7. The adenovirus detected by this kit includes only the subtypes of group B/E, and the ability to detect other subtypes is unknown.
8. The test nucleic acid may remain in the body for a long period of time, regardless of viral activity. A positive test result does not necessarily mean that the virus is infectious, or that the virus is a pathogen that causes clinical symptoms.
9. The performance of the product has not been established on immunocompromised patients.

Performance Characteristics

1. Limit of Detection

The pathogens were diluted in the negative swab matrix and tested. LoD is the lowest reproducible sample concentration that can be distinguished from negative samples at 95% confidence level; or the lowest concentration for 19 positive samples out of 20 repeated tests.

Index type	LoD concentration	Positives/tests(percentage)
Influenza A H1N1 virus	5.00×10^2copies/mL	19/20(95%)
Influenza A H3N2 virus	5.00×10^2copies/mL	19/20(95%)
Influenza A H1N1 (2009) virus	5.00×10^2copies/mL	19/20(95%)
Influenza B virus	5.00×10^2copies/mL	19/20(95%)
Group A streptococcus	5.00×10^2copies/mL	20/20(100%)
Respiratory syncytial virus type A	5.00×10^2copies/mL	20/20(100%)
Respiratory syncytial virus type B	5.00×10^2copies/mL	20/20(100%)
Human adenovirus (type 7)	5.00×10^2copies/mL	19/20(95%)
Mycoplasma pneumoniae	5.00×10^2copies/mL	20/20(100%)

2. Analysis specificity

No cross-reactivity were observed with parainfluenza-1 virus, parainfluenza-2 virus, parainfluenza-3 virus, parainfluenza-4b virus, coronavirus HCoV-OC43, coronavirus HCoV-HKU1, coronavirus HCoV-229E, coronavirus HCoV-NL63, Severe acute respiratory syndrome coronavirus, Middle East Respiratory Syndrome Symptomatic Virus, Group A rotavirus, Norovirus, Human metapneumovirus, EB virus, Human cytomegalovirus, streptococcus pneumoniae, Streptococcus pyogenes, chlamydia pneumoniae , klebsiella pneumoniae, Haemophilus influenzae , Staphylococcus Aureus, mycobacterium tuberculosis . Meanwhile, endogenous substances and therapeutic drugs have no effect on the test. The details are as follows:

Test substance	Test concentration	Result
Endogenous interfering substance		
Human whole blood	10% v/v	No interference
Mucin	2.5% w/v	No interference
Drug		
Oxymetazoline	10% v/v	No interference
Dexamethasone	5μg/mL	No interference
Budesonide	5μg/mL	No interference
Promethazine hydrochloride	5mg/mL	No interference
Watermelon frost	1.7mg/mL	No interference
Ribavirin	7.5mg/mL	No interference
Tobramycin	4μg/mL	No interference

3. Precision

The precision reference products were tested with Take three batches of Respiratory Pathogen Panel 2. Each batch of reagents is tested by 2 people per day, and each person test once in the morning and afternoon. Each concentration is tested 2 times in parallel, and is tested continuously for 5 days. The results met the following criteria.

3.1 Negative sample: The concentration of the test substance is below the minimum detection limit or zero concentration, and the negative detection rate should be 95%.

3.2 Critical positive sample: The concentration of the test substance is slightly higher than the minimum detection limit of the kit, and the positive detection rate should be higher than 95%.

3.3 Medium/strong positive sample: The concentration of the test substance leads to moderate to strong positive result, and the positive detection rate is 100%.

Precautions

1. This product is only suitable for in vitro diagnostic testing, and the experimenter should have received professional training and obtained the relevant work permit.
2. It shall be implemented strictly in accordance with the management norms of gene amplification laboratory promulgated by the relevant competent authorities. The laboratory is divided into three areas for operation (reagent preparation area, sample preparation area and amplification area), items in each area shall not be cross-used, and special instrument and equipment shall be used in each area.
3. The performance characteristics of this test kit for the sample types listed in the section "Intended use" have been identified. The performance of this test kit for other sample types has not been evaluated.
4. The tip with filter and centrifuge tube used in the experiment should be autoclaved, and without DNase and RNase. After use, it is directly driven into the disposal bottle containing 1% sodium hypochlorite.
5. All clinical samples should be treated as infectious substances, operation and disposal shall comply with relevant regulations.

