DRAFT
SUMMARY BASIS OF APPROVAL

1.0 GENERAL INFORMATION

Product Trade Name:	Procleix® WNV Assay
Other Name:	Nucleic Acid Test (NAT) for West Nile virus
Name and Address of Sponsor:	Gen-Probe Incorporated 10210 Genetic Center Drive San Diego, CA 92121
Biologics License Application Tracking Number:	STN BL 125121/0
Date of Submission:	January 27, 2005

2.0 INDICATIONS FOR USE

The Procleix® WNV Assay is a qualitative *in vitro* nucleic acid assay system 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 also is 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.

3.0 BRIEF DESCRIPTION OF TEST

The Procleix WNV Assay utilizes target amplification nucleic acid probe technology for the detection of WNV RNA in blood specimens. All of the assay reactions take place in a single tube. Sample preparation releases virion nucleic acid which hybridizes to oligonucleotides in the Target Capture Reagent that are complementary to conserved viral sequences. The hybridized target is then captured onto magnetic microparticles that are separated from plasma in a magnetic field. Amplification of the captured target occurs via a transcription-based nucleic acid amplification method that produces RNA amplification products through DNA intermediates. Detection of the amplified target is achieved by using chemiluminescent labeled nucleic acid probes that are complementary

to the amplification product. Hybridized and unhybridized probes are distinguished by inactivating the label on unhybridized probes. The chemiluminescent signal from the hybridized probe is measured in a luminometer as relative light units (RLU).

Each assay reaction incorporates an Internal Control (IC) to monitor and validate specimen processing, amplification and detection steps in each reaction. The IC signal is discriminated from the target signal by utilizing separate labels, with distinct light emission kinetics, for the target and IC probes. The Dual Kinetic Assay (DKA) method is used to deconvolute the chemiluminescent signals from the hybridized target (if present) and IC amplicons. A positive result in the WNV assay indicates the presence of WNV in the specimen.

Components of the Procleix WNV Assay Kit are listed below:

- Internal Control Reagent: A HEPES buffered solution containing detergent and an RNA transcript.

- Target Capture Reagent: A HEPES buffered solution containing detergent, capture oligonucleotides and magnetic microparticles.

- Amplification Reagent: Primers, dNTPs, NTPs and co-factors in TRIS-buffered solution containing PROCLIN 300 as preservative.

- Enzyme Reagent: MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS-buffered solution containing 0.05% sodium azide as preservative.

- Probe Reagent: Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.

- Selection Reagent: Borate buffered solution containing surfactant.

- Negative Calibrator: A HEPES buffered solution containing detergent.

- Positive Calibrator: A HEPES buffered solution containing detergent and a WNV RNA transcript.

- Procleix Assay Fluids
  Wash Solution: HEPES buffered solution
  Oil: ----------oil
  Buffer for Deactivation Fluid: ---------- buffered solution

- Procleix Auto Detect Reagents
  Auto Detect 1: ----------
  Auto Detect 2: ----------

4.0 MANUFACTURING AND CONTROLS

A. Manufacturing Overview

The Procleix WNV Assay components are manufactured by Gen-Probe Incorporated. The manufactured components of the assay include Oligonucleotides, Calibrators, Internal Control (IC) and Reagents.

Raw materials intended for the manufacture and use in these assay components are subject to appropriate quality control evaluations before they are accepted for use in manufacturing. The quality of the product components is assessed at multiple stages during manufacture using tests to ensure conformance to acceptable specifications. Acceptance criteria and specifications have been established for all kit components. Components are assembled into kits, each lot of which is subjected to a final performance test with an in-house panel of samples containing ---------- levels of WNV. Meeting the established performance parameters is required for kit release.

B. Stability Studies

The stability of the Procleix WNV Assay kit reagents has been established based upon the results of Real Time studies. All reagents are stored at their recommended long-term storage conditions as defined by the Procleix WNV package insert procedures. The results of these stability studies indicate no compromise in product performance and support the 15-month dating period for the Procleix WNV Assay kit.

C. Methods Validation

Production of test kit components is monitored by in-process testing. Product purity and potency are assured through evaluation of multiple parameters including product appearance, ---------- and performance testing. Assay performance of test kits is assessed through laboratory evaluations using an in-house panel of samples containing ---------- levels of WNV.

