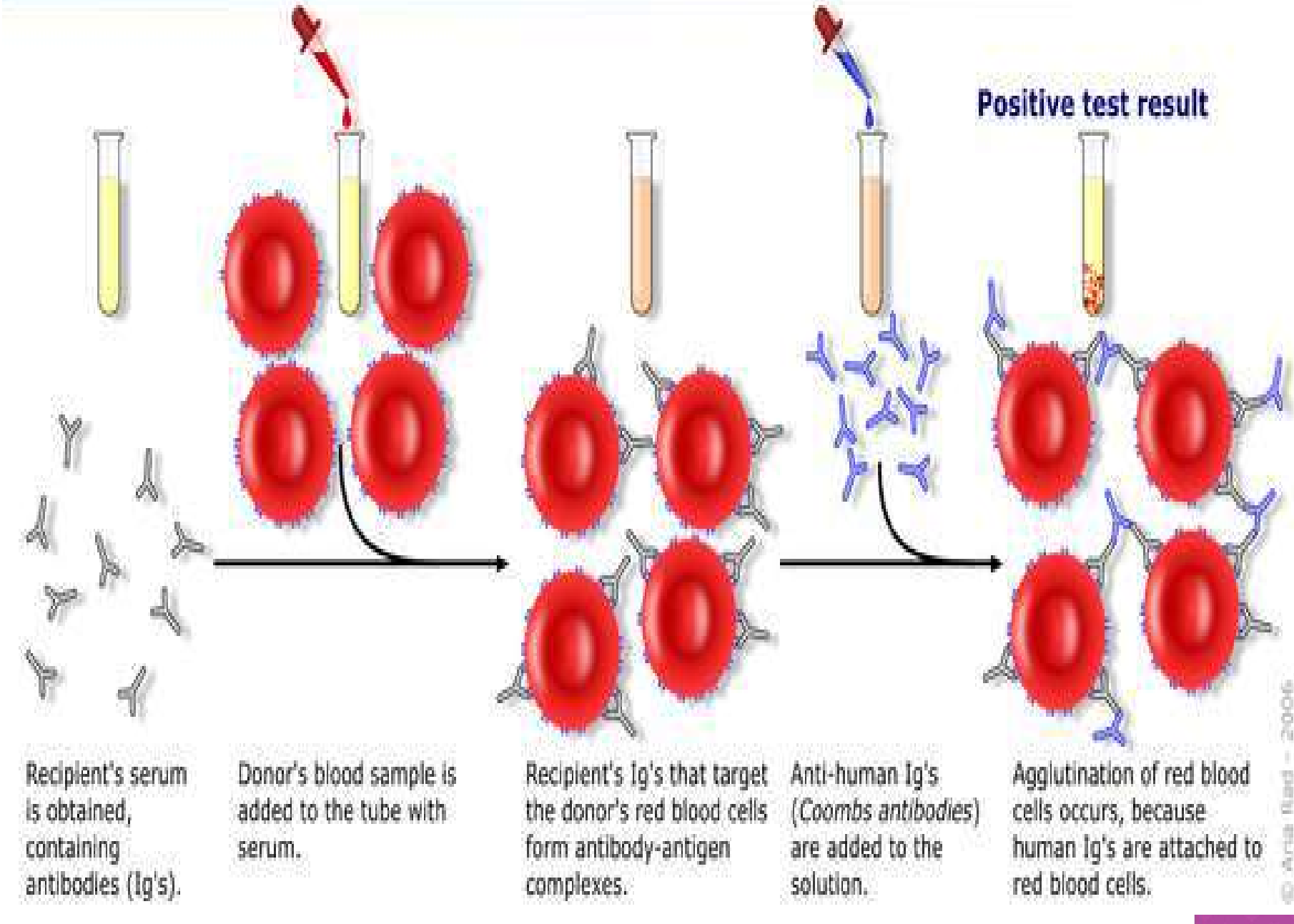
RBCs agglutinate: antihuman antibodies form links between RBCs by binding to the human antibodies on the RBCs.


