Procleix® WNV Assay on the **Procleix®** Panther System

For *In Vitro* Diagnostic Use

IVD

Rx Only

5000 Test Kit

CONTENTS

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 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 donors of whole 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 initial 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 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] Two major lineages of WNV have been defined.[8] In North America, Lineage 1 predominates although some genetic drift has been documented relative to the NY99 strain at the root of the current epidemic.[9] Recent outbreaks of WNV in Central and Southern Europe include a number of cases of Lineage 2 WNV.[10] Human WNV infection was reported in Italy, Romania, and Greece on a seasonal basis between 2008 and 2011.[11,12,13] Analysis of many of these cases reveals homology to WNV Lineage 2 identified in Hungary in 2004.[14] Other studies have proposed additional WNV Lineages in Europe and Southeast Asia.[15]

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-transmitted infection (TTI) were reported to CDC; of these, six cases were classified as confirmed TTI cases.[8-14] Since the implementation of WNV screening, 12 TTI cases have been documented.[15] As of January 12, 2016, 2,060 human WNV cases were reported to the CDC for the 2015 calendar year, of which 66% were characterized by neuroinvasive disease. For this same period, 332 presumptive positive blood donations were identified.[16]

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.[17] The Procleix WNV Assay detects WNV RNA and uses the same technology as the Procleix HIV-1/HCV Assay, Procleix Ultrio Assay, and Procleix Ultrio Plus Assay. The assay has been utilized in the United States for prospective blood screening since June 19, 2003 and was licensed in 2005.

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);[26] 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 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.

The Procleix WNV Assay Calibrators are to be used to determine the assay cutoff and assess assay run validity in the Procleix WNV Assay.

REAGENTS

Procleix WNV Assay Reagents

Internal Control Reagent

A HEPES buffered solution containing detergent and an RNA transcript.

*Store **unopened reagent** at –35° to –15°C.*

Target Capture Reagent

A HEPES buffered solution containing detergent, capture oligonucleotides, and magnetic microparticles. Internal Control Reagent must be added to Target Capture Reagent before use in the assay.

Store at 2° to 8°C. (Do not freeze)

Amplification Reagent

Primers, dNTPs, NTPs, and cofactors in TRIS buffered solution containing ProClin® 300 preservative.

*Store **unopened reagent** at –35° to –15°C.*

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 –35° to –15°C.*

Probe Reagent

Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.

*Store **unopened reagent** at –35° to –15°C.*

Selection Reagent

Borate buffered solution containing surfactant.

Store at 15° to 30°C.

Procleix WNV Assay Calibrators

C0

Procleix WNV Assay Negative Calibrator

A HEPES buffered solution containing detergent.

Store at –35° to –15°C.

C1

Procleix WNV Assay Positive Calibrator

A HEPES buffered solution containing detergent and a WNV RNA transcript.

Store at –35° to –15°C.

Procleix Panther System Reagents

R1

Auto Detect 1

Aqueous solution containing hydrogen peroxide and nitric acid.

*Store **unopened reagent** at 15° to 30°C.*

R2

Auto Detect 2

1.6 N Sodium Hydroxide.

*Store **unopened reagent** at 15° to 30°C.*

W

Wash Solution

HEPES buffered solution.

*Store **unopened reagent** at 15° to 30°C.*

O

Oil

Silicone oil.

*Store **unopened reagent** at 15° to 30°C.*

DF

Buffer for Deactivation Fluid

Sodium bicarbonate buffered solution.

*Store **unopened reagent** at 15° to 30°C.*

STORAGE AND HANDLING 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.

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

D. 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.

E. Do not use assay-specific reagents from any other Procleix assay.

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. For instructions on preparation of reagents, see instructions under REAGENT PREPARATION and the *Procleix Reagent Preparation Incubator Operator's Manual*.

Note: The Procleix WNV Assay is a 500 test kit and is not validated for use with the RPI 250.

K. Consult the following table for storage information.

Reagent/Fluid	**Unopened Reagent**	**Opened Reagent (Opened/Thawed Stability)***		
	Storage Temperature	**Room Temperature**	**Onboard Stability**	**Storage Temperature**
Internal Control Reagent (IC)	-35° to -15°C	Up to 8 hours at RT prior to combining with TCR		
Target Capture Reagent (TCR)	2° to 8°C			
working Target Capture Reagent (wTCR)		75 hours	60 hours	60 days at 2° to 8°C
Amplification Reagent	-35° to -15°C	75 hours	60 hours	60 days at 2° to 8°C
Enzyme Reagent	-35° to -15°C	75 hours	60 hours	60 days at 2° to 8°C
Probe Reagent	-35° to -15°C	75 hours	60 hours	60 days at 2° to 8°C
Selection Reagent	RT	60 days	60 hours	60 days at RT
Calibrators	-35° to -15°C	8 hours, single-use reagent		
Auto Detect Reagents	RT	60 days at RT		
Buffer for Deactivation Fluid	RT	60 days at RT		
Oil	RT	60 days at RT		
Wash Solution	RT	60 days at RT		

RT = Room Temperature

RT stability includes onboard stability time on the Procleix Panther System.

- The RT stability period starts as soon as the reagents are removed from the Procleix Reagent Preparation Incubator (RPI) after the preparation program is completed.
- If opened reagents are placed in the RPI at the room temperature program, the time duration is included in the total RT stability.
- The RT stability time must occur within 60 days, which includes onboard stability. See REAGENT PREPARATION, Item C for more information.

