Summary Basis for Regulatory Action

From: Ricardo Espinola, Chair of the Review Committee

BLA/ STN#: Anti-Human Globulin (Formulated for Automated Testing) STN125098/88

Applicant Name: Bio-Rad Medical Diagnostics GmbH License No. 1845

Date of Submission: February 27, 2014

MDUFA Goal Date: October 21, 2016

Proprietary Name: IH-Card AHG Anti-IgG

Established Name (common or usual name): Anti-Human Globulin (Formulated for Automated Testing).

Intended Use: The Anti-Human Globulin reagent is intended to be used as a component to manufacture the IH gel card products. The final in-vitro products (IH-Cards) that contain Anti-IgG are intended to be used on the IH-1000 analyzer for the detection and identification of clinical relevant antibodies, cross matching, and Direct Antiglobulin Testing (DAT), based on the principles of agglutination and gel filtration.

Recommended Action: Approval

Signatory Authorities Action:

Offices Signatory Authority: Jay Epstein, MD, Director, Office of Blood Research and Review

☐ **I concur with the summary review.**

☐ **I concur with the summary review and include a separate review to add further analysis.**

☐ **I do not concur with the summary review and include a separate review.**

Offices Signatory Authority: Mary Malarkey, Director, Office of Compliance and Biologics Quality

☐ **I concur with the summary review.**

☐ **I concur with the summary review and include a separate review to add further analysis.**

☐ **I do not concur with the summary review and include a separate review.**

Material Reviewed/ Consulted - Specific documentation used in developing the SBRA	Reviewer Name – Document(s) Date
Clinical Review	Joyce Rockwell November 10, 2015 Ricardo Espinola August 08, 2016
Statistical Review	Zhen Jiang, PhD November 26, 2014 July 14, 2015 October 30, 2015 August 30, 2016
CMC Facility Review	Chad Burger December 17, 2014 November 13, 2015
CMC Product Review	Joyce Rockwell June 23, 2014, August 21, 2014, November 13, 2014 Ricardo Espinola August 08, 2016
Labeling	Dana Jones August 26, 2014
Bioresearch Monitoring Review	Bioresearch monitoring inspections were not conducted for this BLA and Efficacy Supplement
Establishment Inspection Report	Chad Burger October 27, 2015
Lot Release Protocols / Testing Plans	Karen Campbell (DBSQC – OCBQ) October 28, 2015 (Laboratory Quality Product Testing Plan) Karen Campbell (DBSQC – OCBQ) November 17, 2015 (Lot Release Protocol Templates)
Advisory Committee Transcript	Not applicable
Other (list)	Not applicable

1. Introduction

Bio-Rad Medical Diagnostics GmbH (BMD), located in Dreieich, Germany (Establishment Registration Number 9610824) submitted to the FDA, 17 applications to obtain approval for an automated immunohematology test system called the IH-System. The submissions consisted of:

- Three Biologics License Applications (BLAs): one Anti-Human Globulin and two Blood Grouping Reagents (BGRs).
- Ten Efficacy Supplements: one Anti-Human Globulin, eight BGRs, and one Reagent Red Blood Cells (RRBCs).

- Four 510(k) premarket notifications for the analyzer, software, control and neutral card.

The following is a list of all submissions associated with the IH-System:

- BMD - BLAs and Efficacy Supplements:
    - Anti-Human Globulin (Rabbit/Murine Monoclonal)(Formulated for Automated Testing), STN 125529/0
    - Anti-Human Globulin (Formulated for Automated Testing) STN 125098/88
    - Blood Grouping Reagent, Anti-B (Murine Monoclonal)(IgG)(Formulated for Automated Testing), STN 125532/0
    - Blood Grouping Reagent, Anti-D (Monoclonal Blend)(Formulated for Automated Testing), STN 125533/0
    - Blood Grouping Reagent, Anti-A (Murine Monoclonal)(Formulated for Automated Testing), STN 125094/113
    - Blood Grouping Reagent, Anti-A,B (Murine Monoclonal)(Formulated for Automated Testing), STN 125096/58
    - Blood Grouping Reagent, Anti-D (Monoclonal)(IgM)(Formulated for Automated Testing), STN 125097/67
    - Blood Grouping Reagent, Anti-E (Monoclonal)(Formulated for Automated Testing), STN 125202/50
    - Blood Grouping Reagent, Anti-e (Monoclonal)(Formulated for Automated Testing), STN 125203/48
    - Blood Grouping Reagent, Anti-K (Monoclonal)(Formulated for Automated Testing), STN 125204/46
    - Blood Grouping Reagent, Anti-c (Monoclonal)(Formulated for Automated Testing), STN 125205/46
    - Blood Grouping Reagent, Anti-C (Monoclonal)(Formulated for Automated Testing), STN 125206/48
    - Reagent Red Blood Cells For Use in Automated Systems, STN 125208/70

- BMD - Companion 510(k) submissions:
    - BK140106 IH-1000 Analyzer System
    - BK140107 IH-COM (data management software)
    - BK140138 IH-Card Neutral
    - BK140139 IH-Card Control

- (b) (4)

The above submissions were grouped as follows: one group containing Anti-Human Globulin reagents, one group containing 10 Blood Grouping Reagents, one group containing eight Reagent Red Blood Cells, and one group containing four 510(k) premarket notifications.

- (b) (4)

Millipore was approved by the FDA on August 14, 2008 to supplement their license to supply the following FFMUs to Biotest Medical Diagnostic GmbH, U.S. License No. 1798 under a shared manufacturing arrangement: Anti-C (clone MS-24), STN 103858/5046, Anti-c (Clone MS-33), STN 103860/5048, Anti-K (clone MS-56), STN 103864/5045 and Anti-e (clones MS-16/MS-21/MS-63), STN103866/5055/5056/5057. BMD U.S. License No. 1845 subsequently acquired portions of Biotest and acquired ownership of these FFMU products.

The IH-System performs ABO grouping, red blood cell antigen typing, detection and identification of clinically significant red blood cell antibodies, crossmatching, and direct antiglobulin testing, based on the principles of agglutination and gel filtration.
It generates results from individual images that must be verified by visual inspection by a qualified operator.

