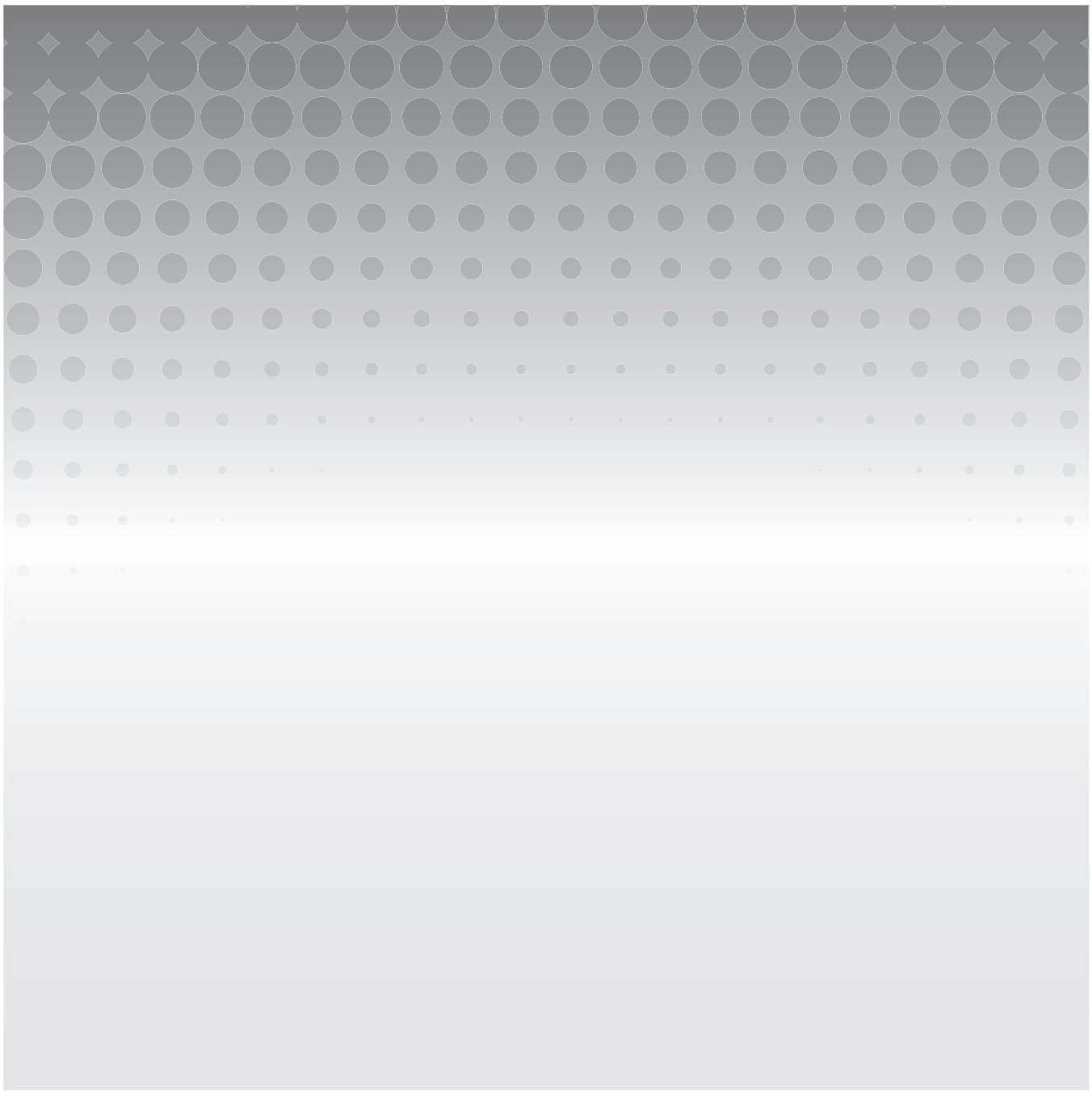
Luminex

Instructions for Use | IVD

xMAP® MultiFlex™ Zika RNA Assay

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

IVD Rx Only



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xMAP® MultiFLEX™ Zika RNA Assay Package Insert
89-30000-00-585 Rev B
May 2017

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For use with MAGPIX® or Luminex® 200™ Instruments.

Refer to *Table 1, "Reagents Supplied in the Kit and Storage Conditions"* for reagent storage conditions.

Kit Components	REF
xMAP® MultiFLEX™ Zika RNA Assay Σ 100	MBP-EUA-01
TDAS LSM Software	CN-SW56-01

Symbols

5.1.5* LOT	Batch Code Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.3.2‡ 	Keep away from sunlight. Indicates a medical device that needs protection from light sources.
5.1.6* REF	Catalog(ue) Number Indicates the manufacturer's catalogue number so that the medical device can be identified.	5.4.4* 	Caution Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the medical device itself.
5.1.1* 	Manufacturer / Date of manufacture Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.	5.5.5* 	Contains Sufficient for <*n*> Tests Indicates the total number of IVD tests that can be performed with the IVD kit reagents.
5.4.3* 	Consult instructions for use Indicates the need for the user to consult the instructions for use.	5.1.3 * 	Date of manufacture Indicates the date when the medical device was manufactured.
5.3.7* 	Temperature Limit Indicates the temperature limits to which the medical device can be safely exposed.	EUA	For use only under Emergency Use Authorization
† Rx Only	Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner (U.S. Only)	5.5.1* IVD	*In vitro* diagnostic medical device Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.

* ANSI/AAMI/ISO 15223-1:2012, Medical devices—Symbols to be used with medical device labels, labeling, and information to be supplied—Part 1: General requirements.

† 21 CFR 809 (FDA Code of Federal Regulations).

‡ ANSI/AAMI/ISO 15223-1:2012, Medical devices—Symbols to be used with medical device labels, labeling, and information to be supplied—Part 1: General requirements.

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89-30000-00-187 Rev C

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89-30000-00-419 Rev. D

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Intended Use

The xMAP® MultiFLEX™ Zika RNA Assay test is a RT-PCR test intended for the qualitative detection of RNA from the Zika virus in serum, plasma, or urine (collected alongside a patient-matched serum or plasma specimen) from individuals meeting Centers for Disease Control and Prevention (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 transmission at the time of travel, or other epidemiologic criteria for which Zika virus testing may be indicated). Test is limited to U.S. laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, and similarly qualified non - U.S. laboratories.

Test results are for the identification of Zika viral RNA. Zika virus RNA is generally detectable in serum 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 Zika virus 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 xMAP MultiFLEX Zika RNA Assay test is intended for use by trained clinical laboratory personnel qualified by state and federal regulations. Specific training on the use of the xMAP MultiFLEX Zika RNA Assay will be made available to end-users at their request. The test is only for use under the Food and Drug Administration's Emergency Use Authorization. The xMAP MultiFLEX Zika RNA Assay is indicated for use on Luminex® IVD xMAP® instruments (MAGPIX® or Luminex® 200™).

