



**LumiraDx™**  
*Fast Lab Solutions*

# SARS-CoV-2 & Flu A/B RNA STAR Complete

Instructions for Use  
For Emergency Use Authorization (EUA) Only



L022180209096



**Rx** Only

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## 1. Intended Use

LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete is a rapid, non-isothermal nucleic acid amplification qSTAR (Selective Temperature Amplification Reaction) *in vitro* diagnostic test intended for the simultaneous qualitative detection and differentiation of RNA from the SARS-CoV-2, Influenza A, and/or Influenza B viruses in nasopharyngeal swabs from individuals with signs and symptoms of respiratory tract infection consistent with COVID-19. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar.

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

Results are for the simultaneous detection and differentiation of SARS-CoV-2, influenza A and influenza B viral RNA in clinical specimens. This test is not intended to detect influenza C virus. RNA from SARS-CoV-2, Influenza A, and Influenza B viruses is generally detectable in nasopharyngeal swab specimens during the acute phase of infection.

Positive results are indicative of the presence of SARS-CoV-2, Influenza A, and/or Influenza B RNA but do not rule out bacterial infection or co-infection with other pathogens 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 definite 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 results do not preclude SARS-CoV-2, Influenza A, and/or Influenza B 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 epidemiological information.

LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete is only for use under the Food and Drug Administration's Emergency Use Authorization.

## 2. Summary and Explanation of the Test

Respiratory tract infections caused by respiratory viruses are a common cause of acute illnesses globally. Timely and accurate diagnosis of the cause of respiratory tract infections is important as disease severity can be increased in young, immunocompromised, or elderly individuals<sup>1</sup>.

### SARS-CoV-2

The World Health Organization (WHO) have named the disease caused by SARS-CoV-2 virus as coronavirus 2019 disease or COVID-19<sup>2</sup>. The common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat or diarrhea. These symptoms are usually mild and begin gradually. Some people become infected but do not develop any symptoms and do not feel unwell (asymptomatic infection). However, the disease can develop rapidly and have high morbidity in certain populations, especially those with underlying health conditions. The disease can spread from person to person through small droplets from the nose or mouth which are spread when a person with COVID-19 coughs or exhales. Most estimates of the incubation period for COVID-19 range from 2-14 days<sup>3</sup>.

### Influenza A/B

Influenza is an acute respiratory illness caused by infection with the influenza virus, primarily influenza A and influenza B. Transmission of the Influenza virus is largely through airborne droplets and is capable of infecting the nose, throat, and lungs. Symptoms arise on average 1 to 2 days post-exposure and may include fever, chills, cough, sore throat, coryza, malaise, muscle or body aches, headaches, fatigue, vomiting, and diarrhea. Both influenza types (A & B) circulate within the human population with type A being the most dominant cause of epidemics annually, often in the winter months. Influenza A viruses can further be categorized into subtypes based upon two major surface protein antigens: hemagglutinin (H) and neuraminidase (N). Influenza B is not categorized into subtypes however, there are two common lineages of influenza B that cause human infection. These are the Victoria and Yamagata lineages. Influenza B virus is capable of epidemic level incidents and may predominate over influenza A virus in some geographies each season, as the Victoria lineage did in the 2020-2021 season in China<sup>4</sup>.

## 3. Principles of the Procedure

LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete is a rapid, non-isothermal nucleic acid amplification test (NAAT) utilizing qSTAR (Selective Temperature Amplification Reaction) technology, which detects and differentiates SARS-CoV-2, influenza A, and influenza B

1 Couch, R.B. and Kasel, J.A. 1995. Influenza in Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. 7th Edition. 431-446.

2 World Health Organization: [www.who.int](http://www.who.int).

3 Center for Disease Control and Prevention: [www.cdc.org](http://www.cdc.org).

4 Huang, Weijuan, 2021. Vital Surveillances: Epidemiological and Virological Surveillance of Seasonal Influenza Viruses — China, 2020–2021. China CDC Weekly. 2021, 3(44): 918-922. <https://weekly.chinacdc.cn/en/article/doi/10.46234/ccdcw2021.224>

viral nucleic acid from nasopharyngeal swab samples collected in transport medium within twenty-five minutes, without the need for up-front sample purification.

The LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Internal Control, Primer and Probe (IC/P) Mix is designed for the qualitative detection and differentiation of nucleic acid from SARS-CoV-2, influenza A, and influenza B in nasopharyngeal swabs collected from individuals suspected by their healthcare provider of respiratory viral infection consistent with COVID-19.

Target virions are lysed and amplified from nasopharyngeal swab samples in a single reaction. Lysis is achieved using detergents found in the Extraction Buffer. Nucleic acids present post-lysis are reverse transcribed and subsequently amplified by qSTAR using specific primers for each assay target. qSTAR amplification is achieved by cycling between two temperatures and using two distinct enzymes, a polymerase and a nicking enzyme. The polymerase is relatively favored at the higher temperature while the nicking enzyme is relatively favored at the lower temperature. Molecular beacons are used to specifically anneal and detect each target amplicon utilizing any of the following 96-Well Configuration open RT-PCR instruments: Applied Biosystems QuantStudio 5 (software version 1.5.1), Applied Biosystems QuantStudio 7 Flex (software version 1.3), or the Bio-Rad CFX Opus System (software version 2.2).

## 4. Reagents and Materials

### 4.1 Materials Required (Provided)

**Table 1. SARS-CoV-2 & Flu A/B RNA STAR Complete Kit Components and Volumes**

Component	96-Well Volume (L022180209096)
SARS-CoV-2 & Flu A/B RNA STAR Complete Positive Control Media (PCM)	250 µL
SARS-CoV-2 & Flu A/B RNA STAR Complete Negative Control Media (NCM)	1.5 mL
SARS-CoV-2 & Flu A/B RNA STAR Complete Salt Mix	1.0 mL
SARS-CoV-2 & Flu A/B RNA STAR Complete Extraction Buffer	500 µL
SARS-CoV-2 & Flu A/B RNA STAR Complete Internal Control & Primer Mix (IC/P Mix)	400 µL
SARS-CoV-2 & Flu A/B RNA STAR Complete Master Mix	2.0 mL

### 4.2 Materials Required (But Not Provided)

**Table 2. Consumables Required (Not Provided)**

Consumables	Source	Catalog #
Appropriate Personal Protective Equipment	General lab supplier	N/A
Aerosol Barrier Pipette Tips with Filters	General lab supplier	N/A
Microcentrifuge Tubes (DNase/Rnase free), 0.6 to 5mL	General lab supplier	N/A
Powder-Free Nitrile Glove	General lab supplier	N/A
Reagent Reservoirs (for minimal dead volume)	General lab supplier	N/A
Sealable Waste Bag or Container	General lab supplier	N/A
Low-Lint Lab Wipes	General lab supplier	N/A

**Table 3. Reagents Required (Not Provided)**

Reagents	Source	Catalog #
Sodium Hypochlorite Solution (Bleach)	General lab supplier	N/A
70% Isopropanol (or 70% Ethanol)	General lab supplier	N/A
DNAZap™ (or equivalent)	ThermoFisher Scientific	AM9890
RnaseZap™ (or equivalent)	ThermoFisher Scientific	AM9782
Molecular Biology Grade Water	Corning	46-000-CM
Compatible Swabs and Transport Medium	General lab supplier	N/A
Saline, 0.85 or 0.90%	General Lab Supplier	N/A
1x Phosphate Buffered Saline (PBS), pH 7.4	General Lab Supplier	N/A
Transport Medium	Corning Transport Medium*	25-500-CM

\*Or media of similar formulation

**Table 4. 96-Well Instruments and Consumables (Not Provided)**

96-Well PCR Instruments & Consumables	Source	Catalog #
Applied Biosystems QuantStudio™ 5, 96-well block, 0.2-mL block (software version 1.5.1)	ThermoFisher Scientific	A28574
Applied Biosystems MicroAmp Optical 96-Well Plate, 0.2 mL	ThermoFisher Scientific	4306737
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690
Applied Biosystems QuantStudio 7 Flex, 96-well block, 0.2-mL block (software version 1.3)	ThermoFisher Scientific	4485698
Applied Biosystems MicroAmp Optical 96-Well Plate, 0.2 mL	ThermoFisher Scientific	4306737
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690
Bio-Rad CFX Opus 96 Real-Time PCR Instrument (software version 2.2)	Bio-Rad	12011319
Eppendorf twin.tec Real-Time PCR Plate 96-Well Skirted	Eppendorf	951022003
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690

**Table 5. Universal Consumables\* (Not Provided)**

Universal Consumables	Source	Catalog #
ThermalSeal A Sealing Film	Research Product International	202545
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690
Utility Sealing Film		
VWR Adhesive Film for Microplates	VWR	60941-070

\*These universal sealing films have been found to be compatible with all instruments listed above.

**Table 6. Equipment (Not Provided)**

Equipment	Source	Catalog #
-80°C Laboratory Freezer	General lab supplier	N/A
-15°C to -25°C Laboratory Freezer	General lab supplier	N/A
2°C to 8°C Laboratory Refrigerator	General lab supplier	N/A
Adjustable Multi-Channel Pipettes (2-20µL, 20-200µL)	General lab supplier	N/A
Adjustable Micropipettes (0.5-10µL, 2-20µL, 20-200µL, 100-1000µL)	General lab supplier	N/A
Centrifuges (for 0.6 to 5mL tubes and 96-well plates)	General lab supplier	N/A
PCR Hood	General lab supplier	N/A
Vortex	General lab supplier	N/A
Cold Block(s) (for microfuge tubes and 96-well) or ice	General lab supplier	N/A
Dry Heat Block (Capable of heating PCR Plate to 65°C +/- 1°C for 5 minutes)	General lab supplier	N/A
IsoFreeze® PCR Racks	Thomas Scientific	1148D61
Racks for Microcentrifuge Tubes	General lab supplier	N/A
USB Flash Drive	General lab supplier	N/A

## 5. Warnings and Precautions

- For use under Emergency Use Authorization (EUA) only.
- For *in vitro* Diagnostic Use (IVD).
- For prescription (Rx) use only.
- This product has not been FDA cleared or approved but has been authorized by FDA for emergency use under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection and differentiation of nucleic acid from SARS-CoV-2, influenza A, and influenza B virus and not for any other viruses or pathogens.
- Results for influenza A and influenza B should be confirmed with an FDA-cleared nucleic acid amplification test if clinically indicated.
- This 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.
- The Salt Mix and the Master Mix contains bovine serum albumin.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV (<https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html>).
- Performance characteristics have been determined with nasopharyngeal specimens from individuals who are suspected of respiratory viral infection consistent with COVID-19.
- Use personal protective equipment such as, but not limited to, gloves and lab coats when handling kit reagents while performing this Test and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Dispose of unused kit reagents and human specimens according to local, regional, national, and international regulations.
- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in this Test.

- To avoid contamination of the environment and / or test reactions with SARS-CoV-2 and / or influenza amplicons, do not open the reactions post-amplification.
- Laboratories are required to report all results for SARS-CoV-2 to the appropriate public health authorities.
- Reagents used with this test include guanidine-containing materials. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite (bleach).
- Only use listed components provided for LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete; other LumiraDx products may not contain the same formulations as needed for this test.
- Do not mix lots, as kits components are not released as individual reagents.
- Deviations from the protocols, parameters, components, instruments, and instrument software versions described in this package insert may give erroneous results.

## 6. Reagent Storage, Handling, and Stability

- Upon receipt, store the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete kit between -15°C and -25°C.
- After initial use, freeze LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Reagents between -15°C and -25°C.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light – probes are a component in the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Internal Control & Primer Mix (IC/P Mix).
- The Extraction Buffer, Salt Mix, Internal Control/Primer Mix, and Master Mix must be thawed and kept on a cold block at all times during preparation and use.
- The external controls, PCM and NCM must be thawed and kept cold at all times during preparation and use.
- Repeated thawing and freezing of the reagents more than 3 times should be avoided, as this might affect the performance of the product.
- Do not mix reagents from kits with different lot numbers.

## 7. Sample Collection, Handling, Storage, and Transport

**Proper collection and handling of specimens critical to laboratory diagnosis of infectious diseases. A specimen that is not collected correctly may lead to incorrect test results.** Testing for respiratory viruses should be conducted in consultation with a healthcare provider. Specimens should be collected as soon as possible once a decision has been made to pursue testing, regardless of the time of symptom onset. Training in specimen collection is highly recommended due to the importance of specimen quality.

