Zika ELITe MGB® Kit U.S.
reagents for RNA reverse transcription and cDNA Real Time amplification

REF **M800552**

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Reagents for RNA Real-Time RT-PCR amplification

FOR USE UNDER AN EMERGENCY USE AUTHORIZATION (EUA) ONLY

REF **M800552** IVD Rx only -20°C

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INTENDED USE

The **Zika ELITe MGB® Kit U.S.** is a real-time RT-PCR test intended for the qualitative detection of RNA from the Zika virus in serum and EDTA plasma 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 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.

Test results are for the identification of Zika virus RNA, which is generally detectable in serum and plasma during the acute phase of infection. 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 **Zika ELITe MGB® Kit U.S.** is intended for use by trained clinical laboratory personnel who have received specific training on the use of the **Zika ELITe MGB® Kit U.S.** on the **ELITe InGenius™** instrument. The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

BACKGROUND

Zika virus (ZIKV) is a member of the virus family Flaviviridae and the genus Flavivirus, transmitted by daytime-active Aedes mosquitoes, such as A. aegypti. Its name comes from the Zika Forest of Uganda, where the virus was first isolated in 1947 from a Rhesus monkey. Until last year Zika was not thought to pose serious health risks. There were multiple small Zika outbreaks from 1947 to 2007, but all were minor without significant clinical presentations. In 2007 75% of the residents of Zap island developed Zika in the largest outbreak to date, with over 80 percent of people who became infected never experiencing symptoms. For example, only one death has been reported in a patient with Zika (Thrombocytopenia). This is in contrast to Dengue, a related virus that can lead to hemorrhagic fever and death. There have been isolated reports for years that Zika can be transmitted by human to human sexual contact.

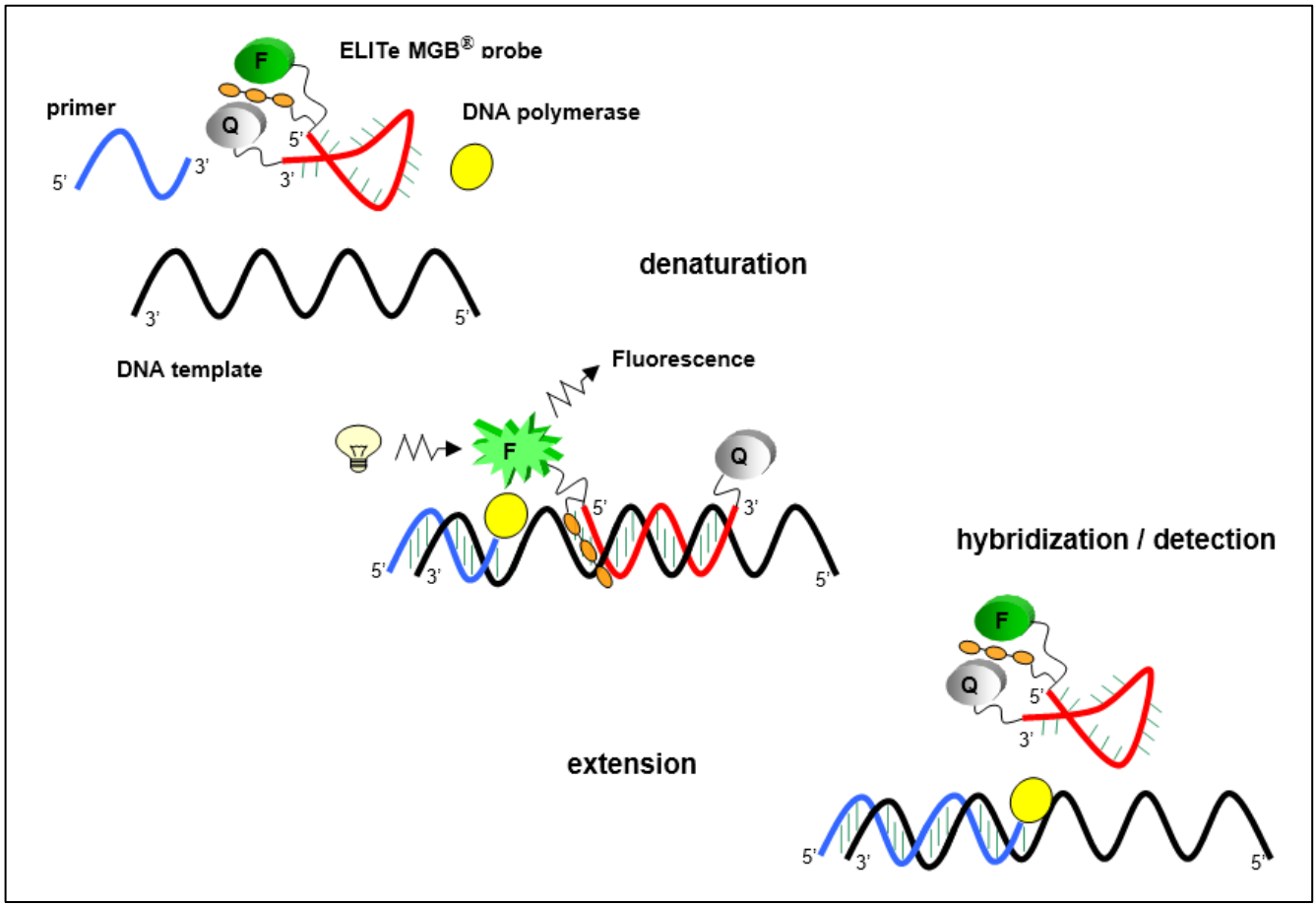
Most Zika infections are asymptomatic. However, symptoms when present may include mild fever, headache, joint pain, rash and conjunctivitis. Other possible outcomes that have been observed include neurological complications, such as Guillain-Barré, and microcephaly in children born to infected mothers. Diagnosis is based on clinical, epidemiological (travel history) and laboratory criteria including viral isolation, rRT-PCR or serology. The spread of Zika virus and its potential involvement in causing microcephaly during pregnancy is a concern worldwide.

ASSAY PRINCIPLE

The Zika ELITe MGB® Kit U.S. has been optimized for use on the ELITe InGenius™ sample-to-result instrument which performs automated nucleic acid extraction, amplification, and detection of up to twelve samples per session. The nucleic acid is isolated and purified from serum or EDTA plasma specimens and combined with the Zika ELITe MGB Kit Monoreagent. The purified nucleic acid is subsequently amplified on the ELITe InGenius™ instrument in a real-time reverse transcription polymerase chain reaction (rRT-PCR) using the Zika ELITe MGB® Kit U.S. reagents.

The Zika ELITe MGB® Kit U.S. has been developed for qualitative rRT-PCR detection of Zika virus RNA. Zika primers and probe target the NS3 protein encoding gene in the presence of an internal control. The Zika virus specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridized with the specific product of the Zika virus amplification reaction. The Internal Control specific probe with ELITe MGB® technology, labelled with AP525 fluorophore (similar to VIC), is activated when hybridized with the specific product of Internal Control amplification reaction. As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data determines the presence of Zika virus RNA in the starting sample.

The picture below depicts the mechanism of activation and fluorescence emission of an ELITe MGB® probe. Note that the probe is not hydrolyzed during the amplification cycle, which allows it to be utilized for dissociation curve analysis.



PRODUCT DESCRIPTION

The **Zika ELITe MGB® Kit U.S.** is a biplexed rRT-PCR assay that uses a primer set and single uniquely labeled probe to amplify and detect the NS3 protein encoding gene. Each **Zika ELITe MGB® Kit U.S.** includes all of the controls and reagents necessary to detect Zika virus. Sufficient material is provided to complete 48 reactions at a final volume of 30 µL each[1].

Zika Virus Detection Reagents

- **20x Zika PreMix**

  The Zika 20x PreMix includes a mixture of primers and probes as shown in the table below. Each tube contains **98 µL** of solution, which is designed to detect the Zika virus as well as bacteriophage MS2, an internal control for monitoring RNA extraction and PCR inhibition.

Table 1. Zika 20x PreMix Primers and Probes

NAME	5' – 3' SEQUENCE	CONC. [µM]	LENGTH (bp)	ABS. MAX/ EM. MAX
ZKV3-L1	CACTGGCTTGAAGCAAGAAT	0.25 µM	20 bp	-
ZKV3-E1	CTTG**A***ACTC**T***CCCTCAAT	1.00 µM	18 bp	-
ZKV3-FAM1	MGB-FAM-**G***ATGGCCTCATAGCCTCG-EDQ	0.20 µM	18 bp	496 nm / 517 nm
MS2-L4	CC**A***TCAA**A***GTCG**A***GGTGCCTAAAGTG	0.20 µM	26 bp	-
MS2-E3	ACGAACGCCATGCGGCTACAGGAAGCTC	0.20 µM	28 bp	-
MS2-AP525-1	MGB-AP525-**G***CTGTTGGTGGTGTAGAGC-EDQ	0.20 µM	19 bp	527 nm / 549 nm
* Denotes proprietary ELITechGroup super bases				

[1] Excess volume is provided in each tube to allow for the "dead volume" that is needed when assays are run using ELITe InGenius™.

- **PCR MasterMix**

  An optimized and stabilized mixture of reagents for reverse transcription and real time amplification. Each tube contains **972 µL** of solution. The PCR MasterMix provides the buffer, magnesium chloride, the nucleotide triphosphates and the DNA polymerase enzyme with hot start capability.

- **RT EnzymeMix**

  An optimized and stabilized mixture of enzymes for reverse transcription. Each tube contains **20 µL** of solution.

- **PCR Grade Water**

  DNase and RNase-free water, included to dilute the 20x Zika PreMix for the final PCR reaction. Each tube contains **208 µL** of solution.

- **Negative Control**

  The Negative Control is comprised of DNase and RNase-free water. Each tube contains **1600 µL** of solution.

Zika Virus Internal Control

- **MS2 RNA Internal Control**

  An optimized MS2 RNA solution stabilized in a guanidinium buffer provided in 4 tubes, and requires extraction. Each tube contains **180 µL** of solution. The MS2 RNA is co-extracted and co-amplified with the target nucleic acid, and controls for integrity of the reagents (polymerase, primers, etc.), equipment function (thermal cycler), and the presence of amplification inhibitors in the samples.

