TM AHG reagent to each tube and gently mix.

Transfer 200µL of serum or serum dilution into appropriately labelled clean, glass serological tubes. Incubate at 37°C for 30 minutes. Add 1 or 2 drops of Epiclone W to all test tubes to validate the result. Centrifuge at low speed (500rcf) for 15 to 20 seconds. Read and record results.

BACKGROUND

Antibody Detection Methods

Red cells that are agglutinated in an ionic solution have a negatively charged membrane which is surrounded by a cloud of corresponding positively charged ions. These charges cause the cells to repel each other. The distance between the cells is dependent on many factors, including the ionic strength of the suspending medium. As IgG antibodies are smaller than IgM antibodies, they are unable to bridge the intercellular gap and thus are generally incapable of causing direct agglutination. The addition of a direct coating of Bovine Albumin increases the dielectric constant of the suspending medium, thereby causing the cells to move closer and may allow agglutination by IgG antibodies.

In the incorporation of Bovine Albumin into a test procedure may also increase the thermal amplitude of some IgM antibodies and in Indirect Antiglobulin Tests (IAT), Bovine Albumin lowers the ionic strength of the suspending medium. As IgG antibodies are smaller than IgM antibodies, they are unable to bridge the intercellular gap and thus are generally incapable of causing direct agglutination. The addition of a direct coating of Bovine Albumin increases the dielectric constant of the suspending medium, thereby causing the cells to move closer and may allow agglutination by IgG antibodies. Bovine Albumin may be used as an antibody enhancing solution for antibody screening. Modern methods using LISS additive reagents, such as bioCSL RAM (Rapid Antibody Medium), are more commonly used due to better specificity, higher sensitivity and cost effectiveness.

Innominative Diagnostics

The serological activity of many antibodies is dependent on a minimal level of protein in the suspending medium. As IgG antibodies are smaller than IgM antibodies, they are unable to bridge the intercellular gap and thus are generally incapable of causing direct agglutination. The addition of a direct coating of Bovine Albumin increases the dielectric constant of the suspending medium, thereby causing the cells to move closer and may allow agglutination by IgG antibodies. Bovine Albumin may be used as an antibody enhancing solution for antibody screening. Modern methods using LISS additive reagents, such as bioCSL RAM (Rapid Antibody Medium), are more commonly used due to better specificity, higher sensitivity and cost effectiveness.

SPECIFIC COLLECTION AND PREPARATION

Tested samples should be obtained aseptically with or without the addition of anticoagulants. Tests should be performed as soon as possible after collection of the sample. If testing the blood sample in delayed, samples should be stored between 2°C to 8°C. Samples collected into EDTA or Hepsera may be stored up to 7 days from the date of withdrawal provided storage has been at 2°C to 8°C. Sera samples may be stored at 4°C. Samples collected into EDTA may be tested up to 14 days from the date of withdrawal provided storage has been at 2°C to 8°C. Sera samples may be stored at 4°C. Samples collected into EDTA may be tested up to 14 days from the date of withdrawal provided storage has been at 2°C to 8°C. Sera samples may be stored at 4°C. Samples collected into EDTA may be tested up to 14 days from the date of withdrawal provided storage has been at 2°C to 8°C. Sera samples may be stored at 4°C.

RECOMMENDED METHODS

Numerous techniques using Bovine Albumin can be found in immunohaematology textbooks, and the choice of a particular method is the prerogative of the user. For this reason, details of actual methods are not included in this leaflet, but guidelines for users are given below. Laboratories should ensure they validate their chosen method.

