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Procleix® Zika Virus Assay

For *In Vitro* Diagnostic Use

IVD

Rx Only
1000 Test Kit, 5000 Test Kit

CONTENTS

INTENDED USE

For *in vitro* diagnostics use

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

SUMMARY AND EXPLANATION OF THE TEST

ZIKV is an RNA virus that is a member of the *Flaviviridae* family and the genus *Flavivirus*.[1] It is transmitted to humans by mosquitoes belonging to the Aedes genus of mosquitoes.[2] ZIKV was first identified in an infected rhesus macaque in 1947 in the Zika Forest of Uganda, followed by the first reported human cases in Uganda and the United Republic of Tanzania in 1952.[3] Since then, sporadic outbreaks of ZIKV have been documented in many areas of Africa and Southeast Asia. The first occurrence of a ZIKV outbreak outside of Asia or Africa occurred in 2007, when a large outbreak occurred on the Pacific island of Yap, in the Federated States of Micronesia.[4]

In 2013 and 2014, a major outbreak of ZIKV disease, associated with clinical complications, was reported in French Polynesia.[5] In May 2015, the first locally acquired cases of ZIKV infection in the Americas were confirmed in Brazil.[6,7] As of early 2016, ZIKV had spread to other countries in South America, Central America, Mexico, and the Caribbean, including the U.S. territories of Puerto Rico and the Virgin Islands.[7] In 2016, 5,102 symptomatic Zika virus disease cases were reported in the U.S. states, including 272 locally acquired cases and 36,079 cases were reported in the U.S. territories, of which 35,937 were locally acquired.[8]

ZIKV has been associated with symptoms ranging from asymptomatic to mild flu-like illnesses, but more recently ZIKV infection has been associated with serious and sometimes fatal cases of Guillain-Barré syndrome.[9] The virus has also been associated with microcephaly and other birth defects in infants born to infected mothers.[10] Although the primary route of infection appears to be through the bite of a mosquito, sexual transmission[11] and possible transfusion-transmission[12] of ZIKV have also been reported.

The Procleix Zika Virus Assay uses the same transcription-mediated nucleic acid amplification (TMA) technology as other Procleix blood screening assays.

PRINCIPLES OF THE PROCEDURE

The Procleix Zika Virus Assay is performed on the fully automated Procleix Panther System.

The Procleix Zika Virus Assay involves three main steps, which take place in a single tube: sample preparation, ZIKV RNA target amplification by TMA,[13] and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).[14]

During sample preparation, RNA is isolated from specimens via target capture. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins, and release viral genomic RNA. Capture oligonucleotides that are homologous to highly conserved regions of ZIKV are hybridized to the ZIKV 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. The T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. The Procleix Zika Virus Assay utilizes the TMA method to amplify regions of ZIKV 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 by a luminometer and is reported as Relative Light Units (RLU).

Internal Control is added to each test specimen, control, and assay calibrator via the working Target Capture Reagent. The Internal Control in the Procleix Zika Virus Assay controls for specimen processing, amplification, and detection steps. Internal Control signal is discriminated from the ZIKV signal by the differential kinetics of light emission from probes with different labels[14] Internal Control-specific amplicon is detected using a probe with rapid emission of light (flasher signal). Amplicon specific to ZIKV 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.[15]

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

REAGENTS

Procleix Zika Virus 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° (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 Zika Virus Assay Calibrators

C0

Procleix Zika Virus Assay Negative Calibrator

A HEPES buffered solution containing detergent.

Store at –35° to –15°C.

C1

Procleix Zika Virus Assay Positive Calibrator

A HEPES buffered solution containing detergent and a ZIKV 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 at 15° to 30°C.

R2

Auto Detect 2

1.6 N sodium hydroxide.

Store at 15° to 30°C.

W

Wash Solution

HEPES buffered solution.

Store at 15° to 30°C.

O

Oil

Silicone oil.

Store at 15° to 30°C.

DF

Buffer for Deactivation Fluid

Sodium bicarbonate buffered solution.

Store 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. 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, 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 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)		72 hours	60 hours	30 days at 2° to 8°C
Amplification Reagent	–35° to –15°C	72 hours	60 hours	30 days at 2° to 8°C
Enzyme Reagent	–35° to –15°C	72 hours	60 hours	30 days at 2° to 8°C
Probe Reagent	–35° to –15°C	72 hours	60 hours	30 days at 2° to 8°C
Selection Reagent	RT	30 days	60 hours	30 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 RPI 250 or RES after the preparation program is completed.
- If opened reagents are placed in the RPI 250 or RES at the room temperature program, the time duration is included in the total RT stability.
- The RT stability time must occur within 30 days, which includes onboard stability. See REAGENT PREPARATION, Item C.1. 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 250 File 3 or RES Room Temperature program for thawing unopened reagents (TCR and Amplification, Enzyme, and Probe Reagents), reagents must remain in the RPI 250 or RES for 4 to 20 hours. Refer to the *Procleix Reagent Preparation Incubator 250 Operator's Manual* or the *Procleix Reagent Equilibration System Operator's Manual* as applicable for additional information.

Caution: ⚠ Only the reagent onboard stability is tracked by the Procleix Panther System software. The time reagents remain at RT when not onboard the Procleix Panther System MUST be manually tracked by the user to ensure maximum allotted RT time is not exceeded.
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 RPI 250, the RES, or the Procleix Panther System. Deviations from storage may impact the performance of the assay.