### 7.1 Sample Collection and Handling

- Follow the sample collection device's instructions for proper collection and handling procedures. Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19) – <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html> – or follow specimen collection device manufacturer instructions for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended.
- **Wet swab:** specimens should be collected and placed into appropriate transport medium. Swabs provided in up to 3 mL of compatible transport medium are acceptable, however, for optimal performance, 1 mL of buffer is suggested.
- For more information on the collection, storage and transport of samples suspected to contain SARS-CoV-2, refer to the World Health Organization Laboratory biosafety guidance related to coronavirus disease (COVID-19): Interim guidance, 28 January 2021. <https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1>

### 7.2 Sample Storage

- Always follow the manufacturer's instructions for proper storage of samples.
- **Wet swab:** specimens should be stored as follows:
  - Room Temperature (15-30°C) for up to 72 hours
  - Refrigerated (2-8°C) for up to 48 hours
  - Frozen ( $\leq$ -20°C) for up to 1 week
- If a delay in testing is expected, store specimens at -20°C or below.
- If specimens cannot be tested within 48 hours for wet-swab, they may be frozen at  $\leq$  -20°C for up to 1 week until tested.
- Repeated thawing and freezing of the swabs should be avoided, as this might affect the performance of the product. Wet swabs may be frozen and thawed up to 2 times.

### 7.3 Sample Transport

- Maintain sample storage conditions as described in the Sample Collection, Handling, Storage, and Transport section of this document.
- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.

- Samples must be shipped in accordance with applicable local, regional, national, and international transportation regulations.

## 8. Real-Time PCR Instrument and Protocol Summary

The LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete contains molecular beacons labeled with fluorophores for the detection and differentiation of nucleic acids from SARS-CoV-2, influenza A, and influenza B as well as an internal control. A summary of the fluorophores and their excitation and emission wavelengths is found in **Table 7. Target/Fluorophores for LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete**. Amplification temperature profiles for instruments are provided **Figure 1.** and **Table 8a - Table 8c**. Optical and analysis settings for instruments are provided in **Table 9a - Table 9b**.

Detailed instructions for the setup of instruments are found in **Appendix A (96-Well Formats)**.

**NOTE:** Heated lids should be used for all cycling reactions.

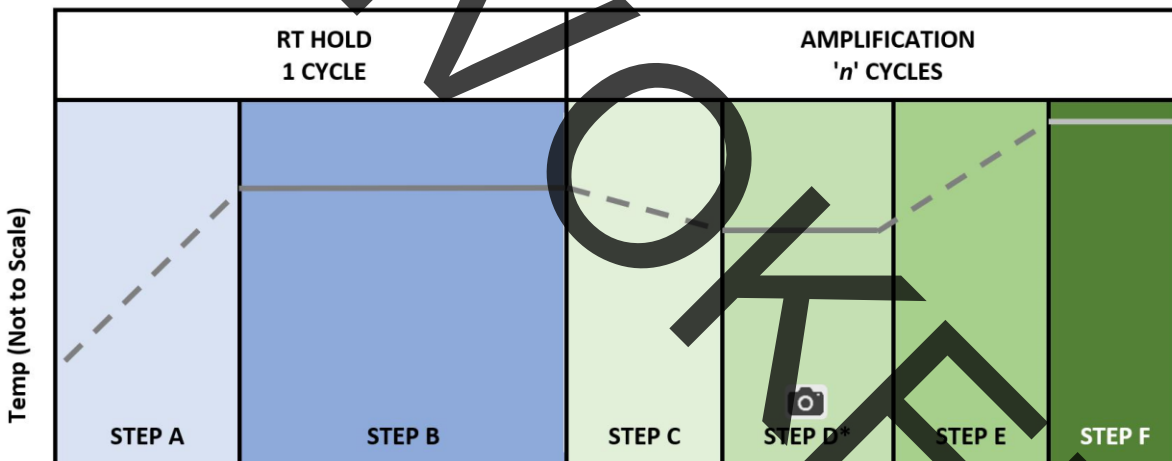
**NOTE:** No passive reference dyes should be selected in instrument settings.

**NOTE:** Thermocyclers should be programmed and queued-up prior to reagent preparation.

**Table 7. Target/Fluorophores for LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete**

Target	Dye	Quencher	Excitation	Emission
SARS-CoV-2	ROX	Dark Quencher	580	623
Influenza A	FAM	Dark Quencher	470	520
Influenza B	Cy5/Mustang Purple™	Dark Quencher	648/640	668/682
Internal Control	HEX/VIC	Dark Quencher	538	555


**Figure 1. General Thermal Profile for LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete**



\* Image acquisition.



**Table 8a. Thermal Profile for LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete: Applied Biosystems QuantStudio 5**

	Instrument	Applied Biosystems QS5	
	Format	96-Wells	
	Volume Setting	60 µL	
	Amp Cycles	'20'	
Ramp Rate	STEP A	2°C/sec	
Temperature/Time	STEP B	52°C	3 min
Ramp Rate	STEP C	2°C/sec	
Temperature/Time	STEP D* 	50°C Enable Auto Delta **	25 sec
Ramp Rate	STEP E	2°C/sec	
Temperature/Time	STEP F	58°C Enable Auto Delta **	1 sec

\* Acquire image during **STEP D** of cycling (50°C).


\*\* Enable auto delta starting at Cycle 1 with a temperature of +0.30 and time of +00.00 for cycling **STEPS D and F**

**NOTE:** Heated lids should be used for all cycling reactions.

**NOTE:** No passive reference dyes should be selected in instrument settings.

**NOTE:** Thermocyclers should be programmed and queued-up prior to reagent preparation.

**Table 8b. Thermal Profile for LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete: Applied Biosystems QuantStudio 7 Flex**

	Instrument	Applied Biosystems QS 7 Flex	
	Format	96-Wells	
	Volume Setting	60 µL	
	Amp Cycles	'20'	
Ramp Rate	STEP A	1.946°C/sec	
Temperature/Time	STEP B	50°C	3 min
Ramp Rate	STEP C	1.946°C/sec	
Temperature/Time	STEP D* 	50°C Enable Auto Delta **	25 sec
Ramp Rate	STEP E	1.946°C/sec	
Temperature/Time	STEP F	58°C Enable Auto Delta **	1 sec

\* Acquire image during **STEP D** of cycling (50°C).


\*\* Enable auto delta starting at Cycle 1 with a temperature of +0.30 and time of +00.00 for cycling **STEPS D and F**

**NOTE:** Heated lids should be used for all cycling reactions.

**NOTE:** No passive reference dyes should be selected in instrument settings.

**NOTE:** Thermocyclers should be programmed and queued-up prior to reagent preparation.

**Table 8c. Thermal Profile for LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete: Bio-Rad CFX OPUS**

	Instrument	CFX OPUS	
	Format	96-Wells	
	Volume Setting	50 µL	
	Amp Cycles	'20'	
Temperature/Time	PRE-STEP 1	45°C	00:00 (Forever)**
Ramp Rate	STEP A	Instrument default	
Temperature/Time	STEP B	51°C	2 min
Ramp Rate	STEP C	Instrument default	
Temperature/Time	STEP D* 	51°C	20 sec
Ramp Rate	STEP E	Instrument default	
Temperature/Time	STEP F	59°C	1 sec

\* Acquire image during STEP D of cycling (51°C).

\*\* PRE-STEP is to allow the lid to heat. Do not load RT-PCR Plate or continue to protocol STEP A until the lid has reached 105°C. Plate is inserted once the temperature is achieved. Once the plate has been loaded the HOLD step must be skipped to proceed with the rest of the protocol

NOTE: No passive reference dyes should be selected in instrument settings.

NOTE: Thermocyclers should be programmed and queued-up prior to reagent preparation.

**Table 9a. Optical and Analysis Settings:  
Applied Biosystems QuantStudio 5 and QuantStudio 7 Flex RT-PCR Instruments**

Plate Format	System Target (Fluor)	Instrument Excitation/Detection Settings	Baseline Cycles	Threshold
QS5 96-Well	SARS-CoV-2 (ROX)	580/623	1-2	50,000
	Influenza A (FAM)	470/520	1-2	50,000
	Influenza B (Cy5/Mustang Purple) Internal Control (VIC)	640/682	1-2	25,000
		520/558	1-2	50,000
QS7 Flex 96-Well	SARS-CoV-2 (ROX)	580/623	1-2	250,000
	Influenza A (FAM)	470/520	1-2	100,000
	Influenza B (Cy5/Mustang Purple) Internal Control (VIC)	640/682	1-2	150,000
		520/558	1-2	100,000

**Table 9b. Optical and Analysis Settings: Bio-Rad CFX OPUS RT-PCR Instrument**

Plate Format	Target (Fluor)	Baseline Cycles	Threshold
96-Well	SARS-CoV-2 (ROX)	1 – 2	2,000
	Influenza A (FAM)	1 – 2	3,000
	Influenza B (Cy5)	1 – 2	3,000
	Internal Control (HEX)	1 – 2	2,000

## 9. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Workflow

### 9.1 Quality Control

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user’s laboratory standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1200.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all Positive and Negative Controls Media when running diagnostic samples and with each new lot of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete to ensure all reagents and kit components are working properly.

- Good laboratory practice (GLP) recommends running a Positive Control (PCM) and Negative Control (NCM) in each amplification plate. All samples include an Internal Control for validation of enzyme, primer, and probe stability.

#### 9.1.1 Internal Controls

- An Internal Control is present in each reaction of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete assay for validation of enzyme, primer, and probe stability and performance.

#### 9.1.2 Positive and Negative Controls

- Test all Positive and Negative Controls Media when running diagnostic samples and with each new lot of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete kit to ensure all reagents and kit components are working properly.

## 9.2 Sample Preparation

LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete removes sample purification and extraction by combining lysis and amplification in a single step. This assay is compatible with swabs stored in compatible transport medium.

**NOTE:** Please handle the PCM with care as it can cause false positives if accidentally spilled or handled carelessly. To reduce the risk of cross-contamination, handle the PCM in a separate area than where samples are processed and use separate pipette tips for all materials.

**NOTE:** Nucleic acid amplification technologies, including qSTAR, are extremely sensitive and accidental introduction of product from previous amplification reactions can result in false positive results. Incorrect results can result if either the sample or the qSTAR reagents used in the amplification step become contaminated by accidental introduction of amplicon. Workflow in the laboratory should always proceed in a unidirectional manner to minimize the potential for such events.

- Maintain separate areas for assay setup and handling of samples.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, filtered pipette tips) for assay setup and handling of samples.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves often and whenever contamination is suspected.
- Keep tubes capped and plates sealed as much as possible.
- It is recommended that a cold block be used to keep materials cold as loose tubes on wet ice may lead to contamination.
- LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Extraction Buffer, LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete IC/P Mix, and LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Master Mix must be thawed and maintained on a cold block equilibrated to 4°C at all times during preparation and use. If the reagents are not entirely consumed in the first use, the reagents may be re-frozen no more than three times.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products (e.g. 10% bleach, DNAZap™, RnaseZap® or Rnase AWAY®, etc.) to minimize risk of nucleic acid contamination. Residual bleach should be removed using Nuclease Free Water and 70% Ethanol.

#### 9.2.1 Wet Swab

- a. No additional preparation is required, however, samples in greater than 3 mL of compatible transport medium may have reduced sensitivity due to lower concentration of virus in the medium. 1 mL of transport medium is recommended.

**NOTE:** An aliquot of this sample will be directly loaded into the RT-PCR plate

## 9.3 qSTAR Reagent Preparation and Plate Setup

Please read and thoroughly understand the following instructions before attempting to prepare the Reaction Mix and controls.

All components should be thawed and kept on a cold block equilibrated between 2 and 8°C to maintain the integrity of the reagents. It is recommended to queue the validated RT-PCR Instrument prior to performing the instructions below to ensure the performance of this assay is maintained.

#### 9.3.1 96-Well Format (60 µL Reaction Volume)

##### 1. Control, Samples, and Extraction Buffer Preparation

- a. Thaw the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete PCM, NCM, and Extraction Buffer on a cold block equilibrated between 2 and 8°C.
- b. Vortex tubes for 5 seconds.
- c. Centrifuge the tubes for 5 seconds to collect reagents at the bottom of each tube.

- d. Directly transfer 21.0  $\mu\text{L}$  of PCM, 21.0  $\mu\text{L}$  of NCM, and 21.0  $\mu\text{L}$  of sample to the appropriate wells of the pre-chilled RT-PCR Plate.
  - e. Add 5.0  $\mu\text{L}$  of Extraction Buffer to each well containing controls and sample(s).
- NOTE:** The addition of Extraction Buffer can be simplified by using a multi-channel pipette.
- f. Mix vigorously for at least 10 seconds (avoid creating bubbles).
  - g. Seal the RT-PCR plate with sealing film and spin down to collect the liquid at the bottom of the wells to ensure no reagent/sample is retained on the side walls of the wells.
  - h. Place the plate at 65°C for 5 minutes then, immediately place the RT-PCR Plate back on the cold block.