Product Description

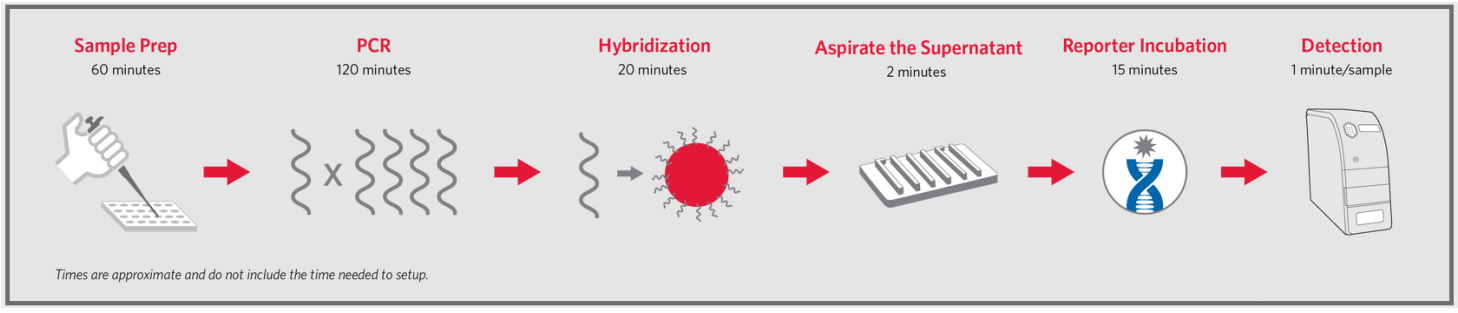
The xMAP® MultiFLEX™ Zika RNA Assay is a qualitative test intended for the detection of Zika virus RNA targets, as well as an internal control. The xMAP MultiFLEX Zika RNA Assay incorporates multiplex Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) in conjunction with the Luminex® xMAP® Technology for target detection. Luminex xMAP Technology uses the principles of fluidics, fluorescent-dyed microspheres, and digital signal processing to enable detection of up to 50 analytes per sample on a MAGPIX instrument and up to 100 analytes per sample on a Luminex® 200™ instrument. xMAP Technology is used extensively in clinical diagnostics for applications in infectious disease, molecular genetics, pharmacogenetics, HLA, and autoimmune diseases.

The xMAP MultiFLEX Zika RNA Assay uses proprietary RT-PCR primers and probes in a panel designed for selective amplification of Zika Virus (6 distinct genomic regions). The 6 distinct Zika genomic regions allow for improved detection of the genetic variation of Zika virus. The panel also includes an internal control: MS2 (bacteriophage RNA extraction control).

Assay Workflow

The xMAP® MultiFLEX™ Zika RNA Assay workflow is shown in *Figure 1* on page 1. Extract samples using a validated automated extraction method/system. Extracted total nucleic acid is then simultaneously amplified using target-specific primers and probes for RT-PCR, followed by amplicon hybridization to probe-coupled microspheres. Detection of the targets of interest are carried out with a Luminex® instrument after adding streptavidin-conjugated R-Phycoerythrin (SAPE).

FIGURE 1. **xMAP® MultiFLEX™ Zika RNA Assay Workflow**



Acceptable Specimens

- Serum (collected in a serum separator tube)
- Plasma (collected in a K3 EDTA tube)
- Urine (collected in specimen cup) and Serum (collected in a serum separator tube)
- Urine (collected in specimen cup) and Plasma (collected in a K3 EDTA tube)

NOTE: Urine alone is not an acceptable specimen type without a matched plasma or serum specimen.

Specimen Handling and Storage

- Separate the serum and plasma specimens according to standard laboratory procedures prior to freezing.
- When transporting human specimens, ensure that all applicable regulations for transport of potentially infectious biological specimens have been met.
- Transport/ship human specimens (serum, plasma, urine) in dry ice, if possible; otherwise use frozen cold packs.
- Process specimens immediately or store specimens at -20°C upon receipt. Thaw sample and keep on ice during sample processing. Store remaining sample at -70°C for long term storage. If specimens will not be tested within the same day of receipt, freeze the specimens immediately upon receipt at -70°C.
- Luminex does not recommend repeat freezing and thawing of specimens.

Materials Provided

Software and CD Contents

Refer to *"Installing TDAS LSM for Zika"* on page 9 for instructions to install and use the data analysis software, TDAS LSM for Zika (IVD-EUA). Ensure that the version of TDAS LSM for Zika specified on the carton containing kit reagents is the version used to analyze data generated with those reagents, unless notified otherwise.

Files provided on the Data Analysis Software LSM for xMAP® MultiFLEX™ Zika RNA Assay CD:

- Set-up executable that installs Data Analysis Software for xMAP MultiFLEX Zika RNA Assay
  - TDAS LSM for Zika
- Data acquisition protocols for xPONENT® software
- Example output files
- TDAS LSM for Zika (IVD-EUA) Version History
- *TDAS LSM 2.4 Software User Manual*

Reagents Kit

Table 1 on page 3 outlines reagents supplied in the kit and their storage conditions.

NOTE: The kit includes 100 reactions worth of reagents.

TABLE 1. **Reagents Supplied in the Kit and Storage Conditions**

Reagents	Volume per Tube	Storage Conditions	Shipping Conditions
Primer Mix	500.0 µL	Store at -20°C, upon receipt	2ºC to 8ºC
Streptavidin-Phycoerythrin (SAPE)	30.0 µL	2ºC to 8ºC, protected from light	2ºC to 8ºC
Microsphere mix	1.0 mL	2ºC to 8ºC, protected from light	2ºC to 8ºC
Buffer A	3.0 mL	2ºC to 8ºC	2ºC to 8ºC
Buffer B	7.5 mL	2ºC to 8ºC	2ºC to 8ºC
MS2 bacteriophage RNA extraction control	1.5 mL	2ºC to 8ºC	2ºC to 8ºC

This kit is shipped at 2°C to 8°C. Upon receipt, the primer mix should be stored at -20°C. All other kit components should be stored at 2°C to 8°C.

NOTE: For a copy of the Safety Data Sheet (SDS), contact Luminex *"Technical Support"* on page 22.

Materials Required but not Provided

- MAGPIX® or Luminex® 200™ System with xPONENT® software, calibrators, and verifiers
- Automated nucleic acid extraction system, BioMerieux® NucliSENS® easyMAG®

  NOTE: Follow the manufacturer instructions for extraction of DNA/RNA from the sample.

  NOTE: There is a product recall for certain lots of easyMAG® extraction reagents. Though no shifts in performance associated with use of these reagents were observed when using them in combination with Luminex Corporation's xMAP® MultiFLEX™ Zika RNA Assay, each lot of affected easyMAG extraction reagents should be evaluated at least weekly before use in extraction of diagnostic specimens. Laboratories should also closely monitor for any trend in Median Fluorescent Intensity (MFI) of the internal MS2 control and the external Zika virus positive control during testing. Laboratories should refer to BioMerieux® Product Safety Correction Notices for a list of impacted lots and advice for end users.

- QIAGEN® OneStep RT-PCR Kit (Catalog # 210210, 210212, or 210215)
- Thermal Cycler (Eppendorf® vapo.protect™ Mastercycler® pro S or Eppendorf® Mastercycler® ep gradient or equivalent)

  NOTE: The thermal cycler should be able to run the thermal profile at 3ºC/sec ramp rate.

- Pipettes (P10, P100, P200, and P1000)
- Vortex mixer
- Mini centrifuge
- Filtered pipette tips
- Bio-Rad® Microseal® 'A' film (Catalog # MSA5001), or equivalent
- USA Scientific® 0.2 mL PCR tubes (Catalog # 1402-3900)
- Bio-Rad Hard-Shell® Low-Profile Thin-Wall 96-Well Skirted PCR Plate (Catalog # HSP9601), or equivalent

- Fisher Scientific DynaMag™-96 Side Skirted Magnet (Catalog # 12027), or equivalent
- PCR-Cooler rack (Catalog # Eppendorf 022510509), or equivalent
- RNase-free water
- External target specific controls, (optional)
- Sonicator bath (Ultrasonic Cleaner, Cole-Parmer®, Catalog # A-08849-00), or equivalent

Personal Computer

A personal computer with the following minimum requirements is required to run TDAS LSM:

- Operating System: Microsoft® Windows® 7
- CPU: 1 gigahertz (GHz) or faster processor
- Monitor: Resolution of 1024 x 768 pixels or better
- Memory: At least 256 megabytes (MB) of available memory
- Disk Space: At least 1 gigabyte (GB) of free space
- CD or DVD-ROM: At least 24X in speed