SPECIMEN COLLECTION, STORAGE, AND HANDLING

Warning: 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, in gel separation tubes (BD Vacutainer PPT or Greiner K_2EDTA Sep Vacuette Blood Collection Tubes), ACD, heparin, sodium citrate, CP2D, or CPDA may be used. Follow sample tube manufacturer's instructions.

Whole blood or plasma may be stored for a total of 13 days from the time of collection to the time of testing with the following conditions:

Whole blood specimens must be centrifuged within 72 hours of draw.

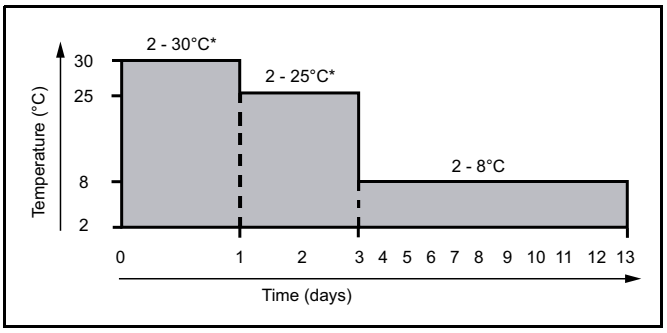
For storage above 8°C, specimens may be stored at any time for 72 hours up to 25°C, and up to 30°C for 24 hours during the 72 hours.

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 30 days at ≤ -20°C before testing.

Do not freeze whole blood.



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

C. Additional blood specimens collected in serum tubes according to the collection container manufacturer's instructions may be used. Serum may be stored as in section B., above.

D. Additional specimens may be taken from whole blood or plasma units containing CPD anticoagulant collected according to the collection container manufacturer's instructions. Whole blood 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:

Whole blood specimens must be centrifuged within 72 hours of draw.

For storage above 8°C, specimens may be stored at any time 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 30 days at ≤ -20°C before testing.

Do not freeze whole blood.

E. Specimen stability may be affected by elevated temperature.

F. No adverse effect on assay performance was observed when plasma or serum were subjected to 3 freeze-thaw cycles.

G. 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.

H. Mix thawed plasma or serum thoroughly and centrifuge, as necessary, for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed gel separation tubes must be validated by the user.

I. Other collection and storage conditions must be validated by the user. 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.

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

K. 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: Plasma specimens from donors of whole blood, blood components, and other living donors, may be pooled.

Note: Studies to validate these conditions were performed on negative specimens spiked with virus. Serum samples and samples collected in K_2EDTA that were evaluated for up to 13 days were spiked with ZIKV at 18 copies/mL; all other samples were spiked with 150 copies/mL, producing a reactivity rate of ≥ 86%.

CADAVERIC BLOOD SPECIMENS

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

B. Specimens should be collected within 24 hours of death if the cadaver was refrigerated (1° to 10°C) within 12 hours of death.

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:

Whole blood specimens must be centrifuged within 72 hours of draw.

For storage above 8°C, specimens may be stored up to 25°C for 24 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 30 days at ≤ -20°C.

Whole blood (clot tube) or serum may be stored a total of 5 days from the time of collection to the time of testing with the following conditions:

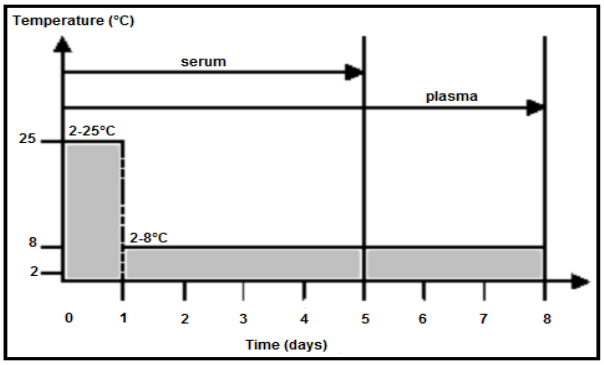
Whole blood specimens must be centrifuged within 72 hours of draw.

For storage above 8°C, specimens may be stored up to 25°C for 24 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 30 days at ≤ -20°C before testing.

Do not freeze whole blood.



C. No adverse effect on assay performance was observed when plasma and serum were subjected to 3 freeze-thaw cycles.

D. Specimen stability may be affected by elevated temperature.

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 or serum thoroughly and centrifuge, as necessary, for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed gel separation tubes must be validated by the user.

G. Other cadaveric blood specimen collection, handling, and storage conditions must be validated by the user. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable international regulations covering the transport of clinical specimens and etiologic agents.

