Summary Basis for Regulatory Action Template

Date: March 30, 2020

From: Susie Lee, Chair of the Review Committee

BLA/ STN#: 125710

Applicant Name: Diagnostic Grifols, S.A.

Date of Submission: May 31, 2019

MDUFA Goal Date: March 30, 2020

Proprietary Name: DG Gel 8 ABO/Rh (2D)

Established Name (common or usual name):

STN	Product Name	Cell Line(s)	Proprietary Name
125710\0	Blood Grouping Reagent, Anti-D (Monoclonal Blend)	P3x61 & ESD1M	DG Gel 8 ABO/Rh (2D)

Intended Use/Indications for Use: The DG Gel 8 ABO/Rh (2D) card is for the determination of ABO forward and reverse group, and RhD antigen on the surface of red blood cells of human blood samples.
For use with the DG Gel System. For in vitro diagnostic use.

Recommended Action: The Review Committee recommends approval of this product.

Review Office Signatory Authority: Nicole Verdun, MD, Director, Office of Blood Research and Review

X 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.

Document Title	Reviewer Name	Document Date
Product Review(s) (product office) • *Clinical* • *Non-Clinical*	Susie Lee, OBRR/DBCD/DRB	March 13, 2020
Statistical Review(s) • *Clinical* • *Non-Clinical*	Paul Hshieh, OBE/DB/TEB	November 06, 2019
CMC Review • *CMC (Product Office)* • *Facilities Review (OCBQ/DMPQ)* • *CMC (DBSQC)*	Susie Lee, OBRR/DBCD/DRB Priscilla M. Pastrana, OCBQ/DMPQ/MRBII Claire Wernly, OCBQ/DBSQC/LMIVTS	March 13, 2020 March 9, 2020 January 16, 2020
Labeling Review(s) • *Product Office* • *APLB (OCBQ/APLB)*	Susie Lee, OBRR/DBCD/DRB Dana Jones, OCBQ/DCM/APLB	March 13, 2020 January 15, 2020
Lot Release Protocols/Testing Plans	Marie Anderson, OCBQ/DBSQC/QAB	February 10, 2020

1. Introduction

Diagnostic Grifols, S.A., (hereafter known as Grifols) located in Barcelona, Spain, submitted an Original Biologic License Application (BLA) for the licensure of Blood Grouping Reagent (BGR), Anti-D (Monoclonal Blend), which will be included in a new DG Gel 8 ABO/Rh (2D) gel card for use with the DG Gel System. The new gel card also contains FDA licensed BGRs, Anti-A (STN 125449), Anti-B (STN 125450), Anti-AB (STN 125456), Anti-D (P3x61) (STN 125452) and neutral gel as a control (BK180262).

The subject BGR, Anti-D (Monoclonal Blend) contains both Anti-D (P3x61) For Further Manufacturing Use (FFMU) supplied by Diagast and Anti-D (ESD1M) FFMU supplied by Alba Bioscience Ltd. (Alba). A manufacturing supplement as a companion submission (STN 125550\9) was submitted by Alba requesting approval to supply Grifols with Anti-D (ESD1M) FFMU.

Intended Use/Indications for Use: (Copied from submission)
The DG Gel 8 ABO/Rh (2D) card is for the determination of ABO forward and reverse group, and RhD antigen on the surface of red blood cells of human blood samples.
For use with the DG Gel System. For in vitro diagnostic use.

Chronology of Events

CBER received this original submission on May 31, 2019. The submission was filed on July 15, 2019. CBER received 16 amendments in response to 14 information requests.

2. Background

Pre-Submission Meeting

The clinical protocol was reviewed in advance by the FDA and changes based on a meeting with the FDA (B170070) were implemented prior to beginning the studies.

Product Description

DG Gel® 8 cards are based on the microtube column agglutination technology with pre-dispensed reagents. The DG Gel 8 ABO/Rh (2D) cards consist of a plastic support with 8 microtubes containing gel solution mixed with different antibody reagents or a buffer. The DG Gel 8 cards are (b) (4) sealed with a bilayer thin film and allocated into a plastic holder of 25 cards. Each cardboard box contains two plastic holders (50 cards).

The microtubes containing specific antibodies incorporated in the gel solution are used as a reaction medium, where red blood cells of unknown phenotype may agglutinate when in contact with the antibodies. The buffer microtubes contain no antibodies and are used as a control for blood grouping reagents (Control) or used in techniques where anti-ABO antibodies present in serum or plasma samples react directly with reagent red blood cells (Neutral). **Table 1** shows the DG Gel 8 ABO/Rh (2D) card configuration.

The gel column acts as a filter that traps agglutinated red blood cells as they pass through the gel column during the centrifugation of the card. The gel column separates agglutinated red blood cells from non-agglutinated red blood cells based on size. Any agglutinated red blood cells are captured at the top of, or along the gel column, and non-agglutinated red blood cells reach the bottom of the microtube forming a pellet.

