

**ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
UTHSC/UCH SARS-COV-2 RT-PCR ASSAY
UTMG PATHOLOGY LABORATORIES**

For *In vitro* Diagnostic Use

Rx Only

For use under Emergency Use Authorization (EUA) only

(The UTHSC/UCH SARS-CoV-2 RT-PCR assay will be performed at UTMG Pathology Laboratory, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, as per the Instructions of Use that were reviewed by the FDA under this EUA.)

INTENDED USE

The UTHSC/UCH SARS-CoV-2 RT-PCR assay is intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasal swabs from individuals suspected of COVID-19 by their healthcare provider. Testing location is limited to UTMG Pathology laboratory that is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratories.

Results are for the qualitative detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasal swabs 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 infective 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 UTHSC /UCH SARS-CoV-2 RT-PCR assay is intended for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The UTHSC/UCH SARS-CoV-2 assay is real time RT-PCR test, which uses real time PCR primer and fluorescent probe based chemistry with CDC developed EUA primer/probe sets purchased from Integrated DNA Technologies (IDT) to detect two regions of SARS-CoV-2 nucleocapsid (N1 & N2) gene and a third primer/probe set to detect human RNase P (RP) in clinical samples, in conjunction with reverse transcription and PCR using Applied Biosystems TaqPath 1-Step RT-qPCR Master Mix, CG (ThermoFisher Catalog # A15299).

In the presence of target sequence (N1, N2 or RP), the FAM coupled probe anneals between primer sites and is cleaved by the 5' nuclease activity of *Taq* polymerase enzyme during primer extension and thereby cleaving the probe and releasing fluorescent reporter dye FAM. Emitted fluorescent signal by free floating FAM molecules are captured by the filter and produce amplification curves for each detected target. With each additional cycle more reporter dye will be released from their matched targets and at the exponential phase the fluorescent intensity will be proportional to the target sequence detected.

INSTRUMENTS USED WITH TEST

The UTHSC/UCH SARS-CoV-2 RT-PCR assay test is to be performed using following equipment:

- Maxwell RSC (Promega)
- Tecan Genesis Liquid Handler (Tecan)
- AriaMx Real Time PCR system and Aria software (Agilent Technologies)

REAGENTS AND MATERIALS

The UTHSC/UCH SARS-CoV-2 RT-PCR assay has been validated using only the components referenced in this submission

The UTHSC/UCH SARS-CoV-2 RT-PCR nucleic acid sequence detection testing is done using ThermoFisher Scientific TaqPath 1-step RT-qPCR Master Mix (ThermoFisher Catalog # A15299) with CDC-developed assay that targets the Nucleocapsid genes N1 and N2 of COVID-19 virus. The sequence of primer/probe set used for the detection of N1 & N2 of the virus and the human specimen control (isolation control) are listed on Table 1.

Table 1: The primer and probe sequences for the N1 and N2 genes, as well as those for the RNase P control (IDT, Catalog # 268845589 EUA Lot)

Name	Description	Oligonucleotide Sequence (5'>3')	Label	Working Concentration
2019-nCoV N1 -F	2019-nCoV N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'	None	6.7 µM
2019-nCoV N1 -R	2019-nCoV N1 Reverse Primer	5' -TCT GGT TAC TGC CAG TTG AAT CTG-3'	None	6.7 µM
2019-nCoV N1 -P	2019-nCoV N1 Probe	5' -FAM-ACC CCG CAT TAC GTT TGG TGG ACC BHQ I-3 ' '	FAM- BHQ-1	1.7 µM
2019-nCoV N2-F	2019-nCoV N2 Forward Primer	5' -TTA CAA ACA TTG GCC GCA AA-3'	None	6.7 µM
2019-nCoV N2-R	2019-nCoV N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'	None	6.7 µM
2019-nCoV N2-P	2019-nCoV N2 Probe	5' -FAM-ACA ATT TGC CCC CAG CGC TTC AG- BHQ1-3'	FAM- BHQ-1	1.7 µM
RP-F	RNaseP Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'	None	6.7 µM
RP-R	RNaseP Reverse Primer	5'-GAG CGG CTG TCT CCA CAA GT-3'	None	6.7 µM
RP-P	RNase P Probe	5' -FAM - TTC TGA CCT GAA GGC TCT GCG CG - BHQ-1-3'	FAM- BHQ-1	1.7 µM