Zika – ELITe Positive Control

- **Zika – Positive Control**

  An optimized synthetic Zika RNA solution stabilized in a guanidinium buffer provided in 2 tubes, and requires extraction. Each tube contains **800 µL** of solution.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Cap Color	Quantity
Zika Virus Detection Reagents			
20x Zika PreMix	Primer/probe oligonucleotides mixture	YELLOW	**1 x 98 µL**
PCR MasterMix	Mixture of reagents for reverse transcription and real-time amplification	NEUTRAL	**1 x 972 µL**
RT EnzymeMix	Reverse transcriptase	BLACK	**1 x 20 µL**
PCR Grade Water	DNase and RNase-free water	WHITE	**1 x 208 µL**
Negative Control	DNase and RNase-free water	WHITE	**1 x 1600 µL**
Zika Virus Internal Control			
MS2 RNA Internal Control	MS2 RNA	NEUTRAL	**4 x 180 µL**
Zika – ELITe Positive Control			
Zika - Positive Control	Synthetic Zika RNA	RED	**2 x 800 µL**

MATERIALS REQUIRED BUT NOT PROVIDED

- Laminar airflow hood or Biological Safety Cabinet.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Sarstedt 2.0 mL tube skirted screw-cap (Sarstedt Ref. 72.694.005).
- Sarstedt 0.5 mL tube skirted screw-cap (Sarstedt Ref. 72.730.005).
- nuclease-free water
- RNase Inhibitor (optional).

OTHER PRODUCTS REQUIRED

The **ELITe InGenius™** instrument, reagents, and consumables **are not** included in this product.

- ELITe InGenius™ (ELITechGroup Inc., ref. INT030-K).
- ELITe InGenius™ SP 200 Extraction Cassette (ELITechGroup Inc., ref. INT032SP200)
- ELITe InGenius™ SP 200 Consumable Set (ELITechGroup Inc., ref. INT032CS)
- ELITe InGenius™ Waste Box (ELITechGroup Inc., ref. F2102-000)
- ELITe InGenius™ PCR Cassette (ELITechGroup Inc., ref. INT035PCR)
- Filter tips 300 Axygen (Axygen BioScience Inc., CA, USA, ref. TR-350-LRS)

For performing the **Zika ELITe MGB® Kit U.S** test on the **ELITe InGenius™** instrument (ELITechGroup Inc., ref. INT030), the following Zika ELITe MGB® Kit U.S. specific protocols are required:

- Assay Profile: Zika ELITe MGB Sample_PL_200_50
- Assay Profile: Zika ELITe MGB_Positive Control
- Assay Profile: Zika ELITe MGB_Negative Control
- Control Definition: Zika - Positive Control
- Control Definition: Zika - Negative Control
- Reagent: MS2 RNA Internal Control
- Reagent: Zika ELITe MGB Monoreagent
- Matrix: Plasma_Serum (IVD)

WARNINGS AND PRECAUTIONS

This product is exclusively designed for *in-vitro* use.

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards.

Wear suitable personal protective equipment.

Never pipette solutions by mouth.

Do not eat, drink, smoke or apply cosmetic products in the work areas.

Carefully wash hands after handling samples and reagents.

Dispose of leftover reagents and waste in compliance with the local, state, and federal regulations.

Carefully read all the instructions provided in the product before running the assay.

While running the assay, follow the instructions provided in the product.

Do not use the product after the indicated expiry date.

Only use the reagents provided in the product and those recommended by the manufacturer.

Do not use reagents from different lots of materials.

Do not use reagents from other manufacturers.

Safety Data Sheets are available upon request.

Warnings and precautions for molecular biology

Molecular biology procedures, such as nucleic acid extraction, reverse transcription, PCR amplification and detection, require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

It is necessary to have separate areas for the preparation of Zika ELITe MGB Monoreagent and internal control mixes and for the extraction/amplification/detection to prevent false positive results.

It is necessary to have lab coats, gloves and tools available which are exclusively used for the extraction/preparation of the amplification reactions and for the amplification / detection of amplification products. Never transfer lab coats, gloves or tools from the area designated for the amplification / detection of amplification products to the area designated for the extraction / preparation of the amplification reactions.

Samples must be handled under a laminar airflow hood or biological safety cabinet. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile and free from DNases and RNases.

The reagents must be handled under a laminar airflow hood or biological safety cabinet. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes used to handle the reagents must be exclusively used for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile and free from DNases and RNases.

Amplification products must be handled in such a way as to reduce as much as possible dispersion into the environment in order to avoid the possibility of contamination. The pipettes used to handle amplification products must be exclusively used for this purpose.

Warnings and precautions specific for the components

20x Zika PreMix

The **20x Zika PreMix** must be stored protected from light at -20 °C, and is stable for up to two years.

The **20x Zika PreMix** can be frozen and thawed no more than **five times**: further freezing / thawing cycles may cause a loss of product performance.

PCR MasterMix

The **PCR MasterMix** must be stored at -20 °C, and is stable for up to two years.

The **PCR MasterMix** can also be stored at 2–8°C for up to 1 month, per manufacturer's instructions. Repeated **PCR MasterMix** freeze/thaw cycles should be avoided (no more than five times) and may cause a loss of product performance.

RT EnzymeMix

The **RT EnzymeMix** must be stored at -20°C, per manufacturer's instructions.

The **RT EnzymeMix** should be taken from –20°C immediately before setting up the Zika ELITe MGB Monoreagent, always kept on ice, and returned to storage at –20°C immediately after use.

Zika - Positive Control

The **Zika - Positive Control** must be stored at -20°C, and is stable for up to two years. Repeated **Zika - Positive Control** freeze/thaw cycles should be avoided (no more than five times) and may cause a loss of product performance. If a spill occurs, the reagent is non-infectious synthetic RNA template.

Note: Do not clean spills with bleach; the buffer will liberate a toxic gas.

MS2 RNA Internal Control

The **MS2 RNA Internal Control** must be stored at -20°C, and is stable for up to two years. Repeated **MS2 RNA Internal Control** freeze/thaw cycles should be avoided (no more than five times) and may cause a loss of product performance. If a spill occurs, the reagent is non-infectious RNA template.

Note: Do not clean spills with bleach; the buffer will liberate a toxic gas.

ELITe InGenius™

SAMPLES AND CONTROLS

Samples

Zika ELITe MGB® Kit U.S. product must be used in conjunction with the ELITe InGenius™ instrument to perform the extraction of RNA from clinical samples of serum or plasma collected in EDTA.

The plasma samples must be collected in EDTA according to laboratory guidelines and stored 4°C (nominal) for a maximum of four hours. The serum samples must be collected according to laboratory guidelines and stored 4°C (nominal) for a maximum of four hours. The plasma and serum samples can be stored at -20°C for a maximum of two days or at -70°C for long term storage.

Note: Use the extraction protocol **Zika ELITe MGB Sample_PL_200_50** to perform RNA extraction from plasma or serum specimens on the **ELITe InGenius™** instrument with the **ELITe InGenius™ Software** version 1.2 (or later equivalent versions). This protocol processes 200 µL of sample, adds the **MS2** Internal Control at 10 µL/extraction and elutes the nucleic acids in 50 µL.

Transfer 200 µL of sample from the primary tube into a sonicator tube. Refer to the ELITe InGenius™ Instructions For Use (INT030-K_IFU_US_EN) for more information.

Amplification Controls

Daily validation and approval of a positive control on the first daily amplification session is required, as well as a negative control on every run.

As a negative control, use the provided **Negative Control** in place of a sample.

As a positive control, use the provided **Zika - Positive Control** in place of a sample.

As a nucleic acid extraction control, use the provided **MS2 RNA Internal Control** to prepare a diluted solution, 10 µL of which will be automatically added from the tube in Inventory Block to each sample or control during the extraction step.

Note: ELITe InGenius™ with **ELITe InGenius™ Software** allows for validation of amplification controls for each lot of amplification reagent to be approved and stored in its database. Amplification validation control results will expire each day and it will be necessary to re-run the Positive and Negative Controls.

The amplification controls must be retested if any of the following events occurs:

- A new lot of amplification reagents is started,
- The results of positive control or negative control analysis are out of specification,
- Any major maintenance is performed on ELITe InGenius™.

PROCEDURE

The procedure consists of three steps:

- System readiness verification
- Set up of the session
- Review and approval of results

System Readiness Verification

Before starting the session it is necessary to:

- Switch on **ELITe InGenius™** and select the mode "**CLOSED**";
- Verify that the amplification Controls (**Zika - Positive Control**, **Negative Control**) have been run, approved and are not expired (status). This can be checked under the "Control" menu in the Home page;
- Choose the type of run and set up the run, following the instructions for the session set up and using the Assay Protocols identified below. These protocols were specifically validated with **ELITe MGB®** kits and **ELITe InGenius™**.

The Assay protocols for the **Zika ELITe MGB® Kit U.S.** are:

- Assay Profile: Zika ELITe MGB Sample_PL_200_50
- Assay Profile: Zika ELITe MGB_Positive Control
- Assay Profile: Zika ELITe MGB_Negative Control
- Control Definition: Zika - Positive Control
- Control Definition: Zika - Negative Control
- Reagent: MS2 RNA Internal Control
- Reagent: Zika ELITe MGB Monoreagent
- Matrix: Plasma_Serum (IVD)

If the Zika ELITe MGB® Kit U.S. protocols required to perform the assay are not installed on the ELITe InGenius™ system, contact your local ELITechGroup Customer Service.

Setup of the rRT-PCR Session

The **Zika ELITe MGB® Kit U.S.,** in association with **ELITe InGenius™,** can be used to perform an integrated run with a Positive Control, a Negative Control, and samples (Extract + PCR).

The Zika ELITe MGB® Kit U.S. amplification thermal profile is included in the protocol available for the ELITe InGenius™ instrument, and is automatically loaded when the assay protocol is selected.

The main steps for the setup of a run is described below.

Integrated run (Extract + PCR) with Positive and Negative Controls.

To set up the integrated run (Extract + PCR) with a Positive control, a Negative Control, and test samples to be analyzed, carry out the steps below. The Positive Control should be included with the first run each day, on each instrument, but can be omitted in subsequent runs. The Negative Control should be included on every run, for each instrument.

1. Remove and thaw at room temperature the tubes containing the samples to be analyzed. Mix by vortexing, centrifuge the tubes to bring the contents to the bottom, and then keep on ice.
2. Remove and thaw at room temperature the tubes containing the Positive and Negative Controls:

a. Remove and warm to room temperature the **Zika - Positive Control** (RED cap) tube. Each tube is sufficient for 4 sessions. Mix by vortexing, centrifuge the tubes to bring the contents to the bottom.

b. Remove and thaw at room temperature the **Negative Control** (WHITE cap) tube. Each tube is sufficient for 8 sessions. Mix by vortexing, centrifuge the tubes to bring the contents to the bottom.

