Blood Grouping Reagent
Anti-A and Anti-B (Murine Monoclonal) Series 1
Anti-B (Murine Monoclonal) Series 3 ● Anti-A,B (Murine Monoclonal Blend) Series 1
For Slide, Tube and Microplate Tests

- IVD
- 1°C 10°C
- Harmful, Preservative: 0.1% Sodium Azide
- Meets FDA potency requirements
- Discard if markedly turbid

CAUTION: DO NOT PIPET BY MOUTH THE ABSENCE OF MURINE VIRUSES HAS NOT BEEN DETERMINED. THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) CONTAINS DRY NATURAL RUBBER.

Immucor, Inc.
3130 Gateway Drive
Norcross, GA 30071 USA
US LICENSE 886

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Blood Grouping Reagent

Anti-A (Murine Monoclonal) Series 1
Anti-B (Murine Monoclonal) Series 1
Anti-B (Murine Monoclonal) Series 3
Anti-A,B (Murine Monoclonal Blend) Series 1

For Slide, Tube and Microplate Tests

IMMUCOR

Intended Use:
Immucor Anti-A Series 1 (Murine Monoclonal), Anti-B Series 1 (Murine Monoclonal), Anti-B Series 3 (Murine Monoclonal) and Anti-A,B Series 1 (Murine Monoclonal Blend) are intended for use in slide, tube and microplate tests.

Summary of the Test:
In 1900, Landsteiner observed that the red blood cells of some of his colleagues were agglutinated by the sera of some of the others.[1] On the basis of observed reactions, Landsteiner divided the bloods of his colleagues into three distinct phenotypes; A, B and O.[2] Decastello and Sturli described the fourth phenotype of this system, AB, in 1902.[3]

The ABO groups of most adults can be determined directly in agglutination tests with Anti-A and Anti-B typing reagents derived from either human serum or supernate of hybridoma cells. Monoclonal antibodies derived from cultured hybridoma cell lines can be used to prepare well-defined, potent, pure Blood Grouping Reagents. That monoclonal antibodies can be used reliably for ABO grouping tests has been shown by several groups of investigators.[4-7]

Immucor Anti-A (Murine Monoclonal) Series 1, Anti-B (Murine Monoclonal) Series 1, Anti-B (Murine Monoclonal) Series 3, and Anti-A,B (Murine Monoclonal Blend) Series 1 are suitable for use in ABO red blood cell typing tests.

Principle of the Test:
Direct agglutination of red blood cells with a particular reagent indicates the presence of the corresponding antigen. No agglutination generally indicates its absence (see LIMITATIONS). The ABO group of a red blood cell specimen is determined from the pattern of reactivity obtained with the reagents tested (see Interpretation of Results).

Because the sera of most individuals older than 6 months of age consistently and predictably contain antibodies to the ABO antigen(s) they are lacking, serum grouping (reverse grouping) tests, employing reagent A_1 and B red blood cells, are used to confirm results obtained in red blood cell grouping tests of individuals other than newborn infants. Discrepancies between red blood cell and serum grouping must be resolved before the blood group is recorded. The resolution of typing discrepancies is discussed in references 8 and 9.

Reagents:
Immucor Anti-A (Murine Monoclonal), Anti-B (Murine Monoclonal) and Anti-A,B (Murine Monoclonal) Blood Grouping Reagents are to be used as supplied. Anti-A Series 1, derived from the single clone line Birma-I, has been colored with FD and C blue #1. Anti-B Series 1, from clone ES4, and Anti-B Series 3, from clone LB-2, are colored with Napthol Yellow. No dye has been added to Anti-A,B Series 1, which is a blend of antibodies from secreting cell lines Birma-1, ES4 and ES15. Antibodies are diluted in a buffered saline solution containing bovine albumin (without stabilizers), ethylenediamine tetraacetate (EDTA), and ingredients to facilitate the resuspension of red blood cell buttons following centrifugation. The Bovine Albumin Solution 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. Sodium azide (0.1% final concentration) has been added to each reagent as a preservative. Series 1 Anti-B has been acidified to approximately pH 6.0. Series 3 Anti-B is not acidified and is of an approximate pH of 7.0, as are Series 1 Anti-A and Anti-A,B reagents.

These reagents contain antibodies derived from cell lines produced by other licensed manufacturers.
Meets FDA potency requirements.

Precautions:
For in vitro diagnostic use.

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

Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into the sink, flush with a large volume of water to prevent azide build-up.

Store at 1-10 C when not in use. Do not freeze or expose to elevated temperatures.

Turbidity may indicate reagent deterioration or contamination. Do not use contaminated reagents. Do not use beyond expiration date. Do not use leaking vials. Avoid contamination of reagent.

Handle and dispose of reagent as if potentially infectious.

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

Certain precautions and limitations apply to this reagent when it is used with automated instrumentation. These conditions are described in detail in the instrument operator manual.

Specimen Collection and Preparation:
Draw a blood specimen using an acceptable phlebotomy technique. Samples may be drawn into EDTA, heparin, ACD, CPD, CPDA-1, CP2D or may be drawn without anticoagulant. Semi automated methods may require the use of samples drawn into an anticoagulant. Consult the instrument's operator manual for specific anticoagulants. All testing should be performed as soon as possible following collection to minimize the chance that falsely positive or falsely negative reactions will occur due to improper storage or contamination of the specimen. Samples that cannot be tested within 24 hours should be stored at 1-10 C. Do not use samples drawn into tubes with neutral gel separators. False positive results may occur with such samples. EDTA samples can be tested up to 10 days, clotted samples up to 21 days. Red blood cells drawn into heparin, ACD, CPD, CPDA-1 or CP2D may be tested up to the expiration of the anticoagulant.

Key:
Underline = Addition or significant change; ▲ = Deletion of text

Procedure:

Materials Provided:

Immucor Series 1 Anti-A (Murine Monoclonal), Anti-B (Murine Monoclonal), Anti-A,B Murine Monoclonal Blend), or Immucor Series 3 Anti-B (Murine Monoclonal).

