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Blood Grouping Reagent
IH-Card RhD(DVI-) + Phenotype
D(DVI-)-C-E-¢-e-Ctl

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: 72090

IH-Card RhD(DVI-) + Phenotype:	[VOL] 12 cards per box	[REF] 813290100
	[VOL] 48 cards per box	[REF] 813291100
	[VOL] 288 cards per box	[REF] 813292100

INTENDED USE
The IH-Card RhD(DVI-) + Phenotype is intended for the detection of D (RH1), C (RH2), E (RH3), ¢ (RH4), and e (RH5) antigens on human red blood cells using the IH-System.

SUMMARY
Landsteiner and Wiener first described the Rhesus blood group system in 1940. More than 50 antigens belong to the Rhesus blood group system. The antigens C (RH2), E (RH3), ¢ (RH4), e (RH5) and D (RH1) are the principle antigens of the Rh system. Although many other antigens have been identified, the antibodies associated with these five antigens are responsible for the majority of hemolytic transfusion reactions and cases of Hemolytic Disease of the Newborn associated with the Rh system.

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 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 IH-Card RhD(DVI-) + Phenotype can be used for the detection of the D, C, E, ¢, and e antigens on human red blood cells. Most weak D antigen expressions will be detected as weak positive reactions with this reagent. However, the partial DVI epitope of the D antigen will not be detected with this monoclonal reagent.

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). 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 RhD(DVI-) + Phenotype consists of six microtubes containing Anti-D, Anti-C, Anti-E, Anti-¢, Anti-e, Ctl. This reagent contains bovine albumin.

Reagent	Source	Antibody Class	Cell lines	Manufacturer
Anti-D(DVI-)	Human Monoclonal	IgM	B9A4	Bio-Rad
Anti-C	Human Monoclonal	IgM	MS24	Millipore (UK) Limited
Anti-E	Human Monoclonal	IgM	DEM-1	Alba Bioscience Limited
Anti-¢	Human Monoclonal	IgM	MS33	Millipore (UK) Limited
Anti-e	Human Monoclonal	IgM	MS16/MS21/MS63	Millipore (UK) Limited
Ctl	Gel containing PVP diluent + preservative	-	-	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, have to 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.

On automated systems if testing is delayed, donor blood collected in CPD or CP2D may be tested up to 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 at 2 to 8°C up to five (5) days post collection for automated testing.

For manual testing, if testing is delayed, donor blood collected in CPD, CP2D and CPDA-1 and donor blood stored in additive solutions AS-1 or AS-3 may be tested up to expiration date indicated on the label of the unit when stored at 1 to 8°C. Cord blood samples may be stored at 2 to 8°C up to ten (10) days post collection for manual testing.

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. Donor segments do not require centrifugation.

TEST PROCEDURE FOR MANUAL AND AUTOMATED SYSTEMS

Material provided

- IH-Card RhD(DVI-) + Phenotype

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, IH-500 and IH-Com User Manual U.S. for testing and reagent handling instructions.

Method for manual testing

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

Prior to use prepare a red blood cell suspension of approximately 1% to be tested in IH-LISS Solution

- 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 shall be used as fast as possible within 24 hours.

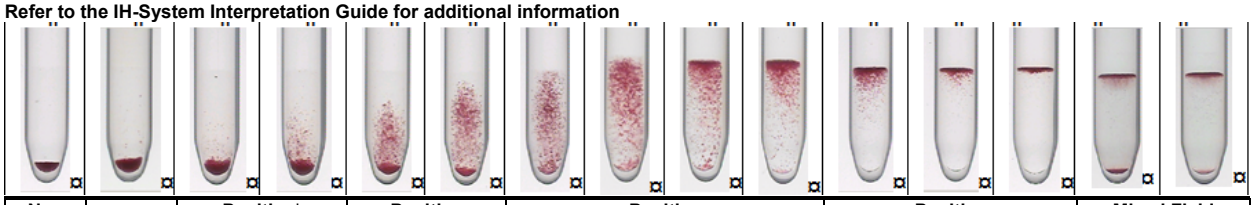
1. Allow reagents and samples to reach room temperature (18 to 25°C) before use.
2. Inspect the condition of the cards before use (see Warnings and Precautions)
3. Label the gel card appropriately.
4. Withdraw the entire foil seal from the card or from the individual microtubes to be used for testing. 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) or hemolysis (in case of serum test) with very few or no red blood cells in the gel column. 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 are 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



Neg (-)	(?)	Positive* (+/- and +)	Positive (++)	Positive (+++)	Positive (++++)	Mixed Field (DP)

* 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.
+	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 the reagents are shown in the following table:

Reagent	Well Reaction	Interpretation
Anti-D	positive	D+
Anti-D	negative	D-
Anti-C	positive	C+
Anti-C	negative	C-
Anti-¢	positive	¢+
Anti-¢	negative	¢-
Anti-E	positive	E+
Anti-E	negative	E-
Anti-e	positive	e+
Anti-e	negative	e-

- For automated and manual test method 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.

STABILITY OF REACTIONS

For visual reading of reactions, best results are obtained within six (6) 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. The Blood Grouping Reagents contained on this card could be controlled by testing rr (¢¢ee) and R1R2 (DC¢Ee) samples (heterozygous when available). Other combinations of samples are possible as long as there is a positive and negative control for each reagent (this does not apply to the control reagent). Each reagent is satisfactory for use if positive and negative samples react as expected. For additional information, please consult the IH-1000, 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.
- If red blood cells (pellet at the bottom of the microtube) are too low in concentration they become difficult to visualize, and, in certain cases, a weak positive reaction can fail to be detected.
- 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. Weak antigen expression may not be detected (e.g. R_z; RH32; C^x). Further investigations may be warranted per site specific procedures.
- Very weak expressions of the D antigen may not be detected. The DVI epitope of the antigen will not be detected with this reagent. No blood grouping reagent of monoclonal origin has yet been found that will detect all parts of the D antigen.If the detection ofweak D and partial D(VI) 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).
- The performance characteristics of these reagents have not been established with chemically modified, frozen/thawed or enzyme treated red blood cells.

