Procleix® WNV Assay

For *In Vitro* Diagnostic Use
5000 Test Kit

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SELECT THE APPROPRIATE SECTION FOR THE INSTRUMENT PLATFORM BEING USED

GENERAL INFORMATION

INTENDED 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 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.

SUMMARY AND EXPLANATION OF THE TEST

WNV is a mosquito-borne flavivirus that is associated with human disease ranging from mild flu-like symptoms to severe neurological disease[1,2]. Most WNV infections are asymptomatic and approximately 20% lead to a mild illness known as West Nile virus fever. Less than 1% of infections are estimated to cause serious neurological disease, with advanced age being the most significant risk factor[3].

WNV was first isolated and identified in 1937 from a febrile person in the West Nile district of Uganda. Prior to 1999, the presence of the virus had not been documented in North America and was found only in the Eastern Hemisphere with wide distribution in Africa, Asia, the Middle East, and Europe[4]. Since the 1999 outbreak in Queens, New York, the virus has continued to expand westward in the United States. During the years 2000 and 2001, geographic spread to about half of the United States was documented via avian mortality surveillance; the virus is now thought to be permanently established in North America[5,6]. A large number of avian species serve as reservoir hosts for the virus, whereas humans and animals, such as horses and other mammals, are believed to be incidental hosts[7].

As of December 28, 2004, 2448 human WNV cases were reported to the CDC for the 2004 calendar year, 87 of which resulted in death. This compares to 9862 human cases, including both mild and severe disease cases, with 264 deaths for 2003, and 4156 WNV cases with 284 fatalities for 2002[8]. The principal route of human WNV infection is through the bite of an infected mosquito, predominantly by the bite of the *Culex, sp.* of mosquitoes. However, in 2002, new mechanisms of person-to-person transmission were documented, including possible mother to infant infection through breast milk, transplacental infection, possible dialysis-related transmission, and transmission through organ donation and blood transfusion. During 2003, twenty-three suspected cases of WNV transfusion-associated transmission (TAT) were reported to CDC; of these, six cases were classified as confirmed TAT cases. As of September 2004, one TAT case has been reported for the year[9-15].

In most human infections, WNV multiplies to a relatively low level producing a transient viremia that can be detected in whole blood, plasma, and serum. Current diagnostic methods for WNV include Immunoglobulin M (IgM) enzyme immunoassays, Plaque Reduction Neutralization assays, and nucleic acid testing (NAT) methods. IgM antibody can be detected in serum or cerebrospinal fluid (CSF) collected within eight days of illness onset but NAT methods are capable of detecting infection prior to the presence of antibodies during the viremic phase. Because serologically based assays detect host immune response after this primary viremic phase and IgM can remain in the body for long periods of time, these tests may not be appropriate for blood screening[3,7].

Screening of whole blood donations with NAT has been in place in the United States since early 1999 and licenses were granted for HIV-1 and HCV screening in 2002[16]. The PROCLEIX® WNV Assay uses the same technology as the PROCLEIX® HIV-1/HCV Assay to detect WNV RNA and has been utilized in the United States for prospective blood screening since June 19, 2003 under IND.

PRINCIPLES OF THE PROCEDURE

The PROCLEIX® WNV Assay involves three main steps, which take place in a single tube: sample preparation; WNV RNA target amplification by Transcription-Mediated Amplification (TMA)[17]; and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA)[18].

During sample preparation, RNA is isolated from specimens via the use of target capture. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins and release viral genomic RNA. Oligonucleotides ("capture oligonucleotides") that are homologous to highly conserved regions of WNV are hybridized to the WNV RNA target, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps are utilized to remove extraneous components from the reaction tube. Magnetic separation and wash steps are performed with a target capture system.

Target amplification occurs via 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 produces multiple copies of RNA amplicon from the DNA copy template. The PROCLEIX WNV Assay utilizes the TMA method to amplify regions of WNV RNA.

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 in a luminometer and is reported as Relative Light Units (RLU).

Internal Control is added to each test specimen, control (if used), and assay calibrator via the working Target Capture Reagent. The Internal Control in the PROCLEIX WNV Assay controls for specimen processing, amplification and detection steps. Internal Control signal is discriminated from the WNV signal by the differential kinetics of light emission from probes with different labels[19]. Internal Control-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[19]. When used for the detection of WNV, the PROCLEIX WNV Assay differentiates between Internal Control and WNV signals.

REAGENTS

PROCLEIX® WNV Assay Kit: P/N 301187 – 5000 Test Kit

CONTENTS

Reagent Name	Number of vials/ Volume per vial
Internal Control Reagent *A HEPES buffered solution containing detergent and an RNA transcript.* *Store **unopened reagent** at –15° to –35°C.*	**10 x 5 mL**

CONTENTS

Reagent Name	Number of vials/ Volume per vial
Target Capture Reagent *A HEPES buffered solution containing detergent, capture oligonucleotides and magnetic microparticles. Store at 2° to 8°C (do not freeze). Internal Control Reagent must be added to Target Capture Reagent before use in the assay.*	**10 x 280 mL**
Amplification Reagent *Primers, dNTPs, NTPs and co-factors in TRIS buffered solution containing PROCLIN 300 as preservative.* *Store **unopened reagent** at –15° to –35°C.*	**10 x 50 mL**
Enzyme Reagent *MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffered solution containing 0.05% sodium azide as preservative.* *Store **unopened reagent** at –15° to –35°C.*	**10 x 18 mL**
Probe Reagent *Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.* *Store **unopened reagent** at –15° to –35°C.*	**10 x 75 mL**
Selection Reagent *Borate buffered solution containing surfactant.* *Store at 15° to 30°C.*	**10 x 180 mL**
PROCLEIX® WNV Negative Calibrator *A HEPES buffered solution containing detergent.* *Store at –15° to –35°C.* C0	**90 x 2 mL**
PROCLEIX® WNV Positive Calibrator *A HEPES buffered solution containing detergent and a WNV RNA transcript.* *Store at –15° to –35°C.* C1	**90 x 2 mL**

STORAGE INSTRUCTIONS

A. Room temperature is defined as 15° to 30°C.

B. The Probe Reagent is light sensitive. Protect this reagent from light during storage and preparation for use.

C. Do not use reagents or fluids after the expiration date.

D. Do not use assay-specific reagents from any other PROCLEIX® assay.

E. If a precipitate forms in the Target Capture Reagent (TCR) during storage, see instructions under REAGENT PREPARATION. DO NOT VORTEX. DO NOT FREEZE TCR.

Note: If after removing the TCR from storage at 2° to 8°C, the precipitate is allowed to settle to the bottom of the container, the likelihood of the formation of a gelatinous precipitate is increased substantially.

F. Do not refreeze Internal Control, Amplification, Enzyme, and Probe Reagents after the initial thaw.

G. Calibrators are single use vials and must be discarded after use.

H. If precipitate forms in the Wash Solution, Amplification Reagent, Selection Reagent, Probe Reagent, Negative Calibrator, or Positive Calibrator, see instructions under REAGENT PREPARATION.

I. Changes in the physical appearance of the reagent supplied may indicate instability or deterioration of these materials. If changes in the physical appearance of the reagents are observed (e.g., obvious changes in reagent color or cloudiness are indicative of microbial contamination), they should not be used.

J. Consult the following table for storage information.

Reagent/Fluid	Unopened Storage	Opened/Thawed Stability (up to expiration date)
Internal Control Reagent (IC)	–15° to –35°C until the expiration date	Prior to combining with TCR, 8 hours at RT*
Target Capture Reagent (TCR), wTCR**	2° to 8°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT***
Probe Reagent	–15° to –35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT***
Amplification Reagent	–15° to –35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT***
Enzyme Reagent	–15° to –35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT***
Selection Reagent	RT until the expiration date	30 days at RT
Calibrators	–15° to –35°C until the expiration date	8 hours at RT
Auto Detect Reagents	RT until the expiration date	30 days at RT
Buffer for Deactivation Fluid	RT until the expiration date	30 days at RT
Deactivation Fluid	N/A	30 days at RT
Oil	RT until the expiration date	30 days at RT
Wash Solution	RT until the expiration date	30 days at RT

* RT = Room Temperature
** Stability time for TCR includes both before and after adding Internal Control
*** The 80 hours must occur within the 30 days.

SPECIMEN COLLECTION, STORAGE AND HANDLING

Note: Handle all specimens as if they are potentially infectious agents.

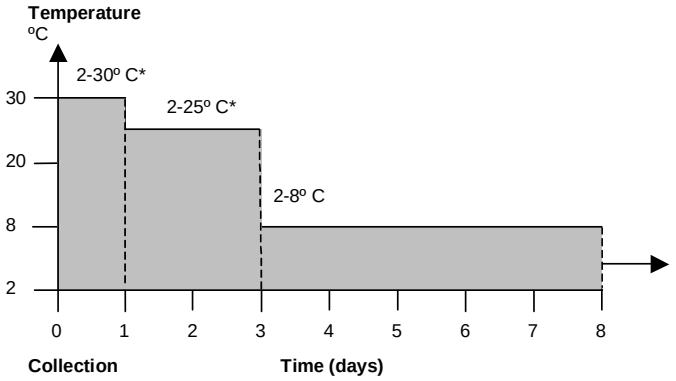
Note: Take care to avoid cross contamination during the sample handling steps. For example, discard used material without passing over open tubes.

LIVING DONOR BLOOD SPECIMENS

A. Blood specimens collected in glass or plastic tubes may be used.

B. Plasma collected in K_2EDTA, K_3EDTA, ACD, heparin, or sodium citrate, or in Becton Dickinson EDTA Plasma Preparation Tubes (BD PPT™), may be used. Follow sample tube manufacturer's instructions. Specimen stability is affected by elevated temperature. Whole blood or plasma from pooled or individual donor specimens may be stored for up to 72 hours from time of draw at ≤ 25°C; temperatures not to exceed 30°C are acceptable for no more than 24 hours. Specimens may be stored an additional five days at 2° to

8°C following centrifugation. Plasma separated from the cells may be stored for longer periods of time at ≤ –20°C before testing. Do not freeze whole blood.



*The 2-30° and 2-25°C periods indicated above may occur at any time.

C. Additional specimens may be taken from whole blood or plasma units containing CPD, CP2D, or CPDA-1 anticoagulants collected according to the collection container manufacturer's instructions. Whole blood (not plasma units) collected in these anticoagulants may be stored for up to 72 hours from time of draw at ≤ 25°C; temperatures not to exceed 30°C are acceptable for no more than 24 hours. Specimens may be stored an additional two days at 2° to 8°C following centrifugation. Plasma separated from the cells may be stored for longer periods of time at ≤ –20°C before testing.

D. No adverse effect on assay performance was observed when plasma was subjected to three freeze-thaw cycles.

E. Specimens with visible precipitates or fibrinous material should be clarified by centrifugation for 10 minutes at 1000 to 3000 x g prior to testing. Do not test specimens that do not have sufficient sample volume above the gel separator or red cell interface.

F. Mix thawed plasma thoroughly and centrifuge for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed BD PPT tubes must be validated by the user.

G. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[20]

H. False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.

I. Specimen Pooling

The pooling software, used in combination with a front end pipettor, performs sample scanning and pooling operations that combine aliquots from individual samples into a single Master Pool Tube, which may be used for further testing.

Note: Only specimens from donors of whole blood or blood components may be pooled.

CADAVERIC BLOOD SPECIMENS

Note: A plasma specimen collected pre-mortem from a non-heart-beating (cadaveric) organ/cell/tissue donor may be tested instead of a cadaveric blood specimen using instructions for cadaveric donors.

A. Cadaveric blood specimens can be collected in clot or EDTA anti-coagulant tubes. Follow sample tube manufacturer's instructions.

B. For collection of specimens from cadaveric donors, follow general standards and/or regulations. Specimen stability is affected by elevated temperature.

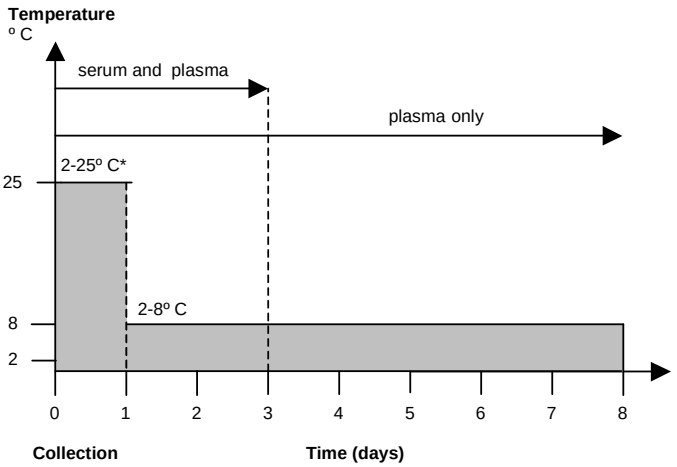
C. Plasma (EDTA collection tubes) may be stored for up to 24 hours at 2° to 25°C. Specimens may be stored for an additional 7 days at 2° to 8°C following centrifugation. Plasma separated from the cells may be stored for longer periods of time at ≤ –20°C before testing. Do not freeze whole blood.

D. Whole blood (clot tubes) and serum may be stored for up to 24 hours at 2° to 25°C. Specimens may be stored for an additional 2 days at 2° to 8°C following centrifugation. Serum removed from the clot may be stored for longer periods of time at ≤ –20°C before testing. Do not freeze whole blood.

E. No adverse effect on assay performance was observed when plasma and serum were subjected to three freeze-thaw cycles.

F. Specimens with visible precipitates or fibrinous material should be clarified by centrifugation for 10 minutes at 1000 to 3000 x g prior to testing. Do not test specimens that do not have sufficient sample volume above the gel separator or red cell interface.

G. Mix thawed plasma or serum thoroughly and centrifuge for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed BD PPT tubes must be validated by the user.



*The 2-25º C period indicated above may occur at any time.

H. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[20]

I. False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.

J. Cadaveric blood specimens may be diluted to overcome potential sample inhibitory substances or specimen shortage. Plasma and/or serum may be diluted 1:5 in saline (0.9% sodium chloride), i.e. 100 µL sample plus 400 µL saline. Diluted specimens should be inverted several times to mix and then may be used in standard assay procedure by pipetting the 500 µL of the diluted specimen into the TTU containing TCR.

