EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE COVID-FLU MULTIPLEX ASSAY

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

The COVID-Flu Multiplex Assay will be performed at Exact Sciences Laboratories located at 650 Forward Drive, Madison, WI 53711, which is certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, and meets the requirements to perform high-complexity tests, as described in the Standard Operating Procedures that were reviewed by the FDA under this EUA.

INTENDED USE

The COVID-Flu Multiplex Assay is a real-time RT-PCR assay intended for the simultaneous qualitative detection and differentiation of nucleic acid from SARS-CoV-2, influenza A virus, and/or influenza B virus in anterior nasal swab specimens self-collected in a healthcare setting by individuals suspected of respiratory viral infection consistent with COVID-19 by a healthcare provider. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar.

This test is also for use with anterior nasal swab specimens that are (1) self-collected at home by individuals age 18 years and older using the Exact Sciences Nasal Swab Home-Collection Kit when home collection is determined to be appropriate by a healthcare provider, or (2) collected using the Everlywell COVID-19 & Flu Test Home Collection Kit when used consistent with its authorization. Specimens collected using the Exact Sciences Nasal Swab Home Collection Kit and Everlywell COVID-19 & Flu Test Home Collection Kit can be transported at ambient temperature for testing.

Testing is limited to Exact Sciences Laboratories, located at 650 Forward Drive, Madison, WI 53711, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meets the requirements to perform high complexity tests.

Results are for the simultaneous detection and differentiation of SARS-CoV-2, influenza A, and/or influenza B viral nucleic acid in clinical specimens, and is not intended to detect influenza C. Nucleic acid from influenza A, influenza B, and SARS-CoV-2 viruses is generally detectable in anterior nasal 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 nucleic acid 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 diagnosis, treatment, or other patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

The COVID-Flu Multiplex assay is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The COVID-Flu Multiplex Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The COVID-Flu Multiplex Assay is a high-throughput real-time reverse transcription polymerase chain reaction (rRT-PCR) test with a testing capacity of 120,000 tests per week. The test is a multiplex assay, run in a single well/vessel that contains three primer/probe sets with identical sequences to those utilized in the previously authorized Center for Disease Control and Prevention Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay for the detection of SARS-CoV-2 virus, influenza A virus, and influenza B virus (SC2, FluA, and FluB, respectively). The assay also contains primers and a probe to detect the human RNase P gene (RP) in clinical specimens or control samples. The oligonucleotide primers and probe for detection of SARS-CoV-2 were selected from an evolutionarily conserved region of the 3' terminus of SARS-CoV-2 genome and include part of the carboxy-terminal portion of the nucleocapsid (N) gene. Primers and probes for the detection of influenza A viruses were selected from an evolutionarily well conserved region of the matrix (M1) gene. The primers and probe selected for detection of influenza B viruses were selected from a conserved region of the nonstructural 2 (NS2) gene.

RNA isolated from anterior nasal swab specimens that are complementary to the oligonucleotide primers are reverse transcribed into cDNA and subsequently amplified using the ThermoFisher TaqPath 1-Step RT- qPCR Master Mix and Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS version 1.4.1 software. During the amplification process, the probe(s) anneal to specific target sequences located between the corresponding forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe bound to the specific target, causing the reporter dyes to separate from the quencher dye, generating a fluorescent signal. Probes specific to each virus generate a fluorescent signal at different wavelengths, enabling the instrument to differentiate between the signals. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by Applied Biosystems 7500 Fast Dx Real-Time PCR System.

INSTRUMENTS USED WITH THE TEST

RNA extraction based on conventional well-known bead-based technology is conducted using the non-commercial, Exact Sciences Corporation extraction procedure on the Hamilton STARlet liquid handler. RT-PCR is performed on the ABI 7500 Fast Dx (Applied Biosystems) Real-Time PCR instrument (software version 1.4.1).

REAGENTS AND MATERIALS USED WITH THE TEST

Reagents	Manufacturer	Catalog #
Nucleic Acid Extraction Reagents		
Carrier / Poly (A)	Exact Sciences Corporation	200885
Elution buffer (TE buffer)	Exact Sciences Corporation	200888
GTC / 10% IGEPAL	Exact Sciences Corporation	200890
Binding Beads (BND BDS)	Exact Sciences Corporation	200886
Conversion Wash	Exact Sciences Corporation	200887
Isopropanol (IPA)	Fisher Scientific Company LLC	500085
PCR Reagents		
Applied Biosystems TaqPath 1-Step Multiplex Master Mix (No ROX)	Thermo Fisher	A28523
Flu/SC Oligo Mix Kit contents: FluA, FluB, SARS-CoV-2, RNase P	Exact Sciences Corporation	200936 200980
DNA Suspension buffer (TE buffer)	TekNova	T0222

The following additional equipment, reagents, and materials are required to run this test:

1. MicroAmp Optical 96-Well Reaction plates with barcode
2. MicroAmp Optical Adhesive Film
3. STARlet CO-RE filtered tips (50, 300 & 1000 µL)
4. ABI Optical Sealing Tool
5. Axygen Deep Well Plate (DWP)
6. Carolina Chill Block
7. Hamilton Cold Block and Tip Carrier
8. ABI Block Cleaning Set
9. Small Trough Carrier
10. Splash Free 96 Well Base