Table 1: The new DG Gel 8 ABO/Rh (2D) card configuration

Microtube 1	Microtube 2	Microtube 3	Microtube 4	Microtube 5	Microtube 6	Microtube 7	Microtube 8
Anti-A	Anti-B	Anti-AB	Anti-D (VI-)	Anti-D (VI+)	Neutral	Neutral	Control
STN 125449	STN 125450	STN 125456	STN 125452	Subject reagent			BK 180262

Marketing History:
The subject Blood Grouping Reagent, Anti-D (Monoclonal blend) obtained the CE marking for the European market in May 2017 and has been commercialized in the European Union since then.

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

1) In Vitro Substances (IVS)
The subject BGR is a monoclonal blend of two clones (P3x61 and ESD1M) of antibody concentrates (IVS) sourced from Diagast located in Loos Cedex, France and Alba located in (b) (4) under shared manufacturing agreements. The Anti-D (P3x61) can be used for the detection of weak D antigens; however, the partial DVI epitope of the D antigen will not be detected with Anti-D (P3x61). Anti-D (ESD1M) can detect weak D antigen as well as the partial DVI epitope.

The IVS are purchased as licensed FFMUs. The FFMU (clone P3x61) has been approved by FDA for Diagast to supply the clone to Grifols (STN 125442). The FFMU (clone ESD1M) was submitted by Alba as a companion supplement to this BLA.

Copies of Certificates of Analysis (CoAs) were reviewed for both FFMUs to include the cell line, product code, bath number, date of manufacture, expiration, preservative, (b) (4), specificity, (b) (4). Grifols performs testing for every new batch of FFMU received. **Table 2 & Table 3** outline the receipt testing and specifications (excerpted from submission).

Table 2: Anti-D antibody solution (clone P3x61) specifications

(b) (4)

Table 3: Anti-D antibody solution (clone ESD1M) specifications

(b) (4)

2) In Vitro Product (IVP)
   a) Manufacturer
      Besides the facility at Passeig Fluvial, 24, Parets del Valles, Barcelona, Spain, six other Grifols subsidiary companies are involved in the manufacture and testing of DG Gel 8 cards as shown in **Table 4**.

Table 4: List of companies involved in manufacturing and testing of DG Gel 8 cards

Name	Address	Responsibility
Grifols Diagnostic Solutions, Inc.	4650 Horton Street, Emeryville, California 94608, USA	The US Agent appointed by Diagnostic Grifols, S.A. belongs to Grifols Diagnostic Solutions, Inc. Warehouse (off site storage). Distribution activities linked to the delivery of products to customers inside the US. Receives the customer feedback performing a preliminary screening and reports to Diagnostic Grifols, S.A. those complaints that need further investigation or are related to events that need to be reported to the FDA.
Grifols International, S.A.	Polígono Levante C/ Can Guasch, s/n, 08150 - Parets del Vallès Barcelona (Spain)	Warehouse (off site storage) Distribution activities (delivery of products to US)
Grifols, S.A.	Avda. Generalitat, 152-158, 08714 - Sant Cugat del Vallès Barcelona (Spain)	Pest control IT systems Cleaning Maintenance of production equipment and installations Personnel recruitment and training Work clothes management Environment Management Personnel Health and Safety Corporate Image & Communications
Laboratorios Grifols, S.A.	Polígono Industrial Sector Z C/ Logística, 2 08150 - Parets del Vallès Barcelona (Spain)	Supplier of the purified water
Instituto Grifols, S.A.	Polígono Industrial Levante C/ Can Guasc, 2 08150 - Parets del Vallès Barcelona (Spain)	HVAC system maintenance and validation Calibration of inspection, measuring and test equipment Special equipment qualification (e.g. autoclave) Raw material for laboratory microbiology and microbiology identification testing
Biomat	Polígono Industrial Levante C/ Llevant, 11 08150 – Parets del Vallès Barcelona (Spain)	Plasma samples supplier

   b) Composition
      All the ingredients, except the antibody concentrates, used for the manufacturing of the new BGR are the same as those used for the manufacturing of other Grifols' licensed BGRs. Certificates of Analyses (CoAs) were reviewed for ingredients and were found to be complete

according to the list provided. The ingredients and receipt testing are shown in **Table 5**.

Table 5: The ingredients and receipt testing

Ingredients	Receipt testing performed for new batch received
Antibody concentrates Anti-D (clone P3x61)	See **Table 2**
Antibody concentrates Anti-D (clone ESD1M)	See **Table 3**
(b) (4)	

c) Method of Manufacturing and Packaging
The manufacturing process includes acceptance or testing of incoming materials, product formulation and filling, labeling, packaging, secondary packaging and release for distribution. In-process testing is performed during the manufacturing process, and lot release testing is performed on the final product.

