**The antiglobulin test (Coombs test)**

The Coombs test was first described in 1945 by [Cambridge](https://en.wikipedia.org/wiki/Cambridge) [immunologists](https://en.wikipedia.org/wiki/Immunology) [Robin Coombs](https://en.wikipedia.org/wiki/Robin_Coombs) (after whom it is named), it was done in [test tubes](https://en.wikipedia.org/wiki/Test_tube). Today, it is commonly done using [microarray](https://en.wikipedia.org/wiki/Protein_microarray) and gel technology.

 **Coombs reagent:** Coombs reagent (also known as **Coombs antiglobulin** or **antihuman globulin**) is used in both the direct Coombs test and the indirect Coombs test. Coombs reagent is antihuman [globulin](https://en.wikipedia.org/wiki/Globulin). It is made by injecting human globulin into animals, which produce [polyclonal](https://en.wikipedia.org/wiki/Polyclonal_response) [antibodies](https://en.wikipedia.org/wiki/Polyclonal_antibodies) specific for human [immunoglobulins](https://en.wikipedia.org/wiki/Immunoglobulins) and human [complement system](https://en.wikipedia.org/wiki/Complement_system) factors. More specific Coombs reagents or [monoclonal antibodies](https://en.wikipedia.org/wiki/Monoclonal_antibodies) can be used.

**Mechanism.**



Schematic showing the **direct and indirect Coombs tests**.

The two Coombs tests are based on the fact that anti-human [antibodies](https://en.wikipedia.org/wiki/Antibody), will bind to human antibodies. Animal anti-human antibodies will also bind to human antibodies that may be fixed onto antigens on the surface of [red blood cells](https://en.wikipedia.org/wiki/Red_blood_cell) (also referred to as RBCs), and in the appropriate test tube conditions this can lead to [agglutination](https://en.wikipedia.org/wiki/Agglutination_%28biology%29) of [RBCs](https://en.wikipedia.org/wiki/Red_blood_cell). The phenomenon of agglutination of [RBCs](https://en.wikipedia.org/wiki/Red_blood_cell) is important here, because the resulting clumping of RBCs can be visualized; when clumping is seen the test is positive and when clumping is not seen the test is negative.

Common clinical uses of the Coombs test include the preparation of blood for [transfusion](https://en.wikipedia.org/wiki/Blood_transfusion) in [cross-matching](https://en.wikipedia.org/wiki/Cross-matching), atypical antibodies in the [blood plasma](https://en.wikipedia.org/wiki/Blood_plasma) of [pregnant](https://en.wikipedia.org/wiki/Pregnant) women as part of [antenatal care](https://en.wikipedia.org/wiki/Obstetrics), and detection of antibodies for the diagnosis of immune-mediated [hemolytic anemias](https://en.wikipedia.org/wiki/Haemolytic_anemia).

Coombs tests are performed using RBCs or serum (direct or indirect, respectively) from venous whole blood samples which are taken from patients by [venipuncture](https://en.wikipedia.org/wiki/Venipuncture). The venous blood is taken to a laboratory (or blood bank), where trained scientific technical staff do the Coombs tests. The clinical significance of the result is assessed by the [physician](https://en.wikipedia.org/wiki/Physician) who requested the Coombs test, perhaps with assistance from a laboratory-based [hematologist](https://en.wikipedia.org/wiki/Hematologist).

**1-Direct Coombs test.**

The direct Coombs test (also known as the **direct antiglobulin test** or DAT) is used to detect if antibodies or [complement system](https://en.wikipedia.org/wiki/Complement_system) factors have bound to [RBCs](https://en.wikipedia.org/wiki/Red_blood_cell) surface antigens [*in vivo*](https://en.wikipedia.org/wiki/In_vivo). The DAT is not currently required for pre-transfusion testing but may be included by some laboratories.

The direct Coombs test is used clinically when immune-mediated [hemolytic anemia](https://en.wikipedia.org/wiki/Hemolytic_anemia) (antibody-mediated destruction of RBCs) is suspected. A positive Coombs test indicates that an immune mechanism is attacking the patient's own [RBCs](https://en.wikipedia.org/wiki/Red_blood_cell). This mechanism could be [autoimmunity](https://en.wikipedia.org/wiki/Autoimmunity), [alloimmunity](https://en.wikipedia.org/wiki/Alloimmunity) ([Hemolytic disease of the newborn](https://en.wikipedia.org/wiki/Hemolytic_disease_of_the_newborn) ;also known as HDN or erythroblastosis fetalis)

**Laboratory method:** The patient's [RBCs](https://en.wikipedia.org/wiki/Red_blood_cell) are washed (removing the patient's own [serum](https://en.wikipedia.org/wiki/Blood_plasma)) and then centrifuged with [antihuman globulin](https://en.wikipedia.org/wiki/Coombs_test#Coombs_reagent) (also known as Coombs reagent). If immunoglobulin or complement factors have been fixed on to the RBC surface [in-vivo](https://en.wikipedia.org/wiki/In-vivo), the antihuman globulin will [agglutinate](https://en.wikipedia.org/wiki/Agglutination_%28biology%29) the RBCs and the direct Coombs test will be positive. (A visual representation of a positive direct Coombs test is shown in the upper half of the schematic).

