SIEMENS

VERSANT® Zika RNA 1.0 Assay (kPCR) Kit

Instructions For Use

For Use Under an Emergency Use Authorization (EUA) Only.

Current Revision and Date	**10731032 Rev. A, 2016-07**	
Product Name	VERSANT® Zika RNA 1.0 Assay (kPCR) Kit, Box 1 VERSANT® Zika RNA 1.0 Assay (kPCR) Kit, Box 2	REF 10974950 REF 10974951

Intended Use

The VERSANT® Zika RNA 1.0 Assay (kPCR) Kit is a real-time PCR based assay intended for the qualitative detection of RNA from the Zika virus in serum, EDTA plasma 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 epidemiologic 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.

Results are used for the identification of Zika virus RNA. Zika virus RNA is generally detectable in serum during the acute phase of infection (approximately 7 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 VERSANT® Zika RNA 1.0 Assay (kPCR) Kit is intended for use by trained clinical laboratory personnel who have received specific training on the use of the VERSANT® Zika RNA 1.0 Assay (kPCR) Kit. The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Caution: Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional.

Background

The Zika virus (ZIKV) is an emerging mosquito-borne flavivirus, which was first isolated in 1947 from a rhesus monkey in Uganda's Zika forest.[1] Prior to 2007, only African countries and Asian countries were reporting a few cases of ZIKV infection in humans. In 2007, the first epidemic of ZIKV infection in humans was documented in Yap, Federated States of Micronesia, an island in the Pacific region. In Yap, it was estimated that 73% of the population 3 years or younger had been infected with ZIKV.[2] In May 2015, the World Health Organization reported the first local transmission of ZIKV in the Americas. Sixteen patients from Brazil were reported ZIKV-positive, and 22 other countries and territories subsequently identified autochthonous transmission.[3]

An estimated 80% of persons infected with the ZIKV show little to no symptoms.[2] Associated symptoms are mild fever; myalgia; headache; conjunctivitis; and cutaneous maculopapular rash.[2] Amid the ZIKV outbreak in Brazil, ZIKV RNA was identified in tissues from a significant number of infants with microcephaly and from fetal losses in women who were infected during pregnancy.[4,5,6] Other outcomes such as Guillain-Barré syndrome are being studied in association with ZIKV infection.[4]

Assay Principle

The VERSANT® Zika RNA 1.0 Assay (kPCR) Kit is a real-time PCR assay for the qualitative detection of RNA from Zika virus.

To perform the VERSANT® Zika RNA 1.0 Assay (kPCR) Kit, samples are first extracted to isolate the Zika virus RNA. Nucleic acids are isolated and purified from serum, plasma or urine using the Siemens automated system, the VERSANT® kPCR Sample Prep (SP) (also referred to as VERSANT® kPCR Molecular System SP) with VERSANT® MiPLX Software Solution and the VERSANT® Sample Preparation 1.0 Reagents. The VERSANT® Sample Preparation 1.0 Reagents use a nucleic acid isolation technology based on iron oxide beads coated with a nanolayer of silica.[7] Magnetic silica technology is a nonspecific capture method and is target independent; it captures any DNA or RNA present in the sample. VERSANT® sample preparation technology employs a classic method of disrupting the cells in chaotropic high salt conditions to release nucleic acids, as well as to protect them from cellular nucleases.[8,9] The nucleic acids are captured on silica coated beads. Using a magnetic field, the beads are separated and washed to remove proteins, nucleases, and other cellular impurities. The nucleic acids are then eluted in a small volume of elution buffer and ready for subsequent analysis.

Manual extraction of samples (serum, plasma or urine) may be performed using the QIAamp viral RNA Mini Kit (QIAGEN). For information on how to perform manual extraction using the QIAamp viral RNA Mini Kit (QIAGEN), refer to the section, *Manual Sample Extraction*.

Purified RNA is added to a PCR plate containing Zika Enzyme Mix and Zika Primer/Probe Mix, and the wells are sealed. The Zika Enzyme Mix contains dNTPs, reference dye, and enzymes for nucleic acid amplification. The assay targets two regions of the Zika virus genome. The Zika Primer/Probe Mix contains synthetic DNA primers and probes and the Zika Internal Control. An assay internal control sequence is included in the kit, and is used as a sample extraction control. The probes include fluorescent dyes and quenchers as modifiers. The dual-labeled probes specifically detect the presence of Zika and Internal Control amplicons during amplification. In their native state, the probes adopt a folded structure, positioning the quencher next to the fluorescent dye. In this condition, most of the fluorescence of the dye is absorbed by the neighboring quencher, minimizing the emitted fluorescence. When amplicons are generated, fluorescent dye-labeled probes uncoil as they hybridize to the amplicons, separating the fluorescent dye from the quencher, thereby increasing the observed fluorescence. During the extension step, the hybridized probe is cleaved through the exonuclease activity of the polymerase. When free in solution, increased fluorescence is continuously detectable. The increased fluorescence of both cleaved and bound probes correlates with the amount of amplicons generated, and is proportional to the amount of Zika RNA in the sample.

Materials Provided in the VERSANT® Zika RNA 1.0 Assay (kPCR)Kit

The VERSANT® Zika RNA 1.0 Assay (kPCR) Kit contains sufficient reagents and materials to perform 48 tests.

VERSANT® Zika RNA 1.0 Assay (kPCR) Box 1 of 2

The VERSANT® Zika Assay Box 1 contains the following components:

Symbol	Component	Quantity	Storage
ENZYME MIX	Zika Enzyme Mix	1 X 340 µL	-20 ± 5°C
PRIMER MIX	Zika Primer/Probe Mix	1 X 170 µL	-20 ± 5°C
CONTROL IC	Zika Internal Control	1 X 720 µL	-20 ± 5°C
CONTROL –	Zika Negative Control	2 X 650 µL	-20 ± 5°C
WATER	Water	1 X 510 µL	-20 ± 5°C

VERSANT® Zika RNA 1.0 Assay (kPCR) Box 2 of 2

The VERSANT® Zika Assay Box 2 contains the following component:

Symbol	Component	Quantity	Storage
CONTROL +	Zika Positive Control	2 X 650 µL	2 to 8°C

Storage Instructions

Zika Enzyme Mix, Zika Primer/Probe Mix, Zika Internal Control, and Water are multi-use reagents and can be accessed up to 2 times. Reagent should not be freeze/thawed more than two times.

Notes:

To avoid the risk of contamination, the kit includes caps that are used to recap the reagents between uses.

After opening, store all of the reagents included in the box at -20° ± 5°C between uses. Avoid repeated thawing and freezing of the Zika Enzyme Mix, the Zika Primer/Probe Mix, and the Zika Internal Control. Do not repeat the process more than twice.

Materials Required but not Provided in the Kit

Note: The following materials are required for performing the VERSANT® Zika RNA 1.0 Assay (kPCR).

REF	Items
REF 04801677 SMN 10286026	VERSANT® Sample Preparation 1.0 Reagents, Box 1
REF 04801685 SMN 10286027	VERSANT® Sample Preparation 1.0 Reagents, Box 2
REF 06635740 SMN 10282928	VERSANT® kPCR Sample Prep with VERSANT® MiPLX Software Solution (system also referred to as VERSANT® kPCR Molecular System SP)
	One of the following validated Real-Time PCR Systems: QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific) Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument (Thermo Fisher Scientific) CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad)
	PCR plate*****
	For QuantStudio 5: MicroAmp EnduraPlate Optical 96-Well Clear Reaction Plate with Barcode (Thermo Fisher) PN: 4483354
	For ABI 7500: MicroAmp Fast 96-Well Reaction Plate (0.1 mL) (Thermo Fisher) PN: 4346907
	For CFX96 Touch: Hard-Shell 96-Well PCR Plates, Low Profile, Semi Skirted, Clear White(Bio-Rad) PN: HSL9605
	Caps or optical adhesive film*****
	For QuantStudio 5: MicroAmp Optical 8-Cap Strips (Thermo Fisher) PN: 4323032 *or* MicroAmp Optical Adhesive Film (Thermo Fisher) PN: 4311971
	For ABI 7500: MicroAmp Optical 8-Cap Strips (Thermo Fisher) PN: 4323032 *or* MicroAmp Optical Adhesive Film (Thermo Fisher) PN: 4311971
	For CFX96 Touch: Microseal 'B' PCR Plate Sealing Film, adhesive, optical (Bio-Rad)** PN: MSB1001

REF	Items
	Quick-spin, bench-top microcentrifuge
	Adjustable micropipettes
	Sterile-packaged, aerosol-resistant disposable tips
	Centrifuge with microtiter plate rotor (optional)
	Vortexer
	Large reagent troughs and small reagent troughs
	1000-µL pipette tips
	300-µL pipette tips
	96-well, 2-mL nuclease free, sterile deep well plates
	Barcoded 96-well semi-skirted polypropylene plates for PCR
	70% ethanol (ethyl alcohol)
	Bleach, unscented (0.5% sodium hypochlorite)
	Microcide SQ
	Deionized water
	QIAamp Viral RNA Mini Kit†
	Ethanol (96-100%)†
	1.5-mL microcentrifuge tubes†
	Microcentrifuge (with rotor for 1.5-mL and 2.0-mL tubes) †

* **Note:** Use PCR plate, PCR caps, and optical adhesive film compatible with each of the validated Real-Time PCR systems. They are not products of Siemens Healthcare Diagnostics.
** Use optical adhesive film with CFX96. For more information, refer to the system manufacturer's manual.
† Materials only required if manual extraction is performed.

