Summary Basis for Regulatory Action

From: Darcel Bigelow, Chair of the Review Committee

***BLA*/STN#:** See the table below

Applicant Name: DIAGAST

Date of Submission: August 17, 2016

MDUFA Goal Date: February 3, 2018

Proprietary Name/ Established Name:

Table 1

Submission Tracking Number	Name of Biological Product	Cell Line(s)	Intended Use
BL 125616/0	Anti Human Globulin Anti-IgG (Murine Monoclonal)	18833 18896	This reagent is intended for use in the direct antiglobulin test to detect in vivo coating on human red blood cells with IgG and for indirect antiglobulin test for antibody screening and identification, and crossmatch and for erythrocyte phenotyping with blood phenotyping reagents requiring an indirect antiglobulin test method.
BL125616/0	Anti Human Globulin Anti-C3d (Murine Monoclonal)	12011D10	This reagent is intended for use in the direct antiglobulin test to detect the *in vivo* coating of human red blood cells with C3d components. Anti-Human Globulin Anti-C3d only recognizes the complement fragment and, consequently, cannot react with component C4.
BL125616/0	Anti Human	12011D10	This reagent is intended for

	Globulin Anti-IgG, C3d (Murine Monoclonal)	18833 18896	use in the direct antiglobulin test to detect the *in vivo* coating on human red blood cells with IgG and/or C3d components. In addition, to recognizing IgG antibodies and the complement fragment C3d, Anti Human Globulin Anti-IgG, C3d is able to recognize IgM antibodies on the surface of red blood cells since IgM antibodies always fix complement *in vivo* (and *in vitro* if the reaction occurs in the presence of complement: i.e., when using a fresh sample).

Recommended Action:
The Review Committee recommends approval of these products.

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.**

The table below indicates the material reviewed when developing the SBRA.

Table 2: Material Reviewed

Document title	**Reviewer name, Document date**
Clinical Review	Ricardo Espinola, OBRR/DBCD/DRB *February 3, 2017* *May 23, 2017* Darcel Bigelow, OBRR/DBCD/DRB *December 20, 2017* *January 29, 2018*
Non-Clinical Review	Ricardo Espinola, OBRR/DBCD/DRB *February 3, 2017* *May 23, 2017*

	Darcel Bigelow, OBRR/DBCD/DRB *December 20, 2017* *January 29, 2018*
Statistical Review	Paul Hshieh, OBE/DB/TEB *January 30, 2017* *April 27, 2017*
CMC Product Review	Ricardo Espinola, OBRR/DBCD/DRB *February 3, 2017* *May 23, 2017* Darcel Bigelow, OBRR/DBCD/DRB *December 20, 2017* *January 29, 2018* Simleen Kaur, OCBQ/DBSQC/LMIVTS Microbiology/Bioburden *April 4, 2017* *September 26, 2017*
CMC Facilities Review	Priscilla Pastrana, OCBQ/ DMPQ/BII *December 14, 2016* *April 3, 2017* *December 21, 2017*
Labeling Review	Ricardo Espinola, OBRR/DBCD/DRB *February 3, 2017* *May 23, 2017* Darcel Bigelow, OBRR/DBCD/ DRB *December 20, 2017* *January 29, 2018* Dana Jones, (OCBQ/DCM/APLB) *March 14, 2017*
Lot Release	Varsha Garnepudi, OCBQ/ DBSQC *April 4, 2017* *December 15, 2017*

1. Introduction

DIAGAST submitted an original Biologics License Application requesting approval to manufacture Anti Human Globulin Anti-IgG (Murine Monoclonal), Anti Human Globulin Anti-C3d (Murine Monoclonal), and Anti Human Globulin Anti-IgG, C3d (Murine Monoclonal). DIAGAST will manufacture these Anti Human Globulin (AHG) reagents at their licensed facility (Establishment Registration Number 3006261638) in Loos, France for Grifols Diagnostic Solutions Inc. who will distribute the products.

Intended Use/Indications for Use:
The Intended Use statements are listed above in Table 1.

Chronology:
CBER received the original submission on August 17, 2016 and received 14 amendments from DIAGAST in response to 11 Information Requests and one Complete Response Letter.

2. Background

Meetings with FDA:
DIAGAST requested a pre-submission meeting (BQ150291) with FDA on July 2, 2015. DIAGAST submitted questions regarding the proposed bundled BLA submissions, and the proposed clinical protocol and clinical study. On September 14, 2015, FDA submitted the responses to DIAGAST and on September 24, 2015 a pre-submission meeting was held regarding the planned clinical study. Based on the discussion at the meeting, an amended protocol was submitted to FDA on October 5, 2015. On March 17, 2016 and May 9, 2016, FDA provided written responses to the subsequent amendments to the protocol.

