INTRODUCTION
Generally antibodies involved in transfusion reactions are of two types, namely the complete and the incomplete, whereas the complete antibodies agglutinate red cells in saline medium, the incomplete type of antibody sensitizes red cell without agglutination. Usually IgM class of antibodies and IgG1 and IgG2 type of IgG antibodies fix complement. Sellysis, in vitro, is mediated through the complement system and the complement component C3b is further acted upon to produce C3d.

In the direct antiglobulin tests, Anti human globulin reagent is used to detect antibodies adsorbed to the red blood cells in vitro. Anti human globulin reagent is useful for compatibility testing, antibody detection, antibody identification, umbilical cord red blood testing and detection of the D-variant of the human red blood cell antigen D (Rho).

REAGENT
Anti human globulin is balanced ready to use blend of highly purified immunoglobulin. It contains Anti human IgG antibodies and antibodies reactive with human complement components C3b and C3d. These anti complement antibodies are IgM class monoclonals and they impart the necessary sensitivity to the reagent.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and titre.

REAGENT STORAGE AND STABILITY
a) Store the reagent at 2-8°C. DO NOT FREEZE. b) The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

PRINCIPLE
Normal human red blood cells, in presence of antibody directed towards the antigen they possess, may fail to agglutinate and become sensitized. This may be due to the particular nature of the antigen and antibody involved. Anti human globulin or components of human complement involved and cause agglutination of the red blood cells.

NOTE
(1). In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use. (2). The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water. (3). Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagents should be discarded. (4). Reagents are not from human sources, hence contamination due to HBsAg and HIV is practically excluded.

SAMPLE COLLECTION AND STORAGE
No special preparation of the patient is required prior to sample collection by approved techniques. Do not use haemolysed samples.

For Direct Antiglobulin Test: Blood drawn into EDTA is preferred but oxalated, citrated or clotted whole blood may be used. The blood samples should be tested as soon
as possible after collection and should not be stored.

**For indirect Antiglobulin Test**: Serum not more than 48 hours old, should be used. Donor units may be tested may be tested up to the end of their dating.

**Preparation of Coombs Control Cells**

1. Dilute Anti-D (IgG)/Anti-D (polyclonal) reagent 1:50 in isotonic saline.
2. Prepare a 5% suspension of group O Rho D positive cells in isotonic saline.
3. Mix equal volumes of diluted Anti-D reagent (as in 1 above) and 5% suspension of O Rho D positive cells (as in 2 above) and incubate at 37°C for 15 minutes.
4. Decant and wash thoroughly with isotonic saline at least thrice.
5. Resuspend in isotonic saline to make a 5% suspension of coombs control cells.

**Additional Material Required**

For **Direct Antiglobulin Test**: Test tubes (10x75mm), Pasteur pipettes, Centrifuge, Isotonic saline, Coombs control cells, Optical aid, for Indirect Antiglobulin Test and Compatibility Test: Test tube (10x75 mm), Pasteur pipettes, Bovine Serum Albumin, Centrifuge, Incubator (37°C), Isotonic saline, Coombs control cells, Optical aid.

**Procedure**

Bring reagent to room temperature before testing.

**Direct Antiglobulin Test**

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Pipette one drop of the cell suspension into a test tube.
3. Fill the tube with fresh isotonic saline and centrifuge for 30 seconds at 3400 rpm (1000g).
4. Decant and repeat this washing at least thrice.
5. Add two drops of Anti human globulin reagent and mix well.
6. Centrifuge for one minute at 1000 rpm (125g) or for 20 seconds at 3400 rpm (1000g).
7. Very gently resuspend the cell button observing for agglutination macroscopically.
8. Proceed to incubation phase.

**Antiglobulin Phase**

1. Only the albumin tubes (A) are tested in the antiglobulin phase.
2. Wash the mixture of red blood cells and serum thoroughly with isotonic saline for three times. Decant completely after the last wash.
3. Place two drops of Anti human globulin reagent into the last tubes containing the sedimented cells and mix well.
4. Centrifuge for one minute at 1000 rpm (125 g) or for 20 seconds at 3400 rpm (1000 g).
5. Very gently resuspend the cell button and observe for agglutination macroscopically.

**Indirect Antiglobulin Test**

In all phases of the compatibility test, if no agglutination or haemolysis is observed then the patient and the donor may be considered compatible, if haemolysis or agglutination at any point till the completion of the antiglobulin phase is observed.

**Remarks**

1. If plasma is used in the indirect antiglobulin test the complement dependent antibodies may not detected due to the absence of calcium.
2. To all negative test results, after the antiglobulin test phase, one drop of Coombs control cells should be added. If Coombs control cells do not agglutinate then the compatibility test must be repeated.
3. In the indirect antiglobulin test procedure an auto control tube (individuals cell in his own serum) should be run.
4. Red blood cells showing a positive direct antiglobulin test cannot be used for the indirect antiglobulin test.
5. It is recommended that Anti- IgG activity of the Anti human globulin reagent be tested from time to time preferably on a daily using Coombs control cells as a positive control.
6. All glassware used in the test should be scrupulously clean dry and free from contamination with human serum.
7. Contaminated Bovine serum albumin, saline or glassware may inactivate anti human globulin reagent.
8. Use of various drugs and certain diseases (such as megaloblastic anaemia) are known to be associated with a positive direct antiglobulin test.
9. Cord cell obtained from a newborn exhibiting hemolytic disease of the newborn, especially due to ABO incompatibility may give false negative results.
10. Anti human globulin reagent does not contain Anti-C, and is free from anti-T activity.
11. As under centrifugation or over centrifugation could lead to erroneous results, it is recommeded that each laboratory calibrate its own equipments and the time required for achieving the desired results.
12. The label minimum titer claim is based on antibody (incomplete) and complement coated red cells for Anti Human reagent. This is based on titration procedure as recommended by the manufacture. Any deviation in test procedure would result in variable results.

**Warranty**

This product is designed to perform as described on the label and package insert. The manufacture disclaims any implied warranty of use and sale for any other purpose.

**Bibliography**

5. Data on File : Global invitro LLP.