

Individuals using assistive technology may not be able to fully access the information contained in this file. For assistance, please send an e-mail to: ocod@fda.hhs.gov and include 508 Accommodation and the title of the document in the subject line of your e-mail.

Blood Grouping Reagent and Anti-Human Globulin Anti-IgG,-C3d; Polyspecific IH-Card ABO/RhD(DVI+)

A-B-A,B-D (DVI+)-Ctl-AHG

FOR IN VITRO DIAGNOSTIC USE
Gel card for use with the IH-System
MEETS FDA POTENCY REQUIREMENTS
U.S. LICENSE NUMBER: 1845
Rx only

Product-Identification: 71020

IH-Card ABO/RhD(DVI+):	[VOL] 12 cards per box	[REF] 813130100
	[VOL] 48 cards per box	[REF] 813131100
	[VOL] 288 cards per box	[REF] 813132100

INTENDED USE

The IH-Card ABO/RhD(DVI+) is intended for the detection of A (ABO1), B (ABO2) and D (RH1) antigens on human red blood cells and for the detection of immunoglobulins and complement on human red blood cells using the Direct Antiglobulin Test using the IH-System.

SUMMARY

Between 1900 and 1902, Karl Landsteiner and associates discovered the ABO system of red blood cell antigens. ABO blood group typing, using Anti-A and Anti-B antisera to detect the A (ABO1) and B (ABO2) antigens, is known as direct or forward grouping.

The Rhesus blood group system was first described by Landsteiner and Wiener in 1940. The antigen discovered by Landsteiner and Wiener is known as the "D" antigen. The terms "Rh-positive" and "Rh-negative" refer to the presence or absence of the D (RH1) red blood cell antigen. The D antigen is probably the most important antigen outside of the ABO blood group system. Most D negative individuals will make anti-D when sensitized by the D antigen. Additionally, D negative females can become sensitized during pregnancy as a result of a fetal-maternal hemorrhage. The sensitization can lead to destruction of fetal red blood cells.

The D antigen is composed of many epitopes. Most of the D positive red blood cells have a conventional RhD protein. Weak D's types are defined by reduced amounts of D antigen and can be classified in different types reflecting the number of D antigens on the red blood cells, which may require an indirect antiglobulin test for their detection. Red cells of individuals with partial D types are lacking one or more epitopes of the D antigen.

This means that individuals with partial DVI may develop anti-D to the missing epitope if exposed to red blood cells that possess the complete D antigen.

The Direct Antiglobulin Test is used for the investigation of Hemolytic Disease of the Newborn (HDN), certain hemolytic anemias, and hemolytic transfusion reactions. The Direct Antiglobulin Test allows the detection of *in vivo* sensitization of human red blood cells with immunoglobulins and/or complement. The IH-Card ABO/RhD (DVI+) is suitable for the detection of A, B and D antigens by direct agglutination. Most D variant expressions will be detected with this reagent although reaction strengths may vary. The DVI epitope of the D antigen will be detected. The IH-Card ABO/RhD(DVI+) will also detect immunoglobulins and/or complement coating on human red blood cells using the Direct Antiglobulin Test.

PRINCIPLES OF THE TEST

The test combines the principles of hemagglutination and gel filtration for detection of blood group antigen-antibody reactions.

The test sample (red blood cell suspension and/or plasma/serum) is distributed into the microtubes containing the appropriate reagent(s). After centrifugation Non-agglutinated red blood cells are collected at the bottom of the microtube while the agglutinates are dispersed throughout the length of the gel, depending upon their size. Their position in the gel determines the intensity of the reaction.

REAGENTS

[IVD]

OBSERVABLE INDICATIONS

Bubbles trapped in the gel, drying of the gel, artifacts, or open or damaged seals may indicate product alteration.

NOTE: INSPECT THE CONDITION OF THE CARDS BEFORE USE (SEE PRECAUTIONS).

IH-Card ABO/RhD(DVI+) consists of six microtubes containing Anti-A, Anti-B, Anti-A,B, Anti-D (DVI+), Ctl, AHG. The anti-IgG component contains antibody activity to IgG light chain and thus may also agglutinate IgA or IgM coated red blood cells. The anti-complement component consists of murine monoclonal IgG anti-C3d antibody reactive with C3b- and C3d coated red blood cells. Antibodies are diluted in an isotonic saline solution containing bovine albumin, absorbed to remove heterospecific antibodies and contains a mixture of colorants Patent Blue and Tartrazin.