**NOTE:** The heat from the 65°C plate could potentially increase the temperature of the cold block to above 8°C. It is recommended that the cold block be swapped with a fresh block (equilibrated to 2 to 8°C) for each setup.

**NOTE:** If condensation is present, the plate may be spun down to collect the liquid at the bottom of the wells.

## 2. Reaction Mix Preparation

- a. Thaw the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Salt Mix, IC/P Mix, and Master Mix on a cold block equilibrated between 2 and 8°C.
- b. Obtain a sterile (Rnase/Dnase free) tube and place on a cold block.
- c. Determine the number of reactions ( $n$ ) to be prepared per assay:

**Table 10. Reaction Mix Volumes (60  $\mu\text{L}$  Reactions)**

Reaction Mix	1 Reaction	100 Reactions	$n$ Reactions
Salt Mix	10.0 $\mu\text{L}$	1000.0 $\mu\text{L}$	$n \times 10.0 \mu\text{L}$
IC/P Mix	4.0 $\mu\text{L}$	400.0 $\mu\text{L}$	$n \times 4.0 \mu\text{L}$
Master Mix	20.0 $\mu\text{L}$	2000.0 $\mu\text{L}$	$n \times 20.0 \mu\text{L}$
<b>Total Volume</b>	<b>34.0 <math>\mu\text{L}</math></b>	<b>3400.0 <math>\mu\text{L}</math></b>	<b><math>n \times 34.0 \mu\text{L}</math></b>

- d. Vigorously vortex the Salt Mix only for 20 seconds, centrifuge for 5 seconds to collect reagent at the bottom of tube, and **IMMEDIATELY** add the appropriate volume to the pre-chilled tube.
- e. Invert the IC/P Mix to mix then centrifuge for 5 seconds to collect reagents at the bottom of the tube (do not vortex), and **IMMEDIATELY** add the appropriate volume to the Salt Mix.
- f. Mix thoroughly for at least 10 seconds (avoid creating bubbles). Centrifuge briefly, then place tube back on the cold block.
- g. Invert the Master Mix to mix then centrifuge for 5 seconds to collect reagents at the bottom of the tube (do not vortex samples), and **IMMEDIATELY** add the appropriate volume to finalize the Reaction Mix.
- h. Mix thoroughly for at least 10 seconds (avoid creating bubbles). Centrifuge briefly, then place tube back on the cold block.
- i. Carefully remove the sealing film from the RT-PCR Plate and add 34.0  $\mu\text{L}$  of Reaction Mix to each well with control or sample. Mix thoroughly for at least 10 seconds (avoid creating bubbles).
- j. Apply the appropriate optical adhesive plate film. Centrifuge the plate for at least 20 seconds at 2000 rpm to collect the reaction at the bottom of each well. After centrifugation confirm that no bubbles persist. If bubbles are observed, centrifuge for 1 minute and repeat as needed until no bubbles are observed.
- k. Immediately place the 96-well plate in the pre-queued RT-PCR instrument.

## 9.4 Amplification

Once the RT-PCR Plate has been placed in a validated RT-PCR Instrument with the appropriate protocol selected based on **Section 8 Real-Time PCR Instrument and Protocol Summary**, start the run.

## 9.5 Interpretation and Reporting of Results

All assay controls should be examined prior to interpretation of patient results. If the controls are not valid, the results cannot be interpreted.

### 9.5.1 LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Controls

The LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Positive Control Media (PCM) is an external positive control needed to ensure test reagents are properly detecting SARS-CoV-2, influenza A and influenza B nucleic acids. It is comprised of a mixture of quantified template for each of the targets in the assay. The control is formulated in a proprietary matrix with purified, intact viral particles containing whole length genomes, which have been rendered non-infectious.

The LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Negative Control Media (NCM) is an external, negative, control needed to monitor cross-contamination, or reagent contamination did not occur during sample processing or reaction setup and is comprised of 1x Phosphate Buffered Saline (PBS), Ph 7.4.

An Internal Control (a component in the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Internal Control and Primer Mix) consists of a synthetic RNA – that the assay primers can bind and amplify from – with a unique probe region for molecular beacon detection in the **HEX/VIC** channel. The Internal Control serves as a control for detection of inhibitors present in the specimen, assures that adequate amplification has taken place, and that the enzymes and primers were not inadvertently damaged during production, shipment, and storage.

The testing algorithm for all instruments is based on the standard practice of determining background fluorescence and calling a sample well positive if the change in fluorescent signal exceeds an established threshold. Background fluorescence levels and thresholds are calculated and applied to each run as shown in **Table 10a – Table 10b**. Positive and negative results are based on a sample well exceeding this threshold. No Cycle Threshold (Ct) cutoff is used for this testing algorithm, and it is expected that all reactions should occur between a Ct value of 3 to 20.

Failure of the PCM or NCM invalidates the qSTAR run and results should not be reported. The qSTAR assay should be repeated with fresh aliquots of external controls and sample. If the results continue to be invalid contact technical support. If the Internal Control (IC) fails to amplify (in the absence of a positive signal), the qSTAR assay should be repeated as described above.

**Table 11. Expected Results from External Controls on Applied Biosystems QuantStudio5, QuantStudio 7 Flex, Bio-Rad CFX Opus 96-Well Instruments**

Control Type/Name	Used to Monitor	SARS-CoV-2 (ROX) Expected Ct Value (Result)	Flu A (FAM) Expected Ct Value (Result)	Flu B (Cy5/Mustang Purple) Expected Ct Value (Result)	IC (HEX/VIC) Expected Ct Value (Result)
PCM	Substantial reagent failure including primer and probe integrity	$3.0 \geq Ct \leq 20.0$ + (Positive)	$3.0 \geq Ct \leq 20.0$ + (Positive)	$3.0 \geq Ct \leq 20.0$ + (Positive)	$3.0 \geq Ct \leq 20.0$ + (Positive) OR - (Negative)*
NCM	Reagent and/or environmental contamination	Ct not detected - (Negative)	Ct not detected - (Negative)	Ct not detected - (Negative)	$3.0 \geq Ct \leq 20.0$ + (Positive)

\*The Internal Control is not required to amplify for the PCM to be deemed positive.

#### 9.5.2 LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Sample Results

Assessment of a sample test result should be performed after the PCM and NCM have been examined and determined to be valid. If the Controls are not valid, the results cannot be interpreted.

**Table 12. Ct Ranges for Positive Assay Results for the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete on Applied Biosystems QuantStudio5, QuantStudio 7 Flex, Bio-Rad CFX Opus 96-Well Instruments**

Sample or Control	Positive Ct Range*	IC (HEX/VIC) Ct Value	Interpretation of Results
<b>SARS-CoV-2 (ROX)</b>	$3.0 \geq Ct \leq 20.0$	$3.0 \geq Ct \leq 20.0$ + (Positive) OR - (Negative)**	SARS-CoV-2 detected
<b>Flu A (FAM)</b>	$3.0 \geq Ct \leq 20.0$	$3.0 \geq Ct \leq 20.0$ + (Positive) OR - (Negative)**	Flu A detected
<b>Flu B (Cy5/Mustang Purple)</b>	$3.0 \geq Ct \leq 20.0$	$3.0 \geq Ct \leq 20.0$ + (Positive) OR - (Negative)**	Flu B detected

\*For samples generating a Ct value greater than 0 and less than 3, perform 1:100 and/or 1:10 dilution using uninoculated compatible medium and process and test according to **Section 7 and Section 8**.

\*\* The Internal Control is not required to amplify.

**Table 13. Results Interpretation for the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete on Applied Biosystems QuantStudio5, QuantStudio 7 Flex, Bio-Rad CFX Opus 96-Well Instruments**

SARS-CoV-2 (ROX)	Flu A (FAM)	Flu B (Cy5/Mustang Purple)	IC (HEX/VIC)	Interpretation and Reporting of Results
+ (Positive)	+ (Positive)	+ (Positive)	+ (Positive) OR - (Negative)*	SARS-CoV-2, Flu A, and Flu B detected. Report results.
+ (Positive)	+ (Positive)	- (Negative)	+ (Positive) OR - (Negative)*	SARS-CoV-2, Flu A detected. Report results.
+ (Positive)	- (Negative)	+ (Positive)	+ (Positive) OR - (Negative)*	SARS-CoV-2, Flu B detected. Report results.
+ (Positive)	- (Negative)	- (Negative)	+ (Positive) OR - (Negative)*	SARS-CoV-2 detected. Report results.
- (Negative)	+ (Positive)	+ (Positive)	+ (Positive) OR - (Negative)*	Flu A, and Flu B detected. Report results.
- (Negative)	+ (Positive)	- (Negative)	+ (Positive) OR - (Negative)*	Flu A detected. Report results.
- (Negative)	- (Negative)	+ (Positive)	+ (Positive) OR - (Negative)*	Flu B detected. Report results.
- (Negative)	- (Negative)	- (Negative)	+ (Positive)	SARS-CoV-2, Flu A, Flu B not detected. Report results. Consider testing for other respiratory pathogens.
- (Negative)	- (Negative)	- (Negative)	- (Negative)	Invalid. Do not report results. Retest the same processed sample. If the retest is also invalid obtain a new specimen and retest.

\* The Internal Control is not required to amplify.

## 10 Limitations

- Do not use reagents past their expiration date.
- This test is qualitative and does not provide quantitative value(s) for target organism(s) in the sample.
- Detection of nucleic acids is dependent upon proper sample collection, handling, transportation, storage, and preparation. Improper collection, transport, or storage of samples may hinder the ability of this test to detect the target sequences.
- This test is for the detection and differentiation of RNA from SARS-CoV-2 and Influenza A/B and cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with a target organism and should not be the sole basis for treatment of patient management decisions.
- The performance of LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete was assessed using nasopharyngeal swab specimens only, performance with other specimen types has not been evaluated.
- There is a risk of false negative results due to the presence of sequence variants in the viral targets of this test.
- Inhibitors present in the specimen and/or errors in following the Test procedure may lead to false negative results.
- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Analyte targets (viral sequences) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, nor that they are the causative agents for clinical symptoms.
- This test performance was not established in immunocompromised patients.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. The clinical performance of this test 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 target viruses and their prevalence, which change over time.
- Interference with LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete was observed with samples containing either 5% (v/v) human blood or  $\geq 1.25$  mg/mL bovine mucin. Interference was not observed with samples containing  $\leq 2.5\%$  v/v human blood or  $\leq 0.625$  mg/mL bovine mucin.

## 11 Conditions of Authorization for the Laboratory

The "LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Letter of Authorization", along with the authorized "Fact Sheet for Healthcare Providers", the authorized "Fact Sheet for Patients", and other 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 LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete, the relevant Conditions of Authorization are listed below:



1. Authorized laboratories <sup>5</sup>using LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete 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.
2. Authorized laboratories using LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete must use the 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.
3. Authorized laboratories that receive the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete test must notify the relevant public health authorities of their intent to run this product prior to initiating testing.
4. Authorized laboratories using LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate
5. Authorized laboratories must collect information on the performance of LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete and report to DMD/OHT7/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and LumiraDx (via email: CustomerServices.US@LumiraDx.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
6. All laboratory personnel using LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete must be appropriately trained in nucleic acid amplification techniques and use appropriate personal protective equipment when handling this Test and use this product in accordance with the authorized labeling.
7. LumiraDx, authorized distributors, and authorized laboratories using LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete must ensure that any records associated with this EUA are maintained until otherwise notified by the FDA. Such records will be made available to the FDA for inspection upon request.

## 12 Performance Characteristics

### 12.1 Analytical Studies

#### 12.1.1 Analytical Sensitivity: (Spiked Sample Matrix)

The analytic sensitivity, or limit of detection (LoD) for each target of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete was determined using virus spiked directly into negative sample matrix consisting of pooled negative nasopharyngeal swab medium and tested on a validated RT-PCR instrument.

To determine the LoD serial dilutions of each template were made and spiked into negative sample matrix. A range-finding study was performed with 5 replicates at each concentration to determine the point at which at least 1 of the 5 replicates was not detected. 96 replicates per concentration were then performed for each target based on the range-finding study. The lowest concentration at which at least 95% of replicates were positive was determined to be the LoD. The confirmed LoD for virus spiked into negative sample matrix for each target of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete is shown in **Table 14-15**.

