Viral Meningitis Panel Kit

USER MANUAL

For *in vitro* Diagnostic Use

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CE IVD

Contents

1. PRODUCT DESCRIPTION

Bosphore® Viral Meningitis Panel Kit detects and characterizes Herpes simplex virus 1, Herpes simplex virus 2, Varicella-Zoster virus, Enterovirus and Parechovirus DNA and RNA in human biological samples. Fluorescence detection is accomplished by using FAM, HEX, Texas RED and Cy5 filters. Herpes simplex virus 1 is amplified and fluorescence detection is accomplished using the FAM filter, Herpes Simplex Virus 2 is amplified and fluorescence detection is accomplished using the Cy5 filter and Varicella-Zoster Virus is amplified and fluorescence detection is accomplished using the HEX filter in the 1st tube with PCR Master Mix 1. In the 2nd tube, Enterovirus is amplified and fluorescence detection is accomplished using the FAM filter and Parechovirus is amplified and fluorescence detection is accomplished using the HEX filter with PCR Master Mix 2.

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 Texas Red filter. The internal control can be added either during DNA extraction or PCR step.

2. CONTENT

Bosphore® Viral Meningitis Panel Kit is composed of Real-Time PCR reagents and positive and negative controls:

Component	REAGENT	100 Reactions	50 Reactions	25 Reactions
1	dH_2O	(1000 µl)	(500 µl)	(500 µl)
2	PCR Master Mix 1	(1650 µl)	(825 µl)	(413 µl)
3	PCR Master Mix 2	(1650 µl)	(825 µl)	(413 µl)
4	Internal Control	(550 µl)	(275 µl)	(138 µl)
5	Positive Control 1	(88 µl)	(44 µl)	(22 µl)
6	Positive Control 2	(88 µl)	(44 µl)	(22 µl)

3. STORAGE

Bosphore® Viral Meningitis Panel Kit 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 or air more than necessary, vials must be kept closed except during pipetting. 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® 484 or Montania® 4896 Real-Time PCR Instrument (Anatolia Geneworks), or another Real-Time PCR system with FAM, HEX, Texas Red and Cy5 filters (iCycler, iQ5, CFX–BioRad, 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, PCR plates or strips
- Magnesia 2448 Nucleic Acid Extraction and PCR Setup Robot and Magnesia 2448 Viral DNA/RNA Extraction Kit (Anatolia Geneworks)/ Magnesia 16 Nucleic Acid Extraction System / Magnesia Viral

Nucleic Acid Extraction Kit / Bosphore Viral DNA Extraction Spin Kit/ Bosphore Viral RNA Extraction Spin Kit (Anatolia Geneworks)/ Magrev Viral DNA/RNA Extraction Kit (Anatolia Geneworks) or other high quality viral DNA/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
- 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 -20°C.
- 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, suitable class microbiological safety cabinet should be used: 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. INFECTION

Meningitis is caused by inflammation of the protective membranes covering brain and spinal cord (meninges). High risk of mortality and morbidity ratios might occur depending on the cause of the infection such as bacterial, viral

agents or use of certain drugs. Viral meningitis is a mild form of disease with higher incidence compared to acute bacterial meningitis. Acute bacterial meningitis is rarely seen, but it may cause hearing loss, brain damage and even death if the symptoms emerging instantly cannot be detected in the early stage.

8. METHOD

Bosphore Viral Meningitis Panel Kit is based on the Real Time PCR method. 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 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. I

The assay utilizes the 5' exonuclease activity of Taq Polymerase to cleave a dual-labeled fluorescent hydrolysis 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 Viral Meningitis Panel Kit 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. HSV 1, HSV 2, VZV, Enterovirus, Parechovirus DNA and internal controls are amplified in 2 PCR tubes, using sequence-specific primers. The fluorescent signals generated by the amplification of the viruses and internal controls, are detected by different probes (labeled at the 5' end with reporter molecules FAM/HEX/Texas RED/Cy5) through the FAM/HEX/Texas RED/Cy5 channel. Herpes simplex virus 1 is amplified and fluorescence detection is accomplished using the FAM filter, Herpes Simplex Virus 2 is amplified and fluorescence detection is accomplished using the Cy5 filter and Varicella-Zoster Virus is amplified and fluorescence detection is accomplished using the HEX filter in the 1st tube with PCR Master Mix 1. In

the 2nd tube, Enterovirus is amplified and fluorescence detection is accomplished using the FAM filter and Parechovirus is amplified and fluorescence detection is accomplished using the HEX filter with PCR Master Mix 2.

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 Texas Red filter.