Anti-A, Anti-B, Anti-A,B and Anti-D blood grouping reagents are provided in a final buffered gel suspension. Anti-A has been colored with FD & C Blue #1 and Anti-B has been colored with FD & C Yellow #5. Anti D is a blend of monoclonal human IgM secreted by mouse/human hybridoma. The Anti-B monoclonal antibody (X9) does not react with acquired B cells. This reagent contains bovine albumin.

Reagent	Source	Antibody Class	Cell lines	Manufacturer
Anti-A	Murine Monoclonal	IgM	15750F7	Bio-Rad
Anti-B	Murine Monoclonal	IgG3	X9	Bio-Rad
Anti-A,B	Murine Monoclonal	IgM	AB5-63-A5-A2/X9	Bio-Rad
Anti-D	Human Monoclonal	IgM	BS226/ESD1M	Bio-Rad / Alba Bioscience Limited
Ctl	Gel containing Dextran diluent + preservative	-	-	Bio-Rad
AHG	Rabbit anti-IgG and murine monoclonal anti-complement	IgG1 and Anti-C3d	053A-714	Bio-Rad

Preservative: Sodium Azide (0.1%)

The bovine albumin used for the production of this reagent is purchased from BSE-free sources.

Each card contains six microtubes.

STORAGE REQUIREMENTS

- Store at 18 to 25°C.
- Do not use beyond expiry on the label, which is expressed as YYYY-MM-DD (Year-Month-Day).
- Store in an upright position.
- Do not freeze or expose cards to excessive heat.
- Do not store near any heat, air conditioning sources or ventilation outlets.

PRECAUTIONS

- All IH-System reagents and test samples must be brought to room temperature (18 to 25°C) prior to use.
- Do not use cards showing signs of drying, discoloration, bubbles, crystals or other artifacts.
- Do not use cards with damaged foil strips.
- Use reagents as furnished.
- Do not use gel cards if the gel matrix is absent or if the liquid level in the microtube is not at or below the gel matrix. A clear liquid layer should be visible on top of the uniform

gel matrix in each microtube.

- Cards with dispersed drops observed at the top of the microtube, due to improper storage or shipping conditions, should be centrifuged with the IH-Centrifuge L or IH-Reader 24 with preset time and speed before use. If drops are still observed on top of the microtube after one centrifugation it is recommended to not use the card.
- The use of diluents other than IH-LISS for the red blood cell suspension may modify the reaction and lead to incorrect test results.
- The use of volumes and/or red blood cell suspension in concentrations other than those indicated in the method, may modify the reaction and lead to incorrect test results, i.e., false positive or false negative results.
- Once the IH-Card has been used for testing, it may contain infectious material and should therefore be handled and disposed of as biohazardous waste in accordance with local, state, and national regulations.
- Warning: Contains sodium azide, which may react with lead or copper plumbing to form explosive azides. If discarded in the sink, flush with large amounts of water to prevent the buildup of explosive metal azides.
- Consult downloads.bio-rad.com to download the valid version of this instruction for use.

Specimen Collection and Preparation

No special preparation of the patient or donor is required prior to specimen collection. Blood samples should be collected following general blood sampling guidelines.

Fresh blood samples collected in anticoagulant are acceptable. Samples should be tested as soon as possible post collection. If testing is delayed EDTA samples may be stored at 2 to 8°C for up to ten (10) days when tested manually and five (5) days when tested on automated systems. In case of testing with samples without anticoagulant only manual testing is accepted and if testing is delayed, these samples may be stored at 2 to 8°C for up to ten (10) days.

Cord blood samples may be stored at 2 to 8°C up to ten (10) days when tested manually and five (5) days when tested on automated system, however, general guidelines for DAT testing recommend testing within 48 hours. Do not use grossly hemolyzed, lipemic or icteric samples.

A distinct separation of red blood cells and plasma is recommended for optimal results. This can be achieved through centrifugation for 10 minutes at 2000g or at a time and speed that consistently produces a distinct cell/plasma interface.

TEST PROCEDURE FOR MANUAL AND AUTOMATED SYSTEMS

Material provided

- IH-Card ABO/RhD(DVI+)

Materials required but not provided

- IH-LISS Rack or IH-LISS Solution
- Dispenser pipette capable of delivering 1 mL
- Pipettes: 10 µL, 50 µL and 1 mL
- Disposable pipette tips
- Glass or plastic test tubes
- IH-Centrifuge L or IH-Reader 24 to centrifuge the IH-Cards at 85g with pre-set time for manual working
- IH-1000 or IH-500 for full automation

Method for automation

Please refer to the IH-1000 or IH-500 and IH-Com User Manual U.S. for testing and reagent handling instructions.

