Instructions for Respiratory SARS-CoV-2 RT-PCR Panel 1

v 6.0

For Prescription Use Only.
For *In Vitro Diagnostic* Use.
For Use Under Emergency Use Authorization Only.

Key to symbols used

IVD	In vitro diagnostic medical device
	Store at -25℃ to -15℃
	Consult instructions for use
	This way up
	Recyclable
	Contains sufficient for (n) test
REF	Catalogue number
LOT	Lot number
	Manufacturer
	Use by date
	Fragile
	Date of manufacture

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Product Name

Respiratory SARS-CoV-2 RT-PCR Panel 1

Kit Contents

96 Tests (SDX-56390) or 384 Tests (SDX-56391)

Intended Use

The Respiratory SARS-CoV-2 RT-PCR Panel 1 is a real-time RT-PCR multiplexed test intended for the simultaneous qualitative detection and differentiation of SARS-CoV-2, influenza A, influenza B and/or respiratory syncytial virus (RSV) nucleic acid from nasopharyngeal swabs, anterior nasal swabs, and mid-turbinate swabs, collected from individuals suspected by a healthcare provider of having respiratory viral infection consistent with COVID-19. Symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high complexity tests.

The Respiratory SARS-CoV-2 RT-PCR Panel 1 is intended for use in the detection and differentiation of SARS-CoV-2, influenza A, influenza B, and/or RSV viral RNA in patient specimens, and is not intended to detect influenza C. RNA from SARS-CoV-2, influenza A, influenza B, and RSV viruses is generally detectable in upper respiratory specimens during the acute phase of infection.

Positive results are indicative of active infection but do not rule out bacterial infection or coinfection with other viruses not detected by the test; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definitive cause of disease. Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

Negative Respiratory SARS-CoV-2 RT-PCR Panel 1 results do not preclude SARS-CoV-2, influenza A, influenza B, and/or RSV infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

Testing with the Respiratory SARS-CoV-2 RT-PCR Panel 1 is intended for use by

qualified laboratory personnel who are specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The Respiratory SARS-CoV-2 RT-PCR Panel 1 is only for use under the Food and Drug Administration's Emergency Use Authorization.

Principles of the Assay

The Respiratory SARS-CoV-2 RT-PCR Panel 1 is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2, Influenza A, Influenza B and RSV primer and probe sets are designed to detect RNA in upper respiratory tract specimens (nasopharyngeal, anterior nasal, and mid-turbinate nasal swabs) from patients suspected by their healthcare provider of respiratory viral infection consistent with COVID-19.

The oligonucleotide primers and probes for detection of SARS-CoV-2 were selected from the regions of the virus' nucleocapsid (N) gene and ORF1ab gene. The primers and probes for detection of influenza A and RSV were selected from regions of the matrix protein. The primers and probes for detection of influenza B were selected from regions of the nuclear export protein (NEP) and nonstructural protein 1 (NS1) genes. An additional primer/probe set to detect the endogenous control gene RNase P is also included in the test. RNase P is used for monitoring sample collection of human biological material and extraction efficiency.

The TaqMan probes for the SARS-CoV-2, Influenza A, Influenza B, RSV and RNase P amplicons are labeled with FAM, ROX, Cy5, Cy5.5 and HEX/VIC fluorescent dyes, respectively to generate target-specific signal.

The assay also uses a dUTP/UNG carryover prevention system to avoid contamination of PCR products and subsequent false positive results.

Kit Components and Packaging Specifications

Catalog Number for 30 µL PCR reaction: SDX-56390 (96 tests per kit)

Component Name	Specifications & Loading		Main Ingredients	Storage Conditions
nCoV Reagent A	950 µL	× 1 tube	Buffers, dNTPs, Mg2+	-25 to -15°C
nCoV Resp Reagent B1	200 µL	× 1 tube	TE buffer, primers, probes	-25 to -15°C
nCoV Resp Reagent B2	40 µL	× 1 tube	TE buffer, primers, probes for RSV only	-25 to -15°C
nCoV Enzyme Mix	170 µL	× 1 tube	Taq DNA polymerase, MMLV, RNasin, UNG	-25 to -15°C

nCoV Resp Positive Control	1.4 mL	× 1 tube	SARS-CoV-2, Influenza A, Influenza B, RSV and RNase P RNA fragments capsulated in bacteriophage	-25 to -15°C
nCoV Negative Control	1.4 mL	× 2 tubes	TE buffer	-25 to -15°C

Catalog Number for 15 µL PCR reaction: SDX-56391 (384 tests per kit)

Component Name	Specifications & Loading		Main Ingredients	Storage Conditions
nCoV Reagent A	950 µL	× 2 tubes	Buffers, dNTPs, Mg2+	-25 to -15°C
nCoV Resp Reagent B1	200 µL	× 2 tubes	TE buffer, primers, probes	-25 to -15°C
nCoV Resp Reagent B2	40 µL	× 2 tubes	TE buffer, primers, probes for RSV only	-25 to -15°C
nCoV Enzyme Mix	170 µL	× 2 tubes	Taq DNA polymerase, MMLV, RNasin, UNG	-25 to -15°C
nCoV Resp Positive Control	1.4 mL	× 4 tubes	SARS-CoV-2, Influenza A, Influenza B, RSV and RNase P RNA fragments capsulated in bacteriophage	-25 to -15°C
nCoV Negative Control	1.4 mL	× 2 tubes	TE buffer	-25 to -15°C

Materials Required but Not Provided

1. RNA extraction reagents and instrument

   RNA extraction reagents, instruments and related software that have been validated with the Respiratory SARS-CoV-2 RT-PCR Panel 1:

   a. chemagic™ Viral DNA/RNA 300 Kit special H96 (Cat # CMG-1033 or CMG-1033-S) and chemagic™ 360 (Cat # 2024-0020) with chemagic™ Rod Head Set 96 (Cat # CMG-370) (software version 6.3.0.3).

2. PCR amplification instruments and software

   a. Applied Biosystems™ QuantStudio™ Dx Real-Time Instrument with 96-well fast block Cat # 4480299 (Test Development Software v1.0.3)

   b. Applied Biosystems™ QuantStudio™ Dx Real-Time Instrument with 384-well block upgrade Cat # 4453545 (Test Development Software v1.0.1 and v1.0.3)

   c. Bio-Rad CFX96™ Touch Real-Time PCR Detection System. (Cat # 1855195) (CFX Maestro Software v4.1.2433.1219) Please see Appendix B for the EUO label for the CFX96™.

   d. ThermoFisher MicroAmp Optical Adhesive Film: Cat # 4311971

   e. Analytik Jena qTower^3 / qTower^3 G Real-Time PCR System: 844-00553-x,

844-00554-x, 844-00555-x, 844-00556-x, 844-00563-x, 844-00564-x, 844 00503-2, 844-00503-4, 844-00504-2 software version qPCRsoft 4.1. Please see Appendix C for the EUO label for the instrument.

f. Analytik Jena qTower^3 84 / qTower^3 84 G Real-Time PCR System: 844-00558-x, 844-00559-x, 844-00568-x, 844-00569-x, 844-00509-2, 844-00509-4 software version qPCRsoft384 1.2. Please see Appendix C for the EUO label for the instrument.

g. Bio-Rad Optical Adhesive Film: Cat # MSB1001

h. 96-well PCR plate:

- Bio-Rad CFX96™ Touch: Cat # MLL9601 or equivalent
- QuantStudio™ Dx 96-well: 4344906 or equivalent
- Bio-Rad CFX96 Touch: MLL9601 or equivalent
- qTower^3 / qTower^3 G: Greiner Bio-one 669285 or equivalent

i. 384-well PCR plate:

- Applied Biosystems™ QuantStudio™ Dx: Cat # 4453545 or equivalent
- qTower^3 84/ qTower^3 84 G: Greiner Bio-one 785235 or equivalent

3. Additional tools and consumables required for automated nucleic acid extraction and PCR setup using chemagic™ 360.

Items	Vendor	Cat #
Centrifuge	Eppendorf	Epp 5810/ 5810 R
Vortex Mixer	VWR	97043-562
Hanging Tip Racks	Revvity	CMG-425
Plate sealing film	Bio-Rad	MSF1001
384-well Spectral Calibration Plate with Cy5 Dye	Revvity	4178-0010
384-well Spectral Calibration Plate with Cy5.5 Dye	Revvity	4179-0010
96-well Spectral Calibration Plate with Cy5 Dye	Revvity	4172-0010
96-well Spectral Calibration Plate with Cy5.5 Dye	Revvity	4173-0010

4. Micropipettors (range between 1 to 20 µL, 20 to 200 µL and 100 to 1000 µL)

5. Non-aerosol pipette tips

6. Plate centrifuge

7. Cy5 and Cy5.5 calibration plate for QuantStudio™ Dx 384 (Revvity: Cat # 4178-0010 for Cy5; Cat # 4179-0010 for Cy5.5)

8. Cy5 and Cy5.5 calibration plate for QuantStudio™ Dx 96 (Revvity: Cat # 4172-0010 for Cy5; Cat # 4173-0010 for Cy5.5)

Storage & Handing Requirements

1. Store all reagents at -25 to -15°C.

2. Completely thaw reagents before use, reagents are stable with six cycles of freeze-thaw. **Reagent A may precipitate upon thawing. Mix reagent at room temperature until fully dissolved.** After thawed, store at 2-8°C.

3. The reagents are estimated to be stable at -25 to -15°C for 24 months based on data from an accelerated stability study. (Real-time stability study is ongoing).

Warnings and Precautions

1. For *in vitro* diagnostic use under Emergency Use Authorization only.
2. For Prescription Use only.
3. This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories.
4. This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus (RSV) not for any other viruses or pathogens.
5. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
6. Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.
7. Keep the kit upright during storage and transportation.

8. Before using the kit, check tubes for leakage or damage. Each component in the kit should be thawed at room temperature, thoroughly mixed, and centrifuged before use.
9. Cross-contamination may occur when inappropriate handling of reference materials and specimens, which will cause inaccurate results. It is recommended to use sterile disposable filter-tips to aspirate reagents and specimens.
10. All specimens to be tested and the reference materials of the kits should be considered as infectious substances and processed strictly in accordance with laboratory biosafety requirements. Sterile centrifuge tubes and filter-tips should be used. After use, the tips should be disposed into a waste bin containing a 10% sodium hypochlorite solution. After the operation, the work area surface and the instrument surface should be disinfected with a freshly prepared 10% sodium hypochlorite solution, and then cleaned with 75% ethanol or pure water. Finally, turn on UV light to disinfect working surfaces for 30 minutes.
11. QuantStudio™ Dx 384 used for this assay should be calibrated regularly according to instrument's instructions to eliminate crosstalk between channels.
12. This kit uses PCR-based technology and experiments should be conducted in three separate areas: reagent preparation area, specimen preparation area, amplification area. Protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.) should be worn during operation and protective equipment accessories should be changed when entering and leaving different work areas. Protective equipment accessories in each work area are not interchangeable.
13. Specimens should be collected with appropriate infection control precautions.
14. Do not use reagents after the expiration date.
15. Do not use the kit if the outer box sealing label is broken upon arrival.
16. Do not use reagents if the tube caps are open or broken upon arrival.
17. Dispose of waste according to local, state, and federal regulations.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable powder-free gloves, hats, protective lab coats and goggles. Change gloves often when handling reagents or samples.
2. Wash hands thoroughly after handling specimens and reagents.

3. Handle all specimens and waste materials as if they could transmit infectious agents in accordance with Universal Precautions.
4. Follow national biological safety recommendations for handling biological samples.
5. Refer to the Clinical and Laboratory Standards Institute (CLSI) Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline (M29), for safety precautions.

Laboratory Precautions for Contamination Prevention

1. Do not handle samples in a biosafety cabinet which is used for other SARS-CoV-2, influenza A, influenza B and RSV culturing purposes.
2. Prior to processing samples, thoroughly clean the work area with freshly prepared 10% bleach or 75% ethanol. Then wipe the work area with water.
3. Avoid excessive handling of the Positive Control to avoid contamination.
4. Change gloves after handling the Positive Control.
5. If spillage of specimen, Positive Control occurs, immediately disinfect the area with freshly prepared 0.5% sodium hypochlorite (bleach) or follow appropriate laboratory biosafety procedures.
6. After amplification is complete, immediately place the PCR plates in a sealable bag; ensure the bag is sealed, then discard the plates in a biohazard container.
7. Change gloves after handling a post PCR plate.
8. All materials used in one area should remain in that area and should not be moved or used in other areas. Never bring post PCR plates to other areas, such as PCR set up area and sample preparation area.
9. Avoid collecting or handling specimens in areas that are exposed to vaccine material. Vaccines may contain PCR-detectable DNA or RNA and particular care should be taken to avoid contamination of the specimen or testing areas (especially with nasal spray vaccines such as FluMist). Contamination of specimens or reagents with vaccine material can cause false positive or false negative results.

Collection, Storage & Shipment of Specimens

1. **Specimen Collection**

   Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate

swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 3 mL of viral transport media. For initial testing, nasopharyngeal swab specimens are recommended.

- Nasopharyngeal swab (NP): Insert a swab into nostril parallel to the palate. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.
- Anterior Nasal Swab (NS): Using a flocked or spun polyester swab, insert the swab at least 1 cm (0.5 inch) inside the nostril (naris) and firmly sample the nasal membrane by rotating the swab and leaving in place for 10 to 15 seconds. Sample both nostrils with same swab.
- Nasal Mid-Turbinate Swab (NMT): Use a flocked tapered swab. Tilt patient's head back 70 degrees. While gently rotating the swab, insert swab less than one inch (about 2 cm) into nostril parallel to the palate (not upwards) until resistance is met at turbinates. Rotate the swab several times against nasal wall and repeat in other nostril using the same swab.

2. **Storage**

   Specimens can be stored at 2-8°C for up to 72 hours after collection, if necessary. If a delay in testing or shipping is expected to extend beyond 72 hours, store specimens at -70°C or below until shipping/processing can proceed.

3. **Shipping**

   Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation External Icon. Store specimens at 2-8°C and ship overnight to the lab on ice pack. If a specimen is frozen at -70°C ship overnight to the lab on dry ice. Additional useful and detailed information on packing, shipping, and transporting specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

4. For more information, refer to:

   - Interim Guidelines for Collecting andHandling of Clinical Specimens for COVID-19 Testing: https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html
   - Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19):

https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html

Assay Procedure

Nucleic Acid Extraction and PCR Setup

Extraction on chemagic™ 360

Please follow chemagic™ 360 User Manual for extraction setup. A quick-start instruction is described below.

1. Both nCoV Resp Positive Control and nCoV Negative Control are required to be included in each extraction run. Input volume of each control is 300 µL.
2. Place specimens and nCoV Resp Positive Control and nCoV Negative Control in a biological safety cabinet. If the specimen or kit controls are frozen, completely thaw them at room temperature before use.
3. In a 2 mL deep-well-plate (riplate SW), add 300 µL Lysis buffer, 300 µL specimen, 4 µL Poly (A) RNA and 10 µL Proteinase K to each well in a sequential order.

   Please note:

   i. Dissolve lyophilized Poly(A) RNA by adding 440 µL of the Poly(A) RNA Buffer to the Poly(A) RNA tube and mix thoroughly before use.

   ii. Dissolve lyophilized Proteinase K in H_2O before use (volume is given on the label).

   iii. An extraction master mix of Poly(A) and Proteinase K can be made for ease of use in sample addition (14 µL of premix is added to each well).
4. In a low-well-plate, add 150 µL magnetic beads into each well.
5. In a new deep-well-plate (riplate SW), add 60 µL Elution Buffer 6 into each well;
6. Turn on the chemagic™ 360, double click the software icon “chemagic_360”, select username and enter password to start. Follow the chemagic™ 360 User Manual to select the appropriate protocol.
7. Load the magnetic rods disposable tips box onto the tracking system (table) according to the instructions given by the chemagic™ software, the tip rack should be in the position indicated in the table below.
8. Load the plates manually onto the tracking system (table) according to the instructions given by the chemagic™ software. The plates should be in the

positions indicated in the table below.

Please note:

i. Specimens and Magnetic Beads should be thoroughly vortex mixed before use.

ii. Never move the tracking system (table) manually. All movements must be performed with the [Turn Table] function in the instrument software.

chemagic™ 360 layout:

Position 1	Magnetic rods disposable tips
Position 2	Low-well-plate (MICROTITER SYSTEM) prefilled with 150 µL Magnetic Beads
Position 3	Deep-well-plate (riplate SW) containing: 300 µL Lysis Buffer 1 300 µL specimen 4 µL Poly(A) RNA 10 µL Proteinase K Binding Buffer 2 (added automatically)
Position 4	Empty deep-well-plate (riplate SW) [Wash Buffer 3 added automatically]
Position 5	Empty deep-well-plate (riplate SW) [Wash Buffer 4 added automatically]
Position 6	Empty deep-well-plate (riplate SW) [Wash Buffer 5 added automatically]
Position 7	Deep-well-plate (riplate SW) prefilled with 60 µL Elution Buffer 6

9. Double check the positions and directions of all consumables according to the tracking system.
10. Click “Start” to start the extraction process.
11. Proceed to downstream assay with the extracted nucleic acids or store the nucleic acids at -25°C to -15°C.

Setup PCR Manually for 30 µL PCR reactions on Bio-Rad CFX96™ instrument, Applied Biosystems™ QuantStudio™ Dx 96 well instrument, and Analytik Jena qTower^3/qTower^3 G Real Time PCR Systems

Note: In order to ensure the performance of multiplex real-time PCR, it requires:

- Prepare PCR mix always when the sample extraction close to finish or finished
- After step 6, start PCR run immediately on the instrument.

Setup PCR manually according the procedures described below after nucleic acid extraction using chemagic™ 360.

1. Prepare PCR mix in Reagent Preparation Area according to the following table. It is recommended to prepare 110% of the calculated amount of PCR mix to account for pipetting carryovers.

