Anti-Human Globulin Anti-IgG
IH-Card AHG Anti-IgG
(Rabbit)(Green)

English, B186359, 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: 74020

IH-Card AHG Anti-IgG:	VOL 12 cards per box..... REF 813 420 100
	VOL 48 cards per box..... REF 813 421 100
	VOL 288cards per box..... REF 813 422 100

INTENDED USE

The IH-Card AHG Anti-IgG is intended for the detection of antibodies 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 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. The Indirect Antiglobulin Tests allows the detection of *in vitro* sensitization of human red blood cells with clinically significant antibodies. The Indirect Antiglobulin Test may be used for antibody detection, identification, IAT crossmatching , and D variant testing. 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 the description of the reaction intensity, please refer to the Reaction Grading Guide in the Interpretation of Results section.

REAGENT

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 consists of six microtubes containing a gel impregnated with rabbit polyclonal antihuman globulin AHG IgG that does not contain antibodies to complement components. The Anti-IgG is light chain specific (sera from hyperimmunised rabbits) and thus may also agglutinate IgA or IgM coated red blood cells. The Anti-IgG is diluted in a phospate 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.

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 build up 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 accepted 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.

Anti-D Testing with IH-Anti-D (RH1) Blend

Fresh blood samples collected in anticoagulants 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 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-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 for up to five (5) days post collection.

TEST PROCEDURE FOR AUTOMATED SYSTEMS

Material provided

• IH-Card AHG Anti-IgG

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-Anti-D (RH1) Blend

• **IH**-1000

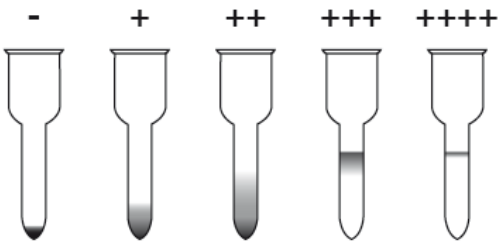
Method

For Indirect Antiglobulin Test (antibody detection and identification, crossmatch and weak D assays) and the Direct Antiglobulin Test, refer to the I**H**-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

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 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 re-suspension 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.
• Fibrin, 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.
• 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 performance characteristics of this reagent have not been established with chemically modified, frozen/thawed or enzyme treated red blood cells with the exception of IH-Anti-D Blend. Frozen/thawed red blood cells may be used with the IH-Anti-D Blend reagent in conjunction with IH-Card AHG Anti-IgG.
• Test results obtained in the Indirect Antiglobulin Test should be carefully evaluated when patient or donor IgG-coated red blood cells are used.
• 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.
• 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.

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. 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 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 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	4,599	98.43% (98.10%)	166	93.98% (90.00%)	Not Tested	NA	192	100% (98.45%)
Antibody Identification	432	90.74% (88.12%)	321	95.02% (92.53%)	709	97.74% (96.59%)	126	100% (97.65%)
DAT	586	97.10% (95.68%)	58	96.55% (89.54%)	Not Tested	NA	67	100% (95.63%)
IAT Crossmatch	467	91.86% (89.47%)	152	98.68% (95.92%)	301	100% (99.01%)	Not Tested	NA

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 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.

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

GLOSSARY OF SYMBOLS

Symbol	Definition	Symbol	Definition
	Batch code		*In vitro* diagnostic medical device
	Caution, consult accompanying documents		Consult instructions for use
	Manufacturer		use by (YYYY-MM-DD)
	Contains sufficient quantity for <n> test.		Catalog number
	Temperature limitation	VOL	Volume

BIBLIOGRAPHY

1. Moreschi C. Neue Tatsache über die Blutkörperchen Agglutinationen. Zbl Bakt. 1908; 46:49,456.
2. Coombs RRA, Mourant AE and Race RR. A new test for the detection of weak and ˝incomplete˝ Rh agglutinins. Br J Exp Pathol. 1945; 26:255.
3. Coombs RRA, Mourant AE and Race RR. In vivo isosensitization of red blood cells in babies with hemolytic disease. Lancet. 1946; i:264-266.
4. Löw B, Messeter L. Antiglobulin test in low-ionic strength salt solution for rapid antibody screening and cross-matching. Vox Sang. 1974; 26:53-61.
5. Kankura T, Kurashina S, Nakao M. A gel filtration technique for separation of erythrocytes from human blood. J Lab Clin Med. 1974; 83:840-844.
6. Rouger Ph, Salmon Ch. La pratique de l'agglutination des érythrocytes et du test de Coombs. Masson. 1981.
7. ISBT/ICSH Working Party. International Reference Polyspecific AntiHuman Globulin Reagents. Engelfriet CP, Voak D. Vox Sang.1987; 53:241-247.
8. Engelfriet CP, Overbeeke MAM, Voak D. The antiglobulin test (Coombs test) and the red cell . In Cash, Progress in Transfusion Medecine. Churchill Livingstone. 1987; vol2:74-98.
9. Voak D. Coordinated report of studies on monoclonal antibodies to complement. Rev Fr Transf Immunohematolol. 1988; XXXI:367-376.
10. Voak D, Downie DN. Serological characterisation of monoclonal antibodies to complement components of C3 and C4. Rev Fr Transf Immunohematol. 1988; XXXI:377-380.
11. Lapierre Y, Rigal D, Adam J et al. The gel test : a new way to detect red cell antigen-antibody reactions. Transfusion. 1990; 30:109-113.
12. Proceedings of the second international workshop and symposium on monoclonal antibodies against human red blood cells and related antigens.IgG/Complement. Lund. 1990; 195-206.
13. Bromilov IM, Adams KE, Hope J, Eggington JA and Duguid JKM. Evaluation of the ID-gel test for antibody screening and identification. Transfusion Medecine. 1991; 1:159-161.
14. Salmon Ch, Cartron JP, Rouger Ph. Les groupes sanguins chez l'homme. 2e ed. Masson. 1991.
15. Pottier C, Quillet P, Baufine-Ducroq H. Gel-test : Interpretation and value of a new technique for the detection of irregular antibodies. Ann Bio Clin. 1992; 50:679-685.
16. Deffune E, Le Pennec PY, Lascaux JM, Rouger Ph. Méthode d'étude des réactifs anti-complément : utilisation d'hématies sensibilisées congelées/décongelées. Rev Fr Transfus Hemobiol. 1992; 35:299-309
17. Burin des Rosiers N, Nasr O. Recherche des anticorps irréguliers érythrocytaires par la méthode du geltest.Analyse de 35882 échantillons. Rev Fr Transfus Hemobiol. 1993; 36:391-399
18. International Forum. What is the best technique for the detection of red cell alloantibodies. Vox Sang. 1995;69:292-300.
19. Issitt PD. Applied Blood Group Serology. 4th ed. Miami: Montgomery Scientific Publications, 1998.
20. John D, Roback MD et al. Technical Manual 17th Edition, Bethesda, MA: AABB, 2011.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