Procleix Zika Virus Assay

For Investigational Use Only. The performance characteristics of this product have not been established.

1000 Test Kit

CONTENTS

INTENDED USE

The Procleix Zika Virus Assay is a qualitative *in vitro* nucleic acid screening test for the direct detection of Zika virus (ZIKV) RNA in plasma specimens from individual human donors, including donors of whole blood and blood components, and other living donors. The assay is intended to test individual donations. 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. The test is not intended for use on cord blood or for screening other body fluids. The test is not for use as an aid in diagnosis.

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] ZIKV is typically associated with human disease ranging from subclinical infections to mild flu-like illnesses, but more recently ZIKV infection has been associated with serious and sometimes fatal cases of Guillain-Barré syndrome.[8] The virus has also been associated with microcephaly and other birth defects in infants born to infected mothers.[9] Although the primary route of infection appears to be through the bite of a mosquito, sexual transmission[10] and possible transfusion-transmission[11] of ZIKV have also been reported. Based on concerns about the potential for transmission of ZIKV by blood transfusion, the US Food and Drug Administration (FDA) released "Recommendations for Donor Screening, Deferral and Product Management to Reduce the Risk of Transfusion-Transmission of Zika Virus," on February 16, 2016.[12]

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

PRINCIPLES OF THE PROCEDURE

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. Oligonucleotides ("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 each run. (See QUALITY CONTROL PROCEDURES for details).

REAGENTS

Procleix Zika Virus Assay

Internal Control Reagent

A HEPES buffered solution containing detergent and an RNA transcript.

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

Target Capture Reagent

A HEPES buffered solution containing detergent, capture oligonucleotides, and magnetic microparticles. Store at 2° to 8°C (do not freeze). Internal Control Reagent must be added to Target Capture Reagent before use in the assay.

Amplification Reagent

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

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

Probe Reagent

Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.

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

Selection Reagent

Borate buffered solution containing surfactant.

Store at 15° to 30°C.

C0 **Procleix Zika Virus Negative Calibrator**

A HEPES buffered solution containing detergent.

Store at –15° to –35°C.

C1 **Procleix Zika Virus Positive Calibrator**

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

Store at –15° to –35°C.

Procleix Panther System Reagents

R1 **Auto Detect 1**
Aqueous solution containing hydrogen peroxide and nitric acid.

R2 **Auto Detect 2**
1.6 N sodium hydroxide.

W **Wash Solution**
HEPES buffered solution.

O **Oil**
Silicone oil.

DF **Buffer for Deactivation Fluid**
Sodium bicarbonate buffered solution.

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 and preparation for use.

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

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

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

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

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

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

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

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

J. Consult the following table for storage information.

Reagent/Fluid	**Unopened Storage***	**Opened/Thawed Stability***
Internal Control Reagent (IC)	–15° to –35°C	Prior to combining with TCR, 8 hours at RT**
Target Capture Reagent (TCR)	2° to 8°C	20 hours at RT (RPI File 3)***
working Target Capture Reagent (wTCR)		30 days at 2° to 8°C*****; 72 hours at RT****
Amplification Reagent	–15° to –35°C	30 days at 2° to 8°C***** 20 hours at RT (RPI File 3)***; 72 hours at RT****
Enzyme Reagent	–15° to –35°C	30 days at 2° to 8°C***** 20 hours at RT (RPI File 3)***; 72 hours at RT****
Probe Reagent	–15° to –35°C	30 days at 2° to 8°C***** 20 hours at RT (RPI File 3)***; 72 hours at RT****
Selection Reagent	RT	30 days at RT
Calibrators	–15° to –35°C	8 hours at RT
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

*Storage and stability conditions are based on similar validated Procleix products.

**RT = Room Temperature

***The 20 hours are only applicable to Reagent Preparation Incubator RPI File 3 for preparation of unopened reagent bottles.

****The 72 hours must occur within 30 days which includes onboard stability. See the onboard stability table in REAGENT PREPARATION, section C.1.

***** ⚠ Reagents should be maintained at the appropriate storage condition when not in use. Unless reagents are in the RPI 250 or the Procleix Panther System, they should be returned to their appropriate storage conditions without delay.

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, or in gel separation tubes (BD Vacutainer PPT) may be used. Follow sample tube manufacturer's instructions. Specimen stability is affected by elevated temperature.

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

Specimens must be centrifuged within 72 hours of draw.

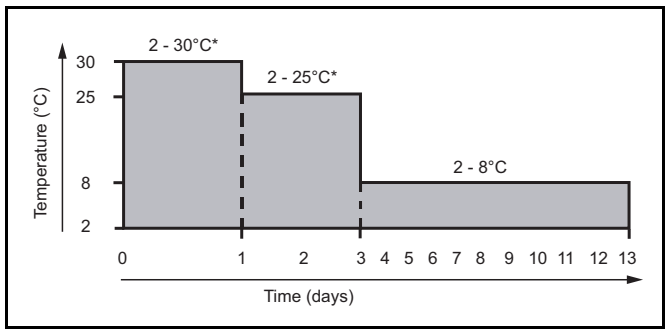
For storage above 8°C, specimens may be stored for 72 hours up to 25°C, and up to 30°C for 24 hours during the 72 hours.

