Respiratory Pathogens Panel Kit v1

USER MANUAL

For *in vitro* Diagnostic Use

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Contents

1. PRODUCT DESCRIPTION

Bosphore® Respiratory Pathogens Panel Kit v1 detects and characterizes Influenza B, *Mycoplasma pneumoniae*, *Klebsiella pneumoniae*, Parainfluenza 2, Parainfluenza 4, Parainfluenza 1, Metapneumovirus, Enterovirus, Influenza A, Parainfluenza 3, RSV A/B, Bocavirus, Rhinovirus, Coronavirus 229E, Pandemic H1N1 influenza A, Seasonal H1N1 influenza A and Salmonella in human respiratory samples. Fluorescence detection is accomplished using the FAM, HEX, Texas RED and Cy5 filters.

In the first PCR tube with PCR Master Mix 1, Influenza B genome is amplified and fluorescence detection is accomplished using the HEX filter, *Mycoplasma pneumoniae* genome is amplified and fluorescence detection is accomplished using the Cy5 filter and *Klebsiella pneumoniae* genome is amplified and fluorescence detection is accomplished using the FAM filter.

In the second PCR tube with PCR Master Mix 2, Parainfluenza 2 genome is amplified and fluorescence detection is accomplished using the HEX filter, Parainfluenza 4 genome is amplified and fluorescence detection is accomplished using the Cy5 filter and Parainfluenza 1 genome is amplified and fluorescence detection is accomplished using the FAM filter.

In the third PCR tube with PCR Master Mix 3, Metapneumovirus genome is amplified and fluorescence detection is accomplished using the HEX filter and Enterovirus genome is amplified and fluorescence detection is accomplished using the FAM filter.

In the fourth PCR tube with PCR Master Mix 4, Influenza A genome is amplified and fluorescence detection is accomplished using the FAM filter, Parainfluenza 3 genome is amplified and fluorescence detection is accomplished using the Texas RED filter and RSV A/B genome is amplified and fluorescence detection is accomplished using the HEX filter.

In the fifth PCR tube with PCR Master Mix 5, Bocavirus genome is amplified and fluorescence detection is accomplished using the FAM filter, Rhinovirus genome is amplified and fluorescence detection is accomplished using the HEX filter, Coronavirus 229E genome is amplified and fluorescence detection is accomplished using the Texas RED filter.

In the sixth PCR tube with PCR Master Mix 6, Pandemic H1N1 influenza A genome is amplified and fluorescence detection is accomplished using the FAM filter, Seasonal H1N1 influenza A genome is amplified and fluorescence detection is accomplished using the HEX filter and Salmonella genome is amplified and fluorescence detection is accomplished using the Cy5 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 Texas RED filter in PCR Master Mix 1, PCR Master Mix 2, PCR Master Mix 3 and PCR Master Mix 6, it is detected with the Cy5 in PCR Master Mix 4 and PCR Master Mix 5. The internal control can be added either during DNA extraction or PCR step.

2. CONTENT

Bosphore® Respiratory Pathogens Panel Kit v1 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)	(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	PCR Master Mix 3	(1650 µl)	(825 µl)	(413 µl)

5	PCR Master Mix 4	(1650 µl)	(825 µl)	(413 µl)
6	PCR Master Mix 5	(1650 µl)	(825 µl)	(413 µl)
7	PCR Master Mix 6	(1650 µl)	(825 µl)	(413 µl)
8	Internal Control	(550 µl)	(275 µl)	(138 µl)
9	Positive Control 1	(88 µl)	(44 µl)	(22 µl)
10	Positive Control 2	(88 µl)	(44 µl)	(22 µl)
11	Positive Control 3	(88 µl)	(44 µl)	(22 µl)
12	Positive Control 4	(88 µl)	(44 µl)	(22 µl)
13	Positive Control 5	(88 µl)	(44 µl)	(22 µl)
14	Positive Control 6	(88 µl)	(44 µl)	(22 µl)

3. STORAGE

Bosphore® Respiratory Pathogens Panel 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 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
- 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 Extraction Kit (Anatolia Geneworks) / Magrev® 24 Stand - Magrev® Viral DNA/RNA Extraction Kit (Anatolia Geneworks) / Bosphore Viral DNA Extraction Spin Kit (Anatolia Geneworks)- Bosphore Viral RNA 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

Respiratory tract infections are typically divided into upper and lower respiratory tract infections which occur mainly in the nose, throat affecting upper respiratory system, windpipe, airways and lungs affecting lower respiratory system. From clinical point of view; symptoms such as nasal obstruction, nasal discharge, sore throat, cough, irritability occur as upper respiratory tract infections, symptoms such as bronchitis and pneumonia occur as lower tract infections.