3. Remove and thaw at room temperature the **20x Zika PreMix** (YELLOW cap) tube for the session. Mix by vortexing and then centrifuge the tubes to bring the content to the bottom.
4. Remove and thaw at room temperature the **PCR MasterMix** (NEUTRAL cap) tube for the session. Mix by vortexing and then centrifuge the tubes to bring the content to the bottom.
5. Remove and thaw at room temperature the **PCR Grade Water** (WHITE cap) tube for the session. Mix by vortexing and then centrifuge the tubes to bring the content to the bottom.
6. When needed, remove the **RT EnzymeMix** (BLACK cap) tube from the -20°C freezer. Gently shake the tubes, centrifuge to bring the contents to the bottom, and then keep on ice.

   Note: The **RT EnzymeMix** should be removed from -20°C freezer and added during the final step of the Zika ELITe MGB Monoreagent formulation and then immediately returned to storage at -20°C to maintain the integrity of the material.
7. Prepare an amount of the **Zika ELITe MGB Monoreagent** sufficient for the required number of reactions, including the positive and/or negative controls according to Table 2, below.

   a. Prepare and label a 2.0 mL Sarstedt tube for the Zika ELITe MGB Monoreagent.

   b. Determine the volumes of the kit components that are required to prepare the Zika ELITe MGB Monoreagent based on the number of samples and controls to be analyzed.

   c. Prepare the complete Zika ELITe MGB Monoreagent by adding the required volumes of the four components to the labeled tube, adding the RT EnzymeMix as the final step

   d. Mix by vortexing, centrifuge the tube to bring the content to the bottom, and then keep in a refrigerator or in ice until ready to transfer to the ELITe InGenius™ instrument.

   Note: In order to determine the volumes of the four components, it is necessary to define the number of reactions (N) of the session by counting the number of the samples and controls to be tested.

Table 2. Preparation of the Zika ELITe MGB Monoreagent[2]

Number of Reactions	20x Zika PreMix Vol. (µL)	PCR MasterMix Vol. (µL)	PCR Grade Water Vol. (µL)	RT EnzymeMix Vol. (µL)	Total Vol. (µL)
1	3.2	31.5	6.7	0.63	42.0
2	4.8	48.0	10.2	0.96	64.0
3	6.5	64.5	13.8	1.29	86.0
4	8.1	81.0	17.3	1.62	108.0
5	9.8	97.5	20.8	1.95	130.0
6	11.4	114.0	24.3	2.28	152.0
7	13.1	130.5	27.8	2.61	174.0
8	14.7	147.0	31.4	2.94	196.0
9	16.4	163.5	34.9	3.27	218.0
10	18.0	180.0	38.4	3.60	240.0
11	19.7	196.5	41.9	3.93	262.0
12	21.3	213.0	45.4	4.26	284.0

Note: The complete reaction mixture should be used within 6 hours if kept on board in the refrigerated block. The complete Zika ELITe MGB Monoreagent **cannot** be stored.

[2] Excess volume is included to allow for the "dead volume" that is needed when assays are run using ELITe InGenius™.

8. Remove the **MS2 RNA Internal Control** tubes in sufficient volume for the session. Prepare an amount of the **Ready-to-Use MS2 RNA Internal Control Mix** sufficient for the required number of reactions, including the positive and/or negative controls, according to Table 3 below.
   a. Prepare and label a 0.5 mL Sarstedt tube for the Ready-to-Use MS2 RNA Internal Control Mix.
   b. Prepare the Ready-to-Use MS2 RNA Internal Control Mix by adding the required volumes of the two components to the labeled tube.
   c. First add the nuclease-free water (not included) and then the MS2 RNA Internal Control template.
   d. After transferring the MS2 RNA Internal Control pipette the mix the solution several times to ensure complete transfer.
   e. Mix by vortexing, centrifuge the tube to bring the contents to the bottom, and then keep the Ready-to-Use MS2 RNA Internal Control mix in a refrigerator or on ice until ready to transfer to the ELITe InGenius™ instrument.

Table 3. Preparation of the Ready-to-Use MS2 RNA Internal Control Mix

Number of Reactions	MS2 RNA Internal Control	Nuclease-Free Water Vol. (µL)	Total Vol. (µL)
1	20.0 µL	20.0 µL	40.0 µL
2	29.0 µL	29.0 µL	58.0 µL
3	36.0 µL	36.0 µL	72.0 µL
4	43.0 µL	43.0 µL	86.0 µL
5	50.0 µL	50.0 µL	100.0 µL
6	57.0 µL	57.0 µL	114.0 µL
7	64.0 µL	64.0 µL	128.0 µL
8	71.0 µL	71.0 µL	142.0 µL
9	78.0 µL	78.0 µL	156.0 µL
10	85.0 µL	85.0 µL	170.0 µL
11	92.0 µL	92.0 µL	184.0 µL
12	99.0 µL	99.0 µL	198.0 µL

9. Using the ELITe InGenius™ Graphical User Interface (GUI), select “Perform Run” from the Home screen, for each track to be loaded.
   a. Check that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 50 µL.
   b. In the “Assay” column select the assay protocol for the Positive Control, Negative Control, and each sample:
      i. In the “Assay” column, select the “**Zika ELITe MGB_Positive Control**” protocol for the ELITe InGenius™ track being prepared for the **Zika – Positive Control**. Fill in the lot number and expiry date, and then click on “Next” to continue the setup.

   Note: This step is optional if the positive control has already been run that day on the ELITe InGenius™ instrument being used.

      ii. In the “Assay” column, select the “**Zika ELITe MGB_Negative Control**” protocol for the ELITe InGenius™ track being prepared for the **Negative Control**. Fill in the lot number and expiry date, and then click on “Next” to continue the setup.
      iii. In the “Assay” column, select the “**Zika ELITe MGB Sample_PL_200_50”** protocol for each ELITe InGenius™ track being prepared for a sample.
   c. In the “SampleID” (SID) column, enter the sample name or scan the sample barcode for each ELITe InGenius™ track being prepared for a sample.

   Note: The SampleID and Protocol columns for the Positive Control and Negative Control are added automatically during the control setup, and cannot be modified.

d. In the "Protocol" column, check that "**Extract + PCR**" is displayed for each ELITe InGenius™ track being prepared for a sample.

e. In the "Sample Position" column, select "**Sonication Tube**" for the sample loading position.

f. Click "Next" to continue the setup.

10. Select an Inventory Block for the **Zika ELITe MGB Monoreagent** and **MS2 RNA Internal Control Mix**

    a. Select a position for the **20X Zika Premix** and enter the lot number, expiration date, and number of tests prepared.

    b. Select a position for the **MS2 Internal Control** and enter the lot number, expiration date, and number of tests prepared.

    Note: Refer to the INT030-K_EN ELITe InGenius™ System Instructions For Use to setup the Inventory block if necessary.

11. Load the ELITe InGenius™ Inventory Area with reagents, tips, and a waste box:

    a. Check that the individual pipettor tip racks in the Inventory Area contain the required number of tips. Replace empty tip racks following the GUI.

    b. Load an ELITe InGenius™ "**Waste Box**" following the GUI

    c. Load the 2.0 mL Sarstedt tube with the prepared **Zika ELITe MGB Monoreagent** into the Inventory Block position selected following the GUI, and remove the cap.

    d. Load the 0.5 mL Sarstedt tube with the prepared **Ready-to-Use MS2 RNA Internal Control Mix** into the Inventory Block position selected following the GUI, and remove the cap.

12. Load the ELITe InGenius™ instrument with cassettes for each track:

    a. Load the required number of "**ELITe InGenius™ SP 200**" extraction cassettes following the GUI.

    b. Load the required number of "**PCR Cassette**" consumables following the GUI.

13. Load the ELITe InGenius™ instrument "**ELITe InGenius™ SP 200 Consumable Set**" cassettes and tubes for each track following the GUI.

    a. Load the required number of uncapped 0.5 mL Sarstedt tubes into the eluate collection rack.

    b. Remove the metal grid cover from the tip cartridge loading position, load the required number of tip cartridges, and then securely replace the metal grid cover.

    c. Load the required number of sonicator tubes into the sonication tube rack, and set aside for sample loading.

14. In laminar flow hood or biological safety cabinet, carefully load the samples into the sonicator rack and transfer it into the ELITe InGenius™ instrument.

    a. Transfer 0.2 mL of Positive Control, Negative Control, and each sample into the sonication tubes in the prepared sonicator rack, following the positions selected in step 9, above.

    Note: The sonication tubes are placed into the sonication rack and are open during the loading process. The sonication rack separates the tubes by 1 cm from tube to tube with a metal splash guard between each tube position. Sonication tube caps can be used if samples need to be stored prior to running the instrument, but need to be removed before starting the procedure.

    b. Load the sonicator rack containing the Positive Control, Negative Control, and samples into the ELITe InGenius™ instrument.

    Note: Check that the all of the consumables, cassettes, tube, and racks are properly loaded and are ready for the run to begin.

15. Close the instrument door.

16. Press "Next" after each step when prompted by the ELITe InGenius™ GUI to confirm that the consumables, cassettes, tube, and racks are loaded in the correct positions, and then the final step is to press "Start" to begin the run.