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

I. 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 the standard assay procedure.

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

MATERIALS REQUIRED

Component	Part Number	Part Number
Procleix Zika Virus Assay Kit	**PRD-04170** (1000 Test Kit)	**PRD-04228** (5000 Test Kit)
Internal Control Reagent	4 x 2.8 mL	20 x 2.8 mL
Amplification Reagent	4 x 26 mL	20 x 26 mL
Enzyme Reagent	4 x 13.4 mL	20 x 13.4 mL
Probe Reagent	4 x 34.7 mL	20 x 34.7 mL
Target Capture Reagent	4 x 161 mL	20 x 161 mL
Selection Reagent	4 x 91 mL	20 x 91 mL
Procleix Zika Virus Assay Calibrators Kit	**PRD-04171** (15 sets)	**PRD-04229** (75 sets)
Negative Calibrator	15 x 2.2 mL	75 x 2.2 mL
Positive Calibrator	15 x 2.2 mL	75 x 2.2 mL
Procleix Assay Fluids Kit	**303344** (1000 Test kit)	
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** (1000 Test Kit)	
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.)*	**Quantity**	**Part Number**
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 and Selection Reagents)	1 bag of 100	**CL0039**
Reagent Spare Caps (Amplification and Probe Reagents)	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 250 (RPI 250) and operator's manual, independent temperature monitor (ITM) and operator's manual, or the Procleix Reagent Equilibration System (RES) and operator's manual.		
Other		
Advanced Cleaning Solution	1 bottle (255 mL)	**PRD-04550**

OTHER MATERIALS AVAILABLE FROM GRIFOLS FOR USE WITH THE PROCLEIX ZIKA VIRUS ASSAY

General Equipment/Software

Front end pipettor for pooling only, pooling software, operator's manual, and quick reference guide

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. 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. Proper handling and disposal methods should be established according to local, state, and federal regulations. Only personnel adequately qualified as proficient in the use of the Procleix Zika Virus 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. 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.

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

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. Thoroughly clean and disinfect all work surfaces.

I. Use only specified disposables.

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

K. Avoid microbial and nuclease 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 AND HANDLING 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. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate, or cloudiness is present. See REAGENT PREPARATION for specific instructions.

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

P. 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 Selection Reagent
 *Boric Acid 3.63 Weight-%* **WARNING** Harmful if inhaled Avoid breathing dust/fume/gas/mist/vapors/spray Use only outdoors or in a well-ventilated area IF INHALED: Remove victim to fresh air and keep rest in a position comfortable for breathing Call a POISON CENTER or doctor/physician if you feel unwell

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 globes/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
Procleix Buffer for Deactivation Fluid
 *Sodium Hydroxide 1–3 Weight-%* *Sodium Hypochlorite <1 Weight-%* **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation P264 - Wash face, hands and any exposed skin thoroughly after handling P280 - Wear protective gloves/protective clothing/eye protection/face protection P362 - Take off contaminated clothing and wash before reuse P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing P337 + P313 - If eye irritation persists: Get medical advice/attention P302 + P352 - IF ON SKIN: Wash with plenty of soap and water P332 + 313 - If skin irritation occurs: Get medical advice/attention

Q. 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; these reagents 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.

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

S. DO NOT heat the Probe Reagent above 35°C when using the RPI 250 or RES. Refer to the *Procleix Reagent Preparation Incubator 250 Operator's Manual* or the *Procleix Equilibration 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. Refer to STORAGE AND HANDLING INSTRUCTIONS.

1. 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. Selection Reagent may form precipitate if it is inadvertenly stored at 2° to 8°C or if the room temperature falls between 2° to 15°C.
2. If cloudiness or precipitate is present, perform Selection Reagent recovery as described in the *Procleix Reagent Preparation Incubator 250 Operator's Manual* or the *Procleix Reagent Equilibration System 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 250 Operator's Manual* if using the RPI 250 or the *Procleix Reagent Equilibration System Operator's Manual* if using the RES to prepare the following reagents: TCR, Probe Reagent, Enzyme Reagent, and Amplification Reagent.

Note: If precipitate is still present after thawing, Probe Reagent can be incubated with RPI File 3 (room temperature) or the RES Room Temperature program 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, H, and I below).

Record the date of thaw (THAW DATE) for each reagent on the space provided on the label.

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 250 or RES, and refer to the applicable *Procleix Reagent Preparation Incubator 250 Operator's Manual* or the *Procleix Reagent Equilibration System 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 250 or the RES 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.

5. Unload TCR from the RPI 250 or the RES and warm the Internal Control Reagent to room temperature.
6. Pour the entire vial of Internal Control Reagent into the TCR bottle. This is now the working Target Capture Reagent (wTCR).
7. Record the date Internal Control Reagent was added, wTCR expiration date (date Internal Control Reagent was added plus 30 days), and lot number used (IC LOT), in the space indicated on the TCR bottle.
8. Retain the IC vial to scan the barcode label into the system.

H. Thaw calibrators at room temperature. **Do not use the RPI 250 or the RES to thaw Procleix Zika Virus Assay Calibrators.**

Note: These are single-use vials which must be thawed prior to each run.

1. Mix calibrators gently by inversion to avoid foaming.
2. If foam is present, remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

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, Oil, Buffer for Deactivation Fluid, 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. Procleix Zika Virus Assay Calibrators are master lotted with the Procleix Zika Virus Assay. The operator must ensure that the Procleix Zika Virus Assay Calibrators are used with the corresponding master lot of kit reagents as indicated on the master lot barcode sheet enclosed with each shipment of Procleix Zika Virus Assay Calibrators.

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

Note: 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. 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 250 or the RES 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.

D. To reduce the risk of invalid results, carefully read the entire package insert for the Procleix Zika Virus Assay prior to performing an assay run. This package insert must be used with the *Procleix Panther System Operator's Manual*, *Procleix Reagent Preparation Incubator 250 Operator's Manual, Procleix Reagent Equilibration System Operator's Manual,* and any applicable technical bulletins.

