Chikungunya Virus Detection Kit v1

USER MANUAL

For *in vitro* Diagnostic Use

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IVD CE

Contents

1. PRODUCT DESCRIPTION

Bosphore® Chikungunya Virus Detection Kit v1 detects all the genotypes of the Chikungunya Virus RNA in various types of human samples such as serum, plasma, cerebrospinal fluid, tissue, swab, feces, etc. The analytic sensitivity is 5 copies/µl. A region within the envelope protein gene is amplified and fluorescence detection is accomplished using the FAM filter.

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

2. CONTENT

Bosphore® Chikungunya Virus Detection Kit v1 is composed of following Real-Time PCR reagents:

Component	REAGENT	100 Tests	50 Tests	25 Tests
1	dH_2O	(1000 µl)	(1000 µl)	(500 µl)
2	PCR Master Mix	(1628 µl)	(814 µl)	(407 µl)
3	Internal Control	(22 µl)	(15 µl)	(15 µl)
4	Positive Control	(88 µl)	(44 µl)	(22 µl)

3. STORAGE

Bosphore® Chikungunya 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 and air 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 (iCycler, iQ5, CFX–BioRad, LightCycler 480-Roche, 7500 Real-Time PCR System -ABI, Stratagene Mx3005P, Mx3000P-Agilent, LineGene 9600-Bioer, Rotorgene 6000, Q-Qiagen)
- 0.2 ml Thin-Wall PCR tubes, plates or strips
- Magnesia® 16 Nucleic Acid Extraction System / Magnesia® Viral Nucleic Acid Extraction Kit / Bosphore® Viral RNA Extraction Spin Kit / Magrev® Viral DNA/RNA Extraction Kit / Magnesia® 2448 Nucleic Acid Extraction & PCR Setup Robot / Magnesia®2448 Viral DNA/RNA Extraction Kit (Anatolia Geneworks) or other high quality viral RNA or 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
- DNAse, 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, DNAse, RNAse, pyrogen free micropipette tips with filters, and DNAse, 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.
- Serum/plasma, all 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 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.
- This product is to be used by personnel specially trained to perform in vitro diagnostic procedures.

7. PATHOGEN

Causative Agents

Chikungunya virus (CHIKV) is an alphavirus and it belongs to the family Togaviridae. It has a single strand positive sense RNA genome. CHIKV was derived from the blood of a febrile patient and it was first isolated in Tanzania in 1953. There are three distinct genotypes: Central/East African, West African and Asian. It has been responsible for major fever epidemics in many parts of the world.

Epidemiology

Chikungunya (CHIK) virus is enzootic in many countries in Asia and throughout tropical Africa. Previous outbreaks in India have been attributed by the Asian genotype of CHIKV. In contrast, due to African genotype, the past recent outbreaks have been revealed in India and on the islands of India Ocean.

Modes of Transmission

The transmission of the virus to humans takes place by the bite of infected Aedes aegypti and A. Albopictus mosquitoes and it can lead to the debilitating disease in humans. The clinical symptoms of the disease are such as high fever, headache, myalgia, severe and prolonged arthralgia, and erythematous skin rashes. The incubation time of Chikungunya fever is determined usually between 3-7 days. However, CHIKV infections do not lead disease in every cases and the cause of these silent infections remains unclear.

8. METHOD

Bosphore® Chikungunya Virus Detection Kit v1 is based on the Real Time RT PCR method. Chikungunya Virus genetic material 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. First, 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 DNA 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 DNA 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 DNA 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® Chikungunya 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. Chikungunya Virus RNA (cDNA) and an internal control are co-amplified in a single reaction, using

sequence-specific primers. The fluorescent signal generated by the Chikungunya Virus amplification is detected by a probe labeled at the 3' end with FAM, through the FAM channel. The fluorescent signal generated by the internal control amplification, is detected by a second probe (labeled at the 5' end with a different reporter molecule, HEX) through the HEX channel.

9. PROCEDURE

9.1. RNA Isolation

We recommend that the Magnesia® 16 Nucleic Acid Extraction System / Magnesia® Viral Nucleic Acid Extraction Kit / Bosphore® Viral RNA Extraction Spin Kit / Magrev® Viral DNA/RNA Extraction Kit / Magnesia® 2448 Nucleic Acid Extraction & PCR Setup Robot / Magnesia® 2448 Viral DNA/RNA Extraction Kit (Anatolia Geneworks) or other high quality viral RNA or extraction kits and systems is used with Bosphore® Chikungunya Virus Detection Kit v1. The RNA isolation should be performed according to the manufacturers' instructions. The amount of internal control that should be used during isolation for each system is 5 µl.

9.2. Kit Components

9.2.1. PCR Master Mix

PCR Master Mix contains; HotStarTaq DNA polymerase, reverse transcriptase, RT-PCR Buffer, Chikungunya Virus specific forward and reverse primers and a dual-labeled probe, internal control-specific forward and reverse primers and a dual-labeled probe, and dNTPs.