Note: If a front-end pipettor will be used to pipette the samples, the minimum volume for the diluted sample should be 1100 µL (220 µL neat sample plus 880 µL saline).

Note: Studies performed to validate these conditions were performed on negative cadaveric specimens spiked with virus. The stability of WNV *in vivo* post-mortem was not assessed.

PROCLEIX® SYSTEM USERS

MATERIALS PROVIDED

PROCLEIX® WNV Assay 5000 Test Kit P/N 301187

- Internal Control Reagent
- Target Capture Reagent
- Amplification Reagent
- Enzyme Reagent
- Probe Reagent
- Selection Reagent
- PROCLEIX® WNV Negative Calibrator
- PROCLEIX® WNV Positive Calibrator

MATERIALS REQUIRED BUT PROVIDED SEPARATELY

PROCLEIX® Assay Fluids P/N 301116

- Wash Solution
- Oil
- Buffer for Deactivation Fluid

PROCLEIX® Auto Detect Reagents P/N 301120

- Auto Detect 1
- Auto Detect 2

Disposables

(Disposables are single use only, do not reuse. Use of other disposables is not recommended.)

Ten-Tube Units (TTUs)	P/N TU0040
Ten Tip Cassettes	P/N 104578
Sealing Cards	P/N 102085

Equipment/Software

PROCLEIX® System:

- TECAN GENESIS RSP instrument (front end pipettor), PROCLEIX® Assay Software, and operator's manual; or PROCLEIX® Worklist Editor software and operator's manual
- PROCLEIX® TCS (target capture system) and operator's manual
- PROCLEIX® HC+ Luminometer, PROCLEIX® System Software, and operator's manual

Multi-tube Vortex Mixer (Vortexer)

Water bath

Dedicated fixed or adjustable repeat pipettors capable of delivering 25-500 µL of liquid with a ± 5% accuracy and a coefficient of variation of ≤ 5%.

Other

PROCLEIX® System Quick Reference Guide (PROCLEIX® System QRG)
Any applicable technical bulletins

OTHER MATERIALS AVAILABLE FROM CHIRON FOR USE WITH PROCLEIX® WNV ASSAY

PROCLEIX® WNV Assay Calibrators P/N 301186

- PROCLEIX® WNV Negative Calibrator
- PROCLEIX® WNV Positive Calibrator

General Equipment/Software

- PROCLEIX® Reagent Preparation Incubator (RPI), independent temperature monitor (ITM), and operator's manual
- PROCLEIX® CPT Pooling Software and operator's manual

For instrument specifics and ordering information, contact Chiron Customer Support.

MATERIALS REQUIRED BUT NOT PROVIDED

Repeat pipettor tips (1.25 mL, 5.0 mL, 10 mL, 12.5 mL)

If using the Manual Sample Pipetting Method: Filtered fixed pipettor tips capable of delivering 500 µL (for samples) and repeat pipettor tips capable of delivering 400 µL (for wTCR)

If using the TECAN GENESIS RSP instrument: Disposable 1000 µL conductive filter tips in rack approved for use with equipment and Front End Pipettor reagent troughs

Bleach

For use in final concentrations of 5% sodium hypochlorite and 0.5% sodium hypochlorite

Bleach alternative (optional)

Contact Chiron Technical Support for a list of bleach alternatives and instructions for use.

Sterile, polypropylene conical tubes with sealing caps. Freestanding tubes are recommended in two different sizes (5 mL to 10 mL tube and ≥ 30 mL tube). The tubes must be able to accommodate the diameter of a repeat pipettor tip.

PRECAUTIONS

A. For *In Vitro* diagnostic use.

B. When performing testing with different PROCLEIX® Assays using shared instrumentation, ensure appropriate segregation is maintained to prevent mix-up of samples during processing (e.g., use of colored TTU racks). In addition, verify that the correct set of reagents is being used for the assay that is being run.

C. Specimens may be infectious. Use Universal Precautions when performing the assay[21]. Proper handling and disposal methods should be established according to local, state and federal regulations[22,23]. Only personnel qualified as proficient in the use of the PROCLEIX® WNV Assay and trained in handling infectious materials should perform this procedure.

D. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

E. To reduce the risk of invalid results, carefully read the entire package insert for the PROCLEIX WNV Assay and instrument and software operator's manuals prior to performing an assay run.

F. Material Safety Data Sheets are available upon request.

G. Avoid contact of Auto Detect Reagents 1 and 2 with skin, eyes and mucous membranes. Wash with water if contact with these reagents occurs. If spills of these reagents occur, dilute with water before wiping dry and follow appropriate site procedures.

H. Dispose of all materials that have come in contact with specimens and reagents according to local, state and federal regulations[22,23]. Thoroughly clean and disinfect all work surfaces.

I. Working TCR (wTCR) remaining in the reagent trough after the completion of the run must be discarded.

J. Use only supplied or specified required disposables.

K. Do not use this kit after its expiration date. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

L. Avoid microbial and ribonuclease contamination of reagents.

M. Store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See STORAGE INSTRUCTIONS and REAGENT PREPARATION.

N. Store all specimens at specified temperatures. The performance of the assay may be affected by use of improperly stored specimens. See SPECIMEN COLLECTION, STORAGE AND HANDLING for specific instructions.

O. Only combine assay reagents or fluids as instructed to by the PROCLEIX WNV Assay package insert.

P. Refer to precautions in the appropriate package inserts, operator's manuals, and the PROCLEIX® System QRG.

REAGENT PREPARATION

These steps should be performed prior to beginning Target Capture in an area that is free of template and amplicon.

A. Room temperature is defined as 15° to 30°C.

B. Verify that reagents have not exceeded the expiration date and/or storage stability times.

C. Remove a bottle of Selection Reagent from room temperature storage.

1. The Selection Reagent must be at room temperature before use.
2. If Selection Reagent has been inadvertently stored at 2° to 8°C or the temperature of the laboratory falls to between 2° and 15°C, precipitate may form.
3. If precipitate forms in the Selection Reagent, heat at 60° ± 1° C for no more than 45 minutes, shaking the bottle frequently (every 5 to 10 minutes). Once all precipitate has gone back into solution, place the bottle in a room temperature water bath and allow the bottle to equilibrate for at least 1 hour. Alternatively, use the Reagent Preparation Incubator (RPI) as described in the PROCLEIX® System QRG. Do not use the Selection Reagent until it has equilibrated.
4. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
5. Do not use if precipitate or cloudiness persists.
6. Record the date that it was first opened (OPEN DATE) on the space provided on the label.

D. Warm all reagents to room temperature and mix thoroughly prior to use. A dedicated water bath at room temperature or the RPI may be used to aid this process. If using the RPI to warm the TCR, Probe Reagent, Enzyme Reagent, and Amplification Reagent, refer to the PROCLEIX System QRG.

1. If using a water bath, thaw reagents upright.
2. Amplification and Probe Reagents may be mixed by vortexing.
3. Enzyme Reagent should be mixed thoroughly by gentle inversion, taking care to avoid excessive foaming.
4. Record the date of thaw (THAW DATE) for each reagent on the space provided on the label.

E. Ensure that precipitates are dissolved. Do not use a reagent if precipitate or cloudiness is present.

F. DO NOT heat Probe Reagent above 30°C if using a water bath. Do not heat Probe Reagent above 35°C if using the RPI. Refer to the PROCLEIX System QRG.

G. Probe Reagent is light-sensitive. Protect this reagent from light during storage and preparation for use.

H. Precipitate will form in the Probe Reagent when stored at 2° to 8°C. Probe Reagent may be warmed in a water bath to facilitate dissolution of precipitate, but temperature in the bath should not exceed 30°C. The Probe Reagent may take up to 4 hours with periodic mixing to allow complete dissolution of precipitate if thawing is conducted on the lab bench. Alternatively, use the RPI to thaw the Probe Reagent at an average temperature of 32° ± 2°C, not to exceed 35°C. Refer to the PROCLEIX System QRG. Ensure that precipitates in the Probe Reagent are dissolved. Do not use if precipitate or cloudiness is present.

I. Prepare working Target Capture Reagent (wTCR):

1. Remove TCR from 2° to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX.
2. After mixing, place the TCR bottle at 22° to 30°C. Approximately every 10 minutes shake the bottle until all precipitate has disappeared. TCR precipitate should normally dissolve in about 30 minutes. Alternatively, use the RPI to thaw the TCR at an average temperature of 32° ± 2°C, not to exceed 35°C. Refer to the PROCLEIX System QRG.

   Note: If a gel is observed after performing this procedure, a new bottle must be used according to the handling recommendations above. Return the bottle with gel back to 2° to 8°C storage for subsequent use.
3. Thaw one vial of Internal Control Reagent up to 24 hours at 2° to 8° C or up to 8 hours at room temperature. Do not use the RPI to thaw Internal Control Reagent.
4. Mix the Internal Control Reagent thoroughly by inversion or vortexing.
5. When the Internal Control (IC) Reagent and TCR have reached room temperature, mix TCR thoroughly by inversion. Pour the entire vial of Internal Control Reagent into the TCR bottle. This is now the working Target Capture Reagent (wTCR). Mix thoroughly.
6. Use the space indicated on the TCR bottle to record the date Internal Control Reagent was added and lot number used (IC LOT). Record the expiration date of the wTCR in the space provided on the label.

J. Thaw calibrators at room temperature. **Do not use the RPI to thaw calibrators.**

1. These are single use vials and must be thawed prior to each run.
2. Mix calibrators gently by inversion to avoid foaming.
3. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

K. Wash Solution is shipped at ambient temperature and stored at room temperature. Precipitates may form in the Wash Solution during shipment or during storage when temperatures fall to between 2° and 15°C. Wash Solution may be warmed to facilitate dissolution of precipitate. **Do not use the RPI to warm the Wash Solution**. Temperature should not exceed 30°C. Ensure that precipitates in the Wash Solution are dissolved prior to use. Do not use if precipitate or cloudiness is present.

L. For Wash Solution, Oil, Selection Reagent, Buffer for Deactivation Fluid, Auto Detect 1, and Auto Detect 2, record the date the reagent was first opened (OPEN DATE) in the space provided on the label.

M. To prepare Deactivation Fluid, mix one part Buffer for Deactivation Fluid with one part 5% sodium hypochlorite. Record the date the Deactivation Fluid was prepared.

PROCEDURAL NOTES

Note: Refer to the PROCLEIX® System QRG for maintenance procedures and information about software operation.

A. To reduce the risk of invalid results, carefully read the entire package insert for the PROCLEIX® WNV Assay prior to performing an assay run. This package insert must be used with the PROCLEIX® System QRG and any applicable technical bulletins.

B. RUN SIZE

1. Kit size is based on an average run size of 55 tests. Smaller run sizes will result in a lower yield.
2. Each run will yield up to 100 test results, including results for three replicates of the Positive Calibrator and three replicates of the Negative Calibrator.

C. EQUIPMENT PREPARATION

1. Three dedicated circulating water baths must be used: one for target capture and pre-amplification (60° ± 1°C), one for amplification (41.5° ± 1°C) and one for hybridization and selection (61° ± 2°C). An additional container of water is required to be maintained at 23° ± 4°C for the step preceding detection.
2. Equilibrate circulating water baths to 60° ± 1°C for target capture and 41.5° ± 1°C for amplification incubations.
3. If using a front end pipettor, set up according to instructions in the PROCLEIX System QRG.
4. Prepare the target capture system for use according to instructions in the PROCLEIX System QRG.
5. Wipe work surfaces and pipettors daily with diluted bleach (0.5% sodium hypochlorite in water). Allow bleach to contact surfaces and pipettors for at least 15 minutes and then follow with a water rinse. **A bleach alternative may be used in pre-amplification areas only. Do not use bleach alternatives in amplification areas or in areas suspected to be contaminated with amplification products. Do not use deactivation fluid on surfaces.**
6. Equilibrate a circulating water bath to 61° ± 2°C for hybridization and selection incubations. Prepare a container of water at 23° ± 4°C for cool down prior to detection.
7. Prepare the luminometer according to instructions in the PROCLEIX System QRG.

D. REAGENTS

1. Add all reagents using a repeat pipettor capable of delivering specified volume with ± 5% accuracy and a precision of ≤ 5% CV. Check pipettor functionality monthly and calibrate regularly.
2. To minimize waste of Amplification, Oil, Enzyme, Probe, and Selection Reagents, aliquot each reagent for a given run size. Aliquoting must be performed after reagent preparation using sterile, polypropylene conical tubes with sealing caps in an area that is template and amplicon free. The aliquoting area must be wiped down with diluted bleach (0.5% sodium hypochlorite in water) before and after the aliquoting process. **A bleach alternative may be used in pre-amplification areas only. Do not use bleach alternatives in amplification areas or in areas suspected to be contaminated with amplification products.** The aliquoted reagents must be used the same day the aliquoting was performed. DO NOT store reagents in the aliquot conical tubes.
3. A color change will occur in the reaction tube after the addition of each of the following reagents: Amplification Reagent, Enzyme Reagent, Probe Reagent, and Selection Reagent.

E. RUN CONFIGURATION

A set of calibrators must be used at the beginning of each worklist. A set of calibrators consists of one vial each of Negative Calibrator and Positive Calibrator.

F. WORK FLOW

1. To minimize the possibility of laboratory areas from becoming contaminated with amplicon, the laboratory area should be arranged with a uni-directional workflow. Proceed from reagent preparation to sample preparation to amplification and then to detection areas. Samples, equipment and reagents should not be returned to the area where a previous step was performed. Also, personnel may not move from the dedicated Hybridization Protection Assay (HPA) area back into previous work areas without proper anti-contamination safeguards.
2. Perform reagent preparation in a clean (amplicon- and template-free) area.
3. Perform Sample Preparation, Target Capture and pre-Amplification steps in an amplicon-free area.
4. Perform HPA in an area separate from the reagent preparation and amplification areas.
5. Upon completion of pipetting specimens (individual samples or pools) into TTUs, the TTUs are removed from the deck and loaded into a TTU rack. If the operator needs to pipette the same specimens (individual samples or pools) for a different PROCLEIX® Assay, the empty calibrator tubes and TCR trough must be discarded but the specimens may be left on the deck. The operator should then change gloves after emptying the used empty calibrator tubes and TCR trough. Clean TTUs should then be loaded into the TTU carriers on the deck. See PROCLEIX® System Users, PRECAUTIONS, Step B, for additional information.

