BLOOD GROUPING REAGENT

Anti-S (Monoclonal) Anti-s̄ (Monoclonal) Gamma-clone®

By Tube Test

Preservative: <0.1% Sodium Azide 1°C-10°C Meets FDA Potency Requirements IVD

CAUTION: THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) MAY CONTAIN DRY NATURAL RUBBER. DO NOT PIPETTE THIS PRODUCT BY MOUTH, AS THE ABSENCE OF MURINE VIRUS HAS NOT BEEN DETERMINED.
DO NOT USE IF MARKEDLY TURBID.

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Intended Use:

Gamma-clone Anti-S (Monoclonal) and Anti-s̄ (Monoclonal) Blood Grouping Reagents are intended for the detection of the S (MNS3) and s (MNS4) antigens, respectively, on red blood cells by tube test.

Summary of the Test:

The S and s antigens are inherited as if they are products of allelic genes.[1,2] The locus at which *S* and s are situated is closely linked to the locus that carries *M* and *N*.[3] Three different red blood cell phenotypes result from the inheritance of either or both of these genes (S+s+, S+s-, S-s+). They can be determined in serologic tests employing the appropriate antibodies. The red blood cells of a fourth phenotype, S-s-, fail to react with these sera altogether.[4,5] Persons with this phenotype inherit genes that do not produce S or s. Some antibodies to S and s antigens may be naturally occurring, while others are formed in response to transfusion or pregnancy. Anti-s̄ antibodies are nearly always immune in origin.[6]

Gamma-clone Anti-S (Monoclonal) and Gamma-clone Anti-s̄ (Monoclonal) Blood Grouping Reagents are used to test patient or donor red blood cells for the presence of the S and s antigen, respectively. Typing of donor red blood cells facilitates the selection of suitable antigen-negative units for transfusion to patients with either of these antibodies. Red blood cell typing also serves as final verification of the identification of allo-anti-S or allo-anti-s̄ in patient or donor samples.

Principle of the Test:

The presence of the S or s antigen is determined by testing with Anti-S or Anti-s̄ , respectively, using the tube test technique. Agglutination of the test red blood cells constitutes a positive test result and indicates the presence of the relevant antigen. No agglutination constitutes a negative test result and indicates that the antigen is not present.

Reagents:

Gamma-clone Anti-S (Monoclonal) Blood Grouping Reagent is prepared from IgM antibodies from the human/murine heterohybridoma cell line MS-94 grown in fluid culture and suitably diluted in a proprietary diluent containing bovine albumin to achieve the appropriate level of potency for the test procedure as described. Sodium azide is added as a preservative (at less than 0.1% w/v). Ready for use as supplied.

Gamma-clone Anti-s̄ (Monoclonal) Blood Grouping Reagent is prepared from IgM antibodies from the human/murine heterohybridoma cell line P3BER grown in fluid culture and suitably diluted in a proprietary diluent containing bovine albumin to achieve the appropriate level of potency for the test procedure as described. Sodium azide is added as a preservative (at less than 0.1% w/v). Ready for use as supplied.

Any Bovine Albumin used in the manufacture of this product is sourced from donor animals of United States origin that have been inspected and certified by USDA Food Safety and Inspection Service inspectors to be disease-free. This ruminant-based product is deemed to have a low-TSE (Transmissible Spongiform Encephalopathy) risk.

Storage:

- Store at 1ºC to 10ºC when not in use.
- Do not use beyond the expiration date which is expressed as CCYY-MM-DD (year-month-date).
- Do not freeze.

Precautions:

- For in vitro diagnostic use.
- Do not dilute.
- Effort should be made to minimize contamination during use.
- Do not use if markedly turbid.

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Sodium azide is added as a preservative (at less than 0.1% w/v). Waste fluids arising from the use of Gamma-clone Anti-S (Monoclonal) and Gamma-clone Anti-s̄ (Monoclonal) must be flushed with large quantities of water to avoid accumulation of potentially explosive compounds in laboratory plumbing.

Handle and dispose of reagent as potentially infectious.

Specimen Collection and Preparation:

No special preparation of the patient is required prior to specimen collection. Blood should be drawn by aseptic technique, with or without an anticoagulant. Samples drawn into EDTA, ACD, CPD, CP2D and CPDA-1, as well as red blood cells that have been stored in the additive solutions AS-1, AS-3 and AS-5 can be used for testing. The specimen should be tested as soon as possible after collection. If delay in testing should occur, the specimen must be stored at 1°C to 10°C. Bacterial contamination of the specimen may cause false test results. Blood drawn into EDTA should not be stored for longer than ten days. Clotted specimens may be tested up to 21 days after collection, and donor blood may be tested up to the expiration date. Storage may result in weaker-than-normal reactions.

Red blood cells that are Direct Antiglobulin Test (DAT) positive can be used for testing. Red blood cells that have been EDTA Glycine-Acid (EGA) treated can be used for testing.

Procedure:

Materials Provided:

Gamma-clone Anti-S (Monoclonal) or Anti-s̄ (Monoclonal)

Additional Materials Required:

1. Test tubes (12x75 mm or 10x75 mm)
2. Pipettes
3. Isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline pH 6.5-7.5
4. Centrifuge*
5. An optical aid such as a hand lens or concave mirror
6. Red blood cells of known S or s phenotypes for use as controls.

*It is the users responsibility to validate an accessory device (either listed or otherwise) for its intended use. Validation results should be maintained as part of the laboratory's records for review by regulatory agencies.

