Summary Basis for Regulatory Action (SBRA)

Date: May 25, 2018

From: *Maria Rios, Ph.D.,* Chair of the Review Committee

BLA/ STN#:125121.80

Applicant Name: Grifols Diagnostic Solutions, Inc.

Date of Submission: June 29, 2016

Complete Response Letter: April 6, 2017

Resubmission date: November 27, 2017

MDUFA Goal Date: May 30, 2018

Proprietary Name: Procleix® WNV, Nucleic Acid Test for use on the Procleix® Panther Systems

Established Name (common or usual name): Procleix® WNV Assay

Intended Use/Indications for Use:
The Procleix WNV assay is a qualitative in vitro NAT for the detection of West Nile Virus (WNV) RNA in plasma specimens from individual human donors, including volunteer donors of whole blood and blood components, and other living donors. It is also intended for use in testing plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, 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 comprised of equal aliquots of not more than 16 individual donations from volunteer donors of whole blood and blood components.

This assay is not intended for use as an aid in the diagnosis of West Nile Virus infection.

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

Review Office Signatory Authority: Hira Nakhasi, Ph.D., Director, DETTD/OBRR/CBER

□ I concur with the summary review.
□ I concur with the summary review and include a separate review to add further analysis.
□ I do not concur with the summary review and include a separate review.

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

Document Title	**Reviewer Name**	**Document Date**
Product Review(s) (*product office*) • *Clinical and Non-Clinical (OBRR)*	 Caren Chancey, Ph.D. Evgeniya Volkova, M.S., M.B.A.	 January 30, 2018 February 28, 2018
Statistical Review(s) • *Clinical and Non-Clinical* (OBE)	 Tie-Hua Ng, Ph.D.	 February 15, 2018
CMC Review • *CMC (OBRR)* • *Facilities Review (OCBQ/DMPQ)* • *Establishment Inspection Report(s) (OCBQ/DMPQ)*	 Caren Chancey, Ph.D. Evgeniya Volkova, M.S., M.B.A. CDR Sean Byrd Deborah Trout	 December 8, 2016 December 8, 2016 March 23, 2017 October 12, 2016
Labeling Review(s) • *APLB (OCBQ/APLB)*	 Dana Jones	 March 10,2017
Bioresearch Monitoring Review	Carla Jordan	January 20, 2017
Software and Instrumentation	Lisa Simone, Ph.D.	February 05, 2018
Consult reviewers for Cadaveric Claim HCT/Ps and Organ Donors Review (OTAT)	 Michelle McClure, Ph.D. Brychan Clark, M.D.	 January 6, 2017 February 2, 2018

1. Introduction

Grifols Diagnostic Solution Inc., (note the submission was originally provided by Hologic, Inc., which has since been acquired by Grifols Diagnostic Solution, Inc.) submitted an efficacy supplement to the Procleix WNV Assay on the Procleix Panther System. Currently, the licensed Procleix WNV Assay is used on the Procleix System (STN BL 125121/0) and the Procleix Tigris System (STN BL 125121/17) platforms in the U.S.

The application was submitted on June 29, 2016, an acknowledgment letter was submitted to the sponsor on July 15, 2016, the submission was filed with deficiencies and the filing notification was sent on August 26, 2016, which included preliminary notification of issues with information requests.

The mid-cycle meeting was held on November 30, 2016. Information requests were sent to the sponsor on October 20, November 6, and December 29 2016, January 19 and December 21, 2017, January 23 and 26, 2018. A Complete Review (CR) letter was sent to the sponsor on April 6, 2017. Resubmission of the application with responses to the CR letter was submitted on December 11, 2017. A total of 10 amendments were received from the sponsor in support of the application.

The clinical studies were performed at 3 testing sites under IND #16234 and were designed to evaluate assay specificity, sensitivity and reproducibility in human plasma specimens tested individually and in 16-sample pools. The findings from the three clinical sites support the proposed intended use for the Procleix WNV Assay on the Procleix Panther

System. The IND #16234 was submitted on October 22, 2014, after communications with CBER through a Type B Pre-IND meeting on May 30, 2014, and other communications by email and teleconference on June 19 and 27, August 1 and 4, and September 5 and 24, 2014. The IND included 5 amendments submitted on October 31, and November 14, 2015, January 30, February 4, April 2, 2015 and an annual report on January 25, 2016.

There are no changes to the principles of the assay technology or procedure as a result of the implementation of a new instrument system. Detailed information about the Procleix Panther System including hardware and software description, verification and validation studies, and risk analysis were provided in this submission.

There are no changes to the chemistry, manufacturing and controls of the in vitro substance components. The same oligonucleotides and the in vitro product reagents used to detect WNV RNA on the Procleix Tigris System will be used to detect WNV RNA using the Procleix Panther System. Minor differences in assay specifications and labeling of the ancillary components were provided in detail in this submission. Information on manufacturing facilities was provided by the applicant, but based on the compliance history of the applicant, the pre-approval inspection of the manufacturing facilities was waived. Labeling that includes a package insert for the Procleix WNV Assay on the Procleix Panther System, Operators' Manual for the Procleix Panther System and labels for the ancillary assay kit and components were submitted. Grifols, Inc. discussed the clinical study design and regulatory strategy with FDA during multiple teleconferences; a summary of the previous communications with FDA was also included in the submission.

2. Background

The process flow of the Procleix WNV Assay run on the Procleix Panther System is similar to the assay run on the Procleix Tigris System. Also, similar to the Procleix Tigris System, the Procleix Panther System is a fully automated system including processing, interpretation and management of nucleic acid test (NAT) results.

The Procleix WNV Assay involves three main steps that take place in a single tube: (a) sample preparation; (b) WNV RNA target amplification by Transcription-Mediated Amplification (TMA); and (c) detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA). The Procleix assays incorporate an Internal Control in each reaction tube for monitoring assay performance in each individual specimen.

During sample preparation, RNA is isolated from specimens through the use of target capture oligonucleotides. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins and release viral genomic RNA. Capture Oligonucleotides are highly homologous to conserved regions of WNV and hybridized to the WNV RNA target, if present, in the test specimen. The hybridized target is then captured by magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps are utilized to remove unbound components from the reaction tube. Target amplification occurs using TMA, which is

a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target RNA sequence. T7 RNA polymerase then produces multiple copies of a RNA amplicon from the DNA copy template.

Detection is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The selection reagent differentiates between hybridized and unhybridized probes by inactivating the label on unhybridized probes. During the detection step, the chemiluminescent signal produced by the hybridized probe is measured by a luminometer and is reported as Relative Light Units (RLU).

The Internal Control (IC) is added to each test specimen and assay calibrator via the working Target Capture Reagent. The IC in the Procleix WNV Assay controls for specimen processing, amplification and detection steps. The IC signal is discriminated from the WNV signal by the differential kinetics of light emission from probes with different labels. IC-specific amplicon is detected using a probe with rapid emission of light (flasher signal). Amplicon specific to WNV is detected using probes with relatively slower kinetics of light emission (glower signal). The Dual Kinetic Assay (DKA) is a method used to differentiate between the signals from flasher and glower labels. When used for the detection of WNV, the DKA differentiates between IC and WNV signals.

The reagents used for the Procleix WNV assay on the Panther System are the same as those used on the licensed Procleix Tigris System except for the Procleix Tigris Controls and Tigris Fluid Preservative, which are not required for the Procleix Panther System.