The IH-System consists of:

- IH-Card: a plastic card, consisting of six microtubes containing the active component, i.e., Blood Grouping Reagent or Anti-Human Globulin, in a buffered (b) (4) gel suspension.
- IH-Anti-D Blend: vialed Anti-D reagent for performing weak D and DVI testing using the IH-AHG Anti-IgG card.
- IH-Cell products: vialed Reagent Red Blood Cells (i.e., reverse grouping cells, screening cells, pool cells, and identification panel cells).
- IH-1000 Automated Analyzer System: an automated, blood grouping and antibody test system analyzer for the IH-Cards.
- IH-COM: stand-alone software to be used for data management, and the evaluation and interpretation of assay results. The software is directly linked to the IH-1000 via a bidirectional interface and can be interfaced with the customer's Laboratory Information System (LIS).
- IH-Card Neutral: a plastic card, consisting of six microtubes containing (b) (4) containing suspension medium, potentiating and preservative medium used for the detection of ABO antibodies during the reverse grouping. (Note: The neutral gel is also contained in single microtubes of certain IH-Cards containing Blood Grouping Reagents).
- IH-Card Control: a plastic card, consisting of six microtubes containing (b) (4) containing buffer, diluent medium, and preservative, and is intended for use as a supplemental control for IH-Cards with monoclonal Blood Grouping Reagent without a control well.
- IH-LISS Rack (Class II Exempt from premarket notification procedures): consists of 10 plastic cards, each with six microtubes, filled with a suspending medium, i.e., modified Low Ionic Strength Solution. The IH-LISS is used for preparing red blood cell suspensions for use with the appropriate IH-Card.

The IH-System is not a first of its kind device. Other manufacturers have been approved/cleared to market manual and automated immunohematology test systems using the column agglutination technique first described by Yves Lapierre in 1985 for the detection of red blood cell agglutination.

2. Background

Meetings with FDA

FDA held a pre-submission (CRMTS # 8105, PTS PS001492) meeting with BMD on October 6, 2011. The discussion items included performance studies design, statistical analysis and data reporting, instrument changes, and submission strategy. The meeting package indicated that the future submissions would include both manual and automated testing methods and instrumentation. Prior to submitting the respective submissions to CBER in February 2014, BMD decided to only submit information and data for automated testing using the IH-1000 Automated Analyzer System.

Marketing History

The design of the IH-Cards is based on the technology transfer from two commercially distributed products manufactured by DiaMed Ltd. and Bio-Rad Laboratories, Inc. The plastic card is used by DiaMed Ltd. (Morat, Switzerland) for the DiaMed ID-Micro Typing System that was introduced to non-US markets in 1988 and is still manufactured and marketed by Bio-Rad in Switzerland to non-US markets. The gel was used by Bio-Rad Laboratories, Inc in (b) (4) for manufacturing the ScanGel® Cards distributed to non-US markets from the late 1990's to 2014.

In 2005, FDA approved the same Anti-IgG (Rabbit) in vitro substance that intended to be used as the active component for the IH-Card for the manufacture of Anti-IgG (Rabbit) (Formulated for Automated Testing) used with BMD's 510(k) cleared TANGO® instrument. However, for the IH-Card AHG Anti-IgG, the formulation of the in vitro substance is different than what was approved in 2005.

Device Description and Function

The IH-System is an immunohematology test system that consists of an analyzer (IH-1000), software (IH-COM), Anti-Human Globulin (IH-Cards AHG), and supplemental reagents (FDA licensed or cleared) for automated testing. The test principle is based on gel filtration and column agglutination. In gel filtration technique, the gel in the microtube acts as a sieve; after centrifugation of the card, non- agglutinated RBCs settle at the bottom of the microtube while the agglutinated cells are dispersed throughout the gel depending on their size.

The IH-Card is a plastic card composed of six microtubes. Each microtube has an incubation chamber at the top of a long and narrow microtube. Each microtube is filled with a mixture of (b) (4) gel, buffer, and a specific antibody i.e., polyclonal anti-IgG. The agglutination occurs when the red blood cells sensitized *in vivo* or *in vitro* by human IgG antibodies comes in contact with the anti-IgG, present in the gel solution. The gel column acts as a sieve that traps agglutinated cells as they pass through the column during the centrifugation of the card. The gel

separates agglutinated red blood cells based on size. Non-agglutinated red blood cells form a pellet at the bottom of the microtube.

IH-Card Presentation – Anti-IgG is contained in each microtube:

Card Name	Microtube Contents					
	1	2	3	4	5	6
IH-Card AHG Anti-IgG	Anti-IgG	Anti-IgG	Anti-IgG	Anti-IgG	Anti-IgG	Anti-IgG

The IH-1000 is a fully automated high throughput analyzer for gel card technology, which allows performing different assays. The gel cards, reagents, and samples are automatically identified by being placed on the analyzer. Sample pipetting, reagent pipetting and incubation of reaction, if applicable, are all performed automatically without interaction from the operator. Reactions in the gel microtube are captured by the camera and analyzed by the image evaluation software for grading. The evaluated images are transferred to the IH-COM external data management software for further interpretation and generation of results. Every individual result that is generated from the instrument is reviewed; validated and equivocal results are edited (changed) as needed.

The various assays performed by the IH-System provide test results for blood collection establishments, transfusion services, and hospitals for managing donors and patients.

Chronology
CBER received the Anti-Human Globulin BLA on March 7, 2014. CBER issued a Filing with No Deficiencies Letter on May 1, 2014. CBER subsequently received thirty amendments in response to fifteen requests for STN 125098/88. A Complete Response Letter was issued on December 31, 2014. A final amendment dated June 30, 2016 completed BMD's responses to all outstanding issues associated with the Anti-Human Globulin BLA.

3. Chemistry Manufacturing and Controls (CMC)

All manufacturing is carried out in a controlled environment.

a) Manufacturing Summary

Manufacturing of the IH-Card AHG Anti-IgG consists of four main manufacturing stages: production of the Anti-IgG components, (b) (4) , preparation of the gel (b) (4) gel, and filling/packaging of the final product. The manufacturing process for the IH-Cards uses the same methodologies, no matter what the antibody type (IgG or IgM), origin (monoclonal or polyclonal), or specificity (i.e., Anti-IgG or Anti-C3d) of in vitro substances are used. The set specifications for the in-process controls and quality control testing demonstrated product homogeneity, reproducibility, and consistency of the manufacturing process.

