



STDs Panel Kit

(Bosphore Sexually Transmitted Diseases Panel Kit v1)

USER MANUAL

For *in vitro* Diagnostic Use



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1. PRODUCT DESCRIPTION

Bosphore® STDs Panel Kit v1 detects and characterizes *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis*, *Neisseria gonorrhea*, *Trichomonas vaginalis* and *Mycoplasma genitalium* in human biological samples. Fluorescence detection is accomplished using the FAM, HEX, Texas RED and Cy5 filters. In the first PCR tube with PCR Master Mix 1, *Ureaplasma urealyticum* genome is amplified and fluorescence detection is accomplished using the FAM filter, *Ureaplasma parvum* genome is amplified and fluorescence detection is accomplished using the HEX filter and *Mycoplasma Hominis* genome is amplified and fluorescence detection is accomplished using the Texas RED filter. In the second PCR tube with PCR Master Mix 2, *Neisseria gonorrhea* genome is amplified and fluorescence detection is accomplished using the FAM filter, *Trichomonas vaginalis* genome is amplified and fluorescence detection is accomplished using the HEX filter and *Mycoplasma genitalium* genome is amplified and fluorescence detection is accomplished using the Texas RED 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 Cy5 filter in both PCR tubes. The internal control can be added either during DNA extraction or PCR step.

2. CONTENT

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

Component	REAGENT	100 Reactions	50 Reactions	25 Reactions
1	dH ₂ O	(1000 µl)	(1000 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® STDs 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, LightCycler 480-Roche, 7500 Real-Time PCR System-ABI, m2000 RealTime System-Abbott Molecular, 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 Bacterial DNA Extraction Kit (Anatolia Geneworks)/ Magnesia® 16 Nucleic Acid Extraction System - Magnesia® Bacterial DNA Extraction Kit (Anatolia Geneworks) / Magrev® 24 Stand - Magrev® Bacterial DNA Extraction Kit (Anatolia Geneworks) / Bosphore Bacterial DNA Extraction Spin Kit (Anatolia Geneworks) or other high quality DNA 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

Prognosis of sexually transmitted diseases is generally latent without any indications. Therefore it is difficult to determine the patients and detect the disease incidence. The patients avoid to consult to the clinicians in many cases even they have the symptoms of STD and the disease lies dormant which leads to the progress of indications such as infertility, ectopic pregnancy, miscarriage and even cancer. Besides it causes a continuous incidence of STD by the transfer of pathogens from the mother to the infant during birth (1, 2).

8. METHOD

Bosphore® STDs Panel Kit v1 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® STDs Panel 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. The reaction is performed in two PCR tubes with PCR Master Mix 1 and PCR Master Mix 2. In the first PCR tube with PCR Master Mix 1, *Ureaplasma urealyticum* genome is amplified and fluorescence detection is accomplished using the FAM filter, *Ureaplasma parvum* genome is amplified and fluorescence detection is accomplished using the HEX filter and *Mycoplasma Hominis* genome is amplified and fluorescence detection is accomplished using the Texas RED filter. In the second PCR tube with PCR Master Mix 2, *Neisseria gonorrhea* genome is amplified and fluorescence detection is accomplished using the FAM filter, *Trichomonas vaginalis* genome is amplified and fluorescence detection is

accomplished using the HEX filter and *Mycoplasma genitalium* genome is amplified and fluorescence detection is accomplished using the Texas RED filter.

The fluorescent signal generated by the internal control amplification, is detected through the Cy5 channel in both tubes with PCR Master Mix 1 and 2.

9. PROCEDURE

9.1. DNA Isolation

We recommend that the Magnesia 2448 Nucleic Acid Extraction and PCR Setup Robot and Magnesia 2448 Bacterial DNA Extraction Kit (Anatolia Geneworks)/ Magnesia® 16 Nucleic Acid Extraction System - Magnesia® Bacterial DNA Extraction Kit (Anatolia Geneworks) / Magrev® 24 Stand - Magrev® Bacterial DNA Extraction Kit (Anatolia Geneworks) / Bosphore Bacterial DNA Extraction Spin Kit (Anatolia Geneworks) or other high quality DNA extraction kits and systems are used with Bosphore® STDs Panel Kit v1. 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 extraction, it is sufficient to start the "STD" protocol in the instrument software.

9.2. Kit Components

9.2.1. PCR Master Mix 1

PCR Master Mix 1 contains a highly specific and accurate HotStarTaq DNA Polymerase, the PCR buffer and the dNTP Mix. PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis* 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 also contains forward and reverse primers and dual-labeled probes specific for *Neisseria gonorrhea*, *Trichomonas vaginalis*, *Mycoplasma genitalium* genomes and for the internal control.

