ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-CoV-2 RT-PCR Assay

Yale New Haven Hospital

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

The SARS-CoV-2 RT-PCR assay will be performed at the Clinical Virology Laboratory at Yale New Haven Hospital, 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 SARS-CoV-2 assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in respiratory specimens (nasopharyngeal swabs, oropharyngeal swabs, nasal swabs, mid-turbinate nasal swabs, BALs, and sputum) from individuals suspected of COVID-19 by a healthcare professional. Testing is limited to the Clinical Virology Laboratory at Yale New Haven Hospital which is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory.

Results are for the 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 definitive 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 assay is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic products. This assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The TaqMan Probe-based chemistry uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR. An oligonucleotide probe is constructed with a fluorescent reporter dye (e.g., 6-FAM) bound to the 5' end and a quencher (e.g., TAMRA or BHQ) on the 3' end. While the probe is intact, the proximity of the quencher greatly reduces the fluorescence emitted by the reporter dye through a process called fluorescence resonance energy transfer (FRET).

If the target sequence is present, the probe anneals between primer sites and is cleaved by the 5' nuclease activity of AmpliTaq Gold during primer extension. This cleavage of the probe separates the reporter dye from the quencher, increasing the reporter dye signal. It also removes the probe from the target strand, allowing primer extension to continue to the end of the template strand. Thus, inclusion of the probe does not inhibit the overall PCR process.

Additional reporter dye molecules are cleaved from their respective probes with each cycle, resulting in an increase in fluorescence intensity proportional to the amount of amplicon produced. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed.

SARS-CoV-2 (originally called 2019 Novel Coronavirus or 2019 nCoV) testing utilizes the CDC-developed assay that targets the Nucleocapsid gene of this virus. Two gene targets, N1 and N2, are used to detect cases of COVID-19. During testing of clinical samples, one or both of the N1 and N2 nucleocapsid genes must be detected in order for the sample to be determined as a positive. The primer and probe sequences for the N1 and N2 genes, as well as those for the RNase P control sequences, are below:

2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes				
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	20 µM
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	None	20 µM
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC BHQ1-3'	FAM-BHQ-1	5 µM
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'	None	20 µM
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'	None	20 µM
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'	FAM-BHQ-1	5 µM
RP-F	RNAse P Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'	None	20 µM

RP-R	RNAse P Reverse Primer	5'-GAG CGG CTG TCT CCA CAA GT-3'	None	20 µM
RP-P	RNAse P Probe	5'-FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1-3'	FAM-BHQ-1	5 µM

INSTRUMENTS USED WITH TEST

RNA extraction is conducted using the NUCLISENS EASYMAG from bioMérieux. The SARS-CoV-2 RT-PCR test is to be used with the TaqPath 1-step RT-qPCR Master Mix on the ABI QuantStudio Flex 6 Real-Time PCR System with software version 1.3

REAGENTS AND MATERIALS

The following reagents/equipment are required to run this test:

1. ABI QuantStudio Flex 6 Real-Time PCR System
2. TaqPath 1-Step RT-qPCR Master Mix, CG (ThermoFisher; cat # A15299 or A15300)
3. 2019-nCoV CDC qPCR Probe primer/probe mix (IDT Technologies)
4. HSC RPP30 positive control and 2019-nCoV-N positive control from IDT (cat 10006625 and 100066236)
5. MicroAmp Fast Reaction Plates (LifeTech Cat No. 4346906)
6. MicroAmp Optical Adhesive Covers (LifeTech Cat No. 205618)
7. MicroAmp Splash Free Support Base (ABI Cat No. 4379590)
8. Eppendorf Pipettors or equivalent (various sizes)
9. Aerosol-Resistant Tips for Eppendorf Pipettors
10. Molecular grade water, nuclease-free
11. Vortex mixer
12. Microcentrifuge
13. Surface disinfectant (10% bleach wipes and alcohol wipes)

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

SARS-CoV-2 Positive Controls:
One positive control is run with each assay on each plate. This control is designed to assess the integrity of the PCR run. The positive control is an RNA transcript that has both the N1 and N2 gene targets and should be used to validate both assays. The ranges of this control are established prior to its use.