(1) Manufacturing Process
(b) (4)

(b) (4)

The date of manufacture (DOM) of the DG Gel card is the date when manufacturing of the (b) (4) is prepared. (b) (4)

(b) (4)

(d) Packaging: Acceptable cards are collected into a 25-card holder, and (b) (4) sets of 25-card holders are combined into an in-process container. (b) (4) 25-card holder out of (b) (4) is checked visually for the presence of bubbles in the microtubes, i.e., absence of gel in the reaction chamber. All gel cards in the in-process containers (the (b) (4) sets of 25-card holders) are tested for seal integrity (b) (4). After the (b) (4) test, cards are visually inspected again for seal integrity by sampling (b) (4) cards out of (b) (4) in-process containers ((b) (4) cards). The final product consists of two 25-card holders manually filled into a carton, along with labeling (secondary packaging). A DG Gel 8 ABO/Rh (2D) card lot size can be up to (b) (4) cards with the same combination of gel lots in each card. Once the product meets all requirements, the Technical Director releases the lot for distribution.

d) Specifications and Test Methods: The in-process and product release test methods and specifications are summarized in **Tables 6-10**.

(1) In-Process Controls and Acceptance Criteria: In-process controls monitor the performance of all processing steps of DG Gel 8 ABO/Rh (2D).

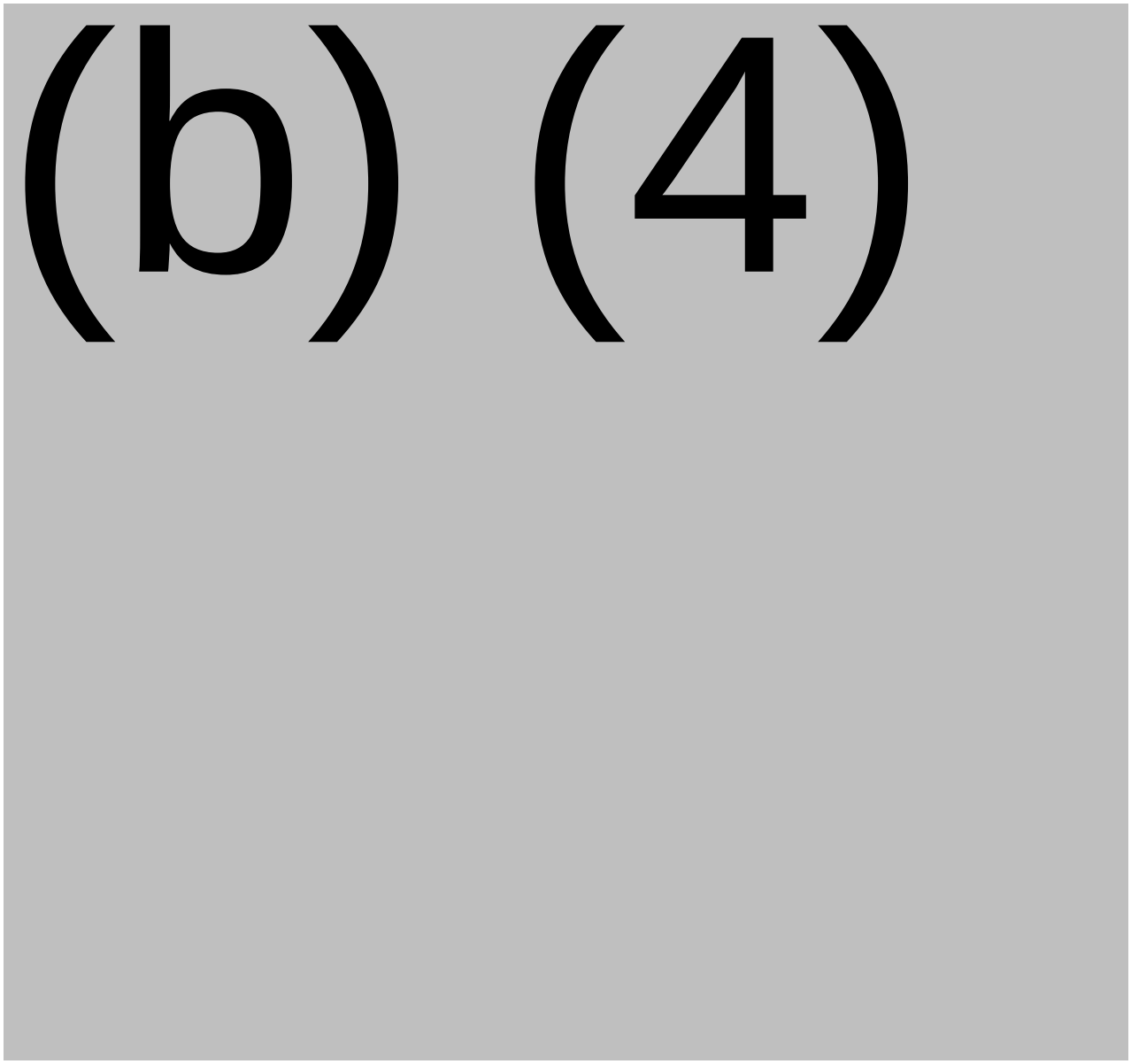
(a) In-Process Controls of (b) (4) Buffer: **Table 6** is a list of QC tests performed before filling and after filling the buffer into the bottles

(b) (4)

Moreover, additional controls are performed by the production department during the manufacturing process, including (b) (4)

(b) In-Process Controls of Gels: (b) (4)

(b) (4)



Additional in-process controls are performed by the production department, including (b) (4)

(2) DG Gel 8 ABO/Rh (2D) Cards Controls: The controls performed by QC in this step of the manufacturing process are final QC testing of the finished DG Gel 8 ABO/Rh (2D) gel card product for lot release.

