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Summary Basis of Regulatory Action (SBRA) for Procleix Babesia assay

Date: January 18, 2019

From: Krishna Mohan V. Ketha, Ph.D., Chair of the Review Committee

BLA/ STN#: 125673/0

Applicant Name: Grifols Diagnostic Solution, Inc.

Date of Submission: March 30, 2018

MDUFA Goal Date: January 28, 2019

Proprietary Name/Established Name: Procleix® Babesia Assay

Intended Use/Indications for Use:

The Procleix® Babesia Assay is a qualitative *in vitro* nucleic acid amplification test for the detection of RNA from *Babesia* species (*B. microti*, *B. duncani*, *B. divergens*, and *B. venatorum*) in whole blood specimens. It is intended for use in screening individual human donors, including volunteer donors of whole blood and blood components, and in screening living donors of organ and tissue. It is also intended for use in screening cadaveric (non-heart-beating) donors for *B. microti*. It is not intended for use on cord blood specimens.

Lysed individual donor whole blood samples are tested either individually or in pools of equal aliquots of not more than 16. Cadaveric donor specimens must be tested individually and not pooled. This assay is not intended for use as an aid in diagnosis of *Babesia* infection.

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

Review Office Signatory Authority: Nicole Verdun, M.D., 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 1 below indicates the material reviewed when developing the SBRA.

Table 1: Reviews Submitted

Document Title	Reviewer Name	Document Date
Product Review(s) (product office) • *Clinical* • *Non-Clinical*	 Julia Lathrop Ranadhir Dey Sreenivas Gannavaram	 December 7, 2018 December 13, 2018 December 21, 2018
Living Organ Donor and Cadaveric Donor Claim	Brychan Clark	December 11, 2018
Statistical Review • *Clinical and Non-Clinical*	 Paul Hshieh	 December 14, 2018
CMC Review • *CMC (Product Office)* • *Facilities Review (OCBQ/DMPQ)* • *Microbiology Review (OCBQ/DBSQC)*	 Babita Mahajan Nitin Verma Bradley Dworak Yen Phan	 December 19, 2018 December 19, 2018 December 20, 2018 January 16, 2019
Labeling Review(s) • *APLB (OCBQ/APLB)*	 Dana Jones	 December 6, 2018
Lot Release Protocols/Testing Plans	Kori Francis Ishrat Sultana Varsha Garnepudi Swati Verma	January 16, 2019 January 16, 2019 December 19, 2018
Bioresearch Monitoring Review	Erin McDowell	August 17, 2018
Software and Instrumentation	Lisa Simone	December 19, 2018

1. Introduction

Grifols Diagnostic Solutions, Inc., located at 10210 Genetic Center Drive, San Diego, CA 92121, submitted an original Biologics License Application (BLA) for licensure of the Procleix® Babesia Assay. The Procleix® Babesia Assay is a nucleic acid test (NAT)-based blood donor screening assay for *Babesia.*

The BLA application from Grifols Diagnostic Solutions, Inc. (GDS) was received on March 30, 2018 through the FDA's Electronic Submissions Gateway with electronic content only (**STN 125673/0**). The BLA had a standard 10-month review status with a Final ADD of January 28, 2019. This submission was filed May 22, 2018 and the mid-cycle meeting was held on August 29, 2018. The chronological summary for the submission (amendments) and FDA correspondence is listed in Table 2.

Table 2: Chronological Summary of Submission and FDA Correspondence

Date	Action	Amendment
June 27, 2016	Type B Pre-IND meeting	BQ160057
September 19, 2016	Teleconference with Grifols	
March 31, 2017	Original IND application	17429/0
May 12, 2017	Issue Advice Letter for IND	

September 14, 2017	Pre-BLA meeting	BQ160057/01
November 24, 2017	Pre-BLA FDA Feedback	BQ160057/01
March 30, 2018	Original BLA submission	125673/0
May 23, 2018	Information Request (IR)	
May 24, 2018	Information Request	
May 30, 2018	Response to FDA IR of May 23	125673/0/01
May 31, 2018	BLA Amendment - Clinical data	125673/0/02
June 21, 2018	Information Request	
July 3, 2018	Response to FDA IR of May 24	125673/0/03
July 6, 2018	Information Request	
July 6, 2018	Response to FDA IR of June 21	125673/0/04
August 17, 2018	Response to FDA IR of July 6	125673/0/05
August 23, 2018	Information Request	
August 30, 2018	Response to FDA IR of August 23	125673/0/07
September 26, 2018	Information Request	
October 29, 2018	BLA Amendment – Labeling	125673/0/08
October 31, 2018	Response to FDA IR of September 26	125673/0/09
November 7, 2018	Information Request – Lot Release	
November 14, 2018	Response to FDA IR of November 7	125673/0/10
November 19, 2018	Teleconference with Grifols	
November 20, 2018	Information Request – Software	
December 13, 2018	Information Request	
December 14, 2018	Response to FDA IR of November 20	125673/0/11
December 17, 2018	Response to FDA IR of December 13	125673/0/12
January 2, 2019	Teleconference with Grifols	
January 7, 2019	Response to FDA IR of January 2	125673/0/13
January 9, 2019	BLA amendment –CBER panel results	125673/0/14
January 9, 2019	Response to FDA IR of January 2	125673/0/15

2. Background

Human babesiosis is a tick-borne zoonotic disease primarily caused by *Babesia microti*, *B. duncani*, *B. divergens*, and *B. venatorum*. Although predominately transmitted by tick bite, babesiosis can also be acquired via blood transfusion and organ transplantation. Babesiosis has been reported in North and South America, Europe, Australia, and southern and eastern Asia with the highest prevalence reported in the United States. The majority of babesiosis cases in the U.S. are caused by *B. microti*, occurring mainly in the Northeast and the upper Midwest. Additional cases of babesiosis caused by other species of *Babesia* occur predominately in the western U.S.; though cases from Missouri and Kentucky have also been reported.

Clinically, babesiosis has a wide spectrum of disease severity. Symptomatic patients experience a viral-like illness that can last weeks to months. However, the majority of infected individuals are entirely asymptomatic. Transmission of *Babesia* is seasonal and coincides with peak tick activity (May-September), although both tick-borne and transfusion-transmitted infections resulting from chronic unresolved parasitemia are reported year-round. Asymptomatic individuals are difficult to recognize and, therefore, transfusion of blood and blood components collected from such individuals may result in

transfusion-transmitted babesiosis (TTB), leading to a potentially fatal clinical outcome in blood transfusion recipients.

The Procleix® Babesia Assay shares assay principles, manufacturing technologies and controls, and common reagents with other FDA-approved Procleix donor screening assays developed for use with the Procleix® Panther System. The Panther System consists of an analyzer, associated hardware/software, assay reagents, and assay-specific software. A total of five kits are required to perform the Procleix® Babesia Assay on the Panther System: two *Babesia*-specific assay kits and three ancillary kits, common to all Procleix® assays.

The Procleix® Babesia Assay targets the 18S ribosomal RNA of the *Babesia* species for amplification and subsequent detection. Whole blood lysate is prepared either manually or on the Procleix® Xpress System by addition of whole blood to the Parasite Transport Medium (PTM). Lysates (0.3 mL) prepared as above can be transferred to a master pool tube to create 16-member pools or analyzed as individual donations. The Procleix® Babesia Assay involves three steps, which take place in a single tube on the Procleix® Panther System: target capture, *Babesia* RNA amplification that utilizes transcription-mediated nucleic acid amplification (TMA) technology, involving production of cDNA by MMLV reverse transcriptase followed by T7 promoter-driven transcription and hybridization with target-specific single-stranded luminescent nucleic acid probes. After luminescence is measured, the reactivity of the specimen is determined by calculating the signal-to-cutoff (S/CO) ratio. The assay performance is ensured through the addition of an Internal Control (IC) to each specimen tube, and the assay cutoff and run validity is determined using assay calibrators. Chemiluminescent probes that hybridize to the IC targets are discriminated from *Babesia*-specific probes by differential kinetics of light emission. The chemiluminescent signal produced by the hybridized probe is measured by a luminometer and reported as Relative Light Units (RLU). The Procleix® Babesia Assay is currently not marketed in any country.

