Anti-Human Globulin
Anti-C3b, -C3d
(Murine Monoclonal)

Revised January 2017
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BioClone®

Does Not Contain Antibodies to Immunoglobulins such as IgG and IgM

Do Not Use Only this Reagent for Compatibility Testing or Antibody Detection Tests

Qualitative Procedure for the Detection of Components of Complement Bound to Red Blood Cells

FOR IN VITRO DIAGNOSTIC USE

REF

716540

Rx ONLY

SUMMARY AND EXPLANATION

Several serum proteins can be bound to the surface of red blood cells and detected by antiglobulin tests. ORTHO® Anti-Human Globulin is a polyspecific reagent which contains antibodies capable of detecting immunoglobulins and/or complement components bound to red blood cells. In contrast, Anti-Human Globulin Anti-C3b, -C3d (Murine Monoclonal) BioClone will react only with red blood cells having the C3b and/or C3d components of complement on their surfaces and will not react with cells which are sensitized with IgG alone.

Anti-C3b, -C3d is an important diagnostic aid in determining the presence or absence of complement components on human red blood cells. Anti-C3b, -C3d can be used to further characterize the proteins on red blood cells which are agglutinated by ORTHO Anti-Human Globulin which has polyspecific activity. Anti-C3b, -C3d may be used in the direct antiglobulin test and in the indirect antiglobulin test for the identification of complement-fixing antibodies.

This table summarizes the indications for use and limitations of application of the following anti-human globulin reagents provided by Ortho-Clinical Diagnostics, Inc.

	Indications for Use			
	Polyspecific			
	Anti-Human Globulin (Rabbit and Murine Monoclonal) BioClone or ORTHO Anti-Human Globulin (Rabbit)	Anti-C3b, -C3d (Murine Monoclonal) BioClone	ORTHO Anti-IgG (Rabbit)	Anti-C3d (Murine Monoclonal) BioClone
Direct Antiglobulin Test				
Diagnosis of Hemolytic Disease of the Newborn	Yes		Yes	
Investigation of Transfusion Reactions	Yes	Yes†	Yes†	Yes†
Detection of Drug-Induced Red Cell Sensitization	Yes	Yes†	Yes†	Yes†
Detection of Autoimmune Hemolytic Anemia	Yes	Yes†	Yes†	Yes†
General Identification of Cell Surface Coat (e.g., complement vs. immunoglobulin)		Yes‡	Yes	
Specific Identification of Cell Surface Coat (e.g., C3d, IgG or IgM)			Yes	Yes
Indirect Antiglobulin Test				
Compatibility Testing	Yes		Yes‡‡	
Donor Screening for Unexpected Antibodies	Yes		Yes‡‡	
Patient Screening for Unexpected Antibodies	Yes		Yes‡‡	
Detection of Antigens	Yes		Yes	
Antibody Identification (serum)	Yes	Yes	Yes	
Antibody Identification (eluates)	Yes		Yes	

ORTHO

† This reagent should not be used as the sole antiglobulin reagent; falsely negative results may be observed unless cells are tested for the presence of both IgG and C3d using suitable reagents.

‡ Except for C4

‡‡ Some literature reports indicate that anti-IgG may occasionally fail to detect antibodies which are demonstrable only by the use of a polyspecific anti-human globulin reagent. Antibodies not detected by anti-IgG may be clinically significant. While ORTHO Anti-IgG can be used for antibody screening tests and for compatibility testing, one must be aware that data available at this time do not support the exclusive use of anti-IgG for these purposes. Antibody identification may be facilitated by using ORTHO Anti-IgG in conjunction with ORTHO Anti-Human Globulin.

PRINCIPLE OF PROCEDURE

The procedures used with this reagent are based on the principle of antibodies directed against components of human serum as described by Moreschi, and Coombs, Mourant and Race and agglutination as described by Landsteiner. Anti-C3b,-C3d will react with red blood cells sensitized with complement components C3b and/or C3d and cause agglutination of the red blood cells. Red blood cells which do not have C3b and/or C3d on them will not agglutinate with this product.

Thorough washing of the red blood cells is essential because the agglutination reaction will be inhibited if anti-human globulin combines with human serum proteins in solution around the red cells.

REAGENT

Anti-C3b, -C3d (Murine Monoclonal) BioClone is prepared by injecting certain mice intraperitoneally with an anti-C3b secreting hybridoma and other mice with an anti-C3d secreting hybridoma. After suitable time, the ascitic fluids are harvested from the mice peritonea, pooled and manufactured into a reagent for use in detecting complement components C3b and/or C3d.

This reagent contains a buffer solution with bovine albumin and rabbit serum, and sodium azide 0.1% as a preservative. Use as furnished. For in vitro diagnostic use.

Meets potency requirements of the FDA. Do not use beyond expiration date. Store at 2 to 8°C. May be at room temperature (25°C ± 5°C) while in use.