The Procleix Panther System operation involves the following steps: (a) *Pre-Assay System Preparation step* requires properly installed and calibrated units to perform assays. The system software alerts the operator when maintenance activities are required and will prevent the use of the system if maintenance is required. The pre-assay system preparation activities include inspection of the waste collection containers, proper preparation of the reagents, tracking of samples, controls and calibrators, loading of pipette tips, Multi-Tube Units (MTUs) and assay specific reagents according to the instructions described in package insert and Procleix Panther System Operator's Manual. (b) *Specimen Transfer and Target Capture steps* include moving each MTU to the pipette position, adding Target Capture Reagent (TCR) and sample to each MTU, software-controlled mixing step, heating, annealing, Adenosine-Thymine Binding (ATB) Incubation and washing steps. (c) *Amplification steps* include moving the MTUs to the re-suspension mixer, adding oil and Amplification Reagent, software-controlled mixing step, heating, annealing, ATB Incubation, stabilizing, adding Enzyme Reagent, mixing and final incubation. (d) *Hybridization and Selection steps* include moving of the MTUs to the HPA Incubator, adding Probe Reagent, mixing and incubation of the reaction mixture, adding Selection Reagent followed by mixing and incubation, moving to

the ATB incubator for Target Binding incubation. (e) *Detection steps* include moving of the MTUs with the reaction mixtures to the parking station for cool-down, verification of barcodes on the MTUs, adding Auto Detect 1 and 2, detection of the chemiluminescent light output as a function of time and deactivation of the solution. (f) *Post-Assay Processing step* includes routine daily cleaning and other maintenance of the Procleix Panther System, results processing and generation of the assay report.

The clinical study was performed under IND 16234, started in November 2014. The study was run in parallel with the licensed Procleix WNV Assay on the Procleix Tigris System.

3. Chemistry Manufacturing and Controls (CMC)

There are no changes in chemistry, manufacture or control of the (b) (4) oligonucleotides that comprise the in vitro substance of the Procleix WNV Assay on the Procleix Panther System as compared to the licensed Procleix WNV Assay on the Procleix Tigris System. Similarly, there are no changes to the reagents required to run the assay with regards to formulation, manufacturing processes and container/closure systems, or to the manufacturer or location of manufacture.

3.1 Product Comparability

Notable changes from the Procleix Tigris System to the Procleix Panther System are as follows:

1. Design of the Panther system obviates the need for System Fluid Preservative and Controls as found on the Tigris system. The Panther system has air based pipetting and does not use system fluid, which makes Fluid Preservative unnecessary. Controls were needed on the Tigris System to mitigate systematic instrument errors, all of which have been addressed (or resolved) through specific built-in hardware/software process controls on the Panther System.
2. The Procleix Panther System has built-in radio frequency identification (RFID) antennas that read the barcode information from the Universal Fluid Bottles: Procleix Assay Fluids Kit and Procleix Auto Detect Reagents Kit, which now require an RFID tag that is applied on a bottle the same way as a regular label. An RFID reader located at the end of the bottle fill and labeling conveyor verifies that the tag has been applied. Catalog numbers for the 2 kits have also been changed to account for this modification.
3. Analytical studies using 2 commercial IVD lots were performed to determine stability, and on-board stability for wash solution, oil, buffer for deactivation fluid, and auto detect reagent was extended from 14 days on Tigris to 60 days on the Panther. WNV Assay calibrators were also shown to be stable for at least 8 hours and up to (b) (4) hours at 30°C.
4. Test methods and release criteria were compared for the Procleix WNV Assay Master Lot, Negative Calibrator, Positive Calibrator, and Internal Control on the Procleix Tigris

and the Procleix Panther Systems. Differences in certain evaluated attributes necessitated changes in release specifications for Procleix WNV Assay on the Procleix Panther platform (Table 1)

Table 1 – Differences in Procleix WNV Assay Release Specifications on Panther and Tigris Instruments

Attribute	Tigris Specification	Panther Specification
Sensitivity – WNV 100 c/mL – Average RLU	(b) (4)	(b) (4)
Sensitivity – WNV 100 c/mL – Individual RLU		
Sensitivity – Average IC RLU		
Positive Calibrator – Average Analyte RLU		

The differences in RLU values between the two instruments are attributed to different (b) (4) and the fact that the Procleix Panther System (b) (4) for all assays it is capable of running.

The release specifications and quality specification documents for the Procleix WNV Assay Master Lot, Negative Calibrator, Positive Calibrator and Internal Control were updated.

3.2 CBER Lot Release

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

3.3 Facilities review/inspection

Facility information and data provided in the supplement were reviewed by CBER and found to be sufficient and acceptable. The manufacturer of the Procleix WNV 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 the table below and are further described in the paragraphs that follow.

Table 2 - Manufacturing facilities for Procleix WNV Assay and Ancillary Kits

Facility Name / Address	FEI Number	Inspection/ waiver	Justification /Results
Manufacturer of: • *Procleix WNV Assay Master Kit (7 components)* 1. *Internal Control Reagent* 2. *Target Capture Reagent (4 components)* 3. *Procleix Probe Reagent (3 Components)* 4. *Selection Reagent* 5. *Enzyme Reagent* 6. *Amplification Reagent (5 components)* 7. *Blank* • *Procleix WNV Assay Fluids Kit (3 components)* 1. *Wash solution* 2. *Oil* 3. *Deactivation Buffer* *Final packaging and shipment of finished assay kit* Grifols Diagnostics Solutions, Inc. (b) (4)	(b) (4)	Waived	Team Biologics inspection, (b) (4) NAI
Manufacturer of: • *Auto Detect Reagents Kit (2 components)* 1. *Auto Detect 1* 2. *Auto Detect 2* • *Procleix WNV Assay Calibrators Kit (2 components)* 1. *WNV Positive Calibrator* 2. *WNV Negative Calibrator* *Procleix Panther System platform acceptance testing* Grifols Diagnostics Solutions, Inc. (b) (4)	(b) (4)	Waived	Team Biologics inspection, (b) (4) VAI

Manufacture of Procleix Panther System platform and final release of finished platform (b) (4)	(b) (4)	Waived	ORA inspection (b) (4) NAI

Team Biologics performed a surveillance inspection of the Grifols Diagnostics Solutions, Inc. manufacturing facility located at (b) (4) No inspectional conditions were noted during the inspection 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) Inspection objectionable conditions were noted on FDA Form 483 and the corrective actions were deemed satisfactory. The inspection was classified as voluntary action indicated (VAI).

ORA performed a surveillance inspection of the (b) (4) manufacturing facility during (b) (4) . No inspectional conditions were noted during the inspection and the inspection was classified as no action indicated (NAI).

3.4 Environmental Assessment

Not applicable.

3.5 Container Closure

Not applicable.

3.6 CMC Review

The review committee identified the following as the major CMC review issues:

1) The Procleix WNV assay on the licensed Tigris system utilizes WNV positive and negative controls and fluid preservatives which are not included for use with the investigational Panther system. The rationale provided for excluding these components was that the Panther had additional process controls and air-based pipetting. The reviewers requested that the sponsor provide a more thorough explanation of the new process controls and pipetting procedure, and indicate how their effect on the workflow obviates the need for the preservative and controls used with the Tigris system.

2) The Panther Instrument is manufactured by (b) (4) for Grifols Diagnostic Solutions, Inc.. There was a lack of documentation on manufacturing in the sponsor's report including (a) quality control systems and activities for (b) (4) (b) description of the manufacturing site, (c) purchasing control procedures, (e) the Incoming and the Final Acceptance Activities for the site where the System is manufactured.