Manufacture of the Anti-IgG in-vitro substance
Polyclonal antibody production consists of (b) (4) of the immune rabbit serum and is performed under a contract agreement with (b) (4), located in (b) (4).

The Anti-IgG component has been used for other BMD Anti-Human Globulin products since 1990. The reactivity of the Anti-IgG is not heavy chain specific, but is directed towards the immunoglobulin light chains of IgG, and thus may also bind to IgM or IgA sensitized red blood cells. There is no reactivity with complement coated red blood cells.

Manufacture of the Anti-IgG in vitro product
Raw materials
Incoming goods used in the manufacture of the in vitro product are initially received at BMD's warehouse. The incoming goods are processed, inspected, verified, labeled as "in quarantine", entered into the SAP System, and reviewed, tested, and released by Quality Assurance. Serological and physicochemical tests are performed on in vitro substances and excipients prior to the start of the manufacturing process to confirm that they meet established acceptance criteria.

The bovine serum albumin ((b) (4)) is derived from bovine blood collected from US sourced cattle slaughtered at a USDA licensed establishment in (b) (4).

Human red blood cells used for purification of the in vitro substance are tested and found negative for anti-HIV1 and 2, anti-HCV, anti-HBV, HBsAg, and syphilis.

Further processing of the Anti-IgG in vitro substance
The rabbit serum received from (b) (4) is further processed by BMD, and includes the addition of 0.1% sodium azide (preservative) and purification by adsorbing with (b) (4). (b) (4) used for purification of the in vitro substance are tested and found negative for anti-HIV1 and 2, anti-HCV, anti-HBV, HBsAg, and syphilis. The purification process requires performing adsorptions with (b) (4) to remove non-specific antibodies.

Production of the (b) (4)
(b) (4)

Production of the gel (b) (4)
(b) (4)

Date of Manufacture / Expiration Date
The date of manufacture (DOM) is the date when the (b) (4) the cards. The expiration date of the filled IH-Card AHG Anti-IgG is 16 months from the DOM.

Filling of the IH-Card
In preparation for filling the IH-Card, the required number of (b) (4) .

(b) (4)

The in-process controls, physical inspections, and testing are performed to ensure all specifications are met throughout the filling, labeling, and packaging operation.

Labeling and Packaging of IH-Cards
(b) (4)

Quality Control Testing
Final serological testing is performed on the filled IH-Cards at least (b) (4) . This validated hold time allows for proper sedimentation of the gel particles and supernatant, resulting in conditions necessary for performing serological tests. Anti-IgG potency testing uses (b) (4) . The indirect antiglobulin test and crossmatching are performed using the IH-1000 analyzer.

The following table summarizes the release testing and acceptance criteria for the IH-Card AHG Anti-IgG:

Table 1: Release testing and acceptance criteria for IH-Card Anti-IgG

Release Testing: IH-Card AHG Anti-IgG			
	Test	**Method**	**Acceptance Criteria**
Positive Specificity	Indirect Antiglobulin Test	(b) (4)	(b) (4)
	Direct Antiglobulin Test		
	Crossmatch		
Negative Specificity	Specificity		
	Indirect Antiglobulin Test		
	Direct Antiglobulin Test		
	Crossmatch		
Potency			
Visual Inspection			
Bioburden			

The information presented in this table was extracted from the submission.

Quality Control Testing
Quality control of packaging and labeling is performed after mechanical labeling and packaging occurs. The number of samples taken for inspection is based on a statistical sampling plan. The inspection includes: label identity, label position, presence of gel and supernatant, check for air bubbles in the gel, sealing area, completeness of packaged carton, and verification of type, quantity, and version of the Instructions for Use.

Microbiology/Bioburden
The AHG Anti-IgG reagent is microbiologically controlled, and as such is not labeled as sterile. The manufacturing of the in-vitro products includes addition of sodium azide as preservative and filtration using a (b) (4) filter. The bioburden test method was qualified in accordance with (b) (4) . The proposed sodium azide formulation concentration was shown to have effective anti-microbial properties in accordance with (b) (4) .

b) Facilities Review/Inspection
Facility information and data provided in the BLA for the manufacture of the Anti-Human Globulin (AHG) were reviewed by CBER and found to be sufficient and acceptable. The facilities involved in the manufacture of AHG Anti-IgG are listed in the table below. The activities performed and inspectional histories are noted in the table and are further described in the paragraphs that follow.

Table 2: Manufacturing Facilities Table for AHG Anti-IgG

Name/address	FEI number	DUNS number	Inspection/ waiver	Results/ Justification
In-Vitro Product Manufacturing and Testing Bio-Rad Medical Diagnostics GmbH Industriestr. 1 Dreieich, Hessen, Germany	3002806595	312576506	Surveillance Inspection Pre-License Inspection	Team Biologics March 16 – 24, 2015 VAI CBER October 1 – 10, 2014 VAI
In-Vitro Drug Substance Manufacturing Bio-Rad Laboratories, (b) (4)	(b) (4)	(b) (4)	N/A*	N/A
In-Vitro Drug Substance Manufacturing (b) (4)	N/A	N/A	N/A*	N/A

* Due to the nature of this product the *in vitro* substance manufacturer facilities were not required to be inspected.

CBER performed a Pre-License Inspection of the Dreieich, Germany facility from October 1-10, 2014. At the end of the inspection, a Form FDA 483 with seven observations was issued. The observations issued were associated with deficiencies in the automated visual inspection and

reconciliation processes of the IH-Cards, inadequate handling of deviations, inadequate production procedures, and inadequate segregation and storage controls for quarantine and released products. The firm responded adequately addressing all 483 observations.