CAUTION: Product deterioration due to excessive heat or freezing may be evidenced by a milky color. Contamination with serum will inactivate anti-human globulin. Bacterial contamination could cause false-positive reactions.

Do not pipette this reagent by mouth as the absence of murine virus has not been determined.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection. Blood should be collected by approved medical techniques. The blood sample should be tested as soon as possible following collection. If the sample is not tested immediately following collection, it may be stored for 24 to 48 hours at 2 to 8°C until tested.

DIRECT ANTIGLOBULIN TEST

For the direct antiglobulin test, blood drawn into EDTA is preferred (to prevent the fixation of complement in vitro) but oxalated, citrated or clotted whole blood may be used.

INDIRECT ANTIGLOBULIN TEST

For the indirect antiglobulin test, serum (no more than 48 hours old) should be used. If plasma is used in the indirect antiglobulin test, complement-dependent antibodies may not be detected because calcium is not available.

PROCEDURES

Required Supplementary Materials

Direct and Indirect Antiglobulin Tests

1. Test tubes, 10 x 75 mm or 12 x 75 mm
2. Pasteur pipettes
3. Centrifuge
4. Isotonic saline
5. Optical aid

Indirect Antiglobulin Test Only

1. Reagent red blood cells for antibody detection or antibody identification
2. Optional enhancing media (such as ORTHO Bovine Albumin or ORTHO Polymerized Bovine Albumin)
3. Incubator, 37°C

Directions for Use

SIMWASH™ Serum/Cell Separation System may be used when performing direct and indirect antiglobulin testing using this reagent.

Follow the procedure in the package insert for SIMWASH.

Direct Antiglobulin Test

CAUTION: Because the levels of complement fixed to red blood cells in vivo may be low, special care should be exercised in performing direct antiglobulin tests with anti-C3b, -C3d reagents.

1. Prepare a 3% to 5% suspension in isotonic saline of the red blood cells to be tested.
2. With a clean Pasteur pipette, add one drop of the prepared cell suspension to a small test tube.
3. Fill the tube with fresh isotonic saline; centrifuge at high speed and decant. Perform this washing a minimum of three times.
4. Decant completely after the last washing. (CAUTION: Do not reintroduce human serum components after washing procedure.)
5. Add two drops of Anti-C3b, -C3d (Murine Monoclonal) BioClone.
6. Mix well and centrifuge.
   Suggested centrifugation: approximately 15 seconds at 3400 rpm (900-1000 rcf) or 1 minute at 1000 rpm (100-125 rcf).*
7. Resuspend the red blood cells by *gentle* agitation and examine macroscopically for agglutination.
8. Examine negative tests with an optical aid.
   NOTE: The sensitivity of complement/anti-complement reactions can be increased by incubation at room temperature for 5 to 10 minutes and recentrifugation.

Interpretation

In the direct antiglobulin test, agglutination of red blood cells in the presence of Anti-C3b, -C3d (Murine Monoclonal) BioClone is a positive test result which indicates the presence of human complement components C3b and/or C3d on the red blood cells. If the blood sample tested was drawn into EDTA, a positive direct antiglobulin test with this reagent indicates in vivo uptake of C3b and/or C3d. If clotted blood was used, a positive result may be due to in vitro uptake of C3b by a cold antibody and the test should be repeated on an EDTA sample (0.01M). EDTA will prevent the fixation of complement in vitro.

Absence of agglutination indicates there are no detectable complement components, C3b or C3d, on the red blood cells.

Red blood cells which agglutinate in the presence of polyspecific anti-human globulin, and do not agglutinate with reagents specific for complement components, may be further tested with anti-IgG to confirm the presence of an IgG blood group antibody.

Indirect Antiglobulin Test

Antibody Identification

When a serum appears to contain complement-binding antibodies, it may be helpful to test it with an antibody identification panel using anti-C3b, -C3d.

1. For each indirect antiglobulin test, label a test tube appropriately.
2. With a clean Pasteur pipette, add two drops of the serum to be tested to each test tube. (Increasing the serum-to-cell ratio, by adding three drops of serum, is an accepted means of enhancing antibody detection.)
3. To each of the test tubes set up in Step 1, add one drop of a 3% to 5% suspension of red blood cells. (Reagent red blood cells should be used as supplied.)
4. If desired, add an enhancing medium according to the manufacturer's directions.
5. Incubate all test tubes at 37°C for the time period specified in the directions which accompany the enhancing medium.
6. If an enhancing medium is not being used, incubate all test tubes at 37°C for 30 minutes.
7. After incubation, wash the cells in each tube three times with tubes full of isotonic saline. Decant and drain completely after the last washing.
8. To each test tube, add two drops of Anti-C3b, -C3d (Murine Monoclonal) BioClone.
9. Mix the contents well of each tube and centrifuge (see Step 6 of Direct Antiglobulin Test for suggested centrifugation).
10. Resuspend the cells by *gentle* agitation and examine tubes macroscopically for agglutination immediately after centrifugation. Record results as "antiglobulin."
    NOTE: The sensitivity of complement/anti-complement reactions can be increased by incubation at room temperature for 5 to 10 minutes and recentrifugation.