When there is no adequate treatment, bronchitis, pneumonia, emphysema and bronchiectasis may occur. Respiratory infections include a diverse group of bacterial, viral and fungal infections which makes the respiratory diseases are very contagious which can spread through different routes of transmission, including contact, droplet, and airborne.

Viral causative agents of respiratory infections include Rhinovirus, Adenovirus, Coxsackievirus, Parainfluenza Virus, Influenza Virus, Respiratory Syncytial Virus and Human Metapneumovirus. Bacterial causative agents of respiratory infections include Streptococcus, Chlamydia, Mycoplasma and Gonococcus. Fungal causative agents of respiratory infections include opportunistic fungal pathogens such as Candidas and Aspergilli [1, 2, 3].

8. METHOD

Bosphore® Respiratory Pathogens 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® Respiratory Pathogens 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 six (6) PCR tubes with PCR Master Mix 1, 2, 3, 4, 5 and 6.

9. PROCEDURE

9.1. DNA/RNA 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. 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 Extraction Kit (Anatolia Geneworks) / Magrev® 24 Stand - Magrev® Viral DNA/RNA Extraction Kit (Anatolia Geneworks) / Bosphore Viral DNA Extraction Spin Kit (Anatolia Geneworks)- Bosphore Viral RNA Extraction Spin Kit (Anatolia Geneworks)- or other high quality DNA extraction kits and systems are used with Bosphore® Respiratory Pathogens 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 "Respiratory" 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 Influenza B, *Mycoplasma pneumoniae*, *Klebsiella pneumonia* 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 Parainfluenza 2, Parainfluenza 4, Parainfluenza 1 genomes and for the internal control.

9.2.3. PCR Master Mix 3

PCR Master Mix 3 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 Metapneumovirus, Enterovirus genomes and for the internal control.

9.2.4. PCR Master Mix 4

PCR Master Mix 4 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 Influenza A, Parainfluenza 3, RSV A/B genomes and for the internal control.

9.2.5. PCR Master Mix 5

PCR Master Mix 5 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 Bocavirus, Rhinovirus, Coronavirus 229E genomes and the internal control.

9.2.6. PCR Master Mix 6

PCR Master Mix 6 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 Pandemic H1N1 influenza A, Seasonal H1N1 influenza A, Salmonella genomes and the internal control.

9.2.7. 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, 2, 3, 4, 5 and 6 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, 2, 3, 4, 5 and 6. **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 target channels in 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:

