Aptima® Zika Virus Assay

For Emergency Use Authorization (EUA) only

For in vitro diagnostic use only

Rx only

General Information

Intended Use

The Aptima® Zika Virus assay is a transcription-mediated amplification test intended for the qualitative detection of RNA from the Zika virus in serum, plasma, processed urine, or processed whole blood K2EDTA (whole blood and urine collected alongside a patient-matched serum or plasma specimen) from individuals meeting CDC Zika virus clinical criteria (e.g., clinical signs and symptoms associated with Zika virus infection) and/or CDC Zika virus epidemiological criteria (e.g., history of residence in or travel to a geographic region with active Zika virus transmission at the time of travel, or other epidemiological criteria for which Zika virus testing may be indicated), by laboratories in the United States that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Specimens are tested using the Panther® System for automated specimen processing, amplification, and detection. Results are for the identification of Zika virus RNA. Zika virus RNA is generally detectable in serum during the acute phase of infection (approximately 14 days following onset of symptoms, if present). Positive results are indicative of current infection. Laboratories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude Zika virus infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Aptima Zika Virus assay is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of nucleic acid amplification and *in vitro* diagnostic procedures. This assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation of the Test

Zika virus (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.[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 ZIKV infection has also been associated with serious and sometimes fatal cases of Guillain-Barré syndrome.[8] The virus has also been linked 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.

Principles of the Procedure

The Aptima Zika Virus assay targets two highly conserved regions in the NS2 and NS4/NS5 regions for increased tolerance to potential mutations. The assay involves three main steps, which take place in a single tube, on the automated Panther system: sample preparation, ZIKV RNA target amplification by Transcription-Mediated Amplification (TMA),[12] and detection of the amplification products (amplicon) by Hybridization Protection Assay (HPA).[13] The assay incorporates an internal control (IC) to monitor nucleic acid capture, amplification, and detection, as well as operator or instrument error.

Serum and plasma specimens are loaded directly onto the Panther system. However, neat urine and whole blood specimens must be manually processed prior to loading onto the Panther system.

On the Panther system, 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") 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 Aptima 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 in a luminometer and is reported as Relative Light Units (RLU).

Internal Control is added to each test specimen and assay calibrator via the working Target Capture Reagent. The Internal Control in the Aptima 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.[13] 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.[14]

The Aptima Zika Virus assay calibrators are used to determine the assay cutoff and assess assay run validity in each run. See *Quality Control* for details.

Warnings and Precautions

A. For *in vitro* diagnostic use. For use under an Emergency Use Authorization (EUA) only.

B. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther/ Panther Fusion Operator's Manual* prior to performing this assay.

Laboratory Related

C. Only personnel adequately trained in the use of the Aptima Zika Virus assay and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures.

D. Use only supplied or specified disposable laboratory ware.

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

F. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution.

G. Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations.[15,16,17,18] Thoroughly clean and disinfect all work surfaces.

H. Enzyme Reagent contains sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing sodium 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.

Specimen Related

I. Specimens may be infectious. Use Universal Precautions[15,16,17] when performing this assay. Proper handling and disposal methods should be established according to local regulations.[18] Only personnel adequately trained in the use of the Aptima Zika Virus assay and trained in handling infectious materials should perform this procedure.

J. Specimen collection, transport, storage and processing procedures outlined in this package insert are required for the optimal performance of this test. Improper collection, transport, or storage of specimens may lead to incorrect results.

K. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

L. Avoid cross-contamination during the specimen handling steps. Be especially careful to avoid contamination by the spread of aerosols when loosening or uncapping specimens. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they come in contact with specimen.

Assay Related

M. Do not use the reagent kit or calibrators after the expiration date.

N. Do not interchange, mix, or combine assay reagents from kits with different master lot numbers. Assay fluids can be from different lot numbers.

O. Avoid microbial and nuclease contamination of reagents.

P. Cap and store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See *Reagent Storage and Handling Requirements* and *Panther System Test Procedure* for more information.

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

R. Some reagents of this kit are labeled with risk and safety symbols.

Note: *For information on any hazard and precautionary statements that may be associated with reagents refer to the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend on www.hologic.com/package-inserts*

US Hazard Information

Selection Reagent
Boric Acid 1 - 5%
WARNING
Causes skin irritation
Causes serious eye irritation
Wash face, hands and any exposed skin thoroughly after handling
Wear protective gloves/protective clothing/eye protection/face protection

Reagent Storage and Handling Requirements

A. The following table shows the storage conditions and stability for reagents and calibrators.

Reagent	Unopened Storage	Open Kit (Thawed)[a]	
		Storage	Stability
Amplification Reagent	–35°C to –15°C	2°C to 8°C	30 days[b]
Enzyme Reagent	–35°C to –15°C	2°C to 8°C	30 days[b]
Probe Reagent	–35°C to –15°C	2°C to 8°C	30 days[b]
Internal Control	–35°C to –15°C	15°C to 30°C	8 hours prior to combining with TCR
Target Capture Reagent (TCR)	2°C to 8°C	n/a	n/a
Working Target Capture Reagent (wTCR)	n/a	2°C to 8°C	30 days[b]
Selection Reagent	15°C to 30°C	15°C to 30°C	30 days[b]
NCAL (Negative Calibrator)	–35°C to –15°C	n/a	Single use vial Use within 8 hours
PCAL (Positive Calibrator)	–35°C to –15°C	n/a	Single use vial Use within 8 hours
Aptima Blood Processing Medium (BPM)	15°C to 30°C	15°C to 30°C	30 days

[a] Open kit storage and stability conditions are based on similar validated assays.

[b] When reagents are removed from the Panther system, they should be immediately returned to their appropriate storage temperatures.

B. Discard any unused, previously prepared reagents and working target capture reagent after 30 days.

C. After opening a bottle of Aptima Blood Processing Medium, it is stable for 30 days.

D. Reagents stored onboard the Panther system have 120 hours (cumulative) of onboard stability. The Panther system logs each time the reagents are loaded.

E. If a precipitate forms in the Target Capture Reagent (TCR) during storage, see instructions under *Preparation of a New Kit*. DO NOT VORTEX. DO NOT FREEZE TCR.

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 Selection Reagent, Probe Reagent, Negative Calibrator, or Positive Calibrator, see instructions under *Panther System Test Procedure*.

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. After thawing the calibrators, the solution must be clear, i.e., not cloudy or have precipitates.

⚠ K. The probe reagent is photosensitive. Protect reagent from light during storage and preparation for use.

Specimen Collection and Storage

The Aptima Zika Virus assay can be used with serum, plasma, processed whole blood, and processed urine specimens.

A processed urine specimen is neat urine added to urine transport media in an Aptima Urine Specimen Transport Tube.

A processed whole blood specimen is whole blood added to Aptima Blood Processing Medium in an Aptima transport tube.

Note: *A urine specimen or whole blood specimen should not be the sole specimen tested from a patient. If a urine specimen or whole blood specimen from a patient is tested, it should be collected alongside a serum or plasma sample from the patient.*

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during sample handling steps. For example, discard used material without passing over open tubes. False-positive results may occur if cross-contamination of specimens is not adequately controlled during specimen handling and processing.*

Note: *The minimum volume of serum or plasma for primary collection tubes is 1200 µL and for specimen aliquot tubes (SATs) the minimum volume is 700 µL to obtain the 500 µL reaction volume. The minimum volume of processed urine or processed whole blood specimens is 1200 µL to obtain the 500 µL reaction volume.*

A. Instructions for Collection

Human serum, plasma, whole blood (K2EDTA), and urine specimens may be used with the Aptima Zika Virus assay on the Panther system.

Refer to the appropriate specimen collection kit package insert for collection instructions.

1. Plasma and Serum Specimens

   Whole blood specimens collected in the following glass or plastic tubes may be used according to manufacturer's instruction:

   - Tubes containing ethylenediaminetetraacetic acid (EDTA) or acid citrate dextrose adenine (ACD-A) anticoagulants or sodium citrate (NAC)
   - Plasma preparation tubes (PPTs)
   - Serum tubes
   - Serum separator tubes (SSTs)

     For serum, allow the clot to form before further processing.

2. Whole Blood Specimens

   Whole blood specimens must be collected in tubes containing K2EDTA.

3. Urine Specimens

   Urine specimens must be collected according to manufacturer's instructions.

B. Specimen Transport and Storage before Testing

1. Plasma and Serum Specimens

   For plasma and serum specimens, whole blood specimens must be centrifuged within 72 hours of collection. Plasma and serum 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 can be stored for 72 hours at temperatures up to 25°C, and up to 24 hours during the 72 hours at temperatures up to 30°C. Do not freeze whole blood prior to centrifugation to obtain plasma and/or serum samples.
   - After whole blood specimens are centrifuged within 72 hours of collection, plasma and serum specimens should be stored at 2°C to 8°C for up to 10 days unless frozen.

   a. If longer storage is needed, freeze plasma and serum separated from the cells and store at –20°C or –70°C.

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

   c. Ensure that plasma and serum specimens have sufficient sample volume above the gel separator or red cell interface.

   d. Specimens with visible precipitates or fibrinous material should be clarified by centrifugation for 10 minutes at 1000 to 3000*g* prior to testing.

2. Whole Blood Specimens

   a. Whole blood may be stored for a total of 14 days from the time of collection to the time of testing with the following conditions: Whole blood can be stored for up to 72 hours at temperatures up to 25°C and up to 24 hours during the 72 hours at temperatures up to 30°C. It can then be stored at 2°C to 8°C for up to 11 days unless frozen.

   b. If longer storage is needed, freeze whole blood at –20°C or –70°C.

   c. Whole blood should not be frozen or thawed more than once.

   d. Whole blood must be processed by adding 1 mL of whole blood to 3 mL of Aptima Blood Processing Medium in an Aptima transport tube. See *Panther System Test Procedure*, step E.2, for more information.

   e. Processed whole blood must be tested within 4 hours of preparation.

3. Urine Specimens

   a. Urine may be stored at 2°C to 30°C and must be transferred to an Aptima Urine Specimen Transport Tube, which contains urine transport media, and thoroughly mixed within 72 hours. See the appropriate collection kit package insert.

   b. Store the mixed, processed urine specimen at 2°C to 30°C and test within 30 days of collection. If longer storage is needed, freeze the processed urine specimen at –20°C or –70°C.

   c. No adverse effect on assay performance was observed when processed urine was subjected to three freeze-thaw cycles.

   d. Ensure that specimens have sufficient sample volume.

   e. Specimens with visible precipitates or fibrinous material should be clarified by centrifugation for 10 minutes at 1000 to 3000*g* prior to testing.