Three conformance lots of the Procleix WNV Assay kit have been submitted to CBER for evaluation. Each master lot of product, along with protocols summarizing pertinent product testing, will be submitted for evaluation and approval by CBER prior to release for distribution.

D. Labeling

The product labeling, including immediate container and package labels and the package insert (directions for use), has been reviewed for compliance with 21 CFR 610.60, 610.61, 610.62, and 809.10 and found to be satisfactory. The package insert for the Procleix WNV Assay states that the intended use of the test is for the detection of WNV RNA in donor plasma. The product trade name,

Procleix WNV Assay, is not known to conflict with other biologic or device trade name.

E. Establishment Inspection

A pre-licensing inspection of the areas where product is manufactured, tested, stored and shipped was not required because the establishment had been inspected in January and February 2005 for a similar BLA. Facilities and procedures are in compliance with current good manufacturing practices (cGMP).

F. Environmental Assessment (EA)

A detailed Environmental Assessment Report was provided in Section 15.0 of the Biologic License Application (STN BL 125121/0). This product has no significant environmental impact. A summary of the procedures taken by the manufacturer to assure that no adverse environment impacts occur is listed below:

1. The WNV positive material is made with RNA transcript only. Human plasma is not used in the production of any WNV reagents.

   Furthermore, all biohazardous waste material is managed in accordance with applicable local, State and Federal regulations.

2. Appropriate precautionary statements are included in the labeling and package insert of the product.

3. Product shipping containers are appropriately labeled and are shipped according to applicable regulations.

Gen-Probe Incorporated, in accordance with current Good Manufacturing Practices, performs all production activities in compliance with applicable environmental regulations.

5.0 BIOLOGICAL PRINCIPLES OF THE TEST

The Procleix WNV Assay is a qualitative *in vitro* assay that utilizes target amplification nucleic acid probe technology for the detection of WNV in donor plasma. The three main steps of the Procleix WNV Assay are (i) Target Capture-based sample preparation, (ii) Internal Control and target amplification by Transcription-Mediated Amplification (TMA) and (iii) detection of the amplification product (amplicon) by the Hybridization Protection Assay (HPA).

(i) Target Capture-based sample preparation
Sample processing allows for the release, stabilization, and capture of viral nucleic acids and the removal of unwanted components of the clinical specimen. Target Capture Reagent, a HEPES-buffered detergent solution, causes lysis of the viral particles and inactivates nucleases. The capture of the released viral nucleic acid is mediated by

oligonucleotides containing a sequence complementary to conserved WNV RNA sequences. The sequences of the capture oligonucleotides hybridize to a second oligonucleotide that is covalently bound to magnetic microparticles resulting in capture and concentration of the nucleic acid targets and internal control transcript from the sample.

(ii) Transcription-Mediated Amplification (TMA)
Target amplification occurs via TMA, which is an isothermal 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 produces multiple copies of RNA amplicon from the DNA copy template. The TMA technology exponentially amplifies regions of the captured WNV viral RNA.

(iii) Hybridization Protection Assay (HPA)
Detection of the amplified viral RNA is accomplished using the HPA, which utilizes chemiluminescent ----------labeled probes. The basis of the HPA is ----------when attached to a hybridized probe. Chemiluminescence of the label on unhybridized probes is destroyed by ---------- during the selection step that follows the hybridization. Hybridized probes maintain chemiluminescence, which is detected by the addition of Auto Detect 1 and Auto Detect 2 that results in photon emission by the label on hybridized detection probes. Photons are measured with a luminometer and are reported as relative light units (RLUs).

The assay incorporates an internal control (IC) to validate each reaction. IC is added to each test specimen, assay control, and assay calibrator reaction via the Target Capture Reagent. It consists of a specific *in vitro* synthesized transcript containing a ----------. Therefore, because the IC-probe detects the ----------, the IC signal is distinguished from WNV analyte signal. Discrimination of the IC signal from the target signal is accomplished via the Dual Kinetic Assay (DKA). DKA utilizes the kinetic discrimination of chemiluminescent emissions. A probe with rapid emission kinetics, known as a “flasher”, is used to detect the Internal Control. A probe with slower emission kinetics, termed “glowers”, is used to detect target viral nucleic acids.