* If using Panther System Software version 7.2 and higher:

- RT stability (wTCR and Amplification, Enzyme, and Probe Reagents) is 84 hours.
- Onboard stability (wTCR and Amplification, Enzyme, Probe, and Selection Reagents) is 72 hours.

If using RPI File 3 for thawing unopened reagents (TCR and Amplification, Enzyme, and Probe Reagents), reagents must remain in the RPI for 4 to 20 hours, Refer to the *Procleix Reagent Preparation Incubator Operator's Manual* for additional information.

Caution: ⚠ Maintain reagents at the appropriate storage condition when not in use. Return reagents to their appropriate storage conditions without delay unless they are on the Procleix RPI or the Procleix Panther System.

SPECIMEN COLLECTION, STORAGE, AND HANDLING

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

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 a total of 8 days from the time of collection to the time of testing with the following conditions:

Specimens must be centrifuged within 72 hours of draw.

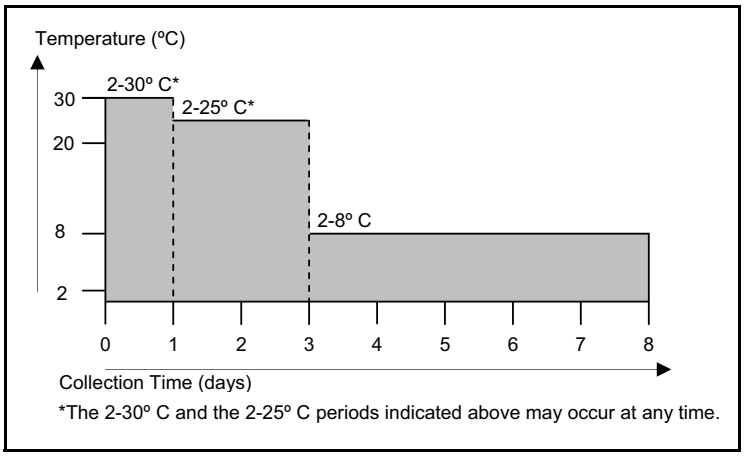
For storage above 8°C, specimens may be stored for 72 hours up to 25°C, and up to 30°C for 24 hours during the 72 hours.

Other than noted above, specimens are stored at 2° to 8°C.

Refer to the example storage temperature chart below.

In addition, plasma separated from the cells may be stored for up to 9 months at ≤ -20°C or up to 15 months at ≤ -70°C before testing.

Do not freeze whole blood.



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) may be stored for a total of 5 days from the time of collection to the time of testing with the following conditions:

Specimens must be centrifuged within 72 hours of draw.

For storage above 8°C, specimens may be stored for 72 hours up to 25°C, and up to 30°C for 24 hours during the 72 hours.

Other than noted above, specimens are stored at 2° to 8°C.

In addition, plasma separated from the cells may be stored for up to 9 months at ≤ -20°C or up to 15 months at ≤ -70°C before testing.

Do not freeze whole blood.

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 must 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 serum or plasma specimen collected from a donor prior to death may be tested instead of a cadaveric blood specimen using either the instructions for cadaveric donor specimens or the instructions for living donor specimens.

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. Whole blood (EDTA collection tube) or plasma may be stored for a total of 8 days from the time of collection to the time of testing with the following conditions:

Specimens must be centrifuged within 72 hours of draw.

For storage above 8°C, specimens may be stored for 24 hours up to 25°C during the 72 hours.

Other than noted above, specimens are stored at 2° to 8°C.

Refer to the example temperature chart below.

In addition, plasma separated from the cells may be stored for up to 11 days at ≤ -70°C before testing.

Do not freeze whole blood.

D. Whole blood (clot tube) or serum may be stored for a total of 3 days from the time of collection to the time of testing with the following conditions:

Specimens must be centrifuged within 72 hours of draw.

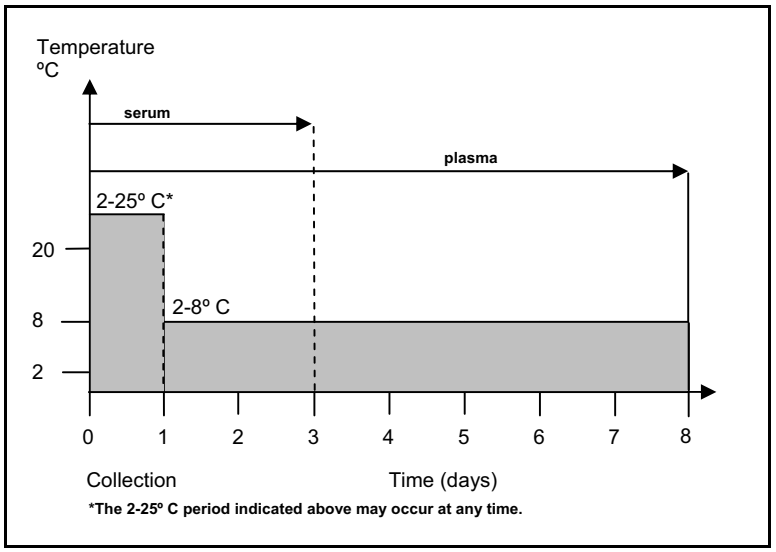
For storage above 8°C, specimens may be stored for 24 hours up to 25°C during the 72 hours.

Other than noted above, specimens are stored at 2° to 8°C.

Refer to the example temperature chart below.

In addition, serum removed from the clot tube may be stored for up to 11 days at ≤ -70°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 must 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.

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.