Additional Materials Required:

All methods:

1. Donor patient red blood cells
2. Marking pens
3. Isotonic saline or phosphate-buffered (approximately 15mM) isotonic saline, pH 6.5-7.5

Slide method:

1. Glass slides
2. Wax marker (optional)
3. Applicator sticks
4. Stopwatch or timer
5. Transfer pipettes

Tube method:

1. Transfer pipettes
2. 10x75mm or 12x75 mm test tubes and a test tube rack
3. Serological centrifuge
4. Interval timer

Microplate or microwell methods:

1. Transfer pipettes or pipetting system*
2. Microplates, microwells, Immucor Hemagglutination/Dilution Strips
3. Centrifuge* with rotor and carriers capable of accommodating rigid 96-well plates or rigid 1x8 strips of wells
4. Mechanical microplate shaker* (optional)
5. Microplate reader* (optional)

*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.

Automated Method:

For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Test Methods:

Tube Test

1. Label 1 test tube for each blood grouping reagent to be tested.
2. Add 1 drop of each blood grouping reagent to the appropriately labeled tube.
3. Using a transfer pipette add 1 drop of a 2-5% suspension of red blood cells prepared in saline, plasma or serum to each tube. (Red blood cells may be washed prior to their resuspension in saline.) Alternatively, applicator sticks may be used to transfer red blood cells from clotted or anticoagulated specimens sufficient to make a 2-5% suspension in each tube containing a reagent.
4. Mix the contents of each tube thoroughly and centrifuge.
5. Gently agitate each tube to resuspend the red blood cells buttons. Examine for agglutination. Record results.

NOTE: Incubation for 5-60 minutes at 18-30 C may be necessary to enhance the reactivity of the blood grouping reagents with some of the weak subgroups of A and B.

Slide Test

1. Place one drop of each blood grouping reagent to be tested on separate clean glass slides or on opposite ends of clean slides. Do not place the slides on a heated illuminated surface.
2. Using a transfer pipette or applicator sticks, add 1 drop of a 35-45% suspension of red blood cells prepared in saline, or group-compatible plasma or serum to each drop of reagent.
3. Using separate clean applicator sticks, mix each red blood cell reagent mixture over an oval area of approximately 20 mm x 40 mm.
4. Slowly rotate each slide and observe for macroscopic agglutination for a period not to exceed 2 minutes. (NOTE: Do not place slides on a heated illuminated surface.) Record results.

Microplate/Microwell Test

1. Label the plates of strips of wells to be used in testing.
2. Add 1 drop of each reagent under test to labeled or identified wells.
3. Prepare a 2% approximate suspension of red blood cells in saline, serum or plasma. (Red blood cells may be washed prior to their resuspension in saline.) Alternatively, applicator stick can be used to transfer red blood cells from clotted or anticoagulated samples to prepare the suspension in each well containing reagent. (NOTE: Centrifugation or agitation speeds needed with serum-or plasma-suspended red blood cells may differ significantly from those used with saline suspended red blood cells.)
4. Using a transfer pipette add 1 drop of each red blood cells suspension to the appropriate wells.
5. Mix the contents of each well thoroughly by tapping the plate manually or by using a mechanical microplate shaker.
6. Centrifuge the plate at 150-250 x g for 60 seconds, or for an appropriate time and speed to produce positive results with antigen-positive red blood cells and negative results with antigen–negative red blood cells.
7. Agitate the plate to resuspend each red blood cell button by manually tapping the plate or placing the plate on a plate agitator. Examine each well for agglutination. If desired, a mirror or reader may be used to examine the reaction in each well. Record results.

NOTE: Incubation for 5-60 minutes at 18-30 C may be necessary to enhance the reactivity of weak subgroups of A and B.

Automated Method

For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Stability of the Reaction:

Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Delays may result in dissociation of antigen-antibody complexes leading to falsely negative or, at most, weakly positive reactions. Slide tests should be completed within the time period specified to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of the reagents. Microplate tests should be interpreted immediately following resuspension to avoid erroneous test results due to settling of red blood cells or dissociation of red blood cell agglutinates.

Automated instrumentation reads results at test completion and stores results for reporting at the completion of the batch operation.

Quality Control:

To confirm the reactivity of Immucor Anti-A, Anti-B or Anti-A,B it is recommended that these reagents be tested each day of use with antigen positive red blood cells, such as A_2B red blood cells. These reagents can be considered to be satisfactory if the antigen-positive red blood cells are agglutinated.

For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Interpretation of Results:

Positive Test: agglutination of red blood cells.

Negative Test: no agglutination of red blood cells.

Instrumentation automatically interprets test results.

Key:
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EXPECTED RED BLOOD CELL TYPING RESULTS

Blood Group	Reagent Anti-A	Anti-B	Anti-A,B	Frequency (%)[10] Whites	Blacks
A	+	0	+	40	27
B	0	+	+	11	20
O	0	0	0	45	49
AB	+	+	+	4	4

Limitations:

Falsely positive or falsely negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, improper storage of materials, or omission of test reagents. Under centrifugation or over centrifugation may result in the occurrence of numerous false-negative or false positives.

Certain subgroups of A and B may produce reactions that are weaker than those obtained with A or B red blood cells of most random donors. Depending on the subgroup involved, some may appear nonreactive in direct agglutination tube, microtitration plate or slide tests.

The red blood cells of people with some disease states may give falsely positive or falsely negative reactions with anti-A or anti-B.[9] Some cord blood specimens may give weakened reactions with these reagents. Cord red blood cells contaminated with Wharton's jelly may give falsely positive reactions.

The ABO system is the only blood group system known where individuals, older than 6 months of age, consistently and predictably produce antibodies to antigens they lack. Serum grouping tests, employing red blood cells of known ABO groups, are used to confirm the results of red blood cell typing procedures. However, discrepancies may occur between serum and red blood cell grouping if the specimen under test possesses unexpected antigens or agglutinins, or if the specimen lacks expected antigens or agglutinins. See reference 9 for a more detailed discussion of ABO grouping discrepancies. Any discrepancies that occur should be resolved before an ABO group is assigned.

Do not use murine monoclonal reagents in indirect antiglobulin tests using antihuman globulin reagents.