Additional controls performed by the production department in this stage include cross-contamination control by visual inspection of (b) (4)

(b) (4) on the filling module, which are placed at the beginning of the filling operation to check for splashes during the filling process; inspection of defects by computer visual inspection; dimensional control of the film using a (b) (4) ; sealing control by (b) (4) test; visual inspection of gel holders for absence of gel in the reaction chamber (bubbles); and reconciliation of materials such as labels, gel, cards, holders and aluminum film.

(a) Lot Release Testing
Potency testing is performed on the (b) (4) Appearance, Specificity Testing (positive) and Test to Confirm the Absence of Contaminating Antibodies are performed on samples of labelled and sealed DG Gel 8 ABO/Rh (2D) cards, before being packaged. A Packaging Control (see **Table 10**) is performed on the finished product packaged in the labeled carton boxes. The test methods, product sampling procedure and acceptance criteria for lot release testing are summarized in **Tables 8 and 9**.

(b) (4)

Table 9: Analytical test performed for DG Gel 8 ABO/Rh (2D) card (lot release testing)

Testing**	Card Samples	Red Blood Cells Sample	Acceptance Criteria
Appearance (visual inspection)	All cards used for Specificity testing and test to confirm absence of contaminant antibodies	NA****	Microtube 1: Anti-A: blue gel Microtube 2: Anti-B: yellow gel Others: colorless gel
Specificity: Positive and Negative samples (according to the method described in the Instructions for Use)	- (b) (4) cards filled from the beginning of each sublot bottle used to fill the card lot. - (b) (4) cards from the end of the last sublot bottle.	At least (b) (4) ABO/Rh red blood cell samples and (b) (4) . <u>Minimum samples:</u> For ABO: (b) (4) samples A1 (b) (4) samples A2 (b) (4) samples B (b) (4) samples A1B (b) (4) samples A2B For Rh (D)*: (b) (4) sample CcDee (R1r) (b) (4) sample ccDee (R0r) (b) (4) sample Ccddee (r'r) (b) (4) sample ccddEe (r"r) (b) (4) sample A1 ccddee (rr) (b) (4) sample B ccddee (rr) (b) (4) sample O ccddee (rr) (b) (4) samples weak D (D^W) (b) (4) sample partial VI (DVI)	<u>Positive results:</u> • ABO/Rh (D): agglutination (b) (4) (normal expression samples) • Anti-A and Anti-AB: agglutination (b) (4) (samples A2 and A2B) • Anti-DVI-/ Anti-DVI+: agglutination (b) (4) (w+) (weak D samples) • Anti-DVI+: agglutination (b) (4) (w+) (partial DVI sample) • Reverse group: agglutination (b) (4) (w+) • Differences (b) (4) between cards when processing the same sample. <u>Negative results:</u> Anti-DVI- (partial VI sample) and other negative results: No agglutination of red blood cells and no hemolysis
Specificity: Absence of contaminant antibodies*** (according to the method described in the Instructions for Use)	(b) (4) cards filled from the beginning of each sublot bottle used to fill the card lot. (b) (4) card from the end of the last sublot bottle.	Red blood cells which express the following antigens: A, B, AB, C, c, D, E, e, C^W, K, IgG (Red blood cells negative for the antigen corresponding to the tested antibody)	Negative results No agglutination of red blood cells and no hemolysis

Note: *Samples of partial D category IV and V are not tested in the final product due to its very low frequency in the population (<0.02%).

**Test for (b) (4) in Rh blood grouping reagents is not performed because correct gel formulation is guaranteed by the potency test performed in (b) (4) against in-house working reference standard, the agglutination strength required in the specificity test, and all the in-process controls performed on each lot of gel.
***The complete set of samples to test the absence of antibodies as recommended by FDA is performed on the (b) (4) instead of on the final product.
****NA=Not applicable

Table 10: Packaging control of DG Gel 8 ABO/Rh (2D) finished product packaged in the labeled carton boxes (lot release testing)

Testing	Card Samples	Acceptance Criteria
Packaging Control	At least (b) (4) cards from (b) (4) Retention Samples from each lot	Verify the following information is correct: - code of gel cards labels - code of box labels - code of the Package Insert - lot number and expiry date shown on the gel cards and on the box labels

B. Product Quality

Testing specifications

The analytical methods and their validations and/or qualifications reviewed for the Anti- DVI+ Blood Grouping Reagent were found to be adequate for their intended use.