Manuals Required for Sample Preparation

Document Number	Items
10994881	VERSANT® Sample Preparation 1.0 Reagents Instructions for Use
11222579	VERSANT® kPCR Molecular System (SP) Application Guide
1090245	QIAamp Viral RNA Mini Handbook (12/2014)*

* The QIAmp Viral RNA Mini Handbook is only required if manual extraction is performed.

Note

For instrument installation, calibration, and maintenance information refer to the manufacturer's manuals and recommendations for each of the instruments.

Warnings and Precautions

- This assay is for *in vitro* diagnostic use under the Food and Drug Administration (FDA) Emergency Use Authorization (EUA) only.
- This product is for use by individuals properly trained in performing this test and in the use of the specified instruments.
- Laboratories are required to report all positive results to the appropriate public health authorities.
- Take precautions to protect against microbiological hazards by sterilizing samples, instruments and all consumables after use. Read and follow all directions carefully.
- Strict adherence to the following warnings and precautions is required when running the VERSANT® Zika RNA 1.0 Assay (kPCR).
- Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.
- Handle contaminated materials as biohazardous.
- Wear personal protective apparel, including disposable gloves, throughout the assay protocol. Thoroughly wash hands after removing gloves, and dispose of gloves as biohazardous waste.
- Do not eat, drink, smoke, or apply cosmetics in areas where reagents or samples are handled.
- Avoid the use of sharp objects wherever possible.
- If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water. Seek medical advice.
- Do not pipette by mouth.
- Use all pipetting devices and instruments with care and follow the manufacturers' instructions for calibration and quality control.
- Avoid contamination of reagents and samples.
- Use aerosol-resistant nuclease free pipette tips and use a new tip every time a volume is dispensed.
- Do not use reagents if they appear turbid or cloudy after bringing to specified temperature.

Assay Protocol

Nucleic acid extraction is performed with the VERSANT® kPCR Sample Prep (SP) (also referred to as VERSANT® kPCR Molecular System Sample Preparation) with VERSANT® MiPLX Software Solution compatible with real-time PCR technology.
Alternatively, manual sample extraction can be performed with the QIAamp Viral RNA kit (QIAGEN).

The VERSANT® Zika RNA 1.0 Assay (kPCR) must be run using one of the following real-time PCR instruments:

- QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific)
- CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad)
- Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument (Thermo Fisher Scientific)

Automated Sample Extraction

The sample extraction protocol consists of setting up and running VERSANT® kPCR Sample Prep. Sample and control extraction are performed with the VERSANT® Sample Preparation 1.0 Reagents and VERSANT® kPCR Sample Prep. For detailed information on operating the VERSANT® kPCR Sample Prep, preparing and loading of consumables and samples, entering sample and reagent lot information, and performing the nucleic acid extraction run, refer to the VERSANT® Sample Preparation 1.0 Reagents product insert and the *VERSANT® kPCR Molecular System Sample Preparation (SP) Application Guide*.

System Preparation

1. Ensure that all required maintenance for VERSANT® kPCR Sample Prep is complete, or the run will not start.
2. Clean the work area and the pipettors.
3. Prepare the consumables and load them onto VERSANT® kPCR Sample Prep.
4. Prepare the VERSANT® Sample Preparation 1.0 Reagents and place them on the VERSANT® kPCR Sample Prep.

   Note: For specific instructions on preparing the reagents, refer to the *VERSANT® Sample Preparation1.0 Reagents* product insert.

Reagent Preparation

The reagents are liquid and are ready to use. To prepare the VERSANT® Zika RNA 1.0 (kPCR) reagents, perform the following steps:

1. Put on clean gloves.
2. Remove the Zika Primer/Probe Mix, the Zika Enzyme Mix, the Zika Internal Control, and the Zika Negative Control from the -20°C freezer. Remove the Zika Positive Control from 2-8°C storage.

   Note: The kit includes 2 tubes of each of the Zika Positive Control and the Zika Negative Control. For a single run, only remove one tube of each reagent from the refrigerator and freezer.
3. Place the Zika Primer/Probe Mix and the Zika Internal Control in cold water to thaw.

Note: To reduce the risk of contamination, place reagents in a rack in the cold water to ensure that the cap remains above the level of the water.

4. To mix the Zika Primer/Probe Mix, the Zika Enzyme Mix, and the Zika RNA Internal Control, invert each tube10 times.

   Note: Do not vortex the Enzyme Mix.

   Maintain the Zika Primer/Probe Mix, the Zika Enzyme Mix, and the Zika Internal Control on ice or refrigerated until you transfer these reagents to the VERSANT® kPCR Sample Prep.

5. Place the Zika Negative Control in cold water to thaw.
6. After thawing, vortex the Zika Positive Control, and the Zika Negative Control for 5‑10 seconds.
7. Briefly (5–10 seconds) centrifuge all of the thawed reagents in a microcentrifuge.
8. Uncap the reagents and dispose of the caps.

   Notes: Zika Primer/Probe Mix, Zika Enzyme Mix and Zika Internal Control may be used up to 2 times.

   New caps for the Zika Primer/Probe Mix, the Zika Enzyme Mix and the Zika Internal Control are included in the kit. Ensure that you use new caps to recap these reagents between uses.

   The positive control and negative control are intended for single use only, discard after use.

9. Assemble the Master Mix for the number of reactions you intend to perform according to the volumes provided in the table below.

   Note: The VERSANT® kPCR Sample Prep requires a dead volume of approximately 150 µL. Include component volume for 10 additional samples when calculating volume of Master Mix. Additional reagent volume is included in the kit to account for the necessary dead volume.

	Number of Reactions	
	1	**12**
Component	**Addition volumes (µL)**	
Zika Enzyme Mix	5	60
Zika Primer/Probe Mix	2.5	30
Water	7.5	90
Total Master Mix	**15**	**180**

10. If saving for additional runs, re-cap Zika Enzyme Mix, Zika Primer/Probe Mix, and Water with provided caps, and return reagents to -20 ±5°C storage.

Sample Preparation

To prepare the samples, follow these steps:

1. If the samples are frozen, thaw them in cold water or in the refrigerator.
2. Briefly vortex the samples (5 to 10 seconds).

   Notes: If samples appear cloudy or if samples contain visible particulates or fibrin, centrifuge the samples for 5 minutes at 2000 g.

   Using good laboratory practices, remove any visible air bubbles above the liquid surface.
3. Load samples in 3.5 mL tubes in the sample carrier racks. When placing the samples in the sample carrier, carefully remove the tube caps.

   Notes: To avoid contamination, use care when removing the caps.

   To reduce risk of cross-contamination, discard sample tube caps as you remove them. Use new caps when recapping the tubes.

   The VERSANT® kPCR Sample Prep requires a dead volume of at least 150 µL. Ensure total sample volume is at least 700 µL.
4. Place the sample carriers on the autoload tray of the VERSANT® kPCR Sample Prep.
5. Run the protocol as described in the *VERSANT® kPCR Molecular System Sample Preparation (SP) Application Guide: Preparing and Loading the Samples*.

Note:
At the end of each run, immediately remove the Zika Internal Control, cap it using a new cap and store at -20° ± 5°C between uses. Do not repeat the process more than twice.

Dispense Volumes for Controls

The following table (Table 1) describes the volume requirements for use on the VERSANT® kPCR Sample Prep (input) and the volume dispensed to the PCR plate (output).

