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Summary Basis for Regulatory Action

Date: January 8, 2025

From: Elias Paz Alonzo, Medical Technologist, Review Committee Chair, OBRR/DBCD

BLA STN#: 125753/0

Applicant Name: Immucor, Inc.

Date of Submission: June 14, 2021

MDUFA Goal Date: January 11, 2025

Proprietary Name: Automated C3d Plate

Established Name (common or usual name): Anti-Human Globulin (Murine Monoclonal)

Device Procode: QHS

Intended Use/Indications for Use: *(copied from page one of the Instructions for Use document for the Automated C3d Plate)*

Automated C3d Plate is intended for use on Echo Lumena and Galileo Echo in automated direct antiglobulin tests (DAT) where detection of C3d is required.

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

Review Office Signatory Authority: Anne Eder, 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.**

Discipline Reviews	Reviewer / Consultant - Office/Division
Product Review(s) (Product Office)	• Elias Paz Alonzo, OBRR/DBCD
CMC • CMC Product (Product Office and OCBQ/DBSQC) • Facilities review (OCBQ/DMPQ) • QC, Test Methods, Product Quality (OCBQ/DBSQC)	• Elias Paz Alonzo, OBRR/DBCD • Hector Carrero, OCBQ/DMPQ • Simleen Kaur, OCBQ/DBSQC
Statistical • Clinical data (OBPV/DB) • Non-clinical data	• Ngoc Ty Nguyen, OBPV/DB
Labeling • Promotional (OCBQ/APLB)	• Elias Paz Alonzo, OBRR/DBCD • Sadhna Khatri, OCBQ/DCM/APLB
Lot Release Protocols/Testing Plans	• Marie Anderson, OCBQ/DBSQC

Table of Contents

1. Introduction

Immucor, Inc. (Immucor) (U.S. License #0886) submitted an original Biological License Application (BLA) requesting approval to manufacture and distribute Anti-Human Globulin (Murine Monoclonal); Anti-C3d. The proprietary name is Automated C3d Plate.

The Automated C3d Plate is intended for use on the Echo Lumena (BK210601) and Galileo Echo v2.0 (BK210608) automated instruments which were submitted as companion submissions with this BLA. The Automated C3d Plate assay is designed to detect C3d on the surface of human red blood cells. The Automated C3d Plate (anti-C3d) includes Immucor's Complement Control Cells (BK210604) as positive control.

The manufacture and assembly of this product is performed at the Immucor facility at 3130 Gateway Drive, Norcross, GA 30071, United States of America.

The in vitro substance (IVS) to manufacture the Automated C3d Plate is provided by (b) (4) ., a wholly-owned subsidiary of Immucor, Inc., located at (b) (4) , under a contract manufacturing arrangement. Immucor submitted the IVS information in this BLA.

2. Background

The direct antiglobulin test (DAT) is used to detect antibodies and/or complement bound to red blood cells *in vivo*. In blood bank settings, the DAT is used to investigate for evidence of immune mediated hemolysis. Testing typically starts with polyspecific anti-human globulin (AHG) containing both anti-IgG and anti-complement with positive reactions repeated using monospecific AHG to individually detect IgG and complement. The Automated C3d Plate is used to perform the DAT on human blood samples, to detect C3d on red blood cells.

The Automated C3d Plate contains anti-C3d bound on the surfaces of polystyrene microwells. A dilute suspension of red blood cells is added to the microwell. Centrifugation brings the red blood cells in contact with the anti-C3d. If C3d is present on the red blood cells, they bind to the anti-C3d, and adhere to the microwell surface (positive test). In a negative test, the red blood cells do not bind to anti-C3d and will form a pellet at the bottom of the microwell.

The Automated C3d Plate consists of 1x8 strips of microwells containing bound anti-C3d derived from murine monoclonal antibodies secreted by the hybridoma cell line F139 4B4-4. Twelve 1x8 strips are packaged with a support frame and enclosed in a foil pouch that contains a desiccant and moisture indicator. Each strip is ready to be used as supplied. Strips can be used singly or in multiples.

Chronology

FDA held a pre-submission meeting with Immucor ((b) (4)) on January 22, 2019, to discuss the proposed study protocols. FDA received the original BLA on June 14, 2021.

