Procedures for recognition of Reagent Deterioration
1. Prepare a 5% suspension in isotonic saline of complement sensitized cells as a positive control, and a 5% suspension in saline of unsensitized cells as a negative control as described in Quality Control in the Blood Bank-Concept Procedures.
2. Label three small test tubes: CCC (Combits Control Cells), CSC (Complement Sensitized Cells), and USC (Unsensitized Cells).
3. To the appropriately labeled test tubes, add one drop of Combits Control Cells, Complement Sensitized Cells, and Unsensitized Cells.
4. To each tube add two drops of Anti-Human Serum.
5. Mix the contents of the tubes and centrifuge. (See Step 6 of Direct Antiglobulin Test for suggested centrifugation.)
6. Read and record results.

B. Negative antiglobulin tests should be controlled with Combits Control Cells, group O red blood cells sensitized with IgG antibody A positive test (agglutination) indicates:
   - The Anti-Human Serum added was capable of reacting in the test.
   - The Anti-Human Serum had not been neutralized by improper washing of the cells' serum mixture.
   - The Anti-Human Serum had, in fact, been added to the test.

C. Cold autoantibodies present in many normal human sera may fix complement to red blood cells at room temperature without agglutinating the cells. If the test has been incubated at room temperature (rather than being centrifuged immediately) and is made through 37°C incubation and the antiglobulin phase, there is possibility that complement fixed at room temperature by an insignificant cold antibody will cause an incompatible crossmatch in the antiglobulin phase. If room temperature incubation is desired, it should be set up as a separate test and should not be carried through the antiglobulin phase.

Caution: Contaminated Buffy Atumine, saline or glassware may inactivate Anti-
Human Serum.

LIMITATIONS OF PROCEDURE
1. The use of various drugs and also certain disease states are known to be associated with positive direct antiglobulin tests.
2. Incompletely washed red blood cells may give false test results.

SPECIFIC PERFORMANCE CHARACTERISTICS
When properly stored and used according to the procedures described under Directions for Use, this reagent will detect IgG, C3d and/or C4 present on red blood cells in greater than normal amounts. This reagent is shown to agglutinate cells sensitized with a standard concentration of IgG blood group antibody and cells sensitized with C3d and C4 components of human complement. This reagent is shown not to agglutinate unsensitized red cells. Semisolid tests (low ionic strength and low ionic strength plus trepton) treatment provides evidence that this reagent will detect IgG, C3d and/or C4 present on red blood cells in greater than normal amounts. This reagent is shown to agglutinate cells sensitized with a standard concentration of IgG blood group antigen and antibodies sensitized with C3d and C4 components of human complement. This reagent is shown not to agglutinate unsensitized red cells.

The Anti-Human Serum is used to demonstrate antibodies absorbed to the red blood cells in vitro. Such tests include antibody screening and identification, the direct test and detection of other red cell antigens e.g. P, K using specific antisera (Anti-A, Anti-B).

The procedures used with this reagent are based on the principle of heteroagglutinins directed against components of human serum as originally described by Moreschi and agglutination as described by Landsteiner. Normal human red blood cells, in the presence of antibody directed toward an antigen they possess, may become sensitized but fail to agglutinate due to the particular nature of the antigen and antibody involved. The procedures used with this reagent will react with red cells sensitized with globulins (red cell antibody) or components of human complement and cause agglutination of the red blood cells.

STORAGE AND PACK SIZE:
The Anti-Human Serum is packed in 5ml & 10ml dropper vials. The antibodies are stable at 2-8°C until the expiry date mentioned on the reagent vial label. The reagent contains 0.1% sodium azide as a preservative. Do not use beyond expiration date. Bring to room temperature (20°C-30°C) prior to use.

BIBLIOGRAPHY
• Moreschi C: Neue tattachen aber die blutkorperchen agglutinationen. Zbl Bakt 46:69 and 456, 1908.