Symbols meaning

	Do not re-use		Manufacturer	EC REP	Authorized representative in the European Community
	Keep dry		Manufacture Date		Do not use if package is damaged
	Caution		Temperature limit(2-8℃)	IVD	In vitro diagnostic medical device
CE	CE Symbol		Keep away from sunlight	LOT	Batch Code
NON STERILE	Non-steril		Consult instructions for use		Symbol for "USE BY"

References

1. Feng L, Li Z, Zhao S, et al. Viral Etiologies of Hospitalized Acute Lower Respiratory Infection Patients in China, 2009-2013[J]. Plos One, 2014, 9(6):e99419.
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3. Zhou Hangyu, et al. Clinical application and evaluation of isothermal amplification technology in the detection of 4 common respiratory viruses in children with pneumonia [D]. Chinese Center for Disease Control and Prevention, 2017.

Basic Information

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Date of approval and amendment of IFU: 2023.10.09
Version number: V1.0

techstar®

Respiratory Pathogen Panel 2 Instruction For Use

Direct detection of Influenza A, Influenza B, Group A streptococcus, Respiratory Syncytial Virus, Human Adenovirus and Mycoplasma pneumoniae by Real-Time PCR

For in vitro diagnostic use only
For Prescription Use only

Name	Specification
Respiratory Pathogen Panel 2	48 reactions/kit

Intend Use

This product is used for the qualitative detection of the nucleic acids of following viruses in the in vitro nasopharyngeal swabs of clinically suspected respiratory infection patients: Influenza A, Influenza B, Group A streptococcus, Respiratory Syncytial Virus, Human Adenovirus and Mycoplasma pneumoniae, which are used to aid the diagnosis of clinical respiratory pathogen infections.

Acute respiratory infection (ARI) is one of the leading causes of morbidity and mortality of infectious diseases at home and abroad. A wide variety of pathogens can cause ARI, including bacteria, viruses, mycoplasma, chlamydia, fungi, and concurrent infections and secondary infections of multiple pathogens, etc., which are common clinical diseases, frequently-occurring diseases, especially in the children, the elderly, and low immunity Individuals. The diseases of this type have similar clinical symptoms and epidemic characteristics, mainly including cough, rhinorrhea, nasal congestion, fever, headache, and muscle pain. It is difficult to identify the type of pathogens infected by clinical symptoms and routine laboratory tests. Harsh pathogen culture conditions result in low positive culture rate, and even some pathogens cannot be cultured under current conditions, which have caused great problems to the patients with respiratory infections and the clinicians. The Respiratory Pathogen Panel 2, combined with other clinical and epidemiological risk factors, provides aid in the diagnosis of respiratory infections. Compared with existing conventional inspection methods, it has significant advantages, such as fast speed, high sensitivity, accurate results, and multi-index parallel test.

Negative results may be caused by other pathogens not detected in the experiment, or lower respiratory tract infections undetectable by nasopharyngeal swabs, which cannot ruled out respiratory infections or be used as the sole basis for diagnosis, treatment, or other management decisions.

Positive results cannot rule out other bacterial or viral infections either.

This kit is for in vitro diagnostic use only. The users should have been professionally trained and obtained relevant qualifications. Some influenza viruses are national legal B-type infectious diseases. The health authority should be contacted in time in case of any positive samples.

Principle

This product uses RT-PCR technology to design specific primers and probes for the Influenza A, Influenza B, Group A streptococcus, Respiratory Syncytial Virus, Human Adenovirus and Mycoplasma pneumoniae. Each test was divided into 2 tubes, one tube for influenza A, influenza B, Group A streptococcus and Reference gene, and the other tube for respiratory syncytial virus, human adenovirus and mycoplasma pneumoniae, as shown in Table 1.

