EMERGENCY USE AUTHORIZATION (EUA) SUMMARY OSUWMC COVID-19 RT-PCR TEST (OHIO STATE UNIVERSITY WEXNER MEDICAL CENTER)

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

(The OSUWMC COVID-19 RT-PCR test will be performed at Ohio State University Wexner Medical Center, Columbus, OH (CLIA# 36D0328940) certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a to perform high complexity tests as per laboratory procedures reviewed by the FDA under this EUA.)

INTENDED USE

The OSUWMC COVID-19 RT-PCR test is a real-time reverse transcription polymerase chain reaction (RT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal, oropharyngeal (OP), and nasopharyngeal (NP) swab specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to the Ohio State University Wexner Medical Center, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARS-Co-V-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infections. 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 OSUWMC COVID-19 RT-PCR test is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The OSUWMC COVID-19 RT-PCR test is only for use under the Food and Drug Administration's Emergency Use Authorization**.**

SPECIAL CONDITIONS FOR USE

For Emergency Use Authorization (EUA) only
For prescription use only
For in vitro diagnostic use

INSTRUMENTS USED WITH TEST

The OSUWMC COVID-19 RT-PCR test is to be used with bioMérieux EasyMAG Extraction System and Applied Biosystems 7500 Fast RealTime PCR instrument (ABI Fast) with software version 1.5.1.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Nucleic acids are isolated and purified from patient sample using the bioMérieux EasyMAG Extraction System: 200 µL sample input, 200 µL lysis buffer, and 50 µL elution volume. 5 µL of the purified nucleic acid is reverse transcribed, using the TaqPath 1-Step RT-qPCR Master Mix, CG, into cDNA, which is then subsequently amplified, all in the ABI Fast. During the amplification 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 DNA Polymerase degrades the bound probe, causing the reporter dye (FAM) to separate from the quencher dye (BHQ1), generating a fluorescent signal. The test contains a third target, RNAse P, a housekeeping gene. RNAse P is detected in all the test specimens by the same mechanism, via a specific probe, labeled with reporter and quencher, which, during the extension phase of the PCR cycle, is degraded by the Taq DNA Polymerase causing the reporter dye to separate from the quencher dye and generate 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 by the ABI fast.

REAGENTS AND MATERIALS

Provided with the OSUWMC COVID-19 RT-PCR test

Reagent	**Manufacturer**	**Catalog #**
TaqPath 1-Step RT-PCR Master Mix, GC	ThermoFisher	A15300
COVID-19_N1-F Primer	IDT	Custom
COVID-19_N1-R Primer	IDT	Custom
COVID-19_N1-P Probe	IDT	Custom
COVID-19_N2-F Primer	IDT	Custom
COVID-19_N2-R Primer	IDT	Custom
COVID-19_N2-P Probe	IDT	Custom
RP-F Primer	IDT	Custom
RP-R Primer	IDT	Custom
RP-P Probe	IDT	Custom
2019 nCoV Positive Control	IDT	10006625

Required but Not Provided with the OSUWMC COVID-19 RT-PCR test

Instrument	**Manufacturer**	**Catalog #**
NucliSens easyMAG Extraction Buffer 1	bioMérieux	280130
NucliSens easyMAG Extraction Buffer 2	bioMérieux	280131
NucliSens easyMAG Extraction Buffer 3	bioMérieux	280132
NucliSens easyMAG Lysis Buffer	bioMérieux	280134
NucliSens easyMAG Magnetic Silica	bioMérieux	280133
NucliSens easyMAG Sample Strip	bioMérieux	280135
ABI 7500 Fast Real-time PCR instrument	Applied Biosystems	4351106

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

a) A no template control (NTC) needed to assess the possibility of sample contamination on the assay run and is used on every assay plate. The NTC is sterile, molecular grade, nuclease-free water.

b) A positive control (PC) is needed to verify that the assay run is performing as intended and is used on every plate. The PC is added directly to the RT-PCR master mix well at a volume of 5 µL (yielding a final concentration of 1000 cp/µL). The PC is 2019 nCoV Positive Control (IDT10006625).

c) An internal control (IC) targeting RNAse P is needed to verify that nucleic acid is present in every sample and is used for every sample processed. As the positive control is DNA, this serves as a control for reverse transcription and also the extraction positive control to ensure that samples resulting as negative for SARS-CoV-2 RNA contain nucleic acid for testing.

d) A Human Specimen Control (HSC) is needed to verify that extraction is performing as intended and to eliminate the possibility of contamination and is used on every plate. The HSC contains the RNAse P target.

