incompatible. If the Coombs Control Cells are not agglutinated the compatibility test must be repeated. Hemolysis or agglutination prior to step 17 indicates that the patient and donor are incompatible. If the Coombs Control Cells are not agglutinated the compatibility test must be repeated.

**ANTIBODY TITRATION PROCEDURE**

1. Label ten small test tubes.
2. Prepare a 5% suspension in Bovine Albumin of appropriate red cells of selected genotype.
3. Prepare a 5% suspension in Bovine Albumin of the patient’s red cells.
4. Make progressively two fold serial dilutions of the patient serum as follows: (a) To each tube, except the first, add 0.1 ml of normal serum. (b) With a clean pipette, deliver 0.1 ml of patient serum to each of the first and second tube. (c) Mix the contents of the second tube with clean pipette, transfer 0.1 ml of the mixture to the next tube, and continue the same procedure until the last tube is reached. Discard 0.1 ml of mixture from the last tube.
5. To Tubes 1 through 9, add 0.1 ml of the albumin suspended selected red cells.
6. To tube 10, add 0.1 ml of the albumin suspended patient cells.
7. Mix the contents of each tube and incubate at 37°C for a minimum of 15 minutes. If desired, incubation may be extended up to 60 minutes.
8. Centrifuge all tubes and read for agglutination.
9. Perform the antiglobulin test on all tubes that are not already strongly agglutinated.

**RESULTS INTERPRETATION**

The reciprocal of the dilution in the last tube containing visible agglutination is the end point of titration.

**LIMITATION OF PROCEDURE**

1. Bovine Albumin will not bring about agglutination of red cells by all IgG blood group antibodies.
2. Red cells that have a positive direct antiglobulin test must not be used for the direct antiglobulin test.
3. Contaminated blood specimen and/or supplementary materials used in the procedure described in this manual may interfere with the test results.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

Bovine Albumin is known to enhance the reactivity of Rh and some other antibodies. The centrifugal force applied to cell/serum mixture should be the minimum required to produce a “button” of red cells and a clear supernate. Overcentrifugation, i.e., the application of force in excess, 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. Under centrifugation, i.e., the failure to apply forces necessary to cause the cells to form a “button” and a clear supernate, may result in weak or negative reactions. 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.

**BIBLIOGRAPHY**


**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, howsoever caused by the product in the use or in the application thereof.

**SUMMARY AND EXPLANATION**

Bovine Albumin is primarily used to enhance the reactivity of blood group antibodies, either in direct antiglobulin tests or indirect antiglobulin test. This fact was confounded by Cameron and Diamond, 1945, who established that certain anti Rho (Anti-D) Sera would agglutinate Rh positive red cells when they were suspended in albumin, however no agglutination was observed when same red cells were suspended in Normal Saline medium.

**PRINCIPLE**

Bovine Albumin is frequently used as a control for Rh typing. Every blood specimen tested by the slide, stick or modified tube method should be controlled by testing simultaneously with a medium such as Bovine Albumin. Since cells coated with an autoantiglutanin or suspended in serum having a protein abnormality may give a false positive result in Rh typing.

**STORAGE AND PACK SIZE**

Bovine Albumin is available in two pack sizes i.e. 5 ml & 10ml dropper vial. The reagent is stable at 2-8°C for 24 months. The reagent contains 0.1% sodium azide as a preservative. Extreme turbidity or precipitation may indicate product alteration.

**DESCRIPTION OF SYMBOLS USED**

The following are graphical symbols used in or found on J. Mitra diagnostic products and packaging. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the British and European Standard BS EN 15223-1:2012.

- **Manufactured By**
- **In vitro diagnostic medical device**
- **Catalogue Number**
- **See Instruction for use**
- **Lot Number**
- **Temperature**
- **Batch Number**
- **Limitation**
- **Manufacturing Date**
- **Caution**
- **Expiry Date**
- **See instruction for use**
SPECIMEN COLLECTION & PREPARATION
For 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 delay in testing, store sample at 2-8°C.