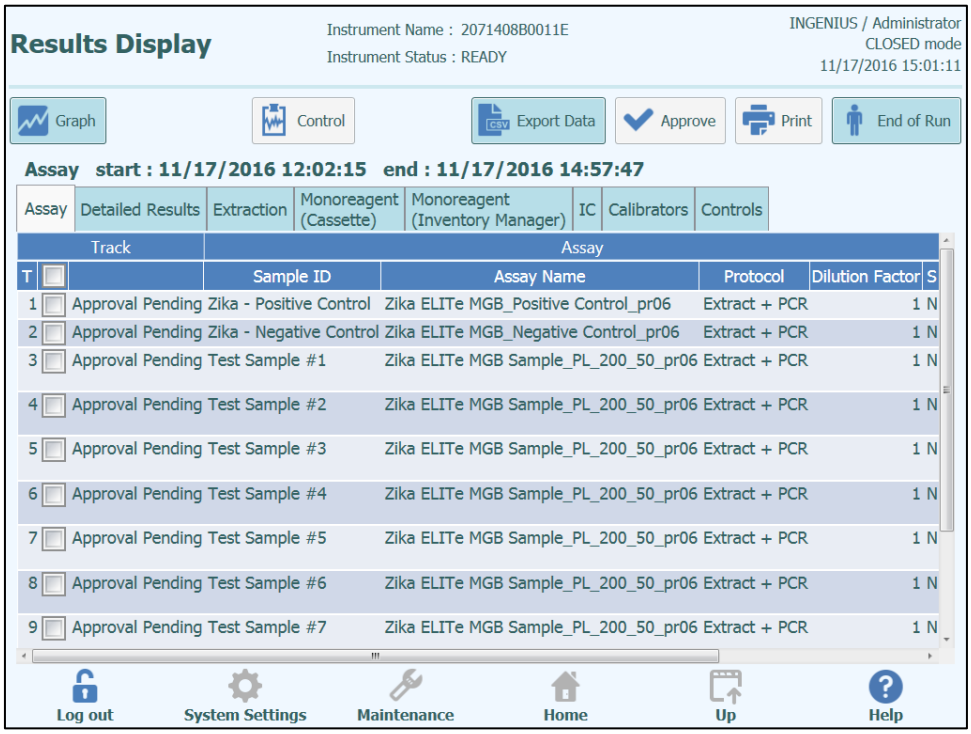
17. Verify the instrument door is closed. Re-adjust the door if the corresponding message appears.

18. After the run is completed, the "Results Display" screen is automatically displayed starting on the "Assay" tab. This window allows the user to view, approve, or store the results and to print and save a report.

Note: At the end of the run the cassettes, consumables, and samples must be removed from the instrument and disposed in accordance with local, state, and federal regulations.

19. Review and approval of results:

At the end of the run, the "Results Display" screen is automatically displayed starting on the "Assay" tab. The example in the figure below shows the sample and control results and additional information regarding the run.



Results Display:

Approval of results is a two-step process. The user must determine if the control & sample results are valid. If any results are invalid, the run (in case of an invalid control) or sample will require retesting.

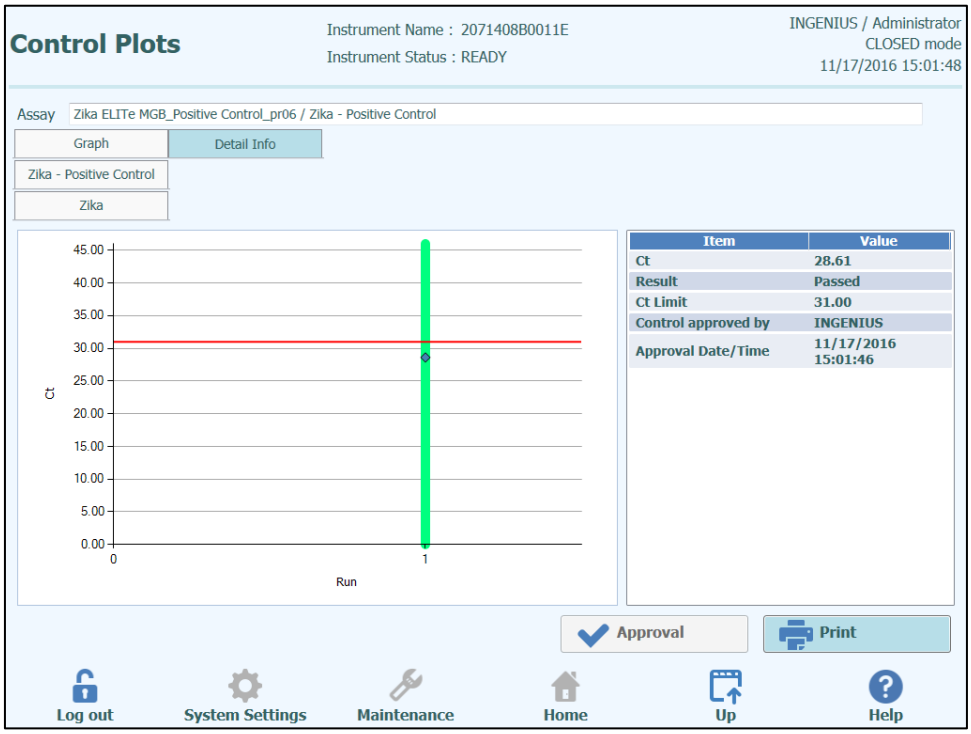
Before analyzing any sample, it is mandatory to generate and approve a Positive Control result for the lot of rRT-PCR Mix used

a. Validation of Positive Control Results (Positive Control Result Approval)

The fluorescence signals emitted by the specific Zika probe (FAM, Channel 1 "Zika") and by the specific MS2 RNA Internal Control probe (AP525, Channel 2 "Internal Control") in the Positive Control reaction are analyzed automatically (Channel 1 C_T < 31.00 and Channel 2 C_T < 35.00) at the end of the run by the ELITe InGenius™ software.

The ELITe InGenius™ software requires approval of the positive controls before any sample results can be approved. The Positive Control results are specific for the reagent lot, and will expire daily.

From the "Results Display" screen, check the box on the left side of the screen for the track containing the "Zika – Positive Control", and then click on the "Control" button. This will open the "Control Plots" window. If the Zika – Positive Control indicates that result is "passed", click on the "Approval" button. The example in the figure below shows the positive control approval screen.



Control Plots: Positive Control Approval

Note: When the Positive Control result does not meet the acceptance criteria, the "not passed" message is shown on the "Controls" menu and it is not possible to approve the result. The Positive Control reaction has to be repeated.

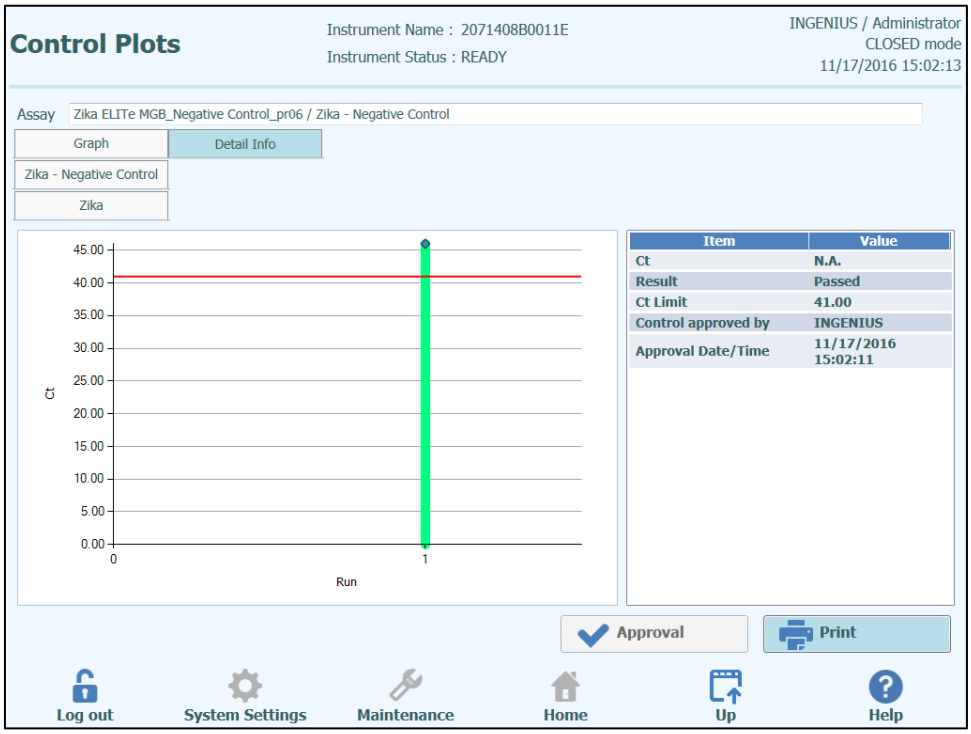
Note: When the Positive Control is run together with samples and its result is invalid, the entire session is invalid and all samples must be repeated.

b. Validation of Negative Control Results (Negative Control Result Approval)

The fluorescence signals emitted by the specific Zika probe (FAM, Channel 1 "Zika") and by the specific MS2 RNA Internal Control probe (AP525, Channel 2 "Internal Control") in the Negative Control reaction are analyzed automatically (Channel 1 C_T > 41.00 and Channel 2 C_T < 35.00) at the end of the run by the ELITe InGenius™ software.

The ELITe InGenius™ software requires approval of the negative control before any sample results can be approved. The Negative Control results are specific for each run.

From the "Results Display" screen, Check the box on the left side of the screen for the track containing the "Negative Control", and then click on the "Control" button. This will open the "Control Plots" window. If the Zika – Positive Control indicates that result is "passed", click on the "Approval" button. The example in the figure below shows the negative control approval screen.



Control Plots: Negative Control Approval

Before analyzing any sample, it is mandatory to generate and to approve a Negative Control result for each run.

Note: When the Negative Control result does not meet the acceptance criteria, the “not passed” message is shown on the “Controls” menu and it is not possible to approve the result. The Negative Control reaction has to be repeated. The entire session is also invalid and all samples must be repeated.

c. Validation of Samples Results (Approval of Sample Results)

The fluorescence signals emitted by the specific Zika probe (FAM, Channel 1 "Zika") and by the specific MS2 RNA Internal Control probe (AP525, Channel 2 "Internal Control") in each sample reaction are analyzed automatically at the end of the run by the ELITe InGenius™ software.

On the “Results Display” screen, check the box on the left side of the screen for each track containing a test sample, and then click on the “Approve” button

Note: Before analyzing any sample, it is absolutely mandatory to generate and to approve the Positive and Negative Controls for the lot of reagent used. In addition, it is also mandatory to generate and approve the **Negative** Control for each specific sample run. The availability of Positive and Negative Control results with “Approved” (Status) is shown in the “Calibration” and “Controls” windows of ELITe InGenius™ software.

The Sample run results are stored in the database and, if valid, can be approved (Result Display) by “Administrator” or “Analyst” personnel using the ELITe InGenius™ software.

Results are described in the reports generated by the instrument ("Result Display").

Note: The results obtained with this assay must be interpreted taking into consideration all the clinical data and the other laboratory test outcomes concerning the patient.

The Sample run is valid when the conditions reported in the table below are met.

1) Positive Control	**Status**
Zika - Positive Control	APPROVED
2) Negative Control	**Status**
Negative Control	APPROVED

The possible Sample result messages are listed the table below.