E. RUN SIZE

For the Procleix Zika Virus Assay, each worklist may contain up to 250 tests, including Procleix Zika Virus Assay Calibrators.

F. EQUIPMENT PREPARATION

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

G. RUN CONFIGURATION

1. Each run must have a set of Procleix Zika Virus Assay Calibrators.
2. For the Procleix Zika Virus 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.

H. WORK FLOW

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

I. DECONTAMINATION

1. The extremely sensitive detection of 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 should be run in singlet in the Procleix Zika Virus Assay.Specimens 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.

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

For equipment preparation, rack setup, and assay procedure information, see instructions in the *Procleix Panther System Operator's Manual*.

QUALITY CONTROL PROCEDURES

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX ZIKA VIRUS ASSAY

A. Run validity:

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

1. In a Procleix Zika Virus 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: Runs/samples may also be invalidated by the operator in cases where there were deviations from the protocol.

II. ACCEPTANCE CRITERIA FOR CALIBRATION AND CALCULATION OF CUTOFF

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate in the Procleix Zika Virus Assay. Each individual Negative Calibrator replicate must have an Internal Control (IC) value greater than or equal to 50,000 RLU and less than or equal to 500,000 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 Zika Virus Assay. Individual Positive Calibrator (PC) analyte values must be less than or equal to 4,000,000 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

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

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 Zika Virus 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 Zika Virus Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 50,000 and ≤ 500,000 RLU
Positive Calibrator	
Analyte	≥ 400,000 and ≤ 4,000,000 RLU
Internal Control	≤ 750,000 RLU

Summary of Cutoff Calculations for Procleix Zika Virus 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 Procleix Panther System Software. 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 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 ≤ 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.

A. Any specimen with an interpretation of Invalid in the Procleix Zika Virus Assay must be retested in singlet.

B. 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.

C. Specimens with a valid Internal Control value and with an Analyte S/CO less than 1.00 in the Procleix Zika Virus Assay are considered Nonreactive for ZIKV RNA.

D. No further testing of a ZIKV Nonreactive specimen is required.

E. 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.

1. If an individual specimen tests Reactive with the Procleix Zika Virus Assay, then the individual specimen is considered Reactive for ZIKV.
2. If a pool tests Reactive with the Procleix Zika Virus Assay, then each of the individual specimens comprising the pool must be tested with the Procleix Zika Virus Assay.
3. Further clarification of the Reactive specimens for informational purposes may be obtained by testing an alternate specimen from the index donation with the Procleix Zika Virus Assay and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of donor eligibility.
4. Any reactive result should be resolved according to the resolution algorithm for reactive specimens, as explained in the INTERPRETATION OF RESULTS section.

LIMITATIONS OF THE PROCEDURE

A. This assay has been developed for use with the Procleix Panther System only.

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

C. Cross-contamination of samples can cause false positive results.

D. Assays must be performed, and results interpreted, according to the procedures provided.

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

F. Failure to meet the acceptance criteria for Procleix Zika 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.

G. Though rare, mutations within the highly conserved regions of the viral genome targeted by the primers and/or probes in the Procleix Zika Virus Assay may result in failure to detect the virus.

H. The Procleix Zika Virus Assay is designed to detect Zika RNA in plasma and serum specimens, and Zika virus RNA may persist in certain organs and tissues, as well as other body fluids, longer than it is detectable in plasma and serum.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS IN LIVING DONOR BLOOD SPECIMENS

Clinical Performance

Clinical Reproducibility of the Procleix Zika Virus Assay

Reproducibility was evaluated on the Procleix Panther System at 3 US sites. Two operators performed testing at each site. Each operator performed 3 runs per day on each of 6 days, using 3 reagent lots equally over the course of testing. Each run had 2 replicates of each panel member.

The negative panel members were made from Zika virus negative plasma. The positive panel members were created by spiking the negative plasma with Zika virus derived from a positive clinical specimen. Low positive, moderate positive, and high positive concentrations were prepared for testing.

Agreement values were 100% in the negative, moderate, and high positive panel members and 98.15% in the low positive panel member. The within run %CV was the main contributor for all conditions tested showing the Procleix Zika Virus Assay is highly reproducible. The total %CV was 21.49, 6.79, and 4.88 for the low, moderate, and high positive panel members, respectively.

Table 1 shows the reproducibility and precision of assay results for each panel member between sites, between operators, between lots, between days, between runs, within run, and overall.

Table 1. Reproducibility of the Procleix Zika Virus Assay on the Procleix Panther System

Panel*	Average S/CO	Agreement (%)	Between Sites		Between Operators		Between Lots		Between Days		Between Runs		Within Run		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
A	<0.01	100	<0.01	97.21	0	0	<0.01	108.95	0	0	0	0	<0.01	738.82	<0.01	753.11
B	30.32	98.15	0	0	0	0	0	0	1.21	3.99	0	0	6.40	21.11	6.52	21.49
C	32.73	100	0.42	1.27	0.21	0.65	0.84	2.57	0.09	0.27	0.48	1.47	1.94	5.93	2.22	6.79
D	32.94	100	0.42	1.26	0	0	0.96	2.93	0	0	0.71	2.15	0.99	3.01	1.61	4.88

CV = Coefficient of variation, S/CO = Signal to cutoff ratio, 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.