3. Chemistry Manufacturing and Controls (CMC)

a. Manufacturing Summary

i) In Vitro Substance

The *in vitro* substances (active ingredient) for the Procleix Babesia Assay are the (b) (4) oligonucleotides contained in the Procleix Babesia Assay kit. The oligonucleotides are categorized into (b) (4) distinct classes based on structure, function, and chemical composition as follows:

(b) (4)

Four of the (b) (4) Procleix Babesia Assay oligonucleotides are identical in structure, composition, manufacturing and control processes, specifications and analytical methods to those of other licensed Procleix Assays. *Babesia*-specific oligos (capture oligos, amplification primers, and probes) target highly conserved regions of 18S ribosomal RNA for all four *Babesia* species. Information on structure, composition, and characterization of the oligonucleotides was provided. The in-process controls during manufacturing involved evaluation of (b) (4) and an in-process test performed to determine (b) (4) The shelf-life dating results were found to be acceptable.

ii) In Vitro Product

The BLA contained information on the common reagents and kits within the Procleix Babesia Assay kit. All components of the Procleix Babesia Assay Kit and Procleix ancillary kits are manufactured and controlled at the (b) (4) All manufacturing facilities are licensed and used to manufacture other Procleix commercialized products. Table 3 lists the five kits required to perform the Procleix Babesia Assay on the Panther System and their manufacturing locations.

Table 3: Procleix Babesia Assay kits, components and manufacturing locations.

Kit	Reagent/Component	Manufacturing Location
Procleix® Babesia Assay Kit 5000 test (Part#9051246)	Enzyme Reagent	(b) (4)
	Internal Control Reagent	
	Amplification Reagent	
	Probe Reagent	
	Selection Reagent	
	Target Capture Reagent	
Procleix® Babesia Assay Calibrators Kit (Part#9051253)	Negative Calibrator	
	Positive Calibrator	
Procleix® PTM Kit (Part#9051577)	Parasite Transport Medium	
Procleix® Assay Fluids Kit (Part#303344)	Wash Solution	
	Oil	
	Buffer for Deactivation Fluid	
Procleix® Auto Detect Reagents Kit (Part#303345)	Auto Detect 1	
	Auto Detect 2	

Manufacturing process information was provided for the Procleix Babesia Assay kit and Procleix Ancillary Kits that included the Internal Control Reagent, Target Capture

Reagent, Amplification Reagent, Probe Reagent, Selection Reagent, *Babesia* Positive Calibrator, Negative Calibrator, Procleix Assay Fluids Kit, Procleix Auto Detect Reagents Kit, and the Procleix Parasite Transport Medium Kit. The information included a brief description of the reagent's function, components and composition, manufacturing process, in-process control validations, lot release specifications and analytical methods, and lot release results. Release testing for the Procleix Babesia Assay Calibrators Kit was based on visual criteria and performance testing was not required for this kit. In-process control validations were performed using representative materials from other Procleix assays, which were found acceptable since the manufacturing process is the same.

Acceptance criteria included specifications and analytical methods to establish the identity, strength, quality, and purity, and lot-to-lot consistency of the Procleix Babesia Assay kit and the Procleix PTM. The ancillary kit reagents are included as part of the master lot release testing performed on the Procleix Babesia Assay Kit. Performance testing was not performed for the Procleix Auto Detect Kit, but individual reagents were tested, and QC released at the component level only.

iii) Stability

Under the formal stability program, the applicant submitted stability data for the Procleix Babesia Kit open-kit conditions for (b) (4) master lots for up to (b) (4) months. At the 3, 6, 9, 12, (b) (4) month real time and open-kit on-board time points, both the High and Low QC panels were 100% reactive. The negative panel was nonreactive at all time points tested. All panel and calibrator results through the open-kit condition time points met the acceptance criteria of the study. The Procleix Babesia Assay reagents exhibited acceptable performance with no unexpected results as of the (b) (4)-month, (b) (4)-day time point for open-kit and 72-hour on-board time point, when stored at the appropriate temperatures. Based on the (b) (4)-month stability data, a 12-month stability claim will be granted at the time of licensure. Based on the real time stability data for the Procleix PTM, a 9-month stability claim for PTM will be granted at the time of licensure. The applicant also submitted data demonstrating that once dispensed in the reaction tubes, the PTM must be used within 48 hours. The shelf-life of the Procleix Assay Fluids kit and Procleix Auto Detect Reagents kit was established under formal stability studies. Stability testing information for the ancillary kits was established previously with the approved expiration dating of 24 months and open-kit and onboard stability of 60 days.

Product Quality

b. Testing specifications

The analytical methods and their validations and/or qualifications reviewed for the Procleix Babesia Assay 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 revision. 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 manufacturer of the Procleix Babesia Assay is Grifols Diagnostics Solutions, Inc. The activities performed, and inspectional histories are noted in Table 4 and are further described in the paragraphs that follow.

Table 4: Manufacturing Facilities Table for Procleix® *Babesia* Assay

Name/Address	FEI number	Inspection/ waiver	Justification /Results
Grifols Diagnostic Solutions, Inc. (b) (4) Final QC release testing, packaging, and shipment of finished assay master kits	(b) (4)	Waived	Team Biologics (b) (4) NAI
Grifols Diagnostic Solutions, Inc. (b) (4) Design specifications, Design Controls, Management Responsibility, CAPA and non-conforming product handling. QC release testing of reagents	(b) (4)	Waived	Team Biologics (b) (4) VAI

Grifols Diagnostic Solutions, Inc. (GDS) manufactures this IVD at two sites: (b) (4) Both sites are owned by GDS and operate under the same Quality System.

Team Biologics performed a week-long surveillance inspection of (b) (4) campus from (b) (4) and the inspection was classified as voluntary action indicated (VAI). All inspectional issues were resolved. The (b) (4) site was inspected on (b) (4) and classified as no action indicated (NAI).

e. Container Closure System

Not Applicable

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.

Review issues

- *Shelf life claims were submitted only for 6 months and GDS was notified that expiration dating will be based on real-time stability data. This issue was resolved after GDS notified FDA that real-time stability studies are on-going and GDS expects an expiration dating of 12-months at the time of licensure.*
- *Stability testing time points of various lots were not consistent, and it was not clear how the final shelf life claim will be established. This issue was resolved by GDS response to FDA IR stating that all lots will be tested up to* (b) (4) *months and final shelf life will be based on the latest time point tested for all* (b) (*lots.*
- *GDS was requested to qualify the Bioburden test method using* (b) (4) *or provide justification for using* (b) (4) *. GDS performed the Bioburden testing using* (b) (4) *and submitted the validation protocol and reports GDSS-VAP-00020 and GDS-VAR-000021.*
- *The responses were found acceptable.*

4. Software and Instrumentation

The Procleix Panther System is used as the instrument platform supporting the Procleix Babesia Assay. The following is a summary overview of software, instrumentation and risk management information provided to support a reasonable assurance that the device is safe and effective for its intended uses and conditions of use.

Versioning: Panther System Software v5.3 (SW v5.3.2.9 and FW 5.3.2.3) and *Babesia* ADM (assay specific software) v0.6 (v0.6.4) running on a Dell OptiPlex XE2 system with Windows 7 operating system and Panther Image 5.7.0. Optional pooler: Procleix Express System v2.0 (v2.0.1 build 2.0.1.67), and Procleix NAT Manager software (v1.2).

Device Description: The Procleix Panther System's process is fully automated from sample loading to results generation and contains a variety of safety features for ID tracking, timing, assay processing steps, liquid level sensing and volume dispense verification for samples, reagents and consumables. The software architecture supports a separation of instrument software and assay specific software. The instrument has connectivity with the outside world via USB, TCP/IP and by removable media, allowing export of data to a USB, hard drive, network destination or customer's Laboratory Information System (LIS).

Risk Management: The highest severity risks associated with the system is transfusion-transmitted babesiosis resulting from a false negative result, and operator infection through exposure. Causes explored include issues with: universal fluids radio frequency ID, user error, ancillaries and accessories, run-time processing, contamination, assay co-existence, installation/maintenance errors, compromised reagents and samples, sample transfer operations, reagent transfer operations, mixing, (b) (4) , temperature control, luminometer issues, and critical software defects and malicious intent (cybersecurity considerations). Pooler-related risks focused on incorrect sample identification during sample pipetting, incorrect sample or lysate pipetting, incorrect

pipetted volume, contamination during pipetting, incorrect mapping of source or lysate tubes for a pool tube, and operator exposure to blood borne pathogens.
The applicant evaluated the overall residual risk and stated that all hazards for the assay and Panther IVD instrument with software v5.3 met the risk acceptability criteria and that no hazards are associated with undesirable or unacceptable residual risk.

