Aptima™ Zika Virus Assay

For *in vitro* diagnostic use.
For U.S. export 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, or processed urine. Specimens are tested using the Panther™ system for automated specimen processing, amplification, and detection. Results are for the identification of Zika virus RNA.

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 December 2016, the World Health Organization reported that 75 countries and territories have reported evidence of mosquito-borne ZIKV transmission since 2007, with 69 of these countries reporting from 2015 onward.[8] 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.[9] The virus has also been linked with microcephaly and other birth defects in infants born to infected mothers.[10] Although the primary route of infection appears to be through the bite of a mosquito, sexual transmission[11] and possible transfusion-transmission[12] of ZIKV have also been reported.

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),[13] and detection of the amplification products (amplicon) by Hybridization Protection Assay (HPA).[14] The assay incorporates an internal control (IC) to monitor nucleic acid capture, amplification, and detection, as well as operator or instrument error.

During sample preparation, RNA is isolated from specimens via target capture. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins, and release viral genomic RNA. Oligonucleotides (“capture oligonucleotides”) 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.[14] Internal Control-specific amplicon is detected using a probe with rapid emission of light (flasher signal). Amplicon specific to ZIKV is detected using probes with relatively slower kinetics of light emission (glower signal). The Dual Kinetic Assay (DKA) is a method used to differentiate between the signals from flasher and glower labels.[15]

The 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.

B. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther System 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.[16,17,18,19] 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[16,17,18] when performing this assay. Proper handling and disposal methods should be established according to local regulations.[19] 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.

Note: Some reagents of this kit are labeled with risk and safety symbols and should be handled accordingly.

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.*

	**Amplification Reagent** **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation
	**Enzyme Reagent** **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation H412 - Harmful to aquatic life with long lasting effects

Selection Reagent
Boric acid 1 - 5%
Sodium Hydroxide <1%
WARNING
H315 - Causes skin irritation
H319 - Causes serious eye irritation
H371 - May cause damage to organs
H373 - May cause damage to organs through prolonged or repeated exposure
P264 - Wash face, hands and any exposed skin thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P337 + P313 - If eye irritation persists: Get medical advice/attention
P302 + P352 - IF ON SKIN: Wash with plenty of soap and water
P332 + P313 - If skin irritation occurs: Get medical advice/attention
P362 - Take off contaminated clothing and wash before reuse

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

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

D. 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.

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

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

G. If precipitate forms in the Selection Reagent, Probe Reagent, Negative Calibrator, or Positive Calibrator, see instructions under *Panther System Test Procedure*.

H. 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.

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

⚠ J. 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, and processed urine specimens.

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

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.*

A. Instructions for Collection

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 instructions:

- 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. Urine Specimens

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

B. Specimen Transport and Storage before Testing

1. Plasma and Serum Specimens

   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 specimens must be centrifuged within 72 hours of collection.
   - Specimens should be stored at 2°C to 8°C unless frozen. However, specimens may 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.

   a. If longer storage is needed, freeze plasma and serum separated from the cells and store at -20°C or -70°C. Do not freeze whole blood.

   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. Urine Specimens

   a. Urine 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-04232 (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	—
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 conductive, liquid sensing	10612513 (Tecan)
Aptima Zika Virus Calibrator Kit *NCAL. Negative calibrator, buffered solution containing detergent, 15 x 2.2 mL* *PCAL. Positive calibrator, RNA transcript in buffered solution containing detergent,15 x 2.2 mL*	PRD-04233
Bleach, 5% to 7% (0.7 M to 1.0 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*	504415
Transfer pipets	—
Cotton-tipped swabs	—
Tube rocker	—
Aptima Urine Specimen Collection Kit	301040
Or Aptima Urine Specimen Transport Tubes	105575
SB100™ Reagent Equilibration System (SB100-RES)	—
Water Bath	—

Panther System Test Procedure

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

Note: *See the SB100 Reagent Equilibration System Application Sheet for optional reagent preparation 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 instrument.
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 the SB100-RES instrument 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 the SB100-RES instrument 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 instrument.

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 - 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. Specimen Handling

1. Allow the specimens and calibrators to reach 15°C to 30°C prior to processing.
2. Ensure each specimen tube contains enough volume for each sample type and tube type.
3. Mix fresh or thawed specimens thoroughly.
4. Just prior to loading samples into a Sample Rack, centrifuge each specimen for 10 minutes at 1000 to 3000*g*. Do not remove caps. 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.