MATERIALS REQUIRED

Component	Quantity	Part Number
Procleix WNV Assay Kit	5000 Test Kit	302181
Internal Control Reagent	10 x 5 mL	
Target Capture Reagent	10 x 280 mL	
Amplification Reagent	10 x 50 mL	
Enzyme Reagent	10 x 18 mL	
Probe Reagent	10 x 75 mL	
Selection Reagent	10 x 180 mL	
Procleix WNV Assay Negative Calibrator	90 x 2 mL	
Procleix WNV Assay Positive Calibrator	90 x 2 mL	
Procleix Assay Fluids Kit		303344
Wash Solution	1 x 2.9 L	
Oil	1 x 260 mL	
Buffer for Deactivation Fluid	1 x 1.4 L	
Procleix Auto Detect Reagents Kit		303345
Auto Detect 1	1 x 245 mL	
Auto Detect 2	1 x 245 mL	
Disposables		
(Disposables are single use only, do not reuse. Use of other disposables is not recommended.)		
Multi-Tube Units (MTUs)	1 case of 100	104772
Waste Bag Kit	1 box of 10	902731
MTU Waste Cover	1 box of 10	504405
Reagent Spare Caps (TCR, Selection, and Probe Reagents)	1 bag of 100	CL0039
Reagent Spare Caps (Amplification Reagent)	1 bag of 100	CL0042
Reagent Spare Caps (Enzyme Reagent)	1 bag of 100	501619
Equipment		
Procleix Panther System and operator's manual Procleix Reagent Preparation Incubator (RPI), independent temperature monitor (ITM), and operator's manual **Note:** The Procleix WNV Assay is a 500 test kit and is not validated for use with the RPI 250		
Other		
Advanced Cleaning Solution	1 bottle (255 mL)	PRD-04550

OTHER MATERIALS AVAILABLE FROM GRIFOLS FOR USE WITH THE PROCLEIX WNV ASSAY

Component	Quantity	Part Number
Procleix WNV Assay Calibrators Kit		301186
Procleix WNV Assay Negative Calibrator	45 x 2 mL	
Procleix WNV Assay Positive Calibrator	45 x 2 mL	

General Equipment/Software

Procleix CPT Pooling Software and operator's manual

Procleix Xpress System (for pooling) and operator's manual

For instrument specifics and ordering information, contact Grifols Technical Service.

MATERIALS REQUIRED BUT NOT PROVIDED

Bleach (for use in final concentrations of 5 to 8.25% sodium hypochlorite and 0.5 to 0.7% sodium hypochlorite)

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

Disposable 1000 µL conductive filter tips (DiTis) in rack approved for use with the Procleix Panther System and pooling instrument

Contact Grifols Technical Service for approved tips.

PRECAUTIONS

A. For *in vitro* diagnostic use.

B. To reduce the risk of invalid results, carefully read the entire package insert for the Procleix WNV Assay and the *Procleix Panther System Operator's Manual* prior to performing an assay.

C. 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.

D. Specimens may be infectious. Use Universal Precautions when performing the assay.[30] Proper handling and disposal methods should be established according to local regulations.[21] Only personnel qualified as proficient in the use of the Procleix WNV Assay and trained in handling infectious materials should perform this procedure.

E. 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, kit reagents, and fluids. Wash hands thoroughly after handling specimens and kit reagents.

F. The Enzyme Reagent contains sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

G. Some reagents of this kit are labeled with risk and safety symbols and should be handled accordingly. Safety Data Sheets are accessible from the manufacturer's website.

Procleix Probe Reagent

Ethyl Alcohol 2.33 Weight-%
DANGER

May cause cancer

Obtain special instructions before use
Do not handle until all safety precautions have been read and understood
Use personal protective equipment as required
IF exposed or concerned: Get medical advice/attention

Store locked up
Dispose of contents/containers to an approved waste disposal plant

Procleix Selection Reagent

Boric Acid 3.63 Weight-%

DANGER

Harmful if inhaled
May damage fertility or the unborn child

Obtain special instructions before use
Do not handle until all safety precautions have been read and understood
Use personal protective equipment as required
Avoid breathing dust/fume/gas/mist/vapors/spray
Use only outdoors or in a well-ventilated area

If exposed or concerned: Get medical advice/attention
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing

Store locked up
Dispose of contents/container to an approved waste disposal plant

Procleix Buffer for Deactivation Fluid

Sodium Hydroxide 1.12 Weight-%
Sodium Hypochlorite 0.49 Weight-%

WARNING

Causes skin irritation
Causes serious eye irritation

Wash face, hands and any exposed skin thoroughly after handling
Wear protective gloves/protective clothing/eye protection/face protection

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention
IF ON SKIN: Wash with plenty of soap and water
If skin irritation occurs: Get medical advice/attention
Take off contaminated clothing and wash before reuse

Procleix Auto Detect 2

Sodium Hydroxide 6.04 Weight-%

DANGER

Causes severe skin burns and eye damage

Do not breathe dust/fume/gas/mist/vapors/spray
Wash face, hands and any exposed skin thoroughly after handling
Wear protective gloves/protective clothing/eye protection/face protection

Immediately call a POISON CENTER or doctor/physician
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing

Immediately call a POISON CENTER or doctor/physician
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
Wash contaminated clothing before reuse
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing

Immediately call a POISON CENTER or doctor/physician
IF SWALLOWED: rinse mouth. Do NOT induce vomiting

Store locked up

Dispose of contents/container to an approved waste disposal plant

H. 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.

I. Dispose of all materials that have come in contact with specimens and reagents according to local regulations.[21] Thoroughly clean and disinfect all work surfaces.