During the amplification process, when the probe is complete, because the 3 'quenched group is close to the 5' reporter group, the fluorescence emitted by the reporter group is absorbed by the quenched group, and no fluorescence signal is emitted. When the nucleic acid of the target was contained in the reaction system, the fluorescent probe bound to the template during primer extension was severed by Taq enzyme (5'-3' exonuclase activity), and the reporter group was separated from the quenched group to produce fluorescence signal. The real-time amplification curve can be plotted by fluorescent quantitative PCR instrument automatically according to the detected fluorescence signal, and implement the qualitative analysis of novel coronavirus nucleic acid in the sample. The assay contains an endogenous internal control (CY5 sign in tube A) for monitor specimen collection, nucleic acid extraction and PCR.

Table 1 Arrangement of the targets

	A tube	B tube
FAM	Influenza A	Respiratory Syncytial Virus
HEX/VIC	Influenza B	Human Adenovirus
ROX	Group A streptococcus	/
CY5	Internal Control	Mycoplasma pneumoniae

Materials Provided

Constituent	Component	48 reactions/kit
Dilution Buffer	Nuclease-free water	2.0mL/vial, 1 vial
RAD RT-qPCR Mix 2A	Primer、Probe、dNTPs、Enzyme	1 vial
RAD RT-qPCR Mix B	Primer、Probe、dNTPs、Enzyme	1 vial
RAD PC2A	Plasmid containing the target gene	1 vial
RAD PCB	Plasmid containing the target gene	1 vial
RAD NC	Nuclease-free water	1.0mL/vial, 1 vial
Mineral oil	Mineral oil	1.5mL/vial, 2 vial

Note: The components in different batch are not interchangeable.

Materials and Equipment Required But Not Provided

- Applied Biosystems® Real-Time PCR System 7500, Roche LightCycler® 480 System, Applied Biosystems® QuantStudio™ 5 Real-Time PCR System, QuantStudio™ 7 Flex.
- QIAamp Virus RNA Mini Kit (cat. #52904 or 52906).
  Alternatively, Nucleic Acid Extraction Kit (cat. SC902). Optionally, High-throughput Automated Sample Preparation System: SC905/SC906
- Vortex mixer.
- Microcentrifuge.
- Micropipettes (2 or 10 μL, 200 μL and 1000 μL).
- Racks for 1.5 mL microcentrifuge tubes.
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach).
- Disposable powder-free gloves and surgical gowns.
- 1.5 mL microcentrifuge tubes (DNase/RNase free).
- 96-well 0.2 mL PCR reaction plates or appropriate optical reaction tube.
- Nuclease-free water

Storage conditions and Expiration period

Store at 2-8 ℃ away from light for 12 months or 20-25 ℃ away from light for 6 months.
Date of manufacture: See label
Expiration Period: See label

Applicable Instrument

Applied Biosystems® Real-Time PCR System 7500, Roche LightCycler® 480 System, Applied Biosystems® QuantStudio™ 5 Real-Time PCR System, QuantStudio™ 7 Flex.

Specimen Collection and Preparation

1. Patient nasopharyngeal swabs are collected in the following standard method and immediately placed in 1-3 mL of transport medium.
1) Tilt the patient's head back by 70 degrees.
2) Insert the swab into the nostril (the depth of the swab should be equal to the distance from the nostril to the outer ear) and leave the swab for a few seconds to absorb the secretions.
3) Retract the swab slowly while rotating it, and both nostrils are sampled with the same swab.
4) Insert the tip of a cotton swab into sterile virus delivery medium and break/sever the swab stick.
2. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test. Improper collection, storage, or transport of specimens may lead to false negative results.

3. The specimen may be tested immediately after collection, or it may be stored at 2-8 ℃ for up to 72 hours before testing. If a delay in testing or shipping is expected, the specimen may be stored at -18 ℃ for no longer than 1 week or at -70 ℃ for no longer than 6 months. Avoid repeated freeze-thaw cycles.
4. The specimen should be shipped in low temperature conditions using dry ice or an ice bag.