The review issues were conveyed to the sponsor in an information request dated January 19, 2016. The sponsor provided the requested documentation on February 9, 2017, and the responses were considered satisfactory by the review committee.

4. Software and Instrumentation

In this submission, the new Procleix Panther System is added as the third instrument platform supporting the existing Procleix WNV 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 WNV ADM (assay specific software) v2.6.5, firmware v5.3.2.3 running on Windows 7 operating system.

Device Description: The Procleix Panther System's process flow is similar to the existing Procleix Tigris System and uses the same algorithm to analyze and interpret results. The 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).

Significant changes were made to the risk processes, which now appear to be more robust in adequately identifying sources of harm in the system and reducing the risks to acceptable levels. Failure Modes Effects Analysis (FMEA) was added to the existing Fault Tree Analysis (FTA) to drive 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: Because the operating environment risk profile has significantly changed since the existing instruments were added in 2005 and 2007, a major focus of this review was on inadequate risk processes and cybersecurity processes, which resulted in several changes to the device and labeling as described below.

Three new processes were added to the overall risk management processes: 1) Design Failure Mode Effects Analysis (DFMEA) process, 2) Cybersecurity Risk Analysis process, and 3) ISO14971 Checklist process to identify medical device characteristics that could affect safety.

Risk analysis processes were updated to supplement the existing Fault Tree Analysis with FMEAs and to better align with ISO 14971 "Medical devices – application of risk management to medical devices." This allowed the applicant to identify existing risks that were not adequately mitigated. Major process changes include removing a non-standard method of assessing Severity of harm, adding/clarifying use of Probability for software-related risks, removing disease prevalence to avoid underestimating calculation of

Probability, and integrating hazards for cybersecurity and critical software defects into the overall risk analysis rather than treating each as unrelated activities.

During this review, the applicant made the following changes to improve safety and effectiveness of the device:

1. Because Windows Vista is now beyond the End of Support (EOS) date (March 2017), the applicant agreed to drop support for this operating system to avoid risks associated with use of an unsupported operating system.
2. An optional firewall was changed to "required" to align with security risks that are mitigated by use of a firewall, as part of the applicant's "layered" approach to security.
3. Guest account on the device has been disabled to remove one avenue of inappropriate access to the device.
4. Service accounts for emergency access used hard-coded passwords, which is a major security risk. Applicant developed a Customer Technical Bulletin to describe a safer "break glass" method to access key service accounts for emergency purposes.
5. To reduce risk of a false negative result, an Independent Temperature Monitor (ITM) is now required equipment for use with the Reagent Preparation Incubator to assure proper temperature profiles in the preparation of assay reagents.
6. Applicant removed an incorrect claim that use of (b) (4) program protects against all zero-day cybersecurity attacks, to avoid misleading the customer about the degree of security protection provided.
7. Cybersecurity processes now include proactive monitoring of independent vulnerability alerts, daily vulnerability scans and passive monitoring.

5. Analytical Studies

The sponsor performed non-clinical/analytical studies to investigate and describe the functionality of the Procleix WNV assay on the Panther system under defined conditions.

5.1 Analytical Reproducibility Studies:

In addition to the Clinical Reproducibility study, the sponsor also performed an analytical reproducibility study to examine the assay performance, percent agreement, and reproducibility of the Procleix WNV assay on the Panther system. Tests were performed using the WNV positive panels at 100 and 30 copies/ml from in-house Quality Control release panels, across 3 lots of WNV IVD reagents, 3 Panther instruments, and 3 operators. Variation was calculated for each of five factors: Inter-Instrument, Inter-Operator, Inter-Lot, Inter-Day and Intra-run) for the analyte and internal control S/CO ratio as well as the RLU signals from the Procleix WNV Assay calibrators. The sponsor noted that the signals from discordant tests were excluded from the variability analysis.

The sponsor found that 108/108 samples with 100 c/ml and 108/108 samples with 30 c/ml WNV were reactive in the Procleix WNV assay on the Panther system (100% for both, 95% CI 96.6-100%). The mean analyte S/COs and 95% CIs for 100 c/ml and 30 c/ml were 32.28 (31.98-32.58) and 32.36 (31.68-32.84) respectively. All 216 WNV-negative samples were non-reactive with a mean S/CO for the internal control of 2.01 (95% CI 2.00-2.02) and analyte 0.00 (0.00-0.01) (Table 3a). The mean analyte RLUs and 95% CIs for 100 c/ml and 30 c/ml were 1,773,740 (1,754,415-1,793,066) and 1,772,354 (1,739,194-1,805,515) respectively (Table 3b).

Table 3a: Summary of Percent Agreement, Internal Control (negative panel only) and Analyte (negative and positive panel) S/CO

Panel WNV	R/N	%R	Lower C.I.	Upper C.I.	Internal control				Analyte			
					Mean S/CO	SD S/CO	Lower C.I.	Upper C.I.	Mean S/CO	SD S/CO	Lower C.I.	Upper C.I.
100 c/mL	108/108	100%	96.6	100					32.28	1.57	31.98	32.58
30 c/mL	108/108	100%	96.6	100					32.26	3.05	31.68	32.84
Negative	0/216	0	0	1.7	2.01	0.09	2.00	2.02	0	0.02	0	0.01

R = Number Reactive; N = Number Valid; C.I. = 95% Confidence Interval; S/CO = Signal to Cutoff Ratio; SD = Standard Deviation; *Mean of analyte S/CO includes 'Reactive' results only.

Table 3b: Summary of Internal Control (negative panel only) and Analyte (negative and positive panel)

Panel WNV	Internal control				Analyte			
	Mean S/CO	SD S/CO	Lower C.I.	Upper C.I.	Mean S/CO	SD S/CO	Lower C.I.	Upper C.I.
100 c/mL					1,773,740	101,311	1,754,415	1,793,066
30 c/mL					1,772,354	173,840	1,739,194	1,805,515
Negative	166.199	18.988	163.0653	168,746	259	912	137	381

C.I. = 95% Confidence Interval; SD = Standard Deviation

Overall, the percent agreement between expected and actual results for the panels and controls was 100%. In the variability analysis, CVs were low overall except for the negative control analyte CV which is expected to be high because of the low background signal. Within-run variability contributed the most to the total variability. Procleix WNV Assay on the Panther system was highly reproducible across operators, instruments, reagent lots, and days tested using a range of panel types with negative and both high and low copy levels of WNV.

5.2 Analytical Sensitivity Studies:

5.2.1 Limit of Detection analysis

The analytical sensitivity for the Procleix WNV assay on the Panther system was assessed using serial dilutions of a WNV lineage 1 stock from the Health Canada and two in-

house transcripts for WNV lineage 1 and lineage 2 (Table 4). Each material was used to produce a six-member panel composed of 100, 30, 10, 3, 1 and o c/ml. Three Panther instruments were used with 3 reagent lots to test 48 replicates each for a total of 144 replicates per panel member. Testing on Panther was performed in parallel with testing on the currently licensed Tigris system.