Table 1: Dispense Volume Information for the Dynamic Assay Preparation 1 (SP 1.0 500) Protocol

Minimum Sample Volume (µL)	Controls (SP) input (µL)	Elution Buffer (µL)	Controls (SP) output (µL) to PCR plate
700	500	100	10

Starting a Sample Preparation Run using a Dynamic Protocol

1. Select the **Protocol** tab in the main screen.
2. Select **Dynamic Assay Preparation 1 (SP 1.0 500)**.
   The assay uses 500-µL input volume and 100-µL eluate volume in sample extraction.

3. Select **Run Protocol**. VERSANT® kPCR Sample Prep initializes system.
4. Select 1 in the **Number of Assays** list to only run one assay.
5. Select the **Dispense IC** checkbox and the **Universal Negative Control** checkbox.
6. Select 1 in the **Controls (SP)** list.
7. Select 0 in the **Controls (No SP)** list.
8. Enter the **Kit Lot Number** and the **Expiration Date**.
9. Load samples.
   The instrument scans the bar codes for samples. Verify the test orders received from LIS.
10. Load consumables and the AR rack.
11. Following sample extraction, the system prompts to load the Post-Extract Analyte Reagent Carrier with the No SP reagents (Master Mix). Refer to the "Preparing the Reagents" section of this document.
12. Once the plate map is generated, the Run Complete window appears. Select View/ Print Report to view the VERSANT® kPCR Sample Preparation Run Report.
13. Select **Finalize Run** to end the run.
14. After the run completes, immediately remove PCR plate from the VERSANT® kPCR Sample Prep and immediately cap the plate before transferring the plate to the thermocycler.

Note: For more information on running a dynamic assay protocol, refer to the *VERSANT® kPCR Molecular System Sample Preparation (SP) Application Guide: Running Dynamic Assay Protocol*.

Manual Sample Extraction

The manual sample extraction can be performed using the QIAamp Viral RNA kit (QIAGEN). The kit combines the selective binding properties of silica- based membrane with the speed of the microspin. The protocol is for purification of viral RNA from 140 µL plasma, serum and urine samples using a mircrocentrifuge. Carefully read the manufacturer's instructions for use (*QIAamp Viral RNA Mini Handbook, 12/2014*) for detailed handling instructions. Prepare Controls as described in the *Reagent Preparation* section of these instructions for use. Prepare samples by following steps 1 and 2 in the *Sample Preparation* section of these instructions for use.

When using the manual extraction procedure, extract positive and negative controls in parallel with the samples.

Note: Reconstitute buffers AW1 and AW2 with 96–100% ethanol (refer to the manufacturer's guidelines for more information).

1. Pipet 560 µL of prepared buffer AVL containing carrier RNA into a 1.5 mL microcentrifuge tube.
2. Add 140 µL of sample (plasma, serum or urine and positive and negative controls) or control to the buffer AVL-carrier RNA in the microcentrifuge tube. Mix by pulse vortexing for 15 seconds.

3. Add 15 µL of Zika Internal Control to each sample. Briefly centrifuge the tube to remove drops from inside the lid.

   Note: Add the Zika Internal Control only after the sample has been added to buffer AVL.

4. Incubate at room temperature (15°–25°C) for 10 minutes.
5. Add 560 µL of ethanol (96–100%) to the sample, and mix by pulse vortexing for 15 seconds. After mixing, briefly centrifuge the tube to remove drops from inside the lid.
6. Carefully apply 630 µL of solution from step 5 to the QIAamp mini column (in a 2-mL collection tube from the QIAamp Viral RNA Mini Kit) without wetting the rim. Close the cap and centrifuge at 6000 x g (8000rpm) for 1minute.
7. Discard the filtrate.
8. Apply the remaining 630 µL of solution from step 5 as described in Step 6.
9. Place the QIAamp mini column into a fresh 2-mL collection tube and discard the tube containing the filtrate.
10. Carefully open the QIAamp mini column and add 500 µL of Buffer AW1. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 minute. Place the QIAamp mini column in a clean 2-mL collection tube and discard the tube containing the filtrate.
11. Carefully open the QIAamp mini column and add 500 µL of Buffer AW2. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 minutes.
12. Place the QIAamp mini column into a fresh 2-mL collection tube and centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 minute.

    Note: This step removes any remaining traces of ethanol.

13. Place the QIAamp mini column in a clean 1.5-mL RNase-free microcentrifuge tube and discard the tube containing the filtrate.
14. Carefully open the QIAamp mini column and add 60 µL of Buffer AVE equilibrated to room temperature (15°–25°C). Close the cap and incubate at room temperature (15°–25°C) for 1 minute. Centrifuge at 6000 x g (8000rpm) for 1 minute. Eluted RNA is stored at 2–8°C until PCR master mixes are prepared.

    Note: For longer term storage of eluates, place at -60 to -80°C.

15. Set up the PCR Plate manually by adding 15 µL of PCR master mix and 10 µL of sample to each well of the PCR plate. Proceed to the "Running the VERSANT® Zika RNA 1.0 Assay (kPCR) on the Real-Time PCR Instruments" section of this document.

Running the VERSANT® Zika RNA 1.0 Assay (kPCR) on the Real-Time PCR Instruments

Set up the Real-Time PCR instruments according to instructions of the instruments you are using. Use the following settings:

Real-Time PCR instrument Settings

Settings	
Reaction Volume	25 µL
Temperature Ramp Rate	Default
Passive Reference Dye	ROX
Zika virus RNA Dye	FAM
Internal Control Dye	CY5

Real-Time PCR instrument Thermal Profile

Function	Temperature	Time	Number of Cycles
Reverse Transcription	50°C	20 minutes	1
TAQ Activation	95°C	5 minutes	1
Denaturation	95°C	15 seconds	45
Amplification & Data Acquisition	60°C	1 minute	45

Special instructions on the setup of authorized real-time PCR instruments

For special instructions on the setup of QuantStudio™ 5 Real-Time PCR System, CFX96 Touch™ Real-Time PCR Detection System, and Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument, review the sections below.

Special instructions on the setup of the QuantStudio™ 5 Real-Time PCR System

1. Open software. Select “New Experiment” tab by clicking either “Template” in the drop-down arrow or “Create New Experiment”.
2. Click the “Properties” tab to name experiment.
3. Click the “Method” tab. Verify the reaction volume is accurate, and the time and temperature steps are correct. To modify a setting, click on that setting and either enter the new value manually or use up/down arrows.
4. Click the “Plate” tab. Click on plate map to select the wells used. Under “Advanced Set Up”, assign fluorophores. Select FAM for testing “Zika virus” and Cy5 for testing “Internal control”.
5. Click the “Run” tab. Click “Start Run” to begin the run.

Special instructions on the setup of the CFX96 Touch™ Real-Time PCR Detection System

1. Open the "Protocol Editor", to create a new protocol or edit an existing one.
2. Select any step in either graphical or text display to edit either time or temperature.
3. Click "Insert Step" or "Delete Step" to add or remove step from the protocol.
4. Click "Add Plate Read" to step to designate the collection of the fluorescence data in the protocol.
5. Click the number of repeats of a GOTO step to change the number of cycles in the protocol
6. Click on "Select Fluorophores". Select FAM™ for testing "Zika virus" and Cy5™ for testing "Internal control".
7. Click the "Start Run" tab and begin the run.

Special instructions on the setup of the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument

1. Open the 7500 software to create a new document or open an existing document.
2. Select "Detectors" on the next tab. Select FAM™ for testing "Zika virus" and Cy5™ for testing "Internal control".
3. Add the assigned detectors (Fluorophores) to "Detectors in Document" section.
4. Click next and "Set up" the sample plate by assigning targets to each well.
5. Following set up, click on the "Instrument" tab and set up the thermal profile.
6. Click "Start" and begin the run.

Quality Control

Zika Negative and Positive Controls

To monitor assay performance, include the Zika Negative Control and the Zika Positive Control with each run.

Run Validity

To produce a valid run; 1) the Zika Negative Control must have a negative Zika result (FAM) and a positive Internal Control result (CY5), and 2) the Zika Positive Control must have a positive Zika result (FAM) and a positive Internal Control result (CY5).

If either the Zika Negative Control or the Zika Positive Control fails, the run is **invalid**.

Note: Disregard results from invalid runs.

If the run is invalid, before repeating the run, perform the following steps:

- Review these instructions for use to ensure that the assay is performed according to the procedure recommended by Siemens Healthcare Diagnostics.
- Verify that the real-time PCR instrument has been correctly maintained and that the correct procedure to set up the run is followed.