Summary of Targets Detected by xMAP® MultiFLEX™ Zika RNA Assay

TABLE 2. **Targets Detected**

Target	Purpose
Zika Virus 1, conserved region 1	Detect Zika Virus
Zika Virus 2, conserved region 2	Detect Zika Virus
Zika Virus 3, conserved region 3	Detect Zika Virus
Zika Virus 4, conserved region 4	Detect Zika Virus
Zika Virus 5, conserved region 5	Detect Zika Virus
Zika Virus 6, conserved region 6	Detect Zika Virus
MS2	Extraction, RT-PCR amplification, and detection control

Warnings and Precautions

1. For *In vitro* diagnostic use under the Emergency Use Authorization only.
2. For prescription use only.
3. In the event of damage to the protective packaging, consult the Safety Data Sheet (SDS) for instructions.
4. Care should be taken when handling, storing, and disposing of potentially infectious materials. Suitable barrier protection against potential pathogens is recommended during all stages of use. Gloves and laboratory coats should be worn at all times. Adherence to appropriate local biosafety and biohazard guidelines or regulations is recommended when working with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. Handle waste disposal in accordance with accepted medical practice and applicable regulations.
5. Luminex recommends that laboratories perform a risk assessment when conducting new tests and safety precautions should be based on the laboratory's risk assessment. Please review CDC guidance for state and local public health laboratories: *http://www.cdc.gov/zika/state-labs/*. See the *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* 5th Edition for additional biosafety information about these viruses and laboratory biosafety practices: *http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.*
6. This procedure should be performed under laboratory safety conditions that take into consideration the potential infectious nature of the serum specimens involved. At a minimum, Luminex recommends that these procedures be performed using BSL-2 facilities and practices. To ensure safety of laboratory personnel, perform all sample manipulations within a Class II (or higher) biological safety cabinet (BSC). Proper disposable gowns, gloves, and eye protection should be worn.
7. Fresh clean gloves must be worn in each area and must be changed before leaving that area.

8. Do not pipette by mouth.
9. For pre-analytical (sample extraction) steps, use the procedure that is provided with the sample extraction system.
10. The nucleic acid extraction procedure should be performed in a separate room from the room where RT-PCR amplification will be performed. The RT-PCR assay should be performed in a separate, template free room. During RT-PCR amplification, portions of the viral genomes are amplified. All original samples should be maintained separately from the PCR room to avoid contamination of samples.
11. Perform the procedure given in this package insert as described. Any deviation from the outlined protocols may result in assay failure or cause erroneous results.

Instrument Setup

Before using the Luminex® instrument for the data acquisition step, follow the procedures for preparation and calibration described in the *xPONENT® for MAGPIX® Software User Manua*l or the *xPONENT® for Luminex® 200™ Software User Manual*.

If using the Luminex® 200™ instrument, manually adjust the probe height for the plate before running the assay.

If using the MAGPIX® instrument, perform the probe height adjustment using the hybridization plate (Bio-Rad® skirted plate) and one alignment sphere. In xPONENT®, the plate name MUST be saved as **MBP_v-bottom plate**.

Prior to hybridization, ensure that the Luminex instrument has been turned on, and run through the daily startup routine. Import the protocol and create a batch containing the appropriate samples for this assay. If using the MAGPIX®, adjust the probe height before turning the plate heater on. Also, ensure that the Luminex instrument plate heater has been set to 52°C.

Instructions for Installing the xPONENT® Data Acquisition Protocol

If the appropriate protocols are already installed on the computer controlling the Luminex® instrument where the assay is being run, skip the following steps.

1. Access the computer that controls the Luminex® system on which the assay will be run.
2. Insert the TDAS LSM for Zika CD into the CD drive of the computer.
3. Start the Luminex® xPONENT® software.
4. From within the Luminex xPONENT software, choose the **Protocols** page.
5. On the **Protocols** page, open the **Protocols** tab, then click **Import**. This launches the **Open** dialog box.
6. In the **Open** dialog box, browse to the **Protocols for Luminex xPONENT** folder on the CD.
7. Select the appropriate protocol file:
   a. If using Luminex® 200™, choose the **xMAP MultiFLEX Zika RNA Assay_LX200[1].lxt** protocol file, then click **Open**.
   b. If using Luminex® MAGPIX®, choose the **xMAP MultiFLEX Zika RNA Assay_MAGPIX[1].lxt** protocol file, then click **Open**.
8. Remove the CD.

Assay Controls

Good laboratory practices recommend using positive and negative controls to assure functionality of reagents and proper performance of the assay procedure.

External Negative Control (Extraction Control) - A confirmed negative sample control should be included in each nucleic acid extraction run/ batch. If the External Negative Control is positive, the specimens processed in the same extraction run should all be re-extracted.

No Template Control (RNase- DNase-free water) - No Template Controls (NTCs) for the amplification/ detection steps are DNase- and RNase free- distilled water in place of specimen nucleic acids and must be included in each assay run. A minimum of one NTC in each assay run is required to monitor for contamination and provide background signals necessary for data processing and making assay calls. The NTC is a control for contamination or improper function of the assay reagents which could result in false positive results.

Internal Control (Bacteriophage MS2) - This internal positive control is added to each test sample, as well as to each external control, prior to extraction. The internal control allows you to ascertain whether the extraction and reverse-transcription/ amplification steps of the assay are functioning correctly.

External Positive Control - In addition to the provided MS2 bacteriophage internal control, additional External Positive Control material that is commercially available from Zeptometrix may be used with the xMAP® MultiFLEX™ Zika RNA Assay based on local guidelines or laboratory SOPs. Commercially available Zika culture fluid (PRVABC59) can be obtained from Zeptometrix (catalog # 0810525CF). This External Positive Control should be processed with the same workflow as clinical specimens. A positive result for the Zika virus specific viral RNA confirms the assay is performing as expected.

Assay Procedure

Sample Preparation

Extract specimens using the BioMerieux® NucliSENS® easyMAG®. Follow manufacturer instructions for instrument set up using the following parameters:

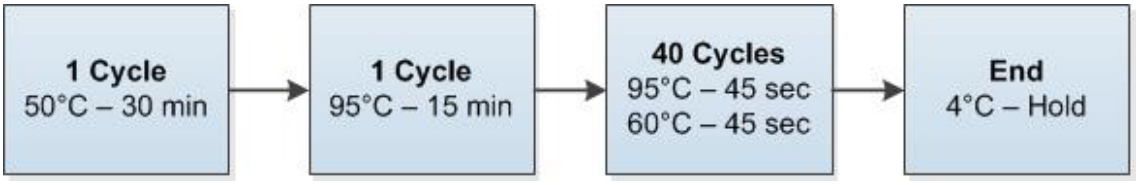
1. Add 5.0 µL of MS2 to 200.0 µL of every specimen and external control.
2. Select **Generic 2.0.1** as the extraction protocol.
3. Select the sample type (for urine, select **other**).
4. Select **0.2 mL** as sample input.
5. Select **25.0 µL** as the elution volume.
6. Select **primary** (on-board) as the lysis method.

RT-PCR Setup

Follow the instructions below to set up and run RT-PCR reactions using the QIAGEN® OneStep RT-PCR Kit.

1. Enter the following RT-PCR program into the thermal cycler with a ramp rate of 3°C/second for all cycling conditions.

FIGURE 2. **RT-PCR Program Setup**



2. Prepare a master mix (MM) according to *Table 3* on page 7. Volumes are for a single reaction.
   a. Multiply each volume by your desired number of reactions, plus one or more No Template Control (NTC) reaction to create an appropriately sized Master Mix.

   NOTE: Volumes do NOT include overage.

   - Vortex and centrifuge each reagent, except the enzyme, for 3 to 5 seconds.

   b. Keep the RT Enzyme Mix and the final Master Mix on a cold block while setting up the RT-PCR reactions. Avoid keeping RT-PCR reagents at room temperature for long periods of time.
   c. Vortex and centrifuge the Master Mix for 3 to 5 seconds before dispensing into individual PCR tubes.