Subsequent to the PLI, Team Biologics performed a surveillance inspection of the Bio-Rad Medical Diagnostics GmbH manufacturing facility from March 16-24, 2015. The corrective actions were found to be acceptable and the inspection was classified as Voluntary Action Indicated (VAI).

c) Environmental Assessment

Bio-Rad Medical Diagnostics GmbH included a request for categorical exclusion from performing an Environmental Assessment under 21CFR Part 25.31(c). The FDA concluded that this request is justified as the manufacturing of this product does not alter significantly the concentration and distribution of naturally occurring substances, and no extraordinary circumstances exist that would require an environmental assessment.

d) Container Closure System

The Anti-Human Globulin is filled into polypropylene plastic cards (IH-Cards) with overall dimensions of 70 x 9 x 53 mm ((b) (4)). Each gel card has six small columns (micro tubes) integrated into them, which is filled with the Anti-Human Globulin and (b) (4) gel. The opening of the columns is covered with a heat sealing lacquered aluminum foil ((b) (4)).

Bio-Rad Medical Diagnostics GmbH conducted the container closure integrity testing for the IH-Cards at their Dreieich location. This testing consisted of (b) (4) IH-Cards at a temperature between 18°C to (b) (4). Then, the cards were tested for serological reactivity, bioburden, and visual inspection for the detection of leaks at 0, 3, 6, 9, 12, 16 (b) (4) of the IH-Cards); all acceptance criteria were met.

4. Analytical Studies

Analytical studies performed included reproducibility/repeatability study, lot-to-lot reproducibility study, stability studies (shelf life and on-board), sample aging and anticoagulant studies and interfering substances study.

Shelf Life Stability

Stability studies were performed on (b) (4) conformance lots of IH-Card AHG Anti-IgG. The lots were used to execute a real-time stability study and (b) (4) lot Card AHG was used in a transport simulation study. Test methods (potency and specificity) used to evaluate the stability of the IH-Card AHG Anti-IgG are the same serological methods used for release testing.

The stability data at the (b) (4) test interval met specifications; however, BMD selected a dating period of 16 months for the IH-Card AHG Anti-IgG when stored at the recommended temperature of 18 – 25 °C.

On-Board Stability
BMD provided data that supports the labeling claim for seven-day storage of unopened and two-hour storage of partially used IH-Cards on the IH-1000 Analyzer. On-board stability testing was performed using one lot of IH-Card AHG Anti-IgG with the following parameters:

Table 3: Parameters for on-board stability study

Test Interval	Card / Storage Conditions
t_{end}	(b) (4) months shelf life
t_{end} + 5 days	(b) (4) months shelf life and five days on-board storage conditions
t_{end} + (b) (4)	(b) (4) months shelf life and (b) (4) on-board storage conditions
t_{end} + (b) (4) + opened	(b) (4) months shelf life and (b) (4) on-board storage conditions and (b) (4) storage of opened cards

t: abbreviation for test interval

The information presented in this table was extracted from the submission.

The stored cards were tested for specificity (positive and negative samples) and potency. The acceptance criteria were the same as established for quality control release testing. There were no differences between results obtain from unstored cards (t_{end}) and the cards stored at the three storage conditions. All acceptance criteria were met after (b) (4) closed and (b) (4) closed and (b) (4) opened, when stored at the maximum allowed IH-1000 Analyzer on-board temperature of (b) (4). The testing used cards stored under worst-case conditions, as the shelf life is established at 16 months, and the on-board storage conditions exceeded the labeling claims of up to seven days (closed) and two hours (opened, mimicking re-use of partially used cards.)

Lot-to-Lot Studies
A Lot-to-Lot Study was conducted internally at Bio-Rad. Three lots of each IH-Card AHG Anti-IgG were tested using a precision panel of known red blood cell phenotypes, complement-coated cells, and antibodies. Each sample was tested in duplicate, with two runs per day on five non-consecutive days over a 20 day period, providing 60 data points per sample (i.e., 60 = 3 lots × 2 duplicates × 2 runs × 5 days). The results for all six lots showed 100% agreement.

Reproducibility and Repeatability Study
A reproducibility study was conducted at three sites (two external and one internal) using one lot of IH-Card AHG Anti-IgG using the same precision panel as used in the lot-to-lot study. Each sample was tested in duplicate, with two runs per day on five non-consecutive test dates over a 20 day period, providing 60 data points per sample (i.e., 60 = 3 sites × 2 duplicates × 2 runs × 5 days). The results for the IH-Card AHG Anti-IgG showed 100% agreement.

Limitation Study
Sensitivity of the crossmatch with the IH-Card AHG Anti-IgG was evaluated internally by BMD. The evaluation was performed by (b) (4) samples containing different clinically significant antibodies (i.e., (b) (4)) that reacted by the indirect antiglobulin test. (b) (4) of each of the (b) (4) antibody samples were prepared and tested with (b) (4) different ABO-compatible donor samples with heterozygous expression of the corresponding antigen with the IH-Card AHG Anti-IgG cards in comparison to a FDA licensed reference. The following table indicates the titer endpoint achieved with the investigational card in comparison with the reference method.

Table 4: Titer endpoint comparison between investigational card and reference method

Antibody Specificity	IH-Card AHG Anti-IgG	Ortho MTS Anti-IgG Card
(b) (4)	32	16
	16	16
	256	256
	256	256
	16	8
	8	8
	512	64
	256	64
	128	64
	64	64

The information presented in this table was extracted from the submission.

The data demonstrate reliable detection of red cell antibodies in the crossmatch assay on the IH-1000 Analyzer with the investigational IH-Card AHG Anti-IgG. In comparison with the reference method, the IH-Card AHG Anti-IgG exceeded titer endpoints in five out of ten tests or had equal titer endpoints.

Sample aging and anticoagulant studies
Data for supporting the sample age and type, and preservative solutions were obtained from the clinical comparison studies and internal studies conducted by BMD to support the labeling claims regarding specimens used in testing. The data demonstrated that the specimen type and age (as listed in the table below) are suitable for use in testing with the IH-Card AHG Anti-IgG. The IH-Card AHG Anti-IgG Instructions for Use and the IH-1000 User Manual were revised to reflect the following sample age restrictions:

Table 5: Sample age restrictions

Test	Specimen type and age
Indirect Antiglobulin Test	Fresh EDTA, citrated, and serum samples are stored at 2 to 8 °C and may be tested up to 10 days post-collection. Frozen serum or plasma that has been stored at -20 °C may be tested: • Sodium citrate samples frozen for up to 674 days • EDTA samples frozen for up to 30 days • Serum samples frozen for up to 26 days
Direct Antiglobulin Test	EDTA and cord blood samples may be tested up to five days post-collection and stored at (b)(to 8 °C
Crossmatching	Donor blood collected in CPD, CP2D, and CPDA-1 may be tested until the expiration of the unit and stored at 2 to 8 °C. Donor blood containing AS-1 or AS-3 may be tested up to 30 days post-collection. Recipient samples collected in EDTA may be tested up to 10 days post-collection.

