ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY UNC Health SARS-CoV-2 real-time RT-PCR test

(University of North Carolina Medical Center)

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

(The UNC Health SARS-CoV-2 real-time RT-PCR test will be performed at the University of North Carolina Medical Center, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The UNC Health SARS-CoV-2 real-time RT-PCR test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal and oropharyngeal specimens, tracheal aspirates, bronchoalveolar lavages (BAL)/bronchial washings (BW), and nasopharyngeal aspirates (NA)/nasal washings (NW) from individuals suspected of COVID-19. Testing is limited to the University of North Carolina Medical Center that is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory.

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 active infection with SARS-CoV-2 but 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 intended for use for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The assay is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines.

Sample Preparation

Two extraction chemistries are validated for COVID-19 PCR testing – the Roche MagNAPure 24 system and the bioMerieux EasyMag system.

The MagNA Pure 24 System is a robotic workstation capable of processing 24 samples in a batch. Nucleic acid is extracted with the Roche MagNA Pure 24 System using the external lysis protocol. Addition of the Lysis/Binding Buffer (green cap) to the patient sample allows for a complete lysis and inactivation of the sample before the patient specimen is removed from the biological safety cabinet. The Lysis/Binding Buffer contains potent denaturing agents including chaotropic salts and proteinase K that act to denature proteins, which releases DNA and RNA. The buffer also acts to bind and stabilize the nucleic acids. Once the external lysis is performed, the lysed sample can be safely loaded onto the MagNA Pure to continue nucleic acid purification. On the MagNA Pure, the addition of magnetic glass particles binds the nucleic acids while several wash steps remove unbound substances. Purified total nucleic acid is eluted with a low salt buffer.

The NucliSENS easyMAG platform is an automated system for the purification and concentration of RNA and DNA from biological specimens. One set of reagents is used, independent of sample type and nature of nucleic acid to be isolated. Samples can be batched, sizes ranging from 1 to 24 samples. The throughput for 24 samples is less than one hour.

The underlying workflow involves adding a lysis buffer that will disrupt cellular material and release nucleic acids. The lysis buffer inactivates nucleases present in the specimen. Magnetic silica is added to the lysed specimen and under high salt concentrations, the nucleic acids bind to the magnetic silica. Following two washes, the nucleic acids are eluted from the magnetic silica into the elution buffer.

Amplification

Detection of SARS-CoV-2 RNA uses reverse transcriptase PCR (RT-PCR) to detect the viral envelope (E) gene. This portion of the genome is conserved in other bat-derived betacoronaviruses and not conserved among other coronaviruses. RT-PCR amplifies RNA targets by first producing cDNA from the RNA target. The cDNA is then amplified by PCR. The SuperScriptIII OneStep RT-PCR System with Platinum Taq DNA Polymerase kit allows this process to proceed without the addition of reagents between the RT and PCR steps.

The addition of a TaqMan probe serves to eliminate detection of nonspecific amplification in the reaction. The probe consists of an oligonucleotide with a 5’-reporter dye (FAM) and a 3’-quencher dye (BBQ). If the target is present, the probe will anneal between the forward and reverse primer sites. In this setting, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. The 3’ end of the probe is blocked so that the probe cannot be extended during PCR. DNA polymerase exonuclease activity cleaves the TaqMan probe during PCR. This separates the reporter dye from the quencher dye, resulting in increased fluorescence of the reporter. This allows detection of the accumulation of PCR products.

Detection

The ABI PRISM 7500 Sequence Detection System is used for qualitative and quantitative detection with fluorescent-based PCR chemistries. During PCR, light from a tungsten-halogen lamp is focused on each well of the microplate. The light excites the fluorescent dye in each well and emission between 500 nm and 600 nm is detected. The system allows data analysis and reporting in a variety of formats.

INSTRUMENTS USED WITH TEST

The UNC SARS-CoV-2 real-time RT-PCR test is to be used with the bioMerieux EasyMag or Roche MagNAPure 24 and the ABI PRISM 7500 instruments.