Three calibrator replicates are run at the beginning of each assay run for the WNV Assay. These are composed of three replicates each of: Negative Calibrator and WNV Positive Calibrator. Negative Calibrators are located in positions 1-3 of the assay run, and WNV Positive Calibrators are located in positions 4-6 of the assay run. Calibrators are used in the determination of the assay cutoff for both the Internal Control and the Analyte, and to determine the validity of the run.

6.0 CLINICAL DATA

A. REPRODUCIBILITY

Reproducibility of the Procleix® WNV Assay was evaluated at three blood testing laboratories. For determination of reproducibility, a 10-member panel comprised of tissue culture-derived WNV was procured (Table 1). Seven panel members were positive for WNV (50, 50, 100, 100, 300, 1000 and 10,000 c/mL) and three panel members were WNV negative.

The reproducibility panels were tested by six operators (two from each testing site) with three different clinical lots over multiple days, using automated (Tecan Genesis RSP instrument) or manual pipetting methods. Twenty-four runs were tested at each site across three clinical lots, with each panel member tested in triplicate per run and each operator performing testing for at least six days.

Assay signal values were expressed as Analyte Signal to Cutoff (S/CO) ratios for panel members containing target and as Internal Control (IC) S/CO ratios for negative panel members. Signals were expressed as analyte Relative Light Units (RLU) for the Positive Calibrators and as IC RLU for the Negative Calibrators in the Procleix WNV Assay. Signal variability of the assays was calculated for intra- and inter-run, inter-lot, and inter-site in terms of standard deviation (SD) and percent coefficient of variation (%CV). Data were also analyzed as percent agreement with expected outcome and mean S/CO ratio or RLU. Since no significant difference in assay reproducibility was observed between automated and manual pipetting, results from the two pipetting methods were combined and shown in Table 1.

The overall percent agreement of test results with expected outcomes was 100% for negative panels and greater than or equal to 99.8% for positive panel members. With regard to variability, intra-run (or random error) and inter-run factors were the largest and second largest contributors to total variance (according to SD values) in the Procleix WNV Assay. While these factors were responsible for the majority of the variance in the assay, the %CV of each of these components by itself did not exceed 13.7% for any positive or negative samples. The inter-site %CVs were 6.1% or less and the inter-lot %CVs were less than 4%, indicating that these factors had little impact on assay performance. Therefore, the reproducibility of the assay is robust and much of the variation that is observed can be attributed to random error.

Table 1. - Reproducibility of the Procleix® WNV Assay*

BBI Panel	n	Concentration Copies/mL	Number of replicates	% Agreement	Mean S/CO	Intra-Run		Inter-Run		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative**	3	0	641	100	1.96	0.11	5.8	0.09	4.7	0.03	1.6	0***	0***
WNV	2	50	426	99.8	26.70	3.44	12.9	2.29	8.6	0***	0***	0.77	2.9
WNV	2	100	431	99.8	26.64	3.65	13.7	2.37	8.9	0.95	3.6	1.25	4.7
WNV	1	300	215	100	28.62	1.38	4.8	2.13	7.4	0.48	1.7	1.55	5.4
WNV	1	1,000	216	100	28.94	1.16	4.0	1.85	6.4	0***	0***	1.74	6.0
WNV	1	10,000	216	100	29.69	1.62	5.5	1.85	6.2	0.52	1.8	1.82	6.1
Sample			**Number of replicates**	**% Agreement**	**Mean RLU**	**Intra-Run**		**Inter-Run**		**Inter-Lot**		**Inter-Site**	
						SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Negative Calibrator**			215	NA	179,345	9,364	5.2	10,248	5.7	4,462	2.5	9,531	5.3
WNV Positive Calibrator			214	NA	1,256,371	45,949	3.7	38,338	3.1	3,559	0.3	35,437	2.8

n = Number of panel members combined for this analysis
* Analysis of analyte signals, unless otherwise noted
** Analysis of internal control signal
*** Per NCCLS guidelines (EP5-A, page 7), numbers <0 are recorded as 0.

