**INTRODUCTION**

The antiglobulin test was first introduced into clinical laboratory in 1945 by Coombs, Mourant and Race.[1,2] Ever since the first application of the test for the detection of Rh antibodies, the sphere of usefulness of the test has continuously expanded. SPAN’s anti-human serum (Coombs sera) is a blend of polyvalent anti-lgG (Rabbit / Goat) and Monoclonal anti-C3d (BRIC-8). Anti-lgG has broad spectrum of reactivity for all subtypes gamma globulins (lgG1, lgG2, lgG3 and lgG4), whereas, anti-C3d reacts with the complement component of C3d. The Coombs sera does not react with C4 fragment of complement.

**DIRECT ANTIGLOBULIN TEST (DAT)** is a diagnostic procedure used to demonstrate in vivo sensitisation of erythrocytes with antibodies and/or complement fragment (C3d). The test is used for:

1. Autoimmune haemolytic anaemia
2. Haemolytic disease of the newborn
3. Drug induced haemolysis
4. Transfusion reactions

Indirect antiglobulin test (IAT) is a diagnostic procedure used to demonstrate in vitro sensitisation of erythrocytes with antibodies.[3]

The test is used for:

1. Antibody screening and identification
2. Compatibility testing
3. Red cell phenotyping

**PRINCIPLE**

Incomplete antibodies directed against the antigens of the erythrocyte surface cause sensitisation (get adsorbed on the surface of red blood cells), but fail to bring about agglutination of these cells. Anti-Human Serum will react with adsorbed antibodies (immunoglobulins) and/or C3d fragment of complement and/or complement fragment (C3d) . The test is used for:

1. Sensitivity of detection of bound complement will be increased
2. Results after immediate centrifugation must also be taken into consideration since Anti-lgG reactions may be adversely affected by incubation at Room Temperature for 10 minutes followed by
3. Note:

   a. Inadvertently omitted.

**SAMPLE**

Blood samples should be collected by standard aseptic technique. Do not use haemolysed samples.

1. For Direct Antiglobulin Test: It is preferable to collect blood with EDTA as anticoagulant because EDTA prevents in vitro adsorption of complement without affecting the complement or antibody which is already attached to the erythrocytes in vivo before withdrawing the blood sample. Erythrocytes from blood collected with other anticoagulants like ACD, CPD or Oxalates can also be used. Cells obtained from clotted whole blood may also be used. Sample should be tested as soon as possible after collection in order to avoid variations in the reactivity of erythrocytes on storage, especially of those sensitised with complement fragments.

2. For Indirect Antiglobulin Test: Serum should be obtained from freshly clotted blood. Complement activity is diminished in sera stored at room temperature for more than 48 hours or at 2-8°C for more than 7 days. Plasma from anticoagulated samples may also be used but it is deficient in complement components.

**KIT COMPONENT**

1. Anti-Human Serum (Polyspecific) is a blend of Polyclonal anti-lgG & Monoclonal anti-C3d containing stabilisers and Sodium Azide (0.1%) as preservative. It is ready to use. Do not dilute.

**MATERIALS REQUIRED BUT (NOT PROVIDED)**

1. Normal Saline
2. Coombs Control Cells
3. Test tubes
4. Centrifuge
5. Waterbath / Incubator block
6. Timer
7. Pipettes

**STORAGE & STABILITY OF THE KIT COMPONENT**

Anti-Human Serum is stable at 2-8°C till the expiry date mentioned on the label of the container (Do not Freeze). Storage at higher temperatures or repeated freezing-thawing cycle will affect stability of the Coombs sera.

**PRECAUTION**

1. Turbidity in the Coombs sera is indicative of deterioration as a result of microbial contamination or improper handling and/or storage. Such kit component must be discarded.
2. Contamination of the Coombs sera with human Serum or gamma globulins will result in loss of potency. Contaminated Coombs sera should not be used.
3. All glassware must be thoroughly cleaned to remove any traces of human Serum.

**PROCEDURE**

Bring the Coombs sera to Room Temperature before use.

**A. DIRECT ANTIGLOBULIN TEST (DAT)**

1. Take 5-7 drops or anticoagulated blood under test in a test tube (10x75mm).
2. Wash the RBCs 3-4 times with normal saline to remove nonspecifically adsorbed plasma proteins.
3. Prepare 3-5% suspension of washed RBCs in normal saline.
4. (a) Place one drop of Anti-Human Serum in a test tube labelled “T” (Test).
   (b) Place one drop of normal saline in a test tube labelled “C” (Control).
5. Add one drop of 3-5% suspension of RBCs to each of the above tubes and mix well.
6. Centrifuge both the tubes at 1000 rpm for 1 minute.
7. Resuspend the packed cells by gentle agitation and examine macroscopically as well as microscopically for agglutination.

**RESULT:** Presence of agglutination indicates positive test result and absence indicates negative test result. Control must show absence of agglutination.