Human Specimen Control (HSC) (Negative Control and Extraction Control)
HSC is a negative control for N1 and N2 and a positive control for RNase P mastermix (MMX). This control must NOT have a detectable Ct with the N1 and N2 reactions. This control should have a reactivity in the RNase P reaction, and that value should fall

within the specified range. The range will be determined with each new MMX preparation. This controls for the extraction process.

RNase P in patient samples (Extraction Control)
RNase P in clinical samples assesses specimen quality. All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 40.00 cycles (< 40.00 Ct), thus indicating the presence of the human RNase P gene.

NTCs (No template controls):
NTC should be run on each plate. The NTC should consist of just 1x working Master Mix.

INTERPRETATION OF RESULTS

1) SARS-CoV-2 RT-PCR test Controls – Positive, Negative, and Internal:

- Negative (no template control) – This control must NOT have a detectable Ct in the N1, N2, or RNase P reactions. If this control has a detectable Ct in any of the reaction wells, this indicates contamination of the PCR run and it is considered invalid and must be repeated.

- Positive control – this control should target Ct values ≥ Ct=28 due to risk of contamination. The control's Ct value should fall within the established range for both genes. If it does not, a supervisor should be contacted to investigate, and the run is not acceptable.

- Negative and extraction control – this control should not have detection for the N1 and N2 targets but should be detected in the RnaseP reaction within the specified Ct range. If this control has a detectable Ct in either N1 or N2, the run is considered invalid and must be repeated.

- Internal control– All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 40.00 cycles (< 40.00 Ct), thus indicating the presence of the human RNase P (RP) gene. Failure to detect RP in any clinical specimens may indicate:
    - Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
    - Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
    - Improper assay set up and execution.
    - Reagent or equipment malfunction.

  If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:
    - If N1 and N2 are positive even in the absence of a positive RP, the result should be considered valid. It is possible that some

samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of 2019-nCoV virus RNA in a clinical specimen.

- If all 2019-nCoV markers AND RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

2019 nCov Assay Control Results Reporting

Control Type	External Control Name	Used to Monitor	2019 nCoV_N1	2019 nCoV_ N2	RP	Expected Ct Values
Positive	nCoVPC	Substantial assay or reagent failure including primer and probe integrity	+	+	-	Ranges established for all lot numbers for both N1 and N2. Will be targeted around 28-32 Cts
NTC	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Negative	HSC	Failure in lysis and extraction procedure, potential contamination during extraction	-	-	+	Ranges established for the lot number in use, targeted around 28-32 Cts

2019 nCoV now designated as SARS CoV-2

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

1. Detected: Positive Specimens:
   Specimens with Ct values of **<40.0** in **both of the N1 and N2 targets**, with or without an acceptable RNAse P, are reported as "Detected" for SARS-CoV-2 RNA.

2. Detected: Low Positive Specimens:
   When **either N1 or N2 is positive, not both**, the sample will be resulted as "Detected" since this is almost always a true but low positive result. These results may be repeated if reagents and time allows and if the diagnosis is in doubt. However, variable results can be seen with true low positive samples. Thus inconsistent detection of one target may still represent a true positive result.

3. Negative Specimens:
   Specimens with undetectable Ct values (i.e. Ct >40) with an acceptable RNAse P (Ct <40) are reported as "Not Detected" for SARS-CoV-2 RNA.

4. Invalid Results:
   With initially invalid results, the sample will be re-extracted and repeated. If result repeats the same, specimen will be resulted as "Invalid" and a comment will trigger (see below).

Interpretation of Patient Results

2019 nCoV_N1	2019 nCoV_N2	RP	Result Interpretation	Report	Actions
+	+	±	2019-nCoV detected	Positive 2019-nCoV	Report results in LIS and to State DPH
+	-	±	2019-nCoV detected	2019-nCoV detected	This usually represents a low positive. If diagnosis in doubt, consider collecting a new specimen.
-	+	±	2019-nCoV detected	2019-nCoV detected	This usually represents a low positive. If diagnosis in doubt, consider collecting a new specimen.
-	-	+	2019-nCoV not detected	Not Detected	Report results. Consider testing for other respiratory viruses.
-	-	-	Invalid Result	Invalid	Repeat extraction and rRT-PCR. If the result remains invalid, consider collecting a new specimen from the patient.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

An LoD study was conducted by spiking in vitro RNA transcripts purchased from Biosynthesis directly into pooled NP/OP clinical matrix. The transcripts were spiked at concentrations between 74-8000 copies/ul. Six serial 2-fold dilutions with 20 replicates per dilution were tested for both the N1 and N2 targets. The claimed LoD for the assay when detection of one target is considered "positive" was 2 copies/µL.

