ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
SARS-CoV-2 ASSAY
(NORTHWESTERN MEDICINE)

For in vitro diagnostic use
Rx only
For use under Emergency Use Authorization (EUA) Only

(The Northwestern Medicine SARS-Cov-2 Assay will be performed in the Northwestern Diagnostic Molecular Laboratory, a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory, per the Instructions for Use that were reviewed by the FDA under this EUA).

INTENDED USE

The Northwestern Medicine SARS-CoV-2 Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab, throat (oropharyngeal) swab, nasal swab, mid-turbinate nasal swab and bronchoalveloar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Northwestern Diagnostic Molecular Laboratory that is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory.

The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 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.

The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Testing of self-collected or healthcare provider-collected nasal and mid-turbinate swabs is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Northwestern Medicine SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test that uses commercially available extraction reagents and primers and probes that were developed and validated by the U.S. Centers for Disease Control and Prevention. The assay is intended to detect RNA from SARS-CoV-2 in nasopharyngeal swab,

throat (oropharyngeal) swab, nasal swab, mid-turbinate swab and BAL specimens from patients, as recommended for testing by public health authority guidelines.

INSTRUMENTS USED WITH THE TEST

The Northwestern Medicine SARS-CoV-2 Assay is for use with the ThermoFisher Scientific Quant Studio 6 Flex, equipped with Quant Studio Real-time PCR software v1.1.

REAGENTS AND MATERIALS

Reagent	Manufacturer	Catalogue #
QIAamp MinElute Virus Spin Kit	QIAGEN, N.V.	57704
TaqPath 1 StepRT-qPCR Master Mix CG	ThermoFisher Scientific	A15300
2019-nCoV Kit	Integrated DNA Technologies	10006606
2019-n-CoV_N Positive Control	Integrated DNA Technologies	10006625
Nuclease-free water	--	--
Ethanol (96-100%)	--	--

CONTROLS

A plasmid carrying the SARS-CoV-2 target sequence diluted to the Limit of Detection (LoD) will be included in every run together with a No Target Control (NTC). The positive plasmid control will be diluted to 100 copies/µL in negative matrix and 200µL of the diluted control will be processed like a patient sample. The RNase P mRNA that is present in all clinical samples will also be used to monitor extraction, reverse transcription amplification/detection. A No template control (NTC) (master mix and water) will be used on every run to detect possible carry over or contamination.

The plasmid Positive Control has an average C_t value of 33.67 with a standard deviation of 0.4213. An acceptable result for the plasmid control will be a Ct value within 2 standard deviations of the mean (i.e., 32.82-34.51).

INTERPRETATION OF RESULTS

Sample	Ct Value		Interpretation	Action
	N1	RNase P		
Positive Control	32.82-34.51	≤35.00	Positive	Report "Positive Run Passed"
	<32.82 or >34.51	≤35.00	Negative	Repeat extraction & RT-PCR for all samples and controls
Negative Control	None	None	Negative	Report "Negative Run Passed"
	None	>35.00	Indeterminate	Repeat extraction & RT-PCR for all samples and controls
	>0	>35.00	Positive	Possible contamination; repeat extraction & RT-PCR for all

Sample	Ct Value		Interpretation	Action
	N1	RNase P		
				samples and controls
Patient Sample	≤34.51	≤35.00	Positive	Report "SARS-CoV-2 Positive"
	>34.51	≤35.00	Indeterminate	Collect new sample and retest
	None	≤35.00	Negative	Report "SARS-CoV-2 Not Detected"
	None	>35.00	Inhibition	Collect new sample and retest

PERFORMANCE EVALUATION

1) Analytical Sensitivity

The LoD was determined by diluting known positive nasopharyngeal swab specimens that had been were characterized by the Illinois Department of Public Health (IDPH) into pooled, negative nasopharyngeal matrix in a series of 10-fold dilutions (3 replicates each). The LoD was then confirmed by testing an additional 20 replicates at the estimated LoD concentration. The highest dilution at which 100% reproducible detection was achieved was 1:5000, with a mean Ct value of 33.67 for the SARS-CoV-2 target.

