

Coombs Test

Dr. Khalid

Direct Coombs

Synonym: Direct antiglobulin testing (DAT).

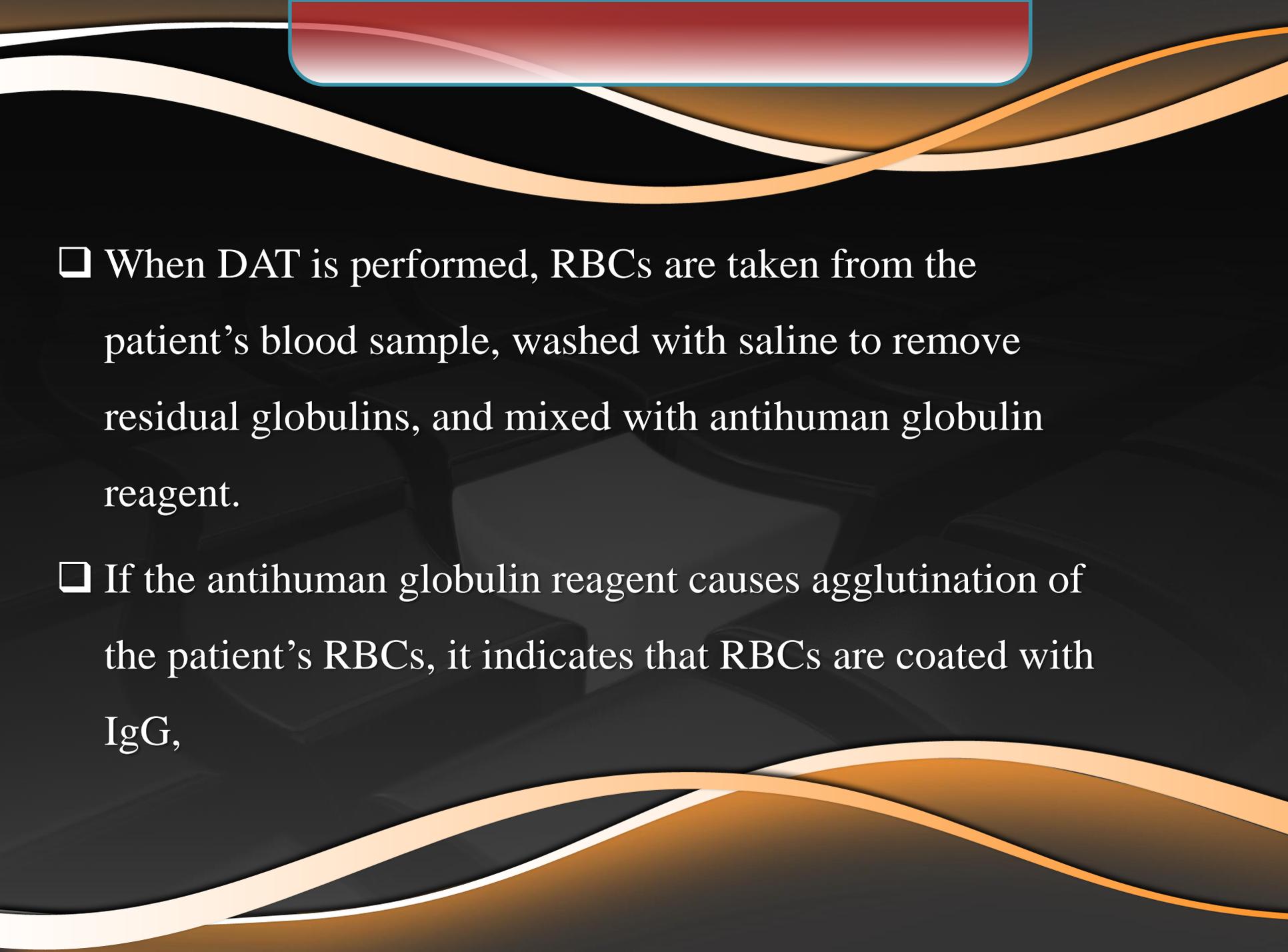
Specimen: Whole blood collected in EDTA tube.

Reference Value: Negative (no agglutination).

Method: Agglutination

Description

- ❑ Direct antiglobulin testing (DAT) detects *in vivo* antibody sensitization of red blood cells.
- ❑ Immunoglobulin G (IgG) produced in certain disease states or in response to certain drugs can coat the surface of RBCs, resulting in cellular damage and hemolysis.