C. Specimen Storage after Testing

1. Specimens that have been assayed must be stored upright in a rack.
2. The specimen tubes should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, place new caps on the specimen tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen tubes must be centrifuged briefly (5 minutes at 500*g*) to bring all of the liquid down to the bottom of the tube. **Avoid splashing and cross-contamination.**

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther System

Reagents for the Aptima Zika Virus assay are listed below for the Panther system. Reagent identification symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima Zika Virus calibrator kits must be purchased separately. See individual catalog kit number below.

Aptima Zika Virus Assay Kit, 1000 tests (4 x 250 tests) Cat. No. PRD-04037-D
(3 assay boxes)

Aptima Zika Virus Assay Box
(store at –35°C to –15°C upon receipt)

Symbol	Component	Quantity
A	**Amplification Reagent** *Non-infectious nucleic acids in buffered solution.*	4 x 26 mL
E	**Enzyme Reagent** *Reverse transcriptase and RNA polymerase in HEPES buffered solution.*	4 x 13.4 mL
P	**Probe Reagent** *Chemiluminescent probes in succinate buffered solution.*	4 x 34.7 mL
IC	**Internal Control Reagent** *A HEPES buffered solution containing detergent and an RNA transcript.*	4 x 2.8 mL
	Master Lot Barcode Sheet	1 sheet

Aptima Zika Virus Assay Box
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
S	**Selection Reagent** *600 mM borate buffered solution containing surfactant.*	4 x 91 mL

Aptima Zika Virus Assay Box
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
TCR	**Target Capture Reagent** *A buffered salt solution containing solid phase, non-infectious nucleic acids.*	4 x 161 mL

Materials Required but Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther System, Diagnostic	303095
Panther Fusion Module	PRD-04173
Panther System Continuous Fluid and Waste (Panther Plus)	PRD-06067
Aptima Assay Fluids Kit (also known as Universal Fluids Kit) *contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent*	303014 (1000 tests)
Aptima Auto Detect Kit	303013 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or, Panther System Run Kit *contains MTUs, waste bags, waste bin covers, auto detects, and assay fluids*	303096 (5000 tests)
Tips, 1000 µL filtered, conductive, liquid sensing, and disposable *Not all products are available in all regions. Contact your representative for region-specific information*	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Aptima Zika Virus Calibrator Kit *Negative calibrator, buffered solution containing detergent, 15 x 2.2 mL* *Positive calibrator, RNA transcript in buffered solution containing detergent, 15 x 2.2 mL*	PRD-04039-D
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Disposable, powderless gloves	—
Replacement non-penetrable caps	103036A
Reagent replacement caps for 250-test bottles *Amplification and Probe reagents* — *CL0041 (100 caps)* *Enzyme reagent* — *501616 (100 caps)* *TCR and Selection reagents* — *CL0040 (100 caps)*	
Plastic-backed laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Tips	—
Primary blood collection tubes of the following dimensions may be used: *13 mm x 100 mm* *13 mm x 75 mm* *16 mm x 100 mm*	—
Centrifuge	—
Vortex mixer	—

Optional Materials

Material	Cat. No.
Aptima Specimen Aliquot Tubes (SAT) (100 pack)	503762
Transport Tube Cap (100 pack) *cap for SAT and Aptima transport tubes*	504415
Aptima Transport Tubes *for processed whole blood specimens*	101738A
Aptima Urine Specimen Collection Kit	301040
Or Aptima Urine Specimen Transport Tubes	105575
Aptima Blood Processing Medium *Tris buffered solution containing detergent, 2 x 1.6 L*	PRD-04744-D
Transfer pipets	—
Cotton-tipped swabs	—
Tube rocker	—
SB100 Reagent Equilibration System (SB100-RES)	—
Water Bath	—

Panther System Test Procedure

Note: *See the Panther/Panther Fusion Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Clean work surfaces where reagents will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared. Use the procedure described above (step A.1).
3. Clean any pipettors. Use the cleaning procedure described above (step A.1).

B. Preparation of a New Kit

Warning: *Avoid creating excessive foam in reagents. Foam compromises the level-sensing by the Panther system.*

Note: *Probe Reagent is photosensitive. Protect it from light during storage and during reagent handling.*

Note: *Amplification, Enzyme, and Probe Reagents may be thawed up to 24 hours at 2°C to 8°C prior to reagent preparation.*

Note: *Internal Control may be thawed up to 24 hours at 2°C to 8°C or up to 8 hours at room temperature (15°C to 30°C) prior to wTCR preparation.*

Target Capture Reagent (TCR), Amplification, Enzyme, and Probe Reagents Preparation

1. Remove a new set of reagents from storage. Check the lot numbers on the reagent bottles to make sure that they match the lot numbers on the Master Lot Barcode Sheet.
2. Allow reagents to reach room temperature (15°C to 30°C) using one of three options described below:

SB100-RES Preparation (Option 1)

1. **Immediately** upon removing from storage (2°C to 8°C), invert TCR bottle vigorously to mix gel into solution (at least 10 inversions and until gel is no longer present on the bottom). DO NOT VORTEX.
2. Prepare the TCR, Amplification, Enzyme, and Probe reagents using the SB100-RES.
3. Upon unload of reagents, record the Thaw Date for the Amplification, Enzyme, and Probe Reagents in the space provided on the label.

Water Bath Preparation (Option 2)

Warning: *Temperature of the water bath should not exceed 30°C.*

Note: *Refer to room temperature (15°C to 30°C) preparation instructions to prepare TCR. Do not use a water bath to prepare TCR.*

1. Upon removing from storage (–35°C to –15°C or 2°C to 8°C), place Amplification, Enzyme, and Probe reagents upright in a dedicated room temperature water bath (15°C to 30°C). At least every 10 minutes, gently invert the reagents to mix thoroughly and visually examine to ensure dissolution of precipitates. Continue to gently invert and visually examine until no precipitates are present.
2. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitation, or cloudiness is present.
3. Record the Thaw Date for the Amplification, Enzyme, and Probe reagents in the space provided on the label.

Room Temperature Preparation (Option 3)

Note: *Probe Reagent from –35°C to –15°C storage, may take up to 4 hours to completely thaw at room temperature (15°C to 30°C) with gentle inversion at least every 10 minutes.*

1. To prepare TCR, perform the following:
   a. **Immediately** upon removing from storage (2°C to 8°C), invert TCR bottle vigorously to mix gel into solution (at least 10 inversions and until gel is no longer present on the bottom). DO NOT VORTEX.
   b. Allow the TCR bottle to remain at room temperature (15°C to 30°C) for at least 45 minutes. At least every 10 minutes, gently invert the TCR bottle (at least 10 inversions) to mix thoroughly and visually examine to ensure no gel is present.
   c. Ensure gel is dissolved and the magnetic particles are suspended before use.

Note: *If gel is present and persists, do not use. Replace TCR bottle into storage (2°C to 8°C) for subsequent use. Remove a new TCR bottle from storage (2°C to 8°C) and repeat steps 1.a to 1.c.*

2. To prepare Amplification, Enzyme, and Probe reagents, perform the following:
   a. Upon removing from storage (–35°C to –15°C or 2°C to 8°C), place reagents upright at room temperature (15°C to 30°C). At least every 10 minutes, gently invert the

reagents to mix thoroughly and visually examine to ensure dissolution of precipitates. Continue to thaw until no precipitates are present.

3. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitation, or cloudiness is present.
4. Record the Thaw Date for the Amplification, Enzyme, and Probe reagents in the space provided on the label.

Internal Control and Working Target Capture Reagent (wTCR) Preparation

Note: *Do not use a SB100-RES to prepare Internal Control.*

1. To prepare Internal Control, perform the following:
   a. Remove one tube of Internal Control from storage (–35°C to –15°C or 2°C to 8°C).
   b. Upon removing from storage (–35°C to –15°C or 2°C to 8°C), allow Internal Control to remain at room temperature (15°C to 30°C) for at least 30 minutes.

Option: *Internal Control tube may be placed in a room temperature (15°C to 30°C) water bath.*

   c. At least every 10 minutes, gently invert the Internal Control tube to mix thoroughly and visually examine for presence of gel. Ensure gel is dissolved prior to use.

Option: *Internal Control tube may be placed on a tube rocker to mix thoroughly during room temperature preparation.*

Note: *If gelling occurs, gel must be dissolved prior to use and within the 8 hour thaw period at room temperature (15°C to 30°C). If gel persists, do not use. Discard the tube, obtain a new tube of Internal Control, and repeat steps 1.a to 1.c.*

2. To prepare wTCR, perform the following:
   a. Once the TCR is ready for use, pour the entire contents of the Internal Control tube into the TCR bottle. Cap the TCR bottle and gently invert to mix thoroughly.
   b. In the space indicated on the TCR bottle, record the date Internal Control was added, the wTCR expiration date (the date Internal Control was added plus 30 days), the Internal Control lot number (IC LOT), and the operator's initials.
   c. Retain the Internal Control tube as it is required to scan the barcode label into the Panther system.

Selection Reagent Preparation

Note: *Do not use if precipitate or cloudiness is present.*

1. To prepare Selection Reagent, perform the following:
   a. Remove a bottle of Selection Reagent from room temperature (15°C to 30°C) storage. Check the lot number on the reagent bottle to make sure it matches the lot number on the Master Lot Barcode Sheet.
   b. Gently invert the bottle to mix thoroughly and visually examine to ensure no precipitate or cloudiness is present.
   c. Record the date that it was first opened (Open Date) on the space provided on the label.

Note: *Selection Reagent Recovery: If Selection Reagent has been inadvertently stored at 2°C to 8°C or the temperature of the laboratory falls below 15°C, precipitate may form. If precipitate forms in the Selection Reagent during storage, heat at 60°C ± 1°C for no more*

than 45 minutes and gently mix the bottle frequently (every 5 to 10 minutes). Once all precipitate has gone back into solution, place the bottle in a room temperature (15°C to 30°C) water bath and allow the bottle to equilibrate for at least 1 hour.

C. Calibrator Preparation

Note: *Avoid creating excessive foam when inverting calibrators. Foam compromises the level-sensing by the Panther system.*

Note: *Do not use SB100-RES to thaw calibrators.*

1. Upon removing calibrators from storage (–35°C to –15°C), allow calibrators to remain at room temperature (15°C to 30°C) for at least 30 minutes.

