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

(Children's Hospital of Philadelphia)

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

(The SARS-CoV-2 RT-PCR test will be performed at the Infectious Disease Diagnostics Laboratory within the Department of Pathology and Laboratory Medicine at the Children's Hospital of Philadelphia Clinical Laboratory, 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 laboratory-developed real-time PCR assay intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal aspirates, nasopharyngeal swabs, nasal swabs, mid-turbinate nasal swabs, tracheal aspirates, and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Infectious Disease Diagnostics Laboratory within the Department of Pathology and Laboratory Medicine at the Children's Hospital of Philadelphia that is 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 definite cause of disease.

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.

This assay is intended for use by CLIA certified high-complexity laboratories with experience in developing molecular diagnostics and is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The SARS-CoV-2 RT-PCR Test is a real-time reverse transcription polymerase chain reaction test. The SARS-CoV-2 PCR assay consists of a primer/probe set that amplifies and detects the N2 gene of the SARS-CoV-2 virus multiplexed with a primer/probe set that amplifies and detects the human β-actin gene as an internal control. RNA is extracted from respiratory specimens using the Roche MagNA Pure LC Total Nucleic Acid automated extraction platform. The RNA is then reverse transcribed to cDNA and subsequently amplified using Applied Biosystems Quant Studio DX automated PCR platform with software version 1.0.1. The real-time PCR reaction exploits the 5' nuclease activity of the DNA polymerase to cleave a TaqMan probe during PCR. The TaqMan probe contains a reporter dye (FAM) at the 5' end of the probe and a quencher dye (BHQ1) at the 3' end of the probe. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. During real-time PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites, and the 5'-3' nucleolytic activity of the DNA polymerase cleaves the probe between the reporter and the quencher. Cleavage results in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye after each cycle.

INSTRUMENTS USED WITH TEST

The SARS-CoV-2 RT-PCR test is to be used with the Roche MagNAPure LC Total Nucleic Acid automated extraction platform and the Applied Biosystems Quant Studio DX automated PCR platform using software version 1.0.1.

REAGENTS AND MATERIALS

Reagent	**Manufacturer**	**Catalog #**
MagNA Pure LC Total Nucleic Acid extraction platform	Roche	03038505001
UltraPlex 1-Step ToughMix Low ROX	QuantaBio	10804-954
N2 Forward Primer, Reverse Primer, Probe	IDT	Custom oligo
β-actin Forward Primer, Reverse Primer, Probe	IDT	Custom oligo
A549 cells	Quidel	56T075

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

1. A negative (no template) control is needed to eliminate the possibility of sample contamination on the assay run and is used on every assay plate. This control is molecular grade, nuclease-free water.

2. A positive template control is needed to verify that the assay run is performing as intended and is used on every assay plate tested. The positive control consists of N positive control plasmid from IDT diluted in A549 cells. The positive control must be positive at a Ct value of 32.96 ± 2.00.

3. An internal control targeting β-actin is needed to verify that nucleic acid is present in every sample and is used for every sample processed. This also serves as the extraction control to ensure that samples resulting as negative contain extracted nucleic acid for testing.

4. A negative control is needed to monitor for any cross- contamination that occurs during the RT-PCR process. The negative control consists of A549 cells that are amplified/detected with each PCR run. This control included on every assay plate that is tested.

INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

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

- Negative (no template control) – the NTC should be negative for all targets detected (Ct Not Detected)

- Positive control – the positive control should be positive for the assay N2 target at a Ct of 32.96 ± 2.00. Results from PCR runs with a negative result for the positive control cannot be reported and must be retested.

- Internal control – the internal control should be positive for every patient sample. Samples that do not have a positive internal control are repeated. If the internal control remains undetected, the sample is reported as invalid.

- Negative control– the negative control must be negative for the N2 target but positive for the β-actin control. Results from PCR runs with a positive result for the negative control must be retested.

If any control does not perform as described above, the run is considered invalid and all specimens are repeated from extraction.

2) Examination and Interpretation of Patient Specimen Results:

The positive and negative controls must yield valid expected results for patient results to be reported. If unexpected control results are obtained, results are not reported, and the samples must be retested after investigation into the source of error. All clinical samples should yield positive results for the β-actin target at < 45 Ct. Samples that fail to show detection of β-actin and the N2 target are invalid and should be repeated

from the extracted product. If the N2 and β-actin targets repeat as negative the sample must be re-extracted and retested.

Interpretation of Patient Results

SARS-CoV-2 N2 Ct value <45	Control	Result Interpretation	Report	Actions
+	Negative: valid Internal: valid Positive: valid	SARS-CoV-2 Detected	Positive	Results reported to sender and appropriate public health authorities
+	Negative: valid Internal: invalid Positive: Valid	SARS-CoV-2 detected	Positive	Results reported to sender and appropriate public health authorities
-	Negative: valid Internal: invalid Positive: Valid	Invalid	Invalid	Repeat from extracted product. If still invalid, re-extract and retest
-	Negative: valid Internal: valid Positive: valid	SARS-CoV-2 not detected	Negative	Results reported to sender

PERFORMANCE EVALUATION

1) ***Analytical Sensitivity:***

Limit of Detection (LoD):

A limit of detection study was performed by spiking negative nasopharyngeal aspirates with a confirmed SARS-CoV-2 positive patient sample. A 10-member dilution series of the contrived sample was performed with concentrations ranging from approximately 63 – 81,000 copies/mL. A total of 3 replicates were tested per dilution. A standard curve was generated to convert the Ct signal to a concentration in copies/mL using a plasmid control from IDT (200,000,000 cp/mL) that was serially diluted in 1:5 increments. The preliminary LoD from this dilution series was confirmed by spiking 20 replicates of negative clinical matrix with a confirmed positive patient sample. Nineteen out of 20 (19/20) replicates were positive with an average of Ct 34.67 and an average quantity of 20,747 cp/mL.

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

The sequences for the N2 primers and probe 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 SARS-CoV-2 assay was evaluated using a combination of contrived samples in a background of individual remnant clinical specimens as well as confirmed positive

patient samples. Thirty negative samples and 30 positive samples were tested. Contrived positive samples were created by spiking individual remnant clinical nasopharyngeal specimens with extracted genomic SARS-CoV-2 RNA. A total of 20 contrived positive samples were tested at a concentration of 1-2X LoD. Ten additional positive samples were tested consisting of 4 confirmed positive patient samples obtained from a peer institution and 6 additional contrived samples spanning the analytical range of the assay. Of the samples spiked at 1-2X LoD, a total of 19/20 samples were positive. Of the 10 remaining positive samples, all 10 were positive. Summary data for all 60 samples tested are shown below:

Clinical Evaluation Summary Data with Contrived and Patient Samples

SARS-CoV-2 concentration	**Number of samples**	**Detection rate**	**Mean Ct N2**	**Mean Ct β-actin**
1-2X LoD	20	19	34.67	36.09
>1-2X LoD	6	6	28.40	31.19
Patient specimens	4	4	27.48	31.32
Negative	30	0	N/A	N/A

PPA = 96.7% (83.3% - 99.4%)
NPA = 100% (88.7 - 100%)