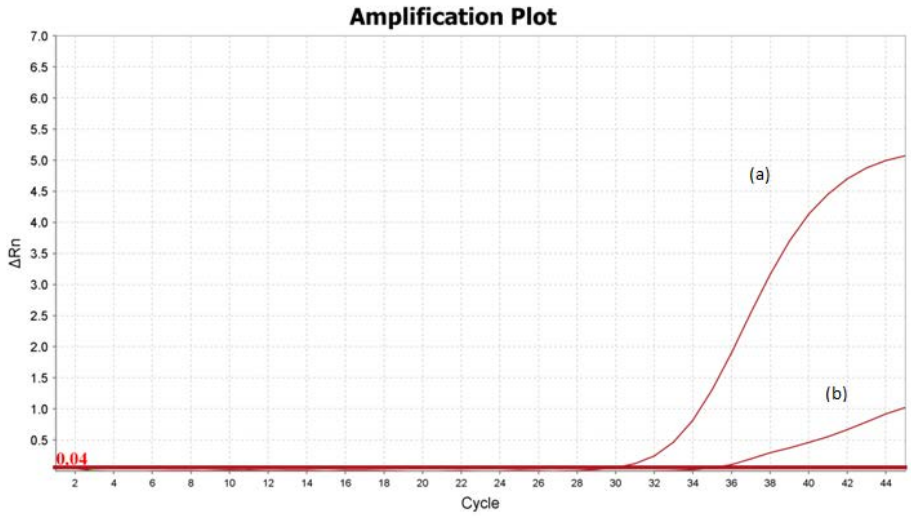
Results

Interpretation of Individual Sample Results

When interpreting the results, carefully review the fluorescence curves. Only true exponential curves should be interpreted as positive. Multi-component or raw fluorescence curves may be helpful in evaluating weak signals for a positive result.

Both Zika target regions produce FAM signal, leading to a single, cumulative FAM fluorescence curve for each specimen. Amplification curves of all test results must be reviewed manually, with careful analysis of weak positive FAM fluorescence curves.

Figure 1: Example of a strong positive FAM curve (a) and a weak positive FAM curve (b).



Note: Only true exponential curves should be interpreted as positive (Figure 1). Multi-component or raw fluorescence curves (Figure 2) can be used in evaluating weak signals such as the FAM curve (b) in Figure 1.

Figure 2: Multicomponent Plot view of FAM curve (b) in Figure 1.

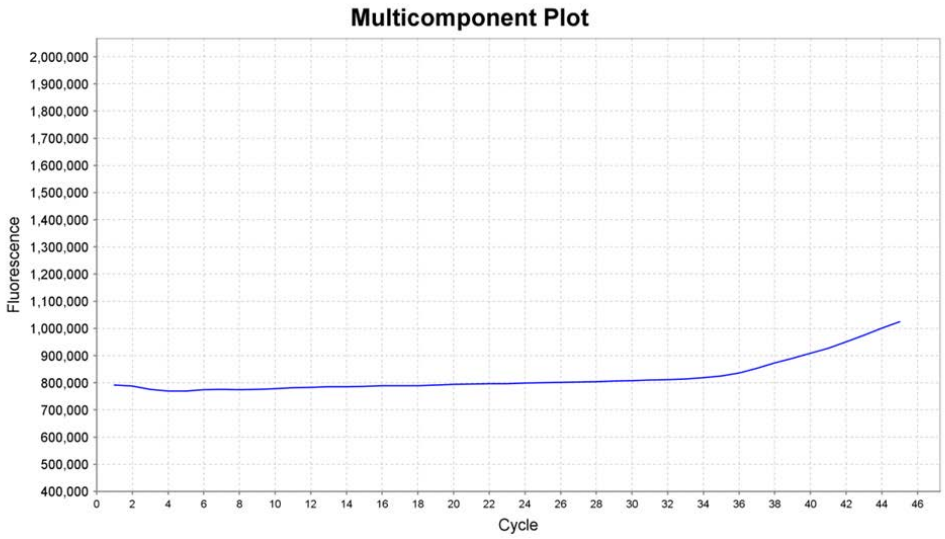
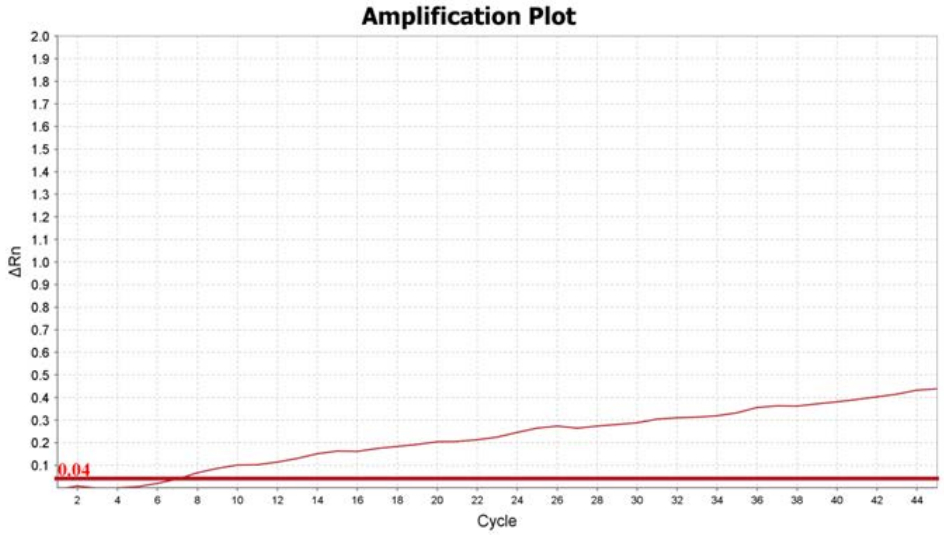
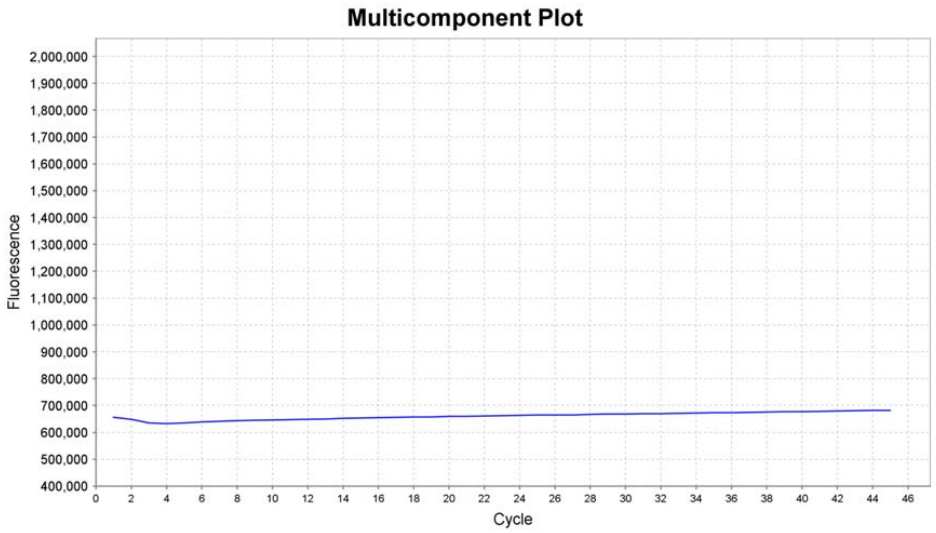


Figure 3: Example of a negative FAM curve that did not indicate an exponential amplification, but crossed the threshold.



Note: A FAM curve that does not display exponential amplification may cross the threshold. Multicomponent view of the curve (Figure 4) can be used to confirm exponential amplification.

Figure 4: Multicomponent Plot view of the negative FAM curve in Figure 3.



For general information on how to interpret the results on a specific real-time PCR instrument, refer to the instrument manufacturer's manual.

Individual Sample Results

Results	Zika (FAM)	Internal Control (CY5)	Interpretation
Zika Detected	Positive	Positive or Negative	Zika virus RNA detected.
Zika Not Detected	Negative	Positive	Zika virus RNA not detected. Follow current CDC guidelines for follow-up testing*
Invalid	Negative	Negative	Individual sample test failure. Repeat testing is required.

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

Limitations

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR procedures.
- Optimal performance of this test requires appropriate specimen collection, transport, storage and processing procedures.
    - Negative results do not preclude infection with Zika virus and should not be used as the sole basis of a patient treatment/management decision. All results should be interpreted by a trained professional in conjunction with review of the patient's history and clinical signs and symptoms.

- Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.
- Improper specimen collection, storage, or transport of specimens may lead to false negative results.
- Specimen collection conducted prior to symptom onset may lead to false negative results.
- Specimen collection after nucleic acid can no longer be found in the patient (approximately 7 days post-onset of symptoms for sera) may lead to false negative results.
- The impact of the administration of Zika virus vaccines and/or therapeutics on the ability to detect Zika Virus RNA in patient specimens has not been evaluated.

- This assay must not be used to directly test clinical specimens. Appropriate nucleic acid extraction of specimens using either the VERSANT® Sample Preparation 1.0 Reagents with the VERSANT® kPCR Sample Prep or the QIAamp viral RNA Mini Kit must be performed prior to running the assay.
- **A patient-matched serum specimen is currently required for serological follow up testing of negative RT-PCR results per the CDC testing algorithm (found at http://www.cdc.gov/zika/index.html).**

Performance Characteristics

Analytical Sensitivity

All sensitivity testing was performed with Zika PRVABC59 strain (ZeptoMetrix) diluted in pooled plasma or serum. Automated extraction was performed using VERSANT® Sample Preparation 1.0 Reagents with the VERSANT® kPCR Sample Prep. A tentative LoD was established with plasma and serum dilution series using the Thermo Fisher QuantStudio™ 5 PCR instrument. Each dilution series tested consisted of 5 replicates of each of 5 levels diluted in either plasma or serum. The lowest level detected at 100% was 0.05 $TCID_{50}$/mL ($TCID_{50}$) or 721 copies/mL (GCE) in both plasma and serum (Table 2) and was considered the tentative LOD.

A. **Plasma and Serum**

Table 2: Tentative LoD of VERSANT® Zika RNA 1.0 Assay (kPCR).

Concentration ($TCID_{50}$/mL)	Genome Copy Equivalent (GCE/mL)	Plasma Percent Detected	Serum Percent Detected	Detected Replicates
0.2	2884	100%	100%	5/5
0.15	2163	100%	100%	5/5
0.1	1442	100%	100%	5/5
0.05	721	100%	100%	5/5
0.025	360	80%	80%	4/5
0.0125	180	60%	60%	3/5

The tentative LoD of 0.05 $TCID_{50}$/mL or 721 copies/mL was confirmed by testing more than 20 replicates of strain PRVABC59 in serum and plasma on each of three thermal cyclers:

QuantStudio™ 5 (Thermo Fisher Scientific), CFX96 Touch™ (BioRad) and Applied Biosystems® 7500 (Thermo Fisher Scientific). The 0.05 $TCID_{50}$/mL or 721 copies/mL level was 100 % detected consistently in both serum and plasma with every instrument, establishing the assay LoD (Table 3).

Table 3: LoD Confirmation of VERSANT® Zika RNA 1.0 Assay (kPCR).

Amplification Detection System*	Sample Type	Concentration ($TCID_{50}$/mL)	Genome Copy Equivalents (GCE/mL)	Percent Detected	Detected Replicates	Average Ct
QS5	Plasma	0.05	721	100%	23/23	33.80
	Serum	0.05	721	100%	22/22	33.59
ABI 7500	Plasma	0.05	721	100%	23/23	33.15
	Serum	0.05	721	100%	22/22	32.85
CFX96	Plasma	0.05	721	100%	23/23	35.86
	Serum	0.05	721	100%	22/22	35.55

* QS5 = QuantStudio 5™ (QS5) Real-Time PCR System (Thermo Fisher Scientific)
CFX96 = CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad)
7500 = Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument (Thermo Fisher Scientific).

B. Urine

The LoD of the assay with urine samples was estimated using Zika PRVABC59 strain (ZeptoMetrix) diluted in pooled urine to target concentrations listed in Table 4. Automated extraction was performed using the Siemens automated system, the VERSANT® kPCR Sample Prep with VERSANT® MiPLX Software Solution and the VERSANT® Sample Preparation 1.0 Reagents. An estimated LoD was established in initial experiments performed on the QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific). Five (5) replicates were tested at each concentration. Similar to plasma and serum, the lowest level with a detection rate of 100% was 0.05 U/mL (50% Tissue Culture Infective Dose $TCID_{50}$) or 721 GCE/mL (Genomic Copy Equivalent/mL) in urine (Table 4). Raw data for the tentative LOD study are included in Appendix 2 of this document.

Table 4: Tentative LoD of the VERSANT® Zika RNA 1.0 Assay (kPCR) in Urine.

Concentration ($TCID_{50}$/mL)	Concentration (GCE/mL)	Hit Rate in Urine (Percent)	Hit Rate (Fraction)
1.25	18024	100%	5/5
0.25	3605	100%	5/5
0.05	721	100%	5/5
0.025	360	40%	2/5
0.010	144	80%	4/5

The estimated LoD of 0.05 $TCID_{50}$/mL (721 GCE/mL) was then confirmed by a detection rate of > 95% on 23 replicates of Zika virus strain PRVABC59 at the target concentration of 0.05 $TCID_{50}$/mL in urine on the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad) and the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument (Thermo Fisher Scientific). However, the QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific) did not achieve a 95% detection rate at 0.05 U/mL. Subsequently, 23 replicates of 0.075 $TCID_{50}$/mL (1081 GCE/mL) confirmed the LoD with this instrument with a >95% detection rate (Table 5).

Table 5: LoD Confirmation of VERSANT® Zika RNA 1.0 Assay (kPCR) in Urine.

Amplification Detection System*	Sample Type	Concentration ($TCID_{50}$/mL)	Concentration (GCE/mL)	Hit Rate (Percent)	Hit Rate (Fraction)	Average Ct
QS5	Urine	0.05	721	87%	20/23	33.53
QS5	Urine	0.075	1081	100%	23/23	31.68
ABI 7500	Urine	0.05	721	100%	23/23	36.56
BioRad CFX96	Urine	0.05	721	96%	22/23	34.70

* QS5 = QuantStudio™ 5 (QS5) Real-Time PCR System (Thermo Fisher Scientific)
CFX96 = CFX96™ Touch Real-Time PCR Detection System (Bio-Rad)
ABI 7500 = Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument (Thermo Fisher Scientific).

Manual extraction of plasma, serum and urine samples was performed using the QIAamp Viral RNA Mini Kit (QIAGEN). An input of 140 µL sample or control was used and extraction was performed following the steps provided in the "Manual Sample Extraction" section of this document. Purified RNA was eluted in 60 µL elution volume. Refer to the *QIAamp Viral RNA Mini Handbook* for more information. Manual extraction of plasma, serum and urine samples confirmed an LoD of 0.075 $TCID_{50}$/mL (Table 6).

Table 6: LoD Confirmation of VERSANT® Zika RNA 1.0 Assay (kPCR) with QIAamp Viral RNA Kit (Manual Extraction).

Amplification Detection System*	Sample Type	Concentration ($TCID_{50}$/mL)	Concentration (GCE/mL)	Hit Rate (Percent)	Hit Rate (Fraction)	Average Ct
QS5	Serum	0.075	1081	95%	19/20	34.76
QS5	Plasma	0.075	1081	100%	22/22	35.09
QS5	Urine	0.075	1081	100%	22/22	34.20

* QS5 = QuantStudio™ 5 (QS5) Real-Time PCR System (Thermo Fisher Scientific)

C. **Analytical Sensitivity – FDA Reference Materials**

An analytical study was performed using FDA reference materials (S1 and S2) following a standard protocol provided by the FDA. The protocol included range-finding and confirmatory LoD studies to evaluate the analytical sensitivity of the VERSANT Zika RNA 1.0 Assay (kPCR) (Table 7).

Table 7: LOD confirmation of VERSANT® Zika RNA 1.0 Assay (kPCR) using the FDA reference materials.

Reference Materials	Specimen Type	Confirmed LoD* in RNA NAAT Detectable Units/mL
S1	Serum	1×10^3
S1	Urine	3×10^3
S2	Serum	5×10^3

S2	Urine	5 x10^3

*Study performed according to an FDA issued protocol

Analytical Specificity

Reactivity/inclusivity wet testing of the VERSANT® Zika RNA 1.0 Assay (kPCR) was performed with two commercially available strains of Zika virus: MR766 and PRVABC59 using the VERSANT® Zika RNA 1.0 Assay (kPCR). The Zeptometrix NATtrol inactivated Zika reference strain MR766 was used as a positive control in these studies, and is included as a positive control in the final kit. A live preparation of the Puerto Rican Zika virus strain PRVABC59 strain was used in the LoD studies in this application. Summary of the inclusivity wet lab testing in Table 8 shows detection of both virus strains diluted in pooled plasma.

