Abbott RealTi*me* ZIKA

en

REF 09N27-090

51-608295/R3

REF 09N27-090

51-608295/R3

NOTE: Changes Highlighted

Rx ONLY

IVD

For use under an Emergency Use Authorization (EUA) Only

Instructions for Use

INTRODUCTION

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NAME

Abbott RealTi*m*e ZIKA

INTENDED USE

The Abbott RealTi*m*e ZIKA assay is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the qualitative detection of RNA from the Zika virus in serum, EDTA plasma, whole blood (EDTA), and urine (whole blood and urine collected alongside a patient-matched serum or plasma specimen) from individuals meeting CDC Zika virus clinical criteria (e.g., clinical signs and symptoms associated with Zika virus infection) and/or CDC Zika virus epidemiological criteria (e.g., history of residence in or travel to a geographic region with active Zika virus transmission at the time of travel, or other 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.

Specimens are tested using the *m*2000*sp* for automated specimen processing and the *m*2000*rt* for amplification and detection.

Results are for the identification of Zika virus RNA. Zika virus RNA is generally detectable in serum, plasma, whole blood, and urine during the acute phase of infection and up to 14 days following onset of symptoms, if present. Positive results are indicative of current infection. Laboratories are required to report all positive results to the appropriate public health authorities.

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

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

For Prescription Use Only.

SUMMARY AND EXPLANATION OF THE TEST

Zika virus infection (Zika fever) is a disease caused by the Zika virus, which is spread to people primarily through the bite of an infected Aedes species mosquito.[1] Zika virus was first isolated in 1947 from the Zika Forest of Uganda.[2] Zika virus is related to dengue, yellow fever, Japanese encephalitis, and West Nile viruses. The illness is usually mild and causes symptoms of fever, rash, joint pain and conjunctivitis (red eyes).[3] The symptoms of Zika virus infection last for several days to a week after being bitten by an infected mosquito.[3] Infected individuals often do not experience symptoms severe enough to seek medical attention, and morbidity is rare. Because the symptoms of Zika virus infection are similar to those of many other infectious diseases, many cases may not have been recognized and hence reported.

In 1952, the first human cases of Zika infection were detected and since then, outbreaks of Zika virus have been reported in tropical Africa, Southeast Asia, and the Pacific Islands.[1] In May 2015, the Pan American Health Organization (PAHO) issued an alert regarding the first confirmed Zika virus infection in Brazil.[4,5] On February 1, 2016, the World Health Organization (WHO) declared Zika virus a Public Health Emergency of International Concern (PHEIC). As of 2016, the infection cannot be prevented by medications or vaccines. Zika virus may spread from a pregnant woman to the baby and can cause microcephaly, a serious birth defect.[6] Zika infections in adults have been reported in association with Guillain-Barre syndrome.[7]

The CDC recommends the use of a nucleic acid based assay to provide faster and accurate detection of Zika virus.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Abbott RealTi*m*e ZIKA assay consists of 2 reagent kits:

- Abbott RealTi*m*e ZIKA Amplification Reagent Kit
- Abbott RealTi*m*e ZIKA Control Kit

The Abbott RealTi*m*e ZIKA assay uses RT-PCR to generate amplified product from the RNA genome of the Zika virus in human serum, plasma (EDTA), whole blood (EDTA), or urine (whole blood and urine collected alongside a patient-matched serum or plasma specimen). The Abbott RealTi*m*e ZIKA assay is a dual target assay. An RNA sequence that is unrelated to the Zika virus target sequence is introduced into each specimen and control at the beginning of sample preparation. This unrelated RNA sequence is simultaneously processed and amplified by RT-PCR, and serves as an internal control (IC) to demonstrate that the process has completed correctly for each sample and control. The Abbott RealTi*m*e ZIKA assay detects the Zika virus and IC target sequences through the use of target-specific fluorescent-labeled oligonucleotide probes. The probes do not generate a signal unless they are specifically bound to the amplified product. The two Zika-specific probes are labeled with the same fluorophore and the IC-specific probe is labeled with a different fluorophore, thus allowing for simultaneous detection of both Zika and IC amplified products in the same reaction well. The Abbott RealTi*m*e ZIKA assay is performed on the Abbott *m*2000 System consisting of a sample preparation unit, the Abbott *m*2000*sp*, and an amplification and detection unit, the Abbott *m*2000*rt*. Application parameters specific to Abbott RealTi*m*e ZIKA are contained on an assay-specific application file, stored on a CD-ROM and loaded onto the Abbott *m*2000*sp* and Abbott *m*2000*rt* instruments.

Sample Preparation

The purpose of sample preparation is to extract and concentrate the target RNA molecules to make the target accessible for amplification, and to remove potential inhibitors of amplification from the extract.

The Abbott *m*2000*sp* provides automated sample preparation using a magnetic microparticle-based protocol and reagents (Abbott *m*Sample Preparation System) to process 0.5 mL samples (serum, plasma [EDTA], whole blood [EDTA], or urine).

During the sample preparation protocol, Zika virions are disrupted by guanidine isothiocyanate, nucleic acids are captured on the magnetic microparticles, and inhibitors and unbound sample components are removed by washing steps. The bound nucleic acids are eluted off the microparticles with buffer and transferred to a 96 deep-well plate. The nucleic acids are then ready for amplification. The Internal Control (IC) is introduced into each specimen at the beginning of the sample preparation process to demonstrate that the process was completed correctly for each specimen and control.

A positive control and a negative control are processed from the start of sample preparation for each test order to evaluate run validity.

Reagent Preparation and Reaction Plate Assembly

The Abbott *m*2000*sp* combines the Abbott RealTi*m*e ZIKA amplification reagent components (ZIKA Amplification Reagent, Thermostable rTth Polymerase Enzyme, and Activation Reagent). The Abbott *m*2000*sp* dispenses the resulting master mix to the Abbott 96-Well Optical Reaction Plate along with aliquots of the nucleic acid samples prepared by the Abbott *m*2000*sp*. Each processed sample is added to one reaction well containing master mix. The plate is ready, after manual application of the optical seal, for transfer to the Abbott *m*2000*rt*.

Amplification

During the amplification reaction on the Abbott *m*2000*rt*, the target RNA is converted to cDNA by the reverse transcriptase activity of the thermostable rTth DNA polymerase. First, the Zika and IC reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded cDNA:RNA product, a second primer anneals to the cDNA strand and is extended by the DNA polymerase activity of the rTth enzyme to create a double-stranded DNA product.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences. Amplification of both targets (Zika virus and IC) takes place simultaneously in the same reaction.

The target sequences for the Abbott RealTi*m*e ZIKA assay are in the prM precursor membrane protein region and the NS3 helicase region of the Zika virus genome. The primers are designed to hybridize to the prM precursor membrane protein region and NS3 helicase region with the fewest possible mismatches among various strains.

The IC target sequence is derived from the hydroxypyruvate reductase gene from the pumpkin plant, Cucurbita pepo, and is delivered in an Armored RNA® particle that has been diluted in negative human plasma

Detection

During each round of PCR amplification, the fluorescent probes anneal to their respective amplification target, if present. All three probes in the Abbott RealTi*m*e ZIKA assay are short linear probes. The 5′ end of each Zika-specific probe and the IC-specific probe is labeled with a fluorescent moiety while the 3′ end is labeled with a quenching moiety. In the absence of the targets, fluorescence is quenched. In the presence of the target sequences, probe hybridization to complementary sequences separates the fluorophore and the quencher and allows fluorescent emission and detection.

The Zika-specific probes and the IC-specific probe are labeled with different fluorophores, thus allowing for simultaneous detection by the Abbott *m*2000*rt* of both Zika and IC amplified products in the same reaction well.

PREVENTION OF NUCLEIC ACID CONTAMINATION

The possibility of nucleic acid contamination is minimized because:

- Reverse transcription, PCR amplification, and oligonucleotide hybridization occur in a sealed Abbott 96-Well Optical Reaction Plate.
- Detection is carried out automatically without the need to open the Abbott 96-Well Optical Reaction Plate.
- Pipettes with aerosol barrier tips or disposable transfer pipettes are used for all pipetting. The disposable pipettes or pipette tips are discarded after use.
- Separate, dedicated areas are used to perform the Abbott RealTi*m*e ZIKA assay. Refer to the **SPECIAL PRECAUTIONS** section of this package insert.

REAGENTS

Abbott RealTi*m*e ZIKA Amplification Reagent Kit (List No. 09N27-090)

1. Abbott RealTi*m*e ZIKA Internal Control (List No. 9N27Y0001)
   (4 vials, 1.2 mL per vial)
   - < 0.01% noninfectious Armored RNA with internal control sequences in negative human plasma. Negative human plasma tested and found to be nonreactive by FDA-licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA-licensed PCR methods for HIV-1 RNA and HCV RNA. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. Abbott RealTi*m*e ZIKA Amplification Reagent Pack (List No. 09N27)
   (4 packs, 24 tests/pack)
   - 1 bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/µL) in buffered solution.
   - 1 bottle (1.0 mL) ZIKA Amplification Reagent.
     < 0.1% synthetic oligonucleotides (6 primers and 3 probes), and < 0.3% dNTPs in a buffered solution with a reference dye.
     Preservative: 0.10% ProClin 300 and 0.15% ProClin 950.
   - 1 bottle (0.400 mL) Activation Reagent. 30 mM manganese chloride solution. Preservatives: 0.10% ProClin 300 and 0.15% ProClin 950.