Description of the Device:
The AHGs are murine monoclonal antibodies derived from in vitro culture of related cell lines listed in table 1. The formulation contains bovine serum albumin, sodium arsenite (0.02%) and sodium azide (<0.1%). The AHGs are manually filled in 14 mL glass vials with a semi-automatic dispenser and dropper capped manually. These AHGs are used for erythrocyte phenotyping with blood phenotyping reagents requiring an indirect antiglobulin test method and for the direct and indirect test (Coombs' test) on human red blood cells as listed in Table 1.

Principles of the Assay:
The direct and indirect antiglobulin test methods are based on the principle of hemagglutination. The addition of the reagents induces agglutination of the red blood cells sensitized *in vivo* (direct antiglobulin test: direct "Coombs" test) or *in vitro* (indirect antiglobulin test).

- **The direct antiglobulin test** determines if red blood cells are coated in vivo with immunoglobulin, complement or both. This test is necessary in the investigation of immune-mediated hemolysis. Immune-mediated hemolysis may be observed in hemolytic transfusion reactions, hemolytic disease of the fetus and newborn, autoimmune hemolytic anemia and drug-induced hemolysis. The red blood cells are washed and mixed directly with the reagent.
- **The indirect antiglobulin test** is used to detect red cell antibodies in patient serum or plasma and is the methodology used for antibody screening, antibody identification, and crossmatch. In the test for the presence of immune antibodies, potentiators may be used according to the manufacturer's instructions for use (IFU). The indirect antiglobulin test reaction is two-stage.

The red blood cells are exposed to the IgG antibodies and the antibodies bind to the red blood cells carrying the corresponding antigen. After washing, Anti-Human Globulin Anti-IgG is added, inducing agglutination of the sensitized red blood cells.

3. Chemistry Manufacturing and Controls (CMC)

The application was submitted in accordance with the recommendations in FDA's Guidance for Industry: *"Content and Format of Chemistry, Manufacturing, and Controls Information and Establishment Description Information for a Biological in-Vitro Diagnostic Product"*.

a) Manufacturing Summary

The AHG Anti-IgG and AHG Anti-C3d in vitro substances (IVS) are produced at DIAGAST's facility in Loos, France from cell culture supernatants containing monoclonal antibodies to IgG or C3d.

DIAGAST submitted representative Certificates of Analysis (CoA) or Technical Data Sheets for the raw materials/components used to manufacture the IVS. Only components that meet incoming raw material requirements are used to produce the AHGs. The raw materials, the components, and the IVS are in-process tested in accordance with the Certificates of Analysis (CoA) or based on in-process testing established at DIAGAST.

(b) (4)

1 Page Determined to be Not Releasable: (b)(4)

In vitro Product (IVP)

All raw materials used for the manufacture of the IVPs are provided by qualified suppliers and accepted based upon the supplier CoA and qualifying tests, as applicable.

Manufacturing Process Description

(b) (4)

The AHG IVPs are filled in 14 mL glass vials in a (b) (4). The vials are filled manually with a semi-automatic dispenser and capped manually with dropper caps in a (b) (4). Caps are tightened using the (b) (4) semi-automatic screwing-capping machine. Cap (b) (4) is checked using (b) (4) equipment. The fill volumes are below:

Table 4: Product and Fill Volume

Antibody	Fill Volume
AHG Anti-IgG	10 ml
AHG Anti-IgG, -C3d	10 ml
AHG Anti-C3d	5 ml

Vial labels are printed. The final product is packed and inspected for proper labeling to assure that vial and kit labels were properly printed. The final products are stored at 2 °C to 8 °C until release. The final batch release is performed by Quality Assurance.

Date of Manufacture

The date of manufacture (DOM) of the IVPs produced from (b) (4) IVS is the date of (b) (4)

Specification and Test Methods

Specificity, activity, titration, appearance, and volume testing are performed on the (b) (4) filled final product vials, using the standard manual tube agglutination method. All acceptance criteria were met.

Table 5: BGR *In vitro* Product Acceptance Criteria

BGR *In vitro* Product Stage	Testing Performed	Acceptance Criteria
	Appearance	Absence of cloudiness and particles
		Color conforms to Technical Product Specifications
	Specificity	No reaction observed with all RBC tested (from

Final QC Testing (Manual Method)		Table 6)
	Activity	Positive reaction with all RBC tested (from Table 6)
	Potency	≥Minimum titer (from Table 6) and within (b) (4) of Reference Standard

Microbiology
Microbiological control of the IVP is accomplished as follows:

- Environmental and in-process controls are in place to limit the presence of micro-organisms, and therefore limit potential contamination of the product through environmental control and aseptic technique.
- The filling process is performed under Class (b) (4) conditions with a Class (b) (4) background environment.
- The final product is (b) (4) to remove microorganisms and tested with a validated bioburden method.
- The final product contains the preservative, (b) (4) sodium azide and 0.02% arsenite, to inhibit growth of micro-organisms.

b) CBER Lot Release

The lot release protocol template was submitted to CBER for review and found to be acceptable after revisions. The lot release testing plan was developed by CBER and will be used for routine lot release.

c) Facilities review/inspection

Facility information and data provided in the BLA bundle were reviewed by CBER and found to be sufficient and acceptable. The facility involved in the manufacture of the products listed in this BLA bundle is listed in the table below. The activities performed and inspectional history is noted in the table.