C. CBER Lot Release

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

D. Facilities review/inspection

Facility information and data provided in the BLA were reviewed by CBER and found to be sufficient and acceptable. The facility involved in the manufacture of the Blood Grouping Reagent (BGR) Anti-DVI+ (Monoclonal Blend, Clones P3x61 and ESD1M) is listed in the table below.

Name/Address	FEI Number	DUNS Number	Inspection/Waiver	Justification/Results
Production and testing of the in vitro Product Diagnostic Grifols S.A. Passeig Fluvial 24, Parets del Vallés Barcelona 08150 Spain	3002772505	466190695	Waiver	December 2018 Surveillance ORA NAI

The pre-license inspection was waived. Team Biologics performed a surveillance inspection of the Diagnostic Grifols facility on December 10-14, 2018. No 483 was issued and the inspection was classified as No Action Indicated (NAI).

E. Container Closure System

The Blood Grouping Reagent (BGR) Anti-DVI+ (Monoclonal Blend, Clones P3x61 and ESD1M) is filled into DG Gel® 8 cards. The DG Gel® 8 cards consist of a polypropylene support with eight microtubes solution (b) (4) The DG Gel® 8 cards are (b) (4) sealed with an aluminum foil strip coated with a polypropylene peel layer (b) (4) The DG Gel® 8 cards are allocated into a polyethylene terephthalate (PET) holder (b) (4)

Diagnostic Grifols S.A. conducted the container closure integrity testing of the DG Gel® 8 cards at their Parets del Vallés Barcelona (Spain) facility using the seal integrity method; all acceptance criteria were met.

F. Environmental Assessment

The BLA 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 will not alter significantly the concentration and distribution of naturally occurring substances and no extraordinary circumstances exist that would require an environmental assessment.

4. Software and Instrumentation

Not applicable

5. Analytical Studies

A. Stability Study

The assigned shelf-life for DG Gel 8 ABO/Rh (2D) card is 12 months stored at 2-25 °C. The stability study includes (1) (b) (4) Buffer Solution stability, (2) Anti-DVI+ reagent stability and (3) Expiration date assignment for DG Gel 8 ABO/Rh (2D) cards.

1) (b) (4)
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Grifols claims the (b) (4) buffer solution is stable up to (b) (4)

2) Anti-DVI+ Reagent: The stability study includes a long-term stability and a (b) (4) transport study. The study was performed on DG Gel 8 Pilot Anti-DVI+ cards, which has (b) (4) The card profile is shown in the **Table 11**.

(b) (4)

Three conformance lots were included in the study and cards were randomly selected from the beginning, middle and end of the lot. The long-term stability samples were stored at (b) (4) controlled temperature and (b) (4) conditions, 5 °C, 25 °C (b) (4) and 25 °C (b) (4), as well as at a moderately accelerated condition, (b) (4) Cards were tested at the planned time points, day zero, 1, 2, 3, 6, 9, 12, (b) (4) months.

(b) (4) transport conditions, (b) (4) were included in the long-term stability. At the beginning of the study, some cards that were intended for 25 °C/(b) (4) and 25 °C/(b) (4) were placed at the (b) (4) conditions for (b) (4) After this time, the cards were returned to the home storage conditions at 25 °C/(b) (4) and 25 °C/(b) (4) (b) (4) **Table 12** shows the temperature (b) (4) conditions and the number of gel card samples included in the stability study.

Table 12: Stability conditions for DG Gel 8 pilot Anti-DVI+ cards

DG Gel 8 card	Stability conditions	(b) (4) transport	Total cards for each lot
DG Gel 8 Pilot Anti-DVI+ Lots: (b) (4)	5 °C	NA	Long term stability: (b) (4) cards (b) (4) duplicates x (b) (4) conditions x (b) (4) intervals
	25 °C/ (b) (4)	(b) (4)	(b) (4) transport: (b) (4) cards x (b) (4) duplicates x (b) (4) conditions x (b) (4) intervals
	25 °C/		Expiry date complementary test: (b) (4) cards (include (b) (4) replicates) x (b) (4) conditions x (b) (4) interval
	(b) (4)	NA	

Testing was performed at each time point, including appearance of gel, supernatant, and functional tests as described in the Instructions for Use of the subject product. **Table 13** shows the testing intervals when the analyses were performed. **Table 14** shows the red blood cells samples tested in each time point.