2) Analytical Specificity

Inclusivity

The target sequences for the Northwestern Medicine SARS-CoV-2 Assay are the N1 region of the viral nucleocapsid gene and the endogenous RNase P mRNA internal control from the CDC 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel. *In silico* analysis of primer and probe inclusivity was performed by CDC using all the publicly available nucleic acid sequences for 2019-nCoV that were deposited in GenBank as of February 1, 2020. The CDC has granted a right of reference to the performance data contained in the CDC's EUA request (FDA submission number EUA200001) to any entity seeking an FDA EUA for a COVID-19 diagnostic device.

Cross-reactivity

In silico BLASTn analysis of primer and probe specificity was performed by CDC, Details are provide din the Package Insert for the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

The analytical specificity for the Northwestern Medicine SARS-CoV-2 Assay was further verified by testing 27 different viruses and bacteria (**Table 1**). All results are from wet testing and all results were negative.

Table 1. Viruses and bacteria used to evaluate the analytical specificity of the Northwestern Medicine SARS-CoV-2 Assay

Viruses	**Bacteria**
Adenovirus (1 and 3)	*Bordetella pertussis*
Human coronavirus 229E	*Chlamydia pneumoniae*
Human coronavirus HKU1	*Haemophilus influenzae*
Human coronavirus NL63	*Legionella pneumophila*
Human coronavirus OC43	*Mycobacterium tuberculosis*
Human Metapneumovirus (hMPV)	*Mycoplasma pneumoniae*
Influenza A & B (H1N1, H3N2, 2009 swine)	*Pseudomonas aeruginosa*
MERS-coronavirus	*Streptococcus pneumoniae*
Parainfluenza virus 1-4	*Streptococcus pyogenes*
Respiratory syncytial virus	
Rhinovirus	
SARS-1 coronavirus	

The concentration of each organism or virus tested was a 1:40 dilution of the parental stock obtained from Zeptometrix. All dilutions were in SARS-CoV-2 negative matrix.

3) Clinical Evaluation

Thirty-one presumptive negative clinical samples (29 nasopharyngeal swabs and 2 BAL) were tested using the Northwestern Medicine SARS-CoV-2 Assay and all were found to be negative (SARS-CoV-2 RNA -detected) (**Table 2**). Four nasopharyngeal specimens that were characterized as SARS-CoV-2-positive by the Illinois Department of Public Health (IPDH) were also tested and each produced a positive result.

In the absence of an abundance of SARS-CoV-2 positive clinical samples, additional testing was also performed using purified SARS-CoV-2 RNA that was extracted from the four SARS-CoV-2-positive clinical specimens described above and spiked into known SARS-CoV-2 negative nasopharyngeal matrix. The target levels used were determined based on the Ct values obtained from testing the undiluted RNA extracts and evaluation of the associated Ct values. Of the 32 contrived samples that were prepared in this manner, 20 were at the estimated LoD, target concentration for the assay. All 32 contrived positive samples produced the expected results (100% agreement; **Table 2**).

Table 2. Summary of results from testing contrived clinical specimens spiked with purified SARS-CoV-2 RNA

SARS-CoV-2 RNA (copies/uL) [1]	Number	Positive	Ct Values			
			SARS-CoV-2		RNase P	
			Mean	SD	Mean	SD
0	31	0	NA	NA	27.0	2.8
20	3	3	35.3	0.6	22.2	0.6
100	20	20	33.7	0.4	28.9	0.1
200	3	3	32.4	0.4	22.4	0.6
2000	3	3	29.3	0.4	22.7	0.6
20000	3	3	25.6	0.1	22.9	0.3

NA: Not applicable; SD: Standard Deviation

[1] Estimated concentration based on expected Ct value at the LoD target concentration