Option: *Calibrators may be placed in a room temperature (15°C to 30°C) water bath to thaw.*

2. At least every 10 minutes, gently invert each tube to mix thoroughly. Ensure tube contents are fully thawed prior to use.

Option: *Calibrators may be placed on a tube rocker to mix thoroughly during room temperature preparation.*

3. If gelling is observed, gently invert the tube until gel is no longer present.

Note: *If gelling occurs, gel must be dissolved prior to use and within the 8 hour thaw period at room temperature (15°C to 30°C). If gel persists, do not use. Discard the tube(s), obtain new tube(s) of calibrators, and repeat steps C.1 to C.3.*

4. When the tube contents have fully thawed, dry the outside of each tube with a clean, dry disposable wipe.
5. To prevent contamination, do not open the calibrator tubes at this time.

D. Reagent Preparation for Previously Prepared Reagents

wTCR, Amplification, Enzyme, and Probe Reagents Preparation

1. Remove wTCR and previously prepared reagents from storage.
2. Allow reagents to reach room temperature (15°C to 30°C) using one of three options described below:

SB100-RES Preparation (Option 1)

1. **Immediately** upon removing from storage (2°C to 8°C), invert TCR bottle vigorously to mix gel into solution (at least 10 inversions and until gel is no longer present on the bottom). DO NOT VORTEX.
2. Prepare the wTCR, Amplification, Enzyme, and Probe reagents using the SB100-RES.

Water Bath Preparation (Option 2)

Warning: *Temperature of the water bath should not exceed 30°C.*

Note: *Refer to room temperature (15°C to 30°C) preparation instructions to prepare wTCR. Do not use a water bath to prepare wTCR.*

1. Upon removing from storage (2°C to 8°C), place Amplification, Enzyme, and Probe reagents upright in a dedicated room temperature water bath (15°C to 30°C). At least every 10 minutes, gently invert the reagents to mix thoroughly and visually examine to ensure dissolution of precipitates. Continue to thaw until no precipitates are present.

2. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitation, or cloudiness is present.

Room Temperature Preparation (Option 3)

1. To prepare wTCR, perform the following:
   a. **Immediately** upon removing from storage (2°C to 8°C), invert wTCR bottle vigorously to mix gel into solution (at least 10 inversions and until gel is no longer present on the bottom). DO NOT VORTEX.
   b. Allow the wTCR bottle to remain at room temperature (15°C to 30°C) for at least 45 minutes. At least every 10 minutes, gently invert the wTCR bottle (at least 10 inversions) to mix thoroughly and visually examine to ensure no gel is present.
   c. Ensure gel is dissolved and the magnetic particles are suspended before use.

Note: *If gel is present and persists, do not use. Replace wTCR bottle and matching reagents into storage (2°C to 8°C) for subsequent use.*

2. To prepare Amplification, Enzyme, and Probe reagents, perform the following:
   a. Upon removing from storage (2°C to 8°C), prepare reagents upright at room temperature (15°C to 30°C). At least every 10 minutes, gently invert the reagents to mix thoroughly and visually examine to ensure dissolution of precipitate. Continue to thaw until no precipitates are present.
3. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitation, or cloudiness is present.

Selection Reagent Preparation

Note: *Do not use if precipitate or cloudiness is present.*

1. Remove matching bottle of Selection Reagent from room temperature (15°C to 30°C) storage.
2. Gently invert the bottle to mix thoroughly and visually examine to ensure no precipitate or cloudiness is present.

Note: *Selection Reagent Recovery: If Selection Reagent has been inadvertently stored at 2°C to 8°C or the temperature of the laboratory falls below 15°C, precipitate may form. If precipitate forms in the Selection Reagent during storage, heat at 60°C ± 1°C for no more than 45 minutes and gently mix the bottle frequently (every 5 to 10 minutes). Once all precipitate has gone back into solution, place the bottle in a room temperature (15°C to 30°C) water bath and allow the bottle to equilibrate for at least 1 hour.*

E. Sample Handling

1. Preparation of Serum, Plasma, and Urine Specimens and Calibrators
   a. Allow the specimens and calibrators to reach 15°C to 30°C prior to processing. Gently invert sample tubes at least 3 times or mix gently on a rocker until the sample is homogeneous.
   b. Ensure each specimen tube contains enough volume for each sample type and tube type.
   c. Mix fresh or thawed specimens thoroughly.

d. Prepare samples for loading into a Sample Rack. Do not remove caps. Review each sample for bubbles, fibrinous material, and precipitates. If necessary, prepare as follows:

i. For serum or plasma samples, centrifuge the samples at 1000 to 3000*g* for 10 minutes.

ii. For whole blood samples, see step E.2.

iii. For processed urine samples, centrifuge if there are bubbles in the tube or liquid in the cap. Bubbles in the tube compromise the level-sensing by the Panther system. Centrifugation times and speeds for pulling down all liquid and precipitates must be validated by the user. If precipitate does not go back into solution, visually ensure that the precipitate does not prevent delivery of the specimen.

2. Whole Blood Specimen Preparation

a. Prior to testing whole blood specimens, the specimens must be processed manually as follows:

i. Allow whole blood specimens to reach 15°C to 30°C which takes approximately 30 minutes for refrigerated samples and 1 hour for frozen samples. Do not use water baths or other incubators.

ii. Gently invert whole blood tubes at least 3 times or mix gently on a rocker until blood is homogeneous.

iii. Pipette 3.0 mL of BPM into Aptima transport tubes.

iv. Into the Aptima transport tube, pipette 1.0 mL of whole blood avoiding any clots. Dispense the specimen just below the surface of the BPM and pipette up and down 2 or 3 times to mix.

v. Cap the Aptima transport tube and invert gently at least 10 times or mix gently on a rocker until homogeneous. Avoid creating excessive foam. This is now referred to as a “processed whole blood specimen.” Do not centrifuge processed whole blood specimens.

See *System Preparation*, step F.2 below, for information about loading the rack and removing the caps.

F. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion Operator’s Manual* and *Procedural Notes.* Make sure that the appropriately sized reagent racks and TCR adapters are used.
2. Load samples into the Sample Rack. Perform the following steps for each sample tube (specimen, and, when necessary, calibrator):

a. Loosen one sample tube cap, but do not remove it yet.

Note: *Be especially careful to avoid contamination by the spread of aerosols. Gently loosen caps on samples.*

b. Load the sample tube into the Sample Rack.

c. Repeat steps 2.a and 2.b for each remaining sample.

d. After the samples have been loaded into the Sample Rack, remove and discard each sample tube cap in one Sample Rack. To avoid contamination, do not pass a cap

over any other Sample Racks or sample tubes. Pierceable caps from the Aptima Urine Specimen Transport Tube must also be removed and discarded.

e. If necessary, use a new, disposable transfer pipet to remove any bubbles or foam.

f. When the last cap has been removed, load the Sample Rack into a Sample Bay.

Note: *If running other assays and sample types at the same time, secure the Sample Retainer prior to loading the Sample Rack into a Sample Bay.*

g. Repeat steps 2.a to 2.f for the next Sample Rack.

Procedural Notes

A. Calibrators

1. The calibrator tubes can be loaded in any position in the Sample Rack and in any Sample Bay Lane on the Panther system. Specimen pipetting will begin when one of the following two conditions has been met:
   a. The calibrators are currently being processed by the system.
   b. Valid results for the calibrator are registered on the system.
2. Once the calibrator tubes have been pipetted and are processing for the Aptima Zika Virus assay reagent kit, specimens can be tested with the associated kit for up to 24 hours **unless:**
   a. The calibrator results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. Each calibrator tube can be used once. Attempts to use the tube more than once can lead to processing errors.

B. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

Quality Control

Acceptance Criteria for the Aptima 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 *Acceptance Criteria for Calibration and Calculation of Cutoff*).

1. In an Aptima 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 Panther System software. If less than the minimum number of calibrator replicates is valid, the 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 *Panther/Panther Fusion Operator's Manual* for details.
3. All individual specimen results that are Invalid in a valid run must be retested.

Acceptance Criteria for Calibration and Calculation of Cutoff

A. Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate in the Aptima 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

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

B. Positive Calibrator Acceptance Criteria

The Positive Calibrator is run in triplicate in the Aptima 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

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

C. 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

D. 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

E. Summary of Acceptance Criteria for the Aptima 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

F. Summary of Cutoff Calculations for the Aptima 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 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 Negative 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 Positive 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. The 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.

Summary of Specimen Interpretation

Specimen Interpretation	Criteria
Negative	Analyte S/CO < 1.00 and IC ≥ IC Cutoff and IC ≤ 750,000 RLU
Positive	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.

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

B. Specimens with a valid Internal Control value and with an Analyte S/CO less than 1.00 in the Aptima Zika Virus assay are considered Negative for ZIKV RNA.

C. 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 Positive for ZIKV RNA.

D. A patient-matched serum specimen is currently required for serological follow up testing of negative NAT (nucleic acid testing) results per the CDC testing algorithm (found at http://www.cdc.gov/zika/index.html).

Limitations

A. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this package insert may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Laboratories are required to report all positive results to the appropriate public health authorities.

D. A patient-matched serum specimen is currently required for serological follow up testing of negative NAT results per the CDC testing algorithm (found at http://www.cdc.gov/zika/index.html).

E. The effect of long term storage of specimens on the performance of the Aptima Zika Virus assay has not been fully evaluated.

F. Though rare, mutations within the highly conserved regions of the viral genome covered by the primers and/or probes in the Aptima Zika Virus assay may result in the failure to detect the virus.

G. This assay has been developed for use with the Panther system only.

H. Performance of this assay with processed whole blood specimens is limited for use on Panther system software version 5.3 only. Performance of the assay with processed whole blood specimens on later Panther system software versions has not been evaluated. Plasma, serum, and urine specimens can be processed with all currently available Panther system software versions.

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

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

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

L. 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.

M. This assay has been tested using only the specimen types indicated. Performance with other specimen types has not been evaluated.

N. Results from the Aptima Zika Virus assay should be interpreted in conjunction with other clinical data available to the clinician.

O. A negative result does not preclude a possible infection because results are dependent on adequate specimen collection. Test results may be affected by improper specimen collection, technical error, specimen mix-up, or target levels below the assay limit of detection.

P. The Aptima Zika Virus assay provides qualitative results. Therefore, a correlation cannot be drawn between the magnitude of a positive assay signal and the number of organisms in a specimen.