Table 13: Stability testing time points for long term stability study and (b) (4) transport study

Time testing after manufacturing (Months)	Long term stability studies			Moderately accelerated study	(b) (4) transport conditions			
	5 °C	25 °C (b) (4)	25 °C	(b) (4)	(b) (4)			
0	✓	✓	✓	✓	✓	✓	✓	✓
1	✓	✓	✓	✓	NA	NA	NA	NA
2	✓	✓	✓	✓	NA	NA	NA	NA
3	✓	✓	✓	✓	✓	✓	✓	✓
6	✓	✓	✓	✓	NA	NA	NA	NA
9	✓	✓	✓	✓	NA	NA	NA	NA
12	✓	✓	✓	✓	✓	✓	✓	✓
(b) (4)								

Table 14: The red blood cells samples tested in each time point

DG Gel 8 cards	Procedure	Samples
DG Gel 8 Pilot Anti-DVI+	Determination of human D antigen	(b) (4)

The acceptance criteria for stability testing includes: positive for expected positive results, agglutination strength (b) (4) for Anti-DVI+ reacted with samples showing normal D expression, agglutination strength (b) (4) w+ for Anti-DVI+ reacted with D variant samples (Weak and DVI), and negative for expected negative results, no agglutination and/or hemolysis observed.

Grifols submitted study data up to (b) (4) months. The study results met all stability requirements. The results show that there were no color changes, no cracked or drying gel, and the supernatant lines met the stability criteria. The functional tests met the stability criteria stated in the protocol up to (b) (4) months stored at 5 °C, 25 °C/(b) (4) 25 °C/(b) (4) (b) (4), including the cards under transport simulation. The negative samples showed no signs of agglutination or hemolysis. All normal expression samples showed a reaction strength of at least (b) (4) with cards stored at 5 °C, 25 °C (b) (4) The DVI variant samples tested along the study showed a reaction strength of at least (b) (4) with cards stored at 5 °C, 25 °C (b) (4)

The assigned shelf-life for the Anti-DVI+ reagent is (b) (4) months stored at 2-25 °C.

3) DG Gel 8 ABO/Rh (2D) Card Stability: The card is composed of the new reagent Anti-DVI+ and the licensed BGRs, Anti-A, Anti-B, Anti-AB and Anti-DVI- (P3x61) and control gel. The reagent with the shortest expiry dating determines the final expiry date and storage condition of the card.

   The assigned shelf-life for DG Gel 8 ABO/Rh (2D) card is 12 months stored at 2-25 °C.

B. Precision Study

1) Repeatability and Reproducibility Study
   (b) (4) of Anti-DVI+ reagent in DG Gel cards was tested against the same precision panel at each of the three clinical study sites as shown in **Table 15**.

Table 15: Cells panel for repeatability and reproducibility study

Sample ID	Repro Rh Samples	D Antigen Test Results
(b) (4)		

The study was performed by all three US sites using the (b) (4) of Anti-DVI+, and according to the following profile:

a) Manual (at LBC): (b) (4) samples x (b) (4) operators x (b) (4) replicates x (b) (4) runs x (b) (4) days = (b) (4) tests.
b) Erytra (at BCW and TRI): (b) (4) samples x (b) (4) days (during (b) (4) days) x (b) (4) runs (b) (4) x (b) (4) replicates (= (b) (4) per Erytra) x (b) (4) sites for a combined total= (b) (4) tests.

The results of the study show that there are (b) (4) data points in total (manual tests + Erytra tests), and overall, 100% agreement between the expected results and the actual results with the new Anti-DVI+ Blood Grouping Reagent.

2) Lot-to-Lot Study
The Lot-to-Lot precision Study was done in-house by Diagnostic Grifols, S.A. (b) (4) samples were selected based on their known antigen expression for the D antigen. The study was carried out according to the following profile: (b) (4) samples x (b) (4) lots x (b) (4) operators x (b) (4) days x (b) (4) replicates = (b) (4) tests. The results of the study show there were no discrepancies between the expected results and the actual results (see **Table 16**).

Table 16: The results of lot-to-lot study

Sample ID	Repro Ph Samples	D Antigen Test Results (Agglutination Grade)
(b) (4)		

C. Sample Aging and Anticoagulant Studies

The sample aging and anticoagulant studies were performed to demonstrate that red blood cell D antigens can be accurately typed with the new Anti-DVI+ reagent both at the beginning of and after the expiration date of the blood product in a variety of anticoagulants (ACD, CPD, CPD-A1, CP2D) or additive solutions (AS-1, AS-3). An EDTA sample was collected at the same time. (b) (4) donors of known D blood group antigens (b) (4) positive and (b) (4) negative) were determined. Results were compared to each other and to the results of the EDTA sample. All the results obtained were as expected. There was no decline in the strength of reactions observed at the end of the testing period versus the freshly-collected units or their EDTA sample tubes. The results demonstrated that for the donor samples collected and stored in EDTA, ACD, CPD, CPD-A1 and CP2D or converted from ACD, CPD or CP2D and stored in AS-1 or AS-3, the D antigen could be successfully typed on the day of collection to 7 days past the expiration date (range (b) (4) days).

Additionally, an In-House Performance testing to confirm the correct performance of the Anti-DVI+ reagent with samples collected with citrate and heparin was carried out. The study demonstrated that samples collected with

citrate and heparin confirm that the reagent Anti-DVI+ can accurately type D antigen up to (b) (4) days after sample collection.

