Blood Grouping Reagents

Anti-A (Murine Monoclonal Blend)

Anti-B (Murine Monoclonal Blend)

Anti-A,B (Murine Monoclonal Blend)

BioClone®

Revised August 2004
e631200448_EN

For Slide, Tube and Microplate Tests

Qualitative Test with Blended Monoclonal Antibodies for Recogntion of the A antigen and its Subgroups and/or the B Antigen on Human Red Blood Cells

REF

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6901935

SUMMARY AND EXPLANATION

Testing with both Anti-A and Anti-B is necessary to determine if red blood cells possess or lack A and/or B blood group antigens. In addition, Anti-A (Murine Monoclonal Blend) BioClone and Anti-A,B (Murine Monoclonal Blend) BioClone have the ability to detect weak subgroups of A (such as A_3, A_x, or $A_{sub}B$). Agglutination is a positive test result indicating the presence of the corresponding antigen. Lack of agglutination is a negative test result indicating the absence of the corresponding antigen.

Anti-A,B reagent agglutinates red blood cells possessing A and/or B blood group antigens. Group O red blood cells will not react with the reagent. In addition, Anti-A,B reagent can be used by transfusion services to confirm blood group O donor units.

The results of red blood cell grouping should be confirmed by reverse (serum or plasma) grouping, i.e., testing the individual's serum or plasma with known A_1 and B red blood cells.

PRINCIPLE OF PROCEDURE

The procedures used with these reagents are based on the principle of agglutination. Normal human red blood cells possessing antigens will agglutinate in the presence of antibody directed toward the antigens.

REAGENTS

Blood Grouping Reagents Anti-A, Anti-B and Anti-A,B (Murine Monoclonal Blend) BioClone for Slide, Tube and Microplate Tests are supplied as three separate reagents by Ortho-Clinical Diagnostics, Inc. and are designed for use in agglutination tests for recognition of the A antigen and its subgroups and/or the B antigen on human red blood cells. To assure broad specificity, antibodies produced from cell cultures of multiple murine hybridomas are blended and pooled to produce each reagent. Anti-A,B (Murine Monoclonal Blend) BioClone is produced by blending Anti-A and Anti-B. The cell cultures containing the antibodies used in the preparation of these reagents are provided by a licensed manufacturer.

These reagents contain sodium azide 0.1% as a preservative, sodium phosphate, sodium chloride, disodium ethylenediamine tetraacetic acid (EDTA), bovine albumin (without stabilizers), and other potentiators.

Reagent	Component Description
Blood Grouping Reagent Anti-A	Anti-A murine monoclonal antibody blend (clones MH04 and A3D3)
	FD&C Blue No. 1
Blood Grouping Reagent Anti-B	Anti-B murine monoclonal antibody blend (clones NB1.19, NB10.5A5, and NB10.3B4)
	FD&C Yellow No. 5
Blood Grouping Reagent Anti-A,B	Anti-A,B murine monoclonal antibody blend (clones MH04, A3D3, NB1.19, NB10.5A5)

Use as furnished. FOR IN VITRO DIAGNOSTIC USE.

MEETS FDA POTENCY REQUIREMENTS.

PRECAUTIONS

Do not use beyond expiration date. Store at 2 to 8°C. May be at room temperature (15 to 30°C) while in use. Replace cap when not in use.

Warning: Contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.

Turbidity may indicate microbial contamination. Serologic testing is necessary to recognize reagent deterioration.

CAUTION: Do not pipette these reagents by mouth as the absence of murine virus has not been determined.

Handle all blood and materials in contact with blood as hazardous or potentially infectious waste. Dispose of all materials according to applicable guidelines and regulations.

CONTROLS

It is recommended that these reagents be tested on each day of use with appropriate positive and negative controls.

Positive control—red blood cells known to possess the antigen toward which the reagent is directed. Group A,B red blood cells are considered an appropriate control.

Negative control—red blood cells known to lack the antigen toward which the reagent is directed.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection. Blood should be collected by approved techniques. Specimen storage should be within applicable regulating agencies' requirements. If specimens are stored before testing, they should be stored at 2 to 8°C. Testing should be performed at room temperature (15 to 30°C). Blood drawn into heparin should be tested within two days. Clotted specimens or blood drawn into sodium citrate or EDTA should be tested within 14 days. Donor blood may be tested up to date of expiration.