Detection of all three panels across all reagent lots was similar. Using the Health Canada Lineage 1 viral stock, results combined across all reagent lots showed 100% detection of the 100 c/ml and 30 c/ml panel members. Detection at 10, 3 and 1 c/ml was 92%, 60% and 34% respectively. Using the Lineage 1 transcript, results combined across all reagent lots showed 100% detection of the 100 c/ml and 30 c/ml panel members. Detection at 10, 3 and 1 c/ml was 90%, 56% and 24% respectively. Using the Lineage 2 transcript, results combined across all reagent lots showed 100% detection of the 100 c/ml and 30 c/ml panel members. Detection at 10, 3 and 1 c/ml was 92%, 47% and 19% respectively. Probit analysis of the performance of the three panels on the investigational Panther system and the licensed Tigris system showed similar 95% LODs.

Table 4 – Limit of Detection analysis

Panel	Platform	**95% LOD (95% Fiducial Limits)**
Health Canada Lineage 1 stock	Panther	11.9 (9.6 - 15.9)
	Tigris	8.9 (7.3 - 11.5)
Lineage 1 transcript	Panther	12.9 (10.6 - 16.7)
	Tigris	9.1 (7.6 - 11.2)
Lineage 2 transcript	Panther	12.0 (10.0 - 15.2)
	Tigris	16.7 (13.9 - 21.0)

5.2.2 Detection of WNV Genetic Types with the Procleix WNV Assay

The sponsor performed further assessment of the ability of the Procleix WNV assay on the Panther system to detect different WNV genetic types with comparable sensitivity to the WNV assay used on the Tigris system. The 2 Lineage 1 tissue culture specimens tested showed identical reactivity on both platforms, with 1 specimen reactive at 100% for both platforms using the 10^{-8} dilution, and 75% for both platforms using the 10^{-9} dilution, and the other reactive at 100% for both 10^{-8} and 10^{-9} dilutions. Results for the Lineage 2 specimens, 1 tissue culture and 3 transcripts, were comparable or better on Panther than on Tigris, with the tissue culture specimen, B-956, showed 100% reactivity on both platforms at the 10^{-6} dilution and at 10^{-7} dilution the reactivity was 75% on Panther and 25% on Tigris. One transcript of a South African isolate was 100% reactive on both platforms at 100 c/ml and 30 c/ml. The other two transcripts of isolates from Greece and Hungary, were 100% reactive on both platforms at 100 c/ml, and at 30 c/ml, they were 100% reactive on Panther and 75% reactive on Tigris (Table 5).

Table 5 – Detection of Genetic Types with WNV Assay on Panther and Tigris System using 2 lots (b) (4)

WNV Lineage	Stain (Accession number) titration method	Level	IVD 18 & 19 Panther		IVD 18 & 19 Tigris	
			Reactive/ tested	% reactive	Reactive/ tested	% reactive
1	NY 2001-6263 (AF533540)	10^{-8} Dilution [a]	4/4	100%	100%	100%
	$TCID_{50}$ units/mL	10^{-9} Dilution [b]	3/4	75%	75%	75%
1	1986 WN02 (DQ164189)	10^{-8} Dilution [c]	4/4	100%	4/4	100%
	TCID50 units/mL	10^{-9} Dilution [d]	4/4	100%	4/4	100%
2	B-956 Uganda (n/a)	10^{-8} Dilution [e]	4/4	100%	4/4	100%
	$TCID_{50}$ units/mL	10^{-9} Dilution [f]	3/4	75%	1/4	25%
2	South Africa 1989 (EF429197)	100 c/mL	4/4	100%	4/4	100%
	Absorbance 260 nm	30 c/mL	4/4	100%	4/4	100%
2	Greece 2010 (HQ537483)	100 c/mL	4/4	100%	100%	100%
	Absorbance 260 nm	30 c/mL	4/4	100%	75%	75%
2	Hungary 2004 (DQ 116961)	100 c/mL	4/4	100%	100%	100%
	Absorbance 260 nm	30 c/mL	4/4	100%	75%	75%

[a] (b) (4) $TCID_{50}$ units/mL; [b] (b) (4) $TCID_{50}$ units/mL; [c] (b) (4) $TCID_{50}$ units/mL; [d] (b) (4) $TCID_{50}$ units/mL; [e] (b) (4) $TCID_{50}$ units/mL; (b) (4) $TCID_{50}$ units/mL.

5.2.3 Detection of WNV in Naturally Infected Samples

The purpose of this study was to assess the clinical sensitivity of the Procleix WNV assay used on the investigational Panther system in detecting WNV in clinical samples, in comparison to the same assay used on the licensed Tigris system. The study tested 352 known-positive clinical specimens with viral loads ranging from 830 to <5 copies/ml, as determined by qualitative and quantitative NAT PCR-based assays. One replicate was run on both Panther and Tigris; further replicates were run for samples that were non-reactive on Panther, Tigris or both. The clinical sensitivity of the Procleix WNV assay across the 352 samples was calculated as 67.0% (95% SCORE CI 62.0-71.9) for Panther and 65.9% (60.8-70.7) for Tigris. Of the 352 known-positive samples, 205 were reactive on both systems, 89 were non-reactive on both systems, and similar numbers were positive on one system or the other (27 vs. 31) (Table 6).

Table 6 – Clinical Sensitivity of the WNV Assay on the Panther System compared to the WNV Assay on the Tigris System

		Procleix Tigris System		
		Non-reactive	Reactive	Totals
Procleix Panther System	Non-reactive	89	27	116
	Reactive	31	205	236
	Totals	120	232	352

The 147 known-positive samples that were initially non-reactive on either system or both systems were retested in duplicate on both systems, and the percentage of reactive results across all three replicates tested was calculated for each system. Out of 441 (147 samples x 3 replicates) tests, 22.9% were reactive on the Panther system and 23.4% were

reactive on the Tigris system, which was not a statistically significant difference. Further retests of the 59 samples that did not yield a reactive result in any of the three replicates tested on either system again showed similar results between the two systems, with 6.2% reactive on Panther vs. 5.3% reactive on Tigris, which was not a statistically significant difference.

Overall, the Procleix WNV Assay on the Panther system performed as well or better than the Tigris system with regards to analytical sensitivity.

5.3 Analytical Performance Studies

5.3.1 Specificity Study

In the specificity study, the Procleix WNV assay on the Panther system was assessed using (b) (4) unlinked EDTA plasma specimens from normal blood donors, evenly divided between fresh and frozen specimens. The specificity result was determined to be 100% with an initial invalid rate of 0.03%, which was within design requirements of equal or better performance than on the Tigris system.

5.3.2 Cross-contamination Study

In the cross-contamination study, high titer (b) (4) copies/ml) WNV-containing samples were interspersed in specimen processing racks to assess the false-positive rate due to sample cross-contamination. Testing was done using an arrangement of alternating positive and negative samples with one production lot of reagents, in (b) (4) different Panther instruments. A total of (b) (4) negative and (b) (4) high-titer positive specimens were tested in the (b) (4) runs. Across all runs, all negative and positive specimens showed 100% concordance with expected negative and positive results. The specificity of the assay in regards to cross-contamination was 100%, and the presence of positive specimens did not affect the analyte cutoff for the negative specimens.