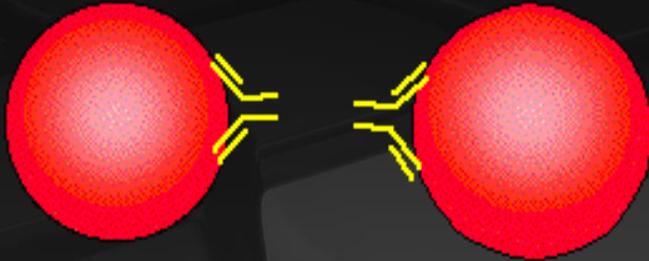
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- ❑ When DAT is performed, RBCs are taken from the patient's blood sample, washed with saline to remove residual globulins, and mixed with antihuman globulin reagent.
 - ❑ If the antihuman globulin reagent causes agglutination of the patient's RBCs, it indicates that RBCs are coated with IgG,

Direct Antiglobulin Test

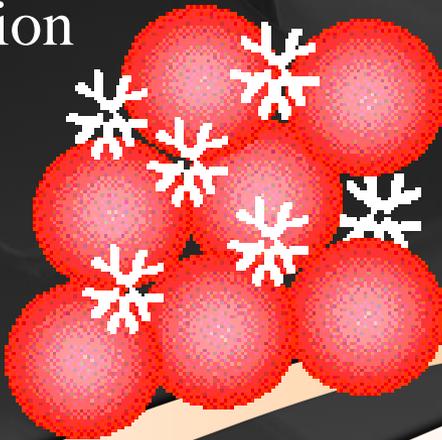
Coombs' test

Tests for antierythrocyte antibodies & C'