Note: Reagent A may precipitate. Keep at room temperature and mix well to ensure complete resuspension**.**

Component	Volume/ test	Volume for N Samples and two Controls	110% of volume
nCoV Reagent A	7.5 µL	7.5 x (n + 2) µL	8.25 x (n + 2) µL
nCoV Resp Reagent B1	1.25 µL	1.25 x (n + 2) µL	1.375 x (n + 2) µL
nCoV Resp Reagent B2	0.25 µL	0.25 x (n + 2) µL	0.375 x (n + 2) µL
nCoV Enzyme mix	1 µL	1 x (n + 2) µL	1.1 x (n + 2) µL

2. Vortex the prepared PCR mix to ensure it is fully mixed, then centrifuge briefly to collect in the bottom of the tube.
3. Pipette 10 µL into each well of a 96-well PCR plate.
4. Add 20 µL of extracted nCoV negative control into each tube or well containing PCR mix.
5. Add 20 µL of extracted nucleic acid (including positive control) into each tube or well containing PCR mix.
6. Seal the PCR plate with an appropriate film.
7. Vortex plate for 10-20 seconds, and centrifuge for 5 minutes at 350 x g.

Setup PCR Manually for 15 µL PCR reactions on Applied Biosystems™ QuantStudio™ Dx 384 well instrument, and Analytik Jena qTower^3 84/qTower^3 84 G Real Time PCR Systems

Note: In order to ensure the performance of multiplex real-time PCR, it requires:

- Prepare PCR mix always when the sample extraction close to finish or finished
- After step 6, start PCR run immediately on the instrument.

Setup PCR manually according the procedures described below after nucleic acid extraction using chemagic™ 360.

1. Prepare PCR mix in Reagent Preparation Area according to the following table. It is recommended to prepare 110% of the calculated amount of PCR mix to account for pipetting carryovers.

Note: Reagent A may precipitate. Keep at room temperature and mix well to ensure complete resuspension**.**

Component	Volume/ test	Volume for N Samples and two Controls	110% of volume
nCoV Reagent A	3.75 µL	3.75 x (n + 2) µL	4.125 x (n + 2) µL
nCoV Resp Reagent B1	0.625 µL	0.625 x (n + 2) µL	0.6875 x (n + 2) µL
nCoV Resp Reagent B2	0.125 µL	0.125 x (n + 2) µL	0.1375 x (n + 2) µL
nCoV Enzyme mix	0.5 µL	0.5 x (n + 2) µL	0.55 x (n + 2) µL

2. Vortex the prepared PCR mix to ensure it is fully mixed, then centrifuge briefly to collect in the bottom of the tube.
3. Pipette 5 µL into each well of a 96-well PCR plate.
4. Add 10 µL of extracted nCoV negative control into each tube or well containing PCR mix.
5. Add 10 µL of extracted nucleic acid (including positive control) into each tube or well containing PCR mix.
6. Seal PCR the plate with an appropriate film.
7. Vortex plate for 10-20 seconds, and centrifuge for 5 minutes at 350 x g.

Amplification

Amplification on the Bio-Rad CFX96™ Real-Time PCR Instrument

1. Set up and run the Bio-Rad CFX96™ Real-Time PCR Instrument. Refer to the CFX96™ manual for detailed instructions. In general, open the CFX Maestro software and New User Defined PCR › Create New Protocol › Create New Plate › then click Run and Start.
2. When setup Create New Protocol, please check the following run settings and choose the correct settings.
    - Create New Protocol
    - Make sure sample volume is 30 µL

Step	Temperature	Time	Number of Cycles
1	37°C	2 minutes	1
2	50°C	15 minutes	1
3	94°C	10 minutes	1
4	94°C	10 seconds	45[a]
	55°C	15 seconds	
	65°C*	45 seconds	

** Collect fluorescence signal during the final 65°C step.*
[a]. 45 cycles are used for validation study. Based on Ct cutoff, 42 cycles can be applied.

3. Next Create the Plate

- Select Create New
- Set Scan Mode to All Channels
- Choose the Fluorophores and assign the target names
- Assign a Task to each well

Target Name or Detector	Channel
SARS CoV-2	FAM
Influenza A	ROX
Influenza B	Cy5
RSV	Quasar 705
RNase P	VIC

4. Set up the plate layout by assigning a unique sample name to each well.
5. Assign a Task to each well.
    - Unknown: for patient samples
    - Standard: for Positive Control
    - NTC: for Negative Control
6. Double check all settings then click Run and Start to initialize amplification

Note: If you desire to suppress the results for a particular target, do not select to read the reporter of that target.

Amplification on the Applied Biosystems™ QuantStudio™ Dx 384-well Real Time PCR Instrument and Applied Biosystems™ QuantStudio™ Dx 96-well Real Time PCR Instrument

1. Set up and run the QuantStudio™ Dx 384-well instrument or QuantStudio™ Dx 96-well instrument. Refer to QuantStudio™ Dx Real Time Instrument User Guide for set up details. In general, double-click QuantStudio™ Dx Test Development software › New experiments › Setup Experiment Properties › Setup the Targets and Samples in Plate Setup › Setup Run Method, then click Run and Start.
2. When setup Experiment Properties, please check the following run settings and choose the correct settings.
    - Block: 384-well (96-well for QuantStudio™ Dx 96)
    - Experiment type: Quantitation – Standard Curve
    - Run Reagent: TaqMan reagents
    - Run Properties: Standard
3. When setting up the Targets and Samples, create the following detectors with the quencher set as none. The passive reference must be set as None.

Target Name or Detector	Reporter	Quencher
SARS CoV-2	FAM	None
Influenza A	ROX	None
Influenza B	Cy5	None
RSV	Cy5.5*	None
RNase P	VIC	None

*Select "OTHER" for Cy5.5 detection based on instrument calibration listed in Appendix.

4. Set up the plate layout by assigning a unique sample name to each well.
5. Assign a Task to each well.
    - Unknown: for patient samples
    - Standard: for Positive Control
    - NTC: for Negative Control
6. Set Run method as following for PCR amplification and fluorescence detection, set the sample volume at 15 µL.

Step	Temperature	Time	Number of Cycles
1	37°C	2 minutes	1
2	50°C	15 minutes	1
3	94°C	10 minutes	1
4	94°C	10 seconds	45[a]
	55°C	15 seconds	
	65°C*	45 seconds	

** Collect fluorescence signal during the final 65°C step.*
[a] 45 cycles are used for validation study. Based on Ct cutoff, 40 cycles can be applied.

Double check all settings then click Run and Start to initialize amplification.

Note: If you desire to suppress the results for a particular target, do not select to read the reporter of that target.

Amplification on the Analytik Jena qTower^3/qTower^3 G and qTower^3 84/qTower^3 84 G Real Time PCR Systems

1. 384-well: Set up and run the Analytik Jena qTOWER^3 84 / qTower^3 84 G Real-Time PCR instrument. Refer to Analytik Jena qTOWER^3 84 / 84 G Real-Time PCR Operating Manual for detailed instructions. In general, double-click qPCRsoft384 software 1.2 › File | New › Settings › Thermal Cycler | Scan | Samples, then click *Start qPCR run*.

   NOTE – the settings described below can be saved and recalled by use of a project template file (.rts384 or *.rtsx384 file)*

96-well: Set up and run the Analytik Jena qTOWER^3 / qTower^3 G Real-Time PCR instrument. Refer to Analytik Jena qTOWER^3 / qTower^3 G Real-Time PCR Operating Manual for detailed instructions. In general, double-click qPCRsoft software 4.1 › File | New › Settings › Thermal Cycler | Scan | Samples, then click Start qPCR run.

NOTE – the settings described below can be saved and recalled by use of a project template file (*.rts or *.rtsx file)

2. Settings | General:
    - Title: as appropriate for this run
    - Operator: appropriate operator designation
    - Start and End: populated automatically as part of the run
    - Comment: any additional information regarding the run
3. Settings | Thermal Cycler:
    - Set Run method as following for PCR amplification and fluorescence detection, using default ramping rate.
    - Lid Temp: 100 °C,

reference (Pass. Ref.) cells/column must be left empty.

Pos.	Channel	Dye	Gain	Measurement
1	Blue	FAM	**3**	X
2	Green	JOE	5	
3	Yellow	**HEX_3**	5	X
4	Orange	ROX	5	X
5	Red	Cy5	5	X
6	NIR1	Cy5.5	5	X

- **Color compensation: refer to Appendix B for details.**

NOTE – all six Pos. and Channel must be activated in Edit color modules before opening any new project files (on software main page, click Extras>Edit color modules). Otherwise, corresponding Pos. and Channel may not show up in Scan setting.

Note: If you desire to suppress the results for a particular target, do not select to read the reporter of that target.

5. Settings | Samples:
    - Set up the plate layout by assigning a unique sample name to each well.
    - Assign a sample type to each well:
        - Positive control
        - Negative control
        - Unknown (patient sample)
6. Double check all settings, save the project, and then click *Start qPCR* run to initialize amplification.

Note – Device selection must match the specific device in thermal cycler setting, otherwise qPCR run will not start, and error message may pop up.

Interpretation of Results

1. **Baseline and threshold setting for CFX96™ and Applied Biosystems™ QuantStudio™ Dx 96 and 384-well instruments**

    After the run completion, save and analyze the data according to PCR instrument instructions.

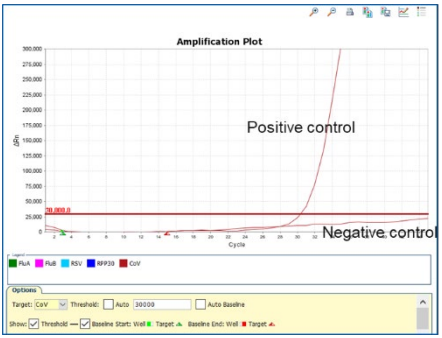
1) Set baseline for each target

View the baseline values, in the Graph Type drop-down list, select Linear. Select the Baseline check box to show the start cycle and end cycle. The horizontal part of the baseline is used for the baseline range, which normally starts from 3-5 cycles and ends at 15-20 cycles. Baseline setting is normally automatically done by instrument. Manual baseline 3-15 is recommended as general.

2) Set threshold for each target

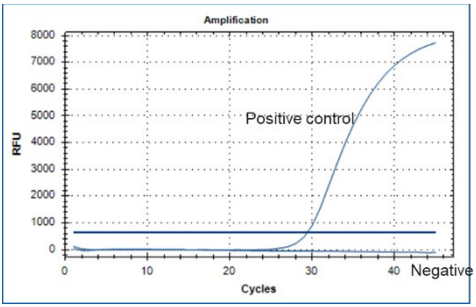
Applied Biosystems™ QuantStudio™ Dx 96 and 384

View the threshold values, In the Graph Type drop-down list, select Linear. In the Target drop-down list, select individual target. Select the Threshold check box to show the threshold. Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background signal (refer to the figure below). The threshold value for different instruments varies due to different signal intensities. Perform data analysis by clicking “Analyze” button of the software.



Bio-Rad CFX96™

Uncheck “Log Scale”, click “Settings”, choose “Baseline Setting”, check “Baseline Subtracted Curve Fit” and “Apply Florescence Drift Correction”. Set up threshold for each fluorophore by checking corresponding box. An example figure is listed below. For CFX96™, the threshold for Cy5 is required to be at least 300 RFU (for example, 500 RFU).



3) Output the data to csv file by the “export” function of the software.

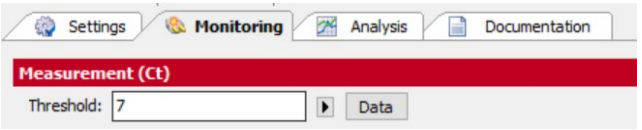
4) Interpret the results based on the tables listed in “Quality Control” and “Examination and Interpretation of Specimen Results”.

2. Baseline and threshold setting for qTower^3/qTower^3 G and qTower^3 84/qTower^3 84 G Real Time PCR Systems

After the run completion, save and analyze the data according to PCR instrument instructions.

Under **Settings** tab, for color compensation configuration, select “Standard 1” or customized settings.

Under **monitoring** tab, click “Calculate Ct”, the following view shows up.

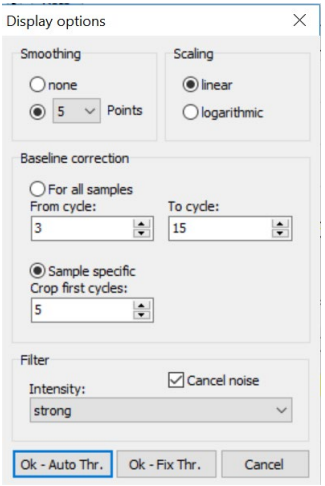


1) Set baseline for each target

In most of the cases, the default baseline can be used. In order to adjust baseline, click icon 

The default setting is “Sample specific crop first cycles”, which is good for most of the cases. The default is 5, which can be adjusted to minimize background noises in some cases. By this setting, every sample has the same baseline start, but different baseline end which is defined by software.

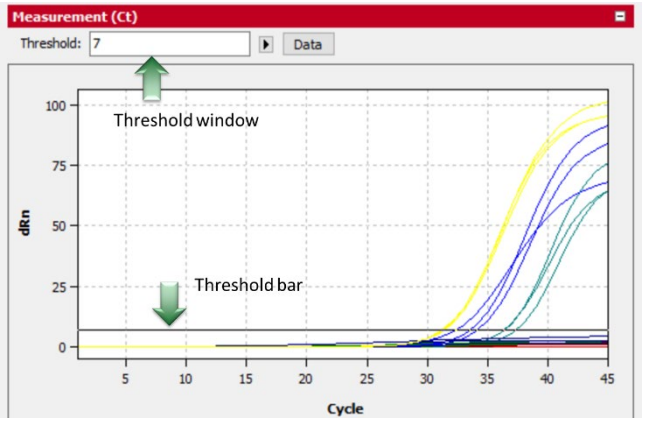
In order to set up universal baseline across samples (same baseline start and same baseline end), click “For all samples”, from cycle X (default 3) to Y (default 15) as the following window.



In order to switch back to the default setting, click “Sample specific Crop first cycles”.

2) Set threshold for each target

Under **Monitoring** tab, view the threshold values under “linear” scaling (showed in above figure) for each target. Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments varies due to different signal intensities. It is recommended to setup threshold manually instead of default settings. For manual threshold setup, it can either remove threshold bar up and down, or manually input threshold number to the threshold window, shown in the following figure. It is recommended to setup the threshold in the range of 5-15 as general.



3. Quality Control

The provided Negative Control (TE buffer) and Positive Control monitor the reliability of the results for the entire batch of specimens from sample extraction to PCR amplification. All test controls must be examined prior to interpretation of patient results. Positive Control and Negative Control must meet the requirements listed in the below table to ensure valid results. If the controls are not valid, the patient results cannot be interpreted.

1) **Negative Control:** A “no template” (negative) control (NTC) is needed to monitor for potential contamination that could occur during extraction or with the RT-PCR reagents. The NTC consists of either TE buffer or nuclease-free water. One NTC must be included per extraction batch and RT-PCR run. The Ct requirements are listed in the following table. If one of the targets fails the Ct requirements, the negative control is invalid and all patient specimens within the run should be retested.

2) **Positive Control:** A positive control is needed to monitor the overall process of extraction, reverse transcriptase and PCR amplification signals for each target at different detection channel/color module. It is a mixture of pseudo viruses, including RNA constructs of SARS-CoV-2, Influenza A, Influenza B, RSV and RNase P.

   It can be used to assist the threshold setup to differentiate the amplification signal vs. instrument background noises or signal drift. The Ct requirements are listed in the following table. If one of the targets fails the Ct requirements, the positive control is invalid and all patient specimens within the run should be retested.

3) **Endogenous Control (Internal Control):** The RNase P serves as an endogenous internal control that is used together with the data from other targets for interpretation of an individual specimen. RNase P must be positive (<=35 Ct) for all clinical specimens in order to report negative results for the target analytes.

   If RNase P is negative in the presence of a positive result for one of the viral targets, the viral target result should be considered valid.

   However, if all viral targets generate negative results and RNase P is also negative (> 35 Ct or not determined), the test is considered as invalid. Failure to detect RNase P in clinical specimens could indicate:

   - Insufficient nucleic acid extraction from clinical samples
   - Poor specimen quality or loss of specimen integrity

- Improper assay execution
- Reagent or equipment malfunction

If the result for any clinical specimen is invalid, repeat testing of specimen nucleic acid and/or re-extract and repeat RT-PCR. If repeat test is invalid, collection of a new specimen and subsequent testing should be considered.

Control Interpretation for the Applied Biosystems™ QuantStudio™ Dx 384, Applied Biosystems™ QuantStudioTM Dx 96, Analytik Jena qTower3/qTower3 G and qTower3 84/qTower3 84 G Real Time PCR Systems

Control name	Ct Value				
	SARS-CoV-2 (FAM)	Influenza A (ROX)	Influenza B (Cy5)	RSV (Cy5.5)	RNase P (HEX/VIC)
Negative control	Undet/blank or > 40	Undet/blank or > 40	Undet/blank or > 40	Undet/blank or > 40	Undet/blank or > 35
Positive control	≤ 35	≤ 35	≤ 35	≤ 35	≤ 35

Undet: Undetermined

Control Interpretation for the Bio-Rad CFX96™Control name	Ct Value				
	SARS-CoV-2 (FAM)	Influenza A (ROX)	Influenza B (Cy5)	RSV (Cy5.5)	RNase P (HEX/VIC)
Negative control	Undet/blank or > 40	Undet/blank or > 40	Undet/blank or > 42	Undet/blank or > 42	Undet/blank or > 35
Positive control	≤ 35	≤ 35	≤ 35	≤ 35	≤ 35

Undet: Undetermined
Note: CFX96™ Cy5 threshold > 300 RFU

4. Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results must be performed after the positive and negative controls have been examined and confirmed to be valid. If the controls are not valid, the patient results cannot be interpreted.

The table below lists the expected results for the assay with valid positive control and negative control. Analysis analyte is defined as SARS-CoV-2, Influenza A, Influenza B, RSV.