   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 System 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.

      Note: *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 System 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.

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

D. 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.

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

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

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

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

I. 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.

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

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

L. 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.

M. 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.

N. Customers must independently validate an LIS transfer process.

Performance

Analytical Sensitivity

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.[20] 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. Two lots of reagents and three Panther instruments were used to test 72 replicates of each copy level per reagent lot for a total of 144 replicates per level, except for the panel member with 90 copies/mL, which was tested in 20 replicates per reagent lot for a total of 40 replicates. The results are summarized in Table 1.

Table 1: Detection of ZIKV in Plasma

Concentration (copies/mL)	% Positivity (95% CI)		
	Lot 1 (n=72)	Lot 2 (n=72)	Combined (n=144)
90	100 (84 - 100)[a]	100 (84 - 100)[a]	100 (91 - 100)[b]
30	100 (95 - 100)	100 (95 - 100)	100 (97 - 100)
10	100 (95 -100)	100 (95 -100)	100 (97 - 100)
3	86 (76 - 92)	92 (83 - 96)	89 (83 - 93)
1	38 (28 - 50)	60 (48 - 71)	49 (41 - 57)
0.3	19 (12 - 30)	14 (8 - 24)	17 (12 - 24)
0.1	1 (0 - 7)	6 (2 - 14)	3 (1 - 7)
0	0 (0 - 5)	0 (0 - 5)	0 (0 - 3)

CI = Confidence Interval.
[a]n=20.
[b]n=40.

The 50% and 95% detection probabilities of ZIKV in plasma were determined by Probit analysis using the data obtained from the analytical sensitivity testing. The limit of detection for ZIKV in the Aptima Zika Virus assay ranged from 0.91 copies/mL to 1.22 copies/mL at the 50% detection probability and from 3.30 copies/mL to 4.41 copies/mL at the 95% detection probability (Table 2).

Table 2: Probit Analysis of Detection of ZIKV in Plasma

Reagent Lot	50% Limit of Detection (95% Fiducial Limits)	95% Limit of Detection (95% Fiducial Limits)
Lot 1	1.22 (1.01 - 1.47)	4.41 (3.46 - 6.14)
Lot 2	0.91 (0.74 - 1.09)	3.30 (2.63 - 4.44)
Combined	1.06 (0.92 - 1.20)	3.87 (3.25 - 4.78)

Limit of Detection for Urine Specimens

The LoD was determined by testing a ZIKV-positive plasma specimen serially diluted in pooled negative urine. The urine sensitivity panel members were prepared by spiking ZIKV-positive plasma specimen into the urine at the stated concentration prior to mixing with UTM at a ratio of 1:1 (processed urine). Two lots of reagents and three Panther instruments were used to test 30 replicates of each copy level per reagent lot for a total of 60 replicates per level. The results are summarized in Table 3.

Table 3: Detection of ZIKV in Urine

Concentration[a] (copies/mL)	% Positivity (95% CI)		
	Lot 1 (n=30)	Lot 2 (n=30)	Combined (n=60)
90	100 (89 - 100)	100 (89 - 100)	100 (94 - 100)
30	100 (89 - 100)	100 (89 - 100)	100 (94 - 100)
10	97 (84 - 100)	83 (66 - 92)	90 (80 - 95)
3	63 (45 - 78)	43 (27 - 60)	53 (41 - 65)
1	27 (14 - 45)	30 (17 - 48)	28 (18 - 40)
0.3	7 (2 - 22)	3 (0 - 16)	5 (2 - 14)
0.1	0 (0 - 11)	0 (0 - 11)	0 (0 - 6)
0	0 (0 - 11)	0 (0 - 11)	0 (0 - 6)

CI = Confidence Interval.

[a] Concentration in urine prior to being processed.

The 50% and 95% detection probabilities of the ZIKV in urine were determined by Probit analysis using the data obtained from the analytical sensitivity testing. The limit of detection for ZIKV in the Aptima Zika Virus assay ranged from 2.26 copies/mL to 3.42 copies/mL at the 50% detection probability and from 8.25 copies/mL to 15.63 copies/mL at the 95% detection probability (Table 4).