**2-Indirect Coombs :**

The indirect Coombs test (also known as the **indirect antiglobulin test** or IAT) is used to detect [in-vitro](https://en.wikipedia.org/wiki/In-vitro) antibody-antigen reactions. It is used to detect very low concentrations of antibodies present in a patient's plasma/serum prior to a blood transfusion. In antenatal care, the IAT is used to screen pregnant women for antibodies that may cause [hemolytic disease of the newborn](https://en.wikipedia.org/wiki/Hemolytic_disease_of_the_newborn). The IAT can also be used for [compatibility testing](https://en.wikipedia.org/wiki/Cross-match), antibody identification, RBC phenotyping, and titration studies.

1. **Blood transfusion preparation:** The indirect Coombs test is used to screen for antibodies in the preparation of [blood](https://en.wikipedia.org/wiki/Blood) for [blood transfusion](https://en.wikipedia.org/wiki/Blood_transfusion). The donor's and recipient's blood must be [ABO](https://en.wikipedia.org/wiki/ABO) and Rh D compatible. In Cross matching, a sample of the recipient's serum incubated with [blood donor's](https://en.wikipedia.org/wiki/Blood_donor) RBCs.and then coombs reagent added to detect the presence of Ab .
2. **Antenatal antibody screening :**The indirect Coombs test is used to screen pregnant women for [IgG](https://en.wikipedia.org/wiki/IgG) [antibodies](https://en.wikipedia.org/wiki/Antibodies) that are likely to pass through the [placenta](https://en.wikipedia.org/wiki/Placenta) into the fetal blood and cause [haemolytic disease of the newborn](https://en.wikipedia.org/wiki/Haemolytic_disease_of_the_newborn).

**Laboratory method:** The IAT is a two-stage test. (A cross match is shown visually in the lower half of the schematic as an example of an indirect Coombs test).

**First stage:** Washed test [red blood cells](https://en.wikipedia.org/wiki/Red_blood_cells) (RBCs) are incubated with a known human serum. If the serum contains [antibodies](https://en.wikipedia.org/wiki/Antibodies) to [antigens](https://en.wikipedia.org/wiki/Antigens) on the RBC surface, the antibodies will bind onto the surface of the RBCs.

**Second stage:** The RBCs are washed three or four times with isotonic saline and then incubated with antihuman globulin. If antibodies have bound to RBC surface antigens in the first stage, RBCs will agglutinate when incubated with the [antihuman globulin](https://en.wikipedia.org/wiki/Coombs_test#Coombs_reagent) (also known Coombs reagent) in this stage, and the indirect Coombs test will be positive.

**Titrations:** By diluting a serum containing antibodies the quantity of the antibody in the serum can be gauged. This is done by using doubling dilutions of the serum and finding the maximum dilution of test serum that is able to produce agglutination of relevant RBCs.

**Enhancement media:**

Both [IgM](https://en.wikipedia.org/wiki/IgM) and [IgG](https://en.wikipedia.org/wiki/IgG) [antibodies](https://en.wikipedia.org/wiki/Antibodies) bind strongly with their [antigens](https://en.wikipedia.org/wiki/Antigen). [IgG](https://en.wikipedia.org/wiki/IgG) antibodies are most reactive at 37 °C. IgM antibodies are easily detected in [saline](https://en.wikipedia.org/wiki/Saline_%28medicine%29) at [room temperature](https://en.wikipedia.org/wiki/Room_temperature) as IgM antibodies are able to bridge between RBC’s owing to their large size, efficiently creating what is seen as [agglutination](https://en.wikipedia.org/wiki/Agglutination_%28biology%29). IgG antibodies are smaller and require assistance to bridge well enough to form a visual [agglutination](https://en.wikipedia.org/wiki/Agglutination_%28biology%29) reaction. Reagents used to enhance IgG detection are referred to as potentiators. RBCs have a net negative charge called zeta potential which causes them to have a natural repulsion for one another. Potentiators reduce the zeta potential of RBC membranes. Common potentiators include low ionic strength solution (LISS), [albumin](https://en.wikipedia.org/wiki/Albumin), [polyethylene glycol](https://en.wikipedia.org/wiki/Polyethylene_glycol) (PEG), and proteolytic enzymes; these can be added to the test to enhance the reaction