# ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY WREN LABORATORIES COVID-19 PCR TEST (WREN LABORATORIES LLC)

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

**(The Wren Laboratories COVID-19 PCR Test will be performed at Wren Laboratories, Inc. located at 688 East Main Street, Branford, CT, 06405, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity tests, as per the Standard Operating Procedure that was reviewed by the FDA under this EUA.)**

## INTENDED USE

The Wren Laboratories COVID-19 PCR Test is a real-time, reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal (throat), anterior nasal, and mid-turbinate nasal swabs, as well as nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to Wren Laboratories located at 688 East Main Street, Branford, CT, 06405 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 identification of SARS-CoV-2 RNA which is generally detected 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.

Testing with the Wren Laboratories COVID-19 PCR Test is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Wren Laboratories COVID-19 PCR Test is only for use under the Food and Drug Administration’s Emergency Use Authorization.

## DEVICE DESCRIPTION AND TEST PRINCIPLE

The Wren Laboratories COVID-19 PCR Test is a two-step real-time, reverse transcription polymerase chain reaction test (rRT-PCR). The assay uses primers and probes that were developed and validated under the Emergency Use Authorization (EUA) for the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel and are designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients suspected of COVID-19 by their healthcare provider. The test uses two primer and probe sets to detect two regions of the nucleocapsid (N) gene; the N1 target is specific to SARS-CoV-2 and the N3 target is specific to Sarbecovirus/SARS-like coronaviruses that includes SARS-CoV-2. The Wren Laboratories Test also includes a primer and probe set to detect human RNase P (RP) in control samples (i.e., positive plate control) and clinical specimens. Three separate master mixes for each target are prepared and run with the Wren Laboratories Test.

RNA is isolated from respiratory specimens including nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swabs as well as nasopharyngeal washes/aspirates or nasal aspirates and BAL specimens using the QIAamp Viral RNA Mini Kit (Qiagen, Cat # 52906). Nucleic acid is manually extracted from 140 µL of acceptable specimen. The final elution volume is 60 µL. RNA is reverse transcribed to cDNA using the ThermoFisher High Capacity cDNA Reverse Transcription Kit (Cat # 4368814) on the Eppendorf Nexus Gradient Mastercycler (software version 3.6.9.0). The cDNA is quantified and diluted to 200 ng/µL and subsequently amplified using the Applied Biosystems QuantStudio 7-Flex Real-Time PCR Instrument with QuantStudio Real-Time PCR software v1.3. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5′ nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye (FAM) to separate from the quencher dye (BHQ-1), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle.

Three technical replicates per assay are run with each patient sample (9 total replicates for 1 sample) to ensure assay accuracy specifically when testing samples with low viral loads.

## INSTRUMENTS USED WITH TEST

The Wren Laboratories COVID-19 PCR Test is to be used with the Eppendorf Nexus Gradient Mastercycler (software version 3.6.9.0) for cDNA synthesis and the Applied Biosystems QuantStudio 7-Flex Real-Time PCR Instrument with QuantStudio Real-Time PCR software v1.3 for PCR amplification.

## REAGENTS AND MATERIALS

| Reagent Manufacturer and Description | Catalog # | Manufacturer |
|---|---|---|
| QIAamp Viral RNA Mini Kit | 52906 | Qiagen |
| High Capacity cDNA Reverse Transcription | 4368814 | ThermoFisher Scientific |
| Universal Master Mix II, with UNG | 4440039 | ThermoFisher Scientific |
| COVID-19_N1-F Primer (forward primer) | 10006606 | Integrated DNA Technologies |

| Reagent Manufacturer and Description | Catalog # | Manufacturer |
|---|---|---|
| COVID-19_N1-R Primer (reverse primer) | 10006606 | Integrated DNA Technologies |
| COVID-19_N1-P Probe (N1 probe) | 10006606 | Integrated DNA Technologies |
| COVID-19_N3-F Primer (forward primer) | 10006606 | Integrated DNA Technologies |
| COVID-19_N3-R Primer (reverse primer) | 10006606 | Integrated DNA Technologies |
| COVID-19_N3-P Probe (N3 probe) | 10006606 | Integrated DNA Technologies |
| RP-F Primer (forward primer) | 10006606 | Integrated DNA Technologies |
| RP-R Primer (reverse primer) | 10006606 | Integrated DNA Technologies |
| RP-P Probe (RNase P probe) | 10006606 | Integrated DNA Technologies |
| 2019-nCoV_N_Positive Control | 10006625 | Integrated DNA Technologies |
| MicroAmp Optical 384-Well PCR plate | 4309849 | ThermoFisher Scientific |
| MicroAmp Optical Adhesive PCR Plate Cover | 4311971 | ThermoFisher Scientific |

## CONTROLS TO BE USED WITH THE WREN LABORATORIES COVID-19 PCR TEST

1) A no template control (NTC) is needed to check for contamination of RT-PCR assay reagents. Molecular grade, nuclease-free DEPC-treated water is used in place of sample nucleic acid for this control. Three NTCs are run on every 384-well assay plate.

