ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-CoV-2 ASSAY (NATIONWIDE CHILDREN'S HOSPITAL)

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

The SARS-CoV-2 Assay will be performed at Nationwide Children's Hospital in Columbus, Ohio certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Laboratory Instructions for Use (CMI-MDL-65) reviewed by the FDA under this EUA.

INTENDED USE

The SARS-CoV-2 assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper and lower respiratory specimens (nasopharyngeal swabs, nasal swabs, mid-turbinate nasal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirates, nasal aspirates, bronchoalveolar lavage, lower respiratory tract aspirates, and sputum) collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Nationwide Children's Hospital, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high-complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in upper and lower 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 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.

Testing with the SARS-CoV-2 assay is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays. The SARS-CoV-2 assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The assay is a real-time reverse transcriptase polymerase chain reaction (RT-PCR) test. The SARS-CoV-2 primer and probe sets are designed to detect RNA from the SARS-

CoV-2 virus in (nasopharyngeal swabs, nasal swabs, mid-turbinate nasal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirates, nasal aspirates, bronchoalveolar lavage, lower respiratory tract aspirates, and sputum) collected from patients as recommended for testing by public health authority guidelines. The NCH SARS-CoV-2 assay is a modification of the CDC 2019-nCoV Real-Time RT-PCR assay which utilizes the same SARS-CoV-2 N1 and N2, and human RNAase P control primers and probes for target amplification and detection.

Nucleic acids are first isolated and purified from upper and lower respiratory specimens using the bioMérieux NucliSENS EasyMAG automated nucleic acid extraction system. To perform the nucleic acid extraction, 400 µl of sample is added to 400 µl of lysis buffer. The final elution volume for each sample is 100 µl. The purified nucleic acid is then reverse transcribed into cDNA using ThemoFisher Scientific TaqPath 1-Step RT-qPCR Master Mix, and subsequently amplified in the Applied Biosystems QuantStudio 7 Flex Real-Time PCR Instrument with QuantStudio Real-Time PCR software v.1.3. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. 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.

Brief Description of Test Steps Including Reagent/Sample Volumes:

- Nucleic Acid Extraction
    - Platform: bioMérieux NucliSENS EasyMAG
    - Protocol: bioMérieux NucliSENS easyMAG system general protocol (not for blood) with an off-board lysis step
    - Recommendation(s): Add 400 µl of sample to 400ul lysis buffer. Elution volume is 100 µl.
- rRT-PCR
    - 5 µl extracted RNA from patient or control samples is added to three separate wells of a MicroAmp Fast Optical 96-well reaction plate, each containing 20 µl RT-PCR mix (one well each for N1, N2, and RP assays).
    - Seal the reaction plate with Optical Adhesive Film and run on the Applied Biosystems QuantStudio 7 Flex Real-Time PCR Detection System (QuantStudio Real-Time PCR software v.1.3).

Table 1: RT-PCR Reaction Components and Parameters

Master mix components		**Cycling parameters**			
Reagent	per reaction	Step	Cycles	Temp	Time
Nuclease-free water	13.5 µl	UNG incubation	1	25ºC	2 min
Combined Primer/Probe Mix	1.5 µl	RT incubation	1	50ºC	15 min
TaqPath™ 1-step MM	5.0 µl	Enzyme activation	1	95ºC	2 min
		Amplification	45	95ºC	3 sec
				55ºC	30 sec
	20 µl				

CONTROLS TO BE USED WITH THE COVID-19 RT-PCR

No Template Control (NTC)

- The NTC consists of nuclease free water and is used to monitor for carry-over of target sequences in PCR reagents. The NTC sample must be included in every PCR run.

SARS-CoV-2 Positive Template Control (PTC)

- The PTC used in this assay is the Integrated NDA Technologies 2019-nCoV DNA plasmid control encoding the SARS-CoV-2 N gene (Cat. No. 10006625) and is used to monitor SARS-CoV-2 specific RT-PCR reagent integrity. The PTC sample must be included in every PCR run.