Interfering substances
A study for interfering substances (hemolysis, icteric, and lipemic) was conducted internally at BMD. The testing of samples was performed with the IH-Card AHG Anti-IgG in comparison to an FDA licensed product. The results obtained with the IH-Card AHG Anti-IgG were concordant with the results obtained with the reference reagent. The results demonstrated that higher than normal concentrations of triglycerides, bilirubin, or hemoglobin did not have an adverse effect on the performance of the IH-Card AHG Anti-IgG.

Samples in the Elderly
More than (b) (4) EDTA samples from patients older than 80 years of age, were tested with the IH-Card AHG Anti-IgG using the antibody detection method. The results were compared with a reference (i.e., FDA licensed Anti-Human Globulin reagent). All results of the antibody detection except for one sample were concordant between the IH-Card AHG Anti-IgG and the reference. The sample with discordant results was negative with the reference and positive with the IH-Card AHG Anti-IgG. The quantity of the sample was insufficient for further resolution testing.

Incubation temperatures used for serological testing
When performing the various assays using the indirect antiglobulin test, the IH-Card AHG Anti-IgG is incubated at 37 ºC. The IH-1000 Analyzer incubator temperature range of 35 to 38 ºC is controlled by the instrument software. A study tested IgG antibodies and complement-coated cells with the IH-Card AHG cards incubated at the minimum and maximum parameters. Data confirmed that the IH-Card AHG Anti-IgG performs as expected when the cards are incubated at the IH-1000 Analyzer minimum and maximum incubator temperatures.

5. Performance Studies

a) Clinical Studies

BMD conducted a clinical comparison study to evaluate the IH-Card AHG Anti-IgG performance in a clinical setting. The studies were performed at four external testing sites: Puget Sound Blood Center (PSBC), Miriam Hospital (MH), Vanderbilt University Medical Center (VUMC), and LifeSource Testing Laboratory (LSTL). In addition, internal studies were conducted at the Bio-Rad Research and Development Laboratory in Cressier, Switzerland.

The clinical comparison study evaluated the performance of the IH-1000 Analyzer for antibody detection, antibody identification, direct antiglobulin testing, and antiglobulin crossmatch testing. The study included the use of BMD IH-Cell products (Reagent Red Blood Cells, 0.6 ± 0.1%), quality control material, IH-LISS (diluent), at least two lots of IH-Card AHG Anti-IgG, and three different IH-1000 Analyzers. The clinical study consisted of testing the IH-Card Anti-IgG in parallel with an FDA-licensed reagent and cleared instruments for concordance. The comparator test method by site is as follows:

Table 6: Comparator test method by site

Test	IH-Card AHG Anti-IgG			
	PSBC	**MH**	**VUMC**	**LSTL**
Antibody Detection	Immucor Galileo Neo (Capture R)	Ortho ID-MTS Anti-IgG Card (ProVue)	Not Performed	BMD TANGO optimo System
Antibody Identification	Not Performed	Ortho ID-MTS Anti-IgG Card (Manual)	Not Performed	BMD Anti-IgG (Tube Test)
Antiglobulin crossmatch	Not Performed	Ortho ID-MTS Anti-IgG Card (ProVue)	Not Performed	Not Performed
Direct Antiglobulin Test	Not Performed	Ortho Anti-IgG (Tube Test)	Not Performed	BMD TANGO optimo System and BMD Anti-IgG (Tube Test)

Note: internal testing used BMD FDA licensed reagents as the reference.

The information provided in this table was extracted from the submission.

Results from the investigational method and the reference assay were compared for concordance. If the results were still discordant after repeat testing, then a third FDA licensed method was used as a referee. If the antibody identification results were discordant after repeat testing, antigen dosage and profile were evaluated as potential cause for discrepancy.

BMD used the following acceptance criteria for the studies:

- Antibody Detection, Antibody Identification, and Direct Antiglobulin Test: The lower bound of the one-sided 95% confidence intervals for the positive percent agreement and the negative percent agreement had to exceed 0.95.

- Antiglobulin Crossmatch: The lower bound of the one-sided 95% confidence intervals for the positive percent agreement and the negative percent agreement had to exceed 0.99.

Some of the IH-Card AHG Anti-IgG testing results described below in the clinical sites failed to meet the acceptance criteria to support approval. In a submission issue meeting held on January 14, 2016, FDA requested BMD to perform additional testing in an In-House Performance Study. The result verification process would include reviewing and editing (if necessary) of the camera images and assay interpretation. Editing of the results was not limited to indeterminate results, initially positive or negative reactions may have to be edited after visual review. The edited results of the studies performed at the clinical sites are described below.

Antibody Detection
The detection of unexpected antibodies was conducted using IH-Card AHG Anti-IgG and three different IH-Cell products used to screen for red blood cell antibodies. The following tables summarize the number of samples collected and analyzed per site:

Table 7: Sample size per site

Site	**Antibody Detection using the IH-Card AHG Anti-IgG**		
	IH-Cell Pool (Pooled Cells)	**IH-Cell I-II** (2-Cell Screen)	**IH-Cell I-II-III** (3-Cell Screen)
PSBC	2,200	0	0
MH	0	800	400
VUMC	0	0	0
LSTL	700	400	0
BMD (internal)	137	0	137
Sub-total	3,037	1,200	537
TOTAL	**4,774**		

The information presented in this table was extracted from the submission.

The edited results for antibody detection performed with the AHG Anti-IgG are shown in the table below.