Ct cutoff for the instrument with FAM, HEX/VIC, ROX, Cy5 and Cy5.5/ Quasar705 detection capability (QuantStudio™ Dx 96 and 384, Bio-Rad CFX96™ Analytik Jena qTower³/qTower³ G and qTower³ 84/qTower³ 84 G Real Time PCR Systems)

The disposition of test results as positive, presumptive positive or negative

must be determined for each analyte independently based on the Ct values for the specific analyte and the RNase P endogenous control. Invalid results are obtained when no amplification is detected for any analyte or the RNase P endogenous control, or when the Ct values obtained for each analyte and the RNase P control fall outside the specified ranges.

QuantStudio™ Dx 384					
Result	**SARS-CoV-2 (FAM)**	**Influenza A (ROX)**	**Influenza B (Cy5)**	**RSV (Cy5.5)**	**RNase P (HEX/VIC)**
Positive (1)	≤ 37	≤ 37	≤ 37	≤ 37	Any or Undet/blank
Presumptive Positive (2)	37 < Ct ≤ 40	37 < Ct ≤ 40	37 < Ct ≤ 40	37 < Ct ≤ 40	Any or Undet/blank
Negative	>40 or Undet	>40 or Undet	>40 or Undet	>40 or Undet	≤ 35
Invalid	>40 or Undet	>40 or Undet	>40 or Undet	>40 or Undet	>35 or Undet/blank
CFX 96™					
Result	**SARS-CoV-2 (FAM)**	**Influenza A (ROX)**	**Influenza B (Cy5)**	**RSV (Cy5.5)**	**RNase P (HEX/VIC)**
Positive (1)	≤ 37	≤ 37	≤ 37	≤ 37	Any or Undet/blank
Presumptive Positive (2)	37 < Ct ≤ 40	37 < Ct ≤ 40	37 < Ct ≤ 42	37 < Ct ≤ 42	Any or Undet/blank
Negative	>40 or Undet	>40 or Undet	>42 or Undet	>42 or Undet	≤ 35
Invalid	>40 or Undet	>40 or Undet	>42 or Undet	>42 or Undet	>35 or Undet/blank

Undet: Undetermined
Note: CFX96™ Cy5 threshold > 300 RFU
1 and 2: The result for each analyte must be determined separately based on its reported Ct value and that of the RNase P control
2. Second result for confirmation as “Positive” for the corresponding analyte is required for the clinical sample. Without a second confirmation result, the sample is considered presumptive positive.

Presumptive positive results must be confirmed by repeat testing either by re-extracting the original clinical sample (recommended if there is sufficient sample remaining) or by performing the RT-PCR from the residual sample extract (repeat RT-PCR only). When repeat testing is required to confirm a Presumptive Positive result obtained on initial testing, the final disposition for each analyte is determined according to the table below.

		First Result
		Presumptive Positive
Second Result	**Positive**	Positive
	Negative	Negative
	Presumptive Positive	Positive

With two test results for a clinical sample, the final report is based on the

combination of the two results for each analyte. Both test results for a given analyte must be non-negative (either positive or presumptive positive) in order to report an analyte as positive. If both test results for the same analyte are "Presumptive Positive", the final report for the analyte is "Positive". Without a second confirmation result, the sample is considered presumptive positive and reported as such.

Kit Limitations

1. The use of this assay as an *in vitro* diagnostic under FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meet requirements to perform high complexity tests.
2. This kit qualitatively detects SARS-CoV-2, influenza A, influenza B, and RSV RNA from human nasopharyngeal swabs, anterior and mid-turbinate nasal swabs. The results cannot directly reflect the viral load in the original specimens.
3. The Respiratory SARS-CoV-2 RT-PCR Panel 1 performance has only been established with nasopharyngeal swabs in the pre-authorization clinical study.
4. Interference was observed with the detection of low levels of RSV in the presence of the three other on-panel analytes and either Fluticasone, Oseltamivir or Mupirocin.
5. Interference was observed with the detection of low levels of SARS-CoV-2, influenza A, influenza B and RSV when mixed together in the presence of Budesonide.
6. Interference was observed with the detection of low levels of RSV in the presence of FluMist.
7. Competitive interference with the detection of low levels of RSV and influenza A was observed on the QuantStudio Dx in the presence of very high levels of the other three analytes.
8. Microbial interference was observed with RSV when multiplexed with other target analytes at 3X LoD and tested in the presence of *Staphylococcus aureus* at high concentration (> 1E8 copies/mL).
9. Recent administration of the FluMist nasal vaccine prior to specimen collection could lead to positive influenza A and influenza B results with the Respiratory SARS-CoV-2 RT-PCR Panel 1 that are not indicative of infection.
10. The specimens to be tested must be collected, processed, stored and transported in accordance with the conditions specified in the instructions.

Inappropriate specimen preparation and operation may lead to inaccurate results.

11. RNA extraction methods other than that listed in the Assay Procedure have not been evaluated. Users should validate any other extraction methods before using them with the Respiratory SARS-CoV-2 RT-PCR Panel 1.
12. Primers and probes for this kit target conserved regions within the genomes of SARS-CoV-2, Influenza A, Influenza B, and RSV. Mutations may occur in these conserved regions and could result in RNA being undetectable.
13. This kit uses an UNG/dUTP PCR products carryover prevention system which can prevent contamination caused by PCR products. However, in the actual operation process, amplicon contamination can be avoided by strictly following the instructions of PCR laboratories.
14. Negative results do not preclude SARS-CoV-2, Influenza A, Influenza B and RSV infections and should not be used as the sole basis for treatment or other management decisions.
15. This kit was not tested for all known/unknown cross-reactants i.e. some fungi, bacteria and virus.
16. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs have not been evaluated.
17. Laboratories are required to report all SARS-CoV-2 results to the appropriate public health authorities.
18. The performance of this test was established based on the evaluation of a limited number of clinical specimens. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
19. As of August 2021, some mutations associated with currently circulating viral escape variants are known to occur in the region of the N gene forward primer for SARS-CoV-2. However, because the Respiratory SARS-CoV-2 RT-PCR Panel 1 targets two different regions of the SARS-CoV-2 genome (N gene and ORF1ab), these mutations are not predicted to affect the inclusivity of the panel.

Conditions of Authorization For Laboratories

The Respiratory SARS-CoV-2 RT-PCR Panel 1 Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for

Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist clinical laboratories using the Respiratory SARS-CoV-2 RT-PCR Panel 1 the relevant Conditions of Authorization are listed below:

A. Authorized laboratories using this product[1] must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using this product must use this product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use this product are not permitted.

C. Authorized laboratories that receive this product will notify the relevant public health authorities of their intent to run this product prior to initiating testing.

D. Authorized laboratories using this product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories must collect information on the performance of this product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Revvity COVID-19 Technical Support (via email: COVID-19TechnicalSupport@Revvity.com and US Toll-Free: 1-888-208-2246) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of this product of which they become aware.

F. All laboratory personnel using this product must be appropriately trained in RT-PCR techniques, the specific processes and instruments used in the Respiratory SARS-CoV-2 RT-PCR Panel 1 and use appropriate laboratory and personal protective equipment when handling this kit, and use this product in accordance with the authorized labeling.

G. Revvity, authorized distributors, and authorized laboratories using this product

[1] This product" refers to the Respiratory SARS-CoV-2 RT-PCR Panel 1. The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests "as "authorized laboratories."

must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

Assay Performance

Limit of Detection (LoD)

Analytical sensitivity of the Respiratory SARS-CoV-2 RT-PCR Panel 1 was determined in limit of detection studies using stocks of live cultured viruses including influenza A virus, influenza B virus, and RSV virus as well as the heat-inactivated form of SARS-CoV-2 (provided from BEI Resources). The LoD study determined the lowest detectable concentration of influenza A, influenza B, RSV, and SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates tested positive. Two different LoD studies using single spiked analytes were completed with different reagents lots and are described below:

Table: Viruses used in LoD study

Viral target	Strain name	Stock titer ($TCID_{50}$/mL)	BEI catalog number
Influenza A (H1N1)	A/Georgia/T51700/2012 (H1N1)pdm09	2.8×10^8	NR-42940
Influenza A (H3N2)	H3N2 (A/Hong Kong/2671/2019)	3.16×10^7	N/A
Influenza B	B/Brisbane/60/2008 (Victoria Lineage)	1.8×10^7	NR-42005
Influenza B	B/Texas/81/2016 (Yamagata lineage)	2.00×10^8	N/A
Human respiratory syncytial virus	A2001/3-12	1.6×10^8	NR-28526
SARS-coronavirus-2	Isolate USA-WA1/2020, Heat Inactivated	1.6×10^5	NR-52286

N/A; Not applicable, strain from the 2020 CDC Human Influenza Virus Panel

a. LoD (Single Formulation Lot #1)

Preliminary range-finding studies were completed to determine an estimated LoD for each individual targeted analyte. Serial dilutions were tested by extracting 300 µL of each virus dilution and eluting with 60 µL of elution buffer provided with the chemagic™ extraction kit. 20 µL was used as input for the PCR reactions on Bio-Rad CFX96™ and 10 µL was used as input for the PCR reactions on Applied Biosystems™ QuantStudio™ Dx 384.

The LoD for each analyte was subsequently confirmed by testing 20 individual extraction replicates at the estimated LoD. The LoD was further confirmed by

testing 20 individual extraction replicates at 1/3X LoD which was below the ≥95% detection rate to show that the LoD was correctly identified.

Summary LoD data are listed in the following two tables with PCR reagent lot 1.

Table: LoD study summary ($TCID_{50}$/mL and EID_{50}/mL) – Single Formulation (Lot 1)

Instrument	SARS-CoV-2	Influenza A H1N1 ($TCID_{50}$/mL)	Influenza A H3N2 (EID_{50}/mL)	Influenza B – Victoria ($TCID_{50}$/mL)	Influenza B – Yamagata (EID_{50}/mL)	RSV
QuantStudio™ 384	0.11*	3.33	2.67	0.33	0.68	3.33
CFX96™	0.11*	3.33	2.67	0.33	0.68	3.33

*0.11 $TCID_{50}$/mL of SARS-CoV-2 is around 258 copies/mL based on supplier's CoA.

Table: Summary of Mean Ct Values for LoD study – Single Formulation (Lot 1)

Instrument	SARS-CoV-2			Influenza A H1N1			Influenza B - Victoria			RSV		
	Mean Ct	Std Dev	N	Mean Ct	Std Dev	N	Mean Ct	Std Dev	N	Mean Ct	Std Dev	N
1X LoD Concentration												
QuantStudio ™ 384	36.87	0.93	20	35.73	0.87	19	36.37	0.95	19	37.92	1.61	19
CFX96™	37.76	0.47	20	37.01	1.52	20	39.27	0.61	20	37.87	1.47	20

Std Dev; Ct Standard Deviation
N; number of replicates detected out of 20
QuantStudio™ 384 1XLoD Presumptive Positive: SARS-CoV-2, 8/20 (40%); Influenza B (Victoria), 4/20 (20%), RSV, 13/20 (65%)
CFX96™ 1XLoD Presumptive Positive: SARS-CoV-2, 2/20 (10%), Influenza A H1N1, 8/20 (40%), Influenza B (Victoria), 20/20 (100%); RSV, 16/20 (80%)

Table: Summary of Mean Ct Values for LoD study (H3N2 and Yamagata lineage) (Lot 1)

Instrument	Influenza A ((H3N2) A/Hong Kong/2671/2019)			Influenza B (B/Texas/81/2016 (Yamagata lineage))		
	Mean Ct	Std Dev	N(hit)	Mean Ct	Std Dev	N(hit)
1X LoD Concentration						
	2.67 EID_{50}/mL			0.68 EID_{50}/mL		
Applied Biosystems QuantStudio 384	37.37	0.79	20/20	37.61	0.66	19/20
Bio-Rad CFX96	36.50	0.40	20/20	38.77	0.72	20/20

Std Dev; Ct Standard Deviation
QuantStudio™ 384 1XLoD Presumptive Positive:H3N2, 13/20 (65%), Yamagata, 4/20 (20%)
CFX96™ 1XLoD Presumptive Positive: H3N2, 2/20 (10%), Yamagata, 20/20 (100%)

In summary, the validated instruments had comparable LoDs for each targeted analyte. QuantStudio™ Dx 384 was chosen as the reference/anchor instrument for further validation, including the clinical study.

b. LoD (Single Formulation Lot #2)

The LoD was further evaluated with a second PCR reagent lot with each analyte in a single formulation on the QuantStudio™ Dx 384 containing the same virus strains shown above. The additional LoD study for lot #2 was completed on a

different day with a different operator and instrument. Summary LoD data for both reagents lots and single spiked analytes are listed in the following table.

Table: Single Formulation summary of two lots on the QuantStudio™ Dx 384

Virus	Panel	Kit Lot	N(Hit)	Hit rate ≥ 95% [$TCID_{50}$/mL]	Hit rate ≥ 95% Mean Ct	Std Dev
Influenza A H1N1	Single-formulated	1	19/20	3.33	35.73	0.87
	Single-formulated	2	20/20	3.33	37.05	0.93
	Single-formulated summary	3.33 $TCID_{50}$/mL				
Influenza B Victoria	Single-formulated	1	19/20	0.33	36.37	0.95
	Single-formulated	2	20/20	0.99	34.44	0.51
	Single-formulated summary	0.99 $TCID_{50}$/mL[a]				
SARS-CoV-2	Single-formulated	1	20/20	0.11	36.87	0.93
	Single-formulated	2	20/20	0.11	35.63	0.5
	Single-formulated summary	0.11 $TCID_{50}$/mL				
Human respiratory syncytial virus	Single-formulated	1	19/20	3.33	37.92	1.61
	Single-formulated	2	20/20	9.99	36.48	2.41
	Single-formulated summary	9.99 $TCID_{50}$/mL[a]				

[a] Represents the worse LoD obtained with the two lots of reagents
Std Dev; Ct Standard Deviation
*Note that the kit lots were used on separate days and in different studies. Different lots were used for analytical validation of the Respiratory SARS-CoV-2 RT-PCR Panel 1 and are mentioned within each study description along with the testing concentration.
Lot #1 Presumptive Positive results: SARS-CoV-2, 8/20 (40%); Influenza B, 4/20 (20%); RSV, 13/20 (65%)
Lot #2 Presumptive Positive results: Influenza A, 11/20 (55%); RSV, 8/20 (40%)

Lot 2 had a 3-fold higher LoD for influenza B and RSV when compared to lot 1. Lot-to-lot variation can be attributed to differences in instruments, operators, or days on which the experiments were performed. All lots met quality control release specifications. Lot 2, being the worse lot (higher LoD for select analytes) was used to determine the co-formulation LoD on both the QuantStudio™ Dx 384 and CFX96™. The co-formulation LoD summary for lot #2 is shown in the following table. In general, the co-formulation LoD was around 2-fold higher than the single-formulation LoD for each targeted analyte when using lot #2. Therefore, it was considered acceptable to use the co-formulated panels or a multiplex of all targeted analytes for analytical studies. The study descriptions below mention if a co-formulation or single formulation LoD was used to determine the appropriate target levels, as well as the lot number of the PCR reagents that was used to complete the study.

Table: Co-Formulation summary of lot 2 on QuantStudio™ Dx 384 and CFX96™

Target	Instrument	N(Hit)	Hit rate ≥ 95% [$TCID_{50}$/mL]	Hit rate ≥ 95% Mean Ct	Std Dev
Influenza A H1N1	QuantStudio™ Dx 384	20/20	6.66	35.93	1.10
	CFX96™	20/20	6.66	36.72	0.81
Influenza B Victoria	QuantStudio™ Dx 384	20/20	1.98	34.21	0.77
	CFX96™	20/20	1.98	36.49	0.65
SARS-CoV-2	QuantStudio™ Dx 384	20/20	0.22	36.13	0.98

	CFX96™	20/20	0.22	36.19	0.57
RSV	QuantStudio™ Dx 384	20/20	19.98	34.47	0.91
	CFX96™	20/20	19.98	36.12	0.86

Std Dev; Standard Deviation of Ct
QuantStudio™ 384 Presumptive Positive results: SARS-CoV-2, 4/20 (20%); Influenza A, 3/20 (15%)
CFX96™ Presumptive Positive results: SARS-CoV-2, 2/20 (10%); Influenza A, 7/20 (35%); Influenza B, 5/20 (25%); RSV, 3/20 (15%)

c. LoD Using Analytik Jena PCR Systems and QuantStudio™ Dx 96 real-time PCR

To expand the use of the Respiratory SARS-CoV-2 RT-PCR Panel 1 Kit for use with the Analytik Jena qTower^3/qTower^3 G and qTower^3 84/qTower^3 84 G PCR Systems and QuantStudio™ Dx 96 real-time PCR instrument, a validation study was conducted using influenza A (A/Georgia/T51700/2012 (H1N1)pdm09), influenza B (B/Brisbane/60/2008 (Victoria Lineage), SARS-CoV-2 (USA-WA1/2020, heat-inactivated) and RSV (A2001/3-12) cultures. Testing on the original Applied Biosystems QuantStudio™ Dx 384 System was included in this study for comparison. The LoD for each targeted analyte was confirmed to be within 3-fold difference on all three instruments compared to the QuantStudio™ Dx 384. Results are summarized below.

Table: LoD verification on alternate Analytik Jena PCR platforms and QuantStudio™ Dx 96.