Laboratory Procedures

1. Sample processing

RNA should be collected from fresh specimen to ensure suitable RNA quality and quantity. RNA should be extracted using the QIAamp Viral RNA Mini Kit (Qiagen) or Nucleic Acid Extraction kit manually (Art.No.SC902/SC905/SC906, Wuxi Tech-star Technology Co.,Ltd) according to the manufacturer`s Instruction of Use.

800μL Nuclease-free water was added to the RAD PC2A and RAD PCB respectively. Vortex and centrifuge briefly. RAD PC2A, RAD PCB and RAD NC after resolution should be processed simultaneously alongside the specimen.

Following extraction, the RNA should be used immediately or stored at -70 ℃ for use later. When handling the positive control, please take precautions to avoid contamination of the specimen sample.

2. Master Mix Setup

To begin, take out the kit contents and thaw thoroughly at ambient temperature. Prepare 96-well plates or appropriate optical reaction tube for real-time RT-PCR based on the estimated number of reactions, two tubes are required for each sample.

980μL Dilution Buffer was added to the RAD RT-qPCR Mix 2A and RAD RT-qPCR Mix B respectively for re-dissolution, and was completely mixed with tip.

Pipette 20μL of PCR-Mix into each well. Cover and transfer the plate into sample processing area.

⚠ ***The remaining PCR-Mix, RAD PC2A, RAD PCB and RAD NC must be stored at under -18 ℃ immediately, and it should be used in 20 days, repeated freezing and thawing times should not exceed 7 times.***

3. Sample Addition

Add 5μL extracted sample RNA, RAD PC2A, RAD PCB and RAD NC, then add 25μL mineral oil over the reagent to prevent contamination , and close the 96-well reaction plate with appropriate lids or optical adhesive film, mix evenly upside down and short spin at 6000rpm.

⚠ ***Make sure that at least one Positive Control and one Negative Control is used per run.***

4. Testing

Double-click 7500 software or select Start>>All Programs>>Applied Biosystems>>7500 Software.

Click New Experiment to enter Experiment menu. In the Experiment Properties screen, enter identifying information for the experiment; you can leave other fields empty.

Select 7500 (96 Wells); Quantitation-Standard Curve (for the experiment type); TaqMan Reagents (forreagent); and Standard (for ramp speed).

Click Plate Setup, in the Targets screen, under the tab Define Targets and Samples, set Target 1 with FAM reporter, Target 2 with VIC/HEX reporter, Target 3 with ROX reporter and Target 4 with CY5 reporter, set Target 5 with FAM reporter, Target 6 with VIC/HEX reporter and Target 7 with CY5 reporter. Quencher Dye all set: None. Define samples according to the samples in this experiment.

Click Assign Targets and Samples tab, in the target(s) screen, for tube A, select target 1, target 2, target 3 and target 4, for tube B, select target 5, target 6 and target 7. In the Samples screen, select samples and controls to include in the reaction plate in corresponding well, and select the sample/target reactions to set up. Select None for passive reference.

Click Run Method. On the Run Method screen, select either the Graphical View tab (default) or the Tabular View to edit the run method. Make sure the thermal profile displays the holding and cycling stages shown below.

Step	Temperature	Time	Fluorescence measured	Cycle
1	50 ℃	20min	No	1
2	95 ℃	3min	No	1
3	95 ℃	15sec	No	45
4	58 ℃	30sec	Yes	

Click Run. In the Run screen, save the experiment. Click START.

After the run completes, unload the instrument and proceed to data analysis.

5. Analysis of result

Click Analysis. In the Amplification Plot screen under Plot Settings tab:

a. In the Plot Type drop-down list, select ΔRn vs Cycle (default).

b. In the Graph Type drop-down list, select Linear.

c. In the Plot Color drop-down list, select Target.