Table 8: Edited results for antibody detection with AHG-Anti-IgG

IH-Cell Products Used (0.6% Reagent Red Blood Cells)	**IH-Card AHG Anti-IgG** (at the sample level)		
	Positive Percent Agreement [Lower 95% CB]	Negative Percent Agreement [Lower 95% CB]	Overall Percent Agreement [Lower 95% CB]
IH-Cell Pool	89.53% 77/86 [82.45%]	99.12% 2916/2942 [98.78%]	98.55% 2993/3037 [98.14%]

IH-Cell I-II	100% 12/12 [77.91%]	97.22% 1155/1188 [96.34%]	97.25% 1167/1200 [96.34%]
IH-Cell I-II-III	98.53% 67/68 [93.21%]	97.23% 456/469 [95.63%]	97.39% 523/537 [95.95%]
TOTAL	*93.98%* *156/166* *[90.00%]*	98.43% 4527/4599 [98.10%]	98.09% 4683/4774 [97.74%]

The positive percent agreement (PPA) did not meet the 95% acceptance criteria when combining results for all screen RRBCs on the IH-Card AHG Anti-IgG.

A summary of the factors contributing to the positive percent agreement not meeting acceptance criteria is listed below:

- Eight samples reacted negative with the investigational screening method and positive with the reference method and no red cell antibodies were detected when antibody identification testing was performed. The reference method for these eight samples was the Immucor Galileo Neo with the Capture Ready Screen Pool assay.
- One sample reacted very weak positive with the reference method and negative with the investigational method, the referee method and the antibody identification tests were not performed because amount of sample was not sufficient.
- One sample that was weakly positive with the reference method and discordant negative with the investigational method was tested at BMD with IH-Cell Pool and IH-Cell I-II-II. The result with both investigational screen RRBCs was negative.
- Two samples - Reacted positive with the reference method and negative with the investigational method, no repeat testing or referee method was performed because the amount of the sample was not sufficient.

Antibody Identification
Antibody identification testing was performed on all samples with positive or equivocal antibody screens by any method. The identification of unexpected antibodies was conducted using IH-Card AHG Anti-IgG with different IH-Cell products used for red blood cell antibody identification.

The following table summarizes the number of samples collected and analyzed per site:

Table 9: Sample size per site

Site	**Antibody Identification**		
	IH-Panel 11 (11-cell panel)	**IH-Panel 11 Papain** (11-cell panel, papain treated)	**IH-Panel 6 Plus** (6-cell panel)
PSBC	0	0	0
MH	59	86	28
VUMC	0	0	0

LSTL	113	114	114
BMD (internal)	84	155	0
Sub-total	**256**	**355**	**142**
TOTAL	**753**		

The information provided in this table was extracted from the submission.

The performance evaluations for the IH-Card AHG Anti-IgG are based on the combined initial results for all three IH-Cell products per IH-Card.

Table 10: Results for antibody identification with AHG-Anti-IgG

	ANTIBODY IDENTIFICATION (per sample)		
	Positive Percent Agreement [Lower 95% CB]	Negative Percent Agreement [Lower 95% CB]	Overall Percent Agreement [Lower 95% CB]
IH-Card AHG Anti-IgG	95.02% 305/321 [92.53%]	87.04% 376/432 [84.07%]	90.44% 681/753 [88.49%]

A total of 321 antibodies were identified in the 753 samples. The positive percent agreement and the negative percent agreement did not meet the 95% acceptance criteria with a 95% lower confidence interval (LCI).

A summary of the factors contributing to not meeting the acceptance criteria is listed below:

Of the 321 antibody positive samples:

- In 63/321 samples only the investigational method identified antibodies.The specificity of these antibodies is not known.
- In 2/321 samples the results were equivocal with the investigational method and positive with the reference method. The samples were tested again, and the results were negative with the investigational method and positive with the reference method. The specificity of these 2 antibodies is not known.

Of the 432 discordant antibody negative results:

- 21/432 samples – Tested negative with the reference method and the investigational method identified antibodies with the following specificity: (b) (4) .

Direct Antiglobulin Test

The direct antiglobulin test (DAT) was evaluated using the IH-Card AHG Anti-IgG in comparison with a licensed Anti-IgG reagent. The comparison testing was performed on a total of 644 samples; 245 at MH and 399 at LSTL.

A total of 58 samples tested positive with the reference method. There was agreement with the investigational method in 56/58 samples.

The results for the DAT testing with IH-Card AHG Anti-IgG are presented in table 10 below. This table was extracted from the revised Statistical Summary dated March 4, 2016, which is included in the submission.

The NPA and overall agreement (OA) exceeded the 95% acceptance criteria, with a 95% LCI. The PPA did not meet the acceptance criteria of 95% with a 95% LCI.

When samples are discrepant ("samples that result in two different interpretations") the laboratory retests the sample using the same comparative method (reference method) and the BMD reagents. If disagreement remains, the discrepancies must be reported to the BMD Study Monitor to determine the appropriate testing for resolution (referee method) such as a third FDA licensed reagent.

Table 11: Results for DAT testing with AHG-Anti-IgG

N = 644 samples

IH-Card AHG Anti-IgG 74020		FDA Licensed AHG Anti-IgG Reference				Positive % Agreement [one-sided Exact 95% Lower CI]	Negative % Agreement [one-sided Exact 95% Lower CI]	Overall % Agreement [one -sided Exact 95% Lower CI]
		Pos	EQV	Neg	Total			
Investigational Anti-IgG N = 644	Pos	56	0	17	73	96.55% 56/58 [89.54%]	97.10% 569/586 [95.68%]	97.05% 625/644 [95.70%]
	EQV	0	0	0	0			
	Neg	2	0	569	571			
	Total	58	0	586	644			

A summary of the factors contributing to not meeting the PPA is listed below:

- The number of positive samples (58) is not large enough to achieve statistical significance.
- 1/58 samples was weakly positive with the reference method and was not tested by the reference method.
- 1/58 samples was negative with the reference method

Antiglobulin Crossmatch

The antiglobulin crossmatch using the IH-Card AHG Anti-IgG was performed with 74 samples (35 antibody positive and 39 antibody negative) that were crossmatched with 760 compatible and incompatible donor units. All 760 crossmatches were performed at the same site (MH).

