**ORTHO® Bovine Albumin Solution**

22% protein concentration, pH 7.2

For Qualitative Use in Antibody Detection, Identification and Titration

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**SUMMARY AND EXPLANATION**

In 1945, Cameron and Diamond established that certain anti-D (anti-Rh\(_o\)) sera would agglutinate Rh positive red cells suspended in albumin but would not agglutinate the same cells in a saline medium. Bovine albumin is used to enhance the reactivity of blood group antibodies.

**PRINCIPLE OF PROCEDURE**

The mechanism of action of bovine albumin was first investigated in 1964 by Pollack. From his experiments he concluded that agglutination of antibody-coated red cells depends on the dimensions of the antibody involved and characteristics of the reaction medium such as ionic strength. IgM blood group antibodies agglutinate red cells in isotonic saline but IgG molecules do not. Some IgG antibodies, especially those with Rh-hr specificities, will agglutinate red cells if the zeta potential, i.e., the effective electrical charge, is carefully adjusted by the addition of colloids and salts.

Stroup and Macllroy, in 1965, showed that albumin also enhances the sensitivity of the indirect antiglobulin test. This test is used to demonstrate antibodies that combine with, but do not agglutinate, red blood cells. The indirect antiglobulin test is used in compatibility testing, antibody screening, identification and titration. Serum and red cells are mixed in vitro to allow attachment of the antibody prior to testing the red cells as in the direct antiglobulin test.

**REAGENT**

ORTHO Bovine Albumin is manufactured and processed from raw bovine serum. The process used yields a bovine albumin solution with a protein concentration of 22%, specifically designed for serological use. The pH is 7.2. This reagent contains 0.1% sodium azide as a preservative. The reagent is to be used as furnished according to the procedures described.

FOR IN VITRO DIAGNOSTIC USE.

No U.S. Standard of Potency. Store at 2 to 8°C. Extreme turbidity or precipitation may indicate product alteration.

**SPECIMEN COLLECTION AND PREPARATION**

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

For the indirect antiglobulin test, serum (no more than 48 hours old) from clotted blood should be used. If plasma is used in the indirect antiglobulin test, complement-binding antibodies may not be detected because calcium is not available. If a delay in testing should occur, the sample should be stored at 2 to 8°C. Donor units may be tested up to the end of their dating.

**PROCEDURES**

Compatibility Testing: See procedure below.

Antibody Detection: For procedure see package insert accompanying SELECTOGEN® Reagent Red Blood Cells or other screening cells.

Antibody Identification: For procedure, see package insert accompanying RESOLVE® Panel A Reagent Red Blood Cells, RESOLVE® Panel B Reagent Red Blood Cells or other panel cells for antibody identification.

Antibody Titration: See procedure below.

**Required Supplementary Materials**

Compatibility Test

1. Test tubes, 10 x 75 mm or 12 x 75 mm
2. Pasteur pipettes
3. Centrifuge
4. Incubator, 37°C
5. Isotonic saline
6. Anti-human globulin, such as ORTHO® Anti-Human Globulin
7. Coombs control cells, such as ORTHO® Coombs Control
8. Optical aid
Antibody Titration (Albumin/Antiglobulin Method)
1. Test tubes, 10 x 75 mm or 12 x 75 mm
2. Antibody-free human serum
3. 0.2 mL serological pipettes
4. Reagent red blood cells which possess the antigen specific for the antibody to be titrated
5. Pasteur pipettes
6. Centrifuge
7. Incubator, 37°C
8. Isotonic saline
9. Anti-human globulin, such as ORTHO Anti-Human Globulin
10. Coombs control cells, such as ORTHO Coombs Control

Directions for Use
The procedures described may be supplemented with tests performed at different temperatures or in different antibody detection media at the discretion of the medical director of the laboratory.