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) ***OSUWMC COVID-19 RT-PCR Test Controls – Positive, Negative, and Internal:***

PC – positive for N1 and N2 SARS-CoV-2 targets (Ct < 40)
IC – positive for RNAse P target (Ct < 40)
NTC – negative for N1 and N2 SARS-CoV-2 targets (Ct ≥ 40), negative for RNAse P target (Ct ≥ 40)

HSC – negative for N1 and N2 SARS-CoV-2 targets (Ct ≥ 40), positive for RNAse P target (Ct < 40).

If any control does not perform as described above, the run is considered invalid and all specimens are repeated from extraction step.

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

Note on the IC: all clinical samples should yield positive results for the RNAse P target at a Ct < 40. Samples that fail to show detection of IC within this range and N1 and N2 SARS-CoV-2 targets should be repeated from the extraction step. If the sample tests positive for the N1 and/or N2 SARS-CoV-2 targets, the lack of amplification of RNAse P target can still be valid.

N1 Target (positive for Ct < 40)	N2 Target (positive for Ct < 40)	RP Target (positive for Ct < 40)	Results Interpretation	Action
If only one or both of targets positive		±	POSITIVE	Report result to sender and appropriate health authorities
-	-	+	NEGATIVE	Report result to sender
-	-	-	INVALID	Repeat extraction and RT-PCR. Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient. If additional clinical sample is unavailable, report INVALID

PERFORMANCE EVALUATION

Analytical Sensitivity

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies(cp)/µL) that can be detected by the OSUWMC COVID-19 RT-PCR test at least 95% of the time. The LoD study was performed on the ABI Fast instrument. The LoD determination was performed in pooled nasopharyngeal (NP) specimen, confirmed negative, collected in VTM. Contrived samples were prepared by spiking Exact Diagnostics SARS-CoV-2 standard into NP matrix, pre-mixed with NucliSens easyMAG Lysis Buffer.

The preliminary LoD was established by testing eight different dilutions of RNA in triplicate (2 cp/µL was tested in sextuplicate). The preliminary LoD was determined to be 2 cp/µL (6/6) (Table 1).

The preliminary LoD was confirmed by testing 21 replicates of contrived samples at 1 cp/µL and 0.25 cp/µL. The study results, that are summarized below, show that the LoD of the OSUWMC COVID-19 RT-PCR test is 0.25 cp/µL (21/21 positive) for NP specimens (Table 2). These results are based on the results interpretation that state that only one target (N1 or N2) need to test positive in order to call a positive for SARS-CoV-2.

Table 1. Results of the Preliminary LoD Experiment

	N1 target			N2 target		
Concentration (cp/µL)	**Positive replicates**	**mean Ct value**	**Standard Deviation**	**Positive replicates**	**mean Ct value**	**Standard Deviation**
50	3/3	27.4	0.1	3/3	30.1	5.7[a]
25	3/3	28.1	0.3	3/3	29.3	2.4
10	3/3	29.6	0.2	3/3	29.5	0.6
5	3/3	30.6	0.5	3/3	30.0	0.4
2	6/6	32.0	0.4	6/6	31.8	0.6
0.2	2/3	36.5	1.4	3/3	36.3	0.6
0.02	0/3	NA	NA	2/3	38.0	1.1
0.002	0/3	NA	NA	0/3	NA	NA

NA = Not Available

[a] one of three replicates tested positive at a Ct value of 36.7. the other two targets tested positive at Ct values of 27.1 and 26.6.