**Important:** All Negative tests should be confirmed by addition of one drop of Coombs Control Cells* to each tube followed by centrifugation at 1000 rpm for 1 minute and should be examined for agglutination under microscope.

Agglutination of Coombs Control Cells* indicates satisfactory performance of Anti-Human Serum in the test and also confirms that the addition of Anti-Human Serum in Step 4 was not inadvertently omitted.

**Note:**

1. Sensitivity of detection of bound complement will be increased by incubation at Room Temperature for 10 minutes followed by recentrifugation at 1000 rpm for 1 minute.
2. Results after immediate centrifugation must also be taken into consideration since Anti-lgG reactions may be adversely affected by second incubation.

**ANTI-HUMAN SERUM**

(Polyspecific)

(Coombs Sera)

(Code No. 11117A, 11117B, 11117C)
B. INDIRECT ANTIGLOBULIN TEST (IAT)

1. Prepare test antigen erythrocytes by taking 5-7 drops of appropriate blood sample (clotted or anticoagulated) in a test tube (10x75mm).
2. Wash the RBCs 3-4 times with normal saline to remove adsorbed plasma proteins.
3. Prepare 3-5% suspension of washed RBCs in normal saline.
4. Place two drops of Serum under test in a test tube labelled "T" (Test).
5. Add two drops or 3-5% suspension of RBCs to the above tube.
6. Incubate the tube at 37°C for 1 hour and centrifuge at 1000 rpm for 1 minute.
7. Remove the supernatant and wash the packed RBCs 3-4 times with Normal Saline.
8. Discard the supernatant and add one drop of Anti-Human Serum to the RBCs and incubate further for 15-30 minutes.
9. Centrifuge the tube at 1000 rpm for 1 minute and examine macroscopically as well as microscopically.

Autocontrol Tube: For every test one autocontrol tube must be included by taking 2 drops of patient's Serum along with 2 drops of 3-5% suspension of patient's own erythrocytes and carrying it through step 6 onwards.

Important: All Negative results must be confirmed by adding to each tube 2 drops of Coombs Control Cells,* followed by centrifugation at 1000 rpm for 1 minute. Agglutination of Coombs Control Cells* indicates satisfactory performance of Anti-Human Serum in the test and also confirms that the addition of Anti-Human Serum in Step 8 was not inadvertently omitted.

Note:
1. Use of low ionic strength solution (LISS) for preparing test cell suspension enables the incubation time to be reduced to 15 minutes. LISS suspended cells will help in detecting cases with low levels of antibodies.
2. In antibody screening and identification test, addition of bovine albumin LISS suspended cells will help in detecting cases with low levels of antibodies. For this purpose, add 2-3 drops of 22% or 30% bovine albumin solution to each tube 2 drops of Serum-cell mixture in Step 5 and centrifuge the tubes at 1000 rpm for 1 minute and note the presence or absence of albumin agglutinating antibodies before proceeding for Steps 6 through 9.

APPLICATION: Within the framework of the test procedure given above, various specific applications are summarised below.

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* PREPARATION OF COOMBS CONTROL CELLS

1. Take approximately 10-15 drops of O(Rh) Positive anticoagulated blood in a test tube.
2. Wash the blood cells twice with excess of normal saline and prepare an approximately 5% suspension of washed RBCs in normal saline.
3. Mix equal volumes of incomplete weak Anti-D and the 5% RBC suspension prepared as per step 2 in a test tube and incubate at 37°C for 60 minutes.
4. Centrifuge and discard the supernatant and wash the cells 3-4 times with excess of normal saline.
5. Decant the supernatant completely and prepare 3-5% suspension of RBCs in normal saline.

SPECIFICITY & POTENCY

The specificity of Polyclonal Anti-Human Serum used in formulation of this product has been checked by immunoelctrophoresis and the potency of both Polyclonal as well as Monoclonal antibodies has been determined by titration using appropriately sensitised human erythrocytes. The Coombs sera has no activity against C4 coated erythrocytes. Due to difference in technique between laboratories, variation in results and interpretation of results can be expected. These variations may become more marked due to fragility of complement fractions.

LIMITATIONS OF THE PROCEDURE

False Negative Results: False negative reactions may be obtained because of one or more of the following reasons:
1. Incomplete washing of patient's cells.
2. Contamination of glassware with human Serum.
3. Too heavy or too weak erythrocyte suspension.
4. Improper temperature, centrifugation or incubation.
5. Inadverted omission of addition of anti-globulin sera.
6. Insufficient time of incubation for proper sensitisation.
7. Fibrin clot in cell suspension.
8. Microbial contamination of Coombs sera leading to inactivation of Anti-Human Serum.

Negative results do not necessarily rule out haemolytic disease of newborn especially when ABO incompatibility is suspected.

False Positive Results: False positive reactions may be obtained because of one or more of the following reasons:
1. Microbial contamination of test cells/Coombs sera.
2. Use in Indirect Antiglobulin Test of erythrocytes which are positive by Direct Antiglobulin Test.
3. Autoagglutination of cells.

REFERENCE