D. DVI Positive Sample Studies

In the Instructions for Use (IFU) document, Grifols claims that the Anti-DVI+ reagent can detect DVI positive samples, however, the in-house performance study, ATT CLI3, included only (b) (4) DVI positive sample. In a September 10th, 2019 information request, FDA asked Grifols to provide additional data to support their IFU claim. Grifols responded on September 18, 2019 (Amendment 3) with an additional three studies (two internal studies and one external study conducted in Spain) which included a total of (b) (4) DVI+ samples.

In the two internal studies Grifols compared their new Anti-DVI+ reagent with several approved Anti-DVI+ reagents, using (b) (4) samples, of which (b) (4) were DVI positive. The test samples were typed previously by the reference centers supplying the samples or by Grifols using FDA licensed reagents.

The external study was carried out in the Hospital Clinical (Barcelona) in January 2014. The study compared their new Anti-DVI+ reagent with several approved Anti-DVI+ reagents using a total of (b) (4) samples, of which (b) (4) were positive for the DVI variant. The D variant samples had been previously tested by Grifols or were categorized by Progenika-Grifols using a molecular technique.

There were no discordant results in the internal and external studies for the DVI positive samples. In conclusion, Grifols provided sufficient data to verify their claim that the Anti-DVI+ reagent, which includes clones P3x61 and ESD1M, can detect the DVI variant.

E. Interfering Substance Study

This study was conducted to demonstrate the effect of potentially interfering substances in blood samples on the performance of Anti-DVI+ reagent in DG Gel 8 ABO/Rh (2D) gel cards. (b) (4) patient blood samples with equal numbers of mild and moderate hemolysis, normal and elevated levels of (b) (4) were included in the study. The blood samples with interfering substance were selected based on the plasma concentration of these metabolites. The levels of hemolysis were selected by visual aspect. All samples were tested by the manual method described in the Instructions for Use.

The results of the study demonstrated that higher than normal concentrations of (b) (4) do not negatively affect the performance of Anti-DVI+ reagent in DG Gel 8 ABO/Rh (2D) gel cards.

6. Clinical Studies

A. Clinical Program

1) Comparison Study

Clinical studies were performed at three US sites and in-house at the Grifols facility. The three US sites were Blood Center of Wisconsin (BCW), TriCore Research Institute (TRI), and Life Share Blood Center (LBC). The clinical studies performed in the US consisted of a method comparison, a precision study and a sample aging and anticoagulant study. The in-house study consisted of a lot-to-lot precision study and an interfering substance study. A total number of 3,046 unique specimens from a diverse population in broad geographical areas and composed of hospital patients (68.48%) and blood donors (31.52%). Testing was performed in accordance with the Instructions for Use documents for both the trial and the comparator reagents.

a) Study Design

The study was designed to obtain more than 50% of the samples from patients and less than 50% from donors; and to be conducted at three sites using two lots of Anti-DVI+ where each lot was tested with at least 500 samples for a combined total of at least 1,000 samples at each site. The Anti-DVI+ reagent was tested on the Erytra® at two sites, Blood Center of Wisconsin (BCW) and TriCore Research Institute (TRI), and the manual gel method was performed at one site, LifeShare Blood Center (LBC). The comparative method was Diagast's BGR, Anti-D (IgM/IgG) using the manual tube method. **Table 17** lists the comparator reagents used in the study. The testing was done in a blinded manner; the code was only broken when the completed test results were ready to be compared; any "doubtful" result (i.e. reaction grades difficult to define as negative result or as weak positive result by the reading algorithm of the instrument) was solved and validated prior to comparison. A direct comparison of grading strength was performed between the new and the comparator method. Differences between grading strengths greater than "2+" were investigated and reported. All discrepancies were re-tested once. The results obtained after the investigation of the discrepancy were not used to make further estimations of agreement.

Table 17: The comparators reagents used in the Study

Comparator Reagents	Studies
BGR, Anti-D (IgM/IgG), Lot (b) (4), Exp. 31Oct2019, manufactured by Diagast, STN 125621	Method Comparison
IUO Anti-DVI+ (clones P3x61 and ESD1M), Lot (b) (4) Exp. 31Oct2019, manufactured by Grifols	50% of Method Comparison All of precision and aging and anticoagulant studies
IUO Anti-DVI+ (clones P3x61 and ESD1M), Lot (b) (4), Exp. 31Oct2019, manufactured by Grifols	50% of Method Comparison

b) Statistical Method and Results

PPA and NPA along with one-sided 95% lower confidence limit (LCL) between the two methods were calculated for each site independently and

pooling the data from all the sites. The acceptance criterion is that the one-sided 95% LCL should exceed 99%.

c) Results of Comparison Study
Among the 3,061 microtubes (each sample used one microtube of DG Gel 8 plastic cards) processed, 15 samples were disqualified which led to 3,046 valid individual unique specimens composed of approximately 68.48% hospital patients and 31.52% blood donors. The valid sample distribution across sites and lots is displayed in **Table 18** (excerpted from Table1, page 1 of 1, Appendix 10 of DG008 Clinical study report).

