Summary Basis for Regulatory Action

Date: 07/02/2018

From: Evgeniya Volkova, Chair of the Review Committee

BLA / STN#: 125667/0

Applicant Name: Grifols Diagnostic Solutions Inc.

Date of Submission: November 29, 2017

MDUFA Goal Date: September 29, 2018

Proprietary Name/ Established Name: Procleix® Zika Virus Assay

Intended Use/Indications for Use:
The Procleix Zika Virus Assay is a qualitative in vitro nucleic acid test for the detection of Zika Virus (ZIKV) RNA in plasma specimens from individual human donors, including volunteer donors of whole blood and blood components for transfusion. It is also intended for use in testing plasma or serum specimens to screen other living (heart-beating) donors of organs and Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps), and in testing blood specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens. The assay is intended for use in testing individual donor samples. It is also intended for use in testing pools of human plasma composed of equal aliquots of not more than 16 individual specimens from volunteer donors of whole blood components. This assay is not intended for use as an aid in the diagnosis of Zika virus infection.

Recommended Action:
The Review Committee recommends approval of this product.
Review Office Signatory Authority: Nicole Verdun, MD, Acting Office Director, Office of Blood Research and Review

☐ **I concur with the summary review.**

☐ **I concur with the summary review and include a separate review to add further analysis.**

☐ **I do not concur with the summary review and include a separate review.**

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

Table 1. Reviews submitted.

Document Title	Reviewer Name	Document Date
Product Review(s) (product office) • *Clinical* • *Non-Clinical*	 Julia Lathrop Krishna Ketha	 06/12/2018 06/01/2018
Statistical Review(s) • *Clinical* • *Non-Clinical*	Tie-Hua Ng	06/08/2018
CMC Review • *CMC (Product Office)* • *Facilities Review (OCBQ/DMPQ)* • *Establishment Inspection Report(s) (OCBQ/DMPQ)* • *Bioburden (OCBQ/DBSQC)*	 Caren Chancey Maria Rios Bradley Dworak Bradley Dworak Claire Wernly	 05/31/2018 06/01/2018 06/29/2018 06/27/2018 04/11/2018
Labeling Review(s) • *APLB (OCBQ/APLB)*	 Dana Jones	 05/17/2018
Lot Release Protocols/Testing Plans	Ishrat Sultana Varsha Garnepudi Swati Verma	06/28/2018 05/23/2018 06/06/2018
Bioresearch Monitoring Review	Erin McDowell	05/18/2018
Software and Instrumentation	Yongqing Chen Lisa Simone	05/23/2018 05/23/2018
HCT/Ps and Organ Donors Review	Bruce Crise	06/25/2018

1. Introduction

Grifols Diagnostic Solutions Inc., located in San Diego, CA, submitted a Biologic License Application for the Procleix® Zika Virus Assay nucleic acid test for use on the Procleix® Panther System. The sponsor requested a priority review designation for the assay; however, the request was not granted by the FDA as the assay was not the first of its kind to be submitted to and licensed by the FDA.
The application was submitted on November 29, 2017 and filed on January 25, 2018. The mid-cycle meeting was held on April 23, 2018. Chronological summary of Information requests (IR) sent to the sponsor and amendments submitted in response is provided in Table 2. Sponsor submitted 11 amendments in response to FDA requests.

Table 2. Chronological Summary of Interactive Review.

Date	Action
January 9, 2018	BIMO IR
January 12, 2018	Amendment 001 in response to Information Request
March 1, 2018	Amendment 002 on filing review comments
March 3, 2018	Software and instrumentation Information Request
April 6, 2018	Amendment 003 in response Information Request
April 25, 2018	Teleconference to discuss review issues

April 26, 2018	Follow-up Information Request
April 30, 2018	Information Request with CMC, analytical and clinical studies, and software and instrumentation questions
May 4, 2018	Amendment 004 in response to Information Request
May 8, 2018	Information Request
May 11, 2018	Amendment 005 in response to CMC, analytical/clinical, software/instrumentation, and DMPQ questions
May 14, 2018	Amendment 006 containing modified LRP Teleconference to discuss DMPQ issues
May 17, 2018	DMPQ IR
May 21, 2018	IR containing questions/comments on CMC, analytical, and clinical studies
May 24, 2018	Amendment 007 in response to Information Request
May 29, 2018	Amendment 008 in response to Information Request for CMC, analytical, and clinical questions
May 30, 2018	Information Request
June 8, 2018	Amendment 009 in response to Information
June 12, 2018	Information Request
June 14, 2018	Information Request
June 15, 2018	Amendment 10 in response to Information Request

*Specific information regarding the information requests and responses can be found in later parts of the SBRA

2. Background

Zika virus (ZIKV) is an enveloped, icosahedral, single-stranded positive sense ribonucleic acid (RNA) arbovirus of the family Flaviviridae, genus *Flavivirus*. ZIKV is primarily transmitted by the bite of an infected *Aedes* species mosquito. It was first isolated in Uganda in 1947, and sporadic infections had been reported in Africa and Asia until 2007, when ZIKV caused an outbreak in Yap State of Micronesia. In 2013-14, ZIKV caused a large outbreak in French Polynesia and emerged in Brazil in 2015, later spreading to other countries in South America, Central America, Mexico, and the Caribbean, including the U.S. territories of Puerto Rico and the Virgin Islands. Most ZIKV infections are asymptomatic or cause mild flu-like illness, but in some cases, infections lead to severe neurological manifestations such as Guillain-Barré syndrome in adults and congenital malformations including microcephaly and ocular defects in fetuses of infected mothers. In addition to the vector-borne route, intrauterine and intrapartum transmission, sexual transmission, transmission through laboratory exposure, and possible transfusion-transmission have been reported.

In February 2016, as a safety measure to reduce the risk of transfusion-transmitted ZIKV, the U.S. FDA issued a guidance that included a recommendation for areas with active transmission to discontinue blood collections unless donations are screened with an FDA-licensed blood donor screening test or treated with an FDA-approved pathogen reduction technology (PRT). No FDA-approved screening tests existed at the time, and FDA-approved PRT was only available for plasma and platelets, which resulted in cessation of blood collection in Puerto Rico. The Procleix® Zika Virus Assay nucleic acid test was developed in response to this recommendation, and the product was available

for use under an Investigational New Drug (IND) application (IND 17003) since June, 2016. In the revised guidance issued in August 2016, the FDA recommended universal testing of donated whole blood and blood components or using PRT in the U.S. and its territories.

The Procleix Zika Virus Assay shares assay principles, manufacturing technologies and controls, and common reagents with FDA-approved Procleix assays, such as Procleix Ultrio Assay (BL 125113, approved on October 3, 2006). It was developed for use with the Procleix Panther System that includes the same Panther instrument and instrument software as the assays previously approved by FDA (Procleix Ultrio Elite Assay, BL 125652, approved on May 3, 2018 and Procleix WNV Assay, BL 125181/80, approved on May 25, 2018). The system consists of an analyzer, associated hardware, assay reagents, and instrument and assay-specific software. It automates sample processing, amplification and detection of target nucleic acid, and report generation. The Procleix Zika Virus Assay is composed of 2 ZIKV-specific kits and 2 Procleix Ancillary kits common for all Procleix assays. The sample is processed automatically in a single tube, where target RNA is first captured with a ZIKV-specific probe, then amplified and hybridized to chemiluminescent probe. The assay utilizes transcription-mediated nucleic acid amplification (TMA) technology, which involves 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 are determined using assay calibrators. Chemiluminescent probes that hybridize to the IC targets are discriminated from ZIKV-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 Zika Virus Assay on the Procleix Panther System is currently commercially available in multiple countries outside of the U.S.

Pre-submission BQ170006 was received by FDA on February 24, 2017, and records of pre-submission communications were provided with the BLA. A teleconference was held on March 7, 2017, preceded by issuance of FDA preliminary written responses to questions posed by the sponsor in the pre-submission and Grifols written comments and follow-up questions. The summary of issues discussed and outcomes are presented below:

- Additional analytical performance evaluation studies to be conducted in support of BLA were discussed and found to be acceptable by FDA provided the type of plasma in all studies is specified and ideally the same across studies. In addition, for studies that were already performed, line data was also to be included in the BLA.

- Cadaveric specificity and sensitivity studies were discussed, and the sponsor was advised on the steps to be performed to ensure adherence to the current FDA Guidance.

- Minor modifications were recommended by FDA to the design of run size validity, calibration set time, and short-term specimen stability studies.

- The issue of inclusion of serum samples for screening for ZIKV in the intended use statement was discussed, and it was decided that the inclusion of serum as a matrix when screening donors for purposes other than blood transfusion may be appropriate, provided certain performance targets are met by serum sensitivity and specificity studies.

- Feasibility of referencing the proposed Intended Use Statement in clinical sensitivity and reproducibility protocols and including data for testing of 16-sample pools in the BLA were discussed, and sponsor's proposals were found to be acceptable; FDA suggested that data from pool testing should be clearly separated.

- The date of December 31, 2016 was proposed by the sponsor as a cut-off date to lock the dataset from the IND study and use the data for the clinical specificity calculation. This was found acceptable by FDA.

- Confirmatory testing for initially reactive samples was discussed, and it was agreed that true positive status can be based on validated alternative NAT testing or on ZIKV serology result if confirmed by (b) (4)

- Grifols proposed to test (b) (4) plasma and (b) (4) serum known-positive samples to determine assay sensitivity. FDA recommended increasing the number of samples to be tested to ensure that the lower bound of the 95% CI is ≥95%, and Grifols confirmed that (b) (4) samples for each matrix will be used.

- Design of the reproducibility study proposed by the sponsor was discussed, and a discrepancy in the sample number calculation was corrected.

- FDA suggested the following studies should be performed in support of the BLA: investigate the potential of a hook effect; include Yellow Fever virus and Japanese Encephalitis virus complex members in the study; and evaluate the effects of exogenous interferents.

A supplement to BQ170006 was submitted on August 18, 2017, which proposed a proportional reduction in the clinical specificity study dataset from 1,600,000 donations to 170,000 donations by random selection of 10,000 individual samples and 10,000 pools from the complete database stratified by lot and site. FDA responded on August 31, 2017 with a memorandum rejecting the proposal and for the Sponsor to perform the study as requested and provided in the submission.

3. Chemistry Manufacturing and Controls (CMC)

a) Manufacturing Summary

In Vitro Substance

The in vitro substances used in the Procleix Zika Virus Assay kit are a set of (b) (4) oligonucleotides, which have been categorized by the sponsor into (b) (distinct classes based on structure, function, and chemical composition, as follows:

(b) (4)

This oligonucleotide classification system has previously been used to classify the in vitro substances in other licensed Procleix products. Four of the oligos are common to other licensed Procleix Assays, including: Procleix HIV-1/HCV Assay (STN BL 103966; product no longer marketed in the US); Procleix Ultrio Assay (STN BL 125113); Procleix Ultrio Plus Assay (STN BL 125113/48); Procleix WNV Assay (STN BL 125121 and 125121/80,); and Procleix Ultrio Elite Assay (STN BL 125652). ZIKV-specific oligos (b) (4) target (b) (4) highly-conserved regions within the ZIKV genome. (b) (4) . Information on structure; composition and quantities; manufacturing process; validated in-process controls; characterization; specifications and validated analytical methods; and clinical lot release results was provided, reviewed, and found acceptable.