Result of Sample Run	Interpretation
Zika: Zika virus RNA Detected	**Zika virus RNA detected**
Zika: Zika virus RNA Not Detected	**Zika virus RNA not detected**[3]
Invalid - Retest Sample	**Not valid assay result** due to Internal Control failure (Incorrect extraction or presence of inhibitor).

Samples not suitable for analysis are reported as “Invalid - Retest Sample” by **ELITe InGenius™ software**. In this case, the Internal Control RNA was not detected due to potential problems in the amplification or extraction phase (degradation of RNA, loss of RNA during the extraction or presence of inhibitor in the extracted eluate) that may cause incorrect or false negative results.

Samples suitable for analysis in which Zika virus RNA is not detected are reported as: “Zika: Zika virus RNA Not Detected". In this case it cannot be excluded that the Zika virus RNA is present at a concentration below the detection capability of the assay (see “performance and characteristic).

20. Sample Result Reporting

After the assay results have been approved, assay result reports can be printed and saved. The sample results are stored in the database and can be exported as “Sample Report” and “Track Report”.

The “Sample Report” shows the details of a sample run sorted by Sample ID (SID).

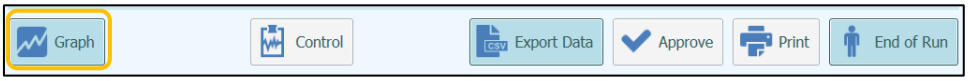
The “Track Report” shows the details of a sample run track by selected track.

The "Sample Report" and "Track Report" can be printed or saved as a PDF document for printing later. The report should be reviewed and then signed by authorized personnel to keep as a record.

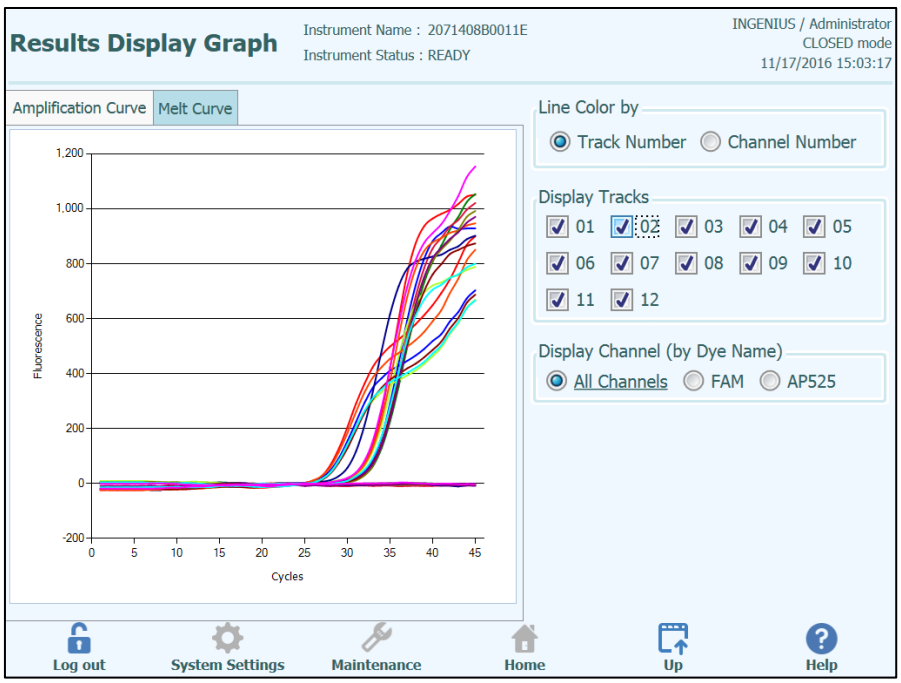
Positive Zika virus RNA results must be reported to the appropriate Public Health authorities.

a. Results Display

From the “Results Display” screen, select the “Graph” button.



This will display the amplification plot for each selected sample. The example in the figure below shows the Results Display Graph (amplification curve) screen.



[3] 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).

Results Display Graph: Amplification Curve

The selected samples can be viewed by track number or channel number. The samples can also be displayed in all channels (default) or a single channel can be selected. Each individual sample can also be individually displayed by either selecting (all samples selected by default) or deselecting a sample.

Fluorescence intensity is monitored at each PCR cycle by the ELITe InGenius™ optics to determine if an amplification curve is positive or negative using the following steps:

i. Determine the raw fluorescence data spread by calculating the standard deviation (SD) of the raw data. If the SD value exceeds a pre-set threshold (SD raw cutoff) the data is taken to the next step, otherwise no amplification is assigned.

ii. Find the inflection (take off) points using an amplification criterion (ratio of the second derivative maximum value and SD). The ratio of 2nd derivative signal to baseline noise must exceed a pre-set value (Amplification Criterion) in order to pass the test. If the requirement is met, a "legitimate" amplification curve is found and the data set is taken to the next step.

iii. The average of fluorescence signals at 5, 6 and 7 cycles before the 2nd derivative maximum (step 2) is calculated and individually subtracted from the data set. The baseline-subtracted data are then taken to the next step.

iv. The C_T value is defined as the cycle value where the corresponding fluorescent signal crosses the defined fluorescence threshold setting.

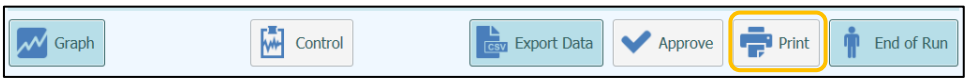
1. On the legitimate amplification curve, PCR cycle numbers with fluorescence just below (Ct-) and just above (Ct+) the user-defined fluorescence threshold (FTH) are determined.
2. Fluorescence values for the Ct- and Ct+ cycles are determined, FCt- and FCt+, respectively.
3. An interpolation procedure is used to calculate the C_T value corresponding to the defined fluorescence threshold setting.

b. Export Data

From the "Results Display" screen, select the "Export Data" button to export the run data.

c. Print

From the "Results Display" screen, select the "Print" button to generate a report, and then print the report or save it as a PDF document for printing later. Verify the controls are valid, and the data is reported correctly, then sign the document to keep as a record.



d. End of Run: After the run is completed and the results have been approved and reported, press the "End of Run" button. This will unlock the ELITe InGenius™ instrument and allow the user to dispose of all of the consumables and reagents.

Note: The cassettes, consumables, and samples must be removed from the instrument and disposed in accordance with local, state, and federal regulations.

Note: For detailed information refer to INT030-K_EN ELITe InGenius™ System Instructions For Use.

PROCEDURE LIMITATIONS

For Use under an Emergency Use Authorization Only

Use this product only with the following clinical samples: Plasma and serum collected in EDTA.

Quantity of extracted RNA higher than 300 ng per reaction may inhibit the reverse transcription reaction and the amplification of nucleic acids.

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

The results obtained with this product depends on an adequate identification, collection, transport, storage and processing of the samples. To avoid incorrect results, it is therefore necessary to take care during these steps and to carefully follow the instructions for use.

False-negative results can arise from:

- poor sample collection
- degradation of the viral RNA during shipping or storage
- specimen collection conducted prior to symptom onset
- specimen collection after nucleic acid can no longer be found in the patient
- mutation in the Zika virus region under the probe or primer sequences
- failure to follow the authorized assay procedures

False-positive results can arise from:

- Cross contamination during specimen acquisition or sample preparation/extraction
- Specimen or sample mix-up
- Cross-reactivity due to mutated organisms
- Cross contamination of patient samples with reagents during assay set-up
- RNA contamination of amplification reagents
- Cross contamination between patient samples

Owing to its high analytical sensitivity, the real time amplification method used in this product is sensitive to cross-contaminations from Zika virus positive clinical samples, the positive controls and the same amplification products. Cross-contamination can cause false positive results. However, cross-contamination can be minimized by using good laboratory practices and following these instructions for use.

This product must be handled by qualified personnel trained in the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

A negative result obtained with this product means that the Zika virus RNA is not detected in the RNA extracted from the sample, but it cannot be excluded that the Zika virus RNA has a lower titer than the product detection limit (see “Performance Characteristics” section). In this case the result could be false negative.

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).

Results obtained with this product may sometimes be invalid due to failed internal control and require retesting, starting from extraction, that can lead to a delay in obtaining final results.

Possible polymorphisms within the region of the viral genome covered by the product primers and probes may impair detection of Zika virus RNA.

The results obtained with this product must be interpreted taking into consideration all the clinical data and other laboratory tests done on the patient.

PERFORMANCE CHARACTERISTICS

A. Limit of Detection (LoD) / Analytical Sensitivity

Limit of detection studies were performed using Zika virus positive culture isolates:

- Strain: PRVABC59; origin – Puerto Rico, 2015 provided by ATCC, and
- Strain: H/PAN/2015/CDC-259359; origin – Panama, 2015 provided by BEI.

This material was quantitated by generating a standard curve using log10 dilutions of RNA transcripts. The quantity of the stock PRVABC59 culture was determined to be 2.6×10^9 copies/mL. The quantity of the stock CDC-259359 culture was determined to be 3.6×10^8 copies/mL.

The materials were then spiked into negative plasma pools. Three-fold dilution series were made, extracted and amplified using the Zika ELITe MGB® Kit U.S. on the ELITe InGenius™ instrument in five replicates at each dilution level per each strain. The lowest concentration detected in all 5 replicates was established as the tentative LoD for the strain in Tables 4-5 below. The LoD was confirmed by extracting and amplifying 20 replicates per each LoD strain with a detection rate of 95% (19/20) and can be found in Table 6. The LoD for plasma is 270 copies/mL.

