Respiratory Panel Real Time PCR Detection

A broad range of respiratory viruses cause acute local and systemic infections that may be severe in children, the elderly and immunocompromised patients. The detection and identification of specific viral type in patients who have symptoms of respiratory tract infection will aid physicians in the timely diagnosis and administering the appropriate treatment for these patients, as well as prevent spreading of the viruses to at risk individuals. Upper and lower respiratory tract infections are caused by a broad range of microbes, including RNA and DNA viruses, bacteria, and even fungi, and yet are often symptomatically similar. This panel is designed for specific and qualitative detection of respiratory viruses in specimens such as oropharyngeal swabs, nasopharyngeal swabs or sputum. The product is intended for use by qualified laboratory personnel well trained in nucleic acid amplification techniques and in vitro diagnostic procedures. Positive results do not rule out bacterial infection or co-infection with other pathogens. Negative results do not preclude microbial infection and should not be used as the sole basis for patient management decisions. Test results must be combined with clinical observations, patient history, and epidemiological information.

SUMMARY AND EXPLANATION OF THE TEST

The respiratory Panel Real Time PCR Detection Kit is designed for simultaneous detection of SARS-CoV-2, Influenza A, Influenza B, Respiratory Syncytial Virus, Adenovirus, Human Metapneumovirus, Rhinovirus, ParaInfluenza 1, ParaInfluenza 2, ParaInfluenza 3 and ParaInfluenza 4. RNA/DNA is extracted from respiratory specimens, amplified using RT-PCR and detected using fluorescent reporter dye probes.

PRINCIPLE

The respiratory viral panel assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The multiples assay contains 11 primer/probe sets that target the RNA/DNA of influenza A, influenza B, SARS-CoV-2, Respiratory Syncytial Virus, Adenovirus, Human Metapneumovirus, Rhinovirus, ParaInfluenza1, ParaInfluenza2, ParaInfluenza3 and ParaInfluenza4. The assay also contains a primer/probe set to detect the human RNase P gene (RP) in clinical specimens or control samples. The kit also contains a positive control (Plasmid DNA) and a negative control for testing process monitoring.

REGENTS AND MATERIALS PROVIDED

Item	Component	Quantity		Volume /T
		100 Tests / Box	500 Tests / Box	
1	PCR Mix I PCR Mix II PCR Mix III	1.5mL/ tube each	1.5 mL/ tube × 5 each	14 µL
2	RT-PCR Enzyme Mix	330 µL/ tube	1550 µL/ tube	1 µL
3	Positive Control 1	50 µL/ tube × 1	250 µL/ tube	5 µL
4	Positive Control 2&3	50 µL/ tube × 1	250 µL/ tube	5 µL
5	Negative Control	50 µL/ tube × 1	250 µL/ tube	5 µL

MATERIALS MAY REQUIRED BUT NOT PROVIDED

1. Real-Time PCR Thermocycler compatible with JOE (***VIC or HEX is equivalent to JOE***), ROX, FAM and Cy5 channels.
2. Specimen Collection Containers
3. RNA extraction reagent
4. PCR tubes compatible with Thermocycler.
5. 1.5 mL centrifuge tubes (RNase-Free)
6. Vortex mixer
7. High-speed centrifuge
8. Micropipette (0.5-2 µL, 1-10 µL)
9. Pipette tips with filters
10. Personal protective material

WARNING AND PRECAUTIONS

1. For use by professionals specifically trained in nucleic acid amplification techniques and in vitro diagnostic procedures.
2. Check the expiration date.
3. Follow Good Laboratory Practices: wear appropriate protective clothing and use disposal gloves and protective eyewear. Do not eat, drink, or smoke in designated work areas. Wash hands thoroughly after handling specimens and kit reagents.
4. The testing workflow must be one-directional to minimize contamination risks (allocate segregated areas for each step): it should be started from RNA Purification Area, then move to the Reaction Setup Area, followed by Amplification and Detection Area. Do not bring samples, equipment, and reagents to the area in which the previous step was performed and always change gloves when changing areas.
5. Regular decontamination of commonly used equipment is recommended, especially for micropipettes and work surfaces.
6. Specimens must be treated as potentially infectious sources as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent. Take appropriate precautions during specimen collection, storage, handling, and disposal in accordance with the guidance from country or region authorities.
7. To ensure optimal performance of the test, always follow appropriate procedures for specimen collection, transport, storage, and processing. Improper procedures may lead to false negative results.
8. Nucleic acids must first be extracted from specimens using a RNA Extraction Kit prior testing. Do not perform a RT-PCR Assay directly using specimens without extraction.
9. Appropriate precautions should be exercised to monitor contamination and preserve the purity of kit components and reactions. Avoid microbial and nuclease (RNase/DNase) contamination of specimens and kit components. Avoid the spread of aerosols when handling or uncapping specimens.
10. To minimize cross-contamination, open only one tube at a time in the process.
11. Always change pipette tips between liquid transfers. To minimize cross contamination, it's recommended to use aerosol-barrier pipette tips.
12. Always use disposable gloves and regularly check that they are not contaminated with sample materials. Discard gloves if they become contaminated.

