BLOOD GROUPING REAGENT

Anti-D (Monoclonal Blend) Gamma-clone®

By Slide, Tube or Microwell Test

IVD **Rx ONLY**

1°C–10°C

Do not use if markedly turbid.

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

Harmful, Preservative: 0.1% Sodium Azide Meets FDA Potency Requirements

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BLOOD GROUPING REAGENT

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INTENDED USE: Gamma-clone Anti-D (Monoclonal Blend) is intended for the detection of the D (RH1) antigen on red blood cells by slide, tube, or microwell test.

SUMMARY OF THE TEST: After the A and B antigens of the ABO blood group system, D is the most important blood group antigen in routine blood banking. To enable measures to be taken to avoid immunization to the D antigen [1], and to assure the identification of all recipients who should be given only D-negative blood, testing for the D antigen is an important laboratory routine. The D-negative phenotype occurs with an incidence of approximately 15% in Whites, and 9-10% in African Americans.

The term D^u was originally coined in 1946 to describe variable reactivity of certain bloods with a battery of sera containing saline-reactive anti-D [2]. It has since been replaced by the term "weak D" to describe forms of the D antigen that may not be agglutinated directly by Anti-D reagents, but require an indirect antiglobulin test to detect them [3,4]. With the introduction of powerful monoclonal Anti-D reagents, many bloods formerly classified as weak D (D^u) have been reclassified because they have been found to show strong direct agglutination with the newer reagents. Individual monoclonal Anti-D reagents may differ in regard to their reactivity with red blood cells of this kind. Some examples of weak D still require an antiglobulin reaction with a suitable Anti-IgG reagent to assure their detection. These include the partial form of D known as Category VI.

The red blood cells of apparently D-negative donors are generally tested for weak D by converting negative tests with Anti-D to an antiglobulin phase and then reading the test again. Testing for weak D on recipients is considered optional [5].

PRINCIPLE OF THE TEST: The presence or absence of the D antigen is determined by testing the red blood cells with Anti-D. Agglutination indicates that the test red blood cells are D-positive. No agglutination indicates that the test red blood cells are D-negative, subject to a negative indirect antiglobulin test for weak D (as in the case of donors). Red blood cells possessing a weak D antigen may give a negative or perceptibly weaker-than-normal reaction in the direct agglutination phases of the test, but will normally yield definite agglutination at the antiglobulin phase. No agglutination at the antiglobulin phase of the test indicates that a D antigen capable of detection by this reagent is not present on the test red blood cells.

REAGENT: Blood Grouping Reagent, Anti-D (Monoclonal Blend) Gamma-clone is manufactured by blending the secretions of two human/murine heterohybridomas [6], grown in fluid culture. The IgM (saline-agglutinating) component is contributed by the cell line GAMA401, and the IgG component by the cell line F8D8. There is no human serum component. The final formulation is in a proprietary buffer to enhance agglutination, which may contain bovine albumin to a total protein concentration that does not exceed 7%. 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 low TSE (Transmissible Spongiform Encephalopathy) risk. Contains 0.1% sodium azide as a preservative.

PRECAUTIONS:

For in vitro diagnostic use. Store at 1° to 10°C when not in use. Do not freeze. Do not dilute. Do not use beyond the expiration date. Effort should be made to minimize contamination during use of the product. Do not use if markedly turbid.

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

This reagent contains 0.1% sodium azide. Warning: H302 Harmful if swallowed.

Warning: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sinks, flush with a large volume of water to prevent azide build-up.

Handle and dispose of reagent as potentially infectious.

The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

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. The specimen should be tested as soon as possible after collection. If delay in testing should occur, the specimen must be stored at 1° to 10°C. It is best to test oxalated or heparinized blood samples within two days of being drawn, and blood drawn into EDTA should not be stored for longer than seven days. Clotted specimens may be tested up to 14 days after collection, and donor blood may be tested up to the expiration date. Storage may result in weaker-than-normal reactions.

PROCEDURE:

Materials Provided: Gamma-clone Anti-D (Monoclonal Blend)

Additional Materials Required: Test tubes (12×75 mm or 10×75 mm), microwell plates with U-bottom wells (those of rigid or flexible construction are equally suitable), pipettes, slides, lighted viewbox, applicator sticks, isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline pH 6.5-7.5, 37°C incubator or waterbath, timer, centrifuge, microwell plate carriers or a mechanical device for the resuspension of tests in microplates, an optical aid such as a hand lens or a concave mirror, red blood cells known to be D-positive and D-negative for use as controls, and Gamma-clone® Control or Monoclonal Control. Anti-Human Globulin containing anti-IgG and IgG-sensitized red blood cells for the test to detect weak D.