Abbott RealTi*m*e ZIKA Control Kit (List No. 09N27-080)

1. Abbott RealTi*m*e ZIKA Negative Control (List No. 9N27Z0001)
   (8 vials, 1.3 mL per vial) Negative human plasma tested and found to be nonreactive by FDA-licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA-licensed PCR methods for HIV-1 RNA and HCV RNA. Preservative: 0.10% ProClin 300 and 0.15% ProClin 950.
2. Abbott RealTi*m*e ZIKA Positive Control (List No. 9N27W0001)
   (8 vials, 1.3 mL per vial) Inactivated Zika virus (strain PRVABC59) in purified protein matrix. Human serum tested and found to be nonreactive by FDA-licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA-licensed PCR methods for HIV-1 RNA and HCV RNA. Preservative: 0.09% Sodium azide.

WARNINGS AND PRECAUTIONS

For Use Under An Emergency Use Authorization Only.

This assay is only for use under the FDA Emergency Use Authorization.

Safety Precautions

Refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Hazard Section, for instructions on safety precautions.

Important information regarding the safe handling, transport and disposal of this product is contained in the Safety Data Sheet.

⚠ **CAUTION**: This preparation contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive by FDA-licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA-licensed PCR methods for HIV-1 RNA and HCV RNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using laboratory safety procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[8] OSHA Standards on Bloodborne Pathogens,[9] CLSI Document M29-A4,[10] and other appropriate biosafety practices.[11] Therefore all human sourced materials should be considered infectious.

These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[8]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[11]

Components of the Abbott RealTi*m*e ZIKA Internal Control, Oligonucleotide Reagent, and Activation Reagent contain the following components:
2-Methyl-4-isothiazol-3-one:

- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one (EC no. 247-500-7) and 2-methyl-2H-isothiazol-3-one (EC no. 220-239-6)(3:1)
- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one (EC no. 247-500-7) and 2-methyl-4-isothiazolin-3-one (EC no. 220-239-6)(3:1)

The following warnings apply:

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/eye protection.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash before reuse.
P501	Dispose of contents/container in accordance with local regulations.

SPECIAL PRECAUTIONS

Handling Precautions for Specimens

The Abbott RealTi*m*e ZIKA assay is only for use with serum, plasma (EDTA), whole blood (EDTA), and urine specimens that have been handled and stored as described in the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section.

During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of ribonucleases (RNases) into samples during and after the extraction procedure.

Proper aseptic technique should always be used when working with RNA.

Amplification technologies such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the reagents used become contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practices.

Work Areas

The *m*2000*sp* and the *m*2000*rt* instruments may be operated in the same location. The use of 2 dedicated areas (Sample Preparation Area and Amplification Area) within the laboratory is recommended when performing the Abbott RealTi*m*e ZIKA assay.

The **Sample Preparation Area** is dedicated to processing samples (specimens and Abbott RealTi*m*e ZIKA Controls) and to adding processed samples and controls to the 96-Well Optical Reaction Plate. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortexers used in the Sample Preparation Area must remain in this area and not be moved to the Amplification Area. Do not bring amplification product into the Sample Preparation Area.

The **Amplification Area** is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to the Sample Preparation Area.

- Components contained within a kit are intended to be used together. Do not mix components from different kit lots. For example, do not use the negative control from control kit lot X with the positive controls from control kit lot Y.
- Do not use kits or reagents after the expiration dates shown on kit labels.
- Work area and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, negative control, positive controls, or specimens. Refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals for instrument cleaning procedures.
- If the Abbott *m*2000*sp* instrument run is aborted, dispose of all commodities and reagents according to the Abbott *m*2000*sp* Operations Manual.
- If the Abbott *m*2000*sp* master mix addition protocol is aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*sp* Operations Manual, Hazards section, along with the gloves used to handle the plate.
- If the Abbott *m*2000*rt* instrument run is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.
- Decontaminate and dispose of all potentially biohazardous materials in accordance with local, state, and federal regulations.[11] All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.

NOTE: Autoclaving the sealed Reaction Plate will not degrade the amplified product and may contribute to the release of the amplified product by opening the sealed plate. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained.

Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used for all manual pipetting. The pipette tips must be used only 1 time. Clean and disinfect spills of specimens and reagents as stated in the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals.

Contamination and Inhibition

The following precautions should be observed to minimize the risks of RNase contamination, cross-contamination between samples, and inhibition:

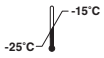
- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves.
- Change gloves after having contact with potential contaminants (such as specimens, eluates, and/or amplified product).
- To reduce the risk of nucleic acid contamination due to aerosols formed during pipetting, pipettes with aerosol barrier tips must be used for all pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Change aerosol barrier pipette tips between ALL manual liquid transfers.
- The Abbott *m*Sample Preparation System (4 × 24 Preps) reagents are single use only. Use new reagent troughs or vessels, reaction vessels, and newly opened reagents for every new Abbott RealTi*m*e ZIKA assay run. At the end of each run, discard all remaining reagents from the worktable as stated in the Abbott *m*2000*sp* Operations Manual and the Abbott *m*Sample Preparation System (4 × 24 Preps) product information sheet.

STORAGE INSTRUCTIONS

Abbott RealTi*m*e ZIKA Amplification Reagent Kit (List No. 09N27-090)

- Abbott RealTi*m*e ZIKA Amplification Reagent Packs and Internal Control (IC) vials must be stored at – 25 to – 15°C when not in use. Care must be taken to separate the Abbott RealTi*m*e ZIKA Amplification Reagent Pack that is in use from direct contact with samples and controls.
- Internal Control (IC) may be used a second time within 14 days of being thawed, if stored capped at – 25 to – 15°C.

Abbott RealTi*m*e ZIKA Control Kit (List No. 09N27-080)



- The Abbott RealTi*m*e ZIKA Negative and Positive Controls must be stored at – 25 to – 15°C.

SHIPPING CONDITIONS

- Abbott RealTi*m*e ZIKA Amplification Reagent Kit: Ship on dry ice.
- Abbott RealTi*m*e ZIKA Control Kit: Ship on dry ice.

If you receive reagents that are in a condition contrary to label recommendation, or that are damaged, contact your Abbott Representative.

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

When a positive or negative control value is out of the expected range, it may indicate deterioration of the reagents. Associated test results are invalid and samples must be retested.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Specimen Collection and Storage

Human serum, plasma (EDTA), whole blood (EDTA), and urine specimens may be used with the Abbott RealTi*m*e ZIKA assay. Follow the manufacturer's instructions for processing serum, plasma, and whole blood collection tubes.

Serum, plasma, and urine specimens may be stored at 15 to 30°C for up to 24 hours or at 2 to 8°C for up to 5 days. If longer storage is required, serum, plasma, and urine specimens must be kept at –70°C or colder. If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if specimens are not being tested immediately, they can be stored at 2 to 8°C for up to 6 hours. Multiple freeze-thaw cycles should be avoided. Serum, plasma, and urine specimens must be shipped frozen on dry ice to the test site.

Whole blood specimens may be stored at 2 to 8°C for up to 5 days. If longer storage is required, whole blood specimens must be kept at –70°C or colder. If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if whole blood specimens are not being tested immediately, they can be stored at 2 to 8°C for up to 6 hours. Multiple freeze-thaw cycles should be avoided. Whole blood specimens must be shipped on cold packs or frozen on dry ice to the test site.

Specimen Transport

Ship specimens according to the recommended storage temperature and time listed in the **Specimen Collection and Storage** section above. For domestic and international shipments, specimens should be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical, diagnostic, or biological specimens.

INSTRUMENT PROCEDURE

The Abbott RealTi*m*e ZIKA EUA application files must be installed on the Abbott *m*2000*sp* and Abbott *m*2000*rt* instruments from the Abbott RealTi*m*e ZIKA Application CD-ROM (List No. 09N27-002 or higher; EUA version) prior to performing the assay. The application file, *m*2000 ZIKA EUA, is required for serum, plasma and urine specimens and the application file, *m*2000 ZIKA WB EUA, is required for whole blood specimens. For a detailed description of how to perform an Abbott *m*2000*sp* instrument and Abbott *m*2000*rt* instrument protocol, refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions sections.

ABBOTT REALTI*M*E ZIKA ASSAY PROCEDURE

This package insert contains instructions for running the Abbott RealTi*m*e ZIKA assay.

Materials Provided

- Abbott RealTi*m*e ZIKA Amplification Reagent Kit (List No. 09N27-090)

Materials Required But Not Provided

- Abbott RealTi*m*e ZIKA Control Kit (List No. 09N27-080)
- Abbott *m*Sample Preparation System (4 × 24 Preps) (List No. 04J70-24)
- Abbott RealTi*m*e ZIKA Application CD-ROM (List No. 09N27-002 or higher; EUA version)

Sample Preparation Area

- Abbott *m*2000*sp* Instrument (*m*2000*sp* software version 6.0 or higher)
- Abbott *m*2000*sp* Operations Manual (List No. 09K20-008 or higher)
- Abbott *m*Sample Preparation System (4 × 24 Preps) (List No. 04J70-24)
- Abbott RealTi*m*e ZIKA Application CD-ROM (List No. 09N27-002 or higher; EUA version)
- 5 mL Reaction Vessels (12 x 75 mm) (List No. 4J71-20)
- Master Mix Tubes (List No. 04J71-80)
- Amplification Reagent Pack Caps (List No. 3N20-01) (Optional)
- Transport Tubes (List No. 04J71-90)
- 200 mL Reagent Vessels (List No. 4J71-60)
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70)
- Abbott 96-Deep-Well Plate (List No. 04J71-30)
- Abbott Splash-Free Support Base (List No. 09K31-01)
- 200 µL and 1000 µL Disposable Tips for Abbott *m*2000*sp* (List No. 4J71-17 and 4J71-10)
- Abbott Optical Adhesive Cover (List No. 04J71-75)
- Abbott Adhesive Cover Applicator (List No. 9K32-01)
- Waste bags (List No. 3N17-01)
- Biohazard bags (List No. 4J71-45)
- Sample racks
- Vortex mixer
- Calibrated precision pipettes capable of delivering 20 µL to 1000 µL
- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes.