LoD (NP/OP matrix)	Mean Ct N1	Mean Ct N2
2 cp/µL	34.86	37.48

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

The sequences for the N1 and N2 primers/probes used in this assay are identical to the N2 primer/probe sequences used in the FDA authorized CDC SARS-CoV-2 assay.

3) ***Clinical Evaluation:***

The clinical evaluation was conducted by testing both patient specimens and contrived samples. For one set of samples, a head to head study was conducted assessing agreement of results with an EUA authorized test. In another set of studies, contrived samples were created and agreement against expected results was assessed.

Comparison to an EUA authorized test:

In this study, 18 confirmed positive samples using the EUA authorized test at the State Department Public Health laboratory (DPH) were tested with the SARs-CoV-2 assay. These positive samples included 12 confirmed positive clinical oropharyngeal/nasopharyngeal specimens and 6 contrived samples. For the clinical specimens, 7 were shared with YNHH from the DPH, and the remaining 5 were the first 5 positive specimens at YNHH that were confirmed with the EUA authorized test at the DPH. The 6 contrived samples were frozen extracted eluates that were shared with YNHH from the DPH laboratory. Briefly, these samples were made at the DPH by spiking matrix from respiratory swab specimens with nCoV PC (positive control provided by CDC) at dilutions targeting low and moderate positive concentrations. The samples were extracted at the DPH, and the extracted eluates were tested with both assays. To assess negative percent agreement, a total of 16 negative samples (13 clinical specimens and 3 contrived) were tested. These 16 samples included the following: 13 clinical specimens consisting of 8 from the DPH and 5 from YNHH (i.e., the first 5 negative specimens at Yale that were confirmed using an EUA authorized test at the DPH), as well as 3 frozen extracted eluates that were spiked with RNase-free water.

The positive percent agreement between the two assays was 100% for both the N1 and N2 targets. Negative percent agreement was 100% for both targets as well. The table below includes a comparison of Ct values for the positive specimens:

Sample		Mean Ct N1		Mean Ct N2	
	# of samples	LDT	EUA authorized	LDT	EUA authorized
Clinical specimens from DPH	7	27.61	28.66	27.9	28.59
Clinical specimens from YNHH	5	23.54	25.12	23.62	26.58
Frozen extracted eluate (low positive)	3	28.67	27.34	30.23	28.37
Frozen extracted eluates (moderate positive)	3	25.27	24.19	26.77	25.31

LDT performed at YNHH; EUA authorized performed at DPH

Comparison against expected values:

An additional clinical evaluation was conducted using a total of 50 contrived positive samples and 30 negative samples in clinical matrix (NP/OP, BAL and sputum). For the positive samples, RNA in vitro transcript in buffer was spiked directly into 30 NP/OP samples, 10 sputum samples, and 10 BAL samples, then extracted. For NP/OP samples, 20 samples were spiked at a concentration targeting 2X LoD and 10 samples were spiked targeting 25x LoD. For BAL and Sputum, 5 samples were spiked targeting 2X LoD and 5 samples targeting 25X LoD, for a total of 10 samples per specimen type. Thirty individual negative NP/OP specimens were also tested. For both the N1 and N2 targets, the PPA and NPA between the SARs-CoV-2 assay and expected results were 100%. The table below summarizes the mean Ct values for each target for each positive specimen type:

	NP/OP Matrix Ct values			BAL Matrix Ct values			Sputum Matrix Ct values		
	N1	**N2**	**RP**	**N1**	**N2**	**RP**	**N1**	**N2**	**RP**
Mean 2x LOD	31.2	33.5	24.2	31.1	33.0	23.2	33.0	35.34	21.1
Std Dev 2x LOD	0.38	0.59	0.53	0.37	0.45	0.14	0.49	1.35	0.14
Mean 25x LOD	28.0	29.7		28.1	29.6		29.8	31.3	
Std Dev 25x LOD	0.18	0.25		0.19	0.15		0.19	0.31	

LIMITATIONS:

- 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 authorized laboratories;
- This product has been authorized only for the detection of nucleic acid from SARSCoV-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 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 declaration is terminated or the authorization is revoked sooner.