Method for manual testing

Refer to the IH-Reader 24 User Manual and IH-Com User Manual U.S. or IH-Centrifuge L User Manual U.S. for equipment operating instructions.

Immediately prior to use prepare a red blood cell suspension of approximately 1% to be tested in IH-LISS.

- Transfer 1 mL of IH-LISS Solution to a labelled disposable tube
- Add 10 µL of red blood cell pellet
- Mix gently
- The red blood cell suspension is ready for use

Note: Red blood cell suspension should be used as soon as possible. If it is not possible red blood cell suspension must be used within 24 hours.

1. Allow reagents and samples to reach room temperature (18 to 25°C) before use.
2. Inspect the condition of the cards before use (see Warnings and Precautions)
3. Label the gel card appropriately.
4. Withdraw the entire foil seal from the card or from the individual microtubes to be used for testing. Carefully peel off the aluminium foil to prevent cross-contamination of the microtube contents.

Note: Once the foil has been removed from the microtubes, testing must be initiated to prevent drying of the gel.

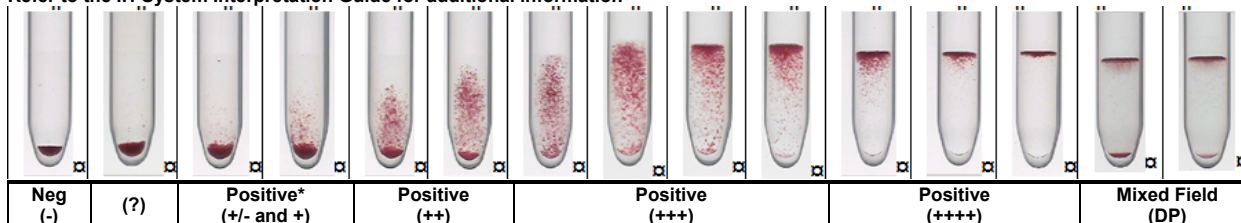
5. Ensure the resuspension of the red blood cells before use.
6. Distribute 50 µL of red blood cell suspension (approximately 1%) into the appropriate wells of microtubes
Note: Carefully dispense the red blood cell suspension, avoiding contact of the pipette tip with the contents of the microtubes to prevent carryover.
7. Centrifuge in the IH-Centrifuge L or IH-Reader 24 at the pre-set conditions as determined by the manufacturer.
8. Read the reactions by visual inspection or automatically with the IH-Reader 24.

INTERPRETATION OF RESULTS

For visual interpretation

- **Positive result** - Agglutinates on the surface of or dispersed through the gel. Report as a positive test result if hemolysis is present in the microtube but not in the sample column. Red blood cells may remain suspended on the top of the gel or dispersed throughout the gel in varying degrees. A few cells may form a button in the microtube bottom in some positive reactions.
- **Negative result** - A compact button of red blood cells at the microtube bottom is a negative test result.

Refer to the IH-System Interpretation Guide for additional information



*A very weak reaction is not an expected result for antigen testing. It may indicate that a false positive or a very weak/partial expression of the antigen is present. Further investigation of this sample should be performed before the antigen status is determined.

Well Reaction Grade	Result Interpretation	Reaction Description
-	Negative	A compact, pellet of RBCs* with a smooth surface at the bottom of the well with no visible agglutination.
+/-	Blood Grouping and Phenotyping including Anti-D Blend = Not interpretable For Reverse (serum) ABO Testing = Positive Direct Antiglobulin Test, Antibody Detection, Autocontrol = Positive Antibody Identification = no overall result interpretation, only well result shown as +/- For Crossmatching = Incompatible	A pellet of RBCs at the bottom of the well with a very few agglutinated RBCs visible above the pellet or an irregular pellet.
Well Reaction Grade	Result Interpretation	Reaction Description