Instrument	**Virus**	**N(Hit)**	**Hit rate ≥ 95% [$TCID_{50}$/mL]**	**Hit rate ≥ 95% Mean Ct**	**Ct Std Dev**
QuantStudio™ Dx 384 (reference)	Influenza A	19/20	3.33	35.73	0.87
	Influenza B	19/20	0.33	36.37	0.95
	SARS-CoV-2	20/20	0.11*	36.87	0.93
	RSV	20/20	3.33	37.92	1.61
QuantStudio™ Dx 96	Influenza A	20/20	3.33	36.65	0.67
	Influenza B	19/20	0.11	36.29	0.64
	SARS-CoV-2	19/20	0.11	36.47	0.57
	RSV	19/20	3.33	37.62	1.14
qTower^3 G	Influenza A	19/20	3.33	37.94	0.63
	Influenza B	20/20	0.11	37.45	0.69
	SARS-CoV-2	20/20	0.11	36.67	0.49
	RSV	20/20	3.33	38.22	1.12
qTower^3 84 G	Influenza A	20/20	6.66	36.74	0.69
	Influenza B	20/20	0.33	36.64	0.89

	SARS-CoV-2	20/20	0.11	36.52	1.05
	RSV	20/20	9.99	36.50	1.52

* 0.11 $TCID_{50}$/mL of SARS-CoV-2 is around 258 copies/mL per supplier's CoA
Ct Std Dev; Ct Standard Deviation
QuantStudio™ Dx 384 1X LoD Presumptive Positive: SARS-CoV-2, 8/20 (40%); Influenza B, 4/20 (20%).
QuantStudio™ Dx 96 1X LoD Presumptive Positive: SARS-CoV-2, 1/20 (5%); Influenza A, 4/20 (20%). Influenza B, 2/20 (20%), RSV 12/20 (60%).
qTower 96 1X LoD Presumptive Positive: SARS-CoV-2, 5/20 (25%); Influenza A, 18/20 (90%). Influenza B, 15/20 (75%), RSV 17/20 (85%).
qTower 384 1XLoD Presumptive Positive: SARS-CoV-2, 5/20 (25%); Influenza A, 9/20 (45%). Influenza B, 6/20 (30%), RSV 3/20 (15%).

Equivalency Between Negative Clinical Matrix and Viral Transport Medium Plus Human Cells

The purpose of this study was to show that the simulated clinical matrix (viral transport medium plus human cells) was equivalent to the negative pooled clinical nasopharyngeal swab (NP) matrix when testing SARS-CoV-2, Influenza A, Influenza B, and RSV with the Respiratory SARS-CoV-2 RT-PCR Panel 1.

The viruses used in the LoD studies were diluted in a simulated clinical matrix and negative pooled clinical NP swab matrix to 3X LOD. Prepared samples underwent the chemagic extraction kit and PCR process (to minimize the run-to-run variations). Each condition was tested with 6 replicates and the study was performed on the QuantStudio™ Dx 384 and CFX96™. Results are summarized in the following table and demonstrated that the reported LoD values established in simulated matrix were reproduced in natural clinical matrix.

Table: Matrix equivalency study on the QuantStudio™ Dx 384 and CFX96™

Instrument	Sample	Pooled NP swab (Clinical)			VTM + human cells		
		Mean Ct	Std Dev	N	Mean Ct	Std Dev	N
QuantStudio™ Dx 384	Influenza A H1N1	35.14	0.36	6	36.14	1.49	6
	Influenza B Victoria	33.81	0.14	6	34.72	0.71	6
	RSV	37.36	0.57	6	37.37	1.49	6
	SARS-CoV-2	33.25	0.21	6	35.30	1.08	6
CFX96™	Influenza A H1N1	36.73	0.45	6	37.44	0.54	6
	Influenza B Victoria	35.60	0.14	6	36.60	0.81	6
	RSV	37.65	0.43	6	38.16	1.87	6
	SARS-CoV-2	34.16	0.12	6	35.78	0.59	6

Note: Lot 1 was used in this study. 3X LoD was based on Lot 1 single-formulation LoD.
Std Dev: Ct Standard Deviation
QuantStudio™ 384 Presumptive Positive results:

- Simulated Matrix: Influenza A, 2/6 (33.3%); RSV,4/6 (66.7%)
- Pooled NP Swab Matrix: RSV, 4/6 (66.7%)

CFX96™ Presumptive Positive results:

- Simulated Matrix: Influenza A, 1/6 (16.7%); Influenza B, 1/6 (16.7%); RSV, 4/6 (66.7%)
- Pooled NP swab Matrix: Influenza A, 5/6 (83.3%), RSV 6/6 (100%)

Co-infection Sensitivity (competitive interference)

To evaluate the co-infection sensitivity (competitive interference) of the Respiratory SARS-CoV-2 RT-PCR Panel 1, testing was completed with inactivated influenza A (A/Georgia/T51700/2012 (H1N1)pdm09), influenza B (B/Brisbane/60/2008 (Victoria Lineage), SARS-CoV-2 (USA-WA1/2020, heat-inactivated), and RSV (A2001/3-12), the same virus strains that were used in the LoD study.

Samples were co-spiked samples in simulated clinical matrix (viral transport medium plus human A549 cells) that contained one interference virus at high concentration (>1E6 $TCID_{50}$/mL or > 1E6 copies/mL or > 3.5E5X LoD) and the other three targets at low concentrations (defined as ~3X LoD single-formulation). Three replicates were tested for each condition. The study was performed on QuantStudio™ Dx 384 with lot 2. Results from study #2 are summarized in the following table and showed that no competitive interference was observed in the evaluated conditions.

Table: Co-infection Summary on QuantStudio™ Dx 384

Condition	SARS-CoV-2	Flu A	Flu B	RSV	SARS-CoV-2		Influenza A		Influenza B			RSV
	[$TCID_{50}$/mL] / [Copies /mL]	[$TCID_{50}$/mL]/[xLoD]			Hit Rate	Mean Ct	Hit Rate	Mean Ct	Hit Rate	Mean Ct	Hit Rate	Mean Ct
SARS-CoV-2 interference	660/1.54E6	9.99	2.97	29.97	3/3	23.65	3/3	35.21	3/3	32.98	3/3	34.23
Flu A H1N1 interference	0.33	1.2E6 /3.63 E5	2.97	29.97	3/3	35.46	3/3	17.62	3/3	32.24	3/3	32.62
Flu B Victoria interference	0.33	9.99	1.2E5 /3.63 E5	29.97	3/3	35.03	3/3	36.13	3/3	17.55	3/3	33.83
RSV interference	0.33	9.99	2.97	1.2E6 /3.63 E5	3/3	34.67	3/3	35.46	3/3	33.04	3/3	15.73

Based on Lot 2 single-formulation LoD.
Interference virus and corresponding hit rates/mean Ct: highlighted in grey.
The copies/mL is based on the ddPCR quantification information provided from supplier of the strains (BEI).
Presumptive Positive results: None.

Analytical Reactivity (Inclusivity)

In silico analysis of SARS-CoV-2 inclusivity:

The inclusivity of the SARS-CoV-2 primers/probes of the Respiratory SARS-CoV-2 RT-PCR Panel 1 was evaluated *in silico* using sequences from NCBI and GISAID databases. The most recent evaluation was in August 2021.

Three filters were applied on the database browsers before downloading the sequences in order to avoid a misleading analysis. The filter definitions included the following:

- Complete sequences (full length): Genomes with >29,000bp

- High quality inclusion: Entries with <1% Ns (unknown sequences) and <0.05% unique amino acid mutations (not seen in other sequences in the database) and no indels unless verified by the submitter
- Low coverage exclusion: Excludes entries with >5% Ns

Primer sequence mutations that were predicted to impact gene detection included the following: (1) primer sequence has at least one non-ambiguous nucleotide mismatch to the genome in the last three base pairs from the primer's 3′ end, or (2) primer sequence has no match to the genome. Mutations in the probe sequence that were predicted to impact detection include the following: (1) probe sequence has greater than two non-ambiguous nucleotide mismatches to the genome, or (2) probe sequence has no match to the genome.

More than 2 million sequences in GISAID and 423,000 sequences in NCBI met the criteria for inclusivity analysis (i.e., "complete," "high quality inclusion," "low coverage exclusion"). The primers/probe for the ORF1ab region were 100% homologous approximately 99% of the sequences. For the N gene probe, more than 99% of the analyzed sequences were 100% homologous; however, for the N gene forward primer, > 76% of sequences exhibited one or more mismatches. The main reason for the reduction in overall homology between the N gene forward primer sequence with strains from the NCBI and GISAID databases is a recent emergence of circulating SARS-CoV-2 strains with a three base pair substitution at the 5′ end of the N forward primer (GGG -> AAC). A summary of the findings is shown in the table below. Also included in the table below is the number of sequences that were predicted to be impacted by each of the mutations identified.

Table: *In silico* Analysis of SARS-CoV-2 Assay Oligonucleotides as of August 2021

Target	N gene						ORF1ab gene					
Database	NCBI			GISAID			NCBI			GISAID		
Oligo	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe
Total Sequences	423584	423584	423584	2210505	2210505	2210505	423584	423584	423584	2210505	2210505	2210505
Sequences with mutations	283077	207781	1545	1725972	970951	5912	783	1560	1530	5962	3559	7814
1 mismatch	39978	206722	1481	607514	965531	5706	651	1503	1362	5921	3514	7224
2 mismatches	323	973	2	9518	4853	102	4	10	55	19	12	146
3 or more mismatches	242776	86	62	1108940	567	104	128	47	113	22	33	444
No match to genome^	1718	1638	1596	2649	2391	1672	187	3408	3469	2767	388	655
Sequences predicted to impact (%)*	1922 (0.4)	1851 (0.4)	1596 (0.4)	3839 (0.2)	3374 (0.1)	1684 (0.08)	187 (0.04)	3408 (0.8)	3469 (0.8)	2769 (0.1)	391 (0.02)	673 (0.03)

For: Forward primer, Rev: Reverse primer

Sequence with mutations includes 1 mismatch, 2 mismatches and 3 or more mismatches. It does not include "No match to genome".

^ Sequences that did not align to any region of interest with the BLAST search

* "Sequences predicted to impact" include those that fall under any of the categories below. Contrary to other categories in the table, this count excludes sequences with mismatches due to ambiguous nucleotides:

- The criteria for primer sequences predicted to be impacted are as follows:
  (1) primer sequence has at least one non-ambiguous nucleotide mismatch to the genome in the last three base pairs from the primer's 3′ end, or

(2) primer sequence has no match to the genome.
- The criteria for probe sequences predicted to be impacted are as follows:
  (1) probe sequence has greater than two non-ambiguous nucleotide mismatches to the genome, or
  (2) probe sequence has no match to the genome.

Detection of SARS-CoV-2 in the Respiratory SARS-CoV-2 RT-PCR Panel 1 is based on amplification/detection of either the N or ORF1ab region sequences. Therefore, the identified sequence variants in the 5′ end of the N gene forward primer are not predicted to affect inclusivity for SARS-CoV-2.

In an independent analysis of 8,400 Delta sequences from NCBI and 324,643 Delta sequences from GISAID, detection of the ORF1ab target was not predicted to be impacted by the presence of this specific variant. The N gene forward primer exhibited a single nucleotide mismatch (first base pair at the 5′ end where G->T) to ~98% (8,245/8,400) of the Delta sequences from NCBI and GISAID (319,398/324,643) that were queried but this is not expected to impact detection of the N gene target. The Respiratory SARS-CoV-2 RT-PCR Panel 1 is predicted to detect the majority of circulating SARS-CoV-2 Delta variant strains.

Table: *In silico* Analysis of SARS-CoV-2 Assay Oligonucleotides Against Delta Variant Sequences

Target	N gene						ORF1ab gene					
Database	NCBI			GISAID			NCBI			GISAID		
Oligo	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe
Total Sequences	8400	8400	8400	324643	324643	324643	8400	8400	8400	324643	324643	324643
Sequences with mutations	8337	108	9	324494	2089	334	11	14	26	310	342	1121
1 mismatch	8245	104	4	320074	2054	329	5	9	16	307	342	1078
2 mismatches	75	1	0	4305	23	1	0	0	1	1	0	14
3 or more mismatches	17	3	5	115	12	4	6	5	9	2	0	29
No match to genome^	62	61	55	57	101	72	15	278	286	45	12	30
Sequences predicted to impact (%)*	64 (0.7)	61 (0.7)	55 (0.6)	112 (0.03)	101 (0.03)	72 (0.02)	15 (0.2)	278 (3.3)	286 (3.4)	45 (0.01)	12 (0.004)	30 (0.009)

For: Forward primer, Rev: Reverse primer
Sequence with mutations includes 1 mismatch, 2 mismatches and 3 or more mismatches. It does not include “No match to genome”.
^ Sequences that did not align to any region of interest with the BLAST search
* “Sequences predicted to impact” include those that fall under any of the categories below. Contrary to other categories in the table, this count excludes sequences with mismatches due to ambiguous nucleotides:
- The criteria for primer sequences predicted to be impacted are as follows:
  (1) primer sequence has at least one non-ambiguous nucleotide mismatch to the genome in the last three base pairs from the primer’s 3′ end, or
  (2) primer sequence has no match to the genome.
- The criteria for probe sequences predicted to be impacted are as follows:
  (1) probe sequence has greater than two non-ambiguous nucleotide mismatches to the genome, or
  (2) probe sequence has no match to the genome.

In silico analysis of Influenza A and Influenza B inclusivity:

The inclusivity of the Respiratory SARS-CoV-2 RT-PCR Panel 1 was also evaluated using *in silico* analysis of the primers and probes for Influenza A and

Influenza B in relation to sequences available in the NCBI and GISAID gene databases as of August 2020.

For Influenza A, there were 41,680 and 87,136 sequences in the NCBI and GISAID databases, respectively. Subtype information was further investigated in the GISAID database. Of the available sequences for Influenza A, 3814 (4.38%), 56,970 (65.38%), and 81,810 (93.89%) showed 100% homology to the forward primer, reverse primer, and probe of the target region, respectively. In total, 5025 Influenza A sequences (3.9%) showed 100% homology to all oligos of the target region. Of the remaining 128,158 sequences, oligo binding is predicted for all but 658 Influenza A sequences for an overall inclusivity of 99.49%.

Specifically, for the 87,136 influenza A sequences obtained from GISAID, 85,185 of the analyzed sequences had mismatches. The majority (~98.1%) of the mismatched sequences occurred with subtypes H1N1 (34,964) and H3N2 (48,628). The most frequently occurring mismatch positions among the influenza A forward and reverse primer sequences obtained from GISAID were located at nucleotide position (from the 5′ end) 8 (58%), 9 (42%), and 18 (42%) for the forward primer and at position 6 (4%), 14 (36%), and 18 (2%) for the reverse primer. The primer annealing temperature is 55°C, and the probe hydrolysis and detection temperature is 65°C. The cycling conditions are designed to tolerate one or more mismatches within the primers without losing detection specificity by the probe and therefore, the mismatches in the H1N1 and H3N2 sequences are not predicted to affect the inclusivity of the assay.

For Influenza B, there were 12,408 and 21,493 sequences in the NCBI and GISAID databases, respectively. Of the available Influenza B sequences that included both the Victoria and Yamagata lineages, 30,558 (90.14%) showed 100% homology to all oligos of the target region. Of the remaining 3343 sequences, oligo binding is predicted for all but 591 for an overall inclusivity of 98.26%.

In-silico analysis of RSV inclusivity:

The *in-silico* analysis for the RSV primers/probe of the Respiratory SARS-CoV-2 RT-PCR Panel 1 was completed in December 2020. For RSV, there were 2,555 sequences found in NCBI nucleotide databases that had names containing either “orthopneumovirus,” “respiratory,” “syncytial,” or “rsv” and were a similar size to RSV genomes. There were no RSV data in the GISAID database. Of the available RSV sequences (including subtypes A, B, and unassigned), 2507 (98.12%), 2295 (89.82%), 1159 (45.36%) showed 100% homology to the forward primer, reverse primer, and probe of the target region. In total, 938 (36.71%) showed 100% homology to all oligos of the target region. Of the remaining 1617 sequences, oligo binding is predicted for all but 61 for an overall inclusivity of 97.61% for the

evaluated sequences.

The forward RSV specific primer (RSV-F) demonstrated that 98.12% of the analyzed sequences had 100% homology. The 48 sequences that did not yield 100% homology with the RSV-F primer had one mismatch. Forty-two of the 48 sequences had a mismatch at position 16 of the primer (total primer length of 24). There is no predicted impact on primer binding because the primer melting temperature (Tm) is around 65ºC and the validated PCR run program annealing temperature is 55ºC. For the reverse RSV specific primer, only single mismatches were identified in either position 3 or 11 from the 5′ end of the primer (total primer length is 28 nucleotides). Of the 1396 (54.64%) probe sequences with mismatches, 1363 (97.64%) had a mismatch at the second to last nucleotide of the 3′ end. The RSV probe hydrolysis and detection temperature is 65 ºC. The probe with 1-mismatch at 3′ end has a Tm around 70 ºC, 5°C higher than the hydrolysis/detection temperature. This is not predicted to have an impact on probe binding efficiency or the detection of RSV.

Wet Testing

Two wet testing studies were performed on the QuantStudio™ Dx 384.

a. Inclusivity study 1: 24-panel evaluation

The inclusivity of the Respiratory SARS-CoV-2 RT-PCR Panel 1 was evaluated using 11 influenza A, 5 influenza B, and 8 RSV strains representing temporal, geographic, and genetic diversity within the subtype and lineage. Each panel member was diluted in simulated matrix and extracted using the chemagic™ 360 instrument and tested in triplicate. Results are presented in the following table. The lowest concentration tested that produced positive results for all the three replicates is indicated in the Table below.