Q. Customers must independently validate an LIS transfer process.

Conditions for Authorization For The Laboratory

The Aptima Zika Virus assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website:
https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm

Use of the Aptima Zika Virus assay must follow the procedures outlined in these manufacturer's Instructions for Use and the conditions of authorization outlined in the Letter of Authorization. Deviations from the procedures outlined are not permitted under the Emergency Use Authorization. To assist clinical laboratories running the Aptima Zika Virus assay, the relevant Conditions of Authorization are listed verbatim below.

- Authorized laboratories will include with reports of the results of the Aptima Zika Virus assay the authorized Fact Sheet for Health Care Providers, the authorized Fact Sheet for Pregnant Women[1], and the authorized Fact Sheet for Patients. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories will perform the Aptima Zika Virus assay on the Panther System or other authorized instruments.
- Authorized laboratories will perform the Aptima Zika Virus assay using the Aptima Auto Detect Reagents kit and Aptima Assay Fluids kit or other authorized ancillary reagents.
- Authorized laboratories will perform the Aptima Zika Virus assay on serum, plasma, or other authorized specimen types.[2]
- Authorized laboratories will have a process in place for reporting test results to health care providers and relevant public health authorities, as appropriate.[3]
- Authorized laboratories will collect information on the performance of the test and report to Hologic, Inc., any suspected occurrence of false positive or false negative results of which they become aware.
- All laboratory personnel using the test should be appropriately trained in nucleic acid amplification techniques and use appropriate laboratory and personal protective equipment when handling this kit.
- Hologic, Inc., its authorized distributor(s), and authorized laboratories will ensure that any records associated with this EUA are maintained until notified by FDA. Such records will be made available to FDA for inspection upon request.

1 Please note, subsequent to the original Letter of Authorization the Pregnant Women and Patient Fact Sheets were combined into one Patient Fact Sheet as of September 2, 2016.
2 Please note, as of March 8, 2018 the Aptima Zika Virus assay is authorized for use with processed whole blood.
3 For questions related to reporting Zika test results to relevant public health authorities, it is recommended that Hologic, Inc. and authorized laboratories consult with the applicable country, state or territory health department(s) and/or CDC. According to CDC, Zika is a nationally notifiable condition. http://www.cdc.gov/zika/.

Performance

Limit of Detection (LoD) for Plasma Specimens

The Limit of Detection (LoD) is defined as the concentration of ZIKV RNA that is detected at 95% or greater probability according to CLSI EP17-A2.[19] The LoD was determined by testing a ZIKV positive plasma specimen serially diluted in defibrinated, delipidated human plasma. The positive plasma specimen was collected from a blood donor during the 2015 Zika outbreak in Brazil. The sample was quantified using a validated real-time RT-PCR assay. The LoD of the Aptima Zika Virus assay was also evaluated by testing an *in vitro* synthesized transcript (corresponding to the appropriate sequence from GenBank accession number AY632535 for Zika isolate MR766). The transcript was serially diluted in buffer. Three Panther instruments were used to test 72 replicates of each target level, except for the panel member with 90 copies/mL, which was tested in 20 replicates. The results are summarized in Table 1 and Table 2.

Table 1: Detection of ZIKV Positive Brazilian Plasma 2015 with the Aptima Zika Virus Assay on the Panther System

Virus copies/mL	#Tested	#Positive	Positivity		
			%P	Lower 95%CI	Upper 95%CI
0	72	0	0	0	5
0.1	72	1	1	0	7
0.3	72	14	19	12	30
1	72	27	38	28	50
3	72	62	86	76	92
10	72	72	100	95	100
30	72	72	100	95	100
90	20	20	100	84	100

%P = percentage positive, CI = Confidence Interval.

Table 2: Detection of Transcripts with the Aptima Zika Virus Assay on the Panther System

Transcript copies/mL	# Tested	#Positive	Positivity		
			%P	Lower 95%CI	Upper 95%CI
0	72	0	0	0	5
0.3	72	2	3	1	10
1	72	16	22	14	33
3	72	39	54	43	65
10	72	66	92	83	96
30	72	72	100	95	100
90	20	20	100	84	100

%P = percentage positive, CI = Confidence Interval.

The 95% detection probabilities were determined using Probit analysis. The limit of detection for the ZIKV positive Brazilian donor plasma 2015 in the Aptima Zika Virus assay was determined to be 5.9 copies/mL at 95% detection probability. The limit of detection for the *in vitro* transcript in

the Aptima Zika Virus assay was determined to be 13.4 copies/mL at 95% detection probability (Table 3).

Table 3: Probit Analysis Detection

Analytes	95% Detection Probability in copies/mL (95% Fiducial Limits)
Zika Virus (Brazilian plasma 2015)	5.9 (4.3–8.9)
Transcript (based on Zika isolate MR766)	13.4 (9.9–20.3)

Inclusivity — *in silico*

Primer and probe conservation with all publicly available Zika virus strains encompassing intended target regions was assessed by direct comparison to multiple sequence alignments. Distance matrices generated for each oligonucleotide against each isolate indicate that none are likely to be missed as each has at least one set of capture oligonucleotide, forward primer, probe, and reverse primer combination with 100% sequence identity. Based on this analysis, false negative results are not likely to occur with the oligonucleotides included in this system. Inclusivity data analysis is provided in Table 4.

Table 4: Inclusivity Data Analysis

Country	Year[a]	Strain/ Isolate	Oligonucleotide Sequence % Identity										
			Capture Oligonucleotides			Forward Primers				Probes		Reverse Primers	
			TCO_1	TCO_2	TCO_3	FP_1	FP_2	FP_3	FP_4	P_1	P_2	RP_1	RP_2
Brazil	2015	Bahia01	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	Bahia07	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	Bahia09	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	BeH815744	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	BeH818995	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	BeH819015	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	BeH819966	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	BeH823339	96	100	100	95	100	91	96	100	100	100	100
Brazil	2015	BeH828305	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	Brazil-ZKV2015	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	Natal RGN	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	PE243	100	100	100	95	100	91	96	100	100	100	100
Brazil	2016	Rio-S1	100	100	100	95	100	91	96	100	100	100	100
Brazil	2016	Rio-U1	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	SSABR1	100	100	100	95	100	91	96	100	100	100	100
Brazil	2015	ZikaSPH2015	100	100	100	95	100	91	96	100	100	100	100
Cambodia	2010	FSS13025	100	100	100	95	100	91	96	95	100	100	100
Canada	2013	From Vero E6 cells	100	100	100	95	100	91	96	100	95	100	100
Central African Republic	1976	ARB13565	100	100	95	100	95	96	91	100	100	96	100
Central African Republic	1968	ArB1362	100	96	100	100	95	96	91	100	100	100	100
Central African Republic	N/A	ARB15076	100	100	100	100	95	96	91	100	100	100	100
Central African Republic	N/A	ARB7701	100	100	95	100	95	96	91	100	100	96	100
China	2016	GD01	100	100	100	95	100	91	96	100	100	100	100
China	2016	GDZ16001	100	100	100	95	100	91	96	100	100	100	100
China	2016	GZ01	100	100	100	95	100	91	96	100	100	100	100

Table 4: Inclusivity Data Analysis (continued)

Country	Year[a]	Strain/ Isolate	Oligonucleotide Sequence % Identity										
			Capture Oligonucleotides			Forward Primers				Probes		Reverse Primers	
			TCO_1	TCO_2	TCO_3	FP_1	FP_2	FP_3	FP_4	P_1	P_2	RP_1	RP_2
China	2016	GZ02/2016	100	100	100	95	100	91	96	100	100	100	100
China	2016	SZ01/2016	100	100	100	95	100	91	96	100	100	100	100
China	2016	SZ02/2016	100	100	100	95	100	91	96	100	100	100	100
China	2016	SZ-WIV01	100	100	100	95	100	91	96	100	100	100	100
China	2016	VE_Ganxian	100	100	100	95	100	91	96	100	95	100	100
China	2016	Z16006	100	100	100	95	100	91	96	100	100	100	100
China	2016	Z16019	100	100	100	95	100	91	96	100	100	100	100
China	2016	Zhejiang04	100	100	100	95	100	91	96	100	100	100	100
China	2016	ZJ03	100	100	100	95	100	91	96	100	100	100	100
China	2016	ZKC2/2016	100	100	100	95	100	91	96	100	100	100	100
Colombia	2015	C1/C2	100	100	100	95	100	91	96	100	100	100	100
Colombia	2015	FLR	100	100	100	95	100	91	96	100	100	100	100
Colombia	2016	UF-1/2016	100	100	100	95	100	91	96	100	100	100	100
French Polynesia	2013	H/PF/2013	100	100	100	95	100	91	96	100	100	96	100
Guatemala	2015	8375	100	100	100	95	100	91	96	95	100	100	100
Guatemala	2015	103344	100	100	100	95	100	91	96	95	100	100	100
Haiti	2014	Haiti/1225/2014	100	100	100	95	100	91	96	100	100	100	100
Honduras	2016	103451	100	100	100	95	100	91	96	95	100	100	100
Italy	2016	Brazil/2016/INMI1	100	100	100	95	100	91	96	100	100	100	100
Italy	2016	Dominican Republic/ 2016/PD1	100	100	100	95	100	91	96	100	100	98	100
Italy	2016	Dominican Republic/ 2016/PD2	100	100	100	95	100	91	96	100	100	100	100
Malaysia	1966	P6-740	100	100	100	95	100	91	96	100	100	96	100
Martinique	2015	MRS_OPY_Martinique _PaRi_2015	100	100	100	95	100	91	96	100	100	100	100
Mexico	2016	MEX/InDRE/Lm/2016	100	100	100	95	100	91	96	100	100	100	100
Mexico	2016	MEX/InDRE/Sm/2016	100	100	100	95	100	91	96	100	100	100	100
Mexico	2015	MEX_I_7	100	100	100	95	100	91	96	95	100	100	100
Micronesia	2007	ZIKV 2007 EC	100	100	100	95	100	91	96	100	100	100	100
Nigeria	1968	IbH_30656	100	100	95	100	95	100	96	100	95	96	100
Nigeria	1968	IbH-30656_SM21V1-V3	100	100	95	100	95	100	96	100	95	96	100
Panama	2016	BEI-259634_V4	100	100	100	95	100	91	96	100	100	100	100
Panama	2015	CDC-259249_V1-V3	100	100	100	95	100	91	96	100	100	100	100
Panama	2015	CDC-259359_V1-V3	100	100	100	95	100	91	96	100	100	100	100
Panama	2015	CDC-259364_V1-V2	100	100	100	95	100	91	96	100	100	100	100
Philippines	2012	CPC-0740	100	100	100	100	95	91	96	100	100	100	100
Puerto Rico	2015	PRVABC59	100	100	100	95	100	91	96	100	100	100	100
Puerto Rico	2015	V3/V2	100	100	100	95	100	91	96	100	100	100	100
Senegal	1984	41525-DAK	100	100	100	100	95	100	96	100	100	100	100
Senegal	1984	41662-DAK	100	100	100	100	95	100	96	100	100	100	100
Senegal	1984	41671-DAK	100	100	100	100	95	100	96	100	100	100	100
Senegal	1984	A1C1/V2	100	100	100	100	95	100	96	100	100	100	100
Senegal	1984	ArD_41519	100	100	100	100	95	100	96	100	100	100	100
Senegal	2000	ArD142623	100	96	90	100	95	100	96	100	100	100	100
Senegal	2001	ArD157995	100	100	100	100	95	100	96	100	100	100	100
Senegal	2001	ArD158084	100	100	100	100	95	100	96	100	100	100	100
Senegal	1968	ArD7117	100	100	100	100	95	100	96	100	100	100	100

