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

English, B186360, Version 05, 2016.07

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

Product-Identification: 71020

IH-Card Group ABO/RhD (DVI+):	VOL 12 cards per box	REF 813 130 100
	VOL 48 cards per box	REF 813 131 100
	VOL 288 cards per box	REF 813 132 100

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 complete protein. Weak D`s are defined by reduced amounts of the 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. Partial D types are missing epitopes of the D antigen. Individuals possessing the DVI epitope may produce an anti-D to the missing epitopes after immunization by fetal or transfused D positive red blood cells.

Direct Antiglobulin Testing is useful in the investigation of Hemolytic Disease of the Newborn (HDN), certain hemolytic anemias, and transfusion reactions. The test will detect immunoglobulins and complement coating human red blood cells.

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 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) is distributed into the microtubes containing the appropriate reagent(s) and centrifuged. 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. For the description of the reaction intensity, please refer to the Reaction Grading Guide in the Interpretation of Results section.

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 reactivity against light IgG chains 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 hybridomas. 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.
• Do not use cards with bubbles.
• Do not use cards with damaged foil strips.
• Use reagents as furnished.
• 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.

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 five (5) days. Cord blood samples may be stored at 2 to 8 °C up to five (5) days post collection, 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 at 10 minutes at 2000g or at a time and speed that consistently produces a distinct cell/plasma interface.

TEST PROCEDURE FOR AUTOMATED SYSTEMS

Material provided
• IH-Card ABO/RhD (DVI+)

Materials required but not provided
• IH-LISS Rack

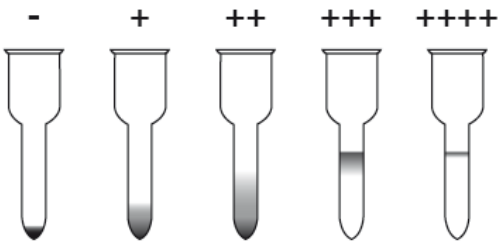
• **IH**-1000

Method
Please refer to the **IH**-1000 User Manual NA for testing and reagent handling instructions.

INTERPRETATION OF RESULTS

For automated systems

Below is a description of the various reaction grades and how the software uses that well reaction to determine the result interpretation.



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, Antisera, 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.
1+	For Blood Grouping, Antisera 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.
2+	For Blood Grouping, Antisera 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.
3+	For Blood Grouping, Antisera 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.
4+	For Blood Grouping, Antisera 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, Antisera, 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, Antisera, 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					Interpretation of the result	
Anti-A	**Anti-B**	**Anti-A,B**	**Anti-D(DVI+)**	**Ctl**	**ABO**	**D**
positive	negative	positive	positive	negative	A	**Positive**
positive	negative	positive	negative	negative	A	**Negative**
negative	positive	positive	positive	negative	B	**Positive**
negative	positive	positive	negative	negative	B	**Negative**
positive	positive	positive	positive	negative	AB	**Positive**
positive	positive	positive	negative	negative	AB	**Negative**
negative	negative	negative	positive	negative	O	**Positive**
negative	negative	negative	negative	negative	O	**Negative**

• 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.

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 Rh(+) and group O Rh(-) 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 reagents should be confirmed by testing with known positive and negative samples using an antiglobulin assay such as antibody screen or direct antiglobulin assays. When using an antibody screen assay to control the antiglobulin reagent, the positive samples should contain known red blood cell antibodies such as anti-D, anti-Fya, etc. When using a direct antiglobulin assay to control the antiglobulin reagent, 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 User Manual NA and the **IH**-COM User Manual NA, 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 may cause an anomalous result.
• A weak reaction is not an expected result and may be indicative of a false positive or weak/partial expression of the antigen. Further investigations may be warranted per site specific procedures.
• 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 expression of the D antigen will be detected with this reagent. If 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).
• A negative Direct Antiglobulin Test does not exclude the diagnosis of Hemolytic Disease of the Newborn, especially if ABO incompatibility is present.

Please refer to the **IH**-1000 User Manual NA 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 on the IH-1000 Analyzer

Testing to determine the performance characteristics of the Bio-Rad IH Blood Grouping Reagents Anti-A, Anti-B, Anti-AB 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 NA and **IH**-COM User Manual NA for more information on verification of results.

Test	Results from Clinical Trials			
	Negative Agreement		Positive Agreement	
	N	Point Estimate (one-sided Exact 95% LCL)	N	Point Estimate (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%)

Testing of the Blood Grouping Reagents was also performed using the Direct Antiglobulin Test with the IH-System, in clinical studies and in supplementary 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 data table below. Also included are the percent agreements and LCL for the additional testing with well-characterized and/or contrived samples.

Test	Results from Clinical Trial				Results with well-characterized and/or contrived samples			
	Negative Agreement		Positive Agreement		Negative Agreement		Positive Agreement	
	N	Point Estimate (one-sided Exact 95% LCL)	N	Point Estimate (one-sided Exact 95% LCL)	N	Point Estimate (one-sided Exact 95% LCL)	N	Point Estimate (one-sided Exact 95% LCL)
DAT	585	97.61% (96.28%)	65	89.23% (80.72%)	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 runs, 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+).

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

GLOSSARY OF SYMBOLS

Symbol	Definition	Symbol	Definition
	Batch code		*In vitro* diagnostic medical device
	Caution, consult accompanying documents		Consult instructions for use
	Manufacturer		use by (YYYY-MM-DD)
	Contains sufficient quantity for <n> test.		Catalog number
	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.
10. John D. Roback, MD et al. Technical Manual 17th Edition, Bethesda, MA: AABB, 2011.

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