Table: Strains used in the 24-panel inclusivity study

No.	Lineage	Strain	Testing Concentration	Unit	Vendor	Cat #	Mean Ct
1	Influenza A (H1N1)	A/Brisbane/59/2007 (H1N1)	8.90E+03	$CEID_{50}$/mL	BEI	NR-12282	31.43
2	Influenza A (H1N1)	A/Denver/1/1957 (H1N1)	1.60E+02	$CEID_{50}$/mL	BEI	NR-4229	34.24
3	Influenza A (H1N2)	A/swine/Ohio/09SW1484E/2009 (H1N2)	1.60E+03	$TCID_{50}$/mL	BEI	NR-36703	33.60
4[a]	Influenza A (H2N2)	A/Japan/305/1957 (H2N2)	3.26E-06	ng/µL	BEI	NR-2775	38.85
5	Influenza A/H3N2	A/Wisconsin/67/2005 (H3N2)	2.80E+02	$CEID_{50}$/mL	BEI	NR-41800	32.00
6	Influenza A (H3N8)	A/equine/Pennsylvania/1/2007 (H3N8)	1.60E-01	$CEID_{50}$/mL	BEI	NR-13426	36.99
7	Influenza A (H5N2)	A/duck/Pennsylvania/10218/1984 (H5N2)	2.55E-03	ng/µL	BEI	NR-2763	36.63
8	Influenza A (H6N2)	A/turkey/Massachusetts/3740/1965 (H6N2)	1.10E+03	EID_{50}/mL	BEI	NR-21654	35.41

9	Influenza A (H7N2)	A/equine/Prague/1/1956 (HA) x A/Aichi/2/1968 (NA) x A/Puerto Rico/8/1934 (H7N2), Reassortant X-32	3.65E-04	ng/µL	BEI	NR-9680	36.98
10	Influenza A (H7N7)	Genomic RNA from Influenza A Virus, A/equine/Prague/1956 (H7N7)	1.50E-03	ng/µL	BEI	NR-35976	36.61
11	Influenza A (H9N2)	A/turkey/Wisconsin/1/1966 (H9N2)	1.60E+03	$CEID_{50}$/mL	BEI	NR-21659	31.03
12	Influenza B	B/Florida/4/2006 (Yamagata Lineage)	1.60E+02	$CEID_{50}$/mL	BEI	NR-41795	32.61
13	B-YAM	Influenza B Virus, B/Texas/06/2011 (Yamagata Lineage)	8.90E+02	$CEID_{50}$/mL	BEI	NR-44024	33.38
14	Influenza B	Influenza B Virus, B/Hong Kong/330/2001 (Victoria Lineage)	1.80E+01	$CEID_{50}$/mL	BEI	NR-41802	30.60
15	Influenza B	B/Malaysia/2506/2004	1.58E+01	EID_{50}/mL	BEI	NR-9723	30.25
16	Influenza B	B/Ohio/01/2005 (Victoria Lineage)	1.60E+03	EID_{50}/mL	BEI	NR-41801	32.30
17	Human respiratory syncytial virus	B1 (NR-4052)	8.90E+00	$TCID_{50}$/mL	BEI	NR-4052	33.60
18	Human respiratory syncytial virus	A2 (NR-12149)	NA	NA	BEI	NR-12149	35.20
19	Human respiratory syncytial virus (A)	A2001/2-20 (NR-28525)	8.90E+00	$TCID_{50}$/mL	BEI	NR-28525	32.74
20	Human respiratory syncytial virus (A)	A1998/12-21 (NR-28528)	2.80E-01	$TCID_{50}$/mL	BEI	NR-28528	35.87
21	Human respiratory syncytial virus (A)	A1998/3-2 (NR-28529)	1.60E-01	$TCID_{50}$/mL	BEI	NR-28529	35.70
22	Human respiratory syncytial virus (B)	B WV/14617/85	10^{-7}	Dilution factor from stock	ATCC	VR-1400	34.49
23	Human respiratory syncytial virus (B)	Strain 18537	10^{-6}	Dilution factor from stock	ATCC	VR-1580	33.30
24	Human respiratory syncytial virus (B)	subgroup B cp23 Clone 1A2	10^{-7}	Dilution factor from stock	ATCC	VR-2579	36.12

[a] Purified RNA. Due to the sample instability with the extraction process, the material was tested directly by PCR.
NA; Concentration not available
Presumptive Positive results: Influenza A (strain #4) 2/3 (66.7%); Influenza A (strain #9,1X LoD) 1/3 (33.3%) RSV (strain #21), 1/3 (33%)

b. Inclusivity study 2: CDC 2020 human influenza virus panel

The 2020-2021 human Influenza annual reactivity panel was used for this study following the CDC protocol. Each of four contemporary influenza A and four influenza B isolates were first serially diluted in VTM with human cells

(simulated clinical matrix) using a 5-fold dilution series. For each concentration, 5 replicates were tested on the QuantStudio™ Dx 384. Testing the dilution series was performed until there was non-reactivity at one to two consecutive five-fold dilution levels, as shown by obtaining zero positive results for all five replicates. The last dilution that produced positive results in at least one out of the five replicates tested is shown in the table below.

Table: CDC panel inclusivity summary showing the lowest concentrations where at least 1/5 replicates were positive

Virus	Strain Designation	EID_{50} titer/mL	Stock (EID_{50}/mL)	Testing (EID_{50}/mL)	Minimum Reactive Concentration (EID_{50}/mL)
Influenza A (H3N2)	A/Perth/16/2009	1E9.3	2.00E+09	5.11E+02	1.02E+02
Influenza A (H3N2)	A/Hong Kong/2671/2019	1E7.5	3.16E+07	8.10E+00	1.62E+00
Influenza A (H1N1)pdm09	A/Christ Church/16/2010	1E10.2	1.58E+10	8.11E+02	1.62E+02
Influenza A (H1N1)pdm09	A/Guandong-Maonan/ 1536/2019	1E9.1	1.26E+09	6.45E+01	1.29E+01
Influenza B (Victoria lineage)	B/Michigan/09/2011	1E6.9	7.94E+06	8.13E-02	3.25E-03
Influenza B (Victoria lineage)	B/Washington/02/2019	1E9.2	7.94E+09	3.25E+00	1.30E-01
Influenza B (Yamagata lineage)	B/Texas/81/2016	1E8.3	2.00E+08	2.04E+00	8.17E-02
Influenza B (Yamagata lineage)	B/Phuket/3073/2013	1E9.9	1.58E+09	1.63E+01	1.30E-01

Testing concentration is the lowest concentration with 5/5 replicates positive.
Presumptive Positive results:

- Influenza A (A/Perth/16/2009); 3/5 (60%)
- Influenza A (A/Hong Kong/2671/2019 (H3N2); 1/5 (20%)
- Influenza A (A/Christ Church/16/2010 (H1N1 pdm09); 4/5 (80%)
- Influenza A (A/Guandong-Maonan/ 1536/2019); 4/5 (80%)
- Influenza B (B/Michigan/09/2011); 2/5 (40%)
- Influenza B (B/Washington/02/2019); 1/5 (20%)
- Influenza B (B/Texas/81/2016); 1/5 (20%)
- Influenza B (B/Phuket/3073/2013); 1/5 (20%)

Influenza A strain, Hong Kong and Influenza B strain, Washington were only tested at one level below the minimum reactive concentration (the lowest concentration at which ⩾1 replicate was reported positive).
Influenza B strain, Phuket was not tested at any level below the minimum reactive concentration.

In summary, the results from inclusivity wet testing 1 and 2 demonstrated that the Respiratory SARS-CoV-2 RT-PCR Panel 1 can detect multiple clinically relevant strains of influenza A, influenza B and RSV, including the strains from the 2020-2021 CDC human influenza panel.

Analytical Specificity (Cross-Reactivity)

Cross-reactivity of the Respiratory SARS-CoV-2 RT-PCR Panel 1 was evaluated using both *in silico* analysis and wet testing against normal and pathogenic

organisms that could be found in the respiratory tract.

In silico evaluation of cross-reactivity

The oligonucleotides for amplification/detection of influenza A, influenza B, and RSV within the Respiratory SARS-CoV-2 RT-PCR Panel 1 are similar to the US CDC design with the addition of degenerate bases to enhance inclusivity. The oligonucleotides for amplification/detection of SARS-CoV-2 within the Respiratory SARS-CoV-2 RT-PCR Panel 1 are the same as those used in the New Coronavirus Nucleic Acid Detection Kit (EUA200055) and were evaluated previously. The sequence alignment of each degenerate oligonucleotide for influenza A, influenza B, and RSV against specific respiratory pathogens is shown in the following table. One representative organism sequence for each pathogen was chosen to map the influenza A, influenza B, and RSV primers/probes against. The match and mismatch scores were 1 and -3, respectively.

- The penalty to create and extend a gap in an alignment was 5 and 2, respectively.
- The search parameters automatically adjusted for short input sequences and the expected threshold was 1000.

In summary, there are no cross-reactivity risks identified for the pathogenic organisms listed in the following table with the oligos for influenza A, influenza B and RSV. The Influenza B reverse primer showed 85% homology to *Candida albicans*. However, there is poor alignment with the Influenza B forward primer and probe. Therefore, the likelihood of the Respiratory SARS-CoV-2 RT-PCR Panel 1 exhibiting cross-reactivity to *Candida albicans* is low. The absence of cross-reactivity to *Candida albicans* was further confirmed via wet testing.

Table: *In silico* cross-reactivity analysis of primers and probes of influenza A, influenza B and RSV

Pathogen	Acc #	% Homology								
		FluA -F	FluA -R	FluA -P	FluB -F	FluB -R	FluB -P	RSV -F	RSV -R	RSV -P
Human coronavirus 229E	NC_002645.1	43.2	47.9	37.5	54.6	52.4	40.7	41.7	41.1	37.0
Human coronavirus OC43	NC_006213.1	40.9	40.6	33.3	59.1	52.4	48.2	41.7	33.9	33.3
Human coronavirus HKU1	NC_006577.2	38.6	37.5	37.5	36.4	52.4	44.4	45.8	35.7	40.7
Human coronavirus NL63	NC_005831.2	38.6	40.6	33.3	36.4	47.6	37.0	37.5	37.5	29.6
SARS-coronavirus	NC_004718.3	40.9	52.1	50.0	45.5	57.1	48.2	41.7	35.7	37.0
MERS-coronavirus	MG987421.1	54.6	43.8	37.5	40.9	47.6	44.4	41.7	35.7	33.3
Adenovirus (e.g. C1 Ad. 71)	J01917.1	40.9	44.8	37.5	40.9	42.9	37.0	50.0	32.1	37.0

Human Metapneumovirus (hMPV)	NC_039199.1	36.4	35.4	33.3	40.9	47.6	40.7	41.7	30.4	44.4
Parainfluenza virus 1 (Human respirovirus 1)	MK167043.1	40.9	37.5	33.3	40.9	42.9	33.3	41.7	35.7	37.0
Parainfluenza virus 2 (Human rubulavirus 2)	MN369034.1	36.4	38.5	37.5	36.4	52.4	33.3	50.0	30.4	33.3
Parainfluenza virus 3 (Human respirovirus 3)	MH330335.1	43.2	39.6	33.3	36.4	42.9	29.6	45.8	46.4	44.4
Parainfluenza virus 4a (Human rubulavirus 4a)	NC_021928.1	45.5	44.8	29.2	45.5	38.1	48.2	54.2	33.9	33.3
Influenza A	NC_007373.1, NC_007372.1, NC_007371.1, NC_007366.1, NC_007369.1, NC_007368.1, NC_007367.1, NC_007370.1	93.2	93.8	100.0	36.4	52.4	29.6	37.5	53.6	33.3
Influenza B	NC_002205.1, NC_002206.1, NC_002207.1, NC_002208.1, NC_002209.1, NC_002210.1, NC_002211.1, NC_002204.1	40.9	37.5	33.3	100.0	100.0	100.0	50.0	37.5	37.0
Enterovirus (e.g. EV68)	NC_001472.1	38.6	44.8	41.7	40.9	42.9	37.0	33.3	32.1	29.6
Respiratory syncytial virus	NC_001803.1	36.4	39.6	33.3	45.5	42.9	33.3	100.0	92.9	92.6
Rhinovirus	NC_038311.1	38.6	41.7	37.5	54.6	52.4	33.3	37.5	42.9	33.3
Chlamydia pneumonia	NC_000922.1	50.0	50.0	54.2	68.2	57.1	48.2	58.3	57.1	44.4
Haemophilus influenzae	NC_000907.1	56.8	54.2	45.8	54.6	61.9	48.2	54.2	44.6	44.4
Legionella pneumophila	NC_002942.5	63.6	61.5	58.3	54.6	61.9	44.4	58.3	50.0	44.4
Mycobacterium tuberculosis	NC_000962.3	56.8	60.4	66.7	59.1	61.9	59.3	50.0	50.0	48.2
Streptococcus pneumoniae	NC_003098.1	59.1	59.4	50.0	50.0	61.9	48.2	54.2	50.0	48.2
Streptococcus pyogenes	NC_002737.2	56.8	54.2	45.8	68.2	61.9	51.9	54.2	53.6	70.4
Bordetella pertussis	NC_002929.2	54.5	62.5	50.0	54.6	76.2	48.2	45.8	41.1	48.2
Mycoplasma pneumoniae	NC_000912.1	50.0	53.1	45.8	54.6	57.1	40.7	50.0	46.4	59.3

Pneumocystis jirovecii	NW_01726477 5.1	54.6	56.3	45.8	63.6	57.1	48.2	62.5	51.8	48.2
Candida albicans	NC_03 2089.1	56.8	62.5	50.0	63.6	85.7	48.2	66.7	53.6	48.2
Pseudomonas aeruginosa	NC_00 2516.2	63.6	50.0	58.3	59.1	66.7	51.9	54.2	44.6	59.3

Acc #; GenBank Accession #
F: Forward primer; R: Reverse primer; P: Probe.

Cross-Reactivity Wet Testing

Wet testing against normal and pathogenic organisms of the respiratory tract was performed to confirm the results of the *in-silico* analysis. Each organism identified in the table below was tested in triplicate with the Respiratory SARS-CoV-2 RT-PCR Panel 1 at the concentrations indicated. The testing was performed on the Applied Biosystems™ QuantStudio™ Dx 384. These organisms were tested in the absence of on-panel targeted analytes. High titer stocks of microorganisms were spiked into negative simulated clinical matrix to a concentration level of at least 1.0E+05 units/mL for viruses and 1.0E+06 units/mL for other microorganisms (i.e., bacteria and fungi), unless otherwise noted. All results for assay targets were negative but generated a positive RNase P signal in the VIC channel as expected.

Table: Organisms tested for cross-reactivity with the Respiratory SARS-CoV-2 RT-PCR Panel 1 (Absence of On-Panel Targets)

Name/Description	Testing concentration	Evaluation Unit	Vendor	Cat #
Human coronavirus 229E	1.60E+05	$TCID_{50}$/mL	BEI	NR-52726
Human coronavirus OC43 (gRNA)	6.34E+08	copies/mL	ATCC	VR-1558D
Human coronavirus HKU1 (gRNA)	5.40E+07	copies/mL	ATCC	VR-3262SD
Human coronavirus NL63	1.60E+04[a]	$TCID_{50}$/mL	BEI	NR-470
SARS-coronavirus	N/A (10-fold dilution of Stock)*	N/A	ZeptoMetrix	NATSARS-ST
MERS-coronavirus	8.90E+04[a]	$TCID_{50}$/mL	BEI	NR-50549
Adenovirus (e.g. C1 Ad. 71)	1.25E+06	$TCID_{50}$/mL	BEI	NR-51436
Human Metapneumovirus (hMPV) (gRNA)	1.82E+08	copies/mL	BEI	NR-49122
Human parainfluenza virus 1 RNA	2.48E+08	copies/mL	ATCC	VR-94D
Human parainfluenza virus 2	5.00E+06	$TCID_{50}$/mL	BEI	NR-3229
Human parainfluenza virus 3 (gRNA)	2.43E+07	copies/mL	ATCC	VR-93D
Human parainfluenza virus 4b	2.50E+05	$TCID_{50}$/mL	BEI	NR-3238
Enterovirus (EV71) RNA	1.46E+09	copies/mL	BEI	NR-4960
Rhinovirus	1.00E+05	$TCID_{50}$/mL	BEI	NR-51453
Haemophilus influenzae gDNA	4.20E+06	copies/mL	ATCC	51907DQ
Legionella pneumophila gDNA	3.27E+07	copies/mL	ATCC	33152D-5
Mycobacterium tuberculosis gDNA	6.78E+08	copies/mL	BEI	NR-14867
Streptococcus pneumoniae gDNA	8.70E+07	copies/mL	ATCC	6308D-5
Streptococcus pyogenes gDNA	6.18E+07	copies/mL	ATCC	BAA-572D-5
Bordetella pertussis gDNA	2.70E+07	copies/mL	ATCC	9797D-5
Mycoplasma pneumoniae gDNA	1.24E+07	copies/mL	ATCC	15531D
Pneumocystis jirovecii (PJP)	N/A (5-fold dilution of Stock)*	N/A	ZeptoMetrix	NATPJI-ERC
Candida albicans gDNA	1.62E+07	copies/mL	BEI	NR-50361
Pseudomonas aeruginosa gDNA	2.33E+07	copies/mL	ATCC	9027D-5
Staphylococcus epidermis gDNA	4.50E+07	copies/mL	ATCC	12228D-5
Streptococcus salivarius gDNA	2.20E+07	copies/mL	BEI	HM-121D

Herpes Simplex virus	1.25E+05	copies/mL	Thermo/ AcroMetrix	954501
Varicella-zoster virus	2.5E+04[a]	copies/mL	Thermo/ AcroMetrix	954512
Epstein Barr virus gDNA	1.01E+09	copies/mL	ATCC	CRL-5957D
Measles Virus gDNA	6.60E+08	copies/mL	BEI	NR-44104
Mumps virus	1.60E+06	$TCID_{50}$/mL	BEI	NR-3846
Cytomegalovirus	5.00E+04[a]	IU/mL	WHO	09/162
Corynebacterium diphtheriae gDNA	9.34E+07	copies/mL	ATCC	700971D-5
Escherichia coli	9.06E+07	copies/mL	BEI	NR-9281
Lactobacillus plantarum	3.16E+06	cfu/mL	ATCC	BAA-793
Moraxella catarrhalis gDNA	2.21E+08	copies/mL	ATCC	25240D-5
Staphylococcus aureus gDNA	1.13E+08	copies/mL	BEI	NR-10320
Neisseria elongata	N/A (20-fold dilution of Stock) *	N/A	ATCC	25295
Neisseria meningitidis gDNA	2.53E+08	copies/mL	BEI	NR-48806

The copy number was calculated based on concentration (ng) and genome size.
[a] Human coronavirus NL63, Varicella-zoster virus, and cytomegalovirus were spiked at a lower concentration than the other organisms, since samples at a higher concentration were not available
*Stock concentration was used directly for spiking into simulated clinical matrix.

Cross-Reactivity (non-targeted influenza types, coronavirus types and RSV)

Cross-reactivity of each primer probe set to viruses targeted by another component of the Respiratory SARS-CoV-2 RT-PCR Panel 1 was also evaluated as part of the cross-reactivity study. The panel of viruses provided by CDC (five replicates) along with one RSV and one SARS-CoV-2 strain (triplicate) were wet tested at high titers as single template input. The performance was evaluated on both the Applied Biosystems™ QuantStudio™ Dx 384 and the Bio-Rad CFX96™.