Unresolved Anomalies: The applicant stated that there are no known software anomalies or cybersecurity related hazards that would contribute to serious injury or death. Four anomalies were described, and justification was provided suggesting that they do not affect safety and effectiveness, related to an unresponsive user interface, loss of communication with a hardware component, insufficient system resources, and incorrect calculation of reagent expiry. Forty-two existing "negligible" severity unresolved anomalies that are not related to serious risk, false negative or false positive results are planned to be addressed in a software revision planned for release by the end of calendar year 2019.

Testing: Panther instrument documentation included: instrument verification, software verification and validation, instrument validation, assay verification, system validation, and clinical evaluation studies. New penetration testing for security mitigations was developed and performed. Xpress documentation included verification and validation plans, protocols and reports. Failed tests and anomalies were adequately explained as deviations or new defects, and the applicant stated that none affect device or assay performance. Adequate risk analyses were provided to justify deferral to future software versions.

Development Management: The software development activities included establishing detailed software requirements, linking requirements with associate verification tests, verification and validation testing, defect tracking, configuration management and maintenance activities to ensure the software conforms to user needs and intended uses.

Review Issues and Software/Device Changes

- *Risk analysis did not include all system components and interactions (instrument, pooler, middleware) that contribute to unacceptable risk. A system-wide hazard analysis was provided to enumerate the risks associated with each instrument and the risks arising from the interactions among the instruments, resulting in several updates to the labeling to ensure all risks are reduced to acceptable levels.*
- *Initial risk analysis stated 53 unacceptable risks associated with incorrect patient results remained in the system. These were resolved by 1) updating the risk process to translate unacceptable risks from the* (b) (4) *analysis into Grifols risk terms to allow reevaluation, 2) eliminating some risks as not applicable to the current intended use, 3) identifying protective measures in the design to demonstrate that risk reduction did not rely exclusively on labeling to reduce risks to acceptable levels, and 4) updating the Xpress Operator's manual with several safety-related changes to clarify safe operation and disclose residual risks.*

Over thirty-five changes were made to the Xpress Operator's manual for safety-related issues:

- Prioritized safer methods to resolve barcode scanning errors to reduce the probability of barcode errors due to tube or rack mishandling.
- Clarified four warnings to describe the cause of potential false negative results.
- Clarified in a warning that false negative results may occur if error prompts are ignored during sample aspiration.
- Added a warning that only new CDROMs should be used to reduce the risk of introducing malware to the Xpress system when using writable CDROMs to transfer output files.
- Added a warning to avoid using USB sticks for file transfers to reduce the risk of introducing malware to the Xpress system.
- Several identical user interface screenshots in the manual were updated to clarify the purposes of each, and to annotate what information is being highlighted for the user.
- Pictures and descriptive text were added to show how the new trough and trough liner (required for lysis) are inserted in the system.
- The responses were found acceptable.

5. Analytical Studies

Based on discussions and FDA feedback (BQ160057 and IND17429), several analytical studies were performed by the Grifols Diagnostic Solutions Inc. to evaluate the performance of the Procleix Babesia Assay on the automated Procleix Panther System.

5.1 Reproducibility Studies

Reproducibility of the Procleix Babesia Assay

Study was designed to evaluate instrument-to-instrument, operator-to-operator, reagent lot-to-reagent lot, day-to-day, and test-to-test variability using 3 reagent lots tested at 3 different sites(b) (4) and Grifols Diagnostic Solutions (GDS)].

(b) (4)

(b) (4)

5.2 Analytical Sensitivity Studies

a. Limits of Detection of Babesia in Sensitivity Panels for B. microti, B. divergens, B. duncani and B. venatorum with the Procleix Babesia Assay

This study evaluated the analytical sensitivity and limit of detection (LOD) of the Procleix Babesia Assay for the detection of *B. microti*, *B. divergens*, *B. duncani* and *B. venatorum in-vitro* transcripts (IVT). The panels consisted of in-house transcripts for *B. microti*, *B. divergens*, *B. duncani* and *B. venatorum* that were serially diluted to a final dilution of 100, 30, 10, 3, 1, and 0 copies/mL and tested using (b) (4) reagent lots. Transcripts of *B. microti*, *B. divergens*, and *B. venatorum* were detected 100% by the Procleix *Babesia* assay at 100 and 30 copies/mL. The *B. duncani* transcript was detected 100% at 100, 30 and 10 copies/mL concentration. The 95% detection probability for IVTs of *B. microti*, *B. divergens*, *B. duncani,* and *B. venatorum* by Probit Analysis was 8.91, 11.58, 6.73, and 12.44 copies/mL (Table 5). The performance was found to be acceptable.

Table 5: Probit analysis for IVT detection

Panel	Detection Probabilities, copies/mL	
	50% (95% Fiducial Limits)	**95% (95% Fiducial Limits)**
B. microti	2.06 (1.55 - 2.56)	8.91 (6.88 - 12.88)
B. divergens	3.90 (3.21 - 4.62)	11.58 (9.38 - 15.44)
B. duncani	2.54 (2.11 - 3.01)	6.73 (5.32 - 9.69)
B. venatorum	2.99 (2.33 - 3.67)	12.44 (9.62 - 17.87)

b. Procleix Babesia Assay limit of detection (LOD) of Babesia parasites

This study, evaluating the analytical sensitivity of the Procleix Babesia Assay, was conducted using *B. microti*-infected hamster blood, *B. divergens*-infected human erythrocytes, and *B. duncani*-infected hamster blood. *B. microti, B. divergens, and B. duncani*-infected samples were serially diluted in human whole blood at 6, 4, 2, 1, 0.5, and 0 parasites/mL. Detection of *B. microti* and *B. divergens* parasites was 100% at 6 and 4 parasites/mL. The average S/CO values for *B. microti* at 6 and 4 parasites/mL was 13.48 and 13.34, and for *B. divergens* was 12.97 and 12.17. For the *B. duncani* parasite, detection was 100% and 98.61% at 6 and 4 parasites/mL with an average S/CO value of 10.73 and 10.13, respectively. Probit analysis (Table 6) indicated the 95% detection probability for *B. microti*, *B. divergens, and B. duncani* to be at 2.98, 1.77, and 3.10 parasites/mL, respectively. The performance was found to be acceptable. LOD studies for *B. venatorum* were not performed due to difficulty in obtaining such samples.

Table 6: Probit analysis for parasite detection

Panel	Detection Probabilities, parasites/mL	
	50% (95% Fiducial Limits)	**95% (95% Fiducial Limits)**
B. microti	0.61 (0.43 - 0.76)	2.98 (2.40 - 4.09)
B. divergens	0.48 (0.34 - 0.60)	1.77 (1.45 - 2.41)
B. duncani	0.72 (0.54 - 0.88)	3.10 (2.53 - 4.11)

c. Detection of Babesia in Naturally-Infected Samples

Sensitivity of the Procleix Babesia Assay in detecting *Babesia* in naturally-infected clinical whole blood specimens was evaluated. Testing was performed to demonstrate assay sensitivity in neat individual specimen lysates and 16-donor lysate pools using (b) (4) reagent lots. (b) (4) clinical, *B. microti*-PCR positive specimens were obtained from the (b) (4) A 16-donor pool lysate was prepared for each specimen and tested in (b) (4) . Whole blood from a single donor was used for making up the volume of (b) (4) negative specimen lysates in the 16-donor pool. The clinical sensitivity of the Procleix Babesia Assay in individual lysates and in 16-pooled lysates was 100% with a 95% CI of (b) (4) respectively. No statistical difference was found in the sensitivity of the assay between neat and pooled naturally-infected lysates. The performance was found to be acceptable.