J. Use only specified disposables.

K. Do not use kit after expiration date.

L. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

M. Avoid microbial and nuclease contamination of reagents and fluids.

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

O. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate, or cloudiness is present. See REAGENT PREPARATION for specific instructions.

P. 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.

Q. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Procleix Panther System verifies reagent levels.

R. The Procleix Panther System groups a kit 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 each subsequent time that they are loaded onto the Procleix Panther System. Bottles belonging to a matched set cannot be swapped with bottles in other matched sets of reagents. Refer to the *Procleix Panther System Operator's Manual* for more information.

S. Refer to additional precautions in the *Procleix Panther System Operator's Manual*.

T. Each calibrator is designed to be run in triplicate and excess material in each vial is to be appropriately discarded.

REAGENT PREPARATION

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

B. Choose a new or opened matched set of reagents. An open set of reagents must be used on either the same Procleix Panther System as used previously, or a Procleix Panther System that is connected to that system via Data Sharing. Do not use reagents that have been used outside the Procleix Panther 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.

The Procleix Panther System tracks the number of hours each reagent and fluid is loaded onboard the analyzer. The Procleix Panther System will not start pipetting specimens if reagents 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, Buffer for Deactivation Fluid, Auto Detect Reagents	60 days

* If using Panther System Software version 7.2 and higher, onboard stability is 72 hours.

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

Note: The Selection Reagent must be at room temperature before use.

1. If Selection Reagent has been inadvertently stored at 2° to 8°C or the temperature of the laboratory falls between 2° and 15°C, use the RPI as described in the *Procleix Reagent Preparation Incubator Operator's Manual*, as precipitate may form.
2. If cloudiness or precipitate is present, use the RPI as described in the *Procleix Reagent Preparation Incubator Operator's Manual.* 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. Refer to the *Procleix Reagent Preparation Incubator Operator's Manual* to equilibrate the following reagents using the RPI*:* TCR, Probe Reagent, Enzyme Reagent, and Amplification Reagent. Record the date of thaw (THAW DATE) for reagent on the space provided on the label.

Note: If precipitate is still present after thawing, Probe Reagent can be incubated with RPI File 3 (room temperature) to facilitate complete dissolution of precipitate. The Probe Reagent may also be warmed in a water bath to facilitate dissolution of precipitate, but temperature in the water bath should not exceed 30°C. If thawing is conducted on the lab bench, Probe Reagent may take up to 4 hours with periodic mixing to allow complete dissolution of precipitate.

F. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate or cloudiness is present (refer to instructions in steps G.4 and H, below).

G. Equilibrate 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 the *Procleix Reagent Preparation Operator's Manual* for instructions.
3. Thaw one vial of Internal Control (IC) 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 gentle manual inversion or mechanical inversion using a laboratory rocker.

   Note: If gelling occurs, gel must be dissolved prior to use and within the 8 hour thaw period at room temperature. To expedite the dissolution of gel, warm the Internal Control Reagent at 25° to 30°C in a water bath. Periodically remove Internal Control Reagent from water bath to gently invert until gel is dissolved. Dry the exterior of the tube prior to opening.

5. After unloading TCR from the RPI and warming the Internal Control to room temperature, pour the entire vial of Internal Control Reagent into the TCR bottle. This is now the working Target Capture Reagent (wTCR). Mix thoroughly.
6. Record the date Internal Control Reagent was added, wTCR expiration date (date Internal Control Reagent was added plus 60 days), and lot number used (IC LOT) in the space indicated on the TCR bottle.
7. Retain the Internal Control vial to scan the barcode label into the system.

H. Thaw calibrators at room temperature. **Do not use the RPI to thaw Procleix WNV Assay Calibrators.**

1. These are single use vials.
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.
4. Once thawed, use Procleix WNV Assay Calibrators within 8 hours.

Note: If gelling occurs, gel must be dissolved prior to use and within the 8 hour thaw period at room temperature. To expedite the dissolution of gel, warm the calibrators at 25° to 30°C in a water bath. Periodically remove calibrators from water bath to gently invert until gel is dissolved.

I. Record the date Wash Solution, Buffer for Deactivation Fluid, Oil, Auto Detect 1, and Auto Detect 2 were first opened and loaded onto the Procleix Panther System (OPEN DATE) in the space provided on the label.

PROCEDURAL NOTES

Note: Refer to the *Procleix Panther System Operator's Manual* for operating instructions.

A. 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.

B. Replace bottles in the Universal Fluids Drawer when notified by the system. Refer to the *Procleix Panther System Operator's Manual*.

Note: Procleix Auto Detect Reagents and Assay Fluids may be used with any master lot of Procleix Assay Reagents that are run on the Procleix Panther System.

C. 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 Panther System Operator's Manual* and any applicable technical bulletins.

D. EQUIPMENT PREPARATION

See the *Procleix Panther System Operator's Manual*.

E. RUN SIZE

For the Procleix WNV Assay, each run may contain up to 500 tests, including Procleix WNV Assay Calibrators.

F. RUN CONFIGURATION

1. Each run must have a set of Procleix WNV Assay Calibrators.
2. For the Procleix WNV Assay, a set of calibrators consists of one vial each of Negative Calibrator and Positive Calibrator. The Negative and Positive Calibrators are run in triplicate.

