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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.
3130 Gateway Drive
Norcross, GA 30071 USA
US LICENSE 0886

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.

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.

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, 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)
6. Galileo Echo* (as applicable)
7. Echo Lumena* (as applicable)
8. NEO Iris* (as applicable)

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

*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.

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

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.

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]
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 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.

Weak or variant expression of the D antigen may result in weak or negative reactivity with Anti-D reagents, including Anti-D Series 4 (Monoclonal Blend).

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.

Performance Characteristics on Galileo Echo and Echo Lumena:

Method comparison studies were performed at four external clinical sites. Specimens were tested on Galileo Echo and/or Echo Lumena and Galileo Neo. Specimens that gave initial equivocal (?) test well results with Anti-D Series 4 (Monoclonal Blend) were retested on the analyzer that gave the initial equivocal results. Test results were evaluated for agreement between analyzers. Combined results from all sites are summarized in the following tables:

Note: Agreement between methods does not indicate which method is correct.

Anti-D N=5682		Galileo Neo				
		Positive	Negative	Equivocal		
Galileo Echo	Positive	4939	8	7	Positive % Agreement	100.0%
	Negative	0	725	0	PPA (95% Lower Bound One-Sided CI)	99.9%
	Equivocal	0	3	0	Negative % Agreement	98.5%
					NPA (95% Lower Bound One-Sided CI)	97.5%

Galileo Echo testing performed with software v2.1. NPA lower 95% CI did not meet 99% due to 8 false-positive and 3 equivocal results. Two of the 8 false-positive discordant samples typed as D+ with manual testing. No Weak D tests were performed on discordant or equivocal samples. False-positive RHD typings carry a risk of transfusing D+ blood to a D- recipient and not administrating RHIG to a D- pregnant female. The Group, Pediatric and Donor assays utilize a second Anti-D reagent for RHD testing, discordant D typings will result in NTD (No Type Determined) test interpretation.

Anti-D N=5962		Galileo Neo				
		Positive	Negative	Equivocal		
Echo Lumena	Positive	5182	1	8	Positive % Agreement	100%
	Negative	1	764	0	PPA (95% Lower Bound One-Sided CI)	99.9%
	Equivocal	0	6	0	Negative % Agreement	99.1%
					NPA (95% Lower Bound One-Sided CI)	98.3%

NPA lower 95% CI did not meet 99% due to 8 false-positive and 3 equivocal results. Two of the 8 false-positive discordant samples typed as D+ with manual testing. No Weak D tests were performed on discordant or equivocal samples. False-positive RHD typings carry a risk of transfusing D+ blood to a D- recipient and not administrating RHIG to a D- pregnant female. The Group, Pediatric and Donor assays utilize a second Anti-D reagent for RHD testing, discordant D typings will result in NTD (No Type Determined) test interpretation.

Weak D Test N=663		Galileo Neo				
		Positive	Negative	Equivocal		
Galileo Echo	Positive	20	1	2	Positive % Agreement	100.0%
	Negative	0	639	1	PPA (95% Lower Bound One-Sided CI)	86.1%
	Equivocal	0	0	0	Negative % Agreement	99.8%
					NPA (95% Lower Bound One-Sided CI)	99.3%

Galileo Echo testing performed with software v2.1. PPA lower 95% CI did not meet 99% due to small weak D positive sample (N) size tested.

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

Weak D Test N=663		Galileo Neo				
		Positive	Negative	Equivocal		
Echo Lumena	Positive	20	1	2	Positive % Agreement	100%
	Negative	0	639	1	PPA (95% Lower Bound One-Sided CI)	86.1%
	Equivocal	0	0	0	Negative % Agreement	99.8%
					NPA (95% Lower Bound One-Sided CI)	99.3%

PPA lower 95% CI did not meet 99% due to small weak D positive sample (N) size tested.

Performance Characteristics on NEO Iris:

Method comparison studies were performed at three external clinical sites, including transfusion services and donor centers. Immucor, Inc., as the manufacturer, was a fourth site. Specimens were tested on NEO Iris and Galileo Neo. Specimens that give initial equivocal (?) test well results with Anti-D Series 4 (Monoclonal Blend) were retested on the analyzer that gave the initial equivocal results. Test results were evaluated for agreement between analyzers. Combined results from all sites are summarized in the following tables:

Note: Agreement between methods does not indicate which method is correct.

N=3948		Galileo Neo			
		Positive	Negative		
NEO Iris	Positive	3556	0	Positive % Agreement	100.0%
				PPA (95% Lower Bound One-Sided CI)	99.9%
	Negative	0	392	Negative % Agreement	100.0%
				NPA (95% Lower Bound One-Sided CI)	99.2%

Results are for North America Market assays.

Weak D Test N=418		Galileo Neo			
		Positive	Negative		
NEO Iris	Positive	10	0	Positive % Agreement	100.0%
				PPA (95% Lower Bound One-Sided CI)	74.1%
	Negative	0	408	Negative % Agreement	100.0%
				NPA (95% Lower Bound One-Sided CI)	99.3%

PPA lower 95% CI did not meet 99% due to small weak D positive sample (N) size tested.
Results are for North America Market assays.

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

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