Other Materials

- Biological safety cabinet approved for working with infectious materials
- Sealable plastic bags
- RNase-free water (Eppendorf or equivalent)†
- 1.7 mL molecular biology grade microcentrifuge tubes (Dot Scientific, Inc. or equivalent)†
- Cotton Tip Applicators (Puritan or equivalent)†

† **Note: These 3 items are used in the procedure for Monitoring the Laboratory for the Presence of Contamination**. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert.

Amplification Area

- Abbott *m*2000*rt* Instrument
- Abbott *m*2000*rt* software version 6.0 or higher
- Abbott *m*2000*rt* Operations Manual (List No. 09K25-007 or higher)
- Abbott RealTi*m*e ZIKA Application CD-ROM (List No. 09N27-002 or higher; EUA version)
- Abbott *m*2000*rt* Optical Calibration Kit (List No. 4J71-93)

Other Materials

- Sealable plastic bags

Procedural Precautions

- Read the instructions in this package insert carefully before processing samples.
- Internal control (IC) may be used up to 2 times, as described in the storage instructions above. The Abbott RealTi*m*e ZIKA Negative Control and Positive Control vials are intended for single-use only and should be discarded after use.
- Use aerosol barrier pipette tips or disposable pipettes only one time when pipetting specimens, IC, or amplification reagents. To prevent contamination to the pipette barrel while pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Monitoring procedures for the presence of amplification product can be found in the **QUALITY CONTROL PROCEDURES** section in this package insert.
- To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.
- The Abbott RealTi*m*e ZIKA Controls must be prepared in conjunction with the specimens to be tested. The use of the Abbott RealTi*m*e ZIKA Controls is integral to the performance of the Abbott RealTi*m*e ZIKA assay. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details.

ASSAY PROTOCOL

For a detailed description of how to perform an Abbott *m*2000*sp* instrument and Abbott *m*2000*rt* instrument protocol, refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions sections.

Laboratory personnel must be trained to operate the Abbott *m*2000*sp* and Abbott *m*2000*rt* instruments. The operator must have a thorough knowledge of the applications run on the instruments and must follow good laboratory practices.

Sample Preparation Area

1. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C.
   - Once thawed, assay controls and IC can be stored at 2 to 8°C for up to 24 hours before use.
   - Vortex each assay control 3 times for 2 to 3 seconds before use. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial.
2. Select amplification reagent packs to be used in the run. Refer to the Abbott *m*2000*sp* Operations Manual (List No. 9K20 version 6 or higher), Operating Instructions section, for instructions pertaining to amplification reagent pack inventory management. All amplification reagent packs used in runs of greater than 24 reactions must have the same lot number. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C until required for the amplification master mix procedure. Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours if not used immediately.

The following table shows the number of sample preparation reagents and internal control vials needed based on the number of reactions.

Sample Preparation Reagents and Internal Control Requirements				
Reagent	**1 to 24 Reactions**	**25 to 48 Reactions**	**49 to 72 Reactions[a]**	**73 to 96 Reactions[a]**
*m*Microparticles	1 bottle	2 bottles	2 bottles	2 bottles
*m*Lysis	1 bottle	2 bottles	3 bottles	4 bottles
*m*Wash 1	1 bottle	2 bottles	3 bottles	4 bottles
*m*Wash 2	1 bottle	2 bottles	3 bottles	4 bottles
*m*Elution Buffer	1 bottle	2 bottles	3 bottles	4 bottles
Internal Control	1 vial	1 vial	2 vial	2 vials

[a] Not applicable for processing whole blood specimens.

Abbott *m*2000*sp* Procedure

3. Gently invert the Abbott *m*Sample Preparation bottles to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved.
4. Vortex each IC 3 times for 2 to 3 seconds before use.
5. Use a calibrated precision **PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 500 µL of IC to each bottle of *m*Lysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming.

6. **A total of 96 serum, plasma, and urine samples or 48 whole blood samples can be processed in each run**. A negative control and a positive control are included in each run, therefore allowing a maximum of 94 serum, plasma, and urine or 46 whole blood specimens to be processed per run.
   - The Abbott RealTi*m*e ZIKA assay minimum sample volume and associated rack requirements on the Abbott *m*2000*sp* are:

Rack	Tube Diameter[a]	0.5 mL
13 mm	11.5 - 14.0 mm	0.7 - 1.2 mL
16 mm	14.5 - 16.0 mm	0.8 - 1.4 mL

[a] Refers to sample tube outer diameter. Minimum sample volume varies with tube geometry and size. Refer to the Abbott *m*2000*sp* Operations Manual and **QUICK REFERENCE GUIDE FOR SAMPLE TUBE SIZES AND VOLUMES** for recommended sample input volume.

   - If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours if not processed immediately.

   NOTE: For every stored specimen, the following actions must be done in the order described: vortex the specimen first and follow with centrifugation of serum, plasma, and urine specimens. If these actions are not performed in this order, then invalid results may occur.

   - Vortex each specimen 3 times for 2 to 3 seconds.

   NOTE: Do not centrifuge whole blood specimens. Vortex only.

   - Centrifuge serum, plasma, and urine specimens only at 2000*g* for 5 minutes before loading onto the Abbott *m*2000*sp* worktable. Aliquot each specimen into clean tubes or vials if necessary. Refer to the Abbott *m*2000*sp* Operations Manual for tube sizes. Avoid touching the inside of the cap when opening tubes.

   Refer to the Abbott *m*2000*sp* Operations Manual for tube sizes. Avoid touching the inside of the cap when opening tubes.
7. Place the positive and negative controls, if applicable, and the patient specimens into the Abbott *m*2000*sp* sample rack. If used, bar codes on tube labels must face right for scanning.
8. Place the 5 mL Reaction Vessels into the Abbott *m*2000*sp* 1 mL subsystem carrier.
9. Load the Abbott *m*Sample Preparation System reagents and the Abbott 96 Deep-Well Plate on the Abbott *m*2000*sp* worktable as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
10. From the Protocol screen, select the appropriate application file and initiate the sample extraction protocol as described in the Abbott *m*2000*sp* Operations Manual, Operating Instruction section.
    - **The application file *m*2000 ZIKA EUA is required for serum, plasma and urine specimens.**
    - **The application file *m*2000 ZIKA WB EUA is required for whole blood specimens.**
    - **The Abbott *m*2000*sp* Master Mix Addition protocol (step 12) must be initiated within 1 hour after completion of Sample Preparation.**

    NOTE: Change gloves before handling the amplification reagents.
11. Load the amplification reagents and the master mix tube (if needed) on the Abbott *m*2000*sp* worktable after sample preparation is completed. The following table shows the number of amplification reagent packs needed based on the number of reactions.
    If only 1 amplification reagent pack is being used, no master mix tube is required.

Amplification Reagent Pack Requirements			
1 to 24 Reactions	**25 to 48 Reactions**	**49 to 72 Reactions[a]**	**73 to 96 Reactions[a]**
1 pack	2 packs	3 packs	4 packs

[a] Not applicable for processing whole blood specimens.

    - All amplification reagent packs used in runs of greater than 24 reactions must have the same lot number.
    - Ensure that the contents of amplification reagent packs are at the bottom of the vials prior to opening the amplification reagents by tapping the vials in an upright position on the bench 5 to 10 times.
    - **Ensure that amplification reagent packs are firmly seated on the instrument.**
12. Select the appropriate deep-well plate that matches the corresponding sample preparation extraction. Initiate the Abbott *m*2000*sp* Master Mix Addition protocol. Follow the instructions as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.

    NOTE: The operator should not manually fill any empty/unfilled wells in the Abbott 96-Well Optical Reaction Plate.

    - After sample extraction is complete, the Abbott *m*2000*sp* automatically fills any empty wells in the Abbott 96-Well Optical Reaction Plate when there are greater than 48 samples processed within a run. Plate fill is not performed for runs containing 48 samples or fewer.
    - If prompted by the instrument, Reagent Carrier 2 should remain in place, minimally containing the reagent vessel for *m*Elution Buffer (Reagent Carrier 2, location 6). If this reagent vessel has been unloaded, place a new reagent vessel with the *m*Elution Buffer label into Reagent Carrier 2, location 6. System fluid will be added to the reagent vessel and used to fill empty wells. Once this process is complete, the system will continue with the master mix addition.

      NOTE: System instructions for use of the automated plate-filling feature are found in the Abbott *m*2000*sp* Operations Manual (List No. 9K20 version 6 or higher), section 5, Operating Instructions, Sample Extraction—Closed Mode.
    - **The Abbott *m*2000*rt* protocol (step 16) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 12).**

      NOTE: If the run is aborted for any reason subsequent to step 12, a new 96-well PCR plate must be used if the Abbott *m*2000*sp* Master Mix Addition Protocol (step 12) will be repeated.

Amplification Area

13. Switch on and initialize the Abbott *m*2000*rt* instrument in the Amplification Area.

    NOTE: The Abbott *m*2000*rt* requires 15 minutes to warm-up.

    NOTE: Remove gloves before returning to the sample preparation area.
14. Seal the Abbott 96-Well Optical Reaction Plate according to the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
15. Place the sealed optical reaction plate into the Abbott Splash-Free Support Base for transfer to the Abbott *m*2000*rt* instrument. Export the completed PCR plate results to a CD (or directly to a mapped Abbott *m*2000*rt* via a network connection).

