October 1, 2020

EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE UMASS MOLECULAR VIROLOGY LABORATORY 2019-nCoV rRT-PCR DX PANEL

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

(The UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel will be performed at the UMass Molecular Virology Laboratory located at UMass Memorial Medical Center University Campus Hospital Labs in Worcester, MA, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests, as per the Standard Operating Procedure that was reviewed by the FDA under this EUA.)

INTENDED USE

The UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal, mid-turbinate nasal, nasopharyngeal and oropharyngeal (throat) swab, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by a healthcare professional.

Testing is limited to the UMass Molecular Virology Laboratory located in Worcester, MA, 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 detection and identification of SARS-CoV-2 RNA. 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 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 UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel 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 UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel 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, (EUA200001). This test uses singleplexed assays that contain two primer/probe sets targeting the N gene of the SARS-CoV-2 virus and primers/probe for the extraction control human RNaseP. Detection of the N gene targets (N1 and N2) is required in order for the sample to be determined as positive.

The UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel follows the procedure in the package insert for the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel with the following product substitutions that were the focus in the validation study. The KingFisher Flex Magnetic Particle Processor (MagMAX Viral/Pathogen Nucleic Acid Isolation Kit) was validated as an alternative to the QIAcube QIAamp DSP Viral RNA Mini Kit. The rRT-PCR Enzyme Mastermix includes the iTaq Universal Probes One-Step Kit from Bio-Rad Laboratories, Inc. and the BioRAD CFX96 Touch Real-Time PCR Detection System was used to perform the assay.

INSTRUMENTS USED WITH THE TEST

The UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel is to be used with the CFX96 Touch Real-Time PCR Detection System. Manual review represents the primary analysis of data and an Excel worksheet serves as a check of the manual analysis and as the method to interface results to the laboratory information system. Extraction is performed on either the QIAGEN QIAcube or the KingFisher Flex Magnetic Particle Processor with 96 Well Head.

REAGENTS AND MATERIALS

Reagent	**Storage Temperature**
QIAcube QIAamp Viral RNA Mini Kits (Cat# 61904, 52904, 52906).	Room temp 15 to 30°C
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, ThermoFisher	Room temp 15 to 30°C
100% ethanol, ACS reagent grade or equivalent	Room temp 15 to 30°C
Nuclease-free water (not DEPC-treated)	Room temp 15 to 30°C
Primer/Probe 2019-nCoV CDC EUA Kit, 500 rxn, Catalog# 10006606 www.idtdna.com	≤ -20°C, one working aliquot at 2-8°C in the dark
iTaq Universal Probes One-Step Kit, 500 x 20 µl rxns, Catalog #1725141, Bio-Rad	-15 to -30°C

Reagent	Storage Temperature
2019-nCoV_N_Positive Control, Catalog# 10006625 www.idtdna.com	≤ -70°C
Negative control: peripheral blood WBCs	≤ -70°C
10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)	Room temp 15 to 30°C

CONTROLS TO BE USED WITH THE TEST

1) **Positive Control:** 2019-nCoV_N_Positive Control (IDT Catalog# 10006625). This plasmid is diluted to 8 copies/µL (4xLOD, or 40 genome copy equivalent (GCE)) in RNA matrix (0.2 ng/µL) extracted from human peripheral blood white blood cells. One positive control is run with each assay on each plate. This control is detectable with the N gene primers/probes. This control is designed to monitor the integrity of the PCR run and identify a reagent failure (e.g., primer and probe).

2) **Negative control:** A negative (no template) control is needed to eliminate the possibility of sample cross-contamination (during RNA extraction or PCR setup) on the assay run and is used on every assay plate. This control is molecular grade, nuclease-free water.

3) **Extraction Control:** The extraction control is included with each extraction run. Aliquots of 25µL of peripheral blood from a SARS-CoV-2 negative individual (previously treated with 250 µL Qiagen erythrocyte lysis (EL) buffer for 10 min, the supernatant removed and 140 µL saline added). This control is not detectable with the N gene primers/probes. This control should be detectable by the human RNase P primer/probes and also serves as an extraction control.

