COOMBS TEST
principles and practice

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The Antiglobulin Test

- **Antiglobulin serum** (Coombs’ Serum) was discovered by Coombs et al in 1945.

- The antiglobulin test can be used to detect red cells sensitized with **IgG alloantibodies**, IgG autoantibodies or complement components.

- Sensitization of red cells can occur in vivo or in vitro. The use of AHG serum to detect sensitization of red cells in vitro is a two stage technique known as indirect antiglobulin test (IAT). The sensitization of red cells in vivo is detected by one stage technique the direct antiglobulin test (DAT).
Principle of Antiglobulin Test

• The incomplete antibodies (IgG) attach to red cell membrane by the Fab portion of the immunoglobulin molecule (IgG).

• The IgG molecules attached to the red cells are unable to bridge the gap between sensitized red cells which are separated from each other by the negative charge on their surface and the sensitized red cells do not agglutinate.
What is Coombs’ Serum

- Serum from a rabbit or other animal previously immunized with purified human globulin to prepare antibodies directed against IgG and complement, used in the direct and indirect Coombs' tests. Also called antihuman globulin.
Showing incomplete and complete Agglutination Reactions
Adding of Antiglobulin serum completes the reaction

- When AHG serum is added to the washed sensitized cells, the Fab portion of the AHG molecule (anti-IgG) reacts with the Fc portions of two adjacent IgG molecules attached to red cells thereby bridge the gap between sensitized red cells and cause agglutination.
Showing a Complete Reaction with Coombs Serum
Showing a Complete Reaction with Coombs Serum
How and Why to prepare for the test

• No special preparation is necessary.
• Infants or young children:
• The preparation you can provide for this test depends on your child's age, previous experiences, and level of trust.

• Why the test is performed
• The indirect Coombs' test detects circulating antibodies against red blood cells (RBCs). The major use of this test is to determine if the patient has antibodies in the blood capable of attaching to RBCs. These antibodies are other than the major ABO system or the Rh type.
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 an Rh-negative women (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-

   • Transfusion of Rh positive blood
   • Pregnancy, if infant is Rh positive (if father is Rh-positive)
   • Abortion of Rh-positive fetus.
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
1. Serum/plasma + Screening cells x2/3/4
2. Incubation at 37°C
3. Wash x3
4. Resuspend, read over light source
5. Centrifugation agglutination
6. Addition of AHG
7. Only bound antibody on RBC
Performing the test ......

Requirements:
- Test tubes: (10x75 mm)
- Pasteur pipettes
- Incubator
- Centrifuge

Specimen: Serum (need not be fasting)

Reagents:
1. Antihuman serum
2. Anti-D serum

Additional Requirements:
- Coombs control cells
  A. Make a pooled ‘O’ Rho (D) positive cells from at least three different ‘O’ positive blood samples.
  B. Wash these cells three times in normal saline (these cells should be completely free from serum with no free antibodies).
**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.
## Test Interpretation:

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<tr>
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<th>Observations</th>
<th>Conclusions</th>
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<tbody>
<tr>
<td>1</td>
<td><strong>Positive Control (PC)</strong></td>
<td>Correctly performed test procedure. Coombs serum may not be proper. Repeat the test again.</td>
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<tr>
<td></td>
<td>(A) Agglutination</td>
<td></td>
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<tr>
<td></td>
<td>(B) No Agglutination</td>
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<tr>
<td>2</td>
<td><strong>Negative control (NC)</strong></td>
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<td></td>
<td>It should show no agglutination, since saline does not contain Anti-D or any other antibodies.</td>
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<td>3</td>
<td><strong>Test (Serum) (T)</strong></td>
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<tr>
<td></td>
<td>(A) Agglutination (and if PC results are correct)</td>
<td>Patients serum contains Anti-D.</td>
</tr>
<tr>
<td></td>
<td>(B) No Agglutination</td>
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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.
Direct Coombs test (direct antiglobulin test):

- **This test is performed to detect anti-D antibody or other antibodies attached to the red cell surface within the blood stream.**
- This occurs in the following circumstances:
  - When there is a Rh-positive baby in the womb of a sensitized Rh-negative women; the antibodies produced in the mother's serum cross the placenta and after entering the baby's blood stream, these antibodies will attach to the baby's Rh-positive red blood cells. These coated (or sensitized) cells are clumped and removed from the circulation, causing hemolytic anemia (Hemolytic Disease of the Newborn: Erythroblastosis Fetalis). When the baby is born, the baby's blood is collected (or cord blood is collected from umbilical cord) and tested by the anti globulin Coombs test (direct) to detect anti D antibodies coated on red blood cells.
  - **Transfusion reactions**
  - **Drug induced red cells sensitization**
  - **Autoimmune hemolytic anemia**
DIRECT ANTIGLOBULIN TEST (DAT)

Cells coated *in vivo*

Washed to remove unbound globulins

Addition of anti-human globulin (AHG) promotes agglutination after centrifugation
Direct antiglobulin test (DAT)

- The direct antiglobulin test (DAT) detects sensitized red cells with IgG and/or complement components C3b and C3d in vivo.
- In vivo coating of red cells with IgG and/or complement may occur in any immune mechanism is attacking the patient's own RBC's.
- This mechanism could be autoimmunity, alloimmunity or a drug-induced immune-mediated mechanism.
Requirements: (same as that for Indirect Coombs test)

- Test tubes: (10x75 mm)
- Pasteur pipettes
- Incubator
- Centrifuge

**Specimen:** Blood drawn into EDTA is preferred but oxalated, or clotted, citrated whole blood may be used (specimen need not be fasting sample)
Procedure

1. Prepare a 5% suspension in isotonic saline of the red blood cells to be tested.
2. With clean Pasture 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 Antihuman 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.
**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.
False positive results: DAT and IAT

- In specimens containing potent cold-reactive antibodies agglutination may occur before adding the AHG reagent.
- Dirty glassware may cause clumping of cells.
- Over centrifugation
- DAT (Direct Agglutination Test)
- A positive DAT from a clotted sample should be repeated on an EDTA sample
- Samples collected from infusion lines may have complement present on the cells.

- IAT Cells with a positive DAT will give a positive result in any indirect antiglobulin procedure.
Coombs Test in Blood Banks

• The test is only rarely used to diagnose a medical condition, but is essential for use by laboratories such as blood banks. Blood banks use the indirect Coombs' test to determine whether there is likely to be an adverse reaction to blood to be transfused.
• Programme Created by Dr.T.V.Rao MD for Medical, Paramedical Students in the Developing World

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