



LAB 7
Coomb's test

Also known as **Anti globulin Test or (AGT)**, The Coombs 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 (**AHG**).

Principle of Coombs Test

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. This will form a “bridge” between the antibodies or complement coating the red cells, causing agglutination.

Types of Coombs tests are:

1- Direct Coombs test 2- Indirect Coombs test

1- Direct Coombs Test

Aim

The direct Coombs test (also known as the direct antiglobulin test or **DAT**) is **used to detect if antibodies or complement system factors have bound to RBC surface antigens in vivo.**

***Used to detect**

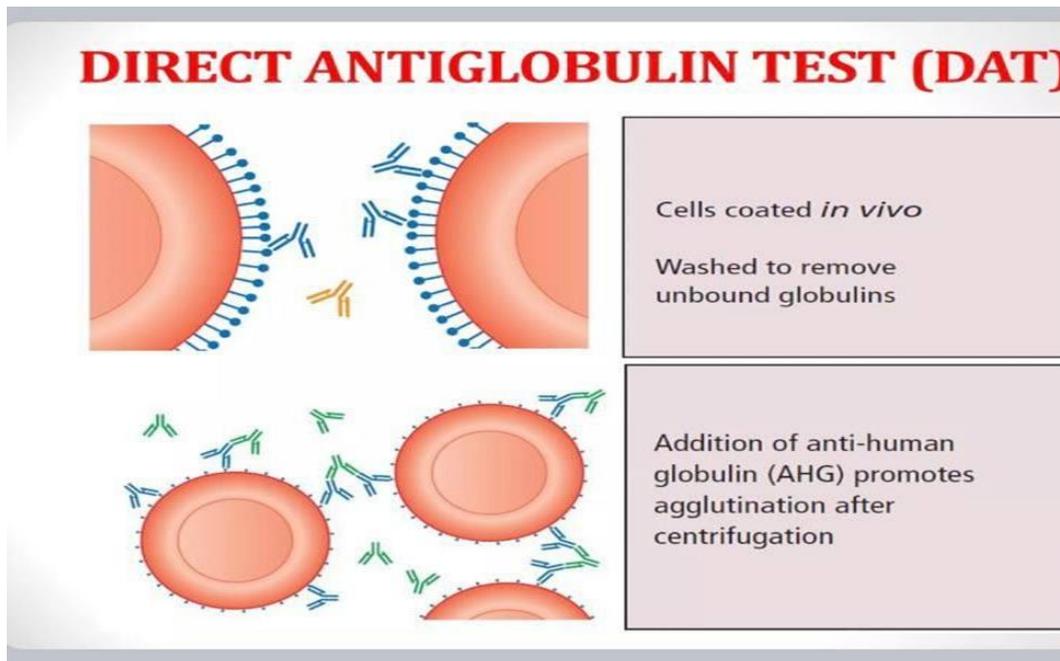
- **autoimmune hemolytic anemias**
- **transfusion reaction**
- **hemolytic disease of the newborn.**

The two most commonly recognized forms of antibody-mediated hemolysis in newborns are Rh incompatibility and ABO incompatibility.

Procedure of Direct Coombs Test

1. Prepare a 5 % suspension of the red blood cells to be tested.
2. With clean pipette add 1 drop of the prepared cell suspension to a test tube.
3. Add 2 drops of Anti-human serum.
4. Mix well and centrifuge for one minute at 1500 RPM.
- 5- Re suspend the cells by gentle agitation and examine macroscopically and microscopically for agglutination.

Agglutination of RBCs, the direct Coombs test is positive, a visual indication that antibodies are bound to the surface of red blood cells.



Indirect Coombs test (Indirect Antiglobulin test):

This test is performed to detect presence of Rh- antibodies or other antibodies in patients' serum in case of the following:

1. To check whether a Rh-negative woman (married to Rh-positive husband) has developed Anti Rh- antibodies
2. Anti D may be produced in the blood of any Rh- negative person by exposure to D antigen by:
 - Pregnancy, if infant is Rh positive (if father is Rh-positive)
 - Abortion of Rh-positive fetus.

Performing the test Requirements:

- Test tubes: (10x75 mm)
- Pasteur pipettes
- Incubator
- Centrifuge
- Specimen: Serum (need not be fasting)

Reagents:

- Antihuman serum
- Anti-D serum

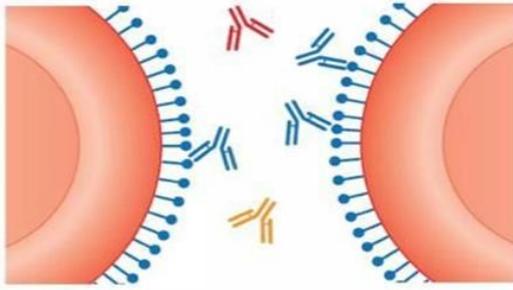
Procedure:

1. Label three test tubes as 'T' (test serum) PC (Positive control) and NC (negative control).
2. In the tube labelled as 'T', add two drops of Anti-D serum
3. In the tube 'PC' add one drop of saline.
4. Add one drop of 5% saline suspension of the pooled 'O' Rho (D) positive cells in each tube.
5. Incubate all the three tubes for one hour at 37°C.

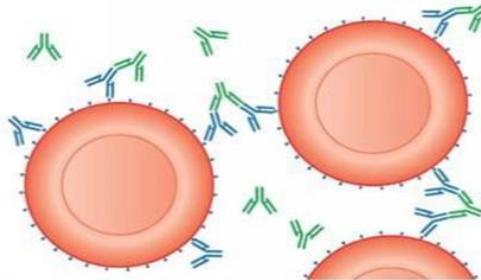
Procedure: (cont.)

- 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).
- Add two drops of Coombs serum (anti human serum) to each tube. Keep for 5 minutes and then centrifuge at 1,500 RPM for one minute.
Resuspend the cells and examine macroscopically as well as microscopically

Indirect antiglobulin test



Serum with specific antibody
mixed with reagent red cells
Washed x3 after incubation to
remove unbound globulins



Anti-human globulin
(AHG) added to promote
agglutination on
centrifugation