In certain clinical situations, group A red blood cells may acquire a B-like antigen *in vivo* due to the action of bacterial enzymes called deacetylases. The enzymes change acetyl galactosamine (the blood group A immunodominant sugar) to galactosamine. Some Anti-B reagents of monoclonal or polyclonal origin can react with galactosamine because it is similar in structure to galactose, the B blood group immunodominant sugar. Depending on the degree of transformation in the particular red blood cells being tested, Series 1 Anti-B (from clone ES4) may react strongly with acquired B red blood cells unless the antibody is prepared in an acidified diluent. Series 1 Anti-B reagent has been formulated to a pH of approximately 6.0 to diminish the frequency and strength of reaction with acquired B red blood cells. It should produce reactions with acquired B red blood cells that are more comparable to those observed with human polyclonal Anti-B. However, in some instances, Series 1 Anti-B may still react more strongly than human Anti-B. In cases where the results with Series 1 Anti-B reagent are questionable, further testing of red blood cells should be carried out using human polyclonal Anti-B or monoclonal Anti-B derived from a hybridoma cell line other than ES4 which is known to be nonreactive with acquired B red blood cells, such as Series 3 Anti-B (from clone LB-2). Further acidification of Series 1 Anti-B should not be attempted.

Autoagglutinins reactive at room temperature are a potential source of error in ABO grouping tests. The presence of these antibodies cannot be predicted. When sufficiently strong they can cause the nonspecific agglutination of reagent A_1 and B cells in serum (reverse) grouping tests. They can also produce nonspecific agglutination in red blood cell (forward) tests with Anti-A, and -B and Anti-A,B when unwashed, plasma-suspended or serum–suspended red blood cells are used. It is for this reason that both forward and reverse grouping tests are performed and the results are compared before ABO interpretations are made. All ABO tests should be read carefully. Discrepancies between forward and reverse results should be investigated thoroughly before an ABO group is assigned, regardless of the strength of the reactions obtained in any red blood cell or serum test. The strong reactions obtained in forward tests cannot be assumed to be more correct than weaker reactions seen in reverse tests with the same sample and vice-versa. Some autoagglutinins reactive at room temperature react best when the test environment is below pH 6.5. Immucor's Series 1 Anti-A, Series 3 Anti-B and Series 1 Anti-A,B are prepared in a diluent at approximately pH 7.0. In contrast, Series 1 Anti-B derived from the clone ES4 is prepared at an approximate pH 6.0 to inhibit the detection of the acquired B antigen. Thus, when unwashed or insufficiently washed red blood cells are being used for testing, acid-dependent autoagglutinins are a potential source of false-positive agglutination in tests with Series 1 Anti-B. The same nonspecific reactivity may not be seen in tests with Series 1 Anti-A, Series 1 Anti-A,B or Series 3 Anti-B reagents, or may be perceptibly weaker than in the test with Series 1 Anti-B. Nonspecific agglutination produced by autoagglutinins can range in strength from weak to strong. When unwashed red blood cells are used and an ABO discrepancy persists on repeat testing, evaluating the red blood cells with other blood grouping reagents (prepared at pH 7.0) or testing the serum or plasma with additional reagent red blood cells may be indicated.

Specific Performance Characteristics:

Prior to release, each lot of Immucor Anti-A, Anti-B and Anti-A,B (Murine Monoclonal) is tested by insert methods against a panel of antigen–positive red blood cells to insure suitable reactivity. The performance of this product is dependent on adhering to the recommended methods found in this insert. The presence of contaminating antibodies to antigens with an incidence of 1% or greater in the random population and including M^g and Wr^a, have been excluded either in direct tests employing ABO compatible red blood cells or in tests employing reagents previously adsorbed to remove anti-A or anti-B. Antibodies to the antigens Le^c and Le^d are not necessarily excluded. For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

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Rev 2/13

Key:
Underline = Addition or significant change; ▲ = Deletion of text

Blood Grouping Reagent
Anti-D (Series 4)
Monoclonal Blend
For Slide, Tube and Microplate Tests

- IVD
- 1°C – 10°C
- Harmful, Preservative: 0.1% Sodium Azide
- Meets FDA potency requirements
- Discard if markedly turbid

CAUTION: THE ABSENCE OF ALL VIRUSES HAS NOT BEEN DETERMINED. THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) CONTAINS DRY NATURAL RUBBER.

Immucor, Inc.
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US LICENSE 886

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Blood Grouping Reagent
Anti-D (Series 4)
(Monoclonal Blend)
For Slide, Tube and Microplate Tests

IMMUCOR

Intended Use:
Immucor Anti-D Series 4 (Monoclonal Blend) is intended for use in slide, tube, microplate and automated tests.

Summary of the Test:
The terms "Rh-positive" and "Rh-negative" refer to the presence or absence of the D (Rh_o) red blood cell antigen. The D determinant is one of the over 40 antigens that comprise the Rhesus system.[1,2] Approximately 85% and 92% of Whites and Blacks, respectively, have inherited the D gene.[3]

The D antigen is, after A and B, the most important antigen in transfusion practice. The likelihood that this antigen will provoke an antibody response in an Rh-negative person is very high.[4] For this reason it is essential that red blood cell typing tests using anti-D be performed with all patient and donor specimens.

Immucor Anti-D blood grouping reagents are used to test red blood cells for the presence, or absence, of D. Most Rh-positive specimens can be easily categorized as D-positive since they show clear-cut agglutination reactions with Anti-D reagents at the immediate spin phase of testing. However, some Rh-positive red blood cells are not immediately agglutinated. To distinguish these D-positive red blood cells from those that are truly Rh-negative, additional testing (weak D test) must be performed. [4]

Principle of the Test:
Agglutination of red blood cells at the immediate spin or 37 C incubated phases of testing with Anti-D indicates the presence of D antigen (see section on QUALITY CONTROL). No agglutination at these phases signifies either the absence of D, or that the red blood cells possess a weakened form of the D antigen. Negative reactions obtained at the antiglobulin phase of testing will confirm the absence of D.