Table 8: Inclusivity Wet Testing.

Zika virus strain	Sample Type	Concentration	Ct*
PRVABC59	Culture Media (Live Virus)	0.05 $TCID_{50}$/mL	33.8
MR766	NATtrol Inactivated Virus	Unknown	28.1
FSS13025**	Culture Media (Heat Inactivated)	5 x10^3 RNA NAAT Detectable Units/mL	34.5

* Evaluation on QuantStudio™ 5.
** Data generated as part of the FDA issued protocol described in Table 7.

An additional 35 strains of Zika virus were tested for reactivity/inclusivity *in silico*. *In silico* analysis was performed with Geneious 8.0.5 software. Strain sequences were downloaded from NCBI into the software application. Zika sequences were interrogated with the two Zika-specific primer/probe sets of the VERSANT® Zika RNA 1.0 Assay (kPCR) (Table 9).

Table 9: *In silico* analysis of inclusivity with the VERSANT® Zika RNA 1.0 Assay (kPCR).

Country of origin	NCBI Accession	Date	ZIKA TARGET REGION 1			ZIKA TARGET REGION 2		
			Forward (%)	Reverse (%)	Probe (%)	Forward (%)	Reverse (%)	Probe (%)
Brazil - ZikaSPH2015	KU321639	2015	100	100	100	100	100	100
Brazil - BeH818995	KU365777	2015	100	100	100	100	100	100
Brazil - BeH819015	KU365778	2015	100	100	100	100	100	100
Brazil - BeH819966	KU365779	2015	100	100	100	100	100	100
Brazil - BeH815744	KU365780	2015	100	100	100	100	100	100
Brazil -ZKV2015	KU497555	2016	100	100	100	100	100	100
Puerto Rico - PRVABC59	KU501215	2015	100	100	100	100	100	100
Haiti - 1225/2014	KU509998	2014	100	100	100	100	100	100
Brazil - Natal	KU527068	2016	100	100	100	100	100	100
Thailand - THA2014	KU681081	2014	100	100	96	100	100	100
Philippines - PHL2012	KU681082	2012	100	100	100	100	95	100
Brazil - SSABR1	KU707826	2016	100	100	100	100	100	100
Brazil - BeH823339	KU729217	2015	100	100	100	100	100	100

Country of origin	NCBI Accession	Date	ZIKA TARGET REGION 1			ZIKA TARGET REGION 2		
			Forward (%)	Reverse (%)	Probe (%)	Forward (%)	Reverse (%)	Probe (%)
Brazil - BeH828305	KU729218	2015	100	100	100	100	100	100
China - VE_Ganxian	KU744693	2016	100	100	100	100	100	100
China - ZJ03	KU820899	2016	100	100	100	100	100	100
Dominican Republic-Dominican Republic/2016/PD1	KU853012	2016	95	100	100	100	100	100
Guatamela - FB-GWUH-2016	KU870645	2016	100	100	100	100	100	100
Mexico - MEX/InDRE/Lm/2016	KU922923	2016	100	100	100	100	100	100
Brazil - Rio-U1	KU926309	2016	100	100	100	100	100	100
Brazil - Rio-S1	KU926310	2016	100	100	100	100	100	100
Brazil - Bahia09	KU940224	2015	100	100	100	100	100	95
Brazil - Bahia07	KU940228	2015	100	100	100	100	100	95
Cambodia - KHM/2010	KU955593	2010	100	100	100	100	95	100
Haiti - Haiti/1/2016	KX051563	2016	100	100	100	100	100	100
Colombia - ZIKAV/Homo sapiens/COL/FLR/2015	KX087102	2015	100	100	100	100	100	100
China - Zhejiang04	KX117076	2016	100	100	100	100	100	100
Panama - BEI-259634	KX198135	2016	100	100	100	100	100	100
Colombia - UF-1	KX247646	2016	100	100	100	100	100	100
China - ZKC2/2016	KX253996	2016	100	100	100	100	100	100
Columbia - FLR	KU820897	2015	100	100	100	100	100	100
Suriname - Z1106033	KU312312	2015	100	100	100	100	100	100
Thailand - THA2014	KU681081	2014	100	100	96	100	100	100
Micronesia - YAP	EU545988	2007	100	100	96	100	100	100
Honduras - 103451	KX262887	2016	100	100	100	100	100	100

Cross Reactivity: Wet Test

Cross-reactivity of the VERSANT® Zika RNA 1.0 Assay (kPCR) was evaluated by testing different organisms with the VERSANT® Zika RNA 1.0 Assay (kPCR). Organisms were obtained from commercial sources, and diluted 1:100 in pooled plasma. Genomic DNA/RNA was extracted from the samples using the VERSANT® Sample Prep 1.0 reagents and VERSANT® Sample Prep instrument. Samples were analyzed with the QuantStudio™ 5 amplification/detection machine.

At the tested concentrations no cross-reactivity was observed with any of the 10 organisms tested with the VERSANT® Zika RNA 1.0 Assay (kPCR). All cross-reactivity samples showed absence of Ct or amplification in the Zika channel and a positive Ct in the Internal Control channel (Table 10).

Table 10: Cross Reactivity. VERSANT® Zika RNA 1.0 Assay (kPCR) Cross Reactivity with Organisms Related to the Zika Virus**.**

Sample Name	Sample Source	Sample Type	Catalog #	Approximate Concentration from Supplier*	Estimated Units/Rxn	Result
Human Parvovirus B19	ZeptoMetrix	Virus	316331	10e8 U/mL	10e5 U	Not Detected
Mayaro Virus	ATCC	Virus	VR-1277	10e8.1 U/mL	10e5.1 U	Not Detected
Plasmodium falciparum	ATCC	Protozoa	30930	10e8.96 U/mL	10e5.96 U	Not Detected
Yellow Fever 17D	ZeptoMetrix	Virus	0810095CF	10e8 U/mL	10e5 U	Not Detected
Dengue Fever 1	ZeptoMetrix	Virus	0810088CF	10e8 U/mL	10e5 U	Not Detected
Dengue Fever 2	ZeptoMetrix	Virus	0810089CF	10e8 U/mL	10e5 U	Not Detected
Dengue Fever 3	ZeptoMetrix	Virus	0810090CF	10e7.06 U/mL	10e4.06 U	Not Detected
Dengue Fever 4	ZeptoMetrix	Virus	0810091CF	10e7.53 U/mL	10e4.53 U	Not Detected
West Nile Virus	ZeptoMetrix	Virus	NATWNV-0005	5e4 C/µL	5e3 C	Not Detected
Chikungunya	ZeptoMetrix	Virus	NATCHIKV-ST	10e8 U/mL	10e5 U	Not Detected

* U/mL were supplied as $TCID_{50}$ from manufacturer. Concentration for West Nile Virus was supplied as Copies/µL from manufacturer.

Cross Reactivity: *In Silico* Test

Additional *in silico* testing was performed on pathogens either distantly related to Zika or whose infection results in symptoms similar to Zika virus infection. NCBI Reference sequences of tested organisms were compared with each individual oligo in the VERSANT® Zika RNA 1.0 Assay (kPCR) using the NCBI BLAST algorithm. No alignments with ≥ 70% homology were found between any of the primers and probes used in the VERSANT® Zika RNA 1.0 Assay (kPCR) and the pathogen sequences tested, indicating no off-target amplification of sequences from these organisms (Table 11).

Table 11: *In silico* Cross Reactivity. Organisms tested In silico for Cross-Reactivity to the VERSANT® Zika RNA 1.0 Assay (kPCR).

