

**ACCELERATED EMERGENCY USE
AUTHORIZATION (EUA) SUMMARY**

**SARS-CoV-2 RT-PCR Assay
(Massachusetts General 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 Massachusetts General Hospital, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a as per Laboratory Standard Operating Procedure that was reviewed by the FDA under this EUA.)

INTENDED USE

The MGH 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 nasal, nasopharyngeal and oropharyngeal swab and bronchoalveolar lavage (BAL) specimens, from individuals suspected of COVID-19 by a healthcare professional. Testing is limited to the Massachusetts General 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 MGH 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 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 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 (i.e., 6-FAM) bound to the 5' end and a

quencher (i.e., 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 Taq polymerase enzyme 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.

The MGH SARS-CoV-2 for 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, both N1 and N2 nucleocapsid genes must be detected in order for the sample to be determined as a positive.

INSTRUMENTS USED WITH TEST

RNA extraction is conducted using the QIAamp Viral RNA Mini Kit (Qiagen). The SARS-CoV-2 RT-PCR test is to be used with the TaqPath 1-step RT-qPCR Master Mix (ThermoFisher Scientific) on the following validated instruments:

- Cobas Z 480 real-time PCR analyzer and analysis software (Roche)
- QuantStudio 7 Flex Real-Time PCR System and analysis software (ThermoFisher)
- ViiA 7 Real-Time PCR System and analysis software (ThermoFisher)

REAGENTS AND MATERIALS

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

1. One of the following Real-Time PCR Instruments:
 - Cobas Z 480 real-time PCR analyzer and analysis software (Roche)
 - QuantStudio 7 Flex Real-Time PCR System and analysis software (ThermoFisher)
 - ViiA 7 Real-Time PCR System and analysis software (ThermoFisher)
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. MicroAmp Fast Reaction Plates (LifeTech Cat No. 4346906)

5. MicroAmp Optical Adhesive Covers (LifeTech Cat No. 205618)
6. MicroAmp Splash Free Support Base (ABI Cat No. 4379590)
7. Eppendorf Pipettors or equivalent (various sizes)
8. Aerosol-Resistant Tips for Eppendorf Pipettors
9. Molecular grade water, nuclease-free
10. Vortex mixer
11. Microcentrifuge
12. 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 diluted into pooled extracted RNA from negative nasopharyngeal swabs in Universal Transport Medium.

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. 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. This control consists of pooled universal transport medium from SARS-CoV-2-negative nasopharyngeal swabs taken through the entire extraction, reverse transcription, and amplification 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 35 cycles (< 35.0 Ct), thus indicating the presence of the human RNase P gene.

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 SARS-CoV-2 virus RNA in a clinical specimen.

NTCs (No template controls):

NTC should be run on each plate. The NTC consists of reaction master mix, primer/probe mix, and nuclease-free water and is included into the PCR reaction only.

INTERPRETATION OF RESULTS

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

Table 2. Expected performance of controls

Control Type	Control Name	Used to Monitor	SARS-CoV-2 N1	SARS-CoV-2 N2	RP	Expected Ct values
Positive	nCoVPC	Substantial reagent failure including primer and probe integrity	+	+	+	< 42.5 Ct for N1 and N2 targets; < 35 Ct for RNase P target.
Negative	NTC	Reagent and/or environmental contamination	–	–	–	None detected
Extraction	HSC	Failure in lysis and extraction procedure, potential contamination during extraction	–	–	+	< 42.5 Ct for N1 and N2 targets; < 35 Ct for RNase P target.

If any of the above controls do not exhibit the expected performance as described, the assay may have been improperly set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

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

1. Positive Specimens:

Specimens with Ct values of <42.5 in **both N1 and N2 targets**, with or without an acceptable RNase P, are reported as “Detected” for SARS-CoV-2 RNA

2. Negative Specimens:

Specimens with undetectable Ct values (i.e. Ct >42.5) for the N1 and N2 targets but with an acceptable RNase P (Ct <35) are reported as “Not Detected” for SARS-CoV-2 RNA.

3. Inconclusive Results:

When all controls exhibit the expected performance and the cycle threshold growth curve for **any one of two markers (N1 or N2) but not both** crosses the threshold line within 42.5 cycles (<42.5 Ct) the result is “inconclusive”.