11. Heat block with 2.0 mL tube insert
12. Molecular grade water, nuclease-free
13. Vortex mixer
14. Mini Plate Spinner Centrifuge
15. Mini Capsule Centrifuge
16. Surface disinfectant (10% bleach wipes and alcohol wipes)
17. 200 mL reagent trough and reagent trough lid
18. 50 and 200 mL reagent carrier
19. CAR-SMP-32 carriers with cryovial
20. Ion bar, 44", 8 emitters, 120 V
21. Aluminum seal tape for 96 well plates

HOME COLLECTION KITS USED WITH THE TEST

The COVID-Flu Multiplex Assay is to be used with the following home collection kits:

- Everlywell COVID-19 & Flu Test Home Collection Kit – to self-collect anterior nasal swab specimens by individuals age 18 years and older (self-collected), 16 years and older (self-collected under adult supervision), or 2 years and older (collected with adult assistance) when determined to be appropriate by a healthcare provider. Collection and inspection of self-collected anterior nasal swabs is performed according to the Accessioning Standard Operating Procedure (SOP) and Instructions for Use of the Everlywell COVID-19 & Flu Test Home Collection Kit. Everlywell has granted ESL a right of reference to the data supporting use of this authorized home collection kit. Additional information about kit components, medical oversight, and accessioning procedures can be found in the Everlywell COVID-19 & Flu Test Home Collection Kit EUA summary.

- Exact Sciences Nasal Swab Home Collection Kit – to self-collect anterior nasal swab specimens by individuals age 18 years and older when determined to be appropriate by a healthcare provider. Testing of self-collected anterior nasal swabs is performed according to the Accessioning SOP and Exact Sciences Laboratories COVID-Flu Multiplex Assay SOP reviewed under this EUA. Additional information about the kit components, medical oversight and accessioning procedures is provided in the sections below.

EXACT SCIENCES NASAL SWAB HOME COLLECTION KIT COMPONENTS

Exact Sciences Nasal Swab Home Collection Kit consists of the following:

Item Name	Description	Quantity	Material Supplier
Anterior Nasal Swab	Synthetic-tipped with a plastic or aluminum shaft	1	Steripak, Fisher Scientific, Puritan Medical Products
Transport media	2 mL Conical Tube, filled with 1.2	1	Exact Sciences

Item Name	Description	Quantity	Material Supplier
(0.9% saline)	mL 0.9% Saline		
Collection tube label	Patient sample identification label	1	Tailored Label Products
Biohazard bag (with absorbent pad)	Leakproof Specimen bag with 3 x 4" Absorbent Sheet	1	Bag: Uline, Fisher Pad: VWR, Fisher
Bubble wrap bag	Air bubble wrap bag with minimum bubble size of 3/16"	1	Uline, Associated Bag
Shipping box with return label	9 ¼ x 3 x 6 ¾" Corrugated Box	1	Green Bay Packaging
Self-collection instructions	Detailed self-collection and shipping instructions with images	1	Exact Sciences

MEDICAL OVERSIGHT AND PROCESS TO BE USED FOR SAMPLE COLLECTION

Medical Oversight of the self-collected Exact Sciences Nasal Swab Home Collection Kit ordering process is provided by the healthcare provider who is ordering the test. When using this kit, patients who are 18 years and older will be evaluated by the healthcare provider either in person or via telemedicine. After the patient is determined to be eligible for at-home self-collection with the Exact Sciences Nasal Swab Home Collection Kit and downstream testing with the COVID-Flu Multiplex Assay based on current CDC screening guidelines, the healthcare provider will place a test order.

When the Exact Sciences Nasal Swab Home Collection Kit is used, the test order is placed by the healthcare provider with Exact Sciences Laboratories (ESL). The Exact Sciences Nasal Swab Home Collection Kits are shipped to patients by ESL with return labels addressed to ESL. The test results are communicated by ESL to the healthcare provider. The healthcare provider is responsible for reporting the result and next steps to the patient.

INSPECTION OF SPECIMENS

Specimens received from patients using the Everlywell COVID-19 & Flu Home Test and Collection Kit should be processed according to the Everlywell Standard Operating Procedures. ESL submitted an SOP for receipt and accessioning of samples collected with the Exact Sciences Nasal Swab Home Collection Kit. The protocol is summarized below.
Specimens received through the Exact Sciences Nasal Swab Home Collection Kit will be checked for the following rejection and exception criteria before entering the workflow. If the sample meets any of the rejection criteria, the sample will be rejected. If the sample meets any of the exception criteria, an attempt will be made to correct the issue. Issues that cannot be resolved will also result in rejection of the sample.

Rejection Criteria

- Labels are not present on sample tube
- Specimen received without two patient identifiers
- Swab not submitted in approved liquid
- Swabs with calcium alginate tips
- Swabs with wooden shafts
- Specimen is grossly contaminated
- Transport device is broken or leaking
- No transport media is within the collection tube, resulting in no sample for testing
- Specimen received >72 hours after collection

Exception Criteria

- Discrepant patient identifiers between the tube and the requisition form or Electronic Data Interchange
- No submitter
- No provider
- No DOC provided on either specimen or requisition form

PATIENT INCLUSION/EXCLUSION CRITERIA

Inclusion of patients 18 years or older suspected of respiratory viral infection consistent with COVID-19, when home collection is determined to be appropriate by a healthcare provider and performed using the Exact Sciences Nasal Swab Home Collection Kit. Individuals with no symptoms are not eligible to be tested using the Exact Sciences Nasal Swab Home Collection Kit with the COVID-Flu Multiplex Assay.