For each evaluated primer/probe set, positive results were only obtained on the corresponding optical channel, with negative results for the other targets. For example, the SARS-CoV-2 primer and probe set generated negative results in the presence of high concentrations of influenza A, influenza B, and RSV viruses. No cross-reactivity risk was identified. Results are summarized in the following table.

Table: Applied Biosystems™ QuantStudio™ Dx 384 summary

Virus	Strain Designation	Testing Concentration (ID_{50}/mL)	Target Ct			Non-target Ct		
			Mean Ct	SD	N	Mean Ct	SD	N
Influenza A (H3N2)	A/Perth/16/2009	3.99E+07	16.64	0.11	5	ND	ND	ND
Influenza A (H3N2)	A/Hong Kong/2671/2019	6.32E+05	19.05	0.42	5	ND	ND	ND
Influenza A (H1N1)pdm09	A/Christ Church/16/2010	3.17E+08	15.63	0.16	5	ND	ND	ND
Influenza A (H1N1)pdm09	A/Guandong-Maonan/1536/2019	2.52E+07	16.93	0.78	5	ND	ND	ND
Influenza B (Victoria lineage)	B/Michigan/09/2011	1.59E+05	15.87	0.1	5	ND	ND	ND
Influenza B (Victoria lineage)	B/Washington/02/2019	3.17E+07	14.94	0.16	5	ND	ND	ND
Influenza B (Yamagata lineage)	B/Texas/81/2016	3.99E+06	15.32	0.09	5	ND	ND	ND
Influenza B (Yamagata lineage)	B/Phuket/3073/2013	1.59E+08	15.27	0.16	5	ND	ND	ND

Human respiratory syncytial virus	A2001/3-12	3.20E+06	15.81	0.04	3	ND	ND	ND
SARS-coronavirus-2	Isolate USA-WA1/2020, Heat Inactivated	3.20E+03	20.87	0.73	3	ND	ND	ND

ND: Not detected. For example, for influenza A input, the target Ct values were from the results of ROX, the non-target Ct values were from the results of FAM, Cy5 and Cy5.5.
N; number of replicates
SD; Ct Standard Deviation
Presumptive Positive results: None.

Table: Bio-Rad CFX 96™ summary

Virus	Strain Designation	Testing Concentration (ID_{50}/mL)	Target Ct			Non-target Ct		
			Mean	SD	N	Mean	SD	N
Influenza A (H3N2)	A/Perth/16/2009	3.99E+07	18.86	0.26	5	ND	ND	ND
Influenza A (H3N2)	A/Hong Kong/2671/2019	6.32E+05	20.89	0.31	5	ND	ND	ND
Influenza A (H1N1)pdm09	A/Christ Church/16/2010	3.17E+08	16.87	0.20	5	ND	ND	ND
Influenza A (H1N1)pdm09	A/Guandong-Maonan/1536/2019	2.52E+07	18.03	0.64	5	ND	ND	ND
Influenza B (Victoria lineage)	B/Michigan/09/2011	1.59E+05	17.02	0.09	5	ND	ND	ND
Influenza B (Victoria lineage)	B/Washington/02/2019	3.17E+07	15.09	0.41	5	ND	ND	ND
Influenza B (Yamagata lineage)	B/Texas/81/2016	3.99E+06	15.93	0.17	5	ND	ND	ND
Influenza B (Yamagata lineage)	B/Phuket/3073/2013	1.59E+08	15.59	0.35	5	ND	ND	ND
Human respiratory syncytial virus	A2001/3-12	3.20E+06	17.11	1.1	3	17.11	1.1	3
SARS-coronavirus-2	Isolate USA-WA1/2020, Heat Inactivated	3.20E+03	22.19	0.96	3	ND	ND	ND

ND; Not detected. For example, for influenza A input, the target Ct values were from the results of ROX, the non-target Ct values were from the results of FAM, Cy5 and Cy5.5.
N; number of replicates
SD; Ct Standard Deviation
Presumptive Positive results: None.

Microbial Interference Study

An additional cross-reactivity/microbial interference study was performed in the presence of high titer non-panel respiratory pathogens to determine whether the pathogens interfered with the detection of the on-panel targets of the Respiratory SARS-CoV-2 RT-PCR Panel 1 (SARS-CoV-2, Influenza A, Influenza B, and RSV). The non-panel organisms were selected because they are considered the most common microbes associated with respiratory infection based on a review of 510(k) cleared respiratory panels.

The study was performed on the QuantStudio™ Dx 384. A four-plex co-spiked mixture of inactivated cultured viruses (influenza A (A/Georgia/T51700/2012 (H1N1)pdm09), influenza B (B/Brisbane/60/2008 (Victoria Lineage), SARS-CoV-2 (USA-WA1/2020, heat-inactivated) and/or RSV (A2001/3-12)) at ~3X LoD (based on single-formulation, Lot #1) in simulated clinical matrix was used as positive

template (panel targets) into which each of the high titer non-panel organisms as spiked at the listed concentration. Each condition was tested in triplicate. *Staphylococcus aureus* at high concentration (>1E8 copies/mL) showed interference with RSV detection (2/3 RSV replicates were detected). None of the other potentially interfering organisms inhibited detection of any panel targets at the concentrations tested. The results are summarized in the following table.

Table: Microbial interference study summary

Organism	Testing concentration	Unit	SARS-CoV-2		Influenza A		Influenza B		RSV	
			Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD
Human coronavirus 229E	1.60E+05	$TCID_{50}$/mL	35.30	0.68	36.78	0.47	34.63	0.08	36.30	0.58
Adenovirus (e.g. C1 Ad. 71)	1.25E+06	$TCID_{50}$/mL	35.49	0.43	36.13	0.16	35.02	0.27	31.18	3.35
Enterovirus (e.g. EV68)	1.46E+09	Copies/mL	35.35	0.30	36.54	0.73	34.47	0.28	36.28	0.53
Haemophilus influenzae	4.20E+06	Copies/mL	36.05	0.63	36.35	0.84	34.40	0.27	35.58	1.20
Streptococcus pneumoniae	8.70E+07	Copies/mL	36.28	0.45	35.96	0.76	34.01	0.41	35.08	2.20
Bordetella pertussis	2.70E+07	Copies/mL	36.17	0.20	35.82	0.69	34.30	0.43	36.73	2.23
Candida albicans	1.62E+07	Copies/mL	35.96	0.37	36.83	1.60	34.70	0.43	36.09	1.95
Herpes Simplex virus	1.25E+05	Copies/mL	35.60	0.32	36.30	0.40	33.73	0.09	35.75	0.98
Cytomegalovirus	5.00E+04	IU/mL	35.62	0.89	36.96	0.46	34.66	0.14	37.21	1.13
Corynebacterium diphtheriae	9.34E+07	Copies/mL	36.34	1.20	37.92	0.46	34.86	0.50	35.59	0.52
Staphylococcus aureus (1)	1.13E+08	Copies/mL	36.97	0.83	36.50	0.62	34.61	0.50	36.24	1.70
Neisseria meningitidis	2.53E+08	Copies/mL	35.94	0.49	35.96	1.32	34.38	0.22	34.29	2.26

(1) *S. aureus* interfered with detection of RSV in 2/3 replicates
Lot 1 was used in this study. 3X LoD is based on Lot 1 single-formulation LoD.
SD; Ct Standard Deviation
Presumptive Positive results stratified by co-infection:
- *Corynebacterium diphtheriae*/SARS-CoV-2; 1/3 (33.3%)
- *Staphylococcus aureus*/SARS-CoV-2; 2/3 (66.7%)
- Human coronavirus 229E/Influenza A; 1/3 (33.3%)
- Enterovirus/Influenza A; 1/3 (33.3%)
- *Haemophilus influenzae*/Influenza A; 1/3 (33.3%)
- *Candida albicans*/Influenza A; 2/3 (66.7%)
- CMV/Influenza A; 2/3 (66.7%)
- *Corynebacterium diphtheriae*/Influenza A; 3/3 (100%)
- *Staphylococcus aureus*/Influenza A; 1/3 (33.3%)
- *Neisseria meningitidis*/Influenza A; 1/3 (33.3%)
- *Corynebacterium diphtheriae*/RSV; 1/3 (33.3%)
- *Staphylococcus aureus*/RSV; 1/3 (33.3%)

Interfering Substances Study

An interfering substances study was performed to determine if common substances that could be present in respiratory samples would impact performance of the Respiratory SARS-CoV-2 RT-PCR Panel 1. The following potential interfering substances listed in the table were evaluated in the absence

(negative samples) or presence (positive samples) of assay targets on the QuantStudio™ Dx 384. All exogenous/endogenous substances were tested at the highest medically relevant concentration (worst case). A four-plex co-spiked mixture of inactivated cultured viruses at 3X LoD (based on single-formulation LoD of lot #1) in simulated clinical matrix was used as positive contrived samples. Prepared samples with each interfering substance were extracted with the chemagic™ system and three replicates were tested using the QuantStudio™ Dx 384.

Table: Substances tested for interference with the Respiratory SARS-CoV-2 RT-PCR Panel 1

Substances	Testing Concentration
Oxymetazoline	15% v/v
Blood	0.5% v/v
Benzocaine, Menthol	3.5 mg/mL
Fluticasone[a]	5% v/v
Menthol	8 mg/mL
Triamcinolone	5% v/v
Phenylephrine hydrochloride	5% v/v
Saline (sodium chloride)	15% v/v
Galphimia glauca, Luffa operculata, Sabadilla	5% v/v
Mucin protein	60 µg/mL
Menthol, thymol, methyl salicylate, and eucalyptol	5% v/v
Budesonide (Glucocorticoid)[b]	1% v/v
Oseltamivir[a]	2.5 mg/mL
Tobramycin	4.0 µg/mL
Zanamivir	3.3 mg/mL
Mupirocin[a]	10 mg/mL
Peramivir	45 ng/mL
FluMist[c]	20% v/v

[a,b,c] Interference was identified.
Note: Blood, FluMist and peramivir were evaluated with single-plex template, triplicate for each condition.

All substances tested in the absence of targeted on-panel analytes except FluMIst generated the expected negative results for SARS-CoV-2, Influenza A, Influenza B, and RSV and positive results for RNase P for all three replicates evaluated. FluMist contains attenuated strains of influenza A and B viruses and therefore, as expected, produced positive results for both these targets. When substances were tested in the presence of positive contrived specimens, all three replicates for each assay target were detected with the following exceptions:

- Interference was observed at 3X LoD for RSV (2/3 replicates detected) when tested with Fluticasone, Oseltamivir, and Mupirocin. All other targets (Influenza A, Influenza B, SARS-CoV-2, and RNase P) were detected in 3/3 replicates.
- Interference was observed at 3X LoD for all assay targets including SARS-

CoV-2, Influenza A, Influenza B, and RSV (2/3 replicates detected) when tested with Budesonide. RNase P was detected in 3/3 replicates.

- Interference was observed at 3X LoD for RSV (0/3 replicates detected) when tested with FluMist. The SARS-CoV-2 and RNase P targets were detected in 3/3 replicates.

Table: Substances tested in the presence of all 4 analytes

Item ID in database	Substance	Mean Ct Value at 3X LoD (4-Plex Mixture of Assay Analytes)				
		SARS-CoV-2	Influenza A H1N1	Influenza B Victoria	RSV	RNase P
1	Oxymetazoline	34.51	33.66	35.91	36.53	31.43
3	Benzocaine, Menthol	34.75	35.95	35.22	37.49	29.70
4	Fluticasone[a]	34.83	34.63	36.24	37.27	32.52
5	Menthol	35.42	33.30	35.26	36.96	31.35
6	Triamcinolone	35.68	34.80	37.70	38.11	32.36
7	Phenylephrine hydrochloride	35.49	33.31	35.62	38.52	31.22
8	Saline (sodium chloride)	34.59	33.13	34.69	35.94	30.68
9	Galphimia glauca, Luffa operculata, Sabadilla	34.90	33.15	35.71	36.09	30.98
10	Mucin protein	34.88	33.46	37.01	35.93	31.60
11	Menthol, thymol, methyl salicylate, and eucalyptol	33.17	32.29	34.89	36.33	30.26
13	Budesonide (Glucocorticoid)[b]	36.89	38.58	37.58	38.39	32.22
14	Oseltamivir[a]	34.99	35.77	35.88	39.34	29.87
15	Tobramycin	34.13	32.88	35.35	38.07	30.28
16	Zanamivir	34.84	33.24	35.87	36.49	31.26
17	Mupirocin[a]	35.23	33.48	35.35	37.32	32.50
18	Peramivir[c]	34.32	34.76	35.42	35.36	28.09[d]
2	Blood[c]	34.37	34.56	35.03	35.90	27.06[d]
22	FluMist Quadrivalent Positive sample[c]	36.08	11.88	12.19	ND	30.34
22	FluMist Quadrivalent Negative sample[c]	ND	11.89	12.20	ND	30.96

[a,b,c] Interference was identified.
[c] single-plex template
ND: Not detected.

Presumptive Positive results:

- SARS-CoV-2
  - Budesonide (Glucocorticoid) 1/3 (33.3%)
- Influenza A
  - Budesonide (Glucocorticoid) 2/3 (66.7%)
- Influenza B
  - Budesonide (Glucocorticoid) 2/3 (66.7%); Triamcinolone 3/3 (100%), Galphimia glauca 1/3 (33.3%); Mucin protein 2/3 (66.7%)
- RSV:
  - Menthol 1/3 (33.3%); Saline (sodium chloride) 1/3 (33.3%); Galphimia glauca 1/3 (33.3%); Menthol, thymol, methyl salicylate, and eucalyptol 1/3 (33.3%); Tobramycin/RVS 1/3 (33.3%); Zanamivir 1/3 (33.3%); Benzocaine, Menthol 2/3 (66.7%); Triamcinolone 2/3 (66.7%);

Phenylephrine hydrochloride 2/3 (66.7%); Budesonide (Glucocorticoid 2/3 (66.7%); Mupirocin 1/3 (33.3%); Fluticasone 1/3 (33.3%); Oseltamivir 2/3 (66.7%)

For Fluticasone, Oseltamivir, Mupirocin, and Budesonide, further evaluation was performed by de-multiplexing the analytes. Testing was conducted in triplicate using single spiked targeted analytes at 3X or 10X LoD. The results are summarized in the following table. No interference was observed.

Table: Additional interference testing using individual targeted analytes

Substances	Testing Concentration	SARS-CoV-2	Influenza A H1N1	Influenza B Victoria	RSV
Fluticasone	5% v/v	NA	NA	NA	3/3 detected at 10XLOD
Budesonide (Glucocorticoid)	1% v/v	3/3 detected at 10XLOD	3/3 detected at 3XLOD	3/3 detected at 3XLOD	3/3 detected at 10XLOD
Oseltamivir	2.5 mg/mL	NA	NA	NA	3/3 detected at 3XLOD
Mupirocin	10 mg/mL	NA	NA	NA	3/3 detected at 3XLOD

NA: not tested as single target because of 3/3 detected in co-infection conditions
3X LoD and 10X LoD are based on Lot 1 single-formulation LoD.
Presumptive Positive results:

- Budesonide (Glucocorticoid)/SARS-CoV-2 (3X LoD) 1/3 (33.3%); RSV (10X LoD) 1/3 (33.3%)
- Mupirocin/RSV (3X LoD) 2/3 (66.7%)
- Fluticasone/RSV (3X LoD) 1/3 (33.3%), (10X LoD) 2/3 (66.7%)
- Oseltamivir/RSV (3X LoD) 2/3 (66.7%)

Precision (Repeatability)

Within-laboratory precision was examined using a panel composed of co-spiked influenza A (A/Georgia/T51700/2012 (H1N1)pdm09), influenza B (B/Brisbane/60/2008 (Victoria Lineage), SARS-CoV-2 (USA-WA1/2020, heat-inactivated) and/or RSV (A2001/3-12) cultures diluted in simulated clinical matrix (viral transport medium plus human A549 cells). Two studies on two instruments (CFX96™ and QuantStudio™ Dx 384) were performed. Sources of variability were examined with a panel consisting of three concentration levels: Negative samples, low positive samples (1.5X LoD co-formulation based on lot #2), moderate positive samples (3X LoD co-formulation based on lot #2).

- Study 1 used two lots of reagents over a time course of 3 days by at least 2 operators. Each sample was tested in duplicate for each lot and each instrument of each run.

- Study 2 was designed to evaluate 20 replicates of each panel for each instrument using one lot of reagents (lot 2).

Study 1: All negative panel members tested negative, and all moderate and low

positive contrived samples tested positive throughout the study. The statistical summary for the two instruments is listed in the following table. Overall agreement for the two instruments: 72/72, 100.0% (95.0%-100.0%).

Study 2: All negative panel members tested negative, and all positive samples tested positive throughout the study. The statistical summary is listed in the following table. Overall agreement for the two instruments: 119/119, 100.0% (96.9%-100.0%).