The in-process controls used during manufacture of the Procleix Zika oligos, the characterization methods used, and validations for those methods are common to other licensed Procleix Assays. The QC release specifications were provided for each category of oligos. Analytical methods and method validations were provided in section 4.4. Lot release results were provided for each lot of oligos used in a clinical lot of the Procleix Zika Assay.

All Procleix Zika Assay-specific oligos for the representative clinical lot were manufactured by Grifols at the (b) (4) , and all common oligos were manufactured at the (b) (4) . However, for future commercial lots and future (b) (4) lots, all oligos and reagents will be manufactured at the (b) (4) facility with the same manufacturing process and equipment

currently implemented at (b) (4). The validated processes used in the manufacture of the Procleix Zika Virus Assay oligonucleotides and reagents are identical to those used for other licensed Procleix Assays.

Stability dating was based on oligo class specific forced degradation studies performed previously for the licensed Procleix Ultrio Assay (BL125113), and was set at (b) (4) months for (b) (4) probes and at (b) (4) months for other oligo classes when stored at (b) (4). This approach was found acceptable based on the review of the original studies.

In Vitro Product

Information on the common kits, as well as common reagents within the Procleix Zika Assay kits, has been provided in previous BLAs. Table 3 lists the four kits required to perform the Procleix Zika Virus Assay on the Panther System and their manufacturing locations (b) (4) – manufacturing facilities).

Table 3. Procleix Zika Virus Assay kits, components and manufacturing locations.

Kit (Clinical Part #/Commercial Part #)	**Component**	**Manufacturing Location**
Procleix Zika Virus Assay Kit 1000 test (PRD-04036-P/PRD-04170) Procleix Zika Virus Assay Kit 5000 test (PRD04088-P/PRD-04228)	Internal Control Reagent	(b) (4)
	Target Capture Reagent	
	Amplification Reagent	
	Probe Reagent	
	Enzyme Reagent	
	Selection Reagent	
Procleix Zika Virus Assay Calibrators Kit – Low Volume (PRD-04038-P/ PRD-04171) High Volume (PRD-04090-P/ PRD-04229)	Positive Calibrator	
	Negative Calibrator	
Procleix Assay Fluids Kit (303344)	Wash Solution	
	Buffer for Deactivation Fluid	
	Oil Reagent	
Procleix Auto-Detect Reagents Kit (303345)	Auto Detect 1	
	Auto Detect 2	

Manufacturing and controls information was provided for the 2 ZIKV-specific kits: the Procleix Zika Virus Assay Kit, the Procleix Zika Virus Assay Calibrators Kit, and their components. The information included brief description of the reagent's function; part numbers for clinical and commercial lots; components and composition; manufacturing process; in-process control validations; lot release specifications and analytical methods; and lot release results. Release testing for the Procleix Zika Virus Assay Calibrators Kit is based on visual criteria, no kit performance testing is required for this kit. In many cases, in-process control validations were performed using representative materials from other Procleix assays, which was found acceptable since the manufacturing process is the same. A proposed plan for CBER lot release testing was also included.

Abbreviated information was submitted for the 2 ancillary kits, the Procleix Assay Fluids Kit and the Procleix Auto Detect Reagents Kit, because they are common to other licensed Procleix Assays. Release of these kits includes specifications for a visual inspection only. Individual reagents are tested and QC released at the component level. The only manufacturing change noted was addition of RFID tags to the labeling system for use with the Procleix Panther System.

Stability

Based on a formal stability program, the sponsor proposed shelf-life dating for the Procleix Zika Virus Assay Kit to be (b) (4) months with open kit dating of up to 30 days including on board the Panther instrument. Stability testing for the Procleix Zika Virus Assay kit was performed using 3 separate protocols, one of which included testing (b) (4) copies/ml (c/ml) and (b) (4) ml QC panel; however, the studies supporting the (b) (4) -month claim have not been completed. The data submitted with the application currently supports 12-month expiration dating based on (b) (4) lots. An (b) (4) monitoring plan was included in the submission to monitor shelf-life for the product in use in the field, to be performed at 6 month intervals throughout the proposed (b) (4) -month shelf life and reported (b) (4) . Stability testing information for the ancillary kits was not included in the submission since their shelf-life was established previously with the approved expiration dating of 24 months and open-kit and onboard stability of 60 days.

Bioburden

Bioburden qualification tests (bacteriostatic and fungistatic qualification) were performed on (b) (4) lots (b) (4) of (b) (4) representative groups of Procleix Ultrio Elite Assay reagents listed in Table 4, for total aerobic microbial count (TAMC) and total combined yeast and mold count (TYMC) to demonstrate the cumulative Procleix Ultrio Elite Assay test matrix does not inhibit bacterial and fungal growth. Since the reagents differ only in the composition of the nucleic acid components, which would not be expected to affect the bioburden qualification tests, and the manufacturing process and raw materials are the same, results of these tests were found acceptable.

Table 4. Procleix Zika Virus Assay Representative Reagent Groups.

Assay Kit	**Kit Component**
Master Assay Kit	Amplification Reagent Enzyme Reagent Probe Reagent Internal Control Reagent Target Capture Reagent Selection Reagent
Calibration Kit	Positive Calibrator Negative Calibrator
Ancillary Fluids Kit	Wash Solution Buffer Oil Reagent
Ancillary Auto Detect Kit	Auto Detect 1 Auto Detect 2

The test methods for TAMC and TYMC were reviewed and qualified in accordance with (b) (4) and the test results showed that there is no product inhibition on microorganism growth, indicating the product matrixes are suitable for the intended test method.

The microbial assurance level (MAL) for the Procleix Zika assay raw materials and reagents was determined based on the results of MAL testing previously performed for similar reagents of the Procleix HIV-1/HCV or Ultrio assays as defined in section 4.2.1.7 of the submission. Bioburden testing was performed as part of the process validations/lot release for each reagent lot to ensure that the maximum contributions of bioburden from all components of a reagent will not exceed the MAL established for that reagent.

Antimicrobial Effectiveness Testing was performed per (b) (4) as part of the process validations for each reagent and met the acceptance criteria of (b) (4)

Testing Specifications

The analytical methods and their validations and/or qualifications reviewed for the Procleix Zika Virus Assay 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. Final Container lots will be submitted to CBER for review.

c) 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 Zika Virus Assay is Grifols Diagnostics Solutions, Inc. The manufacturer of the Procleix Panther System platform is (b) (4) The activities performed and inspectional histories are noted in Table 5 and are further described in the paragraphs that follow.

Table 5. Manufacturing Facilities for Procleix Zika Virus Assay and Procleix Panther System platform.

Name/Address	FEI number	Inspection/ waiver	Justification /Results

Grifols Diagnostic Solutions, Inc. (b) (4) Manufacturer of: • 5 of 6 components of the Procleix Zika Virus Assay Master Kit: enzyme, amplification, probe, selection, and target capture reagents • 1 of 2 components of the Procleix Zika Assay Calibrators Kit: Negative Calibrator • 2 of 3 components of the Procleix Assay Fluids Kit: wash solution and oil reagent Final kitting of finished assay components Shipment of finished assay kit and Panther platform	(b) (4)	Waived	Team Biologics (b) (4) NAI
Grifols Diagnostic Solutions, Inc. (b) (4) Manufacturer of: • 1 of 6 components of the Procleix Zika Assay Master Kit: internal control reagent • 1 of 2 components of the Procleix Zika Assay Calibrators Kit: Positive Calibrator • 1 of 3 components of the Procleix Assay Fluids Kit: buffer for deactivation fluid • All components of the Procleix Auto Detect Reagents Kit: Auto Detect 1 and 2	(b) (4)	Waived	Team Biologics (b) (4) VAI

Perform quality control release testing of assay			
(b) (4) Manufacture of Procleix Panther System platform QC testing and final release of finished platform (excluding Zika software and testing with assay)	(b) (4)	Waived	ORA (b) (4) NAI

Team Biologics performed a surveillance inspection of the Grifols Diagnostics Solutions, Inc. manufacturing facility located at (b) (4) . No inspectional observations were noted and the inspection was classified as no action indicated (NAI).

Team Biologics performed a surveillance inspection of the Grifols Diagnostics Solutions, Inc. manufacturing facility located at (b) (4) . Inspectional observations were satisfactorily resolved and the inspection was classified as voluntary action indicated (VAI).

ORA performed a surveillance inspection of the (b) (4) No inspectional observations were noted and the inspection was classified as NAI.

d) 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.

e) Container Closure

Not applicable.

Review Issues

- In the initial phases of review, Grifols was asked to confirm information concerning some datasets and provide study datasets in exportable format. Requested information was submitted in Amendments 1 and 2.

- The questions of whether testing of the 2 development lots and testing of a (b) (4) QC panel is sufficient to support a stability claim were discussed at the mid-cycle meeting. It was agreed that 2 lots are sufficient; however, data produced using (b) (4) QC panel is preferable and should be requested from the sponsor. The request was included in the IR sent on April 4, 2018, advising Grifols to revise the expiration dating to reflect the stability endpoint determined in their on-going real-time study protocol QCR-00239 at the time of licensure. The response submitted in Amendment 5 included data that would support 12-month expiration dating based on 3 lots, and was found acceptable.

- The sponsor was asked to clarify the notation used for the time points in stability testing, and the clarification was provided in Amendment 5.

- Validation SQ_P_3101-PQR-001, Process Performance Qualification for the Zika Virus Assay Reagents, Controls and Panels, was requested from Grifols, was submitted in Amendment 5, reviewed, and all deviations and non-conformance reports were found to be appropriately resolved.

- A justification for the use of alternate time points in the real-time stability study was requested from the sponsor along with a confirmation that subsequent time points will be collected as specified in the study protocol. The justification, provided in Amendment 8, was indicative of improperly executed quality management procedures; however, since the sponsor confirmed that the testing at the later time points will be conducted per protocol and the post-licensure stability protocol will be executed, the response was found acceptable.

- Minor modifications to the CBER Panel Results section of Lot Release protocol LRP), including populating it with panel numbers and expected results, were requested by the Lot Release reviewers and provided in Amendment 6 to the satisfaction of reviewers.