CONTROLS TO BE USED WITH THE TEST

Controls for each run include:

- Extraction No Target (ENT) serves as an extraction control to monitor for any cross-contamination that could occur during the extraction process. The ENT consists of saline (ESL specimen collection media) and is included in each extraction batch and PCR run.

- A No Target Control (NTC) is used to monitor the possibility of reagent contamination and sample contamination in the assay run and is used once on every PCR assay plate. The control is TE buffer.

- A Positive Extraction Control (PEC) is used to verify that the assay extraction and RT-PCR assay run is performing as intended. The PEC contains targets for FluA, FluB, SARS-CoV-2 N1, and human RNase P, and consists of Influenza A/B NATtrol Positive

Control (Zeptometrix), AccuPlex SARS-CoV-2 Reference material (Seracare), AccuPlex SARS-CoV-2 Negative Reference Material (Human RNase P; Seracare), in TE buffer. The positive extraction control is included in each extraction batch and PCR run.

- Internal Control: Each sample that contains nucleic acid must demonstrate the presence of the internal control (IC) amplicon. The IC is created from PCR amplification of a locus within the human RNase P gene and monitors adequate amounts and quality of RNA in the sample and correct sample processing.

INTERPRETATION OF RESULTS

Control Result Interpretation

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. If any of the above controls do not exhibit the expected performance as described in the below table, the assay may have been improperly set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

Table 1. Expected Results of Controls

Control	2019 nCoV_N1	FluA	FluB	RP	Expected Ct Values
ENT	-	-	-	-	< 10 and ≥ 35 for RP, < 8 and ≥ 40 for nCoV N1, FluA and FluB
NTC	-	-	-	-	< 10 and ≥ 35 for RP, < 8 and ≥ 40 for nCoV N1, FluA and FluB
PEC	+	+	+	+	≥10 and < 35 for RP, ≥ 8 and < 40 for nCoV N1, FluA and FluB

Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. The Ct cutoff values shown above for each assay target when running the controls should also be applied for the interpretation of patient results.

Table 2. Results Interpretation Table for the Exact Sciences COVID-Flu Multiplex Assay

RP	nCoV_N1	Flu A	Flu B	Result
+	-	-	-	**Negative**: SARS-CoV-2 (COVID-19) RNA NOT detected. Negative results do not preclude SARS-CoV-2 (COVID-19) infection and should not be used as the sole basis for treatment for other patient management decisions. Flu A or Flu B RNA NOT detected. Negative results do not preclude Flu A or Flu B infection and should not be used as the sole basis for treatment for other patient management decisions.
+	+	-	-	**Positive**: SARS-CoV-2 (COVID-19) RNA detected.
+	-	+	-	**Positive:** Flu A RNA detected
+	-	-	+	**Positive:** Flu B RNA detected
+	+	+	-	**Positive:** SARS-CoV-2 (COVID-19) RNA detected. **Positive:** Flu A RNA detected
+	+	-	+	**Positive:** SARS-CoV-2 (COVID-19) RNA detected. **Positive:** Flu B RNA detected
+	-	+	+	**Positive:** Flu A RNA detected **Positive:** Flu B RNA detected
+	+	+	+	**Positive**: SARS-CoV-2 (COVID-19) RNA detected. **Positive:** Flu A RNA detected **Positive:** Flu B RNA detected
-	+/-	+/-	+/-	**Invalid**: This specimen exhibited inhibition in the PCR assay, or the specimen contained an inadequate amount of clinical material. Repeat testing is suggested if clinically indicated.

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) - Analytical Sensitivity:

To determine the sensitivity of the COVID-Flu Multiplex Assay, preliminary and confirmatory limit of detection (LoD) studies were conducted using one strain each of inactivated SARS-CoV-2 virus and live influenza A and influenza B virus. Strain information is provided in **Table 3** below.

Table 3. Viral Strains Used in Limit of Detection Studies*

Analyte	Strain/Source
SARS-CoV-2	USA-WA1/2020 (NATSARS(COV2)-ST)
Influenza A	FluA Brisbane/59/07
Influenza B	FluB Florida/02/06

*Although contemporary influenza A and B strains were not used for analytical studies, primer and probe sequences included in the Exact Sciences COVID-Flu Multiplex Assay are identical to those used in the CDC SARS-CoV-2 (Flu SC2) Multiplex Assay. The CDC has provided a right of reference to all validation data contained in the CDC SARS-CoV-2 (Flu SC2) Multiplex Assay EUA and the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel 510(k).

In the preliminary LoD study anterior nasal swab specimens collected from individuals negative for SARS-CoV-2, influenza A and influenza B virus were eluted in 0.9% saline and combined to create pooled negative clinical matrix. The pooled negative clinical matrix was spiked with inactivated SARS-CoV-2 virus or live influenza A or influenza B virus, and five-fold serial dilutions were tested in triplicate with the COVID-Flu Multiplex assay using the Hamilton STARlet liquid handler and the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument as indicated in the laboratory instructions for use. The preliminary LoD, where 100% of the replicates tested positive for the individual targets, was determined to be 18.208 copies/mL, 0.049 $TCID_{50}$/mL, and 0.040 $TCID_{50}$/mL for SARS-CoV-2, influenza A and influenza B, respectively.