**Table 14. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete LOD Determination (Spiked Sample Matrix)**

Organism	Strain	LoD Concentration
SARS-CoV-2	USA-WA1/2020	$8.27 \times 10^1$ TCID <sub>50</sub> /mL
Influenza A H1N1	California/07/09	$9.12 \times 10^{-2}$ TCID <sub>50</sub> /mL
Influenza A H3N2	Switzerland/9715293/13	$2.45 \times 10^{-1}$ TCID <sub>50</sub> /mL
Influenza B (Victoria Lineage)	Washington/02/2019	$7.82 \times 10^{-2}$ TCID <sub>50</sub> /mL
Influenza B (Yamagata Lineage)	Phuket/3073/13	$2.08 \times 10^{-2}$ TCID <sub>50</sub> /mL

#### 12.1.2 Analytical Sensitivity: LoD (Spiked Swab)

The analytic sensitivity, or limit of detection (LoD) for each target of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete was determined using virus diluted in negative sample matrix and spiked onto swabs which were then eluted into 1x PBS, Ph 7.4 and tested in a validated RT-PCR instrument.

96 replicates per concentration were performed for each target. The lowest concentration at which at least 95% of replicates were positive was determined to be the LoD. The confirmed LoD for swabs spiked with virus and eluted into negative sample matrix for each target of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete is shown in

<sup>5</sup> 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."

**Table 15. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete LOD Determination (Spiked Swab)**

Organism	Strain	LoD Concentration
SARS-CoV-2	USA-WA1/2020	8.27 x 10 <sup>1</sup> TCID <sub>50</sub> /mL
Influenza A H1N1	California/07/09	1.82 x 10 <sup>-1</sup> TCID <sub>50</sub> /mL
Influenza A H3N2	Switzerland/9715293/13	2.45 x 10 <sup>-1</sup> TCID <sub>50</sub> /mL
Influenza B (Victoria lineage)	Washington/02/2019	7.82 x 10 <sup>-1</sup> TCID <sub>50</sub> /mL
Influenza B (Yamagata lineage)	Phuket/3073/13	2.08 10 <sup>-2</sup> TCID <sub>50</sub> /mL

**12.1.3 Inclusivity**

Inclusivity of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete was assessed by testing low concentrations (~3x LoD) using 3 replicates each for several strains of each of the target organisms, including several important variants of SARS-CoV-2 tested on a validated RT-PCR instrument. A summary of the results from inclusivity testing is shown in **Table 16**. The LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete successfully detected all inclusivity strains at the concentrations tested.

**Table 16. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Inclusivity**

Organism	Strain	Testing Concentration
SARS-CoV-2	Variant B.1.1.7 (Alpha)	2.48 x 10 <sup>2</sup> TCID <sub>50</sub> /mL
	Variant B.1.351 (Beta)	2.48 x 10 <sup>2</sup> TCID <sub>50</sub> /mL
	Variant B.1.617.2 (Delta)	2.48 x 10 <sup>2</sup> TCID <sub>50</sub> /mL
	Variant B.1.1.529 (Omicron)	2.48 x 10 <sup>2</sup> TCID <sub>50</sub> /mL
Influenza A H1N1	Brisbane/59/07	2.47 x 10 <sup>-1</sup> TCID <sub>50</sub> /mL
	New Cal/20/99	1.09 x 10 <sup>0</sup> TCID <sub>50</sub> /mL
	PR/8/34	4.38 x 10 <sup>1</sup> TCID <sub>50</sub> /mL
	Singapore/63/04	4.38 x 10 <sup>0</sup> TCID <sub>50</sub> /mL
	Taiwan/42/06	1.09 x 10 <sup>2</sup> TCID <sub>50</sub> /mL
Influenza A H3N2	Hong Kong/4801/14	2.94 x 10 <sup>0</sup> TCID <sub>50</sub> /mL
	Perth/16/09	8.82 x 10 <sup>1</sup> TCID <sub>50</sub> /mL
	Singapore/INEFIMH	2.94 x 10 <sup>0</sup> TCID <sub>50</sub> /mL
	South Australia/55/14	1.47 x 10 <sup>0</sup> TCID <sub>50</sub> /mL
	Stockholm/6/14	7.35 x 10 <sup>-1</sup> TCID <sub>50</sub> /mL
	Texas/50/12	7.35 x 10 <sup>-1</sup> TCID <sub>50</sub> /mL
Influenza B (Victoria Lineage)	Alabama/2/17	2.35 x 10 <sup>0</sup> TCID <sub>50</sub> /mL
	Brisbane/33/08	2.35 x 10 <sup>0</sup> TCID <sub>50</sub> /mL
	Brisbane/46/15	2.35 x 10 <sup>0</sup> TCID <sub>50</sub> /mL
	Colorado/06/17	2.35 x 10 <sup>0</sup> TCID <sub>50</sub> /mL
	Hawaii/10/01	2.35 x 10 <sup>0</sup> TCID <sub>50</sub> /mL
Influenza B (Yamagata Lineage)	Massachusetts/2/12	6.24 x 10 <sup>-2</sup> TCID <sub>50</sub> /mL
	Texas/6/11	1.25 x 10 <sup>-1</sup> TCID <sub>50</sub> /mL
	Utah/9/14	1.25 x 10 <sup>-1</sup> TCID <sub>50</sub> /mL
	Wisconsin/1/10	10 <sup>-1</sup> TCID <sub>50</sub> /mL

**12.1.4 Inclusivity (In Silico)**

Inclusivity of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete was assessed in silico as follows:

**SARS-CoV-2**

A sampling of approximately 2,000,000 sequences from the SARS-CoV-19 GISAID database was pulled randomly and processed for quality and completeness in September of 2022. High-coverage and full-length sequences were used in the analysis. The LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete showed perfect homology to >99% of all sequences



assessed in each oligonucleotide region and 1 mismatch or less in 100% of sequences assessed. The LumiraDx SARS-CoV-2 & Flu AB RNA STAR Complete is predicted to detect ~99.71% of all SARS-CoV-2 sequences assessed at the time of analysis.

The LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete showed perfect homology to >98.9% of all sequences assessed in each oligonucleotide region for analyzed Delta strains and homology with 1 mismatch or fewer for >99.8% of all sequences assessed in each oligonucleotide region for analyzed Omicron strains

**Table 17. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete In Silico Inclusivity: SARS-CoV-2**

SARS-CoV-2 Variant/Strain	Oligonucleotide Homology with 1 mismatch or less to Analyzed Sequences
Omicron	100%
Delta	100%
All strains analyzed	>99%

Independent analysis conducted on January 25, 2023 using 19,402 SARS-CoV-2 sequences deposited in the GISAID database within the preceding 4 weeks showed that the frequency of mutations associated with the individual SARS-CoV-2-specific oligonucleotides of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete was < 0.5%. Therefore, the likelihood of there being an adverse effect on device performance due to currently circulating variants was predicted to be low.

**Influenza A**

Two samplings were considered for influenza A, one included the entire database, while a second included only sequences from the previous 5 years. For the complete data set, the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete showed 1 mismatch or less to primers and probes for >96.6% of sequences assessed for influenza A and is predicted to detect >96.9% of influenza A sequences assessed at the time of analysis.

For the data set encompassing the previous 5 years, the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete showed 1 mismatch or less to primers and probes for >98.2% of influenza A sequences assessed and is predicted to detect >99.8% of influenza A sequences assessed at the time of analysis.

**Table 18. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete In Silico Inclusivity: Influenza A**

Target	Oligonucleotide Homology with 1 mismatch or less to Analyzed Sequences	Predicted Detection Rate
Influenza A (Complete data set)	>96.6%	>96.9%
Influenza A (Past 5 years)	>98.2%	>99.8%

**Influenza B**

Two samplings were considered for influenza B, one included the entire database, while a second included only sequences from the previous 5 years. For the complete data set, the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete showed 1 mismatch or less to primers and probes for >99.2% of sequences assessed for influenza B and is predicted to detect ~98.7% of influenza A sequences assessed at the time of analysis.

For the data set encompassing the previous 5 years, the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete showed 1 mismatch or less to primers and probes for >99.9% of influenza B sequences assessed and is predicted to detect ~98.8% of influenza B sequences assessed at the time of analysis.

**Table 19. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete In Silico Inclusivity: Influenza B**

Target	Oligonucleotide Homology with 1 mismatch or less to Analyzed Sequences	Predicted Detection Rate
Influenza B (Complete data set)	>99.2%	>98.7%
Influenza B (Past 5 years)	>99.9%	>98.8%

**12.1.5 Analytical Specificity: Cross-Reactivity and Microbial Interference**

Cross-reactivity and microbial interference for the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete were assessed by testing a panel of 41 microorganisms commonly found in the upper respiratory tract or which are genetically similar to on-panel organisms at high titers (as summarized in **Table 20**). Each organism was diluted in negative sample matrix and tested in triplicate. Testing was performed for each of the off-panel organisms alone and in the presence of each of the on-panel organisms at 3x LOD on a validated RT-PCR instrument. The results of this study are shown in **Table 20**. None of the organisms produced false positive results at the concentrations tested and all replicates of on-panel organisms were detected at 3x LOD in the presence of high-levels of off-panel organism.

**NOTE:** Concentrations are reported in TCID<sub>50</sub> (Median Tissue Culture Infectious Dose), CFU (Colony Forming Units), CCU (Colony Changing Units), Copies (genomic copies or genomic equivalents), and IFU (Inclusion Forming Units).

**Table 20. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Cross-Reactivity and Microbial Interference**

Off-Panel Organism	Strain	Off-Panel Organism Concentration Tested	Off-Panel Organism Testing Result	On-Panel Organism Testing Result
Adenovirus	Type 1	1.00 x 10 <sup>5</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Adenovirus	Type 5	1.00 x 10 <sup>5</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Adenovirus	Type 7	1.00 x 10 <sup>5</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Cytomegalovirus		2.00 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Enterovirus	Type D68	6.20 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Epstein-Barr Virus		1.00 x 10 <sup>5</sup> copies/mL	Not Detected	All organisms detected
Human coronavirus	229 E	1.70 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Human coronavirus	HKU1	2.70 x 10 <sup>4</sup> Copies/mL	Not Detected	All organisms detected
Human coronavirus	NL63	5.00 x 10 <sup>3</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Human coronavirus	OC43	1.00 x 10 <sup>5</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Human metapneumovirus (hMPV)		1.00 x 10 <sup>5</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Measles		2.00 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
MERS coronavirus		2.00 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Mumps		1.00 x 10 <sup>5</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Parainfluenza virus	Type 1	6.20 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Parainfluenza virus	Type 2	7.50 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Parainfluenza virus	Type 3	1.00 x 10 <sup>5</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Parainfluenza virus	Type 4a	2.70 x 10 <sup>3</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Respiratory syncytial virus (RSV)		1.70 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
Rhinovirus	Type 1A	8.00 x 10 <sup>3</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
SARS coronavirus		2.60 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	Not Detected	All organisms detected
<i>Bordetella pertussis</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Chlamydia pneumoniae</i>		1.00 x 10 <sup>6</sup> IFU/mL	Not Detected	All organisms detected
<i>Corynebacterium diphtheriae</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Escherichia coli</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Haemophilus influenzae</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Lactobacillus salivarius</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Legionella pneumophila</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Moraxella catarrhalis</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Mycobacterium tuberculosis</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Mycoplasma pneumoniae</i>		1.00 x 10 <sup>6</sup> CCU/mL	Not Detected	All organisms detected
<i>Neisseria gonorrhoeae</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Neisseria meningitidis</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Pneumocystis jirovecii</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Pseudomonas aeruginosa</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Staphylococcus aureus</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Staphylococcus epidermidis</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Streptococcus pneumoniae</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Streptococcus pyogenes</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Streptococcus salivarius</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected
<i>Candida albicans</i>		1.00 x 10 <sup>6</sup> CFU/mL	Not Detected	All organisms detected

### 12.1.6 Cross-reactivity (In Silico)

Cross-reactivity for the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete was assessed in silico by evaluating sequences of pathogens potentially present in respiratory specimens and/or with genetic similarities to target organisms. A summary of the organisms assessed for cross-reactivity is shown in **Table 21**. No cross-reactivity is predicted based on the in silico analysis.