On April 14, 2022, FDA sent Immucor a Complete Response (CR) Letter. Immucor requested a one-year extension on April 4, 2023, which FDA granted on April 11, 2023. FDA received Immucor's response to the CR letter on April 12, 2024. On September 30, 2024, Immucor submitted a major amendment to the file.

Marketing History
Per Immucor, the anti-C3d murine monoclonal antibodies secreted by the hybridoma cell line F139 4B4-4 and used to manufacture the Automated C3d Plate, were previously licensed in the U.S. to (b) (4) However, (b) (4) is not currently licensed for distribution of AHG reagents using cell line F139 4B4-4 in the U.S. market. The cell line F139 4B4-4 is currently licensed in Canada, CE marked in Europe, and by other regulatory authorities worldwide.

3. Chemistry Manufacturing and Controls (CMC)

a. Manufacturing Summary

Immucor submitted the CMC information in 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". Immucor manufactures the Automated C3d Plate in a controlled environment.

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

(b) (4)

In Vitro Product (IVP)
All raw materials used for the manufacture of the IVP are provided by (b) (4) and accepted based upon the supplier's CoA and qualifying tests, as applicable.

The IVP final product, Automated C3d Plate, consists of 1x8 plastic strip-well microplates coated with anti-C3d (b) (4) . The C3d antibody solution used to coat the Automated C3d Plate test wells is comprised of anti-C3d (b) (4) . The (b) (4) of the anti-C3d is dependent on a predetermined serological assessment. (b) (4)

Manufacturing Process Description
The IVP and Automated C3d Plate manufacturing process consists of (b) (4) main categories: (b) (4)

(b) (4)

Microplate Labeling Inspection

(b) (4), the microplates and pouches are inspected for correct lot number, expiration date, proper product name label, desiccant, indicator card, and plate. Once the microplate labeling is satisfactory, post-plate production testing is performed. The post-plate production testing consists of (b) (4).

Component Packaging and Configuration

Packaging: After the microplate coating and labeling passes inspection and QC testing, each plate is inserted into a labeled foil pouch together with one desiccant and one humidity indicator card. The pouch is sealed using a (b) (4) and the lot number and expiration date are printed on the pouch.

Configuration: Each Automated C3d Plate is made up of 12, 1 x 8 microwell strips that are inserted into a pouch, then sealed. Five pouches and two inserts (Glossary of Symbols) are placed into a paperboard box that is printed with product code, barcode label, and UDI. The packaged Automated C3d Plate final product is stored at 1-30 °C.

Date of Manufacture (DOM)

The first day of production on the (b) (4) instrument is defined as the Automated C3d Plate and IVP DOM. This represents a worst-case scenario as required under 21 CFR 660.21 (e) because the (b) (4) testing is performed later in the manufacturing process during the (b) (4) processing.

Dating Period
The Automated C3d Plate has a shelf life of 12-months, when stored at 1-30 °C.

Specifications and Test Methods
Immucor evaluates the final product's specificity, potency, and sterility before packaging and final labeling. Table 3 below lists the microplate samples, and reagents used for testing and the acceptance criteria.

Table 1: Immucor's Automated C3d Plate testing methods and acceptance criteria.

Testing	Testing Materials	Acceptance Criteria	Expected Results
(b) (4)			Pass
			Pass
			Pass
			Pass
			Pass
			Pass
			Pass
			Pass

Product Sterility Testing
The C3d (b) (4) product should have no growth.

Testing Specifications
The analytical methods and their validations and/or qualifications reviewed for the Anti-Human Globulin (Murine Monoclonal) drug substance(s) and drug product were found to be adequate for their intended use.

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 Globulin (Murine Monoclonal) is listed in the table below. The activities performed and inspectional history is noted in the table and further described in the paragraphs that follow.