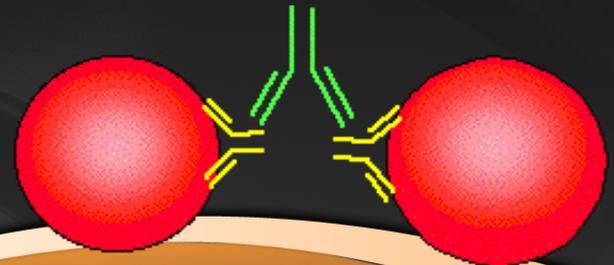
Antierthrocyte antibody



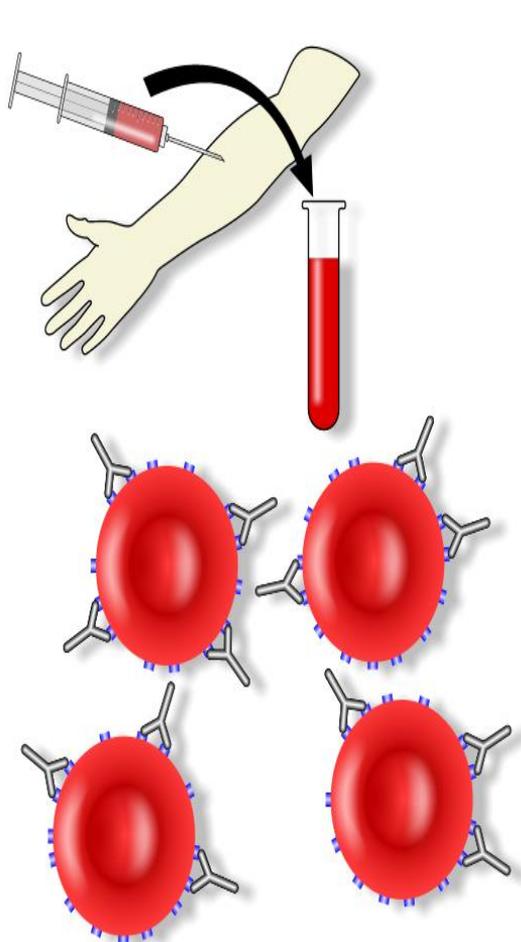
Agglutination



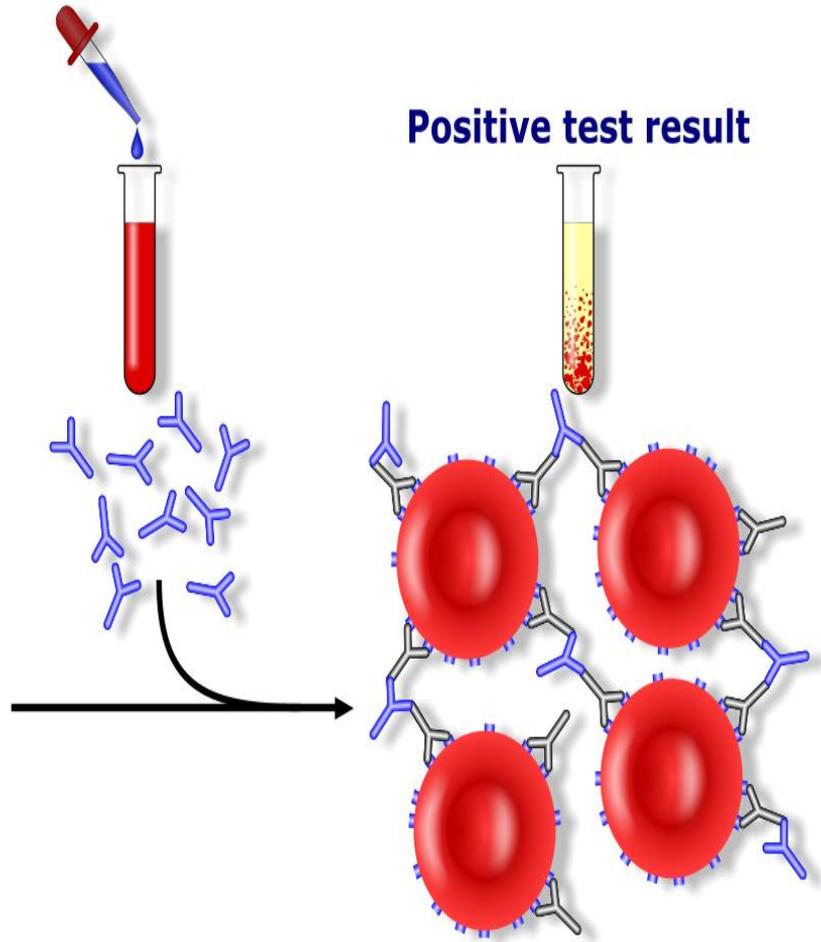
Positive coomb's test



Direct Coombs test / Direct antiglobulin test



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.

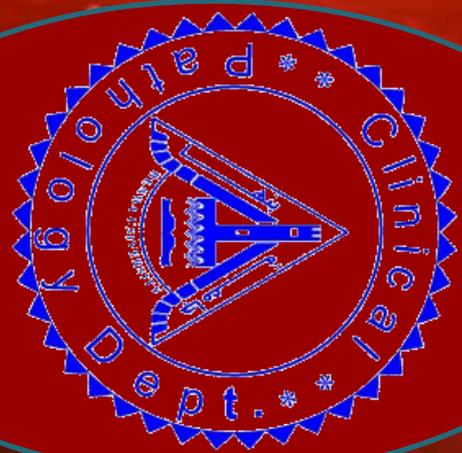
Legend	
	Antigens on the red blood cell's surface
	Human anti-RBC antibody
	Antihuman antibody (Coombs reagent)

INDICATIONS

- Detect autoimmune hemolytic anemia or hemolytic disease of the newborn
- Evaluate suspected drug-induced hemolytic anemia
- Evaluate transfusion reaction

Positive in:

1. Anemia (autoimmune hemolytic, drug-induced)
2. Hemolytic disease of the newborn
3. Lymphomas
4. Passively acquired antibodies from plasma products
5. Transfusion reactions (blood incompatibility)



Indirect Coombs test

Indirect coombs test

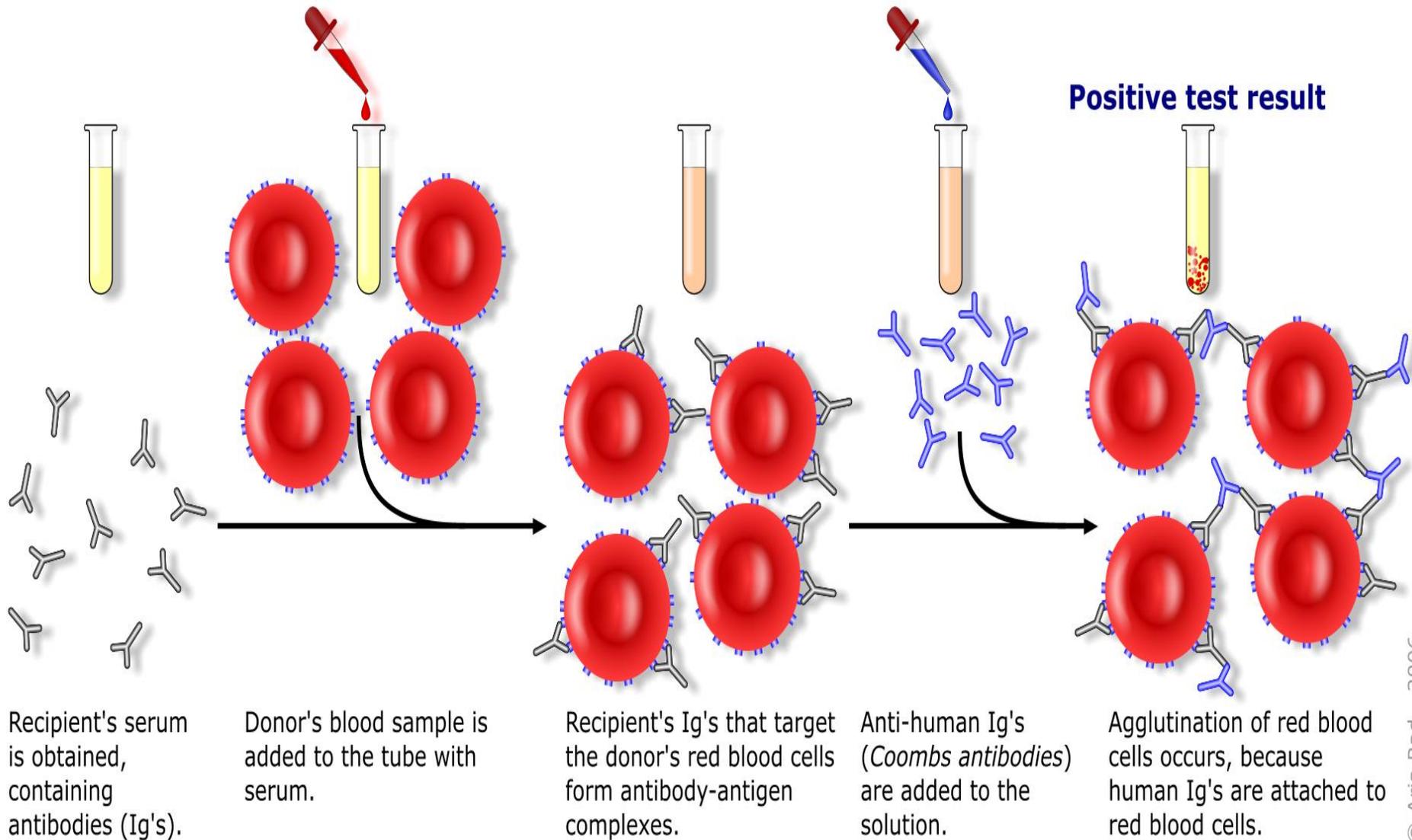
Synonyms: Indirect antiglobulin test (IAT), antibody screen.