Reagents:
Immucor Anti-D Series 4 (Monoclonal Blend) Blood Grouping Reagent is prepared by blending IgM monoclonal anti-D secreted by a human/murine heterohybridoma (MS201) with IgG anti-D of another heterohybridoma (MS26). The antibodies are diluted in a buffered saline solution that contains bovine albumin (without stabilizers), ethylenediamine tetraacetate (EDTA) and ingredients to facilitate the resuspension of red blood cell buttons following centrifugation. The Bovine Albumin Solution 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. Most D+ red blood cell samples will be agglutinated in immediate spin tests by the IgM monoclonal anti-D. The detection of certain weakly reactive D+ or D mosaic samples that are not detected by the IgM monoclonal component will be facilitated by the IgG component in weak D tests.

Sodium azide (0.1% final concentration) has been added as a preservative. The reagent is to be used as supplied.

This reagent may contain antibodies derived from cell lines produced by other licensed manufacturers.

Anti-D (Monoclonal Blend) Series 4 meets FDA potency requirements.

Key:
Underline = Addition or significant change; ▲ = Deletion of text

Precautions:
For in vitro diagnostic use.

Store at 1-10 C when not in use. Do not freeze or expose to elevated temperatures. Turbidity may indicate reagent deterioration or contamination. Do not use contaminated reagents. Do not use beyond expiration date. Do not use leaking vials. Avoid contamination of reagent.

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

Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into the sink, flush with a large volume of water to prevent azide build-up.

Handle and dispose of reagent as if potentially infectious.

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

Specimen Collection and Preparation:
Draw a blood specimen using an acceptable phlebotomy technique. In manual tests, sample drawn into EDTA, heparin, ACD, AS-1, AS-3, AS-5, CPD, CPDA-1, CP2D or without anticoagulant may be used. Semiautomated methods may require the use of samples drawn into an anticoagulant. Consult the instrument's operator manual for specific anticoagulants. All testing should be performed as soon as possible following collection to minimize the chance that falsely positive or falsely negative reactions will occur due to improper storage or contamination of the specimen. Samples that cannot be tested within 24 hours should be stored at 1-10 C. Do not use samples drawn into tubes with neutral gel separation. False positive results may occur with such samples. EDTA samples can be tested up to 10 days, clotted samples up to 21 days. Red blood cells drawn into heparin, ACD, AS-1, AS-3, AS-5, CPD, CPDA-1 or CP2D may be tested up to the expiration of the anticoagulant.

Procedure:
Materials Provided
Immucor Anti-D Series 4 (Monoclonal Blend)
Additional Materials Required
All methods:
1. Donor or patient red blood cells
2. Marking pens
3. Isotonic saline or phosphate-buffered (approximately 15mM) isotonic saline, pH 6.5-7.5

Slide method:
1. Glass slides
2. Wax marker (optional)
3. Transfer pipettes
4. Applicator sticks

5. Lighted, heated viewbox
6. Stopwatch or timer

Tube method:

1. Transfer pipettes
2. 10 x 75 mm or 12 x 75 mm test tubes and test tube rack
3. Serological centrifuge
4. Interval timer
5. Anti-human globulin reagent containing anti-IgG (for weak D test)
6. Coomb's control cells (IgG-coated red blood cells) (for weak D test)
7. 37 C dry heat incubator or water bath
8. Monoclonal Control

Microplate or microwell methods:

1. Transfer pipettes or pipetting system*
2. Microplates, microwells or Immucor Hemagglutination/Dilution Strips
3. Centrifuge* with rotor and carriers capable of accommodating rigid 96-well plates or rigid 1 x 8 strips of wells
4. Mechanical microplate shaker* (e.g., IBG Systems) (optional)
5. Microplate reader* (e.g., IBG Systems Reader) (optional)

*It is the user's 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.

Automated method:

For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Test Methods:

Slide Test

1. Prewarm a clean glass slide to 40-50 C on a lighted viewbox.
2. Place 1 drop of Immucor Anti-D Series 4 (Monoclonal Blend) on the slide.
3. Using a transfer pipette, add 2 drops of a 35-45% suspension of red blood cells, prepared in their own or group compatible plasma or serum, to the reagent.
4. Using a clean applicator stick, mix the red blood cell-reagent mixture over an oval area of approximately 20 mm x 40 mm.
5. Rock the viewbox back and forth and observe for macroscopic agglutination for a period not to exceed 2 minutes (see section on **STABILITY**). Record results.
6. In order to detect weak forms of the D antigen, a weak D test should be performed on all samples that give negative or doubtful positive reactions by the slide test procedure. (See section on **WEAK D TEST**)

Tube Test

NOTE: Red blood cells coated in vivo with IgG molecules often agglutinate spontaneously in tests with reagents that contain more than 12% protein. This is a low protein blood grouping reagent. Therefore, antibody-coated red blood cells are less likely to agglutinate in the presence of the lower protein environment. However, in some instances (as when the patient has produced potent cold-reactive agglutinins or in conditions associated with serum protein abnormalities such as multiple myeloma) spontaneous aggregation or agglutination may still occur leading to falsely positive test results. In these cases, the aggregation or agglutination will, most likely, also be observed in saline tests such as those employing Immucor monoclonal ABO grouping reagents. It is not essential to test a control in parallel with this reagent unless the sample behaves as if it is group AB, D+. In this case, Monoclonal Control can serve as a control reagent when needed in immediate spin or weak D tests. A direct antiglobulin test can also serve as a control weak D test. Weak D test results cannot be considered valid when the red blood cells under test produce positive results in direct antiglobulin tests.

1. Add 1 drop of Immucor Anti-D Series 4 (Monoclonal Blend) to an appropriately labeled test tube.
2. Using a transfer pipette, add 1 drop of 2-5% suspension of red blood cells prepared in saline, or in their own group compatible plasma or serum, to the tube. Alternatively, applicator sticks may be used to transfer cells from clotted or anticoagulated specimens sufficient to make a 2-5% suspension in the tube.
3. Mix the contents of the tube thoroughly and centrifuge the tube.
4. Gently agitate the tube to resuspend the red blood cell button. Examine for agglutination. Record results.
5. A weak D test should be performed on all donor samples that give negative or doubtful positive reactions. (Proceed to weak D TEST).