PROCEDURE

Material Provided

Blood Grouping Reagent Anti-A (Murine Monoclonal Blend) BioClone for Slide, Tube and Microplate Tests

or

Blood Grouping Reagent Anti-B (Murine Monoclonal Blend) BioClone for Slide, Tube and Microplate Tests

or

Blood Grouping Reagent Anti-A,B (Murine Monoclonal Blend) BioClone for Slide, Tube and Microplate Tests

Required Supplementary Materials

Slide Method

1. Glass slides
2. Pipettes
3. Applicator sticks
4. Isotonic saline, 0.85%–0.9% sodium chloride
5. Centrifuge
6. Test tubes

Tube Method

1. Test tubes, 10 x 75 mm or 12 x 75 mm
2. Pipettes
3. Centrifuge
4. Isotonic saline, 0.85%–0.9% sodium chloride

Microplate Method

1. U-bottom microplates, pretreated if desired
2. Laboratory centrifuge adaptable to microplate carriers
3. Centrifuge microplate carriers
4. Microplate shaker (optional)
5. Microplate test reading mirror (optional)
6. Pipettes
7. Isotonic saline, 0.85%–0.9% sodium chloride

Directions for Use

Slide Method

1. Wash red blood cells at least one time in isotonic saline.
2. Prepare a 35% red blood cell suspension in isotonic saline.
3. On a glass slide, at room temperature (15 to 30°C), place one drop of the appropriate reagent. When Anti-A and Anti-B are used concurrently, Anti-A may be added to one half of a slide, and Anti-B to the other half of the slide.
4. Add one drop of the prepared red blood cell suspension to each drop of reagent on the slide(s).
5. With separate applicator sticks, mix the cell/reagent mixtures well.
6. Tilt the slide(s) back and forth and observe for agglutination. Tests that show no agglutination within 2 minutes are considered negative. Do not interpret peripheral drying or fibrin strands as agglutination.

   CAUTION: Care must be used to avoid cross-contamination of reagents.

Tube Method

1. Prepare a 3% to 5% suspension of red blood cells in isotonic saline.
2. To each test tube, add one drop of the appropriate reagent.
3. Add one drop of the cell suspension to each test tube.
4. Mix well and centrifuge the test tube(s).

   Suggested centrifugation: approximately 15 to 30 seconds at 3400 rpm (900–1000 rcf) or 1 minute at 1000 rpm (100–124 rcf).*
5. Completely resuspend the cells by agitation and examine macroscopically for agglutination. Record results.

   NOTE: Use of a centrifuge with a lid-locking device is recommended to reduce exposure to potentially infectious material.

Microplate Method

1. Prepare a 3% to 5% suspension of red blood cells in isotonic saline.
2. Add one drop of the appropriate reagent to each test well.
3. Add one drop of the cell suspension to the appropriate test well.
4. Mix the contents of each well thoroughly by manually tapping the plate or by using a microplate shaker.

   Suggested times for mechanical shaker: (1) mixing 10–30 seconds on a medium agitation setting; (2) resuspension 10–30 seconds on a medium setting or at a time and speed that allows complete resuspension of the entire cell button without destroying positive reactions.
5. Centrifuge the plate.

   Suggested centrifugation time: 15–30 seconds at approximately 400 rcf or a time appropriate for the centrifuge and microplate used that produces the strongest reaction of antibody with antigen-positive red blood cells, yet allows easy resuspension of antigen-negative cells.*
6. Position plate for reading. (See Reading Methods and Interpretation.)
7. Read and record test results.

   NOTE: Use of a centrifuge with a lid-locking device is recommended to reduce exposure to potentially infectious material.

Microplate Reading Methods and Interpretation

In the "gentle agitation" method, a positive reaction is indicated by the presence of agglutination, whereas a negative reaction will appear as a smooth cell suspension in the microwell.

In the "tilt and stream" method, the plate is tilted at a 60 to 90° angle to the bench top for 2 to 4 minutes. If the reaction is negative, the button will flow as a uniform stream down the side of the well. A positive reaction usually remains at the bottom of the well as an intact button; however, the intact button may occasionally slide down the side of the well.