B. SPECIFICITY IN NORMAL BLOOD DONORS

Specificity of the Procleix® WNV Assay

The clinical specificity of the Procleix® WNV Assay was determined in prospectively collected samples tested linked as 16-sample pools and as individual plasma samples from voluntary blood or blood component donors.

Specificity of the Procleix WNV Assay was calculated from 16,885 16-sample pools and 43,503 individual donor samples (IDS) from whole blood donations. For calculations of clinical specificity, reactive results from the Procleix WNV Assay were compared to results from a commercial WNV IgM assay and/or validated WNV Alternate NAT. The overall clinical specificity results are summarized in Table 2. Donors whose samples were initially reactive in the Procleix WNV Assay were pursued for enrollment into a follow-up study for additional testing.

The study was conducted at four blood testing laboratories using samples from donors representing geographically diverse regions of the United States. During this study, all testing was performed linked using three clinical lots of Procleix WNV Assay reagent kits. All 16-member samples from a Procleix WNV Assay reactive pool were tested individually in the Procleix WNV Assay. Reactive samples, whether identified from pool testing or individual donor testing, were retested with the Procleix WNV Assay and also tested with a validated WNV nucleic acid test (Alternate NAT) and a commercial assay for WNV immunoglobulin M (IgM)

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Specificity of the Procleix® WNV Assay in 16-Sample Pools

A total of 16,885 pools were tested in the Procleix® WNV Assay at two blood testing sites. Of these, 16,855 tested non-reactive and were considered true negative. Thirty pools were reactive in the Procleix WNV Assay. Of these, 21 pools contained at least one reactive sample when the constituents of the pool were tested individually. The 21 reactive pools were determined to be true positive pools as the Procleix WNV Assay reactive results were confirmed by reactive Alternate NAT and/or positive IgM antibody results. Nine reactive pools were considered false positive as all individual samples of the pool tested non-reactive in the Procleix WNV Assay. The overall specificity of 16-sample pools from whole blood donations in these studies was 16,876/16,885 = 99.95% (95%CI: 99.90-99.98%).

Specificity of the Procleix® WNV Assay in Individual Donor Samples

For the evaluation of individual donor samples (IDS) specificity of the Procleix® WNV Assay, a total of 43,503 IDS were tested at four blood testing laboratories. There were 43,427 IDS that tested non-reactive and were considered true negative. There were 76 IDS that tested reactive in the Procleix WNV Assay. Of

these, 30 reactive IDS results were confirmed by Alternate NAT and/or IgM immunoassay results and were considered true positive and the remaining 46 IDS were considered false positive. The overall specificity of IDS from whole blood donations in these studies was 43,457/43,503 = 99.89% (95%CI: 99.86-99.92%).

Combining the results from 16-sample pools and individual donor testing, the overall specificity of the Procleix WNV Assay in these studies was 60,333/60,388 = 99.91% (95% CI: 99.86-99.96%).

Table 2- Clinical Specificity of the Procleix® WNV Assay in Pools and IDS from Whole Blood Donations

Sample	n	TN	TP	FP	Specificity (%)	95% CI
16-Sample Pools	16,885	16,855	21	9	99.95	99.90-99.98
IDS	43,503	43,427	30	46	99.89	99.86-99.92
Overall	60,388	60,282	51	55	99.91	99.86-99.96

n = Number of Samples
TN = True Negative
TP = True Positive
FP = False Positive
CI = Confidence Interval

Comparison of the Procleix® WNV Assay with IgM Serology and Alternate NAT

Results generated from pooled and individual donation testing for the clinical specificity study allow comparison of the Procleix® WNV Assay results with WNV serology and Alternate NAT results (Table 3). Of the 97 individual donor samples that were reactive in the Procleix WNV Assay, 50 (51.5%) were Alternate NAT reactive and/or IgM positive at index. Of these, 8 samples were both IgM positive and Alternate NAT reactive, 11 samples were IgM positive only, and 31 samples were Alternate NAT reactive only. One additional sample, which tested non-reactive in Alternate NAT and negative for WNV IgM at index, demonstrated seroconversion at follow up. These 51 reactive results were classified as true positive. For the 31 donors with IgM-negative results at index, follow-up sample results were IgM positive. Thus, seroconversion was observed for all 51 donors with true positive Procleix WNV Assay results.