+	For Blood Grouping and Phenotyping including Anti-D Blend = Not interpretable For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification = no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	A pellet of RBCs at the bottom of the well with agglutinated RBCs visible in the lower half of the gel column.
++	For Blood Grouping and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification = no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Agglutinated RBCs distributed throughout the entire length of the gel column, with no line of RBCs on the top of the well.
+++	For Blood Grouping and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification = no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Most agglutinated RBCs concentrated at the top of the gel or upper half of the gel column.
++++	For Blood Grouping and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification = no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Agglutinated RBCs concentrated as a line on the top of the gel column with a few agglutinated RBCs just underneath the gel surface.
Mixed Field (DP)	Blood Grouping and Phenotyping including Anti-D Blend = Not interpretable For Reverse (serum) ABO Testing = Positive Direct Antiglobulin Test, Antibody Detection, Autocontrol = Positive Antibody Identification = no overall result interpretation, only well result shown as DP For Crossmatching = Incompatible	Agglutinated RBCs as a line at the top of the gel or dispersed in upper part of the gel and non-agglutinated RBCs forming a pellet at the bottom of the well. The instrument interpretation software displays "DP" (double population) for a mixed field result.
?	For Blood Grouping including Reverse ABO Testing and Phenotyping including Anti-D Blend, Antibody Detection and Identification, Direct Antiglobulin Testing = Not interpretable For Crossmatching = Incompatible	Ambiguous result.

For automated reading

Below is a description of the various reaction grades and how the software uses that well reaction to determine the result interpretation. Please refer to the IH-Reader 24 User Manual or IH-1000, IH-500 and IH-Com User Manual U.S. for further information.

Well Reaction Grade	Result Interpretation	Reaction Description
-	Negative	A compact, pellet of RBCs* with a smooth surface at the bottom of the well with no visible agglutination.
+/-	Blood Grouping and Phenotyping including Anti-D Blend = Not interpretable For Reverse (serum) ABO Testing = Positive Direct Antiglobulin Test, Antibody Detection, Autocontrol = Positive Antibody Identification = no overall result interpretation, only well result shown as +/- For Crossmatching = Incompatible	A pellet of RBCs at the bottom of the well with a very few agglutinated RBCs visible above the pellet or an irregular pellet.
+	For Blood Grouping and Phenotyping including Anti-D Blend = Not interpretable For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification = no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	A pellet of RBCs at the bottom of the well with agglutinated RBCs visible in the lower half of the gel column.
++	For Blood Grouping and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification = no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Agglutinated RBCs distributed throughout the entire length of the gel column, with no line of RBCs on the top of the well.
+++	For Blood Grouping and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification = no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Most agglutinated RBCs concentrated at the top of the gel or upper half of the gel column.
++++	For Blood Grouping and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification = no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Agglutinated RBCs concentrated as a line on the top of the gel column with a few agglutinated RBCs just underneath the gel surface.
Mixed Field (DP)	Blood Grouping and Phenotyping including Anti-D Blend = Not interpretable For Reverse (serum) ABO Testing = Positive Direct Antiglobulin Test, Antibody Detection, Autocontrol = Positive Antibody Identification = no overall result interpretation, only well result shown as DP For Crossmatching = Incompatible	Agglutinated RBCs as a line at the top of the gel or dispersed in upper part of the gel and non-agglutinated RBCs forming a pellet at the bottom of the well. The instrument interpretation software displays "DP" (double population) for a mixed field result.
?	For Blood Grouping including Reverse ABO Testing and Phenotyping including Anti-D Blend, Antibody Detection and Identification, Direct Antiglobulin Testing = Not interpretable For Crossmatching = Incompatible	Ambiguous result.

* RBCs = Red Blood Cells

Expected reactions with Anti-A, Anti-B, Anti-A,B, Anti-D(DVI+) and their interpretation are shown in the following table:

Blood Grouping	Blood Grouping	Blood Grouping	Blood Grouping	Interpretation of the result	Interpretation of the result
Anti-A	Anti-B	Anti-A,B	Anti-D(DVI+)	ABO	D
positive	negative	positive	positive	A	Positive
positive	negative	positive	negative	A	Negative
negative	positive	positive	positive	B	Positive
negative	positive	positive	negative	B	Negative
positive	positive	positive	positive	AB	Positive
positive	positive	positive	negative	AB	Negative
Blood Grouping	Blood Grouping	Blood Grouping	Blood Grouping	Interpretation of the result	Interpretation of the result
negative	negative	negative	positive	O	Positive

negative	negative	negative	negative	negative	O	Negative
----------	----------	----------	----------	----------	---	-----------------

