SC2 Flu RSV Real Time PCR Detection

The Sc2 Flu RSV assay is a real-time RT-PCR multiplexed test intended for the simultaneous qualitative detection and differentiation of SARS-CoV-2, influenza A virus, influenza B virus, and RSV-A /RSV-B nucleic acid in upper or lower respiratory specimens (such as nasopharyngeal, oropharyngeal and nasal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) collected from individuals suspected of respiratory viral infection consistent with COVID-19 by a healthcare provider. Symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar. Test results must be combined with clinical observations, patient history, and epidemiological information.

SUMMARY AND EXPLANATION OF THE TEST

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. The Flu SC2 Multiplex Assay is a molecular *in vitro* diagnostic test that aids in the detection and differentiation of RNA from SARS-CoV-2, influenza A virus, and/or influenza B virus and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan®), and control material used in qRT-PCR for the *in vitro* qualitative detection and differentiation of SARS-CoV-2 virus RNA, influenza A virus RNA influenza B virus RNA and RSV-A/RSV-B virus RNA in upper and lower respiratory specimens.

PRINCIPLE

REGENTS AND MATERIALS PROVIDED

Item	Component	Quantity		Volume /T
		100 Tests / Box	500 Tests / Box	
1	PCR Mix	1.5 mL/ tube × 1	1.5 mL/ tube × 5	14 µL
2	RT-PCR Enzyme Mix	110 µL/ tube × 1	550 µL/ tube × 1	1 µL
3	Positive Control	50 µL/ tube × 1	250 µL/ tube × 1	5 µL
4	Negative Control	50 µL/ tube × 1	250 µL/ tube × 1	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, Cy5 and Cy5.5 (Quasar 705) 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 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

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. Cy5 is the infA indicator channel, ROX is the infB indicator, FAM is the ORF1ab indicator channel, and JOE is the RNase P gene indicator channel (internal control).
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.

PCR Program

Cycles	Step	Time	Temperature
1	Reverse transcription	5 minutes	50 ºC
1	Initial denaturation	2 minutes	95 ºC
40	Denaturation	10 seconds	95 ºC
	Annealing/Extension	30 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, Cy5.5 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, Cy5.5 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, Cy5 and Cy5.5 channels are negative.

Channel	Bacteria	Results Interpretation
JOE	RNase P	If JOE has an exponential increase with Ct ≤37. SC2 positive: If FAM has an exponential increase curves with Ct ≤ 37. Flu A positive: If ROX has an exponential increase curves with Ct ≤ 37. Flu B positive: If Cy5 has an exponential increase curves with Ct ≤ 37.
FAM	SC2	
ROX	Flu A	
Cy5	Flu B	
Cy5.5 (Quasar 705)	RSV-A or RSV-B	RSV-A or RSV-B positive: if Cy5.5 has an exponential increase with Ct ≤37 No differentiation of RSV-A and RSV-B

COMPATIBILITY OF REAL-TIME PCR THERMOCYCLERS

Manufacturer	Model
BioRad	CFX 96 or 384

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 novelinfluenza A (H1N1-2009) virus, influenza AH3N2, H5N1, H7N9, , RSV A, RSV B, parainfluenza I, parainfluenza II, parainfluenza III, adenovirus types 1, 2, 3, 4, 5, 7 & 55, enterovirus types A, B, C and D, hMPV (human metapneumovirus), 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, MERS coronavirus.

LIMITATIONS OF TEST

1. This test provides a presumptive diagnosis of SARS-CoV-2 and flu infection. Negative test results do not preclude SC2/Flu 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 SARS-CoV-2, SARS-CoV-2 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 test
	Caution
	Use by date
	Consult instruction for use
	Date of manufacture
	Storage temperature limit

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In the USA, this product is for Research Use Only (RUO) and is not to be used for diagnostic purposes of any kind without validation.