*n = 216 for each panel member; target concentration for each panel member:

A: negative = 0 copies/mL

B: low positive = 11.8 copies/mL

C: moderate positive = 23.6 copies/mL

D: high positive = 59.0 copies/mL

Clinical Specificity of the Procleix Zika Virus Assay

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. Twelve US blood testing laboratories performed testing using the Procleix Panther System. Four reagent kit lots were used over the course of the study; up to 3 reagent kit lots were used at each testing laboratory.

Pools were created by combining aliquots from 16 individual donations. Pools and individual donations were tested with the Procleix Zika Virus Assay on the Procleix Panther System, in accordance with package insert instructions. Samples from individual donations with initial reactive result(s) were confirmed as true or false positive by testing with alternate nucleic acid tests (NATs) for Zika virus (plasma and/or red blood cells), and serologic tests for Zika virus IgM (immunoglobulin M). Donors with initial reactive Procleix Zika Virus Assay results for the index donation were asked to enroll in follow-up testing. Donor status was based on the Procleix Zika Virus Assay result for Procleix Zika Virus Assay nonreactive donations, and testing of the index donation with the comparator assays and/or follow-up testing, when samples were reactive with the Procleix Zika Virus Assay on the Procleix Panther System. Final status was based on results obtained with index or follow-up donation testing.

Of the 7088 Procleix Zika Virus Assay runs, 50 runs (0.7%, 50/7088) were invalid. Of the 24,516 pools and 1,257,359 individual donations with final Procleix Zika Virus Assay results, no pools (0.0%, 0/24,516) and 80 individual donations (< 0.01%, 80/1,257,359) had final invalid or error results and were excluded from the analyses.

An additional 11 individual donations were excluded from the analyses because they had unknown Zika virus donor status. A total of 1,649,524 donations, comprising 1,257,268 individual donations and 24,516 pools of 16 donations, had final outcomes for the specificity analyses.

Table 2 shows the specificity of the Procleix Zika Virus Assay on the Procleix Panther System in 24,516 pools and 1,257,268 individual donations. Specificity was 100% in 16-sample pools and 99.999% in individual donations.

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

Sample Type	n	True Negative	True Positive	False Positive	Specificity (%)	95% CI
16-sample pools	24,516	24,516	0	0	100	99.985–100
Individual donations	1,257,268	1,257,239	12	17	99.999	99.998–> 99.999

n = number of specimens
CI = Clopper-Pearson confidence interval

Evaluation of Yield During Specificity Study Testing

A Zika virus yield case is defined as an individual whose Zika virus infection was not detected by serologic methods at index (IgM test seronegative) but was correctly identified using the Procleix Zika Virus Assay (as determined by seroconversion in follow-up samples).

During specificity testing of whole blood donors from June 2016 to December 2016, individual donation testing in the US identified 12 (0.001%) initially reactive and confirmed samples from 1,257,268 donations with known outcomes. Of these, 2 were confirmed yield cases. Follow-up samples collected from the donors 8 and 17 days after the respective index donations were confirmed IgM seropositive, indicating seroconversion.

Clinical Sensitivity of the Procleix Zika Virus Assay

Clinical Sensitivity on Known-Positive Plasma and Serum Samples

Zika virus known-positive plasma and serum samples were procured from clinical specimen suppliers. Known positive status was verified by testing with an alternative NAT when tested neat. The positive samples were prepared neat (i.e., undiluted; n=108 for both plasma and serum) and in a 1:16 dilution (n=108 for both plasma and serum) and tested with the Procleix Zika Virus Assay at three laboratories (one in-house and two external). 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 (100%, 10/10) were valid. All neat and diluted plasma and serum samples had final valid results.

The sensitivity in neat (undiluted) Zika virus known-positive plasma samples was 98.15% (106/108; 95% CI: 93.47% to 99.77%). The 2 neat plasma samples with false negative results were inconsistently reactive when tested with an alternate NAT and the Procleix Zika Virus Assay (in multiple replicates), suggesting the samples may have had low viral loads.

The sensitivity in neat (undiluted) Zika virus known-positive serum samples was 96.30% (104/108; 95% CI: 90.79% to 98.98%). The 4 neat serum samples with false negative results were nonreactive when tested with an alternate NAT and were inconsistently reactive when tested with Procleix Zika Virus Assay (in multiple replicates), suggesting the samples may have had low viral loads.

The sensitivity in diluted (1:16) Zika virus known-positive plasma samples was 85.19% (92/108; 95% CI: 77.06% to 91.29%).

The sensitivity in diluted (1:16) Zika virus known-positive serum samples was 79.63% (86/108; 95% CI: 70.80% to 86.77%).

Table 3. Clinical Sensitivity of the Procleix Zika Virus Assay in Known-Positive Samples

Sample Type	Matrix	n	True Positive	False Negative	Sensitivity (%)	95% CI
Neat	Plasma	108	106	2	98.15	93.47–99.77
	Serum	108	104	4	96.30	90.79–98.98
Diluted	Plasma	108	92	16	85.19	77.06–91.29
	Serum	108	86	22	79.63	70.80–86.77

n = number of samples
CI =Clopper-Pearson Confidence Interval

Analytical Performance

Specificity of the Procleix Zika Virus Assay in Normal Blood Donor Serum Specimens

Serum specimens from normal blood donors were obtained from volunteer whole blood donations and purchased from a commercial vendor. Specimens were tested in the Procleix Zika Virus Assay on the Procleix Panther System.