Table 4: PRVABC59 Preliminary LoD

COPIES/ML	REP 1 C_T	REP 2 C_T	REP 3 C_T	REP 4 C_T	REP 5 C_T	AVG. C_T	CALL RATE
2,430	34.96	35.70	35.93	35.33	35.47	35.48	5/5 (100%)
810	36.32	37.12	37.66	36.71	39.03	37.37	5/5 (100%)
270	**38.05**	**38.10**	**40.08**	**40.90**	**38.25**	**39.08**	**5/5 (100%)**
90	39.67	40.37	40.74	NEG	41.38	40.54	3/5 (60%)
30	NEG	NEG	NEG	40.72	NEG	40.72	1/5 (20%)
10	NEG	NEG	40.89	40.17	NEG	40.53	2/5 (40%)
Data Analysis: Determination of the last dilution that gives 100% positivity							

Table 5: CDC-259359 Preliminary LoD

COPIES/ML	REP 1 C_T	REP 2 C_T	REP 3 C_T	REP 4 C_T	REP 5 C_T	AVG. C_T	CALL RATE
2,430	35.44	35.12	35.81	35.31	35.30	35.40	5/5 (100%)
810	36.89	37.47	35.61	38.19	38.09	37.25	5/5 (100%)
270	**38.82**	**40.85**	**39.50**	**40.71**	**39.39**	**39.85**	**5/5 (100%)**
90	40.31	NEG	NEG	NEG	NEG	40.31	1/5 (20%)
30	NEG	NEG	40.48	40.52	NEG	40.50	2/5 (40%)
10	NEG	NEG	NEG	NEG	NEG	NEG	0/5 (0%)
Data Analysis: Determination of the last dilution that gives 100% positivity							

Table 6: LoD Confirmation for Both Zika Strains in Plasma at 270 copies/mL

LOD CONFIRMATION	PRVABC59		CDC-259359	
	ZIKA C_T	IC C_T	ZIKA C_T	IC C_T
1	35.87	33.12	37.02	32.93
2	38.29	33.24	40.60	32.02
3	36.39	32.66	40.44	33.32
4	38.00	32.81	38.34	32.38
5	38.69	33.37	38.80	33.50
6	37.90	33.85	38.28	33.46
7	36.29	33.55	NEG	33.18
8	36.85	32.93	38.16	32.93
9	36.63	33.12	38.12	32.42
10	37.24	33.35	40.68	33.54
11	36.30	31.48	37.63	31.33
12	36.63	31.48	39.21	31.16
13	36.80	31.67	39.70	30.91
14	38.09	31.39	39.52	31.77

LOD CONFIRMATION	PRVABC59		CDC-259359	
	ZIKA C_T	IC C_T	ZIKA C_T	IC C_T
15	37.40	31.98	39.52	31.89
16	37.76	31.44	37.40	31.59
17	37.40	31.24	38.58	30.95
18	37.44	31.09	38.50	31.18
19	37.62	32.37	37.87	31.52
20	36.35	32.87	40.02	31.19
Mean	37.20	32.45	38.86	32.16
StDev	0.78	0.89	1.10	0.94
%CV	2.11%	2.75%	2.83%	2.91%
Result	20/20 (100%)		19/20 (95%)	

B. Analytical Sensitivity – FDA Reference Materials

An analytical study was performed using FDA reference materials (S1 and S2) following a standard protocol provided by the FDA, which includes a LoD range finding study and a confirmatory LoD study, to evaluate the analytical sensitivity of the Zika ELITe MGB® Kit U.S. on the ELITe InGenius™ instrument (Table 7 below).

Table 7: LoD Confirmation Results – FDA Reference Materials

Reference Materials	Specimen Type	Confirmed LoD[4] in RNA NAAT Detectable Units/mL
S1 (FSS)	Plasma	3.33×10^3
S2 (PRV)	Plasma	5.56×10^3

C. Inclusivity (Analytical Reactivity)

In addition to testing two Zika LOD strains (PRVABC59 and CDC-259359), reactivity of the Zika ELITe MGB® Kit U.S. was evaluated by wet testing of 8 additional isolates of Zika virus spiked into Zika negative plasma in triplicates for a total of ten wet tested isolates (see Table 8 below).

Table 8: Wet Tested Isolates

ID	Isolate	Source	Test Conc. (Copies/mL)	Positivity	Avg.[5] Zika C_T
1	Zika virus, strain MR 766	ATCC	5.40×10^2	3/3	36.41
2	Zika virus, strain PRVABC59 (LoD)	ATCC	5.40×10^2	3/3	37.66
3	Zika virus, strain IB H 30656	ATCC	5.40×10^2	3/3	37.27
4	Zika virus, strain DakArD 41662	ZeptoMetrix	5.40×10^2	3/3	35.89
5	Zika virus, FLR	BEI Resource	5.40×10^2	3/3	36.35
6	Zika virus, H/PAN/2016/BEI-259634	BEI Resource	5.40×10^2	3/3	36.88
7	Zika Virus, H/PAN/2015/CDC-259359 (LoD)	BEI Resource	5.40×10^2	3/3	36.90
8	Zika Virus, H/PAN/2015/CDC-259249	BEI Resource	5.40×10^2	3/3	39.17
9	Zika Virus, H/PAN/2015/CDC-259364	BEI Resource	5.40×10^2	3/3	35.63
10	Zika virus, MEX 2-81 (Mosquito/2016/Mexico)	BEI Resource	5.40×10^2	3/3	34.91

The Zika ELITe MGB Kit U.S. inclusivity was also evaluated using *in silico* analysis of published Zika virus sequences shown in Table 9 below.

Table 9: Zika Virus Sequences Used for In Silico Analysis

GenBank #	Strain/Isolate	Country	Year	ZKV3-L1	ZKV3-FAM1	ZKV3-E1
KU501215	PRVABC59	Puerto Rico	2015	100%	100%	100%
KX087101	PRVABC59	Puerto Rico	2015	100%	100%	100%
AY632535	MR 766	Uganda	1947	100%	100%	94%

[4] Study preformed according to an FDA issued protocol
[5] IC was detected in all inclusivity samples.

GenBank #	Strain/Isolate	Country	Year	ZKV3-L1	ZKV3-FAM1	ZKV3-E1
KU963573	MR 766	Uganda	1947	100%	100%	94%
KU955594	MR 766	Uganda	1947	100%	100%	94%
KU720415	MR 766	Uganda	1947	100%	100%	94%
LC002520	MR 766	Uganda	1947	100%	100%	94%
HQ234498	MR 766	Uganda	1947	100%	100%	94%
DQ859059	MR 766	Uganda	1947	95%	100%	94%
KU820897	FLR	Colombia	2015	100%	100%	100%
KX087102	C1/C2	Colombia	2051	100%	100%	100%
HQ234500	IbH_30656	Nigeria	1968	100%	100%	100%
KU963574	IbH-30656_SM21V1-V3	Nigeria	1968	100%	100%	100%
KU955592	A.taylori-tc/SEN/1984/41662-DAK	Senegal	1984	100%	100%	100%
KU758877	17271	French Guiana	2015	100%	100%	100%
KX280026	Paraiba_01	Brazil	2015	100%	100%	100%
KX262887	103451	Honduras	2016	100%	100%	100%
KX253996	ZKC	China	2016	100%	100%	100%
KX247646	COL/UF-1	Colombia	2016	100%	100%	100%
KX247632	MEX_I_7	Mexico	2015	100%	100%	100%
KX198135	PAN/BEI-259634_V4	Panama	2016	100%	100%	100%
KX197192	Brazil/PE243	Brazil	2015	100%	100%	100%
KX185891	CN/SZ02	China	2016	100%	100%	100%
KU937936	ZIKVNL00013	Suriname	2016	100%	100%	100%
KX156776	PAN/CDC-259364_V1-V2	Panama	2015	100%	100%	100%
KX156775	/PAN/CDC-259249_V1-V3	Panama	2015	100%	100%	100%
KX156774	PAN/CDC-259359_V1-V3	Panama	2015	100%	100%	100%
KX101066	Bahia01	Brazil	2015	100%	100%	100%
KX117076	Zhejiang04	China	2016	100%	100%	100%
KX051563	Haiti/1/2016	USA	2016	100%	100%	100%
KU509998	Haiti/1225/2014	Haiti	2014	100%	100%	100%
KU963796	SZ-WIV01	China	2016	100%	100%	100%
KU991811	Brazil/2016/INMI1	Italy	2016	100%	100%	100%
KU940228	Bahia07	Brazil	2015	100%	100%	100%
KU940224	Bahia09	Brazil	2015	100%	100%	100%
KU955589	Z16006	China	2016	100%	100%	100%
KU870645	FB-GWUH-2016	Guatemala	2016	100%	100%	100%
KU926310	Rio-S1	Brazil	2016	100%	100%	100%
KU926309	Rio-U1	Brazil	2016	100%	100%	100%
KU922960	MEX/InDRE/Sm/2016	Mexico	2016	100%	100%	100%
KU922923	MEX/InDRE/Lm/2016	Mexico	2016	100%	100%	100%
KU866423	SZ01	China	2016	100%	100%	100%
KU820898	GZ01	China	2016	100%	100%	100%
KU740184	GD01	China	2016	100%	100%	100%
KU853013	Dominican Republic/2016/PD2	Italy	2016	100%	100%	100%
KU853012	Dominican Republic/2016/PD1	Italy	2016	100%	100%	100%
KU820899	ZJ03	China	2016	100%	100%	100%
KU729217	BeH823339	Brazil	2015	100%	100%	100%
KU729218	BeH828305	Brazil	2015	100%	100%	100%
KU761564	GDZ16001	China	2016	100%	100%	100%
KU681081	H.sapiens-tc/THA/2014/SV0127- 14	Thailand	2014	100%	100%	100%
KU744693	VE_Ganxian	Venezuela	2016	100%	100%	100%

GenBank #	Strain/Isolate	Country	Year	ZKV3-L1	ZKV3-FAM1	ZKV3-E1
KU497555	Brazil-ZKV2015	Brazil	2015	100%	100%	100%
KU707826	SSABR1,	Brazil	2015	100%	100%	100%
KU527068	Natal RGN	Brazil	2015	100%	100%	100%
KU647676	MRS_OPY_Martinique_PaRi	Martinique	2015	100%	100%	100%
KU501217	8375	Guatemala	2015	100%	100%	100%
KU501216	103344	Guatemala	2015	100%	100%	100%
KU365780	BeH815744	Brazil	2015	100%	100%	100%
KU365779	BeH819966	Brazil	2015	100%	100%	100%
KU365778	BeH819015	Brazil	2015	100%	100%	100%
KU365777	BeH818995	Brazil	2015	100%	100%	100%
KU312312	Z1106033	Suriname	2015	100%	100%	100%
KU321639	ZikaSPH2015,	Brazil	2015	100%	100%	100%
KJ776791	H/PF/2013	French Polynesia	2013	100%	100%	100%
KX056898	GZ02/2016	China	2016	95%	100%	100%
KU955590	Z16019	China	2016	95%	100%	100%
KU955593	/H.sapiens-tc/KHM/2010/FSS13025	Cambodia	2010	100%	100%	100%
KF993678	PLCal_ZV	Canada	2013	100%	100%	100%
JN860885	FSS13025	Cambodia	2010	100%	100%	100%
EU545988	-	Micronesia	2007	100%	100%	100%
KU681082	H.sapiens-tc/PHL/2012/CPC-0740	Philippines	2012	100%	94%	100%
KJ461621	Tahiti	Norway	2013	100%	100%	100%
KF383117	ArD128000	Senegal	1994	100%	100%	100%
HQ234499	P6-740	Malaysia	1966	100%	100%	100%
KX198134	SEN/DAK-AR-41524_A1C1-V2	Senegal	1984	100%	100%	100%
KU955595	A.taylori-tc/SEN/1984/41671-DAK	Senegal	1984	100%	100%	100%
KU955591	A.africanus-tc/SEN/1984/41525-DAK	Senegal	1984	100%	100%	100%
KF383120	ArD142623	Senegal	2000	100%	100%	100%
KF383116	ArD7117	Senegal	1968	100%	100%	100%
HQ234501	ArD_41519	Senegal	1984	100%	100%	100%
KF383121	ArD158095	-	-	100%	100%	94%
KF383119	ArD158084	Senegal	2001	100%	100%	94%
KF383118	ArD157995	Senegal	2001	100%	100%	94%
KF268950	ARB7701	Central African Republic	-	100%	100%	100%
KF268949	ARB15076	Central African Republic	-	100%	100%	100%
KF268948	ARB13565	Central African Republic	1976	100%	100%	100%
KF383115	ArB1362	Central African Republic	1968	100%	100%	94%