Interpretation

Following the indirect antiglobulin test, agglutination in the presence of Anti-C3b, -C3d (Murine Monoclonal) BioClone is a positive test result which indicates the uptake of complement components C3b and/or C3d by the red blood cells, presumably as a result of antibody. Absence of agglutination indicates there is no detectable C3b and/or C3d on the red blood cells.

CONTROL OF ERROR

Serologic testing is necessary to recognize reagent deterioration. It is recommended that the reagent be tested with appropriate positive and negative controls on each day of use according to approved standard operating procedures.

Positive Control - Use red cells sensitized with the specific complement component (for example, a low ionic strength or low ionic strength plus trypsin treatment method as published by FDA in Docket No. 84S-0182 Recommended Methods for Anti-Human Globulin Evaluation).

Negative Control - Use washed, unsensitized red blood cells (for the referenced FDA document, the appropriate negative control(s) are described in the document).

LIMITATIONS OF PROCEDURE

1. This reagent is not a polyspecific anti-human globulin. It does not contain antibodies to immunoglobulins. It may be used in addition to, but not in place of, a polyspecific anti-human globulin for antiglobulin tests.
2. This reagent cannot be used for D^u testing.
3. This reagent is unsatisfactory for detecting hemolytic disease of the newborn.
4. Incompletely washed red blood cells may give false test results.
5. Contaminated supplementary materials used in the procedures described may interfere with the test results.
6. The use of various drugs and also certain disease states are known to be associated with positive direct antiglobulin tests.
7. In the direct antiglobulin test, weaker reactions may be obtained if the cell/serum mixtures are incubated for less than five minutes or more than ten minutes.
8. Hemolysis should not be expected following addition of anti-human globulin.

SPECIFIC PERFORMANCE CHARACTERISTICS

When properly stored and used according to the procedures described under Directions for Use, this reagent will detect C3b and/or C3d present on red blood cells in excess of the small amounts normally found on red blood cells.

Serologic tests (low ionic strength and low ionic strength plus trypsin treatment methods, as published by FDA in Docket No. 84S-0182 Recommended Methods for Anti-Human Globulin Evaluation) provide evidence that this reagent will detect C3b and C3d. Minimal acceptable levels of activity have not been established for these specificities by the FDA.

Serologic tests are also used to confirm the absence of anti-C4 and anti-IgG activity. Red cells sensitized only with incomplete anti-D (anti-Rh_o) and unsensitized red cells will not be agglutinated by this product. This serum has adequate levels of antibody to complement components to meet the present potency and specificity requirements of the FDA.

Technical questions concerning this reagent should be directed to Ortho Care™ Technical Solutions Center at 1-800-421-3311.

* The centrifugal force applied to cell/serum mixtures should be the minimum required to produce a "button" of red 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 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.

No one speed and time of centrifugation can be recommended which 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.

SUMMARY OF REVISIONS

Section	Revision
SPECIFIC PERFORMANCE CHARACTERISTICS	Changed Customer Technical Support to Ortho Care™ Technical Solutions Center and updated phone number.
Back Page	Updated copyright information.

BIBLIOGRAPHY

Moreschi C. Neue tatsachen uber die blutkorperchen agglutinationen. Zbl Bakt 1908;46:49,456.

Coombs RRA, Mourant AE, Race RR. A new test for the detection of weak and 'incomplete' Rh agglutinins. Br J Exp Pathol 1945;26:255.

Landsteiner K. Uber agglutinationserscheinungen normalen menschlichen blutes. Klin Wschr 1901;14:1132.

Bell CA, Zwicker H, Sacks HJ. Autoimmune hemolytic anemia: routine serologic evaluation in a general hospital population. Am J Clin Pathol 1973;60:903.

Chaplin H. Clinical usefulness of specific antiglobulin reagents in autoimmune hemolytic anemias. Prog Hematol 1973;8:25.

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

Issitt PD, Smith TR. Evaluation of antiglobulin reagents. In: A seminar on performance evaluation. Washington, DC: American Association of Blood Banks, 1976;61,65-6.

A seminar on laboratory management of hemolysis. Washington, DC: American Association of Blood Banks, 1979.

Garratty G. Laboratory investigation of drug-induced immune hemolytic anemia and/or positive direct antiglobulin tests. Washington, DC: American Association of Blood Banks, 1980.

Chaplin H Jr, Freedman J, Massey A et al. Characterization of red blood cells strongly coated in vitro by C3 via the alternative pathway. Transfusion 1980;20:256.

Technical manual. 9th ed. Arlington, VA: American Association of Blood Banks, 1985:93,99.

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