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

English, B186358, Version 07, 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: 74010

IH-Card AHG Anti-IgG,-C3d:

VOL 12 cards per box..... REF 813 410 100
VOL 48 cards per box..... REF 813 411 100
VOL 288cards per box..... REF 813 412 100

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 or plasma/serum) 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 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 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 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 a phosphat 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.

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.
• 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.
• 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.

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, citrated and serum samples are acceptable; however serum separator tubes may not be used. For Autocontrol only samples with anticoagulants are acceptable for automated testing.
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.

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 five (5) days. 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. 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.

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 AUTOMATED SYSTEMS

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

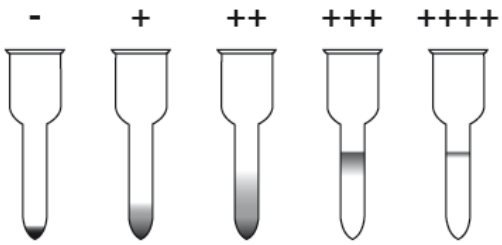
Materials required but not provided
• Reagent Red Blood Cells (IH-Cells or IH-Panels) or red blood cells
• IH-LISS Rack (when using cells other than IH-Cells or IH-Panels)

• **IH**-1000

Method
For Indirect Antiglobulin Test (antibody detection and identification and crossmatch assays) and the Direct Antiglobulin Test, 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 softward 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	Ambiguous result.

	Testing = Not interpretable For Crossmatching = Incompatible	

* RBCs = Red Blood Cells

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 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.
• Fibrins, clots, particulates or other artifacts may cause some red blood cells to be trapped at the top of the gel that may cause an anomalous result.
• 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
• 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**-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 Reagent is tested against antigen positive and negative samples to ensure suitable reactivity and specificity.

Performance characteristics on the IH-1000 Analyzer

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 NA and **IH**-COM User Manual NA for more information on verification of results.

Test	Results from Clinical Trials				Results from In-House Study 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)
Antibody Detection	3,821	98.77% (98.43%)	153	95.42% (91.58%)	Not Tested	NA	191	100% (98.44%)
Antibody Identification	247	91.50% (87.99%)	48	97.92% (90.49%)	425	98.35% (96.93%)	128	100% (97.69%)
DAT	585	97.61% (96.28%)	65	89.23% (80.72%)	Not Tested	NA	69	100% (95.75%)
IAT Crossmatch	422	95.73% (93.74%)	198	92.42% (88.57%)	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.

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

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