- A question was raised regarding the planned transfer of reagents manufacture to (b) (4) facility occurring during the submission review cycle. A teleconference was held on May 14, 2018, during which the sponsor indicated that the timeframe for the move is (b) (4) of 2018 and confirmed that a CBE-30 or a post-approval supplement will be submitted to indicate that change. In addition, in Amendment 7, Grifols revised sections of the submission to remove references to shipping of product from the (b) (4) facility.

- Inconsistencies in the room numbers listed for equipment locations were noted and resolved through an IR (Amendment 4).

- The following was requested from the sponsor and provided with Amendments 4 and 5, which resolved the issues:
    - A list of any new equipment used in the product manufacturing
    - The risk assessment for the assay
    - Information related to the Quality Systems management

- Additional risk assessment addressing cross-contamination and false positive/negative results was requested and provided with Amendment 7.

- Questions regarding the use of (b) (4) were resolved via an IR (Amendment 7).

- The protocols and reports for Analytical Sensitivity and Specificity demonstration and the quality assurance testing plans for the Panther System were requested and provided in Amendment 9.

- A list of changes to the Panther System hardware since the last system verification report was requested from Grifols and submitted in Amendment 11.

- Questions regarding the organization and roles of Grifols, Hologic, and (b) (4) in relationship to the Panther System and levels of control of each company over the system and software were clarified with the sponsor via an IR (Amendment 10).

- The sponsor was asked to confirm that a Grifols employee field service engineer performs the final IQ/OQ/PQ at the customer site, and the confirmation was provided in Amendment 10.

- Grifols was asked to remove an incorrect statement concerning system validation from a document, and the revised document was submitted with Amendment 11.

4. Software and Instrumentation

4.1. Summary

In this submission, the Procleix Panther System is used as the instrument platform supporting the Procleix Zika 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 and Zika (b) (4) (assay specific software) v1.4, firmware v5.3.2.3 running on Windows 7 operating system.

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 are multiple infections 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, magnetic parking, temperature control, luminometer issues, and critical software defects and malicious intent (cybersecurity considerations).

Failure Modes Effects Analysis (FMEA) was recently added to Fault Tree Analysis (FTA) to support a Risk Assessment process better aligned with ISO 14971 "Medical devices – application of risk management to medical devices." Regarding overall residual risk, the applicant states 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 states that there are no known software anomalies or cybersecurity related hazards that would contribute to serious injury or death. Four anomalies were described and discussed. Existing "negligible" severity unresolved anomalies are planned to be addressed in a software revision planned for release by the end of calendar year 2019.

Testing

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.

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.

Major Issues and Software/Device Changes
No changes were made to the software, instrument or labeling as a result of this review.

4.2. Review Issues

- Several software and instrumentation-specific questions were issued and resolved in previous related submissions (BL125652 and BL125121/80). In the IR sent out on March 16, 2018, the sponsor was asked to confirm that the changes implemented during the resolution of issues for these 2 submissions were made and implemented on Panther System Software v5.3 for Procleix Zika Virus Assay and provide any new/updated documentation. The responses submitted by Grifols in Amendment 3 were found acceptable and the issues were considered resolved.

- In the IR issued on April 30, 2018, sponsor was asked to:
    - Indicate how the opportunity of cross-contamination during sample preparation through target signal detection steps from both the user side is prevented or reduced and how the correct target signals will be correctly generated and shown on both screen/monitor and final report where the target assay result (like Zika) is obviously separated from other assays' result.
    - Provide information on random access sample loading to evaluate the effect of this feature on the system.
    - Submit the final version of the Panther System Operator's Manual.

  The responses submitted by the sponsor in Amendment 5 were considered acceptable, and the discipline of software and instrumentation in the submission was found approvable.

5. Analytical Studies

5.1. Reproducibility Studies

Repeatability Study
Testing was designed to evaluate instrument-to-instrument, operator-to-operator, day-to-day, and test-to-test variability. Overall coefficients of variance (CV) were very low for all panels, showing very good repeatability. Mean analyte S/CO ratios in the ZIKV panels at approximately 3xLoD, 5xLoD, 10xLoD, 100xLoD, 1000xLoD were 33.1 (4% CV), 33.2 (4% CV), 33.2 (4%CV), 33.0 (4% CV), and 33.0 (4% CV), respectively. For all panels, intra-run difference contributed most of the variability

(4% CV). Repeatability of the Analyte (Assay Positive and Assay Negative calibrators) and IC (Assay Negative calibrator only) RLU signal of ZIKV Assay calibrators indicated that the overall variability for all calibrator signals was very low. The mean RLU for the ZIKV Assay Positive Calibrator was 1,902,124 (4% CV). The mean IC and Analyte RLU for the Assay Negative Calibrator were 267,585 (6% CV) and 0 respectively. Inter-instrument differences were the main contributors of variance for the IC signal of the Assay Negative Calibrators and the analyte signal of the Assay Positive Calibrators, at 5% CV and 3% CV, respectively.

Reproducibility Study

The reproducibility of the Zika Virus (ZIKV) Assay on the Panther System was demonstrated by evaluating the variability of the assay between different days, different operators, different reagent lots, different instruments, and within run.

All negative panels in plasma and HEPES buffered solution containing detergent were 100% nonreactive. Low panel (30 c/mL) members and high panel (100 c/mL) members in both matrices were 100% reactive. Overall the percent agreement of test results was 100% for negative members in plasma and buffer. The percent agreement of test results for low panel in both matrices was 100%. The percent agreement of test results for high panel (100 c/mL) in both matrices was 100%. The CV for the mean analyte S/CO ratios was 4% for all positive panel members. The mean IC S/CO ratios for the negative panels was also low: 2% CV for HEPES buffered solution containing detergent and 3% for plasma.

Reproducibility of the assay was determined by an evaluation of mean and standard deviation of the analyte signal to cut-off (S/CO) for each positive panel and IC S/CO ratio for each negative panel for each of five variance factors: Inter-Reagent Lot, Inter-Instrument, Inter-Operator, Inter-Day, and Intra-Run. The greatest source of variability observed was in the intra-run factor across all panels in both matrices. The overall variability of the analyte S/CO was low with a total of 4% CV observed in the positive panels. The overall variability of the IC S/CO was also low with the highest total of 3% CV observed in the negative plasma panels.

5.2. Sensitivity Studies

Limit of Detection

This study evaluated the analytical sensitivity and LoD of the Procleix Zika Virus Assay on the Panther instrument with the 1st World Health Organization (WHO) International Standard (IS) for Zika Virus RNA for NAT-based assays (PEI product code 11468/16) and a ZIKV positive specimen in clinical plasma and clinical serum. Sensitivity and LoD was also evaluated for a ZIKV *in vitro* transcript (IVT) which was also used to determine the copies to infectious units (c/IU) conversion factor. Concentrations ranging from 0 to 30 IU/ml for the WHO IS, 0 to 30 c/ml for the positive plasma, and 0 to 90 c/ml for the IVT were tested. Three lots of reagents were used to test the panels. All negative panel members were nonreactive. The 50% and 95% LoD determined by Probit analysis and 95% fiducial limits are presented in Table 6. Using the results from the Zika WHO IS and Zika IVT, the conversion factor

was determined to be (b) (4) c/IU and (b) (4) c/IU for plasma and serum, respectively. The performance was found to be acceptable.

Table 6. Combined LoD results for the Procleix Zika Virus Assay.

	WHO IS panels (IU/mL)		(b) (4) panels (c/mL)		IVT panel (c/mL)
	Plasma	Serum	Plasma	Serum	
50% LoD	0.64 (0.54 – 0.76)	0.56 (0.48 – 0.65)	0.86 (0.72 – 1.02)	0.73 (0.61 – 0.86)	3.01 (2.67 – 3.37)
95% LoD	2.90 (2.22 – 4.18)	1.91 (1.52 – 2.63)	4.28 (3.27 – 6.15)	3.39 (2.59 – 4.90)	12.05 (10.39 – 14.34)

Detection of ZIKV in Naturally Infected Samples

This testing was performed to demonstrate assay sensitivity in neat individual donations and 16-specimen pools. Twenty-six clinical ZIKV-positive plasma specimens were obtained from various vendors (specimens were collected from Colombia and Dominican Republic).

The clinical sensitivity of the Procleix ZIKV Assay is shown in Table 7. One out of 26 pools was 75% reactive (3/4), and one out of 26 pools was 25% reactive (1/4). Since replicate testing is not indicated in the intended use, a sample was considered non-reactive even if only one of the four replicates was non-reactive. The performance was found to be acceptable.

Table 7. Clinical sensitivity of the Procleix Zika Virus Assay in individual donation samples and 16-sample pools.

Specimen	**Total number**	**Reactive**	**Percent reactive**	**95% CI**
Neat	26	26	100	87 - 100
16-sample pool	26	24	92	76 - 98

ZIKV Inclusivity

This study evaluated the sensitivity of the Procleix Zika Virus Assay on the Panther System for detection of various strains of the Zika virus. The IVT, which was prepared in-house and represented multiple strains (MR66, P 6-740, and H/PF/2013) of ZIKV, was used to evaluate the sensitivity of the assay. Results are presented in Table 8. The validity rate was 100% for all panels. For all 3 strains tested, the ZIKV Assay met the requirement of (b) (4) reactivity at (b) (4) The performance was found to be acceptable.

Table 8. Inclusivity of the Procleix Zika Virus Assay.

ZIKV Strain	**Target c/mL**	**Reactive**	**Total number**	**Reactivity (95% CI)**
MR-66	18	20	20	100 (84 – 100)
	33	20	20	100 (84 – 100)
P 6-740	18	20	20	100 (84 – 100)
	33	19	20	95 (76 – 99)
H/PF/2013	18	20	20	100 (84 – 100)

	33	20	20	100 (84 – 100)
Negative sample	0	0	0	0 (0 – 16)

5.3. Specificity Studies

Specificity in Normal Blood Donor Plasma and Serum Specimens
This study evaluated the specificity of the assay by testing (b) (4) plasma and (b) (4) serum samples collected from normal donors using 1 reagent Master Lot. The overall specificity was 100% (1015/1015) with a lower 95% score CI of 99.6%, which met the applicant's design goals of ≥99% specificity for normal blood donor plasma specimens. Performance of IC was also evaluated and shown to be robust. The performance was found to be acceptable.

Specificity in Frozen Serum Specimens
A total of (b) (4) unique serum specimens from negative normal donors were tested in singlet with the Procleix ZIKV Assay on Panther. The specimens tested were split amongst three reagent lots. The overall specificity for the ZIKV Assay was 100% with a lower 95% score CI of 99.85%. There were no initial reactive results. There was a total of 7 invalid results due to firmware or hardware errors (b) (4) analysis error) and clot detection. The initial invalid specimens were valid and nonreactive upon retest, indicating that none of the specimens tested exhibited inhibitory effects on the assay. Hardware/software errors are not included in the initial invalid rate. Overall, the initial invalid rate due to IC failure was 0%. The performance was found to be acceptable.