Proceed with Section A, Sample Preparation under ASSAY PROCEDURE.

G. ENVIRONMENTAL CONDITIONS

1. The Target Capture, Amplification, HPA and Selection steps are temperature dependent. Therefore, it is imperative that the water baths are maintained within the specified temperature range. Use a calibrated thermometer.
2. Room temperature is defined as 15° to 30°C.
3. Detection is sensitive to temperature. The laboratory temperature in the detection area must be 21° to 27°C.
4. The operational conditions of the room in which the RPI runs must be within a temperature of 15° to 25°C.
5. Refer to instrument and software operator's manuals for additional environmental conditions requirements.

H. TIME

The Target Capture, Amplification, and HPA steps are all time dependent. Adhere to specific times outlined in ASSAY PROCEDURE.

I. VORTEXING

Proper vortexing is important to the successful performance of the PROCLEIX WNV Assay. Vortex equipment speed settings may vary. The vortexer speed should start at a low level and increase until the speed is adequate to achieve the desired results without allowing the reaction mixture to touch the sealing cards. **For each step that requires vortexing, it is critical that the content of the tubes be well-mixed.**

J. PIPETTING

1. All the pipettes used in the Target Capture, Amplification and HPA steps must be dedicated for these purposes only to avoid cross contamination.
2. Take care to deliver reagents, excluding working Target Capture Reagent (wTCR), to each tube without inserting pipette tip into the tube or touching the rim of the tube to minimize the chance of carryover from one tube to another.
3. When adding Oil, Probe Reagent, and Selection Reagent, angle the pipette tip toward the sides of the tube, not straight to the bottom, to avoid splashback.

K. MANUAL SPECIMEN PIPETTING

1. When using the manual sample/wTCR pipetting method, improper pipetting technique will affect the results of the assay. In order to avoid the loss of Positive ID Tracking, verification of correct sample ID by a second individual is recommended.
2. Ensure that the TTU is oriented in the rack with the pointed end on the left side and the rounded end on the right side of the rack. Pipette the first calibrator into the first tube next to the pointed end of the TTU. Samples are pipetted from left to right.
3. Use a new pipette tip for each sample and dispose of the tip in a biological waste container after use. Take care to avoid cross contamination by pipetting the specimens and discarding the used pipette tips without passing over open tubes or touching laboratory surfaces or other pieces of equipment.
4. To avoid the risk of contamination, clean and decontaminate manual sample pipettors between assay runs.
5. Ensure proper sample placement into the correct TTU position as indicated on the manual work list record.

L. DECONTAMINATION

1. The extremely sensitive nature of the test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces and pipettes must be decontaminated daily with diluted bleach (0.5% sodium hypochlorite in water). Allow bleach to contact surfaces for at least 15 minutes and then follow with a water rinse. Chlorine solutions may pit equipment and metal. Thoroughly rinse bleached equipment to avoid pitting.
2. **A bleach alternative may be used in pre-Amplification areas only. Do not use bleach alternatives in Amplification areas or in areas suspected to be contaminated with amplification products.**
3. Reaction tubes must be decontaminated with Deactivation Fluid as described in the PROCLEIX System QRG.
4. Follow instructions provided in the PROCLEIX System QRG for instrument decontamination and maintenance procedures.

M. SEALING CARDS

1. When applying sealing cards, cover the TTUs with the sealing card and press gently to ensure complete contact with all of the tubes. Always use a new sealing card. DO NOT re-use sealing cards.
2. When removing sealing cards, carefully lift and peel in one continuous motion to avoid aerosols and cross contamination. Immediately dispose of card in appropriate waste container.

ASSAY PROCEDURE

PROCLEIX® WNV ASSAY ON INDIVIDUAL DONOR SPECIMENS OR POOLED SPECIMENS

All specimens (individual donations or pooled specimens) should be run in singlet in the PROCLEIX® WNV Assay.

PROCLEIX® WNV Assay Calibrators are to be used with the corresponding master lot of the PROCLEIX WNV Assay. The operator must check to ensure that the PROCLEIX WNV Assay Calibrators are used with the corresponding master lot of kit reagents as indicated on the PROCLEIX WNV Assay master lot sheet in use.

Specimens from other living donors (except whole blood or blood components) and from cadaveric donors must be tested neat using the individual donor testing method only. If the initial test result from a cadaveric blood specimen is invalid, the specimen may be diluted to overcome potential inhibitory substances as described in SPECIMEN COLLECTION, STORAGE AND HANDLING, Cadaveric Blood Specimens, and retested in singlet.

To run the PROCLEIX WNV Assay for the detection of WNV RNA, follow the steps below for Target Capture, Amplification and Hybridization Protection Assay.

Note: For instrument and software steps, refer to the PROCLEIX® System QRG.

Note: Continuous Process Flow: All process steps described below are intended to be completed in a continuous flow with a minimal, if any, delay between steps.

A. SAMPLE PREPARATION/TARGET CAPTURE

Sample Preparation

The PROCLEIX® WNV Assay has been validated using manual pipetting and a front end pipettor. The use of manual pipetting requires additional operator training and demonstration of proficiency. Repeat pipettors used in this step must be dedicated for use only in the TARGET CAPTURE steps.

IF USING THE MANUAL SAMPLE PIPETTING METHOD:

For sample tracking, an electronic worklist must be created using the PROCLEIX® Worklist Editor software. Refer to the PROCLEIX® System QRG for instructions, or contact Chiron Technical Support. Verification of correct sample ID on the worklist with the specimen tubes and with the detailed assay run report by a second individual is recommended. The assay results within the run report will be marked *M* indicating that the specimens were manually pipetted.

1. Load sufficient Ten Tube Units (TTUs) for the run into a TTU rack.
2. Thoroughly mix the wTCR immediately before use to resuspend microparticles.
3. Refer to the worklist and carefully pipette 400 µL of wTCR to each tube that will contain a sample. To dispense, insert the tip approximately one quarter of the way into the tube at an angle and pipette wTCR down the side of the tube. Take care to avoid touching the rim or the side of the tube with the pipette tip. Always pipette the wTCR first, followed by the sample.
4. Pipette samples.
   a. Refer to the worklist to identify the TTU number with the corresponding calibrator and test specimen identification numbers.
   b. Aspirate 500 µL of each calibrator, external quality control or test specimen from its collection tube using a single channel pipettor with corresponding filtered disposable tip. Insert only the end of the pipette tip into the sample. Do not disturb the sediment, if any.
   c. To dispense, insert the pipette tip halfway into the tube taking care not to touch the sides of the upper half of the tube with the pipette tip. At an angle, pipette the sample down the side of the bottom half of the tube. Hold down the plunger of the pipettor while removing it from the tube. Take care to avoid touching the rim or the side of the tube with the pipette tip when removing it from the tube.
5. Replace the pipette tip with a new tip and repeat Step 4 until all samples have been pipetted.
6. Visually inspect tubes to ensure proper sample volume and wTCR volume have been dispensed.
7. Cover the TTUs with sealing cards. See PROCLEIX® System Users, PROCEDURAL NOTES. Proceed to the Target Capture section.

IF USING A FRONT END PIPETTOR:

1. Prepare front end pipettor for automatic pipetting of calibrators, samples, and wTCR; refer to the PROCLEIX® System QRG.
2. Instrument will add 400 µL of wTCR to reaction tubes.
3. Instrument will add 500 µL each of calibrators and test samples into assigned reaction tubes.
4. When all samples have been pipetted, transfer the TTUs to a TTU rack. Cover the TTUs with sealing cards. See PROCLEIX®

System Users, PROCEDURAL NOTES, Step M, SEALING CARDS for additional information.

5. Proceed to the Target Capture section.

Target Capture

1. Vortex the rack of TTUs a minimum of 20 seconds and until magnetic microparticles are resuspended. See PROCLEIX® System Users, PROCEDURAL NOTES on vortexing.
2. The rack may remain at room temperature up to 75 minutes prior to proceeding to the 60° ± 1°C incubation.
3. Incubate the tubes in a water bath at 60° ± 1°C for 20 minutes ± 1 minute.
4. Remove the rack of TTUs and transfer to the Target Capture area.
5. Incubate the rack of TTUs on the lab bench at room temperature for 14 to 20 minutes.
6. Transfer the rack of TTUs to the target capture system (TCS) for 9 to 20 minutes.
7. Carefully remove and dispose of the sealing cards.
8. To aspirate and wash, refer to the Target Capture section of the PROCLEIX System QRG.
9. Cover the TTUs with sealing cards.
10. Vortex to resuspend the microparticle pellets, then inspect the reaction tubes to make sure that all of the magnetic particles have been uniformly suspended.
11. Place the rack of TTUs on the TCS for 4 to 10 minutes.
12. Carefully remove and dispose of the sealing cards.
13. Repeat Steps 8 through 12.
14. Completely aspirate the solution from each tube. Refer to the Target Capture section of the PROCLEIX System QRG.
15. Cover the TTUs with sealing cards.
16. Proceed directly to Amplification.

B. AMPLIFICATION

Do not use bleach alternatives in this area.

The repeat pipettors used in this step must be dedicated for use only in AMPLIFICATION steps.

1. Carefully remove and dispose of the sealing cards.
2. Add 75 µL of Amplification Reagent to each tube (a color change can be observed in the reaction tube). See PROCLEIX® System Users, PROCEDURAL NOTES on pipetting.
3. Add 200 µL of Oil to each tube.
4. Cover the TTUs with sealing cards.
5. Vortex the rack of TTUs a minimum of 20 seconds until well-mixed and all microparticles are resuspended. Ensure that magnetic particles are no longer adhering to the walls of the tube, and are uniformly resuspended.
6. Incubate the TTUs in a water bath at 60° ± 1°C for 10 minutes ± 1 minute.
7. Incubate the TTUs in a water bath at 41.5° ± 1°C for 9 to 20 minutes.
8. Leaving the rack of TTUs at 41.5° ± 1°C, carefully remove and dispose of the sealing cards. Immediately add 25 µL of the Enzyme Reagent into each tube (a color change can be observed in the reaction tube). Place new sealing cards over the TTUs.
9. Remove the rack of TTUs from the water bath and shake to mix. DO NOT VORTEX. Minimize the time the tubes are out of the water bath.
10. Incubate the rack of TTUs in the water bath at 41.5° ± 1°C for 60 minutes ± 5 minutes.
11. Remove the rack of TTUs from the water bath and transfer it to the HPA area. Rack may remain at room temperature for up to 30 minutes prior to the addition of Probe Reagent.

C. HYBRIDIZATION PROTECTION ASSAY (HPA)

A separate, dedicated location for the Hybridization Protection Assay (HPA) step is recommended to minimize amplicon contamination in the assay. This dedicated area should be on a separate bench in a separate area from the reagent and sample preparation and amplification areas. **Do not use bleach alternatives in this area.**

The repeat pipettor used in this step must be dedicated for use only in HYBRIDIZATION PROTECTION ASSAY.

1. Hybridization
   a. Carefully remove and dispose of the sealing cards. See PROCLEIX® System Users, PROCEDURAL NOTES on pipetting.
   b. Add 100 µL of Probe Reagent into each tube (a color change can be observed in the reaction tube). See PROCLEIX® System Users, PROCEDURAL NOTES.
   c. Cover the TTUs with sealing cards. Vortex the rack of TTUs a minimum of 20 seconds and until contents are well-mixed. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card. See PROCLEIX® System Users, PROCEDURAL NOTES on vortexing.
   d. Incubate the rack of TTUs in a dedicated water bath at 61° ± 2°C for 15 minutes ± 1 minute.
2. Selection
   a. Remove the rack of TTUs from the 61° ± 2°C water bath. Carefully remove and dispose of the sealing cards.
   b. Add 250 µL of Selection Reagent to each tube (a color change can be observed in the reaction tube).
   c. Cover the TTUs with sealing cards. Vortex the rack of TTUs a minimum of 20 seconds and until contents are well-mixed. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card. Return the rack of TTUs to the 61° ± 2°C water bath for 10 minutes ± 1 minute.
   d. Cool the rack of TTUs in a 23° ± 4°C container of water for a minimum of 10 minutes while preparing for Detection.
   e. Remove the rack of TTUs from the 23° ± 4°C container of water onto absorbent material.
3. Detection

Note: Tube readings should be completed within 75 minutes after completing the selection reaction.

For Detection and decontamination, refer to the PROCLEIX® System QRG.

QUALITY CONTROL PROCEDURES

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX® WNV ASSAY

A. A run is valid if the minimum number of calibrators is valid and calibrators meet acceptance criteria (see section II below).

1. In a PROCLEIX® WNV Assay run, at least four of the six calibrator replicates must be valid. At least two of the three Negative Calibrator replicates and two of the three Positive Calibrator replicates must be valid.
2. The PROCLEIX® System Software will automatically invalidate a run if less than the minimum number of calibrator replicates is valid. All specimens in an invalid run due to calibrators must be retested.
3. Cutoff values will be automatically calculated for Internal Control (flasher) and analyte (glower) in a valid run (see section II).
4. In a valid run, specimens with an analyte signal (glower signal)

greater than the analyte cutoff are not invalidated even if the Internal Control signal is below the cutoff. Specimens with an Internal Control (IC) signal above 500,000 RLU are invalidated by the software and the reactive status cannot be assessed. Positive Calibrators with an IC signal above 500,000 RLU are invalidated by the software.

B. An assay run or an individual sample may be invalidated by an operator if specific technical/operator/instrument difficulties were observed and documented. If individual samples are invalidated by an operator, then the percent invalid rate must be manually calculated.

C. The PROCLEIX System Software will print an alert on the run report when more than 10% of the calibrators and specimens in a run are invalid (see the PROCLEIX® System QRG for details). Specimens that are invalid solely due to insufficient sample or wTCR are not included in the calculation of the 10% invalid rate.