Abbott *m*2000*rt* Procedures

For a detailed description of how to perform the Abbott *m*2000*rt* ZIKA protocol, refer to the Operating Instructions section in the Abbott *m*2000*rt* Operations Manual.

16. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument. Initiate the Abbott RealTi*me* ZIKA protocol (***m*2000 ZIKA EUA or *m*2000 ZIKA WB EUA)**, as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

    NOTE: Test order transfer through the use of CD-ROM or network connection with export and import features of the *m*2000*sp* and *m*2000*rt* software is recommended. If creating the Abbott *m*2000*rt* test order manually, enter sample IDs in the corresponding PCR tray locations according to the "Wells for Selected Plate" grid, found on the detail screen of the "PCR Plate Results" on the Abbott *m*2000*sp*. See Section 5 of the Abbott *m*2000*sp* Operations Manual.

POST PROCESSING PROCEDURES

1. Remove the Abbott 96 Deep-Well Plate from the worktable and dispose of according to the Abbott *m*2000*sp* Operations Manual.
2. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.
3. Clean the Abbott Splash-Free Support Base before next use, according to the Abbott *m*2000*rt* Operations Manual.

QUALITY CONTROL PROCEDURES

Abbott *m*2000*rt* Optical Calibration

Refer to the Calibration Procedures section in the Abbott *m*2000*rt* Operations Manual for a detailed description of when and how to perform an Abbott *m*2000*rt* Optical Calibration.

Optical calibration of the Abbott *m*2000*rt* instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTi*me* ZIKA assay.

The following Abbott *m*2000*rt* Optical Calibration Plates are used to calibrate the Abbott *m*2000*rt* instrument for the Abbott RealTi*me* ZIKA assay:

- FAM™ Plate (Carboxyfluorescein)
- ROX™ Plate (Carboxy-X-rhodamine)
- VIC® Plate (Proprietary dye)

Detection of Inhibition

A defined, consistent quantity of IC nucleic acid is introduced into each specimen and control at the beginning of sample preparation and measured on the Abbott *m*2000*rt* to demonstrate proper specimen processing and assay validity. The IC is comprised of a RNA sequence unrelated to the Zika virus target sequences.

An IC CN validity range is defined within the Abbott RealTi*me* ZIKA Assay Application File and must be met by all specimens and controls in order to generate a valid result.

An error is displayed when a specimen or control fails to meet the IC specification. Specimens whose IC CN value exceeds the established range must be retested starting with sample preparation. Whole blood specimens that generate invalid results may be diluted 2 fold with negative plasma and retested. If the retest also returns an invalid result, testing of the patient-matched serum or EDTA plasma specimen should be considered. Refer to RESULTS section of this package insert and the Abbott *m*2000*rt* System Operations Manual for a list of error codes and flags.

Negative and Positive Controls

A negative control and a positive control are included in each test order to evaluate run validity in order to generate a valid result.

The Abbott *m*2000*rt* instrument automatically reports the control results and interpretations on the Abbott *m*2000*rt* workstation. An error control flag is displayed when a control result is out of range. Refer to the Abbott *m*2000*rt* Operations Manual for an explanation of the corrective actions for the error control flag. If negative or positive controls are out of range, all of the specimens and controls from that run must be reprocessed, beginning with sample preparation.

The presence of the Zika virus must not be detected in the negative control. Zika virus detected in the negative control is indicative of contamination by other samples or by amplified product introduced during sample preparation or during preparation of the Abbott 96-Well Optical Reaction Plate. To avoid contamination, clean the Abbott *m*2000*sp* instrument and the Abbott *m*2000*rt* instrument and repeat sample processing for controls and specimens following the **Procedural Precautions**. If negative controls are persistently reactive, contact your Abbott representative.

Monitoring the Laboratory for the Presence of Contamination

It is recommended that this test be done at least once a month to monitor laboratory surfaces and equipment for contamination by amplification product. It is very important to test all areas that may have been exposed to processed specimens, controls, and/or amplification product. This includes routinely handled objects such as pipettes, the Abbott *m*2000*sp* and Abbott *m*2000*rt* function keys, laboratory bench surfaces, microcentrifuges, and centrifuge adaptors.

1. Add 0.8 mL RNase-free water to a 1.7 mL molecular biology grade microcentrifuge tube.
2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the RNase-free water from the microcentrifuge tube.
3. Using the saturated cotton tip of the applicator, wipe the area to be monitored using a sweeping motion. Place the applicator into the microcentrifuge tube.
4. Swirl the cotton tip in RNase-free water 10 times, and then press the applicator along the inside of the tube so that the liquid drains back into the solution at the bottom of the microcentrifuge tube. Discard the applicator.
5. Pipette 0.5 mL of *m*Wash 1 buffer to a clean tube using the pipette dedicated for Internal Control use.
6. Add 20 µL of the *m*Wash 1 buffer to each microcentrifuge tube.
7. Cap the microcentrifuge tube.
8. Test this sample according to the assay procedure section of this study brochure.
    - Transfer liquid from the microcentrifuge tube to a 5 mL Reaction Vessel.
    - Bring the volume to 1.5 mL with RNase-free water.
9. The presence of contamination is indicated by the detection of Zika nucleic acid in the swab samples.
10. If Zika nucleic acid is detected on equipment, follow the cleaning and decontaminating guidelines given in that equipment's operations manual. If Zika nucleic acid is detected on surfaces, clean the contaminated areas with 1.0% (v/v) sodium hypochlorite solution, followed by 70% ethanol or water.

    NOTE: Chlorine solutions may pit equipment and metal. Use sufficient amounts or repeated applications of 70% ethanol or water until chlorine residue is no longer visible.

11. Repeat testing of the contaminated area by following steps 1 through 10.

INTERPRETATION OF RESULTS

The Abbott *m*2000*rt* instrument automatically reports the results and interpretations on the Abbott *m*2000*rt* workstation. An error is displayed when a result is invalid. Assay results and interpretations will look similar to the following examples:

Location	Sample ID	Sample Type	Assay	Result	Interpret	Flags	Error Code
A1	ZIKA_NEG	Control	ZIKA	Not Detected			
		Control	ZIKA				XXXX[1]
B1	ZIKA_POS	Control	ZIKA	XX.XX CN			
		Control	ZIKA				XXXX[2]
C1	Sample 1		ZIKA	XX.XX CN	Positive		
D1	Sample 2		ZIKA	Not Detected	Negative[3]		
E1	Sample 3		ZIKA	XX.XX CN	Positive	IC[4]	
F1	Sample 4		ZIKA				XXXX[5]
G1	Sample 5		ZIKA				XXXX[6]
H1	Sample 6		ZIKA				XXXX[6]

[1] Error code generated due to negative control failure.

[2] Error code generated due to positive control failure

[3] **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).**

[4] Patient sample with positive amplification of target but failed internal control will produce valid result with a flag for internal control failure.

[5] Error code generated due to no amplification of target and internal control failure.
Whole blood specimens that generate invalid results may be diluted 2 fold with negative plasma and retested. If the retest also returns an invalid result, testing of the patient-matched serum or EDTA plasma specimen should be considered.

[6] Indicates a failed control, invalidating all results in the run. Users are instructed to rerun the samples starting at sample preparation.

For more information about error codes and flags, refer to the Abbott *m*2000*rt* Operations Manual.

LIMITATIONS OF THE PROCEDURE

For Use Under An Emergency Use Authorization Only.

- This assay is for *in vitro* diagnostic use under FDA Emergency Use Authorization only.
- Use of the Abbott RealTi*m*e ZIKA assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and the Abbott *m*2000 system.
- Laboratories are required to report all positive results to the appropriate public health authorities.
- The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive controls or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this package insert.
- Optimal performance of this test requires appropriate specimen collection, storage, and transport to the test site (refer to the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section of this package insert).
- 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.
- Specimen collection conducted prior to symptom onset or after the acute phase of infection (approximately 14 days post-onset of symptoms) may lead to false negative results.
- Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
- Negative results do not preclude infection with Zika virus and should not be the sole basis of a patient treatment/management or public health decision. Follow up testing should be performed according to the current CDC recommendations.
- **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)**.
- 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.

SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

Limit of detection (LoD) studies determined the lowest detectable concentration of Zika virus at which ≥ 95% of replicates tested positive in the Abbott RealTi*m*e ZIKA assay.

The LoD for each sample matrix was determined by testing dilutions of Zika virus Puerto Rico strain (PRVABC59) prepared in negative human plasma, serum, whole blood, and urine. The viral stock concentration was calculated based on a standard curve derived from in vitro transcribed RNA of known concentration containing the prM and NS3 regions of interest.

Tentative Limit of Detection (LoD)

The tentative LoD for human serum, plasma, and urine was determined by testing dilutions of Zika virus in 8-member panels with the following target concentrations: 100, 75, 50, 40, 30, 20, 10, and 0 copies/mL. The tentative LoD for human whole blood was determined by testing dilutions of Zika virus in a 5-member panel with the following target concentrations: 160, 120, 80, 40, and 0 copies/mL.

Each panel member was tested in replicates of 5. The results are summarized in Table 1.

LOD Confirmation

The LoD for human serum, plasma, and urine was confirmed by testing six-member panels with target concentrations ranging from 50 to 0 copies/mL for each sample matrix. The LoD for human whole blood was confirmed by testing a 4-member panel with target concentrations ranging from 140 to 0 copies/mL. The final LoD of each sample matrix was determined to be the lowest concentration resulting in ≥ 95% detection of replicates.