Results for the investigational Anti-IgG with 760 crossmatch tests are compared to test results with the FDA licensed methods in the table listed below. The table was extracted from the submission.

Table 12: Results for crossmatch testing with AHG-Anti-IgG

N = 760 crossmatches

IH-Card AHG Anti-IgG		FDA Licensed Anti-IgG Reference				Positive % Agreement [one-sided Exact 95% Lower CI]	Negative % Agreement [one-sided Exact 95% Lower CI]	Overall % Agreement [one -sided Exact 95% Lower CI]
		Pos	EQV	Neg	Total			
Investigational Anti-IgG 74020 N = 760	Pos	279	1	39	319	99.29% 279/281 [97.78%]	91.84% 439/478 [89.48%]	94.47% 718/760 [92.91%]
	EQV	0	0	0	0			
	Neg	2	0	439	441			
	Total	281	1	478	760			

The NPA and the PPA agreement did not meet the 99% acceptance criteria with a 95% LCI.

Failure to meet the acceptance criteria was due to a high number (39) of false negative results with the reference method when the expected result was positive (incompatible). In all of these crossmatches, the investigational method (IH-Card AHG Anti-IgG) was positive (incompatible) as expected. One recipient sample with Anti-M was not detected by the investigational method but was detected by the reference method in two crossmatches with M+ donor units.

Summary of Clinical Studies Results
During the clinical study, the test results obtained with the following assays failed to meet the primary endpoint:

- Detection of Unexpected Antibodies with IH-Card AHG Anti-IgG.
  - The PPA did not meet the 95% acceptance criteria.
- Antibody-identification with IH-Card AHG Anti-IgG.
  - The PPA and the NPAnegative did not meet the 95% acceptance criteria.
- Direct Antiglobulin Test (DAT) with IH-Card AHG Anti-IgG.
  - The PPA did not meet the 95% acceptance criteria.
- AHG Crossmatch with IH-Card AHG Anti-IgG, C3d
  - The PPA and the NPA did not meet the 99% acceptance criteria.

Because the assays mentioned above failed to meet the primary endpoint, on January 14, 2016, FDA requested BMD to do additional testing in an In-House Performance Study, to support the approval of the IH-Card AHG Anti-IgG for detection and identification of unexpected antibodies, DAT and AHG compatibility testing on the IH-1000. For the in-house performance study, BMD used known positive and negative samples and/or contrived samples.

b) In-House Performance Study
The additional studies needed for the Anti-Human Globulin reagent are shown in the table below.

- Green indicates the study endpoint was met in the clinical study and no further study is required
- Red indicates that the study endpoint was not met in the clinical study and testing of additional samples in the in-house performance study is required to demonstrate that the predetermined study endpoints are met.

Table 13: Additional studies for IH-Card Anti-IgG

Assay	Antibody screening		Antibody Identification		Direct Antiglobulin Testing (DAT)		AHG Crossmatching*	
Study Type	PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA
IH-Card AHG Anti-IgG								
IH-Card AHG Anti-IgG, -C3d								

Card	IH-Card AHG Anti-IgG		IH-Card AHG Anti-IgG, -C3d	
	PPA	NPA	PPA	NPA
IH-Cell Pool				
IH-Cell I-II				
IH-Cell I-II-III				
IH-Panel 11				
IH-Panel 6 Plus				
IH-Panel 11 Papain			NA	

* detection of incompatibility due to IgG antibodies

Sample Size Summary

1) Testing with antibody positive and negative samples – detection and identification of antibodies.
    - Antibody Screening: 192 antibody positive samples
    - Antibody Identification: 709 antibody negative samples and 126 antibody positive samples.

2) Testing with well-characterized antibody positive and antibody negative samples –AHG crossmatching.
    - Number of samples by characterization result: 38 antibody positive frozen EDTA samples.
    - Total number of crossmatches: 301 samples (with 19 ABO compatible donor units collected in sodium citrate)

3) Testing with well-characterized and/or contrived DAT positive samples
    - Forty six (46) contrived samples and 1 Coombscell-E (IgG Coated Control Cells)
    - 22 well-characterized samples

Testing Algorithm
After the sample characterization with at least two FDA licensed methods was completed, the samples were tested with the investigational reagents using the same sample aliquot used for the characterization. Before final test interpretation, all results were validated by trained operators.

For this study, BMD adopted the same acceptance criteria used for the clinical study.

Results
The result tables presented in this section are extracted from the In-House Performance Study report, which is included in this submission.

1. **Detection of unexpected antibodies with IH-Card AHG Anti-IgG and IH-Cell Pool, IH-Cell I-II and IH-Cell I-II-III**

Sixty four well-characterized antibody positive samples were tested with IH-Card AHG Anti-IgG and IH-screening cells (IH-Cell Pool, IH-Cell I-II and IH-Cell I-II-III) on the IH-1000. The 64 samples were tested in each of the three panels.

Table 14: Testing results with IH-Cell Pool

Ab screen IH-Card AHG Anti-IgG IH-Cell Pool		Expected result	
		Positive	Negative
Investigational method	Positive	64	NA
	EQV	0	
	Negative	0	
	Total	**64**	
% Agreement		**100%**	
One-sided Exact 95% LCL		**95.43%**	

Table 15: Testing results with IH-Cell I-II

Ab screen IH-Card AHG Anti-IgG IH-Cell I-II		Expected result	
		Positive	Negative
Investigational method	Positive	64	NA
	EQV	0	
	Negative	0	
	Total	**64**	
% Agreement		**100%**	
One-sided Exact 95% LCL		**95.43%**	

Table 16: Testing results with IH-Cell I-II-III

Ab screen IH-Card AHG Anti-IgG IH-Cell I-II-III		Expected result	
		Positive	Negative
Investigational method	Positive	64	NA
	EQV	0	
	Negative	0	
	Total	**64**	
% Agreement		**100%**	
One-sided Exact 95% LCL		**95.43%**	

Conclusion: The results presented in table 12, 13 and 14, met the acceptance criteria.