Inconclusive results should be repeated since inconsistent detection of one target may still represent a true positive result.

4. Invalid Results:

Samples without any growth curves in the SARS-CoV-2 targets AND the RNase P (RP) are invalid. With initially invalid results, the sample will be re-extracted and repeated. If the result remains invalid, consider collecting a new specimen from the patient.

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
+	-	±	Inconclusive Result	Inconclusive	This is an inconclusive result Repeat extraction and RT-PCR. If the repeated result remains indeterminant, contact CDC for instructions for transfer of the specimen to CDC for additional testing and further guidance.
-	+	±	Inconclusive Result	Inconclusive	
-	-	+	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 into pooled Nasopharyngeal swab clinical matrix. The transcripts were spiked at concentrations of 10000 copies/µl, serial dilutions were made down to 2 copy/µl and tested with the cobas z 480 instrument system. The tentative LoD was identified at 5 copies/µl. For 5 and 10 copies/µl 20 replicates per dilution were tested for both the N1, N2 and RNase P targets. The LOD was confirmed at 5 copies/µl for both, N1 and N2 targets.

The LoD was then verified for two additional instruments, the QuantStudio 7 Flex Real-Time PCR System and the 7 Real-Time PCR System. For both instrument 20 replicates at 5 copies/µl were tested and all were detected, confirming the LoD of 5 copies/µl for all instruments.

2) **Analytical Inclusivity/Specificity:**

The sequences for the N1 and N2 primers/probes used in this assay are identical to the N1 and N2 primer/probe sequences used in the FDA authorized CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

3) **Clinical Evaluation:**

The clinical evaluation was conducted by testing patient specimens that were confirmed positive and negative by local Public Health Authorities with an EUA authorized test.

The clinical performance assessment is based on a total of n=60 nasopharyngeal swabs. Thirty (30) confirmed positive and 30 negative NP swab samples were tested according to the laboratories SOP on the Roche Cobas z480 platform. All positive samples and 2 negative samples underwent confirmatory testing from the State Department Public Health laboratory (DPH). The remaining n=28 negative samples were collected prior to the COVID-19 outbreak and the MGH COVID-19 qPCR Assay result was compared to the expected value (i.e., negative). All positive samples in a blinded randomized manner with negatives

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 all clinical specimens:

	DPH Test		Total	Mean Ct [N1]	Mean Ct [N2]	Mean Ct [RP]
	Positive	Negative				
MGH Positive	30	0	30	20.1	22.3	25.1
MGH Negative	0	30	30	N/A	N/A	25.7
Total	30	30	60	N/A	N/A	25.4
PPA	100% (88.7%-100%)*					
NPA	100% (88.7%-100%)*					

For validation of the different instruments, a head to head study was conducted with a subset of samples assessing agreement of the results on the additional instruments with the results from the cobas z 480 instrument. From the original 30 samples tested on the cobas z 480 a subset of 15 were tested on the additional instruments. However, the 15 samples were not identical between all additional instruments due to volume constraints.

Massachusetts General Hospital SARS-CoV-2 Test EUA Summary

Instrument	cobas z 480*	QuantStudio 7 Flex (Instrument A)	QuantStudio 7 Flex (Instrument B)	ViiA 7
2019-nCoV-N1				
Number Tested	30	15	15	15
Positives/Total	30	15	15	15
Mean Ct	19.7	21.5	22.0	22.8
Standard Deviation	4.59	4.12	4.40	3.87
Ct (range)	(10.6-31.8)	(13.7-28.3)	(13.3-29.6)	(17.1-30.1)
2019-nCoV-N2				
Number Tested	30	15	15	15
Positives/Total	30	15	15	15
Mean Ct	21.9	23.7	24.8	23.8
Standard Deviation	4.66	4.59	5.10	4.30
Ct (range)	(12.3-31.7)	(14.8-31.3)	(14.9-33.8)	(17.5-32.1)
RNaseP				
Number Tested	30	15	15	15
Positives/Total	30	15	15	15
Mean Ct	25.2	27.5	27.7	27.5
Standard Deviation	1.59	1.38	1.29	1.35
Ct (range)	(22.5-29.2)	(26-30.8)	(25.9-30.8)	(24-30.6)