Specimen: Serum

Reference Value: Negative (no agglutination).

Method: Agglutination

Indirect Coombs test / Indirect antiglobulin test



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.

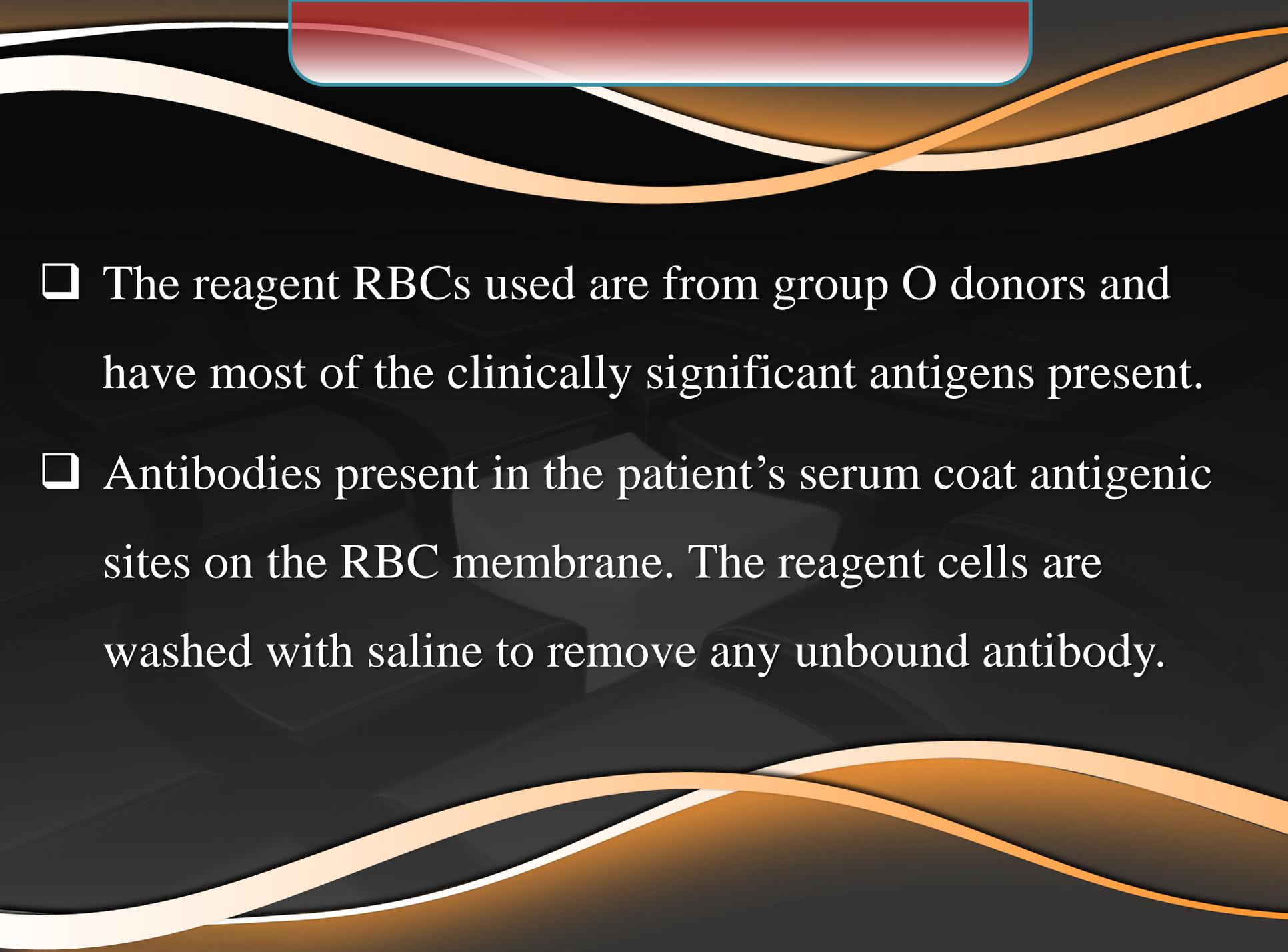
Anti-human Ig's (Coombs antibodies) are added to the solution.