Specificity in High Titer Samples
This study evaluated the carryover contamination rate of the Procleix Zika Virus Assay on the Panther Instrument by using negative and high-titer positive ZIKV (targeted at (b) (4) c/mL) panels. The panels were prepared using buffer and the ZIKV IVT, and tubes from both panels were (b) (4) with the Procleix ZIKV Assay.

There were no invalid runs; however, there were three invalid tests out of (b) (4) non-calibrator tests, resulting in an invalid reaction rate of 0.28%. All were due to specimen volume issues not associated with the test conditions of this study. The reactivity rate for (b) (4) valid high titer specimens tested with the ZIKV Assay was 100%. The false positive rate (no high-titer positive panels) for (b) (4) valid results tested with the ZIKV Assay was 0% with a mean analyte S/CO of 0.00 (SD 0.00). The false positive rate (in presence of high-titer positive panels) for (b) (4) valid results tested with the ZIKV Assay was 0% with a mean analyte S/CO of 0.00 (SD 0.00). The performance was found to be acceptable.

5.4. Effect of Donor and Donation Factors on Specificity and Sensitivity

Infected or virus-spiked samples were divided into 2 aliquots, one of which was used for the specificity evaluation, and the other one was spiked with ZIKV at 3xLoD and used for sensitivity evaluation. Control conditions for the study were negative plasma

prepared in the absence or presence of Zika virus. Ten samples from donors infected with each pathogen and 10 replicates of the specimens spiked with microorganisms were tested.

Specimens Containing Blood borne Pathogens Other than ZIKV
Multiple infected patient specimens from each group of patients with the following viral infections were evaluated:

- Dengue Virus (DENV)
- Hepatitis A Virus (HAV)
- Hepatitis B Virus (HBV)
- Hepatitis C Virus (HCV)
- Human Immunodeficiency Virus 1 and 2 (HIV-1/2)
- Parvovirus B19
- West Nile Virus (WNV)

Individuals that had received HBV vaccine were also tested.

Specimens were spiked with Hepatitis E Virus (HEV) at a concentration of (b) (4) c/mL and Chikungunya at (b) (4) units per mL (U/mL) were also evaluated. Additional specimens spiked with the following viruses were also evaluated:

- Adenovirus type 5
- BK Human Polyomavirus
- Cytomegalovirus (CMV)
- DENV 1-4
- Epstein-Barr Virus (EBV)
- Flu H1N1
- HAV
- HBV
- HCV
- Hepatitis G Virus (HGV)
- Human Herpes Virus Type 6B
- Human Herpes Virus Type 8
- HIV-1/2
- Human Papillomavirus
- Herpes Simplex Virus Type 1
- Herpes Simplex Virus Type 2
- Human T-lymphotropic Virus 1
- Human T-lymphotropic Virus 2
- Japanese Encephalitis Virus (JEV)
- Murray Valley Encephalitis Virus
- Parvo B19
- Rubella Virus
- St. Louis Encephalitis Virus
- Vaccinia Virus
- WNV (b) (4)
- Yellow Fever Virus

Specificity: Results obtained for the ZIKV negative control specimen and those with other blood borne pathogens in the ZIKV Assay on the Panther System showed 100% and 99.8% specificity, respectively, and the average IC S/CO value was 1.90 for both conditions.

Sensitivity: Results obtained for the ZIKV Specimens containing other blood borne pathogens resulted in 100% detection of Zika virus at the spiked concentration in the presence of all other infectious organisms tested.

There were no invalid runs, however there was one invalid result from an unspiked HCV positive sample for specificity testing, which was invalid upon retest. This test

sample did yield a valid result upon removal of precipitant and retesting. The overall invalid reaction rate was 0.8% (2/242). All other invalids were due to a QNS (Quantity Not Sufficient) error and yielded valid results upon retest. The performance was found to be acceptable.

Specimens Contaminated with Bacterial, Yeast and Fungal Pathogens
The specificity and sensitivity of the assay in specimens contaminated with bacteria, yeast, or fungi was evaluated.

Negative plasma was used to prepare specimens spiked to (b) (4) colony forming units per mL (CFU/mL) or inclusion forming units per mL (IFU/mL) with each of the following microorganisms:

- *Staphylococcus epidermidis*
- *Staphylococcus aureus*
- *Corynebacterium diphtheriae*
- *Propionibacterium acnes*
- *Candida albicans*
- *Neisseria gonorrhoeae*
- *Chlamydia trachomatis*
- *Pneumocystis carinii*

The microorganism spiked plasma was then divided into two aliquots; the first aliquot was left unspiked and the second aliquot was spiked with ZIKV positive plasma targeted to (b) (4). Specimens were stored at or below (b) (4) until testing. (b) (4) replicates of each specimen were tested. Control conditions for the study were negative plasma prepared in the absence and presence of Zika virus.

Specificity: Testing of the control and microorganism contaminated specimens resulted in 100% specificity, and the average IC S/CO values were 1.93 and 1.92, respectively.

Sensitivity: Testing of the ZIKV spiked control and specimens contaminated with microorganisms resulted in a sensitivity rate of 100%, and the average analyte S/CO values were 32.81 and 31.67, respectively. Additional testing of the ZIKV spiked control and the *Pneumocystis carinii* spiked specimen also resulted in a reactivity rate of 100% with the average analyte S/CO values of 31.83 and 33.01, respectively. There were no invalid runs and no invalid reactions of test specimens. The performance was found to be acceptable.

Hemolyzed, Icteric and Lipemic Specimens
This study evaluated the performance of the Procleix Zika Virus Assay in specimens containing potentially interfering substances such as bilirubin, lipids, hemoglobin, and protein (albumin) on the Panther instrument platform.

Two different panels of specimens (analytical and clinical) were tested with one reagent lot of the ZIKV Assay. The analytical panel was prepared by spiking a pool of defibrinated, delipidated, Zika virus negative human plasma with the following substances: (a) hemoglobin at 2,000 mg/L; (b) bilirubin at 200 mg/L; (c) lipids at 30,000 mg/L; and (d) albumin at 60 g/L. The clinical panel consisted of clinical specimens from HIV-1, HCV, and HBV serology negative patients with icteric, hemolyzed, or lipemic plasma obtained from a vendor. Aliquots of the normal plasma controls from the analytical panel served as controls for both panels. The

controls, interference panels, and clinical specimens were divided into two aliquots, one of which was used for specificity evaluation, and the other one was spiked with ZIKV at 3xLoD and used for sensitivity evaluation. Ten donors for each specimen type and 10 replicates of each analytical sample were tested.

Specificity: Testing resulted in 100% specificity for both analytical and clinical panels. The average IC S/CO values are presented in Tables 9 and 10.

Sensitivity: Testing resulted in a sensitivity rate of 100% for both analytical and clinical panels. The average analyte S/CO values are presented in Tables 9 and 10.

Table 9. Performance of the Procleix Zika Virus Assay in samples containing potentially interfering substances.

Specimen	Normal control	NaOH control	Albumin	Bilirubin	Hemoglobin	Lipids
IC S/CO for specificity study	1.89	1.86	1.92	1.86	1.85	1.93
Analyte S/CO for sensitivity study	32.20	33.77	33.02	31.94	32.41	32.86

Table 10. Performance of Procleix Zika Virus Assay in specimens from patients with hemolyzed, icteric, and lipemic plasma.

Specimen	Control	Hemolyzed	Lipemic	Icteric
IC S/CO for specificity study	1.89	1.86	1.84	1.88
Analyte S/CO for sensitivity study	31.89	31.51	31.96	31.69

There were no invalid runs. There were 3 invalid reactions due to sample quantity not sufficient (QNS). All invalid reactions were retested with valid results. There were no invalid reactions due to IC failure. The overall invalid rate due to IC failure was 0% (0/203), and total invalid rate was 1.5% (3/203). The performance was found to be acceptable.

Specimens from Patients with Autoimmune and Other Diseases

Specimens from patients with autoimmune and other diseases were obtained from a vendor. The following pathological conditions or laboratory findings were evaluated: rheumatoid factor (RF), antinuclear antibody (ANA), systemic lupus erythematosus (SLE), and multiple myeloma (MM). Ten donors for each condition were tested. The control condition for the study was a pool of defibrinated, delipidated, Zika virus negative human plasma. The control specimens and specimens from patients with autoimmune and other diseases were divided into two aliquots, one of which was used for specificity evaluation, and the other one was spiked with ZIKV at 3xLoD and used for sensitivity evaluation.

Specificity: Testing resulted in 100% specificity. The average IC S/CO values for normal control and clinical specimens were 1.89 and 1.90, respectively.
Sensitivity: Testing of the ZIKV spiked specimens resulted in a reactivity rate of 100% for the control and 97.6% for the clinical specimens. Fisher's Exact test showed no significant difference between the control and test conditions. The average analyte S/CO values for control and clinical specimens were 31.89 and 32.30, respectively.

One reaction from an SLE donor specimen spiked with Zika virus initially gave a nonreactive result. This was determined to be an operator error where the sample was not spiked with Zika virus. This donor specimen was re-prepared and was reactive upon retest. The initial and retest results were included in the data analysis. There were no invalid runs and no invalid reactions. The performance was found to be acceptable.

Presence of Exogenous Substances
Testing panels were prepared from defibrinated, delipidated, Zika virus negative human plasma spiked with exogenous substances listed in Table 11 in their respective solvents. The panels were divided into two aliquots, one of which was left unspiked and the other one was spiked with ZIKV at 3xLoD. Two reagent kit lots were used for the testing. Ten replicates per exogenous substance panel were tested for specificity and sensitivity per reagent lot. All non-spiked panels were nonreactive, and testing of spiked specimens in the presence of potentially interfering exogenous substances demonstrated 100% sensitivity. The performance was found to be acceptable.

Table 11. Exogenous Substances Evaluated for Interference with Procleix Zika Assay

Substance Name	Concentration	Solvent
Acetaminophen	1324 µmol/L	Water
Acetylsalicylic Acid	3620 µmol /L	Ethanol
Ascorbic Acid	342 µmol/L	Water
Atorvastatin	600 µg Eq/L	Methanol
Ibuprofen	2425 µmol/L	Ethanol
Loratadine	0.78 µmol/L	Ethanol
Naproxen	2170 µmol/L	Methanol
Phenylephrine HCL	491 µmol/L	Water

Pools of Donor and Donation Factors
Specimens from abnormal (containing various donor and donation factors) blood donors were tested in pools of 16 to evaluate specificity and sensitivity of the Zika Virus assay on Panther. 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 infections other than Dengue; and specimens from donors with autoimmune or other diseases. Specificity and sensitivity of Zika Virus assay in 9 pools (pool size of 16) of specimens from donors with putative interfering substances (test pools) were compared to those of specimens from normal donors. One reagent

lot of ZIKV Assay reagents was used. Each test pool was divided into two aliquots, one of which evaluated the analytical specificity in the absence of Zika virus and the other one evaluated sensitivity in the presence of Zika virus targeted at 3xLoD. One control pool was prepared from 16 different Zika negative plasma from normal volunteer blood donors. This pool was divided into 2 aliquots for sensitivity and specificity evaluations, with and without spiking with ZIKV, respectively. (b) (4) replicates for each of the 9 test pools and (b) (4) replicates from the control pool were evaluated.

