Zika Virus Detection Kit v1

USER MANUAL

For *in vitro* Diagnostic Use

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IVD CE

Contents

1. PRODUCT DESCRIPTION

Bosphore® Zika Virus Detection Kit v1 detects Zika virus RNA in human biological samples such as blood, serum or plasma, body fluids, urine, tissue, swap specimens. Analytic sensitivity is 2.4 copies/µl. A region within the polyprotein gene of Zika virus genome is amplified and detected with FAM filter.

The internal control has been integrated into the kit in order to check nucleic acid extraction and PCR inhibition. The amplification data of the internal control is detected with the HEX filter. The internal control is added during nucleic acid extraction step or PCR step.

2. CONTENT

Bosphore® Zika Virus Detection Kit v1 is composed of Real-Time PCR reagents listed in the table below:

Component	REAGENT	100 Reactions	50 Reactions	25 Reactions
1	dH_2O	(1000 µl)	(500 µl)	(500 µl)
2	PCR Master Mix	(1628 µl)	(814 µl)	(407 µl)
3	Internal Control	(550 µl)	(275 µl)	(138 µl)
4	Positive Control	(88 µl)	(44 µl)	(22 µl)

3. STORAGE

Bosphore® Zika Virus Detection Kit v1 PCR reagents should be stored at -20°C. Repeated thawing and freezing (>3x) should be avoided since it may reduce sensitivity. If the components are to be used in small amounts, they should be frozen in aliquots.

While preparing the PCR; the components should not be exposed to room temperature for more than 10 min. and the detection mix components should not be exposed to light more than 1-2 min. We recommend preparing the PCR on a cooling block and keeping the detection mixes within a closed container.

The components maintain their stability until the expiry dates on the labels, if they are stored at advised conditions.

4. REQUIRED MATERIALS AND DEVICES

- Montania® 483, Montania® 484 or Montania® 4896 Real-Time PCR Instrument (Anatolia Geneworks), or another Real-Time PCR system with FAM and HEX filters (such as iCycler, iQ5, CFX–BioRad, LightCycler 480-Roche, 7500 Real-Time PCR System-ABI, Stratagene Mx3005P, Mx3000P-Agilent, LineGeneK, LineGene 9600-Bioer, Rotorgene 6000, Q-Qiagen)
- 0.2 ml Thin-Wall PCR tubes, strips or PCR plates
- Magnesia® 16 Nucleic Acid Extraction System / Magnesia® Viral Nucleic Acid Extraction Kit/ Magrev® 24 stand and Magrev® Viral DNA/RNA Extraction Kit/Bosphore® Viral RNA Extraction Spin Kit (Anatolia Geneworks) or other high quality viral RNA extraction kits and systems
- Deep freezer (-20°C)
- Desktop centrifuge with rotor for 2 ml. microcentrifuge tubes
- Calibrated adjustable micropipettes
- DNAse, RNAse, pyrogen free micropipette tips with filters
- RNAse, RNAse, pyrogen free 1.5 or 2 ml. microcentrifuge tubes
- Disposable laboratory gloves

5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

Important!:

- The product should be delivered on dry ice. Check for presence of dry ice upon arrival.
- Check for the expiry dates on the box and tube labels, upon arrival. Do not use expired products or components.
- Calibrated or verified micropipettes, RNAse, RNAse, pyrogen free micropipette tips with filters, and RNAse, RNAse, pyrogen free microcentrifuge tubes should be used.
- Before starting a test procedure, all components should be thoroughly thawed. After thawing, all components should be centrifuged briefly (spin-down for 3-5 seconds), and mixed well to ensure homogeneity prior to use.
- The kit components should be kept on ice or a cooling block until the reaction is prepared, and they should be quickly returned to -20ºC.
- PCR and nucleic acid isolation must be performed in different compartments. Samples should be stored separately to avoid contact with the kit components.
- Pathogen information should be reviewed to be aware of the health related risks.
- Biological samples should be handled with extreme caution: Physical contact with pathogens should be avoided by; wearing lab coats and gloves, no allowance for eating or drinking within the workspace, prevention of unauthorized individuals' access to the working area.
- All the pathogenic wastes produced during the nucleic acid isolation step; including the serum/plasma samples and material contacted with them, should be discarded into medical waste and disposed safely.

6. PRODUCT USE LIMITATIONS

- All the components may exclusively be used for in vitro diagnostics.
- This product should be used in accordance with this user manual, by personnel specially trained to perform in vitro diagnostic procedures.