Weak D TEST

1. Add 1 drop of Anti-D Series 4 and 1 drop of red blood cells as prepared in step 2 (of tube test) to a clean test tube and proceed to step 2 below. Alternatively, the negative test obtained in step 4 (of tube test) can be taken to step 2 below. If desired, one additional drop of Anti-D Series 4 can be added to the test before proceeding to step 2.
2. Mix the contents of the tube thoroughly. Incubate the tube at 36-38 C for 15-60 minutes. Incubating for the upper end of the time range may enhance reactivity. (OPTIONAL: Tests can be centrifuged and read after 37 C incubation.)
3. Wash at least three times with isotonic saline.
4. Add anti-human globulin in the amount specified by the manufacturer's insert. Mix the contents thoroughly.
5. Centrifuge the tube. Gently resuspend the red blood cell button and examine macroscopically for agglutination. Record results.
6. Use IgG-coated red blood cells to confirm the validity of a negative antiglobulin test.

CAUTION: POSITIVE WEAK D TEST RESULTS ARE VALID ONLY IF IT CAN BE SHOWN THAT THE RED BLOOD CELLS UNDER TEST PRODUCED NEGATIVE RESULTS IN DIRECT ANTIGLOBULIN TESTS.

MICROPLATE TEST

Microplate / microwell method:

1. Label the plates or strips of wells to be used in testing.
2. Add 1 drop of Immucor Anti-D Series 4 (Monoclonal Blend) to labeled or identified well.
3. Prepare a 2% (approximate) suspension in saline of the red blood cells under test.
4. Using a transfer pipette, add 1 drop of the red blood cell suspension to the appropriate well.
5. Mix the contents of the well thoroughly by tapping the plate manually or by using a mechanical microplate shaker.
6. Centrifuge the plate.
7. Agitate to resuspend the red blood cell button by manually tapping the plate or by the use of a microplate shaker. Examine the well for agglutination. If desired, a microplate test reading mirror may be used to examine the reactions in the well. Record results.
8. A weak D test should be performed on all donor samples that give negative or doubtful positive reactions (proceed to WEAK D TEST).

Automated method:

For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Stability of the Reaction:

Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Delays may result in dissociation of antigen-antibody complexes leading to falsely negative or, at most, weakly positive reactions. Slide tests should be completed within the time period specified to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of the reagents. Microplate tests should be interpreted immediately following resuspension to avoid erroneous test results due to settling of red blood cells or dissociation of red blood cell agglutinates.

Automated instrumentation reads results at test completion and stores results for reporting at the completion of the batch operation.

Quality Control:

To confirm the reactivity of Immucor Anti-D Series 4 (Monoclonal Blend) it is recommended that this reagent be tested each day of use with D-positive and D-negative red blood cells. The reagent can be considered to be satisfactory for use if it reacts suitably with D-positive red blood cells.

For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Key:
Underline = Addition or significant change; ▲ = Deletion of text

Interpretation of Results:

Positive Test: agglutination of red blood cells at the immediate spin, or 37 C or antiglobulin phases

Negative Test: no agglutination of red blood cells at any test phase

NOTE: Agglutinates in microplate wells are indicative of a positive reaction. Properly resuspended negative reactions will appear as a uniform red blood cell suspension without agglutinates.

Instrumentation automatically interprets test results.

EXPECTED RED BLOOD CELL TYPING RESULTS

		Interpretation			Frequency %[3]
		Weak D			Whites
Test	Control	Test	DAT		
+	0	/	/	D-positive }	85
0	0	+	0	D-positive	
0	0	0	0	D-negative	15
0	0	+	+	Test invalid	
+	+	/	/	Test invalid	

Limitations:

Falsely positive or falsely negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time and temperature, improper centrifugation, improper storage of materials, or omission of test reagents. Proper centrifuge calibration is particularly important to the proper performance of microplate test methods. Undercentrifugation or overcentrifugation may result in the occurrence of numerous false-negatives or false-positives.

Positive reactions obtained with stored specimens may be weaker than those obtained with fresh specimens.

It is the user's 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.

Red blood cells demonstrating a positive direct antiglobulin test cannot be accurately tested in the weak D test with this reagent.

Manually Interpreted tests

Red blood cells that carry the low-incidence antigen Rh33 and are of the R_o^{Har} phenotype are classified as carrying a depressed D antigen based on results in tests with human polyclonal Anti-D. The weakened D antigen is not easily detected even at the antiglobulin phase of D typing tests and the classification of D+ is often made only when it is shown the red blood cells with adsorb and elute anti-D. The IgM portion of this reagent, derived from MS201 anti-D, reacts well with the D antigen of R_o^{Har} red blood cells at the immediate spin phase of tests. Thus, when testing such red blood cells it is possible to find them nonreactive with human polyclonal Anti-D, yet reactive with Anti-D Series 4.

The D+ red blood cells of most people will produce strong reactions (3-4+) with Anti-D Series 4 (Monoclonal Blend). Reactions of less than 2+ in immediate spin tests should be evaluated thoroughly since such reactions may not be due to interaction between reagent Anti-D and D antigen on the test red blood cells. Falsely positive results may occur in direct tests with Anti-D Series 4 in the presence of strong cold-reactive agglutinins or strong rouleaux-forming factors. Such factors lead to cellular aggregation that can be misinterpreted as a positive result when unwashed, serum- or plasma-suspended red blood cells are used. The same factors usually lead to discrepant results in ABO red blood cell typing when similarly prepared red blood cells are used. To determine the validity of positive results obtained in the presence of potent cold-reactive agglutinins or rouleaux-forming proteins, controls of 6-30% bovine albumin in saline can be tested in parallel. Positive results obtained with the albumin control indicate reactions obtained with Anti-D may be invalid. Such problems can be eliminated if test red blood cells are washed thoroughly with warmed saline and resuspended in saline before testing.