Seven tests that were invalid due to instrument hardware, software, or sample processing errors were retested. Only the valid retest results are included in the data analysis. All specimens were valid and non-reactive upon retest. There were no initial invalid results due to assay chemistry errors, for an initial invalid rate of 0% (0/2500). Table 4 shows the reactivity and specificity rates for the Procleix Zika Virus Assay.

Table 4. Specificity of Procleix Zika Virus Assay in Frozen Normal Blood Donor Serum Specimens

n, Valid Results	2500
n, Initial Reactive	0
n, True Positive	0
n, False Positive	0
%Specificity (95% CI)	100 (99.8–100)
Combined Mean Analyte S/CO of Negative Specimens ± standard deviation	0.00 + 0.01

n = Number of Specimens
CI = Score Confidence Interval

Analytical Sensitivity

Analytical sensitivity panels, consisting of Zika virus World Health Organization (WHO) standard (11468/16; Asian Strain)[16] serially diluted in Zika virus negative plasma or serum, were used to evaluate assay sensitivity. Assay sensitivity was also evaluated with a serially diluted *in vitro* transcript based on the sequence corresponding to Zika isolate MR766 (GenBank Accession number AY632535; African Strain). The panels were tested with the Procleix Zika Virus Assay on the Procleix Panther System.

The average analyte S/CO ratio and percent coefficient of variation (%CV) for samples containing viral RNA or *in vitro* transcript were calculated from concordant results only (S/CO >1.0). The 95% confidence intervals of the reactivity rates were based on the Score method.[17] Estimations of 50% and 95% detection rates were determined by Probit Analysis.

The detection rates in plasma and serum for the Zika virus WHO standard (11468/16) are shown in Table 5 and 6, respectively. The detection rates for the Zika virus *in vitro* transcript are shown in Table 7.

Table 5. Detection of Zika Virus WHO Standard in Plasma

ZIKV WHO (11468/16) Prepared in Plasma, IU/mL	Number of Reactive /Tested	% Reactive (95%CI)	Average S/CO	% CV
30	90/90	100 (95.9–100)	32.71	7
10	90/90	100 (95.9–100)	31.89	9
3	87/90	96.7 (90.7–98.9)	27.19	28
1	58/90	64.4 (54.2–73.6)	20.79	29
0.3	20/90	22.2 (14.9–31.8)	20.23	32
0	0/90	0 (0–4.1)	0	N/A

CI = Score Confidence Interval, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio in concordant replicates only, N/A = Not Applicable

Table 6. Detection of Zika Virus WHO Standard in Serum

ZIKV WHO (11468/16) Prepared in Serum, IU/mL	Number of Reactive /Tested	% Reactive (95% CI)	Average S/CO	% CV
30	90/90	100 (95.9–100)	33.15	6
10	90/90	100 (95.9–100)	33.05	6
3	89/90	98.9 (94.0–99.8)	29.61	21
1	70/90	77.8 (68.2–85.1)	23.86	30
0.3	18/90	20.0(13.0–29.4)	19.34	53
0	0/90	0 (0–4.1)	0	N/A

CI = Score Confidence Interval, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio in concordant replicates only, N/A = Not Applicable

Table 7. Detection of Zika Virus *In Vitro* Transcript

ZIKV *In Vitro* Transcript, copies/mL	Number of Reactive /Tested	% Reactive (95% CI)	Average S/CO	% CV
90	60/60	100 (94.0–100)	33.35	4
30	216/216	100 (98.3–100)	33.16	5
10	195/216	90.3 (85.6–93.6)	31.78	15
3	116/216	53.7 (47.0–60.2)	28.55	27
1	38/216	17.6 (13.1–23.2)	27.28	25
0.3	12/216	5.6 (3.2–9.5)	27.87	25
0	0/216	0 (0–1.7)	0	N/A

CI = Score Confidence Interval, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio in concordant replicates only, N/A = Not Applicable

Probit Analysis

The 50% and 95% detection probabilities for the Zika virus WHO standard (11468/16) in plasma and serum and Zika virus *in vitro* transcript were determined by Probit Analysis.[18] The 50% LOD point estimates from testing dilutions of the WHO Standard were 0.64 IU/mL in plasma and 0.56 IU/mL in serum. The 95% LOD estimates for the WHO Standard were 2.90 and 1.91 IU/mL in plasma and serum, respectively (Table 8). For the Zika virus transcript, the 50% and 95% LOD estimates were 3.01 and 12.05 copies/mL, respectively (Table 9). The Normal model was used for Probit Analysis of the Zika virus WHO standard and the Gompertz model for the Zika virus *in vitro* transcript.

Table 8. Detection Probabilities of Zika Virus WHO Standard in Plasma and Serum

Matrix	Detection Probabilities, IU/mL	
	50% (95% Fiducial Limits)	**95% (95% Fiducial Limits)**
Plasma	0.64 (0.54 to 0.76)	2.90 (2.22 to 4.18)
Serum	0.56 (0.48 to 0.65)	1.91 (1.52 to 2.63)

Table 9. Detection Probabilities of Zika Virus *In Vitro* Transcript

Detection Probabilities, copies/mL	
50% (95% Fiducial Limits)	**95% (95% Fiducial Limits)**
3.01 (2.67 - 3.37)	12.05 (10.39 - 14.34)

Specificity and Sensitivity of the Procleix Zika Virus Assay in the Presence of Donor and Donation Factors

When tested with the Procleix Zika Virus Assay, no cross-reactivity or interference was observed for naturally occurring icteric, hemolyzed, or lipemic specimens or plasma containing the following substances: albumin (60,000 mg/L), hemoglobin (2,000 mg/L), bilirubin (200 mg/L), and lipids (30,000 mg/L).