TEST METHODS:

Slide Method

1. Place 1 drop of Gamma-clone Anti-D (Monoclonal Blend) on a warm (approx. 45°C) slide on a lighted viewbox. Note: The slide test may also be carried out at room temperature (23°±3°C), but the strength of agglutination may be slightly weaker in the case of a positive test.
2. Add 2 drops of an approximately 35-45% suspension of the red blood cells to be tested to the drop of reagent. The red blood cells may be suspended in saline, in their own (or group-compatible) serum or plasma, or the proper amount of whole blood may be transferred to the slide by the use of applicator sticks or a dropper. If the test sample shows visible hemolysis, lipemia, or

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Underline = Addition or significant change ▲ = Deletion of text

icterus, the red blood cells should be washed at least one time in physiologic saline and resuspended in saline for testing.

3. Mix thoroughly with an applicator stick over an oval area approximately 20×40 mm in size. Rock the viewbox (slowly) back and forth for a period not exceeding two minutes. Caution: Extending the period before interpretation beyond two minutes may result in drying of the reactants on the slide, and may cause false-positive results.
4. Read macroscopically for agglutination and record test results.
5. If the test result is negative or doubtful and a test for weak D is required, repeat the test using the Tube Method, preparatory to carrying out the test for weak D.

Stability of Reaction: Test results must be interpreted immediately upon completion of the test.

Tube Method

1. Place 1 drop of Gamma-clone Anti-D (Monoclonal Blend) in a properly labeled test tube.
2. Add 1 drop of an approximate 3-4% suspension of the red blood cells to be tested (washed or unwashed) to each tube. The red blood cells may be transferred directly from the clot with applicator sticks or may be a prepared suspension in saline, or in their own serum or plasma. If the test sample shows visible hemolysis, lipemia, or icterus, the red blood cells should be washed at least one time in physiologic saline and resuspended in saline for testing.
3. Mix thoroughly by shaking and centrifuge for:
   (a) 1 minute at 1,000 rpm (rcf 100 to 125) or
   (b) 15 seconds at 3,400 rpm (rcf 900 to 1,000) or
   (c) a time appropriate to the calibration of the centrifuge.
   NOTE: It is not essential to centrifuge immediately. Centrifugation may be delayed for up to thirty minutes at room temperature (23°±3°C), if desired.
4. Examine for the absence of hemolysis. *NOTE: Hemolysis may be the consequence of bacterial contamination and should not be interpreted as a positive result.*
5. Resuspend the red blood cells by gentle shaking and read macroscopically for agglutination. An optical aid may be used. Record results.
6. If the test result is negative or doubtful and a test for weak D is required, proceed to carry out the test for weak D, noting the need for a direct antiglobulin test (or an indirect antiglobulin test after incubating the red blood cells with a serologically inert control reagent) before interpreting the result of the test for weak D as positive.

Stability of Reaction: Test results must be interpreted immediately upon completion of the test.

Microwell Method

(in conventional microplates)

NOTE: In some cases, plastic microplates may require pretreatment before use, such as rinsing in distilled water, or may need to stand on a damp towel when being filled in order to dissipate static electricity. It is the laboratory's responsibility to develop its own procedures for the pretreatment of microplates, if needed.