4) **Internal Control:** The assay includes primers and a probe for detection of endogenous RNase P nucleic acid that is extracted and amplified from every patient sample.

INTERPRETATION OF RESULTS

Test controls are examined prior to interpretation of patient results. If the controls are Equivocal or Invalid, a qualified Laboratory Director will review the assay to confirm thresholds are set correctly (i.e. above background and within the exponential phase). Invalid controls will trigger repeat testing. Similarly, an invalid patient result will trigger a re-extraction and repeat testing. See Figures 1and 2.

Figure 1. Expected Performance of Controls Included in the UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel (based on the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel)

Control Type	External Control Name	Used to Monitor	2019 nCoV_N1	2019 nCoV_N2	RP	Expected Ct Values
Positive	nCoVPC	Substantial reagent failure including primer and probe integrity	+	+	+	< 40.00 Ct
Negative	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Extraction	HSC	Failure in lysis and extraction procedure, potential contamination during extraction	-	-	+	< 40.00 Ct

Figure 2. Patient Results Interpretation Guide for the UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel (based on the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel)

2019 nCoV_N1	2019 nCoV_N2	RP	Result Interpretation[a]	Report	Actions
+	+	±	2019-nCoV detected	Positive 2019-nCoV	Report results
If only one of the two targets is positive		±	Inconclusive Result	Inconclusive	Repeat testing of nucleic acid and/or re-extract and repeat rRT-PCR. Re-collect specimen from patient
-	-	+	2019-nCoV not detected	Not Detected	Report results to sender. Consider testing for other respiratory viruses.[b]
-	-	-	Invalid Result	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

PERFORMANCE EVALUATION OF THE UMASS MOLECULAR VIROLOGY LABORATORY 2019-nCoV rRT-PCR DX PANEL

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

Since no quantified virus isolates of the 2019-nCoV were initially available, the LoD of the UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel was initially evaluated using a commercial positive control (IDT plasmid) (N gene; GenBank accession: MN908947.2) of known titer (plasmid copies/µL) spiked into a diluent consisting of a suspension of peripheral blood WBCs and saline to mimic a clinical specimen. Samples were extracted using the QIAGEN QIAcube Kit, QIAGEN QIAamp DSP Viral RNA Mini Kit, and RT-PCR was performed using the Bio-Rad iTaq Universal Probes One-Step Kit on the Bio-Rad CFX96 Real-Time PCR Detection System.

A preliminary LoD for each assay was determined followed by LoD confirmation with 20 extracted replicates. Using the positive control plasmid, the LoD was determined as the lowest concentration where ≥ 95% (19/20) of the replicates were positive, 4 plasmid copies/µL. The results are summarized in Table 1 below.

Table 1. Limit of Detection Confirmation UMass 2019-nCoV Real-Time RT-PCR Diagnostic Panel with QiaCube QIAGEN QIAmp DSP Viral RNA Mini Kit Using a Positive Control Plasmid

Targets	2019-nCov_N1			2019-nCov_N2			2019-nCov_N3*			Negative[3]
Plasmid Concentration[1]	4	2	1	4	2	1	4	2	1	0
Positives/Total	20/20	18/20	14/20	20/20	20/20	17/20	20/20	20/20	15/20	0/9
Mean Ct[2]	34.8	35.7	36.4	35.1	36.2	37.2	35.3	36.4	37.0	>40
Standard Deviation	0.7	0.9	0.8	0.8	0.5	0.7	0.5	0.7	0.9	NA

[1]Concentration is presented in plasmid copies/µL. 5uL used in each reaction yields a LoD (genome copy equivalent or GCE) of 5 times the LoD in copies/µL (i.e. 10-20 GCE)
[2]Mean Ct reported for dilutions that are ≥ 60% positive. Calculations only include positive results
[3] Performed as duplicates with RNA concentrations of 100, 50, 25, 12.5, 3.1, 1.6, 0.8, 0.4 ng/µL
NA, not applicable
* Note: N3 primers are not used.