Organisms Tested *In silico*	Taxonomic ID	Ref Seq ID	NCBI/Zika Assay Primers and Probes Cross Reactivity
St. Louis Encephalitis Virus	11080	NC_007580.2	ND
Eastern Equine Encephalitis Virus	11021	EF568607.1	ND
Western Equine Encephalitis Virus	11039	NC_003908.1	ND
Ross River virus	11029	NC_001544.1	ND
Barmah Forest virus	11020	NC_001786.1	ND
O'nyong-nyong virus	11027	NC_001512.1	ND
Sindbis virus	11034	NC_001547.1	ND

Organisms Tested *In silico*	**Taxonomic ID**	**Ref Seq ID**	**NCBI/Zika Assay Primers and Probes Cross Reactivity**
Tonate virus	60877	AF398384.1	ND
Una virus	59304	HM147992.1	ND
Japanese Encephalitis Virus	11072	NC_001437.1	ND
Measles Virus	11234	NC_001498.1	ND
Spondweni virus	64318	NC_029055.1	ND
Hepatitis C virus genotype 2	40271	NC_009823.1	ND
Hepatitis C virus genotype 3	356114	NC_009824.1	ND
Hepatitis C virus genotype 6	42182	NC_009827.1	ND
Hepatitis C virus genotype 5	33746	NC_009826.1	ND
Hepatitis C virus genotype 1	11103	NC_004102.1	ND
Hepatitis C virus genotype 4	33745	NC_009825.1	ND
Rubella Virus	11041	NC_001545.2	ND
Enterovirus A	138948	NC_001612.1	ND
Enterovirus B	138949	NC_001472.1	ND
Enterovirus C (Polio virus)	138950	NC_002058.3	ND
Enterovirus D	138951	NC_001430.1	ND
Enterovirus E (Bovine enterovirus)	12064	NC_001859.1	ND
Enterovirus F	1330520	NC_021220.1	ND
Enterovirus G (Porcine)	64141	NC_004441.1	ND
Enterovirus H (Simian enterovirus A)	310907	NC_003988.1	ND
Enterovirus I	230161	EU672965.1	ND
Enterovirus J 1631	1330521	NC_010415.1	ND
Enterovirus J N203	1330521	NC_013695.1	ND
Adenovirus B	108098	KP279748.1	ND
Adenovirus D	130310	KU170749.1	ND
Adenovirus C	129951	JX173081.1	ND
Adenovirus B1	565302	JX117835.1	ND
Adenovirus 7	10519	KP670856.2	ND
Hepatitis C Virus	11102	BD314310.1	ND
Hepatitis B Virus (Strain ayw)	10404	NC_003977.2	ND
HIV 1	11676	NC_001802.1	ND
HIV 2	11079	KP890355.1	ND
Varicella Zoster Virus (Human Herpesvirus 3)	10335	NC_001348.1	ND
Cytomegalovirus (Human herpesvirus 5)	10358	NC_006273.2	ND
Epstein Barr Virus (Human herpesvirus 4)	10376	NC_009334.1	ND
Rikettsia sp	780	HW294506.1	ND
Borrelia burgdorferi	139	AB872956.1	ND
Group A Streptococcus	36470	DM001444.1	ND
Leptospirosis	171	S76603.1	ND
Plasmodium vivax	5855	KU318072.1	ND

Organisms Tested *In silico*	Taxonomic ID	Ref Seq ID	NCBI/Zika Assay Primers and Probes Cross Reactivity
Trypanosoma cruzi (Chagas)	5693	KR350585.1	ND
Schistosomiasis	6183	HE601627.1	ND
Hepatitis A Virus Vaccine	208726	AH003953.2	ND
Salmonella typhi str. Ty21a (Typhoid - Ty21a vaccine)	527001	CP002099.1	ND
Escherichia coli strain NGF1	562	NZ_CP016007.1	ND
Escherichia coli O157:H7	386585	NC_002695.1	ND

Clinical Performance Evaluation

The clinical performance of the VERSANT® Zika RNA 1.0 Assay (kPCR) was evaluated against a comparator assay described in Lanciotti et al (2008)[10]. The VERSANT® Zika RNA 1.0 Assay (kPCR) was initially evaluated with a total of 349 clinical samples.
Two (2) samples were excluded from the study due to sample preparation error and two additional samples were excluded because they were in CPD Plasma. Out of the remaining 345 clinical samples, 245 samples were from symptomatic individuals from endemic populations, and 100 samples were from Zika virus negative individuals that were from low prevalence populations.

Among the 245 potentially Zika positive samples from endemic populations, 156 samples were from 78 matched serum/plasma and urine samples, and both the serum/plasma and urine samples were tested in this study. The remaining
89 plasma/serum samples did not have matching urine samples. Among the 100 Zika negative samples from low prevalence populations, 50 were serum/plasma and 50 were urine.

The clinical sample results for the VERSANT® Zika RNA 1.0 Assay (kPCR) and the Lanciotti et al. Assay are summarized in Tables 12-17 by sample type (plasma, serum, urine) and by population type (endemic populations and low prevalence populations).

Table 12: Comparison of the VERSANT® Zika RNA 1.0 Assay (kPCR) and Lanciotti et al. Assay Results for Clinical Plasma Samples Collected from Endemic Populations.

		Lanciotti et al. Assay	
		Positive	Negative
VERSANT® Zika RNA 1.0 Assay (kPCR)	Positive	35	7*
	Negative	0	26

* The 7 “false positive” samples had Ct values > Ct 34 with the VERSANT assay. This Ct range suggests that the analyte levels in these samples were close to or below the LOD of the VERSANT assay. Repeat testing of the 7 “false positive” samples was performed with 3 replicates each for the comparator assay. 4 out of 7 “false positive” samples had at least one positive replicates with the comparator test.

Table 13: Comparison of the VERSANT® Zika RNA 1.0 Assay (kPCR) and Lanciotti et al. Assay Results for Clinical Plasma Samples Collected from Low Prevalence Populations.

		Lanciotti et al. Assay	
		Positive	Negative
VERSANT® Zika RNA 1.0 Assay (kPCR)	Positive	0	0
	Negative	0	28

Table 14: Comparison of the VERSANT® Zika RNA 1.0 Assay (kPCR) and Lanciotti et al. Assay Results for Clinical Serum Samples Collected from Endemic Populations.

		Lanciotti et al. Assay	
		Positive	Negative
VERSANT® Zika RNA 1.0 Assay (kPCR)	Positive	57	8**
	Negative	9*	24

* The 9 "false negative" samples had Ct values > Ct 35 with the comparator assay. This Ct range suggests that the analyte levels in these samples were close to or below the LoD of the comparator assay. Repeat testing of the 9 "false negative" samples was performed with 3 replicates each for the VERSANT assay. 4 out of 9 "false negative" samples had at least one positive replicate with the VERSANT assay.
** The 8 "false positive" samples had Ct values > Ct 34 with the VERSANT assay. This Ct range suggests that the analyte levels in these samples were close to or below the LoD of the VERSANT assay. Repeat testing of the 8 "false positive" samples was performed with 3 replicates each for the comparator assay. 5 out of 8 "false positive" samples had at least one positive replicate with the comparator test.

Table 15: Comparison of the VERSANT® Zika RNA 1.0 Assay (kPCR) and Lanciotti et al. Assay Results for Clinical Serum Samples Collected from Low Prevalence Populations.

		Lanciotti et al. Assay	
		Positive	Negative
VERSANT® Zika RNA 1.0 Assay (kPCR)	Positive	0	0
	Negative	0	22

Table 16: Comparison of the VERSANT® Zika RNA 1.0 Assay (kPCR) and Lanciotti et al. Assay Results for Clinical Urine Samples Collected from Endemic Populations.

		Lanciotti et al. Assay	
		Positive	Negative
VERSANT® Zika RNA 1.0 Assay (kPCR)	Positive	39	13*
	Negative	6*	21

* The 6 "false negative" samples had Ct values > Ct 36 with the comparator assay. This Ct range suggests that the analyte levels in these samples were close to or below the LoD of the comparator assay. Repeat testing of the 6 "false negative" samples was performed with 3 replicates each for the VERSANT assay. 5 out of 6 "false negative" samples had at least one positive replicate with the VERSANT assay.
** The 13 "false positive" samples had Ct values > Ct 34 with the VERSANT assay. This Ct range suggests that the analyte levels in these samples were close to or below the LoD of the VERSANT assay. Repeat testing of the 13 "false positive" samples was performed with 3 replicates each for the comparator assay. 10 out of 13 "false positive" samples had at least one positive replicate with the comparator test.

Table 17: Comparison of the VERSANT® Zika RNA 1.0 Assay (kPCR) and Lanciotti et al. Assay Results for Clinical Urine Samples Collected from Low Prevalence Populations.

		Lanciotti et al. Assay	
		Positive	Negative
VERSANT® Zika RNA 1.0 Assay (kPCR)	Positive	0	0
	Negative	0	50

Contrived Sample Evaluation

Performance of the VERSANT® Zika RNA 1.0 Assay (kPCR) with contrived samples was evaluated using 30 unique negative serum and 30 unique negative K_2EDTA plasma specimens from uninfected donors. The negative specimens for each matrix were spiked with the Zika

PRVABC59 strain (ZeptoMetrix) as follows: five at 5X LoD (0.25 $TCID_{50}$/mL), five at 3X LoD (0.15 $TCID_{50}$ /mL), five at 2X LoD (0.10 $TCID_{50}$/mL) and fifteen specimens at LoD (0.05 $TCID_{50}$/mL). Samples were extracted using the VERSANT® Sample Preparation 1.0 Reagents automated with the VERSANT® kPCR SP with VERSANT® MiPLX Software Solution. The QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific) instrument was used for amplification and detection. One sample in plasma at 0.25 $TCID_{50}$/mL or 5X LoD had a sample preparation error. Data from the remaining
59 samples are summarized (Table 18).