Table 2: Assay Composition for TaqPath I-Step RT-qPCR Master Mix per reaction

Reagent	Volume / Reaction
Nuclease Free Water	N x 8.5 uL
Combined Primer/Probe Mix	N x 1.5 uL
TaqPath 1-Step RT-qPCR Master Mix (4x)	N x 5.0 uL
Total Volume	N x 15.0 uL

CONTROLS TO BE USED WITH THE UTHSC/UCH SARS-COV-2 ASSAY

Controls that will be provided with the test kit include:

- No template controls (NTC): The NTC for each assay set should be run on each plate. The NTC consists of TaqPath 1-step RT- qPCR Master Mix, respective primer/probe mix (N1/N2/RP), and nuclease-free water replacing the nucleic acid template.
- 2019-nCoV N Positive Control (nCoVPC) (IDT, Catalog# 268845587): nCoVPC is an *in vitro* transcribed RNA with both N1 and N2 gene targets and should be run for each assay as a positive control. This control is designed to assess the efficiency of each primer/probe set on the PCR run.
- Human Specimen Control (HSC) (Extraction Control): HSC is a negative control for N1 and N2 and a positive control for human RNase P, a single copy human gene. This is an extraction control that is used to show the integrity of the extraction protocol. This control should have a reactivity in the RNase P reaction, and that value should fall within the specified value of <40 Ct. Laboratory-made frozen batches of human pancreatic cell line derived cells, that were previously tested, were used as HSC for extraction controls in each of the Maxwell RNA isolation runs.
- RNase P in patient samples (Internal Positive Control): RNase P in patient sample is considered as an internal positive control for extraction and amplification of clinical samples. All patient samples were expected to have exponential fluorescence growth curves for RNase P reaction that cross the threshold line a before 40 cycles (< 40.0 Ct), thus indicating the presence of required amount of template material.

However, some clinical samples that have strong fluorescence signal for the SARS-CoV-2 virus RNA, may fail to exhibit RNase P fluorescence growth curves due to low cell numbers. The absence of an RNase P fluorescence growth curve and virus specific N1 or N2 probe signal is an invalid result, which requires recollection of sample.

INTERPRETATION OF RESULTS

The UTHSC/UCH SARS-CoV-2 assay indicates that if any of the controls do not exhibit the expected performance as stated, the assay set up and/or extraction protocol may have been executed improperly, or reagent or equipment malfunctions could have occurred. Invalidate the run and retest.

Table 3: Performance Expectations for Controls

Control Type	Control Name	Purpose	SARS-VoV-2 N1	SARS-CoV-2 N2	RP	Expected Ct Value
Positive	nCoVPC	Amplification efficiency, Primer probe integrity	+	+	-	< 40.00 Ct
Negative	NTC	Contamination Monitoring	-	-	-	None
Extraction	HSC	Extraction monitoring	-	-	+	< 40.00 Ct
Internal	RP	Quality and Quantity of Clinical sample	-	-	+	< 40.00 Ct

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

Table 4: Interpretation of Clinical Samples and Result Reporting

2019 nCoV-N1	2019 nCoV-N2	RP	RT-PCR Results Interpretation	Report	Action
+	+	+	Positive 2019 nCoV	DETECTED	Report Results Confirmation of Infection
One of the two targets, N1 or N2, positive		+	Inconclusive	INCONCLUSIVE	Report also contains an interpretive comment "presumptive positive of SARS-CoV-2" . Clinical correlation recommended.