D. For runs that exceed the 10% invalid rate, further evaluation is required. Review package insert procedures to identify operator errors. In addition, the run report should be reviewed using the criteria described below.

1. If the invalid specimens are all from the same TTU, those specimens contributing to the 10% invalid rate may have been inadequately washed, or erroneous reagent addition may have occurred. All nonreactive and invalid specimens in the affected TTU should be retested.
2. If the invalid specimens are randomly located throughout the run and no specific cause can be identified, the nonreactive and invalid specimens must be retested.
3. If the invalid specimens are randomly located throughout the run, a specific cause that explains the invalid result can be identified, and the remaining valid results have consistent IC RLU values, only invalid specimens may be retested.

Note: Specimens with an overall interpretation of Reactive, as determined by the software, must become the test of record. The specimens should be resolved according to the resolution algorithm for reactive specimens, as explained in the INTERPRETATION OF RESULTS section.

II. ACCEPTANCE CRITERIA FOR THE CALIBRATION AND CALCULATION OF CUTOFF FOR THE PROCLEIX® WNV ASSAY

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate in the PROCLEIX® WNV Assay. Each individual Negative Calibrator replicate must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 400,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 40,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid due to an IC value or an analyte value outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator replicate values have IC values or analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator values (NC_x) for Internal Control [NC_x (Internal Control)]

Example:

Negative Calibrator	Internal Control Relative Light Units
1	235,000
2	200,000
3	210,000
Total Internal Control RLU =	645,000

$$NC_x \text{ (Internal Control)} = \frac{\text{Total Internal Control RLU}}{3} = 215{,}000$$

Determination of the mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)]

Example:

Negative Calibrator	Analyte Relative Light Units
1	14,000
2	16,000
3	15,000
Total Analyte RLU =	45,000

$$NC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 15{,}000$$

Positive Calibrator Acceptance Criteria

The Positive Calibrator (PC) is run in triplicate in the PROCLEIX WNV Assay. Individual Positive Calibrator analyte values must be less than or equal to 2,700,000 RLU and greater than or equal to 400,000 RLU. IC values may not exceed 500,000 RLU. If one of the Positive Calibrator values is outside these limits, the Positive Calibrator mean (PC_x) will be recalculated based upon the two acceptable Positive Calibrator values. The run is invalid and must be repeated if two or more of the three Positive Calibrator analyte values are outside of these limits.

Determination of the mean of the Positive Calibrator (PC_x) values for Analyte [PC_x (Analyte)]

Example:

Positive Calibrator	Analyte Relative Light Units
1	1,250,000
2	1,500,000
3	1,150,000
Total Analyte RLU =	3,900,000

$$PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 1{,}300{,}000$$

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = 0.5 X [NC_x (Internal Control)]

Using values given in the Negative Calibrator example above:

Internal Control Cutoff Value = 0.5 X (215,000)

Internal Control Cutoff Value = 107,500 RLU

Calculation of the WNV Analyte Cutoff Value

Analyte Cutoff Value = NC_x (Analyte) + [0.03 X PC_x (Analyte)]

Using values given in the Negative Calibrator and Positive Calibrator examples above:

Analyte Cutoff Value = 15,000 + (0.03 X 1,300,000)

Analyte Cutoff Value = 54,000 RLU

Summary of Acceptance Criteria for PROCLEIX® WNV Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 75,000 and ≤ 400,000 RLU
Positive Calibrator	
Analyte	≥ 400,000 and ≤ 2,700,000 RLU
Internal Control	≤ 500,000 RLU

Summary of Cutoff Calculations for PROCLEIX® WNV Assay

Analyte Cutoff =	NC Analyte Mean RLU + [0.03 X (PC Analyte Mean RLU)]
Internal Control Cutoff =	0.5 X (Negative Calibrator IC Mean RLU)

INTERPRETATION OF RESULTS

All calculations described above are performed by the luminometer software. Two cutoffs are determined for the PROCLEIX® WNV Assay: one for the Analyte Signal (glower signal) termed the Analyte Cutoff and one for the Internal Control Signal (flasher signal) termed the Internal Control Cutoff (IC Cutoff). The calculation of these cutoffs is shown above. For each sample, an Analyte Signal RLU value and Internal Control Signal RLU value is determined. Analyte Signal RLU divided by the Analyte Cutoff is abbreviated as the Analyte Signal/Cutoff (S/CO) on the report.

A specimen is Nonreactive if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO < 1.00) and the Internal Control (IC) Signal is greater than or equal to the Internal Control Cutoff (IC Cutoff) and less than or equal to 500,000 RLU. A specimen is Reactive if the Analyte Signal is greater than or equal to the Analyte Cutoff (i.e., Analyte S/CO ≥1.00) and the Internal Control Signal is less than or equal to 500,000 RLU. Reactive results will be designated by the software. A specimen is Invalid if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO < 1.00) and the Internal Control Signal is less than the Internal Control Cutoff. Any specimen with Internal Control values greater than 500,000 RLU is considered Invalid and the reactive status cannot be assessed.

Cadaveric blood specimens, when tested neat, may be invalid due to inhibitory substances within the specimen. These invalid specimens may be diluted as described in SPECIMEN COLLECTION, STORAGE AND HANDLING, Cadaveric Blood Specimens, and retested in singlet.

Summary of Specimen Interpretation:

Specimen Interpretation	Criteria
NonReactive	Analyte S/CO < 1.00 and IC ≥ IC Cutoff and IC ≤ 500,000 RLU
Reactive	Analyte S/CO ≥ 1.00 and IC ≤ 500,000 RLU
Invalid*	IC > 500,000 RLU or Analyte S/CO < 1.00 and IC < IC Cutoff

*For specimens with IC signal greater than 500,000 RLU, the specimen will be invalidated by the software and the reactive status cannot be assessed.

1. Any specimen, including cadaveric specimens, with an interpretation of Invalid in the PROCLEIX WNV Assay must be retested in singlet. Cadaveric specimens previously diluted 1:5 may be retested diluted 1:5.
2. If at any point in the testing algorithm there is insufficient volume to complete the testing then an alternate specimen from the index donation may be used as long as the storage criteria in the package insert are met.
3. Specimens with a valid Internal Control value and with an Analyte S/CO less than 1.00 in the PROCLEIX WNV Assay are considered Nonreactive for WNV RNA.
    a. IF THE NONREACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool is considered Nonreactive and no further testing is required.
    b. IF THE NONREACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION, then the individual specimen is considered Nonreactive for WNV and no further testing is required.
4. Specimens with an Analyte S/CO greater than or equal to 1.00 with IC signal less than or equal to 500,000 RLU are considered Reactive.
    a. IF THE REACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool must be tested with the PROCLEIX WNV Assay.
        1. If an individual specimen tests Nonreactive with the PROCLEIX WNV Assay, then the specimen is considered Nonreactive for WNV and no further testing is required.
        2. If an individual specimen tests Reactive with the PROCLEIX WNV Assay, then the individual specimen is considered Reactive for WNV. Further clarification of the Reactive specimens for informational purposes may be obtained by testing an alternate specimen from the index donation with the PROCLEIX WNV Assay and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of donor eligibility.
    b. IF THE REACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION, then the individual specimen is considered Reactive for WNV. Further clarification of the Reactive specimens for informational purposes may be obtained by testing an alternate specimen from the index donation with the PROCLEIX WNV Assay and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of donor eligibility.
5. Reactive specimens in an operator-invalidated run due to the 10% invalid rate are identified by the software as reactive and must become the test of record. Any reactive result should be resolved according to the resolution algorithm for reactive specimens, as explained in the INTERPRETATION OF RESULTS section, step 4.

PROCLEIX® TIGRIS® SYSTEM USERS

MATERIALS PROVIDED

PROCLEIX® WNV Assay 5000 Test Kit P/N 301187

Internal Control Reagent
Target Capture Reagent
Amplification Reagent
Enzyme Reagent
Probe Reagent
Selection Reagent
PROCLEIX® WNV Negative Calibrator
PROCLEIX® WNV Positive Calibrator

MATERIALS REQUIRED BUT PROVIDED SEPARATELY

PROCLEIX® Assay Fluids P/N 301116

Wash Solution
Oil
Buffer for Deactivation Fluid

PROCLEIX® Auto Detect Reagents P/N 301120

Auto Detect 1
Auto Detect 2

PROCLEIX® System Fluid Preservative P/N 301175

PROCLEIX® WNV TIGRIS® Controls P/N 301185

PROCLEIX® WNV TIGRIS® Negative Control
PROCLEIX® WNV TIGRIS® Positive Control

Disposables

(Disposables are single use only, do not reuse. Use of other disposables is not recommended.)

Multi-Tube Units (MTUs) – case of 100 P/N 104772

Waste Bag Kit (MTU and Tiplet) – 30 of each P/N 900907

MTU Waste Cover P/N 105523

MTU Waste Deflector P/N 900931

Reagent Spare Caps (TCR, Selection, Probe Reagent) P/N CL0039

Reagent Spare Caps (Amplification Reagent) P/N CL0042

Reagent Spare Caps (Enzyme Reagent) P/N CL0043

PROCLEIX® TIGRIS® System Maintenance Bottle Kit P/N 105655

Equipment/Software

PROCLEIX® TIGRIS® System, PROCLEIX® TIGRIS® System Software, PROCLEIX® WNV Assay Software, and operator's manual

PROCLEIX® Reagent Preparation Incubator (RPI), independent temperature monitor (ITM), and operator's manual

Other

PROCLEIX® TIGRIS® System Quick Reference Guide (PROCLEIX® TIGRIS® System QRG)

Any applicable technical bulletins

OTHER MATERIALS AVAILABLE FROM CHIRON FOR USE WITH PROCLEIX® WNV ASSAY

PROCLEIX® WNV Assay Calibrators P/N 301186

PROCLEIX® WNV Negative Calibrator
PROCLEIX® WNV Positive Calibrator

General Equipment/Software

TECAN GENESIS RSP instrument (front end pipettor) for pooling only, PROCLEIX® CPT Pooling Software, operator's manual, and quick reference guide

For instrument specifics and ordering information, contact Chiron Customer Support.

MATERIALS REQUIRED BUT NOT PROVIDED

Disposable conductive filter tips (DiTis) in rack approved for use with equipment (required for pooling only)

Bleach

For use in final concentrations of 5% sodium hypochlorite and 0.5% sodium hypochlorite

Bleach alternative (optional)

Contact Chiron Technical Support for a list of bleach alternatives and instructions for use.

Alcohol (70% ethanol, 70% isopropyl alcohol solution, or 70% isopropyl alcohol wipes)

CLSI Type 1 water

Disposable 1000 µL conductive filter tips in rack approved for use with the PROCLEIX® TIGRIS® System. Contact Chiron Technical Support for approved tips.

PRECAUTIONS

A. For *In Vitro* diagnostic use.

B. When performing testing with different PROCLEIX® Assays using shared instrumentation, ensure appropriate segregation is maintained to prevent mix-up of samples during processing. In addition, verify that the correct set of reagents is being used for the assay that is being run.

C. Specimens may be infectious. Use Universal Precautions when performing the assay[21]. Proper handling and disposal methods should be established according to local, state and federal regulations[22,23]. Only personnel qualified as proficient in the use of the PROCLEIX® WNV Assay and trained in handling infectious materials should perform this procedure.

D. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

E. To reduce the risk of invalid results, carefully read the entire package insert for the PROCLEIX WNV Assay and the operator's manual for the PROCLEIX® TIGRIS® System prior to performing an assay run.

F. Material Safety Data Sheets are available upon request.

G. Avoid contact of Auto Detect Reagents 1 and 2 with skin, eyes and mucous membranes. Wash with water if contact with these reagents occurs. If spills of these reagents occur, dilute with water before wiping dry and follow appropriate site procedures.

H. Dispose of all materials that have come in contact with specimens and reagents according to local, state and federal regulations[22,23]. Thoroughly clean and disinfect all work surfaces.

I. Use only supplied or specified required disposables.

J. Do not use this kit after its expiration date. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

K. Avoid microbial and ribonuclease contamination of reagents.

L. Store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See STORAGE INSTRUCTIONS and REAGENT PREPARATION.

M. Store all specimens at specified temperatures. The performance of the assay may be affected by use of improperly stored specimens. See SPECIMEN COLLECTION, STORAGE AND HANDLING for specific instructions.

N. Only combine assay reagents or fluids as instructed to by the PROCLEIX WNV Assay package insert. Do not top off reagents or fluids. The PROCLEIX TIGRIS System verifies reagent levels.

O. The PROCLEIX TIGRIS System groups a quadrant of reagents into a matched set the first time that it scans their barcodes during the inventory process and are required to be run as a set in all subsequent worklists. Bottles belonging to a matched set cannot be swapped with bottles in other kits of reagents. Refer to the PROCLEIX® TIGRIS® System QRG for more information.

P. When running a worklist or control bracket of 50 or fewer specimens, the software does not apply the 10% invalid rate. See PROCLEIX TIGRIS System Users, QUALITY CONTROL PROCEDURES.

Q. Resolution of pools is not performed by the PROCLEIX TIGRIS System. Follow laboratory procedures for resolving pools.

R. Refer to precautions in the appropriate PROCLEIX® Assay package inserts and the PROCLEIX TIGRIS System operator's manual and QRG.

REAGENT PREPARATION

A. Room temperature is defined as 15° to 30°C.

B. Choose a new or opened matched set of reagents that will be sufficient to complete testing of the number of samples in a worklist. Do not use reagents that have been used outside the PROCLEIX® TIGRIS® System or on another PROCLEIX TIGRIS System, as the instrument verifies reagent volumes.

C. Verify that the reagents have not exceeded the expiration date and/or storage stability times, including onboard stability.

1. The PROCLEIX TIGRIS System does not track the room temperature stability of reagents or fluids. However, it does track the number of hours each reagent and fluid is loaded onboard the analyzer. The PROCLEIX TIGRIS System will not allow an assay to be run using reagents that have expired or exceeded their onboard stability. Consult the following table for onboard stability information.