Specificity: For all nine test pools and the control pool the specificity was 100%. No statistical difference was observed between the control pool and the test pools.
Sensitivity: Sensitivity was 100% for all nine test pools and the control pool. No statistical difference was observed between the control pool and the test pools. The performance was found to be acceptable.

5.5. Serum and Plasma Specimens Collected in Various Anticoagulants and Tube Types

Ten donor samples were collected using the following anticoagulants and tube types: K2EDTA, K3 EDTA, ACD-A, NaC, PPT, SST, Serum Tube (Serum), CPD, CP2D, CPDA-1, and LiH. Whole blood was collected in house for the first 7 conditions, and plasma was obtained from a vendor for the last four conditions. An aliquot of 10 donor specimens from each of the 11 specimen types (separated serum or plasma) was tested with the ZIKV Assay for specificity. A second aliquot of each specimen was spiked with ZIKV positive plasma targeted to 3x LoD to evaluate sensitivity.

Specificity: Testing of specimens collected in different anticoagulants and tube types demonstrated 100% (110/110) specificity across all conditions. The mean analyte S/CO of negative specimens for the different anticoagulants and tube types ranged from 0 to 0.18. The mean IC S/CO ratios ranged from 1.77 to 1.90 with CVs ranging from 2 to 4%.
Sensitivity: In all specimen types tested, sensitivity was 100% (110/110). The mean analyte S/CO ratios ranged from 28.50 to 34.20 with CVs ranging from 0 to 4%.

There were 2 invalid reactions due to volume verification failure (VVFS), 1 invalid reaction due to sample clot (CLT), and 1 invalid reaction due to sample dispense error (RDFS). All invalid replicates were retested with valid results. There were no invalids due to IC failure. The overall invalid reaction rate due to IC failure was 0% (0/244), and total invalid rate was 1.79% (4/224). The performance was found to be acceptable.

5.6. Matched Pair Serum/Plasma Samples

Matched plasma and serum specimens were procured from vendors. At least 24% of the specimens (8/33) were determined to be near the LOD of the assay based on dilutional testing. For dilutional testing, samples were tested neat, diluted 1:10, 1:100 and 1:1,000, and results were used to estimate sample value assignments based on the preliminary LoD and reactivity results. Testing was split evenly between 3 lots of reagent kits on one Panther instrument. (b) (4) replicate of each neat sample was

tested. Results were evaluated to determine the positive and negative agreement between matched serum and plasma specimens.

A total of three runs were completed with a total of 66 valid results. There were two nonreactive serum samples, giving a detection rate of 96.97% (score CI 89.61% - 99.17%) for all samples tested. Fisher's exact test for all samples yielded a p-value of 0.4923 between the plasma and serum reactivities, which supports the null hypothesis that there is no significant difference between the matrices. Two nonreactive serum samples were repeated in (b) (4) neat and diluted 1:5 to determine if their concentration is greater than (b) (4) c/mL. Results of these tests are presented in Table 12, demonstrating a starting concentration of less than (b) (4) copies/mL for both samples due to expected 100% reactivity near (b) (4) c/mL. The performance was found to be acceptable.

Table 12. Additional testing results.

Sample	**1**		**2**	
Matrix	**Plasma**	**Serum**	**Plasma**	**Serum**
Neat reactivity	100%	100%	100%	33%
1:5 reactivity	33%	67%	67%	0%

5.7. Statistical Analysis of Specificity and Sensitivity Data

Run Size Validity

This study demonstrated the maximum assay run size and throughput of ZIKV Assay on the Panther instrument platform.

Testing was designed to validate that the system can complete at least (b) (4) tests (acceptance criteria) prior to regularly scheduled maintenance. This consisted of (b) (4) 250 test runs and one (b) (4) test run on the Panther System. One lot of the ZIKV Assay kit was tested on two different instruments, each with (b) (4) total tests. Instruments were expected to complete processing of at least (b) (4) tests without any user activity beyond the loading of disposables. QC Panel members were used to evaluate the performance of the assay. Timing and throughput for completion of (b) (4) 250, (b) (4) tests were evaluated.

Both instruments demonstrated a capability of completing (b) (4)-test worklists without any user activity beyond the loading of disposables, and a throughput of (b) (for processing of 250 tests. For both instruments, processing duration for 1 test was less than (b) (4) . The performance was found to be acceptable.

5.8. Internal Control Validity and Inhibition

This study evaluated the performance of the IC for the Procleix Zika Virus Assay under known inhibitory conditions. Assay results were evaluated to determine if the IC performs acceptably as a control for false negative results.

Two QC Panels (Panel A and Panel C) were tested using reagents under three inhibitory conditions that affect IC performance, which included:

- Thawing the reagent kit in the Reagent Preparation Incubator (RPI) on (b) (4) instead of the frozen storage condition (b) (4)
- Not adding the IC to the Target Capture Reagent (TCR)
- Using Probe reagent contaminated with (b) (4) by volume, prior to loading the reagent on to the Panther instrument.

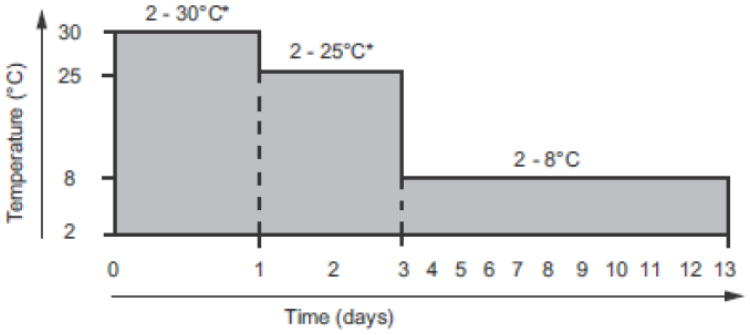
These runs were compared against a baseline run that used normally prepared reagents. Two lots of ZIKV Assay reagents were used. QC panels were tested with (b) (4) replicates each for each condition. A total of (b) (runs were performed per lot. Both baseline runs were valid; 0% reactivity was observed for all Panel A samples and 100% reactivity was observed for all Panel C samples in these runs. As expected, the runs under inhibitory conditions were invalidated by the IC and resulted in invalid runs. Within each valid run there were a total of (b) (4) replicates tested. The performance was found to be acceptable.

5.9. Stability Studies

Plasma and Serum Specimen Stability

Stability of Zika virus was evaluated in 10 donors each of plasma and serum and one pool of 16 of each plasma and serum donors. Each was tested for sensitivity at 3xLoD of the ZIKV Assay. Testing was performed on five Panther Systems using two reagent lots. Clinical Zika virus positive plasma diluted in negative matrix was used to make all spiked-positive samples. Whole blood samples were collected in-house from 10 individual donors in serum and K2EDTA plasma primary tubes; one pool of 16 plasma and one pool of 16 serum samples were made. Each neat or pooled sample was spiked with Zika virus in the collection tube and then incubated according to Figure 1.

Figure 1. Storage Conditions of Plasma and Serum Specimens



Time points also included 10-20% additional time for confidence margin. Whole blood was separated from plasma/serum in the primary tube by centrifugation on Day 3. At Day 0 (Baseline), Day 3, and Day 13 time points, an aliquot of each specimen was removed from the collection tube, transferred to SAT tubes and centrifuged at 3000g for 10 min to separate the supernatant from the blood cells. The aliquots were then frozen at -20°C prior to testing. Specimens that completed 13 days of incubation were also tested after three freeze/thaw (F/T) cycles. On the day of testing, samples were thawed and diluted with their respective negative matrices

to attain a final concentration of 18 c/mL (3xLoD). For each matrix at each timepoint, (b) (4) replicates were analyzed (b) (4) replicates per 10 donors and one pool).

Sensitivity results for detection of Zika virus indicated a reactivity rate of 100% for Baseline, Day 3, and Day 13 (3F/T) time points for plasma and Baseline, Day 3, and Day 13 time points for serum. Reactivity rate for Day 13 timepoint for plasma and Day 13 (3F/T) timepoint for serum was 97%.

Stability testing results demonstrate the Zika Virus Assay detected Zika virus with no statistically significant difference in sensitivity of specimens in plasma and serum stored under different conditions. The performance was found to be acceptable.

Anticoagulants Specimen Stability

Results of this study were evaluated to determine if the Procleix ZIKV Assay on the Panther instrument platform could detect ZIKV in specimens collected in various anticoagulants and stored at different temperatures for extended time periods.

Whole blood from normal donors was collected into the following anticoagulants and tube types: K3EDTA, ACD, NaC, LiH, CPD, CP2D, CPDA-1, Greiner K2EDTA gel separator, and plasma and serum separation tubes. Twelve pools of 16 plasma donors were also tested. Normal whole blood from 12 individual donors for each anticoagulant were obtained from vendors. Whole blood samples in collection tubes were either spiked with Zika isolate or Zika plasma to attain a final concentration of approximately (b) (4) c/mL. The exception was K3EDTA having two donor sets, with one set spiked with Zika isolate and the second set spiked with Zika plasma. After viral spiking and mixing, an aliquot of whole blood from each specimen was removed and transferred to a new tube. The whole blood was centrifuged to separate the plasma or serum, which was frozen at ≤-20°C until testing. The Day 0 aliquots were used as the baseline control. Spiked whole blood in the original collection tubes then followed a series of incubations and time points' specimen collections as shown in Figure 1 (above). Twelve anticoagulant pools were created by pooling spiked plasma of 16 donors. The pools were incubated at the same temperatures and time points as the individual anticoagulant tubes. Plasma/serum aliquots were removed from each specimen at each timepoint and then frozen at ≤- 20°C. Specimens completing 13 days of incubation were additionally tested after 3 F/T cycles. Specimen aliquots from each timepoint were diluted on the day of testing with negative plasma or serum to attain a final concentration of approximately 150 c/mL. One lot of Zika assay reagents was used for testing. Reactivity rates from time points were compared to the baseline data to determine if there was a statistical difference between the data sets. Different matrices were also compared to determine if there were differences in stability due to tube type.

Stability of ZIKV in Various Anticoagulants. A reactivity rate of 100% was observed at baseline for all specimens. A reactivity rate of ≥86% was observed for Day 3, Day 13, and Day 13 F/T time points in specimens collected in all anticoagulants as well as serum tubes, except for the CPD collection tube having a reactivity rate of 64% for Day 13 F/T. Statistical analysis indicated significant difference between the reactivity rate of CPD specimens at Day 13 F/T timepoint and that at baseline. Since 64%

reactivity was below the acceptance criteria, specimens from an intermediate timepoint (Day 8 & Day 8 F/T) were tested. A reactivity rate of 92% was observed for Day 8 F/T, and results had no significant statistical difference in comparison with baseline.