   NOTE: To avoid carry-over contamination from previously amplified RT-PCR products that could potentially generate false positive calls, avoid setting-up RT-PCR Master Mix reactions in the same area where post-amplification is processed. Designate separate areas for pre-amplification and post-amplification, with separate pipettes and reagents. Do not store or add template in the area designated for pre-amplification set-up.

TABLE 3. **Master Mix Volumes**

Reagents	Volume µL Per Reaction
QIAGEN® One-Step RT-PCR Buffer, 5X	5.0 µL
Primer Mix	5.0 µL
RNase-free water	3.0 µL
QIAGEN® One-Step 10 mM dNTP mix	1.0 µL
QIAGEN® One-Step RT-PCR Enzyme	1.0 µL
Total Volume	**15.0 µL**

NOTE: Volumes do NOT include overage.

3. Aliquot 15.0 µL of the Master Mix into the desired number of PCR tubes.
4. Add 10.0 µL of extracted sample (DNA/RNA) or RNase-free water into each PCR tube, using a different tip for each tube.

NOTE: Avoid letting Master Mix and template sit at room temperature while adding template to individual PCR tubes. Use cold blocks to set up RT-PCR reactions.

5. Cap PCR tubes.
6. Vortex and centrifuge the PCR tubes for 5 seconds to ensure all reaction components are at the bottom of the PCR tubes.
7. Place the PCR tubes into the thermal cycler and run the RT-PCR program in *Figure 2* on page 6.
8. Proceed to *"Assay Hybridization"* on page 7.

Assay Hybridization

Follow the instructions below to hybridize the amplified product to Luminex® microspheres and read on the Luminex instrument (MAGPIX® or Luminex® 200™).

1. Ensure the instrument plate heater is set to 52°C prior to sample acquisition.

NOTE: Refer to *"Instrument Setup"* on page 5 for more information regarding to instrument setup.

2. Vortex the stock microsphere mix for 5 to 10 seconds. Sonicate for 10 seconds.
3. Prepare a microsphere reaction that is sufficient for all samples and controls. Prepare the diluted microsphere mixture as follows:

NOTE: Microsphere mixture is light sensitive. Care should be taken to minimize exposure to light.

TABLE 4. **Diluted Microsphere Mix Volumes**

Reagents	Volume
Microsphere Mix	10.0 µL
Buffer A	30.0 µL
Total Volume	**40.0 µL**

NOTE: Volumes do NOT include overage.

4. Obtain the Bio-Rad® 96-Well Skirted PCR Plate to run in your thermal cycler for the hybridization reaction.
5. Vortex the diluted microsphere mixture for 3 to 5 seconds.
6. Pipette 40 µL of the diluted microsphere mixture into each of the wells.
7. Centrifuge the RT-PCR product for 5 to 10 seconds before opening the tubes.
8. Pipette 10 µL of RT-PCR product into each well, using a different tip for each well. Pipette up and down 4 to 5 times to mix.

9. Seal the 96-well PCR plate with Microseal® 'A' film.
10. Incubate the 96-well PCR plate in a thermal cycler with the heated lid enabled, and program the following:

NOTE: During the incubation, prepare the fresh SAPE mixture. Remove the plate immediately after the 19-minute incubation.

11. Prepare a fresh SAPE mix sufficient for all samples and controls as follows:

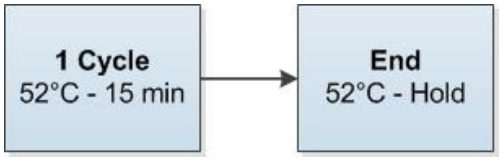
NOTE: SAPE is light sensitive. Care should be taken to minimize exposure to light.

TABLE 5. **Diluted SAPE Volumes**

Reagents	Volume
Stock SAPE	0.3 µL
Buffer B	74.7 µL
Total Volume	**75.0 µL**

NOTE: Volumes do NOT include overage.

12. Vortex the diluted stock SAPE for 3 to 5 seconds.
13. Remove the 96-well PCR plate from the thermal cycler. Carefully remove the Microseal 'A' film from the plate to avoid cross-contamination.
14. Place the 96-well PCR plate onto a magnetic plate separator and allow microspheres to separate into a pellet for 2 minutes.
15. Remove all supernatant from each well, using a different tip for each well. Take care not to disturb the microsphere pellet.
16. Remove the 96-well PCR plate from the magnetic plate separator.
17. Pipette 75 µL of freshly made SAPE dilution mixture into each well containing microspheres.
18. Resuspend the microsphere pellet by pipetting up and down 4 to 5 times.
19. Seal the 96-well PCR plate with a new Microseal 'A' film and return the plate to the thermal cycler, and program the following:



NOTE: Remove plate immediately after the 15 minute incubation.

20. Remove the 96-well PCR plate from the thermal cycler. Carefully remove the Microseal 'A' film from the plate to avoid cross-contamination.
21. Transfer the 96-well PCR plate directly to the pre-heated block on the Luminex instrument.

NOTE: Ensure that the Luminex instrument plate heater is pre-heated to 52°C before running the assay.

22. Analyze the plate using the included xPONENT® protocol.

Data Analysis

Once the data is acquired from the previous steps, an output file is created under the Luminex® batch run folder. Use the Data Analysis Software (TDAS LSM for Zika) to analyze this file.

Installing TDAS LSM for Zika

NOTE: Skip this section if the proper version of **TDAS LSM for Zika** is already installed.

To install the software, complete the following:

1. Ensure that you have sufficient Windows® administrative privileges to install software on the computer.
2. Insert the **TDAS LSM for Zika** CD.
3. Launch **My Computer**.
4. Browse to the CD drive, then double-click **TDAS LSM for Zika** set-up.
5. Follow the on-screen instructions to complete the installation.

Verifying the Version of TDAS LSM for Zika

To confirm that the correct version of TDAS is installed, complete the following.

1. Double-click the **TDAS LSM for Zika** icon. The **Log-on TDAS LSM for Zika** dialog box opens. Ensure that the TDAS version in the dialog box matches the software version on the kit label.
2. Log into **TDAS LSM for Zika**. Use a password if password protection was enabled during installation.
3. On the **Help** menu, click **About TDAS…**.
4. Verify that the correct software version appears in the **About TDAS LSM for Zika** dialog box.
5. Verify that the installed assay is the xMAP® MultiFLEX™ Zika RNA Assay.

   NOTE: Verifying the installed assay is critical for proper analysis.

6. Click **Close** in the **About TDAS LSM for Zika** dialog box.
7. If the above steps fail, uninstall the software. Follow the steps in the *"Installing TDAS LSM for Zika"* on page 9 procedure to re-install the software.
8. Remove the CD.

Analyzing Data with TDAS LSM for Zika

To perform data analysis, complete the following:

1. Verify that the Luminex® output file is accessible by the computer where TDAS LSM for Zika is installed.
2. Launch TDAS LSM for Zika on your computer through **Start** > **All Programs** or by double-clicking the desktop icon.
3. On the **File** menu, click **Open**.
4. Browse and click on the output file. Ensure that TDAS LSM for Zika recognized the selected file and will analyze it using the xMAP® MultiFLEX™ Zika RNA Assay (IVD-EUA) configuration file.
5. Click **Open**.
6. If the batch data is opened for the first time on the computer for analysis, identify the no template controls (NTC).
   a. In the **Identify Samples** dialog box, choose the **Sample ID** for each NTC. (Click the **Sample ID** again to clear.)
   b. Click **Next**.
7. Verify your NTC selections in the **Confirmation** dialog box. Confirm the selections by selecting check box **All sample settings are correct**.

   NOTE: Once the selections are confirmed and applied, all subsequent analyses of this batch data are performed using the settings selected for the first analysis. Click the **Back** button if modification is necessary.

8. Click **Apply** to see the analysis results.

Results Interpretation

All interpretation of test results must be performed using Data Analysis Software LSM (TDAS LSM for Zika) in conjunction with the xMAP® MultiFLEX™ Zika RNA Assay configuration file.