G. WORK FLOW

1. Prepare reagent in a clean area.
2. The sample loading area must be amplicon free.

H. DECONTAMINATION

1. The extremely sensitive detection of the analytes by this test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces must be decontaminated daily with 0.5 to 0.7% 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. Follow instructions provided in the *Procleix Panther System Operator's Manual* for instrument decontamination and maintenance procedures.

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 barcode 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 and further assay processing information, see instructions in the *Procleix Panther System Operator's Manual*.

QUALITY CONTROL PROCEDURES

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX WNV ASSAY

A. Run validity:
A run 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 Panther System Software. If less than the minimum number of calibrator replicates is valid, the Procleix Panther 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 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 *Procleix Panther System Operator's Manual* for details.
3. All individual specimen results that are Invalid in a valid run must be retested.

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 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 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 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 (WNV PC_x) values for Analyte [WNV 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

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

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 WNV 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

INTERPRETATION OF RESULTS

All calculations described above are performed by the assay software of the Procleix Panther 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) but 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.

LIMITATIONS OF THE PROCEDURE

A. 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. Please note that majority of the samples in the clinical sensitivity study (71/109) had a WNV viral load of 1,600 copies/mL or higher and only 14 of them had a WNV viral load of 100 copies/mL prior to dilution.

B. Certain substances may interfere with the performance of the assay. See PERFORMANCE CHARACTERISTICS, Specificity and Sensitivity of the Procleix WNV Assay in the Presence of Donor and Donation Factors on the Procleix Panther System section.

C. Assay performance characteristics for use in testing plasma specimens from paid source plasma donors have not been determined.

D. The Procleix WNV Assay primers and probes target a highly conserved region of WNV RNA genome. However, in rare instances, mutations in this region may affect the sensitivity for detection of WNV.

E. Test results may be affected by improper specimen collection, storage, or specimen processing.

F. 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.

G. Failure to meet the acceptance criteria for Procleix WNV Assay Negative and Positive Calibrator as specified in QUALITY CONTROL PROCEDURES is an indication of an invalid run. Possible sources of error include test kit deterioration, operator error, faulty performance of equipment, calibrator deterioration, or contamination of reagents.

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

Reproducibility was evaluated on the Procleix Panther System at 3 US sites. Two operators performed testing at each site. Each operator performed 2 runs per day over 9 days, using 3 reagent lots over the course of testing. Each run had 2 replicates of each panel member.

Reproducibility was tested using panel members made from WNV negative plasma. The positive panel members were created by spiking the negative plasma with WNV stock composed of positive clinical plasma specimens. One positive panel member was spiked with a very low concentration of WNV (approximately 2 copies/mL; below the 95% LOD of the assay).

Table 1 shows the reproducibility and precision of assay results for each positive panel member between sites, between operators, between lots, between days, between runs, within runs, and overall. The very low positive panel member had a total %CV of 114.8% due to the inconsistent results that are expected when testing samples with very low concentrations.

Table 1. Reproducibility of the Procleix WNV Assay on the Procleix Panther System

Panel[1,2]	Mean S/CO	Agreement (%)	Between Sites		Between Operators		Between Lots		Between Days		Between Runs		Within Runs		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
A	<0.1	100	<0.1	69.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<0.1	465.3	<0.1	470.5
B	12.8	51.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.7	114.8	14.7	114.8
C	30.8	98.1	0.0	0.0	0.6	2.0	0.0	0.0	0.6	1.8	0.0	0.0	4.7	15.2	4.8	15.4
D	31.5	100	0.3	0.9	0.0	0.0	0.3	0.9	0.8	2.5	0.5	1.7	1.2	4.0	1.6	5.1
E	31.6	100	0.5	1.6	0.0	0.0	<0.1	0.2	0.3	1.0	0.9	2.7	1.2	3.9	1.6	5.2

CV = Coefficient of Variation

SD = Standard Deviation

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.0.

S/CO = Signal to Cutoff ratio

[1]n=216 for each panel member.

[2]Estimated concentration (c/mL) for each panel member: A=0 copies/mL, B=2 copies/mL, C=22 copies/mL, D=199 copies/mL, E=994 copies/mL

SPECIFICITY IN NORMAL BLOOD DONORS

A prospective, multicenter clinical trial was conducted. Plasma samples from voluntary whole blood donors were tested in 16-sample pools or individually. Two blood testing laboratories performed testing using two Procleix Panther System instruments each. Three reagent kit lots were used by each laboratory.

Pools were created by combining aliquots from 16 individual donations. Pools and individual donations were tested with the Procleix WNV Assay on the Procleix Panther System and on the licensed Procleix Tigris System in accordance with package insert instructions. Pools with reactive results were resolved by testing the individual donations within the reactive pool. Individual donations with reactive result(s) were tested with an alternate licensed WNV nucleic acid test (NAT). All alternate NAT testing was performed on the index donation. Donor status was based on testing with the Procleix WNV Assay on the Procleix Tigris System and on the licensed alternate NAT when samples were reactive with the Procleix WNV Assay on the Procleix Panther System or had discordant Procleix WNV Assay results on the Procleix Panther System and Procleix Tigris System.

Of the 249 Procleix WNV Assay runs on the Procleix Panther System, 8 runs (3.2%, 8/249) were invalid. Of the 10,744 pools and 13,619 individual donations (includes samples that were never pooled and the 16 constituent samples from a reactive pool) processed in valid runs, 104 pools (1.0%, 104/10,744) and 196 individual donations (1.4%, 196/13,619) had final invalid or error results and were excluded from the analyses. Note: most of the final invalid and error results occurred in pools (104/104) or individual donations (189/196) that had initial invalid or error results and were never retested due to the laboratories' need to release product.