5.4 Effect of Donor and Donation Factors on Sensitivity and Specificity

Multiple studies were conducted to assess the impact of donor and donation factors on the sensitivity and specificity of the Procleix WNV Assay on the Panther System. These studies found that the sensitivity (for WNV-spiked specimens) or specificity (for specimens not spiked with WNV) of the WNV assay on the Panther System was not affected by the following:

(a) the presence of other blood borne pathogens (Herpes Simplex Virus 1 and 2, Human T-cell Lymphotrophic Virus Types I and II, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Human Immunodeficiency Virus 1 and 2, Cytomegalovirus, Epstein-Barr Virus, Rubella Virus, Parvovirus B19, Hepatitis G virus, St. Louis Encephalitis Virus, Murray Valley Encephalitis Virus, Japanese Encephalitis Virus, Yellow Fever Virus, Dengue (types 1-4) or vaccinations (Hepatitis B or influenza);

(b) donations from individuals with autoimmune and other diseases (rheumatoid factor, antinuclear antibody, lupus, multiple myeloma, multiple sclerosis, rheumatoid arthritis, hyperglobulinemia, alcoholic cirrhosis, elevated alanine aminotransferase);
(c) the presence of bacterial (*Staphylococcus epidermidis, S. aureus, Micrococcus luteus, Corynebacterium diphtheriae, Propionibacterium acnes*), yeast (*Candida albicans*) or fungal (*Pneumocystis carinii*) contamination;
(d) the presence of hemoglobin, bilirubin, lipids or albumin.

To serve as a control group for this set of studies, specimens from 90 normal (negative for HIV-1, HBV, HCV and WNV by NAT) blood donors were obtained from a supplier and aliquotted. One set of aliquots was tested without spiking for the specificity analysis; another set was spiked with WNV-positive plasma to a final concentration of 150 copies/ml for use in the sensitivity analysis.

Results of the specificity study showed no invalid results and 100% specificity. In the sensitivity study, two discrepant results occurred, resulting in false negative results on the Procleix WNV Assay on Panther for WNV-spiked specimens from two donors. These two specimens were retested with the Procleix WNV Assay on Panther, with valid, reactive results on both repeats. The sponsor attributed the initial discrepant results to a failure to spike the specimens properly. As the two repeat test results were positive, it was concluded that the initial discrepant results were not due to inhibitory substances in the specimens, excluded the discrepant results, and used the results from the new WNV-spiked specimens in their analysis. Overall, the sensitivity and specificity of the Procleix WNV Assay on the Panther system were not affected by the donor and donation factors tested.

5.5 Statistical Analysis of Specificity and Sensitivity Data

The purpose of this study was to determine whether run time length or the positioning of calibrators affects the accuracy of the West Nile Virus (WNV) Assay on the investigational Panther system. The run length component of the assay was designed to test the accuracy of the assay on the Panther system using the maximum possible number of calibrators and specimens that can be processed using a single 500-test reagent kit, without daily maintenance. Two 500-test runs were performed, each using a different Panther instrument and IVD reagent kit. Sets of WNV-negative samples were analyzed alongside sets of samples positive for WNV at 100 copies/ml, as determined by alternative NAT, to test for positional effects within the 500-test runs. The Internal Control (IC) and analyte cutoff values were analyzed by position across both runs with no significant differences found. Both 500-test runs yielded 100% agreement with expected results for each set of samples. The overall %CV for S/CO values for positive tests was 8.5%. These results are acceptable, and this testing validates the maximum length run of 500 tests per worklist

(limited by the reagent kit volume and by the required daily maintenance) on the Panther System for the WNV Assay.

5.6 Panther Calibration Set Time

This study was performed to validate the Panther system's calibration set time of (b) (4) and determine whether the placement of the assay calibrators at different time points throughout the period covering on-board stability testing affected the performance of the Procleix WNV assay on the Panther system. This testing was performed in concert with the "Panther On-board Stability" Study using 2 WNV IVD reagent lots. Assay calibrators, WNV-negative samples and WNV-positive samples were placed at (b) (4) intervals for up to 60 hours at baseline (0 days), 32 days, and (b) (4) days of on-board stability. Samples and assay calibrators were tested in sets to determine any positional effects.

No differences were observed in the IC or analyte cutoff calculated from calibrators in each of the six calibration sets throughout all on-board stability time points. The rate of agreement with expected results for WNV-positive and negative panel sets across all (b) (4) intervals and stability time points was 100%. Analysis of the S/COs showed that no trends or positional effects were observed in the analyte or IC S/COs for each group of sets across all three stability timepoints. Five invalid results were generated due to a system check of the AutoDetect reagent 2 at the final stability time point (b) (4) days), which were excluded from the analysis. This testing validates that a calibration set time of (b) (4) is appropriate for the WNV Assay on the Panther System.

5.7 (b) (4) Analysis and Justification of Analyte Cutoff Calculation

This section describes the validation of the floating Analyte (glower signal) Cutoff calculation using a statistical analysis of sensitivity and specificity of the Procleix WNV Assay on the investigational Panther system. To perform this validation, (b) (4) curves were generated for the sensitivity and specificity data to determine optimal cutoff values, and the formulas that would be applied to the calibrators to calculate the floating Analyte Cutoff were verified to ensure that they generated values falling within the calculated optimal range.

Values used to generate the floating Analyte Cutoff are drawn from the 3 replicates each of the Positive and Negative calibrators used for each assay run. The algorithm used to generate this cutoff value is the same algorithm used on the licensed Tigris system, shown below.

WNV Assay Analyte Cutoff calculation:

Analyte (A) Cutoff = [Avg. Negative Calibrator A RLU] + [0.03 x (Avg. WNV Calibrator A RLU].

The sensitivity and specificity data used for these analyses came from a combination of the analytical studies performed using reagent IVD lots (b) (4), described elsewhere in the application. Briefly, sensitivity data was obtained from performance of the Procleix WNV assay on the Panther System with spiked samples, naturally infected samples and RNA transcripts, and specificity data was obtained from plasma and serum from analytical panels, normal blood donors, and blood donors with potentially inhibitory or interfering factors. All valid assay Positive and Negative calibrator results were included in the analyses. For the (b) (4) calculation, each result was tagged with an identifier, the associated IC and analyte cutoff values and either a result of '0' for true negatives or '1' for true positives. Data were sorted by analyte RLU and then summarized using Analyte Cutoff Tables and a (b) (4) plot.

A study was performed to determine the optimal range of Analyte Cutoff values using data generated with the Procleix WNV Assay on the Procleix Panther System. The optimal cutoff value was determined using (b) (4) curves of sensitivity and specificity data.

However, the (b) (4) analysis of (b) (4) true negative and (b) (4) true positive samples, generated an Average of the Observed Cutoff Value different from the predicted Optimal Cutoff value. That was probably due to the inclusion of true positive samples with viral loads below the assay's 95% LOD were included, at 10, 3, or 1 copies/ml resulting in specificity slightly lower and sensitivity slightly higher than that of the calculated optimal value.

Table 7 – (b) (4) analysis of Predicted Optimal and Observed Mean Analyte RLU in Procleix WNV Assay with Reagents Lots (b) (4)

Cutoff	Location on Range	Value (RLU)	Percent Specificity	Percent Sensitivity
Predicted	Optimal	(b) (4)	(b) (4)	(b) (4)
Observed	Minimum			
	Average			
	Maximum			

They then assessed the potential alterations to assay sensitivity and specificity that would result from lowering the assay cutoff by recalculating the assay's performance on the subset of samples with RLUs in the range between the calculated optimal cutoff and the mean observed cutoff, and concluded that lowering the assay cutoff to a value closer to the calculated optimal cutoff would increase the false positive rate without substantially improving the sensitivity of the assay. Therefore, the sponsor kept the algorithm used for calculation of floating cutoff value, which is determined for each Procleix WNV Assay run for the Analyte signal (glower signal) unchanged.

Overall, the algorithm to set the floating Analyte Cutoff was performed appropriately when the Procleix WNV Assay was performed on the Procleix Panther System.