Table 2: Facility Information

Name/Address	FEI Number	DUNS Number	Inspection/Waiver	Justification/Results
Immucor, Inc. 3130 Gateway Drive, Norcross, GA 30071 US *In vitro Product Manufacturing and release testing*	1034569	061446282	Waiver	ORA/OBPO July 21-30, 2021 Voluntary Action Indicated (VAI)

CBER: Center for Biologics Evaluation and Research; OBPO: formerly the Office of Biological Products Operations; ORA: formerly the Office of Regulatory Affairs; FEI: Facility Establishment Identifier; DUNS: Data Universal Numbering System; VAI: Voluntary Action Indicated

ORA/OBPO performed a surveillance inspection of the Immucor facility from July 21-30, 2021. The inspection was classified as Voluntary Action Indicated (VAI).

d. Container Closure System

The IVP container closure system is a sealed foil laminate pouch. The pouch is identical to the one currently used for other approved microtiter plate-based (solid phase) Reagent Red Blood Cells licensed under STN BL (b) (4) products). The container closure integrity testing was performed by (b) (4) test; all acceptance criteria were met.

e. Environmental Assessment

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

4. Software and Instrumentation

Not applicable.

5. Analytical Studies

a. Stability Studies

To support the dating period of 12 months, when shipped or stored at 1-30 °C, Immucor performed stability testing using (b) (4) lots that passed their final release testing and met their specificity/reactivity and potency requirements. The stability testing was conducted under (b) (4) pouch conditions. For the (b) (4) conditions, (b) (4)

Additionally, the (b) (4)

The table below shows the different storage conditions and testing performed.

Table 3: Stability Testing Timepoints and Storage Conditions

(b) (4)

Immucor performed temperature excursion studies on all (b) (4) lots used for the real-time stability studies to simulate shipping conditions. (b) (4) different anti-C3d plates from each lot were exposed at different temperature conditions.

(b) (4)

The Automated C3d Plate met the stability criteria at the following shipping and storage conditions:

- 365 days storage at 1-30°C.

(b) (4)

The (b) (4) conditions which simulate transport (b) (4) did not alter the functionality of the Automated C3d Plate reagent.

b. Anticoagulant Studies

Immucor performed anticoagulant studies using the (b) (4) automated methods on the Echo Lumena version 2 system. (b) (4) donor samples were collected in (b) (4) EDTA tubes: (b) (4) EDTA samples for a total of (b) (4) samples.

- Negative C3d samples: (b) (4)
- Positive C3d samples: (b) (4)

Acceptance criteria: Samples should generate expected positive and negative results when testing samples in EDTA (b) (4) anticoagulants.

The anticoagulant study met the acceptance criteria for the donor samples collected and stored in (b) (4) EDTA. The Automated C3d Plate detected the presence of complement bound to the red blood cells from samples collected in EDTA up to 7 days and up to (b) (4) days with (b) (4)

c. Interference Study

Immucor performed interfering substances studies with the Automated C3d Plate on the (b) (4) Echo v2.0 Lumena instrument systems using samples that contained known concentrations of the following interfering substances:

- Lipemia (triglycerides) up to 600 mg/dl,
- Icterus (bilirubin) up to 30 mg/dl,
- (b) (4)

Results showed that samples with a 3+ or greater hemolysis resulted in “no interpretation” testing results. Normal and elevated concentrations of lipids, bilirubin, and (b) (4) up to the values tested above did not negatively affect the performance of the Automated C3d Plate.

d. In-House Performance Study

Immucor performed an in-house comparison study with (b) (4) patient and donor samples. Immucor also performed an in-house pediatric study with an additional 29 samples. The patient samples were collected in EDTA. Samples from donor bags collected in (b) (4), (b) (4) were also used in the study. Due to the low frequency of DAT positive samples, red blood cells sensitized *in vitro* with C3d were included in the study.

The samples were tested according to the IFU of the Automated C3d Plate. The testing results from the Automated C3d Plate on the Echo Lumena were compared with the (b) (4) method using the (b) (4)

Acceptance Criteria for In-House Comparison Study:
The lower bound of the one-sided 95% confidence intervals for the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) should exceed 95%. For the selected and contrived samples, 100% agreement by point estimate is expected. The results of the in-house comparison study are shown in the Table below.