7. PATHOGEN

CAUSATIVE AGENTS

Zika virus (ZIKV) is a flavivirus belonging to the Flaviviridae family that was first isolated in 1947 in 'Zika Forest' of Uganda and named after this region. ZIKV virions are approximately 40 nm in diameter and are composed of a single-stranded, positive sense RNA genome of length between 10 kb and 11kb. [1]

EPIDEMIOLOGY

The human cases of Zika Virus were first detected in 1952. Since then outbreaks of Zika have been reported in tropical Africa, Southeast Asia, and the Pacific Islands. In 2015, there were between 440000-1300000 cases reported in South America, mainly in Brazil. On February 1, 2016, Zika virus was declared as a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO). [2], [3]

MODES OF TRANSMISSION

It is transmitted to people predominately through the use mosquitoes (species of *Aedes)* as vectors. However transmission via sexual contact and through blood transfusion can be observed. Symptoms such as fever, rash, joint pain, muscle pain are the most common described ones. The virus can be transmitted from an infected mother to her fetus during pregnancy or at delivery and may cause microcephaly or other brain malformations. [4], [5]

8. METHOD

Bosphore® Zika Virus Detection Kit v1 is based on the Real-Time RT-PCR method. Genetic material of Zika Virus is amplified by reverse transcription technique since it is composed of RNA. RT-PCR which is also referred as RNA PCR, is a two-step reaction. Firstly complementary DNA is synthesized from RNA by reverse transcription and then complementary DNA is amplified by standard PCR. The primer binds to the target RNA region in RT-PCR and RNA-DNA double strand is synthesized by reverse transcriptase enzyme using the RNA template for complementary DNA. Afterwards, standard PCR continues.

Polymerase chain reaction is a technique that is used for amplification of a RNA region. The reaction occurs by the repeating cycles of heating and cooling. The main components of PCR are primers, dNTPs, Taq polymerase enzyme, buffer solution and template. As a brief explanation, primers are small synthetic RNA those anneal to the specific regions of the template in order to start the synthesis. dNTPs are the building blocks of the amplified products. Taq polymerase amplifies the RNA template. Buffer solution provides the pH adjustment required for the reaction and template, as referred, is the target region for synthesis. In addition to these components, in RT PCR, reverse transcriptase is added to the reaction and cDNA synthesis from the RNA template is acquired

In Real Time PCR technique, in contrast to conventional PCR, PCR product can be monitored during the reaction. Therefore Real-Time PCR obviates the need for further analysis methods like gel electrophoresis, whereby minimizing the risk of contamination. Dual labeled probes employed in the reaction in addition to the conventional PCR reagents, enable detection of the amplified target with increased sensitivity.

The assay utilizes the 5′ exonuclease activity of Taq Polymerase to cleave a dual-labeled fluorescent hybridization probe during the extension phase of PCR.

The probe is labeled at the 5′ end with a fluorescent ‘reporter’ molecule, and at the 3′end with another fluorescent molecule that acts as a ‘quencher’ for the ‘reporter’. When the two fluorophores are in close proximity, and the reporter is excited by light, no reporter fluorescence can be detected. During the elongation step of PCR, Taq Polymerase encounters and cleaves the probe bound to the template. As the reporter is freed from the suppressing effect of the quencher, fluorescence signal can be detected.

The fluorescence generated by the reporter increases as the PCR product is accumulated; the point at which the signal rises above background level and becomes distinguishable, is called the threshold cycle (C_T). There is a linear relationship between the log of the starting amount of a template and its threshold cycle, thus starting amount of unknown templates can be determined using standard curves constructed using C_T values of the known starting amounts of target templates.

Bosphore® Zika Virus Detection Kit v1 employs multiplex PCR, and an internal control is incorporated into the system in order to control the isolation procedure and to check for possible PCR inhibition. Zika Virus RNA is amplified and detected with FAM filter.

The internal control has been integrated into the kit in order to check nucleic acid extraction and PCR inhibition. The amplification data of the internal control is detected with the HEX filter. The internal control is added during nucleic acid extraction or PCR step.

9. PROCEDURE

9.1. RNA Isolation

We recommend that Magnesia® 16 Nucleic Acid Extraction System / Magnesia® Viral Nucleic Acid Extraction Kit/ Magrev® 24 stand and Magrev® Viral DNA/RNA Extraction Kit/Bosphore® Viral RNA Extraction Spin Kit (Anatolia Geneworks) or other high quality viral RNA extraction kit and system is used with Bosphore® Zika Virus Detection Kit v1. The RNA isolation should be performed according to the manufacturers' instructions.