Test Method:

1. Place one (1) drop of Gamma-clone Anti-S (Monoclonal) or Gamma-clone Anti-s̄ (Monoclonal) into a properly labeled test tube.
2. Add one (1) drop of an approximate 2-5% suspension of the red blood cells to be tested to the test tube (from step 1 above). The red blood cells to be tested should previously have been washed at least one time and resuspended in saline.
3. Mix the test tube contents well by gently shaking the tube and incubate the tube for five (5) to fifteen (15) minutes at room temperature (15ºC to 30° C). Incubating for the upper end of the time range may enhance reactivity.
4. Centrifuge the test tube.*
5. After centrifugation, immediately resuspend the red blood cells by gently shaking the test tube and examine for macroscopic agglutination. Negative reactions may be examined

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with an optical aid; however, microscopic reading is not recommended. Record the results.

*Suggested centrifugation time and RCF: 15 to 30 seconds at 900-1000 xg or a time and speed, appropriate for the centrifuge used, that produces the strongest reaction of antibody with antigen-positive red blood cells, yet allows easy suspension of antigen-negative red blood cells.

Stability of Reaction:

Following centrifugation, the tube test should be read immediately and interpreted without delay.

Quality Control:

The reactivity of blood grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigens. S+s+ red blood cells are the most suitable positive control red blood cells for Gamma-clone Anti-S (Monoclonal) and Gamma-clone Anti-$\bar{s}$ (Monoclonal). Each reagent is satisfactory for use if it reacts only with antigen-positive red blood cells. Immucor Reagent Red Blood Cells are a convenient source of control cells and may be used as supplied.

Interpretation of Results:

Agglutination of the red blood cells constitutes a positive test result and indicates the presence of the S or s antigen, dependent on the specific reagent used.

No agglutination constitutes a negative test result, and indicates the absence of the S or s antigen, dependent on the specific reagent used.

The reaction patterns possible with Anti-S and Anti-$\bar{s}$ are shown in Table 1, together with the frequencies of the resulting phenotypes in some ethnic populations.

Phenotype	Reagent		Frequency (%) [7]	
	Anti-S	Anti-$\bar{s}$	Whites	Blacks
S+s-	+	0	10	6
S+s+	+	+	42	24
S-s+	0	+	48	68
S-s-	0	0	0	2

Table 1: The reaction patterns of Anti-S and Anti-$\bar{s}$ and the approximate frequencies of the resulting phenotypes in some ethnic populations.

Limitations:

1. Factors that may cause false test results include the following:
   a. Bacterial or chemical contamination of blood specimens, reagent and/or supplementary materials.
   b. Improper storage of materials.
   c. Aged or stored blood specimens. Such specimens may yield weaker reactions than those obtained with fresh red blood cells.
   d. Too heavy a red blood cell suspension of the specimen.
   e. Improper incubation time or temperature.
   f. Improper centrifugation. Proper centrifuge calibration is particularly important to the proper performance of the test. Excessive centrifugation may lead to difficulty in resuspending the red blood cell button in the tube test leading to a possible false positive result. At the same time, inadequate centrifugation may yield unclear red blood cell button patterns and agglutinates that are too readily dispersed leading to a possible false negative result.
   g. Improper examination for agglutination (usually too vigorous shaking). The resuspension of reactions in the tube test procedure must be carried out by gentle shaking. Shaking too vigorously may cause agglutinates to be dispersed.
   h. Deviation from the recommended test procedure such as the omission of test reagents.
2. Red blood cells with a weak expression of the S or s antigen (such as those from persons who have inherited the S^2 gene and no *S* gene) may give results weaker than those obtained with S+s+ control red blood cells.[6]
3. The red cell sialoglycoprotein that carries the S and s antigens also carries the U determinant. Red cells that lack this structure entirely will type as S-s-U-. Depending on the structure involved, red blood cells that carry deleted, hybridized or unusual forms of this protein may type as S-s-U+; or S+s+ but U-.[8]
4. Red blood cells from individuals with unusual MN system phenotypes may produce reactions with Anti-S that are considerably weaker than those of control red blood cells tested in parallel. Diminished expression of the S antigen has been reported with red blood cells that carry the low frequency antigen Mitchell (Mit).[9] The S antibodies secreted by the MS-94 cell line do not react with the partial S antigen on GP.Hop and GP.JL red blood cells. Weakened expression of s has been noted in tests with red blood cells of the M^v phenotype.[10] Red blood cells that carry unusual forms of S or s may react with only certain examples of Anti-S or Anti-$\bar{s}$ reagents. One such example is Dantu, a low frequency antigen associated with blacks.[11,12] Dantu is carried on a novel membrane structure that differs from that which normally carries S and s, yet it is thought of as a type of s antigen. However, Dantu+ red cells may not react with all examples of Anti-$\bar{s}$.
5. False negative reactivity may occur with Anti-S if test red blood cells or positive control red blood cells are exposed to sodium hypochlorite.[13]
6. Red blood cells that have been enzyme-treated must not be used for testing as either red blood cells under investigation or as a source of control red blood cells because use of these enzyme-treated red blood cells may yield erroneous results.

Specific Performance Characteristics:

Gamma-clone Anti-S (Monoclonal) and Anti-$\bar{s}$ (Monoclonal) meets FDA potency requirements. Each lot is tested by insert methods against a panel of antigen-positive and antigen-negative red blood cells to ensure suitable reactivity and specificity. The specificity of the murine monoclonal antibodies secreted by the cell lines used to manufacture these Blood Grouping Reagents has been determined by testing with red blood cells of varying phenotypes.

The performance of this product is dependent upon adhering to the package insert recommended methodology.

For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

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