Table 4: Random In-House Study Testing Results for Automated C3d Plate

	(b) (4)			
Automated C3D Plate (Random Samples)		Positive	Negative	Total
	Positive	5	3	8
	Negative	0	337	337
	Total	5	340	345
PPA (95% 1-sided LCI)		100% (54.9%)		
NPA (95% 1-sided LCI)		99.1% (97.7%)		

PPA = Positive Percent Agreement; NPA = Negative Percent Agreement; LCI = Lower Confidence Interval

The NPA results of the in-house study met Immucor's acceptance criteria. The PPA (95% 1-sided LCI) did not meet acceptance criteria due to the low number of positive samples found in a random population. Contrived samples were tested to augment the positive percent agreement, see the method comparison section for a discussion of the contrived samples.

Three discordant samples gave initial positive results with the Automated C3d Plate assay on the Echo Lumena and negative results with comparator method, (b) (4) . Referee testing confirmed that these samples were negative with C3d.

6. Clinical Studies

a. Method Comparison Study

The method comparison study was executed at two external sites and one internal site. The external study sites were (b) (4) which is a donor center and the (b) (4) , which is a Transfusion Service. The internal site was Immucor's Norcross GA facility. Parallel testing between the Automated C3d Plate on Echo Lumena and (b) (4) method using (b) (4) was performed with de-identified samples by trained operators at each site.

The Automated C3d Plate assay was used to test (b) (4) random samples on the Echo Lumena. The results obtained from Echo Lumena were compared with the (b) (4) method using (b) (4) , the licensed comparator. Due to the low number of positive DAT samples in a random population, 87 contrived samples were also tested to increase the number of C3d positive samples for this study. One sample was excluded from analysis due to invalid (no interpretation) results after retesting.

The acceptance criterion for the study were as follows:

- For random samples: The lower bound of the one-sided 95% confidence interval for the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) should be ≥ 95%.
- For the contrived samples: 100%-point estimate agreement is expected.

The method comparison study had 44 sample deviations. The 44 deviations were due to 39 sample collection dates not being provided by two sites and 5 (b) (4) results being read (b) (4) by two sites, which deviates from the IFU. The Automated C3d Plate had 41 samples with equivocal results, and one invalid result after retest. Twenty-five of the 41 equivocal results gave valid results after retest. Sixteen samples remained equivocal and were excluded from analysis. Immucor calculated the equivocal rate to be at 1.55% (16/1032).

Automated C3d Plate Method Comparison Results

The testing results with the Automated C3d Plate, and the (b) (4) comparator using random samples are provided in the table below. The study results combined the three study sites with a total of 1016 random samples.

Table 5: Random Sample Results for Automated C3d Plate

Automated C3d Plate 1016 Random Specimens		(b) (4)	
		Positive	Negative
Galileo Echo/ Echo Lumena	Positive	10	7
	Negative	4	995
PPA (95% 1-sided LCI)			71.43% (46%)
NPA (95% 1-sided LCI)			99.30% (98.69%)

The 11 discordant results were investigated, and the investigation summary is provided below:

- Two out of the 11 discordant samples gave initial negative results with the Automated C3d Plate assay on the Echo Lumena and positive results with comparator method. Referee testing confirmed that these samples were negative, agreeing with the Automated C3d Plate assay.
- Seven out of the 11 discordant samples gave initial positive results with the Automated C3d Plate assay on the Echo Lumena and negative results with comparator method. Referee testing confirmed that these samples were negative, agreeing with the comparator.
- One out of the 11 discordant samples gave initial negative results with the Automated C3d Plate assay on the Echo Lumena and positive results (1+) with comparator method. Referee testing confirmed that this sample was positive (1+) agreeing with the comparator.

- One sample gave initial negative result with the Automated C3d Plate assay on the Echo Lumena and positive result with comparator method. Referee testing with anti-C3d (Murine Monoclonal) reagent gave a negative result. Testing with Immucor Anti-C3b, -C3d (Murine Monoclonal) (b) (4) reagent gave a positive result, indicating the comparator method demonstrated cross-reactivity with C3b.

Using random samples, the method comparison study met the acceptance criteria for NPA, but not for PPA. Seven discordant samples were resolved in favor of the comparator reagent and appeared to be false positive. These false positive results were likely due to variation in reagent and method sensitivity. The overall false positive rate of 0.70% for the Automated C3d Plate was low and demonstrated acceptable performance within the known limitations of the test method.