PCR Master Mix 1	Klebsiella pneumoniae (FAM)	Influenza B (HEX)	Mycoplasma pneumoniae (Cy5)	Internal Control (Texas RED)	Result
	+	-	-	+/-	Sample is **Klebsiella pneumoniae** positive
	-	+	-	+/-	Sample is **Influenza B** positive
	-	-	+	+/-	Sample is **Mycoplasma pneumoniae** positive
	-	-	-	+	Sample is negative
	+	+	-	+/-	Sample is **Klebsiella pneumoniae** and **Influenza B** positive
	-	+	+	+/-	Sample is **Influenza B** and **Mycoplasma pneumoniae** positive
	+	-	+	+/-	Sample is **Klebsiella pneumoniae** and **Mycoplasma pneumoniae** positive
	+	+	+	+/-	Sample is **Klebsiella pneumoniae, Influenza B** and **Mycoplasma pneumoniae** positive
	-	-	-	-	Test should be repeated!
PCR Master Mix 2	**Parainfluenza 1 (FAM)**	**Parainfluenza 2 (HEX)**	**Parainfluenza 4 (Cy5)**	**Internal Control (Texas RED)**	**Result**
	+	-	-	+/-	Sample is **Parainfluenza 1** positive
	-	+	-	+/-	Sample is **Parainfluenza 2** positive
	-	-	+	+/-	Sample is **Parainfluenza 4** positive
	-	-	-	+	Sample is negative
	+	+	-	+/-	Sample is **Parainfluenza 1** and **Parainfluenza 2** positive
	-	+	+	+/-	Sample is **Parainfluenza 2** and **Parainfluenza 4** positive
	+	-	+	+/-	Sample is **Parainfluenza 1** and **Parainfluenza 4** positive
	+	+	+	+/-	Sample is **Parainfluenza 1, Parainfluenza 2** and **Parainfluenza 4** positive
	-	-	-	-	Test should be repeated!
PCR Master Mix 3	**Enterovirus (FAM)**	**Metapneumovirus (HEX)**	**x**	**Internal Control (Texas RED)**	**Result**
	+	-	x	+/-	Sample is **Enterovirus** positive
	-	+	x	+/-	Sample is **Metapneumovirus** positive
	-	-	x	+	Sample is negative
	+	+	x	+/-	Sample is **Enterovirus** and **Metapneumovirus** positive
	-	-	-	-	Test should be repeated!
PCR Master Mix 4	**Influenza A (FAM)**	**RSV A/B (HEX)**	**Parainfluenza 3 (Texas RED)**	**Internal Control (Cy5)**	**Result**
	+	-	-	+/-	Sample is **Influenza A** positive
	-	+	-	+/-	Sample is **RSV A/B** positive
	-	-	+	+/-	Sample is **Parainfluenza 3**

					positive
	-	-	-	+	Sample is negative
	+	+	-	+/-	Sample is **Influenza A** and **RSV A/B** positive
	-	+	+	+/-	Sample is **RSV A/B** and **Parainfluenza 3** positive
	+	-	+	+/-	Sample is **Influenza A** and **Parainfluenza 3** positive
	+	+	+	+/-	Sample is **Influenza A, RSV A/B** and **Parainfluenza 3** positive
	-	-	-	-	Test should be repeated!
PCR Master Mix 5 *	**Bocavirus (FAM)**	**Rhinovirus (HEX)**	**Coronavirus 229E (Texas RED)**	**Internal Control (Cy5)**	**Result**
	+	-	-	+/-	Sample is **Bocavirus** positive
	-	+	-	+/-	Sample is **Rhinovirus** positive
	-	-	+	+/-	Sample is **Coronavirus 229E** positive
	-	-	-	+	Sample is negative
	+	+	-	+/-	Sample is **Bocavirus** and **Rhinovirus** positive
	-	+	+	+/-	Sample is **Rhinovirus** and **Coronavirus 229E** positive
	+	-	+	+/-	Sample is **Bocavirus** and **Coronavirus 229E** positive
	+	+	+	+/-	Sample is **Bocavirus, Rhinovirus** and **Coronavirus 229E** positive
	-	-	-	-	Test should be repeated!
PCR Master Mix 6	**Pandemic H1N1 influenza A (FAM)**	**Seasonal H1N1 influenza A (HEX)**	**Salmonella (Cy5)**	**Internal Control (Texas RED)**	
	+	-	-	+/-	Sample is **Pandemic H1N1 influenza A** positive
	-	+	-	+/-	Sample is **Seasonal H1N1 influenza A** positive
	-	-	+	+/-	Sample is **Salmonella** positive
	-	-	-	+	Sample is negative
	+	-	+	+/-	Sample is **Pandemic H1N1 influenza A** and **Salmonella** positive
	+	+	-	+/-	Sample is **Pandemic H1N1 influenza A** and **Seasonal H1N1 influenza A** positive
	-	+	+	+/-	Sample is **Seasonal H1N1 influenza A** and **Salmonella** positive
	+	+	+	+/-	Sample is **Pandemic H1N1 influenza A, Seasonal H1N1 influenza A** and **Salmonella** positive
	-	-	-	-	Test should be repeated!

* Enterovirus positive samples also give positive signal for Rhinovirus assay. The positive samples on both Rhinovirus and Enterovirus assays should be regarded as enterovirus positive only.