9. PROCEDURE

9.1. DNA Isolation

We recommend that the Magnesia 2448 Nucleic Acid Extraction and PCR Setup Robot and Magnesia 2448 Viral DNA/RNA Extraction Kit (Anatolia Geneworks)/ Magnesia 16 Nucleic Acid Extraction System / Magnesia Viral Nucleic Acid Extraction Kit / Bosphore Viral DNA Extraction Spin Kit/ Bosphore Viral RNA Extraction Spin Kit (Anatolia Geneworks)/ Magrev Viral DNA/RNA Extraction Kit (Anatolia Geneworks) or other high quality viral DNA/RNA extraction kits and systems are used with Bosphore® Viral Meningitis Panel Kit. The DNA isolation should be performed according to the manufacturers' instructions.

When Magnesia 2448 Nucleic Acid Extraction and PCR Setup Robot is used for DNA/RNA extraction, it is sufficient to start the "Viral Meningitis" protocol in the instrument software.

9.2. Kit Components

9.2.1. PCR Master Mix1

PCR Master Mix 1 contains a highly specific and accurate HotStarTaq DNA Polymerase, the PCR buffer and the dNTP Mix. PCR Master Mix 1 also contains forward and reverse primers and dual-labeled probes specific for HSV 1, HSV 2, VZV genomes and for the internal control.

9.2.2. PCR Master Mix 2

PCR Master Mix 2 contains a highly specific and accurate HotStarTaq DNA Polymerase, the PCR buffer and the dNTP Mix. PCR Master Mix 2 also contains forward and reverse primers and dual-labeled probes specific for Enterovirus, Parechovirus genomes and for the internal control.

9.2.3. 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 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/HEX/Cy5 negative samples in the 1st tube and lack of internal control amplification in the FAM/HEX negative samples in the 2nd tube, 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. Internal control amplification should be evaluated according to the following table:

	HSV 1 (FAM)	HSV 2 (Cy5)	VZV (HEX)	Internal Control (Texas RED)	Result
1. Tube	+	-	-	+/-	Sample HSV1 positive
	-	+	-	+/-	Sample HSV2 positive
	-	-	+	+/-	Sample VZV positive
	+	+	-	+/-	Sample HSV1 and HSV 2 positive
	+	-	+	+/-	Sample HSV1 and VZV positive
	-	+	+	+/-	Sample HSV2 and VZV positive
	+	+	+	+/-	Sample HSV1, HSV2 and VZV positive
	-	-	-	+	Sample negative
	-	-	-	-	Test should be repeated!
	Enterovirus (FAM)	**Parechovirus (HEX)**		**Internal Control (Texas Red)**	**Result**
2. Tube	+	-		+/-	Sample Enterovirus positive
	-	+		+/-	Sample Parechovirus positive
	+	+		+/-	Sample Enterovirus and Parechovirus positive
	-	-		+	Sample negative
	-	-		-	Test should be repeated!

9.2.4.Positive Control

The kit includes three synthetic DNA positive controls including:

Positive Control 1: HSV 1, HSV 2 and VZV DNA,

Positive Control 2: Enterovirus and Parechovirus RNA,

They must be included in every experiment 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 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 (1/2)	15 µl
Internal Control *	0.2 µl
Sample DNA (Negative/Positive Control)	10 µl
Total Volume	25 µl

* Internal control should not be added in the reaction if it has already been added during the extraction step

Pipette 15 µl of the master mix into the PCR tubes or strips, and add 10 µl of DNA (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® Viral Meningitis Panel Kit 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.

The thermal protocol to be applied for all the 2 reactions; the PCR Master Mix 1 and 2 is indicated below:

Incubation (RT)	50°C	30:00 min.	
Initial denaturation	95°C	14:30 min.	
Denaturation	97°C	00:30 min.	50 cycles
Annealing (Data Collection)	58°C	01:30 min.	
Hold	22°C	02:00 min.	

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

- Choose all the filters to be used (FAM, HEX, Texas RED and Cy5),
- Identify unknown samples, positive and negative controls,
- Select the correct thermal protocol.
- Start the experiment.

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.