**Table 21. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete In Silico Cross-Reactivity Organisms**

Viruses	Bacteria	Eukaryotes
Adenovirus (Type 1)	<i>Bordetella pertussis</i>	<i>Candida albicans</i>
Adenovirus (Type 5)	<i>Chlamydia pneumoniae</i>	<i>Pneumocystis jirovecii</i>
Adenovirus (Type 7)	<i>Corynebacterium diphtheriae</i>	Human genome
Cytomegalovirus	<i>Escherichia coli</i>	
Enterovirus D68	<i>Haemophilus influenzae</i>	
Epstein-Barr Virus	<i>Lactobacillus salivarius</i>	
Human coronavirus (229E)	<i>Legionella pneumophila</i>	
Human coronavirus (HKU1)	<i>Moraxella catarrhalis</i>	
Human coronavirus (NL63)	<i>Mycobacterium tuberculosis</i>	
Human coronavirus (OC43)	<i>Mycoplasma pneumoniae</i>	
Human metapneumovirus (hMPV)	<i>Neisseria gonorrhoeae</i>	
Influenza A*	<i>Neisseria meningitidis</i>	
Influenza B*		
Measles	<i>Pseudomonas aeruginosa</i>	
MERS coronavirus	<i>Staphylococcus aureus</i>	
Mumps	<i>Staphylococcus epidermidis</i>	
Parainfluenza virus (Type 1)	<i>Streptococcus pneumoniae</i>	
Parainfluenza virus (Type 2)	<i>Streptococcus pyogenes</i>	
Parainfluenza virus (Type 3)	<i>Streptococcus salivarius</i>	
Parainfluenza virus (Type 4a)		
Respiratory syncytial virus (RSV)		
Rhinovirus (Type 1A)		
SARS Coronavirus		
SARS-CoV-2*		

\*Used as negative for non-target assays and expected positive for target assays

### 12.1.7 Competitive Interference

Potential competitive interference amongst on-panel organisms was assessed by testing (heat-inactivated or live virus) of each on-panel organism at 3x LoD in the presence of the other on-panel organisms at high titer (1.00 x 10<sup>5</sup> TCID<sub>50</sub>/mL or 1.00 x 10<sup>5</sup> copies/mL) in triplicate on a validated RT-PCR instrument. The results of this study are shown in **Table 22**. No competitive inhibition between on-panel organisms was observed at the concentrations tested.

**Table 22. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Competitive Inhibition Study Results**

Low Titer Organism (3x LoD)	High Titer Organism	High Titer Organism Concentration	Low Titer Organism Detection Rate
SARS-CoV-2	Influenza A	1.00 x 10 <sup>5</sup> CEID50/mL	100%
	Influenza B	1.00 x 10 <sup>5</sup> CEID50/mL	100%
Influenza A	SARS-CoV-2	1.00 x 10 <sup>5</sup> TCID50/mL	100%
	Influenza B	1.00 x 10 <sup>5</sup> CEID50/mL	100%
Influenza B	SARS-CoV-2	1.00 x 10 <sup>5</sup> TCID50/mL	100%
	Influenza A	1.00 x 10 <sup>5</sup> CEID50/mL	100%

### 12.1.8 Potentially Interfering Substance

Substances commonly found in respiratory samples, substances that could be introduced during specimen collection, and medications commonly used to treat congestion, allergies, or asthma symptoms which could potentially interfere with the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete were individually evaluated at clinically relevant concentrations against low titer (3x LoD) on-panel organisms in negative clinical matrix on a validated RT-PCR instrument. A summary of substances tested is shown in **Table 23**. Interference was observed with human blood at 5% (v/v) and > 2.5 mg/mL bovine mucin. No interference was observed at lower concentrations of these substances.

**Table 23. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Potentially Interfering Substances**

Potentially Interfering Substance	Active Ingredient	Testing Concentration
Beclomethasone	Beclomethasone	0.1 mg/mL
Budesonide	Budesonide	0.1 mg/mL
Chloraseptic® Max	Phenol (1.5%), Glycerin (33%)	5% (v/v), or 0.075% Phenol, 1.65% Glycerin
Cold & Flu Relief Cough Syrup	Acetaminophen (325 mg), Dextromethorphan HBr (10 mg), Guaifenesin (200 mg), Phenylephrine (5 mg) per 15mL	5% (v/v), or Acetaminophen 1.08 mg/mL, Dextromethorphan 0.033 mg/mL, Guaifenesin 0.667 mg/mL, Phenylephrine 0.0167 mg/mL
Dexamethasone	Dexamethasone	0.1 mg/mL
Flonase	Fluticasone Propionate (50 mcg)	5% (v/v)
Flunisolide	Flunisolide (0.025%)	0.1 mg/mL
Mometasone Furoate	Mometasone Furoate	0.1 mg/mL
Mucin <sup>1</sup> , Type I-S (bovine)	Purified mucin protein	2.5, 1.25, 0.625, and 0.25mg/mL
Mupirocin	Mupirocin	0.1 mg/mL
Nasacort	Triamcinolone acetonide (55 mcg)	5% (v/v)
Saline Nasal Spray	Sodium chloride (0.65%)	5% (v/v) or 0.0325% NaCl
Oxymetazoline Nasal Spray	Oxymetazoline HCl (0.05%)	5% (v/v) or 0.0025% Oxymetazoline HCl
Phenylephrine Nasal Spray	Phenylephrine HCl (1%)	5% (v/v) or 0.05% Phenylephrine HCl
Tobramycin	Tobramycin	0.033 mg/mL
Vicks® VapoCOOL™ (throat lozenge)	Benzocaine, Menthol	15 mg/mL, 20 mg/mL
Zanamivir	Zanamivir	0.75 mg/mL
Zicam® (Nasal gel, homeopathic allergy relief)	Galphimia glauca, Luffa operculata, Sabailla	5% (v/v)
Whole Blood <sup>2</sup> (Human)	N/A	5, 2.5, and 1.25% (v/v)
Nicotine	N/A	0.03mg/mL

<sup>1</sup> Mucin inhibited only SARS-CoV-2 at 2.5 mg/ml and 1.25mg/mL. Mucin was diluted to 0.625mg/mL and 0.25mg/mL and showed 3 positives out of 3 replicates for all targets (100%).

<sup>2</sup> Whole blood was shown to interfere with Flu A, FluB, and SARS-CoV-2 targets at a 5% concentration. No interference was observed when diluted to a 2.5% or lower concentration.

#### 12.1.9 Repeatability

To assess the repeatability of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete, SARS-CoV-2 virus, influenza A virus, and influenza B virus (all rendered non-infectious) were spiked, individually, into negative clinical matrix at low and moderate positive concentrations (2x and 5x LoD) and tested over 6 days by two operators with two distinct reagent lots with four replicates per condition along with negative controls on a validated RT-PCR instrument. 100% of replicates were in agreement with the expected result demonstrating the repeatability of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete. A summary of repeatability testing conditions is shown in **Table 24**.

**Table 24. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Repeatability Conditions (Per Each Target)**

Condition	Negative Replicates	2x LoD Replicates	5x LoD Replicates
Days	6	6	6
Reagent Lots	2	2	2
Operators	2	2	2
Replicates/Condition	4	4	4
<b>Totals</b>	<b>96</b>	<b>96</b>	<b>96</b>

#### 12.1.10 Reproducibility

To assess the reproducibility of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete, SARS-CoV-2 virus, influenza A virus, and influenza B virus (all rendered non-infectious) were spiked, individually, into negative clinical matrix at low and moderate positive concentrations (2x and 5x LoD) and tested over 6 days by two operators at three distinct sites with three replicates per condition along with negative controls using a validated RT-PCR instrument. 100% of replicates were in agreement with the expected result demonstrating the reproducibility of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete. A summary of reproducibility testing conditions is shown in **Table 25**.

**Table 25. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Reproducibility Conditions**

Condition	Negative Replicates	2x LoD Replicates	5x LoD Replicates
Number of Sites	3	3	3
Days	6	6	6
Operators	2	2	2
Replicates/Condition	3	3	3
<b>Totals</b>	<b>108</b>	<b>108</b>	<b>108</b>

## 12.2 Clinical Study

An analysis was performed to demonstrate positive and negative percent agreement of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete. The performance of LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete was evaluated in comparison to an FDA-authorized method for SARS-CoV-2 and an FDA-cleared assay for influenza A/B, using a combination of prospectively collected and archived (retrospective) nasopharyngeal swab specimens. Positive and negative percent agreement between the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete and the comparator test are summarized in **Table 26-28**.

**Table 26. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Positive and Negative Percent Agreement: SARS-CoV-2**

		Comparator		
		Positive	Negative	Total
LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete <b>SARS-CoV-2</b>	Positive	285	1	286
	Negative	15	182	197
	<b>Total</b>	<b>300</b>	<b>183</b>	<b>483</b>
Percent Agreement		95.00% (Pos)	99.45% (Neg)	
95% Confidence Interval		91.89 – 97.17%	96.99-99.99%	

**Table 27. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Positive and Negative Percent: Influenza A**

		Comparator		
		Positive	Negative	Total
LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete <b>Influenza A</b>	Positive	94	3	97
	Negative	7	85	92
	<b>Total</b>	<b>101</b>	<b>88</b>	<b>189</b>
Percent Agreement		93.07% (Pos)	96.59% (Neg)	
95% Confidence Interval		86.24 – 97.17%	90.36 – 99.29%	

**Table 28. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Positive and Negative Percent Agreement: Influenza B**

		Comparator		
		Positive	Negative	Total
LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete <b>Influenza B</b>	Positive	40	2	42
	Negative	1	146	147
	<b>Total</b>	<b>41</b>	<b>148</b>	<b>189</b>
Percent Agreement		97.56% (Pos)	98.65% (Neg)	
95% Confidence Interval		87.14 – 99.94%	95.20 – 99.84%	

## 12.3 Equivalency Studies

### 12.3.1 Transport Medium Equivalency

A sample matrix equivalency study was performed to demonstrate the equivalency between four transport media. Several transport media were spiked with heat-inactivated virus at 1x LoD (a minimum of 20 replicates) and run on a validated RT-PCR instrument. Detection of ≥95% of positive samples at 1x LoD and 100% detection of IC in negative samples with no false positive results were necessary to determine equivalency for each of the media tested. A summary of media tested and determined to be equivalent is shown in **Table 29**.

**Table 29. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Medium Equivalency**

Equivalent Media
1X PBS, pH 7.4
Saline (0.85%)
Saline (0.90%)
Transport Medium*

\*CDC Viral Transport Medium or medium of similar formulation

### 12.3.2 Instrument Equivalency (96-Well)

An instrument equivalency study was performed to verify equivalent performance between four 96-well instruments. A minimum of 20 replicates of SARS-CoV-2, influenza A, and influenza B (each rendered non-infectious) were individually tested in negative sample matrix to verify the LoD for each instrument. All instruments tested demonstrated  $\geq 95\%$  detection at LoD for each assay target and 0% detection of negative samples with the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete. A summary of equivalent instruments is shown in **Table 30**.

**Table 30. LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete 96-Well Instrument Equivalency**

Instrument	Manufacturer
CFX Opus	Bio-Rad
QuantStudio5 (96-Well)	Applied Biosystems
QuantStudio 7 Flex (96-Well)	Applied Biosystems

## 13 Contact Information, Ordering, and Product Support

### For ordering, contact LumiraDx at:

Website: [www.LumiraDx.com](http://www.LumiraDx.com)

Email (US): [CustomerServices.US@LumiraDx.com](mailto:CustomerServices.US@LumiraDx.com).

Email (International): [CustomerServices@LumiraDx.com](mailto:CustomerServices@LumiraDx.com).

### For product information, contact LumiraDx at:

Email: [CustomerServices.US@LumiraDx.com](mailto:CustomerServices.US@LumiraDx.com). Include "LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete" in the subject line.

Phone: 1-888-586-4721.

### For technical support, contact LumiraDx at:

Email: [TechnicalServices@LumiraDx.com](mailto:TechnicalServices@LumiraDx.com). Include "LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete" in the subject line.

Phone: 1-888-586-4721

### For return policy, contact LumiraDx at:

If there is a problem with LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete you may be asked to return the item. Before returning the kit, please obtain a return authorization number from LumiraDx Customer Services ([CustomerServices.US@LumiraDx.com](mailto:CustomerServices.US@LumiraDx.com)). This return authorization number must be on the shipping carton for return. For ordinary returns following purchase, please contact LumiraDx Customer Services for terms and conditions.

### Intellectual property

The LumiraDx test and all provided LumiraDx documentation ('Products') are protected by law. The Intellectual Property of the LumiraDx Products remains at LumiraDx. Details of relevant Intellectual Property regarding our products can be found at [LumiraDx.com/IP](http://LumiraDx.com/IP).

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### Manufacturer Information

LumiraDx UK Ltd, Building 115, Bedford Technology Park, Thurleigh, Bedford MK44 2YA, UK Company Number 09206123.

### LumiraDx US Office

221 Crescent St, Suite 502, Waltham, MA 02453. Telephone: 1-617-621-9775.

### Made in the USA

LumiraDx 6650 Nancy Ridge Drive, San Diego, CA 92121.













### Limited Warranty

LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete – As per shelf life.