9.2.8. Positive Control

The kit includes six synthetic DNA positive controls including:

Positive Control 1: Influenza B RNA, *Mycoplasma pneumoniae* and *Klebsiella pneumoniae* DNA,

Positive Control 2: Parainfluenza 2, Parainfluenza 4, Parainfluenza 1 DNA,

Positive Control 3: Metapneumovirus and Enterovirus DNA,

Positive Control 4: Influenza A, Parainfluenza 3, RSV A/B DNA,

Positive Control 5: Bocavirus, Rhinovirus and Coronavirus 229E DNA,

Positive Control 6: Pandemic H1N1 influenza A, Seasonal H1N1 influenza A and Salmonella 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 (1/2/3/4/5/6)	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/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® Respiratory Pathogens Panel 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.

The thermal protocol to be applied for the reaction is indicated below:

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)	55°C	01:20 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.

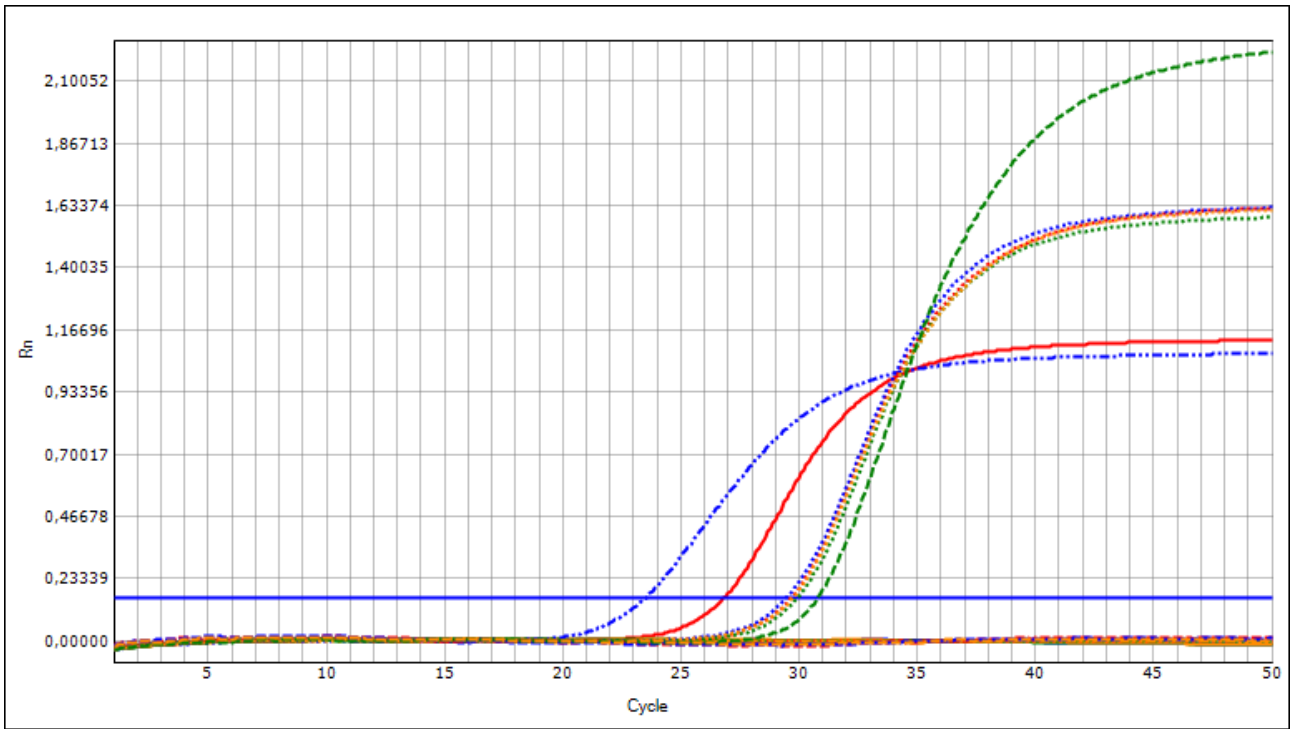

Fig. 1: Amplification Curve of a Bosphore® Respiratory Pathogens Panel Kit v1

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® Respiratory Pathogens Panel Kit v1 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 1/2/3/4/5/6	27±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/viral load below the detection limit of the assay. For these undetectable samples, the Texas RED (for PCR Master Mix 1, 2, 3 and 6) and Cy5 (for PCR Master Mix 4 and 5) data of the internal control should also be checked to avoid false negative results.

