Anti-Human Globulin Anti-IgG,-C3d; Polyspecific
IH-Card AHG Anti-IgG,-C3d
(Rabbit / Murine Monoclonal)(Green)

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: 74010

IH-Card AHG Anti-IgG,-C3d:	[VOL] 12 cards per box	[REF] 813410100
	[VOL] 48 cards per box	[REF] 813411100
	[VOL] 288 cards per box	[REF] 813412100

INTENDED USE
The IH-Card AHG Anti-IgG,-C3d is intended for the detection of antibodies and complement on human red blood cells using the Direct and Indirect Antiglobulin Tests.

SUMMARY
Moreschi first described the use of Anti-Human Globulin in 1908. [1] Coombs rediscovered the test in 1945.[2,3] By injecting rabbits with human IgG, they were able to produce a protein (Anti-IgG) that reacted with "incomplete" antibodies (IgG). Most "incomplete" antibodies (IgG) fail to agglutinate red blood cells suspended in saline.[4] Most clinically significant antibodies in red blood cell serology are of the IgG class and can only be detected by the use of Anti-IgG.

The IH-Card AHG Anti-IgG,-C3d is suitable for the Direct and Indirect Antiglobulin Tests. The Direct Antiglobulin Test allows the detection of *in vivo* sensitization of human red blood cells with immunoglobulins and/or complement components. The Indirect Antiglobulin Tests allows the detection of *in vitro* sensitization of human red blood cells with clinically significant antibodies and/or complement components. The Indirect Antiglobulin Test may be used for antibody detection, identification and IAT crossmatching. An optional autocontrol may help to distinguish autoantibodies and alloantibodies.

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 AHG Anti-IgG,-C3d consists of six microtubes containing a gel impregnated with polyspecific Anti-Human Globulin (AHG) containing a blend of rabbit anti-IgG and murine monoclonal anti-complement. The anti-IgG component contains antibody reactivity against 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 a phosphate buffered saline solution containing bovine albumin, absorbed to remove heterospecific antibodies and contains a mixture of colorants Patent Blue and Tartrazin.
This reagent contains bovine albumin.

Reagent	Source	Antibody Class	Cell line	Manufacturer
Anti-C3d	Murine Monoclonal	IgG	053A-714	Bio-Rad
Anti-IgG	Rabbit	Polyclonal	-	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. Depending on the test profile, individual wells of this card can be used by carefully peeling off the aluminum foil from the individual microtubes.

STORAGE REQUIREMENTS
- Store at 18 to 25°C.
- Do not use beyond expiration date 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 reagents beyond their expiration date.
- 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.
- Caution: This product is derived from animal source material and was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from animals will not transmit infectious agents.
- 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. Do not use grossly hemolyzed, lipemic or icteric samples.

Samples should be centrifuged for 10 minutes at 2000g or at a time and speed that consistently produces cell-free plasma. Frozen and thawed plasma and serum samples should be centrifuged for 10 minutes at 1500g or at a time and speed sufficient to remove particulate matter. Donor segments do not require centrifugation.

Detection and Identification of Unexpected Antibodies including Autocontrol
Fresh EDTA, ACD, CPDA and serum samples are acceptable; however serum separator tubes may not be used.
For Autocontrol all sample types are acceptable when testing manually. For automated testing only samples with anticoagulants are acceptable for autocontrol. Samples should be tested as soon as possible after collection. If testing is delayed, samples may be stored at 2 to 8 °C for up to ten (10) days post collection. Frozen samples can be used within the instrument when plasma and serum is separated from the red blood cells and stored frozen (at -20 °C or colder). In clinical studies, samples collected in sodium citrate were tested after storage at -20 °C for up to 674 days, samples collected in EDTA were tested after 1 month at -20 ˚C, and serum samples were tested after 26 days at -20 ˚C. 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.

DAT
Fresh blood samples collected in anticoagulant are acceptable. Samples should be tested as soon as possible post collection. If testing is delayed, EDTA samples and cord blood 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. However, general guidelines for DAT testing recommend testing within 48 hours.