Red blood cells possessing comparatively weak expressions of the D antigen may not react well within the 2-minute limit of the slide test or on immediate centrifugation in tube tests.[3]

Specific Performance Characteristics:

Prior to release, each lot of Immucor Anti-D Series 4 (Monoclonal Blend) is tested by insert methods against a panel of antigen-positive red blood cells to insure suitable reactivity. The performance of this product is dependent upon adhering to the insert's recommended methodology. The reactions of Anti-D Series 4 with red blood cells of the rare phenotypes –D-, .D., Rh_{mod} and Rh_{null} have not been determined. Anti-D Series 4 reaction characteristics with enzyme-premodified red blood cells is not known. The presence of contaminating antibodies to antigens with an incidence of 1% or greater in the random population, and including M^g or Wr^a have been excluded either in direct tests employing the appropriate D-negative red blood cells or in tests employing the reagent previously adsorbed to remove anti-D. Antibodies to the antigens Le^c and Le^d are not necessarily excluded.

Certain rare D+ red blood cells will react unexpectedly with this reagent. R_o^{Har} red blood cells produce weak to strong reactions at the immediate spin test phase, even though such red blood cells are generally nonreactive at the weak D phase with Anti-D derived from polyclonal or other monoclonal sources. Some D+w red blood cells, including some DVa, DVc red blood cells, may react at immediate spin with this reagent, but only at the Weak D phase with alternative reagents. No blood grouping reagent of monoclonal origin has yet been found that detects all parts of the D antigen.

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

Bibliography:

1. Issitt PD. Serology and genetics of the Rhesus blood group system. Cincinnati: Montgomery Scientific, 1979.
2. Race RR, Sanger R. Blood groups in man. 6th ed. Oxford: Blackwell Scientific, 1975: 179-260.
3. Brecher ME, ed. Technical manual. 15th ed. Bethesda MD: AABB, 2005.
4. Mollison PL, Englefriet CP, Contreras M. Blood transfusion in clinical medicine. 9th ed. Oxford: Blackwell Scientific, 1992.

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Rev 02/13

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Blood Grouping Reagent
Anti-D (Series 5)
Monoclonal Blend
For Slide, Tube and Microplate Tests

- IVD
- 1°C 10°C
- Meets FDA potency requirements
- Harmful, Preservative: 0.1% Sodium Azide
- Discard if markedly turbid

CAUTION: THE ABSENCE OF ALL VIRUSES HAS NOT BEEN DETERMINED. THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) CONTAINS DRY NATURAL RUBBER.

Immucor, Inc.
3130 Gateway Drive
Norcross, GA 30071 USA
US LICENSE 886

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Blood Grouping Reagent
Anti-D (Series 5)
(Monoclonal Blend)
For Slide, Tube and Microplate Tests

IMMUCOR

Intended Use:
Immucor Anti-D Series 5 (Monoclonal Blend) is intended for use in slide, tube and microplate tests.

Summary of the Test:
The terms "Rh-positive" and Rh-negative" refer to the presence or absence of the D (Rh_o) red blood cell antigen. The D determinant is one of over 40 antigens that comprise the Rhesus system.[1, 2] Approximately 85% and 92% of Whites and Blacks, respectively, have inherited the D gene.[3]

The D antigen is, after A and B, the most important antigen in transfusion practice. The likelihood that this antigen will provoke an antibody response in an Rh-negative person is very high.[4] For this reason it is essential that red blood cell typing tests using anti-D be performed with all patient and donor specimens.

Immucor Anti-D blood grouping reagents are used to test red blood cells for the presence, or absence, of D. Most Rh-positive specimens can be easily categorized as D-positive since they show clear-cut agglutination reactions with Anti-D reagents at the immediate spin phase of testing. However, some Rh-positive red blood cells are not immediately agglutinated. To distinguish these D-positive cells from those that are truly Rh-negative, additional testing (weak D test) must be performed.[4]

Principle of the Test:
Agglutination of red blood cells at the immediate spin or 37 C incubated phases of testing with Anti-D indicates the presence of D antigen (see section on QUALITY CONTROL). No agglutination at these phases signifies either the absence of D, or that the red blood cells possess a weakened form of the D antigen. Negative reactions obtained at the antiglobulin phase of testing will confirm the absence of D.

Reagents:
Immucor Anti-D Series 5 (Monoclonal Blend) Blood Grouping Reagent is prepared by blending IgM monoclonal anti-D secreted by a human/murine heterohybridoma (TH28) with IgG anti-D of another heterohybridoma (MS26). The antibodies are diluted in a buffered saline solution that contains bovine albumin (without stabilizers), ethylenediamine tetraacetate (EDTA) and ingredients to facilitate the resuspension of red cell buttons following centrifugation. The bovine albumin solution is sourced from donor animals of United States origin that have been inspected and certified by US Veterinary Service inspectors to be disease-free. This ruminant-based product is deemed to have low-TSE (Transmissible Spongiform Encephalopathy) risk. Most D+ red blood cell samples will be agglutinated in immediate spin tests by the IgM monoclonal anti-D. The detection of certain weakly reactive D+ or D mosaic samples that are not detected by the IgM monoclonal component will be facilitated by the IgG component in weak D tests.

Sodium azide (0.1% final concentration) has been added as a preservative. The reagent is to be used as supplied.

This reagent may contain antibodies derived from cell lines produced by other licensed manufacturers.

Anti-D (Monoclonal Blend) Series 5 meets FDA potency requirements.

Precautions:

Key:
Underline = Addition or significant change; ▲ = Deletion of text

For in vitro diagnostic use.

Store at 1-10 C when not in use. Do not freeze or expose to elevated temperatures.

Turbidity may indicate reagent deterioration or contamination. Do not use contaminated reagents. Do not use beyond expiration date. Do not use leaking vials. Avoid contamination of reagent.

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

Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into the sink, flush with a large volume of water to prevent azide build-up.

Handle and dispose of reagent as if potentially infectious.

CAUTION: THE ABSENCE OF ALL VIRUSES HAS NOT BEEN DETERMINED. THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) CONTAINS DRY NATURAL RUBBER.