Table 4: Inclusivity Data Analysis (continued)

Country	Year[a]	Strain/ Isolate	Oligonucleotide Sequence % Identity										
			Capture Oligonucleotides			Forward Primers				Probes		Reverse Primers	
			TCO_1	TCO_2	TCO_3	FP_1	FP_2	FP_3	FP_4	P_1	P_2	RP_1	RP_2
Suriname	2015	Z1106033	100	100	100	95	100	91	96	100	100	100	100
Suriname	2016	ZIKVNL00013	100	100	100	95	100	91	96	100	100	100	100
Thailand	2014	SV0127-14	100	100	100	95	100	91	96	100	100	100	100
Uganda	1947	MR 766	100	100	100	100	95	100	96	100	100	100	100
USA	2016	FB-GWUH-2016	100	100	100	95	100	91	96	95	100	100	100
USA	2016	Haiti/1/2016	100	100	100	95	100	91	96	100	100	100	100
—	—	ArD158095	100	100	100	100	95	100	96	100	100	100	100

[a] Year collected.

Repeatability for Plasma Specimens

To assess the repeatability of the Aptima Zika Virus assay, a panel that was made by spiking virus stock into negative plasma (18 copies/mL, 30 copies/mL, 60 copies/mL, and 600 copies/mL) and transcripts in buffer solution (6000 copies/mL) was tested by two operators using three Panther systems over 2 days. The repeatability panel was tested with 108 replicates for each panel member of 18 copies/mL and 30 copies/mL, and 54 replicates for each panel member of 60 copies/mL, 600 copies/mL, and 6000 copies/mL, for a total of 378 replicates for all five panel members.

Repeatability analyses included evaluation of percent agreement of the observed result to the expected result and mean signal to cutoff (S/CO) ratios for panel members. The results were analyzed to assess total variance as well as variance within each run and between days, operators, and instruments. The standard deviation (SD) and percent coefficient of variation (%CV) of the S/CO ratios are shown in Table 5. The mean analyte S/CO ratios were analyzed for all panel members. The percent agreement between the assay results and the true status of each panel member was calculated using the analyte S/CO for all panel members.

The overall percent agreement of test results was 100% for all panel members. There was no correlation of positive rate to the variance factors tested in this study. With regard to signal variability, intra-run was the largest contributor to total variance (as measured by SD values) in the Aptima Zika Virus assay.

Table 5: Repeatability of the Aptima Zika Virus Assay

Panel	N	#P	%A	Analyte Mean S/CO	Inter-Day		Inter-Operator		Inter-Instrument		Intra-Run		Total	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
18 c/mL	108	108	100%	33.12	0.15	0%	0.15	0%	0.25	1%	1.29	4%	1.33	4%
30 c/mL	108	108	100%	33.24	0.24	1%	0.16	0%	0.18	1%	1.32	4%	1.36	4%
60 c/mL	54	54	100%	33.20	0.24	1%	0.19	1%	0.25	1%	1.20	4%	1.26	4%
600 c/mL	54	54	100%	32.96	0.24	1%	0.21	1%	0.27	1%	1.32	4%	1.38	4%
6000 c/mL	54	54	100%	32.97	0.33	1%	0.21	1%	0.23	1%	1.27	4%	1.35	4%

N= Number of panel members combined for this analysis, P = Number of Positives, A = Agreement, S/CO = Signal to Cutoff Ratio, SD = Standard Deviation, CV = coefficient of variance, c/mL = copies per mL.

Cross-Reactivity with Other Blood-Borne Pathogens for Plasma Specimens

Cross-reactivity of the Aptima Zika Virus assay was evaluated by testing clinical specimens from 10 patients with each of the following viral infections: Dengue virus, Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human Immunodeficiency virus 1 and 2 (HIV-1/2), Parvovirus B19, and West Nile virus (WNV). Specimens from 10 individuals that had received HBV vaccine were also tested. The specimens were obtained from a commercial source and characterized by vendors using validated methods. In addition, pooled negative plasma spiked with Hepatitis E virus (HEV) at 1 x 10^5 copies/mL and pooled negative plasma spiked with Chikungunya virus at 1 x 10^5 U/mL were evaluated. Each sample described above was split into two aliquots. One aliquot was used for the cross-reactivity evaluation. The other aliquot was spiked with ZIKV positive plasma and used as contrived specimens in the Clinical Evaluation. For cross-reactivity, aliquots from donor samples with naturally occurring infections or who had received the HBV vaccine were tested once. The samples spiked with HEV and Chikungunya were tested in replicates of 10.

Aptima Zika Virus assay results were negative for all samples. No cross-reactivity was observed in the specimens from subjects infected with other blood-borne pathogens or specimens from individuals that had received HBV vaccines or in specimens spiked with virus.

Table 6: Aptima Zika Virus Assay Results Summary of Cross-Reactivity with Other Blood-borne Pathogens

Pathogen	N	#P	%P	IC S/CO			Analyte S/CO		
				Mean	SD	CV	Mean	SD	CV
HCV	10	0	0%	1.91	0.07	4%	0.00	0.00	N/A
WNV	10	0	0%	1.95	0.05	3%	0.00	0.00	N/A
HAV	10	0	0%	1.95	0.04	2%	0.01	0.02	316%
HIV 1-2	10	0	0%	1.87	0.09	5%	0.00	0.01	N/A
Dengue	10	0	0%	1.91	0.05	3%	0.04	0.08	219%
Parvo B19	10	0	0%	1.93	0.04	2%	0.00	0.00	N/A
HBV	10	0	0%	1.92	0.05	3%	0.01	0.03	316%
HBV Vaccinated	10	0	0%	1.88	0.06	3%	0.00	0.00	N/A
Chikungunya	10	0	0%	1.89	0.05	3%	0.01	0.02	316%
HEV	10	0	0%	1.93	0.03	2%	0.00	0.01	N/A

N = number of specimens, #P = number of positives, %P = percentage of positives, IC = Internal Control, S/CO = Signal to Cutoff ratio, SD = Standard Deviation, CV = Coefficient of Variation, N/A = not available.

Cross-Reactivity and Interference with Other Microorganisms for Plasma Specimens

Negative plasma was used to prepare specimens spiked to 1 x 10^6 colony forming units (CFU/mL) or inclusion forming unit per mL (IFU/mL) with each of the following microorganisms: *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Corynebacterium diphtheriae*, *Propionibacterium acnes*, *Candida albicans*, *Neisseria gonorrhoeae*, or *Chlamydia trachomatis*. Negative urine was used to prepare specimens spiked to 1 x 10^6 CFU/mL of *Escherichia coli* or *Staphylococcus aureus*. Cross-reactivity was tested using ZIKV unspiked specimens, and all results were negative. The potential for microbial interference was tested using an aliquot of each specimen, spiked with ZIKV at 18 copies/mL, and all results were positive. No cross-reactivity or interference was observed in the specimens containing bacteria or fungi.

Cross-Reactivity Analysis – *in silico*

The sequences of the primers, probes, and capture oligonucleotides in the Aptima Zika Virus assay were subjected to a BLAST (Basic Local Alignment Search Tool) analysis against the species shown in Table 7. The Aptima Zika Virus assay does not appear to be capable of significantly cross-reacting with the examined subject sequences when evaluated by in-silico Blast (blastn, max target seq=10,000, word size=7, e-val threshold=1,000, default penalties) and proximity analysis (correct orientation and common hit proximity of all oligonucleotides within 300bp). Hits that are ≥ 45% identical to the query oligonucleotide and within 300bp of one another on the same subject sequence are considered potentially problematic. None of the subjects included in the specific cross-reactivity datasets (751,867 entries and 486,723 entries) or in the GenBank non-redundant viral, plant, bacterial, invertebrate, environmental, other vertebrate or bacteriophage divisions were found to meet all these criteria to a complete set of oligonucleotides to produce false positive results.

The *in silico* analysis included genomic RNA/DNA sequences of the viruses and organisms listed in Table 7.

