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# Summary Basis for Regulatory Action

**Date:** May 17, 2022

**From:** Kimberly Bigler, Medical Technologist, Review Committee Chair, OBRR/DBCD

**BLA/ STN#:** 125705/0 & 125711/0

**Applicant Name:** Diagnostic Grifols, S.A.

**Date of Submission:** 125705/0 May 6, 2019  
125711/0 May 22, 2019

**MDUFA Goal Date:** May 21, 2022

**Proprietary Name:** DG Gel 8 Direct Coombs

**Established Name (common or usual name):**

125705/0 Anti-Human Globulin Anti-IgG, -C3d (Rabbit/Murine Monoclonal)  
125711/0 Anti-Human Globulin Anti-C3d (Murine Monoclonal)

**Device Procode:** QHS

**Intended Use/Indications for Use:** *(copied from page one of the Instructions for Use document for DG Gel 8 Direct Coombs card)*

The DG Gel 8 Direct Coombs card is used for the evaluation of the Direct Antiglobulin Test of two different human blood samples. It allows to differentiate red blood cells sensitized in vivo by IgG type immunoglobulins or the complement C3d fractions. For use with the DG Gel System.

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

**Review Office Signatory Authority:** Nicole Verdun, 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.

| <b>Document Title</b>   | <b>Reviewer Name</b>   | <b>Document Date</b>   |
|---|--|--|
| Product Review(s) (product office)  | Kimberly Bigler, OBRR/DBCD/DRB   | April 7, 2022  |
| Statistical Review(s)   | Paul Hshieh, OBE/DB/TEB  | March 17, 2022   |
| CMC Review <ul style="list-style-type: none"> <li>• CMC (Product Office)</li> <li>• Facilities Review (OCBQ/DMPQ)</li> <li>• QC, Test Methods and Product Quality (OCBQ/DBSQC)</li> </ul> | <ul style="list-style-type: none"> <li>• Kimberly Bigler, DBCD/DRB</li> <li>• Priscilla Pastrana, OCBQ/DMPQ</li> <li>• Yen Phan, OCBQ/DBSQC</li> </ul> | <ul style="list-style-type: none"> <li>• April 7, 2022</li> <li>• January 8, 2020</li> <li>• January 29, 2020</li> </ul> |
| Labeling Review(s) <ul style="list-style-type: none"> <li>• Product Office</li> <li>• Promotional (OCBQ/APLB)</li> </ul>  | <ul style="list-style-type: none"> <li>• Kimberly Bigler, DBCD/DRB</li> <li>• Dana Jones OCBQ/DCM/APLB</li> </ul>                                      | <ul style="list-style-type: none"> <li>• April 7, 2022</li> <li>• March 30, 2022</li> </ul>                              |
| Lot Release Protocols/Testing Plans   | Varsha Gharnepudi OCBQ/DBSQC   | <ul style="list-style-type: none"> <li>• May 16, 2022</li> </ul>   |

## 1. Introduction

Diagnostic Grifols (U.S. License #1887) (Grifols) submitted two original Biological License Applications (BLAs) requesting the approval to manufacture and distribute the following Anti-Human Globulin (AHG) reagents:

Table 2: STNs and Proper Names

| <b>STN</b> | <b>Proper Name</b>                            |
|------------|---|
| 125705/0   | AHG Anti-IgG, -C3d (Rabbit/Murine Monoclonal) |
| 125711/0   | AHG Anti-C3d (Murine Monoclonal)              |

The AHG reagents are designed to detect IgG and C3d components on the surface of human red blood cells when used with the DG Gel 8 Direct Coombs card. The manufacture and assembly of these products are performed at the Diagnostic Grifols facility at S.A., Passeig Fluvial, 24, 08150 Parets del Vallès, Barcelona, Spain.

The in vitro substances (IVS) for manufacture of these products are provided to Diagnostic Grifols by Diagast, located in Loos, France, under a shared manufacturing arrangement. Diagast submitted companion BLA applications for the IVS For Further Manufacturing Use (FFMU) products, Anti-IgG, -C3d (STN 125708/0) and Anti-C3d (STN 125707/0).