9.2. Kit Components

9.2.1. PCR Master Mix

PCR mix contains; HotStarTaq DNA Polymerase, RT-PCR Buffer, Zika Virus-specific forward and reverse primers and dual-labeled probes, internal control-specific forward and reverse primers and a dual-labeled probe, RT-Mix and dNTPs.

9.2.2. Internal Control

An internal control is included in the kit to control nucleic acid extraction and PCR inhibition. The internal control is a synthetic DNA molecule derived from human genome. If it is added from extraction step, it should be added 5µl/sample.If it is added directly into the PCR master mix to control the PCR inhibition, 0.2 µl of internal control should be added for each reaction into the master mix. Lack of internal control amplification in the HEX negative samples, may indicate a problem in isolation or PCR inhibition. In this case, isolation and PCR should be repeated. In samples that contain a high viral load, internal control can be suppressed and no increase of the signal is detected. Please use the table below for the interpretation of internal control data:

Zika Virus /FAM	Internal control/HEX	Result
+	+	Sample positive for Zika Virus
+	-	Sample positive for Zika Virus
-	+	Sample negative for Zika Virus
-	-	Repeat the test!

9.2.3. Positive Control

Positive control contains Zika Virus RNA. It can be included in the PCR to test the efficiency of the PCR exclusively. The threshold cycle for the positive control is given in the acceptance criteria table (Section 10. Analysis). Threshold cycles higher than the acceptance criteria may indicate an efficiency loss in the reaction.

9.3. Preparing the RT-PCR

Make sure that all the kit components are thawed before use. Refer to the table below for preparing the PCR. It is for only one reaction, multiply these values with the sample number to find the values required for the master mix. While preparing master mixes for more than 5 samples, an extra 10% should be added to the total sample number.

PCR Master Mix	14.8 µl
Internal Control*	0.2 µl
Sample RNA **Negative/Positive Control**	10 µl
Total Volume	25 µl

*If the internal control is added from extraction step, it is not required to add in PCR step.

Pipette 15 µl of the master mix into the PCR tubes or strips, and add 10 µl of RNA (sample/positive or negative control). Close the tube cap. Make sure that the solution in each tube is at the bottom of the tube. Centrifuge if necessary.

9.4. Programming the Real-Time PCR Instrument

The thermal protocol for Bosphore® Zika Virus Detection Kit v1 is composed of an initial denaturation for activation the HotStarTaq RNA Polymerase, a two-step amplification cycle and a terminal hold. The real-time data is collected at the second step of the amplification cycle.

Reverse Transcription	50°C	30:00 min.	
Initial denaturation	95°C	14:30 min.	
Denaturation	97°C	00:30 min.	50 cycles
Annealing and Synthesis	55°C	01:20 min.	
(Data Collection)	72°C	00:15 min.	
Hold	22°C	05:00 min.	

To start a Real-Time PCR reaction using the Bosphore® Kits, the following steps should be completed:

- Choose the filter pairs to be used (FAM and HEX),
- Identify unknown samples, positive and negative controls,
- Select the correct thermal protocol.
- Start the protocol

10. ANALYSIS

By the end of the thermal protocol, the Real-Time PCR Instrument software automatically calculates the baseline cycles and the threshold.

Example of an amplification curve is given in Fig. 1.