Direct Antiglobulin Test
Antigen
Erythrocyte
In vivo antibody coating of erythrocytes
Anti-IgG AHG reagent added after erythrocytes are washed
AHG reagent causes IgG-coated erythrocytes to agglutinate
Indirect Antiglobulin Test
Antibodies in serum
Reagent erythrocyte

## b. Indirect Coomb's Test

- If it is necessary to know whether a serum sample has antibodies directed against particular red blood cell and you want to be sure that you also detect potential non- agglutinating antibodies in the sample.
- **Indirect Coomb's test is performed.**
- This test is done by **incubating the red blood cells with the serum sample,**
- **washing** out any unbound antibodies and then adding **a second anti-immunoglobulin reagent to cross link the cells.**

- **The indirect Coombs test is used in:**
- prenatal testing of pregnant women
- and in testing prior to blood transfusion .
- The test detects antibodies against foreign red blood cells.
- In this case, serum is extracted from a blood sample taken from the patient.
- The serum is incubated with foreign red blood cells of known antigenicity.
- Finally, anti-human globulin is added.
- If agglutination occurs, the indirect Coombs test is positive.


Positive test result
Recipient's serum is obtained, containing antibodies (Ig's).
Donor's blood sample is added to the tube with serum.
Recipient's Ig's that target the donor's red blood cells form antibody-antigen complexes.
Anti-human Ig's (*Coombs antibodies*) are added to the solution.
Agglutination of red blood cells occurs, because human Ig's are attached to red blood cells.
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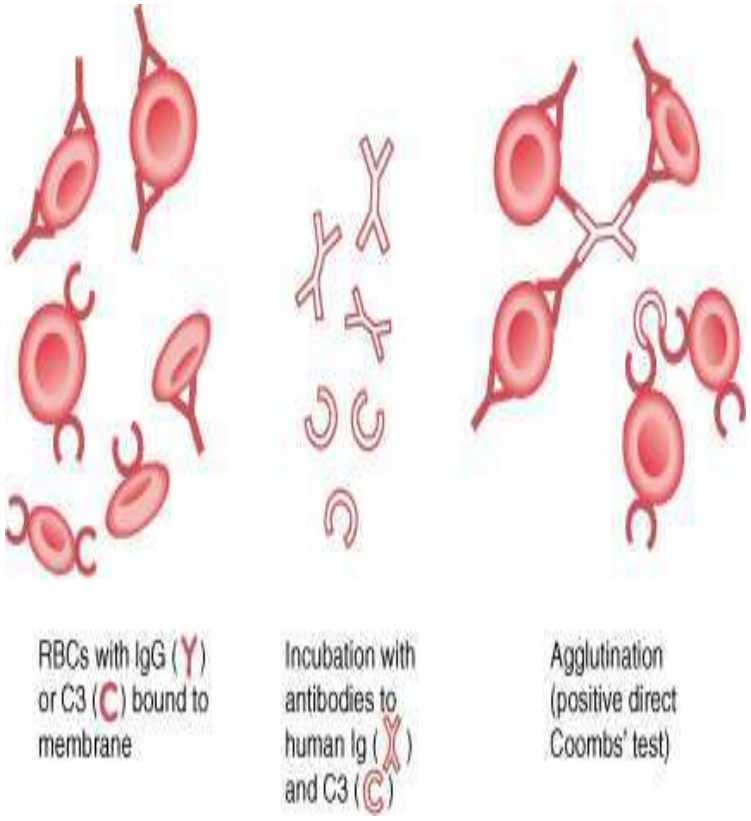
# Applications of Coomb's Test

- These include detection of anti-rhesus factor (Rh) antibodies. Antibodies to the Rh factor generally do not agglutinate red blood cells.
- Thus, red cells from Rh+ children born to Rh- mothers, who have anti-Rh antibodies, may be coated with these antibodies.
- To check for this, a direct Coombs test is performed. To see if the mother has anti-Rh antibodies in her serum an Indirect Coombs test is performed



# THANK YOU