Table 4. Preliminary LoD Study Summary

Target	Concentration	Concentration Units	Ratio Detected	Avg. Ct of Positives
SARS-CoV-2	91.04	cp/mL	3/3	34.0
SARS-CoV-2	**18.208**	**cp/mL**	**3/3**	**34.4**
SARS-CoV-2	3.642	cp/mL	0/3	ND
Flu A	0.243	$TCID_{50}$/mL	3/3	33.7
Flu A	**0.049**	**$TCID_{50}$/mL**	**3/3**	**33.8**
Flu A	0.010	$TCID_{50}$/mL	0/3	ND
Flu B	0.202	$TCID_{50}$/mL	3/3	33.7
Flu B	**0.040**	**$TCID_{50}$/mL**	**3/3**	**34.2**
Flu B	0.008	$TCID_{50}$/mL	1/3	34.1

Additional extraction replicates (n=20) of SARS-CoV-2, influenza A and influenza B virus in clinical matrix were tested at and above the preliminary LoD concentrations to confirm the assay LoD for each target. Samples were individually extracted and tested following the COVID-Flu Multiplex Assay protocol. The LoD for SARS-CoV-2, influenza A, and influenza B virus was determined to be 182 copies/mL, 0.24 $TCID_{50}$/mL and 0.40 $TCID_{50}$/mL, respectively, as defined by the lowest concentration which yielded ≥95% (19/20) positivity. A summary of the LoD confirmatory study results is provided in **Table 5**.

Table 5. Confirmatory LoD Study Summary:

Target	Conc.	Units	Replicates Positive for target	Percent Positivity	Avg. Ct of Positives
SARS-CoV-2	**182**	**cp/mL**	**20/20**	**100%**	**34.0**
SARS-CoV-2	91.04	cp/mL	16/20	80%	34.3
SARS-CoV-2	18.12	cp/mL	9/20	45%	34.9
Flu A	**0.243**	**$TCID_{50}$/mL**	**19/20**	**95%**	**33.8**
Flu A	0.049	$TCID_{50}$/mL	15/20	75%	34.8
Flu B	**0.404**	**$TCID_{50}$/mL**	**19/20**	**95%**	**33.9**
Flu B	0.202	$TCID_{50}$/mL	13/20	65%	34.5
Flu B	0.040	$TCID_{50}$/mL	5/20	25%	35.0

To support combining all three assay targets into one sample for downstream analytical studies, an additional study was conducted to demonstrate that the LoD of each analyte is similar when present alone or in combination with the other analytes in the panel. The study was performed with the same SARS-CoV-2, influenza A and influenza B strains as above spiked into negative clinical matrix near (within 2-fold the confirmed LoD). Twenty individual extraction replicates were tested. The results confirmed that combining targets does not significantly alter the LoD of any individual target.

Table 6: Results of Combined Target LoD in negative clinical matrix:

Target	Conc.	Units	Ratio Confirmed	Avg. Ct
SARS-CoV-2	364	cp/mL	20/20	34.0
Flu A	0.486	$TCID_{50}$/mL	20/20	33.8
Flu B	0.809	$TCID_{50}$/mL	19/20	34.0

LoD Bridging Study to Validate Use of HEX-labeled Flu B Probes in the Flu/SC Oligo Mix

The COVID-Flu Multiplex Assay was initially authorized with a Flu/SC Oligo Mix that contains Yakima Yellow (YAKYEL) labeled Flu B oligo probe. As an alternative source, a Flu B probe labeled with HEX dye was validated. The Flu B probe sequence is identical and there is no change to the fluorophore emission channel. Therefore, no calibration is needed for the instrument and software used to run the COVID-Flu Multiplex Assay.

An LoD bridging study was performed to establish equivalent performance between parallel testing of same nasal swab specimens with the HEX Labeled probe mix and the YAK labeled probe mix. Testing consisted of three-fold serial dilutions of viral materials (**Table 3**) in pooled nasal swab specimen matrix in twenty replicates until a hit rate of <95% was achieved. Results are summarized in **Table 7** and show that the LoD with HEX-labeled oligo mix for each target was the same or lower than the LoD concentration with the YAKYEL-labeled oligo mix (within 3 Ct).

Table 7: LoD Bridging Study Results Summary

Target*	Conc.	Number of reps	Covid/Flu Oligo mix with YAKYEL				Covid/Flu Oligo mix with HEX Probe			
			Avg CT	SD	# Pos	% Pos	Avg CT	SD	# Pos	% Pos
SARS-CoV-2 (Copies/mL)	1092	20	31.87	0.33	20	100%	31.69	0.53	20	100%
	364	20	**32.55**	**0.29**	**20**	**100%**	32.47	0.41	20	100%
	121	20	33.18	0.37	18	90%	**33.01**	**0.46**	**20**	**100%**
	40	20	33.54	0.33	8	40%	33.44	0.24	8	40%
	13	20	33.10	0.30	3	15%	33.43	0.00	2	10%
Flu A $TCID_{50}$/mL	1.458	20	29.44	0.74	20	100%	29.27	0.78	20	100%
	0.486	20	30.57	0.51	20	100%	30.60	0.45	20	100%
	0.162	20	**31.71**	**0.41**	**20**	**100%**	31.73	0.53	20	100%
	0.054	20	33.15	0.16	15	75%	**33.09**	**0.30**	**20**	**100%**
	0.018	20	33.13	0.12	4	20%	NA	NA	0	0%
Flu B $TCID_{50}$/mL	2.427	20	29.84	0.37	20	100%	29.84	0.42	20	100%
	0.809	20	31.08	0.37	20	100%	31.33	0.30	20	100%
	0.270	**20**	**32.23**	**0.29**	**20**	**100%**	**32.39**	**0.31**	**20**	**100%**
	0.090	20	34.00	0.07	3	15%	33.95	0.10	2	10%
	0.030	20	NA	NA	0	0%	33.86	NA	1	5%
RPP30	N/A	20	24.59	0.28	20	100%	24.52	0.42	20	100%

