INTENDED USE
The Anti-S reagent is for the in vitro detection and identification of human S positive red blood cells by the indirect antiglobulin test.

PRINCIPLE OF THE TEST
When used by the recommended technique, this reagent will cause agglutination (clumping) of red blood cells carrying the s antigen. Lack of agglutination of the red blood cells demonstrates the absence of the s antigen.

REAGENT DESCRIPTION
This reagent has been prepared from plasma collected from blood donors. ABC hemagglutinins were removed by adsorption. Conversion to serum was achieved by the addition of calcium chloride and where necessary, thrombin. Excess calcium was removed by the addition of sodium oxalate. The formulation also contains 0.1% (w/v) sodium azide.

The volume delivered by the reagent dropper bottle is approximately 40 μL. Bearing this in mind, care should be taken to ensure that appropriate serum: cell ratios are maintained in all test systems.

SUMMARY AND EXPLANATION
Anti-S and anti-s were described in 1947 and 1951 respectively and define a pair of alleles on the long arm of chromosome 4. The S/s locus is closely linked to the MN locus and consequently, like the CDE antigens in the Rh system, the MNSs genetic contribution from each parent is inherited as a haplotype e.g., MS, NS etc.

Ss antigens are carried on a red cell glycoprotein, glycophorin B, where they are characterised by a single amino acid substitution at position 29. Methionine is responsible for S antigen expression, threonine for s antigen expression.

Ss antigens are generally destroyed when red cells are exposed to papain, bromelin or ficin. Trypsin generally has no adverse effect.

Ss antibodies are generally best detected in the indirect antiglobulin test where their reactions are normally improved by incubating at 18-24 °C rather than 37 °C. The phenotype S-s is extremely rare in Caucasians but occurs in approx. 2% of African Americans. Complexities within the MNS system also produce a number of phenotypes in which S/s expression may be modified.

PRECAUTIONS FOR USE AND DISPOSAL
This reagent contains 0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into sink, flush with a large volume of water to prevent azide buildup.

Handle and dispose of reagents as potentially infectious.

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.

This product has components (dropper bulbs) containing dry natural rubber.

This reagent is for in vitro diagnostic use only.

SPECIMEN COLLECTION AND PREPARATION
Specimens should be collected by a standard collection technique. The specimen should be tested as soon as possible after collection. If testing is delayed, the specimen should be stored at refrigerated temperatures. Blood specimens exhibiting contamination should not be used. Extreme care should be taken if hemolyzed samples must be tested. Clotted samples or those collected in EDTA should be tested within fourteen days from collection. Donor blood may be tested until the expiry date of the donation.

MATERIALS
Materials provided
- ALBa sera® Anti-S

Materials required but not provided
- Isotonic saline
- Reagent red blood cells suitable for the control of Anti-S
- Polyspecific Anti-Human Globulin / Monospecific Anti-Human IgG
- IgG-sensitized red blood cells
- 10 x 75 mm or 12 x 75 mm glass test tubes
- Pipettes
- Centrifuge
- Optical aid

TEST PROCEDURE
General Information
This reagent has been standardized for use by the technique described below and therefore its suitability for use in other techniques cannot be guaranteed. When a test is required to be incubated for a specific period of time, a timer should be used.

RECOMMENDED TECHNIQUES
20°C Indirect Antiglobulin
- Add 2 drops of blood grouping reagent to a test tube.
- Add 1 drop of red blood cells suspended to 2-4% in isotonic saline. Reagent red blood cells may be used as provided (preservative suspended).
- Mix the test well and incubate for 15-45 minutes at 18-24 °C.
- Wash the test at least 3 times with a large excess of isotonic saline e.g. 4 mL of saline per 12 (or 10) x 75 mm glass tube

NOTE: (i) allow adequate spin time to sediment the red blood cells.
(ii) make sure that most of the residual saline is removed at the end of each wash.
- Add Anti-Human Globulin to each test tube in the amount specified in the manufacturer’s product insert.
- Mix the contents of the test tube well and centrifuge.

Suggested centrifugation: 900-1000g (~3400 rpm) for 10-20 seconds or a time and speed appropriate for the centrifuge used that produces the strongest reaction of antibody with
antigen-positive cells, yet allows easy re-suspension of antigen-negative cells.

- Gently shake the test tube to dislodge the cell button from the bottom and observe macroscopically for agglutination. Negative reactions may be examined with an optical aid.
- Record results.
- Add IgG sensitized antiglobulin control cells to confirm the validity of negative test results.

STABILITY OF REACTION
Test results should be read and interpreted immediately after centrifugation. Delays may cause dissociation of antigen-antibody complexes resulting in weak positive or false negative reactions.

INTERPRETATION OF RESULTS
Agglutination = positive test result
No agglutination = negative test result

Frequency (%):

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Caucasians</th>
<th>African Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td>S+s-</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>S+s+</td>
<td>42</td>
<td>24</td>
</tr>
<tr>
<td>S-s+</td>
<td>48</td>
<td>68</td>
</tr>
<tr>
<td>S-s-</td>
<td>0</td>
<td>2</td>
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</tbody>
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SUPPLEMENT 1

STABILITY OF REACTION
Test results should be read and interpreted immediately after centrifugation. Delays may cause dissociation of antigen-antibody complexes resulting in weak positive or false negative reactions.

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Suppressive or weak expression of blood group antigens may give rise to false-negative reactions.

False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials, omission of test reagents and certain disease states.

SPECIFIC PERFORMANCE CHARACTERISTICS
Prior to release, each lot of ALBAsera Anti-s is tested by FDA recommended methods against a panel of antigen-positive and antigen-negative red blood cells to ensure suitable reactivity.

For additional information or technical support, contact Product Technical Support at 1-888-228-1990.

BIBLIOGRAPHY

DATE OF ISSUE
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