2019 nCoV-N1	2019 nCoV-N2	RP	RT-PCR Results Interpretation	Report	Action
-	-	+	Not Detected	NOT DETECTED	Report results. Absence does not rule out SARS-CoV 2 infection. Consider additional testing, if clinically warranted.
-	-	-	Invalid Results	INVALID	Recommend specimen Recollection

Detected: Clinical samples with Ct values of <40 in both N1 and N2 targets, with or without an acceptable RNase P, are reported as Detected for SARS-CoV-2 RNA

Inconclusive Results: Clinical samples with one of the two targets, either N1 or N2, with Ct value < 40 and with or without acceptable Ct value for RNase P is inconclusive. One of two nucleocapsid targets is detected, reported as Inconclusive for SARS-CoV-2 RNA.

Negative Specimens: Clinical samples with undetectable Ct values (i.e. Ct >40.0) for both N1 and N2 targets but with an acceptable RNase P (Ct <40) are reported as Not Detected for SARS- CoV-2 RNA.

Invalid Results: Clinical samples without any growth curves in the SARS-CoV-2 targets AND the RNase P are invalid. Reported as Invalid and recommend specimen recollection

LIMITATIONS

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to UTMG Pathology laboratory that is a certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

- False-negative results may arise from:
 - Improper sample collection
 - Degradation of the viral RNA during shipping/storage
 - Using unauthorized extraction or assay reagents
 - The presence of RT-PCR inhibitors
 - Mutation in the SARS-CoV-2 virus
 - Failure to follow instructions for use
- False-positive results may arise from:
 - Cross contamination during specimen handling or preparation
 - Cross contamination between patient samples
 - Specimen mix-up
 - RNA contamination during product handling
- The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not yet been evaluated.
- Please note, Negative results do not preclude infection of SARS-CoV-2 virus and should not be the sole basis of a patient management decision. A positive result indicates detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable. Laboratories are required to report all positive results to the appropriate public health authorities.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

To determine the Limit of Detection (LoD) and analytical sensitivity of the UTHSC/UCH SARS-CoV-2 assay, studies were performed using serial dilutions of analyte and the LoD was determined to be the lowest concentration of template that could reliably be detected with 95% of all tested positive.

The LoD of each target assay in the UTHSC/UCH SARS-CoV-2 assay was evaluated and verified using SARS-Related Coronavirus 2 Isolate USA- WAI/2020 (Catalog # NR-52281) viral cell supernatant. Using a 10-fold dilution series, SARS-CoV-2 viral cell supernatant was spiked into nasal swab specimens that were confirmed negative for SARS-CoV-2. Nucleic acid was extracted from the swabs using a modified RNA isolation protocol on the Maxwell RSC semi-automated system and the reverse transcription RT-PCR was performed using the AriaMx Real Time PCR system.

The LOD was confirmed by spiking 1:100 dilution containing 100 PFUs spiked SARS-Related Coronavirus 2 Isolate USA- WAI/2020 (Catalog # NR-52281) viral cell supernatant. Nucleic acid was extracted from the swabs using the modified RNA

isolation protocol on Maxwell RSC semi-automated system and the reverse transcription RT-PCR was performed using the AriaMx Real Time PCR system.

Table 5: LoD Confirmation

Targets	2019-nCoV_N1	2019-nCoV_N2
Analyte Concentration	100 PFUs	100 PFUs
Positives/Total	20/20	20/20
% Detected	100%	100%
Mean Ct	36.1	38.1
Mean SD	0.5	0.9
CV	1.4	2.2

The data confirmed the assay analytical sensitivity was 100 PFUs.

2) **Analytical Inclusivity:**

An alignment was performed with the oligonucleotide primer and probe sequences of the UTHSC/UCH SARS-CoV-2 assay with 1544 publicly available SARS-CoV-2 sequences from NCBI to demonstrate the predicted inclusivity of the assay. All the alignments show 100% identity of the assay to the top available SARS-CoV-2 sequences on April 28, 2020.