In silico analysis of the primer and probe sequences was performed to verify reagent sequence homology with the target region. An extensive list of current and historical Zika strains was selected for *in-silico* analysis. For each Zika strain analyzed, at least one primer and probe set had sequences with ≥94% sequence identity.

D. Cross-Reactivity (Analytical Specificity)

A panel of viruses and organisms known to cause similar signs and symptoms of Zika virus infection were selected for analysis in the cross reactivity study for the Zika ELITe MGB Kit U.S. on the ELITe InGenius™ instrument. Purified nucleic acid from Chikungunya, Dengue 1, Dengue 2, Dengue 3, Dengue 4, West Nile, Yellow Fever, *plasmodium falciparum*, Parvo virus, and Mayaro virus were tested at high concentrations to assess for potential cross reactivity with the assay primers and probes.

Table 10 below demonstrates lack of cross reactivity among symptom-similar organisms in plasma. Strains and concentrations tested are identified in the table below.

Table 10: Pathogens Wet Tested in Cross-Reactivity Study

Organism	Source	Zika Ct[6]	Test Conc.
Chikungunya	ARUP Laboratories	NEG	1.00×10^5 PFU/mL
Dengue 1 virus	ATCC VR-1254	NEG	1.00×10^5 PFU/mL
Dengue 2 virus	ATCC VR-345	NEG	1.00×10^5 1e5 PFU/mL
Dengue 3 virus	ATCC VR-1256	NEG	1.00×10^5 PFU/mL
Dengue 4 virus	ATCC VR-1257	NEG	1.00×10^5 PFU/mL
Mayaro virus	ATCC VR-1277	NEG	1.00×10^5 PFU/mL
Plasmodium falciparum	ARUP Laboratories	NEG	1.00×10^5 protozoa/mL
Parvovirus B-19	ZeptoMetrix	NEG	1.00×10^5 IU/mL
West Nile	ARUP Laboratories	NEG	1.00×10^5 PFU/mL
Yellow fever, strain 17D	ZeptoMetrix	NEG	1.00×10^5 PFU/mL

Additional potential cross-reactivity of the Zika ELITe MGB® Kit U.S. primers and probe was evaluated by *in silico* NCBI BLAST analysis to determine if additional organisms have significant combined homology that would predict potential false positive results. Percent homology for each primer and probe is presented in Table 11.

Table 11: Primer and Probe Percent Homology

Organism	Tax ID	ZKV3 L1	ZKV3 FAM1	ZKV3 E1
Japanese encephalitis virus	11072	70%	56%	61%
St. Louis encephalitis virus	11080	70%	50%	56%
Yellow Fever	11089	50%	56%	56%
Dengue 1	11053	55%	56%	61%
Dengue 2	11060	55%	56%	61%
Dengue 3	11069	55%	56%	61%
Dengue 4	11070	55%	56%	78%
West Nile virus	11802	60%	56%	61%
Chikungunya virus	37124	55%	50%	50%
Mayaro virus	59301	55%	61%	50%
Spondweni virus	64318	70%	50%	50%
Eastern Equine encephalitis virus	11021	55%	61%	61%
Western Equine encephalitis virus	11039	50%	56%	44%
Ross River virus	11029	45%	50%	44%
O'nyong-nyong virus	11027	55%	50%	61%
Hepatitis C virus	11103	60%	83%	37%
Barmah Forest virus	NC_00 1786	45%	44%	44%
Measles virus	11234	50%	50%	61%
Rubella virus	11041	40%	44%	50%
Enterovirus	12059	80%	61%	78%
Adenovirus	10508	60%	61%	72%
Hepatitis B virus	10407	75%	55%	67%
HIV	11652	75%	67%	72%

[6] IC was detected in all cross-reactivity samples.

Organism	Tax ID	ZKV3 L1	ZKV3 FAM1	ZKV3 E1
Varicella Zoster virus	10335	45%	50%	50%
Cytomegalovirus	10358	55%	61%	61%
Epstein Barr virus	10376	60%	72%	56%
Rickettsia sp	780	65%	67%	67%
Borrelia burgdorferi	64895	65%	61%	72%
Group A *Streptococcus*	1314	80%	72%	67%
Leptospirosis	1643688	70%	67%	83%
Plasmodium sp	5820	70%	72%	83%
Trypanosoma cruzi	5693	65%	78%	78%
Schistosomiasis	6181	75%	78%	83%
Hepatitis A virus vaccine	208726	35%	44%	50%
Salmonella	590	65%	78%	78%
Salmonella typhi vaccine	527001	55%	67%	72%
Streptococcus	1301	80%	78%	83%
E. coli	562	75%	78%	83%

Hits were evaluated for potential formation of PCR products via binding of primers in close proximity, with the correct orientation to each other and within the same strain. Cross-reactivity is not expected for any organisms listed in Table 11.

E. Potential Microbial Interference Risk Assessment

In several instances, the primers or probe show ≥ 70% homology to other organisms based on BLAST analysis of the taxon for that pathogen. In several cases, the top hits are for pathogens that do not infect humans but are part of that taxon. Because the amount of primers and probe that are added to the master mix are in excess of $1x10^{12}$ copies per reaction for each oligo, the presence of a significant co-infection pathogen at the highest known clinically relevant concentrations are not expected to impact the ability of the Zika ELITe MGB® Kit U.S. detecting a low titer Zika virus positive.

In addition, microbial interference wet-testing was conducted at ARUP using a Zika assay under an EUA that utilizes the exact same primers/probe as the Zika ELITe MGB® Kit U.S. In this study, Zika virus RNA at low concentration (i.e., 3×LoD) in the presence of high titer organisms for Dengue 1-4, Chikungunya, West Nile, Hepatitis B, HIV, *Escherichia coli* and Plasmodium species : *P. knowlesi, P. falciparum, P. ovale, P. vivax*, and *P. malariae* were detected in all samples. No microbial interference was observed in this study.

F. Matrix Equivalency Study

A total of 40 paired serum and plasma samples were tested at ARUP using a Zika assay under an EUA that utilizes the exact same primers/probe as the Zika ELITe MGB® Kit U.S. in order to support the claim of serum as an additional specimen type to plasma. Twenty paired serum and plasma samples were spiked at 1.5×LoD, 10 paired serum and plasma samples were spiked at 5×LoD, and 10 paired serum and plasma samples were run as unspiked negatives. All samples were blinded and randomized prior to testing. Results of the matrix equivalency study indicate equivalent performance in serum and plasma samples.

G. QCMD 2016 Zika Virus EQA Pilot Study (ZIKA16)

The 2016 Zika Virus EQA Pilot Study panel consists of ten vials containing freeze dried transport medium samples with various concentrations of Zika virus or samples negative for Zika virus. The samples were labeled ZIKA16-01 to ZIKA16-10, and blinded for all test sites until the end of the study. All panel components were produced and certified according to the guidelines for the production and certification of BCR reference materials (EU document BCR/48/93) and the ISO 17043: 2010, Proficiency testing by inter-laboratory comparisons. The 2016 Zika Virus EQA Pilot Study panel was

received from QCMD along with detailed instructions/protocol on how to dilute and test the reference material. Once results were returned to QCMD, a report was received.

A summary of results for this testing is provided below in Table 12 below. The "Detection/ Frequency" column describes the frequency of detection for each sample based on the peer group (115 participants) consensus of all qualitative results returned from participants within the QCMD 2016 Zika Virus EQA challenge / distribution.

Table 12: QCMD 2016 Zika Virus EQA Pilot Study (ZIKA16) Results

Sample Code	Sample Content	Detection/ Frequency	Zika ELITe MGB Kit/Zika C_T	Final Interpretation
ZIKA16-01	Dengue Virus Type 2, West Nile Virus (NY99), Yellow Fever Virus	Negative	NEG	Zika:Zika Virus RNA not detected
ZIKA16-02	Zika Virus (African)	Detected	36.43	Zika:Zika Virus RNA detected
ZIKA16-03	Zika Virus (French Polynesian)	Frequently Detected	31.48	Zika:Zika Virus RNA detected
ZIKA16-04	Zika Virus (French Polynesian)	Frequently Detected	30.55	Zika:Zika Virus RNA detected
ZIKA16-05	Zika Virus Negative	Negative	NEG	Zika:Zika Virus RNA not detected
ZIKA16-06	Zika Virus (French Polynesian)	Frequently Detected	28.01	Zika:Zika Virus RNA detected
ZIKA16-07	Chikungunya Virus	Negative	NEG	Zika:Zika Virus RNA not detected
ZIKA16-08	Zika Virus (African)	Frequently Detected	34.92	Zika:Zika Virus RNA detected
ZIKA16-09	Zika Virus (African)	Frequently Detected	30.32	Zika:Zika Virus RNA detected
ZIKA16-10	Zika Virus (African)	Frequently Detected	32.83	Zika:Zika Virus RNA detected

Out of ten blinded samples, all ten were identified correctly. Three test samples were either Zika virus negative or contained other viruses, and seven were Zika virus positive. The Zika ELITe MGB® Kit U.S. results were 100% correct.