5.3 Specificity Studies

a. Procleix Babesia Assay specificity in fresh whole blood specimens

This study evaluated the specificity of the Procleix Babesia Assay on the Panther System. Normal whole blood donor specimens were lysed and tested neat (IDL) and in pools of 16 (PDL) using (b) (4) reagent lots for IDL and (b) (4) for PDL. A total of (b) (4) IDL and (b) (4) PDL were tested with the Procleix Babesia Assay. Two initial reactive results in IDL and corresponding PDL were confirmed by repeat testing and serology by IFA testing. Overall specificity for the Procleix Babesia Assay was 100% both in IDL (b) (4) and PDL (b) (4) with a 95% CI of (b) (4) respectively. The performance of the Internal Control was robust, with an initial invalid rate due to assay chemistry errors of 0% (b) (4) in IDL and 0% (b) (4) for the Procleix Babesia Assay. This met the design goal of (b) (4) invalid rate. The performance was found to be acceptable.

b. Procleix Babesia Assay specificity in presence of high titer specimens

The carryover contamination rate of the Procleix Babesia Assay on the Panther Instrument was evaluated in this study by using negative and high-titer *Babesia*-positive (at approximatively (b) (4) parasites/mL) specimens prepared on the Procleix Xpress system that were interspersed throughout the specimen processing racks containing negatives samples. Lysates and pools of 16 were prepared on (b) (4) Xpress systems and were tested using (b) (4) across (b) (4) Panther Systems. There were no invalid runs. The reactivity rate for (b) (4) valid high titer specimens (b) (4) for IDL and (b) (4) for PDL) tested with the *Babesia* Assay was 100%. False positive rate for (b) (4) negative lysates tested (b) (4) for IDL and (b) (4) for PDL) with the Procleix Babesia Assay was 0% with a mean analyte S/CO of (b) (4) (SD (b) (4). The specificity of the Procleix Babesia Assay on the Panther System was 100% which was found to be acceptable.

5.4 Effect of Donor and Donation Factors on Specificity and Sensitivity

a. Sensitivity and Specificity in specimens with microorganisms

The specificity and sensitivity of the Procleix Babesia Assay in specimens spiked with microorganisms was evaluated. Whole blood specimens were spiked with microorganisms at the indicated concentration: *Staphylococcus epidermidis* (b) (4) CFU/mL), *Staphylococcus*

aureus (b) (4) CFU/mL), *Micrococcus luteus* (b) (4) CFU/mL), *Corynebacterium diphtheriae* (b) (4) CFU/mL), *Propionibacterium acnes* (b) (4) CFU/mL), *Candida albicans* (b) (4) CFU/mL), *Pneumocystis carinii* (b) (4) nuclei/mL), *Borrelia burgdorferi* (b) (4) CFU/mL), *Trypanosoma cruzi* (b) (4) cells/mL), *Trypanosoma rangeli* (b) (4) cells/mL), and *Leptospira interrogans* (b) (4) cells/mL).

The microorganism spiked whole blood was then divided into two aliquots, the first aliquot was left untreated and the second aliquot was spiked with *B. microti*-infected hamster blood. Control conditions for the study consisted of normal negative whole blood specimens prepared similarly in the absence and presence of *Babesia*.

Sensitivity: *Babesia*-spiked controls and *Babesia*-spiked specimens containing microorganisms were used for evaluating sensitivity of the assay and the testing demonstrated 100% reactivity. The average analyte S/CO values were (b) (4) for the control specimens and *Babesia*-spiked specimens containing microorganisms, respectively.

Specificity: None of the negative control specimens or specimens containing microorganisms were reactive by the Procleix Babesia assay and demonstrated 100% specificity. The average IC S/CO values were (b) (4) for the control specimens and specimens containing microorganisms, respectively. There were no invalid runs and no invalid reactions out of (b) (4) total reactions tested. The results demonstrated that specimens containing the other microorganisms tested do not affect the specificity or sensitivity of the Procleix Babesia Assay.

b. Sensitivity and Specificity in specimens with blood borne pathogens

Whole blood specimens spiked with various blood borne pathogens (Human Immunodeficiency Virus 1 and 2 (HIV-1/2), Hepatitis A Virus (HAV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Hepatitis E Virus (HEV), Dengue Virus 1-4 (DENV), Chikungunya virus (CHIKV), Parvovirus B19, Influenza (H1N1), *Plasmodium falciparum*, Cytomegalovirus (CMV), Human T-lymphotropic Virus 1 and 2 (HTLV 1/2), Influenza vaccine, and HBV vaccine) were included in this study. The pathogen-spiked whole blood was then divided into two aliquots, the first aliquot was left untreated and the second aliquot was spiked with *B. microti*-infected hamster blood. Control conditions for the study consisted of normal negative whole blood specimens prepared similarly in the absence and presence of *Babesia*.

Sensitivity: Testing of the *Babesia*-spiked control specimens and *Babesia*-spiked specimens containing other blood borne pathogens with the *Babesia* assay demonstrated 100% reactivity with average analyte S/CO values of (b) (4), respectively.

Specificity: Testing of the negative control specimens and specimens containing other blood borne pathogens with the Procleix Babesia Assay resulted in 100% specificity. The average IC S/CO values were (b) (4) for the control specimens and specimens containing other blood borne pathogens, respectively. The results demonstrated that specificity or sensitivity of the Procleix Babesia Assay was not affected by specimens containing other blood borne pathogens.

c. Sensitivity and specificity in specimens with potentially interfering substances

The specificity and sensitivity of the Procleix Babesia Assay in specimens containing potentially interfering substances was evaluated in this study. Whole blood specimens were spiked with the following substances: (a) albumin at 60 g/L, (b) bilirubin at 200 mg/L, (c) hemoglobin at 5g/L, (d) lipids at 30 g/L, and (e) human gamma globulin at 60 g/L. The interfering substances-spiked whole blood was then divided into two aliquots, the first aliquot was left untreated and the second aliquot was spiked with *B. microti*. Control conditions for the study consisted of normal negative whole blood specimens prepared similarly in the absence and presence of *Babesia*.

Sensitivity: Testing of the *Babesia*-spiked control specimens and *Babesia*-spiked specimens containing potentially interfering substances resulted in 100% reactivity with average analyte S/CO values of (b) (4), respectively. There were no invalid runs, false-reactives, or false-negatives in the study. The performance was found to be acceptable.

Specificity: The Procleix Babesia Assay demonstrated 100% specificity in testing of the negative control specimens and specimens containing potentially interfering substances with an average IC S/CO values of (b) (4), respectively.

d. Specificity in Specimens from Patients with Autoimmune and Other Diseases

Samples from patients with the following pathological conditions were evaluated: rheumatoid factor (RF), antinuclear antibody (ANA), systemic lupus erythematosus (SLE), multiple myeloma (MM), multiple sclerosis (MS), rheumatoid arthritis (RA), alcoholic cirrhosis (AC) and elevated alanine aminotransferase (ALT). Control specimens for this study consisted of fifty individual normal whole blood specimens.

Specificity: Analysis of the *Babesia*-negative specimens with autoimmune and other diseases with the Procleix Babesia Assay demonstrated 100% specificity with average IC S/CO values of (b) (4). Control specimens testing revealed 100% specificity with an average IC S/CO value of (b) (4) There were no invalid results.

Sensitivity: The Procleix Babesia assay demonstrated a sensitivity of 98.81% in *Babesia*-spiked specimens with autoimmune and other diseases with an average analyte S/CO of (b) (4) The reactivity rate observed for *Babesia*-spiked control specimens was 100% with average analyte S/CO values of (b) (4) Six samples that resulted in discordant results (invalid/nonreactive) were found reactive upon retest. The reason for retesting was that one sample had an Internal Control invalid result and the others were found to be improperly spiked. Statistical analysis indicated no difference between testing specimens from normal control donors or donors with autoimmune and other diseases. The performance was found to be acceptable.

e. Specificity and sensitivity of Pools of Donor Donation Factors

Specimens from blood donors containing various donor and donation factors were tested in pools to evaluate specificity and sensitivity of the Procleix Babesia assay. The donation factor samples included the following: icteric, lipemic, and hemolyzed clinical specimens; specimens spiked with microorganisms; specimens from donors that had received flu and HBV vaccinations; specimens from donors with other blood-borne

infections; and specimens from donors with autoimmune or other diseases. Specificity and sensitivity of Procleix Babesia assay in (b) (4) pools of specimens from donors with putative interfering substances (test pools) were compared to those of specimens (16 pools) from normal donors using (b) (4) reagent lots. Each test and control pool were divided into two aliquots, one of which evaluated the analytical specificity in the absence of *Babesia* and the other evaluated sensitivity in the presence of *Babesia*.