Target*	Conc.	Number of reps	Covid/Flu Oligo mix with YAKYEL				Covid/Flu Oligo mix with HEX Probe			
			Avg CT	SD	# Pos	% Pos	Avg CT	SD	# Pos	% Pos
(Internal Control)	N/A	20	24.99	0.24	20	100%	24.78	0.26	20	100%
	N/A	20	25.41	0.27	20	100%	25.24	0.38	20	100%
	N/A	20	26.13	0.34	20	100%	26.01	0.22	20	100%
	N/A	20	26.12	0.21	20	100%	25.83	0.34	20	100%

*Study was performed with all 3 targets mixed as one specimen, at the specified concentration of each target.

2) ***Inclusivity (analytical sensitivity):***

The COVID-Flu Multiplex Assay primer and probe set sequences are identical to the CDC SARS-CoV-2 (Flu SC2) Multiplex Assay and, although the remaining COVID-Flu Multiplex Assay reagents and workflow differ slightly from the CDC SARS-CoV-2 (Flu SC2) Multiple Assay, the differences are not expected to impact inclusivity of the device. Therefore, the inclusivity data provided by the CDC by Right of Reference are sufficient to support inclusivity of the COVID-Flu Multiplex Assay. Please refer to the CDC SARS-CoV-2 (Flu SC2) Multiplex Assay EUA summary for detailed *in silico* and wet testing SARS-CoV-2, influenza A and influenza B inclusivity information.

An additional *in silico* analysis assessing the impact of the recently emerged SARS-CoV-2 variants on target detection show that none of the observed mutations would be expected to interfere with N gene detection.

3) ***Cross-reactivity (Analytical Specificity)***

The COVID-Flu Multiplex Assay primer and probe set sequences are identical to the CDC SARS-CoV-2 (Flu SC2) Multiplex Assay and, although the remaining COVID-Flu Multiplex Assay reagents and workflow differ slightly from the CDC SARS-CoV-2 (Flu SC2) Multiple Assay, the differences are not expected to impact cross-reactivity of the device. Therefore, the cross-reactivity data provided by the CDC by Right of Reference are sufficient to support cross-reactivity of the COVID-Flu Multiplex Assay. Please refer to the CDC SARS-CoV-2 (Flu SC2) Multiplex Assay EUA summary for detailed *in silico* and wet testing SARS-CoV-2, influenza A and influenza B cross-reactivity information.

4) ***Competitive Interference:***

Competitive inhibition testing was performed to assess the effects of co-infection with SARS-CoV-2, and influenza A or influenza B on test results. For this study, live influenza A and influenza B strains with viral titers reported in copies/mL were used to ensure that the

concentrations tested in the study were appropriately challenging. Because these strains differed from those used in the original limit of detection (LoD) study, the sensitivity of the assay was re-established with the new influenza strains. The LoD of the assay for influenza A (H3N2) was 1,563 copies/mL with a mean Ct of 32.8 and the LoD for influenza B (Yamagata), 1,000 copies/mL with a mean Ct of 32.79.

The first set of samples were created to evaluate the impact of high influenza A or influenza B concentrations on detection of the other panel analytes. To create the contrived co-infection specimens, negative clinical nasal swab matrix was spiked with high concentrations of either influenza A ($1x10^6$ copies/mL and $1x10^7$ copies/mL) or influenza B ($1x10^6$ copies/mL) virus, to which low concentrations of SARS-CoV-2, influenza A or influenza B were added (3x or 5xLoD).

The samples were tested with the COVID-19-Flu Multiplex Assay in triplicate. No inhibition of SARS-CoV-2 detection was observed in the presence of high influenza A or influenza B concentrations. Similarly, no inhibition was observed in samples containing low influenza A paired with high influenza B concentrations, or low influenza B paired with high influenza A concentrations. Results from the study are summarized in **Tables 8-9** below.

Table 8: High Flu A with low SARS-COV-2 (SC2) or Flu B

Test condition			Average CT				Percent Positives		
High Target	**Low Target**	**n**	**RP**	**FluA**	**FluB**	**SC2**	**FluA**	**FluB**	**SC2**
Flu A- 10^6 copies/mL	3x LoD SC2	3	26.10	22.15	ND	33.50	100%		100%
Flu A- 10^6 copies/mL	5x LoD SC2	3	25.97	22.14	ND	32.71	100%		100%
Flu A- 10^6 copies/mL	3x LoD Flu B	3	26.15	21.97	29.43	ND	100%	100%	
Flu A- 10^6 copies/mL	5x LoD Flu B	3	26.14	21.91	28.71	ND	100%	100%	
Flu A- 10^7 copies/mL	3x LoD SC2	3	24.80	19.13	ND	33.63	100%		100%
Flu A- 10^7 copies/mL	5x LoD SC2	3	24.26	18.96	ND	33.61	100%		100%
Flu A- 10^7 copies/mL	3x LoD Flu B	3	24.35	18.92	31.07	ND	100%	100%	
Flu A- 10^7 copies/mL	5x LoD Flu B	3	25.01	18.92	30.36	ND	100%	100%	