Forty-six (46) samples, which tested initially reactive in the Procleix WNV Assay, had non-reactive Procleix WNV Assay results upon retest. These samples were IgM negative and Alternate NAT non-reactive at index and were considered false positive. Follow-up samples were obtained from 38 of the 46 donors with false positive Procleix WNV Assay results; all samples were Procleix WNV Assay and Alternate NAT non-reactive and were IgM negative.

Of the 51 samples with true positive Procleix WNV Assay results, eight samples were both IgM positive and Alternate NAT reactive at index. This pattern is

consistent with individuals infected with WNV in the early stage of immune response. Thirty-one samples were Alternate NAT reactive and IgM negative, consistent with individuals in the viremic phase of infection with little to no antibody production. Eleven samples were IgM positive, but were non-reactive in the Alternate NAT at index. Five of these 11 samples were repeat reactive in the Procleix WNV Assay and nine of the 11 samples were reactive in the Procleix WNV Assay at follow-up. The variability between Procleix WNV Assay and Alternate NAT results in this set of samples is consistent with low levels of WNV RNA during a later stage of infection.

Table 3 - Clinical Specificity Study: Comparison with WNV Serology and Alternate NAT

Outcome	Test Results for Index Donation		n	%
TP	Alternate NAT+	IgM +	8	8.2
TP	Alternate NAT+	IgM-	31	32.0
TP	Alternate NAT -	IgM+	11	11.3
TP	Alternate NAT -	IgM-	1*	1.0
Subtotal			51	52.6
FP	Alternate NAT -	IgM-	46	47.4
Total			**97**	**100**

* Sample was IgM positive at follow up
TP = True Positive
FP = False Positive
IgM+ = positive for WNV Immunoglobulin M antibody
IgM- = negative for WNV Immunoglobulin M antibody
n = number of samples

C. NON-SPECIFICITY STUDIES

Specificity And Sensitivity Of The Procleix® WNV Assay In The Presence Of Donor And Donation Factors

When tested with the Procleix® WNV Assay, no cross-reactivity or interference was observed for naturally occurring hemolyzed, icteric or lipemic specimens or plasma containing the following substances: serum albumin (up to 6 g/dL), hemoglobin (up to 500 mg/dL) and lipids (up to 3,000 mg/dL), and plasma containing bilirubin up to 20 mg/dL.

No cross-reactivity or interference was observed in specimens from patients with autoimmune diseases or with liver diseases not caused by hepatitis C virus or hepatitis B virus infection. Multiple specimens from each group of patients with the following autoimmune conditions were evaluated: rheumatoid arthritis (n = 10), rheumatoid factor (n = 10), antinuclear antibody (n = 10), multiple sclerosis (n = 6), lupus (n = 10) and multiple myeloma (n = 10). Also tested were samples from patients with hyperglobulinemia (n = 10), with elevated ALT (n = 10) and from patients with alcoholic liver cirrhosis (n = 10).

No cross-reactivity or interference was observed in bacterially contaminated plasma or in specimens from patients infected with other blood borne pathogens. Multiple specimens from each group of patients with the following viral infections were evaluated: herpes simplex virus 1/2 (n = 10, human T-cell lymphotropic virus type I/II (n = 10), hepatitis A virus (n = 10), hepatitis B virus (n = 10), hepatitis C virus (n = 10), hepatitis G virus (n = 10), cytomegalovirus (n = 10), Epstein-Barr virus (n = 10), rubella virus (n = 10), parvovirus B-19 (n = 4) and human immunodeficiency virus type 1 and type 2 (n = 10). Also tested were donor samples from influenza virus and HBV vaccinees (n = 10), and samples spiked with tissue culture-derived viruses related to members of the Japanese encephalitis virus (JEV) sero-complex, including dengue virus (n = 1), Saint Louis encephalitis virus (n = 1), Murray Valley encephalitis virus n= 1), and yellow fever virus (n = 1) with no cross-reactivity or interference. The Procleix WNV Assay detected Kunjin virus (n = 1), a variant of WNV.