Table 2. Results of the Analytical Sensitivity for the OSUWMC COVID-19 RT-PCR test

	N1 target			N2 target		
Concentration (cp/µL)	**Positive replicates**	**mean Ct value**	**Standard Deviation**	**Positive replicates**	**mean Ct value**	**Standard Deviation**
1	21/21	32.1	0.9	21/21	32.1	0.7
0.25[b]	17/21	34.6	1.1	20/21	36.1	0.8

[b]Conducted June 9, 2020

Inclusivity

The primer/probe sets for the N1 and N2 SARS-CoV-2 targets were designed by the CDC which conducted the *in silico* inclusivity analysis on known sequences of SARS-CoV-2. The data from this analysis are available in the FDA EUA EUA200001 "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel"

On May 14, 2020, FDA analyzed 5,457 US sequences uploaded to the GISAID database. Table 4 shows the number of sequences that found nucleotide mismatches against the

primers/probes for the N1 and N2 SARS-CoV-2 targets. From this analysis, there was a single sequence with three mismatches to the N2 probe, shown below:

N2 Probe	ACAATTTGCCCCCAGCGCTTCAG
EPI_ISL 426088	ACAATTTGCCCCCAATGTTTCAG

Table 3. *In Silico* Inclusivity Analysis

Target	Primer/Probe	Position	Number of sequences with mismatch at Position
N1 Target	Forward primer	28289	1
		28300	2
		TOTAL	3
	Reverse Primer	28340	1
		28344	3
		28356	1
		28357	1
		TOTAL	6
	Probe	28310	2
		28311	13
		28313	1
		28321	2
		28326	1
		28329	1
		TOTAL	20

Target	Primer/Probe	Position	Number of sequences with mismatch at Position
N2 Target	Forward primer	29165	2
		29167	1
		29179	1
		TOTAL	4
	Reverse Primer	29218	5
		29219	1
		29230	1
		TOTAL	7
	Probe	29188	34
		29194	3
		29199	1
		29200	3
		29203	4
		29205	1
		29206	1
		TOTAL	47

Analytical Specificity

The primer/probe sets for the N1 and N2 SARS-CoV-2 targets were designed by the CDC, which conducted the cross-reactivity testing *in silico.* In addition to the *in silico* analysis, several organisms were extracted and tested with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel to demonstrate analytical specificity and exclusivity. The data from these analyses is available in the FDA EUA EUA200001 “CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel”

Clinical Evaluation

Clinical Evaluation Using Contrived Samples

A contrived clinical study was conducted to evaluate the performance of the OSUWMC COVID-19 RT-PCR test. A total of 60 individual NP clinical specimens, confirmed negative for SARS-CoV-2, were used in this study. One natural NP clinical specimen, confirmed positive (confirmed by the Ohio State Public health lab), was also included in this study. Thirty contrived positive samples were prepared by spiking Exact Diagnostics SARS-CoV-2 standard into the media of the individual negative specimens at 1X, 2X, 5X, 10X, and 25X LoD. Samples were extracted and tested in a randomized and blinded fashion using the NucliSens easyMAG system and ABI Fast instrument. The result from the single natural clinical specimen was concordant. The positive and negative percent agreements between the OSUWMC COVID-19 RT-PCR test and the expected results for contrived clinical specimens are shown below.

Table 4. Clinical Performance of the OSUWMC COVID-19 RT-PCR test

SARS-CoV-2 RNA concentration	Number of NP swabs	N1 target % Positive (95% CIs)	N1 Mean Ct Value	N2 target % Positive (95% CIs)	N2 Mean Ct Value
1x LoD	10	10/10 100% (72.3 – 100)	32.1 ± 0.6	10/10 100% (72.3 – 100)	32.3 ± 1.2
2x LoD	10	10/10 100% (72.3 – 100)	32.7 ± 0.6	10/10 100% (72.3 – 100)	31.6 ± 0.4
5x LoD	4	4/4 100% (51.0 – 100)	30.4 ± 0.3	4/4 100% (51.0 – 100)	30.8 ± 0.4
10x LoD	4	4/4 100% (51.0 – 100)	30.1 ± 0.2	4/4 100% (51.0 – 100)	29.8 ± 0.8
25x LOD	2	2/2 100% (32.2 – 100)	28.9 ± 0.2	2/2 100% (32.2 – 100)	30.0 ± 1.4
Negative	30	0/30 (N/A)	N/A	0/30 (N/A)	N/A

N/A = Not available.