Reagent/Fluid	Onboard Stability*
wTCR, Probe Reagent, Enzyme Reagent, Amplification Reagent, Selection Reagent	60 hours**
Wash Solution, Oil, System Fluid, Deactivation Fluid, Auto Detect Reagents	14 days

* The onboard time must occur within the room temperature times listed in General Information, STORAGE INSTRUCTIONS.

** Worklists cannot be queued using reagents that have been onboard for more than 48 hours.

2. Print an Assay Reagent Status Report to check the stability remaining for unexpired reagent sets in the system's database.

D. Remove a bottle of Selection Reagent from room temperature storage.

1. The Selection Reagent must be at room temperature before use.
2. If cloudiness or precipitate is present, use the RPI as described in the PROCLEIX® TIGRIS® System QRG. Do not use if precipitate or cloudiness persists.
3. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
4. Record the date that it was first opened (OPEN DATE) on the space provided on the label.

E. To prepare the following reagents using the RPI, refer to the PROCLEIX TIGRIS System QRG: TCR, Probe Reagent, Enzyme Reagent, and Amplification Reagent. Record the date of thaw (THAW DATE) for reagent on the space provided on the label.

F. Ensure that precipitates are dissolved. Do not use a reagent if precipitate or cloudiness is present.

G. Prepare working Target Capture Reagent (wTCR):

1. Remove TCR from 2° to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX.
2. Place TCR into the RPI, and refer to PROCLEIX TIGRIS System QRG for instructions.
3. Thaw one vial of Internal Control Reagent up to 24 hours at 2° to 8° C or up to 8 hours at room temperature. **Do not use the RPI to thaw Internal Control Reagent.**
4. Mix the Internal Control (IC) Reagent thoroughly by gentle inversion or vortexing.
5. After unloading TCR from the RPI and warming the IC Reagent to room temperature, pour the entire vial of IC Reagent into the TCR bottle. This is now the working Target Capture Reagent (wTCR). Mix thoroughly.
6. Use the space indicated on the TCR bottle to record the date Internal Control Reagent was added and lot number used (IC LOT). Record the expiration date of the wTCR in the space provided on the label.
7. Retain the IC vial to scan the barcode label into the system.

H. Thaw calibrators at room temperature. **Do not use the RPI to thaw calibrators.**

1. These are single use vials, which must be thawed prior to each run.
2. Mix calibrators gently by inversion to avoid foaming.
3. If foam is present, remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

I. Follow instructions provided in the PROCLEIX® WNV TIGRIS® Controls package insert for preparation of PROCLEIX WNV TIGRIS Controls. **Do not use the RPI to thaw PROCLEIX WNV TIGRIS Controls.**

1. Avoid reagent foaming.

2. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

J. Wash Solution is shipped at ambient temperature and stored at room temperature. Precipitates may form in the Wash Solution during shipment or during storage when temperatures fall to between 2° and 15ºC. Wash Solution may be warmed to facilitate dissolution of precipitate. **Do not use the RPI to warm the Wash Solution**. Temperature should not exceed 30ºC. Ensure that precipitates in the Wash Solution are dissolved prior to use. Do not use if precipitate or cloudiness is present.

K. For the Wash Solution, Oil, Auto Detect 1, and Auto Detect 2, record the date the fluid was first opened and loaded onto the PROCLEIX TIGRIS System (OPEN DATE) in the space provided on the label.

L. To prepare Deactivation Fluid, combine Buffer for Deactivation Fluid with 5% sodium hypochlorite in the Deactivation Fluid bottle.

1. Fill the Deactivation Fluid bottle with 5% sodium hypochlorite to a level between the liquid fill lines.
2. Pour entire contents of one bottle of Buffer for Deactivation Fluid into the Deactivation Fluid bottle.
3. Place the barcode label from the Buffer for Deactivation Fluid bottle on the top of the Deactivation Fluid bottle. This barcode is required to be scanned into the system during Fluid Inventory.
4. Record the date the Deactivation Fluid was prepared on the Buffer for Deactivation Fluid label.

M. To prepare System Fluid, combine PROCLEIX® System Fluid Preservative with CLSI Type 1 water in the System Fluid Bottle.

1. Fill the System Fluid Bottle to the liquid fill line with CLSI Type 1 water.
2. Pour entire contents of one bottle of PROCLEIX System Fluid Preservative into the System Fluid bottle.
3. Place the barcode label from the PROCLEIX System Fluid Preservative on the top of the System Fluid bottle. This barcode is required to be scanned into the system during Fluid Inventory.
4. Record the date the System Fluid was prepared on the System Fluid Preservative label.

N. Load Fluids on the PROCLEIX TIGRIS System according to instructions provided in the PROCLEIX TIGRIS System QRG.

PROCEDURAL NOTES

Note: Refer to the PROCLEIX® TIGRIS® System QRG for maintenance procedures and information about software operation.

A. To reduce the risk of invalid results, carefully read the entire package insert for the PROCLEIX® WNV Assay prior to performing an assay run. This package insert must be used with the PROCLEIX TIGRIS System QRG and any applicable technical bulletins.

B. RUN SIZE

1. Kit size is based on an average run size of 55 tests. Smaller run sizes will result in a lower yield.
2. Each run (also identified as a worklist) may contain up to 500 tests.

C. EQUIPMENT PREPARATION

See the PROCLEIX TIGRIS System QRG.

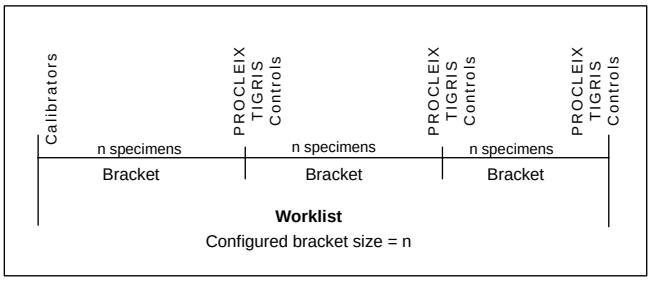
D. RUN CONFIGURATION

1. Each run (also identified as a worklist) must have a set of PROCLEIX® WNV Assay Calibrators at the beginning and a set of PROCLEIX® WNV TIGRIS® Controls at the end.

   a. A set of calibrators consists of one vial each of PROCLEIX® WNV Negative Calibrator and PROCLEIX® WNV Positive Calibrator. Each calibrator is run in triplicate.

   b. A set of PROCLEIX WNV TIGRIS Controls consists of one vial each of PROCLEIX® WNV TIGRIS® Negative Control and PROCLEIX® WNV TIGRIS® Positive Control. Each PROCLEIX WNV TIGRIS Control is run in singlet.

2. Using additional sets of PROCLEIX WNV TIGRIS Controls, each run (worklist) can be divided into smaller subsets called control brackets. A control bracket is defined as a group of specimens within a worklist that has a set of PROCLEIX WNV TIGRIS Controls at each end. The results of each bracket are reported based on the validity criteria of each control set (see PROCLEIX TIGRIS System Users, QUALITY CONTROL PROCEDURES for more details). The default bracket size is 172, but this feature is configurable in the PROCLEIX® TIGRIS® System Software and can be changed to any value between 1 and 492. In the first bracket of a worklist, PROCLEIX WNV TIGRIS Controls are not required at the beginning of the bracket.



3. A printed worklist report may assist operators in locating the rack and tube position where calibrators and controls are to be placed in a worklist. Refer to the PROCLEIX TIGRIS System QRG for instructions on how to view/print a worklist report.
4. Calibrator and PROCLEIX WNV TIGRIS Control tube placement is automatically read and verified by the PROCLEIX TIGRIS System. The PROCLEIX® TIGRIS® System will not allow assay processing if a calibrator or PROCLEIX WNV TIGRIS Control is placed in an incorrect tube position in a worklist or has an unreadable or missing barcode.
5. Test results from completed brackets of in-process run (worklist) can be viewed or printed by the operator before processing of the entire run is finished. Refer to the PROCLEIX TIGRIS System QRG for instruction on how to view/print test results.

E. WORK FLOW

1. Perform reagent preparation in a clean (amplicon- and template-free) area.
2. The sample loading area must be amplicon free.

F. ENVIRONMENTAL CONDITIONS

1. The operational conditions of the room in which the PROCLEIX TIGRIS System runs must be within a temperature of 15° to 25°C and humidity of 20 to 85%.
2. Refer to instrument and software operator's manuals for additional environmental conditions requirements.

G. DECONTAMINATION

1. The extremely sensitive nature of the test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces must be decontaminated daily with 0.5% sodium hypochlorite in water (diluted bleach). Allow bleach to contact surfaces for at least 15 minutes, then follow with a water rinse. Chlorine solutions may pit equipment and metal. Thoroughly rinse bleached equipment to avoid pitting.
2. A bleach alternative may be used in the sample preparation/ RPI areas only. **Do not use bleach alternatives on the PROCLEIX TIGRIS System.**
3. The PROCLEIX TIGRIS System automates the decontamination step by adding Deactivation Fluid to MTUs prior to disposal.

4. Follow instructions provided in the PROCLEIX TIGRIS System QRG for instrument decontamination and maintenance procedures.

H. CLSI TYPE 1 WATER

CLSI Type 1 water is required. Excursions up to 100 cfu/mL do not adversely affect assay results. Refer to manufacturer instructions for maintaining the water system.

ASSAY PROCEDURE

All specimens (individual donations or pooled specimens) should be run in singlet in the PROCLEIX® WNV Assay.

PROCLEIX® WNV Assay Calibrators are to be used with the corresponding master lot of the PROCLEIX WNV Assay. The operator must check to ensure that the PROCLEIX WNV Assay Calibrators are used with the corresponding master lot of kit reagents as indicated on the PROCLEIX WNV Assay master lot sheet in use. The software will generate an error if calibrators from a different master lot are used.

Specimens from other living donors (except whole blood or blood components) and from cadaveric donors must be tested neat using the individual donor testing method only. If the initial test result from a cadaveric blood specimen is invalid, the specimen may be diluted to overcome potential inhibitory substances as described in SPECIMEN COLLECTION, STORAGE AND HANDLING, Cadaveric Blood Specimens, and retested in singlet.

For equipment preparation, rack setup, and assay procedure information, see instructions in the PROCLEIX® TIGRIS® System QRG.

QUALITY CONTROL PROCEDURES

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX® WNV ASSAY

A. Run validity:
A run (also identified as a worklist) is valid if the minimum numbers of calibrators meet their acceptance criteria and are valid (see section II below).

1. In a PROCLEIX® WNV Assay run, at least four of the six calibrator replicates must be valid. At least two of the three Negative Calibrator replicates and two of the three Positive Calibrator replicates must be valid.
2. Calibrator acceptance criteria are automatically verified by the PROCLEIX® TIGRIS® System Software. If less than the minimum number of calibrator replicates is valid, the PROCLEIX TIGRIS System Software will automatically invalidate the run.
3. In a valid run, cutoff values will be automatically calculated for Internal Control (flasher) and analyte (glower).
4. If a run is invalid, sample results are reported as Invalid and all specimens must be retested.

B. Sample validity:

1. In a valid run, a sample result is valid if the IC signal is equal to or above the IC cutoff, with the following exceptions:
   a. Specimens with an analyte signal (glower signal) greater than the analyte cutoff are not invalidated even if the Internal Control (IC) signal is below the cutoff.
   b. Specimens with an IC signal above 750,000 RLU are invalidated by the software and their reactive status cannot be assessed. The software also automatically invalidates positive Calibrators and Positive PROCLEIX® WNV TIGRIS® Controls with an IC signal above 750,000 RLU.
2. A sample may also be invalidated due to instrument and results processing errors. Refer to the QRG for details.
3. All individual specimen results that are Invalid in a valid run or control bracket must be retested.

C. Control bracket validity:

1. A valid control bracket requires valid PROCLEIX® WNV TIGRIS® Control sets at the beginning and end of the bracket (excluding the first bracket which has calibrators at the beginning and PROCLEIX WNV TIGRIS Controls at the end). A set of PROCLEIX WNV TIGRIS Controls consists of one vial each of PROCLEIX® WNV TIGRIS® Negative Control and PROCLEIX® WNV TIGRIS® Positive Control. Each PROCLEIX WNV TIGRIS Control is run in singlet. A valid control set requires that all PROCLEIX WNV TIGRIS Controls in the set be valid. Controls acceptance criteria are automatically verified by the PROCLEIX TIGRIS System Software. Instructions for handling specimens in brackets with invalid PROCLEIX WNV TIGRIS Control sets are described in item E below.
2. In addition, a valid bracket requires that no more than 10% of the specimens in the bracket are invalid. If control bracketing is not being used, the 10% invalid rate is determined from all the specimens in the run. For runs or brackets of more than 50 specimens, the PROCLEIX TIGRIS System Software automatically applies the 10% invalid rate and nonreactive specimens are labeled as “Suspect” (see item D3 below). For runs or brackets of 50 or fewer specimens, the PROCLEIX TIGRIS System Software does not automatically apply the 10% invalid rate. The invalid rate must be manually calculated by the operator (see instructions in section F.2.). If individual specimens are invalidated by an operator outside the PROCLEIX TIGRIS System Software, then the 10% invalid rate must be manually recalculated. Instructions for handling suspect specimens due to greater than 10% invalid results are described in item F below.

D. Specimen results interpretation when bracket acceptance criteria are not met:

1. Specimens with an analyte S/CO <1.00 and IC RLU less than the IC cutoff will be marked as Invalid by the PROCLEIX TIGRIS System Software.
2. Specimens with an analyte S/CO greater than or equal to 1.00 and with IC signal between 0 and 750,000 RLU will be marked as Reactive by the PROCLEIX TIGRIS System Software and are the test of record.
3. Specimens with an analyte S/CO <1.00 and IC RLU greater than or equal to the IC cutoff will be flagged as Suspect by the PROCLEIX TIGRIS System Software. For the PROCLEIX® TIGRIS® System, the term “Suspect” refers to nonreactive specimens that are not automatically invalid, but must be further evaluated and resolved (see sections E and F).