3) **Cross-reactivity:**

Analytical specificity of the primer/probe combination for the UTHSC/UCH SARS-CoV-2 assay was evaluated by wet laboratory testing using real time PCR analysis of the ZeptoMetrix NATrol Respiratory Verification Panel (ZeptoMetrix, Catalog # NATRVP-NNS). The following organisms were tested with NI, N2 and RP primer probe set.

Table 6: Organisms Analyzed by Wet Laboratory Testing for Cross Reactivity

Organism	Strain	Target NI	Target N2
Parainfluenza 1	N/A	Not Detected	Not Detected
RSVB	CH93(18)-18	Not Detected	Not Detected
Rhinovirus I A	N/A	Not Detected	Not Detected
Influenza AH3	A/Brisbane/ 10/07	Not Detected	Not Detected
Influenza B	B/Florida/02/06	Not Detected	Not Detected
Influenza AH I	A/Singapore/63/04	Not Detected	Not Detected
RSVA	N/A	Not Detected	Not Detected

Organism	Strain	Target N1	Target N2
hMPV-8	Paru6-2003	Not Detected	Not Detected
Adenovirus 3	N/A	Not Detected	Not Detected
Parainfluenza 4	N/A	Not Detected	Not Detected
Parainfluenza 3	N/A	Not Detected	Not Detected
Parainfluenza 2	N/A	Not Detected	Not Detected
<i>B. Parapertussis</i>	A747	Not Detected	Not Detected
<i>B. pertussis</i>	A639	Not Detected	Not Detected
<i>B. holmesii</i>	F061	Not Detected	Not Detected
Negative Control	NnA	Not Detected	Not Detected

No cross reactivity was observed with common respiratory panel organisms

4) **Clinical Evaluation:**

Clinical evaluation of the UTHSC/UCH SARS-CoV-2 assay was conducted with contrived nasal swab samples including 30 positive and 30 negative samples. Nasal swab samples that were confirmed to be negative for SARS-CoV-2 were contrived at 1x LoD and 1.5x LoD with SARS-Related Coronavirus 2 Isolate USA- WAI/2020 (Catalog # NR-52281) viral cell supernatant. Nucleic acid was extracted from the swabs using modified RNA isolation protocol on Maxwell RSC semi-automated system and the reverse transcription RT-PCR was performed AriaMx Real Time PCR system. Data is summarized in the Table below:

Table 7: Summary of Contrived Clinical Sample Evaluation

Specimen Type	SARS-CoV-2			Mean Ct Values			Performance Agreement	95% CI
	N1 +	N2 +	RP +	N1 +	N2 +	RP +		
Negative Samples	0/30	0/30	30/30	N/A	NA	31.5	100%	88.7-100%
Positive Samples 1.0x LOD (100 PFU's) Contrived swabs	20/20	19/20	20/20	34.0	37.1	33.1	95%	76.4-100%

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Positive Samples 1.5x LOD (150 PFU's) Contrived Swabs	10/10	10/10	10/10	32.30	35.1	31.6	100%	72.2-100%
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The results of 4/5 positive and 5 negative specimens tested with the Roche cobas SARS-CoV-2 (EUA200009) at Poplar Healthcare, PLLC Laboratory was confirmed using an alternative assay and fulfills the requirement for confirmatory testing of at least five positive and five negative specimens.

An additional study was conducted with 20 blinded samples (10 positive and 10 negative) provided by the reference laboratory that were tested with the Roche cobas SARS-CoV-2 (EUA200009). Samples were received as remnant material from swabs placed in 3 ml of VTM.

Results for 10/10 positive samples correlated, although for 1 sample only N1 was positive (inconclusive). Results for 9/10 negative samples correlated with the key provided for the blinded samples. In a single sample, UTHSC/UCH SARS-CoV-2 assay reported an inconclusive result (positive for NI only). One false negative and one false positive sample were believed to be a low positive sample.