Name/Address	FEI number	DUNS number	Results/Justification
in vitro Substance *in vitro Product* *Release Testing* Diagast EuraSante Parc 215 Avenue Eugène Avinée 59374 LOOS, Cedex, France	3006261638	381527001	Team Biologics February 13-21, 2017 VAI

Team Biologics performed a surveillance inspection of the LOOS, Cedex, France facility February 13-21, 2017. All 483 issues were resolved and the inspection was classified as Voluntary Action Indicated (VAI).

d) Environmental Assessment

This BLA bundle included a request for categorical exclusion from an Environmental Assessment under 21 CFR 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.

e) Container Closure

The *in vitro* products are filled into a 14mL (b) (4) Glass Vial (b) (4) supplied by (b) (4) and 14 mL glass dropper assembly cap supplied by (b) (4) Diagast conducted the container closure integrity testing at the LOOS, Cedex, France facility, employing (b) (4) verification and (b) (4) test; all acceptance criteria were met.

4. Analytical Studies

Analytical studies included stability, anticoagulant, and precision studies.

Stability Studies

Three lots of each AHG IVP produced were tested to support the shelf life of up to 24 months stored at 2 °C to 8 °C in 14 mL glass vial. DIAGAST used the standard manual tube agglutination methods for AHG for testing potency and specificity of the stability samples.

AHG Anti-IgG, AHG Anti-C3d and AHG Anti-IgG-C3d products were tested at 6, 9, 12, 15, 18, 21 and (b) (4) months for validation of the current shelf life and an extended target shelf life of 24 months.

Table 6 shows details of the red blood cells used for specificity, activity, and titration testing and the corresponding acceptable minimum titer for each antibody.

DIAGAST provided 24 months of potency and specification test results for the real-time stability study. The acceptance criteria were met for all time points for each of the three conformance lots.

Table 6: AHG In-Vitro Products Testing Specificity

In vitro Product	Specificity RBC Used (1)	Activity RBC Used	Minimum Titer	Potency (Neat)
AHG Anti-IgG	(b) (4)			
AHG Anti-C3d				

AHG Anti-IgG, C3d	(b) (4)

(b) (4)

Microbiology testing is performed on all DIAGAST (b) (4) AHG IVP.

In addition to the real time stability study on the IVP, DIAGAST also performed a simulated transport stability study. This study was performed between DIAGAST (Loos, France) and a Grifols Diagnostic Solutions Inc. (GDS) warehouse provider in the US (b) (4)) from (b) (4) Each shipment included samples of three conformance lots of each AHG IVP, (b) (4) -packed in a corrugated carton filled with packing paper. A (b) (4) temperature recorder was packed in the carton along with the product. DIAGAST tested the AHG IVP for appearance, specificity, and potency (b) (4)

At GDS-(b) (4) the shipment was checked for integrity and stored unopened at 2 °C to 8 °C until it was shipped back to DIAGAST. Once back at DIAGAST, the shipment was checked for integrity and the data recorder was read and analyzed. The product was removed and stored at 2 °C to 8 °C until the performance of stability testing. Specificity, potency and acceptance criteria are the same as for the real time stability testing as previously described in this review memo. The testing results met the acceptance criteria for the time period included in the stability reports.

Based on the results of the Stress Testing, DIAGAST determined that the recorded temperatures during shipment from DIAGAST to Grifols must remain below (b) (4) with (b) (4) and must take no more than (b) (4) for the shipping method to be acceptable.

Anticoagulant Studies
Two anticoagulant studies were performed at (b) (4) . In the first study, whole blood donor samples (EDTA vs Sodium Citrate and EDTA vs Lithium Heparin) were used. Samples were provided by the (b) (4) , which were tested at 1-3 days and (b) (4) days of collection using direct and indirect antihuman globulin tests. There were no differences between the results obtained at the beginning of the study and at the end of the study.

In the second study, (b) (4) whole blood donations were collected in different anticoagulants (CPD, CP2D, CPDA-1 and ACD) and then (b) (4) of these donations were used to manufacture red blood cells (RBCs). For these (b) (4) products, storage solutions were added (b) (4) AS-1, and AS-3).

All results for all the samples tested with the DIAGAST AHG throughout the study

obtained 100% agreement with the positive or negative results initially obtained with the FDA licensed reagents and the initial EDTA samples tested with the DIAGAST AHG. No discrepancies were observed and no large differences in positive results (greater than 2) from the initial results or DIAGAST results were observed.