Negative Extraction Control (NEC)

- The NEC consists of pooled negative patient specimen matrix or viral transfer media (Remel M6) and is used to monitor for potential reagent and/or environmental contamination. The NEC sample must be included in every extraction batch and PCR run.

Positive Extraction Control (PEC)

- The PEC consists of a SARS-CoV-2 positive patient sample or a negative patient sample spiked with SARS-CoV-2 viral RNA and is used to control for every step of the test procedure including nucleic acid extraction, reverse transcription, and RT-PCR reagent integrity. The PEC sample must be extracted at least once per day and included in every PCR run.
- Also serving as a positive extraction control, as well as an internal positive control for each specimen, is the RNase P (RP) specific primers and probes. Detection of the RP gene in patient test samples and NEC samples comprised of pooled negative patient sample matrix verifies successful extraction of the sample, proper assay setup, sample integrity, and efficient sample collection.

INTERPRETATION OF RESULTS

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

1) ***NCH SARS-CoV-2 Assay Controls –No Template Control, Positive Template Control, Negative Extraction Control and Positive Extraction Control***

Before the results can be determined for each clinical specimen, the PCR run must be determined to be valid. For a run to be valid, the controls must yield the expected results:

- The NTC sample should be negative for all assay targets.
- The PTC sample should have a positive Ct value of < 40 for both SARS-CoV-2 targets (N1 and N2) and a negative result for the RP target.
- If the NEC sample used consists of pooled negative sample matrix, the sample should be negative for both SARS-CoV-2 targets (N1 and N2) and positive for the RP target (Ct < 40). If the NEC sample used is viral transport media only, the sample should be negative for all assay targets.
- The PEC sample should be positive for all assay targets. A positive Ct value is defined as < 40. For N1, N2 the Ct value must also fall within the acceptable QC range (mean ± 2α).
    - The acceptable criteria for N1 and N2 targets are evaluated for each PEC lot. The mean Cts and standard deviation are determined using data from ≥ 5 independent runs.

Table 2: Assay Control Results Interpretation

Control Type	Control Name	Used to Monitor	Expected result and Ct values		
			2019 nCoV N1	2019 nCoV N2	RP
No Template Control	NTC	Carry-over, contamination	- 0 Ct	- 0 Ct	- 0 Ct
Positive Template Control	PTC	Assay set-up, PCR, SARS-CoV-2 reagent integrity	+ < 40 Ct	+ <40 Ct	- 0 Ct
Negative Extraction Control	NEC	Matrix: Extraction, assay set-up, reverse transcription, PCR, RP reagent integrity	- 0 Ct	- 0 Ct	+ < 40 Ct
		Media: reagent and/or environmental contamination	- 0 Ct	- 0 Ct	- 0 Ct
Positive Extraction Control	PEC	Total process control	+ < 40 Ct*	+ < 40 Ct*	+ < 40 Ct

*N1 and N2 Ct values for the PEC must be < 40.00 and within the acceptable QC range. The acceptable criteria are evaluated on each lot. The mean Cts and standard deviation (α) are determined using data from ≥ 5 independent runs. The Ct of PEC should fall within the range of mean ± 2α.

If unexpected results are obtained for any of the controls, invalidate the run and repeat the RT-PCR assay with stricter adherence to the procedure guidelines.

2) ***Examination and Interpretation of Patient Specimen Results:***

Assessment of clinical specimen test results should be performed after the controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

SARS-CoV-2 Markers (N1 and N2)

- When both SARS-CoV-2 targets are negative and RP is positive (Ct < 40), the result should be considered as negative.
- When both SARS-CoV-2 targets are positive (Ct < 40) and RP is positive (Ct < 40) or negative, the result should be considered as valid and positive.
- If only one SARS-CoV-2 target is positive (Ct < 40) and RP is positive (Ct < 40) or negative, the results should be considered as valid and positive.
- When both SARS-CoV-2 targets are negative and RP is negative, the result is invalid. Repeat the test with residual RNA extract, or if residual RNA is not available, re-extract RNA from the original specimen and repeat the test. If the repeat result is the same, report as invalid.