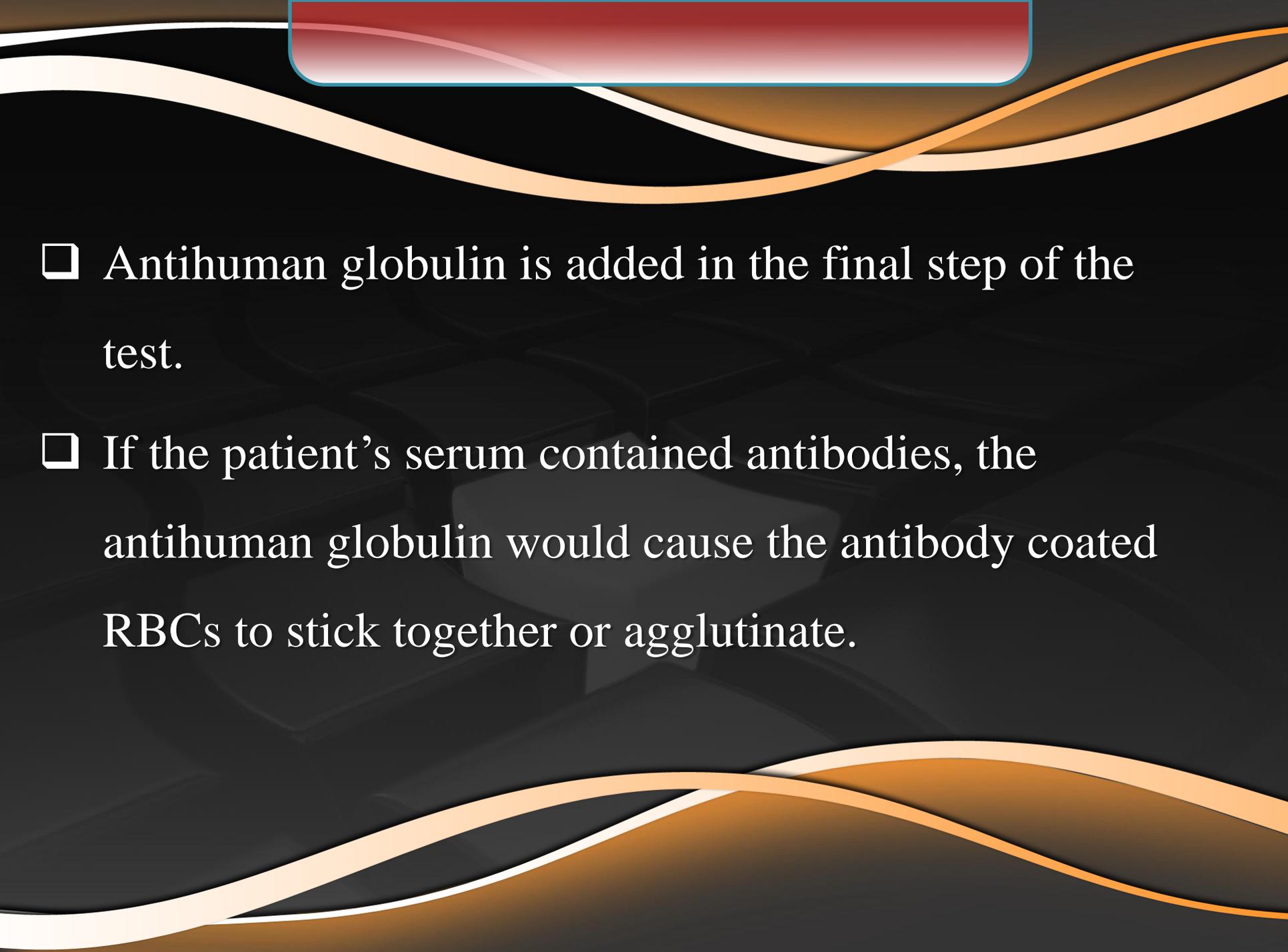
Agglutination of red blood cells occurs, because human Ig's are attached to red blood cells.