Stability of ZIKV in Pools. A reactivity rate of 100% was observed at Day 0 and Day 3 time points. Reactivity rates of 94% and 97% were observed for Day 13 and Day 13 F/T, respectively. Statistical analysis indicated no statistically significant differences. Initial non-reactive results were retested in duplicate for informational purpose only. Specimen stability was demonstrated up to 13 days post-collection for all tested anticoagulants except CPD. Specimen stability was demonstrated up to 8 days post collection for CPD. No adverse effect on assay performance was observed when plasma or serum was subjected to three freeze-thaw cycles.
The performance was found to be acceptable.

On-board Specimen Stability
The assay performance with specimens that have been stored on-board the Panther System for a minimum of 8 hours, including up to 2 sample loading cycles was evaluated in this study using one Panther instrument and one ZIKV Assay reagent lot. Zika positive specimens were prepared by spiking plasma or serum with 3x required LoD (150 c/mL) of Zika virus. Ten donors each of serum and K2EDTA plasma were tested. Each donor was used to prepare a spiked and an unspiked sample. One replicate of each specimen was tested at 0 hours and at ≥8 hours.

Specificity: All tested replicates were valid, and all valid replicates were nonreactive across all matrix types and time points.
Sensitivity: All tested replicates were valid and reactive across all matrix types and time points.

Stability testing results showed that the Zika Virus Assay detected Zika virus with no statistically significant difference in sensitivity and specificity of specimens stored on-board the Panther System for a minimum of 8 hours, with up to 2 loading cycles. The performance was found to be acceptable.

Long-term Frozen Specimen Stability
This study evaluated the long-term frozen stability of Zika virus specimens from living and cadaveric donors stored up to 30 days at -20°C when tested with the Procleix Zika Virus Assay on Panther.

Living donor specimens evaluated included plasma collected in K3 EDTA, ACD, CPD, and PPT anticoagulants/tube types. Cadaveric specimens evaluated included serum and EDTA plasma. Samples from 12 unique living donors and 11 unique cadaveric donors were collected for each matrix. Donor specimens were spiked with Zika plasma or isolate to attain a final concentration of 150 c/mL (cadaveric donors) or 750 c/mL (living donors). After viral spiking, each type of specimen was separated into at least 2 aliquots (a separate aliquot for each timepoint). Baseline (Day 0) aliquots were frozen at -20°C and tested on same day. The remaining aliquots were stored at -20°C for 14 days and 30 days before testing. On the day of testing, the

living donor specimens were diluted with negative human plasma to attain a final concentration of 150 c/mL of Zika virus. (b) (4) replicates were tested for each matrix type and timepoint, except baseline (Day 0) specimens, which were tested with (b) (4) replicates. One Zika Assay reagent lot was used for testing. Reactivity at different time points was compared to the baseline data to determine if there was a statistical difference between the data sets. Different matrices were also compared to determine if there were differences in stability due to tube type.

For each timepoint (Day 14 and Day 30) and each anticoagulant used for living and cadaveric donor specimens, 100% reactivity was observed. The results demonstrated that the Procleix Zika Assay can detect ZIKV in living donor and cadaveric donor specimens frozen at -20°C for up to 30 days with no statistically significant differences in sensitivity from baseline testing. The performance was found to be acceptable.

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

The assay performance of the Zika Virus Assay reagents after being stored on-board the Panther System and at open kit conditions was evaluated in this study using one Panther instrument and one lot of reagents. Accuracy and precision of the assay were assessed for reagents up to 250 hours onboard (open-kit) and at 38 days after reconstitution. Panel members consisted of Zika IVT 1 and Zika IVT 2 (corresponding to the two distinct target regions of the assay) at 100c/ml and 30 c/mL and a negative panel.

All valid replicates of the negative panels were nonreactive across all time points. All Zika IVT 1 and Zika IVT 2 replicates at 30 c/mL and 100 c/mL tested were valid and reactive across all time points. The assay reagents demonstrated acceptable performance when the reagents were prepared and stored per the package insert specifications. These results validated the stability of open kit reagents after 20 hours of 25°C incubation in the RPI File 3, up to 30 days after opening, and up to 90 hours onboard the Panther instrument. Results also validated the 24-hour stability of calibrators after incubating them for 30 hours at 15-30°C. The performance was found to be acceptable.

Procleix ZIKV Assay Environmental Conditions

Performance of the Procleix Zika Virus Assay was evaluated on one Panther System in an environmental chamber, using one lot of Zika Assay reagents and one lot of Zika calibrators. Positive samples consisted of QC Zika panels (ZIKV-1 100 c/mL and 30 c/mL, ZIKV-2 100 c/mL and 30 c/mL), negative samples consisted of delipidated, defibrinated human plasma. Test runs were performed at each temperature and humidity condition. Each panel was tested with (b) (4) replicates per condition. A baseline condition was performed at ambient lab temperature and humidity.

There were no reactive results with negative panel at any of the environmental conditions. All replicates at 100 c/mL were 100% reactive for Zika-1 and Zika-2 QC panels. Replicates at 30 c/mL were 100% reactive for Zika-2 QC panel for all

conditions and 96.7% reactive for Zika-1 QC panel for the 30°C (b) (4) condition and 100% reactive for the remaining 3 conditions. The Zika Virus Assay met the performance acceptance criteria at the environmental specification limits for external ambient temperature (b) (4) and relative humidity (b) (4) for the Panther System. The performance was found to be acceptable.

Real-Time and Open Kit Reagent Stability

Shelf-life evaluation will be based on real-time performance, which sponsor is planning to monitor for (b) (4) months to support 24-month stability. This study evaluated the shelf-life stability of the Procleix Zika Virus Assay reagents on the Panther instrument platform stored under real-time conditions.

Two lots of reagents were used to evaluate reagent stability on at least one Panther instrument. During shelf-life storage, the ZIKV Assay reagents were stored in an inverted position at intended storage temperatures. During open-kit stability, the ZIKV Assay kit is opened and exposed to conditions of use and stored as recommended. Two development lots of QC panels were used for testing. A ZIKV negative panel as well as ZIKV-1 and ZIKV-2 at 100 c/mL and 30 c/mL were tested at each time point. The 30 c/mL panels were tested for information purposes only.

Current results include stability data up to 12 months, open-kit conditions. Reactivity of the ZIKV QC panels at 100 c/mL was 100% at all time points. The negative panel was 100% nonreactive for all time points. The Procleix Zika Virus Assay reagents exhibited acceptable performance with no unexpected results as of the 12 month, 38 day open-kit time point. The TCR, Amplification, Enzyme, Probe and Selection reagents demonstrated acceptable performance as of the 12-month time point with regards to 38 days open-kit stability with 72 hours onboard stability when stored at the appropriate temperatures.

5.10. Cadaveric Studies

Reproducibility

The objective of this study was to determine the reproducibility of the ZIKV results when testing cadaveric samples and compare the ZIKV reproducibility observed when testing cadaveric samples with that observed when testing living-donor samples under the same conditions. This study included 20 cadaveric and 20 living donor plasma specimens and 20 cadaveric and 20 living donor serum specimens with hemolysis ranging from (b) (4). Each specimen was spiked to 10xLoD, divided into 6 aliquots, and tested individually, in 6 separate runs on 6 separate days using 3 different reagent lots.

The assay for reproducibility in plasma spiked with 60 c/mL demonstrated differences in reactivity rates between normal and cadaveric plasma, so an additional study was conducted for reproducibility with spiked cadaveric plasma samples containing 150 c/mL.

In the reproducibility study using cadaveric plasma samples spiked with 150 c/mL and normal plasma spiked with 60 c/mL the reactivity rates were 100%. The 95% CI was 99.0-100% for 60 c/mL spiked normal plasma specimens and 150 c/mL spiked cadaveric plasma specimens.

In 60 c/mL spiked serum specimens, the observed reactivity rates were 100% for normal specimens, and 99.4% for cadaveric serum specimens. The 95% CI was 99.0-100% for normal serum specimens and 98.0-99.9% for cadaveric serum specimens. The p-value for Analyte S/CO variance showed significant statistical difference exists between conditions, but the difference was functionally insignificant as the p-value for % reactivity was 0.4993.

Specificity and Sensitivity

The objective of this study was to determine the specificity and sensitivity of the Procleix Zika Virus Assay for testing cadaveric plasma and serum specimens and compare the specificity and sensitivity with those observed when testing living donor samples. Cadaveric donor plasma and serum samples were assessed for plasma dilution and met the guidance. Additionally, samples included hemolyzed specimens ranging from (b) (4) .

For this study 50 cadaveric plasma, 50 cadaveric serum, 50 living donor plasma and 50 living donor serum specimens were obtained from an outside vendor. For living donor specimens, 40 specimens were tested in (b) (4) and 10 were tested in (b) (4) for each plasma and serum specimen, but only the first replicate was included in the analysis. Fifty unique cadaveric specimens were tested in (b) (4) for each plasma and serum specimen. Samples for sensitivity study were spiked with ZIKV positive plasma to 3xLoD. Six unique, naturally infected specimens were used as spiking material.

Specificity. The estimated specificity for cadaveric plasma was 98.0%. The estimated specificity for cadaveric serum and living donor serum and plasma was 100%.

Sensitivity. The estimated sensitivity for both cadaveric and living donor plasma was 98.0%. The estimated sensitivity for living donor serum was 96.0%, while for cadaveric serum it was 100%.

ZIKV Stability

The objective of this study was to determine the stability of Zika virus in whole blood from cadaveric donor specimens. Ten each cadaveric plasma and serum specimens were obtained from a vendor and spiked with clinical ZIKV positive plasma. They were tested for sensitivity at 150 c/mL (3 times the required LoD). Plasma cadaveric specimens tested were subjected to 1 day at 30˚C, 2 days at 25 ˚C, and either 2 days at 2˚ to 8˚C followed by 3 freeze/thaw cycles or 5 days at 2˚ to 8˚C followed by 3 F/T cycles for the Day 5 F/T timepoint and Day 8 F/T timepoint, respectively.

Serum cadaveric specimens tested were subjected to 1 day at 30˚C, 2 days at 25 ˚C, and either 3 freeze/thaw cycles or 2 days at 2˚ to 8˚C followed by 3 F/T cycles for the Day 3 F/T timepoint and Day 5 F/T timepoint, respectively.

Three replicates per donor were analyzed for each sample timepoint, which were as follows: Day 0 plasma, Day 0 serum, Day 3 serum, Day 5 plasma, Day 5 serum, Day 8 plasma. A total of 180 replicates were tested. There were 0 invalid replicates and the overall invalid reaction rate was 0.00%.