- For the automated and manual test methods, the control (Ctl) should be negative for the antigen tests on this card to be considered valid.
- This product does not contain ingredients that enhance 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 tests with all the IH System Monoclonal Blood Grouping Reagents. If the control test is positive, laboratories are advised to consult their approved site specific procedures. The test cells can be washed several times in warm saline and retested.¹ If the control test again gives a positive reaction, a valid interpretation of the results obtained cannot be made. Additional testing will be necessary to resolve the false positive reaction according to site-specific procedures.
- Caution must be taken in interpreting a reaction as a mixed field. Additional patient history and testing may be necessary for resolution. Not all mixed field populations have a sufficient minor population to be detected.
- A positive direct antiglobulin test is indicative of immunoglobulins and/or complement on the red blood cells tested. Negative direct antiglobulin test results do not necessarily rule out hemolytic disease of the newborn, especially if ABO incompatibility is suspected.

STABILITY OF REACTIONS

Please refer to the instrument operator's manual for results interpretation process. For visual reading of reactions, best results are obtained within six hours of centrifugation. Interpretation may be affected by drying of the gel, hemolysis of red blood cells and slanting of reaction patterns due to storage in a non-upright position. Processed cards that are stored in the refrigerator (2 to 8°C) and properly sealed to protect from evaporation may be interpreted for up to one (1) day. Gel cards should not be interpreted after the first sign of drying, or if hemolysis is observed. The age and condition of red blood cells, as well as the temperature at which the card is stored, will affect how long cards can be stored. The presence of sodium azide in the gel may cause the red blood cells to become dark in color over time. This darkening does not interfere with the test result.

QUALITY CONTROL

On each day of use, the reactivity of all Blood Grouping Reagents should be confirmed by testing with known positive and negative samples. For example, the Blood Grouping Reagents contained on this card could be controlled by testing group AB D positive and group O D negative samples. Other combinations of ABO and Rh types are possible as long as there is a positive and negative control for each reagent (this does not apply to the control reagent).

On each day of use, the reactivity of antiglobulin reagent should be confirmed by testing with known positive and negative samples. As positive control red blood cells coated with IgG may be used.

Each reagent is satisfactory for use if positive and negative samples react as expected. For additional information, please consult the IH-1000 or IH-500 User Manual U.S. and the IH-Com User Manual U.S., Quality Control Sections.

LIMITATIONS

- Erroneous and abnormal results may be caused by:
 - Bacterial or chemical contamination of the blood specimens, reagents, supplementary materials and/or equipment.
 - Patient medication or disease yielding a cross-reaction.
 - A red blood cell concentration or suspension medium different from that recommended.
 - Incomplete resuspension of the red blood cells.
 - Sample hemolysis prior to testing.
 - Contamination between microtubes through pipetting errors.
 - Use of procedure other than the one described above.
- Grossly icteric blood samples, blood samples with abnormally high concentrations of protein or blood samples from patients who have received plasma expanders of high molecular weight may give false positive results.
- Fibrin, clots, particulates or other artifacts may cause some red blood cells to be trapped at the top of the gel and cause an anomalous result. They may appear as a pinkish layer. In a negative reaction the false appearance of a mixed field could lead to misinterpretation.
- Since A and B antigens are not fully developed at birth, cord blood cells may give weaker reactions than red cells of adults and subgroups often cannot be identified. They may appear as a pinkish layer. In a negative reaction the false appearance of a mixed field could lead to misinterpretation.
- A weak reaction is not an expected result for antigen typing and may be indicative of a false positive or weak/partial expression of the antigen. Further investigations may be warranted per site specific procedures.
- Very weak ABO subgroups may not be detected with the Anti-A, Anti-B and Anti-AB reagents used in this gel card.
- The Anti-B reagent does not react with the acquired B antigen.
- Very weak expressions of the D antigen may not be detected. The DVI epitope of the antigen will be detected with this reagent. If the detection of weak D samples is required, the samples producing negative results with this Anti-D reagent should be further tested with an Anti-D reagent known to detect weak D antigen expression (i.e. IH-Anti-D (RH1) Blend). No blood grouping reagent of monoclonal origin has yet been found that will detect all parts of the D antigen.
- A negative Direct Antiglobulin Test does not exclude the diagnosis of Hemolytic Disease of the Newborn, especially if ABO incompatibility is present.
- A false positive result in the Direct Antiglobulin Test may be caused by complement attached to red blood cells in specimens collected from infusion lines used to administer dextrose-containing solutions.
- Very weak reactions in the Direct Antiglobulin Test may not be detected.