Table 18: Performance of VERSANT® Zika RNA 1.0 Assay (kPCR) with Contrived Samples.

Matrix	$TCID_{50}$ /mL	Copies/ mL	Concentration	Average Ct	Hit Rate	Percent Detection
Serum	0.25	3605	5X LoD	32.11	5/5	100%
	0.15	2163	3X LoD	33.09	5/5	100%
	0.10	1442	2X LoD	33.47	5/5	100%
	0.05	721	LoD	35.14	15/15	100%
Total Serum					**30/30**	**100%**
Plasma	0.25	3605	5X LoD*	32.45	4/4	100%
	0.15	2163	3X LoD	32.97	5/5	100%
	0.10	1442	2X LoD	33.94	5/5	100%
	0.05	721	LoD	34.69	15/15	100%
Total Plasma					**29/29**	**100%**

* One plasma sample at 5X LoD had an extraction error

The VERSANT® Zika RNA 1.0 Assay (kPCR) detected all (at 5X LoD, 2X LoD, 3X LoD and LoD) spiked samples for both serum and plasma matrices. The detection rates were 100%.

Percent Agreement

The Positive Percent Agreement (PPA) and the Negative Percent Agreement (NPA) in the test results generated with the VERSANT® Zika RNA 1.0 Assay (kPCR) and the comparator Lanciotti et al. assay were calculated and summarized (Tables 19–21).

Table19: Percent Agreement between the VERSANT® Zika RNA 1.0 Assay (kPCR) and the Lanciotti et al. Assay Results for Clinical Plasma Samples.

Agreement Type**	**Test Population**	**# of Agreements** (correctly detected # of specimens/total # of tested specimens)	**Percent Agreement**	**95% Confidence Interval (CI)**
PPA	Endemic	35/35	100%	(90.1%, 100%)
	Low Prevalence (U.S.)	N/A	N/A	N/A
	Contrived Samples*	1x LoD: 15/15 2x LoD: 5/5 3x LoD: 5/5 5x LoD: 4/4	100%	(88.3%, 100%)
PPA	**ALL**	**64/64**	**100%**	**(94.3%, 100%)**
NPA	Endemic	26/33	78.8%	(62.2%, 89.3%)
	Low Prevalence (U.S.)	28/28	100%	(87.9%, 100%)
	Contrived Samples*	N/A	N/A	N/A
NPA	**ALL**	**54/61**	**88.5%**	**(78.2%, 94.3%)**

* Samples were from low prevalence populations collected in 2009 and were not tested prior to spiking. ** PPA=Positive Percent Agreement; NPA=Negative Percent Agreement

Table 20: Percent Agreement between the VERSANT® Zika RNA 1.0 Assay (kPCR) and the Lanciotti et al. Assay Results for Clinical Serum Samples.

Agreement Type**	**Test Population**	**# of Agreements** (correctly detected # of specimens/total # of tested specimens)	**Percent Agreement**	**95% Confidence Interval (CI)**
PPA	Endemic	57/66	86.4%	(76.1%, 92.7%)
	Low Prevalence (U.S.)	N/A	N/A	N/A
	Contrived Samples*	1x LoD: 15/15 2x LoD: 5/5 3x LoD: 5/5 5x LoD: 5/5	100%	(88.6%, 100%)
PPA	**ALL**	**87/96**	**90.6%**	**(83.1%, 95.0%)**
NPA	Endemic	24/32	75.0%	(57.9%, 86.7%)
	Low Prevalence (U.S.)	22/22	100%	(85.1%, 100%)
	Contrived Samples*	N/A	N/A	N/A
NPA	**ALL**	**46/54**	**85.2%**	**(73.4%, 92.3%)**

* Samples were from low prevalence populations collected in 2009 and were not tested prior to spiking. ** PPA=Positive Percent Agreement; NPA=Negative Percent Agreement

Table 21: Percent Agreement between the VERSANT® Zika RNA 1.0 Assay (kPCR) and the Lanciotti et al. Assay Results for Clinical Urine Samples.

Agreement Type**	Test Population	# of Agreements (correctly detected # of specimens/total # of tested specimens)	Percent Agreement	95% Confidence Interval (CI)
PPA	Endemic	39/45	86.7%	(73.8%, 93.7%)
	Low Prevalence (U.S.)	N/A	N/A	N/A
	Contrived Samples*	N/A	N/A	N/A
PPA	**ALL**	**39/45**	**86.7%**	**(73.8%, 93.7%)**
NPA	Endemic	21/34	61.8%	(45.0%, 76.1%)
	Low Prevalence (U.S.)	50/50	100%	(92.9%, 100%)
	Contrived Samples*	N/A	N/A	N/A
NPA	**ALL**	**71/84**	**84.5%**	**(75.3%, 90.7%)**

* Samples were from low prevalence populations collected in 2009 and were **n**ot tested prior to spiking. ** PPA=Positive Percent Agreement; NPA=Negative Percent Agreement

Analysis of Matched Plasma/Serum and Urine Sample Sets

A total of 78 matched plasma/serum and urine sample sets were analyzed in the VERSANT® Zika RNA 1.0 Assay (kPCR) and the Lanciotti et al. Assay.

The patient infection status (PIS) from the Lanciotti et al. Assay was compared with the PIS from the VERSANT® Zika RNA 1.0 Assay (kPCR) (Tables 22 and 23). PIS positivity is defined as plasma/serum positive and/or urine positive. PIS negativity is defined as negative in both plasma/serum and the corresponding matched urine sample. From a total of 78 matched sets analyzed, 68/71 matched sets agreed as PIS positive between the two assays (positive agreement of 95.8%). From the remaining 7 matched sets, 3/7 agreed as PIS negative between the two assays (negative agreement of 42.9%).

Table 22: Comparison of the VERSANT® Zika RNA 1.0 Assay (kPCR) and Lanciotti et al. Assay PIS Results for Matched Sample Sets.

		Lanciotti et al. Assay	
		Positive	Negative
VERSANT® Zika RNA 1.0 Assay (kPCR)	Positive	68	4**
	Negative	3*	3

* The 3 “false negative” matched sample sets had one matched sample with a Ct value > Ct 36 with the comparator assay. This Ct range suggests that the analyte levels in these samples were close to or below the LoD of the comparator assay. Repeat testing of the 3 “false negative” matched sample sets was performed with 3 replicates each for the VERSANT assay. Two out of three “false negative” samples had at least one positive replicate with the VERSANT test.

** The 4 “false positive” matched sample sets had at least one matched sample with a Ct value > Ct 34 with the VERSANT assay. This Ct range suggests that the analyte levels in these samples were close to or below the LoD of the VERSANT assay. Repeat testing of the 4 “false positive” matched sample sets was performed with 3 replicates each for the comparator assay. Two out of four “false positive” samples had at least one positive replicate with the comparator test.

Table 23: Percent Agreement in VERSANT® Zika RNA 1.0 Assay (kPCR) and Lanciotti et al. Assay Results for PIS in Matched Sample Set Results.

Agreement Type	Number of Agreements	Percent Agreement	95% Confidence Interval (CI)
Positive Percent Agreement	68/71	95.8	88.3, 98.6
Negative Percent Agreement	3/7	42.9	15.8, 75.0

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all local and regulatory requirements.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

Contact Number: +1-(877)-229-3711
Website: http://www.siemens.com/diagnostics

Understanding the Symbols

The following symbols may appear on the labeling and packaging:

Symbol	Definition
EUA	For Use Under an Emergency Authorization (EUA) Only
RxOnly	Prescription Use Only
REF	Catalog Number
SMN	Catalog Number
	Consult instructions for use
	Manufacturer
	Use By
	Temperature limitation
	Contains sufficient for (n) tests
LOT	Batch Code
	Keep away from sunlight
2008-11-17	Date format (year-month-day)
2008-11	Date format (year-month)
	Store upright

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Legal Manufacturer
Siemens Healthcare Diagnostics Inc.
511 Benedict Avenue
Tarrytown, NY 10591-5097 USA

Siemens Healthcare Headquarters
Siemens Healthcare GmbH
Henkestr. 127
91052 Erlangen
Germany
Phone: +49 9131 84-0
siemens.com/healthcare