Table 7: List of Organisms for Cross-Reactivity Analysis

Flaviviruses	Other Organisms	
Dengue virus 1, 2, 3 and 4	Adenovirus	*Acinetobacter lwoffii*
Hepatitis C virus	Barmah Forest virus	*Actinomyces israelii*
Japanese encephalitis virus	Borrelia burgdorferi	*Alcaligenes faecalis*
Spondweni virus	Chikungunya virus	*Atopobium vaginae*
St. Louis encephalitis virus	Cytomegalovirus	*Bacteroides fragilis*
West Nile virus	Eastern Equine Encephalitis virus	*Bifidobacterium adolescentis*
Yellow fever	Enterovirus	*Campylobacter jejuni*
—	Epstein Barr virus	*Candida albicans*
—	Group A *Streptococcus*	*Chlamydia trachomatis*
—	Hepatitis B virus	*Clostridium difficile*
—	Human Immunodeficiency virus	*Corynebacterium genitalium*
—	Leptospirosis	*Cryptococcus neoformans*
—	Mayaro virus	*Enterobacter cloacae*
—	Measles virus	*Enterococcus faecalis*
—	O'nyong-nyong virus (Sindbis virus and Una virus)	*Escherichia coli*
—	Parvovirus (B19)	*Finegoldia magnus*
—	Plasmodium falciparum	*Fusobacterium nucleatum*
—	Plasmodium sp. (Plasmodium vivax)	*Gardnerella vaginalis*
—	*Rickettsia* sp.	*Haemophilus ducreyi*
—	Ross River virus	*Klebsiella pneumoniae*
—	Rubella virus	*Lactobacillus acidophilus*
—	Schistosoma sp.	*Lactobacillus crispatus*
—	*Trypanosoma cruzi*	*Leptotrichia buccalis*
—	Varicella Zoster virus	*Listeria monocytogenes*

Table 7: List of Organisms for Cross-Reactivity Analysis (continued)

Flaviviruses	Other Organisms	
—	Western Equine Encephalitis virus	*Mobiluncus curtisii*
—	Herpes simplex virus type 1	*Mycoplasma hominis*
—	Herpes simplex virus type 2	*Mycoplasma pneumoniae*
—	Human papillomavirus type 16	*Neisseria gonorrhoeae*
—	*Trichomonas vaginalis*	*Prevotella bivia*
—	*Streptococcus agalactiae*	*Propionibacterium acnes*
—	*Streptococcus pyogenes*	*Proteus vulgaris*
—	*Ureaplasma parvum*	*Pseudomonas aeruginosa*
—	*Ureaplasma urealyticum*	*Staphylococcus aureus*
—	—	*Staphylococcus epidermidis*

Interference for Plasma Specimens

The potential for interference from endogenous substances was evaluated by testing patient plasma samples with the characteristics shown in Table 8. Ten plasma specimens were included for each characteristic. Each specimen was split into two aliquots. One aliquot was spiked with ZIKV positive plasma to a concentration of 18 copies/mL. The spiked and unspiked aliquots were tested with the Aptima Zika Virus assay. All unspiked samples were negative. One spiked aliquot was negative on initial testing. The negative result was determined to be due to a spiking error. A fresh aliquot of the sample was spiked and retested. The result was positive upon retesting.

Table 8: Aptima Zika Virus Assay Results Summary of Interference from Endogenous Substances

Zika Virus	Specimen	N	#P	%P	IC S/CO			Analyte S/CO		
					Mean	SD	CV	Mean	SD	CV
Unspiked	Icteric	10	0	0%	1.88	0.03	2%	0.00	0.00	N/A
	Lipemic	10	0	0%	1.84	0.05	3%	0.00	0.00	N/A
	Hemolyzed	10	0	0%	1.86	0.05	3%	0.00	0.00	N/A
	Antinuclear antibody	10	0	0%	1.92	0.06	3%	0.00	0.00	N/A
	Multiple Myeloma	10	0	0%	1.87	0.05	3%	0.00	0.00	N/A
	Systemic Lupus Erythematosus	10	0	0%	1.89	0.07	4%	0.00	0.00	N/A
	Rheumatoid Factor	10	0	0%	1.93	0.04	2%	0.00	0.00	N/A
Spiked (18 copies/mL)	Icteric	10	10	100%	2.25	0.21	9%	31.69	0.69	2%
	Lipemic	10	10	100%	2.19	0.26	12%	31.96	1.58	5%
	Hemolyzed	10	10	100%	2.12	0.25	12%	31.51	0.49	2%
	Antinuclear Antibody	10	10	100%	2.09	0.42	20%	31.84	5.18	16%
	Multiple Myeloma	10	10	100%	2.14	0.23	11%	31.73	1.28	4%
	Systemic Lupus Erythematosus	10	9[a]	90%	2.35[b]	0.39[b]	17%	32.92[b]	0.69[b]	2%
	Rheumatoid Factor	10	10	100%	2.13	0.34	16%	32.86	0.66	2%

N = number of specimens, #P = number of positives, %P = percentage of positives, IC = Internal Control, S/CO = Signal to Cutoff ratio, SD = Standard Deviation, CV = Coefficient of Variation, N/A = not available.

[a] A fresh aliquot of the sample was spiked and retested. The result on retesting was positive

[b] Calculated using positive results.

Matrix Equivalency for Serum and Plasma Specimens

To compare the performance of serum and plasma specimens in the Aptima Zika Virus assay, blood from 10 normal donors was collected using the following anticoagulants and tube types: 1) dipotassium ethylenediaminetetraacetic acid (K2 EDTA), 2) tripotassium ethylenediaminetetraacetic acid (K3 EDTA), 3) Acid Citrate Dextrose Adenine (ACD-A), 4) Sodium Citrate (NAC), 5) Plasma Preparation Tubes (PPT), 6) Serum Separation Tube (SST), and 7) Serum Tube (Serum). For each of the 10 donors, blood was collected using each of the 7 tube types. Each donor sample was split into two aliquots. One aliquot was spiked with ZIKV positive plasma at 18 copies/mL. Both the spiked and unspiked aliquots were tested with the Aptima Zika Virus assay.

For the unspiked aliquots, all 70 samples were negative in the Aptima Zika Virus assay. The mean IC S/CO ratios ranged from 1.83 to 1.90 with %CVs ranging from 2% to 3% for each tube type (Table 9). For the spiked aliquots, all 70 samples were positive in the Aptima Zika Virus assay. The mean analyte S/CO ratio for each of the 7 tube types ranged from 31.90 to 34.20 with %CVs ranging from 3% to 4% (Table 10).

Table 9: Aptima Zika Virus Assay Results for Unspiked Plasma and Serum Samples Collected in Various Tube Types

Collection Tube	N	#P	%P	IC S/CO			Analyte S/CO		
				Mean	SD	CV	Mean	SD	CV
K2EDTA	10	0	0%	1.89	0.06	3%	0.00	0.01	N/A
K3EDTA	10	0	0%	1.87	0.04	2%	0.00	0.00	N/A
ACD-A	10	0	0%	1.87	0.04	2%	0.00	0.00	N/A
PPT	10	0	0%	1.83	0.04	2%	0.00	0.00	N/A
NAC	10	0	0%	1.84	0.06	3%	0.00	0.00	N/A
Serum	10	0	0%	1.85	0.06	3%	0.00	0.00	N/A
SST	10	0	0%	1.90	0.05	3%	0.00	0.00	N/A

N = number of specimens, #P = number of positives, %P = percentage of positives, IC = Internal Control, S/CO = Signal to Cutoff ratio, SD = Standard Deviation, CV = Coefficient of Variation, N/A = not available.

Table 10: Aptima Zika Virus Assay Results for Spiked Plasma and Serum Samples Collected in Various Tube Types

Collection Tube	N	#P	%P	IC S/CO			Analyte S/CO		
				Mean	SD	CV	Mean	SD	CV
K2EDTA	10	10	100%	2.05	0.45	22%	32.77	1.23	4%
K3EDTA	10	10	100%	1.99	0.39	20%	32.63	0.82	3%
ACD-A	10	10	100%	1.88	0.44	23%	32.02	1.32	4%
PPT	10	10	100%	1.92	0.25	13%	32.32	1.24	4%
NAC	10	10	100%	1.91	0.50	26%	31.90	1.31	4%
Serum	10	10	100%	1.78	0.31	18%	34.20	1.34	4%
SST	10	10	100%	1.77	0.51	29%	32.52	1.32	4%

N = number of specimens, #P = number of positives, %P = percentage of positives, IC = Internal Control, S/CO = Signal to Cutoff ratio, SD = Standard Deviation, CV = Coefficient of Variation.

Clinical Evaluation for Plasma Specimens

Twenty six (26) plasma specimens were obtained from three commercial resources. The 26 specimens were determined by the vendors to be positive for ZIKV based on the results of the CDC TrioPlex Assay (two vendors) or a validated real-time RT-PCR test. The specimens were re-tested using a different validated real-time RT-PCR test and 24 of 26 specimens were confirmed positive. The two specimens that were negative on re-testing are considered negative for the reference result in the analyses below. The Aptima Zika Virus assay was positive for all 26 clinical specimens. Table 11 shows the results for the 24 reference positive specimens.

Table 11: Aptima Zika Virus Assay Results of 24 ZIKV Positive Clinical Specimens

Specimen ID	Country of Origin	Reference Ct/Cp	Aptima Result	Aptima S/CO
08847156	Colombia	34.14	Positive	30.5
08847163	Colombia	34.90	Positive	31.3
08847229	Colombia	31.43	Positive	31.3
08847260	Colombia	32.75	Positive	32.5
08847264	Colombia	36.32	Positive	32.8
08847284	Colombia	33.14	Positive	32.5
08847325	Colombia	36.22	Positive	31.2
08847716	Colombia	31.76	Positive	29.8
1043-TDS-0112	Dominican Republic	31.80	Positive	30.9
1043-TDS-0114	Dominican Republic	35.20	Positive	31.8
1043-TDS-0115	Dominican Republic	24.74	Positive	32.3
1043-TDS-0119	Dominican Republic	30.69	Positive	32.1
1043-TDS-0122	Dominican Republic	35.05	Positive	30.6
1043-TDS-0129	Dominican Republic	37.24	Positive	31.8
1043-TDS-0130	Dominican Republic	34.23	Positive	33.4
1043-TDS-0131	Dominican Republic	29.66	Positive	30.3
1043-TDS-0134	Dominican Republic	37.30	Positive	31.0
1043-TDS-0135	Dominican Republic	34.07	Positive	32.1
1043-TDS-0137	Dominican Republic	29.54	Positive	31.7
1043-TDS-0141	Dominican Republic	30.71	Positive	32.0
1043-TDS-0143	Dominican Republic	28.73	Positive	29.6
1043-TDS-0144	Dominican Republic	34.19	Positive	29.8
1043023924	Colombia	34.69	Positive	30.3
8798593	Colombia	22.75	Positive	31.7

A total of 90 contrived specimens were prepared by spiking ZIKV positive plasma into individual plasma specimens to a concentration of 18 copies/mL. The 90 specimens include 10 individual plasma specimens from patients who are positive for Parvovirus B19, Dengue, HAV, HBV, HCV, HIV, or WNV; 10 plasma specimens from an HBV vaccinated donor; and 10 plasma specimens from normal donors.

A total of 72 individual plasma samples were used as ZIKV negative specimens. Seventy (70) specimens include 10 individual plasma specimens each that are antinuclear antibody positive, hemolyzed (elevated hemoglobin), Icteric (elevated bilirubin), lipemic (elevated lipid), multiple myeloma, rheumatoid arthritis, or systemic lupus erythematosus. Two specimens positive by initial reference testing but negative on re-testing are also included. These two specimens were

positive by the Aptima Zika Virus assay. The clinical evaluation results are summarized in Table 12.