Specificity: For all 16 control pools and (b) (4) test pools the specificity was 99.02% and 100%, respectively. No statistical difference was observed between the control pools and the test pools.
Sensitivity: Sensitivity was 100% for all test pools and control pools. The performance was found to be acceptable.

f. Sensitivity and specificity in specimens containing Exogenous Substances

Panels were prepared by spiking exogenous substances (acetaminophen, acetylsalicylic acid, ascorbic acid, atorvastatin, ibuprofen, loratadine, naproxen, and phenylephrine HCl) (b) (4) in human whole blood. Each diluent and solvent control panel, and each exogenous substance panel was split into two aliquots; one remained absent of *Babesia* to evaluate specificity while the other was spiked with *B. microti*-infected hamster blood to evaluate sensitivity. The diluent control was human whole blood in the absence of potential interfering substances.

All negative panels were nonreactive and 100% agreement with corresponding controls for all exogenous substances was observed. All *Babesia*-spiked panels were reactive (100%). Percent agreements with corresponding controls were 100% for all exogenous substances tested. There were no invalid runs or results. The performance was found to be acceptable.

g. Testing in various anticoagulants

Whole blood from (b) (4) normal donors was collected in the following anticoagulants: K2EDTA, K3EDTA, sodium citrate and CPDA. An aliquot of each of the (b) (4) donor specimens from each of the four tube types was tested to evaluate the specificity of the Procleix Babesia Assay. A second aliquot of each specimen was spiked with *B. microti*-infected hamster whole blood to evaluate the sensitivity of the Procleix Babesia Assay. Individual *Babesia*-negative specimens collected in different anticoagulants demonstrated 100% (b) (4) specificity for the Procleix Babesia Assay. Sensitivity of the Procleix Babesia Assay was also found to be 100% (b) (4) following testing of individual *Babesia*-spiked specimens collected in different anticoagulants. Additionally, detection was 100% (b) (4) for all pools of 16 tested. There were no invalid runs. Assay performance, demonstrating no effect of the blood collection tube types (K2EDTA, K3EDTA, sodium citrate, and CPDA) on the sensitivity or specificity of the Procleix Babesia Assay, was deemed acceptable.

5.5 Equivalency Between Lysate made on Automated Xpress System and Manually

Normal or *Babesia*-spiked whole blood specimens were lysed and pooled on the Xpress system or manually and tested on the Panther System to compare specificity and sensitivity. IDLs were tested in (b) (4) and PDLs were tested in (b) (4) using a (b) (4)

(b) (4). For both methods, all IDL and PDL replicates were reactive for the *B. microti*-spiked samples. There were no reactive replicates in any of the individual or pooled normal negative whole blood lysates. Sensitivity and specificity with the automated sample preparation and with the manual method were 100% demonstrating equivalence between the two methods for the Procleix Babesia Assay on Panther. Additionally, (b) (4) studies using normal and *Babesia*-spiked whole blood specimens manually lysed at (b) (4) whole blood specimen-to-PTM volume ratios demonstrated 100% sensitivity and specificity across the (b) (4) ratios.

5.6 Statistical Analysis of Specificity and Sensitivity Data

a. Run Size Validity

(b) (4)

b. Internal Control Validity and Inhibition

Performance was evaluated for the Procleix Babesia Assay IC on the Panther system by testing the following conditions using (b) (4) reagent lots: (1) thawing the reagent kit in (b) (4)

5.7 Specimen Stability Studies

Specimen Stability

Stability of *Babesia* parasite was evaluated in (b) (4) individual whole blood donor samples spiked with *Babesia*-infected hamster whole blood at about (b) (4) LOD of the Procleix Babesia Assay and collected in each of the following anticoagulants: K2EDTA, K3EDTA, sodium citrate, and CPDA. Stability was also evaluated in IDLs prepared from each of the (b) (4) *Babesia*-spiked individual donor specimens collected in the 4 different anticoagulants. Additionally, stability was evaluated in (b) (4) unique pools of (b) (4) donors comprised of (b) (4)

negative lysates and (b) (4) *Babesia*-spiked lysate. After spiking the (b) (4) individual whole blood specimens with *Babesia* parasite on the same day as specimen collection, each collection tube was subjected to a series of incubations depicted in Figure 1 and Figure 2.

Figure 1: Storage conditions of Whole blood.

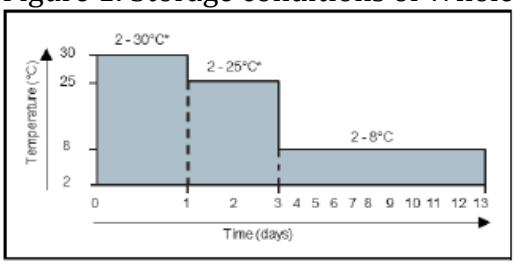
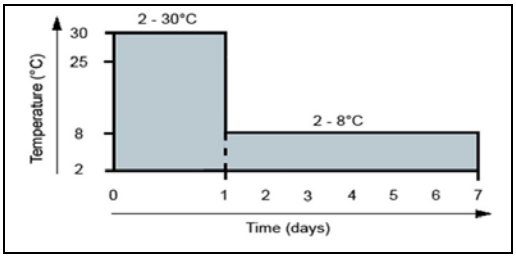


Figure 2: Storage conditions of PDLs.



Whole Blood: Baseline (Day 0) reactivity rate was 100% in whole blood specimens collected in K3EDTA, CPDA, and sodium citrate anticoagulants as well as in lysate pools of 16 donors. Specimens collected in K2EDTA demonstrated a reactivity rate of 90% at Day 0. The lower reactivity rate (90%) in K2EDTA was found to be due to improper sample preparation of one of the donor replicates at baseline because lysates prepared from this donor was reactive at later time point. The Day (b) (4) testing of specimens collected in K2EDTA, K3EDTA, sodium citrate, CPDA anticoagulants, and lysate pools of 16 demonstrated a reactivity rate of 100%, 100%, 96.67%, 90%, and 90%, respectively.

IDLs: Day (b) (4) whole blood stability results were used as the results for Baseline lysate stability. Day 7 testing in lysates from whole blood specimens collected in K2EDTA, K3EDTA, sodium citrate, and CPDA anticoagulants revealed a reactivity rate of 100%, 100%, 96.67% and 90%, respectively. Testing after 3 F/T cycles, lysates from whole blood specimens collected in K2EDTA, K3EDTA and sodium citrate anticoagulants had a reactivity rate of 100%; while the specimens in CPDA had a reactivity rate of 93.33%.

PDLs: Pooled lysates were stored at temperatures ranging between (b) (4) 30°C up to 7 days with or without freeze-thaw cycling. Results from the stability studies indicated that pooled donor lysates may be stored a total of 7 days from the time of the lysis to the time of testing under two conditions: i) for storage above 8°C, PDLs may be stored at up to 30°C for a maximum time limit of 24 hours, and ii) for the remaining time they must be stored between 2 - 8°C (Fig.2). Freeze-thawing of PDLs resulted in loss of sensitivity and therefore was not acceptable. Storage conditions for pooled donor lysates described in Fig.2 were acceptable.