The LoD was then assessed using a SARS-CoV-2 positive patient sample. A pool of negative nasopharyngeal (NP) specimens was spiked with a positive patient sample at a 1:10000 dilution. The concentration of the RNA extracted from the 1:10000 diluted positive patient specimen was determined from the Ct signal converted to a concentration in copies/µL by employing a standard curve generated using the positive control (IDT plasmid 200,000 copies/µL) and ATCC VR-3276SD (Quantitative Synthetic SARS-CoV-2 RNA containing ORF1ab, N, E, RdRp, and ORF1b-nsp14 genes). These results were used to calculate the dilution and copies/µL to be used in this LoD study. Residual SARS-CoV-2 negative NP samples were then pooled and spiked with the positive patient sample described above at a 1:50,000 and a 1:500,000 dilution and processed using the

QIAGEN QIAamp DSP Viral RNA Mini Kit. Subsequently, 24 replicates of each dilution (spiked to approximate a 2xLoD (8 copies/µL) and 0.25xLoD (1 copy/µL)) were tested. Based on this evaluation, LoD is 8 copies/µL. The results are summarized in Table 1A below.

Table 1A. Limit of Detection Confirmation UMass 2019-nCoV Real-Time RT-PCR Diagnostic Panel with QiaCube QIAGEN QIAmp DSP Viral RNA Mini Kit

Targets	2019-nCov_N1		2019-nCov_N2	
RNA Concentration[1]	8.6	0.9	8.6	0.9
Positives/Total	24/24	05/24	24/24	10/24
Mean Ct[2]	35.2	37.7	34.7	36.5
Standard Deviation	0.6	0.3	0.5	1.1

[1]Concentration is presented in RNA copies/µL. 5uL used in each reaction yields a GCE of 5 times RNA concentration.
[2]Mean Ct reported for dilutions that are ≥ 20% positive. Calculations only include positive results.

A comparable analysis using SARS-CoV-2 negative clinical matrix spiked with a positive patient specimen was performed using the KingFisher Flex Magnetic Particle Processor (MagMAX Viral/Pathogen Nucleic Acid Isolation Kit) extraction method. The LoD results were confirmed by testing 26 replicates, with LoD determined to be 2 copies/µL. The results are summarized below in Table 2.

Table 2. Limit of Detection Confirmation UMass 2019-nCoV Real-Time RT-PCR Diagnostic Panel with KingFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit

Targets	2019-nCov_N1				2019-nCov_N2			
RNA Concentration[1]	2	1	0.5	0	2	1	0.5	0
Positives/Total	26/26	21/26	18/26	0/10	26/26	26/26	19/26	0/10
Mean Ct[2]	35.5	36.3	37.1	>40	35.5	36.5	37.1	>40
Standard Deviation	1.1	0.9	0.7	NA	0.9	1.1	0.9	NA

[1]Concentration is presented in RNA copies/µL. 5µL used in each reaction yields a LoD (genome copy equivalent or GCE) of 5 times the LoD in copies/µL (i.e. 5-10 GCE)
[2]Mean Ct reported for dilutions that are ≥ 60% positive. Calculations only include positive results.

2) Inclusivity/Cross-reactivity:

The sequences for the N1, N2 primers/probes and the endogenous RNase P mRNA internal control are identical to the primer/probe sequences used in the FDA authorized CDC 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Panel. *In Silico* Analysis of Primer and Probe Sequences for both Reactivity/Inclusivity and Specificity/Exclusivity has been reported by the CDC who has granted a right to reference to the performance data contained in the CDC's EUA 200001 to any entity seeking an FDA EUA for a COVID-19 diagnostic device.

