

Anti-S and Anti-s

For Indirect Antiglobulin Techniques.

SUMMARY

The S and s antigens were reported in 1947 and 1951 respectively and form part of the MNS system. Anti-S and anti-s have both been implicated in Haemolytic Transfusion Reactions and Haemolytic Disease of the Newborn.

Anti-S	Anti-s	Phenotype	Caucasians %	Afro-Americans %
+	0	S+s-	11	3
+	+	S+s+	44	28
0	+	S-s+	45	69
0	0	S-s-	0	<1

PRINCIPLE

The reagents will cause indirect agglutination (clumping) of test red cells, that carry the corresponding specific antigen, in the antiglobulin phase of testing. No agglutination generally indicates the absence of the corresponding specific antigen (see Limitations).

REAGENTS

Atlas Human Anti-S and Anti-s blood grouping reagents are prepared from human serum diluted in a sodium chloride solution containing macromolecular potentiators and bovine albumin. Each reagent is supplied at optimal dilution for use with all recommended techniques stated below without the need for further dilution or addition. For lot reference number and expiry date see Vial Label.

STORAGE

Do not freeze. Reagent vials should be stored at 2 - 8°C 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 30°C.

SAMPLE COLLECTION AND PREPARATION

Blood samples drawn with or without anticoagulant may be used for antigen typing. If testing is delayed then store specimens at 2-8°C. EDTA and citrate samples should be typed within 48 hours. Samples collected into ACD, CPD or CPDA-1 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.

PRECAUTIONS

- The reagents are intended for in vitro diagnostic use only.
- If a reagent vial is cracked or leaking, discard the contents immediately.
- Do not use the reagents past the expiration date (see Vial Label).
- Do not use the reagents if a precipitate is present.
- Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
- 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.
- 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.
- Materials used to produce the reagents were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HBsAg using approved microbiological tests.
- No known tests can guarantee that products derived from human or animal sources are free from infections agents. Care must be taken in the use and disposal of each vial and its contents.

CONTROLS AND ADVICE

- It is recommended a positive control (ideally heterozygous cells) and a negative control be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
- The antiglobulin techniques can only be considered valid if all negative tests react positively with IgG sensitised red cells.
- In the Tube Technique one volume is approximately 40µl when using the vial dropper provided.
- 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.
- The user must determine the suitability of the reagents for use in other

techniques.

REAGENTS AND MATERIALS REQUIRED

- Anti-human globulin i.e. Atlas Polyspecific AHG Elite or anti-IgG i.e. Atlas Monospecific Anti-IgG.
- Coombs cell washer.
- DiaMed ID-Cards (LISS/Coombs),
- DiaMed ID-Centrifuge.
- DiaMed ID-Diluent: e.g. ID-CellStab.
- DiaMed ID-Incubator equilibrated to 37°C - 2°C.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- IgG sensitised red cells i.e. Atlas Coombs Control Cells.
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22°C ± 1°C
- Positive (ideally heterozygous) and negative control red cells.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37°C ± 2°C.

RECOMMENDED TECHNIQUES

A. Indirect Antiglobulin Technique (IAT)

- Prepare a 2-3% suspension of washed test red cells in PBS.
- Place in a labelled test tube: 1 volume of Atlas reagent and 1 volume of test red cell suspension.
- Mix thoroughly and incubate at 37°C for 15 minutes.
- Wash test red cells 4 times with PBS, taking care to decant saline between washes and resuspend each red cell button after each wash. Completely decant saline after last wash.
- Add 2 volumes of anti-human globulin or anti-IgG to each dry cell button.
- Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
- Gently resuspend red cell button and read macroscopically for agglutination
- Confirm validity of all negative reactions with IgG sensitised red cells.

B. DiaMed-ID Micro Typing Technique

- Prepare a 0.8% suspension of washed test red cells in an ID-Diluent.
- Remove aluminium foil from as many microtubes as needed.
- Place in appropriate microtubes: 50µl of test red cell suspension and 25µl of Atlas reagent.
- Incubate the ID-Card(s) for 15 minutes at 37°C.
- Centrifuge the ID-Card(s) for 10 minutes at 90 rcf or for a suitable alternative time and force.
- Read macroscopically for agglutination.

INTERPRETATION OF TEST RESULTS

- Positive: Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the appropriate antigen on the test red cells.
- Negative: No agglutination of the test red cells constitutes a negative result and within the accepted limitation of the test procedure, indicates the absence of the appropriate antigen on the test red cells.

STABILITY OF THE REACTIONS

- Washing steps should be completed without interruption and tests centrifuged and read immediately after addition of the reagent. Delays may result in dissociation of antigen-antibody complexes, causing false negative or weak positive results.
- Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended

LIMITATIONS

- Red cells that have a positive DAT due to a coating of IgG cannot be typed by the Indirect Antiglobulin Technique.
- Antibodies directed at low frequency antigens may occur as unsuspected contaminants in blood grouping antisera. In addition, certain antigens (eg. Bg, Sd^b) can be present in an exalted state on red blood cells. These phenomena may be the source of rare false positive reactions, which may occur with more than one lot of a given specificity.
- It is not possible to claim the absence of all contaminating antibodies, as red cells carrying antigens of low frequency or exalted antigens are not always available for testing.
- Suppressed or diminished expression of certain blood group antigens- may conversely give rise to false negative reactions and so caution should always be exercised when assigning genotypes on the basis of test results.
- False positive or false negative results may also occur due to.
 - Contamination of test materials
 - Improper storage, cell concentration, incubation time or temperature
 - Improper or excessive centrifugation.
- Deviation from the recommended techniques

SPECIFIC PERFORMANCE CHARACTERISTICS

1. The reagents have been characterised by the procedures mentioned in the Recommended Techniques.
2. Prior to release, each lot of Atlas Anti-S and Anti-s reagent is tested by the Recommended Techniques against a panel of antigen-positive red cells to ensure suitable reactivity.
3. The presence of contaminating antibodies to antigens with an incidence of 1% or greater within the random population has been excluded either in tests employing the appropriate antigen-negative red cells or in tests employing the reagents previously absorbed to remove the interfering specificities.
4. Antibodies to Xg^a, Do^a, Yt^a, Co^a, Wr^a, Bg^a and V^w may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cell. This can also be said for YE, M^g and Vⁿ and other low frequency antigens which may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cells
5. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS prior to use.

DISCLAIMER

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

BIBLIOGRAPHY

1. Widman FK. Technical Manual, 9th Edition. American Association of Blood Banks, Arlington, VA, 1985; Chapter 8
2. Race RR, Sanger R. Blood Groups in Man. 4th Edition. Blackwell Scientific, Oxford 1975; Chapter 2
3. Mollison PL. Blood Transfusion in Clinical Medicine, 8th Edition. Blackwell Scientific, Oxford 1987; Chapter 7
4. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 6
5. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
6. British Committee for Standards in Haematology, Blood Transfusion Task Force. Recommendations for evaluation, validation and implementation of new techniques for blood groupin^g, antibody screening and cross matching. Transfusion Medicine, 1995, 5, 145-150.

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