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

Specimen Collection and Preparation:
Draw a blood specimen using an acceptable phlebotomy technique. In manual tests, samples drawn into EDTA, heparin, ACD, AS-1, AS-3, AS-5, CPD, CPDA-1, CP2D or without anticoagulant may be used. Semiautomated methods may require the use of samples drawn into an anticoagulant. Consult the instrument's operator manual for specified anticoagulants. All testing should be performed as soon as possible following collection to minimize the chance that falsely positive or falsely negative reactions will occur due to improper storage or contamination of the specimen. Samples that cannot be tested within 24 hours should be stored at 1-10 C as soon as possible. Do not use samples drawn into tubes with neutral gel separators. False-positive results may occur with such samples. EDTA samples can be tested up to 10 days, clotted samples up to 21 days. Red blood cells drawn into heparin, ACD, AS-1, AS-3, AS-5, CPD, CPDA-1 or CP2D may be tested up to the expiration of the anticoagulant.

Procedure:
Materials Provided
Immucor Anti-D Series 5 (Monoclonal Blend)

Additional Materials Required
All methods:
1. Donor or patient red blood cells
2. Marking pens
3. Isotonic saline or phosphate-buffered (approximately 15mM) isotonic saline, pH 6.5-7.5

Slide method:
1. Glass slides
2. Wax paper (optional)
3. Transfer pipettes

4. Applicator sticks
5. Lighted, heated viewbox
6. Stopwatch or timer

Tube method:

1. Transfer pipettes
2. 10 x 75 mm or 12 x 75 mm test tubes and test tube rack
3. Serological centrifuge
4. Interval timer
5. Anti-human globulin reagent containing anti-IgG (for weak D test)
6. Coomb's control red blood cells (IgG-coated cells) (for weak D test)
7. 37 C dry heat incubator or water bath
8. Monoclonal Control

Microplate or microwell methods:

1. Transfer pipettes or pipetting system*
2. Microplates, microwells or Immucor Hemagglutination/Dilution Strips
3. Centrifuge* with rotor and carriers capable of accommodating rigid 96-well plates of rigid 1 x 8 strips of wells
4. Mechanical microplate shaker*
5. Microplate reader*

*It is the user's 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.

Automated method:

For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Test Methods:

SLIDE TEST

1. Prewarm a clean glass slide to 40-50 C on a lighted viewbox.
2. Place 1 drop of Immucor Anti-D Series 5 (Monoclonal Blend) on the slide.
3. Using a transfer pipette, add 2 drops of a 35-45% suspension of red blood cells, prepared in their own or group compatible plasma or serum, to the reagent.
4. Using a clean applicator stick, mix the red blood cell-reagent mixture over an oval area of approximately 20 mm x 40 mm.
5. Rock the viewbox back and forth and observe for macroscopic agglutination for a period not to exceed 2 minutes (see section on **STABILITY**). Record results.
6. In order to detect weak form of the D antigen, a weak D test should be performed on all samples that give negative or doubtful positive reactions by the slide test procedure. (See section on **weak D TEST.)**

TUBE TEST

NOTE: Red blood cells coated in vivo with IgG molecules often agglutinate spontaneously in tests with reagents that contain more than 12% protein. This is a low protein blood grouping reagent. Therefore, antibody-coated red blood cells are less likely to agglutinate in the presence of the lower protein environment. However, in some instances (as when the patient has produced potent cold-reactive agglutinins or in conditions associated with serum protein abnormalities such as multiple myeloma) spontaneous aggregation or agglutination may still occur leading to falsely positive test results. In these cases, the aggregation or agglutination will, most likely, also be observed in saline tests such as those employing Immucor monoclonal ABO grouping reagents. It is not essential to test a control in parallel with this reagent unless the sample behaves as if it is group AB, D+. In this case, Monoclonal Control can serve as a control reagent when needed in immediate spin or weak D tests. A direct antiglobulin test can also serve as a weak D control test. Weak D test results cannot be considered valid when the red blood cells under test produce positive results in direct antiglobulin tests.

1. Add 1 drop of Immucor Anti-D Series 5 (Monoclonal Blend) to an appropriately labeled test tube.
2. Using a transfer pipette, add 1 drop of 2-5% suspension of red blood cells prepared in saline, or in their own group compatible plasma or serum, to the tube. Alternatively, applicator sticks may be used to transfer red blood cells from clotted or anticoagulated specimens sufficient to make a 2-5% suspension in the tube.
3. Mix the contents of the tube thoroughly and centrifuge* the tube.
4. Gently agitate the tube to resuspend the red blood cell button. Examine for agglutination. Record results.
5. A weak D test should be performed on all donor samples that give negative or doubtful positive reactions. (Proceed to weak D TEST).

WEAK D TEST

1. Add 1 drop of Anti-D Series 5 and 1 drop of red blood cells as prepared in step 2 (of Tube Test) to a clean test tube and proceed to step 2 below. Alternatively, the negative test obtained in step 4 (of Tube Test) can be taken to step 2 below. If desired, one additional drop of Anti-D Series 5 can be added to the test before proceeding to step 2.
2. Mix the contents of the tube thoroughly. Incubate the tube at 36-38 C for 15-60 minutes. Incubating for the upper end of the time range may enhance reactivity. (OPTIONAL: Tests can be centrifuged and read after 37 C incubation.)
3. Wash at least three times with isotonic saline.
4. Add anti-human globulin in the amount specified by the manufacturer's insert. Mix the contents thoroughly.
5. Centrifuge the tube.* Gently resuspend the red blood cell button and examine macroscopically for agglutination. Record results.
6. Use IgG-coated red blood cells to confirm the validity of a negative antiglobulin test.

CAUTION: POSITIVE WEAK D TEST RESULTS ARE VALID ONLY IF IT CAN BE SHOWN THAT THE RED BLOOD CELLS UNDER TEST PRODUCED NEGATIVE RESULTS IN DIRECT ANTIGLOBULIN TESTS.

*Suggested centrifugation time: a time, appropriate for the centrifuge used, that produces the strongest reaction of antibody with antigen-positive red blood cells, yet allows easy resuspension of antigen-negative red blood cells.

