EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
PCL SARS-CoV-2 Real-Time RT-PCR
Patients Choice Laboratories

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

The PCL SARS-CoV-2 Real-Time RT-PCR will be performed at the Patients Choice Laboratories located at 7026 Corporate Drive, Indianapolis, IN 46278, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, as per the PCL SARS-CoV-2 Real-Time RT-PCR Standard Operating Procedure that was reviewed by the FDA under this EUA.

INTENDED USE

1) Intended Use:

The PCL SARS-CoV-2 Real-Time RT-PCR assay is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal (NP) and oropharyngeal (OP) swab specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to Patients Choice Laboratories located at 7026 Corporate Drive, Indianapolis, IN 46278, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets requirements to perform high-complexity tests.

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 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 PCL SARS-CoV-2 Real-Time RT-PCR assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The PCL SARS-CoV-2 Real-Time RT-PCR assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TESTPRINCIPLE

The PCL SARS-CoV-2 Real-Time RT-PCR assay is a real-time, reverse transcription polymerase chain reaction (RT-PCR) test. The PCL SARS-CoV-2 Real-Time RT-PCR assay is a modified version of the ThermoFisher TaqPath COVID-19 Combo Kit, which is an authorized test. The PCL SARS-CoV-2 assay has the following modifications:

- Reaction volume has been decreased from 25 µL to 12.5 µL to allow high throughput processing in 384-well plates
- The assay is run on the QuantStudio 12K Flex instrument
- Results are analyzed using the QuantStudio 12K Flex software, v1.4

The SARS-CoV-2 primer and probe set(s) are designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients recommended for testing by public health authority guidelines. The assay simultaneously detects four targets: three SARS-CoV-2 viral targets, the Nucleocapsid (N) gene, the ORF1ab gene and the Spike protein (S) gene, and one primer/probe set detecting MS2 RNA spiked into the reaction as an extraction and process control.

Upper respiratory specimens should be collected, transported and stored according to standard procedures. Nucleic acid is extracted from patient specimens using an automated extraction procedure that consists of the Hamilton Vantage Liquid Handling System (serial number:1630) and Omega BiotekMag-Bind Viral RNA Xpress Kit (Omega Biotek, Cat# M6219-2304). Isolated viral RNA is reverse transcribed and amplified on the QuantStudio 12 K Flex instrument, software, v1.4.

INSTRUMENTS USED WITH THE TEST

The PCL SARS-CoV-2 Real-Time RT-PCR assay is to be used with the Hamilton Vantage Liquid Handling System (serial number:1630) and the QuantStudio 12 K Flex instrument (software version 1.4).

REAGENTS AND MATERIALS

Reagent Description	Catalog #	Manufacturer
Mag-Bind Viral Xpress Kit	M6219-2304	Omega Biotek
TaqPath-1-Step Multiplex Master Mix (No ROX) (4X)	A28523	Applied Biosystems
2019-nCoV Assays Multiplex Assay (20X0	A47814	Applied Biosystems
MS2 Phage Control (IPC) (2.5X)	A47814	Applied Biosystems
2019-nCoV DNA (Positive Control, 1:100 dilution)	A47814	Applied Biosystems
TaqPath Control Dilution Buffer	A47814	Applied Biosystems
MicroAmp Optical Adhesive Film	4311971	Applied Biosystems
Nuclease Free Water (Non-DEPC Treated)	4387936	Ambion
MicroAmp Optical 384-well Reaction Plate with Barcode	4326270	Applied Biosystems

CONTROLS TO BE USED WITH THE TEST

1. **Positive Control:** The ThermoFisher TaqPath COVID-19 Combo Kit positive control serves as the positive control for the PCL SARS-CoV-2 Real-Time RT-PCR assay. The positive control contains the SARS-CoV-2 RNA genomic regions (N, ORF1ab, and S genes) targeted by the assay and monitors the RT-PCR reaction setup and reagent integrity. The positive control is run with each batch of patient specimens.

2. **Negative Control:** Molecular grade, nuclease free water is used as a negative control to monitor cross-contamination during RNA extraction and reaction setup. The negative control is run with each batch of patient specimens.

3. **No Template Control:** A no template control is included in every extraction batch to monitor for cross-contamination during RNA extraction and the reaction setup.

4. **Internal Control:** The assay includes primers and probes for detection of an exogenous MS2 phage control (from the TaqPath COVID-19 Combo Kit) that is used to monitor the RNA extraction process. The internal control is extracted and amplified with every patient specimen.

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.

Table 1. Expected Results of Controls for the PCL SARS-CoV-2 Real-Time RT-PCR Assay

Control	Expected Results				Expected Crt values
	ORF1ab	N	S	MS2 (IC)	
Positive Control	Positive	Positive	Positive	Positive	<35
Negative Control	Negative	Negative	Negative	Positive	<35
No Template Control	Negative	Negative	Negative	Positive	<35

Assessment of clinical specimens should be performed after the positive, negative, no template, and internal controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Cycle relative thresholds (Crt) for each of the three SARS-CoV-2 target genes, in addition to the MS2 internal control Crt, will be used to determine control results and patient infected status. The PCL SARS-CoV-2 Real-Time RT-PCR assay employs the Relative Threshold estimation algorithm (Crt), which calculates a relative threshold from a fitted model for gene expression. The Relative Threshold method was employed to avoid Baseline Thresholding (Ct), which requires modification/interpretation of the threshold and subsequently can impact detection of

specimens with relatively low viral titers.

The interpretation and reporting of clinical specimens is summarized in **Table 2**.