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-D(DVI-), Anti-C, Anti-¢, Anti-E, and Anti-e 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-D(DVI-)	550	99.64% (98.86%)	2,944	99.86% (99.69%)
Anti-C	498	99.40% (98.45%)	1,010	100% (99.70%)
Anti-¢	302	99.67% (98.44%)	1,207	100% (99.75%)
Anti-E	1,078	99.72% (99.28%)	431	100% (99.31%)
Anti-e	94	97.87% (93.45%)	1,470	100% (99.80%)

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-D(DVI-), Anti-C, Anti-¢, Anti-E and Anti-e 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-D(DVI-), Anti-C, Anti-¢, Anti-E and Anti-e.

Performance characteristics using the IH-500

Testing to determine the performance characteristics of the Bio-Rad IH Blood Grouping Reagents Anti-D(DVI-), Anti-C, Anti-¢, Anti-E, and Anti-e 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-D(DVI-)	Random samples	343	100% (99.13%)	1,918	100% (99.84%)
Anti-D(DVI-)	Known RhD neg	257	100% (98.84%)	NA	NA
Anti-D(DVI-)	All samples	600	100% (99.50%)	1,918	100% (99.84%)
Anti-C	Random samples	581	100% (99.49%)	1,037	100% (99.71%)
Anti-C	Known ¢ Ag neg	NA	NA	105	100% (97.19%)
Anti-C	All samples	581	100% (99.49%)	1,142	100% (99.74%)
Anti-¢	Random samples	296	100% (98.99%)	1,322	100% (99.77%)
Anti-¢	Known ¢ Ag neg	104	100% (97.16%)	1[1]	100% (5.00%)
Anti-¢	All samples	400	100% (99.25%)	1,323	100% (99.77%)

Test	Sample type	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Anti-E	Random samples	1,245	100% (99.76%)	448	100% (99.33%)
Anti-E	Known e Ag neg	NA	NA	30	100% (90.50%)
Anti-E	All samples	1,245	100% (99.76%)	478	100% (99.38%)
Anti-e	Random samples	71	100% (95.87%)	1,622	100% (99.82%)
Anti-e	Known e Ag neg	30	100% (90.50%)	NA	NA
Anti-e	All samples	101	100% (97.08%)	1,622	100% (99.82%)

[1] One (1) sample enrolled in the study as known Anti-c negative was positive by both the investigational and reference method during study testing.
The sample was enrolled in the study based on historical testing performed on a sample from a previous donation. The negative result was not confirmed for the sample enrolled in the study.

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-D(DVI-), Anti-C, Anti-¢, Anti-E and Anti-e 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-D(VI-)	Random samples	185	100% (98.39%)	668	100% (99.55%)
Anti-D(VI-)	Known RhDneg	134	100% (97.79%)	NA	NA
Anti-D(VI-)	All samples	319	100% (99.07%)	668	100% (99.55%)
Anti-C	Random samples	561	100% (99.47%)	846	100% (99.65%)
Anti-¢	Random samples	176	100% (98.31%)	1101	100% (99.73%)
Anti-¢	Known ¢ neg	136	100% (97.82%)	NA	NA
Anti-¢	All samples	312	100% (99.04%)	1101	100% (99.73%)
Anti-E	Random samples	1044	100% (99.71%)	222	100% (98.66%)
Anti-E	Known E positive	NA	NA	142	100% (97.91%)
Anti-E	All samples	1044	100% (99.71%)	364	100% (99.18%)
Anti-e	Random samples	36	100% (92.02%)	1377	100% (99.78%)

NA = Not Applicable

Performance characteristics for manual testing
Testing to determine the performance characteristics of the Bio-Rad IH Blood Grouping Reagents Anti-C, Anti-¢ Anti-E, Anti-e and Anti-D 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.

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Anti-D(DVI-)	628	100% (99.52%)	1,901	100% (99.84%)
Anti-C	417	99.76% (98.87%)	837	100% (99.64%)
Anti-¢	316	99.68% (98.51%)	938	100% (99.68%)
Anti-E	882	99.43% (98.81%)	372	100% (99.20%)
Anti-e	50	100% (94.18%)	1,204	100% (99.75%)

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-D(DVI-), Anti-C, Anti-¢ Anti-E and Anti-e and Anti-D 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-D(DVI-), Anti-C, Anti-¢, Anti-E and Anti-e 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.

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Anti-D(DVI-)	624	99.84% (99.24%)	1,905	100% (99.84%)
Anti-C	369	99.46% (98.30%)	835	100% (99.65%)
Anti-¢	316	99.68% (98.51%)	888	100% (99.66%)
Anti-E	834	99.64% (99.07%)	370	100% (99.19%)
Anti-e	50	100% (94.18%)	1,154	100% (99.74%)

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-D(DVI-), Anti-C, Anti-¢, Anti-E and Anti-e 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.

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GLOSSARY OF SYMBOLS

Symbol	Definition	Symbol	Definition
[LOT]	Batch Code	[IVD]	*In vitro* diagnostic medical device
!	Consult the instructions for use for important cautionary information such as warnings and precautions	I	Consult instructions for use
M	Manufacturer	e	Use by YYYY-MM-DD
s	Contains sufficient quantity for <n> tests	[REF]	Catalog number
t	Temperature limitation	[VOL]	Volume

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Key: **Underline** = Addition of changes ◄ = Deletion of text