CONTROLS TO BE USED WITH THE SARS-CoV-2 TEST:

The following controls will be included in this assay:

Control	Extracted (Y/N)	Process
Past positive patient (SARS-CoV-2 RNA)	Yes	Extraction, RT-PCR, E gene primers/probe
MRC-5 cells	Yes	Extraction, inhibition, albumin gene primers/probe
No template control (water blank)	No	Contamination

The SARS-CoV-2 positive control is a past positive patient that will be extracted along with every extraction of patient samples. This will control for both the extraction and RT- PCR. It will be positive for the SARS-CoV-2 E gene. The specimen is diluted to reach a quantity of SARS-CoV-2 RNA that is 3-5X the LOD.

The albumin positive control is a cellular lysate of MRC-5 cells that is extracted with every extraction of patient samples. Albumin is assayed for every specimen as an extraction, inhibition and cellularity control. The cellular lysate is the positive control for these primers and probes. It also serves as the negative extracted control for the E gene primers and probes.

The no template control (NTC) will be a water blank that is not extracted but will be performed with every rt-RT-PCR. The number of NTCs will be ~10% of the total number of reactions.

INTERPRETATION OF RESULTS

Examine the results of each patient for both SARS-CoV-2 and albumin. If controls in each assay are acceptable, report as follows based on possible scenarios:

A. SARS-CoV-2 E gene detected, albumin detected or not detected.
   SARS-CoV-2 test is resulted as Detected.

B. SARS-CoV-2 E gene not detected, albumin detected.

SARS-CoV-2 test is resulted as Not Detected.

C. SARS-CoV-2 E gene not detected, albumin not detected after repeat and 1:10 dilution.

SARS-CoV-2 test is Indeterminate.

An additional comment is released for indeterminate results: **"**Indeterminate indicates that an internal control was not detected in this specimen. This could be due to poor specimen collection or the presence of inhibitory substances. The presence of SARS-CoV-2 RNA cannot be determined. Consider collecting additional specimens if testing is still warranted."

The following test comment is added to every reported sample:

This test has been validated by the CLIA-certified, CAP-inspected UNC Medical Center Clinical Molecular Microbiology Laboratory. FDA's independent review of this validation is pending. This real-time RT-PCR test detects SARS-CoV-2 by targeting the E gene. 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. Information for providers and patients can be found here: https://www.uncmedicalcenter.org/mclendon-clinical-laboratories/available-tests/covid-19-pcr/

PERFORMANCE EVALUATION

Limit of Detection (LoD) - Analytical Sensitivity:

Serial dilutions of plasmid DNA containing the targeted E gene segment of SARS-CoV-2 were tested in triplicate. Probit regression analysis was used to estimate the limit of detection as 1000 copies/mL, extracted RNA was then used to verify the LoD.

Extracted nucleic acid of a positive COVID-19 sample (M-XNA) diluted to 550,000 copies/mL was obtained from Mayo Medical Laboratories. The M-XNA was quantified using RNA material from BEI to generate a standard curve and quantify the patient material.

Swab Specimens:

NP swabs and OP swabs in UTM were separately pooled and spiked with the quantified M-XNA. The final concentration of M-XNA was 880 copies/mL (17.6 copies/reaction). All pooled specimens were detected at this LOD (20 NP swabs and 5 OP swabs).

Lower Respiratory Specimens:

Tracheal aspirates in UTM were individually tested to ensure negativity for SARS-CoV-2. Negative specimen were pooled and spiked with serial dilutions of M-XNA. Spiked samples were extracted and SARS-CoV-2 was detected per protocol. Probit regression analysis of serial dilution replicates calculated the LoD to be 2055 copies/mL for M-XNA spiked into pooled tracheal aspirates. The LoD was verified using quantified remnant SARS-CoV-2 positive specimen at a final concentration of 3210 copies/mL (64.2 copies/reaction; 1X LOD). All spiked

pooled specimens were detected at this LoD (20 tracheal aspirates in UTM, 5 tracheal aspirates in PBS, and 5 BAL/BW).