Please refer to the IH-Reader 24 User Manual or IH-1000, IH-500 and IH-Com User Manual U.S. for instrument-specific assay limitations.

SPECIFIC PERFORMANCE CHARACTERISTICS

The final release testing is performed according to the product specific Standard Operating Procedures. As part of the lot release process, each lot of Bio-Rad Blood Grouping Reagents is tested against antigen positive and negative samples to ensure suitable reactivity and specificity.

Performance characteristics using the IH-1000

Testing to determine the performance characteristics of the Bio-Rad IH Blood Grouping Reagents Anti-A, Anti-B, Anti-A,B and Anti-D(DVI+) was performed at four different US clinical sites and included patient, cord blood and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH Blood Grouping Reagents in comparison to the FDA-licensed reference reagents. Microtube results for a given reagent were combined across applicable IH-Cards.

Results of the positive percent agreement and negative percent agreement, with the one-sided Exact 95% Lower Confidence Limit (LCL) are listed in the data table below. Note: See the IH-1000 User Manual U.S. and IH-Com User Manual U.S. for more information on verification of results.

Results from Clinical Trials

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Anti-A	4,392	99.91% (99.79%)	2,942	99.93% (99.79%)
Anti-B	6,172	100% (99.95%)	1,161	99.83% (99.46%)
Anti-A,B	1,593	99.94% (99.70%)	1,603	99.88% (99.61%)
Anti-D(DVI+)	672	99.40% (98.64%)	3,169	100% (99.91%)

Direct Antiglobulin Testing to determine the performance characteristics of AHG Anti-IgG, -C3d was also performed in clinical studies and in internal studies with well-characterized and/or contrived samples. The clinical trial results of positive percent agreement and negative percent agreement, as well as the one-sided Exact 95% Lower Confidence Limit (LCL) for DAT testing, are listed in the table below. Also included are the percent agreements and LCL for the additional testing with well-characterized and/or contrived samples.

Note: See the IH-1000 User Manual U.S. and IH-Com User Manual U.S. for more information on verification of results.

Results from Clinical Trial

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
DAT	585	97.61% (96.28%)	65	89.23% (80.72%)

Results with well-characterized and/or contrived samples

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
DAT	Not Tested	NA	69	100% (95.75%)

NA= not applicable

Agreement between the methods does not imply which method obtained the correct result. The above results do not reflect any discrepancy resolution between the methods.

Reproducibility was evaluated at two external sites and one internal site by testing a reproducibility panel according to the following scheme: one lot of reagent x 3 sites x 1 operator x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days using the IH-1000 Analyzer. Reproducibility was demonstrated for the Blood Grouping Reagents Anti-A, Anti-B, Anti-A,B and Anti-D(DVI+) within run, between runs and between sites.

A precision study was conducted internally using three reagent lots x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days using the IH-1000 Analyzer. Precision was demonstrated with all three lots of Blood Grouping Reagents Anti-A, Anti-B, Anti-A,B and Anti-D(DVI+).

Performance characteristics using the IH-500

Testing to determine the performance characteristics of the Bio-Rad IH Blood Grouping Reagents Anti-A, Anti-B, Anti-A,B and Anti-D(DVI+) using IH-500 v.2.1.14 was performed at three different US clinical sites and included patient and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH Blood Grouping Reagents in comparison to the FDA-licensed reference reagents. Microtube results for a given reagent were combined across applicable IH-Cards.

Results of the positive percent agreement and negative percent agreement, with the one-sided Exact 95% Lower Confidence Limit (LCL) are listed in the data table below. Note: See the IH-500 User Manual U.S. and IH-Com User Manual U.S. for more information on verification of results.

Results from Clinical Trials with IH-500 v.2.1.14

Test	Sample type	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Anti-A	Random samples	1,270	100% (99.76%)	993	100% (99.70%)
Anti-A	Known group B	255	100% (98.83%)	NA	NA
Anti-A	All samples	1,525	100% (99.80%)	993	100% (99.70%)
Anti-B	Random samples	1,914	99.95% (99.75%)	349	100% (99.15%)
Anti-B	Known group B	NA	NA	255	100% (98.83%)
Anti-B	All samples	1,914	99.95% (99.75%)	604	100% (99.51%)
Anti-D(DVI+)	Random samples	131	100% (97.74%)	691	100% (99.57%)
Anti-D(DVI+)	Known RhD neg	255	100% (98.83%)	NA	NA
Anti-D(DVI+)	All samples	386	100% (99.23%)	691	100% (99.57%)

NA= not applicable

Results from Clinical Trials with IH-500 v.2.1.14

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Anti-A,B	675	100% (99.56%)	815	100% (99.63%)

Direct Antiglobulin Testing to determine the performance characteristics of AHG Anti-IgG, -C3d was also performed in clinical studies. The clinical trial results of positive percent agreement and negative percent agreement, as well as the one-sided Exact 95% Lower Confidence Limit (LCL) for DAT testing, are listed in the table below. Note: See the IH-500 User Manual for U.S. and IH-Com User Manual for U.S. for more information on verification of results.