2. Antibody identification with IH-Card AHG Anti-IgG and IH-Panel 11

Table 17: Test results with IH-Panel 11

AbID IH-Card AHG Anti-IgG IH-Panel Panel 11		Expected result	
		Positive	Negative
Investigational method	Positive	64	4[a]
	EQV	0	0
	Negative	0	208[b,c]
	Total	**64**	**212**
% Agreement		**100%**	**98.11%**
One-sided Exact 95% LCL		**95.43%**	**95.73%**

Conclusion: The results met the acceptance criteria.

3. Antibody Identification with IH-Card AHG Anti-IgG and IH-Panel Plus 6

Table 18: Test results with IH-Panel Plus 6

AbID IH-Card AHG Anti-IgG IH-Panel Plus 6		Expected result	
		Positive	Negative
Investigational method	Positive	NA	2[a]
	EQV		0
	Negative		211[b,c]
	Total		**213**
% Agreement			**99.06%**
One-sided Exact 95% LCL			**97.07%**

Conclusion: The results met the acceptance criteria.

4. Direct Antiglobulin Test (DAT) with IH-Card AHG Anti-IgG

Table 19: Testing results for DAT

DAT IH-Card AHG Anti-IgG		Expected result	
		Positive	Negative
Investigational method	Positive	67	NA
	EQV	0	
	Negative	0	
	Total	**67**	
% Agreement		**100%**	
One-sided Exact 95% LCL		**95.63%**	

Conclusion: The results met the acceptance criteria.

5. AHG Crossmatch with IH-Card AHG Anti-IgG

The testing included 301 compatible crossmatches.

Table 20: Testing results for AHG Crossmatch

AHG crossmatch IH-Card AHG Anti-IgG		Expected result	
		Positive	Negative
Investigational method	Positive	NA	0
	EQV		0
	Negative		301[a]
	Total		**301**
% Agreement			**100%**
One-sided Exact 95% LCL			**99.01%**

[a] One recipient plasma sample was crossmatched with 8 donor units. In 5 of these 8 crossmatches the reaction was positive (DP). The operator edited the results to negative upon visual review. He noted cell localizations at the top of the reaction well most likely caused by fibrin residues in the recipient sample causing a DP reaction interpretation by IH-1000.

Conclusion: The results met the acceptance criteria.

Summary of in-house performance study results

During the in-house performance study, the test results obtained for the following assays met the acceptance criteria:

1. Detection of Unexpected Antibodies with IH-Card AHG Anti-IgG (PPA)
2. Antibody-identification with IH-Card AHG Anti-IgG (PPA and NPA)

3. Direct Antiglobulin Test (DAT) with IH-Card AHG Anti-IgG (PPA)
4. AHG Crossmatch with IH-Card AHG Anti-IgG (PPA and NPA)

b) Pediatrics

Data from the clinical studies found that cord blood samples could be properly tested using the IH-Card AHG Anti-IgG with the IH-System.

c) Other Special Populations

In the Sample Aging and Anticoagulant analytical studies, cord blood samples and samples from elderly patients were included.

d) Overall Comparability Assessment

The results of the clinical comparison study and product labeling support the conclusion that the IH-Card AHG Anti-IgG formulated for automated testing on the IH-1000 Analyzer is safe and effective. In addition, the lots manufactured in support of the submission, demonstrate the reliability of the manufacturing process to consistently produce IH-Cards AHG that meet established specifications, perform as intended, and remains stable throughout its shelf life.

6. Advisory Committee Meeting

The Bio-Rad Medical Diagnostics GmbH IH-System does not include novel technology; therefore, an advisory committee meeting was not held or required.

7. Other Relevant Regulatory Issues

The review committee members from DBCD, DMPQ, DB, DCM, and DBSQC reviewed their specific sections of the BLA and Efficacy Supplement and resolved any issues through information requests and teleconferences with BMD. The Review Team sought the expertise of their respective management, when warranted. No internal or external disagreements were communicated to the regulatory project manager or chairperson. All reviewers recommended approval of the Anti-Human Globulin (Formulated for Automated Testing) BLA and Efficacy Supplement.

No postmarketing commitments are associated with the Anti-Human Globulin (Formulated for Automated Testing) BLA and Efficacy Supplement.

8. Labeling

The labeling for Anti-Human Globulin (Formulated for Automated Testing) complies with Title 21 CFR 610.62, 610.63, 610.64, 610.65, 660.55 and 809.10. In the teleconference held on June 8, 2016. BMD agreed to include in the final labeling all the recommendations provided by FDA. The final labeling for IH-Card AHG Anti-IgG includes the following changes.

- The use of a table format to represent the results from the clinical study and the in-house performance study in the package inserts of AHG under the Specific Performance Characteristics section.
- All packages inserts (BGRs, AHGs, and RRBCs) are revised to define visual reading and editing (if applicable) as a required process. All package inserts are revised to include the “edited results” rather than the “initial results”.

Unique Device Identification (UDI) review performed by CBER found the required elements to comply with Title 21 CFR 830. The labeling met the UDI requirements ahead of the September 24, 2016 compliance date for this classification of medical devices.

9. Recommendations and Risk/ Benefit Assessment

a) Recommended Regulatory Action

The review committee members, representing the necessary review disciplines (DBCD, DMPQ, DB, DCM, and DBSQC) recommend approval. These were independent conclusions based on content of the BLA, issues satisfactorily resolved during the review cycle, and concurrence by their respective management. No internal or external disagreements were brought to the attention of the chairperson.

b) Risk/ Benefit Assessment

The IH-1000 Automated Analyzer and the reagents used by the IH-System, provide potential advantages to support transfusion medicine.

- The clinical benefits using the IH-System include greater patient safety and timely availability of transfusion products to the patient through improved productivity.

- Features that impact patient safety include reduction in errors associated with subjective interpretation due to manual testing, transcription errors, test errors (i.e., using expired reagents or the wrong reagent), and the capability to review of stored test results, if necessary.

- Features that impact timely availability of transfusion products include reduction in hands-on technologist time by automating the process, time required for recording assay reagents, controls, and equipment, as well as turn-around time.

c) Recommendation for Postmarketing Activities

There are no postmarketing commitments associated with the Anti-Human Globulin (Formulated for Automated Testing) BLA or Efficacy Supplement.