D. CLINICAL SENSITIVITY

Testing of Known Positive Samples

Two hundred and three (203) WNV known-positive samples were procured from a blood bank repository. These samples were determined to be positive for WNV RNA by testing with two validated NAT methods. In addition to NAT, the samples were tested for the presence of IgM antibodies to WNV. The clinical sensitivity study was performed at two blood testing laboratories using three clinical reagent kit lots of the Procleix® WNV Assay. The positive samples were tested neat (i.e., undiluted; n=202) and in a 1:16 dilution (n=203) in the Procleix WNV Assay. Negative plasma samples were also tested in the Procleix WNV Assay at each clinical site as a control for potential study bias. For determination of clinical sensitivity, neat and diluted sample test results from the Procleix WNV

Assay were compared to the known viral status of each sample when tested neat (Table 4).

For the sensitivity study, neat samples had known WNV RNA concentrations equal to or greater than 100 copies/mL. Known-positive samples with WNV RNA copy levels below the sensitivity claim of 100 copies/mL after the 1:16 dilution were included in the clinical sensitivity analyses.

The sensitivity of the Procleix WNV Assay in neat (undiluted) WNV known-positive samples in this study was 100% (95% CI: 98.2-100%). The sensitivity of the Procleix WNV Assay in diluted (1:16) WNV known-positive samples in this study was 91.6% (95% CI: 86.9-95.0%). All of the 17 diluted samples with false negative results were derived from neat samples that had low WNV viral loads. The sensitivity of the Procleix WNV Assay in diluted samples with copy levels greater than or equal to the sensitivity claim of 100 copies/mL in this study was 100%.

Table 4 - Clinical Sensitivity of the Procleix® WNV Assay in Known-Positive Samples

Assay	n	TP	FN	Sensitivity (%)	95% CI
Neat	202*	202	0	100	98.2-100
Diluted 1:16	203	186	17	91.6	86.9-95.0

* One neat sample not tested
n = Number of samples
TP = True Positive
FN = False Negative
CI = Confidence Interval

Testing of Known-Positive 16-Sample Pools

The clinical sensitivity of the Procleix® WNV Assay in pooled samples was determined by testing 98 sixteen-sample pools composed of 1 to 3 WNV positive samples and 13 to 15 negative samples. The 98 positive samples from different blood donors were procured from a blood bank repository. These specimens were determined to be positive for WNV RNA by testing with two validated NAT methods. In addition to NAT, the samples were tested for the presence of IgM antibodies to WNV. Two clinical sites participated in the study using three clinical reagent kit lots. Pools contained known-positive samples with neat viral concentrations ranging from 200 to 430,000 copies/mL. Six of the 98 pools contained less than 100 copies/mL after pooling. The sensitivity of the Procleix WNV Assay in 98 known-positive pools in this study was 100% (95% CI: 96.3-100%) (Table 5).

Table 5 - Clinical Sensitivity of the Procleix® WNV Assay in 16-Sample Pools Containing Known-Positive Samples

n	TP	FN	Sensitivity (%)	95% CI
98*	98	0	100	96.3 - 100

* Included 61 pools with 1 positive sample, 25 pools with 2 positive samples, and 12 pools with 3 positive samples
n = Number of samples
TP = True Positive
FN = False Negative
CI = Confidence Interval

ANALYTICAL SENSITIVITY

Determination of Analytical Sensitivity Using a Dilutional Sensitivity Panel Made From the Health Canada WNV Reference Standard

An analytical sensitivity panel comprised of serially diluted WNV provided by Health Canada was used to evaluate assay sensitivity. The WNV panel was prepared by serial dilution of heat-treated tissue culture-derived viral stock (1,000 copies/mL). Three operators tested 30 replicates of each copy level with three clinical lots using the Procleix® System for a total of 90 replicates. The 95% confidence intervals (CI) of the reactive rates were based on the exact binomial distribution. Estimations of 50% and 95% detection rates by probit analysis are provided.

In this study, WNV RNA detection with the Procleix® WNV Assay was 100% at 100 copies/mL and at 30 copies/mL. Reactivity at 10 copies/mL was 97% and 53% at 3 copies/mL (Table 6).