The LoD of the Abbott RealTi*m*e ZIKA assay is 30 copies/mL in serum, 40 copies/mL in plasma, 40 copies/mL in urine, and 120 copies/mL in whole blood. The results, representative of the analytical sensitivity of the Abbott RealTi*m*e ZIKA assay, are summarized in Table 2.

Table 1. Abbott RealTi*me* ZIKA Tentative Limit of Detection Summary for Serum, Plasma, Urine, and Whole Blood Sample

Matrix	Panel Member	Target Zika RNA Concentration (copies/mL)	Number of Replicates		Percent Detected	Mean CN
			Tested	Detected		
Serum	1	100	5	5	100	36.68
	2	75	5	5	100	37.06
	3	50	5	5	100	38.00
	4	40	5	5	100	38.12
	5	**30**	**5**	**5**	**100**	**39.37**
	6	20	5	4	80	39.54
	7	10	5	2	40	40.72
	8	0	5	0	0	-1.00
Plasma	1	100	5	5	100	36.57
	2	75	5	5	100	37.25
	3	50	5	5	100	38.12
	4	**40**	**5**	**5**	**100**	**38.89**
	5	30	5	4	80	39.37
	6	20	5	4	80	38.65
	7	10	5	1	20	38.97
	8	0	5	0	0	-1.00
Urine	1	100	5	5	100	36.57
	2	75	5	5	100	37.44
	3	50	5	5	100	38.35
	4	40	5	5	100	37.74
	5	30	5	5	100	38.39
	6	20	5	5	100	40.76
	7	**10**	**5**	**5**	**100**	**40.36**
	8	0	5	0	0	-1.00
Whole Blood	1	160	5	5	100	37.84
	2	**120**	**5**	**5**	**100**	**38.35**
	3	80	5	3	60	37.80
	4	40	5	1	20	41.56
	5	0	2	0	0	-1.00

Table 2. Abbott RealTi*me* ZIKA Limit of Detection Confirmation Summary for Serum, Plasma, Urine, and Whole Blood Sample

Matrix	Panel Member	Target Zika RNA Concentration (copies/mL)	Number of Replicates		Percent Detected	Mean CN
			Tested	Detected		
Serum	1	50	20	20	100	38.28
	2	40	20	20	100	38.65
	3	**30**	**20**	**20**	**100**	**38.96**
	4	20	20	10	50	41.73
	5	10	10	1	10	42.83
	6	0	4	0	0	-1.00
Plasma	1	50	20	20	100	38.57
	2	**40**	**20**	**20**	**100**	**38.67**
	3	30	20	17	85	39.03
	4	20	20	16	80	40.17
	5	10	10	3	10	40.91
	6	0	4	0	0	-1.00
Urine	1	50	40	38	95	38.96
	2	**40**	**40**	**40**	**100**	**38.84**
	3	30	40	34	85	38.89
	4	20	40	36	90	40.07
	5	10	20	11	55	41.34
	6	0	8	0	0	-1.00
Whole Blood	1	140	20	20	100	38.01
	2	**120**	**20**	**20**	**100**	**38.20**
	3	100	20	16	80	39.78
	4	0	8	0	0	-1.00

Analytical Sensitivity - FDA Reference Materials

An analytical sensitivity study was performed using FDA reference material following a standard protocol provided by the FDA. The study included range-finding and confirmatory LoD studies. The results are summarized in Table 3.

Table 3. Summary of LoD Confirmation Results using the FDA Reference Materials

FDA Reference Material	Specimen Type	Confirmed LoD per FDA Protocol (RNA NAAT Detectable Units/mL)
S1	Serum	1000
	Urine	300
	Whole Blood	1000
S2	Serum	500
	Urine	500
	Whole Blood	1500

Reactivity / Inclusivity

Reactivity of the Abbott RealTi*me* ZIKA assay was assessed by testing three unique strains of Zika virus, comprising the Asian and African lineages. Each virus culture supernatant was diluted in negative human plasma to a target concentration of 2 x LoD (80 copies/mL) and tested in replicates of 5. All replicates tested were detected. The results are summarized in Table 4.

Table 4. Abbott RealTi*me* ZIKA Reactivity Summary

Zika Strain / Isolate	Source / Lineage	Mean CN
Zika virus PRVABC59[a]	Puerto Rico / Asian	37.26
Zika virus SPH 2015	Brazil / Asian	37.23
Zika virus MR766	Uganda / African	37.00

[a] Zika virus PRVABC59 is the same viral isolate that was used for the LoD studies.

In silico analysis of the Abbott RealTi*me* ZIKA assay primer and probe sequences was performed to verify sequence homology with the corresponding target regions found in 48 complete Zika virus genome sequences. The results of the *in silico* inclusivity analysis are provided in Table 5. Percent identify is noted within the table. Overall, primer and probe sequences demonstrated 94 to 100% sequence identity with the prM targets and demonstrated 86 to 100% sequence identity with the NS3 targets.

Table 5. Abbott RealTi*me* ZIKA *In Silico* Inclusivity Analysis Summary

		Sequence % Identity					
		prM Homology			NS3 Homology		
Zika Strain / Isolate	Strain Location	Forward Primer	Reverse Primer	Probe	Forward Primer	Reverse Primer	Probe
Zika-Zeptometrix	Uganda	95	100	94	86	95	100
Zika virus MR766	Uganda	95	100	94	86	95	100
KU720415	Uganda	95	100	94	86	95	100
KF383118	Senegal	95	100	94	86	95	100
KF383119	Senegal	95	100	94	86	95	100
HQ234500	Nigeria	95	100	94	95	95	100
HQ234501	Senegal	95	100	100	95	95	100
KF383117	NA (African)	95	100	94	86	100	100
KF383116	NA (African)	95	100	94	95	95	100
DQ859059	Uganda	95	100	94	86	95	100
KF383115	Central African Republic	95	100	94	86	95	100
KF268950	Central African Republic	95	100	94	86	95	100
KF268948	Central African Republic	95	100	94	86	95	100
KF268949	Central African Republic	95	100	94	86	95	100
HQ234499	Malaysia	100	100	94	100	100	100
JN860885	Cambodia	100	100	100	95	100	100
EU545988	Micronesia	100	100	94	100	100	100
KU681082	Philippines	100	95	94	100	100	100
KU681081	Thailand	100	100	94	100	100	100
KU365777	Brazil	100	100	100	100	100	100
KU365778	Brazil	100	100	100	100	100	100
KU365779	Brazil	100	100	100	100	100	100
KU365780	Brazil	100	100	100	100	100	100
KU312312	Suriname	100	100	100	100	100	100
KU647676	Martinique	100	100	100	95	100	100
KU820897	Columbia	100	100	100	95	100	100
KU321639	Brazil	100	100	100	100	100	100
KU509998	Haiti	100	100	100	100	100	100
KU501217	Guatemala	100	100	100	100	100	100
KU501216	Guatemala	100	100	100	100	100	100
KU501215	Puerto Rico	100	100	100	100	100	100
KU707826	Brazil	100	100	100	100	100	100
KU527068	Brazil	100	100	100	100	100	100
KU497555	Brazil	100	100	100	100	100	100
KU744693	Venezuela	100	100	100	100	100	100
KU729218	Brazil	100	100	100	100	100	100

Table 5. Abbott RealTi*m*e ZIKA *In Silico* Inclusivity Analysis Summary

		Sequence % Identity					
		prM Homology			NS3 Homology		
Zika Strain / Isolate	**Strain Location**	**Forward Primer**	**Reverse Primer**	**Probe**	**Forward Primer**	**Reverse Primer**	**Probe**
KU681081	Thailand	100	100	100	100	100	100
KU761564	China	100	100	100	100	100	100
KU853013	Dominican Republic	100	100	100	100	100	100
KU853012	Dominican Republic	100	100	100	100	100	100
KU729217	Brazil	100	100	100	100	100	100
KU820899	China	100	100	100	100	100	100
KU740184	China	100	100	100	100	100	100
KJ776791	French Polynesia	100	100	100	100	100	100
ZIKV_BR_ Salvador_Sample07	Brazil	100	100	100	100	100	100
ZIKV_BR_ Salvador_Sample09	Brazil	100	100	100	100	100	100
Zika virus SPH2015	Brazil	100	100	100	100	100	100
Zika virus PRVABC59	Puerto Rico	100	100	100	100	100	100

Cross-reactivity

Cross-reactivity of the Abbott RealTi*m*e ZIKA assay was evaluated by testing viruses or purified nucleic acid from Dengue virus (1, 2, 3 and 4), Yellow fever virus, Mayaro virus, West Nile virus, Chikungunya, Human parvovirus B19, and *Plasmodium falciparum* in replicates of 3 at high concentrations. All samples were processed with the RealTi*m*e ZIKA assay beginning with sample preparation on the *m*2000*sp* instrument, with the exception of *P. falciparum* DNA which was added directly to the PCR reaction. No cross-reactivity of the RealTi*m*e ZIKA Assay with the selected pathogens was observed at the concentrations tested. The results are summarized in Table 6.