Results from Clinical Trial with IH-500 v.2.1.14

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
DAT	440	100% (99.32%)	111	93.69% (88.48%)

Agreement between the methods does not imply which method obtained the correct result. The above results do not reflect any discrepancy resolution between the methods.

Reproducibility was evaluated at three external sites by testing a reproducibility panel according to the following scheme: one lot of reagent x 3 sites x 1 operator x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days. Reproducibility for the Blood Grouping Reagents Anti-A, Anti-B and Anti-D(DVI+) using the IH-500 was demonstrated within run, between runs and between sites.

Internal comparison studies have been performed with IH-500 v.2.1.14 and IH-500 v.3.0. The study included testing of patient and donor samples as well as known samples. The results of positive percent agreement and negative percent agreement, as well as the one-sided Exact 95% Lower Confidence Limit (LCL), are listed in the data table below.

Results from In-House Study comparing IH-500 v.2.1.14 with IH-500 v.3.0

Test	Sample type	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Anti-A	Random samples	436	100% (99.32%)	365	100% (99.18%)
Anti-A	Known group B	125	100% (97.63%)	NA	NA
Anti-A	Known group AB and Ax	2	100% (22.36%)	62	100% (95.28%)
Anti-A	All samples	563	100% (99.47%)	427	100% (99.30%)
Anti-B	Random samples	683	100% (99.56%)	115	100% (97.43%)
Anti-B	Known group B and AB	NA	NA	187	100% (98.41%)
Anti-B	All samples	683	100% (99.56%)	302	100% (99.01%)
Anti-D(VI+)	Random samples	181	100% (98.36%)	669	100% (99.55%)
Anti-D(VI+)	Known RhD neg	143	100% (97.93%)	NA	NA
Anti-D(VI+)	Known D.VI pos	NA	NA	5	100% (54.93%)

Anti-D(VI+)	All samples	324	100% (99.08%)	674	100% (99.56%)
Anti-A,B	Random samples	364	100% (99.18%)	448	99.55% (98.6%)
Anti-A,B	Known group A, AB and Ax	NA	NA	187	100% (98.41%)
Anti-A,B	All samples	364	100% (99.18%)	635	99.49% (99.01%)
DAT	Random samples	984	99.8% (99.36%)	17	100% (83.84%)
DAT	Known DAT_pos	NA	NA	63	100% (95.36%)
DAT	All samples	984	99.8% (99.36%)	80	100% (96.32%)

NA = Not Applicable

The above results do not reflect any discrepancy resolution between the methods.

Performance characteristics for manual testing

Testing to determine the performance characteristics of the Bio-Rad IH Blood Grouping Reagents Anti-A, Anti-B, Anti-A,B and Anti-D(DVI+) was performed at five different US clinical sites and one internal site and included patient, cord blood and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH Blood Grouping Reagents in comparison to the FDA-licensed reference reagents. Microtube results for a given reagent were combined across applicable IH-Cards.

Results of the positive percent agreement and negative percent agreement, with the one-sided Exact 95% Lower Confidence Limit (LCL), are listed in the data table below.

Results from Clinical Trials

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Anti-A	1,506	100% (99.80%)	1,060	100% (99.72%)
Anti-B	1,999	99.95% (99.76%)	567	100% (99.47%)
Anti-A,B	623	100% (99.52%)	842	99.88% (99.44%)
Anti-D(DVI+)	466	99.79% (98.99%)	893	100% (99.67%)

Direct Antiglobulin Testing to determine the performance characteristics of AHG Anti-IgG, -C3d was also performed in clinical studies. The clinical trial results of positive percent agreement and negative percent agreement, as well as the one-sided Exact 95% Lower Confidence Limit (LCL), are listed in the table below.