E. Resolution of Suspect specimens due to invalid PROCLEIX WNV TIGRIS Control sets:

1. Suspect specimens that result from invalid PROCLEIX WNV TIGRIS Control sets are flagged with error code ”x” on the Assay Results Run Report. PROCLEIX WNV TIGRIS Controls may be invalid for one of two reasons (see the PROCLEIX® TIGRIS® System QRG for definitions):
   a. Instrument processing errors (error codes in UPPER CASE letters)
   b. Results processing errors (error codes in lower case letters)
2. If PROCLEIX WNV TIGRIS Control sets are invalidated due to instrument processing errors, results from all Suspect specimens should be considered valid non-reactive if the next set of PROCLEIX WNV TIGRIS Controls is valid. If no valid PROCLEIX WNV TIGRIS Control results are available in the subsequent bracket(s), all Suspect specimens should be considered invalid and be retested.
3. If PROCLEIX WNV TIGRIS Control results are invalidated due to results processing errors, all Suspect specimens should be considered invalid and be retested regardless of the status of subsequent PROCLEIX WNV TIGRIS Controls.

F. Resolution of Suspect specimens due to >10% invalid results:

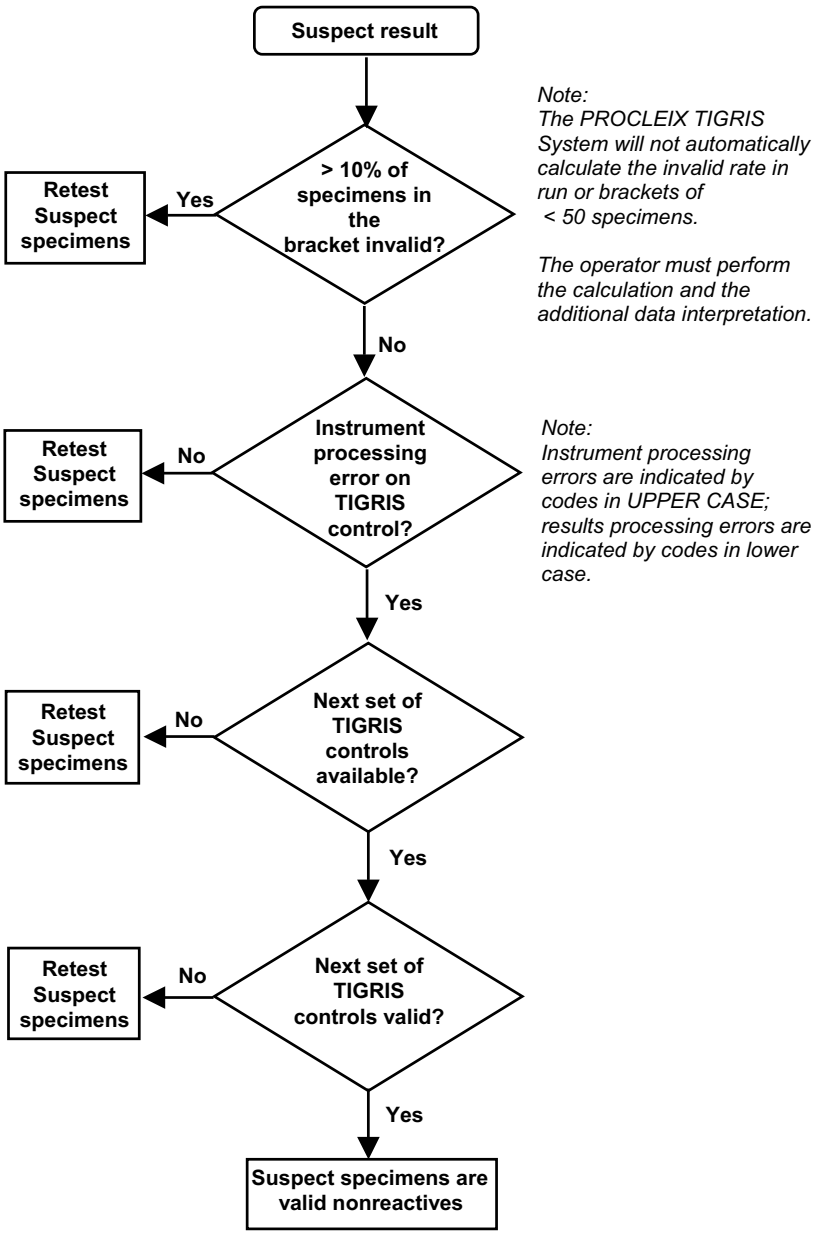
1. In a PROCLEIX WNV Assay bracket or run of more than 50 specimens, when more than 10% of the specimens in the bracket are invalid, specimens with an analyte S/CO less than 1.00 and IC RLU greater than or equal to the IC cutoff will be marked Suspect and flagged with error code “v” in the Run Report. All Suspect specimens in such runs or brackets must be retested.
2. For runs or brackets of 50 or fewer specimens, the operator must manually calculate the invalid rate. If more than 10% of the specimens in a run or bracket of 50 or fewer specimens are invalid due to multiple occurrences of the same error, the bracket(s) should be invalidated and any specimens that the software has not identified as reactive should be retested.

G. Summary of Specimen Result Interpretation
The following table and flow chart summarize results interpretation on the PROCLEIX TIGRIS System:

Interpretation assigned by PROCLEIX TIGRIS System Software on run report	Status of PROCLEIX WNV TIGRIS Controls for the bracket	Percent invalid specimens per bracket	Analyte S/CO	IC result	User Action Required
Reactive (test of record)	Valid or Invalid	NA	≥1.00	0 to 750,000 RLU	None
Valid, Non-reactive	Valid	≤10%	<1.00	≥IC C/O, ≤ 750,000 RLU	None
Valid, Non-reactive (for brackets less than 50 specimens)*	Valid	>10% (user calculated)	<1.00	≥ IC C/O, ≤750,000 RLU	Follow instructions in section F, step 2.
Suspect (marked with error code “v”)	Valid	>10%	<1.00	≥IC C/O, ≤750,000 RLU	Retest (see section F and flow chart below for Suspect results).
Suspect (marked with error code “x”)	Invalid	≤10%	<1.00	≥IC C/O, ≤750,000 RLU	Follow instructions in section E and flow chart below for Suspect results.
Invalid	NA	NA	NA	NA	Retest

* User must calculate the percent invalid for brackets with less than 50 specimens.
NA = Not applicable.

If Suspect results are observed in the Run Report, consult the following chart for direction:



Note: Specimens with an overall interpretation of Reactive, as determined by the software, must become the test of record. The specimens should be resolved according to the resolution algorithm for the reactive specimens, as explained in the PROCLEIX TIGRIS System USERS, INTERPRETATION OF RESULTS section.

Note: A run or an individual sample may also be invalidated by an operator if package insert instructions for specimen or reagent handling were not followed.

II. ACCEPTANCE CRITERIA FOR CALIBRATION AND CALCULATION OF CUTOFF

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate in the PROCLEIX® WNV Assay. Each individual Negative Calibrator replicate must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 400,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 40,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid due to an IC value or an analyte value outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator replicate values have IC values or analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator values (NC_x) for Internal Control [NC_x (Internal Control)]

Example:

Negative Calibrator	Internal Control Relative Light Units
1	235,000
2	200,000
3	210,000
Total Internal Control RLU =	645,000

$$NC_x \text{ (Internal Control)} = \frac{\text{Total Internal Control RLU}}{3} = 215{,}000$$

Determination of the mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)]

Example:

Negative Calibrator	Analyte Relative Light Units
1	14,000
2	16,000
3	15,000
Total Analyte RLU =	45,000

$$NC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 15{,}000$$

Positive Calibrator Acceptance Criteria

The Positive Calibrator is run in triplicate in the PROCLEIX® WNV Assay. Individual Positive Calibrator (PC) analyte values must be less than or equal to 2,700,000 RLU and greater than or equal to 400,000 RLU. IC values may not exceed 750,000 RLU. If one of the Positive Calibrator replicate values is outside these limits, the Positive Calibrator mean (PC_x) will be recalculated based upon the two acceptable Positive Calibrator replicate values. The run is invalid and must be repeated if two or more of the three Positive Calibrator analyte values are outside of these limits.

Determination of the mean of the Positive Calibrator (PC_x) values for Analyte [PC_x (Analyte)]

Example:

Positive Calibrator	Analyte Relative Light Units
1	1,250,000
2	1,500,000
3	1,150,000
Total Analyte RLU =	3,900,000

$$PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} \quad 1{,}300{,}000$$

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = 0.5 X [NC_x (Internal Control)]

Using values given in the Negative Calibrator example above:

Internal Control Cutoff Value = 0.5 X (215,000)

Internal Control Cutoff Value = 107,500 RLU

Calculation of the WNV Analyte Cutoff Value

Analyte Cutoff Value = NC_x (Analyte) + [0.03 X PC_x (Analyte)]

Using values given in the Negative Calibrator and Positive Calibrator examples above:

Analyte Cutoff Value = 15,000 + (0.03 X 1,300,000)

Analyte Cutoff Value = 54,000 RLU

Summary of Acceptance Criteria for PROCLEIX® WNV Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 75,000 and ≤ 400,000 RLU
Positive Calibrator	
Analyte	≥ 400,000 and ≤ 2,700,000 RLU
Internal Control	≤ 750,000 RLU

Summary of Cutoff Calculations for PROCLEIX® WNV Assay

Analyte Cutoff =	NC Analyte Mean RLU + [0.03 X (PC Analyte Mean RLU)]
Internal Control Cutoff =	0.5 X (Negative Calibrator IC Mean RLU)

III. ACCEPTANCE CRITERIA FOR PROCLEIX® WNV TIGRIS® CONTROLS IN THE PROCLEIX® WNV ASSAY

In the PROCLEIX® WNV Assay, a valid set of controls is required at the beginning and end of a bracket (excluding the first bracket, which only has controls at the end) for the results for that bracket to be valid. The PROCLEIX® WNV TIGRIS® Negative Control must have an S/CO less than 1.00 (nonreactive) to be accepted. The PROCLEIX® WNV TIGRIS® Positive Control must have an S/CO greater than or equal to 1.00 (reactive) and less than 100.00 to be accepted.

Acceptance Criteria:			
Negative Control			
Analyte	≥ 0	and	≤ 150,000 RLU
Analyte S/CO		< 1.00	
Internal Control	≥ 75,000	and	≤ 400,000 RLU
Internal Control S/CO		≥ 1.00	
Positive Control			
Analyte	≥ 0	and	≤ 2,700,000 RLU
Analyte S/CO	≥ 1.00	and	< 100.00
Internal Control		≤ 750,000 RLU	

INTERPRETATION OF RESULTS

All calculations described above are performed by the assay software of the PROCLEIX® TIGRIS® System. Two cutoffs are determined for each assay: one for the Analyte Signal (glower signal) termed the Analyte Cutoff and one for the Internal Control Signal (flasher signal) termed the Internal Control Cutoff. The calculation of these cutoffs is shown above. For each sample, an Analyte Signal RLU value and Internal Control Signal RLU value are determined. Analyte Signal RLU divided by the Analyte Cutoff is abbreviated as the Analyte Signal/Cutoff (S/CO) on the report.

A specimen is Nonreactive if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO <1.00) and the Internal Control (IC) Signal is greater than or equal to the Internal Control Cutoff (IC Cutoff) and less than or equal to 750,000 RLU. A specimen is Reactive if the Analyte Signal is greater than or equal to the Analyte Cutoff (i.e., Analyte S/CO ≥ 1.00) and the IC Signal is less than or equal to 750,000 RLU. Reactive results will be designated by the software. A specimen is invalid if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO <1.00) and the Internal Control Signal is less than the Internal Control Cutoff. Any specimen with Internal Control values greater than 750,000 RLU is considered Invalid and the reactive status cannot be assessed.

Cadaveric blood specimens, when tested neat, may be invalid due to inhibitory substances within the specimen. These invalid specimens may be diluted as in SPECIMEN COLLECTION, STORAGE AND HANDLING, Cadaveric Blood Specimens, and retested in singlet.

Summary of Specimen Interpretation:

Specimen Interpretation	Criteria
NonReactive	Analyte S/CO < 1.00 and IC ≥ IC Cutoff and IC ≤ 750,000 RLU
Reactive	Analyte S/CO ≥ 1.00 and IC ≤ 750,000 RLU
Invalid*	IC > 750,000 RLU or Analyte S/CO < 1.00 and IC < Cutoff

*For specimens with IC signal greater than 750,000 RLU, the specimen will be invalidated by the software and the reactive status cannot be assessed.

1. Any specimen, including cadaveric specimens, with an interpretation of Invalid in the PROCLEIX WNV Assay must be retested in singlet. Cadaveric specimens previously diluted 1:5 may be retested diluted 1:5.
2. If at any point in the testing algorithm there is insufficient volume to complete the testing then an alternate specimen from the index donation may be used as long as the storage criteria in the package insert are met.
3. Specimens with a valid Internal Control value and with an Analyte S/CO less than 1.00 in the PROCLEIX WNV Assay are considered Nonreactive for WNV RNA.
    a. IF THE NONREACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool is considered Nonreactive and no further testing is required.
    b. IF THE NONREACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION, then the individual specimen is considered Nonreactive for WNV and no further testing is required.
4. Specimens with an Analyte S/CO greater than or equal to 1.00 with IC Signal less than or equal to 750,000 RLU are considered Reactive.
    a. IF THE REACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool must be tested with the PROCLEIX WNV Assay.
        1. If an individual specimen tests Nonreactive with the PROCLEIX WNV Assay, then the specimen is considered Nonreactive for WNV and no further testing is required.
        2. If an individual specimen tests Reactive with the PROCLEIX WNV Assay, then the individual specimen is considered Reactive for WNV. Further clarification of the Reactive specimens for informational purposes may be obtained by testing an alternate specimen from the index donation with the PROCLEIX WNV Assay and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of donor eligibility.
    b. IF THE REACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION, then the individual specimen is considered Reactive for WNV. Further clarification of the Reactive specimens for informational purposes may be obtained by testing an alternate specimen from the index donation with the PROCLEIX WNV Assay and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of donor eligibility.
5. Reactive specimens in an operator-invalidated run due to the 10% invalid rate (see QUALITY CONTROL PROCEDURES for PROCLEIX® TIGRIS® SYSTEM USERS, step F) are identified by the software as reactive and must become the test of record. Any reactive result should be resolved according to the resolution algorithm for reactive specimens, as explained in the INTERPRETATION OF RESULTS section, step 4 above.