Table 6. Abbott RealTi*m*e ZIKA Cross-reactivity Summary

Organism	**Strain**	**Source / Sample Type**	**Concentration**	**Result**
Dengue virus 1 (DENV-1)	CDC	Zeptometrix / culture fluid	1.19 x 10^5 PFU/mL	Not detected
Dengue virus 2 (DENV-2)	New Guinea C		2.92 x 10^5 PFU/mL	Not detected
Dengue virus 3 (DENV-3)	H87		1.53 x 10^6 PFU/mL	Not detected
Dengue virus 4 (DENV-4)	H241		8.81 x 10^5 PFU/mL	Not detected
Mayaro virus	TRVL 15537	ATCC / lyophilized suspension	1.26 × 10^8 U/mL	Not detected
Yellow fever virus	17D	Zeptometrix / culture fluid	2.92 x 10^5 PFU/mL	Not detected
Chikungunya	R80422	Zeptometrix / culture fluid	1.97 x 10^7 PFU/mL	Not detected
Human parvovirus B19	Isolated from Plasma, no strain information available	Zeptometrix / NATtrol inactivated virus	1 x 10^5 IU/mL	Not detected
West Nile virus	NY2001-6263	Zeptometrix / culture fluid	8.04 x 10^6 PFU/mL	Not detected
Plasmodium falciparum	3D7	ATCC / Purified DNA	100 picograms/reaction	Not detected

The primer and probe sequences in the Abbott RealTi*m*e ZIKA assay were subject to a BLAST analysis against all available sequences for the organisms listed in Table 7, with the following pararameters: blastn, maximum target sequence = 10,000, word size = 7, expect threshold = 1,000, Match/Mismatch score = 1,-3, and Gap Costs = Existence: 5 Extension: 2. The results of this analysis predict no significant Abbott RealTi*m*e ZIKA cross-reactivity with the sequences available considering percent homology with all 3 oligonucleotides, orientation of forward primer, reverse primer, and probe, and predicted amplification product size.

Table 7. Abbott RealTi*m*e ZIKA *In Silico* Cross-reactivity Analysis Summary

Organism		**Tax ID**	**Organism**		**Tax ID**
Flavivirus	Dengue virus 1 (DENV-1)	11053	**Alphavirus**	Barmah Forest virus	11020
	Dengue virus 2 (DENV-2)	11060		Chikungunya	37124
	Dengue virus 3 (DENV-3)	11069		Eastern equine encephalitis virus	11021
	Dengue virus 4 (DENV-4) 1	11070		Mayaro virus	59301
	Hepatitis C virus	11102		O'nyong-nyong virus	11027
	Japanese encephalitis virus	11071		Ross River virus	11029
	St Louis encephalitis virus	11080		Western equine encephalitis virus	11039
	Spondweni virus	64318			
	Yellow fever virus	40005			
	Yellow fever virus vaccine strain	11090			
	West Nile virus	11082			

Table 7. Abbott RealTi*m*e ZIKA *In Silico* Cross-reactivity Analysis Summary

Organism		Tax ID	Organism		Tax ID
Other Virus	Adenovirus B	108098		Hepatitis B virus	10407
	Adenovirus B1	565302		Human Cytomegalovirus	10358
	Adenovirus C	129951		Human immunodeficiency virus 1	11676
	Adenovirus D	130310		Human parvovirus	10798
	Adenovirus 7	10519		Measles virus	11234
	Enterovirus	12059		Rubella virus	11041
	Epstein-Barr virus	10376		Varicella-zoster virus	10335
	Hepatitis A virus vaccine	208726			
Bacteria	*Borrelia burgforferi*	64895	**Protozoa**	*Plasmodium falciparum*	5833
	E coli NGF1	562		*Plasmodium* sp	5820
	E coli 0157:H7	386585		*Tyrpanosoma cruzi*	5693
	Leptospira	171			
	Rickettsia	780	**Trematode**	*Schistosoma*	6183
	Salmonella typhi	527001			
	Group A *Streptococcus*	36470			

Microbial Interference Evaulation

Microbial Interference of the Abbott RealTi*m*e ZIKA assay from high levels of Dengue virus that may be present in clinical specimens was evaluated by testing Zika negative and positive human plasma samples containing Dengue virus type 1 at a concentration of 1 x 10^5 copies/mL in replicates of 5.

No cross-reactivity of the RealTi*m*e ZIKA Assay was observed at the concentration tested. The data confirms that specimens positive for Dengue virus type 1 do not interfere with the results of the Abbott RealTi*m*e ZIKA assay. The results are summarized in Table 8.

Table 8

	Concentration (copies/mL)		Number of Replicates			
Panel Member	**Zika Virus**	**Dengue Virus**	**Tested**	**Detected**	**Percent Detected**	**Average CN**
1	0	1 x 10^5	5	0	0	-1.00
2	1000	1 x 10^5	5	5	100	33.33
3	80	1 x 10^5	5	5	100	37.75
4	80	0	5	5	100	37.33

Sample Matrix Equivalence Study

To demonstrate matrix equivalence between serum and EDTA plasma, paired serum and plasma samples from individual donors were spiked using Zika virus Puerto Rico strain (PRVABC59). Ten samples were spiked at 60 copies/mL and twenty samples were spiked at 150 copies/mL.

Serum and plasma are considered equivalent as Zika virus RNA was detected in all spiked samples of both matrices and in none of the negative samples in both matrices. The results are summarized in Table 9.

Table 9 Comparison Results for Contrived Clinical Plasma and Serum Samples

Agreement Type	Number of Agreements	Percent Agreement	95% Confidence Interval (CI)
PPA	30/30	100%	(88.4%, 100%)
NPA	10/10	100%	(69.2%, 100%)

Clinical Performance Evaluation

The Abbott RealTi*m*e ZIKA assay was evaluated by testing:

- 60 individual serum, plasma, and urine samples and 50 individual whole blood samples from low prevalence populations.
- 25 contrived serum, plasma, whole blood samples, and 50 contrived urine samples.
- 36 patient matched clinical serum, plasma and urine samples and 25 clinical whole blood samples collected from endemic populations.

The performance of the Abbott RealTi*m*e ZIKA assay with the matched serum and urine samples and with the whole blood samples was compared against EUA Comparator assay results.

Low Prevalence Sample Evaluation

Sixty non-matched serum, plasma and urine samples from a low prevalence population were tested in the Abbott RealTi*m*e ZIKA assay and the serum and urine samples were confirmed negative when tested with the EUA Comparator assay. The plasma sample results were compared to the expected negative results.

Zika virus RNA was detected in one urine specimen. The specimen tested positive in two subsequent runs of the Abbott RealTi*m*e ZIKA assay. No additional volume of this specimen was available from the vendor for further investigation.

All negative serum and plasma samples were correctly reported as negative. One false positive urine sample was detected. The false positive was repeat positive in duplicate re-test samples.

Fifty whole blood normal donor samples were tested in the Abbott RealTi*m*e ZIKA assay and compared to the expected result for normal donor status. All negative whole blood samples were correctly reported as negative.

The results for the low prevalence samples are summarized in Tables 18 to 21 at the end of the clinical study section.

Contrived Sample Evaluation

Performance of the Abbott RealTi*m*e ZIKA assay was assessed using contrived serum, plasma (EDTA), whole blood (EDTA), and urine samples. A total of 125 samples (25 serum, 25 plasma, 25 whole blood, and 50 urine) were tested with the Abbott RealTi*m*e ZIKA assay on the Abbott *m*2000 System. The positive samples for each matrix were prepared by diluting Zika virus strain PRVABC59 in unique negative specimens. The results are summarized in Table 10.

Table 10. Performance of Abbott RealTi*me* ZIKA with Contrived Samples

Matrix	Copies/mL	Concentration	Avg. CN	Hit Rate	Percent Detection
Serum	150	5 × LoD	36.69	5/5	100%
	60	2 × LoD	38.55	10/10	100%
	30	1 × LoD	39.77	7/10	70%[a]
	Total			22/25	88%
Plasma	200	5 × LoD	36.36	5/5	100%
	80	2 × LoD	37.87	10/10	100%
	40	1 × LoD	38.85	9/10	90%
	Total			24/25	96%
Urine	200	5 × LoD	36.54	24/25	96%
	80	2 × LoD	38.66	24/25	96%
	Total			48/50	96%
Whole Blood	600	5 × LoD	36.17	10/10	100%
	240	2 × LoD	37.07	15/15	100%
	Total			25/25	100%

[a] Based on the comparison of the mean CN for the detected replicates at this level (39.77) to the mean CN at LOD in the confirmatory LOD study for serum (38.96), it appears that the actual Zika virus concentration at this level was close to or below LOD, and therefore a hit rate lower than 95% can be explained.

Matched Clinical Sample Evaluation

Thirty-six matched serum, plasma, and urine clinical samples were tested with the Abbott RealTi*me* ZIKA assay and the serum and urine samples were tested with the emergency use authorized Comparator PCR assay.