9.2.3. Internal Control

An internal control is included in the kit to control DNA isolation and PCR inhibition. The internal control is a synthetic DNA molecule. It is added into the biological sample, 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 1 and 2 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 1 and 2. **Caution: It is not necessary to include the internal control in the reaction if it has already been added during the extraction step.** Lack of internal control amplification in the FAM/HEX/Texas RED 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 bacterial 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:

1. Tube	<i>Ureaplasma urealyticum</i> (FAM)	<i>Ureaplasma parvum</i> (HEX)	<i>Mycoplasma hominis</i> (Texas RED)	Internal Control (Cy5)	Result
	+	-	-	+/-	Sample is <i>Ureaplasma urealyticum</i> positive
	-	+	-	+/-	Sample is <i>Ureaplasma parvum</i> positive

	-	-	+	+/-	Sample is Mycoplasma hominis positive
	-	-	-	+/-	Sample is negative
	+	+	-	+/-	Sample is Ureaplasma urealyticum and Ureaplasma parvum positive
	-	+	+	+/-	Sample is Ureaplasma parvum and Mycoplasma hominis positive
	+	-	+	+/-	Sample is Ureaplasma urealyticum and Mycoplasma hominis positive
	+	+	+	+/-	Sample is Ureaplasma urealyticum , Ureaplasma parvum and Mycoplasma hominis positive
	-	-	-	-	Test should be repeated!
2. Tube	Neisseria gonorrhea (FAM)	Trichomonas Vaginalis (HEX)	Mycoplasma Genitalium (Texas RED)	Internal Control (Cy5)	Result
	+	-	-	+/-	Sample is Neisseria gonorrhea positive
	-	+	-	+/-	Sample is Trichomonas Vaginalis positive
	-	-	+	+/-	Sample is Mycoplasma Genitalium positive
	-	-	-	+/-	Sample is negative
	+	+	-	+/-	Sample is Neisseria gonorrhea and Trichomonas Vaginalis positive
	-	+	+	+/-	Sample is Trichomonas Vaginalis and Mycoplasma Genitalium positive
	+	-	+	+/-	Sample is Neisseria gonorrhea and Mycoplasma Genitalium positive
	+	+	+	+/-	Sample is Neisseria gonorrhea , Trichomonas Vaginalis and Mycoplasma Genitalium positive
	-	-	-	-	Test should be repeated!

9.2.4. Positive Control

The kit includes two synthetic DNA positive controls including:

Positive Control 1: *Ureaplasma urealyticum*, *Ureaplasma parvum* and *Mycoplasma hominis* DNA,

Positive Control 2: *Neisseria gonorrhea*, *Trichomonas vaginalis* and *Mycoplasma genitalium* DNA,

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® STDs 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 the reaction is indicated below:

Initial denaturation	95°C	14:30 min.	
Denaturation	97°C	00:30 min.	} 45 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.