## 2. Background

### Product Description:

The AHG reagents will be filled into a DG Gel 8 Direct Coombs card along with the

already licensed AHG Anti-IgG (BL 125445/0) and the 510(k) cleared neutral gel control (BK180262). The DG Gel 8 Direct Coombs card is used to perform the Direct Antiglobulin Test on human blood samples. The gel card is used to determine if red blood cells are sensitized in vivo by IgG type or the complement C3d.

Table 3 displays the configuration of the DG Gel 8 Direct Coombs card

Table 3: DG Gel 8 Direct Coombs Microtube Description

| 1         | 2   | 3   | 4    | 5         | 6   | 7   | 8    |
|-----------|-----|-----|------|-----------|-----|-----|------|
| IgG, -C3d | IgG | C3d | Ctrl | IgG, -C3d | IgG | C3d | Ctrl |

Table 4 displays a list of the microtubes identification, description, clone, clone supplier and the reagent color of each reagent in the microtube.

Table 4: DG Gel 8 Direct Coombs microtubes identification, clone, clone supplier and reagent color

| Microtube Identification and Description    | Clone                          | Clone Supplier | Reagent Color |
|---|--------------------------------|----------------|---------------|
| IgG, -C3d<br>Contains Anti-IgG and Anti-C3d | Polyclonal and clone 12011 D10 | Diagast        | Green         |
| IgG*<br>Contains Anti-IgG                   | Polyclonal                     | Diagast        | Clear         |
| C3d<br>Contains Anti-C3d                    | Clone 12011D10                 | Diagast        | Clear         |

\* Anti-IgG previously approved by FDA (BL 125445)

### Chronology:

FDA held a pre-submission meeting with Grifols (BQ170088) on September 28, 2017, where the proposed study protocol was discussed. CBER received the original application for the Anti-IgG, -C3d reagent on May 6, 2019 and the application for the Anti-C3d reagent on May 22, 2019. Grifols submitted nine amendments in response to eight information requests from FDA. On February 28, 2020, FDA sent Grifols a Complete Response (CR) Letter. Grifols requested a one-year extension on November 4, 2020. In addition, a submission issue request meeting (BQ 200534) was held on December 2, 2020, to discuss a proposed method comparison study. FDA received the CR Response on November 19, 2021.

### Marketing History:

According to Grifols, the Anti-IgG, -C3d and Anti-C3d reagents referenced in this submission are included in different gel cards distributed throughout Europe (under the CE mark), as well as Argentina, Australia, Brazil, Canada, Columbia, Costa Rica, China, Ecuador, El Salvador, Guatemala, Honduras, India, Indonesia, Israel, Japan, Korea, Malaysia, Morocco, Mexico, Pakistan, Panama, Peru, Philippines, Russia, Saudi Arabia,

Singapore, Thailand, Turkey, Uruguay, Venezuela, and Vietnam.

### 3. Chemistry Manufacturing and Controls (CMC)

Grifols submitted the application 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". Grifols manufactures the products in a controlled environment.

#### a) Manufacturing Summary

##### In Vitro Substance (IVS) (b) (4)

Diagast produces the Anti-IgG, -C3d and Anti-C3d FFMU products at their manufacturing facility in Loos Cedex France.

The IVS for the Anti-IgG, -C3d reagent is a blend of murine monoclonal antibody anti-C3d and rabbit polyclonal Anti-IgG antibody. The Anti-IgG antibody is currently licensed and Diagast has supplied this product to Grifols since January 2011 for the EU market. A contract manufacturer, (b) (4)

The AHG Anti-C3d IVS (b) (4)

The Anti-C3d (b) (4)

(b) (4)

Upon receipt, Grifols conducts testing according to specifications for (b) (4)

##### In Vitro Product (IVP)

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

*Manufacturing Process Description:*

Grifols divides the manufacturing process into (b) (4)

- (b) (4)