Table: Precision – summary of detection rates

Strain	Target	Panel Member	Concentration [$TCID_{50}$/mL]	Detection or Agreement Rate (95% CI) for study 1	Detection or Agreement Rate (95% CI) for study 2
CFX96™*					
Influenza A (A/Georgia/T5170 0/2012 (H1N1)pdm09)	Influenza A	Moderate Positive	19.98	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Low Positive	9.99	12/12, 100.0% (75.6-100.0%)	19/19, 100% (83.1%-100.0%)
		Negative	0	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Positive Control	NA	5/5, 100.0% (56.6-100.0%)	NA
Influenza B (B/Brisbane/60/20 08 (Victoria Lineage)	Influenza B	Moderate Positive	5.94	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Low Positive	2.97	12/12, 100.0% (75.6-100.0%)	19/19, 100% (83.1%-100.0%)
		Negative	0	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Positive Control	NA	5/5, 100.0% (56.6-100.0%)	NA
SARS-CoV-2 Isolate USA-WA1/2020, heat-inactivated	SARS-CoV-2	Moderate Positive	0.66	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Low Positive	0.33	12/12, 100.0% (75.6-100.0%)	19/19, 100% (83.1%-100.0%)
		Negative	0	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Positive Control	NA	5/5, 100.0% (56.6-100.0%)	NA
Human respiratory syncytial virus (A2001/3-12)	RSV	Moderate Positive	59.94	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Low Positive	29.97	12/12, 100.0% (75.6-100.0%)	19/19, 100% (83.1%-100.0%)
		Negative	0	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Positive Control	NA	5/5, 100.0% (56.6-100.0%)	NA
QuantStudio™ Dx 384					
Influenza A (A/Georgia/T5170 0/2012 (H1N1)pdm09)	Influenza A	Moderate Positive	19.98	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Low Positive	9.99	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Negative	0	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Positive Control	NA	5/5, 100.0% (56.6-100.0%)	NA
Influenza B (B/Brisbane/60/20 08 (Victoria Lineage)	Influenza B	Moderate Positive	5.94	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Low Positive	2.97	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Negative	0	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Positive Control	NA	5/5, 100.0% (56.6-100.0%)	NA
SARS-CoV-2 Isolate USA-WA1/2020, heat-inactivated	SARS-CoV-2	Moderate Positive	0.66	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Low Positive	0.33	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Negative	0	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Positive Control	NA	5/5, 100.0% (56.6-100.0%)	NA
Human respiratory syncytial virus (A2001/3-12)	RSV	Moderate Positive	59.94	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Low Positive	29.97	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Negative	0	12/12, 100.0% (75.6-100.0%)	20/20, 100% (83.9%-100.0%)
		Positive	NA	5/5, 100.0% (56.6-100.0%)	NA

		Control			

*One well in study 2 is invalid.
LP: Low Positive; MP: Moderate Positive
QuantStudio™ Dx 384 Presumptive Positive:

- Study 1: Influenza A, 1/12 (8.3%) from LP; RSV, 2/12 (16.7%) from LP, 2/12 (16.7%) from MP
- Study 2: Influenza A, 1/20 (5%) from LP.

CFX96 ™ Presumptive Positive:

- Study 1: SARS-CoV-2, 3/12 (25%) from LP; Influenza A, 4/12 (33.3%) from LP, 2/12 (16.7%) from MP; Influenza B, 1/12 (8.3%) from LP; RSV, 3/12 (25%) from LP, 4/12 (33.3%) from MP.
- Study 2: Influenza A, 3/20 (15%) from LP, 1/20 (5%) from MP; RSV, 8/20 (40%) from LP.

Table: Precision/repeatability study - overall mean, standard deviations (SD), and coefficients of variation (CV%) for target Ct values (QuantStudio™ Dx 384)

Target	Panel Member	Detection Rate	Mean Ct	Instrument-to-Instrument		Lot-to-Lot		Day-to-Day		Run-to-Run		Within Run		Total	
				SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Influenza A H1N1	Moderate Positive	32/32	34.22	NA	NA	0	0.00%	1.131	3.30%	1.131	3.30%	0.44	1.29%	1.21	3.55%
	Low Positive	32/32	35.27	NA	NA	0	0.00%	0.911	2.58%	0.911	2.58%	0.83	2.34%	1.23	3.49%
	Negative	32/32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Influenza B Victoria	Moderate Positive	32/32	31.64	NA	NA	0.86	2.73%	0.49	1.54%	0.49	1.54%	0.82	2.60%	1.29	4.07%
	Low Positive	32/32	32.86	NA	NA	0.96	2.92%	0.51	1.54%	0.51	1.54%	0.94	2.87%	1.44	4.37%
	Negative	32/32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
SARS-CoV-2	Moderate Positive	32/32	33.65	NA	NA	0.46	1.38%	0.40	1.19%	0.40	1.19%	0.32	0.95%	0.69	2.06%
	Low Positive	32/32	34.63	NA	NA	0	0.00%	0.61	1.76%	0.61	1.76%	0.50	1.45%	0.79	2.28%
	Negative	32/32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
RSV	Moderate Positive	32/32	33.14	NA	NA	2.63	7.94%	1.549	4.67%	1.549	4.67%	0.80	2.40%	3.15	9.52%
	Low Positive	32/32	33.61	NA	NA	2.61	7.76%	0.53	1.57%	0.53	1.57%	1.93	5.76%	3.29	9.79%
	Negative	32/32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

SD; Ct Standard Deviation
% CV; Coefficient of Variation
NA; Not Applicable
Presumptive Positive results: Refer to table “Precision – summary of detection rates”

Table: Precision/repeatability study - overall mean, standard deviations (SD), and coefficients of variation (CV%) for target Ct values (CFX96™)

Target	Panel Member	Detection Rate	Mean Ct	Instrument-to-Instrument		Lot-to-Lot		Day-to-Day		Run-to-Run		Within Run		Total	
				SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Influenza A H1N1	Moderate Positive	32/32	35.92	NA	NA	0.96	2.66%	0.28	0.79%	0.28	0.79%	0.44	1.22%	1.09	3.03%
	Low Positive	31/32	36.66	NA	NA	0	0.00%	0	0.00%	0	0.00%	0.62	1.70%	0.62	1.70%
	Negative	32/32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Influenza B Victoria	Moderate Positive	32/32	34.08	NA	NA	0	0.00%	1.83	5.37%	1.83	5.37%	0.74	2.16%	1.97	5.79%
	Low Positive	31/32	35.06	NA	NA	0	0.00%	1.40	3.98%	1.40	3.98%	0.81	2.32%	1.62	4.61%
	Negative	32/32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
SARS-CoV-2	Moderate Positive	32/32	34.86	NA	NA	1.25	3.59%	0.10	0.30%	0.10	0.30%	0.38	1.08%	1.31	3.76%
	Low Positive	31/32	35.69	NA	NA	0.81	2.28%	0.12	0.35%	0.12	0.35%	0.56	1.57%	1.00	2.79%
	Negative	32/32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
RSV	Moderate Positive	32/32	35.77	NA	NA	1.11	3.10%	0.721	2.02%	0.721	2.02%	0.42	1.16%	1.39	3.88%
	Low Positive	31/32	36.22	NA	NA	0.77	2.12%	0.00	0.00%	0.00	0.00%	1.92	5.30%	2.07	5.71%
	Negative	32/32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

SD; Ct Standard Deviation
% CV; Coefficient of Variation
NA; Not Applicable
*One well in study #2 on the CFX96 instrument was invalid
Presumptive Positive results: Refer to table “Precision – summary of detection rates”

Fresh vs. Frozen Study

Because frozen samples were tested in the clinical study, a fresh versus frozen specimen equivalency study was necessary to demonstrate that the performance of the Respiratory SARS-CoV-2 RT-PCR Panel 1 was not impacted by multiple

freeze/thaw cycles. Contrived positive specimens consisted of matrix spiked with mixtures of targeted organisms at low positive (1.5X LoD co-formulation using lot #2) and moderate positive (3X LoD co-formulation using lot #2) testing concentrations. Ten replicates were evaluated per concentration on the QuantStudio™ Dx 384. The same viruses evaluated in the LoD study were used for this study influenza A (A/Georgia/T51700/2012 (H1N1)pdm09), influenza B (B/Brisbane/60/2008 (Victoria Lineage), SARS-CoV-2 (USA-WA1/2020, heat-inactivated), and RSV (A2001/3-12). The samples were tested as fresh dilution and then underwent two separate freeze-thaw cycles (frozen at <-70°C, thawed at room temperature). Results are summarized in the table below and demonstrated that there was no Ct trend or increase in standard deviation following the freezing-thawing conditions. In summary, preservation of samples by freezing at <-70°C did not affect the accuracy of test results compared to freshly prepared samples when evaluated with the Respiratory SARS-CoV-2 RT-PCR Panel 1.

Table: Fresh vs. frozen study Ct summary on QuantStudio™ Dx 384

Panel Name	SARS-CoV-2		Influenza A H1N1		Influenza B Victoria		RNase P		RSV	
	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD	Mean Ct	SD
LP (No Freeze-thaw)	35.20	0.38	35.69	1.08	33.79	0.53	28.61	0.30	34.61	1.62
LP (Freeze-thaw Once)	35.50	0.47	36.45	1.14	33.88	0.50	28.69	0.35	35.34	1.20
LP (Freeze-thaw Twice)	35.61	0.59	36.70	1.13	33.35	0.73	28.79	0.33	36.31	0.99
MP (No Freeze-thaw)	35.20	0.38	35.69	1.08	33.79	0.53	28.61	0.30	34.61	1.62
MP (Freeze-thaw Once)	34.25	0.51	34.47	0.39	32.32	0.73	28.68	0.27	32.41	1.19
MP (Freeze-thaw Twice)	34.45	0.37	34.59	0.47	32.90	0.41	28.71	0.28	33.84	1.09

LP: Low positive; MP: Moderate positive.
Based on lot 2 co-formulation LoD.
SD; Ct Standard Deviation
Presumptive Positive results stratified by concentration level and number of freeze-thaws:

- 7 Influenza A (Low Positives)
  - No freeze-thaw, 1/10 (10%)
  - 1 freeze-thaw, 2/10 (20%)
  - 2 freeze-thaws, 4/10 (40%)
- 4 RSV (Low Positives)
  - No freeze-thaw, 1/10 (10%)
  - 1 freeze-thaw, 1/10 (10%)
  - 2 freeze-thaws, 2/10 (20%)

Clinical Study

Clinical Performance with Retrospective (Archived) Specimens

The clinical performance of the Respiratory SARS-CoV-2 RT-PCR Panel 1 with retrospective specimens was evaluated at one external site using 286 archived nasopharyngeal swab (NPS) samples collected in viral transport medium (VTM/UTM/UVT) from patients suspected of a respiratory infection by their healthcare provider.

One (1) NPS sample did not have a valid result because the endogenous control was out of specification. In this scenario the results interpretation table indicates to re-extract nucleic acid from the clinical samples and repeat the RT-PCR. This follow-up procedure was not completed due to insufficient sample volume. Two samples had a Ct in the range of >37 but ≤40 that were required to be retested as instructed in the Respiratory SARS-CoV-2 RT-PCR Panel 1 IFU to confirm the samples as true positives. There was insufficient residual sample for retesting, thus these three samples were excluded from the analysis of performance. Taking these exclusions into consideration, the study was performed on a total of 283 retrospective NPS samples that had been previously tested with an FDA authorized assay for detection of SARS-CoV-2 or an FDA cleared molecular comparator method for detection of influenza A, B or RSV. Testing of the archived specimens was conducted on the QuantStudio™ Dx 384 platform and the results are summarized in the table below.

Table: Clinical Performance Study summary: retrospective specimens

Virus	# of Samples	Test Results				Agreement Statistics		
		Concordant Positive (N)	Discordant Negative (N)	Concordant Negative (N)	Discordant Positive (N)	Agreement Parameter	Percent Agreement (%)	95% CI LCL, UCL
Inf A	170	62	0	107	1	PPA	100%	94.2%, 100.0%
						NPA	99.1%	94.9%, 99.8%
Inf B	170	34	0	136	0	PPA	100%	90.0%, 100%
						NPA	100%	97.3%, 100%
RSV	183	55	0	128	0	PPA	100%	93.5%, 100%
						NPA	100%	97.1%, 100%
SARS-CoV-2	69	39	0	30	0	PPA	100%	91.0%. 100%
						NPA	100%	88.7%. 100%

PPA = Positive Percent Agreement; NPA = Negative Percent Agreement
CI = confidence interval; LCL = Lower confidence limit; UCL = Upper confidence limit
Confidence interval is calculated using Wilson's Score method

Clinical Performance with Prospectively Collected Specimens

The clinical performance of the Respiratory SARS-CoV-2 RT-PCR Panel 1 was evaluated at three external sites using 150 prospectively collected NPS samples in VTM that were collected at three different sites from patients suspected of a respiratory infection by a healthcare provider.

The prospectively collected specimens were assessed using an FDA-cleared comparator method and by the candidate assay on the Applied Biosystems™ QuantStudio™ Dx 384 thermocycler. Three samples initially resulted in a presumptive positive call by the Respiratory SARS-CoV-2 RT-PCR Panel 1, with

Ct values in the range of >37 but ≤40 for SARS-CoV-2. Subsequent retesting on the residual sample extract using the Respiratory SARS-CoV-2 RT-PCR Panel 1 resulted in one positive and two negative calls for SARS-CoV-2. The NPA for each target, and the PPA for SARS-CoV-2 are shown in the prospective clinical performance study summary below.

Table: Clinical Performance Study Summary: prospectively collected specimens

Virus	# of Samples	Test Results				Agreement Statistics		
		Concordant Positive (N)	Discordant Negative (N)	Concordant Negative (N)	Discordant Positive (N)	Agreement Parameter	Percent Agreement (%)	95% CI LCL, UCL
Inf A	150	1	0	149	0	PPA	100%	20.7%, 100%
						NPA	100%	97.5%, 100%
Inf B	150	0	0	150	0	PPA	N/A	N/A
						NPA	100%	97.5%, 100%
RSV	150	0	0	150	0	PPA	N/A	N/A
						NPA	100%	97.5%, 100%
SARS-CoV-2	150	52	0	97	1	PPA	100.0%	93.1%, 100.0%
						NPA	99.0%	94.4%, 99.8%

PPA = Positive Percent Agreement; NPA = Negative Percent Agreement
CI = confidence interval; LCL = Lower confidence limit; UCL = Upper confidence limit
Confidence interval is calculated using Wilson's Score method

Appendix

A. Applied Biosystems™ QuantStudio™ Dx Calibration

This PCR assay utilizes all the detector channels available in the instrument including Cy5.5. This plate is only available through Revvity. Revvity also recommends using our Cy5 calibration plate order to ensure Cy5 detection quality.

Calibration plate name	Vendor	Part No.
384-well Spectral Calibration Plate, Background	ThermoFisher	4432320
384-well Spectral Calibration Plate, ROI	ThermoFisher	4432320
384-well Spectral Calibration Plate, Uniformity	ThermoFisher	4432320

Calibration plate name	Vendor	Part No.
384-well Spectral Calibration Plate, FAM/ROX	ThermoFisher	4432308
384-well Spectral Calibration Plate, VIC/ROX	ThermoFisher	4432308
384-well Spectral Calibration Plate, FAM	ThermoFisher	4432271
384-well Spectral Calibration Plate, VIC	ThermoFisher	4432278
384-well Spectral Calibration Plate, ROX	ThermoFisher	4432284
384-well Spectral Calibration Plate, Cy5	Revvity	4178-0010
384-well Spectral Calibration Plate, Cy5.5	Revvity	4179-0010
96-well Spectral Calibration Plate, Cy5	Revvity	4172-0010
96-well Spectral Calibration Plate, Cy5.5	Revvity	4173-0010

Additionally, special attention must be given to the calibration curve results for the background calibration. Even if the background calibration passes, there must be no outlier well curves in the Background Calibration. Outlier wells should be identified, and the block should be removed and cleaned thoroughly before proceeding with additional calibrations. A new ROI and background calibration are recommended at that point.

Calibration procedure:

NOTE: Calibration plates should be thawed 30 minutes before use and should not be used after 2 hours of being thawed so do not thaw more than 2-3 plates at a time. Calibration plates cannot be frozen/re-used again.

NOTE: Before any calibration, vortex the calibration plate for 5 seconds and centrifuge the plate for 2 minutes at < 1500 rpm.

NOTE: Do not allow the bottoms of the plates to become dirty. Fluids and other contaminants that adhere to the bottoms of the consumables can contaminate the sample block and cause an abnormally high background signal.

Calibrate the instrument in IVD or RUO mode following the specific plate order and instructions below (if it is calibrated in IVD mode, it can cover both IVD and RUO mode):

1. ROI calibration

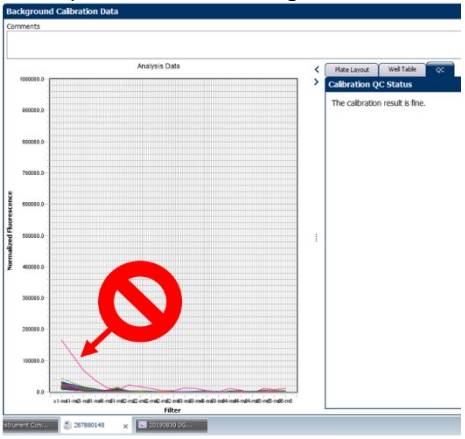
2. Background calibration – regardless of the pass/fail outcome of the

calibration, all the traces should be in a tight bundle. Check for outlier wells in the calibration results (I.e. any well whose calibration trace is significantly different from the rest of the wells, see Figure below). Even if the calibration "Passes", wells with curves that are obviously higher than most curves may produce a shift in the Ct calls for specific analytes. If outlier wells are apparent, the block and/or cover must be cleaned, and the ROI and Background calibration performed again using the same plates but inverting them for the repeat calibration.

NOTE: Recommended cleaning procedure:

- Identify outlier well(s) in the block (rotated 180° if compared with the plate calibration results). Visually check if there's any debris in the well(s) and clean that well(s) with appropriate QTIP.
- Pipette 20µL of 10% bleach solution into the well. Let it sit for 1 minute and dry/clean with appropriate QTIP.
- Repeat the previous step with water.
- Repeat the previous step with 70% Ethanol.

Figure: example of outlier in Background Calibration:



If after cleaning the block the background calibration results in the same outlier(s), the optical cover/path may need to be cleaned/checked.

3. Uniformity calibration
   Note: Change the instrument to RUO mode to check if Uniformity transferred. If not, repeat Uniformity calibration in RUO mode. The switch back to IVD mode.

4. CY5 calibration (Revvity Brand calibration plate)*

5. OTHER calibration – CY5.5 (Revvity Brand calibration plate)*

6. Calibrate FAM, VIC and ROX.

7. Normalization calibrations (FAM/ROX and VIC/ROX)

* If calibration fails, check the QC tab and check whether there is any outlier in the calibration plot. Make sure that the bottom of the plate is clean and re-run the calibration inverting the plate up to two additional times. If the calibration still fails after two repeated attempts, a new CY5 or CY5.5 plate must be used. If failure still occurs with the new plate(s), clean the instrument block and start over from Step 1.

B. Analytik Jena PCR Systems Calibration and Verification

It is required to calibrate and verify the real-time PCR instrument according to the instrument's user manual. The links to the calibration reagent order information, user guides and OQ/PQ ("Operational Qualification"/"Performance Qualification) procedures for the instruments are provided in the following table below.