Table 9: High Flu B with low SARS-COV-2 (SC2) or Flu A

Test condition			Average CT				Percent Positives		
High Target	**Low Target**	**n**	**RP**	**FluA**	**FluB**	**SC2**	**FluA**	**FluB**	**SC2**
Flu B- 10^6	3x LoD SC2	3	25.41	ND	22.01	32.72		100%	100%

Test condition			Average CT				Percent Positives		
High Target	**Low Target**	**n**	**RP**	**FluA**	**FluB**	**SC2**	**FluA**	**FluB**	**SC2**
copies/mL									
Flu B- 10^6 copies/mL	5x LoD SC2	3	25.51	ND	22.17	32.24		100%	100%
Flu B- 10^6 copies/mL	3x LoD Flu A	3	25.35	29.27	21.99	ND	100%	100%	
Flu B- 10^6 copies/mL	5x LoD Flu A	3	25.04	28.40	22.06	ND	100%	100%	

Additional studies were conducted to evaluate the impact of high SARS-CoV-2 concentrations on influenza A and influenza B detection. In one study, samples containing inactivated SARS-CoV-2 virus spiked into clinical nasal swab matrix at $1x10^5$ copies/mL were mixed with low concentrations of either influenza A or influenza B virus stocks (3x and 5x LoD). The mean Ct value for the SARS-CoV-2 target in these samples was 27.2, compared to the mean Ct value of 34.0 observed at LoD. Under these conditions, no inhibition of influenza A or influenza B virus was observed.

Further evaluation of higher concentrations of SARS-CoV-2 virus that are known to occur in clinical samples was conducted to determine the potential for competitive inhibition across panel analytes. For these studies, two separate pools of SARS-CoV-2 positive specimens were diluted to obtain a target Ct value of 20. Low concentrations of influenza A and influenza B virus were spiked into the specimen pools at 3x and 5x LoD concentrations and tested in triplicate. Data from the studies showed variable results between the two specimen pools. The first SARS-CoV-2 positive specimen pool inhibited influenza A detection at 3x concentrations in all three replicates tested (100% inhibition) and in two of three replicates at 5x concentrations (67% inhibition). Influenza B virus was also inhibited at 3x (2/3 replicates) and 5x (3/3 replicates) concentrations. Conversely, the second SARS-CoV-2 specimen pool had no impact on influenza A or influenza B detection.

Data from these studies **(Tables 10-11)** suggest that SARS-CoV-2 virus, when present at concentrations above $1x10^5$ copies/mL can inhibit the detection and amplification of influenza A and B virus if influenza A or influenza B virus are present at concentrations near the assay limit of detection.

Table 10: High concentration of SARS-COV-2 virus (inactivated virus) with low concentration of Flu A or Flu B

Test condition			Average CT				Percent Positives		
High Target	**Low Target**	**n**	**RP**	**FluA**	**FluB**	**SC2**	**FluA**	**FluB**	**SC2**
SC2- 10^5 copies/mL	3x LoD Flu A	3	26.81	30.54	ND	27.32	100%		100%
SC2- 10^5 copies/mL	5x LoD Flu A	3	26.12	28.06	ND	27.22	100%		100%
SC2- 10^5	3x LoD Flu B	3	26.83	ND	29.05	27.35		100%	100%

Test condition			Average CT				Percent Positives		
High Target	**Low Target**	**n**	**RP**	**FluA**	**FluB**	**SC2**	**FluA**	**FluB**	**SC2**
copies/mL									
SC2- 10^5 copies/mL	5x LoD Flu B	3	26.60	ND	28.81	26.90		100%	100%

Table 11: High concentration of SARS-COV-2 (positive specimen pools) with low concentration of Flu A or Flu B

Test Condition			Average CT				Percent Positives		
High Target	**Low Target**	**n**	**RP**	**FluA**	**FluB**	**SC2**	**FluA**	**FluB**	**SC2**
SC2 Positive Pool #1	3x LoD Flu A	3	24.69	ND	ND	20.42	**0%**	-	100%
SC2 Positive Pool #1	5x LoD Flu A	3	24.69	29.0	ND	20.59	**33%**	-	100%
SC2 Positive Pool #1	3x LoD Flu B	3	24.79	ND	32.74	20.49	-	**33%**	100%
SC2 Positive Pool #1	5x LoD Flu B	3	24.80	ND	ND	20.59	-	**0%**	100%
SC2 Positive Pool #2	3x LoD Flu A	3	25.02	31.33	ND	21.28	100%		100%
SC2 Positive Pool #2	5x LoD Flu A	3	24.81	29.99	ND	20.77	100%		100%
SC2 Positive Pool #2	3x LoD Flu B	3	24.68	ND	32.85	19.64		100%	100%
SC2 Positive Pool #2	5x LoD Flu B	3	24.87	ND	30.83	20.98		100%	100%

5) Matrix Equivalency

To support the use of the COVID-Flu Multiplex Assay with anterior nasal swab specimens collected in the clinic in universal transport media (UTM), a matrix equivalency study was performed to demonstrate that assay performance is the same when testing samples eluted in both saline and UTM matrices for all three analytes in the assay panel. To create the contrived specimens, matrix consisting of negative clinical swabs eluted in either saline or universal transport media (UTM) was spiked with inactivated SARS-CoV-2 virus, or live influenza A or influenza B virus at concentrations equivalent to 3x the established LoD. Twelve replicates were tested for each target. All samples yielded the expected results.