Target Calls

- POS: Zika virus RNA detected.
- NEG: Zika virus RNA not detected.
- No Call: there is a failure in one or more assay parameters/controls.

Control Calls

- PRES: the recommended Internal Control is detected.
- ABS: the recommended Internal Control is not detected.
- No Call: unable to determine presence or absence of the Internal Control due to an assay-specific criterion not being met.

Using TDAS LSM for Zika, controls and individual test sample results are then interpreted according to the following algorithm.

- Consider all targets for the negative control(s).
  - An External Negative Control (Extraction Control) should be included in each nucleic acid extraction run/ batch. If the External Negative Control is positive, the specimens processed in the same extraction run should all be re-extracted.
  - If all of the NTCs fail to meet the criteria, then the plate fails. All samples will be labeled as “No Call”.
  - If any NTC meets all criteria, then the software will select the last successful control as the primary negative control and use this control to define background levels for the entire plate.
  - If any result is positive for any target for an NTC, then the run is considered invalid and should be re-run.
  - If all NTCs meet all criteria, then the plate will be considered free of contamination and all positive results for samples will be considered valid.
- Consider all targets for the External Positive Control
  - In addition, an External Positive Control may be used with the test based on local guidelines or laboratory SOPs. If the External Positive Control is negative, the specimens processed in the same extraction run should all be re-extracted.
- Consider all targets for samples.
  - If any result is positive for any target other than MS2, then the positive targets will be identified as “POS” and the target is considered “Detected” for that sample.
    - “”Detected” results at least one of the six Zika virus specific target regions (beads) are considered Zika RNA positive.
  - If all non MS2 targets are negative for a sample, then consider MS2.
    - If MS2 is present, then all other targets will be identified as “NEG” and no targets are detected for that sample.
    - If MS2 is absent, then all other targets are identified as “No Call” for that sample and the sample fails.

NOTE: A patient matched serum specimen is required for serological follow up testing of all Zika virus RNA negative results, per the CDC testing algorithm. (Found at *http://www.cdc.gov/zika/index.html*).

Assay Limitations

1. All results should be interpreted by a trained professional in conjunction with the review of the patient’s history, clinical signs, and symptoms.
2. The assay is for *In vitro* diagnostic use under the FDA Emergency Use Authorization (EUA) and is limited to U.S. laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests, and similarly qualified non-U.S. laboratories only.
3. Interpretation of results must account for the possibility of false-negative and false-positive results.
4. False positive results can be caused by:
   - Contamination with previously amplified nucleic acids

- Carry-over from samples processed in the same batch

5. False negative results can be caused by:
   - Poor sample collection
   - Degradation of viral nucleic acids during shipping or storage
   - Specimen collection prior to symptom onset
   - Specimen collection after the viremic phase (up to 14 days post-onset of symptoms for serum samples)
   - Failure to follow assay procedures
   - Use of unauthorized extraction method or platform
6. Adverse interfering effects have been observed on detecting low levels of Zika virus RNA when elevated levels of Dengue 2 virus RNA are also present in the same specimen.
7. Negative results do not preclude infection and should not be used as the sole basis of patient treatment/management decisions.
8. Proper collection, storage, and transport of samples are critical for achieving accurate results.
9. Extraction of nucleic acid from clinical specimens must be performed with the specified extraction methods listed in this procedure. Extraction methods not listed in this procedure have not been evaluated for use with this assay.
10. All specimens should be handled as if they are infectious. Proper biosafety precautions must be used.
11. Performance has only been established with the specimen types listed in the *"Intended Use"* on page 1. Other specimen types are not acceptable for use with this assay.
12. The Luminex® xMAP® MultiFLEX™ Zika RNA Assay described here has not been extensively tested with clinical specimens. Modifications of these assays (i.e., use of platforms or chemistries other than those described) is not permitted. These assays should not be further distributed without the explicit consent from Luminex.
13. **A patient matched serum specimen is required for serological follow up testing of negative results, per the CDC testing algorithm. (Found at *http://www.cdc.gov/zika/index.html*).**
14. This test cannot rule out diseases caused by other bacterial or viral pathogens.

Performance Characteristics

Analytical Sensitivity - Limit of Detection

The Limit of Detection (LoD) for Zika virus (Asian lineage) using the xMAP® MultiFLEX™ Zika RNA Assay was established and verified in a series of studies.

Limit of Detection (LoD) for Zika virus on the xMAP MultiFLEX Zika RNA Assay was determined evaluating a Zika virus strain of the Asian Zika virus lineage.

- Zika Virus Asian lineage strain H/PF/2013 - Zika standard #1, inactivated. GenBank ID KJ776791.1 provided by European Virus Archives (EVA Marseille, France).

Determination of Preliminary Limit of Detection

The Zika virus initial stock concentration was determined using the titer provided by the vendor of 5.5×10^6 copies RNA/200 µL. The Zika virus was quantified by the vendor in $TCID_{50}$ before inactivation and then the stock concentration was determined by the vendor in RNA copies by qRT-PCR after inactivation. The Zika virus standard was spiked into 200 µL of negative human serum, plasma, and urine. Serial dilutions were prepared, followed by nucleic acid purification of 200 µL input volume on the BioMerieux® NucliSENS® easyMAG® extraction system followed by a 25 µL elution. From this elution, 10 µL eluate was used as input into the xMAP® MultiFLEX™ Zika RNA Assay RT-PCR reaction. The assay was performed and then detected on MAGPIX® system. Each concentration was extracted, amplified and hybridized in triplicate (n=3). The lowest concentration at which all 3 replicates were detected was used to determine the preliminary Zika LoD concentration.

TABLE 6. **Determination of the Preliminary LoD**

Target	Matrix	Dilution Factor	Testing Concentration Copies/mL	Call Rate
Zika virus (strain H/PF/2013) Stock at 5.5 x 10^6 copies RNA/200uL	Serum	1:40,000	687	3/3
		1:160,000	172	2/3
		1:320,000	86	1/3
		1:640,000	43	3/3
	Plasma	1:12,649	2,174	3/3
		1:40,000	687	3/3
		1:126,491	217	3/3
		1:400,000	68	1/3
	Urine	1:40,000	687	3/3
		1:160,000	172	2/3
		1:320,000	86	2/3
		1:640,000	43	3/3

Confirmation of the Limit of Detection (LoD)

Based on the preliminary Zika LoD determination, a second study was performed by spiking the Zika virus standard stock into pooled negative human plasma, serum, and urine samples. Twenty (20) independent (n=20) extractions were performed as previously described, followed by amplifications, hybridizations, and analysis on the Luminex® 200™ and MAGPIX® instruments at the preliminary LoD concentration and at 2 to 3 fold lower. Replicates were considered positive if the Median Fluorescent Intensity (MFI) was greater than or equal to 150 MFI and greater than or equal to two times the MFI of the No Template Control (NTC).

The final LoD for Zika virus was confirmed in all matrices when the results of the 20 replicates were positive with a 95.0% Confidence Interval as presented in *Table 7* on page 12. The LoD was established at a final concentration of 687 copies/mL of the Zika RNA.