NOTICE
• All efforts are made to supply ordered consignment as per the sample submitted but due to continuous development, the company reserves the right to improve/change any specifications/"products without prior information notice to the buyer.

LIMITED EXPRESSED WARRANTY DISCLAIMER
The manufacturer limits the warranty to the test kit, in as much as that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer’s liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, however caused by the product in the use or in the application thereof.

PRINCIPLE
The procedures used with this reagent are based on the principle of heteroagglutinins directed against components of human serum as originally described by Moreschi and agglutination as described by Landsteiner. Normal human red blood cells, in the presence of antibody directed toward an antigen they possess, may become sensitized but fail to agglutinate due to the particular nature of the antigen and antibody involved. The procedures used with this reagent will react with red cells sensitized with globulins (red cell antibody) or components of human complement and cause agglutination of the red blood cells.

SUMMARY AND EXPLANATION
The principle of the antigen-antibody reaction was first described in 1908 by Moreschi. The antiglobulin test (Combits test) was first used in blood serology in 1945 when Coombs and Mantour and Race described the reaction of antiglobulin serum with components of human complement.

Anti-human serum is an important diagnostic aid in determining the presence or absence of red blood cell antibody or components of human complement on red blood cells. Accordingly, Anti-human Serum is used for compatibility testing, antibody detection, antibody identification, testing for the variant of the Rh (D) antigen, I' test and unrelated red blood cell testing.

Agglutination of red blood cells in the presence of Anti-human Serum is a positive test result which indicates the presence of an antibody on the red blood cells. Absence of agglutination indicates there is no detectable antibody or human complement component on the red blood cells. Anti-human Serum is polyspecific since contains required Anti-IgG and Anti-complement components.

Anti-human Serum may be used in the direct antiglobulin test and in the indirect antiglobulin test to detect antibodies and/or complement on red blood cells. In the direct antiglobulin test, Anti-human Serum is used to demonstrate antibodies absorbed to the red blood cells in vitro. Anti-human serum may also be used for some indirect antiglobulin tests to demonstrate antibodies absorbed to the red blood cells in vitro. Such tests include antibody screening and identification, the direct test and detection of other red cell antigens e.g. P, K using specific antisera (Anti-A, Anti-B).

The following table summarizes the indications for use of Anti-human Serum

<table>
<thead>
<tr>
<th>Direct Antiglobulin Test</th>
<th>Indirect Antiglobulin Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Identification (Serum)</td>
<td>Antibody Identification (Serum)</td>
</tr>
<tr>
<td>Antibody Identification (Saliva)</td>
<td>Antibody Identification (Saliva)</td>
</tr>
<tr>
<td>Detection of Antigens</td>
<td>Detection of Antigens</td>
</tr>
<tr>
<td>Detection of Drug-Induced</td>
<td>Detection of Drug-Induced</td>
</tr>
<tr>
<td>Red Cell Sensitization</td>
<td>Red Cell Sensitization</td>
</tr>
<tr>
<td>Investigation of Transfusion Reactions</td>
<td>Investigation of Transfusion Reactions</td>
</tr>
<tr>
<td>Detection of Unexpected Antibodies</td>
<td>Detection of Unexpected Antibodies</td>
</tr>
<tr>
<td>Detection of Unexpected Antibodies</td>
<td>Detection of Unexpected Antibodies</td>
</tr>
<tr>
<td>Donor Screening for Unexpected Antibodies</td>
<td>Donor Screening for Unexpected Antibodies</td>
</tr>
<tr>
<td>Compatability Testing</td>
<td>Compatability Testing</td>
</tr>
</tbody>
</table>

For in-vitro diagnostic use only, not for medicinal use
**DIRECT ANTIGLOBULIN TEST**

For the direct antiglobulin test, blood drawn into EDTA is preferred (to prevent the fixation of complement in vitro). In cases of severe hemolysis, citrated or clotted whole blood may be used. The blood sample should be tested as soon as possible following collection and should not be stored.