Crossmatching
Donor cells
Fresh blood samples collected in anticoagulant are acceptable. Samples should be tested as soon as possible post collection.

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.

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.

Recipient's sample
Fresh EDTA samples are acceptable; however serum separator tubes may not be used. Samples should be tested as soon as possible after collection. If testing is delayed, samples may be stored at 2 to 8 °C for up to ten (10) days post collection.

TEST PROCEDURE FOR MANUAL AND AUTOMATED SYSTEMS

Material provided
- IH-Card AHG Anti-IgG,-C3d

Materials required but not provided
- Reagent Red Blood Cells (IH-Cells or IH-Panels) or red blood cells
- IH-LISS Rack or IH-LISS Solution (when using red blood cells other than IH-Cells or IH-Panels)
- Dispenser pipette capable of delivering 1.0 mL
- Pipettes: 10 µL, 25 µL, 50 µL and 1 mL
- Disposable pipette tips
- Glass or plastic test tubes
- IH-Incubator L for manual working
- 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
For Indirect Antiglobulin Test (antibody detection and identification, crossmatch and weak D assays) and the Direct Antiglobulin Test, refer to the IH-1000 or IH-500 User Manual U.S. 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. and IH-Incubator L User Manual U.S. for equipment operating instructions.

Direct Antiglobulin Test (DAT)
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 should be used as soon 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. Add 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.

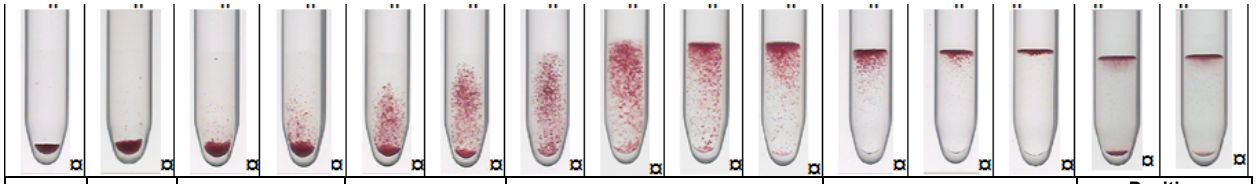
Indirect Antiglobulin Test (IAT)
1. Allow reagents and samples to reach room temperature (18 to 25°C) before use.
2. Inspect the condition of the cards before use (see Warnings and Precautions)
3. Label the gel card appropriately.
4. Withdraw the entire foil seal from the card or from the individual 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. Add 50 µL of test cells into the appropriate well of the microtubes
   *Note: **If not using** IH-Cells, a cell suspension of* approximately *1% must be prepared with 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)*
   Note: Red blood cell suspension should be used as soon as possible within 24 hours.
7. Add 25 µL of plasma or serum into the appropriate wells of microtubes
   Note: Carefully dispense the red blood cell suspension and the serum or plasma, avoiding contact of the pipette tip with the contents of the microtubes to prevent carryover. After pipetting an air gap between the supernatant of the microtubes and the red blood cells / serum and or plasma should be visible.
8. Incubate for 15 to 20 minutes in the IH-Incubator L (with pre-set temperature).
9. Centrifuge in the IH-Centrifuge L or IH-Reader 24 at the pre-set conditions as determined by the manufacturer.
10. 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 (++++)	Positive Mixed Field (DP)

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.

Well Reaction Grade	Result Interpretation	Reaction Description
++++	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

- When recording the reactions, ensure that the lot number of the Antigen Profile corresponds with the lot number of the Reagent Red Blood Cells used for testing.
- Identification of the antibody present in the serum or plasma may be made by matching the reactions obtained with the Antigen Profile furnished with the reagent. If the antibody specificity is not evident, testing with additional cells may be required.
- In case of complete or partial hemolysis (pinkish supernatant and/or gel) in microtubes, the interpretation should be positive if there is no problem of cell collection and/or handling of the sample.

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 antiglobulin reagents should be confirmed by testing with known positive and negative samples. The IH-Card AHG Anti-IgG,-C3d is satisfactory for use if negative and positive samples react as expected.