9.2.2. Internal Control

An internal control is included in the kit to control RNA isolation and PCR inhibition. The internal control is a synthetic DNA molecule. It is added into the serum/plasma, proteinase K and carrier RNA mixture during DNA isolation, to control the isolation efficiency and PCR inhibition. The amount of IC that should be added during isolation is 5 µl per serum/plasma sample. Alternatively, the internal control can be added directly into the PCR master mix to control the PCR inhibition exclusively. For this purpose, 0.2 µl of internal control should be added for each reaction into the master mix. Lack of internal control amplification in the FAM negative samples, may indicate a problem or PCR inhibition. In this case 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:

Chikungunya Virus (FAM)	Internal Control (HEX)	Interpretation
+	+	Sample positive
-	+	Sample negative
+	-	Sample positive
-	-	Repeat the test!

9.2.3. Positive Control

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

9.3. Preparing the RT-PCR

Positive control should be added into the PCR reaction together with the samples and the negative control (PCR-grade water). 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

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® Chikungunya Virus Detection Kit v1 is composed of an initial denaturation for activation the HotStarTaq DNA 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 (Data Collection)	54°C	01:20 min.	
Hold	22°C	05:00 min.	

Before starting 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.

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.

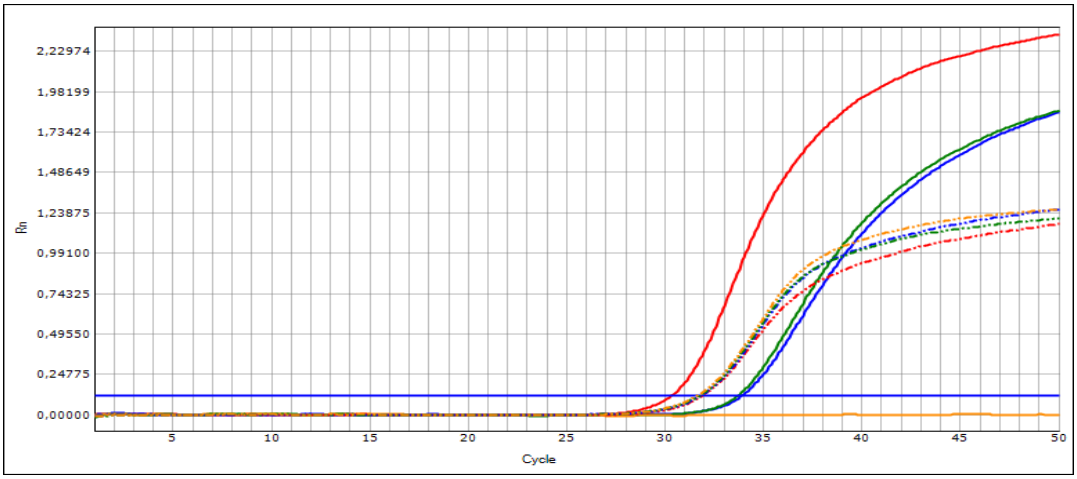

Fig. 1: Amplification Curve of a Bosphore® Chikungunya Virus v1 test

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 allows, while 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.)

The table below displays the acceptance criteria for Bosphore® Chikungunya Virus Detection Kit v1.

Component/Parameter	Cycle Threshold (C_T)
Positive Control	32±4

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

The qualitative results of the test are displayed as a spread sheet containing the calculated starting quantities of the unknown samples in each tube is shown. The samples that cross the threshold in Channel 1 (FAM) are displayed with a calculated starting quantity, samples that do not cut the threshold are displayed as "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:

Signal detected in FAM filter pair	The sample contains Chikungunya Virus RNA, the result is positive	No need to check the internal control since the sample is positive (high positive samples may suppress the signal from the internal control)
No signal in FAM, signal in HEX	The Chikungunya Virus RNA in the sample is not detectable	Signal from HEX filter pair rules out the possibility of PCR inhibition
No signal in FAM and HEX	The diagnosis is inconclusive	No signal in HEX points out to PCR inhibition or to a problem in RNA isolation

In rare cases of PCR inhibition due to medication or other PCR inhibitors in the sample, we recommend to repeat the test of inhibited samples, by freezing and thawing the RNA samples and using them in the PCR after diluting them 1:2 or 1:4 with dH_2O. (Caution: The dilution factor must be taken into account while reporting the Real-Time PCR quantitative results.)

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® Chikungunya Virus was found to be 5 copies/µl. The sensitivity was determined using serial dilutions of previously quantitated viral positive control 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 HIV, HCV, HDV, HBV, JCV, Enterovirus, West Nile Virus, Dengue Virus with known high positivity were tested, and found negative.

12. REFERENCES

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3. Niyas et al.: Molecular characterization of Chikungunya virus isolates from clinical samples and adult Aedes albopictus mosquitoes emerged from larvae from Kerala, South India. Virology Journal 2010 7:189

4. Powers AM, Logue CH: Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J Gen Virol 2007, 88 :2363-2377

13. SYMBOLS


Use by date

Batch Code

Catalog number


Temperature limitation


Caution, consult accompanying documents


Manufacturer

In Vitro Diagnostic Medical Device

14. CONTACT INFORMATION

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