- (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

The final product consists of a 25-gel card holder packaged within a single labeled box. Grifols manually applies the labels on the boxes and inserts all Instructions for Use and holders manually into the boxes. All gel cards are sampled (b) (4) final packaging to check for identity using a (b) (4)

*Date of Manufacture (DOM)*

The date of manufacture for the gel cards is the date when Grifols manufacturers the gel (b) (4). Grifols states that each DG Gel 8 card includes different types of reagents based on the antibody profile of each gel card. The date of manufacture is associated with the first gel reagent being manufactured, as required under 21 CFR 660.21 (e). This represents a worst-case scenario because the potency testing is performed (b) (4) manufacturing process (b) (4). The delay between the day of manufacture and the potency test is expected not to exceed (b) (4) days.

*Dating Period*

For both AHG Anti-IgG, -C3d and AHG Anti-C3d, the dating period is 25 months when stored at 2-8 °C.

*Specifications and Test Methods*

Before Grifols fills the (b) (4) gel into cards the gel is sampled and analyzed.

Grifols performs the following testing:

- (b) (4)

Acceptance criteria for the AHG Anti-IgG, -C3d reagent:

- (b) (4)

Acceptance criteria for the AHG Anti-C3d reagent:

- (b) (4)

Once the DG Gel 8 cards are labeled and sealed Grifols performs appearance, specificity, and testing to confirm absence of contaminant antibodies. Table 5 lists the card samples, red cells used for testing and acceptance criteria.

Table 5: Grifols Test Method and Acceptance Criteria on Labeled Gel Cards

| Testing                                | Card Samples   | Red Blood Cells Type | Acceptance Criteria |
|--|--|----------------------|---------------------|
| Appearance                             | <div style="display: flex; justify-content: space-around; font-size: 48px; font-weight: bold;"> <span>(b)</span> <span>(4)</span> </div> |                      |                     |
| Specificity Testing (positive samples) |  |                      |                     |
| Specificity Testing (positive samples) |  |                      |                     |
| Specificity Testing (negative samples) |  |                      |                     |



|  |  |
|--|--|
| Specificity Testing (negative samples) |  |
| Absence of contaminating antibodies    |  |

Finally, Grifols performs identity testing on the finished and packaged labeled DG Gel 8 cards. The Quality Control Department verifies the code of the DG Gel 8 card labels, the code of the box labels, the code of the Package Inserts, and the lot number and expiration date shown on the DG Gel 8 cards and on the box label.

**b) 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.

**c) 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 Anti-Human Globulins (AHGs) Anti-IgG, -C3d (Rabbit Polyclonal/Murine Monoclonal) and Anti-C3d (IgM Murine Monoclonal) is listed in the table below.

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

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

#### **d) Environmental Assessment**

The BLA included a request for categorical exclusion from an Environmental Assessment under 21 CFR 25.31. The FDA concluded that this request is justified, and no extraordinary circumstances exist that would require an environmental assessment.

#### **e) Container Closure**

The Anti-Human Globulins (AHGs) Anti-IgG, -C3d (Rabbit Polyclonal/Murine Monoclonal) and Anti-C3d (IgM Murine Monoclonal) are filled into DG Gel® 8 cards. The DG Gel® 8 cards consist of a (b) (4) with (b) (4) microtubes solution

(b) (4) and (b) (4). The DG Gel® 8 cards are (b) (4)

The DG Gel® 8 cards are allocated into a (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 a seal integrity method; all acceptance criteria were met.

### **4. Software and Instrumentation**

Not applicable for this submission.

### **5. Analytical Studies**

#### **a) Stability Studies**

Grifols performed the stability study on (b) (4) pilot lots of DG Gel 8 AHG Anti-IgG, -C3d and Anti-C3d cards. This pilot card has (b) (4) microtubes filled with AHG Anti-IgG, -C3d gel reagent and (b) (4) microtubes filled with AHG Anti-C3d. The study was conducted using the following temperature and relative humidity (RH) conditions:

- 5 °C ± 3 ° C (b) (4)

- (b) (4)

Grifols stated the reagents can be transported at 2-8 °C. They performed testing for the (b) (4)

Grifols defined the acceptance criteria for all conditions as:

- (b) (4)

The AHG Anti-IgG, -C3d and Anti-C3d met the stability criteria for (b) (4) months at the storage condition of 5 °C ± 3 °C.