Positive test result

Description

- ❑ (IAT) detects and identifies unexpected circulating complement molecules or antibodies in the patient's serum.
- ❑ The test is used to screen a patient's serum for the presence of antibodies that may react against transfused red blood cells.
- ❑ During testing, the patient's serum is allowed to incubate with reagent RBCs.

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- ❑ The reagent RBCs used are from group O donors and have most of the clinically significant antigens present.
 - ❑ Antibodies present in the patient's serum coat antigenic sites on the RBC membrane. The reagent cells are washed with saline to remove any unbound antibody.

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- ❑ Antihuman globulin is added in the final step of the test.
 - ❑ If the patient's serum contained antibodies, the antihuman globulin would cause the antibody coated RBCs to stick together or agglutinate.

Indications

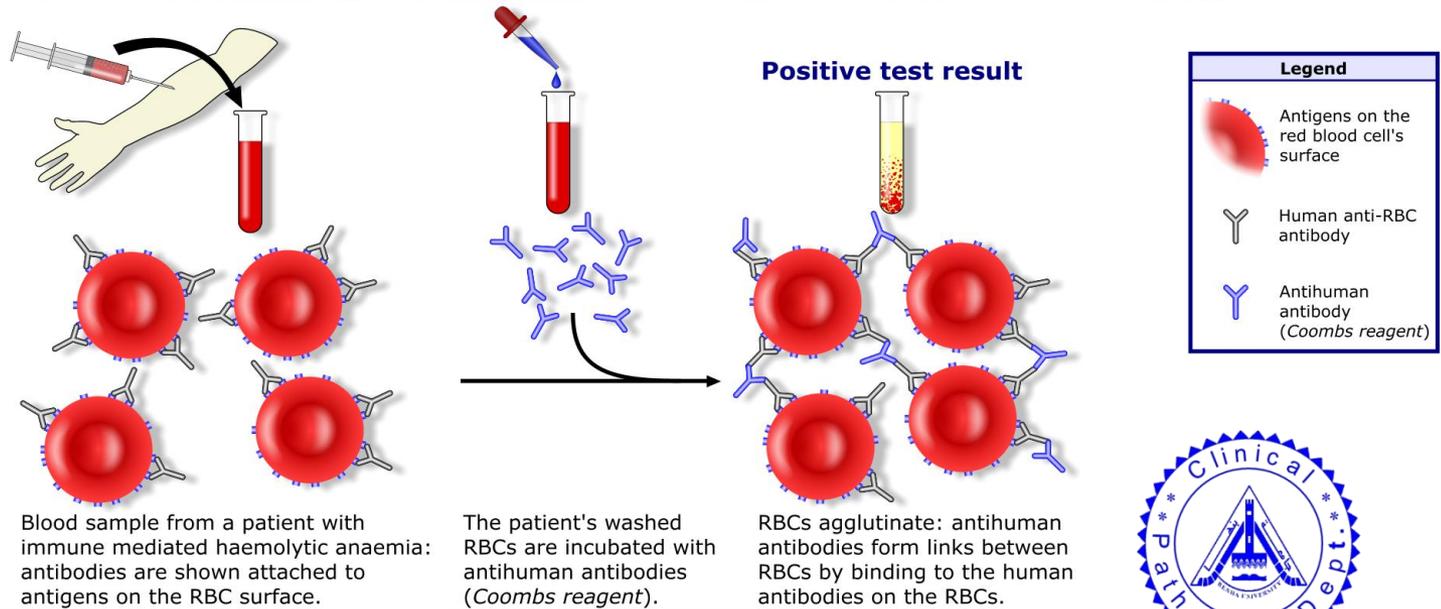
1. Detect other antibodies in maternal blood that can be potentially harmful to the fetus
2. Determine antibody titers in Rh negative women sensitized by an Rh positive fetus
3. Screen for antibodies before blood transfusions