Contrived samples
Due to the low number of positive DAT samples in a random population, Immucor augmented its positive DAT samples by testing contrived C3d DAT positive samples. The table below shows test results of the contrived samples.

Table 6: Automated C3d Plate Contrived Sample Results

Automated C3d Plate 86 C3d Positive Samples		Anti-C3d, Expected Positive	
		Positive	Negative
Galileo Echo/ Echo Lumena	Positive	86	0
	Negative	0	0
PPA (95% 1-sided LCI)			100% (96.58%)
NPA (95% 1-sided LCI)			N/A

Immucor met 100%-point estimate agreement for their contrived samples.

b. Precision Study

The reproducibility and repeatability studies were performed to demonstrate that the Automated C3d Plate generates reproducible and accurate results. The studies were conducted using a panel of well-characterized samples across different sites, using different operators, and on different days. The acceptance criterion was 100% agreement between the test and expected results. Immucor performed precision studies at the same three sites as their method comparison study. Each site ran (b) (4) samples, (b) (4) runs per day over a (b) (4)-day period for a total of (b) (4) test runs for each site on the Echo Lumena.

Immucor initially demonstrated a 99.4%-point estimate for PPA and 100%-point estimate for NPA. One expected positive sample gave an initial equivocal result. However, after retesting, Immucor demonstrated 100% agreement between the expected results and the actual results for all sites, operators, and equipment for the Automated C3d Plate. Precision study results demonstrated no discordant results; all expected positive tests generated unequivocal positive reactions and all expected negative tests generated

unequivocal negative reactions.

c. Lot-to-Lot Study

Immucor performed the lot-to lot study in-house using the Echo Lumena instrument. They tested (b) (4) different lots of the Automated C3d Plates for (b) (4) consecutive days with (b) (4) samples that included (b) (4) positive (b) (4) and (b) (4) negative samples tested in (b) (4) . The acceptance criterion for PPA and NPA between the C3d coated plates should be 100% agreement. There were a total of (b) (4) testing results for the (b) (4) positive, (b) (4) positive, and (b) (4) negative samples for a combined total of (b) (4) test results. The study achieved 100% agreement between expected results and actual results for both PPA and NPA.

d. Pediatrics

Pediatric Study
Immucor performed a pediatric study with 29 pediatric samples. The pediatric samples were collected on patients ranging from ages 2 up to 17 years of age. The acceptance criterion was 100% agreement between the test and expected results. The table below lists the pediatric study and its statistical results for NPA and PPA.

Table 7: Pediatric Testing Results for Automated C3d Plate

	(b) (4)			
Automated C3d Plate (Pediatric samples)		Positive	Negative	Total
	Positive	1	0	1
	Negative	0	28	28
	Total	1	28	29
PPA (95% 1-sided LCI)		100 % (5 %)		
NPA (95% 1-sided LCI)		100 % (89.85 %)		

The selected pediatric study met 100%-point estimate agreement.

Immucor did not include cord blood samples in their performance studies. The IFU contains a warning indicating the Automated C3d Plate assay is “not for use with cord blood specimens.”

e. 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

and a 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 Instructions for Use (IFU) on July 30 and December 12, 2024, and the container labels on Dec 9, 2024, and found them acceptable from a promotional and comprehension perspective.

DBCD reviewed the IFU performance claims and content. Immucor submitted container labels, packaging labels, and the IFU documents for review. All labels met the requirements outlined in 21 CFR 660 and 21 CFR 809.10.

10. Recommendations and Benefit/Risk 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 were concurred by their respective management. No internal or external disagreements were brought to the attention of the chairperson.

b. Benefit/Risk Assessment

Licensing the automated AHG C3d reagent provides additional options for DAT testing in immunohematology settings. This assay, which is developed from a new cell line, allows end-users to determine if red blood cells are sensitized in vivo by complement C3d. Additionally, use of the Automated C3d Plate allows for testing of multiple samples and can improve turnaround times when performing complex immunohematology testing .

The evaluation of the validation studies, manufacturing processes, and method comparison studies reduce the risk associated with licensing the new AHG C3d reagent. In addition, the AHG C3d reagent 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

The review committee members did not recommend post-marketing activities for this submission.