

Blood Grouping Reagent

IH-Card ABD(DVI+)-Conf

A-B-D(DVI+) / A-B-D(DVI+)

English, B186354, 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: 71080

IH-Card ABD(DVI+)-Conf:

| | |
|------------|-------------------|
| VOL | 12 cards per box |
| VOL | 48 cards per box |
| VOL | 288 cards per box |

| | |
|------------|-------------|
| REF | 813 190 100 |
| REF | 813 191 100 |
| REF | 813 192 100 |

INTENDED USE

The IH-Card ABD(DVI+)-Conf is intended for the detection of A (ABO1), B (ABO2) and D (RH1) antigens on human red blood cells using the IH-System.

SUMMARY

Between 1900 and 1902, Karl Landsteiner and associates discovered the ABO system of red blood cell antigens. The importance of this discovery is the recognition that antibodies are present when the corresponding antigens are lacking. The ABO system is the only blood group system in which the reciprocal antibodies are consistently and predictably present in most people. ABO blood group typing, using Anti-A and Anti-B antisera to detect 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 one of many that comprise the Rh blood group system. Approximately 85% of random donors in Caucasian populations have inherited the D gene and will phenotype as D positive.

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.

The IH-Card ABD(DVI+)-Conf is suitable for the detection of ABO/ RhD antigens. 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.

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 ABD(DVI+)-Conf consists of six microtubes containing Anti-A, Anti-B, Anti-D (DVI+). Anti-A, Anti-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 | 157050F7 | Bio-Rad |
| Anti-B | Murine Monoclonal | IgG3 | X9 | Bio-Rad |
| Anti-D | Human Monoclonal | IgM | BS226/ESD1M | Bio-Rad/ Alba Bioscience Limited |

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 or donor blood collected in CPD or CP2D may be tested up to the expiration date of the unit when stored at 1 to 8 °C. Donor blood stored in additive solutions AS-1 or AS-3 may be tested up to thirty (30) days post collection when stored at 1 to 8 °C. Cord blood samples may be stored 2 to 8 °C up to five (5) days post collection. 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. Donor segments do not require centrifugation.

TEST PROCEDURE FOR AUTOMATED SYSTEMS

Material provided

- IH-Card ABD(DVI+)-Conf

Materials required but not provided

- IH-LISS Rack

- IH-1000

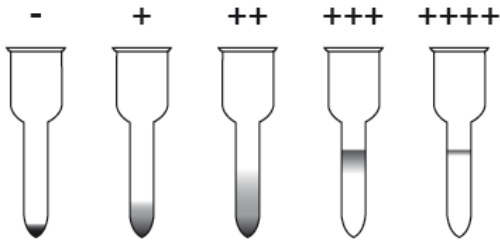
Method

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-D(DVI+) and their interpretation are shown in the following table:

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

• A control test to detect spontaneous agglutination is not essential in routine testing because the IH-System Monoclonal Blood Grouping Reagents do not contain ingredients that enhance spontaneous agglutination of immunoglobulin-coated red blood cells. In some circumstances, a false positive test result may 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 all blood grouping results for a given sample are positive, a control may be indicated. The IH-Card Control can be used for this purpose. 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.

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. 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 re-suspension 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 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.
- 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 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).
- The performance characteristics of this product with chemically modified, frozen/thawed or enzyme treated red blood cells have not been established.

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 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.

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-D(DVI+) | 672 | 99.40% (98.64%) | 3,169 | 100% (99.91%) |










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 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 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 |  | <i>In vitro</i> 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 |

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