INNO Diagnostics Reference Laboratory at Ponce Medical School Foundation, Inc.
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ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
SARS-CoV-2 Real Time RT-PCR Diagnostic Panel
(INNO Diagnostics Reference Laboratory)

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

The SARS-CoV-2 Assay will be performed at INNO Diagnostics Reference Laboratory at Ponce Medical School Foundation, Inc. certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Laboratory Instructions for Use reviewed by the Office of the Assistant Secretary for Health at the Department for Health and Human Services (DHHS) 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 obtained from samples collected in upper respiratory specimens (nasopharyngeal swabs) from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to INNO Diagnostics Reference Laboratory at Ponce Medical School Foundation, Inc. in Ponce, Puerto Rico that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper 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 test 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 PMSF-INNO SARS-CoV-2 assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The PMSF-INNO SARS-CoV-2 assay is only for use under the U.S. Department of Health and Human Services-issued Emergency Use Authorization (HHS-EUA).

DEVICE DESCRIPTION AND TEST PRINCIPLE

The assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines.

The INNO SARS-CoV-2 Real-Time RT-PCR Diagnostic assay uses primers and probes implemented previously by CDC that targets the N gene. They are designed to detect two regions in the viral nucleocapsid gene (N gene) and additional set of primers/probe are used to identify the internal control. Viral RNA is extracted from nasopharyngeal samples by using QIAamp Viral RNA kit (Qiagen), according to the manufacturer's instructions. The viral RNA is reverse transcribed to cDNA and amplified (reverse transcription polymerase chain reaction) by using real time PCR procedure by using the qScript XLT 1-Step RT-qPCR kit (Quantabio) and the Roche Lightcycler 480 II instrument. The TaqMan Probe-based chemistry uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR. During the extension phase of the procedure (real-time PCR), the Taq polymerase cleaves the bound probes by using the 5' nuclease activity. This cleavage of the probe separates the reporter dye from the quencher, increasing the reporter dye signal. The fluorescent signal is monitored by the PCR instrument. The primer and probe sequences for the N1 and N2 genes, as well as those for the RNase P control sequences, is as follows:

Reagents, Equipment and Materials

- QIAamp Viral RNA Mini Kit (Qiagen, cat# 52904 or 52906)
- Roche LightCycler 480 II
- qScript XLT 1-Step RT-qPCR ToughMix
- SARS-CoV-2 qPCR primers and probes

Control Material(s) to be Used:

- SARS-CoV-2 positive control (PC) is an RNA transcript that contains both the N1 and N2 gene targets. The PC is included in each assay plate and is used to assess the integrity of the assay.
- Human Specimen Control (HSC) Internal Control (IC): RNase P is used to confirm that nucleic acid is present in every sample as an extraction control. This control is used to verify that negative samples contain nucleic acid for testing. All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line (see below Assay interpretation and results).
- Negative Extraction Control (NEC) is included in each assay run. The NEC is a previously characterized negative vehicle transport medium (VTM), which help us to monitor cross contamination and nucleic acid contamination of reagents. This sample included IC.

- A no template control (NTC) is used to monitor sample contamination on the assay and is used on every assay plate. The control used is molecular grade (nuclease-free water) and master mix.

Assay results and interpretation

SARS-CoV-2 RT-PCR Test Controls – Positive, Negative, and Internal:

The test controls should always be evaluated prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted (invalid assay), and all specimens must be repeated from the extraction step.

- NTC – Must not have detectable Cp in the N1, N2, or RNase P reactions. If this control has a detectable Cp in any reaction well, this indicates contamination of the PCR run and it is considered an invalid result.
- PC – positive for SARS-CoV-2 N1 and N2 target detected (Cp < 27). If the target is out of range the run is not acceptable.
- HSC IC– This control should not have detection for SARS-CoV-2 N1 and N2 targets (Cp ≥45), but RNase P (RP) target should be detected (Cp < 35). 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.

    However, a sample with negative for RP but positive to N1 and N2 should be considered valid. It is possible that RNase P may not be detected 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. If all SARS-CoV-2 markers and RP are negative for the clinical specimen, the result should be considered invalid. If residual specimen is available, the extraction must be repeated and the sample re-tested. If after re-testing all markers remain negative, the results should be reported as invalid and a new specimen should be collected if possible.
- NEC – negative for SARS-CoV-2 targets N1 and N2 and positive for RNase P (RP) target (Cp < 35).