Interpretation

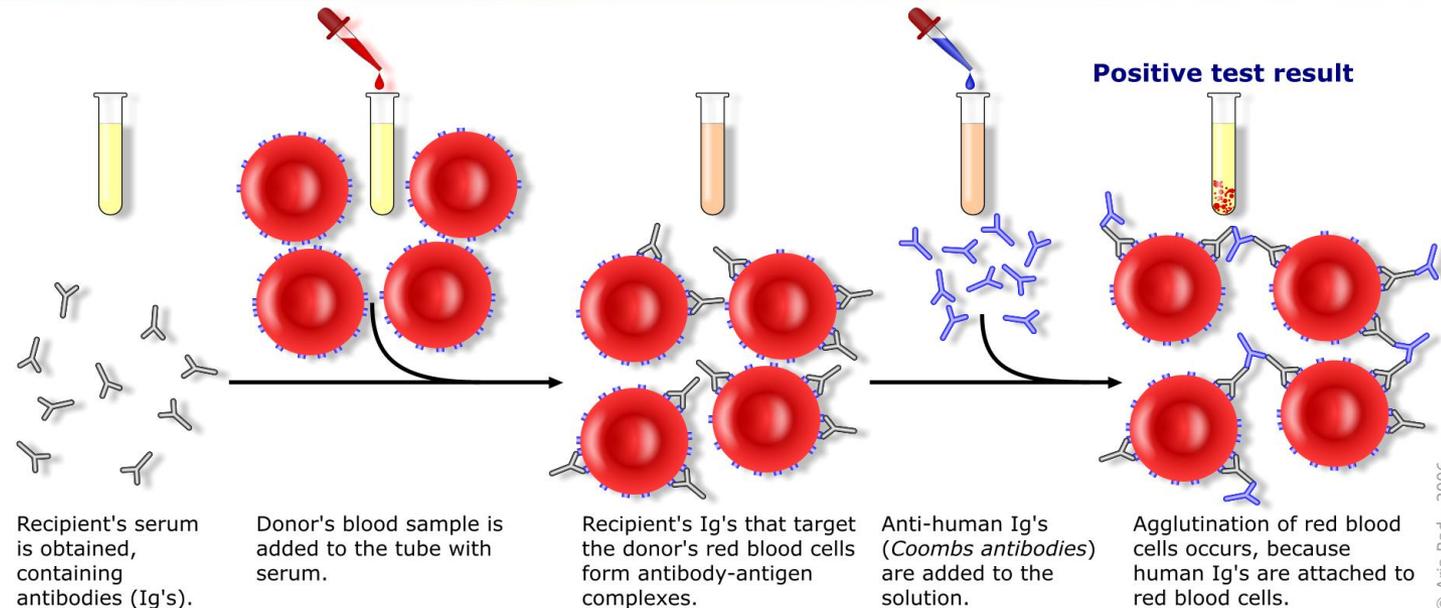
Positive in:

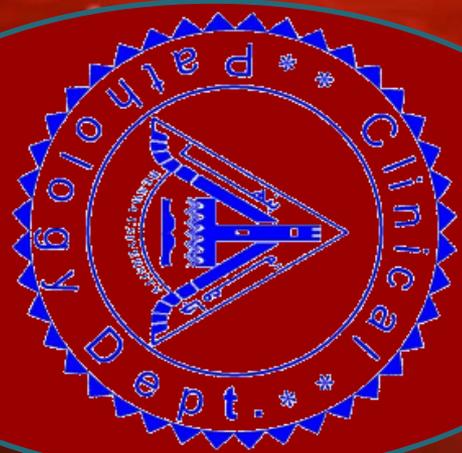
1. Hemolytic anemia (drug-induced or autoimmune)
2. Hemolytic disease of the newborn
3. Incompatible crossmatch
4. Maternal-fetal Rh incompatibility

Direct Coombs test / Direct antiglobulin test



Indirect Coombs test / Indirect antiglobulin test





Osmotic Fragility

Dr. Khalid

Osmotic Fragility

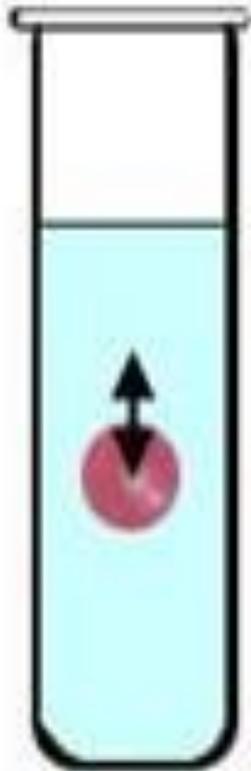
- ❑ **Synonym:** Red blood cell osmotic fragility, (OF)
- ❑ **Specimen:** Whole blood.
- ❑ **Reference Value:** Hemolysis begins at 0.5 w/v sodium chloride solution and is complete at 0.3 w/v NaCl solution. Results are compared to a normal curve.
- ❑ **Method:** Spectrophotometry



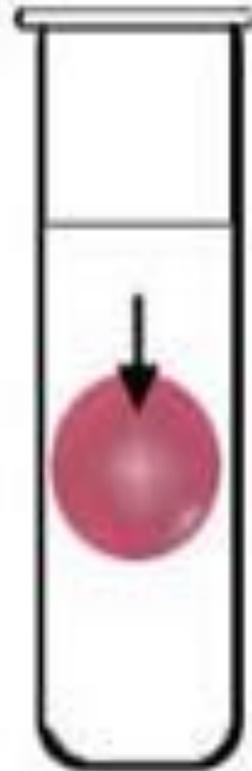
Description

- ❑ Osmotic fragility is an indication of the ability of red blood cells (RBCs) to take on water without lysing.
- ❑ In this test, RBCs are placed in graded dilutions of sodium chloride.