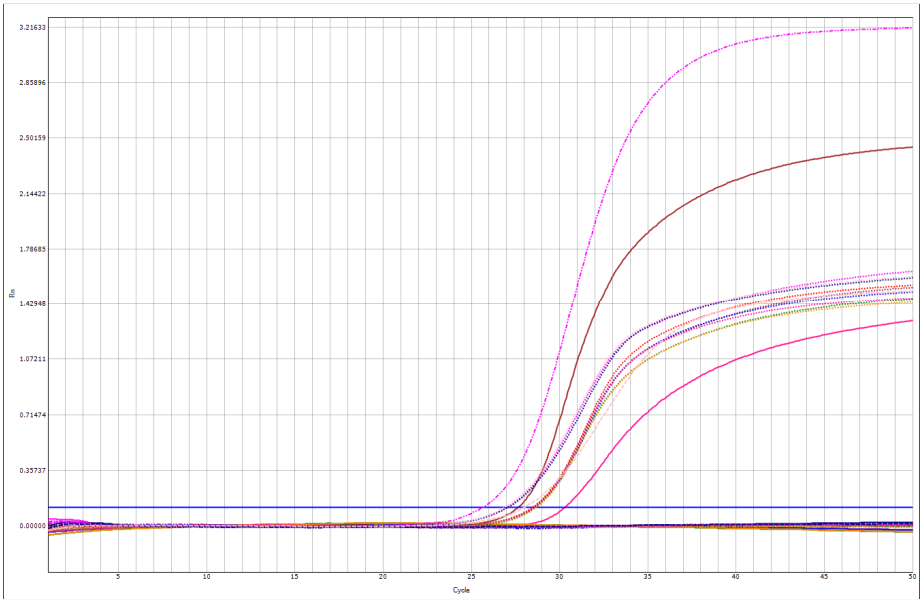

Fig. 1: Amplification Curve of a Bosphore® Viral Meningitis Panel Kit

Analysis of the results should be performed by trained personnel who have received the required training for analyzing 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, if the system allows pulling down the threshold as much as possible in order to detect slight amplifications, attention should be paid to keep the threshold line above the background.

Positive control of Bosphore Viral Meningitis Panel Kit is essential for accurate result analysis. The cycle threshold acceptance criteria for the positive controls are given below:

Parameter	Cycle Threshold (C_T)
Positive Control 1	30±3
Positive Control 2	30±3

Test results should not be reported unless there is amplification of the internal control in negative samples. Please contact the manufacturer if an impairment in the product's performance is observed (See the last page for contact information).

The samples that cross the threshold in FAM Channel, HEX Channel, Texas RED Channel and Cy5 Channel are displayed with their positive/negative results, 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, Texas Red for PCR Master Mix 1 and Cy5 for PCR Master Mix 2 data of the internal control should also be checked to avoid false negative results.

The table below shows the possible results and their interpretation:

	HSV 1 (FAM)	**HSV 2 (Cy5)**	**VZV (HEX)**	**Internal Control (Texas RED)**	**Result**
1. Tube	+	-	-	+/-	Sample HSV1 positive
	-	+	-	+/-	Sample HSV2 positive
	-	-	+	+/-	Sample VZV positive
	+	+	-	+/-	Sample HSV1 and HSV 2 positive
	+	-	+	+/-	Sample HSV1 and VZV positive
	-	+	+	+/-	Sample HSV2 and VZV positive
	+	+	+	+/-	Sample HSV1, HSV2 and VZV positive
	-	-	-	+	Sample negative
	-	-	-	-	Test should be repeated!
	Enterovirus (FAM)	**Parechovirus (HEX)**		**Internal Control (Texas Red)**	**Result**
2. Tube	+	-		+/-	Sample Enterovirus positive
	-	+		+/-	Sample Parechovirus positive
	+	+		+/-	Sample Enterovirus and Parechovirus positive
	-	-		+	Sample negative
	-	-		-	Test should be repeated!

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® Viral Meningitis Panel Kit was found to 11 copies/reaction for HSV 1, 7 copies/reaction for HSV 2, 9 copies/reaction for VZV, 39 copies/reaction for Enterovirus and 500 copies/reaction for Parechovirus. 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. To eliminate the risk of cross-reactivity; JCV, CMV, EBV, HBV, Parvovirus B19 ve BKV samples with known high positivity were tested, and found negative.

12. REFERENCES

1. Logan SAE and MacMahon E (2008). Viral meningitis. BMJ 336(7634): 36–40.
2. Tunkel AR, Scheld WM (2005). Acute meningitis. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett's principles and practice of infectious diseases 6th ed. Philadelphia: Elsevier Churchill Livingstone: 1083-1126.

13. SYMBOLS


Use by

Lot/Batch

Catalog number


Temperature limitation


Caution, consult accompanying documents


Manufacturer

In Vitro Diagnostic Medical Device

14. CONTACT INFORMATION

Anatolia Tanı ve Biyoteknoloji A.Ş.

Address: Egitim Mah. Kasap İsmail Sokak No:10/23
34722 - Kadıköy / İstanbul-TÜRKİYE
Tel: +90 216 330 04 55
Faks: +90 216 330 00 42

e-mail: info@ anatoliagenetik.com
www.anatoliagenetik.com