**INDIRECT ANTIGLOBULIN TEST**

For the indirect antiglobulin test, serum (not more than 48 hours old) from clotted blood should be used. If plasma is used in the indirect antiglobulin test, complement-dependent antibodies may not be detected because acriflavine is not available. If a delay in testing should occur, the sample should be stored at 2-8°C. Donor units may be tested up to the end of their dating.

---

**PROCEDURES**

**Required Supplementary Materials**

- Test tubes, 10x75mm or 12x75mm
- Centrifuge
- Coombs' Control Cells
- Optical aid

**Direct Antiglobulin Test**

1. Prepare a 5% suspension in isotonic saline of the red blood cells to be tested.
2. With a clean Pasteur pipette, add one drop of the prepared cell suspension to a small test tube.
3. Fill the tube with fresh isotonic saline, centrifuge at high speed and decant. Perform this washing a minimum of three times.
4. Decant completely after the last washing. (Caution: Do not introduce human serum components while running the test.)
5. Mix contents well and centrifuge at 1000 rpm for 1 minute.
6. Resuspend the red blood cells by gentle agitatin and examine macroscopically for agglutination. If negative, examine with an optical aid.
7. Control negative antiglobulin tests by adding one drop of Coombs' Control Cells.

**Indirect Antiglobulin Test**

1. Prepare a 5% suspension in isotonic saline of the donor red blood cells to be tested.
2. Place 1 drop of Pastur pipette.
3. Place 1 drop of Centrifuge.
4. Place 1 drop of Incubator, 37°C

**Compatible Coombs' Control Cells**

Dilute Anti-D (IgG) Monoclonal Antibodies 1:10 in isotonic saline. Add an equal volume of this dilution to a 10% cell suspension of Group O Rh1 (D) positive (D+) red blood cells at 37°C for 30 minutes for sensitization. After incubation, wash the cells three times with fresh isotonic saline (Fill the tube about 50 volumes) and again make 3% suspension of sensitized cells in isotonic saline. Add one drop of these Coombs Control Cells suspension to negative antiglobulin tests, mix gently and centrifuge for 13 seconds at 3400 rpm (950-1000 rcf) or 1 minute at 1000 rpm (100-125 rcf). All patients should be adequately informed of the antiglobulin test.

**Compatibility Test**

1. Prepare a 5% suspension in isotonic saline of the donor red blood cells.
2. Place 1 drop of Pastur pipette.
3. Place 1 drop of Centrifuge.
4. Place 1 drop of Incubator, 37°C

**REQUIRED SUPPLEMENTARY MATERIALS**

- Test tubes, 10x75mm or 12x75mm
- Pasteur pipette
- Centrifuge
- Isotonic saline
- Optical aid

**COMPATIBILITY TEST**

1. Prepare a 5% suspension in isotonic saline of the donor red blood cells.
2. Place 1 drop of Pastur pipette.
3. Place 1 drop of Centrifuge.
4. Place 1 drop of Incubator, 37°C

**INDIRECT ANTIGLOBULIN TEST**

1. Place 1 drop of Pastur pipette.
2. Place 1 drop of Centrifuge.
3. Place 1 drop of Incubator, 37°C

**INTERPRETATION**

If agglutination is present through Step 16 and the Coombs control cells are agglutinated, the patient and donor may be considered compatible.

If the Coombs control cells are not agglutinated, the Compatibility Test must be repeated. Agglutination prior to Step 16 indicates that the patient and the donor are incompatible.

**CONTROL OF ERROR**

A. Serologic testing is necessary to recognize reagent deterioration. The function of Anti-Human Serum is to agglutinate red cells sensitized with antibody (gamma globulin) and/or components of complement (beta globulin). Anti-Human Serum should not agglutinate unsensitized red cells. Anti-Human Serum should be tested with Coombs Control Cells as a positive control of anti-gamma activity of the reagent. The positive control of anti-beta activity is a test with red cells sensitized with complement. The negative control for Anti-Human Serum is a test with washed, unsensitized red cells.