The table below shows the possible results and their interpretation:

PCR Master Mix 1	**Klebsiella pneumoniae (FAM)**	**Influenza B (HEX)**	**Mycoplasma pneumoniae (Cy5)**	**Internal Control (Texas RED)**	**Result**
	+	**-**	**-**	**+/-**	Sample is **Klebsiella pneumoniae** positive
	-	**+**	**-**	**+/-**	Sample is **Influenza B** positive
	-	**-**	**+**	**+/-**	Sample is **Mycoplasma pneumoniae** positive
	-	**-**	**-**	**+**	Sample is negative
	+	**+**	**-**	**+/-**	Sample is **Klebsiella pneumoniae** and **Influenza B** positive
	-	**+**	**+**	**+/-**	Sample is **Influenza B** and **Mycoplasma pneumoniae** positive
	+	**-**	**+**	**+/-**	Sample is **Klebsiella pneumoniae** and **Mycoplasma pneumoniae** positive
	+	**+**	**+**	**+/-**	Sample is **Klebsiella pneumoniae, Influenza B** and **Mycoplasma pneumoniae** positive
	-	**-**	**-**	**-**	Test should be repeated!
PCR Master Mix 2	**Parainfluenza 1 (FAM)**	**Parainfluenza 2 (HEX)**	**Parainfluenza 4 (Cy5)**	**Internal Control (Texas RED)**	**Result**
	+	**-**	**-**	**+/-**	Sample is **Parainfluenza 1** positive
	-	**+**	**-**	**+/-**	Sample is **Parainfluenza 2** positive
	-	**-**	**+**	**+/-**	Sample is **Parainfluenza 4** positive
	-	**-**	**-**	**+**	Sample is negative
	+	**+**	**-**	**+/-**	Sample is **Parainfluenza 1** and **Parainfluenza 2** positive
	-	**+**	**+**	**+/-**	Sample is **Parainfluenza 2** and **Parainfluenza 4** positive
	+	**-**	**+**	**+/-**	Sample is **Parainfluenza 1** and **Parainfluenza 4** positive

	+	**+**	**+**	**+/-**	Sample is **Parainfluenza 1, Parainfluenza 2** and **Parainfluenza 4** positive
	-	**-**	**-**	**-**	Test should be repeated!
PCR Master Mix 3	**Enterovirus (FAM)**	**Metapneumovirus (HEX)**	**x**	**Internal Control (Texas RED)**	**Result**
	+	**-**	**x**	**+/-**	Sample is **Enterovirus** positive
	-	**+**	**x**	**+/-**	Sample is **Metapneumovirus** positive
	-	**-**	**x**	**+**	Sample is negative
	+	**+**	**x**	**+/-**	Sample is **Enterovirus** and **Metapneumovirus** positive
	-	**-**	**-**	**-**	Test should be repeated!
PCR Master Mix 4	**Influenza A (FAM)**	**RSV A/B (HEX)**	**Parainfluenza 3 (Texas RED)**	**Internal Control (Cy5)**	**Result**
	+	**-**	**-**	**+/-**	Sample is **Influenza A** positive
	-	**+**	**-**	**+/-**	Sample is **RSV A/B** positive
	-	**-**	**+**	**+/-**	Sample is **Parainfluenza 3** positive
	-	**-**	**-**	**+**	Sample is negative
	+	**+**	**-**	**+/-**	Sample is **Influenza A** and **RSV A/B** positive
	-	**+**	**+**	**+/-**	Sample is **RSV A/B** and **Parainfluenza 3** positive
	+	**-**	**+**	**+/-**	Sample is **Influenza A** and **Parainfluenza 3** positive
	+	**+**	**+**	**+/-**	Sample is **Influenza A, RSV A/B** and **Parainfluenza 3** positive
	-	**-**	**-**	**-**	Test should be repeated!
PCR Master Mix 5 *	**Bocavirus (FAM)**	**Rhinovirus (HEX)**	**Coronavirus 229E (Texas RED)**	**Internal Control (Cy5)**	**Result**
	+	**-**	**-**	**+/-**	Sample is **Bocavirus** positive
	-	**+**	**-**	**+/-**	Sample is **Rhinovirus** positive
	-	**-**	**+**	**+/-**	Sample is **Coronavirus 229E** positive
	-	**-**	**-**	**+**	Sample is negative
	+	**+**	**-**	**+/-**	Sample is **Bocavirus** and **Rhinovirus** positive
	-	**+**	**+**	**+/-**	Sample is **Rhinovirus** and **Coronavirus 229E** positive
	+	**-**	**+**	**+/-**	Sample is **Bocavirus** and **Coronavirus 229E** positive
	+	**+**	**+**	**+/-**	Sample is **Bocavirus, Rhinovirus** and **Coronavirus 229E** positive
	-	**-**	**-**	**-**	Test should be repeated!
PCR Master Mix 6	**Pandemic H1N1 influenza A (FAM)**	**Seasonal H1N1 influenza A (HEX)**	**Salmonella (Cy5)**	**Internal Control (Texas RED)**	
	+	**-**	**-**	**+/-**	Sample is **Pandemic H1N1 influenza A** positive
	-	**+**	**-**	**+/-**	Sample is **Seasonal H1N1 influenza A** positive
	-	**-**	**+**	**+/-**	Sample is **Salmonella** positive