5.8 Analytical Review:

During the review of this BLA, the review committee identified deficiencies in the analytical review area of the original submission. These issues were discussed during review committee meetings, meetings with senior management of the Division and the Office and were conveyed to the sponsor in an information request dated January 19, 2017. The following were the major analytical review issues identified by the committee:

1. In the analytical sensitivity study in Section 8.2.5.5, two discrepant false negative results for WNV-spiked samples in the control group were excluded from the analysis and results of re-tests of new WNV-spiked specimens for those two donors were included. FDA requested that the sponsor include the initial false negative results and perform a root cause analysis.
   The sponsor responded that while re-tests of the discrepant samples did not follow the procedures outlined in the product insert, it did adhere to the pre-approved technical protocol. The sponsor did not redo the analysis to include the discrepant results and noted that the set of samples involved served as controls for all of the analytical donor and donation factor studies. Instead, a brief root cause analysis was provided concluding that insufficient material was spiked into the two samples in question.
   This point was included in the CR letter sent on April 6, 2017. The sponsor response to the CR letter on November 27 was reviewed by the committee as found acceptable, the issue was considered resolved.
2. The LoD based on the analytical sensitivity study (Section 8.2.2.2) showed data to support a 100% reactive rate for all panel members of 30 copies/mL and a high % reactive rate for panel members of 10 copies/mL. The sponsor was asked to comment on the lack of criteria set for diluted samples which may have had <100 copies/mL (the LoD). The sponsor response was submitted on February 9 2017, and the review committee considered their responses acceptable and the issues resolved.

6. Clinical Studies

6.1 Clinical Specificity:

The objective of this study was to evaluate the clinical specificity of the Procleix WNV assay on the Procleix Panther system in plasma samples from U.S. donors of whole blood and blood components. Donations were tested individually and in 16-sample pools.

The clinical specificity of the Procleix WNV assay on the Procleix Panther system in comparison to the licensed Procleix WNV assay on the Procleix Tigris system was tested at two external testing sites, with two Panther systems at each site and three Procleix WNV assay reagent kit master lots used approximately equally by each site. All samples that had

reactive Procleix WNV assay results on the Panther system or discordant results between the licensed assay on Tigris performed on the Tigris System and the assay on the investigational Panther System were sent for testing with the FDA-licensed (b) (4) WNV assay (b) (4) Results of the Procleix WNV assay on the Procleix Panther system were compared to results of the Procleix WNV assay on the licensed Tigris system and the (b) (4) assay to estimate the clinical specificity using the 2-sided 95% Clopper-Pearson Exact confidence interval [CI]. Specificity was calculated separately for individual donor samples (never pooled) and 16-sample pools. Testing with the Procleix WNV assay on the Panther system was conducted between February 16 and August 21, 2015. There were 10,631 pools and 13,371 individual donor samples that had valid results on both the licensed Tigris system and investigational Panther system and were included in the specificity calculations.

6.1.1 Pooled Testing Results

There were 10,744 pools processed in valid Procleix WNV Assay runs on the Procleix Panther System generating a total number of 104 pools with invalid results and 10,640 pools with final valid results. Of those, 9 pools had final invalid Procleix WNV assay results on the licensed Procleix Tigris system and were excluded from the specificity analysis, leaving 10,631 pools. (Table 8) The clinical specificity of the Procleix WNV Assay on the Procleix Panther System in 16-sample pools was calculated across all sites and lots as 100% with a 95% CI of (99.965-100).

Table 8 – Clinical Specificity of the Procleix WNV Assay on the Procleix Panther System in 16-Sample Pools

Site	Lot	N	TN	FN	TP	FP	Specificity% (95% CI)[1]
All	All	10631	10630	0	1	0	100 (99.965 - 100)
	Lot 1	3566	3566	0	0	0	100 (99.897 - 100)
	Lot 2	4029	4028	0	1	0	100 (99.908 - 100)
	Lot 3	3036	3036	0	0	0	100 (99.879 - 100)
(b) (4)	All	4971	4970	0	1	0	100 (99.926 - 100)
	Lot 1	1651	1651	0	0	0	100 (99.777 - 100)
	Lot 2	1719	1718	0	1	0	100 (99.786 - 100)
	Lot 3	1601	1601	0	0	0	100 (99.770 - 100)
(b) (4)	All	5660	5660	0	0	0	100 (99.935 - 100)
	Lot 1	1915	1915	0	0	0	100 (99.808 - 100)
	Lot 2	2310	2310	0	0	0	100 (99.840 - 100)
	Lot 3	1435	1435	0	0	0	100 (99.743 - 100)

[1] Clopper-Pearson Exact CI

6.1.2 Individual Testing Results

There were 13,619 individual donations processed in valid Procleix WNV Assay runs on the Procleix Panther System generating a total number of 13,423 samples with final valid

results. Of those, 36 samples that had final invalid Procleix WNV assay results on the licensed Procleix Tigris system and the 16 samples that were constituents of a positive pool were excluded from the specificity analysis, leaving 13,371 samples (Table 9). The clinical specificity of the Procleix WNV Assay on the Procleix Panther System in individual samples (neat) was calculated across all sites and lots as 100% with a 95% CI of (99.965-100).

Table 9 – Clinical Specificity of the Procleix WNV Assay on the Procleix Panther System in Individual Donations (IDS – Never Pooled)

Site	Lot	N	TN	FN	TP	FP	Specificity% (95% CI)[1]
All	All	13,371	13,371	0	0	0	100 (99.972 - 100)
	Lot 1	3,898	3,898	0	0	0	100 (99.905 - 100)
	Lot 2	5,324	5,324	0	0	0	100 (99.931- 100)
	Lot 3	4149	4149	0	0	0	100 (99.911 - 100)
(b) (4)	All	5,772	5,772	0	0	0	100 (99.936 - 100)
	Lot 1	1,709	1,709	0	0	0	100 (99.784 - 100)
	Lot 2	2,004	2,004	0	0	0	100 (99.816 - 100)
	Lot 3	2,059	2,059	0	0	0	100 (99.821 - 100)
(b) (4)	All	7,599	7,599	0	0	0	100 (99.951 - 100)
	Lot 1	2,189	2,189	0	0	0	100 (99.832 - 100)
	Lot 2	3,320	3,320	0	0	0	100 (99.889 - 100)
	Lot 3	2,090	2,090	0	0	0	100 (99.824 - 100)

[1] Clopper-Pearson Exact CI

6.2 Clinical Sensitivity:

The objective of this study was to evaluate the clinical sensitivity of the Procleix WNV assay on the Procleix Panther system in known WNV RNA-positive plasma samples. Frozen WNV-positive samples (as determined by an FDA-licensed NAT) were obtained from a clinical specimen supplier (b) (4) and sent by the sponsor to an external site for aliquotting; neat aliquots were then sent by the sponsor to (b) (4) for qualification by the (b) (4) Assay to verify that WNV nucleic acid was still detectable after frozen storage and aliquotting. Since the (b) (4) assay has a LoD of (b) (4), only samples with quantitative results (b) (4) qualified for the study. At an external site, samples were prepared neat and diluted 1:16 with known-negative bulk plasma samples to mimic 16-sample pools. Known-negative samples were also provided to mask operators to the expected results. Neat and diluted samples were tested with the Procleix WNV assay on the Procleix Panther system. A total of 100 neat and 100 diluted qualified plasma samples were tested, distributed among 3 testing sites (2 external and 1 in-house); sites did not test the same samples. Testing was performed under IRB approval for each testing site. Each site performed testing using 3 Procleix WNV assay reagent kit master lots. All statistical analyses were performed by the sponsor, and only known-positive samples with valid assay results were included in the sensitivity calculation. The sensitivity (with corresponding 2-sided 95% Clopper-Pearson

Exact confidence intervals [CIs]) was calculated relative to the known-positive status of the samples.