Table 11. Matched Clinical Sample Line Listing

ID	Days Collected Post Symptom Onset	Abbott Results			Comparator Results*	
		Plasma CN	Serum CN	Urine CN	Serum	Urine
1043-TDS-0112	2	31.70	35.21	33.95	POS	POS
1043-TDS-0114	3	37.62	36.50	40.79	POS	POS
1043-TDS-0115	2	26.13	25.22	32.67	POS	POS
1043-TDS-0119	2	30.59	31.39	37.18	POS	POS
1043-TDS-0122	3	35.43	36.44	42.81	POS	NEG
1043-TDS-0130	1	35.41	36.97	37.86	POS	POS
1043-TDS-0131	1	30.55	30.93	38.33	POS	POS
1043-TDS-0134	2	38.64	39.07	35.76	POS	POS
1043-TDS-0135	2	35.64	35.27	-1.00	POS	NEG
1043-TDS-0200	3	-1.00[a]	-1.00	-1.00	POS	POS
1043-TDS-0201	3	35.18	34.27	35.19	POS	POS
1043-TDS-0203	4	32.87	33.24	37.27	POS	POS
1043-TDS-0052	3	28.67	28.85	36.29	POS	POS
1043-TDS-0058	4	33.04	35.51	-1.00	POS	POS
1043-TDS-0059	3	33.25	33.86	35.34	POS	POS
1043-TDS-0060	2	36.31	38.00	35.27	POS	POS
1043-TDS-0064	3	34.63	35.22	37.92	POS	POS
1043-TDS-0269	4	30.59	29.86	-1.00	POS	POS
1043-TDS-0323	2	27.99	28.62	-1.00	POS	NEG
1043-TDS-0332	3	28.24	29.23	39.10	POS	POS
1043-TDS-0348	2	29.37	29.70	36.92	POS	POS
1043-TDS-0354	3	36.04	30.89	42.59	POS	NEG
1043-TDS-0428	3	30.19	37.20	30.17	POS	POS
1043-TDS-0433	4	26.86	30.73	-1.00	POS	POS
1043-TDS-0436	2	26.58	29.21	-1.00	POS	POS
1043-TDS-0440	3	30.39	31.16	-1.00	POS	NEG
1043-TDS-0474	2	28.09	29.54	-1.00	POS	NEG
1043-TDS-0486	3	27.13	27.60	-1.00	POS	NEG
1043-TDS-0499	5[b]	29.07	29.50	-1.00	POS	POS
1043-TDS-0202	3	-1.00	40.20	-1.00	NEG	POS
1043-TDS-0206	6	-1.00	-1.00	36.67	NEG	POS
1043-TDS-0053	4	35.71	37.60	-1.00	NEG	POS
1043-TDS-0054	8	38.27	-1.00	41.84	NEG	POS
1043-TDS-0056	7	40.32	-1.00	36.81	NEG	POS
1043-TDS-0057	4	41.26	-1.00	34.24	NEG	POS
1043-TDS-0065	7	38.65	-1.00	37.63	NEG	POS

[a] Tested positive on repeat

[b] Pregnant patient

* The comparator assay was a real-time RT-PCR assay authorized by FDA for detection of Zika RNA with analytical sensitivity in the range 3162-5000 RNA NAAT Detectable Units/mL for serum and 1581-5000 RNA NAAT Detectable Units/mL for urine using the FDA Reference Materials S1 and S2.

Whole Blood Clinical Sample Evaluation

Twenty-five whole blood clinical samples were tested with the Abbott RealTi*m*e ZIKA assay and the serum samples were tested with the emergency use authorized Comparator PCR assay.

Table 12. Clinical Sample Line Listing

ID	Days Collected Post Symptom Onset	Abbott Result Whole Blood CN	Comparator Results* Serum
1043-TDS-0472	4	31.68	POS
1043-TDS-0474	2	26.00	POS
1043-TDS-0475	4	36.36	POS
1043-TDS-0477	4	34.16	POS
1043-TDS-0478	3	30.27	POS
1043-TDS-0480	3	34.50	POS
1043-TDS-0482	4	37.01	POS
1043-TDS-0483	4	33.17	POS
1043-TDS-0486	3	25.89	POS
1043-TDS-0488	4	35.09	POS
1043-TDS-0489	5	31.64	POS
1043-TDS-0490	5	28.00	POS
1043-TDS-0491	4	33.06	POS
1043-TDS-0495	3	33.11	POS
1043-TDS-0498	5	34.72	POS
1043-TDS-0499	5	33.25	POS
1043-TDS-0500	5	36.67	POS
1043-TDS-0501	4	-1.00a	POS
1043-TDS-0503	4	-1.00b	POS
1043-TDS-0505	4	35.23	POS
1043-TDS-0506	3	32.94	POS
1043-TDS-0509	1	36.57	POS
1043-TDS-0510	3	32.33	POS
1043-TDS-0511	3	35.83	POS
1043-TDS-0514	3	36.34	POS

a The sample was invalid due to an Internal Control failure at initial and repeat testing. Dilution of this sample with an equal volume of negative plasma resulted in a valid, negative result.

b Tested positive on repeat.

*Comparator method was a transcription-mediated amplification (TMA) IVD assay authorized by FDA for detection of ZIKV RNA in plasma with analytical sensitivity in the range 100-150 NAAT Detectable Units/mL using the FDA Reference Materials S1 and S2. Equivalency between serum and plasma specimens was demonstrated.

Clinical Serum Sample Evaluation

Comparison of the Abbott RealTi*m*e ZIKA assay and the EUA Comparator assay for clinical serum samples is presented in Table 13.

Table 13. Comparison Results for Clinical Serum Samples

Serum Samples		EUA Comparator Result* Positive	EUA Comparator Result* Negative
Abbott RealTime ZIKA Result	Positive	28	2[b]
	Negative	1[a]	5

[a] The Abbott PIS for this sample was negative as determined by negative results for both the serum and urine samples.

[b] The 2 false positives had CN values of 40.20 and 37.60 with the Abbott assay, indicating virus concentrations close to or below the LOD. The EUA Comparator PIS for these samples was positive as determined by positive urine results.

*The comparator assay was a real-time RT-PCR assay authorized by FDA for detection of Zika RNA with analytical sensitivity in the range 3162-5000 RNA NAAT Detectable Units/mL for serum using the FDA Reference Materials S1 and S2.

The Positive Percent Agreement (PPA) and the Negative Percent Agreement (NPA) of the test results generated with the Abbott RealTi*m*e ZIKA assay and the EUA Comparator assay for the clinical serum samples are summarized in Table 18.

Clinical Urine Sample Evaluation

Comparison of the Abbott RealTi*m*e ZIKA assay and the EUA Comparator assay for clinical urine samples is presented in Table 14.

Table 14. Comparison Results for Clinical Urine Samples

Urine Samples		EUA Comparator Result* Positive	EUA Comparator Result* Negative
Abbott RealTi*m*e ZIKA Result	Positive	21	2[b]
	Negative	8[a]	5

[a] The Abbott PIS for 7 / 8 of these samples was positive as determined by positive serum results.

[b] The 2 false positives had CN values of 42.59 and 42.81 with the Abbott assay, indicating virus concentrations close to or below the LOD. The EUA Comparator PIS for these samples was positive as determined by positive serum results.

*The comparator assay was a real-time RT-PCR assay authorized by FDA for detection of Zika RNA with analytical sensitivity in the range 1581-5000 RNA NAAT Detectable Units/mL for urine using the FDA Reference Materials S1 and S2.

The Positive Percent Agreement (PPA) and the Negative Percent Agreement (NPA) of the test results generated with the Abbott RealTi*m*e ZIKA assay and the EUA Comparator assay for the clinical urine samples are summarized in Table 19.

Clinical Plasma Sample Evaluation and Percent Agreement Summary

Table 15. Comparison of the Abbott Zika RealTi*m*e Assay Results for Matched Clinical Plasma and Serum Samples

		Serum Result	
		Positive	**Negative**
Plasma Result	**Positive**	29	4[b]
	Negative	1[a]	2
	Total	30	6

Agreement Type	**Number of Agreements**	**Percent Agreement**	**95% Confidence Interval (CI)**
PPA	29/30	96.7%	(82.8%, 99.9%)
NPA	2/6	33.3%	(4.3%, 77.7%)

[a] Serum sample CN of 40.20 indicating a virus concentration close to or below the LoD.

[b] Plasma samples CN of 38.27, 40.32, 41.26, 38.65 indicating a virus concentration close to or below the LoD.

Comparison of the Abbott RealTi*m*e ZIKA assay and the EUA Comparator PIS determination for clinical plasma samples is presented in Table 16.

Table 16. Comparison Results for Clinical Plasma Samples

		EUA Comparator PIS	
		Positive	**Negative**
Abbott RealTi*m*e ZIKA Plasma Result	**Positive**	34	0
	Negative	2[a]	0
	Total	36	0

Agreement Type	**Number of Agreements**	**Percent Agreement**	**95% Confidence Interval (CI)**
PPA	34/36	94.4%	(81.3%, 99.3%)
NPA	N/A		

[a] The 2 negative plasma samples were negative by comparator serum testing; urine was positive by comparator to yield the positive PIS.

Clinical Whole Blood Sample Evaluation

Comparison of the Abbott RealTi*m*e ZIKA assay and the EUA Comparator PIS (determined by a positive serum result) for clinical whole blood samples is presented in Table 17.

Table 17. Comparison Results for Clinical Whole Blood Samples

		EUA Comparator PIS	
		Positive	**Negative**
Abbott RealTi*m*e ZIKA Whole Blood Result	**Positive**	23	0
	Negative	2[a,b]	0
	Total	25	0

[a] One sample initially negative was positive upon retest.

[b] One sample was invalid due to an Internal Control failure at initial and repeat testing. Dilution of this sample with an equal volume of negative plasma resulted in a valid, negative result.

The Positive Percent Agreement (PPA) and the Negative Percent Agreement (NPA) of the test results generated with the Abbott RealTi*m*e ZIKA assay and the EUA Comparator assay for the clinical whole blood samples are summarized in Table 21.

Clinical Sample Percent Agreement Summary

The PPA and NPA in the test results generated with the Abbott RealTi*m*e ZIKA assay and the EUA Comparator assay for clinical serum samples are summarized in Table 18.