Upper Respiratory Specimens:
LoD was also determined for nasal aspirates (NA) and nasal washes (NW). Negative NA/NW specimens were pooled and spiked at 880 copies/mL (17.6 copies/reaction; 1X LoD), the LoD determined for NP swabs. All pooled specimens were detected at this LoD.

Conclusion:

The final LoD for this assay is:
NP and OP swabs: 17.6 genome copies/reaction
Tracheal aspirates and BAL/BW: 64.2 genome copies/reaction
Nasal aspirates and Nasal washes:17.6 genome copies/reaction

Reactivity (Inclusivity):

We aligned all available SARS-CoV-2 sequences in the NCBI database as of 03/03/2020 to each E gene primer and probe. After excluding partial gene sequences that did not include the targets, 57 sequences remained (Appendix B). 56 whole genome sequences and 1 partial sequence were included in the alignment performed using CLC Genomics Workbench v.20.0.2. The alignment table is in Appendix C. The percent identity is shown below.

Primer/probe	Sequence (mismatch highlighted in red)	Percent nucleotide identity
E gene F primer	ACAGGTACGTTAATAGTTAATAGCGT	100%
E gene R primer	ATATTGCAGCAGTACGCACACA	100%
E gene probe	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	100%

To monitor for evolving genomic mutations in SARS-CoV-2 that may affect our primers and probe binding sites, we will realign our sequences to the NCBI public database weekly.

Cross-reactivity (Analytical Specificity):

Specificity studies were performed by testing positive specimens or culture isolates. The only detections were those that were expected (SARS, bat CoV). The organisms included in specificity studies are in the following table.

Organism	Material tested (number)
SARS-CoV*	Plasmid
MERS-CoV (JX869059)	Purified RNA from viral stock

BtSCoV-WIV1 (KF367457)*	Purified RNA from viral stock
BtSCoV-SHC014 (KC881005)*	Purified RNA from viral stock
CoV 229E	Positive specimens (8)
CoV HKU1	Positive specimens (6)
CoV NL63	Positive specimens (8)
CoV OC43	Positive specimens (6)
Influenza A	Positive specimens (4)
Influenza B	Positive specimens (4)
RSV	Positive specimens (4)
Rhinovirus/enterovirus	Positive specimens (6)
Metapneumovirus	Positive specimens (4)
Adenovirus	Positive specimens (4)
Parainfluenza virus 1	Positive specimens (4)
Parainfluenza virus 2	Positive specimens (4)
Parainfluenza virus 3	Positive specimens (4)
Parainfluenza virus 4	Positive specimens (4)
Chlamydia pneumoniae	Positive specimens (4)
Mycoplasma pneumoniae	Positive specimens (4)
Mycobacterium tuberculosis	Positive specimen (1)
Legionella pneumophila	Cultured organisms (3)
Haemophilus influenzae	Cultured organism (1)
Streptococcus pneumoniae	Cultured organism (1)
Streptococcus pyogenes	Cultured organism (1)
Bordetella pertussis	Positive specimen (1)
Moraxella catarrhalis	Cultured organism (1)
Staphylococcus epidermidis	Cultured organism (1)
Streptococcus salivarius	Cultured organism (1)
Pseudomonas aeruginosa	Cultured organism (1)
Candida albicans	Cultured organism (1)
Pneumocystis jiroveci	Positive specimens (2)
Mixture of oropharyngeal flora	Cultured organisms (3)

*detected in E gene assay, as expected.

No cross reactivity was detected for any organisms tested in the table above.