For cadaveric serum specimens, a reactivity rate of 100% was observed for day 0, and lower reactivity rates of 96.7% and 83.3% for days 3 F/T and day 5 F/T, respectively. For cadaveric plasma specimens, a reactivity rate of 100% was

observed for day 0, and lower reactivity rates of 83.3% and 90% for day 5 F/T and day 8 F/T specimens, respectively.

5.11. Review Issues and their Resolution

- Initially, the level of hemolysis for cadaveric specimens was erroneously reported in mg/dL, which would make hemolysis grade too low. The issue was clarified with the sponsor during a teleconference and a follow-up IR (Data provided in Amendment 4).

- The type of anticoagulant was not specified for the living and cadaveric plasma specimens used in Cadaveric Reproducibility, Specificity, and Sensitivity studies, which was clarified with the sponsor via IR (Data provided in Amendment 4).

- Initially, the 10 living donor specimens that were tested in triplicates in Cadaveric Specificity and Sensitivity studies were analyzed as 30 separate specimens, producing N=70. At the conference call held on April 25, 2018, Grifols was advised to reanalyze the data reporting each sample, but not each replicate, separately, and use only the result of the initial run for each sample, therefore using N=50. The reanalysis was submitted in Amendment 4.

- Similarly, in the study of detection of ZIKV in samples from naturally infected individuals, the 95% CI for the pooled samples was reported using the sample size of 104, whereas only 26 samples were analyzed in the replicates of 4. The sponsor was advised to recalculate the sensitivity of the assay using the actual number of samples tested rather than the number of replicates, and the reanalysis was submitted with Amendment 5, where the sample was considered positive when ≥1 replicate was positive. A follow-up IR was issued recommending the reanalysis of the data where the sample would be considered negative if ≥1 replicate is negative. Reanalyzed data was submitted with Amendment 8.

- In the original submission, samples that tested false positive or false negative in Cadaveric Specificity and Sensitivity studies were retested and those retests analyzed as separate samples. Sponsor was asked to reanalyze the data using only initial results for calculations. The reanalysis was submitted in Amendment 4.

- In Individual Results for the Specificity in Specimens Containing Blood borne Pathogens Other than ZIKV or in Specimens from HBV Vaccinated Persons (Additional Testing), a false positive result was reported in line listings but not mentioned in the text, and the reported specificity was 100%. The discrepancy was reconciled with the sponsor, specificity was recalculated, and reanalysis submitted with Amendment 5.

- A discrepancy in calculated reactivities between the table and the text was noted for the stability study and was reconciled via an IR (Amendment 8).

- Sponsor was asked by the statistical reviewer to provide additional details regarding the cadaveric reproducibility study, and the data was provided in Amendment 4.

- The spiking concentration of JEV in the study of specimens containing Blood borne Pathogens was initially not specified. The issue was clarified with the sponsor through an IR (Amendment 5).

PI-Related Issues

- The information to support storage conditions of cadavers prior to sample collection specified in the PI was not included in the submission and was requested from sponsor. In Amendment 4, Grifols replied that the storage conditions included in the PI were more strict than those used in analytical testing.

- The PI contained sample storage instructions inconsistent with the data in the cadaveric stability study. When asked to clarify, sponsor reanalyzed the data using the number of donors, rather than the number of replicates, as the denominator, and considering the donor reactive if ≥1 out of 3 replicates was reactive, which raised the reactivity rate at various time points to 100%, therefore supporting the claimed storage conditions (Amendment 4). The sponsor was advised that the data should be reported using the number of replicates, and the reanalysis was submitted with Amendment 9. The original sample storage issue was resolved by consultations between OTAT and OBRR.

- It was suggested that statements describing the proper procedures pertinent to dilution of cadaveric specimens be added to the Assay Procedure and the Interpretation of Results sections of the PI, and the sponsor submitted the revised PI in Amendment 9.

- A suggestion to reiterate the fact that the assay is intended for use with the Procleix Panther System only in the Limitations of the Procedure section was discussed, and it was decided not to proceed with an IR since the intended use clearly states that the assay has been developed for use with the Panther, and the same wording is used in already cleared assays.

- FDA recommended to revise the package insert (PI) as follows:
    - Update to reflect the new analyses of the cadaveric specificity, sensitivity, and reproducibility studies.
    - Add a procedural limitation reflecting that the assay is designed to detect ZIKV RNA in plasma and serum specimens, and the RNA may persist in certain organs, tissues, and body fluids longer than it is detectable in plasma and serum.

- Describe the procedure for the resolution testing of the reactive pools.
- Update to reflect the revised intended use.
- Add a statement clarifying that the Procleix Zika Virus Assay is performed on the Procleix Panther System.
- Revise Specimen Collection, Storage and Handling instructions to be consistent with the intended use statement and with the submitted results of analytical studies, also noting that the studies were performed on samples spiked at certain LoD.
- Add a note stating that runs/samples may be invalidated by the operator.
- Revise the procedural limitation to refer to the failure to meet the acceptance criteria for calibrators instead of the failure to achieve expected results.

6. Clinical Studies

The clinical studies were performed under IND #17003 with testing initiated in June, 2016 at (b) (4) facilities and later expanded to additional sites. Testing was originally performed in pools of 16 samples and transitioned to individual-donation testing (IDT) in September, 2016. Two clinical protocols were submitted with the IND, and blood screening under this IND is still ongoing. Design of clinical studies was based on FDA feedback provided for pre-submission BQ170006.

Clinical Specificity Study

The specificity study was performed under 2 protocols: Pre-pivotal Procleix Zika Virus Assay Testing of Donations from Donors of Whole Blood and Blood Components (B10383-ZIKVPS-CSP-01), and Prospective Screening of Donations from Donors of Whole Blood and Blood Components with Procleix Zika Virus Assay by the (b) (4) (B10383-ZIKVPS-CSP-02). These protocols describe procedures and analyses for the specificity study, which included results pertaining to index testing up to Dec. 31, 2016, and ongoing blood screening under the IND.

Study Design. A prospective, multicenter clinical trial was conducted in the United States. Twelve testing sites obtained samples from blood donations from affiliated U.S. blood collection centers. Samples were linked to allow for donor identification, deferment, and follow-up for donors with reactive results. Samples were tested individually (not pooled) with the investigational Procleix Zika Virus assay on the Procleix Panther system at all 12 testing sites. Under the original protocol and protocol amendment 1 for protocol number B10383-ZIKVPS-CSP-02, 16-sample pools were also tested at 3 of the 12 testing sites. Up to three Procleix Zika Virus assay reagent kit master lots were used by each testing site. Samples with nonreactive Procleix Zika Virus assay results were considered negative for Zika virus and were not tested further. Donors of samples with initially reactive results were contacted for follow-up. Additional testing was

performed for donations with initial reactive results, volume permitting, including the following:

- Replicate testing with the Procleix Zika Virus assay from the original sample (≥ 3 replicates (b) (4) testing sites] or ≥ 2 replicates [all other sites]).
- Replicate testing with the Procleix Zika Virus assay from the plasma unit (10 replicates [(b) (4) sites] or 21 replicates (b) (4) sites] for information only).
- In addition, serum was sent for testing with an immunoglobulin M (IgM) antibody test for Zika virus; if the IgM antibody test was positive, the sample was tested with a plaque neutralization test. Under protocol B10383-ZIKVPS-CSP-01, serum was also sent for testing with an immunoglobulin G (IgG) antibody test for Zika virus and an IgG antibody test for Dengue virus. Available plasma (e.g., from the plasma units) and/or whole blood from donors with initial reactive Procleix Zika Virus assay results was sent for testing with an alternate NAT and/or the Procleix Zika Virus assay.

Donors and donor products were managed based on the investigational Procleix Zika Virus assay results and in accordance with FDA guidance. Procleix Zika virus assay results were compared with the true negative status or results of the comparator assays and/or follow-up testing (when appropriate) to estimate clinical specificity (with 2-sided 95% Clopper-Pearson Exact CIs). Specificity was calculated separately for individual donor samples and 16-sample pools.

Results. Numbers of samples tested during the study and results validity are represented in Table 13. Forty donations had initial reactive results in individual testing and no 16-member pools had initially reactive results. The true status of the samples with initial reactive results is presented in Table 14.

Table 13. Samples Tested for Specificity Study and Results Validity.

Individual donations	N	%
Total runs on Procleix Panther system	7088	
Valid runs on Procleix Panther system	7038	99.3
Individual donor samples-initial	1,257,594	
Inconclusive results	235	0.02
Individual donor samples-final	1,257,359	
Invalid/error	80	< 0.01%
Individual donor final, valid results	**1,257,279**	
16-donor pools	N	%
Pooled final, valid results	**24,516**	**100**

Table 14. Initially Reactive Results and their True Status.

Initial reactive	40
Repeat reactive with Procleix Zika	40
Unconfirmed IR (no follow up, true status not confirmed)	11
False positive (not reactive by reference test Alt NAT or serology, not reactive by follow-up)	17
Confirmed True positive (reference test reactive, reference test unreactive but follow up reactive)	12

Out of 1,257,279 individual final valid results, 1,257,239 were non-reactive. Eleven individual samples with unknown Zika virus donor status were excluded from the analysis (1,257,279 – 11 = 1,257,268). Specificity therefore was 99.999% (1,257,239/1,257,268, 95% CI: 99.998% to 99.999%) for individual donations and 100% (24,516/24,516, 95% CI: 99.985%–100%) for pools.

Evaluation of Yield during Specificity Study Testing. A Zika virus yield case is defined as an individual whose infection was not detected by serologic methods at index (IgM test seronegative) but was correctly identified using the Procleix Zika Virus assay (as determined by seroconversion in follow-up samples).
During specificity testing of whole blood donors from June 2016 to December 2016, individual donation testing in the US identified 12 (0.001%) initially reactive and confirmed samples from 1,257,268 donations with known outcomes. Of these, 2 were confirmed yield cases. Follow-up samples collected from the donors 8 and 17 days after the respective index donations were IgM seropositive and confirmed by neutralization, indicating seroconversion.

Clinical Sensitivity Study
The study estimated the clinical sensitivity of the Procleix Zika Virus Assay on the Procleix Panther System in known ZIKV-positive plasma and serum samples. Testing was performed by two external sites (b) (4) and one in-house (GDS) using three investigational reagent kit master lots. Neat and 1:16 diluted samples made from qualified positive samples were included for study testing (simulated pools). The clinical protocol, statistical analysis plan and final clinical report were included in Section 8: Clinical Protocol #B10381-ZIKVPS-CSP-01, Statistical Analysis Plan for ZIKV Sensitivity Study, Final Clinical Report B10381-ZIKVPS-CSR-01 for ZIKV Sensitivity Study in the submission.