An additional 9 pools and 36 individual donations were excluded from the analyses because they had final invalid Procleix WNV Assay results on the Procleix Tigris System.

Table 2 shows the specificity of the Procleix WNV Assay on the Procleix Panther System in 10,631 pools and 13,371 individual donations. Specificity was 100% in 16-sample pools and individual donations.

Table 2. Specificity of the Procleix WNV Assay on the Procleix Panther System in 16-Sample Pools and Individual Donations

Sample Type	n	True Negative	False Negative	True Positive[1]	False Positive	Specificity (%)	95% CI[2]
16-sample pool	10,631	10,630	0	1	0	100	99.965–100
Individual Donation	13,371	13,371	0	0	0	100	99.972–100

n = number of specimens

[1]The 1 pool with a true positive result contained an individual donation that was reactive when tested with the alternate NAT.

[2]Clopper-Pearson confidence interval

CROSS-REACTIVITY/INTERFERENCE STUDIES

Specificity and Sensitivity of the Procleix WNV Assay in the Presence of Donor and Donation Factors on the Procleix Panther System

When tested with the Procleix WNV Assay, no cross-reactivity (100% specificity) or interference (100% sensitivity) 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, rheumatoid factor, antinuclear antibody, multiple sclerosis, lupus and multiple myeloma. Also tested were samples from patients with hyperglobulinemia, with elevated alanine transaminase (ALT) and from patients with alcoholic liver cirrhosis.

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, human T-cell lymphotropic virus type I/II, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis G virus, cytomegalovirus, Epstein-Barr virus, rubella virus, parvovirus B-19 and human immunodeficiency virus type 1 and type 2. Also tested were donor samples from influenza virus and HBV vaccinees, and samples spiked with tissue culture-derived viruses related to members of the Japanese encephalitis virus (JEV) sero-complex, including dengue virus, Yellow Fever virus, Saint Louis encephalitis virus, and Murray Valley encephalitis virus with no cross-reactivity or interference. The Procleix WNV Assay detected Kunjin virus, a variant of WNV. No cross-reactivity or interference was observed when equal volume pools of 16 donor and donation factor specimens were tested with the Procleix WNV Assay.

The control study, performed in absence of any potentially known interfering substances, microorganisms or blood borne pathogens showed 100% specificity (90/90) and 97.92% sensitivity (94/96).

CLINICAL SENSITIVITY

Clinical Sensitivity on Known-Positive Samples

One hundred and nine (109) WNV known-positive samples were procured from a blood bank repository. Samples had known WNV RNA concentrations of 100 copies/mL or more when tested neat. The positive samples were prepared neat (i.e., undiluted; n=109) and in a 1:16 dilution (n=109) and tested with the Procleix WNV Assay at three blood testing laboratories. Three clinical reagent kit lots were used at each site. Results were compared to the known viral status and clinical sensitivity was calculated (Table 3).

Of the 10 runs performed, all were valid. Of the test results from neat (n=109) and diluted (n=109) samples, all were valid.

The sensitivity of the Procleix WNV Assay in neat (undiluted) WNV known-positive samples was 99.1% (108/109; 95% CI: 95.0% to 100%). The 1 sample with a false negative result had a viral load of 100 copies/mL by a validated quantitative NAT, but the sample was nonreactive when tested neat with a licensed alternate NAT.

The sensitivity of the Procleix WNV Assay in diluted (1:16) WNV known-positive samples was 98.2% (107/109; 95% CI: 93.5% to 99.8%). The 2 diluted samples with false negative results contained a known-positive sample with 100 copies/mL, thus, the diluted samples had approximately 6 copies/mL (100 copies/mL ÷ 16). When the diluted samples were tested with a licensed alternate NAT, results were also nonreactive.

Table 3. Procleix Panther System – Clinical Sensitivity of the Procleix WNV Assay in Known-Positive Samples

Assay	n	TP	FN	Sensitivity (%)	95% CI[1]
Neat	109	108	1[2]	99.08	94.99–99.98
Diluted	109	107	2[3]	98.17	93.53–99.78

n = number of specimens
FN = false negative
TP = true positive

[1]Clopper-Pearson confidence interval

[2]Undiluted sample produced a false negative result with a viral load of 100 copies/mL on a validated quantitative NAT. This sample was nonreactive when tested neat with a licensed alternate NAT.

[3]Diluted samples had concentrations of approximately 6 copies/mL.

ANALYTICAL SENSITIVITY

Determination of Analytical Sensitivity of the Procleix WNV Assay Using a Sensitivity Panel Made From the Health Canada WNV Reference Standard on the Procleix Panther System

An analytical sensitivity panel comprising 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). The panel was tested on the Procleix Panther System. Three systems were used, testing 48 replicates of each copy level evenly with each of three reagent lots for a total of 144 replicates. The 95% confidence intervals (CI) of the reactive rates were based on the score method[32]. Estimations of 50% and 95% detection rates by probit analysis are provided.

WNV detection with the Procleix WNV Assay was 100% at 100 copies/mL and 30 copies/mL. Reactivity at 10 copies/mL and 3 copies/mL was 92% and 60%, respectively (Table 4).