1. Place 1 drop of Gamma-clone Anti-D (Monoclonal Blend) into an identified microwell. *NOTE: If reactions are to be interpreted from the streaming patterns of the test red blood cells, a parallel test with Gamma-clone Control or Monoclonal Control is needed, in which case 1 drop of the Control reagent should be placed into a second microwell. NOTE: The control test, though not essential in a low-protein test system to recognize spontaneous agglutination, facilitates interpretation by enabling streaming of the red blood cells in the test with Anti-D to be compared with that in a known negative test for each red blood cell suspension tested.*
2. To each microwell, add 1 drop of an approximate 3-4% suspension of the red blood cells to be tested, previously prepared in saline. For optimal reactions when the test is to be read by streaming, the drop of red blood cell suspension should be equal in volume to that of the serum added at Step 1. The red blood cells may be used washed or unwashed. If the test sample shows visible hemolysis, lipemia, or icterus, however, the red blood cells should be washed at least one time in physiologic saline and resuspended in saline for testing.
3. Tests performed in a conventional microplate should be mixed, either manually or by using a mechanical device. *NOTE: It is not essential to centrifuge immediately. Centrifugation may be delayed for up to thirty minutes at room temperature (23°±3°C), if desired.*
4. Centrifuge at an appropriate speed and time for the centrifuge being used. As a guide, a speed of 1,000 rpm (rcf 280-300) for 15 seconds is suggested for the Sorvall GLC-2B centrifuge. *NOTE: Centrifugation is critical for proper test results. Each laboratory should calibrate its own centrifuges to determine the optimum time and speed of centrifugation required to achieve acceptable reaction patterns by the microwell test procedure with red blood cell suspensions of known phenotypes. The speed of centrifugation will vary with flexible or rigid plates, and with different centrifuges. Correct interpretation of test reactions is dependent on the application of appropriate centrifugal force to the microplate to produce distinct red blood cell buttons with clear supernatant backgrounds. Streaming of the red blood cells in negative tests should commence within 15 to 60 seconds of tilting the microwell plate to a 60 to 90° angle, and streaming patterns should be interpretable within 2 to 4 minutes.*
5. Examine for the absence of hemolysis. *NOTE: Hemolysis may be caused by bacterial contamination and should not be interpreted as a positive result.*
6. (a) In the case of some microplates, the Anti-D becomes fixed to the plastic, with the result that D-positive test red blood cells may form into a mat across the entire curved surface of the microwell, while D-negative test red blood cells form a small, discrete button in the base. Resuspension, as described in (c) below, usually dislodges only with difficulty the mat of red blood cells in a positive test, while in a negative test the red blood cells readily resuspend. In other cases, the test results may be interpreted from streaming patterns, as described in (b) below, or the red blood cell buttons may be resuspended (by hand or with the aid of a mechanical device), as described in (c).
   (b) *Reading by Streaming:* Tilt the microwell plate at a 60 to 90º angle to the bench top and examine the streaming pattern of the red blood cells in each microwell. Distinct red blood cell streaming patterns will be observed within 2 to 4 minutes, although streaming may commence in as little time as 15 to 60 seconds. It is recommended that test results should not be interpreted after four minutes have elapsed. In a negative reaction, the button of red blood cells will stream downwards. In a positive reaction, the red blood cells remain as a distinct button in the bottom of the microwell, as deposited during centrifugation, but may become dislodged if disturbed and may then fall as a large clump, without streaming, or may fold down to present a "half-moon" appearance. Doubtful results in microplates may be confirmed by gently resuspending the red blood cells as described in (c) below.
   (c) *Reading by Resuspension:* Resuspend the deposited red blood cell button by hand or with a mechanical device, while observing the resuspension to detect weak agglutination. Each laboratory should determine the optimum speed and time for its own mechanical resuspension device. In general, only sufficient force should be applied to bring the red blood cell buttons off the base of the wells. On resuspending, positive reactions are interpreted based on the normal appearance of agglutinated red blood cells in a conventional tube test, while a negative reaction will appear as a smooth red blood cell suspension in the microwell. An optical aid may be used, if desired.
7. Record test results.
8. If the test result is negative or doubtful and a test for weak D is required, set up the test again in a tube, and proceed as described under the Test Method for the Detection of weak D.

Stability of Reaction: Test results must be interpreted immediately upon completion of the test.

Test Method for the Detection of Weak D (D^u)

NOTE: The following method recommends that the test for weak D should be performed in a test tube. This, however, does not preclude the use of microplates for this test if an institution has developed a suitable procedure and has documented evidence of its efficacy.

1. Incubate the reactants in the Tube Method for 15 minutes at 37°±1°C. Incubation may be extended to 30 minutes, if desired. Incubating for the upper end of the time range may enhance reactivity. Proceed to Step 2. Alternatively, centrifuge and read test results as described under Steps 3 through 5 of the Tube Method. If definite macroscopic agglutination is observed, it is unnecessary to continue. The test red blood cells are D-positive. Otherwise, proceed to Step 2.
2. Wash the red blood cells showing a negative or doubtful result at least 3 times with saline, being careful to decant the saline between washes and to resuspend the red blood cells thoroughly when adding saline for the next wash.
3. Decant the saline completely following the last wash.
4. To each tube add 1 or 2 drops of Gamma-clone Anti-Human Globulin (Anti-IgG or Anti-IgG,-C3d; Polyspecific), or follow the directions of the AHG manufacturer. Adding 2 drops of AHG may enhance reactivity.
5. Mix thoroughly and centrifuge immediately as detailed under Step 3 of the Tube Method.
6. Read and record test results as detailed under Step 5 of the Tube Method.

Stability of Reaction: The washing phase of the antiglobulin test must be carried out without interruption, and test results must be interpreted immediately upon completion of the test.

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 antigen. The red blood cells used for the positive control test should preferably have heterozygous expression of the antigen. A control test to detect spontaneous agglutination of immunoglobulin-coated red blood cells is not essential in routine testing with Gamma-clone reagents, because these are prepared in a diluent that does not potentiate this phenomenon. However, a control test is recommended when performing the Microwell Method and interpreting the result by examining for streaming. A parallel control test may be indicated in certain other situations, as discussed below under Interpretation of Test Results.

A negative reaction at the antiglobulin phase of the test for weak D should be confirmed by adding IgG-sensitized red blood cells, such as Checkcell®, and then repeating centrifugation and reading. A positive test result at this point confirms that active antiglobulin (anti-IgG) was added to the test system and was present when the original test was interpreted as negative.