WARNING FOR USERS
CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THROUGH 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. 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 at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed of 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 skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. The reagent contain Sodium Azide as a preservative. If these material are ingested or come in contact with skin puncture or wounds. Consult a physician immediately in case of accident or contact.
10. Do not pipette by mouth.
11. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
12. Tests are for in vitro diagnostic use only and should be run by competent person only.
13. Do not pipette by mouth.
14. All materials used in the assay and samples should be decontaminated in 2% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed of in accordance with established safety procedures.

PROCEDURES AND USES OF BOVINE ALBUMIN
A. Compatibility Testing
See procedure below.
B. Antibody Detection.
C. Antibody Identification
For B & C. See direction circular accompanying Anti-Human Serum.

D. Antibody Titration
See procedure below.
E. Control of Rh typing
For procedure, refer instruction manual of Anti-D (IgM) Monoclonal Antibodies for Slide Test and Modified Test Tube.
F. D+ Testing
For procedure, refer instruction manual of Anti-D (IgG) Monoclonal and Anti-D (IgM+IgG) Monoclonal Antibodies for Slide test and Modified Tube test.

REQUIRED SUPPLEMENTARY MATERIALS
For tube test method
1. Test Tubes, 10x75 mm or 12x75mm.
2. Pasteur pipettes.
3. Red Blood Cells (Human) of specific genotypes for anti body detection and for identification Anti-D (IgM) Monoclonal antibodies for slide test and Modified Tube test.
4. Centrifuge
5. Incubator, 37°C
6. Isotonic saline.
8. Coombs Control Cells.

Additional Material for Titration
10. 0.2ml. serological pipettes.
11. Red Blood cells (Human) which possesses the antigen specific for the antibody to be titrated.

SLIDE TEST METHOD
1. Glass Slides
2. Pasteur pipettes
3. Applicator Sticks
4. Anti-D (IgM) Monoclonal antibodies for Slide Test and Modified Tube Test

DIRECTIONS FOR USE OF BROAD SPECTRUM COMPATIBILITY TEST
MAGN CROSSMATCH PROCEDURE
INITIAL PHASE
1. For each donor to be cross matched, label two small test tubes. Suggested labelling: S, (for saline) and A (for Albumin)
2. Prepare a 5% suspension of the donor red blood cells in isotonic saline or their own serum or plasma.
3. With a clean Pasteur pipette, add two drops of the recipient serum to each test tube.
4. With a clean Pasteur pipette, add one drop of the donor red cells to each test tube.
5. To the Albumin tube only add two drops of Bovine Albumin.
6. Mix well.
7. Centrifuge both tubes. Suggested centrifugation: 15 seconds at 3400 rpm (900-1000rcf) or 1 minute at 1000 rpm (100-125rcf).
8. Examine both tubes macroscopically for hemolysis and/or agglutination.

INCUBATION PHASE
9. Incubate the saline tube at room temperature and the Albumin tube at 37°C for a minimum of 15 minutes. If desired, incubation may be extended up to 60 minutes.
10. Centrifuge both tubes.
11. Examine both tubes macroscopically for hemolysis and/or agglutination.
12. Proceed to Antibody Phase with the Albumin tube.

ANTIGLOBULIN PHASE
13. Utilizing the albumin tube only, wash the cell-serum mixture thoroughly a minimum of three times with tubes full of Saline. Decant and drain completely after use.
14. Add two drops of Anti Human Serum to the sedimented cells.
15. Mix well and centrifuge.
16. Resuspend the cells by gently agitation and examine macroscopically for agglutination. If negative examine with an optical aid.
17. To all negative antiglobulin tests add one drop of Coombs Control Cells. Refer instruction manual of Anti Human Serum for details.

COOMBS’ CONTROL CELLS
Dilute Anti-D (IgG) Monoclonal Antibodies for Slide Test and Modified Tube Test 1:10 in isotonic saline. Add an equal volume of this dilution to a 10% cell suspension of Group O Rh1 (D) positive (O+) red blood cells and incubate at 37°C 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 negative 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.

MINOR CROSS MATCH PROCEDURE
This procedure is the same as that used for the major side of the Broad Spectrum Compatibility test except that the serum or plasma of the donor is tested against the red cells of the recipient.

RESULTS INTERPRETATION
If no hemolysis or agglutination is present through step 16 and the Coombs control cells are aglutinated, the patient and donor may be considered