Table 18: The valid sample distribution across site and lot

Site	Source Patient/donor	Required No	Actual	# of patients	Tested with		Age of Sample	> 79 Y/O	ped/ cord
					Lot 1	Lot 2	Days 7 to 9		
BCW	50% pat. /50% dnr.	1,000	1,030	510	519	511	397	30	57
LBC	50% pat. /50% dnr.	1,000	1,013	573	507	506	68	23	26
TRI	100% patient	1,000	1,003	1,003	502	501	156	65	49
TOTAL		**3,000**	**3,046**	**2,086**	**1,528**	**1,518**	**621**	**118**	**132**

Thirteen out of 15 samples were disqualified due to positive reactions obtained with the testing of the Negative Control (Neg CTL), which indicated samples were not suitable for testing with Anti-DVI+ or by tube comparative method. The thirteen samples were all from patient samples. Two discrepancies were originally disqualified as the applicant considered they were due to operator error. However, FDA did not agree with the exclusion and requested re-analysis with the two discrepancies included.

The results of the study showed positive and negative percent agreements (PPA and NPA) of 99.96% and 99.76%, respectively; with corresponding one-sided 95% LCLs at 99.82% and 98.89%. The PPA met the acceptance criterion while the NPA did not because of the two discrepancies. These results are considered acceptable, because the NPA LCL was 99% after rounding decimals to the nearest whole number, and as Grifols stated, since the discrepancies were due to operator error. The comparison study results of 3,048 samples are displayed in **Table 19** (excerpted from Table 1, page 1 of 2, 002_Response_Amendment dated October 10, 2019).

Table 19: Site Individual and Pooled 2x2 Tables for Anti-DVI+

		anti-D (IgM clones (b) (4) B10 IgG clones (b) (4) by Tube Method							
		BCW		LBC		TRI		Pooled	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
anti-D^{VI+} - (P3x61 and ESD1M) in Gel	Positive:	859	1	884	0	880	0	2,623	1
	Negative:	0	171	1	129	0	123	1	423
PPA (Lower 95% CB):		100.00% (99.65%)		99.89% (99.47%)		100.00% (99.66%)		99.96% (99.82%)	
NPA (Lower 95% CB):		99.42% (97.27%)		100.00% (97.70%)		100.00% (97.59%)		99.76% (98.89%)	
OPA (Lower 95% CB):		99.90% (99.54%)		99.90% (99.53%)		100.00% (99.70%)		99.93% (99.79%)	

d) Deviations: Seven types of deviations were observed; five of them were sponsor-approved and two were unapproved.

Deviations 1-3: Moved Saturday test date to either Friday or Monday. Approved deviations and no impact on study.

Deviations 4-5: TRI lost the reproducibility run due to a fluidics error on Day 5, and after leak was repaired completed testing on Day 8. BCW moved Day 10 testing to Day 12 due to repairs needed for Erytra instrument. Approved deviations and no impact on study.

Deviations 6-7: Due to problems with Erytra requiring servicing, 56 of 60 samples were not tested in the same 8 hours shift with the comparator on January 18, 2018 but completed on January 22, 2018. On February 23, 2018, BCW did not complete investigation of grading discrepancy until the following day. Grifols believed the delay of testing didn't affect the validity of the test results.

2) Clinical/Statistical/Pharmacovigilance
Not Applicable

B. Pediatrics

The comparator studies for each of the blood grouping reagents included neonate and cord blood samples. Test results demonstrate that these samples do not affect the performance of the reagents.

Hospital patients include subjects of all ages and special efforts were made to include a significant number of cord or pediatric blood samples (132 samples) and persons older than 79 years of age (118 samples).

C. Other Special Populations

Not applicable

7. Advisory Committee Meeting

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

8. Other Relevant Regulatory Issues

The review committee members from DBCD, DB, DMPQ, DCM and DBSQC reviewed their specific sections of the BLA and resolved any issues through information requests with Grifols. 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.

9. Labeling

The Advertising and Promotional Labeling Branch (APLB) found the proposed Instructions for Use (IFU), and the package and container labeling, acceptable from a promotional and comprehension perspective.

Grifols submitted container labels, packing labels, and the Instructions for Use (IFU) documents for review. All labels met the requirements outlined in 21 CFR 660 and 21 CFR 809.10.

10. Recommendations and Risk/ Benefit Assessment

A. Recommended Regulatory Action

The review committee members, representing the necessary review disciplines (DBCD, DB, DMPQ, 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 subject BGR is a blend of two monoclonal antibody products that were previously approved by the FDA in October 2016 and in July 2013. The licensing of the subject BGR for use with the DG Gel System will not alter the benefit-risk profile of the product because the same manufacturing controls and processes are in place.

C. Recommendation for Postmarketing Activities

There are no post-marketing activities associated with this submission.