Nevertheless, the inclusivity of the CDC N1 and N2 primer sets (EUA200001) was re-evaluated at UMass by *in silico* analysis. An alignment was performed with the oligonucleotide primer and probe sequences of the UMass CDC LDT against all publicly available nucleic acid sequences for 2019-nCoV in the U.S. National Library of Medicine, National Center of Biotechnology Information GenBank as of June 15, 2020. A primer blastn search used parameters for highly similar sequences (Megablast) in the nucleotide collection for SARS-CoV-2 (Taxid:2697049) with output set to the maximum target sequences (20000). In summary, 99.9%, 99.7%, and 99.6% of all alignments show 100% identity to the N1F, N1R and N1P primers and probe, respectively. Similarly, 99.8%, 99.8%, and 99.8% of all alignments show 100% identity to the N2F, N2R and N2P primers and probe, respectively. The impact of a nucleotide mismatch on reactivity, and a false negative result is partly dependent on the location of the mismatch in the primer/probe. A mismatch at the 3' prime end of the primer is unlikely to be tolerated (large negative impact) whereas a mismatch at the 5' prime or center of the primer may be tolerated (small impact). In the N1 primer set, two viral sequences (Accession # MT598635.1 & MT451406.1) show a mismatch in both the N1R primer and N1 probe. In the N2 primer set, one viral sequence (Accession # MT451637.1) shows a mismatch in both the N2F and the N2R primers. None of the viral sequences showed a mismatch in both N1 and N2 primer/probe sets, indicating at least one primer/probe set will detect the virus when present.

3) Clinical Evaluation:

A total of 148 unique clinical specimens were tested using the UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel and one of two EUA-authorized tests that target separate genes (Table 3). After testing, results were assessed to determine agreement. The UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel results demonstrated 100% (37/37) positive percent agreement (PPA) and 100% (111/111) negative percent agreement (NPA).

Table 3. Results of Clinical Evaluation

		EUA Authorized Assay	
		Pos	Neg
UMass Modified CDC Assay	Pos	37	0
	Neg	0	111
	Total	37	111

PPA: 100% (37/37) (95% CI: 90.51% to 100.00%)
NPA: 100% (111/111) (95% CI: 96.73% to 100.00%)

The mean Ct values for each target detected by the UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel were as follows in Table 4:

Table 4. Ct values for 37 Positive Clinical Specimens

UMass modified CDC assay	Primer set		
	N1	N2	RNase P
Mean +/- Std dev	24.1 +/- 5.9	24.7 +/- 6.2	24.4 +/- 2.0
Range [Min-Max]	13.6 - 33.6	13.5 - 35.2	20.5 - 29.4

4) Matrix Equivalency

Due to difficulties obtaining viral transport media/universal transport media (VTM/UTM) to collect and transport patients' samples, the laboratory evaluated saline as an alternative to VTM. An equivalency study was performed to evaluate the performance of the UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel for nasopharyngeal swabs collected in viral transport medium (VTM) and sterile saline. A total of sixty (60) contrived positive specimens were tested:

- 30 contrived positive (2x-4x LoD) and 10 negative NP specimens collected in VTM
- 30 contrived positive (2x-4x LoD) and 10 negative NP specimens collected in saline

Samples were contrived by spiking known concentrations of SARS-CoV-2 virus into individual VTM or saline NP specimens with a negative SARS-CoV-2 result. Individual samples were processed through the KingFisher Flex Magnetic Particle Processor (MagMAX Viral/Pathogen Nucleic Acid Isolation Kit) extraction method. All positive samples yielded positive results and all negative samples yielded negative results. Results are shown in Table 5.

Table 5: Summary of results from testing contrived clinical specimens spiked with SARS-CoV-2 virus.

[SARS-CoV-2] (LoD)	Number of samples	Samples Detected	UMass MVL 2019-nCoV rRT-PCR Dx Panel (BioRad CFX96)					
			SARS-CoV2 N1 primer		SARS-CoV2 N2 primer		RNaseP primer	
			VTM	Saline	VTM	Saline	VTM	Saline
2xLoD	20	20	34.0 +/- 0.4	34.9 +/- 0.7	33.9 +/- 0.4	34.3 +/- 0.3	25.9 +/- 1.5	26.5 +/- 1.6
4xLoD	10	10	33.3 +/- 0.3	33.8 +/- 0.3	33.1 +/- 0.3	33.1 +/- 0.3	26.2 +/- 1.2	26.1 +/- 1.7
NEGATIVE	10	0	N/A	N/A	N/A	N/A	23.2 +/- 1.8	25.1 +/- 1.2

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 SARS-CoV-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 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 authorization is terminated or revoked sooner.