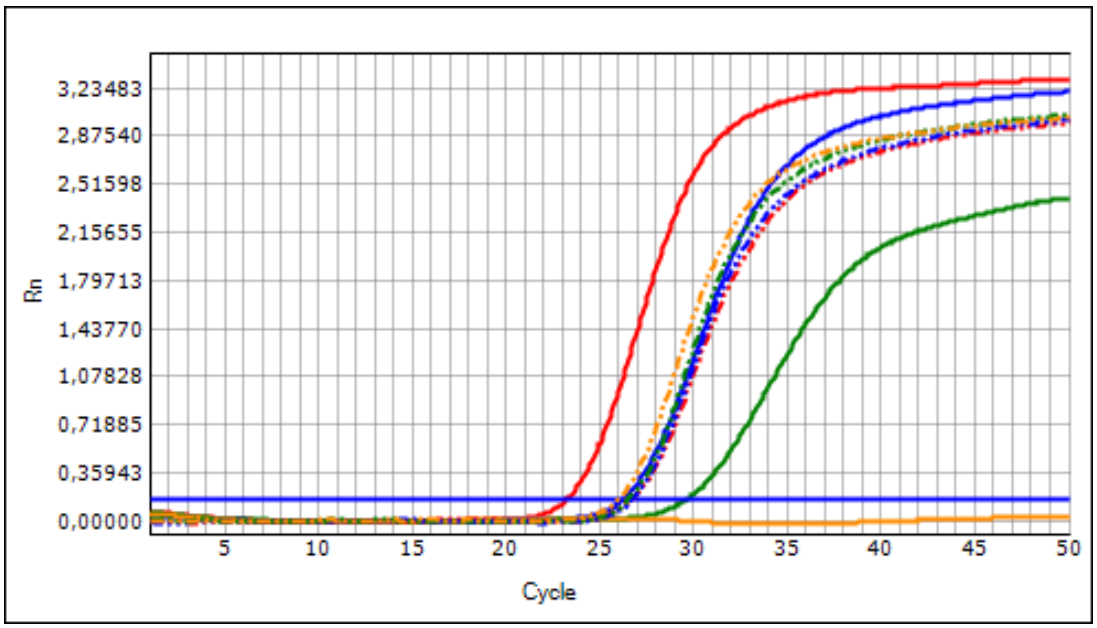

Fig. 1 : Amplification Curves of a Bosphore® Zika Virus v1 test

Analysis of the results should be performed by trained personnel who have received the required training for analysing Real-Time PCR data. We recommend that the test results must be evaluated by an expert clinician, taking the patient's clinical findings and the results of other tests into consideration.

All analysis is done automatically in routine use. However, when the trained personnel, who have received the required training from manufacturer, consider it as necessary, and if the system's software allows pulling down the threshold as much as possible in order to detect low positive samples, attention should be paid to keep the threshold line above the background and to keep the correlation coefficient at the maximum possible value (and within its acceptance criteria.)

Positive control of Bosphore® Zika Virus v1 are essential for accurate result analysis. The table below displays the acceptance criteria for positive control:

Component/Parameter	Ct
Positive Control	25±4

Test results should not be reported unless the assay results meet the criteria stated above. Please contact the manufacturer if impairment in the product's performance is observed (See the last page for contact information).

The qualitative results of the test are displayed on the report screen. The samples that cross the threshold in FAM are displayed as positive for Zika Virus whereas samples that do not cut the threshold are displayed as "Negative" or "No Ct. These samples are regarded as negative or having a viral load below the detection limit of the assay. For these undetectable samples, the HEX data of the internal control should also be checked to avoid false negative results.

The following table shows the possible results and their interpretation:

Zika Virus /FAM	**Internal control HEX**	**Result**
+	+	Sample positive for Zika Virus
+	-	Sample positive for Zika Virus
-	+	Sample negative for Zika Virus
-	-	Repeat the test!

11. SPECIFICATIONS

11.1. Sensitivity

Analytical sensitivity may be expressed as the limit of detection: i.e. the smallest amount of the target marker that can be precisely detected. The detection limit of an individual analytical procedure is the lowest amount of nucleic acid in a sample which can be detected but not necessarily quantitated as an exact value. The analytical sensitivity or detection limit for NAT assays is expressed by the 95% positive cut-off value. The analytical detection limit for Bosphore Zika Virus Detection Kit v1 was found to be 2.4 copies/µl. The sensitivity was determined using serial dilutions of Zika Virus RNA. The dilutions were tested in different runs in replicates. The results were analyzed by probit method.

11.2. Cross-Reactivity

To eliminate potential cross-reactivity, both assay design evidence and experimental studies were employed. Primer and probe sequences were checked for possible homology to other known pathogen sequences by sequence comparison analysis using database alignment. Samples of HCV, CCHFV, HIV, hMPV, RSV, HPIVs, Influenza virus A, Influenza virus B, CMV, EBV, Parvovirus B19 and BKV with known high positivity were tested, and found negative.

12. REFERENCES

1. Faye O, Freire C, Iamarino A, Faye O, de Oliveira J, Diallo M, Zanotto P, Sall A. (2014) Molecular Evolution of Zika Virus during Its Emergence in the 20th Century. PLOS Neglected Tropical Diseases
2. Campos GS, Bandeira AC, Sardi SI. (2015) Zika virus outbreak, Bahia, Brazil. CDC Emerging Infectious Diseases
3. Goldsmith, Cynthia (18 March 2005). "Zika Virus". Centers for Disease Control and Prevention. Retrieved 4 March 2016.
4. "CDC Concludes Zika Causes Microcephaly and Other Birth Defects". CDC. 13 April 2016. Retrieved 14 April 2016.
5. "CDC Zika: Transmission". U.S. Centers for Disease Control and Prevention. 15 April 2016. Retrieved 17 April 2016.

13. SYMBOLS


Use by

Lot/Batch

Catalog number


Temperature limitation


Caution, consult accompanying documents


Manufacturer

In Vitro Diagnostic Medical Device

14. CONTACT INFORMATION

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