6) Fresh vs. Frozen:

A study was performed to demonstrate that freeze/thawing has no impact on the sensitivity of the assay to detect influenza A and influenza B target analytes. For this study, contrived specimens

were prepared by spiking negative nasal swab matrix eluted in saline with quantitated live influenza A and influenza B viral stocks at concentrations equivalent to 1.5x (n=20) and 4x (n=20) the established LoD. Half the samples were tested fresh, and half were stored at -20°C for 24 hours prior to testing with the COVID-Flu Multiplex Assay protocol. Negatives (n=10) were also included in the study. All specimens generated the expected results and frozen samples exhibited average Ct values within 3 Cts of samples tested at baseline, indicating fresh and frozen samples perform similarly with the test device.

7) Clinical Evaluation:

A total of 195 de-identified and blinded clinical specimens were used for clinical evaluation. One set of 80 specimens consisted of 45 SARS-CoV-2 positive and 35 SARS-CoV-2 negative anterior nasal swab specimens collected in 0.9% saline. This set of 80 specimens was tested with the COVID-Flu Multiplex Assay, and performance of the test for SARS-CoV-2 detection was estimated based on the positive and negative percent agreement of the results with a highly sensitive molecular EUA authorized comparator method. Among the 45 specimens, 10 represented weak positive samples. Positive and negative percent agreement for the SARS-CoV-2 analyte was 95.56% and 100%, respectively, as shown in **Table 12** below.

A second set of 115 specimens consisted of the following: 80 influenza A and influenza B positive and negative nasopharyngeal specimens collected in UTM; and 35 negative anterior nasal swab specimens collected in 0.9% saline and tested with the COVID-Flu Multiplex Assay. Performance of the test for influenza A and influenza B detection was estimated based on the positive and negative percent agreement of the results with an FDA-cleared influenza A and influenza B molecular comparator method. Two of the specimens were indeterminate for all analytes when tested with the comparator method and were excluded from the analysis. The Ct values for influenza A and influenza B ranged from 16.4-33.6 and 17.7-39.5. respectively, when tested with the comparator method. The positive percent agreement for influenza A and influenza B of the remaining 113 samples was 94% (34/36) and 97% (32/33), respectively. The negative percent agreement for both analytes was 100%. Results are shown in **Tables 13-14** below.

Table 12: SARS-CoV-2 Performance Evaluation with Clinical Specimens

COVID-Flu multiplex assay		EUA Authorized Comparator Method	
		Positive	Negative
SARS-CoV-2	Positive	43	0
	Negative	2*	35
PPA: 35/35 = 95.56% (95% CI: 85.17% - 98.77%); NPA: 35/35 = 100% (95% CI: 90.11% - 100%)			

* Two specimens were negative for SARS-CoV-2 with the COVID-Flu Multiplex Assay but were positive with the comparator assay (Ct values: 38.8 and 39.3, respectively).

Table 13: Influenza A Performance Evaluation with Clinical Specimens

COVID-Flu Multiplex Assay		FDA-cleared Comparator Method	
		Positive	Negative
FluA	Positive	34	0
	Negative	2*	77
PPA: 34/36 = 94.44% (95% CI: 81.86% - 98.46%; NPA: 77/77 = 100% (95% CI: 95.25% - 100%)			

* Two specimens were negative for FluA with the COVID-Flu Multiplex Assay but were positive with the comparator assay (Ct values: 38.4 and 39.5, respectively).

Table 14: Influenza B Performance Evaluation with Clinical Specimens

COVID-Flu Multiplex Assay		FDA-cleared Comparator Method	
		Positive	Negative
FluB	Positive	32	0
	Negative	1*	80
PPA: 32/33 = 96.97% (95% CI: 84.68% - 99.46%); NPA: 80/80 = 100% (95% CI: 95.42% - 100%)			

* One specimen was negative for Flu B with the COVID-Flu Multiplex Assay but was positive with the comparator assay (Ct value: 39.5).

8) Sample Stability/Shipping Studies for the Exact Sciences Nasal Swab Home Collection Kit

A sample stability study was performed to verify stability of SARS-CoV-2, influenza A and influenza B virus in anterior nasal swab specimens collected at home in saline and shipped to Exact Sciences Laboratories.

Samples were prepared using pooled negative clinical anterior nasal swab matrix and live viral stocks of influenza A or influenza B, or an inactivated stock of SARS-CoV-2 at concentrations equivalent to 2x (low positive) or 7.5x (medium positive) their respective LoDs. To prepare each sample, spiked clinical anterior nasal swab matrix was loaded onto a swab, and the swab was eluted in 0.9% saline per the Exact Sciences Nasal Swab Home Collection Kit Instructions for Use. Negative clinical samples consisting of only pooled negative clinical anterior nasal swab matrix in 0.9% saline were also included in the study. Samples, as prepared in **Table 15**, were evaluated to simulate winter and summer conditions following the shipping condition recommendations outlined in the ISTA7D 2007. After the temperature challenges were completed, all samples were stored at 2-8 ºC and tested within 24 hours with the COVID-Flu Multiplex Assay.