Precision Studies (Reproducibility and Repeatability)
The Reproducibility and Repeatability Study was performed to demonstrate that the test reagent generates reproducible and accurate results using a panel of well-characterized samples across different sites, using different operators, and on different days. The acceptance criterion stated there should be 100% agreement between the test outcomes and the expected results.

The Precision Panel was shipped to the three clinical study sites. The testing was performed by (b) (4) operators over (b) (4) non-consecutive days, on one lot of product each with replicate testing performed by each operator within each run.

There were no discrepancies observed among the three sites. Results showed 100% agreement for all the AHGs. No variability was observed in the strength of reactions among the operators.

5. Clinical Studies

a) Clinical Performance Studies (Comparison Study)

DIAGAST conducted a clinical study to evaluate the performance of the AHGs for their intended use in the hands of end-users in clinical settings. The clinical study was performed at five United States (US) clinical sites which included Blood Center of Wisconsin (BCW), LifeShare Blood Centers (LBC), American Red Cross Blood Center Pacific Northwest (PRC), American Red Cross Blood Center Northeast Pennsylvania (NRC), and Emory University Hospital (EUH). The individual AHGs were tested in parallel with currently licensed US products using de-identified leftover clinical (patient or donor) samples.

The studies involved three lots of each of the AHGs. A total of 11,604 de-identified clinical specimen samples were tested in the comparison study, resulting in 45,695 actual tests. Overall, 63.2% of the test profiles were conducted on patient samples and 36.8% were donor samples. The testing was performed in a blind manner. The study covered all the testing included in the intended use statement; DAT, IAT used in antibody screening and identification, and the crossmatch test.

Positive Percentages Agreement (PPA) and Negative Percentages Agreement (NPA) between the DIAGAST and the comparison methods were calculated for each reagent's specificity. The analysis of the results was performed on pooled data from all sites. The results of the study are shown in the table below.

Table 7: Overall Statistical Analysis Results of the Comparison Study

		Number	Lower 95% CI	Point Estimate	Acceptance Criteria
Antibody Screening Anti-IgG	NPA	817/817	99.63%	100%	95%
	PPA	301/302	98.44%	99.67%	95%
Antibody Identification Anti-IgG	PPA	108/108	97.26% (1)	100%	95%
AHG Crossmatch Anti-IgG	NPA	185/185	98.39% (2)	100%	99%
	PPA	185/185	98.39% (2)	100%	99%
DAT Anti-IgG-C3d	NPA	184/184	98.39%	100%	95%
	PPA	104/104	97.16%	100%	95%
DAT Anti-IgG	NPA	192/193	97.57%	99.48%	95%
	PPA	95/95	96.90%	100%	95%
DAT Anti-C3d	NPA	243/243	98.77%	100%	95%
	PPA	44/45	89.89% (3)	97.78%	95%

(1) There was 100% agreement for the antibody identification.
(2) Both the PPA and NPA had 100% agreement but the sample size (NPA-185 and PPA-185) is smaller than what is required to meet the 99% acceptance criteria.
(3) Lower value for the PPA lower confidence bound was obtained due to the limited quantity of samples (45) with complement activated and bound to the red blood cells.

b) Other Special Populations

Cord blood samples were included in the comparator study. Test results demonstrate that this sample type does not affect the reagent's performance.

6. Advisory Committee Meeting

This supplement does not include novel technology; therefore, an advisory committee meeting was not required.

7. Other Relevant Regulatory Issues

There are no other relevant regulatory issues for this submission. The review committee members reviewed their specific sections of the BLA and resolved any issues through information requests with DIAGAST. 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 AHG.

8. Labeling

The Product Office and the Advertising and Promotional Labeling Branch reviewed the container labels, the Instructions for Use (IFU) document, and generic packing labels. All labels met the requirements outlined in 21 CFR Part 610.62, 610.64, 660.28 and 21 CFR Part 809.10.

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 concurred by their respective management. No internal or external disagreements were brought to the attention of the chairperson.

b) Risk/ Benefit Assessment

The benefit of licensing DIAGAST AHG Anti-IgG (Murine Monoclonal), AHG Anti-C3d (Murine Monoclonal), and AHG Anti-IgG-C3d (Murine Monoclonal) is to improve the safety of the blood supply by providing a wide range of anti-human globulin reagents which can increase the probability of the detection of clinically significant antibodies which have coated red blood cells in vivo or *in vitro*. The evaluation of the validation and clinical studies and the manufacturing process reduces the risks associated with licensing these new AHG reagents. In addition, these reagents will be subject to post market surveillance (Medical Device Reporting) which will identify adverse events associated with the product.

c) Recommendation for Postmarketing Activities

We did not recommend any postmarketing activities.