GENERAL INFORMATION

LIMITATIONS OF THE PROCEDURE

This assay has been evaluated with the PROCLEIX® System and PROCLEIX® TIGRIS® 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/mL. Samples with less than 100 copies/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 the procedures provided.

Deviations from these procedures, adverse shipping and/or storage conditions, or use of outdated calibrators and/or reagents may produce unreliable results.

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

PROCLEIX® System

Reproducibility of the PROCLEIX® WNV Assay was evaluated at three blood testing laboratories. For determination of reproducibility, a ten-member panel comprised of tissue culture-derived WNV was procured from Boston Biomedica (BBI, West Bridgewater, MA) (Table 1). Seven panel members were positive for WNV (50, 50, 100, 100, 300, 1,000, and 10,000 copies/mL) and three panel members were WNV negative.

The reproducibility panel members were tested by six operators (two from each testing site) with three different clinical reagent kit 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.

Of the 79 runs generated on the PROCLEIX® System, 6 (7.6%) were invalid. Of these invalidated runs, 4 were invalidated by the instrument due to an insufficient number of valid calibrators. The remaining 2 of 6 runs were invalidated by the operator: 1 was due to operator error and 1 was invalidated because the run contained greater than 10% invalid test results that were due to Internal Control (IC) failures. From the valid assay runs, 2,162 test results were generated. Of these, 17 (0.8%) were invalid due to IC failures.

In Table 1, assay signal values were expressed as Analyte Signal to Cutoff (S/CO) ratios for panel members containing target and as IC S/CO ratios for negative panel members. Signals were expressed as analyte Relative Light Units (RLU) for the Positive Calibrator and as IC RLU for the Negative Calibrator 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 CLSI guidelines (EP5-A, page 7), numbers <0 are recorded as 0.

PROCLEIX® TIGRIS® System

Reproducibility of the PROCLEIX® WNV Assay was evaluated at three blood testing laboratories. For determination of the reproducibility of each assay, 10 members from a reproducibility panel were tested as individual samples (Table 2). The panel, comprised of tissue culture-derived WNV, was procured from Boston Biomedica (BBI, West Bridgewater, MA). Seven panel members were positive for WNV (50, 50, 100, 100, 300, 1,000, and 10,000 copies/mL) and three panel members were WNV negative.

The reproducibility panel members were tested by a total of six operators (two from each testing site) with three different clinical reagent kit lots over multiple days using three PROCLEIX® TIGRIS® System instruments. Each operator performed three worklists (i.e., runs) per PROCLEIX WNV Assay clinical reagent kit lot on one of the three PROCLEIX TIGRIS System instruments. Nine worklists were completed by each operator for a total of 54 worklists overall. The worklists were repeated three times, totaling 162 results per panel member.

Of the 62 runs generated on the PROCLEIX TIGRIS System, 7 (11.3%) were invalid. Of these invalid runs, 6 were due to one incident of a hardware error in 1 run, which was invalidated by the operator: the error caused the instrument to shut down and the 5 subsequent runs in the queue were invalidated by the instrument. The remaining 1 of 7 invalidated runs was invalidated by the operator because the run contained greater than 10% invalid test results that were due to an instrument communication failure. From the valid assay runs, 1,620 test results were generated. Of these, 1 (0.1%) was invalid due to an assay processing error.

In Table 2, 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 Calibrator and as IC RLU for the Negative Calibrator in the PROCLEIX WNV Assay. Signal variability of the runs 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 to expected outcome and mean S/CO ratio or RLU.

The overall percent agreement of test results with expected outcomes was 99.8% for negative panels and greater than or equal to 99.7% for positive panel members. With regard to variability, inter-run and intra-run (or random error) factors were the largest and second largest contributors, respectively, 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 11.2% for any positive or negative samples. The inter-lot %CVs were 4.1% or less and the inter-instrument %CVs were 9.9% or less, indicating that these factors had less impact on assay performance. Therefore, the reproducibility of the assay is robust.

Table 2. PROCLEIX® TIGRIS® System - 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-Instrument	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative**	3	0	486	99.8	2.1	0.1	5.9	0.1	2.8	0.0***	0	0.0***	0
WNV	2	50	324	99.7	28.6	3.2	11.2	2.8	9.8	1.2	4.1	2.2	7.8
WNV	2	100	323	99.7	29.1	3.0	10.2	2.8	9.6	0.7	2.4	2.0	6.9
WNV	1	300	162	100	28.9	1.2	4.1	2.5	8.8	1.1	3.8	2.9	9.9
WNV	1	1,000	162	100	28.8	1.2	4.1	2.5	8.8	1.1	3.9	2.8	9.7
WNV	1	10,000	162	100	30.1	1.4	4.6	2.5	8.4	0.8	2.6	2.0	6.7
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**			156	N/A	148,970.3	9,051.6	6.1	3,888.5	2.6	3,741.8	2.5	11,715.6	7.9
WNV Positive Calibrator			161	N/A	1,514,280.8	68,309.2	4.5	0.0***	0.0	12,630.8	0.8	20,206.2	1.3

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

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. Nine hundred four (904) runs were generated from testing of the pools and individual donor samples (IDS) on the PROCLEIX System. Of these, 14 (1.5%) runs were invalid. Ten of the 14 runs were invalidated by the instrument due to an insufficient number of valid calibrators. The remaining 4 of 14 runs were invalidated by the operator: 3 contained greater than 10% invalid test results due to Internal Control failures, and 1 was due to operator error. From the valid assay runs, 16,885 and 43,503 test results were generated from pools and IDS, respectively; none were invalid.

Specificity of the PROCLEIX WNV Assay was calculated from 16,885 16-sample pools and 43,503 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 3. 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 immunoglobulin M (IgM) assay.

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 nonreactive 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 nonreactive 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 nonreactive 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 3. PROCLEIX® System - 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 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 4). 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 nonreactive 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 nonreactive PROCLEIX WNV Assay results upon retest. These samples were IgM negative and Alternate NAT nonreactive 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 nonreactive 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 nonreactive 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 4. PROCLEIX® System- 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

NON-SPECIFICITY STUDIES

SPECIFICITY AND SENSITIVITY OF THE PROCLEIX® WNV ASSAY IN THE PRESENCE OF DONOR AND DONATION FACTORS

PROCLEIX® System

To test for cross-reactivity, specimens with various donor and donation factors were tested with the PROCLEIX® WNV Assay. To test for interference, detection (sensitivity) of the PROCLEIX WNV Assay was evaluated by spiking the various donor and donation specimens to a final concentration of 150 copies/mL of WNV.

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, 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 (n=10) and type 2 (n=10). Also tested were donor samples from influenza virus (n=10) 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=4), 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.

PROCLEIX® TIGRIS® System

To test for cross-reactivity, specimens with various donor and donation factors were tested with the PROCLEIX® WNV Assay. To test for interference, detection (sensitivity) of the PROCLEIX WNV Assay was evaluated by spiking the various donor and donation specimens to a final concentration of 150 copies/mL of WNV.

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), multiple sclerosis (n=6), rheumatoid factor (n=10), antinuclear antibody (n=10), lupus (n=10) and multiple myeloma (n=10). Also tested were samples from patients with elevated hyperglobulinemia, 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. No cross-reactivity was observed 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 (n=9) and 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=4), cytomegalovirus (n=11), Epstein-Barr virus (n=10), rubella virus (n=10), and human immunodeficiency virus type 1 (n=11). Donor samples from influenza virus vaccinees (n=10) were also tested with no cross-reactivity or interference.

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 5).

Of the 15 runs generated on the PROCLEIX System for the clinical sensitivity study, none were invalid. Of the 202 and 203 test results generated from neat and 1:16 diluted samples, respectively, none were invalid.

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 5. PROCLEIX® System - 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.

Known-positive pools were tested in the same runs with the neat and 1:16 diluted known-positive samples described above; all runs were valid. Of the 98 test results generated from pooled samples tested, none were invalid. The sensitivity of the PROCLEIX WNV Assay in 98 known-positive pools in this study was 100% (95% CI: 96.3-100%) (Table 6).

Table 6. PROCLEIX® System - 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 for both the PROCLEIX® System and the PROCLEIX® TIGRIS® System. Reactivity at 10 copies/mL was 97% and 91% for the PROCLEIX System and the PROCLEIX TIGRIS System, respectively. At 3 copies/mL, the detection rates were 53% and 58% for the PROCLEIX System and the PROCLEIX TIGRIS System, respectively (Tables 7a and 7b).

Table 7a. PROCLEIX® System - Detection of WNV RNA in Health Canada Analytical Sensitivity Panel

WNV RNA 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	42	63	23.43	35
1	26/89	29	20	40	21.10	49
0	0/89	0	0	3	0.06	120

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

Table 7b. PROCLEIX® TIGRIS® System - Detection of WNV RNA in Health Canada Analytical Sensitivity Panel

WNV RNA copies/mL	Number of reactive/ tested*	% Reactive	95% CI		Average S/CO**	%CV
			Lower	Upper		
100	77/77	100	96	100	30.22	14
30	74/74	100	96	100	29.21	18
10	82/90	91	83	96	26.52	27
3	52/90	58	47	68	24.16	36
1	19/90	21	13	31	17.03	61
0	0/90	0	0	3	0.11	101

*Samples were QNS for 30 replicates with one of the three clinical lots
**Average of reactive replicates unless all tests were nonreactive, in which case the average analyte S/CO of nonreactive 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 RNA in this study was 8.2 copies/mL for the PROCLEIX® System and 9.8 copies/mL for the PROCLEIX® TIGRIS® System with the Health Canada Sensitivity Panel (Table 8).

Table 8. Detection Probabilities of WNV RNA 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)
PROCLEIX® TIGRIS® System	4.0 (1.7 – 8.8)	9.8 (6.5 – 27.3)

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. The same panel was tested in ten replicates using the PROCLEIX® TIGRIS® System with one instrument and one lot of reagents. Detection of all panel members with a WNV RNA titer of 100 copies/mL or greater was 100% with both the PROCLEIX System and the PROCLEIX TIGRIS System (Tables 9a and 9b).

Table 9a. PROCLEIX® System - 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 nonreactive, in which case the average analyte S/CO of nonreactive replicates is shown.

Table 9b. PROCLEIX® TIGRIS® System - 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	10/10	100	31.88	4
2	NY99	10	8/10	80	28.82	24
3	Hu2002	0	0/9	0	0.03	131
4	Hu2002	50	9/10	90	31.15	3
5	NY99	0	0/9	0	0.04	115
6	NY99	1000	10/10	100	31.43	4
7	Hu2002	100	10/10	100	30.64	8
8	Hu2002	1000	9/9	100	29.73	6
9	Hu2002	5	10/10	100	29.67	12
10	NY99	5	7/10	70	20.80	50
11	NY99	500	10/10	100	30.66	4
12	Hu2002	10	10/10	100	29.40	11
13	NY99	50	9/10	90	31.14	4
14	Hu2002	500	10/10	100	31.30	6

*Only valid reactions were included
**Average of reactive replicates unless all tests were nonreactive, in which case the average analyte S/CO of nonreactive 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 each tested 10 to 20 replicates of each copy level with each of the three clinical lots using the PROCLEIX® System for a total of 100 replicates. The same panel was tested using the PROCLEIX TIGRIS System. Three instruments were each used to test 10 replicates of each copy level with each of the three clinical lots for a total of 90 replicates. The 95% confidence intervals of the reactive rates were based on the exact binomial distribution.

WNV RNA detection with the PROCLEIX® WNV Assay was 98% and 100% at 100 copies/mL for the PROCLEIX System and the PROCLEIX® TIGRIS® System, respectively. Reactivity at 30 copies/mL was 99% for the PROCLEIX System and 97% for the PROCLEIX TIGRIS System. At 10 copies/mL, the detection rates were 89% for the PROCLEIX System and 82% for the PROCLEIX TIGRIS System (Tables 10a and 10b).

Table 10a. PROCLEIX® System - Detection of Lineage 2 WNV in BBI Analytical Sensitivity Panel

WNV RNA 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 nonreactive, in which case the average analyte S/CO of nonreactive replicates is shown
CI = Confidence Interval

Table 10b. PROCLEIX® TIGRIS® System - Detection of Lineage 2 WNV in BBI Analytical Sensitivity Panel

WNV RNA copies/mL	Number reactive/ tested*	% Reactive	95% CI		Average S/CO**	%CV
			Lower	Upper		
100	90/90	100	97	100	10.72	19
30	85/88	97	90	99	5.78	45
10	73/89	82	72	89	2.60	66
3	15/88	17	10	27	1.64	45
1	1/90	1	0	6	1.09	n/a
0	0/90	0	0	3	0.08	113

*Only valid reactions included
**Average of reactive replicates unless all tests were nonreactive, in which case the average analyte S/CO of nonreactive 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 spiked with 150 copies/mL WNV were tested with 3 clinical reagent kit lots: one lot was tested on only the PROCLEIX® System, a second lot was tested on both the PROCLEIX System and the PROCLEIX® TIGRIS® System, and a third lot was tested on only the PROCLEIX TIGRIS System. The reactive rates, S/COs, and %CVs are shown in Table 11. For the WNV spiked specimens tested with the PROCLEIX® System, the %CVs for the cadaveric and control specimens were 18% and 14%, respectively. For the WNV spiked specimens tested with the PROCLEIX TIGRIS System, the cadaveric and controls specimen %CVs were 8% and 7%, respectively. The percent reactive rate for cadaveric specimens and control specimens in this study was 100% for both the PROCLEIX System and the PROCLEIX TIGRIS System.