	-	-	-	+	Sample is negative
	+	-	+	+/-	Sample is **Pandemic H1N1 influenza A** and **Salmonella** positive
	+	+	-	+/-	Sample is **Pandemic H1N1 influenza A** and **Seasonal H1N1 influenza A** positive
	-	+	+	+/-	Sample is **Seasonal H1N1 influenza A** and **Salmonella** positive
	+	+	+	+/-	Sample is **Pandemic H1N1 influenza A, Seasonal H1N1 influenza A** and **Salmonella** positive
	-	-	-	-	Test should be repeated!

* Enterovirus positive samples also give positive signal for Rhinovirus assay. The positive samples on both Rhinovirus and Enterovirus assays should be regarded as enterovirus positive only.

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® Respiratory Pathogens Panel Kit v1 was found to 22 copies/reaction for Influenza B, 2 copies/reaction for *Mycoplasma pneumoniae,* 20 copies/reaction for *Klebsiella pneumoniae*, 14 copies/reaction for RSV-A, 6770 copies/reaction for Influenza A, 87 copies/reaction for Parainfluenza-3, 18 copies/reaction for Parainfluenza-2, 782 copies/reaction Parainfluenza-4, 508 copies/reaction for Parainfluenza-1, 123 copies/reaction for Enterovirus, 229 copies/reaction for Metapneumovirus, 98 copies/reaction for RSV-B, 40 copies/reaction for Bocavirus, 24 copies/reaction for Rhinovirus, 112 copies/reaction for Coronavirus 229E, 72 copies/reaction for Salmonella, 3195 copies/reaction for Pandemic H1N1 and 170 copies/reaction for Seasonal H1N1. 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 pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenza*, *Bordetella pertussis*, *Streptococcus pneumoniae*, Legionella, VZV, HSV-1,HSV-2, Parechovirus and *Moraxella catarrhalis* samples with known high positivity were tested and found negative.

* Enterovirus positive samples also give positive signal for Rhinovirus assay. The positive samples on both Rhinovirus and Enterovirus assays should be regarded as Enterovirus positive only.

12. REFERENCES

1. Mahony, James B. "Detection of respiratory viruses by molecular methods." Clinical microbiology reviews 21.4 (2008): 716-747.
2. Bulla, A., and K. L. Hitze. "Acute respiratory infections: a review." Bulletin of the World Health Organization 56.3 (1978): 481
3. Kon, Kateryna, and Mahendra Rai, eds. The Microbiology of Respiratory System Infections. Vol. 1. Academic Press, 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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