TABLE 7. **Zika Virus (in Human Serum, Urine, and Plasma)**

Matrix Tested	Conc. Tested	Number Positive/ Number Tested	
EVA Zika Virus Standard H/PF/2013 (copies/mL)		MAGPIX®	Luminex® 200™
Serum	687	20/20	20/20
Serum	343	15/20	15/20
Urine	687	20/20	20/20
Urine	343	17/20	17/20
Plasma	687	20/20	20/20
Plasma	217	18/20	NA

Analytical Sensitivity- FDA Reference Material

An analytical study was performed using reference material (S1 and S2) and a standard protocol provided by the FDA, which includes a LoD range finding study and a confirmatory LoD study, to evaluate the analytical sensitivity of the xMAP® MultiFLEX™ Zika RNA Assay. The results are presented in *Table 8* on page 13:

TABLE 8. **Summary of LoD Confirmation Results Using the FDA Reference Materials**

Reference Materials	Specimen Type	Confirmed LOD* in RNA NAAT Detectable Units/mL
S1	Serum	3000
S1	Urine	3330
S2	Serum	5000
S2	Urine	5000

*Study preformed according to the FDA issued protocol

Analytical Reactivity - Inclusivity

Inclusivity was evaluated for the xMAP® MultiFLEX™ Zika RNA Assay empirically with both African and Asian lineage Zika virus strains acquired from vendors. For the Martinique and ZIKV 2007 strains, stocks were prepared by spiking the acquired stock material with known titers into plasma, serum, and urine and serially diluted. Nucleic acid from each serially diluted stock was purified in triplicate (n=3) using 200 µL input volume on the BioMerieux® NucliSENS® easyMAG® extraction system, with a 25 µL elution. From this elution, 10 µL was used as input into the xMAP MultiFLEX Zika RNA Assay RT-PCR reaction. The MR766 Zika strain acquired from EVA was added directly to the xMAP MultiFLEX Zika RNA Assay RT-PCR reaction. The RT-PCR reaction was performed and detected with the MAGPIX® instrument in triplicate (n=3). The Zika virus concentrations were determined using the copies/mL provided by the vendor. The test results are shown in *Table 9* on page 13.

TABLE 9. **Zika Inclusivity Empirical Testing Summary**

Zika Strain Tested	Concentration (copies/mL)	Matrix	Number Positive/ Number Tested
ZIKV 2007 - synthetic material from Sera Care GenBank EU545988.1	1000	serum	3/3
	500		3/3
	250		2/3
	1000	plasma	3/3
	500		3/3
	250		2/3
	1000	urine	3/3
	500		3/3
	250		3/3
Zika virus - MRS_OPY_Martinique_PaRi_2015 (EVA / 33219) GenBank KJ647676	700	serum	2/3
		plasma	3/3
		urine	3/3
Zika virus - MR 766 - BEI /NR-50065 GenBank AY632535.2	30400	N/A	3/3
	9600		3/3
	3000		3/3
	1000		3/3

In silico analysis of the primer and probe sequences was performed to verify reagent sequence homology with each target region. A total of 28 current and historical Zika strains were selected for *in silico* analysis. For each Zika strain analyzed, at least one primer and probe set had sequences with >95.0% sequence identity. In addition, many Zika strains show a high degree of homology, with multiple primer and probe sets allowing for redundancy and assurance that false negative Zika results are not likely to occur.

TABLE 10. **Zika *in silico* Primer and Probe Sequence Identity Summary**

	Zika 5			Zika 6			Zika 1			Zika 2			Zika 3			Zika 3 modified			Zika 4		
	fwd	**rev**	**probe**	**fwd**	**rev**	**probe**	**fwd**	**rev**	**probe**	**fwd**	**rev**	**probe**	**fwd**	**rev**	**probe**	**fwd**	**rev**	**probe**	**fwd**	**rev**	**probe**
AY632535.2	100%	90%	87%	96%	100%	85%	100%	100%	100%	100%	100%	100%	100%	100%	100%	86%	100%	100%	100%	100%	100%
HQ234498.1	100%	90%	87%	96%	100%	85%	100%	100%	100%	100%	100%	100%	100%	100%	--	86%	100%	--	100%	100%	100%
KU720415.1	100%	90%	87%	96%	100%	85%	100%	100%	100%	100%	100%	100%	100%	100%	100%	86%	100%	100%	100%	100%	100%
LC002520.1	100%	90%	87%	96%	100%	85%	100%	100%	100%	100%	100%	100%	100%	100%	100%	86%	100%	100%	100%	100%	100%
KF383118.1	100%	90%	87%	92%	100%	90%	100%	100%	100%	95%	100%	100%	100%	100%	--	86%	100%	--	95%	100%	95%
KF383119.1	100%	90%	87%	96%	100%	85%	100%	100%	100%	100%	100%	100%	100%	100%	--	86%	100%	--	100%	100%	100%
EU545988.1	100%	95%	100%	96%	100%	100%	100%	88%	87%	92%	100%	91%	92%	100%	--	93%	100%	--	91%	95%	85%
KU681082.3	100%	95%	100%	92%	100%	100%	100%	88%	87%	92%	100%	88%	88%	100%	97%	96%	100%	97%	94%	91%	85%
KJ776791.1	100%	100%	100%	96%	100%	100%	100%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU681081.3	100%	100%	98%	96%	100%	98%	100%	90%	86%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	88%
KU509998.1	100%	100%	100%	96%	100%	100%	100%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU365778.1	100%	100%	100%	96%	100%	100%	92%	90%	87%	92%	100%	91%	88%	96%	97%	96%	96%	97%	91%	91%	85%
KU527068.1	95%	100%	100%	96%	100%	98%	100%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU707826.1	100%	100%	100%	96%	100%	100%	100%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU312312.1	100%	100%	100%	96%	100%	98%	92%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	--
KU501217.1	100%	100%	100%	96%	100%	100%	100%	90%	87%	92%	100%	91%	88%	100%	--	96%	100%	--	91%	91%	85%
KU497555.1	100%	100%	100%	100%	100%	100%	100%	90%	87%	92%	100%	91%	100%	100%	97%	100%	100%	97%	95%	91%	85%
KU501215.1	100%	100%	100%	96%	100%	100%	92%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU321639.1	100%	100%	100%	96%	100%	100%	100%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU647676.1	100%	100%	100%	96%	100%	100%	100%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU853012.1	95%	100%	100%	96%	100%	100%	93%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU853013.1	95%	100%	100%	96%	100%	100%	93%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU744693.1	100%	96%	100%	96%	100%	98%	100%	95%	87%	100%	100%	94%	88%	100%	97%	96%	100%	97%	91%	91%	--
KU820899.2	100%	100%	100%	96%	100%	100%	100%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU922923.1	100%	100%	100%	96%	100%	100%	100%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU922960.1	100%	100%	100%	96%	100%	100%	100%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KU740184.2	100%	100%	100%	96%	100%	100%	92%	90%	87%	92%	100%	91%	88%	100%	97%	96%	100%	97%	91%	91%	85%
KF383121.1	100%	90%	87%	96%	100%	85%	100%	100%	100%	100%	100%	100%	100%	100%	--	100%	100%	--	100%	100%	100%

Exclusivity – Cross Reactivity

A panel of viruses and organisms known to cause similar signs and symptoms of the Zika virus infection were selected for analysis in the exclusivity/cross reactivity study for the xMAP® MultiFLEX™ Zika RNA Assay. Zika virus, Dengue 1, Dengue 2, Dengue 3, Dengue 4, Chikungunya, West Nile, Yellow Fever, plasmodium falciparum, Parvo, and Mayaro were tested at high concentrations to monitor for cross reactivity with the assay primers and probes.

Dilutions were prepared by spiking tissue culture fluid purchased from a vendor (Zeptometrix) for each organism into negative human serum, plasma, and urine. Nucleic acid was purified with 200 µL input volume on the BioMerieux® NucliSENS® easyMAG® extraction system, with a 25 µL elution. From the elution, 10 µL was used as input into the xMAP MultiFLEX Zika RNA Assay RT-PCR reaction. The assay was performed and then detected with the MAGPIX® instrument. Each prepared sample was extracted, amplified and hybridized in triplicate (n=3). No cross reactivity was observed with any potential cross reacting organisms tested.

The data below demonstrates lack of cross reactivity among symptom-similar organisms in serum, plasma, and urine. Strains and concentrations tested are identified in *Table 11* on page 15.