RESULTS

Interpretation

1. Agglutination of the red blood cells in the presence of reagent is a positive (+) test result and indicates the presence of the corresponding antigen.
2. No agglutination of the red blood cells is a negative (0) test result and indicates the corresponding antigen is not demonstrable.
3. Expected reactions with Anti-A, Anti-B, and Anti-A,B reagents, and reverse grouping with A_1 and B cells with proper interpretation, are shown in the following table.

Reactions with					Blood
Anti-A	Anti-B	Anti-A,B	A_1 cells	B cells	Group
0	0	0	+	+	O
+	0	+	0	+	A
0	+	+	+	0	B
+	+	+	0	0	AB

4. SERUM/PLASMA GROUPING TESTS PERFORMED IN CONJUNCTION WITH RED CELL GROUPING SHOULD ALWAYS AGREE. DISCREPANCIES BETWEEN SERUM/PLASMA AND CELL GROUPING SHOULD BE RESOLVED BEFORE TRANSFUSION.
5. In some patients (e.g., newborns, elderly or immunocompromised patients) the expected ABO antibodies may be weak or missing.

Stability of Final Reaction Mixture

Slide Method

All results must be interpreted within 2 minutes.

Tube and Microplate Methods

All results should be interpreted immediately following centrifugation and resuspension.

LIMITATIONS OF PROCEDURE

1. Tests using these reagents should not be routinely performed at temperatures below 15°C or higher than 30°C. Reactions with Anti-A (Murine Monoclonal Blend) BioClone and Anti-A,B (Murine Monoclonal Blend) BioClone may be enhanced at lower temperatures.
2. The Slide Method should be performed with washed red blood cells at a 35% concentration in isotonic saline. The use of whole blood may interfere with assay performance.
3. **These reagents are not intended for use with enzyme-treated cells.**
4. Group A_1 red blood cells and subgroups of A may demonstrate equally strong reactions with Anti-A reagent. If desired, they can be differentiated by using a specific reagent, such as ORTHO® Anti-A_1 Lectin.
5. Some subgroups of the A antigen may not be detected by these Anti-A and Anti-A,B reagents.
6. The Anti-A reagent may also detect previously unrecognized A antigen in a small number of group B individuals now identified as B(A) cells. Reported studies indicate that incidence of this occurrence is approximately 0.1% of group B cells tested. The agglutination is usually weaker than expected, mixed-field and is easily dispersed. In cases where the results with this Anti-A reagent are questionable, further testing of the red blood cells with human polyclonal anti-A or monoclonal anti-A derived from a hybridoma cell line other than MH04 that is known to be nonreactive with B(A) red blood cells may be useful in discrepancy resolution.
7. Due to antigen deterioration, aged red blood cells may exhibit weaker reactivity than fresh cells.
8. Contaminated blood specimens and/or supplementary materials used in the procedures described may interfere with test results.

9. Failure to completely resuspend the cell button before interpreting results may lead to error in determining the ABO group. However, vigorous agitation during resuspension should be avoided because weak agglutination may be dispersed causing a weak positive reaction to be missed.
10. Tests with these reagents should not be read microscopically. Microscopic evaluation may cause conditions such as rouleaux to be misinterpreted as a positive result.
11. No one speed and time of centrifugation can be recommended that will cover the wide variety of centrifuges available; each laboratory must calibrate its own equipment and determine the time required at a given speed to achieve the desired result.
12. The Test Procedure and Interpretation of Results must be followed closely to ensure the accuracy of the test results. Each laboratory should have a program that provides training on the proper use and handling of these products.
13. When using test methods other than those described in this package insert, laboratories must follow their institution's approved validation procedures to demonstrate the compatibility of these products with predicate methods.
14. The Slide test method is less efficient at detecting weak blood group antigens on red cells.

SPECIFIC PERFORMANCE CHARACTERISTICS

Anti-A (Murine Monoclonal Blend) BioClone and Anti-A,B (Murine Monoclonal Blend) BioClone have the ability to detect many of the weak subgroups of the A antigen such as A_3, A_x or $A_{sub}B$. Anti-A has been shown to be nonreactive with Tn polyagglutinable cells. Anti-B (Murine Monoclonal Blend) BioClone has been shown to be nonreactive with acquired B antigens.