Table 4: Probit Analysis of Detection of ZIKV in Urine[a]

Reagent Lot	50% Limit of Detection (95% Fiducial Limits)	95% Limit of Detection (95% Fiducial Limits)
Lot 1	2.26 (1.67 - 3.00)	8.25 (5.89 - 13.53)
Lot 2	3.42 (2.42 - 4.64)	15.63 (10.70 - 27.30)
Combined	2.81 (2.23 - 3.47)	11.99 (9.17 - 17.04)

[a] Concentration in urine prior to being processed.

Reproducibility

Reproducibility for Blood Specimens

Reproducibility of the Aptima Zika Virus assay on the Panther System was evaluated by testing a ZIKV panel composed of positive panel members at 100 copies/mL and 30 copies/mL, and one negative panel member made from negative plasma (Table 5). Positive panel members were created by spiking ZIKV-positive plasma specimen into negative plasma. The panel was tested by three operators with three different reagent lots and on three Panther instruments over multiple days. A total of 27 valid runs were generated with the Aptima Zika

Virus assay. Each panel member was tested in 486 replicates in total. The overall invalid rate is 0% (0/1458).

Reproducibility analyses included evaluation of percent agreement and mean signal to cutoff (S/CO) ratios for panel members, and evaluation of standard deviation (SD) and percent coefficient of variation (%CV) of the S/CO ratios for each of the five variance factors (Table 5). The mean analyte S/CO ratios were analyzed for the positive panel members, and the mean Internal Control S/CO ratios were analyzed for the negative panel member. 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 positive panel members and 100% for the negative panel member. There was no correlation of reactive rate to the variance factors tested in this study. With regard to signal variability, within run was the largest contributor to total variance (as measured by SD values) in the Aptima Zika Virus assay.

Table 5: Reproducibility of the Aptima Zika Virus Assay for Blood Specimens

Panel Member	N	#P	% A	Mean S/CO[a]	Between Lots		Between Instruments		Between Operators		Between Days		Within Run		Total	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
High (100 copies/mL)	486	486	100	33.22	0.02	0%	0.34	1%	0.17	1%	0.12	0%	1.33	4%	1.38	4%
Low (30 copies/mL)	486	486	100	33.35	0.17	1%	0.27	1%	0.08	0%	0.08	0%	1.27	4%	1.31	4%
Negative	486	0	100	1.94	0.02	1%	0.01	1%	0.01	0%	0.00	0%	0.05	2%	0.05	3%

N = number of panel members combined for this analysis; #P = number of positives; %A = percentage of agreement; S/CO = Signal to Cutoff ratio in reactive replicates only; SD = Standard Deviation; CV = Coefficient of Variation.

[a] Mean analyte S/CO for the positive panel members (High and Low); mean Internal Control S/CO for the negative panel member.

Reproducibility for Urine Specimens

Reproducibility of the Aptima Zika Virus assay on the Panther System was evaluated by testing a ZIKV panel composed of positive panel members at 100 copies/mL and 30 copies/mL, and one negative panel member made from pooled negative urine (Table 6). Positive panel members were prepared by spiking ZIKV-positive plasma specimen into pooled negative urine at the stated concentration. All panel members were mixed with UTM at 1:1 ratio to create reproducibility panels of processed urine. The panel was tested by three operators with three different reagent lots and on three Panther instruments over multiple days. A total of 27 valid runs were generated with the Aptima Zika Virus assay. Each panel member was tested in 486 replicates in total. The overall invalid rate is 0% (0/1458).

Reproducibility analyses included evaluation of percent agreement and mean signal to cutoff (S/CO) ratios for panel members, and evaluation of standard deviation (SD) and percent coefficient of variation (%CV) of the S/CO ratios for each of the five variance factors (Table 6). The mean analyte S/CO ratios were analyzed for the positive panel members, and the mean Internal Control S/CO ratios were analyzed for the negative panel member. 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 the high positive panel member, 96.5% for the low positive panel member, and 100% for the negative panel member. There was no correlation of reactive rate to the variance factors tested in this study. With regard to

signal variability, within run was the largest contributor to total variance (as measured by SD values) in the Aptima Zika Virus assay.

