Anti-Human Serum (Coomb’s Antiserum)

Qualitative test procedure for the detection of cell bound blood group antibody or components of human complement

**SUMMARY AND EXPLANATION**

The principle of the anti-globulin reaction was first described in 1908 by Moreschi. The antiglobulin test (Coomb’s test) was first used in blood group serology in 1945 when Coombs, Mourant 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 Rho (D) antigen (D- tests), and unclotable cord 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. Absence of agglutination indicates there is no detectable antibody or human complement components 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 vivo. 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, and D- and D’ test and detection of other red cell antigens e.g. Fy’, K using specific antisera (anti-Fya, Anti-K) by the indirect Antiglobulin test procedure.

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

<table>
<thead>
<tr>
<th>Indirect Antiglobulin Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Detection (Serum)</td>
</tr>
<tr>
<td>Antibody Identification (Serum)</td>
</tr>
<tr>
<td>Antibody Identification (Situates)</td>
</tr>
</tbody>
</table>

**BIBLIOGRAPHY**


Moreschi C: Neue tatsachen uber die bluterkerchen agglutinationen. zbl Bak 46:49 and 456. 1908.


Austen KT et al: Nomenclature of complement. Immunchemistry 7:137, 1970 Drug interferences to continuous development, the company reserves the right to improve/change any specifications/ components without prior information/note 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 purpose for any product. 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 there of.

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**PRINCIPLE**

The procedures used with this reagent are based on the principle of heteroagglutination directed against components of human serum as originally described by Moreschin 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 fail to agglutinate due to the particular nature of the antigen and antibody involved. Anti-Human Serum will react with red cells sensitized with gamma globulin (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 (20-30°C) prior to use.
PROTOCOLS

Required Supplementary Materials

1. Test tubes, 10x75mm or 12x75mm
2. Pasteur pipettes
3. Centrifuge
4. Optical aid
5. Coombs Control Cells
6. Incubator, 37°C
7. Outlet pipettes
8. Examine negative tests with an optical aid.
9. Centrifuge Tube A (and Tube S, if desired) at 1000 rpm for 1 minute.
10. Remove the tube carefully from centrifuge, observe supernatant for hemolysis, and

INTERPRETATION:

If no agglutination is present through Step 16 and the Coombs control cells are agglutinated, the patient and
donor may be considered incompatible.
It 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 may be tested with Coombs Control Cells, as a positive control of antigamma 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.

MINOR CROSSMATCH PROCEDURE

The basic procedure described under "Broad Spectrum" Compatibility Test may be used with the
following change:
In Tube A and Tube S, use donor serum in place of recipient serum and recipient red blood cells in
donor of donor red blood cells.

Caution: Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.

The use of disposable gloves and proper bihaazardous clothing is STRONGLY RECOMMENDED while running the test.

1. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C for 15 min. for 60 min. Do not use autoclave or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material is ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. The reagent contains Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Supplies to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia. April 30, 1976.)

DIRECT ANTIGLOBULIN TEST

For the direct antiglobulin test, blood drawn into EDTA is preferred (to prevent the fixation of complement
in vitro), but oxalated, clotted, or crotated 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, blood (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 calcium 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.

Once the compatibility of the recipient and the potential donor is determined by sequential testing at temperatures and in media known to be optimum for the detection of those antibodies which are clinically significant.

MAJOR CROSSMATCH PROCEDURE

Initial Phase
1. Prepare a 5% suspension in isotonic saline of the donor red blood cells to be tested. (Alternatively, the donor red blood cells may by suspended in their own plasma.)
2. For each major crossmatch, label two small test tubes: S (for saline) and A (for albumin).
3. With a clean Pasteur pipette, add two drops of fresh recipient serum to each test tube.
4. With a clean Pasteur pipette, add one drop of 5% suspension of donor red blood cells to each test tube.
5. Mix well.
6. Centrifuge both tubes at 1000 rpm for 1 minute.
7. Examine both tubes macroscopically for hemolysis and/or agglutination. Tube S may be discarded. To provide useful information if incompatibility is present, it may be incubated at room temperature and/or 37°C for 15 minutes (incubation may be extended up to 60 minutes, if desired.)

Incubation Phase

1. Incubate Tube A at 37°C for a minimum of 15 minutes. (Incubation may be extended up to 60 minutes, if desired.)
2. Centrifuge Tube A (and Tube S, if desired) at 1000 rpm for 1 minute.
3. Remove the tube carefully from centrifuge, observe supernatant for hemolysis, and

The sensitivity of complement/anti-complement reactions can be increased by incubation
for 30 minutes for sensitization. After incubation, wash the cells thrice with fresh isotonic saline (Fill the tube about 50 volumes) and again make 3-4% suspension of sensitized cells in isotonic saline.

If no agglutination is present through Step 16 and the Coombs control cells are agglutinated, the patient and
donor may be considered incompatible.
It 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.