Procedure 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 (Coombs Control Cells), CSC (Complement Sensitized Cells), and USC (Unsensitized Cells)
3. To the appropriately labeled test tubes, add one drop of Coombs 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 Coombs 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 cell/ 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 carried 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 incuba- tion is desired, it should be set up as a separate test and should not be carried through the antiglobulin procedure.
Caution : Contaminated Bovine Albumin, 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, C3b, 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 C3b, C3d and C4 components of human complement. This reagent is shown not to agglutinate unsensitized red cells.

Serological tests (low ionic strength and low ionic strength plus trypsin treatment) provide evidence that this reagent will detect C3b, and/or C4, Minimal acceptable levels of activity have not been established for these specificities. Red cells sensitized with incomplete anti-Rho (anti-D) are shown to be agglutinated by this product.

Overcentrifugation, i.e., the application of forces in excess of the minimum, causes the cells to adhere to the bottom of the test tube so that vigorous agitation is necessary before they can be resuspended. During such agitation weak agglutination may be dispersed causing a positive reaction to be missed.

Undercentrifugation, i.e., the failure to apply forces necessary to cause the cells to form a "button" and a clear supernatant, may result in weak or negative reaction.

No one speed and time of centrifugation can be recommended which will cover the wide variety of centrifuges available; each laboratory must calibrate its own equipment and determine the time required at a given speed to achieve the desired result.

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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 for use, 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, howsoever caused by the product in the use or in the application there of.

VER-02 R-03

in vitro diagnostic Reagent, not for medicinal use

J. Mitra & Co. Pvt. Ltd.

A-180-181, Okhla Ind. Area, Phase - 1, New Delhi - 110 020, INDIA

Ph.: +91-11-47130300, 47130500

E-mail: jmitra@jmitra.co.in **Internet:** www.jmitra.co.in

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J. Mitra & Co. Pvt. Ltd.

ANTI-HUMAN SERUM
(COOMB'S ANTISERA)

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

SUMMARY AND EXPLANATION

The principle of the antiglobulin 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^u tests), and umbilical 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 on the red blood cells. 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, the D^u test and detection of other red cell antigens e.g. Fy^a, 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

Direct Antiglobulin Test	Indirect Antiglobulin Test
• Diagnosis of Hemolytic Disease of the Newborn	• Compatibility Testing
• Investigation of Transfusion Reactions	• Donor Screening for Unexpected Antibodies
• Detection of Drug-Induced Red Cell Sensitization	• Patient Screening for Unexpected Antibodies
• Detection of Autoimmune Hemolytic Anemia	• Detection of Antigens
	• Antibody Identification (Serum)
	• Antibody Identification (eluates)

PRINCIPLE

The procedures used with this reagent are based on the principle of heteroagglutinins 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 sensitize but 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 blood cell antibody) or components of human complement and cause agglutinaion 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.

Caution: Product deterioration due to excessive heat or freezing may be evidenced by a milky colour. Contamination with serum or gamma globulins will inactivate Anti-Human Serum. Bacterial contamination could cause false positive reactions.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection. Blood should be collected by approved medical techniques.

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2021.

	Manufactured By	IVD	*In vitro* diagnostic medical device
REF	Catalogue Number		Instruction for use
LOT	Lot Number Batch Number	8°C 2°C	Temperature Limitation
	Manufacturing Date		Caution See instruction for use
	Expiry Date		Keep away from sunlight
	Do not use if package is damaged	BIO	Contains biological Material of Animal Origin
	Keep Dry	IN	Country of Manufacture

WARNING FOR USERS

CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running 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. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7. Bring the reagent at room temperature (20ºC to 30ºC) before use.
8. 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 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
9. 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 are ingested or come in contact with skin puncture or wounds.
10. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
11. 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 Sink Drains 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, 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 calcium is not available. If a delay in testing should occur, the sample should be stored at 2-8ºC. Donor units may be tested up to the end of their dating.

PROCEDURES

Required Supplementary Materials

Direct Antiglobulin Test

1. Test tubes, 10x75mm or 12x75mm
2. Pasteur pipettes
3. Centrifuge
4. Isotonic saline
5. Coombs Control Cells
6. Optical aid

Indirect Antiglobulin Test

1. Test tubes, 10x75mm or 12x75mm
2. Pasteur pipettes
3. Red Blood Cells (human) for antibody detection or antibody identification
4. Centrifuge
5. Isotonic Saline
6. Incubator, 37ºC
7. Coombs Control Cells

Compatibility Test

1. Test tubes, 10x75mm or 12x75mm
2. Pasteur pipettes
3. Centrifuge
4. Isotonic saline
5. Coomb's Control Cells
6. Incubator 37ºC
7. Optical aid

Coomb's Control Cells

Dilute Anti-D (IgG) Monoclonal Antibodies 1:10 in isotonic saline. Add an equal volume ot this dilution to a 10% cell suspension of Group O Rh1 (D) positive (O+) red blood cells and incubate at 37ºC 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. Add one drop of these Coombs Control Cells suspension to negaive antiglobulin tests, mix gently and centrifuge for 15 seconds at 3400 rpm (900-1000 rcf) or 1 minute at 1000 rpm (100-125 rcf). Agglutination validates the antiglobulin test.

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 reintroduce human serum components after washing procedure.)
5. Add two drops of Anti-Human Serum.
6. Mix well and centrifuge at 1000 rpm for 1 minute.
7. Resuspend the red blood cells by gentle agitation and examine macroscopically for agglutination.
8. Examine negative tests with an optical aid.
   NOTE: The sensitivity of complement/anti-complement reactions can be increased by incubation at room temperature for 5 to 10 minutes and recentrifugation. However, the results obtained following immediate centrifugation should not be ignored because anti-IgG reactions may be adversely affected by incubation.
9. Control all negative antiglobulin tests by adding one drop of Coomb's Control Cells (See Coomb's Control Cells procedure).

Indirect Antiglobulin Test

The indirect antiglobulin test is used to demonstrate antibodies that combine with , but do not agglutinate, red blood cells. Direct antiglobulin positive red blood cells cannot be used. This test is performed by mixing serum and red blood cells in vitro to allow attachment of the antibody prior to testing the red cells as in the direct antiglobulin test.

An auto-control tube, i.e., a test of the individual's serum with his own red cells, should be set up with every indirect antiglobulin procedure.

BROAD SPECTRUM' Compatibility Test

Experience has shown that no single test is capable of detecting all blood group antibodies. In the following procedure 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 37ºC for 15 minutes (Incubation may be extended up to 60 minutes, if desired.)

Incubation Phase

8. Incubate Tube A at 37ºC for a minimum of 15 minutes. (Incubation may be extended up to 60 minutes, if desired.)
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 read macroscopically for agglutination.
11. Proceed to Antiglobulin Phase with Tube A.

Antiglobulin Phase

12. Wash cells in Tube A three times with tubes full of isotonic saline. Decant and drain completely after the last washing.
13. Add two drops of Anti-Human Serum.
14. Mix contents well and centrifuge at 1000 rpm for 1 minute.
15. Resuspend the red blood cells by gentle agitation and examine macroscopically for aggultination. If negative. examine with an optical aid.
16. Control negative antiglobulin tests by adding one drop of Coombs Centrol Cells. (See Coomb's Control Cells procedure).

MINOR CROSSMATCH PROCEDURE

The basic procedure described under 'Broad Spectrum' Compatibility Test may by 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 place of donor red blood cells.

INTERPRETATION:

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

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 should 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.