5.8 Reagent Stability Studies

a. On-Board Reagent Stability, Calibrator Set Time, and RPI 250T File 3 Stability on Panther

This study assessed the accuracy and precision of the assay for reagents up to (b) (4) hours onboard (open-kit) and at (b) (4) days after reconstitution. Panel members consisted of in-house *Babesia* High QC panel at (b) (4) copies/mL, Negative QC panel, Negative and Positive Calibrator incubated at (b) (4) hours. Results demonstrated no invalid replicates for the negative panel throughout the study and all valid replicates of the Negative QC panel were nonreactive across all time points. All *Babesia* High QC Panel (b) (4)

copies/mL) tested were valid and reactive (100% reactive) across all time points. The assay reagents demonstrated acceptable performance when the reagents were prepared and stored per package insert specifications. Results from this study verified the stability claims of open kit reagents after 20 hours of 25°C incubation in the Reagent Preparation Incubator (RPI) using File 3, up to 30 days after opening, and 60 hours on-board the Panther system. Results also verified the 8-hour stability of calibrators after incubating them at 15-30°C.

b. Procleix Babesia Assay Environmental Conditions

This study demonstrated that the performance of Procleix Babesia Assay on the Panther System was not affected at the environmental specification limits for external ambient temperature (b) (4) and relative humidity (RH) (b) (4) for the Panther System. Positive specimens consisted of in-house *Babesia* QC panels (High QC at (b) (4) copies/mL and Low QC at (b) (4) copies/mL), positive lysate at about (b) (4) LOD, and positive (b) (4) pooled lysates. Negative specimens consisted of Negative QC panel, negative lysate, and negative (b) (4) pooled lysates. Runs on Panther and Xpress systems in the environmental chamber were performed using (b) (4) reagent lots at each of (b) (4) temperature and humidity conditions (b) (4)

Results indicated that for all environmental conditions tested there were no false reactive replicates in any of the negative specimens and all replicates of the *Babesia* High and Low QC panel, *Babesia*-spiked IDLs and PDLs were 100% reactive. The performance of the Procleix Babesia Assay was not affected at the environmental specification limits for external ambient temperature (b) (4) and relative humidity (b) (4) for the Panther and Xpress Systems and was found to be acceptable.

c. Real-Time and Open Kit Reagent Stability

The shelf-life stability of the Procleix Babesia Assay reagents was assessed when stored for up to (b) (4) months at the intended storage conditions using (b) (4) lots of reagents. The applicant submitted a proposed performance testing plan at Baseline (0), 3, 6, 9, 12, (b) (4) months for both real-time and open-kit (Panther on-board) conditions to support a (b) (4)-month stability claim. This study is on-going and stability data up to (b) (4) months, open-kit conditions were submitted. Baseline testing results demonstrated 100% reactivity for the High QC panel at (b) (4) copies/mL and 98.3% reactive for the Low QC panel at (b) (4) copies/mL. Both High and Low QC panel were 100% reactive up to the (b) (4)-month testing point. The negative panel was 0% reactive at all time points tested. All panel and calibrator results through the (b) (months open-kit condition time point met the acceptance criteria of the study. The Procleix Babesia Assay reagents exhibited acceptable performance with no unexpected results as of the (b) (4)-month, (b) (4) day open-kit 72-hour on-board time point when stored at the appropriate temperatures. Based on the (b) (4)-month stability data, a 12-month stability claim will be granted at the time of licensure. The study will be completed at (b) (4)-month testing for future extension of the stability claim.

Review issues

- *An IR was communicated seeking clarification on the different acceptance criteria for the Low QC panel in three different stability studies. GDS provided the following explanation. The acceptance criteria for the Low QC panels was* (b) (4) *reactivity up to 6 months and*

was reduced to (b) (4) *beyond 6 months of stability study time points. This was acceptable since the Low QC panel is expected to lose sensitivity over extended periods of time and the acceptance criteria for the High QC panel was consistently set at* (b) (4) *across all timepoints and this issue was resolved.*

- *Certain study documents were missing in the submission. GDS provided all requested documentation (SQ_P_3132-PQR-001; VAP-04625, QCR—00309, QCRTSS-MP, GDSS-VAR-000002) and this issue was resolved.*

5.9 Parasite Transport Medium (PTM) Stability Studies

a. PTM Shipping stability

The shipping stability of PTM was evaluated by storing (b) (lots of PTM at 15 (b) (4) and at 30 (b) (4) for (b) (4) hours to support the (b) (4) -hour shipping claim.

Specificity rate of 100% was observed for Baseline (Day 0) testing in whole blood specimens prepared with PTM stored at room temperature. A specificity rate of 100% was observed for (b) (4) -hour and (b) (4) -hour testing in whole blood specimens prepared with PTM stored at 15^0C or 30^0C.

Sensitivity rate of 100% was observed for Baseline (Day 0) testing in *Babesia*-spiked whole blood specimens prepared with PTM stored at room temperature; for (b) (4) -hour and (b) (4) -hour testing in *Babesia*-spiked whole blood specimens prepared with PTM stored at 15^0 C or 30^0C; and for the pooled lysates (b) (4) . Average (b) (4) concentration was within acceptable limits at Baseline, (b) (4) Hours at 30^0C, and (b) (4) Hours at 15^0C, respectively.

b. *PTM Real-Time Stability*

The purpose of this study was to evaluate the real-time (long-term), open-bottle, and open-tube stability of the PTM used for the Procleix Babesia Assay when stored for up to (b) (4) months at the intended storage.

Specificity rate of 100% was observed for Baseline testing in whole blood specimens prepared with PTM stored at room temperature. Testing at 3-months demonstrated a specificity rate of 99.72%, while testing at 6, 9, and (b) (4) months demonstrated a specificity of 100% for whole blood specimens prepared with PTM stored at the real-time, open-bottle and open-tube stability conditions.

Sensitivity: a reactivity rate of 100% was observed for Baseline testing in *Babesia*-spiked whole blood specimens prepared with PTM stored at room temperature. A reactivity rate of 100% was observed for 3, 6, 9, and (b) (4) -months testing in *Babesia*-spiked whole blood specimens prepared with PTM stored at the real-time, open-bottle and open-tube stability conditions. The average (b) (4) concentration was within specification at Baseline, 6-month and (b) (4) month testing. PTM real-time stability results demonstrated acceptable performance of the PTM reagent at various storage conditions. The study will be completed at (b) (4) -month testing. Based on the real time stability data for the Procleix PTM, a 9-month stability claim for PTM will be granted at the time of licensure.

Review issues

- *Shelf life for PTM indicated an out-of-specification* (b) (4) *concentration at* (b) (4) *-month and* (b) (4) *-month time point. GDS submitted that the* (b) (4) *concentration indicated as* (b) (4) *was a typo and provided the corrected values.*

5.10 Cadaveric studies

a. Procleix Babesia Assay testing in cadaveric specimens

Cadaveric whole blood specimens collected from donors from 1 hour and 13 minutes to 23 hours and 43 minutes post-mortem, were appropriately evaluated for plasma dilution, and included hemolyzed specimens ranging from (b) (4) To determine assay specificity, 50 cadaveric specimens were tested with the *Babesia* assay. To determine assay sensitivity, a second aliquot of 50 cadaveric specimens spiked with *B. microti* at about 3-fold the 95% LOD of the Babesia Assay was tested with the Procleix Babesia Assay. Additionally, a total of 50 *Babesia*-spiked and unspiked normal whole blood control donors were evaluated. Specificity was 100% for the negative control and cadaveric specimens testing with average IC S/CO values of 2.06 and 2.08 for the control and cadaveric specimens, respectively. Testing of the *Babesia*-spiked control and cadaveric specimens in the Procleix Babesia assay demonstrated a sensitivity of 100% and 98.00%, respectively. The average analyte S/CO values of combined reagent lots for the control and cadaveric specimens were 13.16 and 13.24, respectively.

b. Reproducibility of the Procleix Babesia Assay in Cadaveric Specimens

Twenty cadaveric specimens were compared to 20 living donors for the detection of *Babesia* parasite with the Procleix Babesia Assay. Whole blood lysates, prepared by diluting *B. microti*-spiked living or cadaveric whole blood at about (b) (4) LOD were tested individually, in six separate runs on six separate days using each of three different reagent lots. Reproducibility results demonstrated reactivity rates of 100% for control specimens, and 99.73% for cadaveric specimens. The % reactivity 95% CI was 98.98-100% for control specimens and 98.50-100% for cadaveric specimens. Mean analyte S/CO values in controls samples was 12.81 and the %CV of analyte S/CO was 4.80%. The mean analyte S/CO value in cadaveric samples for all lots combined was 12.99 and the %CV of analyte S/CO was 11.79%. Mean and %CV calculations for S/CO values were from reactive results only. The p-value for analyte S/CO variance was <0.0001, showing some significant statistical difference exists between conditions, but the difference is functionally insignificant as the p-values for % Reactivity showed no significant statistical difference with a p-value of 1.00. Reproducibility Analysis of Analyte S/CO indicated intra-run (random error) as the largest source of variability.

c. Cadaveric Specimen Stability

The stability of *Babesia* parasite was evaluated in whole blood, collected within 24 hours post-mortem, from 10 individual cadaveric donors spiked with *B. microti* at about 3-fold the 95% LOD of the *Babesia* Assay. The stability was also evaluated in individual lysates.

After spiking the individual whole blood cadaveric specimens with *Babesia* parasite, each collection tube was subjected to the series of incubations depicted in Figure 3 and 4.