Table 3. Clinical Sample Results Interpretation

2019 nCoV N1	2019 nCoV N2	RP	Result Interpretation	Report	Actions
-	-	+	SARS-CoV-2 RNA not detected	Negative	N/A
+	+	±	SARS-CoV-2 RNA detected	Positive	Report result to state and/or local health department
If only one target is positive		±	SARS-CoV-2 detected	Positive	Report result to state and/or local health department
-	-	-	Invalid	Invalid	Repeat the test with residual RNA extract. If the same result is obtained as the first run, report as invalid

The assay reports Ct values for each individual target from which the user will need to interpret independently. For all targets, a Ct value < 40 indicates a positive result.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD)

A preliminary LoD for each SARS-CoV-2 target in the NCH SARS-CoV-2 assay was determined using quantified genomic RNA from SARS-Related Coronavirus 2 Isolate USA_WA1/2020 cultured in Vero cells (University of Texas Medical Branch, World Reference Center for Emerging Viruses and Arboviruses). Studies were conducted in both negative clinical nasopharyngeal swab (NP) matrix and sputum samples.

NP matrix pre-mixed with easyMAG Lysis Buffer was spiked with 37.5 or 3.75 genomic equivalents/reaction of SARS-CoV-2 RNA, and sputum pre-mixed with lysis buffer was spiked with 375 or 37.5 genomic equivalents/reaction of SARS-CoV-2 RNA. Ten

replicates of each dilution in each sample matrix were extracted using the bioMérieux NucliSENS easyMAG automated extraction system and amplified on the Applied Biosystems QuantStudio 7 Flex Real-Time PCR Detection System.
Twenty additional replicates were tested containing 12.5 genomic equivalents/reaction in NP matrix or 125 genomic equivalents/reaction in sputum. Each set of 20 replicates produced >95% positive results (20/20). Data from the study are summarized below.

Table 4: LoD Study Data Summary

Specimen Type	Conc. RNA (GE/rxn)	No. Replicates Positive N1	Mean N1 Ct value positives	No. Replicates Positive N2	Mean N2 Ct value positives
NP	37.5	10/10	34.8	10/10	34.5
	3.75	15/20	37.2	19/20	37.7
	12.5	**20/20**	**35.9**	**20/20**	**35.9**
Sputum	375	10/10	34.8	10/10	34.3
	37.5	6/10	38.6	10/10	38.2
	125	**20/20**	**35.2**	**20/20**	**34.7**

2) ***Analytical Inclusivity/Specificity:***

Inclusivity in silico Analysis

To assess inclusivity of the NCH SARS-CoV-2 assay, NI and N2 primer/probes were subjected to BLASTn (NCBI) against the NCBI database. As of April 13, 2020, 380 SARS-CoV2 sequences that span the amplicon of N1 assay were available for comparison. The N1 primer/probe set demonstrated 100% homology to all available sequences. Similarly, 380 SARS-CoV2 sequences that span the amplicon of N2 assay were available for comparison. Among these, 3 (0.8%) SARS-CoV-2 strains with mis-matches were identified. Two strains had an identical single nucleotide mis-match as the 5'end of the probe and one strain had a single nucleotide mis-match at the 4th position from the 5'end of the forward primer. These single-nucleotide mis-matches at the 5' end of the N2 probe and forward primer sequences are not expected to compromise N2 reactivity.

Specificity/Exclusivity in silico Analysis

The NCH SARS-CoV-2 test utilizes identical oligonucleotide sequences for the N1 and N2 SARS-CoV-2 target genes as those used in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

As reported under the CDC EUA, *in silico* analysis of the N1 primer/probe set showed high sequence homology of the N1 probe with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome.