Instrument	Calibration reagent order information (part number)	Calibration and OQ or PQ procedure
qTower^3/qTower^3 G (qTower 96)	NA	Schedule install service (OQ): Analytik Jena US Phone: (909) 946-3197 Email: covid19support@us.analytik-jena.com
qTower^3 84/ qTower^3 84 G (qTower 384)	NA	Schedule install service (OQ): Analytik Jena US Phone: (909) 946-3197 Email: covid19support@us.analytik-jena.com

After instrument installation and service of OQ and/or PQ from Analytik Jena, follow the procedure for calibration or color compensation for using the Respiratory SARS-CoV-2 RT-PCR Panel 1.

The following reagents are required for the calibration or color compensation process, available through Revvity.

Part number: SDX-56501

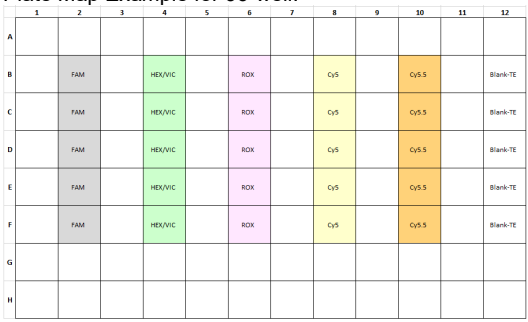
Component Name	Volume provided (uL)	Notes
Calibration FAM	300 µL	Calibration dye for instrument calibration

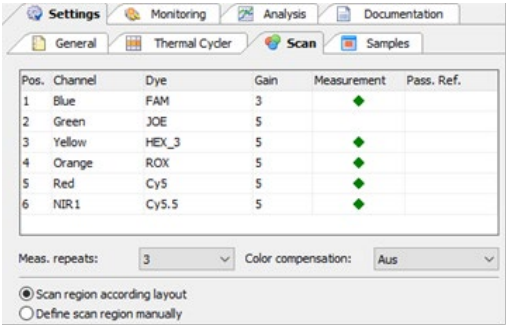
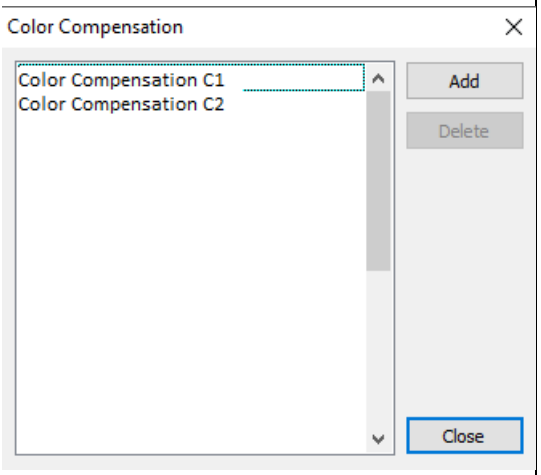
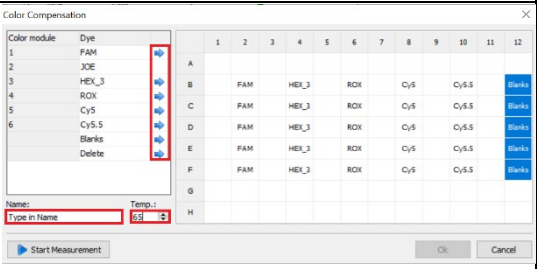
Calibration HEX/VIC	300 µL	Calibration dye for instrument calibration
Calibration ROX	300 µL	Calibration dye for instrument calibration
Calibration Cy5	300 µL	Calibration dye for instrument calibration
Calibration Cy5.5	300 µL	Calibration dye for instrument calibration
Template FAM	100 µL	Plasmid template, can be amplified and detected in FAM
Template HEX/VIC	100 µL	Plasmid template, can be amplified and detected in HEX/VIC
Template ROX	100 µL	Plasmid template, can be amplified and detected in ROX
Template Cy5	100 µL	Plasmid template, can be amplified and detected in Cy5
Template Cy5.5	100 µL	Plasmid template, can be amplified and detected in Cy5.5
1X TE Buffer or nCoV Negative Control	1.4 mL	For dilution, NTC, or blanks during calibration

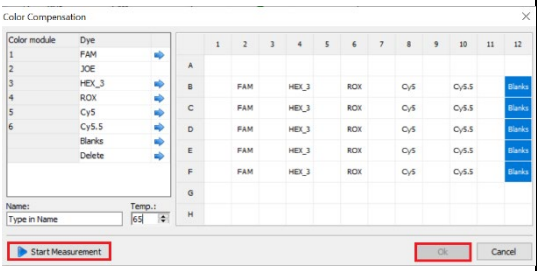
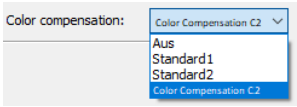
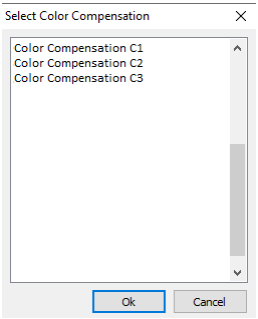
1. Color compensation

   Follow the procedure listed in the following table for color compensation or calibration for 5-color detection.

   Table: 5-color calibration procedure

1. Pipette 30 uL of individual dyes into 96-well plates or 15 uL per dye for 394-well plate. ● 5 to 10 replicates for each dye is recommended. 1X TE, standard EDTA, pH 8.0 can be used for blank wells. 2. Seal the plate tight, followed by quick vortex at 2500 rpm for 10 second. Spin down the plate for 5 mins at 350 x g.	Plate Map Example for 96-well: 

3. Open qTower software, click on  to create new run; or click on  to open template protocol stored in the computer. • In the “Setting” > “Scan” tab, make sure all dyes are selected in measurement, and their names and gain settings are the same with the assay.	Scan Setting: 
4. Click on  , previous color compensations are listed on the left. 5. Click “Add” to create a new color compensation file.	
6. Select the wells on the right and click  to assign dye labels. • Be sure to add “Blanks” when doing multiple color compensations in a row. 7. Enter a name for the file and set temperature to which the assay uses to capture fluorescent signal.	

8. Click on “Start Measurement”. • The instrument should start making some noise and the indication light should be blinking during the run. • For qTower 384, a “Saved as” window would pop up toward finish, click “Close” and wait a few seconds. 9. Click “ok” once the run is done.	
10. To make sure the new file is saved, click on the “Color compensation” drop down menu, select a previously calibrated file after “Standard 2”.  • The new color compensation should have been added to the list at the bottom.	
11. To make a copy of the color compensation file “compensation.cmp”, go to “Dev3” folder. • The file name cannot be changed; therefore, it is recommended to backup your original .cmp file before and after each color calibration.	Examples depending on the softwre version For qTower 96: C:\ProgramData\Analytik Jena\qPCRsoft\4.1\Dev3 For qTower 384: C:\ProgramData\Analytik Jena\qPCRsoft384\1.2\Dev3 

Note: If the instrument shows: Signal is too strong. Dilute the calibration dye to 0.5X with TE buffer (1:1 dilution) for use.

2. PQ process

The purpose of the study is to select and validate the right color compensation configuration for 5-color detection on qTower real-time PCR systems.

In order to select and validate the right color compensation configuration for a particular qTower instrument or plates for using 5-color detection, it is required to perform a single template and a panel template (4-plex template mix) run at a single concentration in triplicate. The acceptance criteria for a color compensation choice (Standard 1 or Standard 2 or the customized configuration established by the procedure described in the section “color compensation”) is listed below:

- No cross-talk with single template
- No signal-loss for panel template

The details procedures are listed below.

1) The templates to be used for this study are listed as following, total 21 reactions (5 µL per PCR reaction, tested in triplicate):
    1) Template FAM
    2) Template HEX/VIC
    3) Template ROX
    4) Template Cy5
    5) Template Cy5.5
    6) Panel Template (four-plex)
    7) NTC (either nCoV Negative control, or nuclease-free water or 1XTE buffer)

2) Prepare PCR Reaction mix according to the following table. Note: Extra volume is prepared to account for pipetting carryovers/variations.

Starting material	Volume/reaction (µL) for 96-well	Volume (for 21+2 extra reactions) (µL) for 96-well
Nuclease-free water	15.0	360.0
nCoV Reagent A	7.5	180.0
nCoV Resp Reagent B1	1.25	30.0
nCoV Resp Reagent B2	0.25	6.0
nCoV Enzyme mix	1.0	24.0
Total vol.	**25.0**	**600.0**

Starting material	Volume/reaction (µL) for 384-well	Volume (for 21+2 extra reactions) (µL) for 384-well
Nuclease-free water	5.0	120.0
nCoV Reagent A	3.75	90.0
nCoV Resp Reagent B1	0.625	15.0
nCoV Resp Reagent B2	0.125	3.0
nCoV Enzyme mix	0.5	12.0
Total vol.	**10.0**	**240.0**

3) Vortex the prepared PCR Master mix, centrifuge and pipette into each well of a PCR plate. For a 96-well PCR plate, pipette 25 µL of PCR Master mix per well and for a 384-well PCR plate, pipette 10 µL of PCR Master mix per well.

4) Prepare Panel template (4-plex) according to the following table.

Panel Template Preparation for 96-well or 384-well		
Starting material	**Volume/reaction (µL)**	**Volume (for 3+2 extra reactions) (µL)**
1X TE Buffer	1.0	5.0
Template FAM	1.0	5.0
Template ROX	1.0	5.0
Template Cy5	1.0	5.0
Template Cy5.5	1.0	5.0
Total vol.	**5.0**	**25.0**

Note: Extra volume is prepared to account for pipetting carryovers.

5) Vortex the template (provided with the kit) and/or the prepared Panel template, centrifuge (short spin) and pipette 5 µL into each well of a PCR plate containing PCR Master mix.

Note: Add TE Buffer (provided with the kit) as negative control instead of the template to the NTC wells.

6) Seal the plate with an appropriate film.

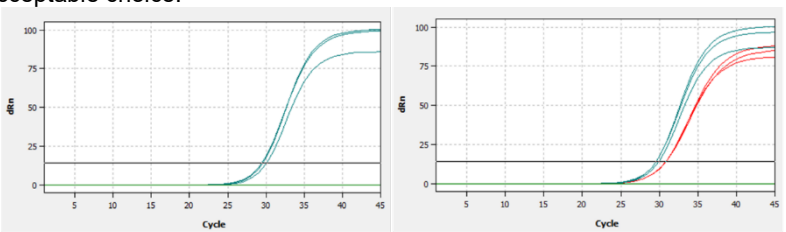
7) Vortex plate for 10-20 seconds, and centrifuge for 5 minutes at 350 x g.

8) Transfer plate to the qPCR instrument to run.

9) Run the PCR with the settings described in the section “Amplification on the Analytik Jena qTower^3/qTower^3 G and qTower^3 84/qTower^3 84 G Real Time PCR Systems”.

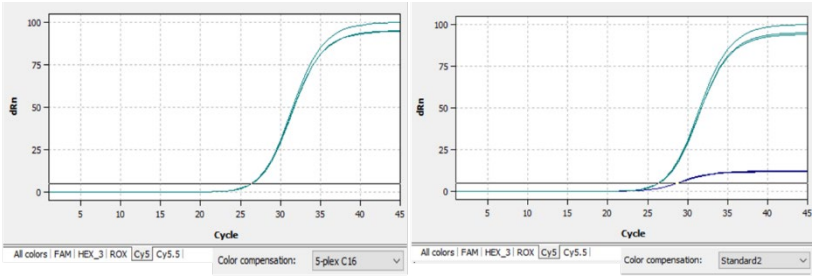
10) Evaluation results based on acceptance criteria. Some examples are listed below.

Example 1: No cross-talk signals from single template. For Cy5.5 color module selection, only ‘Template Cy5.5’ (green amplifications) is detected in the Cy5.5 channel by setting color compensation mode as C16 (established during procedure described in the section “color compensation”). However, both the ‘Template Cy5.5’ (green amplifications) and the ‘Template Cy5’ (red amplifications) are detected in the CY5.5 channel when the color compensation mode is set at AUS. Therefore, C16 is an acceptable choice, AUS is not an acceptable choice.



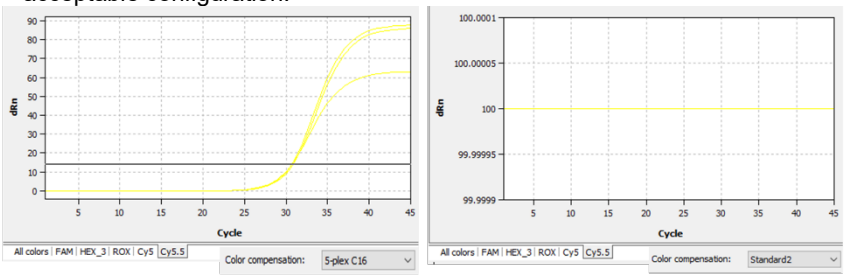
E.g. 1- Cy5.5: No cross-talk at 5-plex C16 color compensation mode (left) while cross-talk at AUS mode (right)

Example 2: No cross-talk signals from single template. For Cy5 color module selection, only 'Template Cy5' (green amplifications) is detected in the Cy5 channel by setting color compensation mode as C16. However, both the 'Template Cy5' (green amplifications) and the 'Template Cy5.5' (blue amplifications) are detected in the CY5 channel when the color compensation mode is set at "Standard 2". Therefore, C16 is an acceptable choice, "Standard 2" is not an acceptable choice.



E.g. 2- Cy5: No cross-talk at 5-plex C16 color compensation mode (left) while cross-talk at Standard2 mode (right)

Example 3: No signal or signal loss for panel input after color compensation. With C16 mode, the amplification (yellow) from panel template is visible for Cy5.5 color module. However, the amplification is not visible when choosing "Standard 2" color compensation setting. Therefore, "Standard 2" is not an acceptable configuration.



E.g. 4- Cy5.5: Signal obtained at 5-plex C16 color compensation mode (left) is lost at Standard2 mode (right)

3. Performance verification

Follow kit IFU, with the validated color compensation determined in the step "PQ Process", perform tests on 3 positive samples (kit positive control: sample 1-3;), 3 negative samples with extraction process (sample 4-6) and 3 NTC (non-template control, sample 7-9) without extraction process, and run on the same real-time PCR plate.

The detail procedure is listed below.

1) Take nCoV Resp Positive Control and nCoV Negative Control, or Negative samples out from freezer, place them in a biological safety cabinet and completely thaw them at room temperature. Vortex the tubes to mix the contents, then centrifuge the tubes briefly at 1000 rpm to collect the liquid to the bottom of the tubes.

2) Prepare the evaluation panel for sample extraction as following:
   - Sample 1-3: 300 µL nCoV Resp Positive Control per sample
   - Sample 4-6: 300 µL nCoV Negative Control per sample.

3) Perform sample extraction on sample 1-6, following the procedure in section "Extraction chemagic 360".

4) Prepare PCR master mix and plate, following the procedure in section "Setup PCR Manually" for either 30uL or 15uL PCR reaction.

   - Sample 1-3: Add 20/10 uL (depending on the PCR instrument) of extracted nCoV Resp Positive Control to PCR plate position 1-3.

   - Sample 4-6: Add 20/10 uL (depending on the PCR instrument) of extracted nCoV Negative Control to PCR plate position 4-6.

   - Sample 7-9: Add 20/10 uL (depending on the PCR instrument) of nCoV Negative Control or nuclease-free water or 1XTE buffer directly to PCR plate position 10-12.

5) Run the PCR program according to the settings for the corresponding verification instrument, following the procedure in section "Amplification", page 18-20

Acceptance criteria

All evaluated 9 samples must meet the criteria for "PASS" based on the following table prior to usage of the Respiratory SARS-CoV-2 RT-PCR Panel 1 for diagnostic testing.

Result Interpretation:

Control name	Ct Value					Result interpretation
	SARS-CoV-2 (FAM)	Influenza A (ROX)	Influenza B (Cy5)	RSV (Cy5.5)	RNase P (HEX/VIC)	
1-3	≤ 35	≤ 35	≤ 35	≤ 35	≤ 35	PASS
4-6	Undet/ blank or > 40	Undet/ blank or > 40	Undet/ blank or > 40	Undet/ blank or > 40	Undet/ blank or > 35	PASS

7-9	Undet/ blank or > 40	Undet/ blank or > 40	Undet/ blank or > 40	Undet/ blank or > 40	Undet/ blank or > 35	PASS

Undet: Undetermined

C. Additional Label for the CFX96™ Touch Real-Time PCR Detection System

Please print the below label and place the label on the front panel of the instrument. If the instrument includes labeling indicating "For Research Use Only", please cover with the below "Emergency Use Only" labeling. The instrument should retain this labeling throughout the EUA use of the Respiratory SARS-CoV-2 RT-PCR Panel 1.

Emergency Use Only

This instrument is authorized for use with the Respiratory SARS-CoV-2 RT-PCR Panel 1

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2. Innis, MA, et Al, and the PCR Protocols Applications: A Laboratory Manual, Academic, New York, 1989.
3. Mahony JB. Detection of Respiratory Viruses by Molecular Methods. Clinical Microbiology Reviews. 2008; 21 (4): 716-747.
4. ASTM F1980 – 16: Standard Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices.
5. Shufa Zheng, Jian Fan, et al., Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study, BMJ 2020, 369:m1443.
6. Roman Wölfel, Victor M. Corman, et al., Virological assessment of hospitalized patients with COVID-2019, Nature 2020, Vol 581: 465-469.

Revision history: Publication Number v1.0

Revision	Date	Description
1.0	October 06, 2021	New document
2.0	November 1, 2021	Added QuantStudio™ Dx 96, qTower 96, qTower 384
3.0	December 14, 2021	Update to the reagent stability claim from 12 to 24 months
4.0	January 21, 2022	Flumist interference study and RSV inclusivity data added
5.0	July 21, 2022	Prospective Clinical Performance added
6.0	January 26, 2024	Update product name, company branding, legal manufacture address, and technical support contact information

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