LIMITATIONS

- Erroneous and abnormal results may be caused by:
  - Bacterial or chemical contamination of the serum, plasma, red blood cells 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 or Reagent Red Blood Cell hemolysis prior to testing.
  - Contamination between microtubes through pipetting errors.
- Use of procedure other than the one described above.
- Grossly icteric, hemolytic or lipemic 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 or questionable results. Icteric blood samples may cause difficulty in interpretation and test results should be used with caution.
- 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.
- The performance characteristics of this reagent have not been established with chemically modified, frozen/thawed or enzyme treated red blood cells.
- Negative Direct Antiglobulin Test results do not necessarily rule out hemolytic disease of the fetus or newborn (HDFN), especially if ABO incompatibility is suspected as the cause.
- 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.
- Test results obtained in the Indirect Antiglobulin Test should be carefully evaluated when patient or donor IgG-coated red blood cells are used.
- Rare antibodies, notably anti-Jk^{a} or anti-Jk^{b}, may be detected only when polyspecific Anti-Human Globulin is used and when active complement is present.
- Negative reaction will be obtained if red blood cells used for testing contains antigens which shows only a weak expression or have only a partial expression due of an antigen variant.
- Negative reactions will be obtained if the sample contains antibodies present in concentrations too low to be detected by the test method employed. No test method is capable of detecting all red cell antibodies.
- Some conditions that may cause false positive results are:
  - Contamination of sample or reagents
  - Autoantibodies
  - Improper storage or preparation of red blood cells
  - Antibodies to antibiotics or other reagents
  - Cold antibodies

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 Reagent is tested against antigen positive and negative samples to ensure suitable reactivity and specificity.

Performance characteristics using the IH-1000

A multi-center clinical trial, which included testing at four different US clinical sites and an internal site, was conducted to evaluate the performance of IH-Card AHG Anti-IgG,-C3d. The clinical trial included testing of patient and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH-Card AHG Anti-IgG,-C3d in comparison to the FDA licensed reference reagents. Additional internal studies have been performed with well-characterized and/or contrived samples to evaluate the performance of IH-Card AHG Anti-IgG,-C3d when tested on the IH-1000.

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 data 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 Trials

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Antibody Detection	3,821	98.46% (98.09%)	153	95.42% (91.58%)
Antibody Identification	247	91.09% (87.53%)	48	97.92% (90.49%)
DAT	585	97.61% (96.28%)	65	89.23% (80.72%)
IAT Crossmatch	422	95.73% (93.74%)	198	92.42% (88.57%)

Results from In-House Study 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)
Antibody Detection	Not Tested	NA	191	100% (98.44%)
Antibody Identification	425	98.35% (96.93%)	128	100% (97.69%)
DAT	Not Tested	NA	69	100% (95.75%)
IAT Crossmatch	301	100% (99.01%)	299	100% (99.00%)

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 IH-Card AHG Anti-IgG,-C3d 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 IH-Card AHG Anti-IgG,-C3d.

Performance characteristics using the IH-500
A multi-center clinical trial, which included testing at three different US clinical sites and an internal site, was conducted to evaluate the performance of IH-Card AHG Anti-IgG,-C3d using IH-500 v.2.1.14. The clinical trial included testing of patient and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH-Card AHG Anti-IgG,-C3d in comparison to the FDA licensed reference reagents. Additional internal studies have been performed with well-characterized samples to evaluate the performance of IH-Card AHG Anti-IgG,-C3d when tested using IH-500.

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)
Antibody Detection	Random samples	863	99.30% (98.63%)	87	94.25% (88.30%)
Antibody Detection	Known Ab pos	5[1]	80.00% (34.26%)	265	99.25% (97.64%)
Antibody Detection	All samples	868	99.19% (98.49%)	352	98.01% (96.30%)
Antibody Identification	Known Ab pos	NA	NA	233	99.57% (97.98%)
DAT	Random samples	440	100% (99.32%)	10	30.00% (8.73%)
DAT	Known DAT pos	NA	NA	101	100% (97.08%)
DAT	All samples	440	100% (99.32%)	111	93.69% (88.48%)
IAT Crossmatch	All samples	468	99.36% (98.35%)	462	98.70% (97.45%)

NA = Not Applicable
1) Four (4) samples enrolled in the study as known antibody positive were negative by both the investigational and reference method during study testing. Historical results were used to determine the antibody status in the inventory samples and were not repeated prior to enrollment in the study. It was unknown if the titer of the antivbody had dropped and if the antibody was still detectable after thawing and enrollment in this study.