TABLE 11. **Exclusivity Summary for (Serum, Plasma, and Urine)**

Specimen/Target	Concentration Tested/Unit	Zika Result
Dengue virus Type 1 - Hawaii	$1 \times 10^{4.39}$ $TCID_{50}$/mL	No cross- reactivity
Dengue virus Type 2 -New Guinea	$1 \times 10^{4.39}$ $TCID_{50}$/mL	No cross- reactivity
Dengue virus Type 3 -H87	$1 \times 10^{4.62}$ $TCID_{50}$/mL	No cross- reactivity
Dengue virus Type 4 -H241	$1 \times 10^{5.53}$ $TCID_{50}$/mL	No cross- reactivity
West Nile virus - 1986	$1 \times 10^{4.82}$ $TCID_{50}$/mL	No cross- reactivity
Yellow fever - 17D	$1 \times 10^{5.96}$ $TCID_{50}$/mL	No cross- reactivity
Chikungunya – R80422	1:10 dilution N/A	No cross- reactivity
Plasmodium falciparum IPC 5202 - Battambang Cambodia 2011	1:10 dilution % infected cells	No cross- reactivity
Mayaro virus -TRVL 15537	$1 \times 10^{5.10}$ LD_{50}/gm in SM	No cross- reactivity
Parvo virus - B19	5×10^{5} IU/mL	No cross- reactivity
Zika Asian Lineage - PRVABC59	$1 \times 10^{4.34}$ $TCID_{50}$/mL	Positive
Zika Asian Lineage - H/PF/2013	$1 \times 10^{5.16}$ $TCID_{50}$/mL	Positive

In silico evaluation of the analytical specificity was performed for each primer and probe sequence. BLAST® analysis queries of the primers were performed against the GenBank public domain nucleotide sequences and showed no significant combined (forward primer, reverse primer, and capture probe) homologies with other conditions that would predict potential false positive results. The BLAST algorithms were set to: blastn, word size 15, maximal target sequences 1000, match/mismatch scores 1,-2. All primers and probes were blasted individually.

In addition, *in silico* analysis (BLAST Results) for each of the organisms in *Table 12* on page 16 was performed. The primer and probe sequences were blasted against selected species (viruses, bacteria, and parasites) and no significant cross-reactivity was identified based on the specific prediction rules applied in the *in silico* analysis.

TABLE 12. **Organisms Analyzed for *in silico* Cross Reactivity**

Organism		Organism (taxid)
Bacteria	*Borrelia burgdorferi*	64895
	Group A Strep	36470
	Salmonella spp.	590
	Escherichia coli	562
	Leptospira spp.	171
	Rickettsia spp.	780
Trematodes	*Schistosoma* spp.	6181
Protozoa	*Plasmodium vivax*	5855
	Plasmodium spp.	5820
	Trypanosoma cruzi	5693
Flavivirus	Zika (DENV and CHIKV)	64320
	Dengue (ZIKV and CHIKV)	11052
	WNV	11082
	YFV	40005
	SLEV	11080
	Spondweni virus	64318
	JEEV	11071
Alphavirus	Chikungunya (ZIKV and DENV)	37124
	EEEV	11021
	WEEV	11039
	Ross River virus	11029
	Barmah Forest virus	11020
	O'nyong-nyong virus	11027

Organism		Organism (taxid)
Other virus	Measles virus	11234
	Rubella virus	11041
	Cytomegalovirus	10358
	VZV	10335
	Epstein-Barr virus	10376
	HIV	11676
	Hepatitis A	12092
	Hepatitis B	10407
	Hepatitis C	11102
	Enterovirus	12059
	Adenovirus	108098
		130310
		129951
		565302
		10519

Interference Study

Interference studies were not performed since conventional, well-established methods were used for both nucleic acid extraction and RT-PCR amplification.

Co-infection/Competitive Interference Study

To determine if competitive interference is present in the assay, co-infection studies were performed using EVA Zika Standard # 1 H/PF/2013 spiked into two different matrices; serum and urine. Zika target was spiked at 3x LoD (2.06×10^3 copies/mL), while the co-infecting target was spiked at the highest concentration possible to determine potential interference.

Zika virus, near LoD, was detected in all triplicates along with the co-infected target in all case scenarios, except in the presence of high levels of Dengue virus type 2 when spiked in serum or urine matrices. This is expected because internal studies indicate that in the presence of high concentrations of Dengue virus type 2 (approximately at 1×10^5 $TCID_{50}$/mL), the RT-PCR demonstrates limited amplification for Zika targets in the panel at concentrations close to their LoD. No cross-reactivity with non-intended targets was observed.

In order to establish the assay's ability for low level Zika virus detection in the presence of high concentrations of Dengue virus type 2, contrived co-infected titration was performed by spiking EVA Zika Std1 at 3x LoD and Dengue virus type 2 at different concentrations to determine at which Dengue 2 concentration Zika RNA is detected.

Dual detection of co-infected specimens with Zika at concentrations near the LoD and high concentration of Dengue virus type 2 can be achieved when Dengue type 2 viral loads are as high as $10^{3.6}$ $TCID_{50}$/mL in both serum and urine matrices. Dengue type 2 viral loads higher than this will preclude detection of low levels of Zika in specimens containing both targets.

Bridging Studies

To demonstrate equivalence among three matrices (serum, plasma, and urine) and to demonstrate equivalence between the MAGPIX® and Luminex® 200™ systems, the LoD study was repeated using all three matrices and both systems. The results are shown in *Table 13* on page 18. Additional matrix equivalency was also demonstrated during LoD range finding, inclusivity, and exclusivity studies as described in prior sections.

TABLE 13. **Matrix (serum, urine, and plasma) and Instrument (MAGPIX® and Luminex® 200™) Equivalence**

Matrix Tested	Conc. tested	Number positive/ number Tested	
EVA Zika Virus Standard H/PF/2013 (copies/mL)		MAGPIX®	Luminex® 200™
Serum	687	20/20	20/20
Serum	343	15/20	15/20
Urine	687	20/20	20/20
Urine	343	17/20	17/20
Plasma	687	20/20	20/20
Plasma	217	18/20	NA

Clinical Evaluation

Clinical performance was evaluated with a combination of retrospective specimens and contrived samples.

Zika Clinical Testing

Matched human serum, urine, and plasma specimens were procured from a commercial source. Matched human specimens were collected in the Dominican Republic from 35 individual patients suspected of Zika virus infection. Plasma samples from these patients were found to be Zika virus RNA positive by the vendor using an RT-PCR assay. Samples were collected from patients between 1-7 days post-onset of symptoms.

The specimens were analyzed using a Zika virus real-time RT-PCR test under an EUA as the comparator method.

TABLE 14. **Zika Human Clinical Testing Results**

Sample ID (1043-TDS)	xMAP® MultiFLEX™ Zika RNA Assay				Comparator Method (Ct Values)		
	Urine	Serum	Plasma	Patient Call	Urine	Serum	Patient Call
0180	POS	POS	POS	POS	36.5	25.4	POS
0183	NEG	POS	POS	POS	Undetermined	31.7	POS
0184	POS	POS	POS	POS	39.1	33.9	POS
0197	POS	POS	POS	POS	39.4	36.0	POS
0203	POS	POS	POS	POS	37.5	35.5	POS
0206	POS	NEG	NEG	POS	Undetermined	39.2	POS
0208	POS	POS	POS	POS	Undetermined	25.5	POS
0211	POS	POS	POS	POS	36.1	35.4	POS
0216	POS	POS	NEG	POS	36.0	29.9	POS
0223	POS	POS	POS	POS	34.0	33.7	POS
0228	POS	NEG	NEG	POS	38.6	Undetermined	POS
0230	POS	NEG	POS	POS	35.3	Undetermined	POS
0257	POS	POS	POS	POS	32.1	32.4	POS
0263	POS	POS	POS	POS	36.3	30.2	POS
0190	NEG	POS	POS	POS	Undetermined	33.8	POS