INTERPRETATION OF TEST RESULTS: Agglutination of the red blood cells indicates the presence of the D antigen. If no agglutination occurs, the test red blood cells may be reported as D-negative, except that in donors a test for weak D is normally done with a suitable Anti-D reagent to confirm the absence of the D antigen. This product is suitable for the detection of weak D by the indirect antiglobulin test, although it may directly agglutinate some red blood cells that would be classified as weak D with some other Anti-D reagents. Such reactions are ordinarily quite weak, and may tend to become progressively weaker with incubation than upon reading after immediate-spin. *NOTE: Red blood cells with a positive direct antiglobulin test due to IgG cannot be tested for weak D by the indirect antiglobulin test.* Mixed-field agglutination when testing the red blood cells of a recently delivered woman for weak D may be an indication that fetal D-positive red blood cells are present in the maternal circulation. No agglutination in the antiglobulin test with this reagent indicates that a weak D antigen is not being detected. The red blood cells may be reported as D-negative.

This product does not contain ingredients that enhance the spontaneous agglutination of immunoglobulin-coated red blood cells, but a false-positive test result may still occur due to strong cold autoagglutinins or to a protein imbalance causing the formation of rouleaux. In such cases, similar phenomena would be likely to occur in the ABO grouping test. In cases where the test sample shows definite or doubtful agglutination with both Anti-A and Anti-B, a control test (using Gamma-clone Control, Monoclonal Control, the patient's own serum, or 7% bovine albumin) should be performed to investigate the reliability of the reactions observed in the tests. If the control test is positive, the test red blood cells should be washed several times in warm saline and retested by the Tube or Microwell Method. If the control test again gives a positive reaction, a valid interpretation of the results obtained with the Anti-D cannot be made. In cases where the red blood cells being tested are previously known to have a positive direct antiglobulin test, or where an unexpectedly weak reaction is observed in any of the test procedures leads to the discovery that the direct antiglobulin test is positive, a control test is strongly recommended when attempting to determine the D antigen status of the red blood cells. In this situation, the Gamma-clone Control or Monoclonal control are recommended as being the most suitable control, as they are similar in formulation to Gamma-clone Anti-D (Monoclonal Blend).

LIMITATIONS: Factors that may cause false test results include the following:

1. Contamination of blood specimens, reagents, and/or supplementary materials.
2. Aged blood specimens, which may yield weaker reactions than those obtained with fresh red blood cells.
3. Too light or too heavy a red blood cell suspension.
4. Improper incubation time or temperature. Do not exceed 2 minutes in the Slide Test Procedure.
5. Calibration of the centrifuge is critical. *Excessive centrifugation may lead to difficulty in resuspending the red blood cell button in the tube or microwell test. Conversely, inadequate centrifugation may yield unclear red blood cell button patterns and agglutinates that are too readily dispersed.*
6. Improper examination for agglutination (usually too vigorous shaking). The resuspension of reactions in the tube and microwell test procedures must be carried out by gentle shaking. Shaking too vigorously may cause agglutinates to be dispersed.
7. Deviation from the recommended test procedure.
8. Proteolytic enzymes may have a deleterious effect on components of this product. Accordingly, its use by a one-stage enzyme test procedure (manual or automated) is not recommended.

SPECIFIC PERFORMANCE CHARACTERISTICS: Gamma-clone Anti-D (Monoclonal Blend) meets FDA potency requirements. Each lot is tested against at least 10 red blood cell samples positive for the D antigen (and representative of different Rh phenotypes), in order to assure adequate reactivity in use. All lots are additionally tested against a panel of at least 10 selected red blood cell samples negative for the D antigen, in order to assure true specificity when used by the recommended test procedures. Certain rare red blood cells that appear to be D-negative with other Anti-D reagents will react unexpectedly with this reagent. Red blood cells of the Crawford phenotype are agglutinated at the immediate-spin test phase and are usually nonreactive at the weak D phase [7]. Red blood cells of the R_o^{Har} phenotype are usually agglutinated strongly by this product, but red blood cells of Category VI of partial D can be expected to give positive reactions only at the antiglobulin phase. Other partial D categories were tested and found to be reactive; but this does not guarantee reactivity with all examples of all partial D antigens. Lots of product formulated from these monoclonal antibodies have been tested and found non-reactive with red blood cells positive (or presumptively positive) for antigens having a frequency of 1% or more in the general population of the U.S., as well as V, VS and Js^a, and such low-incidence antigens as Wr^a, M^g, Di^a and Vw. The behavior of this product with polyagglutinable red blood cells has not been evaluated. The performance of this product is dependent on adhering to the recommended methods found in this insert.

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

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