Figure 3: Whole blood stability

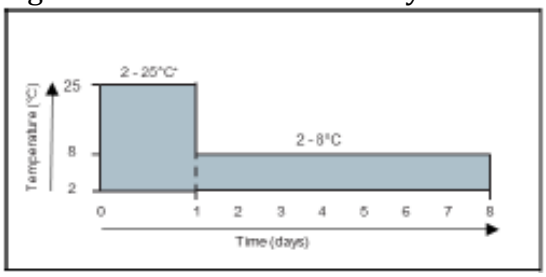
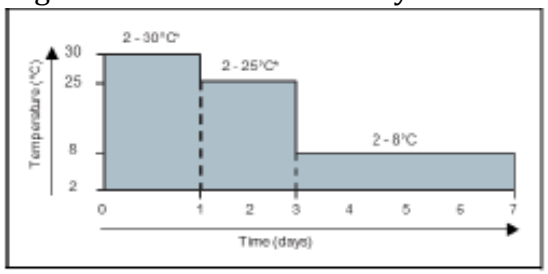


Figure 4: Individual Donor Lysate stability



Whole blood: A reactivity rate of 95.45% was observed for Baseline testing and 100% for Day 1, Day 5 and Day 8 testing in cadaveric whole blood specimens.
IDLs: Day 5 and Day 8 cadaveric whole blood stability results were used as the results for Baseline lysate stability. A reactivity rate of 100% was observed for Baseline (Day 0), Day 4, and Day 7 testing and for testing after 3 F/T cycles in lysates from 5-day old and 8-day old cadaveric individual lysates.

d. Long Term Storage Specimen Stability

This study evaluated the long-term frozen stability of *Babesia* specimens in living and cadaveric donors stored up to 1 year at ≤-20°C when tested with the Procleix Babesia Assay. Living donor specimens included whole blood collected using K2EDTA, K3EDTA, sodium citrate (4%), and CPDA anticoagulant tube types. Cadaveric specimens included whole blood collected using K2EDTA. Collected specimens were spiked with *B. microti* and separate aliquots were made for each time-point including Day 0 (Baseline), 6, 9, and 12 months post baseline. Day 0 aliquots were frozen at -20°C before testing. The samples maintained 100% and 93.75% reactivity through 9-month and 12-month testing, respectively. Nineteen individual invalid results out of (b) (4) tests were in the cadaveric samples tested due to failure of internal control to amplify. Invalid replicates were retested either neat or diluted in 1:4 ratio in PTM and only valid results were used in the analysis. The overall invalid rates of living and cadaveric samples were 0% (0/(b) (4) and 17.57% (b) (4) , respectively.

Review issues

- *Originally, the Intended Use included cadaveric claims for all four Babesia species while submitting validation results for only B. microti. Grifols was advised to either provide validation data for all four species or revise the intended use language appropriately to reflect claims only against B. microti. Grifols responded satisfactorily by revising the Intended Use claim.*
- *Cadaveric studies were analyzed by the Score method instead of Exact (Clopper-Pearson) method. Grifols submitted the reanalyzed data and this issue was resolved.*
- *Grifols was asked to explain the discrepancy and occurrence of high invalid rates in cadaveric studies. This was resolved by Grifols response to the IR.*

- *For the cadaveric reproducibility and sensitivity studies, Grifols included the following statements along with the invalid rate: "A higher incidence of initial invalid results was observed in cadaveric specimens. All specimens were valid upon retest." Grifols was notified to provide the same statement and invalid rate for the cadaveric specificity study, Cadaveric Blood Specimen Storage Study and Cadaveric Specimen Storage-Frozen study.*
- *Grifols was notified to add a summary of the Cadaveric Blood Specimen Storage Study with results and Cadaveric Specimen Storage Frozen study with results to the "Performance Characteristics of Cadaveric Specimen Testing" section of the Package Insert.*
- *Grifols was notified to add a statement in the Assay Procedure section of the Package Insert clarifying when to dilute cadaveric specimens 1:4 in PTM, (e.g. when an invalid result is obtained during a valid run.). This was resolved by Grifols response to the IR.*
- *Originally, the Intended Use included the statement, "Lysed individual donor whole blood samples are tested either individually or in pools of equal aliquots of not more than 16." Grifols was asked to revise the Intended Use to include the statement "Cadaveric donor specimens must be tested individually and not pooled." Grifols also revised the "Specimen Collection, Storage, and Handling" section of the Package Insert by adding "Lysates from cadaveric specimens should not be pooled."*
- *All the above issues were resolved by Grifols in response to the IR.*

6. Clinical Studies

The clinical studies supporting this application were performed under IND #17429. Testing was initiated on June 28, 2017 and blood donors in regions predicted to be highly endemic, low-medium endemic, and non-endemic for *B. microti* were included in the clinical study to evaluate the performance of the Procleix Babesia Assay in both individual and pooled lysates format. Testing was performed at American Red Cross, Creative Testing Solutions, and the Rhode Island Blood Center.

Clinical Specificity Study

The specificity study was performed under a single protocol. The protocol describes procedures and analyses with an objective to estimate the specificity of the Procleix Babesia Assay on the Procleix Panther System in donations from donors of whole blood and blood components. Additional objectives were to investigate potential yield cases and evaluate assay performance in samples tested in 16-lysate pools and 8-lysate pools vs performance in lysates tested individually.

A prospective, multicenter clinical trial was conducted in the United States. Three (3) testing sites obtained samples from blood donations from affiliated United States blood collection centers. Samples were linked to allow for donor identification, deferment, and donor follow-up if reactive. Prior to testing with the Procleix Babesia assay, the investigational Procleix Xpress system was used to create individual donor lysates (IDLs) by combining fresh whole blood with PTM, and to create pooled lysates by combining equal volumes of individual IDLs from up to 16 donations.

In the first phase of the study, samples were tested simultaneously as IDLs and in 16-lysate pools with the Procleix Babesia assay. In phase two, when approximately 3300 IDLs had been tested, IDL testing ceased and screening was performed using 16-lysate pools, with resolution testing of any pools with reactive results. Pools with reactive results were

resolved by testing the IDLs comprising the pool with the Procleix Babesia assay to identify the reactive donation(s) in the pool. Up to (b) (4) Procleix Babesia assay reagent kit master lots were used by each testing site. Lysates with nonreactive Procleix Babesia assay results were considered negative for *Babesia* and were not tested further. Donors with reactive *Babesia* test results were contacted for follow-up. Additional testing was performed for donations with reactive results, volume permitting, including the following:

- Replicate testing with the Procleix Babesia assay from the original lysate/pool in (b) (4) replicates,
- Primary comparator NAT testing of a whole blood sample with a real-time polymerase chain reaction (PCR) assay that detects *B. microti*,
- Serologic testing of a serum sample, if available, or plasma sample with an immunoglobulin G (IgG) immunofluorescence assay (IFA) that detects *B. microti*, and
- If the primary comparator NAT and serologic test had nonreactive results, secondary comparator NAT testing of a whole blood sample with a real-time PCR assay that detects *B. microti*, *B. duncani*, *B. divergens*, and *B. venatorum* was performed.

IDLs that had a reactive Procleix Babesia *assay* result were also checked in the 16-lysate pool in which the lysate was tested and confirmed to have a reactive result. If the pool had a nonreactive result, a 8-lysate pool was created and tested in (b) (replicates with the Procleix Babesia assay.

There were 509 Procleix Babesia assay runs; of these, 502 (98.62%) were valid and 7 (1.38%) were invalidated by the instrument due to operator or hardware errors. There were 11,068 IDLs tested in valid runs. Of these, 11,067 IDLs (99.99%) had final valid results and 1 IDL (<0.01%) had a final invalid result (Table 7). There were 11,058 pools tested in valid runs. Of these, 11,038 pools (99.83%) had final valid results and 19 pools (0.17%) had final invalid results (Table 8).