Table 12: Clinical Evaluation Results for the Aptima Zika Virus Assay

Specimen Category	Aptima Zika Virus Assay		
	Number Tested	ZIKV Positive	ZIKV Negative
Natural Zika Positive Specimens	24	24 / 24	0 / 24
Contrived Zika Positive Clinical Specimens (3 x LoD)	90[a]	90 / 90	0 / 90
Expected Zika Negative Clinical Specimens	72[b]	2 / 72	70 / 72
Positive Percent Agreement	100% (114 / 114) 95% CI: 96.7% to 100%		
Negative Percent Agreement	97.2% (70 / 72)[b] 95% CI: 90.4 to 99.2%		

CI = Confidence Interval.

[a] Includes the Zika spiked aliquots from the 80 plasma specimens evaluated in the Cross-Reactivity study and the Zika spiked aliquots from 10 plasma specimens evaluated in the Matrix Equivalency study.

[b] Includes two patient samples that were positive on initial reference testing and negative on re-testing by an alternate PCR method and was considered a false positive.

Additional Specificity Testing for Plasma and Serum Specimens

The specificity of the Aptima Zika Virus assay on the Panther instrument platform was further evaluated by testing 775 plasma specimens and 240 serum specimens from normal blood donors. All results were negative. The specificity was 100% (1015/1015) for the Aptima Zika Virus assay with a lower 95% confidence interval of 99.6%. There were no invalid results out of 1015 samples tested with the Aptima Zika Virus assay (Table 13).

Table 13: Plasma and Serum Specificity

	Specimen Type		Total
	Plasma	Serum	
Number Valid	775	240	1015
Number Negative	775	240	1015
Specificity	100.0	100.0	100.0
95% CI, Lower Limit	99.5	98.4	99.6
95% CI, Upper Limit	100.0	100.0	100.0

CI = Confidence Interval.

Invalid Rate for Serum and Plasma Specimens

The invalid rate due to assay chemistry errors for all analytical and clinical specimen testing was 0.09% (3/3375). There were 12 invalid reactions due to hardware or sample issues: 1 CLT (sample clot), 1 RDFS (sample dispense error), 2 VVFS (volume verification failure), 3 QNS (sample quantity not sufficient), and 5 PTF (pipettor arm is unable to pick a tip due to tip loading error by operator). The total invalid rate was 0.44% (15/3375). All invalid replicates were retested with valid results.

Clinical Evaluation for Processed Whole Blood Specimens

The clinical evaluation for whole blood for the Aptima Zika Virus assay on the Panther system consisted of evaluating 25 natural Zika positive samples, 25 contrived Zika positive samples, and 50 negative samples.

From 25 individual donors, 25 index plasma samples as well as matched plasma and whole blood samples from a subsequent time point were received from a vendor. These samples were obtained from Puerto Rico and Florida from ZIKV positive donors. At the time of collection, the index plasma specimen from each study donor was determined to be positive for ZIKV RNA using IND assays. For the 25 index plasma positive samples, repeat testing was performed with the primary screening IND assays and an additional validated assay. For the matched plasma and whole blood samples at follow up time points, testing was initially done using an IND assay and a validated assay. For Aptima Zika Virus assay testing, whole blood samples were lysed in order to make processed whole blood. The 25 processed whole blood specimens were tested in one replicate. The plasma specimens were tested neat.

A patient infected status (PIS) was used as the comparator for evaluating the whole blood performance. The PIS was determined based on the combined index plasma testing results of the original IND result and the Aptima Zika Virus assay duplicate testing, where ≥ 2/3 is positive and 1/3 is considered indeterminate.

The Aptima Zika Virus assay results are provided in Table 14. Out of 25 index plasma samples tested, 22 were positives. For the 3 negatives seen in the index plasma specimens, the comparator results at time of collection also were negative for repeat testing results. Indeterminate PIS specimens, ARBO8135 and ARBO2165, were excluded from the performance evaluation, giving a total of 23 clinical positives based on the PIS. At the follow up time point, there were 10 Aptima Zika positive plasma results and 24 positive whole blood results. For the 1 out of 25 whole blood sample that tested negative, the matched plasma follow up time point was also negative.

Table 14: Aptima Zika Virus Assay Results of 25 ZIKV Positive Clinical Processed Whole Blood Specimens

Specimen ID	Index Plasma			Follow up Time Point Plasma		Follow up Time Point Whole Blood	
	S/CO	Result	Patient Infected Status	S/CO	Result	S/CO	Result
ARBO5707	31.73	POSITIVE	Positive	0	Negative	10.50	POSITIVE
ARBO1184	34.00	POSITIVE	Positive	0	Negative	31.79	POSITIVE
ARBO1501	32.92	POSITIVE	Positive	0	Negative	21.43	POSITIVE
ARBO8852	18.63	POSITIVE	Positive	0	Negative	31.89	POSITIVE
ARBO5970	32.35	POSITIVE	Positive	34	POSITIVE	33.34	POSITIVE
ARBO0560	16.87	POSITIVE	Positive	32	POSITIVE	31.26	POSITIVE
ARBO3777	17.57	POSITIVE	Positive	0	Negative	30.98	POSITIVE
ARBO8135[a]	0.00	Negative	Indeterminate	0	Negative	30.80	POSITIVE
ARBO7608	32.57	POSITIVE	Positive	31	POSITIVE	33.27	POSITIVE
ARBO6411	32.74	POSITIVE	Positive	33	POSITIVE	20.89	POSITIVE
ARBO1358	30.61	POSITIVE	Positive	0	Negative	30.75	POSITIVE
ARBO2952	32.19	POSITIVE	Positive	0	Negative	30.64	POSITIVE
ARBO3249	32.15	POSITIVE	Positive	31	POSITIVE	30.55	POSITIVE
ARBO1389	32.26	POSITIVE	Positive	32	POSITIVE	31.42	POSITIVE
ARBO3371	17.13	POSITIVE	Positive	0	Negative	31.18	POSITIVE

Table 14: Aptima Zika Virus Assay Results of 25 ZIKV Positive Clinical Processed Whole Blood Specimens

Specimen ID	Index Plasma			Follow up Time Point Plasma		Follow up Time Point Whole Blood	
	S/CO	Result	Patient Infected Status	S/CO	Result	S/CO	Result
ARBO5191	32.62	POSITIVE	Positive	0	Negative	0.00	Negative
ARBO9066[b]	0.00	Negative	Positive	33	POSITIVE	32.00	POSITIVE
ARBO8167	31.41	POSITIVE	Positive	0	Negative	30.87	POSITIVE
ARBO1505	30.77	POSITIVE	Positive	0	Negative	19.68	POSITIVE
ARBO8314	31.81	POSITIVE	Positive	16	POSITIVE	31.17	POSITIVE
ARBO6837	31.91	POSITIVE	Positive	0	Negative	32.93	POSITIVE
ARBO3264	32.73	POSITIVE	Positive	0	Negative	30.44	POSITIVE
ARBO5760	15.95	POSITIVE	Positive	32	POSITIVE	30.41	POSITIVE
ARBO8597	28.31	POSITIVE	Positive	3	POSITIVE	22.62	POSITIVE
ARBO2165[c]	0.00	Negative	Indeterminate	0	Negative	30.30	POSITIVE

[a] Index plasma negative in 2/2 original repeat testing and equivocal in BSRI test.
[b] Index plasma negative in original 1/2 repeat testing and negative in BSRI test.
[c] Index plasma negative in original 2/2 repeat testing and negative in BSRI test.

A total of 25 contrived specimens were prepared by spiking the FDA ZIKV reference S2 positive into individual whole blood specimens. Ten samples were spiked at 10X LOD (1670 RNA NAAT units/mL) and 15 samples were spiked at 2X LOD (334 RNA NAAT units/mL). A total of 50 individual whole blood samples were used as ZIKV negative specimens. The clinical evaluation results based on the PIS are summarized in Table 15.

Table 15: Clinical Evaluation Results for the Aptima Zika Virus Assay: Processed Whole Blood Specimens

Specimen Category	Aptima Zika Virus Assay		
	Number Tested	ZIKV Positive	ZIKV Negative
Natural Zika Positive Specimens	23[a]	22/23	1[b]/23
Contrived Zika Positive Clinical Specimens	25	24/25	1/25[c]
Expected Zika Negative Clinical Specimens	50	0/50	50/50
Positive Percent Agreement	95.3% (46/48) 95% CI: 86.0% to 98.9%		
Negative Percent Agreement	100% (50/50) 95% CI: 92.9% to 100%		

[a] Of the 25 individuals whose index plasma specimen initially tested positive by an IND assay, 2 individuals patient infected status testing resulted in an indeterminate PIS and the samples were excluded from the performance analysis.
[b] The paired plasma result was negative by the Aptima Zika Virus assay; therefore, the negative whole blood result may not be a false negative result.
[c] Negative contrived sample tested positive upon repeat.

Invalid Rate for Processed Whole Blood Specimens

The invalid rate due to assay chemistry errors for all analytical and clinical specimen testing described in this section for processed whole blood specimen was 0.92% (6/655). Upon retesting all invalid results repeated as valid results. There were no invalid reactions due to hardware or sample issues. The total invalid rate was 0.92% (6/655).

Performance Evaluation for Urine Specimens

The Aptima Zika Virus assay performance was evaluated using processed urine specimens. For processing, urine was mixed with Aptima Urine Transport Medium at the ratio of 1:1 (2 mL of urine was added into Aptima Urine Specimen Transport Tube, which contained 2 mL of urine transport media).

Limit of Detection (LoD) for Urine Specimens

The Limit of Detection (LoD) is defined as the concentration of ZIKV RNA that is detected at 95% or greater probability according to CLSI EP17-A2.[19] The LoD was determined by testing a ZIKV positive plasma specimen serially diluted in pooled negative urine. The highest volume of the positive plasma spiked into urine was 5.5%. The positive plasma specimen was collected from a blood donor during the 2015 Zika outbreak in Brazil. The sample was quantified using a validated real-time RT-PCR assay. The urine sensitivity panel members were prepared by spiking ZIKV positive plasma specimen into the urine at the stated concentration. The spiked panel members were processed by mixing with UTM at a ratio of 1:1 prior to testing. Three Panther instruments were used to test 30 replicates of each target level. The results are summarized in Table 16.