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 at -20°C for longer periods of time.

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 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 and may be stored as in step B., above.

D. Additional specimens may be taken from whole blood or plasma units containing ACD or sodium citrate according to the collection container manufacturer's instructions and may be stored as in step B., above.

E. Additional blood specimens may be collected in tubes and heparin according to the collection container manufacturer's instructions.

Whole blood or plasma from 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.

F. No adverse effect on assay performance was observed when plasma was subjected to three 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 thoroughly and centrifuge 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 should 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.

MATERIALS REQUIRED

Component	Quantity	Part Number
Procleix Zika Virus Assay Kit	1000 tests	PRD-04036-D
Internal Control Reagent	4 x 2.8 mL	
Amplification Reagent	4 x 26 mL	
Enzyme Reagent	4 x 13.4 mL	
Probe Reagent	4 x 34.7 mL	
Target Capture Reagent	4 x 161 mL	
Selection Reagent	4 x 91 mL	
Procleix Zika Virus Assay Calibrators Kit		PRD-04038-D
Negative Calibrator	15 x 2 mL	
Positive Calibrator	15 x 2 mL	
Assay Fluids Kit, Aptima, AS		303014
Wash Solution	1 x 2.9 L	
Oil	1 x 260 mL	
Buffer for Deactivation Fluid	1 x 1.4 L	
Auto Detect Reagents Kit, Aptima, AS		303013
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, Selection and Probe Reagents)	1 bag of 100	CL0039
Reagent Spare Caps (Amplification Reagent)	1 bag of 100	CL0042
Reagent Spare Caps (Enzyme Reagents)	1 bag of 100	501619
Equipment		
Procleix Panther System and operator's manual Reagent Preparation Incubator 250 (RPI 250) and operator's manual Independent Temperature Monitor (ITM)		
Other		
Advanced Cleaning Solution	1 bottle (255 mL)	303085

OTHER MATERIALS AVAILABLE FROM GRIFOLS FOR USE WITH PROCLEIX ZIKA 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

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

Bleach (for use in final concentrations of 5 to 7% 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 in rack approved for use with the Procleix Panther System. Contact Grifols Technical Service for approved tips.

PRECAUTIONS

A. For Investigational Use Only. The performance characteristics of this product have not been established.

B. Provided that the blood specimen have been tested and found negative for Zika virus by investigational Procleix Zika Virus Assay, it is recommended that an appropriate acknowledgement or consent be obtained prior to transfusion of high risk recipient (e.g., a fetus in utero or pregnant woman).

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. 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.

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 and kit reagents. 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. 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.

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, state, and federal regulations. Thoroughly clean and disinfect all work surfaces.

J. Use only specified disposables.

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

L. Avoid microbial and nuclease contamination of reagents.

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

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

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

P. 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.

Q. 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.

The Selection Reagent contains boric acid and sodium hydroxide.
 **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation
Oil contains polydimethylsiloxane.
 **WARNING** H315 - Causes skin irritation H320 - Causes eye irritation
Auto Detect 2 contains 6% sodium hydroxide.
 **DANGER** H314 - Causes severe skin burns and eye damage

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. DO NOT heat the Probe Reagent above 35°C when using the RPI 250. Refer to the *Procleix RPI 250 Operator's Manual*.

U. 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. 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.

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

*The onboard time must occur within the room temperature times listed in the STORAGE AND HANDLING INSTRUCTIONS.

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, precipitate may form.
2. If cloudiness or precipitate is present, perform Selection Reagent recovery as described in the *Procleix RPI 250 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 RPI 250 Operator's Manual* to prepare the following reagents using the RPI 250: TCR, Probe Reagent, Enzyme Reagent, and Amplification Reagent.

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, and refer to the *Procleix RPI 250 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 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 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 to thaw 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, 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. Proficiency panel members or external quality controls must not be used as substitutes for the Procleix Zika Virus Assay Calibrators.

C. 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.

D. 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 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.

E. 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 RPI 250 Operator's Manual,* and any applicable technical bulletins.

F. RUN SIZE

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

G. EQUIPMENT PREPARATION

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

H. 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.

I. WORK FLOW

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

J. 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 (individual donations) should be run in singlet in the Procleix Zika Virus Assay.

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.

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.

Summary of Specimen Interpretation:

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

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

1. Any specimen with an interpretation of Invalid in the Procleix Zika Virus Assay must be retested in singlet.
2. If at any point in the testing algorithm there is insufficient volume to complete the testing then an alternate specimen from the index donation may be used as long as the storage criteria in the package insert are met.
3. Specimens with a valid Internal Control value and with an Analyte S/CO less than 1.00 in the Procleix Zika Virus Assay are considered Nonreactive for ZIKV RNA.

   No further testing of a ZIKV Nonreactive specimen is required.
4. Specimens with an Analyte S/CO greater than or equal to 1.00 with IC Signal less than or equal to 750,000 RLU are considered Reactive.
   a. If an individual specimen tests Reactive with the Procleix Zika Virus Assay, then the individual specimen is considered Reactive for ZIKV. 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.
   b. 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 achieve expected results is an indication of an invalid run. Possible sources of error include test kit deterioration, operator error, faulty performance of equipment, specimen deterioration, or contamination of reagents.

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

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