Control	Used to monitor	SARS-CoV-2 N1	SARS-CoV-2 N2	RP	Expected Cp Values
Positive	SARS-CoV-2 gene target detection	+	+	-	Ranges established for the control in use for both N1 and N2. Will be targeted around Cp < 27.
NTC	Reagent and/or environmental contamination	-	-	-	None detected
NEC (HSC, IC)	Failure in extraction procedure. Monitor cross contamination and nucleic acid contamination of reagents	-	-	+	Ranges established for the lot number in use, targeted around Cp <35.

Examination and Interpretation of Patient Specimen Results:

1. Positive Specimens: Specimens with Cp values ≤40 in both N1 and N2 targets, with or without an acceptable RNAse P, are reported as "Detected" for SARS-CoV-2 RNA. Cp values are recorded for internal use only and are not reported.
2. Negative Specimens: Specimens with undetectable Cp values (i.e. Cp >40) with an acceptable RNAse P (Cp < 35) are reported as "Not Detected" for SARS-CoV-2 RNA.
3. Inconclusive Results: With inconclusive results (either N1 or N2 positive, not both), the sample will be resulted as "Inconclusive" and a comment will trigger (see below). Inconclusive results may be repeated if reagents and time allows. However, variable results can be seen with true low positive samples. Thus, inconsistent detection of one target may still represent a true positive result.
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).

SARS-CoV-2 N1	SARS-CoV-2 N2	RP	Interpretation	Report	Action
+	+	±	SARS-CoV-2 detected	Positive SARS-CoV-2	Report results
-	+	±	Inconclusive positive results	Inconclusive	Repeat nucleic acid extraction and rRT-PCR once. This result could represent low positive. Is recommendable collecting a new sample.
+	-	±	Inconclusive positive results	Inconclusive	Repeat nucleic acid extraction and rRT-PCR once. This result could represent low positive. Is recommendable collecting a new sample.
-	-	+	SARS-CoV-2 not detected	Not detected	Report results
-	-	-	Invalid results	Invalid	Repeat nucleic acid extraction and rRT-PCR once. If results remain is recommendable collecting a new sample.

PERFORMANCE EVALUATION

The following validation studies should be performed during your assay development:

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

The limit of detection (LoD) is established by using the lowest concentration of virus (SARS-CoV-2) that can be detected by the test at least 95% of the time. Preliminary data were obtained by evaluating 10-fold serial dilution of SARS-CoV-2 spiked directly into clinical matrix and extracted. The SARS-CoV-2 was previously quantified by using quantified RNA transcripts obtained from Biosynthesis, Inc. (donated by Yale New Haven Hospital). Confirmation was performed by using serial dilutions (10 cp/µL, 6 cp/µL, 5 cp/µL, 6 and 1 cp/µL) and evaluated 20 samples at each concentration. The preliminary LoD was 6,000 copies/mL for the N1 target and 5,000 copies/mL for the N2 target.

2) Analytical Sensitivity and 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. The evaluation of the sequences for the N1 and N2 primers/probes used in the assay were performed by the FDA authorized CDC SARS-CoV-2 assay. The information has been provided in the HHS-EUA granted to this organization.

3) Clinical Evaluation:

Method Comparison:

In this study, a total of 66 nasopharyngeal specimens (32 positives and 34 negatives) were collected and evaluated. The samples were analyzed by EUA authorized cobas SARS-CoV-2 test at Laboratorio Clínico Toledo and Laboratory Corporation of America (LabCorp). The positive agreement was 28/32 and the negative agreement was 34/34 with 4 inconclusive results on the PMSF-INNO SARS-CoV-2 assay.

Limitation

The performance of the PMSF-INNO SARS-CoV-2 assay was established using nasopharyngeal swab specimens and sputum specimens. Nasal swabs, mid-turbinate swabs, oropharyngeal swabs, nasopharyngeal wash/aspirates, nasal aspirates, bronchoalveolar lavage, and lower respiratory tract aspirates are also considered acceptable specimen types for use with the NCH SARS-CoV-2 assay. Testing of nasal and mid-turbinate nasal swabs (self-collected at a healthcare site or collected by a healthcare provider) is limited to patients with symptoms of COVID-19.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by the Office of the Assistant Secretary for Health, U.S. Department of Health and Human Services, under an EUA for use by the authorized laboratory;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-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 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 authorization is terminated or revoked sooner.