Table 7: Clinical Specificity Study Results in IDLs

Site	Number	True Negative	False Positive	True Positive	Specificity % (95% CI)
ARC	4126	4124	0	2	100 (99.91-100)
CTS	3487	3487	0	0	100 (99.89-100)
RIBC	3454	3449	0	5	100 (99.89-100)
Total	11,067	11,060	0	7	100 (99.97-100)

Table 8: Clinical Specificity Study Results in PDLs

Site	Number	True Negative	False Positive	False Negative	True Positive	Specificity % (95% CI)
ARC	3843	3820	0	1	22	100 (99.90-100)
CTS	3201	3200	0	0	1	100 (99.89-100)
RIBC	3994	3957	0	0	37	100 (99.90-100)
Total	11,038	10,977	0	1	60	100 (99.97-100)

Specificity was 100% (11,060/11,060, 95% CI: 99.97% to 100%) in IDLs (Table 7), and 100% (10,977/10,977, 95% CI: 99.97% to 100%) in 16-lysate pools (Table 8).

Seven IDLs and 60 pools had reactive Procleix Babesia assay results and were confirmed to have true positive outcomes. One donor from Florida had a reactive result, where the risk of *Babesia* infection is expected to be much lower than in the Northeast and Upper Midwest. Two PDLs had reactive Procleix Babesia assay results and negative serologic test results at index (i.e., potential yield cases over serology). One of the two pools contained a sample from a donation with a positive primary NAT comparator test result at index, confirming the Procleix Babesia assay result as true positive. One pool contained a sample from a donation that had negative primary and secondary comparator NAT results at index. This donor had one follow-up visit approximately 6 weeks after the index donation and had a reactive Procleix Babesia assay result and positive primary comparator NAT and serologic test results. There was one pool with a false negative result. This pool contained an IDL that had a true positive status; results for the IDL are described below.

All IDLs with repeat reactive Procleix Babesia assay results were also detected in 16-lysate pools (6/6). One of the 7 confirmed positive IDLs was initially reactive in the Procleix Babesia assay but nonreactive in all subsequent repeat IDL testing and in its corresponding 16-lysate and 8-lysate pools. Additional testing of a newly prepared IDL and pooled lysates for this donation was performed in-house. This additional testing yielded all nonreactive Procleix Babesia assay results for (b)(replicates each of the new IDL, 8-lysate pool, and 16-lysate pool samples. This donation also had a negative primary comparator NAT result at index but had a positive serologic test result. At follow-up, the donor remained NAT nonreactive but was positive by serology, suggesting a resolving infection. The clustered matched-pair analysis for the pool effectiveness testing results indicated that the variance of the difference between results for IDLs and PDLs was not statistically significant.

Review issues

A root cause analysis report was requested for the 84 IDLs with initial invalid results. GDS provided adequate response and this issue was resolved. 79/84 of the IDLs were attributed to the same failed run ("other" reasons under invalid), which appeared to be an isolated incident since it was not repeated during the clinical trial or under the IND. 5 other invalids were either hardware errors or specimen errors. The responses were found to be adequate and this issue was resolved.

Clinical Sensitivity Study

Clinical sensitivity of the Procleix Babesia assay in known-positive whole blood samples was evaluated in accordance with the protocol B10385-BABPS-CSP-02 [Clinical Sensitivity Evaluation of the Procleix Babesia Assay Using the Procleix Panther System in Known-Positive Samples]. Testing was performed at 2 external sites (ARC and CTS) and one in-house (GDS) site using 3 reagent kit master lots. Known-positive samples of *B. microti*-infected human blood samples were used. For *B. duncani*-infected hamster blood, *B. venatorum*-infected (b) (4) whole blood samples, and *B. divergens*-cultured erythrocytes diluted in *Babesia*-negative human whole blood were used in this study. Both IDL and PDLs were prepared from these whole blood specimens. Testing sites were provided with

known-positive whole blood lysate samples that were prepared neat (original undiluted concentration) and diluted 1:16 with known-negative whole blood lysate samples (to mimic 16-sample pools). Known-negative lysate samples were also provided to mask operators to the expected results. The neat and diluted samples were split approximately equally among 3 testing sites.

Results: All 131 neat and 131 diluted known positive samples had positive Procleix Babesia assay results (Table 9). Overall sensitivity of the Procleix Babesia assay was 100% (131/131, 95% CI: 97.22% - 100%) in both neat and diluted samples.

Table 9: Clinical Sensitivity Study Results

	IDL		**16-lysate pool**	
	# Reactive/ # Tested	Sensitivity % (95% CI)	# Reactive/ # Tested	Sensitivity % (95% CI)
B. microti	90/90	100 (95.98-100)	90/90	100 (95.98-100)
B. duncani	15/15	100 (78.19-100)	15/15	100 (78.19-100)
B. divergens	15/15	100 (78.19-100)	15/15	100 (78.19-100)
B. venatorum	11/11	100 (71.50-100)	11/11	100 (71.50-100)
Total	131/131	100 (97.22-100)	131/131	100 (97.22-100)

Review issues

Details about sample preparation, methodology and work flow were requested. Grifols provided all the above details and the responses were found to be adequate and this issue was resolved.

Clinical Reproducibility Study

The clinical reproducibility study for Procleix Babesia assay was performed under protocol B10385-BABPS-CSR-03 under IND17429. Testing was performed at three sites, one internal and two external, ARC and CTS. A five-member reproducibility panel was prepared that included one *Babesia*-negative panel member and 4 *Babesia*-positive panel members (b) (4) LOD, (b) (4) LOD, (b) (4) LOD, and (b) (4) LOD) created using lysate made from *B microti*-infected hamster blood diluted in human whole blood and lysed in Parasite Transport Medium. Reproducibility was assessed across the following factors: 3 reagent lots x 3 test sites x 2 operators (1 instrument per site) x 6 days of testing x 2 runs x 2 replicates of each panel member.

Table 10: Clinical Reproducibility Study Results

Panel Member	**Description**	**Concentration (parasites/mL)**	**Agreement (%)**	**95% CI (%)**
A	Negative	0	100	98.3-100
B	Very Low Positive	2.6 (b) (4) LoD)	67.91	61.22-74.09
C	Low Positive	15.61 (b) (4) LoD)	100	98.29-100
D	Moderate Positive	52.05 (b) (4) LoD)	100	98.31-100
E	High Positive	260.23 (b) (4) LoD)	100	98.29-100

There were 108 runs initiated; all were valid. Of the 1080 samples tested in valid runs, 1074 samples (99.44%) had valid results. Agreement values are reported in Table 10. As expected, the very low positive panel member (panel B) had a positive agreement value of 67.91% due to the probability that approximately half of the tubes for this panel member would not contain a *Babesia* parasite due to the low concentration of the bulk panel member (targeted at (b) (4) LOD parasites/mL).

BIMO – Clinical/Statistical/Pharmacovigilance

A Bioresearch Monitoring (BIMO) inspection was conducted at one clinical investigator site that participated in the conduct of Study B10385-BABPS-CSP-01. The inspection did not reveal significant problems that impact the data submitted in the Biologics License Application (BLA).

7. Advisory Committee Meeting

It was determined that this submission did not require presentation at an Advisory Committee meeting prior to approval.

8. Other Relevant Regulatory Issues

None

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.

10. Recommendations and Risk/ Benefit Assessment

a) Recommended Regulatory Action

The Review Committee reviewed the original submission and related amendments. All review issues have been resolved therefore; the Review Committee recommends licensure of the Procleix Babesia Assay.

b) Risk/ Benefit Assessment

The Procleix Babesia Assay is intended for detection of RNA from *Babesia* species (*B. microti*, *B. duncani*, *B. divergens*, and *B. venatorum*) in whole blood specimens. Adverse events that may occur would be a false negative test result that would permit *Babesia*-infected blood to be transfused; or false positive result that would result in discarding healthy, usable blood and loss of the donor who would be deferred. The assay has an estimated 95% LOD range of 6.73-12.44 *Babesia* transcript copies/mL and 1.77-3.10 *Babesia* parasites/mL. The clinical studies demonstrated a sensitivity of 100% (95% CI of 97.22-100.00%), indicating low probability of a false negative result. Among ~170,000 units of blood tested with the Procleix Babesia Assay, no cases of transfusion transmitted babesiosis have occurred. The assay specificity of 100% (95% CI of 99.97-100%) in clinical trials suggests the low probability of false positives. The Procleix Babesia Assay with high clinical sensitivity and specificity will significantly improve blood safety and public health by reducing the transfusion of *Babesia*-

infected blood, which can be fatal in susceptible recipients. Therefore, licensure of this test ensures further protection of the nation's blood supply.

c) Recommendation for Postmarketing Activities

No postmarketing activities have been proposed for this application.