TRANSPORT AND STORAGE INSTRUCTIONS

Store the kit frozen at -20±5°C and avoid repeated freeze-thaw cycles (less than 5 frozen-thaw times allowed). The product is valid for 6 months, and the expiry date of the product is shown on the label.

SPECIMEN TYPE and PRESERVATION

Specimen type: oropharyngeal swabs, nasopharyngeal swabs and sputum.

Specimen preservation: process specimens for viral RNA extraction and nucleic acid detection as soon as possible. Specimens can be stored at 4°C for 24 hours, or frozen at -80°C for 1 year, if not processed immediately. Avoid repeated freeze-thaw cycles during transport and storage of the specimens.

ASSAY PCR REACTION SETUP (at reagent preparation area)

A. Reagent Preparation

To calculate the number (n) of PCR reaction tubes (n= number of specimens to be tested + number of positive controls + number of negative controls), add n x 14 µL of PCR Mix and n x 1 µL of RT-PCR Enzyme Mix to a clean tube, vortex to mix well, centrifuge and aliquot 15 µL of the mix into each PCR reaction tube.

B. Addition of Viral RNA

Add 5 µL of viral RNA extracts of the specimen or positive/negative control to each PCR tube according to the reaction preparation table below for testing. Then immediately cap the tube tightly, centrifuge the mixture and move it to the amplification area for RT-PCR detection.

PCR Reaction Preparation:

Component	Volume per test
PCR Mix	14 µL
RT-PCR Enzyme Mix	1 µL
Negative Control / Positive Control / RNA of specimen to be tested	5 µL
Total Reaction Volume	20 µL

C. RT-PCR amplification (at Amplification Area, use Applied Biosystems 7500 Real-Time PCR System as an example)

1. Place the complete PCR reaction tubes into the fluorescent quantitative PCR analyzer and label positive control, negative control and testing specimen on the Thermocycler software.

2. Enter the PCR reaction volume: 20 μL.
3. Select fluorescence detection channels: JOE, ROX, FAM and Cy5 channels.
4. Select PCR cycle parameter setting following the instructions below, save the file and start the PCR run.
5. Analyze the results according to your thermocycler manufacturer's instructions. If the positive and negative control samples do not meet their respective requirements, the PCR run must be repeated.

Note: The positive and negative control can be used directly without purification.

Cycles	Step	Time	Temperature
1	Reverse transcription	15 minutes	50 ºC
1	Initial denaturation	30 seconds	95 ºC
40	Denaturation	30 seconds	95 ºC
	Annealing/Extension	45 seconds	60 ºC

QUALITY CONTROL

Quality control requirements must be performed in conformance with local, accreditation requirements and the user's laboratory's standard quality control procedures.

NC (negative control) material: no obvious amplification curve for Cy5, ROX and FAM detection channels, and Ct value ≤37 for JOE channel.

PC (positive control) material: obvious amplification curves for Cy5, ROX and FAM detection channels (Ct value ≤37), and obvious amplification curve for JOE channel.

Pay attention that the above requirements must be met each time when the experiment is performed; otherwise, the experiment is considered as invalid. The reason of invalidation needs to be resolved and the test result needs to be repeated using residual specimens stored appropriately.

DETERMINATION OF RESULTS

1. The results can only be determined when the results of both controls meet their requirements.
2. Criterion for a positive specimen: a specimen can be reported as positive when FAM, Cy5, ROX and JOE channels are all positive.
3. Criterion for a negative specimen: a specimen can be reported as negative when the JOE channel is positive and the ROX, FAM and Cy5 channels are negative.

Multiplex qPCR setup 1

InfA (ROX)	InfB (Cy5)	RdRp (FAM)	RNase P (JOE)	Interpretation
+	-	-	+or -	Influenza A detected
-	+	-	+or -	Influenza B detected
-	-	+	+or -	SC2 detected
-	+	+	+or -	Influenza B and SC2 detected
+	-	+	+or -	Influenza B and SC2 detected
+	+	+	+or -	Influenza A& B and SC2 detected
-	-	-	+ (<35 Ct)	Not detected
-	-	-	-(>=35 Ct)	Invalid result

Multiplex qPCR setup 2

RSV JOE	AV FAM	HMPV ROX	Rhinovirus Cy5	Interpretation
+	-	-	-	RSV detected
-	+	-	-	AV detected
-	-	+	-	HMPV detected
-	-	-	+	Rhinovirus detected
+	+	-	-	RSV, and AV detected
-	-	+	+	HMPV and Rhinovirus detected
-	+	+	-	AV, HMPV detected
+	+	+	-	RSA, AV, HMPV detected
+	+	+	+	RSV, AV. HMPV, Rhino detected
-	-	-	-	None detected