MICROPLATE / MICROWELL METHOD

1. Label the plates or strips of wells to be used in testing.
2. Add 1 drop of Immucor Anti-D Series 5 (Monoclonal Blend) to labeled or identified well.
3. Prepare a 2% (approximate) suspension in saline of the red blood cells under test.
4. Using a transfer pipette, add 1 drop of the red blood cell suspension to the appropriate well.
5. Mix the contents of the well thoroughly by tapping the plate manually or by using a mechanical microplate shaker.
6. Centrifuge the plate*
7. Agitate to resuspend the red blood cell button by manually tapping the plate or by the use of a microplate shaker. Examine the well for agglutination. If desired, a microplate test reading mirror may be used to examine the reactions in the well. Record results.
8. A Weak D test should be performed on all donor samples that give negative or doubtful positive reactions. (Proceed to weak D TEST).

*Suggested centrifugation time: a time, appropriate for the centrifuge used, that produces the strongest reaction of antibody with antigen-positive red blood cells, yet allows easy resuspension of antigen-negative blood cells.

AUTOMATED METHOD

For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Stability of Reaction:

Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Delays may result in dissociation of antigen-antibody

complexes leading to falsely negative or, at most, weakly positive reactions. Slide tests should be completed within the time period specified to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of the reagents. Microplate tests should be interpreted immediately following resuspension to avoid erroneous test results due to settling of red blood cells or dissociation of red blood cell agglutinates.

Automated instrumentation reads results at test completion and stores results for reporting at the completion of the batch operation.

Quality Control:
To confirm the reactivity of Immucor Anti-D Series 5 (Monoclonal Blend) it is recommended that this reagent be tested each day of use with D-positive and D-negative red blood cells. The reagent can be considered to be satisfactory for use if it reacts suitably with D-positive red blood cells.
For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Results:
Positive Test: agglutination of red blood cells at the immediate spin, or 37 C or antiglobulin phases
Negative Test: no agglutination of red blood cells at any phase

NOTE: Agglutinates in microplate wells are indicative of a positive reaction. Properly resuspended negative reactions will appear as a uniform red blood cell suspension without agglutinates.

Instrumentation automatically interprets test results.

EXPECTED RED BLOOD CELL TYPING RESULTS

Interpretation						Frequency %[3]
Test	Control	Weak D Test	DAT			Whites
+	0	/	/	D-positive	}	85
0	0	+	0	D-positive		
0	0	0	0	D-negative		15
0	0	+	+	Test Invalid		
+	+	/	/	Test invalid		

Limitations:
Falsely positive or falsely negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time and temperature, improper centrifugation, improper storage of materials, or omission of test reagents. Proper centrifuge calibration is particularly important to the proper performance of microplate test methods. Undercentrifugation or overcentrifugation may result in the occurrence of numerous false negatives or false positives.

Positive reactions obtained with stored specimens may be weaker than those obtained with fresh specimens.

It is the user's 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 record for review by regulatory agencies.

Red blood cells demonstrating a positive direct antiglobulin test cannot be accurately tested in the weak D test with this reagent.

Manually Interpreted tests
Red blood cells that carry the low-incidence antigen Rh33 and are of the R_o^{Har} phenotype are classified as carrying a depressed D antigen based on results in tests with human polyclonal Anti-D. The weakened D antigen is not easily detected even at the antiglobulin phase of D typing tests and the classification of D+ is often made only when it is shown the red blood cells will adsorb and elute anti-D. The IgM portion of this reagent, derived from TH28 anti-D, reacts well with the D antigen of R_o^{Har} cells at the immediate spin phase of tests. Thus, when testing such red blood cells it is possible to find them nonreactive with human polyclonal Anti-D, yet reactive with Anti-D Series 5.

The D+ red blood cells of most people will produce strong reactions (3-4+) with Anti-D Series 5 (Monoclonal Blend). Reactions of less than 2+ in immediate spin tests should be evaluated thoroughly since such reactions may not be due to interaction between reagent Anti-D and D antigen on the test red blood cells. Falsely positive results may occur in direct tests with Anti-D Series 5 in the presence of strong cold-reactive antiglobulins or strong roleaux-forming factors. Such factors lead to cellular aggregation that can be misinterpreted as a positive result when unwashed, serum or plasma-suspended red blood cells are used. The same factors usually lead to discrepant results in ABO cell typing when similarly prepared red blood cells are used.

To determine the validity of positive results obtained in the presence of potent cold-reactive agglutinins or roleaux-forming proteins, controls of 6-30% bovine albumin in saline can be tested in parallel. Positive results obtained with the albumin control indicate reactions obtained with Anti-D may be invalid. Such problems can be eliminated if test red blood cells are washed thoroughly with warmed saline and resuspended in saline before testing.

Red blood cells possessing comparatively weak expressions of the D antigen may not react well within the 2-minute limit of the slide test or on immediate centrifugation in tube tests.[3]

Specific Performance Characteristics:
Prior to release, each lot of Immucor Anti-D Series 5 (Monoclonal Blend) is tested by insert methods against a panel of antigen-positive red blood cells to insure suitable reactivity. The performance of this product is dependent upon adhering to the insert's recommended methodology. The reactions of Anti-D Series 5 with red blood cells of the rare phenotypes –D-, .D., Rh_{mod} and Rh_{null} have not been determined. Anti-D Series 5 reactions characteristics with enzyme-premodified red blood cells is not known. The presence of contaminating antibodies to antigens with an incidence of 1% or greater in the random population, and including M^g or Wr^a have been excluded either in direct tests employing the appropriate D-negative red blood cells or in tests employing the reagent previously adsorbed to remove anti-D. Antibodies to the antigens Le^c and Le^d are not necessarily excluded.

Certain rare D+ red blood cells will react unexpectedly with this reagent. R_o^{Har} red blood cells produce weak to strong reactions at the immediate spin test phase, even though such red blood cells are generally nonreactive at the weak D phase with Anti-D derived from polyclonal or other monoclonal sources. Some D+w red blood cells, including some DVa, DVc red blood cells, may react at immediate spin with this reagent but only at the weak D phase with alternative reagents. No blood grouping reagent of monoclonal origin has yet been found that detects all parts of the D antigen.

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