Set the baseline starting point at cycle 3 and ending at cycle 15

Adjust the threshold to be equal to the maximum level of the no-template control curve (i.e., equal to the maximum value of the random noise curve).

Click Analyze. The software analyzes the data with the settings.

Interpretation of Result

1. Quality control standards

1.1 RAD NC: No typical S-type amplification curve or Ct>40 for FAM, HEX (VIC), ROX and CY5 channels.

1.2 RAD PC2A :FAM, HEX (VIC) , ROX and CY5 channels showed a typical S-type amplification curve and Ct≤33.

RAD PCB: FAM, HEX (VIC) and CY5 channels showed a typical S-type amplification curve and Ct≤33. No typical S-type amplification curve or Ct>40 for ROX channels.

Positive control and Negative control are used to monitor the effectiveness of the instrument and test reagents. The test results of all indexes can only be interpreted when the test results meet the conditions in section 1.1 and 1.2 at the same time.

Internal Control: To ensure the correctness of sample processing, Reference gene designs a set of primers based on the mRNA sequence of human ribonuclease subunit 30. Reference gene is mainly used for quality control of samples for proper processing and to monitor sample-related inhibition during amplification.

2. Result interpretation

Under the premise that Positive control and Negative control meet the requirements, the following analysis is carried out:

2.1 If the Ct values of any of the 5 indicators detected by this product in their respective channel meet the requirements in the below, the indicator is positive, As shown below.

1) Influenza A positive: Ct of FAM channel in tube A was less than 40, and the curve was s-shaped with obvious exponential growth period.
2) Influenza B positive: Ct of HEX/VIC channel in tube A was less than 40, and the curve was s-shaped with obvious exponential growth period.
3) Group A streptococcus positive: Ct of ROX channel in tube A was less than 40, and the curve was s-shaped with obvious exponential growth period.
4) Respiratory Syncytial Virus positive: Ct of FAM channel in tube B was less than 40, and the curve was s-shaped with obvious exponential growth period.
5) Human Adenovirus positive: Ct of HEX/VIC channel in tube B was less than 40, and the curve was s-shaped with obvious exponential growth period.
6) Mycoplasma pneumoniae positive: Ct of CY5 channel in tube B was less than 40, and the curve was s-shaped with obvious exponential growth period.

⚠ ***If two or more of the above conditions are present in one sample at the same time, it is indicated as multiple infection.***

2.2 Negative: All the 5 indicators of the samples to be tested had no Ct value, and the CY5 channel of tube A showed a typical S-type amplification curve, which was judged to be negative. As shown in the following table.

	Tube A				Tube B		
	Influenza A	Influenza B	Group A streptococcus	IC	Respiratory Syncytial Virus	Human Adenovirus	Mycoplasma Pneumoniae
	FAM	HEX (VIC)	ROX	CY5	FAM	HEX (VIC)	CY5
Negative	NoCt	NoCt	NoCt	＜40	NoCt	NoCt	NoCt

2.3 If there is no typical S-type amplification curve in the FAM, HEX(VIC) , ROX and CY5 channels, it indicates that the system is inhibited or misoperated, and the test is invalid. Therefore, the samples should be reexamined.

Limitations

1. The results obtained by this kit should be interpreted in conjunction with other laboratory data and clinical data obtained by the doctor. It should not be used alone as a basis for patient management.
2. Negative results cannot completely exclude pathogen infection, because a concentration of target gene in the sample lower than the test limit or mutation in target sequence can lead to negative results. Improper sample collection, delivery, and handling, as well as improper test operation and experimental environment, can all lead to false negative or false positive results.
3. False positive results may be attributable to non-specific amplification caused by the inhibitors in the sample or cross-reactions of other microorganisms in the respiratory tract. The cross-reactions observed and detected by this kit and the highest concentrations of some inhibitors are already stated in "Analysis specificity".
4. Diseases caused by other bacterial or viral pathogens cannot be ruled out.
5. The samples collected from the patients who have been vaccinated with live attenuated vaccines may result in positive test results.
6. The samples collected from the patients who used drug therapy may result in false negative results.