Table 6 - Detection of WNV in Health Canada Analytical Sensitivity Panel

WNV copies/mL	Number reactive/ tested*	% Reactive	95% CI		Average S/CO**	%CV
			Lower	Upper		
100	**89/89**	**100**	**97**	**100**	**30.05**	**9**
30	**90/90**	**100**	**97**	**100**	**29.46**	**10**
10	**87/90**	**97**	**91**	**99**	**27.16**	**25**
3	**47/89**	**53**	**41**	**63**	**23.43**	**35**
1	**26/89**	**29**	**20**	**40**	**21.10**	**49**
0	**0/89**	**0**	**0**	**0**	**0.06**	**120**

*Only valid reactions were included
**Average of reactive replicates unless all tests were non-reactive, in which case the average analyte S/CO of non-reactive replicates is shown
CI = Confidence Interval

Probit Analysis

The predicted 50% and 95% detection rates in copies/mL were determined by probit analysis of the analytical sensitivity results. The predicted 95% detection level for WNV in this study was 8.2 copies/mL with the Health Canada Sensitivity Panel (Table 7).

Table 7 - Detection Probabilities of WNV using a Sensitivity Panel from Health Canada Reference Standard

Assay System	Detection Probabilities (copies/mL)	
	50% (95% CI)	95% (95% CI)
Procleix® System	3.4 (1.8 – 7.2)	8.2 (5.5 – 21.5)

CI = Confidence Interval

Determination of Analytical Sensitivity Using an FDA WNV Reference Panel

An analytical sensitivity panel provided by the Center for Biologics Evaluation and Research (CBER) and manufactured by Boston Biomedica (BBI, West Bridgewater, MA) was used to evaluate assay sensitivity. Performance of the assay was evaluated by testing four replicates of each copy level with three clinical lots using the Procleix® System for a total of 12 replicates. Detection of all panel members with a WNV titer of 100 copies/mL or greater was 100% (Table 8).

Table 8 – Detection of Lineage 1 WNV in an FDA WNV Reference Panel

Panel I.D.	WNV Strain	Copy Level (copies/ mL)	Number reactive/ tested	% Reactive	Average S/CO*	%CV
1	**NY99**	**100**	**12/12**	**100**	**31.53**	**4**
2	**NY99**	**10**	**12/12**	**100**	**29.24**	**8**
3	**Hu2002**	**0**	**0/12**	**0**	**0.10**	**85**
4	**Hu2002**	**50**	**12/12**	**100**	**31.62**	**4**
5	**NY99**	**0**	**0/12**	**0**	**0.05**	**70**
6	**NY99**	**1000**	**12/12**	**100**	**32.48**	**3**
7	**Hu2002**	**100**	**12/12**	**100**	**32.34**	**6**
8	**Hu2002**	**1000**	**12/12**	**100**	**31.74**	**9**
9	**Hu2002**	**5**	**12/12**	**100**	**25.07**	**43**
10	**NY99**	**5**	**11/12**	**92**	**23.64**	**44**
11	**NY99**	**500**	**12/12**	**100**	**32.22**	**4**
12	**Hu2002**	**10**	**12/12**	**100**	**28.48**	**26**
13	**NY99**	**50**	**12/12**	**100**	**31.61**	**6**
14	**Hu2002**	**500**	**12/12**	**100**	**32.19**	**5**

*Average of reactive replicates unless all tests were non-reactive, in which case the average analyte S/CO of non-reactive replicates is shown.

Determination of Analytical Sensitivity Using a Dilutional Sensitivity Panel made from Lineage 2 WNV from Boston Biomedica (BBI)

An analytical sensitivity panel comprised of serially diluted WNV provided by BBI was used to evaluate assay sensitivity. Heat-inactivated, lineage 2 virus from the Qualification Panel QWN701 (10,000 copies/mL) was used to make a serially diluted analytical sensitivity panel. Three operators tested 30-40 replicates of each copy level with three clinical lots for a total of 100 replicates. The 95% confidence intervals of the reactive rates were based on the exact binomial distribution.

WNV detection with the Procleix® WNV Assay was 98% at 100 copies/mL. Reactivity at 30 copies/mL was 99.0%. At 10 copies/mL, the detection rates were 89% (Table 9).