Table 18. Percent Agreement between Abbott RealTi*m*e ZIKA and EUA Comparator Assay or Expected Results for all tested Serum Samples (Clinical Study, Low Prevalence, Contrived Samples)

Agreement Type	**Test Population**	**Number of Agreements**	**Percent Agreement**	**95% Confidence Interval (CI)**
PPA	Endemic	28/29[a]	96.6%	(82.8%, 99.4%)
	Contrived Samples			
	5 X LoD	5/5		
	2 X LoD	10/10	88.0%	(68.8%, 97.5%)
	1 X LoD	7/10		
PPA	**ALL**	**50/54**	**92.6%**	**(82.1%, 97.9%)**
NPA	Endemic	5/7	71.4%	(29.0%, 96.3%)
	Low Prevalence	60/60	100%	(94.0%, 100%)
NPA	**ALL**	**65/67**	**97.0%**	**(89.6%, 99.6%)**

[a] There was insufficient volume for the false negative for retest.

The PPA and NPA in the test results generated with the Abbott RealTi*m*e ZIKA assay and the EUA Comparator assay for clinical urine samples are summarized in Table 19.

Table 19. Percent Agreement between Abbott RealTi*m*e ZIKA and EUA Comparator Assay or Expected Results for Clinical Urine Samples

Agreement Type	Test Population	Number of Agreements	Percent Agreement	95% Confidence Interval (CI)
PPA	Endemic	21/29	72.4%	(54.3%, 85.3%)
	Contrived Samples			
	5 X LoD	24/25	96%	(86.3%, 99.5%)
	2 X LoD	24/25		
PPA	**ALL**	**69/79**	**87.3%**	**(78.0%, 93.8%)**
NPA	Endemic	5/7	71.4%	(29.0%, 96.3%)
	Low Prevalence	59/60[a]	98.3%	(91.1%, 99.7%)
NPA	**ALL**	**64/67**	**95.5%**	**(87.5%, 99.1%)**

[a] The false positive was repeat positive on two retests.

The PPA and NPA in the test results generated with the Abbott RealTi*m*e ZIKA assay and Comparator for clinical plasma samples are summarized in Table 20.

Table 20. Percent Agreement between Abbott RealTi*m*e ZIKA and Comparator[a] for all tested Plasma Samples (Clinical samples, Low Prevalence, and Contrived)

Agreement Type	Test Population	Number of Agreements	Percent Agreement	95% Confidence Interval (CI)
PPA	Endemic	33/36[b]	91.7%	(78.2%, 97.1%)
	Contrived Samples			
	5 X LoD	5/5	96.0%	(79.6%, 99.9%)
	2 X LoD	10/10		
	1 X LoD	9/10		
PPA	**ALL**	**57/61**	**93.4%**	**(84.1%, 98.2%)**
NPA	Endemic	0/0	N/A	N/A
	Low Prevalence	60/60	100%	(94.0%, 100%)
NPA	**ALL**	60/60	100%	(94.0%, 100%)

[a] Evaluated against comparator (Comparator for contrived samples and the low prevalence population was the expected result; comparator for endemic population samples was the patient infected status (PIS) as determined by testing of patient matched serum and urine samples with the EUA comparator assay).
[b] One false negative was positive on retest; there was insufficient volume for the remaining 2 for retest.

The Positive Percent Agreement (PPA) and the Negative Percent Agreement (NPA) in the test results generated with the Abbott RealTi*m*e ZIKA assay and the expected results for clinical whole blood samples are summarized in Table 21.

Table 21. Percent Agreement between Abbott RealTi*m*e ZIKA and Comparator Assay PIS for Whole Blood Samples (Clinical samples, Low Prevalence, and Contrived)

Agreement Type	Test Population	Number of Agreements	Percent Agreement	95% Confidence Interval (CI)
PPA	Endemic	23/25 [a,b]	92.0%	(74.0%, 99.0%)
	Contrived Samples			
	5 X LoD	10/10	100.0%	(86.3%, 100.0%)
	2 X LoD	15/15		
PPA	**ALL**	**48/50**	**96.0%**	**(86.3%, 99.5%)**
NPA	Low Prevalence	50/50	100%	(92.9%, 100.0%)
	ALL	**50/50**	**100%**	**(92.9%, 100.0%)**

[a] One sample initially negative was positive upon retest.
[b] One sample was invalid due to an Internal Control failure at initial and repeat testing. Dilution of this sample with an equal volume of negative plasma resulted in a valid, negative result.

Analysis of Matched Serum, Plasma, and Urine Sample Sets

The patient infected status (PIS) of the Abbott RealTi*m*e ZIKA assay was compared with the patient infected status from the EUA Comparator assay.

A total of 36 matched sample sets were analyzed.

Results from the Abbott RealTi*m*e ZIKA assay and the EUA Comparator assay were each used to construct a patient infected status based upon the following algorithm:

- A subject was defined as infected for Zika virus if one positive assay result was reported in serum or urine.
- A subject was defined as not infected for Zika virus if negative assay results were reported in both serum and urine.

The patient infected status was also determined in the same way using the Abbott RealTi*m*e ZIKA assay plasma and urine results.

From a total of 36 matched sets analyzed, 35/36 matched sets agreed as serum and urine PIS positive between the two assays with a positive agreement of 97.2% (95% CI. 85.8%, 99.9%). The remaining matched set was PIS negative by the Abbott RealTi*m*e ZIKA assay and PIS positive by the EUA Comparator assay.

Comparison of the Abbott RealTi*m*e ZIKA assay and the EUA Comparator assay determined serum and urine patient infected status is presented in Table 22.

Table 22. Comparison of patient infected status between Abbott RealTi*m*e ZIKA and the Comparator – Serum & Urine

Abbott Serum and Urine PIS		**EUA Comparator PIS**	
		Positive	**Negative**
Abbott RealTi*m*e ZIKA PIS	**Positive**	35	0
	Negative	1	0
Agreement Type	**Number of Agreements**	**Percent Agreement**	**95% Confidence Interval (CI)**
PPA	35/36	97.2%	(85.8%, 99.9%)
NPA	N/A		

[a] The PIS by the EUA Comparator assay is determined by serum and urine testing.

From the total of 36 matched sets analyzed, 34/36 matched sets agreed as PIS positive between the two assays based upon Abbott plasma and urine PIS determination and EUA Comparator serum and urine PIS determination, with a positive agreement of 94.4% (95% CI. 81.3%, 99.3%).

Comparison of the Abbott RealTi*m*e ZIKA assay determined plasma and urine patient Infected Status and the EUA Comparator assay determined serum and urine PIS is presented in Table 23.

Table 23. Comparison of patient infected status between Abbott RealTi*m*e ZIKA and the Comparator – Plasma & Urine

Abbott Plasma and Urine PIS		**EUA Comparator PIS**	
		Positive	**Negative**
Abbott RealTi*m*e ZIKA PIS	**Positive**	34	0
	Negative	2	0
Agreement Type	**Number of Agreements**	**Percent Agreement**	**95% Confidence Interval (CI)**
PPA	34/36	94.4%	(81.3%, 99.3%)
NPA	N/A		

[a] The PIS by the EUA Comparator assay is determined by serum and urine testing.

BIBLIOGRAPHY

1. Musso D, Gubler DJ. Zika Virus. *Clin Microbiol Rev*. 2016;29(3):487–524
2. Kitchen SF, Haddow AJ. Zika virus I. Isolation and serological specificity. *Trans R Soc Trop Med Hyg*. 1952;46:509–20.
3. https://www.cdc.gov/zika/symptoms/ accessed 6/27/2016.
4. Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak, Bahia, Brazil. *Emerg Infect Dis* 2015;21:1885–6. 10.3201/eid2110.150847.
5. Zanluca C, Melo VC, Mosimann AL, et al. First report of autochthonous transmission of Zika virus in Brazil. *Mem Inst Oswaldo Cruz* 2015;110:569–72.
6. Rasmussen SA, Jamieson DJ, Honein MA, et al. Zika virus and Birth Defects – Reviewing the Evidence for Causallity. *N Engl J Med*. 2016;374:1981–7. 10.1056/NEJMsr1604338.
7. Cao-Lormeau, V.-M. et al. Guillain-Barré syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* http://dx.doi.org/10.1016/S0140-6736(16)00562–6.
8. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. *Type*> www.cdc.gov, *search*>BMBL5>look up sections III and IV.]
9. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
10. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Fourth Edition*. CLSI Document M29-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
11. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.

Key to Symbols Used

For Prescription Use Only.


Caution


Warning


Manufacturer


Temperature Limit


Consult Instructions For Use

In Vitro Diagnostic Medical Device

Global Trade Item Number

Lot Number


Use By

Reference Number

IN VITRO DIAGNOSTIC MEDICAL DEVICE

CUSTOMER SERVICE INTERNATIONAL: CALL YOUR ABBOTT REPRESENTATIVE

For technical assistance, call Abbott Technical Services at 1-800-553-7042 (within the US), or +49-6122-580 (outside the US), email molecularsupport@abbott.com, or visit the Abbott website at www.molecular.abbott.

Armored RNA® is a patented technology jointly developed by Ambion, Inc. and Cenetron Diagnostics, LLC. US patents #5,677,124, #5,919,625, #5,939,262 and patents pending.

Armored RNA is a registered trademark of Ambion.

ProClin is a registered trademark of Rohm and Haas.

FAM and ROX are trademarks of Life Technologies Corporation or its subsidiaries in the US and/or certain other countries.

VIC is a registered trademark of Life Technologies Corporation or its subsidiaries in the US and/or certain other countries.

Abbott *m*, *m*2000, *m*2000*rt*, and *m*2000*sp* are trademarks of Abbott.

Abbott Molecular Inc. is the legal manufacturer of the:

Abbott RealTi*m*e ZIKA Amplification Reagent Kit (List No. 09N27-090)

Abbott RealTi*m*e ZIKA Control Kit (List No. 09N27-080)

Abbott Molecular Inc.
1300 East Touhy Avenue
Des Plaines, IL 60018 USA

February 2024
51-608295/R3

Abbott