Table 11. 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
PROCLEIX® TIGRIS® System	Cadaveric	20	120	100% (97.5-100)	28.56	8
	Control	20	120	100% (97.5-100)	29.03	7

CI = Confidence Interval

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 and 51 cadaveric specimens and 51 normal blood donor specimens were tested on the PROCLEIX® TIGRIS® System. The cadaveric and control specimens 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%) for both the PROCLEIX System and the PROCLEIX TIGRIS System (Tables 12a and 12b). No invalid results were observed with the cadaveric specimens.

Table 12a. PROCLEIX® System - 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

Table 12b. PROCLEIX® TIGRIS® System - Specificity of PROCLEIX® WNV Assay with Cadaveric Blood Specimens

	Control	Cadaveric
n	51	51
Mean IC S/CO	2.05	2.16
Analyte S/CO	0.20	0.16
Percent Specificity	100	100
95% CI	94-100	94-100

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 6 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 and 51 cadaveric specimens and 51 normal blood donor specimens were tested on the PROCLEIX® TIGRIS® 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%) for both the PROCLEIX System and the PROCLEIX TIGRIS System (Tables 13a and 13b). No invalid results were observed with the cadaveric samples.

Table 13a. PROCLEIX® System - 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

Table 13b. PROCLEIX® TIGRIS® System - Sensitivity of the PROCLEIX® WNV Assay in Cadaveric Blood Specimens

	Control	Cadaveric
n	50*	51
Analyte S/CO	23.81	26.55
Percent Sensitivity	100	100
95% CI	94-100	94-100

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

COMPARABILITY OF THE PROCLEIX® TIGRIS® SYSTEM AND THE PROCLEIX® SYSTEM

The comparability of the PROCLEIX® TIGRIS® System and the PROCLEIX® System was evaluated in panels composed of WNV positive and negative samples. The panels tested in the PROCLEIX® WNV Assay (n=510) contained positive members that were IgM-positive with RNA copy levels greater than 300 copies/mL, IgM-negative with RNA copy levels greater than 300 copies/mL, IgM-positive with RNA copy levels less than or equal to 300 copies/mL, IgM-negative with RNA copy levels less than or equal to 300 copies/mL, and negative members with various anticoagulants, interfering substances, and blood-borne pathogens. Three replicates of each panel were tested on the PROCLEIX TIGRIS System at three sites and on the PROCLEIX System at one site. The contents of the panels were masked during testing to control for bias. Testing was performed using one PROCLEIX WNV Assay clinical lot.

Of the 20 runs generated on the PROCLEIX System, 2 (10.0%) were invalidated by the operator because of operator error. From the valid assay runs, 2 of 1,526 (0.1%) test results were invalid on the PROCLEIX System; both were due to Internal Control failures. For the PROCLEIX TIGRIS System, 2 of 26 (7.7%) runs were invalid; both runs were invalidated by the operator because they contained more than 10% invalid test results. From the valid assay runs, 16 of 4,570 (0.4%) test results were invalid on the PROCLEIX TIGRIS System. Of the 16 invalid test results, 1 was due to Internal Control failure, 7 were due to instrument failures, 7 were due to clots in the samples, and 1 was due to insufficient sample volume.

To demonstrate equivalent performance in the samples with valid test results, the accuracy was calculated for each system using the PROCLEIX WNV Assay. The accuracies of the two systems were compared for all positive samples, the subcategories of the positive samples, all negative samples, and all samples combined. In addition, analysis of the S/CO values (IC for negative samples and analyte for positive samples) was performed for each system using the PROCLEIX WNV Assay. The S/CO values of the two systems were compared for all positive samples, the subcategories of the positive samples, and all negative samples.

Performance of the PROCLEIX WNV Assay on the PROCLEIX TIGRIS System was equivalent to that of the PROCLEIX System. The accuracy for all sample types was 99.6% (95% CI: 99.1%-99.9%) for the PROCLEIX System and 99.9% (95% CI: 99.8%-100%) for the PROCLEIX TIGRIS System (Table 14a). The accuracies were also similar between the two systems when using the PROCLEIX WNV Assay for the positive and negative samples. The mean analyte S/CO values for the positive samples generated from the PROCLEIX WNV Assay were 29.32 for the PROCLEIX System and 30.35 for the PROCLEIX TIGRIS System (Table 14b). The mean analyte S/CO values were also similar between the two systems for the various positive sample subcategories. The mean IC S/CO values for the negative samples were 1.96 for the PROCLEIX System and 2.11 for the PROCLEIX TIGRIS System.

Table 14a. Comparison of PROCLEIX® WNV Assay Performance with the PROCLEIX® TIGRIS® System and the PROCLEIX® System - Analysis of Accuracy

Sample Type	PROCLEIX® System			PROCLEIX® TIGRIS® System		
	Correct	Total	Accuracy (%) (95% CI)	Correct	Total	Accuracy (%) (95% CI)
All Samples	1518	1524	99.6 (99.1, 99.9)	4551	4554	99.9 (99.8, 100)
Positive Samples	297	299	99.3 (97.6, 99.9)	887	887	100 (99.6, 100)
> 300 copies/mL, IgM +	9	9	100 (66.4, 100)	27	27	100 (87.2, 100)
> 300 copies/mL, IgM -	146	146	100 (97.5, 100)	436	436	100 (99.2, 100)
≤ 300 copies/mL, IgM +	25	27	92.6 (75.7, 99.1)	80	80	100 (95.5, 100)
≤ 300 copies/mL, IgM -	117	117	100 (96.9, 100)	344	344	100 (98.9, 100)
Negative Samples	1221	1225	99.7 (99.2, 99.9)	3664	3667	99.9 (99.8, 100)

IgM+ = IgM positive
IgM- = IgM negative
CI = Confidence Interval
N/A = not applicable

Table 14b. Comparison of the PROCLEIX® WNV Assay Signal to Cutoff Values for the PROCLEIX® TIGRIS® System and the PROCLEIX® System

Sample Type	n		Mean S/CO		SD		%CV	
	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System
Positive Samples	297	887	29.32	30.35	4.26	3.60	14.52	11.87
> 300 copies/mL, IgM +	9	27	31.31	30.32	4.58	3.40	14.62	11.21
> 300 copies/mL, IgM -	146	436	29.80	30.66	3.76	3.41	12.62	11.14
≤ 300 copies/mL, IgM +	25	80	27.46	27.86	5.89	6.20	21.44	22.27
≤ 300 copies/mL, IgM -	117	344	28.97	30.55	4.30	2.72	14.85	8.89
Negative Samples*	1221	3664	1.96	2.11	0.15	0.15	7.68	7.15

n = Number of samples
S/CO = Signal to cutoff ratio
SD = Standard deviation
CV = Coefficient of variation
* Analysis of internal control signal

An additional migration study sensitivity panel in which approximately 50% of the specimens were below 100 copies/mL was tested on the PROCLEIX System and the PROCLEIX TIGRIS System at 1 site. An analysis of accuracy of both systems for detection of the 414 replicates tested (3 replicates of 138 unique WNV-positive clinical specimens) is shown in Table 15a. With samples at or above 100 copies/mL, 178/180 (98.9%) of the replicates tested were detected on the PROCLEIX System, compared to 179/180 (99.4%) on the PROCLEIX TIGRIS System. With samples below 100 copies/mL, 183/234 replicates (78.2%) were detected on the PROCLEIX System and 170/234 (72.6%) on the PROCLEIX TIGRIS System. Although small differences were seen in the overall results (e.g. greater detection with the PROCLEIX TIGRIS System at or above 100 copies/mL and greater detection with the PROCLEIX System below 100 copies/mL) there were no statistically significant differences between the performances of the two platforms, as the 95% confidence intervals for the percent differences in each case included 0. In addition to the WNV-positive samples tested, 20 unique WNV-negative specimens were tested in 3 replicates each (60 replicates on each platform), yielding all non-reactive results on both the PROCLEIX System and PROCLEIX TIGRIS System platforms (Table 15a).

The mean analyte S/CO values for the positive samples generated from the PROCLEIX WNV Assay were 25.71 for the PROCLEIX System and 23.92 for the PROCLEIX TIGRIS System (Table 15b). The mean analyte S/CO values for the positive samples with less 100 copies/mL WNV were 19.90 for the PROCLEIX System and 18.51 for the PROCLEIX TIGRIS System. The mean analyte S/CO values for samples with greater than or equal to 100 copies/mL WNV were 33.27 for the PROCLEIX System and 30.95 for the PROCLEIX TIGRIS System. The mean IC S/CO values for the negative samples were 1.98 for the PROCLEIX System and 2.05 for the PROCELIX TIGRIS System.

Table 15a. Additional Migration Study: Accuracy of the PROCLEIX® WNV Assay on the PROCLEIX® TIGRIS® System Compared to the PROCLEIX® System

Sample Type	PROCLEIX® System			PROCLEIX® TIGRIS® System			
	Correct	Total	Accuracy (%) (95% CI)	Correct	Total	Accuracy (%) (95% CI)	Difference (%) (95% CI)
All Positive Sample Replicates	361	414	87.2 (82.8, 91.6)	349	414	84.3 (79.5, 89.1)	2.90 (–0.48, 6.28)
< 100 copies/mL	183	234	78.2 (71.1, 85.2)	170	234	72.6 (65.2, 80.1)	5.56 (–0.05, 11.16)
≥ 100 copies/mL	178	180	98.9 (96.3, 100)	179	180	99.4 (98.4, 100)	–0.56 (–2.99, 1.88)
Negative Samples	60	60	100 (95.1, 100)	60	60	100 (95.1, 100)	N/A

CI = Confidence Interval
N/A = not applicable

Table 15b. Additional Migration Study: Comparison of the PROCLEIX® WNV Assay Signal to Cutoff Values for the PROCLEIX® TIGRIS® System and the PROCLEIX® System

Sample Type	n		Mean S/CO		SD		%CV	
	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System
All Positive Sample Replicates	414	414	25.71	23.92	12.85	12.09	49.96	50.56
< 100 copies/mL	234	234	19.90	18.51	12.87	13.51	64.65	72.99
≥ 100 copies/mL	180	180	33.27	30.95	7.98	3.45	23.99	11.15
Negative Samples*	60	60	1.98	2.05	0.11	0.11	5.63	5.34

n = number of samples
S/CO = Signal to cutoff ratio
SD = Standard deviation
CV = Coefficient of variation
* Analysis of internal control signal

BIBLIOGRAPHY

1. **Campbell G. L., A. A. Marfin, R. S. Lanciotti, and D. J. Gubler.** 2002. West Nile Virus. Lancet Infect Dis. **2**:519-529.
2. **Petersen L. R., and A. A. Marfin.** 2002. West Nile Virus: A Primer for the Clinician. Ann Intern Med. **137**:173-179.
3. **Centers for Disease Control.** January 24, 2002. West Nile Virus (WNV) infection: information for clinicians. Clinical features. Website posting.
4. **Centers for Disease Control.** September 29, 2004. West Nile Virus: Background Information for Clinicians. Website posting.
5. **Centers for Disease Control.** January 8, 2003. West Nile Virus: Virus History and Distribution. Website posting.
6. **Center for Biologics Evaluation and Research (CBER).** May 2003. Guidance for Industry: Revised Recommendations for the Assessment of Donor Suitability and Blood and Blood Product Safety in Cases of Known or Suspected West Nile Virus Infection, Final Guidance.
7. **Centers for Disease Control.** December 23, 2002. West Nile Virus: Questions and Answers. Website posting.
8. **Centers for Disease Control.** Statistics, Surveillance, and Control: Case Count 2002 (posted September 17, 2003), 2003 (posted May 21, 2004), and 2004 (posted November 30, 2004). Website postings.
9. **Centers for Disease Control.** March 13, 2003. Update on Investigations of West Nile Virus Transfusion Transmitted Cases. Presentation at the Blood Products Advisory Board.
10. **Centers for Disease Control.** September 13, 2002. Public Health Dispatch: Investigation of Blood Transfusion Recipients with West Nile Virus Infections. MMWR. **51**:823.
11. **Centers for Disease Control.** 2002. CDC Public Health Dispatch: Possible West Nile Virus Transmission to an Infant through Breast-Feeding. MMWR. **51**:877-878.
12. **Centers for Disease Control.** February 27, 2004. Interim Guidelines for the Evaluation of Infants Born to Mothers Infected with West Nile Virus During Pregnancy. MMWR. **53**:154-157.
13. **Centers for Disease Control.** August 20, 2004. Possible Dialysis-Related West Nile Virus Transmission. MMWR. **53**:738-739.
14. **Centers for Disease Control.** April 9, 2004. Update: West Nile Virus Screening of Blood Donations and Transfusion-Associated Transmission. MMWR. **53**:281-284.
15. **Centers for Disease Control.** September 17, 2004. Transfusion-Associated Transmission of West Nile Virus. MMWR. **53**:842-844.
16. **Giachetti, C., J. M. Linnen, D. P. Kolk, J. Dockter, et al.** 2002. Highly Sensitive Multiplex Assay for Detection of Human Immunodeficiency Virus Type 1 and Hepatitis C Virus RNA. J Clin Microbiol. **40**:2408-2419.
17. **Kacian, D. L. and T. J. Fultz.** 1995. Nucleic acid sequence amplification methods. U.S. Patent 5,399,491.
18. **Arnold, L. J., P. W. Hammond, W. A. Wiese, and N. C. Nelson.** 1989. Assay formats involving acridinium-ester-labeled DNA probes. Clin Chem **35**:1588-1594.
19. **Nelson, N. C., A. Cheikh, E. Matsuda and M. Becker.** 1996. Simultaneous detection of multiple nucleic acid targets in a homogeneous format. Biochem. **35**:8429-8438.
20. **Title 42, Code of Federal Regulations**, Part 72, 2004.
21. **29 CFR Part 1910.1030.** Occupational exposure to bloodborne pathogens; Final Rule, Federal Register/Vol. **56**, No. 235/ December 6, 1991.
22. **National Committee for Clinical Laboratory Standards.** 1986. Clinical laboratory hazardous waste; proposed guidelines. NCCLS Document GP5-P. Villanova, PA.
23. **U. S. Environmental Protection Agency.** EPA guide for infectious waste management. Washington, DC: U.S. Environmental Protection Agency, Publication No. EPA/530-SW-86-014, 1986.

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