The AHG Anti-C3d reagent met the stability criteria up to 18 months for the (b) (4) and 24 months for the (b) (4).

The AHG Anti-IgG, -C3d reagent met the stability criteria at both (b) (4) up to 9 months.

The (b) (4) conditions which simulate transport (b) (4) did not alter the functionality of the AHG Anti-IgG, -C3d and AHG Anti-C3d reagents.

#### **b) Anticoagulant Studies**

Grifols performed the anticoagulant study to demonstrate that red blood cells with a positive or negative DAT can be accurately detected with the AHG Anti-IgG, -C3d and AHG Anti-C3d in a variety of anticoagulants and blood product storage solutions.

The DG Gel 8 package inserts include the following sample limitations:

- Blood samples collected in EDTA and Sodium Citrate should be tested as soon as possible but may be used up to 24 hours after their collection.
- Donor blood collected in ACD, CPD, CPDA-1, CP2D, with AS-1 or AS-3, may be used up to the expiration date indicated on the label of the bag.

Grifols expected that all samples would demonstrate agreement for DAT results between the donor red blood cells in EDTA and the red blood cells collected in the blood product anticoagulant or when converted to an AS product after collection and beyond expiration date. Differences between grading strengths greater than (b) (4) were not expected and were investigated and reported in the study report.

Grifols tested samples to the end of the expiration date plus (b) (4) for samples using the Manual Gel method.

The anticoagulant study met the acceptance criteria for the donor samples collected and stored in ACD, CPD, CPD-A1 and CP2D or converted from ACD, CPD or CP2D and stored in AS-1 and AS-3 the DAT could accurately detect the presence of IgG or C3d bound to the stored red blood cells on the day of collection to (b) (4) past the expiration date (range (b) (4))

**c) Interference Study**

Grifols performed an interference study to evaluate the effects of potential interfering substances. Grifols used (b) (4) patient blood samples collected in EDTA in the study. They (b) (4) of the (b) (4) samples with high concentrations of proteins, lipids, bilirubin, and hemoglobin while (b) (4) of the (b) (4) samples contained normal to elevated levels of the above-mentioned substances. Grifols tested all samples with the manual method described in the Instructions for Use document. Results showed that high plasma protein values are associated with positive DATs. Normal and elevated concentrations of lipids, bilirubin, and hemoglobin did not negatively affect the performance of the AHG Anti-IgG, -C3d and Anti-C3d reagents.

**d) In-House Performance Study**

Grifols performed an in-house comparison study with (b) (4) patient, donor, and newborn samples. The samples were collected in EDTA, heparin and citrate. Samples from bags collected in CPD, (b) (4), CPD/ADSOL, CPDA-1 and ACD were also used in the study. Due to the low frequency of DAT positive samples, red blood cells sensitized *in vitro* with IgG or C3d were included in the study. (b) (4) samples preserved in (b) (4) were also included; these samples were washed with physiologic saline before processing.

The samples were tested according to the Instructions for Use of DG Gel 8 cards. The results were compared with Bio-Rad for the IgG and C3d components and MTS reagents for IgG only (Anti-C3d is not available in the MTS system).

Acceptance Criteria for In-House Comparison Study:

The lower confidence bound of the overall percentage of agreement (OPA) between the investigational reagents Anti-IgG, -C3d and comparator reagents should be 95%.