Results from In-House Studywith well-characterized samples 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)
Antibody Detection	Random samples	Not tested	NA	Not tested	NA
Antibody Detection	Known Ab pos	Not tested	NA	Not tested	NA
Antibody Detection	All samples	Not tested	NA	Not tested	NA
Antibody Identification	Known Ab pos	Not tested	NA	60	100% (95.13%)
DAT	Random samples	Not tested	NA	Not tested	NA
DAT	Known DAT pos	Not tested	NA	Not tested	NA
DAT	All samples	Not tested	NA	Not tested	NA
IAT Crossmatch	All samples	300	100% (99.01%)	314	100% (99.05%)

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 three external sites by testing a reproducibility panel according to the following scheme: one lot of reagent x 3 sites x 1 operators x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days. Reproducibility for the IH-Card AHG Anti-IgG,-C3d using the IH-500 was demonstrated within runs, 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 or contrived 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)
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
A multi-center clinical trial, which included testing at five different US clinical sites and an internal site, was conducted to evaluate the performance of IH-Card AHG Anti-IgG,-C3d. The clinical trial included testing of patient and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH-Card AHG Anti-IgG,-C3d in comparison to the FDA licensed reference reagents. Additional internal studies have been performed with well-characterized samples to evaluate the performance of IH-Card AHG Anti-IgG when tested manually using IH-Centrifuge L and IH-Incubator L.

The clinical trial results for 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 Clinical Trials

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Antibody Detection	1,290	98.14% (97.39%)	378	99.47% (98,34%)
Antibody Identification	633	98.58% (97.53%)	258	95.35% (92.57%)
DAT	245	99.18% (97.45%)	143	100% (97.93%)
IAT Crossmatch	235	100% (98.73%)	308	98.05% (96.19%)

Results from In-House Studywith well-characterized samples

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Antibody Detection	Not Tested	NA	Not Tested	NA
Antibody Identification	Not Tested	NA	61	100% (95.21%)
DAT	Not Tested	NA	Not Tested	NA
IAT Crossmatch	344	100% (99.13%)	320	100% (99.07%)

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 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 IH-Card AHG Anti-IgG,-C3d using the IH-Centrifuge L and IH-Incubator L was demonstrated within runs, between runs and between sites.

Performance characteristics using the IH-Reader 24

A multi-center clinical trial, which included testing at five different US clinical sites and an internal site, was conducted to evaluate the performance of IH-Card AHG Anti-IgG,-C3d. The clinical trial included testing of patient and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH-Card AHG Anti-IgG,-C3d in comparison to the FDA licensed reference reagents. Additional internal studies have been performed with well-characterized samples to evaluate the performance of IH-Card AHG Anti-IgG when tested manually using IH-Reader 24.

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)
Antibody Detection	1,183	96.79% (95.81%)	338	98.82% (97.31%)
Antibody Identification	527	97.72% (96.34%)	188	95.74% (92.45%)
DAT	245	97.96% (95.76%)	165	100% (98.20%)
IAT Crossmatch	264	98.86% (97.09%)	126	97.62% (93.96%)

Results from In-House Studywith well-characterized samples

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
Antibody Detection	60	100% (95.13%)	60	100% (95.13%)
Antibody Identification	Not Tested	NA	60	100% (95.13%)
DAT	Not Tested	NA	Not Tested	NA
IAT Crossmatch	300	100% (99.01%)	300	100% (99.01%)

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 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 IH-Card AHG Anti-IgG,-C3d using the IH-Reader 24 was demonstrated within runs, 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]	*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