Testing with the Procleix WNV assay on the Procleix Panther system began on 19 May 2015 and was completed on 29 September 2015. Sensitivity testing was performed using Procleix Panther system software version 5.2.0.50 and Procleix WNV assay system software version 2.6.4. The algorithm used to interpret assay results for the sensitivity analysis is shown in table 10.

Table 10 – Interpretation of Procleix WNV Assay Results for the Sensitivity Analysis

Procleix WNV Assay Result	Known-Positive Status	Interpretation
Reactive	Reactive or Positive	True Positive (TP)
Non-Reactive	Reactive or Positive	False Negative (FN)
Invalid	Reactive or Positive	Unknown

Sensitivity was calculated as (TP/ [TP + FN]) x 100%. All study runs on the Procleix Panther system were valid, and all 109 WNV known-positive neat samples and 109 known-positive diluted samples had valid initial and final testing results.

For the neat samples (Table 11), 108/109 had TP results with 1 FN result, for an overall sensitivity in neat samples of 99.1% (95% CI 95.0-100%). This met the acceptance criteria set for the study of ≥99.0% for neat samples with the lower bound of the 2-sided 95% Exact CI ≥94.6%. The one FN result was generated from a sample that passed viral load qualification (100 copies/ml), but was negative upon retest with the cobas WNV assay, which the sponsor attributes to a viral load under 100 copies/ml.

Table 11 - Clinical Sensitivity of the Procleix Assay using Known-Positive Sample tested neat as individual donations

	Neat sample testing - representing individual donation			
Lot	n	True Positive	False Negative	Sensitivity (95% CI[1])
All	109	108	1	99.1 (95.0 – 100)
Lot 1	30	29	1	96.7 (82.8 – 99.9)
Lot 2	50	50	0	100 (92.9 – 100)
Lot 3	29	29	0	100 (88.1 – 100)

CI = Confidence Interval; [1]Exact CI

For the diluted samples (Table 12), 107/109 had TP results with 2 FN results, for an overall sensitivity in diluted samples of 98.2% (95% CI 93.5-99.8%). Acceptance criteria were not set for the diluted samples because of the chance that dilution could reduce the sample concentration below the test limit of detection (LoD). The two FN results were generated from samples that retested as negative on the cobas WNV assay. Both diluted samples were produced from neat samples that had passed viral load qualification; one neat

sample was positive by Procleix WNV on Panther, and the other was diluted from the same sample that produced a FN result in the neat sample study. Sensitivity did not differ across lots and sites for either neat or diluted samples.

Table 12 - Clinical Sensitivity of the Procleix Assay using Known-Positive Sample tested in 1:16 dilution to simulate pool

Lot	Diluted sample* testing - representing of pool of 16 donations			
	n	True Positive	False Negative	Sensitivity (95% CI[1])
All	109	107	2	96.7 (82.8 – 99.8)
Lot 1	30	29	1	96.7 (82.8 – 99.9)
Lot 2	50	50	0	100 (92.9 – 100)
Lot 3	29	28	1	96.6 (82.2 – 99.9)

* Samples with viral loads under limit of detection (LOD) after dilution were included
CI = Confidence Interval; [1]Exact CI;

The data from this study validated the use of the Procleix WNV assay on the Procleix Panther system.

6.3 Clinical Reproducibility:

The objective of this study was to estimate the reproducibility and repeatability of the Procleix WNV assay on the Procleix Panther system. Testing was performed at 3 sites, 2 external and 1 internal, with 2 test runs per day over at least 9 days, for a total of 36 runs per site, using 3 reagent lots equally. Two operators and one Panther system were used per site. Each testing run contained 2 replicates of a 5-member reproducibility panel (1 WNV-negative, 4 WNV-positive, created using WNV-positive clinical plasma specimens spiked into bulk normal human plasma) (Table 13).

Table 13 – Panel Composition

Panel Member	Designation	Estimated concentration[1]	Expected Reactivity[2]
A	Negative	0	0%
B	High negative	2 copies/mL	5% to 95%
C	Low positive	22 copies/mL	>95%
D	Low moderate positive	199 copies/mL	100%
E	High moderate positive	994 copies/mL	100%

[1]Estimated concentrations (rounded up to the next whole number) were calculated by multiplying the concentration of the WNV spiking stock (b) (4) by the dilution factor. The concentration of the WNV stock was estimated by triplicate testing with the (b) (4) assay (b) (4) .

[2]Quantification testing preformed in house verified that all the panel members met the expected percent reactivity specifications.

The results were reported as the 2-sided 95% Score confidence interval of the agreement with the expected positive (reactive) or negative (non-reactive) results. The variability was

calculated for the following: 1) within runs, 2) between runs, 3) between operators, 4) between sites/instruments, 5) between reagent kit lots, 6) between days, and 7) total. The results showed (Table 14) that agreement with expected results for the panel was high for 4/5 members; 100% (95% CI 98.3-100) for the negative, low moderate positive (199 copies/ml, as determined by the (b) (4) Assay) and high moderate positive (994 copies/ml) members and 98.1% (95% CI 95.3-99.3) for the low positive member (22 copies/ml). Agreement for panel member B, the high negative panel member was 51.9% (95% CI 45.2-58.4) with the expected non-reactive result; this panel member had been spiked with WNV, but at an amount lower than the 95% LOD of the assay (2 copies/ml).

Table 14 – Reproducibility Study: Overall Agreement of Procleix WNV Assay Results on the Procleix Panther System with Expected Results

Panel Member	Description	Expected Result	Agreement /number test	% Agreement 95% CI[1]
A	Negative	Non-reactive	216/216	100 (98.3 – 100)
B	High negative[2]	Non-reactive	112/216	51.9 (45.2 – 58.4)
C	Low positive	Reactive	212/216	98.1 (95.3 – 99.3)
D	Low moderate positive	Reactive	216/216	100 (98.3 – 100)
E	High moderate positive	Reactive	216/216	100 (98.3 – 100)

[1] Confidence Interval; [2] This panel member was manufactured to target a concentration below LOD with reactivity in the range of 5% to 95%.

Within-run variability in the mean signal/cutoff was the largest source of variation for each panel member and across the study. Total variability for the 3 positive panel members was 15.4% for C, 5.1% for D and 5.2% for E. The reproducibility and variability results for the Procleix WNV Assay on the Panther system supports the proposed intended use.

6.4 Clinical Review:

During the review of this BLA, the review committee identified deficiencies in the clinical review area of the original submission. These issues were discussed during review committee meetings, meetings with senior management of the Division and the Office and were conveyed to the sponsor in the filing letter dated August 26, 2016 and in information requests dated December 29, 2016 and January 19, 2017 and in a CR letter dated April 6, 2017.