Results from Clinical Trial

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
DAT	245	99.18% (97.45%)	143	100% (97.93%)

Agreement between the methods does not imply which method obtained the correct result. The above results do not reflect any discrepancy resolution between the methods.

Reproducibility was evaluated at three external sites by testing a reproducibility panel according to the following scheme: one lot of reagent x 3 sites x 2 operators x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days. Reproducibility for the Blood Grouping Reagents Anti-A, Anti-B, Anti-A,B and Anti-D(DVI+) using the IH-Centrifuge L was demonstrated within run, between runs and between sites.

Performance characteristics using the IH-Reader 24

Testing to determine the performance characteristics of the Bio-Rad IH Blood Grouping Reagents Anti-A, Anti-B, Anti-A,B and Anti-D(DVI+) was performed at five different US clinical sites and one internal site and included patient and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH Blood Grouping Reagents in comparison to the FDA licensed reference reagents. Microtube results for a given reagent were combined across applicable IH-Cards.

Results of the positive percent agreement and negative percent agreement, with the one-sided Exact 95% Lower Confidence Limit (LCL) are listed in the data table below. Note: See the IH-Reader 24 User Manual and IH-COM User Manual U.S. for more information on verification of results.

Results from Clinical Trials

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Anti-A	1,503	99.93% (99.68%)	1,063	99.91% (99.55%)
Anti-B	2,003	99.80% (99.54%)	563	100% (99.47%)
Anti-A,B	623	99.84% (99.24%)	842	99.88% (99.44%)
Anti-D(DVI+)	471	99.79% (99.00%)	888	100% (99.66%)

Direct Antiglobulin Testing to determine the performance characteristics of AHG Anti-IgG, -C3d was also performed in clinical studies. The clinical trial results of positive percent agreement and negative percent agreement, as well as the one-sided Exact 95% Lower Confidence Limit (LCL), are listed in the table below.

Results from Clinical Trials

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
DAT	245	97.96% (95.76%)	165	100% (98.20%)

Agreement between the methods does not imply which method obtained the correct result. The above results do not reflect any discrepancy resolution between the methods.

Reproducibility was evaluated at three external sites by testing a reproducibility panel according to the following scheme: one lot of reagent x 3 sites x 2 operators x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days. Reproducibility for the Blood Grouping Reagents Anti-A, Anti-B, Anti-A,B and Anti-D(DVI+) using the IH-Reader 24 was demonstrated within run, between runs and between sites.

For technical support or further product information, contact Bio-Rad Laboratories, Inc. at 800-224-6723.

GLOSSARY OF SYMBOLS

Symbol	Definition	Symbol	Definition
[LOT]	Batch Code	[IVD]	<i>In vitro</i> diagnostic medical device
!	Consult the instructions for use for important cautionary information such as warnings and precautions	!	Consult instructions for use
M	Manufacturer	e	Use by YYYY-MM-DD

Symbol	Definition	Symbol	Definition
S	Contains sufficient quantity for <n> tests	[REF]	Catalog number
t	Temperature limitation	[VOL]	Volume

BIBLIOGRAPHY

1. Kankura T, Kurashina S, Nakao M.: A gel filtration technique for separation of erythrocytes from human blood. J Lab Clin Med 1974;83: 840-844.
2. Rouger Ph, Salmon Ch.:La pratique de l'agglutination des érythrocytes et du test de Coombs. Masson 1981.
3. Lapierre Y, Rigal D, Adam J et al: The gel test: a new way to detect red cell antigen-antibody reactions. Transfusion 1990;30:109-113.
4. Salmon Ch, Cartron JP, Rouger Ph.: Les groupes sanguins chez l'homme. 2é ed. Masson 1991.
5. Pottier C, Quillet P, Banfine-Ducroq H.: Gel-test: Interpretation and value of a new technique for the detection of irregular antibodies. Ann Bio Clin 1992;50:679-685.
6. Burin des Rosiers N, Nasr O.: Recherche des anticorps irréguliers érythrocytaires par la méthode du gel-test. Analyse de 35882 échantillons. Rev. Fr. Transfus. Hemobiol., 1993;36:391-399.
7. International Forum. What is the best technique for the detection of red cell alloantibodies. Vox Sang 1995;69:292-300.
8. Issitt PD.: Applied Blood Group Serology. 4th ed. Miami: Montgomery Scientific Publications, 1998.
9. John D. Roback, MD et al. Technical Manual 17th Edition, Bethesda, MA: AABB, 2011.

Key: Underline = Addition of changes ◀ = Deletion of text