Table 2. Result Interpretation for Patient Samples

ORF1ab	N	S	MS2	Status	Result	Action
NEG (Crt>37)	NEG (Crt>37)	NEG (Crt>37)	NEG (Crt>35)	Invalid	NA	Repeat test.[1] If the repeat result remains invalid, consider collecting a new specimen.
NEG (Crt>37)	NEG (Crt>37)	NEG (Crt>37)	POS (Crt<35)	Valid	SARS-CoV-2 Not Detected	Report results to healthcare provider. Consider testing for other viruses.
Only one SARS-CoV-2 target = POS (Crt<37)			POS (Crt≤35) or NEG (ND or Crt>35)	Valid	SARS-CoV-2 Inconclusive	Repeat test.[2] If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.
Two or more SARS-CoV-2 targets = POS (Crt<37)			POS (Crt≤35) or NEG (ND or Crt>35	Valid	Positive SARS-CoV-2	Report results to healthcare provider and appropriate public health authorities.

[1] Retesting should be performed by re-extracting the original sample and repeating the RT-PCR
[2] Samples with a result of SARS-CoV-2 Inconclusive shall be retested once

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) - Analytical sensitivity:

The LoD was determined by spiking negative nasopharyngeal specimens with SARS-CoV-2 genomic RNA (ATCC, cat#VR-1986D) in concentrations ranging from 10^6 to 100 copies/µL. These samples were extracted using the Omega Biotek Mag-Bind Viral RNA Xpress Kit on the Hamilton Vantage Liquid Handling System. To estimate the LoD, samples were tested in quadruplicate on the QuantStudio 12K Flex. The LoD was estimated to be 100 copies/µL. The LoD was confirmed by testing 20 negative nasopharyngeal specimens spiked with SARS-CoV-2 genomic RNA (ATCC, cat#VR-1986D) at 100 copies/µL. 100% detection (20/20) was observed at this concentration, confirming an LoD of 100 copies/µL. Results of the confirmatory LoD study are illustrated in **Table 3**.

Table 3. LoD Verification Study Results

Concentration (copies/µL)	Mean Crt Values				% Detection (# Detected/Reps Tested)
	ORF1ab	N	S	MS2	
100	33.8	33.6	33.5	28.4	100% (20/20)
10	36.7	36.0	36.2	28.4	75% (3/4)

2) Inclusivity - Analytical Sensitivity:

The PCL SARS-CoV-2 Real-Time RT-PCR assay utilizes identical oligonucleotide sequences as the Thermo Fisher TaqPath COVID-19 Combo Kit authorized assay. The inclusivity has been evaluated previously and therefore, additional evaluation was not necessary. Thermo Fisher has granted a right of reference to Patients Choice Laboratories for the inclusivity performance data contained in the Thermo Fisher EUA request (EUA200010).

3) Cross-reactivity - Analytical Specificity:

The PCL SARS-CoV-2 Real-Time RT-PCR assay utilizes identical oligonucleotide sequences as the Thermo Fisher TaqPath COVID-19 Combo Kit authorized assay. The cross-reactivity has been evaluated previously and therefore, additional evaluation was not necessary. Thermo Fisher has granted a right of reference to Patients Choice Laboratories for the cross-reactivity performance data contained in the Thermo Fisher EUA request (EUA200010).

4) Clinical Evaluation:

Clinical Performance of the PCL SARS-CoV-2 Real-Time RT-PCR assay was evaluated using 75 residual nasopharyngeal (NP) swabs (40 positives and 35 negatives) collected from patients suspected of COVID-19. These specimens were previously characterized at three external reference labs – each using a different FDA emergency use authorized SARS-CoV-2 molecular test. For the positive residual NP swab samples, the positive percent agreement between the PCL SARS-CoV-2 Real-Time RT-PCR assay and the comparator assays was 100% (40/40). For the 35 negative residual NP swab samples, the negative percent agreement between the PCL SARS-CoV-2 Real-Time RT-PCR assay and the comparator assays was 100% (35/35). Qualitative results of the clinical evaluation are shown in **Table 4**.

Table 4. Clinical Performance of the PCL SARS-CoV-2 Real-time RT-PCR Assay

		Authorized Comparator -1		
		Positive	**Negative**	**Total**
PCL SARS-CoV-2 Real-Time RT PCR assay	**Positive**	11	0	11
	Negative	0	0	0
	Total	11	19	30
Positive Agreement		100% (11/11), 95% CI: (74.1, 100.0%)[1]		
Negative Agreement		100% (19/19), 95% CI: (83.2, 100.0%)		
		Authorized Comparator -2		
		Positive	**Negative**	**Total**
PCL SARS-CoV-2 Real-Time RT PCR assay	**Positive**	12	0	12
	Negative	0	8	8
	Total	12	8	20
Positive Agreement		100% (12/12), 95% CI: (75.8, 100.0%)		
Negative Agreement		100% (8/8), 95% CI: (67.6, 100.0%)		
		Authorized Comparator -3		
		Positive	**Negative**	**Total**
PCL SARS-CoV-2 Real-Time RT PCR assay	**Positive**	17	0	17
	Negative	0	8	8
	Total	17	8	25
Positive Agreement		100% (17/17), 95% CI: (81.6, 100.0%)		
Negative Agreement		100% (8/8), 95% CI: (67.6, 100.0%)		

[1] Two-sided 95% confidence intervals

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by Patients Choice Laboratories located at 7026 Corporate Drive, Indianapolis, IN 46278;
- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

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 used was the Omega BiotekMag-Bind Viral RNA Xpress Kit (Omega Biotek, Cat# M6219-2304) performed on the Hamilton Vantage Robotic Liquid Handler System. Isolated viral RNA is reverse transcribed and amplified on the QuantStudio 12 K Flex instrumentThe results are summarized in the following Table.

Table 5. 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 in VTM	$5.4x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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