Sample ID (1043-TDS)	xMAP® MultiFLEX™ Zika RNA Assay				Comparator Method (Ct Values)		
	Urine	Serum	Plasma	Patient Call	Urine	Serum	Patient Call
0195	POS	POS	POS	POS	36	31.9	POS
0218	NEG	POS	POS	POS	Undetermined	37.2	POS
0232	POS	NEG	POS	POS	36.4	38.5	POS
0254	NEG	POS	POS	POS	Undetermined	34.8	POS
0182	POS	POS	POS	POS	34.7	34.2	POS
0196	POS	POS	POS	POS	34.0	35.1	POS
0201	POS	POS	POS	POS	33.7	36.6	POS
0214	POS	POS	POS	POS	34.9	38.1	POS
0220	NEG	POS	NEG	POS	Undetermined	Undetermined	NEG
0221	POS	POS	NEG	POS	35.4	Undetermined	POS
0226	NEG	NEG	NEG	NEG	39.4	Undetermined	POS
0231	POS	NEG	POS	POS	30.0	Undetermined	POS
0238	POS	POS	POS	POS	39.0	34.3	POS
0243	NEG	POS	POS	POS	Undetermined	33.8	POS
0247	POS	POS	POS	POS	36.2	35.1	POS
0255	NEG	NEG	POS	POS	37.8	Undetermined	POS
0265	POS	POS	POS	POS	36.9	35.2	POS
0269	POS	POS	POS	POS	37.0	30.6	POS
0270	POS	POS	POS	POS	36.3	35.7	POS
0224	POS	POS	POS	POS	35.9	35.4	POS

Seven Zika virus RNA negative serum specimens were identified by the comparator method. In addition, 99 individual expected Zika virus RNA negative serum specimens collected from U.S. patients by a U.S. vendor were tested using the xMAP® MultiFLEX™ Zika RNA Assay.

A total of 20 contrived Zika samples were also prepared by spiking Zika strain H/PF/2013 into individual serum samples. Ten (10) samples were prepared at 2x LoD (low), seven samples were prepared at 4x LoD (mid), and three samples were prepared at 10x LoD (high). These contrived serum samples were used to supplement the 28 individual Zika virus RNA positive clinical serum specimens (as determined by the comparator method) for a total of 48 Zika positive serum samples.

Using the xMAP MultiFLEX Zika RNA Assay, Zika virus RNA was detected at very low levels in two specimens (specimen 0220 and 0221) of the 106 negative clinical specimens. Zika virus was detected in all 20 contrived serum positives and 26 out of 28 natural clinical positive serum specimens. The comparator method gave high CT values of 39.2 and 38.5 for the two serum specimens (specimen 0206 and 0232) which were not detected in the xMAP MultiFLEX Zika RNA Assay.

TABLE 15. **Clinical Evaluation Results for Detecting Zika Virus RNA in Human Serum**

Serum Category	Number Tested	xMAP® MultiFLEX™ Zika RNA Assay	
		Zika Positive	Zika Negative
Natural Zika Positive Serum Specimens (as determined by comparator result in serum)	28	26/28	2/28
Contrived Zika Positive (2x LoD)	10	10/10	0/10
Contrived Zika Positive (4x LoD)	7	7/7	0/7
Contrived Zika Positive (10x LoD)	3	3/3	0/3
Zika Negative Clinical Serum Specimens from U.S. Donors	99	0/99	99/99
Zika Negative Clinical Serum Specimens (as determined by comparator from suspected cases in endemic areas)	7	2/7	5/7
Positive Percent Agreement	95.8% (46/48) 95.0% CI: 85.1% - 99.5%		
Negative Percent Agreement	98.1% (104/106) 95.0% CI: 93.4% - 99.5%		

Eight (8) Zika virus RNA negative urine specimens were identified by the comparator method. In addition, 54 individual negative urine specimens collected from U.S. patients by a U.S. vendor were tested using the xMAP MultiFLEX Zika RNA Assay.

A total of 25 contrived Zika urine samples also were prepared by spiking Zika strain H/PF/2013 into individual urine samples. Twelve (12) samples were prepared at 2x LoD (low), 8 samples were prepared at 4x LoD (mid), and 5 samples were prepared at 10x LoD (high). These contrived urine samples were used to supplement the 27 individual Zika virus RNA positive clinical urine specimens (as determined by the comparator method) for a total of 52 Zika positive urine samples.

Using the xMAP MultiFLEX Zika RNA Assay, Zika virus RNA was detected at very low levels in two specimens (specimen 0206 and 0208) of the 63 negative clinical urine specimens. Zika virus RNA was detected in 24 out of 25 contrived urine positives and 26 out of 28 natural clinical positive urine specimens. The comparator method gave CT values of 39.4 and 37.8 for the two urine specimens (specimen 0226 and 0255) which were not detected in the xMAP MultiFLEX Zika RNA Assay.

TABLE 16. **Clinical Evaluation Results for Detecting Zika Virus RNA in Human Urine**

Urine Category	Number Tested	xMAP® MultiFLEX™ Zika RNA Assay	
		Zika Positive	Zika Negative
Natural Zika Positive Urine Specimens (as determined by comparator result in urine)	28	26/28	2/28
Contrived Zika Positive (2x LOD)	12	11/12	1/12
Contrived Zika Positive (4x LOD)	8	8/8	0/8
Contrived Zika Positive (10x LOD)	5	5/5	0/5
Zika Negative Clinical Urine Specimens from US donors	54	0/54	54/54
Zika negative Urine Specimens as determined by comparator from suspected Zika cases in endemic areas.	8	2/8	6/8
Positive Percent Agreement	94.3% (50/53) 95.0% CI: 84.6% -98.1%		
Negative Percent Agreement	96.8% (60/62) 95.0% CI: 89.3% - 99.1%		

Additional analysis was performed based on the comparator method's call algorithm whereby a positive result for either serum or urine (patient matched specimens) results in a positive patient infected status. Thirty-four (34) out of 35 patients were confirmed positive by having either a positive serum or urine result in the comparator test. Based on the xMAP® results using serum, and urine, the xMAP MultiFLEX Zika RNA Assay had one false positive (specimen 0220) and two false negatives (specimen 0226 and 0255) when compared to the comparator method. Based on the xMAP results using plasma and urine, the xMAP MultiFLEX Zika RNA Assay had no false positives and one false negative (specimen 0226) when compared to the comparator.

One Zika negative was identified by the comparator's call algorithm. In addition, performance data generated from testing 99 individual expected Zika virus negative serum specimens (collected from U.S. patients by a U.S. vendor) using the xMAP MultiFLEX Zika RNA Assay was also included in this additional analysis.

TABLE 17. **Clinical Evaluation Results for xMAP Zika RNA Clinical Call Based on Serum, and Urine**

Specimen Category	Number Tested	xMAP® MultiFLEX™ Zika RNA Assay	
		Zika Positive	Zika Negative
Natural Zika Positive Patient Result (as determined by comparator patient infected status)	34	32/34	2/34
Zika Negative Patient Results from US donors	99	1/99	99/99
Zika Negative Patient Results as determined by comparator patient infected status for suspected Zika cases in endemic areas.	1	1/1	0/1
Positive Percent Agreement	94.1% (32/34) 95.0% CI: 80.9% - 98.4%		
Negative Percent Agreement	99.0% (99/100) 95.0% CI: 94.6% - 99.8%		

TABLE 18. **Clinical Evaluation Results for xMAP Zika RNA Clinical Call Based on Plasma, and Urine**

Specimen Category	Number Tested	xMAP® MultiFLEX™ Zika RNA Assay	
		Zika Positive	Zika Negative
Natural Zika Positive Patient Result (as determined by comparator patient infected status)	34	33/34	1/34
Zika Negative Clinical Patient Results from US donors	99	0/99	99/99
Zika negative Patient Results as determined by comparator patient infected status for suspected Zika cases in endemic areas.	1	0/1	1/1
Positive Percent Agreement	97.1% (33/34) 95.0% CI: 85.1% - 99.5%		
Negative Percent Agreement	100.0% (100/100) 95.0% CI: 96.3% - 100.0%		

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