Multiplex qPCR setup 3

PIV4 JOE	PIV3 FAM	PIV2 ROX	PIV1 Cy5	Interpretation
+	-	-	-	PIV4 detected
-	+	-	-	PIV3 detected
-	-	+	-	PIV2 detected
-	-	-	+	PIV1 detected
+	+	-	-	PIV4, PIV3 detected
-	+	+	-	PIV3, PIV2 detected
-	-	+	+	PIV2, PIV1 detected
-	+	-	+	PIV3, PIV1 detected
+	-	+	-	PIV4, PIV2 detected
+	-	-	+	PIV4, PIV1 detected
+	+	+	-	PIV4, PIV3, PIV2 detected
+	+	+	+	All detected
-	-	-	-	None detected

Abbreviation of Viruses:

Name of Viruses	Abbreviation
SARS-CoV-2	SC2
Influenza A	InfA
Influenza B	InfB
Respiratory Syncytial Virus	RSV
Adenovirus	AV
Human Metapneumovirus	HMPV
Rhinovirus	RV
ParaInfluenza 1	PIV1
ParaInfluenza 2	PIV2
ParaInfluenza 3	PIV3
ParaInfluenza 4	PIV4

COMPATIBILITY OF REAL-TIME PCR THERMOCYCLERS

Manufacturer	Model
Applied Biosystems	7500 Real-Time PCR System
	7300 plus Real-Time PCR System

PRODUCT PERFORMANCE

Product analysis performance evaluation results:

1. The analytical sensitivity of this kit is 250 copies/mL.
2. Cross-reaction: no cross-reaction with other pathogens such as, enterovirus types A, B, C and D, EB virus, measles virus, human cytomegalovirus, rotavirus, norovirus, mumps virus, varicella zoster virus, *mycoplasma pneumoniae, chlamydia pneumoniae, legionella, bordetella pertussis, haemophilus influenzae, staphylococcus aureus, streptococcus pneumoniae, streptococcus pyogenes, klebsiella pneumoniae, mycobacterium tuberculosis, aspergillus fumigatus, candida albicans, candida glabrata, cryptococcus neoformans*, coronavirus (HKU1, OC43, NL63, 229E), SARS-CoV-1, and MERS coronavirus.

LIMITATIONS OF TEST

1. This test provides a presumptive diagnosis of SARS-CoV-2, Influenza A, Influenza B, Respiratory Syncytial Virus, Adenovirus, Human Metapneumovirus, Rhinovirus, ParaInfluenza1, ParaInfluenza2, ParaInfluenza3 and ParaInfluenza4. Negative test results do not preclude SARS-CoV-2 infection. All test results should be evaluated by healthcare professionals in the context of clinical symptoms, epidemiological information, patient history, and other diagnostic test results as the basis for patient management decisions.
2. Factors that may lead to false negative results must be excluded, including poor specimen quality; specimens collected too early or too late; specimens not properly stored, transported or processed; virus variation, PCR inhibitors, etc.
3. If test results are negative but clinical symptoms persist, follow up with additional serological diagnostic testing.
4. The Assay Procedure and the Interpretation of Test Results sections must be followed closely when testing. Failure to follow the procedure may lead to inaccurate results.
5. This test should be used only with respiratory specimens. The use of other specimen types has not been validated.
6. The quality of the sample impacts the quality of the test; improper specimen collection, storage and/or transport, and improper RNA purification may yield false negative results.
7. In some samples, extremely low levels of target (below the limit of detection) may yield an amplification signal, but results may not be reproducible.

8. Cross-contamination by samples containing high copies of viral particles or amplification products from previous reactions can yield false positive results. Take proper precautions to monitor contamination and preserve the purity of the kit component

REFERENCE

1. National Health Commission & State Administration of Traditional Chinese Medicine. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7). [R]. March 3, 2020

Symbols used

Symbol	Meaning
	Manufacturer
	Authorized representative in the European Community
	In vitro diagnostic medical device
	Meet the requirements of EC Directive 98/79/EC
	Catalogue number
	Batch code
	The number of tests
	Caution
	Use by date
	Consult instruction for use
	Date of manufacture
	Storage temperature limit

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Company name: Biomiga, Inc.
Address: 10637 Roselle Street, Suite C, San Diego, CA 92121 USA
Tel: 858-603-3219
Email: info@biomiga.com

Company name: Lotus NL B.V.
Address: Koningin Julianaplein 10, 1e Verd, 2595AA, The Hague, Netherlands.
Email: peter@lotusnl.com