Combining primers and probe, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

For the N2 primer/probe set, *in silico* analysis of the forward primer sequence of showed high sequence homology to Bat SARS-like coronaviruses. The reverse primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora. Combining primers and probe, there is no prediction of potential false positive rRT-PCR results.

3) ***Clinical Evaluation:***

The performance of the NCH SARS-CoV-2 assay was evaluated using contrived clinical samples. A total of 60 contrived positive samples were prepared for testing by spiking 30 individual negative clinical NP specimens and 30 individual negative sputum samples with known concentrations of genomic RNA from SARS-Related Coronavirus 2 Isolate USA_WA1/2020 cultured in Vero cells (University of Texas Medical Branch, World Reference Center for Emerging Viruses and Arboviruses).

Of the 30 contrived positive samples per clinical matrix, 20 contained SARS-CoV-2 RNA at 1X-2X the LoD, 4 contained SARS-CoV-2 RNA at 10X the LoD, 3 contained SARS-CoV-2 RNA at 20X the LoD, and the remaining 3 contained SARS-CoV-2 RNA at 100X the LoD. An additional 30 individual negative NP specimens and 30 negative sputum samples were also included in the study. RNA from each sample was extracted using the bioMérieux NucliSENS easyMAG automated extraction system and amplified on the Applied Biosystems QuantStudio 7 Flex Real-Time PCR Detection System. The results of the NCH SARS-CoV-2 assay are shown below.

Table 5. Clinical Evaluation with Contrived Nasopharyngeal Swab Specimens

RNA Concentration (relative to LoD)	RNA Concentration (GE/rxn)	Detection Rate (# Pos/Total)	Mean Ct	
			N1	N2
~1X	15	100% (10/10)	34.3	35.3
2X	25	100% (10/10)	33.8	34.2
10X	125	100% (4/4)	32.1	32.7
20X	250	100% (3/3)	31.2	31.7
100X	1250	100% (3/3)	28.8	28.8
Negative	0	0% (0/30)	-	-

PPA = 100% (88.7-100%)
NPA= 100% (88.7-100%)

Table 6. Clinical Evaluation with Contrived Sputum Specimens

RNA Concentration (relative to LoD)	RNA Concentration (GE/µl)	Detection Rate (# Pos/Total)	Mean Ct	
			N1	N2
~1X	200	100% (10/10)	30.8	30.4
2X	250	100% (10/10)	31.3	30.8
10X	1250	100% (4/4)	29.7	29.1
20X	2500	100% (3/3)	30.1	29.9
100X	12500	100% (3/3)	26.2	25.3
Negative	0	100% (0/30)	-	-

PPA = 100% (88.7-100%)
NPA= 100% (88.7-100%)

The Nationwide Children's Hospital Clinical Microbiology and Immunoserology lab sent the first 5 positive and 5 negative patient samples to the Ohio Department of Health for confirmatory testing. All samples produced concordant results.

Limitation

- The performance of the NCH SARS-CoV-2 assay was established using nasopharyngeal swab specimens and sputum specimens. Nasal swabs, mid-turbinate swabs, oropharyngeal swabs, nasopharyngeal wash/aspirates, nasal aspirates, bronchoalveolar lavage, and lower respiratory tract aspirates are also considered acceptable specimen types for use with the NCH SARS-CoV-2 assay. Testing of nasal and mid-turbinate nasal swabs (self collected at a healthcare site or collected by a healthcare provider) is limited to patients with symptoms of COVID-19.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with 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:

- This product has not been FDA cleared or approved by FDA, but has been authorized by FDA under an EUA for use by the authorized laboratory;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or the authorization is revoked sooner

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish

specificity and to confirm the LoD. The extraction method and instrument used were the bioMérieux NucliSENS EasyMAG extraction platform and the Applied Biosystems QuantStudio 7 Flex Real- Time PCR detection system with QuantStudioTM Real-Time PCR software v.1.3. repectively. The results are summarized in the following Table.

Table 7. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provide d by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not Detected