When properly stored and used according to the procedures described under Directions for Use, these reagents will agglutinate red blood cells that have the antigen(s) against which they are directed. The potency of these reagents meets FDA requirements. The reactivity and identity of each lot is demonstrated in tests with the recommended procedure using cells from different donors. The specificity of the source murine monoclonal antibodies used in the manufacture of these products has been demonstrated using a panel of cells that lack the antigen against which the reagent is directed. Specificity test results submitted to the FDA for release of product will be furnished upon request.

Technical questions concerning these reagents should be directed to Customer Technical Support at 1-800-421-3311.

* The centrifugal force applied to cell/reagent mixtures should be the minimum required to produce a "button" of red blood cells and a clear supernate.

Overcentrifugation, i.e., the application of forces in excess of the minimum, causes the cells to adhere to the bottom of the test tube or microplate well so that vigorous agitation is necessary before they can be resuspended. During such agitation, weak agglutination may be dispersed causing a positive reaction to be missed.

Undercentrifugation, i.e., the failure to apply forces necessary to cause the cells to form a "button" and a clear supernate, may result in a weak or negative reaction.

SUMMARY OF REVISIONS

Section	Revision
SUMMARY AND EXPLANATION	Removed frequency of blood types in caucasians. Changed "serum" to "serum or plasma".
REAGENTS	Presented information in chart format, added clone designations. Added statement regarding disposal of materials. Added "Replace cap…". Modifed the sequence of text.
PRECAUTIONS	Added new section "Precautions" and moved warnings and other cautionary statements to this new section. Added statement regarding handling of blood.
SPECIMEN COLLECTION AND PREPARATION	Revised specimen storage conditions for operator clarity. Removed oxalate and finger puncture.
PROCEDURE	Additional required supplementary materials included under Slide Method. Changed "transfer pipettes" to "pipettes" throughout Procedure.
Slide Method	Revised procedure to remove the use of whole blood. New method includes wash step and use of 35% red cell suspension. Added "CAUTION…".
Tube Method	Revised to remove "use of cells suspended in serum or plasma". Added statement regarding use of centrifuge with locking lid.
Microplate Method	Added statement regarding the use of a centrifuge with locking lid.
LIMITATIONS OF PROCEDURE	Added limitations 3, 10, 12, 13 and 14. Revised limitation 2 to reflect change in Slide Method. Moved limitation regarding centrifigation to this section (limitation 11).
SPECIFIC PERFORMANCE CHARACTERISTICS	Updated telephone number for Customer Technical Support.
BIBLIOGRAPHY	Updated edition of Technical Manual.

Store at 2 to 8°C

Preservative:
Sodium azide 0.1%

Turbidity or precipitation may indicate product alteration

Blood Grouping Reagents
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Anti-B
Anti-A,B
(Murine Monoclonal Blend)
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MEETS FDA POTENCY REQUIREMENTS

FOR IN VITRO DIAGNOSTIC USE

CAUTION: HANDLE AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS

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BIBLIOGRAPHY

Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495.

Race RR, Sanger R. Blood Groups in Man. 6th ed. Oxford: Blackwell Scientific Publications, 1975:8–13.

Stroup M, Treacy M. Blood Group Antigens and Antibodies. Raritan, NJ: Ortho Diagnostic Systems Inc., 1982:117–9.

Goding JW. Monoclonal Antibodies: Principles and Practice. New York: Academic Press, 1983:56–97.

Yates AD, Feeney J, Donald ASR, Watkins WM. Characterization of a blood group A-active tetrasaccharide synthesized by a blood-group B gene specified glycosyltransferase. Carbohydr Res 1984;130:251–60.

Greenwell P, Yates AD, Watkins WM. UDP-N-acetyl-D-galactosamine as a donor substrate for the glycosyltransferase encoded by the "B" gene at the human blood group ABO locus. Carbohydr Res 1986;149:149–70.

Goldstein F, Lenny L, Davies D, Voak D. Further evidence for the presence of A antigen on group B erythrocytes through the use of specific exoglycosidases. Vox Sang 1989;57:142–6.

American Association of Blood Banks, Technical Manual. 14th ed. Bethesda, MD: 2002:271-85.

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