Table 4. Detection of WNV in a Health Canada Analytical Sensitivity Panel

Panel	Procleix Panther System					
WNV Copies/mL	#Reactive/ #Tested*	% Reactive	95% CI		Average S/CO**	%CV
			Lower	Upper		
100	144/144	100	97	100	31.89	7
30	144/144	100	97	100	32.01	6
10	133/144	92	87	96	31.00	15
3	87/144	60	52	68	28.02	33
1	49/144	34	27	42	28.51	27
0	0/144	0	0	3	0.01	267

CI = Score Confidence Interval
S/CO = Signal to Cutoff ratio
CV = Coefficient of Variation
*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

Probit Analysis

The predicted 50% and 95% detection rates in copies/mL were determined by probit analysis[22] of the analytical sensitivity results. The predicted 50% and 95% detection level for WNV with the Procleix WNV Assay were 2.0 copies/mL and 11.9 copies/mL, respectively, with the Health Canada Sensitivity Panel (Table 5).

Table 5. Detection Probabilities of WNV using a Sensitivity Panel from Health Canada Reference Standard*

System	Detection Probabilities (Copies/mL)	
	50% (95% FL)	95% (95% FL)
Procleix Panther System	2.0 (1.6–2.4)	11.9 (9.6–15.9)

FL = Fiducial Limit

*All probit results reflect application of the Gompertz model.

Determination of Analytical Sensitivity of the Procleix WNV Assay Using a Sensitivity Panel made from Lineage 1 WNV *In-Vitro* Transcribed RNA on the Procleix Panther System

An analytical sensitivity panel comprising serially diluted WNV *in vitro* transcript (IVT) RNA from a clone of Lineage 1 WNV RNA used in-house was used to evaluate assay sensitivity. A total of 48 replicates of each copy level were tested evenly with each of three reagent lots on the Procleix Panther System for a total of 144 replicates. The 95% confidence intervals of the reactive rates were based on the score method.

WNV detection with the Procleix WNV Assay was 100% at 100 and 30 copies/mL. Reactivity at 10 copies/mL and 3 copies/mL was 90% and 56%, respectively (Table 6).

Table 6. Detection of Lineage WNV 1 IVT RNA

Panel	Procleix Panther System					
WNV Copies/mL	#Reactive/ #Tested*	% Reactive	95% CI		Average S/CO**	%CV
			Lower	Upper		
100	144/144	100	97	100	32.88	6
30	144/144	100	97	100	32.85	7
10	129/144	90	84	94	31.75	18
3	80/144	56	47	63	30.45	23
1	34/144	24	17	31	26.58	41
0	0/144	0	0	3	0.01	282

CI = Score Confidence Interval

S/CO = Signal to Cutoff ratio

CV = Coefficient of Variation

*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

Probit Analysis

The predicted 50% and 95% detection rates in copies/mL were determined by probit analysis of the analytical sensitivity results. The predicted 50% and 95% detection level for WNV Lineage 1 IVT RNA with the Procleix WNV Assay were 2.8 copies/mL and 12.9 copies/mL, respectively (Table 7).

Table 7. Detection Probabilities of WNV Lineage 1 IVT RNA*

System	Detection Probabilities (Copies/mL)	
	50% (95% FL)	95% (95% FL)
Procleix Panther System	2.8 (2.3–3.2)	12.9 (10.6–16.7)

FL = Fiducial Limit

*All probit results reflect application of the Gompertz model.

Determination of Analytical Sensitivity of the Procleix WNV Assay Using a Dilutional Sensitivity Panel made from Lineage 2 WNV *In-Vitro* Transcribed RNA on the Procleix Panther System

An analytical sensitivity panel composed of serially diluted WNV IVT RNA from a sequence corresponding to WNV Lineage 2 (GenBank accession number: EF429197) was used to evaluate assay sensitivity. Synthetic gene construction (IDT, Coralville, IA) was used to create a DNA clone from which IVT RNA was transcribed. A total of 48 replicates of each copy level were tested evenly with each of three reagent lots using the Procleix Panther System for a total of 144 replicates. The 95% confidence intervals of the reactive rates were based on the score method.

WNV detection with the Procleix WNV Assay was 100% at 100 and 30 copies/mL. Reactivity at 10 copies/mL and 3 copies/mL was 92% and 47%, respectively (Table 8).

Table 8. Detection of Lineage 2 WNV IVT RNA

Panel	Procleix Panther System					
WNV Copies/mL	#Reactive/ #Tested*	% Reactive	95% CI		Average S/CO**	%CV
			Lower	Upper		
100	144/144	100	97	100	11.86	9
30	144/144	100	97	100	11.69	15
10	132/144	92	86	95	9.89	31
3	67/144	47	39	55	7.61	50
1	28/144	19	14	27	7.76	57
0	0/144	0	0	3	0.01	304

CI = Score Confidence Interval
S/CO = Signal to Cutoff ratio
CV = Coefficient of Variation
*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

Probit Analysis

The predicted 50% and 95% detection rates in copies/mL were determined by probit analysis of the analytical sensitivity results. The predicted 50% and 95% detection level for WNV Lineage 2 IVT RNA with the Procleix WNV Assay were 3.1 copies/mL and 12.0 copies/mL, respectively (Table 9).

Table 9. Detection Probabilities of WNV Lineage 2 IVT RNA*

System	Detection Probabilities (Copies/mL)	
	50% (95% FL)	95% (95%FL)
Procleix Panther System	3.1 (2.7–3.6)	12.0 (10.0–15.2)

FL = Fiducial Limit
*All probit results reflect application of the Gompertz model.

