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 0.1ml. of normal serum.
   (b) With a clean autopipette, 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 37ºC for a minimum of 15 minutes. If desired, incubation may be extended upto 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 procedures 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 supernatant.

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

a) Cameron, J.W. and Diamond, L.K. "Chemical, Clinical and Immunological studies on the Products of Human Plasma.

b) Fractionation; Serum Albumin as a Diluent for Rh Typing Regeants". J. Clin. Invest. 24:793, 1945.

in vitro diagnostic Reagent, not for medicinal use

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BOVINE ALBUMIN

22% Protein Concentration

For qualitative use in antibody detection, identification, titration and control of Rh typing

SUMMARY AND EXPLANATION

Bovine Albumin is primarily used to enhance the reactivity of blood group antibodies, either in direct agglutination 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 autoagglutinin 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 2-80C 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 sysmbols used in or found on J. Mitra diagnostic products and packing. These sysbols 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


Catalogue Number


Lot Number
Batch Number


Manufacturing Date


Expiry Date


Do not use if package is damaged


Keep Dry


In vitro diagnostic medical device


Instruction for use


Temperature Limitation


Caution
See instruction for use


Keep away from sunlight


Contains biological Material of Animal Origin


Country of Manufacture

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 2-8ºC.

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 of 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 contain 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" (Center for Disease Control, Atlanta, Georgia, April 30, 1976.)

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 for use of Anti-D (IgM) Monoclonal Antibodies for Slide Test And Modified Test Tube.

F. D^{u} Testing
For procedure refer, instruction for use 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, 37ºC
6. Isotonic saline.
7. Anti-human serum.
8. Coombs Control Cells.

Additional Material for Titration

9. Antibody-free human serum.
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

MAJOR 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 (900-1000 rcf) or 1 minute at 1000 rpm (100-125 rcf).
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 37ºC for a minimum of 15 minutes. If desired, incubation may be extended upto 60 minutes.
10. Centrifuge both tubes.
11. Examine both tubes macroscopically for hemolysis and/or agglutination.
12. Proceed to Antiglobulin 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 for use of Anti Human Serum for details.

COOMB'S CONTROL CELLS

Dilute Anti-D (IgG) Monoclonal Antibodies for Slide Test and Modified Tube Test 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 negative antiblobulin 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 compatible. 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.

ANTIBODY TITRATION PROCEDURE

1. Label ten small test tubes.
2. Prepare a 5% suspension in Bovine Albumin of appropriate red cells of selected genotype.