No cross-reactivity or interference was observed in specimens from patients with autoimmune or other diseases not caused by Zika virus infection. Multiple specimens from each group of patients with the following autoimmune or other conditions were evaluated: rheumatoid factor, antinuclear antibody, systemic lupus erythematosus, and multiple myeloma.

No cross-reactivity or interference was observed in plasma contaminated with bacteria or fungi, or in specimens from subjects infected with other blood-borne pathogens, or those that had received HBV vaccines, with the exception of one replicate of a Dengue virus type3 sample with a false positive result for an overall specificity of 99.8% (95% score confidence interval: 98.6–100%). Plasma spiked with the following microorganisms was evaluated: *Staphylococcus epidermidis, Staphylococcus aureus, Corynebacterium diphtheriae, Propionibacterium acnes, Candida albicans, Neisseria gonorrhoeae, Pneumocystis carinii,* or *Chlamydia trachomatis*. Multiple specimens from each group of patients with the following viral infections were evaluated: dengue virus types 1–4, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus 1 and 2 (HIV 1/2), parvovirus B-19, and West Nile virus (WNV). Plasma spiked with the following viruses was also evaluated: hepatitis E virus (HEV), chikungunya, adenovirus type 5, BK human polyomavirus, cytomegalovirus, Epstein-Barr virus, influenza virus H1N1, hepatitis G virus (HGV), human herpesvirus Type 6B and 8, human papillomavirus (HPV), herpes simplex virus 1 and 2, human T-lymphotropic virus 1 and 2, Japanese encephalitis virus (JEV), Murray Valley encephalitis virus, rubella virus, St. Louis Encephalitis virus, vaccinia virus, and Yellow Fever virus.

No cross-reactivity or interference was observed when equal volume pools of 16 donor and donation factor specimens were tested in the Procleix Zika Virus Assay.

Specificity and Sensitivity of the Procleix Zika Virus Assay in the Presence of Exogenous Substances

No cross-reactivity or interference was observed in plasma containing the following substances: Acetaminophen (1324 µmol/L), Acetylsalicylic Acid (3620 µmol/L), Ascorbic Acid (342 µmol/L), Atorvastatin (600 µg Eq/L), Ibuprofen (2425 µmol/L), Loratadine (0.78 µmol/L), Naproxen (2170 µmol/L), and Phenylephrine HCl (491 µmol/L).

PERFORMANCE CHARACTERISTICS IN CADAVERIC DONOR BLOOD SPECIMENS

Reproducibility of the Procleix Zika Virus in Cadaveric Blood Specimens on the Procleix Panther System

The inter-assay reproducibility of the Procleix Zika Virus Assay with cadaveric blood specimens was assessed by determining the %CVs obtained when each of 20 cadaveric plasma, 20 control plasma, 20 cadaveric serum, and 20 control serum specimens, spiked with either 60 copies/mL or 150 copies/mL Zika Virus were tested on the Procleix Panther System. Each specimen was tested individually, in 6 separate runs, on 6 separate days using 3 reagent lots with a minimum of 2 operators and 2 Procleix Panther Systems. All specimens were initially spiked with 60 copies/mL of Zika virus. A statistically significant difference between the reactivity of control and cadaveric plasma specimens was observed at 60 copies/mL using Fisher's exact test. The cadaveric plasma condition was then repeated at 150 copies/mL. The reactive rates, S/COs, and %CVs are shown in Table 10.

The %CVs for cadaveric plasma 60 copies/mL, cadaveric plasma 150 copies/mL, control plasma 60 copies/mL, cadaveric serum 60 copies/mL, and control serum 60 copies/mL specimens were 16%, 5%, 5%, 11%, and 5%, respectively. The percent reactive rates for the cadaveric plasma 60 copies/mL, cadaveric plasma 150 copies/mL, control plasma 60 copies/mL, cadaveric serum 60 copies/mL, and control serum 60 copies/mL specimens in this study were 97.5%, 100%, 100%, 99.4%, and 100%, respectively.

Table 10. Summary of Reproducibility Testing of the Procleix Zika Virus Assay in Cadaveric and Control Specimens

Sample	Copies/mL	# Donors	# Replicates	% Reactivity (95% CI)	Mean Analyte S/CO	%CV
Cadaveric Plasma	60	20	360	97.5 (95.3–98.9)	32.08	16
Cadaveric Plasma	150	20	360	100 (99.0–100)	34.63	5
Control Plasma	60	20	360	100 (99.0–100)	33.09	5
Cadaveric Serum	60	20	360	99.4 (98.0–99.9)	32.95	11
Control Serum	60	20	360	100 (99.0–100)	33.06	5

CI = Clopper-Pearson Confidence Interval

S/CO = Signal to Cutoff ratio in concordant replicates only

CV = Coefficient of Variation in concordant replicates only

Specificity of the Procleix Zika Virus in Cadaveric Blood Specimens on the Procleix Panther System

Cadaveric serum and plasma specimens were tested to determine the specificity of the Procleix Zika Virus Assay. One hundred cadaveric specimens[19] (50 unique cadaveric serum specimens and 50 unique cadaveric plasma specimens) and 100 normal blood donor (control) specimens (50 serum and 50 plasma specimens) were tested. The specificity for the control plasma and serum specimens was 100% (95% CI: 92.9–100%). The specificity for the cadaveric plasma and serum specimens was 98.0% (95% CI: 89.4–99.9%) and 100% (95% CI: 92.9–100%) respectively (Table 11).

