Bovine Serum Albumin 22% / Bovine Serum Albumin 30%

INTENDED USE

Vitro bovine serum albumin reagent is intended to be used as potentiating reagent for the detection of red cell antibodies in human serum

METHOD

Antigen-antibody interactions enhancement.

CLINICAL SIGNIFICANCE

Serological albumin was first recognized as a potentiator of certain antigen-antibody interactions in 1945 by Diamond. Since then, methods employing serological albumin have been widely used for the detection or quantitation of antibodies. Serological albumin has also been shown to enhance the sensitivity of the indirect antiglobulin test for some antibody specificities.

ASSAY PRINCIPLE

When used by the recommended techniques, the reagent will not affect the first stage of haemagglutination (antibody uptake) but it will enhance the second stage (agglutination) by allowing the antibody-coated red cells to come closer together than they would in a saline medium without additives (see **Limitations**).

REAGENTS

BSA 22% and 30% are prepared from a mixture of bovine serum albumin, and buffered saline. The polymer content of the Polymer Enhanced BSA is increased naturally by a process modification. No artificial avidity enhancers or high molecular weight agglutination potentiators are added to any BSA preparation. None of the BSA reagents do contain sodium caprylate. Each BSA reagent is supplied at optimal dilution for use with all the recommended techniques stated below without the need for further dilution or addition.

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

Do not freeze. Reagent vials should be stored at 2 - 8ºC on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity. Reagent will remain stable for up to 7 days when subjected to temperatures not exceeding 30ºC

SPECIMENE

Blood samples should be drawn aseptically into EDTA and tested within 48 hours. If EDTA is unavailable, samples drawn into ACD, CPD or CPDA-1 are acceptable and may be tested up to 35 days from the date of withdrawal.
All blood samples should be washed at least twice with PBS before being tested.

WARNING & PRECAUTION

1. The reagents are intended for *in vitro* diagnostic use only.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagents past the expiration date (see **Vial Label**).
4. Do not use the reagents if a precipitate is present.
5. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
6. The reagents have been filtered through a 0.2 µm capsule to reduce the bio-burden.
Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
7. The BSA has been obtained from a closed herd in the female line since 1980, in which no animal has been clinically suspected of having Bovine Spongiform Encephalopathy (BSE), and which has not been fed rations containing ruminant derived protein during that period.
8. The reagents contain 0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
9. For information on disposal of the reagents and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

NOTES

1. Red cells sensitised with an *in vitro* or *in vivo* autoantibody may agglutinate spontaneously in concentrations of serological albumin as low as 6%. It is therefore necessarily set up control tests in which the test red cells are mixed with the appropriate serological albumin solution alone.
2. The antiglobulin techniques can only be considered valid if all negative tests react positively with IgG sensitised red cells.
3. In the **Recommended Techniques** one volume is approximately 40µl when using the vial dropper provided.
4. The use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
5. The user must determine the suitability of the reagents for use in other techniques.

MATERIAL REQUIRED

- Anti-human globulin or anti-IgG.
- Coombs cell washer.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- IgG sensitised red cells.
- Inert AB serum.
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22ºC ± 1ºC
- Test tube centrifuge.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.

PROCEDURE

A. Albumin Immediate Spin Technique

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 2 volumes each of test serum, test red cell suspension and 22% BSA.
3. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
4. Examine supernatant for haemolysis, then gently resuspend cell button and examine macroscopically for agglutination.

B. Albumin Room Temperature Saline Phase Technique

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 2 volumes test serum, 1 volume test cell suspension and 2 volumes 22% BSA.
3. Mix thoroughly and incubate at 18-25ºC for 5-30 minutes.
4. Centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
5. Examine supernatant for haemolysis, then gently resuspend cell button and examine macroscopically for agglutination.

C. Albumin 37ºC Technique

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 2 volumes test serum, 1 volume test cell suspension and 2 volumes 22% BSA.
3. Mix thoroughly and incubate at 37ºC for 15-60 minutes.
4. Centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
5. Examine supernatant for haemolysis, then gently resuspend cell button and examine macroscopically for agglutination.

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D. Indirect Antiglobulin Technique (IAT)

1. Follow steps 1 to 3 of Albumin 37ºC Technique above.
2. Wash test red cells 4 times with PBS, taking care to decant saline between washes and resuspend each cell button after each wash. Completely decant saline after last wash.
3. Add 2 volumes of anti-human globulin to each dry cell button.
4. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination.

E. Antibody Titration Technique

1. Prepare a 2-3% suspension of washed test red cells in 22% BSA.
2. Prepare doubling dilutions of test serum in inert AB serum.
3. Add 1 volume of test red cell suspension to 1 volume of each dilution.
4. Mix thoroughly and incubate at 37ºC for 15-60 minutes.
5. Centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
6. Gently resuspend each cell button and read macroscopically for agglutination.

INTERPRERATION OF TEST RESULTS

1. **Positive:** Agglutination of test red cells constitutes positive test result within accepted limitations of the test procedure.
2. **Negative:** No agglutination of the test red cells constitutes negative test result within accepted limitations.

Stability of the reactions

1. Tube tests should be read immediately after centrifugation.
2. Washing steps should be completed without interruption and tests should be centrifuged and read immediately after addition of anti-human globulin. Delays may result in dissociation of antigen-antibody complexes, leading to false negative or weak positive reactions.
3. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those **recommended**.

QUALITY CONTROL

It is recommended that controls be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

LIMITATIONS

1. Red cells with a positive DAT due to a coating of IgG cannot be typed by the indirect antiglobulin technique.
2. False positive results may occur due to the fact that agglutinins to albumin are found in a small proportion of serum samples.
3. The efficacy of albumin reagent is to be controlled throughout their use.
4. Serological Albumin will no enhance the reactivity of all blood group antibodies.
5. Serological Albumin should not be used as negative controls for potentiated IgG blood grouping reagents.
6. False positive or false negative results may occur due to:

Contamination of test materials Improper storage, cell concentration, incubation time or temperature Improper or excessive centrifugation Introduction of human serum/gamma globulins into test

7. The user is responsible for the performance of the reagents by any methods other than those mentioned in the **Recommended Techniques**.
8. Any deviations from the **Recommended Techniques** should be validated prior to use.

PERFORMANCE CHARACTERISTICS

1. The reagents have been characterised by the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of 22% and 30% BSA have been shown to enhance agglutination of Rh and other antibodies when used according to **Recommended Techniques**.
3. Each lot is tested to assure specificity in an antibody-free system with red cells known to possess the most frequently inherited blood group antigens.
4. The Quality Control of the reagents was performed using red cells that had been washed with PBS prior to use.

BIBLIOGRAPHY

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

Item	REF	SIZE
Bovine Serum Albumin 22%	20501	10 x 10 ml
Bovine Serum Albumin 30%	20701	10 x 10 ml

ISO 13485:2016 | CE

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