H. Clinical Evaluation

Clinical Performance of the Zika ELITe MGB® Kit U.S. on the ELITe InGenius™ instrument was evaluated with a combination of retrospective natural clinical specimens and contrived samples.

a. Natural Clinical Specimens Testing:
A total of 75 retrospective natural EDTA plasma samples were collected from patients in Colombia and/or acquired from Boca Biolistics. Twenty (20) natural EDTA plasma specimens were collected from symptomatic patients in Dominican Republic (showing Zika symptoms such as fever, conjunctivitis, joint/muscle pain, etc.) where Zika is currently endemic. These specimens were tested positive for Zika virus RNA by a Zika RT-PCR test under an EUA before archiving. Fifty (55) natural EDTA plasma specimens were collected from Zika endemic areas in Colombia from high risk asymptomatic patients. These 55 EDTA plasma samples from asymptomatic patients were characterized with the use of laboratory developed tests (LDTs) before archiving, where 25 of the 55 samples were determined to be positive of Zika RNA and 30 of the 55 samples were determined to be negative of Zika RNA.

In addition, a total of 30 retrospective natural EDTA plasma samples were collected from individual normal donors from Salt Lake City, Utah (non-endemic for Zika). Zika RNA is not expected to be

detected in these samples since they were collected from a non-endemic area in the US.

All samples were run in the ARUP Laboratories with a comparator method authorized for use under an EUA, and in the ELITechGroup MDx Laboratory with Zika ELITe MGB® Kit U.S on the ELITe InGenius™ instrument. All samples were blinded and randomized prior to testing.

i. Of the 25 positive asymptomatic plasma specimens, 11 had matched positive results between Zika ELITe MGB® Kit U.S. and EUA comparator.

ii. Of the 20 positive symptomatic plasma specimens, 16 had matched positive results between Zika ELITe MGB® Kit U.S. and EUA comparator.

iii. Of the 30 asymptomatic plasma specimens that were Zika RNA negative (as determined by the LDTs) before archiving, 29 had matched negative results between the Zika ELITe MGB® Kit U.S. and the EUA comparator, and one was negative by the Zika ELITe MGB® Kit U.S. assay only, and positive by the EUA comparator.

iv. Of the 30 plasma specimens collected from normal donors from the U.S., all 30 had matched negative results between the Zika ELITe MGB® Kit U.S. and the expected Zika RNA negative results.

In addition, the Biomedical Advanced Research and Development Authority (BARDA) and the Office of the Assistant Secretary for Preparedness and Response (ASPR) in conjunction with the CDC sent a panel of Zika PCR positive and negative plasma samples to be tested. The molecular panel consisted of 16 plasma samples (approx. 1 mL each) including low and high C_Ts (based on the Roche cobas Zika assay under an IND) and two negatives. The panel was sent to EG MDx blinded so that testing was unbiased. The positive plasma specimens were obtained from the Zika PCR positive donor units collected in Puerto Rico. The panel was tested with the Zika ELITe MGB® Kit U.S. on the ELITe InGenius™ system in the EG MDx laboratories in Bothell, WA. Once results were returned to ASPR/BARDA, a key was obtained.

Performance of the Zika ELITe MGB® Kit U.S. on the ELITe InGenius™ system testing natural clinical specimens (data from all studies testing natural clinical specimens combined) are presented in Table 13 below.

Table 13: Natural Plasma Samples Results Summary (vs. the comparator results or expected results)

Plasma – Specimen Category	Zika ELITe MGB® Kit U.S.					
	Number Tested	Zika RNA Positive	Zika RNA Negative	Invalid	PPA (95% CI)	NPA (95% CI)
Natural specimens collected from symptomatic patients in Dominican Republic where Zika is currently endemic. These specimens were tested positive for Zika virus RNA by a Zika RT-PCR test under an EUA. [c]	20	17	3	0	88.9% (16/18) (67.2% - 96.9%)	50% (1/2 [a]) (9.5% - 90.5%)
Natural specimens collected from Colombia from high risk asymptomatic patients that were tested positive for Zika virus RNA by laboratory developed Zika RT-PCR tests. [c]	25	17	8	0	100% (11/11) (74.1% - 100%)	57.1 % (8/14 [b]) (32.6% - 78.6%)
Natural specimens collected from Colombia from high risk asymptomatic patients that were tested negative for Zika virus RNA by an investigational Zika RT-PCR test. [c]	30	0	30	0	NA (0/1)	100% (29/29) (88.3% - 100%)
Expected Zika RNA negative specimens that were collected from normal donors from ARUP in Utah.	30	0	30	0	NA (0/0)	100% (30/30) (88.6% - 100%)
Natural specimens from a panel provided by the Biomedical Advanced Research and Development Authority (BARDA) and the Office of the Assistant Secretary for Preparedness and Response (ASPR) in conjunction with the CDC. The specimens in the panel were characterized by the Roche cobas Zika assay under an IND.	16	12	4	0	85.7% (12/14) (60.1% - 96.0%)	100% (2/2) (34.2% - 100%)
Positive Percent Agreement	**88.6% (39/44) 95% CI (76.0% - 95.0%)**					
Negative Percent Agreement	**90.9% (70/77) [a,b] 95% CI (82.4% - 95.5%)**					

[a] 1/1 "false positive" plasma specimens against the EUA comparator results were tested ZIKV RNA positive using the Roche LightMix Zika Assay under an EUA.
[b] 6/6 "false positive" plasma specimens against the EUA comparator results were tested ZIKV RNA positive using laboratory developed Zika RT-PCR assays.
[c] The comparator assay was an rRT-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.

b. Contrived Clinical Specimens Testing

Accuracy for the Zika ELITe MGB® Kit U.S. assay was also assessed by testing contrived plasma samples collected from individual donors. For the contrived panel, a different set of negative plasma samples (not the negative samples that were used for the natural clinical specimens testing) were used, 25 from Colombia and 25 from ARUP. A total of 50 samples were tested.

The contrived panel consisted of five levels (10 replicates at each level) spiked at 1×LoD, 2×LoD, 3×LoD, 4×LoD, and 5×LoD. Zika virus strain PRVABC59 was used to spike all contrived specimens. All samples were blinded and randomized prior to testing. As shown in Table 14, Zika virus RNA was detected in 100% of the contrived samples.

Table 14: Summary Results and % Agreement for Contrived Specimens

Spiked Level	No. Tested	No. Detected	% Agreement	95% CI
1×LoD	10	10	100%	72.2% - 100%
2×LoD	10	10	100%	72.2% - 100%
3×LoD	10	10	100%	72.2% - 100%
4×LoD	10	10	100%	72.2% - 100%
5×LoD	10	10	100%	72.2% - 100%
All	50	50	100%	92.9% - 100%

Performance of the Zika ELITe MGB® Kit U.S. on the ELITe InGenius™ system testing all Clinical Specimens (Data from all studies testing natural and contrived clinical specimens combined) are presented in Table 15 below.

Table 15: Natural and Contrived Plasma Samples Results Summary (vs. the comparator results or expected results)

Plasma – Specimen Category	Zika ELITe MGB® Kit U.S.					
	Number Tested	Zika RNA Positive	Zika RNA Negative	Invalid	PPA (95% CI)	NPA (95% CI)
Natural specimens collected from symptomatic patients in Dominican Republic where Zika is currently endemic. These specimens were tested positive for Zika virus RNA by a Zika RT-PCR test under an EUA. [c]	20	17	3	0	88.9% (16/18) (67.2% - 96.9%)	50% (1/2 [a]) (9.5% - 90.5%)
Natural specimens collected from Colombia from high risk asymptomatic patients that were tested positive for Zika virus RNA by laboratory developed Zika RT-PCR tests. [c]	25	17	8	0	100% (11/11) (74.1% - 100%)	57.1 % (8/14 [b]) (32.6% - 78.6%)
Natural specimens collected from Colombia from high risk asymptomatic patients that were tested negative for Zika virus RNA by an investigational Zika RT-PCR test. [c]	30	0	30	0	NA (0/1)	100% (29/29) (88.3% - 100%)
Expected Zika RNA negative specimens that were collected from normal donors from ARUP in Utah.	30	0	30	0	NA (0/0)	100% (30/30) (88.6% - 100%)
Natural specimens from a panel provided by the Biomedical Advanced Research and Development Authority (BARDA) and the Office of the Assistant Secretary for Preparedness and Response (ASPR) in conjunction with the CDC. The specimens in the panel were characterized by the Roche cobas Zika assay under an IND.	16	12	4	0	85.7% (12/14) (60.1% - 96.0%)	100% (2/2) (34.2% - 100%)
Contrived specimens (1xLoD)	10	10	0	0	100% (10/10) (72.2% - 100%)	NA (0/0)
Contrived specimens (2xLoD)	10	10	0	0	100% (10/10) (72.2% - 100%)	NA (0/0)
Contrived specimens (3xLoD)	10	10	0	0	100% (10/10)	NA (0/0)

Plasma – Specimen Category	Zika ELITe MGB® Kit U.S.					
	Number Tested	Zika RNA Positive	Zika RNA Negative	Invalid	PPA (95% CI)	NPA (95% CI)
					(72.2% - 100%)	
Contrived specimens (4xLoD)	10	10	0	0	100% (10/10) (72.2% - 100%)	NA (0/0)
Contrived specimens (5xLoD)	10	10	0	0	100% (10/10) (72.2% - 100%)	NA (0/0)
Positive Percent Agreement	**94.7% (89/94) 95% CI (88.1% - 97.7%)**					
Negative Percent Agreement	**90.9% (70/77) [a,b] 95% CI (82.4% - 95.5%)**					

[a] 1/1 "false positive" plasma specimens against the EUA comparator results were tested ZIKV RNA positive using the Roche LightMix Zika Assay under an EUA.
[b] 6/6 "false positive" plasma specimens against the EUA comparator results were tested ZIKV RNA positive using laboratory developed Zika RT-PCR assays.
[c] The comparator assay was an rRT-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.

REFERENCES

W. A. Verstrepen et al. (2001) *J Clin Microbiology* 39: 4093 - 4096

E. A. Lukhtanov et al. (2007) *Nucleic Acids Res*. 35: e30

SYMBOLS

REF	Catalog number	IVD	*In vitro* diagnostic medical device
	Upper temperature limit		Contains sufficient material for <n> tests
LOT	Batch code		Consult instructions for use
	Use by		Manufacturer
Rx only	For prescription use only		Warning
	Positive Control		Control
CONTROL -	Negative Control		Keep away from sunlight
CONT	Contents		

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