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

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	1.92	2.01	1.91	1.99
Mean Analyte S/CO	0.00	0.01	0.00	0.00
%Specificity (95%CI)	100 (92.9–100)	98.0 (89.4–99.9)*	100 (92.9–100)	100 (92.9–100)
n	50	50	50	50

n = Number of tests

IC = Internal Control

CI = Clopper-Pearson Confidence Interval

S/CO = Signal to Cutoff ratio in concordant replicates only

* False positive sample retested in singlet resulting in Nonreactive result (not included in final analysis)

Sensitivity of the Procleix Zika Virus Assay in Cadaveric Blood Specimens Spiked with Zika Virus

Cadaveric serum and plasma specimens spiked with a low level of Zika virus (approximately 18 copies/mL from 6 unique donors) were tested to determine the sensitivity of the Procleix Zika Virus Assay. One hundred cadaveric specimens[19] (50 unique cadaveric serum specimens and 50 unique cadaveric plasma specimens), and 100 normal blood donor (control) specimens (50 serum and 50 plasma specimens) were tested. The spiked cadaveric and control samples were tested across four reagent lots. The reactive rate for the control plasma and serum specimens was 98.0% (95% CI: 89.4–99.9%) and 96.0% (95% CI: 86.3–99.5%), respectively. The reactive rate for the cadaveric plasma and serum specimens was 98.0% (95% CI:89.4–99.9%) and 100% (95% CI: 92.9–100%), respectively (Table 12).

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

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	2.25	2.20	2.08	2.20
Mean Analyte S/CO	31.75	31.73	30.93	30.40
%Reactivity (95%CI)	98.0 (89.4–99.9)*	98.0 (89.4–99.9)*	96.0 (86.3–99.5)*	100 (92.9–100)
n	50	50	50	50

n = Number of tests
IC = Internal Control
CI = Clopper-Pearson Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only
* False negative samples retested in duplicate resulting in at least one Reactive result (not included in final analysis)

Stability of Zika Virus in Cadaveric Plasma and Serum Specimens

Stability of Zika virus was evaluated in 10 unique cadaveric plasma and 10 unique cadaveric serum specimens by spiking whole blood with Zika virus to a concentration of approximately 150 copies/mL. After spiking, specimens were subjected to a series of incubations.

On Day 0 an aliquot of each whole blood specimen was transferred to a specimen aliquot tube and centrifuged. The separated plasma or serum was then frozen, thawed, and tested on the same day. The remaining volume of whole blood was incubated at 30˚C for 1 day followed by 2 days at 25˚C. On Day 3, each whole blood specimen was centrifuged to separate the plasma or serum, and then returned to incubation at 2° to 8˚C. On Day 3 for serum, Day 5 for plasma and serum, and Day 8 for plasma, an aliquot of each specimen was transferred to a specimen aliquot tube and frozen. These time point specimens were each frozen and thawed 3 times prior to testing to incorporate the effect of multiple freeze/thaw cycles. Three replicates of each donor specimen were tested at each time point.

The reactive rates for cadaveric plasma at Day 0, Day 5, and Day 8 were 100%, 83.3%, and 90.0%, respectively (Table 13). The reactive rates for cadaveric serum at Day 0, Day 3, and Day 5 were 100%, 96.7%, and 83.3%, respectively (Table 14). These reactivity results were compared to Day 0 results using Fisher's Exact Test and gave p-values greater than or equal to 0.0522, showing no statistically significant differences.

Table 13. Stability of Zika Virus in Cadaveric Plasma Specimens

	Day 0*	Day 5**	Day 8**
# Valid	30	30	30
# Reactive	30	25	27
% Reactivity (95% CI)	100 (88.4–100)	83.3 (65.3–94.4)	90.0 (73.5–97.9)

CI = Clopper-Pearson Confidence Interval
*Specimens were subjected to 1 freeze/thaw cycle prior to testing
**Specimens were subjected to 3 freeze/thaw cycles prior to testing

Table 14. Stability of Zika Virus in Cadaveric Serum Specimens

	Day 0*	Day 3**	Day 5**
# Valid	30	30	30
# Reactive	30	29	25
% Reactivity (95% CI)	100 (88.4–100)	96.7 (82.8–99.9)	83.3 (65.3–94.4)

CI = Clopper-Pearson Confidence Interval
*Specimens were subjected to 1 freeze/thaw cycle prior to testing
**Specimens were subjected to 3 freeze/thaw cycles prior to testing

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REVISION HISTORY

Document Number	Revision Date	Revisions
GDSS-IFU-000005 v. 7.0	2023-04	• Updated Caution Statement in Storage and Handling Instructions to include the requirements for manual reagent tracking. • Under Reagent Preparation, Selection Reagent temperature reverted back to 2°C to 8°C and 2 to 15°C vs. below 15°C. • Note added: PI available at Grifols Technical Library. • Grifols Technical Service information updated. • Addition of revision history.