Isotonic

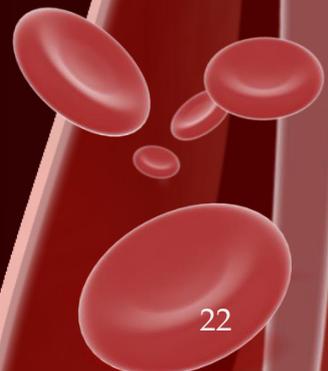


Hypotonic



Hypertonic





- ❑ Swelling of the cells occurs at lower concentrations of NaCl as they take on water in the hypotonic solution.
- ❑ Thicker cells, such as spherocytes, have an increased OF; thinner cells have a decreased OF



Indications

- ❑ Evaluate hemolytic anemia



Interpretation

Increased in:

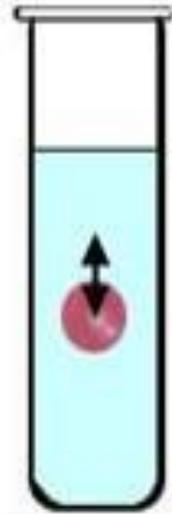
- Acquired immune hemolytic anemias
- Hemolytic disease of the newborn
- Hereditary spherocytosis
- Malaria
- Pyruvate kinase deficiency



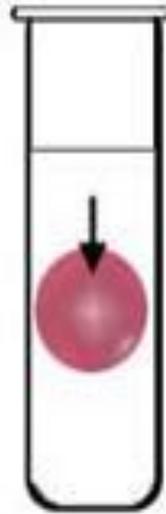
Decreased in:

- Hemoglobinopathies
- Iron deficiency anemia
- Liver disease
- Reticulocytosis

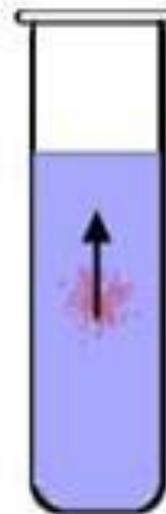




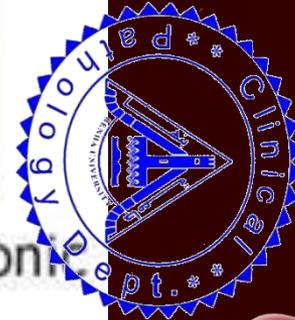
Isotonic



Hypotonic



Hypertonic



Glucose-6- Phosphate Dehydrogenase



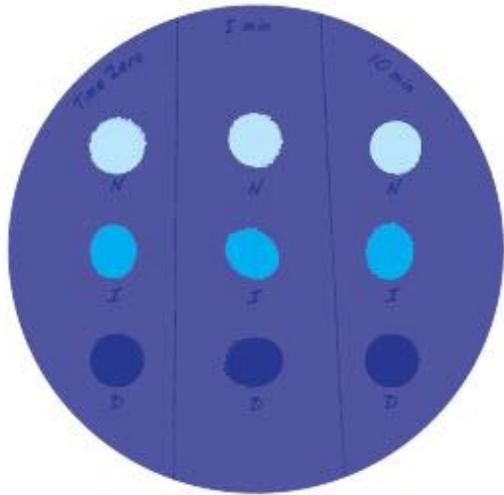
Dr. Khalid

- **Synonym:** G6PD.
- **Specimen:** Whole blood
- **Reference value:**
 - ❑ Newborn 7.8–14.4 U/g hemoglobin
 - ❑ Adult 5.5–9.3 U/g hemoglobin
- **Method:** Fluorescent

View spots under UV light

1. View the filter papers under a long-wave UV light (365 nm) after spots have dried completely. Use a viewing box with UV tempered glass to protect your eyes. View in a dark room so that you can see the fluorescence.





CareStart™
G6PD



Normal

S

CareStart™
G6PD



Deficient

S

Description

- ❑ G6PD is a red blood cell enzyme.
- ❑ It is involved in the hexose monophosphate shunt, and its function is to protect hemoglobin from oxidation.
- ❑ G6PD deficiency is an inherited abnormality.
- ❑ This deficiency results in hemolysis.

Interpretation

Decreased in:

1. Congenital non-spherocytic anemia
2. G6PD deficiency
3. Non-immunologic hemolytic disease of the newborn

Increased in:

1. Hepatic coma
2. Hyperthyroidism
3. Myocardial infarction
4. Pernicious anemia
5. Viral hepatitis