Table 9 - Detection of Lineage 2 WNV in BBI Analytical Sensitivity Panel

WNV copies/mL	Number reactive/ tested*	% Reactive	95% CI		Average S/CO**	%CV
			Lower	Upper		
100	98/100	98	93	100	10.73	18
30	99/100	99	95	100	6.10	45
10	89/100	89	81	94	2.92	69
3	30/100	30	21	40	2.09	54
1	4/99	4	1	10	1.95	39
0	0/100	0	0	3	0.08	82

*Invalid reactions were not included
**Average of reactive replicates unless all tests were non-reactive, in which case the average analyte S/CO of non-reactive replicates is shown
CI = Confidence Interval

PERFORMANCE OF THE PROCLEIX WNV ASSAY IN CADAVERIC BLOOD SPECIMENS FROM TISSUE DONORS

REPRODUCIBILITY

The inter-assay reproducibility of the Procleix® WNV Assay with cadaveric blood specimens was assessed by determining the %CVs obtained when each of 20 cadaveric and 20 control specimens were tested with 2 clinical reagent kit lots. The reactive rates, S/COs, and %CVs are shown in Table 10. For the WNV spiked specimens tested with the Procleix System, the %CVs for the cadaveric and control specimens were 18% and 14%, respectively. The percent reactive rate for cadaveric specimens and control specimens in this study was 100%.

Table 10 - Procleix® WNV Assay with Cadaveric and Control Specimens Spiked with 150 copies/mL of WNV

	Sample	Number of donors	Number of replicates	% Reactive (95% CI)	Mean Analyte S/CO	%CV
Procleix® System	Cadaveric	20	120	100% (97.5-100)	27.46	18
	Control	20	120	100% (97.5-100)	28.30	14

SPECIFICITY

WNV-negative cadaveric serum specimens were tested to determine the specificity of the Procleix® WNV Assay. Forty-five cadaveric specimens and 45 normal blood donor specimens were tested on the Procleix® System. The cadaveric and control samples were tested using three clinical lots. The specificity of the Procleix WNV Assay for the cadaveric specimens in this study was 100% (95% CI: 94%-100%) (Table11). No invalid results were observed with the cadaveric samples.

Table 11 - Specificity of Procleix® WNV Assay with Cadaveric Blood Specimens

	Control	Cadaveric
n	44*	45
Mean IC S/CO	2.13	2.07
Analyte S/CO	0.12	0.15
Percent Specificity	100	100
95% CI	94-100	94-100

*45 samples were tested. One sample was invalid and was not used in the results analysis.
n = Number of samples
CI = Confidence Interval

SENSITIVITY

WNV-negative cadaveric serum specimens spiked with a low level of WNV (approximately 150 copies/mL) were tested within 5 hours of spiking to determine the sensitivity of the Procleix® WNV Assay. Forty-five cadaveric specimens and 45 normal blood donor specimens were tested on the Procleix® System. The spiked cadaveric and control samples were tested using three clinical lots. The reactive rate of the Procleix WNV Assay for the cadaveric specimens in this study was 100% (95% CI: 94%-100%) (Table 12). No invalid results were observed with the cadaveric samples.)

Table 12 - Sensitivity of the Procleix® WNV Assay with Cadaveric Blood Specimens

	Control	Cadaveric
n	45	45
Analyte S/CO	33.16	29.55
Percent Sensitivity	100	100
95% CI	94-100	94-100

n = Number of samples
CI = Confidence Interval

7.0 LIMITATIONS OF THE PROCEDURE

- This assay has been evaluated with the Procleix® System only.
- The clinical sensitivity for the Procleix WNV Assay has been demonstrated for specimens with WNV viral concentrations equal to or greater than 100 copies per mL. Samples with less than 100 copies per mL may not yield reproducible results.
- Assay performance characteristics for use in testing plasma specimens from paid source plasma donors have not been determined.
- Assays must be performed and results interpreted according to procedures provided.
- Deviation from these procedures, adverse shipping and/or storage conditions, or use of outdated calibrators and/or reagents may produce unreliable results.

8.0 Package Inserts

A copy of the following package insert is attached:

- IN0169EN, Revision A