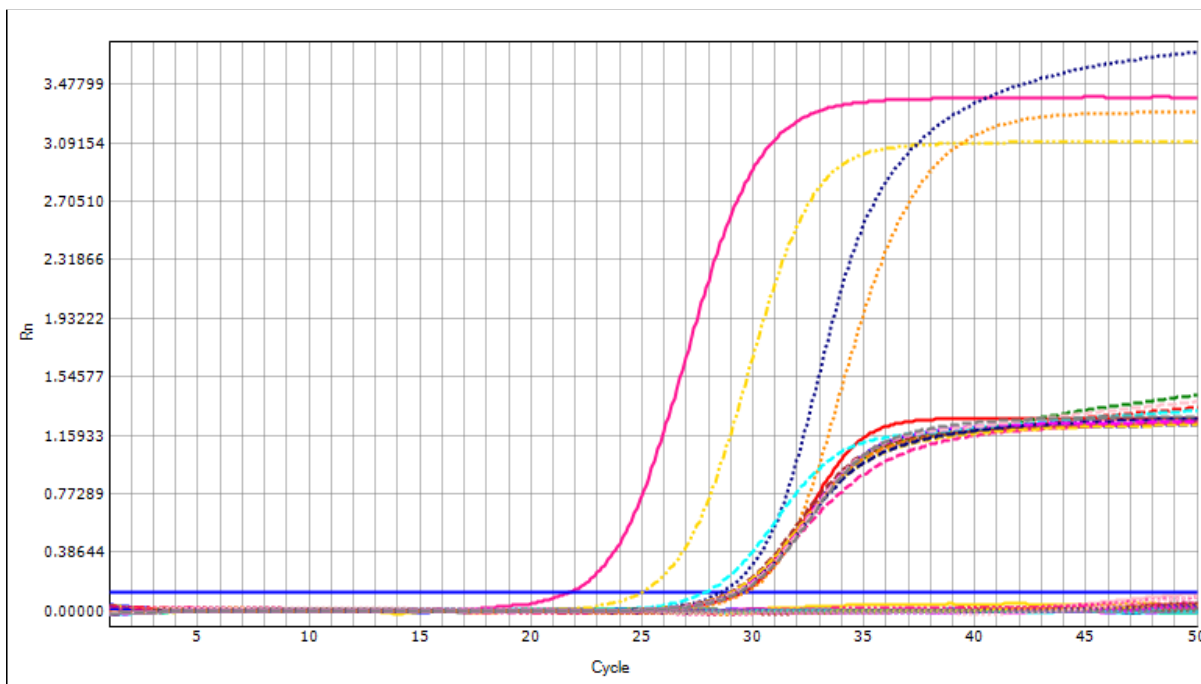


Fig. 1: Amplification Curve of a Bosphore® STDs Panel Kit

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 STDs Panel Kit is essential for accurate result analysis. The cycle threshold acceptance criteria for the positive control is listed below:

Component/Parameter	Threshold value (C_T)
Positive Control 1	25±3
Positive Control 2	25±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 bacterial load below the detection limit of the assay. For these undetectable samples, the Cy5 data of the internal control should also be checked to avoid false negative results.

The table below shows the possible results and their interpretation:

1. Tube	Ureaplasma urealyticum (FAM)	Ureaplasma parvum (HEX)	Mycoplasma hominis (Texas RED)	Internal Control (Cy5)	Result
	+	-	-	+/-	Sample is Ureaplasma urealyticum positive
	-	+	-	+/-	Sample is Ureaplasma parvum positive
	-	-	+	+/-	Sample is Mycoplasma hominis positive
	-	-	-	+/-	Sample is negative
	+	+	-	+/-	Sample is Ureaplasma urealyticum and Ureaplasma parvum positive
	-	+	+	+/-	Sample is Ureaplasma parvum and Mycoplasma hominis positive
	+	-	+	+/-	Sample is Ureaplasma urealyticum and Mycoplasma hominis positive
	+	+	+	+/-	Sample is Ureaplasma urealyticum , Ureaplasma parvum and Mycoplasma hominis positive
	-	-	-	-	Test should be repeated!
2. Tube	Neisseria gonorrhea (FAM)	Trichomonas Vaginalis (HEX)	Mycoplasma Genitalium (Texas RED)	Internal Control (Cy5)	Result
	+	-	-	+/-	Sample is Neisseria gonorrhea positive
	-	+	-	+/-	Sample is Trichomonas Vaginalis positive
	-	-	+	+/-	Sample is Mycoplasma Genitalium positive
	-	-	-	+/-	Sample is negative
	+	+	-	+/-	Sample is Neisseria gonorrhea and Trichomonas Vaginalis positive
	-	+	+	+/-	Sample is Trichomonas Vaginalis and Mycoplasma Genitalium positive
	+	-	+	+/-	Sample is Neisseria gonorrhea and Mycoplasma Genitalium positive
	+	+	+	+/-	Sample is Neisseria gonorrhea , Trichomonas Vaginalis and Mycoplasma Genitalium positive
	-	-	-	-	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® STDs Panel Kit was found to 12 copies/reaction for *Ureaplasma urealyticum*, 50 copies/reaction for *Ureaplasma parvum*, 19 copies/reaction for *Mycoplasma hominis*, 17 copies/reaction for *Mycoplasma genitalium*, 22 fg/reaction for *Neisseria gonorrhea* and 32 fg/reaction for *Trichomonas vaginalis*. 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; *Chlamydia trachomatis*, *Neisseria meningitidis*, *Klebsiella pneumoniae*, *Haemophilus influenza*, *Bordetella pertussis*, *Salmonella*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae* samples with known high positivity were tested, and found negative.

12. REFERENCES

1. Sexually transmitted infections (STIs), Fact Sheet, August 2016, WHO media center, from <http://www.who.int/mediacentre/factsheets/fs110/en/>.
2. Centers for Disease Control and Prevention. (2011, January 28). Sexually transmitted diseases (STDs). Sexually transmitted diseases treatment guidelines, 2010. Vaccine-preventable STDs. Retrieved June 3, 2012, from <http://www.cdc.gov/std/treatment/2010/vaccine.htm#a1>

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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