Reagents must be stored according to the required storage conditions as printed in this Instructions for use and they can be used only up to the expiry date printed on the kit box. For the applicable warranty period, LumiraDx warrants that each product shall be (i) of good quality and free of material defects, (ii) function in accordance with the material specifications referenced in the product insert, and (iii) approved by the proper governmental agencies required for the sale of products for their intended use (the "limited warranty"). If the product fails to meet the requirements of the limited warranty, then as customer's sole remedy, LumiraDx shall either repair or replace, at LumiraDx's discretion. Except for the limited warranty stated in this section, LumiraDx disclaims any and all warranties, express or implied, including but not limited to, any warranty of merchantability, fitness for

a particular purpose and non-infringement regarding the product. LumiraDx’s maximum liability with any customer claim shall not exceed the net product price paid by the customer. Neither party shall be liable to the other party for special, incidental or consequential damages, including, without limitation, loss of business, profits, data or revenue, even if a party receives notice in advance that these kinds of damages might result. The Limited Warranty above shall not apply if the customer has subjected the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Kit to physical abuse, misuse, abnormal use, use inconsistent with the LumiraDx instructions for use, fraud, tampering, unusual physical stress, negligence or accidents. Any warranty claim by Customer pursuant to the Limited Warranty shall be made in writing within the applicable Limited Warranty period.

## 14 Glossary of Symbols

	Temperature limitation		Use-by Date – The date after which the unopened reagent cannot be used.
	In vitro diagnostic medical device		Contains sufficient reagent for ‘n’ reactions
	Catalog Reference Number		Refer to <a href="http://www.lumiradx.com">www.lumiradx.com</a> for the electronic form of the instructions for use.
	Lot Number/ Batch Code		Negative Control Media
	Manufacturer		Positive Control Media
	Uncontaminated recycled content packaging, kit box, Instructions for Use is recyclable if it can be collected, separated, or otherwise recovered from the waste stream through an established recycling program.		Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner



## 15 Appendix A: Detailed Instructions for Instrument Setup (96-Well Format)

This appendix is intended to assist the user with the programming of instruments for use with the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete.

### 15.1 Instrument Setup for Applied Biosystems™ QuantStudio 5 (96-Well Format)

Refer to User Manual Part Number MAN0010407 for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate.

#### 15.1.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to Step 13, below before preparing the Reagent Mix as defined in **Section 9.3 qSTAR Reagent Preparation and Plate Setup**. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use only 96-well plates and seals that are compatible with the instrument manufacturer.

**NOTE:** If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a plate.

1. Launch the 'QuantStudio Design & Analysis' Desktop Software (version v1.5.1)
2. Select 'Create New Experiment'. A window will open with the 'Properties' tab selected.
3. Enter the 'Experiment Properties' as follows:
  - a. Name the experiment "SARS-CoV-2 Flu AB RNA STAR Complete Template"
  - b. Set 'Instrument type' to 'QuantStudio 5 System'
  - c. Set 'Block type' to '96-Well 0.2mL Block'
  - d. Set 'Experiment type' to 'Standard Curve'
  - e. Set 'Chemistry' to 'Other'
  - f. Set 'Run mode' to 'Standard'
4. Proceed to the 'Method' tab.
  - a. Click 'Action'
  - b. Select 'Optical filter settings' from the drop-down menu.
  - c. In the 'PCR Filter' table, uncheck all filter combinations except the following:
    - x1(470±15), m1(520±15) FAM
    - x2(520±10), m2(558±11) VIC
    - x4(580±10), m4(623±14) ROX
    - x5(640±10), m5(682±14) Cy5/Mustang Purple
  - d. Click 'Close' to return to the 'Method' tab.
  - e. Set the 'Volume' to '60 µL'.
5. In the 'Hold Stage' section, hover over 'Step 2' and click the '-' button that appears just above the wording to delete this plateau.
6. In the 'Hold Stage' section, change 'Step 1' settings as follows:
  - a. Set ramp rate to '2°C/s'
  - b. Set temp to '52°C'
  - c. Set time to '03:00'
7. In the 'PCR Stage' section, change 'Step 1' settings as follows:
  - a. Set ramp rate to '2°C/s'
  - b. Set temp to '50°C'
  - c. Set time to '00:25'
  - d. Set the data collection for this stage by ensuring that the camera icon is active (not grayed out)
  - e. Click the gear icon (within Step 1), an 'Advanced Settings' window will pop up
  - f. Check the 'Auto Delta' tab to enable auto delta
  - g. Set the 'Starting Cycle' to '1'
  - h. Set the 'Auto Delta Temperature' to + 0.30 (ensure that the '+' is selected under the 'v' dropdown adjacent to the settings box)
  - i. Leave the 'Auto Delta Time' at 00:00
  - j. Click 'Save'
8. In the 'PCR Stage' section, change 'Step 2' settings as follows:
  - a. Set ramp rate to '2°C/s'
  - b. Set temp to '58°C'
  - c. Set time to '00:01'
  - d. Click the gear icon (within Step 2), an 'Advanced Settings' window will pop up
  - e. Check the 'Auto Delta' tab to enable auto delta



- f. Set the 'Starting Cycle' to '1'
  - g. Set the 'Auto Delta Temperature' to + 0.30 (ensure that the '+' is selected under the 'v' dropdown adjacent to the settings box)
  - h. Leave the 'Auto Delta Time' at 00:00
  - i. Click 'Save'
9. At the bottom of the 'PCR Stage' section, set 'Cycles' to '20'.
  10. Proceed to the 'Plate' tab.
    - a. Change 'Passive Reference' to 'None' in the 'Plate Attributes' window.
    - b. Select all the wells from the plate layout.
  11. Proceed to the 'Advanced Setup' tab to set up the targets and controls.

#### COVID

- a. Under the 'Targets' section, rename 'Target 1' to '**COVID**'.
- b. Change the default color to red .
- c. Select '**ROX**' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB').
- d. Checkmark the empty box to the left of the colored box to apply it to the wells.

#### Influenza A

- a. 'Add' a second target then rename to '**Flu A**'
- b. Change the default color to blue
- c. Select '**FAM**' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB')
- d. Checkmark the empty box to the left of the colored box to apply it to the wells.

#### Influenza B

- a. 'Add' a third target then rename to '**Flu B**'
- b. Change the default color to purple
- c. Select '**Cy5**' or '**Mustang Purple**' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB')
- d. Checkmark the empty box to the left of the colored box to apply it to the wells.

#### Internal Control

- a. 'Add' a fourth target then rename to '**IC**'
- b. Change the default color to green
- c. Select '**VIC**' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB')
- d. Checkmark the empty box to the left of the colored box to apply it to the wells.

**Optional:** Under the 'Samples' section, the 'Sample Name' can be added individually or pasted into the 96-well plate layout from an Excel file.

12. Proceed to the 'Run' tab.
  - a. Save the experiment as a template for subsequent runs by clicking the 'Save' down arrow and selecting the 'Save As' option.
  - b. Name the template "SARS-CoV-2 Flu AB RNA STAR Complete RUO Template" and click 'Save'.
13. Load the plate as follows:
  - a. Press the 'eject' icon on the QuantStudio 5 instrument touchscreen at the top right of the window.
  - b. Place the sample plate on the amplification block. Make sure to align A1 on the 96-well plate with the defined A1 position on the instrument drawer.
  - c. Press the 'eject' icon on the instrument touchscreen to close the drawer.
14. Return to the desktop software 'Run' tab and click the 'START RUN' button. When instrument connection is made, the instrument number will appear in a drop-down menu below the 'START RUN' button.
15. Select the instrument number to initiate the run.
16. Change the 'File Name' to "LumiraDx SARS-CoV-2 Flu AB RNA STAR Complete [YYMMDD\_Plate#]" and click 'Save'.
17. Following the run, the instrument touchscreen indicates when the run is 'Complete'. Close the run screen by pressing 'Done'.
18. Remove the plate and discard the plate in a sealable waste bag or container.

**NOTE:** Wells containing PCM as well as those which were positive for a target will contain high levels of amplicon which can cause false positive results in future tests if allowed into the laboratory environment. Never remove the optical seal from the plate after amplification.

#### 15.1.2 Analysis Instructions

1. Modify the analysis settings using the 'Results' tab in the 'QuantStudio Design & Analysis' Desktop Software (version v1.5.1).
2. Select the desired wells for analysis in the Plate Layout then click on the Eye symbol above the 'Amplification Plot' to configure the plot as follows:
  - a. Set 'Plot Type' to 'ΔRn vs Cycle'
  - b. Set 'Graph Type' to 'Log'
  - c. Set 'Plot Color' to 'Target'
  - d. Click out of the window to accept changes (All other content remains unchanged)

3. Click the 'Gear' icon to the right of the 'Analyze' button in the top left of the software to open the 'Analysis Settings' window.

#### **COVID**

- a. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for COVID' section.
- b. Uncheck the 'Automatic Threshold' box
- c. Uncheck the 'Automatic Baseline' box
- d. Enter "50,000" for the 'Threshold'
- e. Set 'Baseline Start Cycle' to '1'
- f. Set 'End Cycle' to '2'
- g. Click 'Apply'

#### **Influenza A**

- a. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for Flu A' section.
- b. Uncheck the 'Automatic Threshold' box
- c. Uncheck the 'Automatic Baseline' box
- d. Enter "50,000" for the 'Threshold'
- e. Set 'Baseline Start Cycle' to '1'
- f. Set 'End Cycle' to '2'
- g. Click 'Apply'

#### **Influenza B**

- a. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for Flu B' section.
- b. Uncheck the 'Automatic Threshold' box
- c. Uncheck the 'Automatic Baseline' box
- d. Enter "25,000" for the 'Threshold'
- e. Set 'Baseline Start Cycle' to '1'
- f. Set 'End Cycle' to '2'
- g. Click 'Apply'

#### **Internal Control**

- a. Select the 'IC' line
  - b. Uncheck the 'Default settings' box
  - c. Uncheck the 'Automatic Threshold' box
  - d. Uncheck the 'Automatic Baseline' box.
  - e. Enter "50,000" for the 'Threshold'
  - f. Set 'Baseline Start Cycle' to '1'
  - g. Set 'End Cycle' to '2'.
  - h. Click 'Apply'.
4. Proceed the 'Export' tab to define the 'File Name' for the exported file ("SARS-CoV-2 Flu AB RNA STAR Complete [YYMMDD\_Plate#]").
  5. Define the 'File name'
    - a. Choose the 'File Type' (default is QuantStudio as an .xlsx file)
    - b. Choose the 'Location' to export the file
    - c. Choose the 'Content' to be exported (the 'Results' box needs to be checked for Ct values).
    - d. Click 'Customize' to proceed to what is to be exported within each Content item.
  6. Go to the 'Results' tab
    - a. Select the 'All Fields' box to uncheck all content
    - b. Check the boxes for the following content:
      - 'Well'
      - 'Well Position'
      - 'Sample Name'
      - 'Target Name'
      - 'Reporter'
      - 'CT'
      - 'Ct Threshold'
      - 'Baseline Start'
      - 'Baseline End'
    - c. Click 'Close' to return to the 'Export' tab screen.
  7. Click 'Save' to save the modified settings.
  8. Click the 'Export' button to generate the export data file. The exported file will include a 'Results' section which contains the sample Ct values.
  9. Close the software.

## 15.2 Instrument Setup for Applied Biosystems QuantStudio 7 Flex (96-Well Format)

Refer to User Manual Part Number 4489821 for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate.

### 15.2.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to Step 13, below before preparing the Reagent Mix as defined in **Section 9.3 qSTAR Reagent Preparation and Plate Setup**. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use only 96-well plates and seals that are compatible with the instrument manufacturer.

**NOTE:** If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a plate.

1. Launch the 'QuantStudio Real-Time PCR Software' Desktop Software (version v1.3).
2. On the toolbar select 'Tools' then 'Preferences' to open the 'Defaults' tab
3. Confirm that the 'Instrument Type:' is set to 'QuantStudio™ 7 Flex System'
4. Confirm that the 'Block Type:' is set to '96-Well Block (0.2mL)'
5. Confirm that the 'Decimal Places to Show:' is set to '3'.
6. Click the box next to 'Show optical filters for run method' and click on 'OK'.
7. Select 'Create New Experiment'. A window will open with the 'Properties' tab selected.
8. Enter the 'Experiment Properties' as follows:
  - a. Name the experiment "SARS-CoV-2 Flu AB RNA STAR Complete Template"
  - b. Set 'Instrument type' to 'QuantStudio™ 7 Flex System'
  - c. Set 'Block type' to '96-Well (0.2mL) Block'
  - d. Set 'Experiment type' to 'Standard Curve'
  - e. Set reagents being used to detect target sequences as 'Other'
  - f. Set the properties for the instrument run as 'Standard'
9. Proceed to the 'Define' tab under the 'Targets' section.

**COVID**

  - a. Under the 'Targets' section, rename 'Target 1' to "COVID"
  - b. Select 'ROX' as the reporter
  - c. Change the 'color' to red
  - d. Confirm that the 'Quencher' is automatically entered as 'NFQ-MGB'.

**Influenza A**

  - a. 'Add' a second target by clicking 'New' (just above the 'Target Name') then rename to 'Flu A'
  - b. Select 'FAM' as the reporter
  - c. Change the default color to blue
  - e. Confirm that the 'Quencher' is automatically entered as 'NFQ-MGB'.