Detection of Various WNV Lineages with the Procleix WNV Assay on the Procleix Panther System

An analytical sensitivity panel was prepared from IVT RNA and WNV viral tissue cultures. Performance of the Procleix WNV Assay was evaluated by testing two replicates of each dilution level with two reagent lots using the Procleix Panther System for a total of four replicates. The concentration of RNA transcript dilutions were determined spectrophotometrically while the viral samples were tested as dilutions from the concentrated source material. Performance of the assay ranged from 75% to 100% reactivity with both WNV Lineage 1 and Lineage 2 for the lowest concentrations tested (Table 10).

Table 10. Detection of WNV Genotypes

WNV Lineage	Origin, strain or year identified, (accession)	Level	Procleix Panther System	
			Reactive / Tested	% Reactive
1	USA, NY 2001-6263, (AF533540)	10^{-8} dilution	4/4	100
		10^{-9} dilution	3/4	75
1	USA, 1986, (DQ164189)	10^{-8} dilution	4/4	100
		10^{-9} dilution	4/4	100
2	Uganda B-956 (N/A)	10^{-6} dilution	4/4	100
		10^{-7} dilution	3/4	75
2	South Africa, 1989, (EF429197)	100 copies/mL	4/4	100
		30 copies/mL	4/4	100
2	Greece 2010 (HQ537483)	100 copies/mL	4/4	100
		30 copies/mL	4/4	100
2	Hungary, 2004 (DQ116961)	100 copies/mL	4/4	100
		30 copies/mL	4/4	100

N/A = Not applicable; no accession number available.

-PERFORMANCE OF CADAVERIC BLOOD SPECIMENS FROM TISSUE DONORS

Specificity of the Procleix WNV Assay in Cadaveric Blood Specimens on the Procleix Panther System

WNV-negative cadaveric serum and plasma specimens were tested to determine the specificity of the Procleix WNV Assay. Fifty cadaveric specimens[23] (25 unique cadaveric serum specimens and 25 unique cadaveric plasma specimens) and fifty normal blood donor (control) specimens (25 serum and 25 plasma specimens) were tested on the Procleix Panther System. The cadaveric and control samples were tested evenly between three reagent lots. The specificity of the Procleix WNV Assay for the cadaveric plasma and serum specimens was 100% (95% CI: 86.3%-100%) for both on the Procleix Panther System (Table 11). No invalid results were observed in the cadaveric or control specimens.

Table 11. Specificity of the Procleix WNV Assay in Cadaveric Blood Specimens

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	1.88	1.93	1.86	1.92
Mean Analyte S/CO	0	0	0	0
Specificity Rate (%)	100	100	100	100
95% CI, Specificity Rate	86.3–100	86.3–100	86.3–100	86.3–100
n	25	25	25	25

n = number of samples
IC = Internal Control
S/CO = Signal to Cutoff ratio
CI = Clopper-Pearson confidence interval

Sensitivity of Detection for the Procleix WNV Assay in Cadaveric Blood Specimens on the Procleix Panther System

WNV-negative cadaveric serum and plasma specimens spiked with a low level of WNV (approximately 150 copies/mL from 5 unique donors[33]) were tested to determine the sensitivity of the Procleix WNV Assay. Fifty cadaveric specimens (25 unique cadaveric serum specimens and 25 unique cadaveric plasma specimens) and fifty normal blood donor plasma specimens were tested on the Procleix Panther System. The spiked cadaveric and control samples were tested evenly between three reagent lots. The reactive rate of the Procleix WNV Assay for the cadaveric plasma and serum specimens was 100% (95% CI: 86.3%-100%) for both on the Procleix Panther System (Table 12). No invalid results were observed in the cadaveric or control specimens.

Table 12. Sensitivity of the Procleix WNV Assay in Cadaveric Blood Specimens

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	1.97	2.21	1.96	2.07
Mean Analyte S/CO	31.99	32.47	32.38	32.98
Specificity Rate (%)	100	100	100	100
95% CI, Reactive Rate	86.3–100	86.3–100	86.3–100	86.3–100
n	25	25	25	25

n = number of samples
S/CO = Signal to Cutoff ratio
CI = Clopper-Pearson confidence interval

Reproducibility in Cadaveric Blood Specimens

The inter-assay reproducibility of the Procleix WNV Assay with cadaveric blood specimens was assessed by determining the %CVs obtained when each of 10 cadaveric plasma, 10 control plasma, 10 cadaveric serum, and 10 control serum specimens were spiked with 150 copies/mL WNV were tested with three clinical reagent kit lots. The reactive rates, S/COs, and %CVs are shown in Table 13. The %CV for cadaveric plasma, control plasma, cadaveric serum, and control serum specimens were 4%, 4%, 5%, and 10%, respectively. The percent reactive rates for the cadaveric plasma, control plasma, cadaveric serum, and control serum specimens in this study were 100%, 100%, 100%, and 97.2%, respectively.

Table 13. Reproducibility of the Procleix WNV Assay in Cadaveric and Control Specimens Spiked with 150 copies/mL of WNV

Sample	# Donors	# Replicates	% Reactivity (95% CI)	Mean Analyte S/CO	%CV
Cadaveric Plasma	10	180	100 (98.0–100)	33.09	4
Control Plasma	10	180	100 (98.0–100)	31.51	4
Cadaveric Serum	10	180	100 (98.0–100)	32.30	5
Control Serum	10	180	97.2 (93.6–99.1)	31.30	10

CI = Clopper-Pearson confidence interval
S/CO = Signal to Cutoff ratio in concordant replicates only
%CV = Coefficient of Variation

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U.S. License Number 2032

GDSS-IFU-000007 v. 5.0

2021-06

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