Table 16: Detection of Zika Virus in Processed Urine Specimen with the Aptima Zika Virus Assay on the Panther System

Virus copies/mL	#Tested	#Positive	Positivity		
			%P	Lower 95%CI	Upper 95%CI
0	30	0	0	0	11
0.3	30	3	10	3	26
1	30	6	20	10	37
3	30	14	47	30	64
10	30	30	100	89	100
30	30	30	100	89	100
90	30	30	100	89	100

%P = percentage positive, CI = Confidence Interval.

The 95% detection probability was determined using Probit analysis. The limit of detection for the Zika virus in processed urine was determined to be 8.5 copies/mL at 95% detection probability (Table 17).

Table 17: Probit Analysis Detection

Urine Specimen	95% Detection Probability in copies/mL (95% Fiducial Limits)
Processed Urine	8.5 (6.0–15.3)

Clinical Evaluation for Urine Specimens

10 paired specimens were obtained (plasma/serum/urine matched specimens collected from 10 symptomatic patients) from a commercial resource. The 10 symptomatic patients were determined by the vendor to be positive for ZIKV based on the results of the serum specimens tested with a validated real-time RT-PCR test. The urine specimens were processed prior to testing. The ten processed urine specimens were tested along with the plasma and serum

samples from each of 10 patients using the Aptima Zika Virus assay. All specimens were positive upon initial testing. Table 18 shows the results for the 10 matched specimens.

Table 18: Aptima Zika Virus Assay Results of 10 Matched ZIKV Positive Clinical Specimens

Specimen ID	Country of Origin	Reference Cp (Serum)	Plasma		Serum		Processed Urine	
			Result	S/CO	Result	S/CO	Result	S/CO
1043-TDS-0159	Dominican Republic	36.31	Positive	33.1	Positive	31.7	Positive	32.9
1043-TDS-0163	Dominican Republic	32.54	Positive	33.4	Positive	33.4	Positive	17.0
1043-TDS-0165	Dominican Republic	40.38	Positive	32.8	Positive	32.6	Positive	33.7
1043-TDS-0173	Dominican Republic	33.15	Positive	32.6	Positive	32.8	Positive	34.1
1043-TDS-0206	Dominican Republic	36.62	Positive	31.6	Positive	30.8	Positive	32.4
1043-TDS-0221	Dominican Republic	38.11	Positive	17.8	Positive	33.5	Positive	32.8
1043-TDS-0223	Dominican Republic	32.50	Positive	34.0	Positive	33.4	Positive	31.9
1043-TDS-0224	Dominican Republic	31.81	Positive	33.8	Positive	31.6	Positive	33.6
1043-TDS-0230	Dominican Republic	30.51	Positive	33.8	Positive	33.7	Positive	34.7
1043-TDS-0231	Dominican Republic	35.63	Positive	31.6	Positive	33.8	Positive	34.4

A total of 99 contrived urine specimens were prepared by spiking ZIKV positive plasma into individual urine specimens: 33 specimens were spiked at 20 copies/mL, 33 specimens were spiked at 36 copies/mL, and 33 specimens were spiked at 100 copies/mL. Each spiked urine specimen was processed prior to testing with the Aptima Zika Virus assay. All contrived processed urine specimens tested positive.

A total of 123 individual urine specimens were used as ZIKV RNA negative specimens. 87 urine specimens were collected from a normal population. 36 individual female urine specimens were collected from a patient population (7 patients with breast cancer, 6 patients with chronic kidney disease, 6 patients with systemic lupus erythematosus, 4 patients with pneumonia, 8 patients with diabetes, and 5 patients with urinary tract infection). Each urine specimen was processed prior to testing with the Aptima Zika Virus assay. All specimens tested negative. The clinical evaluation results are summarized in Table 19.

Table 19: Clinical Evaluation Results for Processed Urine Specimens

Specimen Category	Aptima Zika Virus Assay		
	Number Tested	ZIKV Positive	ZIKV Negative
Natural Zika Positive Specimens	10	10/10	0/10
Contrived Zika Positive Clinical Specimens	99	99/99	0/99
Expected Zika Negative Clinical Specimens	123	0/123	123/123
Positive Percent Agreement	100% (109/109) 95% CI: 96.6% to 100%		
Negative Percent Agreement	100% (123/123) 95% CI: 97.0% to 100%		

CI = Confidence Interval

Invalid Rate for Urine Specimens

The invalid rate due to assay chemistry errors for all analytical and clinical specimen testing described in this section for urine specimen was 0% (0/482). There were no invalid reactions due to hardware or sample issues. The total invalid rate was 0% (0/482).

Comparator Assay Information

The Aptima Zika Virus assay performance with processed whole blood (PWB) specimens was evaluated in comparison to two qualitative RT-PCR tests approved by FDA for the detection of Zika Virus (ZIKV) RNA in plasma specimens (cobas Zika and Procleix Zika Virus Assay approved by CBER) and one RT-PCR lab developed test for the detection of Zika Virus RNA in plasma specimens (Vitalant Research Institute, formerly known as Blood Systems Research Institute).

The Aptima Zika Virus assay performance with plasma specimens was evaluated in comparison to two qualitative RT-PCR tests for use with plasma specimens. The first test was authorized by FDA for use by clinical laboratories for the qualitative detection of RNA from Zika Virus (claimed LoD 10,000 RNA NAAT Detectable Units/mL (FDA Reference Material Testing S1) and 5,000 RNA NAAT Detectable Units/mL (FDA Reference Material Testing S2)). The second test was a RT-PCR lab developed test for the detection of Zika Virus RNA in plasma specimens (Vitalant Research Institute, formerly known as Blood Systems Research Institute). One additional qualitative RT-PCR test authorized by FDA for the detection of Zika Virus RNA in serum was also used. The claimed LoD for the comparator assay with serum specimens was 3,300 RNA NAAT Detectable Units/mL (FDA Reference Material Testing S1), and 1,670 RNA NAAT Detectable Units/mL (FDA Reference Material Testing S2).

The Aptima Zika Virus assay performance with processed urine specimens was evaluated in comparison to a qualitative RT-PCR test that was authorized by FDA for use by clinical laboratories for the qualitative detection of RNA from Zika Virus. The claimed limit of detection for the comparator assay with plasma specimens is 10,000 RNA NAAT Detectable Units/mL (FDA Reference Material Testing S1) and 5000 RNA NAAT Detectable Units/mL (FDA Reference Material Testing S2). Units chose for LoD claims were selected by the comparator assay developers. Not all comparator assays were compared to FDA reference panel S1 and S2.

Analytical Sensitivity using FDA Reference Material

The evaluation of analytical sensitivity was performed using reference material (S1 and S2) and standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. The results are summarized in the Table 20.

Table 20: Summary of LoD Confirmation Result using the FDA Reference Materials

Reference Materials Provided by FDA	Specimen Type	Confirmed LoD per FDA Protocol (RNA NAAT Detectable Units/mL)
S1 ($1x10^6$ RNA NAAT detectable units/mL)	Plasma	100
	Processed Urine	300
	Processed Whole Blood	1000
S2 ($5x10^6$ RNA NAAT detectable units/mL)	Plasma	150
	Processed Urine	150
	Processed Whole Blood	167

Bibliography

1. **International Committee on Taxonomy of Viruses.** http://www.ictvonline.org/
2. **Musso D, Gubler DJ.** 2016. Zika virus. Clin Microbiol Rev. Jul;29(3):487-524.
3. **Kitchen SF, Haddow AJ.** 1952. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg. 46:509–520.
4. **Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, Pretrick M, Marfel M, Holzbauer S, Dubray C, Guillaumot L, Griggs A, Bel M, Lambert AJ, Laven J, Kosoy O, Panella A, Biggerstaff BJ, Fischer M, Hayes EB.** 2009. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 360:2536–2543. doi: 10.1056/NEJMoa0805715
5. **Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, Sall AA, Musso D.** 2014. Zika virus, French Polynesia, South Pacific, 2013. Emerg Infect Dis 20:1085–1086.
6. **Campos GS, Bandeira AC, Sardi SI.** 2015. Zika virus outbreak, Bahia, Brazil. Emerg Infect Dis 21:1885–1886. doi: 10.3201/eid2110.150847.
7. **Zanluca C, Melo VC, Mosimann AL, Santos GI, Santos CN, Luz K.** 2015. First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz 110:569-572.
8. **Cao-Lormeau, V.-M. et al.** Guillain–Barré syndrome outbreak associated with Zika virus infection in French Polynesia: a case–control study. Lancet https://doi.org/10.1016/S0140-6736(16)00562-6.
9. **Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR.** 2016. Zika virus and birth defects — reviewing the evidence for causality. N Engl J Med. 2016 May 19;374(20):1981-1987. doi: 10.1056/NEJMsr1604338. PubMed PMID: 27074377.
10. **Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM.** 2015. Potential sexual transmission of Zika virus. Emerg Infect Dis 21: 359-61.
11. **U.S. Centers for Disease Control and Prevention.** http://www.cdc.gov/zika/transmission/blood-transfusion.html
12. **Kacian DL, Fultz T J.** 1995. Nucleic acid sequence amplification methods. U.S. Patent 5,399,491.
13. **Arnold LJ, Hammond PW, Wiese WA, Nelson NC.** 1989. Assay formats involving acridinium-ester-labeled DNA probes. Clin Chem 35:1588-1594.
14. **Nelson NC, Cheikh A, Matsuda E, Becker M.** 1996. Simultaneous detection of multiple nucleic acid targets in a homogeneous format. Biochem. 35:8429-8438.
15. **Clinical and Laboratory Standards Institute.** 2005. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline. CLSI Document MM13-A. Wayne, PA.
16. **29 CFR Part 1910.1030.** Occupational Exposure to Bloodborne Pathogens; current version.
17. **Centers for Disease Control and Prevention/National Institutes of Health.** Biosafety in Microbiological and Biomedical Laboratories (BMBL); current version.
18. **Clinical and Laboratory Standards Institute.** 2002. Clinical Laboratory Waste Management. CLSI Document GP5-A2. Villanova, PA.
19. **Clinical and Laboratory Standards Institute (CLSI)**. 2012. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—2nd ed. CLSI Document EP17-A2. Clinical and Laboratory Standards Institute, Wayne, PA.

IVD

Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

Customer Support: +1 800 442 9892
customersupport@hologic.com
Technical Support: +1 888 484 4747
molecularsupport@hologic.com

For more contact information, visit www.hologic.com.

AW-15406 Rev. 005

2023-05