**Influenza B**

  - a. 'Add' a third target then rename to 'Flu B'
  - b. Select 'Cy5' or 'Mustang Purple' as the reporter
  - c. Change the default color to violet
  - d. Confirm that the 'Quencher' is automatically entered as 'NFQ-MGB'.

**Internal Control**

  - a. 'Add' a fourth target then rename to 'IC'
  - b. Select 'VIC' as the reporter
  - c. Change the default color to green
  - d. Confirm that the 'Quencher' is automatically entered as 'NFQ-MGB'.

**Passive Reference Dye:**

  - a. Use the drop-down menu in the 'Passive Reference' section to change the passive reference dye to 'None'

**Optional:**

  - a. Under the 'Samples' section, the 'Sample Name' can be added individually.
10. Proceed to the 'Assign' tab.
  - a. Select all wells from the plate layout by clicking the box between 'A' and '1'.
  - b. On the left of the screen in the 'Targets' section, check the boxes next to 'COVID', 'Flu A', 'Flu B', and 'IC' to apply these targets to the wells.
  - c. If 'Samples' have been defined for each well, select individual wells and check the box next to the appropriate 'Name' in the 'Samples' section.
11. Proceed to the 'Run Method' tab.
  - a. Under the 'Run Method' section, define the 'Reaction Volume per Well' to "60" (µL).

- b. In the 'Graphical View' section, select the 'Hold Stage' and delete one step by right-clicking the step and clicking 'Delete Selected' so one 'Hold Stage' segment remains.
  12. Change the 'Hold Stage' 'Step 1' settings as follows:
    - a. Set the ramp rate to '1.946°C/s'
    - b. Set the temp to '50.0°C'
    - c. Set time to '03:00'.
  13. Change 'Step 1' in 'PCR Stage' as follows:
    - a. Set the ramp rate (middle) to '1.946°C/s'
    - b. Set the temp (upper middle) to '50°C'
    - c. Set the time (lower middle) to '00:25'
    - d. Check the 'Enable Auto Delta' box to enable auto delta
    - e. Set the 'Starting Cycle' to '1'
    - f. Click on the green up-arrow located in 'Step 1' to open the 'Auto Delta Settings' window
    - g. Set the 'Auto Delta Temperature' to + 0.30 (ensure that the '+' is selected under the 'v' dropdown adjacent to the settings box)
    - h. Leave the 'Auto Delta Time' at '0:00'
    - i. Click 'Save Setting' to exit the window
  14. Change 'Step 2' in 'PCR Stage' as follows:
    - a. Set the ramp rate (left) to '1.946°C/s'
    - b. Set the temp (upper right) to '58°C'
    - c. Set the time (lower right) to '00:01'
    - d. Check the 'Enable Auto Delta' box to enable auto delta.
    - e. Set the 'Starting Cycle' to '1'
    - f. Click on the green up-arrow located in 'Step 1' to open the 'Auto Delta Settings' window.
    - g. Set the 'Auto Delta Temperature' to + 0.30 (ensure that the '+' is selected under the 'v' dropdown adjacent to the settings box)
    - h. Leave the 'Auto Delta Time' at '0:00'
    - i. Click 'Save Setting' to exit the window
  15. 'Step 1' in the 'PCR Stage' is the data collection step. At the top of the 'PCR Stage', set 'Number of Cycles' to '20'.
  16. Near the top of the window next to the 'Graphical View' tab select 'Optical Filters'. Just below in the 'PCR Filter' section, uncheck all 'Emission Filter' combinations except the following:
    - x1(470±15), m1(520±15) FAM
    - x2(520±10), m2(558±11)VIC
    - x4(580±10), m4(623±14)ROX
    - x5(640±10), m5(682±14)Cy/Mustang Purple
  17. Save the experiment as a template for subsequent runs as follows:
    - a. Click the 'Save' drop-down menu and select 'Save As Template'.
    - b. Name the template "SARS-CoV-2 Flu AB RNA STAR Complete Template" and click 'Save'. The experiment must be saved again to connect to the instrument.
    - c. Click the drop-down menu next to 'Save' and select 'Save As'.
    - d. Save the 'File Name' as "LumiraDx SARS-CoV-2 Flu AB RNA STAR Complete [YYMMDD\_Plate#]" and click 'Save'.
  18. Load the plate as follows:
    - a. Press the red 'Arrow' icon on the QuantStudio 7 Flex instrument touchscreen at the bottom right of the window.
    - b. Place the sample plate on the amplification block that automatically comes out. Make sure to align A1 on the 96 well plate with the defined A1 position on the instrument drawer.
    - c. Press the red 'Arrow' icon on the instrument touchscreen to close the drawer.
  19. Begin the run as follows:
    - a. Return to the desktop software 'Run' tab and click the 'START RUN' button. The instrument number will appear in a drop-down menu below the 'START RUN' button.
    - b. Select the instrument number to initiate the run.
    - c. A pop-up window may appear indicating the ramp rate in Stage 1 and 2 is above the maximum. Select 'Yes' to proceed.
  20. Following the run, the instrument touchscreen indicates when the run is 'Complete'. Remove the plate and discard the plate in a sealable waste bag or container.
- NOTE:** Wells containing PCM as well as those which were positive for a target will contain high levels of amplicon which can cause false positive results in future tests if allowed into the laboratory environment. Never remove the optical seal from the plate after amplification.

### 15.2.2 Analysis Instructions

1. Modify the analysis settings using the 'Analysis' 'Amplification Plot' tab in the 'QuantStudio Real-Time PCR Software' Desktop Software (version v1.3).

2. Select the desired wells for analysis in the 'Plate Layout'
3. Configure the 'Amplification Plot' window on the left as follows:
  - a. Set 'Plot Type' to 'ΔRn vs Cycle'
  - b. Set 'Graph Type' to 'Log'
  - c. Set 'Plot Color' to 'Target'
4. On the top right of the software to the right of the 'Analyze' button, click the 'Analysis Settings' to open the 'Analysis Settings' window.

**COVID**

- a. In the 'Ct settings' tab in the bottom right window for 'CT Settings for COVID', uncheck the 'CT Settings to Use' box next to 'Default Settings'. (This will toggle the 'Automatic Threshold' and 'Automatic Baseline' boxes to be checked).
- b. Uncheck the box to the left of 'Automatic Threshold'
- c. Uncheck the box to the left of 'Automatic Baseline'
- d. Define the 'Threshold' as "250,000"
- e. Set the 'Baseline Start Cycle' to '1'
- f. Set 'End Cycle' to '2'.

**Influenza A**

- a. In the 'Ct settings' tab in the bottom right window for 'CT Settings for Flu A', uncheck the 'CT Settings to Use' box next to 'Default Settings'. (This will toggle the 'Automatic Threshold' and 'Automatic Baseline' boxes to be checked).
- b. Uncheck the box to the left of 'Automatic Threshold'
- c. Uncheck the box to the left of 'Automatic Baseline'
- d. Define the 'Threshold' as "100,000"
- e. Set the 'Baseline Start Cycle' to '1'
- f. Set 'End Cycle' to '2'.

**Influenza B**

- a. In the 'Ct settings' tab in the bottom right window for 'CT Settings for Flu B', uncheck the 'CT Settings to Use' box next to 'Default Settings'. (This will toggle the 'Automatic Threshold' and 'Automatic Baseline' boxes to be checked).
- b. Uncheck the box to the left of 'Automatic Threshold'
- c. Uncheck the box to the left of 'Automatic Baseline'
- d. Define the 'Threshold' as "150,000"
- e. Set the 'Baseline Start Cycle' to '1'
- f. Set 'End Cycle' to '2'.

**Internal Control**

- a. In the 'Select a Target' window (on the bottom left), select the 'IC'
- b. In the 'CT Settings for IC' uncheck the 'Default settings'
- c. In the 'CT Settings for IC' uncheck the 'Automatic Threshold'
- d. In the 'CT Settings for IC' uncheck the 'Automatic Baseline'
- e. Define the 'Threshold' as "100,000"
- f. Set 'Baseline Start Cycle' to '1'
- g. Set 'End Cycle' to '2'.
- h. On the bottom of the window, select 'Apply Analysis Settings' to close out of the 'Analysis Settings' pop-up window.

5. On the left of the screen, proceed to the 'Export' tab:
  - a. Define the 'Export File Name' as "SARS-CoV-2 Flu AB RNA STAR Complete [YYMMDD Plate#]"
  - b. Choose the 'File Type' (default is QuantStudio as an .xls file)
  - c. Choose the 'Export File Location' to export the file
  - d. Select the desired tabs below 'Export File Location' to determine what data is exported.

**Note:** The box next to the 'Results' tab needs to be checked to export CT values.

6. Within the 'Results' tab select the following:
  - a. 'Well'
  - b. 'Well Position'
  - c. 'Sample Name'
  - d. 'Target Name'
  - e. 'Reporter'
  - f. 'Quencher'
  - g. 'CT'
  - h. 'Ct Threshold'
  - i. 'Baseline Start'

- j. 'Baseline End'
  - k. Click 'Start Export' at the bottom of the screen to generate the export data file. (The exported file will include a 'Results' section which contains the sample Ct values).
7. Click 'Save' to save the modified settings.
  8. Close the software.

### 15.3 Instrument Setup for Bio-Rad CFX OPUS (96-Well Format)

Refer to User Manual Part Number 10000119983 for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate.

#### 15.3.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to set up the instrument up to Step 16, below before preparing the Reagent Mix as defined in **Section 9.3 qSTAR Reagent Preparation and Plate Setup**. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use only 96-well plates and seals that are compatible with the instrument manufacturer.

1. Launch the 'Bio-Rad CFX Maestro™' Software (version v2.2). In the 'Startup Wizard' pop-up window, select 'User-defined' as the run type.
2. In the 'Run Setup' pop-up window, select 'Edit Selected...' on the right of the window under the 'Protocol' tab to begin setup.
3. The 'Protocol Editor' pop-up window will open. Set the 'Sample Volume' to "50"  $\mu$ L (the sample volume should not be set higher than this).
4. Click 'Insert Step' to add a total of five steps to the protocol.
5. Edit steps '1' through '5' as follows:
  - a. Set step '1' temp to '45.0' C
  - b. Set '1' time to '0:00' (this sets the time to 'Forever')
  - c. Set step '2' temp to '51.0' C, set step '2' time to '2:00'
  - d. Set step '3' temp to '51.0' C, set '3' time to '0:20' and 'Add Plate Read' by clicking the icon on the left.
  - e. Set step '4' temp to '59.0' C, set '4' time to '0:01'
  - f. Set step '5 GO TO' to '3' and '19' x (this defines that step 3 through step 4 will be repeated a total of 20 times).
  - g. Select 'OK' and then select 'Yes' to save the changes to the protocol file.
  - h. Define the 'File name' as "SARS-CoV-2 Flu AB RNA STAR Complete Template.prcl" in the appropriate file path and select 'Save'.
6. Proceed to the 'Plate' tab by selecting the 'Next' button on the bottom right of the 'Protocol Editor' window.
7. Select 'Edit Selected...' on the right of the window.
8. Select the entire plate by selecting the gray box between position 'A' and '1' on the plate.
9. Ensure that 'Scan Mode' is set to 'All Channels' by using the drop-down menu.
10. Set up the fluorophores by clicking 'Select Fluorophores...' on the far top right of the window.
  - a. Ensure the only fluorophores selected are 'FAM', 'ROX', 'HEX', and 'Cy5'
  - b. Change the 'Color' for 'FAM' to blue.
  - c. Change the 'Color' for 'ROX' to red.
  - d. Change the 'Color' for 'HEX' to green.
  - e. Change the 'Color' for 'Cy5' to purple.
  - f. Select 'OK' to close out of the pop-up window.
11. To define targets select 'Experiment Settings...' near the bottom right of the window to open the 'Experiment Settings' pop-up window and do the following as follows:
  - a. Under the 'Targets' tab, add a new target by defining 'New' as "COVID" and selecting 'Add'.
  - b. Add a second target and define 'New' as "Flu A" then select 'Add'.
  - c. Add a third target and define 'New' as "Flu B" then select 'Add'.
  - d. Add a fourth target and define 'New' as "IC" then select 'Add'.
  - e. Under the 'Exclude the following sample types from Gene Expression analysis' at the bottom of the pop-up window, deselect all checkboxes and select 'OK' to close out the pop-up window.
  - f. Select the entire plate again by selecting the gray box between position 'A' and '1' on the plate and ensure the check boxes to the left of the 'Load' selection are checked for 'FAM', 'ROX', 'HEX', and 'Cy5'.
  - g. Using the drop-down menu under 'Target Name' define the 'Target Name' for '**ROX**' as 'COVID'
  - h. Define the 'Target Name' for '**FAM**' as 'Flu A'.
  - i. Define the 'Target Name' for '**Cy5**' as 'Flu B'.
  - j. Define the 'Target Name' for '**HEX**' as 'IC'.
12. Select the plate type as follows:
  - a. Click 'Settings' from 'Plate Editor' menu bar
  - b. Select 'Plate Type' from the dropdown list.