The following were the major clinical review issues identified by the committee and their resolution:

1. In the clinical specificity study, reviewers noted an increase in the number of invalid test results generated when using the Procleix WNV Assay on the investigational Panther system relative to the licensed Tigris system, as well as a high percentage of errors resulting from hardware error. The sponsor was asked to explain the discrepancy, to provide a full accounting of the sources of errors for samples with initial invalid results

and valid retests, and provide a plan to reduce the number of invalid test results produced on the Procleix Panther system. In their response dated February 9, 2017, the sponsor resolved an apparent text discrepancy and provided the requested summary (Table 15) of error sources and a mitigation plan which included "more accurate categorization of the root cause of invalid results" in order to reduce the number of errors classified as "hardware errors", and measures already taken to address failures that occurred due to one specific cause, placement of the foam inserts in the amplification incubator. The sponsor also provided a table showing that in testing done to support the CE-mark for use outside the U.S., the invalid test rate was similar between the two platforms. In the CR letter dated April 6, 2017, reviewers requested that the sponsor clarify the reclassification of errors as a suggested mitigation for excessive hardware failures and the number of samples affected by the amp incubator foam insert error. Reviewers found the accounting of the invalid result causes acceptable.

Table 15 - Comparison of WNV Initial Invalid Tests for Panther and Tigris

Procleix WNV Assay on Procleix Panther System [1,2]		Procleix WNV Assay on Procleix Tigris System [3]	
# Tested (%)	3933 (100%)	# Tested (%)	4570 (100%)
# Valid Tests (%)	3921 (99.69%)	# Valid Tests (%)	4554 (99.65%)
# Initial Invalid Test (%)	12 (0.31%)	# Initial Invalid Test (%)	16 (0.35%)
# Internal Control Failure	1	# Internal Control Failure	1
# VVFS RDFS	4	# Instrument Error	7
# NTI	2	# Clot Error	7
# RVHA	5	# QNS	1

VVFS = LLS measurement of TCR/Sample in MTU tube falls outside the expected volume
RDFS = Sample dispense verification failed
NTI = Sample not tested due to a fatal hardware error
RVHA = Amplification reagent volume check is too high
QNS = Quantity not sufficient; insufficient sample volume

2. In the clinical specificity study, there was one pool and four individual donor samples with reactive Procleix WNV assay results on the Procleix Tigris system, but non-reactive results on both Procleix Panther System and on the FDA-licensed (b) (4) . The sponsor was asked to reclassify these samples from false positive to true negative and include them in the specificity calculations. For pools, this did not result in a change to the calculated specificity. For ID-NAT, this only resulted in a change of the 95% CI for the affected lot and location from 100% (99.823-100) to 100% (99.824-100) and did not affect the overall specificity. In their response dated February 9, 2017, the sponsor made the changes requested and submitted tables showing the updated results and calculations, which the reviewers considered acceptable.

6.5 Cadaveric specimens

6.5.1 Summary

Testing of cadaveric donors is included in the intended use for the Procleix WNV assay on the Panther system. To support this intended use, the original submission contained data on specificity and sensitivity in cadaveric samples. For the specificity study, a set of 50 cadaveric specimens (25 unique serum and 25 unique plasma specimens) were tested using the WNV assay on the Panther system. These tests showed a specificity of 100%. For the sensitivity study, an additional set of 50 cadaveric specimens (25 unique serum and 25 unique plasma specimens) were spiked with WNV-infected plasma targeted to 150 copies/ml and tested along with 50 normal (non-cadaveric) controls using the WNV assay on the Panther system. These tests showed an assay sensitivity of 100%.

The reproducibility study was performed using 20 cadaveric specimens (10 unique serum and 10 unique plasma cadaveric specimens) that had been spiked with WNV-infected plasma targeted to 150 copies/ml and tested along with 20 normal (non-cadaveric) controls (10 unique serum and 10 unique plasma samples) using the WNV assay on the Panther system. These tests showed an assay reproducibility of 100% for cadaveric plasma, control plasma and cadaveric serum and an assay reproducibility of 97.2% for control serum.

6.5.2 Cadaveric specimen review

During filing review of the submission, reviewers noted the lack of a cadaveric reproducibility study. In the filing notification letter sent to the sponsor on August 26, 2016 and in information requests dated October 20, 2016 and November 9, 2016, FDA requested that reproducibility study data including a full dataset be submitted for review. The sponsor submitted a data summary for the reproducibility study on October 26, 2016 and the full dataset on November 14, 2016.

Additionally, in the information request on January 19, 2017, the review committee requested that the sponsor provide data on hemolysis and plasma dilution for the cadaveric specimens used for the specificity, sensitivity and reproducibility studies. The sponsor provided the requested documentation on February 9, 2017, and the responses were considered satisfactory by the review committee.

7. Advisory Committee Meeting

For this submission, it was determined that the discussion at the Advisory Committee Meeting was not required

8. Other Relevant Regulatory Issues

8.1 Bioresearch Monitoring Inspection

Bioresearch Monitoring (BIMO) inspections were conducted at two clinical sites that participated in the conduct of both Study# B10241-WNVPS-CSP-01 and Study# B10241-WNVPS-CSP-02. The inspections did not reveal any issues that impact the data submitted in this application.

9. Labeling

Proprietary Name: Procleix® WNV, Nucleic Acid Test for use on the Procleix® Panther Systems

The labeling for the Procleix WNV Assay used on the Panther system was provided with the original submission, and included the package insert (PI), component labeling including the Procleix Panther Auto Detect and Assay Fluids reagents which are sold separately from the Procleix WNV Assay reagents, kit labeling, software labeling and operator's manual for the Procleix Panther System. The sponsor also included a highlighted copy comparing the PI for use with the Panther system with the PI for the approved Tigris system.

9.1 Labeling Review

The review committee noted some issues with the Package Insert (PI) provided at the time of the submission. In the CR letter dated April 6, 2017, the committee requested the following updates to the PI, along with some minor editorial changes:

1) That updated data provided by the sponsor during review of the clinical sensitivity and cadaveric reproducibility study be included in the PI;
2) That the clinical sensitivity and 95% CIs be reported to two decimal places to be consistent with the approved Tigris PI;
3) That the false negative results from the analytical study "Specificity and Sensitivity of the Procleix WNV assay in the Presence of Donor and Donation Factors on the Procleix Panther System" discussed in section 5.8 of this document be included in the PI.

A revised PI incorporating the requested revisions, along with additional changes to support the rebranding from Hologic, Inc. to Grifols Diagnostic Solutions, Inc., was provided to FDA on November 28, 2017.

Additional clarification and modifications were requested from the sponsor on January 23 and on February 16, 2018. The sponsor provided the responses on February 22, 2018, which included a revised version of the PI incorporating the requested changes, and the responses were considered satisfactory by the review committee.

10. Recommendations and Risk/ Benefit Assessment

a) **Recommended Regulatory Action**

The Review Committee reviewed the original submission and related amendments submitted by Grifols, Inc. All review issues have been resolved; therefore, the Review

Committee recommends licensure of the Procleix WNV Assay for use on the Procleix Panther System.

b) **Risk/ Benefit Assessment**

The Procleix WNV, Nucleic Acid Test for use on the Procleix Panther Systems has very high sensitivity for the detection of WNV RNA in plasma specimens. The limit of detection for WNV RNA in the Procleix WNV assay performed on the Procleix Panther System is equivalent to that performed on the Procleix Tigris System. The performance data provided supporting the Procleix WNV, Nucleic Acid Test for use on the fully automated with high throughput Procleix Panther System has demonstrated that the risk/benefits analysis of the assay outweighs any risk to the blood donor and the safety and availability of the nation's blood supply.

c) **Recommendation for Postmarketing Activities**

No postmarketing activities have been proposed for this application.