Results are summarized in **Table 16**. Acceptance criteria were defined as mean Ct of the test conditions are not greater than 3 Ct of the control condition and ≥ 95% of intended calls match

actual calls. All acceptance criteria were met. No shifts in the mean Ct for the test condition compared to the mean control condition Ct were greater than 3 Ct.

Results of this study support the stability of SARS-CoV-2, influenza A, influenza B specimens through Summer and Winter shipping conditions up to 72 hours.

Table 15: Summary of sample population by type, condition, and replicate

Sample	Replicates	Condition
Low Positive each target (2x LoD)	20	Summer Temperature Challenge (22 to 35 ºC)
Medium Positive each target (7.5x LoD)	10	
Negative	10	
Low Positive each target (2x LoD)	20	Control (2 to 8 ºC)
Medium Positive each target (7.5x LoD)	10	
Negative	10	
Low Positive each target (2x LoD)	20	Winter Temperature Challenge (-20 ºC to ambient)
Medium Positive each target (7.5x LoD)	10	
Negative	10	
Total:	300	

Table 16: Specimen Stability Ct Result Summary

Condition	Sample Type	Mean Ct CV	Mean Ct Flu A	Mean Ct Flu B	Mean Ct RP	Acceptance Pass / Fail (< 3 Ct Shift)
Control Condition	Low Positive SARS-CoV-2	33.38	NA	NA	30.52	Pass
Summer Profile		33.54	NA	NA	30.34	Pass
Winter Profile		33.60	NA	NA	30.23	Pass
Control Condition	Medium Positive SARS-CoV-2	32.46	NA	NA	30.20	Pass
Summer Profile		32.27	NA	NA	30.21	Pass
Winter Profile		32.46	NA	NA	30.08	Pass
Control Condition	Low Positive Flu A	NA	31.88	NA	30.11	Pass
Summer Profile		NA	32.13	NA	29.90	Pass
Winter Profile		NA	32.40	NA	30.10	Pass
Control Condition	Medium Positive Flu A	NA	30.59	NA	30.24	Pass
Summer Profile		NA	31.03	NA	29.86	Pass
Winter Profile		NA	31.70	NA	30.21	Pass
Control Condition	Low Positive Flu B	NA	NA	31.80	29.81	Pass
Summer Profile		NA	NA	31.80	29.48	Pass
Winter Profile		NA	NA	32.86	29.59	Pass
Control Condition	Medium Positive Flu B	NA	NA	30.84	29.94	Pass
Summer Profile		NA	NA	30.99	29.48	Pass

Condition	Sample Type	Mean Ct CV	Mean Ct Flu A	Mean Ct Flu B	Mean Ct RP	Acceptance Pass / Fail (< 3 Ct Shift)
Winter Profile		NA	NA	31.97	29.88	Pass
Control Condition	Negative	NA	NA	NA	30.42	Pass
Summer Profile		NA	NA	NA	30.06	Pass
Winter Profile		NA	NA	NA	29.95	Pass

9) Human Usability study

The kit components and self-collection instructions that are provided with the Exact Sciences Nasal Swab Home Collection Kit are identical to those in the Exact Sciences COVID-19 Nasal Swab Home-Collection Kit previously reviewed and authorized for use with the SARS-CoV-2 (N gene detection) Test.

Based on the previously reviewed and similarly applicable usability study data (refer to the EUA summary for the SARS-CoV-2 (N gene detection) Test), the Exact Sciences Nasal Swab Home Collection Kit instructions appear understandable, the kit appears easy to use, and samples were successfully self-collected, which demonstrated acceptable usability in the hands of the intended user.

LIMITATIONS:

- Competitive inhibition studies showed that SARS-CoV-2 virus, when present at concentrations above $1x10^5$ copies/mL, can inhibit the detection and amplification of influenza A and influenza B virus RNA if present at or below 4,689 copies/mL and 3,000 copies/mL, respectively, and may lead to false negative influenza virus results. If co-infection with influenza A or influenza B virus is suspected in samples with a positive SARS-Cov-2 result, the sample should be re-tested with another FDA cleared, approved, or authorized influenza test, if influenza virus detection would change clinical management.
- Results (positive and negative) for influenza should be interpreted with caution. If an influenza result is inconsistent with clinical presentation and/or other clinical and epidemiological information, FDA-cleared influenza NAATs are available for confirmation if clinically indicated.
- While it has been determined, based on reviewing studies evaluating specimen adequacy (e.g., by evaluating RNase P), that unobserved self-collected anterior nasal swab samples using the Exact Sciences Nasal Swab Home Collection Kit will likely contain similar levels of human cellular genetic material as HCP-collected anterior nasal swab samples, performance of testing self-collected anterior nasal swab samples using the Exact Sciences Nasal Swab Home Collection Kit has not been specifically evaluated.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established in all circulating variants

but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

WARNINGS:

- For in vitro diagnostic use under FDA Emergency Use Authorization only.
- For Prescription Use only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by the authorized laboratory.
- This product has been authorized only for the detection and differentiation of nucleic acid from SARS-CoV-2, influenza A and/or influenza B, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.