**Table 6 Results for AHG Anti-IgG, -C3d (DG Gel 8 vs Bio-Rad)**

|                              |          | Bio-Rad, Anti- IgG, -C3d |          |       |
|------------------------------|----------|--------------------------|----------|-------|
|                              |          | Positive                 | Negative | Total |
| DG Gel 8,<br>Anti- IgG, -C3d | Positive | 63                       | 1        | 64    |
|                              | Negative | 0                        | 69       | 69    |
|                              | Total    | 63                       | 70       | 133   |
| PPA (Lower 95% CB)           |          | 100 % (95.4 %)           |          |       |
| NPA (Lower 95% CB)           |          | 98.6 % (93.4 %)          |          |       |
| OPA (Lower 95% CB)           |          | 99.2 % (96.5 %)          |          |       |

**Table 7 Results for AHG Anti-IgG, -C3d (DG Gel 8 vs MTS)**

|                              |          | MTS, Anti- IgG, -C3d   |           |            |
|------------------------------|----------|------------------------|-----------|------------|
| DG Gel 8,<br>Anti- IgG, -C3d |          | Positive               | Negative  | Total      |
|                              | Positive | 63                     | 1         | <b>64</b>  |
|                              | Negative | 1                      | 68        | <b>69</b>  |
|                              | Total    | <b>64</b>              | <b>69</b> | <b>133</b> |
| <b>PPA (Lower 95% CB)</b>    |          | 98.4 % (92.8 %)        |           |            |
| <b>NPA (Lower 95% CB)</b>    |          | 98.6 % (93.3 %)        |           |            |
| <b>OPA (Lower 95% CB)</b>    |          | <b>98.5 % (95.3 %)</b> |           |            |

The results of the study met Grifols' acceptance criteria. One of the discrepant samples was classified as DAT positive by the donor center's supplier laboratory. This sample was positive in the DG Gel Method but was negative with both the MTS and Bio-Rad Gel Method. The sample was tested with a referee method and found to be negative. The second discrepant sample was negative in the DG Gel Method but positive with the MTS Gel Method. This sample had a negative result with the referee method.

**Table 8 Results for AHG Anti-C3d (DG Gel 8 vs Bio-Rad)**

|                           |          | Bio-Rad, Anti-C3d      |            |            |
|---------------------------|----------|------------------------|------------|------------|
| DG Gel 8,<br>Anti-C3d     |          | Positive               | Negative   | Total      |
|                           | Positive | 23                     | 2          | <b>25</b>  |
|                           | Negative | 0                      | 108        | <b>108</b> |
|                           | Total    | <b>23</b>              | <b>110</b> | <b>133</b> |
| <b>PPA (Lower 95% CB)</b> |          | 100.0 % (87.8 %)       |            |            |
| <b>NPA (Lower 95% CB)</b> |          | 98.2 % (94.4 %)        |            |            |
| <b>OPA (Lower 95% CB)</b> |          | <b>98.5 % (95.3 %)</b> |            |            |

The results of the study met Grifols' acceptance criteria for OPA. Two discrepant samples were classified as positive by the donor center's supplier laboratory. Both samples tested positive with the DG Gel Method but negative with the Bio-Rad Gel Method. Both samples were positive with the referee method.

## 6. Clinical Studies

### a. Method Comparison Study

Grifols compared the results of the licensed AHG Anti-IgG, -C3d MTS Gel Card and AHG Anti-C3b, -C3d Immucor tube reagent with those results obtained with the DG Gel

8 Cards containing the investigative AHG reagents, Anti-IgG, -C3d and Anti-C3d, utilizing the manual gel method or the Erytra® automated instrument.

The external clinical testing was conducted at three US blood establishment facilities, two of which are blood centers, American Red Cross Johnstown Region (ARC) and LifeShare Blood Center (LBC). The third site is a regional laboratory's research department TriCore Research Institute (TRI). Discrepancies were sent to a referee laboratory for analysis and resolution (LifeShare Blood Centers Reference Laboratory).

Three lots of the investigative AHG reagents were tested against the licensed comparator using random de-identified samples. Testing was performed using the Erytra® instrument at two sites and the manual gel method at one of the sites.

Due to the low number of positive DAT samples in the random population, LBC and ARC created DAT positive contrived samples for use in this study. In addition, they included selected samples known to have positive DAT results. All clinical sites tested contrived and selected samples.

The acceptance criterion for the study were as follows:

- For random samples:  $\geq 95\%$  concordance at the lower bound of the one-sided 95% confidence interval for both negative and positive percent agreements.
- For the selected and contrived samples: 100% point estimate agreement.