Table 6: Reproducibility of the Aptima Zika Virus Assay for Urine Specimens

Panel Member[a]	N	#P	% A	Mean S/CO[b]	Between Lots		Between Instruments		Between Operators		Between Days		Within Run		Total	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
High (100 copies/mL)	486	486	100	34.36	0.09	0%	0.25	1%	0.08	0%	0.07	0%	1.31	4%	1.34	4%
Low (30 copies/mL)	486	469	96.5	32.02	1.01	3%	0.30	1%	0.46	1%	1.46	5%	5.21	16%	5.53	17%
Negative	486	0	100	1.99	0.02	1%	0.01	1%	0.02	1%	0.01	0%	0.04	2%	0.05	2%

N = number of panel members combined for this analysis; #P = number of positives; %A = percentage of agreement; S/CO = Signal to Cutoff ratio in reactive replicates only; SD = Standard Deviation; CV = Coefficient of Variation.

[a] Concentration in urine prior to being processed.

[b] Mean analyte S/CO for the positive panel members (High and Low); mean Internal Control S/CO for the negative panel member.

Interference

Interfering Substances for Blood Specimens

The potential for interference from endogenous substances was evaluated by testing specimens from patients with autoimmune and other diseases. Ten plasma specimens from each group of patients with the following autoimmune and other conditions were evaluated: icteric, lipemic, hemolyzed, antinuclear antibody, multiple myeloma, systemic lupus erythematosus, and rheumatoid factor. 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. All spiked samples were positive, except one spiked aliquot from one patient with systemic lupus erythematosus. A fresh aliquot of the sample was spiked and retested. The result was positive upon retesting.

The potential for interference from endogenous substances was further evaluated by testing plasma spiking the following substances: albumin (60,000 mg/L), hemoglobin (2,000 mg/L), bilirubin (200 mg/L), and lipids (30,000 mg/L). No interference was observed by evaluating the specificity and sensitivity.

To evaluate the interference from anticoagulants and collection device, the performance of serum and plasma specimens in the Aptima Zika Virus assay was compared. 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 seven 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 7). 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 seven tube types ranged from

31.90 to 34.20 with %CVs ranging from 3% to 4% (Table 8). No interference from anticoagulants and collection device is observed.

Table 7: 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 8: 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; N/A = not available.

Interfering Substances for Urine Specimens

To test the effects of urine metabolites, KOVA-Trol I High Abnormal with Urobilinogen Urinalysis Control was diluted into urine transport medium (UTM) in place of urine. This human urine-based urinalysis control material contains potential interferents such as protein (albumin), glucose, ketones, bilirubin, red blood cells, nitrite, urobilinogen, and leukocytes. In additional, urine containing whole blood was tested at a concentration of 5% volume/volume. No interference was observed with any of the substances when spiked with ZIKV to a final concentration of 18 copies/mL or unspiked with ZIKV, and tested in the Aptima Zika Virus assay.

Cross-Reactivity

Cross-Reactivity for Blood Specimens

Other blood-borne pathogens for blood specimens were tested for cross-reactivity and interference. 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. The potential for 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 other blood-borne pathogens.

Additional microorganisms were tested for cross-reactivity and interference. 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*. 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 for Urine Specimens

The cross-reactivity and interference of microorganisms in urine were tested for Aptima Zika Virus assay. Pooled negative urine 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: *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans, Chlamydia trachomatis* (5 x 10^5 IFU/mL), *Trichomonas vaginalis, Mycoplasma genitalium, Gardnerella vaginalis, Lactobacillus gasseri, Lactobacillus crispatus, Lactobacillus jensenii*, or *Proteus mirabilis* (1 x 10^6 rRNA/mL). 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 those microorganisms.

Clinical Evaluation

Clinical Evaluation for Blood 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 9 shows the results for the 24 reference positive specimens.

Table 9: 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 10.

Table 10: 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 ZIKV-spiked aliquots from the 90 plasma specimens evaluated in the Interference studies.

[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.

Clinical Evaluation for Urine Specimens

Ten (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 the 10 patients using the Aptima Zika Virus assay. All specimens were positive upon initial testing. Table 11 shows the results for the 10 matched specimens.

Table 11: 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

Table 11: Aptima Zika Virus Assay Results of 10 Matched ZIKV-Positive Clinical Specimens (continued)

Specimen ID	Country of Origin	Reference Cp (Serum)	Plasma		Serum		Processed Urine	
			Result	S/CO	Result	S/CO	Result	S/CO
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. Of these, 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 12.

Table 12: 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

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