Antibody Detection and Identification Method

Bovine Albumin Addition Method using 30% Bovine Albumin:

1. Place a drop of 3% normal cells in a test tube containing 1 drop of 30% Bovine Albumin solution and 1 drop of the appropriate 1.5% Reagent Red Blood Cells (0.75% or 1.5%).
2. Mix the contents and incubate at 37°C for 30 minutes.
3. Centrifuge at low speed (500rcf) for 15 to 20 seconds.
4. Gently resuspend the cell suspension. Read and record results.
5. Test using a negative reaction may be continued in an Indirect Antiglobulin Test (IAT).
6. Add 1 or 2 drops of Epiclone W to all test tubes to validate the result.
7. Add 1 or 2 drops of bioCSL AHG Control Cells 3% to all negative test tubes to validate the result.
8. Centrifuge at low speed (500rcf) for 15 to 20 seconds.
9. Gently resuspend the cell suspension. Read and record results.
10. Add 1 drop of bioCSL AHG Control Cells 3% to all negative test tubes to validate the result.
11. Centrifuge at low speed (500rcf) for 15 to 20 seconds. Read and record results.

Note: Do not centrifuge at a speed and time appropriate for the centrifuge in use.

Antibody Dilution for Reagent Potency Testing and Antibody Titration

Preparation of a protein diluent (5% w/v) in Phosphate Buffered Saline (PBS) or Ice-Cold Buffered Saline (IBS).

Add 1 volume of Bovine Albumin 30% w/v Solution to 5 volumes of PBS (or IBS). Ep. Add 10 volume of Bovine Albumin 30% w/v Solution to 5 volumes of PBS (or IBS).

Note: Reagents to be potency tested should be within expiry date and stored at all times under conditions recommended by the manufacturer.

Antibody Titration Method

This method is the ‘Australian NICE (National Immunohaematology Continuing Education) Method’ agreed and published in 1995.

1. In a labelled set of test tubes, a suitable range of doubling dilutions of the test serum is made in a diluent comprising 5% w/v Bovine Albumin in saline or buffered saline.
2. Add 1 drop of serum to each tube in the appropriate range. Each tube should contain 40µL of serum.
3. Add 20µL of buffer or serum dilution into appropriately labelled clean, glass serological test tubes (340µL or 310µm).
4. Add 15µL of the cell suspension to each tube.
5. A negative control consisting of 20µL of 5% w/v Bovine Albumin solution and 50µL of the test cell suspension should always be included as a control.
6. Gently mix and incubate at 37°C for 30 minutes.
7. Read and record results.
8. Add 1 drop of bioCSL AHG Control Cells 3% to all negative test tubes to validate the result.
9. Centrifuge at low speed (500rcf) for 15 to 20 seconds.
10. Gently resuspend the cell suspension. Read and record results.

Note: Do not centrifuge at a speed and time appropriate for the centrifuge in use.

The titre is expressed as the reciprocal of the last dilution showing a score of 5 (on the 0 to 12 scale), or the last positive result greater than 5 where no further reactions were recorded.

LIMITATIONS OF PROCEDURE

Routine contamination of Bovine Albumin 30% w/v Solution may give rise to false results. High concentrations of protein in patient samples may cause autoagglutination or modulus.

Discrepant results may occur due to:

1. Incorrect technique.
2. Presence of gross modulus.
3. Use of aged blood samples, reagents or supplementary materials.
4. Contaminated blood samples, reagents or supplementary materials.
5. Haplos, that is a negative Direct Antiglobulin Test (DAT).
6. The use of LA or non-specific Binding.
7. Incorrect concentrations of red cells.
8. Incorrect readings of results (ie. failure to detect haemolysis, etc.)
PRECAUTIONS
1. For in vitro diagnostic use only.
2. No known testing method can assure that products derived from blood will not transmit infectious agents. This product is derived from bovine serum and as such should be treated as a biological product and treated as potentially infectious.
3. Sodium Azide 0.1% w/v is added as a preservative. Users should be aware of the toxicity and cumulative explosive nature of Sodium Azide and take appropriate precautions when handling and discarding this reagent.
4. Turbidity may indicate bacterial contamination. The reagent should not be used if a precipitate or particles are present.
5. The bovine material used is from an approved source free of Bovine Spongiform Encephalopathy (BSE).

REFERENCES

BioCSL, Epiclone and Celpresol are trademarks of CSL Limited.