The clinical study had six deviations and two events. The two events were temperature excursions during the shipment of reagents to the clinical sites, when the reagents were exposed to temperatures outside of the required storage temperature range. Grifols determined that this had no effect on the stability of the products based on their product stability data.

The following is a description of each of the six deviations:

- A DG Gel DC Scan card for was used for the investigative AHG Anti-C3d reagent instead of the DG 8 Gel card. Not all the microtubes in this gel card were part of the study but the entire set had to be pipetted with sample due to testing on the Erytra.
- One site saved 47 cord blood samples in preservative because they would have been too old to test otherwise.
- Two sites tested more cord blood samples than required by the clinical protocol.
- One site tested sixteen donor samples after 72 hours in error. The protocol required samples to be less than 72 hours.
- One site tested four cord blood samples after 72 hours in error. The protocol required samples to be less than 72 hours.
- One site didn't load a selected sample on the Erytra within the same 8-hour shift as the comparative testing as required by the protocol.

### AHG Anti-IgG, -C3d Method Comparison Results

Table 9: Random Sample Results for AHG Anti-IgG, -C3d

| <b>Anti-IgG, -C3d</b>                            |          | Anti-IgG, -C3d MTS Gel Method Results |                 |
|--|----------|---------------------------------------|-----------------|
|  |          | Positive                              | Negative        |
| DG Gel 8<br>Coombs<br>Card                       | Positive | 60                                    | 16              |
|  | Negative | 1                                     | 971             |
| Positive Point Estimate Agreement (Lower 95% CB) |          |                                       | 98.36% (92.46%) |
| Negative Point Estimate Agreement (Lower 95% CB) |          |                                       | 98.38% (97.55%) |

Table 10: Selected and Contrived Sample Results for AHG Anti-IgG, -C3d

| <b>Anti-IgG, -C3d</b>                            |          | Anti-IgG, -C3d MTS Gel Method Results |          |
|--|----------|---------------------------------------|----------|
|  |          | Positive                              | Negative |
| DG Gel 8<br>Coombs<br>Card                       | Positive | 118                                   | 0        |
|  | Negative | 0                                     | 6        |
| Positive Point Estimate Agreement (Lower 95% CB) |          |                                       | 100%     |
| Negative Point Estimate Agreement (Lower 95% CB) |          |                                       | 100%     |

FDA assessment of Method Comparison Study Data:

For the random samples the comparison study data met the acceptance criteria for NPA but did not meet the acceptance criteria for PPA due to the small sample size and 16 discrepancies. The one discrepancy which appeared false negative was resolved in favor of the investigational reagent. Five samples which appeared false positive were resolved in favor of the investigational reagent. Eleven samples were resolved in favor of the comparator reagent suggesting the investigational reagent obtained false positive results. However, a positive result with an AHG Anti-IgG-, C3d reagent would likely require additional testing and not lead to patient harm.

For the selected and contrived samples, the comparison data met the acceptance criteria.

In summary, the study results are acceptable and all discrepancies were resolved. It should be noted that for the random samples the results are affected by the small number of available positive DAT (IgG, -C3d) samples in the random population. The results demonstrate that AHG Anti-IgG, -C3d reagent is comparable to US licensed products with the same intended use.

AHG Anti-C3d Method Comparison Results

Table 11: Random sample results for AHG Anti-C3d

| <b>Anti-C3d</b> | Anti-C3d MTS Gel Method Results |
|-----------------|---------------------------------|
|-----------------|---------------------------------|

|  |          | Positive | Negative         |
|--|----------|----------|------------------|
| DG Gel 8<br>Coombs<br>Card                       | Positive | 2        | 0                |
|  | Negative | 1        | 1045             |
| Positive Point Estimate Agreement (Lower 95% CB) |          |          | 66.67% (13.54%)  |
| Negative Point Estimate Agreement (Lower 95% CB) |          |          | 100.00% (99.71%) |