Study Design. Testing sites were provided with known-positive plasma and serum samples that were prepared neat (original undiluted concentration) and diluted 1:16 with known-negative bulk plasma or serum samples (simulated pools). Known-negative samples were blinded and provided to operators to the expected results. The neat and diluted plasma samples were split approximately equally among three testing sites (two external and one in-house); different samples were tested at each site. Neat and diluted known-positive samples were tested with the Procleix Zika Virus assay on the Procleix Panther system; known-

negative samples were also tested. Each site performed testing using three Procleix Zika Virus assay reagent kit master lots; samples were split approximately equally among lots at each site. All test results were provided to the sponsor. The sponsor performed statistical analyses. Only known-positive samples with valid assay results were included in the calculation of sensitivity. Results were compared with the known-positive status of the samples and sensitivity was calculated (with corresponding 2-sided 95% Clopper-Pearson Exact CIs).

Results. Of 126 known-positive plasma and 114 known-positive serum samples, 112 samples of each were qualified. Of those, serum sample had insufficient volume for 1 aliquot and 4 plasma samples and 3 serum samples were retained at the sponsor's Research and Development laboratory. The remaining 108 samples of each matrix were tested with the Procleix Zika Virus assay both neat and in simulated 16-donor pools. There were 10 runs initiated; all were valid. All 432 samples tested had final valid results. Two neat samples and one diluted sample had initial invalid results due to specimen issues, but had valid results upon retesting. Assay sensitivity is summarized in Table 15.

Table 15. Sensitivity Study Results.

Individual donations	Positive/N	Sensitivity (%)	95% CI (%)
Plasma [a]	106/108	98.15	93.47–99.77
Serum [b]	104/108	96.30	90.79–98.98
Simulated 16-donor pools			
Plasma [c]	92/108	85.19	77.06–91.29
Serum [d]	86/108	79.63	70.80–86.77

True positive designation and confirmatory testing:
[a]: One sample had Zika virus RNA detected by an alternate NAT (Zika Virus by PCR, Blood; (b) (4) alternate NAT), and 4 of 9 replicates with valid results were Procleix Zika Virus assay reactive upon retesting and one sample did not have Zika virus RNA detected by an alternate NAT; all 9 replicates with valid results were Procleix Zika Virus assay reactive upon retesting.
[b] None had Zika virus RNA detected by an alternate NAT. These samples had inconsistently reactive results when retested in nine replicates with the Procleix Zika Virus assay.
[c] Seven samples were qualified for the study because they had positive (b) (4) Zika Virus Real-Time RT-PCR (b) (4) polymerase chain reaction [PCR] assay; (b) (4) results in initial qualification testing and nine samples were qualified for the study because they had positive Aptima Zika Virus assay results for both replicates after the initial PCR test results were negative or equivocal.
[d] Eight samples were qualified for the study because they had positive (b) (4) PCR assay results in initial qualification testing and 14 samples were qualified for the study because they had positive Aptima Zika Virus assay results for both replicates after the initial (b) (4) PCR assay results were negative or equivocal.

The neat samples with discordant results were evaluated further per the protocol with alternative NAT and additional Procleix replicate testing. Results of this testing were inconsistently reactive for all 6 samples. The majority (23/38) of diluted samples with discordant results had negative or equivocal results from initial qualification testing with the (b) (4) PCR assay.

Clinical Reproducibility Study

This study estimated the reproducibility and repeatability of the Procleix Zika Virus Assay on the Procleix Panther System. Testing was performed by 2 external sites (b) (4) , and 1 in-house site (GDS). A 4-member reproducibility panel containing one negative panel member and three Zika virus-positive panel members was tested using three investigational reagent kit master lots. The clinical protocol, statistical analysis plan and final clinical report were included in Section 8 of the submission: Clinical Protocol # B10381-ZIKVPS-CSP-02 Amendment 2 Statistical Analysis Plan for ZIKV Reproducibility Study Final Clinical Report B10381-ZIKVPS-CSR-02 for ZIKV Reproducibility Study.

Study Design. Testing sites were provided with a four-member reproducibility panel that included one Zika virus-negative panel member and three Zika virus-positive panel members. Positive panel members were created using Zika virus stock solution by spiking the negative plasma with Zika virus stock composed of virus isolate derived from a Zika virus–positive plasma specimen collected from a blood donor during the 2015 Zika virus outbreak in Brazil.

Procleix Zika Virus assay testing on the Procleix Panther system was conducted at 3 sites; at each site, 2 operators each performed 3 runs per day over at least 6 days (days did not need to be consecutive and only 1 operator performed testing each day) to obtain a total of 36 valid runs.. One Procleix Panther system was used to perform testing at each site. Each run contained 2 replicates of each reproducibility panel member (2 x 3 x 6 x 2 = 72 replicates per panel member/lot) with 8 samples per run. Three reagent kit lots were used equally by each operator (72 x 3 = 216 replicates per panel member total). Agreement with expected positive (reactive) or negative (nonreactive) results was calculated with 2-sided 95% Clopper-Pearson CI for each panel member by site, by reagent kit lot, by operator at each site, and overall. For each panel member, results were reported using descriptive statistics of the signal to cutoff ratio (S/CO), including mean, standard deviation (SD), and CV. Variability was calculated using the random effect linear model for the following sources of variation: 1) within runs, 2) between runs, 3) between operators, 4) between sites/instruments, 5) between reagent kit lots, and 6) between days. The total variability was determined from these 6 sources.

At each of the three sites, 288 samples were tested for a total of 864 samples tested overall in valid runs. Each panel member had 216 replicates tested in valid runs. Agreement was expected to be 100% except for the low positive samples (11.8 copies/mL), in which the concentration is 1–2xLoD (~ 6 c/mL).

Results. There were 108 (108/116, 93.1%) valid runs initiated. Of the 864 samples tested in valid runs, all samples (100%) had valid results. Table 16 summarizes results from the agreement analyses:

Table 16. Clinical Reproducibility Study Results.

Panel Member	Description	Concentration (copies/mL)	Expected Result	Agreed/N	Agreement (%) 95% CI
A	Negative	0	Nonreactive	216/216	100 (98.31–100)
B	ZIKV Low Positive	11.8	Reactive	212/216	98.15 (95.33–99.49)
C	ZIKV Moderate Positive	23.6	Reactive	216/216	100 (98.31–100)
D	ZIKV High Positive	59.0	Reactive	216/216	100 (98.31–100)

The low positive panel member (panel member B) had a positive agreement of 98.15%, which was as expected. The remaining samples demonstrated 100% agreement, as expected.

The CV was greatest for within-run (other than for the negative sample) for the low positive sample (21.11%), yielding a total CV of 21.49%. The low concentration of this sample renders this result acceptable. The remaining CV for the different samples and for the different variables are all <10%, which is acceptable. The results indicate the repeatability and reproducibility of the Procleix Zika Virus assay using the Procleix Panther system are robust. These findings are acceptable and support the proposed intended use.

Review Issues and their Resolution

- The Procleix Zika test failed to meet the performance target agreed upon during the pre-submission (BQ170006) discussions, specifically that the lower bound of the 95% CI has to be ≥ 95% for both plasma and serum. However, because the performance of the Grifols Zika test with plasma samples (93.47%) is equivalent to that of the already licensed test, it was found acceptable.

- Since serum is not used as a matrix for donor screening, Grifols was asked to provide a rationale and justification for the inclusion of this matrix in the intended use. Sponsor argued that the serum claim could be kept for donors of HCT/Ps if at least 30 matched serum/plasma samples are tested for sensitivity and the lower bound of the 95% CI in the specificity study is ≥99.8%. Additional IR was issued recommending to revise the intended use statement in a way that would make it clear that serum specimens can only be used to screen organ and HCT/P donors, living or cadaveric; the sponsor submitted the revised statement in Amendment 8, where FDA recommendations were incorporated.

- Sponsor was asked to provide a summary of the test results for initially reactive samples in the specificity study and classify them by outcome. The information was provided with Amendment 5, and the opinion of the statistical reviewer was that one true positive result (confirmed by a reactive follow-up Procleix Zika Virus Assay result) and 11 samples with unknown outcomes due to missing follow-up information should be considered false positive because the index samples were nonreactive by both the alternate NAT and serology testing. The issue was discussed and resolved.

- Sponsor was asked by the statistical reviewer to provide additional details regarding the clinical sensitivity study, and the data provided with Amendment 5 was found to be acceptable.

- Sponsor was asked to clarify whether any samples tested in pools for the specificity study were also tested individually and why some samples in the sensitivity study were retained at the sponsor's R&D laboratory. The information was submitted with Amendment 5, and the response was found to be acceptable.

- Sponsor was asked by the statistical reviewer to provide additional details regarding the random effects analysis used for reproducibility study, and the data was provided in Amendment 5. It was concluded that the results were robust enough.

Label Considerations

There are no labeling restrictions other than those noted in the intended use statement.

BIMO

Bioresearch Monitoring (BIMO) inspections were conducted at three clinical study sites that participated in the conduct of clinical study *Pre-pivotal Procleix Zika Virus Assay Testing of Donations from Donors of Whole Blood and Blood Components* (Protocol No. B10383-ZIKVPS-CSP-01). The BIMO inspections did not reveal substantive problems that impact the data submitted in this application.

a) Pediatrics

N/A

b) Other Special Populations

N/A

7. Advisory Committee Meeting

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

8. Other Relevant Regulatory Issues

None.

9. Labeling

Proprietary name: Procleix® Zika Virus Assay.

APLB Review: 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.

Carton and immediate container labels:

- Sponsor was asked to revise the kit outer box labeling and other labeling that contains references to the intended use so that it reflects the updated intended use statement. Since the sponsor plans to eventually market the product globally, to ensure continuity of labeling the following language for box labels was proposed by Grifols: "The Procleix Zika Virus Assay is a qualitative in vitro nucleic acid amplification test for the detection of Zika virus (ZIKV) RNA in specimens from human donors", which was found acceptable by FDA.

10. Recommendations and Risk/ Benefit Assessment

a) Recommended Regulatory Action

The Review Committee reviewed the original submission and related amendments submitted by Grifols. All review issues have been resolved; therefore, the Review Committee recommends licensure of the Procleix® Zika Virus Assay on the Procleix® Panther System.

b) Risk/ Benefit Assessment

The Procleix® Zika Virus Assay is intended for detection of Zika virus nucleic acid in blood donations. The specificity of the assay is 99.999% for individual donations and 100% for 16-sample pools, and its sensitivity is 98.15% and 96.30% for neat plasma and serum samples, respectively, and 85.19% and 79.63% for diluted plasma and serum samples, respectively. While not the first assay of its kind, the Procleix Zika Virus Assay has comparable characteristics as demonstrated by clinical studies, and considering possible adverse effects of ZIKV infection on the safety of the blood supply, this test will likely offer additional significant public health benefit.

c) Recommendation for Postmarketing Activities

No postmarketing activities recommended.