- c. Select 'BR White' from the dropdown sub-list.
  - d. Select 'OK' and save the changes by clicking 'Yes'.
13. Define the 'File name' as "SARS-CoV-2 Flu AB RNA STAR Complete Template.pltd" in the appropriate file path and select 'Save' to save the plate file.
14. The 'Plate Editor' pop-up window will close and the new plate will be loaded in the 'Plate' tab of the 'Run Setup' window. Proceed to the 'Start Run' tab by selecting 'Next >>' on the bottom right of the window.
15. On the 'Start Run' tab, click 'Start Run'
16. Save the experiment by defining the 'File name' as "SARS-CoV-2 Flu AB RNA STAR Complete [YYMMDD\_Plate#].pcrd" in the appropriate file path then selecting 'Save'. The run will begin initializing and lid will begin preheating.

**NOTE:** The run will stay paused on Step 1 until the 'Skip Step' button is clicked. When the sample plate is ready and the lid is preheated, proceed to the next step to add the sample plate to the amplification block and begin the run.

17. To load the plate do the following:
  - a. At the bottom left of the screen, select 'Open Lid'. A pop-up window may appear indicating the run will pause to open the lid.
  - b. Click 'OK' to proceed. The lid will automatically open.
  - c. Place the sample plate on the amplification block
  - d. Select 'Close Lid' to close the lid.
  - e. Once the lid has finished closing, the 'Skip Step' button will become available.
  - f. Select 'Skip Step' to initiate the run.
18. When the run finishes, the instrument will become idle and the 'Data Analysis' window will pop up.
19. Refer to the 'Bio-Rad CFX Maestro' Software (version v2.2) and select 'Open Lid'. The lid will automatically open.
20. Remove the plate and discard the plate in a sealable waste bag or container. Select 'Close Lid' to close the lid.

**NOTE:** Wells containing PCM as well as those which were positive for a target will contain high levels of amplicon which can cause false positive results in future tests if allowed into the laboratory environment. Never remove the optical seal from the plate after amplification.

### 15.3.2 Analysis Instructions

1. In the 'Data Analysis' pop-up window under the 'Quantification' tab, select 'Settings' in the main menu then select 'Baseline Setting' and then 'Baseline Subtracted Curve Fit'.
2. Edit the baseline and cycle threshold settings for **COVID** by performing the followings steps:
  - a. Under the 'Amplification' curves, uncheck all fluorophore boxes **except 'ROX'**.
  - b. From the main menu, select 'Settings'
  - c. Select 'Baseline Threshold...' to open the 'Baseline Threshold' pop-up window.
  - d. Under the 'Baseline Cycles' section, select 'User Defined' then, immediately below this, click the square between 'Well' and '1' to select all the wells.
  - e. Edit 'All Selected Rows: Begin' to "1" and 'End:' to "2".
  - f. In the 'Single Threshold' section, select 'User Defined:' and set this to "2000.00".
  - g. Click 'OK' to proceed.
3. Edit the baseline and cycle threshold settings for **Flu A** by performing the followings steps:
  - a. Under the 'Amplification' curves, uncheck all fluorophore boxes **except 'FAM'**.
  - b. From the main menu, select 'Settings'
  - c. Select 'Baseline Threshold...' to open the 'Baseline Threshold' pop-up window.
  - d. Under the 'Baseline Cycles' section, select 'User Defined' then, immediately below this, click the square between 'Well' and '1' to select all the wells.
  - e. Edit 'All Selected Rows: Begin' to "1" and 'End:' to "2".
  - f. In the 'Single Threshold' section, select 'User Defined:' and set this to "3000.00".
  - g. Click 'OK' to proceed.
4. Edit the baseline and cycle threshold settings for **Flu B** by performing the followings steps:
  - a. Under the 'Amplification' curves, uncheck all fluorophore boxes **except 'Cy5'**.
  - b. From the main menu, select 'Settings'
  - c. Select 'Baseline Threshold...' to open the 'Baseline Threshold' pop-up window.
  - d. Under the 'Baseline Cycles' section, select 'User Defined' then, immediately below this, click the square between 'Well' and '1' to select all the wells.
  - e. Edit 'All Selected Rows: Begin' to "1" and 'End:' to "2".
  - f. In the 'Single Threshold' section, select 'User Defined:' and set this to "3000.00".
  - g. Click 'OK' to proceed.
5. Edit the baseline and cycle threshold settings for **IC** by performing the followings steps:
  - a. Under the 'Amplification' curves, uncheck all fluorophore boxes **except 'HEX'**.
  - b. From the main menu, select 'Settings'
  - c. Select 'Baseline Threshold...' to open the 'Baseline Threshold' pop-up window.

- d. Under the 'Baseline Cycles' section, select 'User Defined' then, immediately below this, click the square between 'Well' and '1' to select all the wells.
  - e. Edit 'All Selected Rows: Begin' to "1" and 'End:' to "2".
  - f. In the 'Single Threshold' section, select 'User Defined:' and set this to "2000.00".
  - g. Click 'OK' to proceed.
6. After defining baseline and cycle threshold settings for COVID, Flu A, Flu B and IC, check both boxes on the left of all channels then proceed to the 'Quantification Data' tab.
  7. Right-click over the 'Results' table, and chose the desired method for exporting the data (e.g. 'Export to Excel...').
  8. Save the report by defining the 'File name' as "SARS-CoV-2 Flu AB RNA STAR Complete Cq Results [YYMMDD\_Plate#].xlsx" in the appropriate file path then selecting 'Save'.
  9. Close out the software. (If prompted, save the changes to the experiment).

## 16 Appendix B: Instrument Qualification

This appendix is intended to provide a qualification procedure describing how to prepare a panel of mock specimens for use in verifying performance of the below RUO instruments with the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete test by the end user. Qualification of these instruments with the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete test must be achieved prior to usage for diagnostic testing.

- Applied Biosystems QuantStudio 5 (software version 1.5.1) (96-Well Format)
- Applied Biosystems QuantStudio 7 Flex (software version 1.3) (96-Well Format)
- Bio-Rad CFX Opus (software version 2.2) (96-Well Format)

### 16.1 Required Materials

**Table 31. SARS-CoV-2 & Flu A/B RNA STAR Complete Kit Components and Volumes (96-Well Kits)**

Item	Quantity
LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Kit (96-Well)	1 Kit

### 16.2 Precautions

The LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete Positive Control Media (PCM) is an external positive control needed to ensure test reagents are properly detecting SARS-CoV-2, influenza A and influenza B nucleic acids. It is comprised of a mixture of quantified template for each of the targets in the assay. The control is formulated in a proprietary matrix with purified, intact viral particles containing whole length genomes, which have been rendered non-infectious.

### 16.3 Procedure and Expected Results

If test results fail to meet expected results, contact LumiraDx Technical Support by telephone at 1-888-586-4721 or by email at [TechnicalServices@LumiraDx.com](mailto:TechnicalServices@LumiraDx.com).

#### Control, Samples, and Extraction Buffer Preparation

A new Positive Control vial should be used with each instrument run. The qualification run will consist of running 5 replicates of positive control media neat (~5x LoD), 5 replicates of positive control material diluted 1:1.66 in NCM (~3x LoD) and 5 replicates of negative control media (Negative). The steps below will provide instructions on how to prepare the 5x Positive, 3x Positive, and Negative Samples to be used in each RNA STAR Complete Reaction.

1. Thaw the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete kit in a cold block equilibrated between 2 and 8°C.
2. Vortex the Negative Control Media (NCM) and Positive Control Media (PCM) for 5 seconds then pulse centrifuge for 5 seconds to collect reagents at the bottom of the tube.
3. Create the "Negative" sample tube as follows:
  - a. Pipette 120 µL of NCM from the SARS-CoV-2 & Flu A/B RNA STAR Complete Kit to the "Negative" tube.
4. Create the 3x LoD Positive tube as follows:
  - a. Pre-chill a tube labelled "3x Positive"
  - b. Dilute 72 µl of PCM from the SARS-CoV-2 & Flu A/B RNA STAR Complete Kit in 48 µl of NCM in the "3x Positive" tube for a final volume of 120 µL.
5. Create the 5x LoD Positive tube as follows:
  - a. Pre-chill a tube labelled "5x Positive"
  - b. Pipette 120 µL of stock (undiluted) PCM from the SARS-CoV-2 & Flu A/B RNA STAR Complete Kit to the "5x Positive" tube.
6. Load the Instrument Qualification PCR Plate with Positive and Negative samples as follows:
  - a. Directly transfer 21.0 µL of "5x Positive" to each of 5 wells of the PCR Plate
  - b. Directly transfer 21.0 µL of "3x Positive" to each of 5 wells of the PCR Plate
  - c. Directly transfer 21.0 µL of "Negative" to each of 5 wells of the PCR Plate



**NOTE:** No external controls are required for the qualification run.

7. Add 5.0 µL of Extraction Buffer to each well containing positive or negative samples.

**NOTE:** The addition of Extraction Buffer can be simplified by using a multi-channel pipette.

8. Mix vigorously for at least 10 seconds (avoid creating bubbles).
9. Seal the RT-PCR plate with sealing film and spin down to collect the liquid at the bottom of the wells to ensure no reagent/sample is retained on the side walls of the wells.
10. Place the plate at 65°C for 5 minutes then, immediately place the RT-PCR Plate back on the cold block.

**NOTE:** The heat from the 65°C plate could potentially increase the temperature of the cold block to above 8°C. It is recommended that the cold block be swapped with a fresh block (equilibrated to 2 to 8°C) for each setup.

**NOTE:** If condensation is present, the plate may be spun down to collect the liquid at the bottom of the wells.

#### Reaction Mix Preparation and Amplification

Prepare the Reaction Mix as described in the qSTAR Reagent Preparation and Plate Setup Section under heading **8.3.1 96-Well Format (60 µL Reaction Volume) Bullets 2 a-k “Reaction Mix Preparation”** and proceed with the amplification appropriate to your instrument of choice using its appropriate thermal profile and analysis(found in **Section 8. Real-Time PCR Instrument and Protocol Summary** of these instructions for use).

Use **Table 32** below to record the results of testing and to verify that the acceptance criteria have been met.

**Table 32. Instrument Qualification Procedure Results**

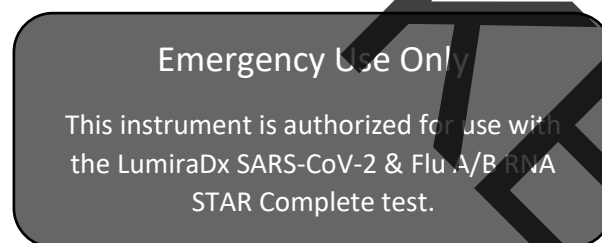
Controls Name	SARS-CoV-2 (ROX) Acceptable Ct Values	Flu A (FAM) Acceptable Ct Values	Flu B (Cy5) Acceptable Ct Values	IC (HEX) Acceptable Ct Values	Acceptance Criteria	Instrument Result
5x Positive	$3.0 \leq Ct \leq 20.0$	$3.0 \leq Ct \leq 20.0$	$3.0 \leq Ct \leq 20.0$	$3.0 \leq Ct \leq 20.0^*$	5/5 (100%)**	
3x Positive	$3.0 \leq Ct \leq 20.0$	$3.0 \leq Ct \leq 20.0$	$3.0 \leq Ct \leq 20.0$	$3.0 \leq Ct \leq 20.0^*$	5/5 (100%)**	
Negative	None Detected	None Detected	None Detected	$3.0 \leq Ct \leq 20.0^*$	0/5 (0%)	

\* The internal control is not required to amplify for the Positive Sample to be deemed positive.

\*\* Requirement must be met for each of the 3 targets independently.

#### 16.4 Additional Label

For all RUO RT-PCR Instruments including the Bio-Rad CFX Opus (software version 2.2), Applied Biosystems QuantStudio 5 (software version 1.5.1), and Applied Biosystems QuantStudio 7 Flex (software version 1.3).



Please print and place this label on the front panel of the above instrument(s). If the instruments include labeling indicating “For Research Use Only”, please cover with the below “Emergency Use Only” labeling. The instruments should retain this labeling throughout the EUA use of the LumiraDx SARS-CoV-2 & Flu A/B RNA STAR Complete test.

Refer to **16 Appendix B: Instrument Qualification** for detailed instrument qualification instructions.