Table 12: Selected and Contrived sample results for AHG Anti-C3d

| <b>Anti- C3d</b>                                 |          | Anti-C3d MTS Gel Method Results |          |
|--|----------|---------------------------------|----------|
|  |          | Positive                        | Negative |
| DG Gel 8<br>Coombs<br>Card                       | Positive | 115                             | 0        |
|  | Negative | 0                               | 26       |
| Positive Point Estimate Agreement (Lower 95% CB) |          |                                 | 100.00%  |
| Negative Point Estimate Agreement (Lower 95% CB) |          |                                 | 100.00%  |

FDA assessment of Method Comparison data:

For the random samples the acceptance criteria met the acceptance criteria for NPA but did not meet the acceptance criteria for PPA due to the small sample size. There was one discrepancy resolved in favor of the investigational reagent

For the selected samples, the comparison data met the acceptance criteria.

In summary, the study results are acceptable and discrepancies were resolved in favor of the investigational reagent. It should also be noted that for the random samples the results are affected by the small number of available positive C3d samples in the random population. The results demonstrate that AHG Anti-C3d reagent is comparable to US licensed products with the same intended use.

**b. Precision Study**

The reproducibility and repeatability studies were 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 was that there should be 100% agreement between the test and expected results.

Grifols performed the study at the same three external US sites that performed the comparison study, using (b) (4) lot against a panel of (b) (4) samples with the Erytra<sup>®</sup> instrument at two sites and the manual gel method at one site. The clinical sites performed testing over (b) (4) days within a (b) (4)-day period, for (b) (4) runs (b) (4) runs),



with (b) (4) testing performed by each operator within each run. The Erytra® sites could use the (b) (4) operator for each precision test event. For the site that used the manual gel method, (b) (4) operators performed (b) (4) runs (b) (4) for each panel sample.

Grifols demonstrated 100% agreement between the expected results and the actual results for all sites, operators, and equipment for the AHG Anti-IgG, -C3d and AHG Anti-C3d reagents. Results for study demonstrated no discordant results; all expected positive tests generated unequivocal positive reactions and all expected negative tests generated unequivocal negative reactions. In addition, there were no differences between grading strengths greater than (b) (4)

### **c. Lot-to-Lot Study**

Grifols performed the lot-to lot study in-house. They tested (b) (4) lots of the reagents at one site with the same samples and test design as the external Precision Study. After the initial reading, the DG Gel 8 cards were held for 24 hours at 2-8 ° C and re-read to demonstrate the ability to re-read the tests. The acceptance criterion was 100% agreement between expected results and actual results. The results of the study met the acceptance criteria.

### **d. Pediatrics**

The method comparison studies for the AHG reagents included neonate and cord blood samples. The test results demonstrate that these samples do not affect the performance of the reagents.

### **e. Other Special Populations**

Grifols included (b) (4) samples for patients older than (b) (4) years. Grifols made note of any disease states for patients used in the method comparison studies. The test results demonstrate that these samples do not affect the performance of the reagents.

## **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 and one Complete Response letter. 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 the chairperson. All reviewers recommend approval.

## **9. Labeling**

The Advertising and Promotional Labeling Branch (APLB) reviewed the proposed Instructions for Use on March 30, 2022 and found them acceptable from a promotional and comprehension perspective.

Grifols submitted container labels, packaging 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 these two BLAs, issues satisfactorily resolved during the review cycle, and were concurred by their respective management. No internal or external disagreements were brought to the attention of the chairperson.

### **b) Risk/ Benefit Assessment**

Licensing these two AHG reagents may increase the safety of performing complex antibody identification procedures by providing new cell lines allowing end-users to differentiate if red blood cells are sensitized in vivo by IgG type immunoglobulins or complement C3d fractions.

The evaluation of the validation studies, manufacturing processes and method comparison studies reduce the risk associated with licensing these new AHG reagents. In addition, these reagents will be subjected to post market surveillance (medical device reporting and biological deviation reporting) which will identify adverse events associated with this product.

### **c) Recommendation for Postmarketing Activities**

We did not recommend post-marketing activities for this submission.