In addition, BLAST was utilized with CLC Genomics Workbench v.20.0.2 to determine any predicted cross-reactivity in primer and probe sequences for the E gene. Briefly, nucleotide sequences were queried after filtering out Wuhan, nCoV, SARS-CoV-2, and severe acute respiratory syndrome coronavirus 2. For the E gene primers and probe, the majority of matches were CoVs that are predicted to react (i.e., SARS, bat CoV). To determine potential cross-reactivity, we compared BLAST results for accession numbers that matched across each primer and probe. For the E gene, after filtering out SARS and bat CoV, there were five remaining organisms (shown below).

E gene matches shared among primer and probe set:

NCBI Accession Number	Description	Max % Identity
MT084071	Pangolin coronavirus isolate MP789 genomic sequence	100
KY352407	Severe acute respiratory syndrome-related coronavirus strain BtKY72, complete genome	100
KF569996	Rhinolophus affinis coronavirus isolate LYRa11, complete genome	100
FJ429166	Recombinant SARS coronavirus, complete sequence	100
AY274119	Severe acute respiratory syndrome-related coronavirus isolate Tor2, complete genome	100

Clinical Evaluation:

30 pooled NP swab specimens in UTM were spiked, 20 at 1X LoD and 10 spanning a range of positivity. The results are shown below where all samples were detected. Five pooled OP swabs in UTM were spiked at 1X; all were detected with tight precision. Thirty respiratory panel or group A Strep, negative specimens (20, NP; 10, OP) were tested, and all were also negative for SARS-CoV-2 RNA.

NP Swab Contrived Clinical Specimen Results

Spiking Titer	Replicate (Sample #)	Ct value for E gene
1xLoD	1	35.18
	2	36.06
	3	35.83
	4	35.8
	5	35.26
	6	36.82
	7	36.08
	8	35.72
	9	36.31
	10	36.54
	11	36.41
	12	36.69
	13	35.85
	14	36.75
	15	36.33
	16	35.65
	17	37.27
	18	36.39

	19	36.38
	20	36.59
10,000 xLoD	21	21.89
10,000 xLoD	22	21.81
1,000 xLoD	23	24.83
1,000 xLoD	24	25.22
100 xLoD	25	28.14
10 xLoD	26	28.97
10 xLoD	27	33.25
10 xLoD	28	25.78
4 xLoD	29	34.46
4 xLoD	30	34.3

Specimen type	Number of samples	Spiked quantity of SARS-CoV-2	Ct value E gene (Precision)
Pooled NP swabs in UTM	20	1X LoD	Average: 36.20 (CV: 1.5%)
	10	4X - 10,000X LoD	Range: 21.81 – 34.46
Pooled OP swabs in UTM	5	1X LoD	Average: 36.29 (CV: 1.1%)

Clinical specimens received by UNC were tested by the UNC SARS-CoV-2 assay were confirmed by another clinical laboratory; either by Mayo Medical Laboratories (n=1, positive nucleic acid extract) or the NC SLPH, public health lab (n=8, specimens). Results are below.

NP and OP swabs	Reference result (MML/NCSLPH)	
	Positive	Negative
Positive	4	0
Negative	0	5

To validate nasal aspirates and nasal washes as an acceptable specimen type additional clinical testing was performed. Clinical NA/NW samples were collected concordantly with NP swabs from 44 patients who were tested by the assay. Results are below.

		NP Swabs	
		Positive	Negative
NA/NW	Positive	1	0
	Negative	0	43

We observed 100% concordance between NA/NW and NP swabs. Per routine laboratory protocol, detection of the albumin gene serves as our internal control to detect possible inhibition and/or poor specimen collection (i.e., low cellularity). 11.4% (5/44) of NA/NW had no detected albumin, which would be resulted as indeterminate. Corresponding NP swabs all had albumin detected.

***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 bioMerieux EasyMag and the ABI 7500 respectively. The results are summarized in the following Table.

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

Reference Materials Provided by FDA	**Specimen Type**	**Product LoD**	**Cross-Reactivity**
SARS-CoV-2	Nasopharyngeal swabs	0.6×10^4 NDU/mL	N/A
MERS-CoV		N/A	ND

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