Performance of the OSUWMC COVID-19 RT-PCR test against the expected results are:

Positive Percent Agreement 30/30 = 100% (95% CI: 88.7% - 100%)
Negative Percent Agreement 30/30 = 100% (95% CI: 88.7% - 100%)

Clinical Evaluation Using Natural Samples

A clinical study, using natural clinical specimens, was conducted to evaluate the performance of the OSUWMC COVID-19 RT-PCR test. A total of 51 NP specimens were tested with both the OSUWMC COVID-19 RT-PCR test and the Simplexa COVID-19 Direct Assay (authorized 3/19/2020). All 26 specimens positive by the Simplexa COVID-19 Direct Assay were positive by the OSUWMC COVID-19 RT-PCR test. All 25 specimens negative by the Simplexa COVID-19 Direct Assay were negative by the OSUWMC COVID-19 RT-PCR test. The positive and negative percent agreements between the OSUWMC COVID-19 RT-PCR test and the Simplexa COVID-19 Direct Assay are shown in Table 5.

Table 5. Clinical performance of the OSUWMC COVID-19 RT-PCR test		**Simplexa COVID-19 Direct Assay**	
		Positive	**Negative**
OSUWMC COVID-19 RT-PCR test	**Positive**	26	0
	Negative	0	25

Performance of the OSUWMC COVID-19 RT-PCR test using natural clinical samples against the Simplexa COVID-19 Direct Assay are:

Positive Percent Agreement 26/26 = 100% (95% CI: 87.1% - 100%)
Negative Percent Agreement 25/25 = 100% (95% CI: 86.7% - 100%)

Additionally, five positive and six negative NP specimens were sent to the Ohio Department of Health and tested using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel. All results were concordant.

Table 6. Clinical Performance of the OSUWMC COVID-19 RT-PCR test Against the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel.

	Ohio Department of Health				OSUWMC COVID-19 RT-PCR test			
Sample ID	Result	N1 (Ct)	N2 (Ct)	RP (Ct)	Result	N1 (Ct)	N2 (Ct)	RP (Ct)
nCoV-1	NEG	ND	ND	25.5	NEG	ND	ND	25.5
nCoV-2	POS	20.1	20.1	25.1	POS	21.4	21.2	26.2
nCoV-3	NEG	ND	ND	24.7	NEG	ND	ND	24.0
nCoV-4	NEG	ND	ND	30.3	NEG	ND	ND	30.1
nCoV-5	NEG	ND	ND	29.3	NEG	ND	ND	29.2
nCoV-6	POS	26.7	27.0	28.8	POS	27.4	26.3	27.9
nCoV-7	NEG	ND	ND	26.1	NEG	ND	ND	24.7
nCoV-8	POS	14.9	15.0	25.3	POS	16.8	16.0	24.4

	Ohio Department of Health				OSUWMC COVID-19 RT-PCR test			
Sample ID	Result	N1 (Ct)	N2 (Ct)	RP (Ct)	Result	N1 (Ct)	N2 (Ct)	RP (Ct)
nCoV-9	POS	16.2	16.2	24.7	POS	18.0	16.8	24.8
nCoV-10	NEG	ND	ND	24.4	NEG	ND	ND	24.0
nCoV-11	POS	26.04	26.56	28.22	POS	24.5	23.7	23.8

ND = Not Detected

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratory;
- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests 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 authorization is terminated or revoked sooner.