Compatibility Test
1. For each “major crossmatch,” label a small test tube.
2. Prepare a 3% to 5% suspension in isotonic saline of the donor red blood cells to be tested. If preferred, the cells may be suspended in the donor’s serum or plasma.
3. With a clean Pasteur pipette, add two drops of fresh recipient serum to the test tube.
4. With a clean Pasteur pipette, add one drop of the donor red blood cells.
5. Add two drops of ORTHO Bovine Albumin.
6. Mix well. If an immediate spin result is desired, centrifuge and read prior to Step 7.
7. Incubate the tube at 37°C for a minimum of 15 minutes. (Incubation may be extended up to 60 minutes, if desired.)
8. Centrifuge the tube and examine macroscopically for hemolysis and/or agglutination. Record result.
   Suggested centrifugation: 15 seconds at 3400 rpm (900-1000 rcf) or one minute at 1000 rpm (100-125 rcf).*
9. Wash the cell/serum mixture thoroughly three times with tubes full of isotonic saline.
10. Add two drops of anti-human globulin to the sedimented cells.
11. Mix well and centrifuge. (See Step 8 for suggested centrifugation.)
12. Resuspend the cells by gentle agitation. Antibody detection and identification results should be examined macroscopically. Compatibility test results should be examined with an optical aid.
13. To all negative antiglobulin tests, add red blood cells sensitized with IgG antibody, e.g., ORTHO Coombs Control. (See package insert for procedure.)

RESULTS
Interpretation
1. Hemolysis or agglutination is a positive test result and reflects the presence of an antibody/antigen reaction.
2. No hemolysis or agglutination is a negative test result and indicates the absence of an antibody/antigen reaction. Negative tests are verified by the addition of Coombs control cells.
   a. No agglutination after the addition of Coombs control cells to a negative test indicates that the anti-human globulin was inactivated, neutralized or omitted. In such a case, the negative test result should be considered invalid and the entire antibody detection or identification test should be repeated.
   b. Agglutination after the addition of Coombs control cells to a negative test indicates that the anti-human globulin added was capable of reacting and the negative test result may be considered valid.

Antibody Titration Procedure
1. Label ten small test tubes.
2. Prepare a 3% to 5% suspension of the appropriate reagent red cells in ORTHO Bovine Albumin. When titrating anti-D, this may be done by pooling 0.6 mL each of SELECTOGEN I and SELECTOGEN II. Mark the top level of the cell suspension, centrifuge, remove the supernate, replace with albumin to the mark and resuspend the cells in the albumin.
3. Make progressively doubled dilutions of the patient serum as follows:
   a. To each tube, except the first, add 0.1 mL of normal serum.
   b. With a 0.2 mL pipette, deliver 0.1 mL of the patient serum to each of the first two tubes.
   c. With a clean 0.2 mL pipette, mix the contents of the second tube, 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 the mixture from the last tube.
4. To tubes 1 through 10, add one drop of the albumin-suspended reagent red cells.
5. 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.
6. Centrifuge* all tubes and read for macroscopic agglutination.
7. 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 the titration.

LIMITATIONS OF PROCEDURE
1. Bovine albumin will not bring about agglutination of red cells by all IgG blood group antibodies.
2. Contaminated blood specimens and/or supplementary materials used in the procedures described may interfere with the test results.
3. Improper technique may invalidate the results obtained with this reagent.
4. Other potentiating media, such as low ionic strength solutions, should not be used in conjunction with ORTHO Bovine Albumin.

SPECIFIC PERFORMANCE CHARACTERISTICS
Bovine albumin is known to enhance the reactivity of Rh and some other antibodies.
Technical questions concerning this reagent should be directed to Customer Technical Support at 1-800-322-6374.

*The centrifugal force applied to cell/serum mixtures should be the minimum required to produce a "button" of red cells and a clear supernate. 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 supernate, may result in a 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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BIBLIOGRAPHY
Cameron JW, Diamond LK. Chemical, clinical and immunological studies on the products of human plasma fractionation; serum albumins as a diluent for Rh typing reagents. J Clin Invest. 1945;24:793.