2) The positive control is the 2019-nCoV_N_Positive Control from Integrated DNA Technologies (IDT) Cat # 10006625). Positive template control is needed to verify PCR reagent integrity as well as proper assay set-up of the RT-PCR reactions for the N1 and N3 genes. The positive control is used on every assay plate starting at PCR master mix addition (not reverse transcription master mix set-up) at a final concentration of 3 copies/µL. The 2019-nCoV_N_Positive Control is commercially supplied from IDT and is made of *in vitro* transcribed and purified plasmid DNA targets that contains one copy each of N1 and N3.

3) A positive plate control is used to evaluate RNase P primers and probe, reagent integrity and amplification. Three wells of cDNA from a human cell line are run on every 384-well assay plate.

4) RNase P is co-extracted and amplified from all patient samples as an internal control. Detection of the RNase P gene in patient test samples verifies successful extraction of the sample, proper assay setup, sample integrity, and collection of human biological material.

## INTERPRETATION OF RESULTS

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted (Refer to Table 1 for a summary of control results).

1) ***COVID-19 RT-PCR Test Controls – NTC, SARS-CoV-2 Positive Viral Control, Positive Plate Control, and Internal RNase P Control:***

- The no template controls (NTC) should be negative (Ct Not Detected) for all assay targets. If the N1, N3, or RNase P targets exhibit positive fluorescence above the threshold (Ct < 38 for N1/N3 and Ct < 34 for RNase P), it is possible that contamination occurred, or that the assay was setup improperly. The RT-PCR run is invalid. The user is instructed to repeat the RT-PCR using residual extracted material for the clinical samples and a fresh no template control. If the repeat NTC results (one for each assay) are positive for any of the assay targets, this indicates contamination with the water or a master mix component. All master mix reagents and water must be replaced and PCR must be re-run. If only one of the NTCs were positive, this would suggest contamination of the primer/probe set and therefore, the primer/probe set must be replaced, and the PCR must be re-run.

- The positive control (2019-nCoV_N_Positive Control) must be positive for the N1 and N3 targets (Ct < 38) and negative (Ct Not Detected or Ct ≥ 28) for RNase P. Negative results with the N1 or N3 targets invalidates the run and suggests the assay may have been set up incorrectly, the integrity of the primers/probes could have been compromised, or potential carry-over of PCR inhibitors. The user is instructed to repeat the RT-PCR step using residual extracted material for clinical samples.

- The positive plate control should be negative for N1 and N3 (Ct Not Detected or Ct ≥ 38), and positive for the RNase P target (Ct < 28). If positive results are obtained for N1 and N3 targets, cross-contamination of samples may have occurred. Failure of the control to yield a RNase P Ct value of < 28 may indicate degradation of primer/probe integrity.

- The Internal RNase P Control must be positive for each clinical sample (Ct < 34). Test samples that fail to show detection of RNaseP are invalid and the RT-PCR assay must be repeated using residual nucleic acid. If repeat testing of the clinical samples are negative for RNase P, all samples must be re-extracted from residual clinical samples and the RT-PCR assay must be re-run with fresh controls.

**Table 1. Ct Values for Controls that Must be Observed to Obtain Valid Results**

| Control | Expected N1 Result | Expected N3 Result | Expected RP Result |
|---|---|---|---|
| 2019-nCoV_N_ Positive Control (N1, N3 template) | Ct < 38 | Ct < 38 | Not Detected; Ct ≥ 28 |
| No Template Control (NTC) | Not Detected; Ct ≥ 38 | Not Detected; Ct ≥ 38 | Not Detected; Ct ≥ 28 |
| Positive Plate Control (Human Cell Line) | Not Detected; Ct ≥ 38 | Not Detected; Ct ≥ 38 | Ct < 28 |
| Internal RNase P Control (Clinical Samples) | N/A | N/A | Ct < 34 |

Not Detected; No detectable signal
N/A; Not Applicable

If the results obtained with the positive control and NTC do not meet the criteria shown, the results from the entire batch of samples are considered invalid and repeat testing must be performed using residual extracted nucleic acid and a fresh NTC. If the internal RNase P control does not meet the acceptability criteria for the tested clinical sample, the RT-PCR assay must be re-run using residual extracted nucleic acid. If repeat testing for the clinical samples shows negative results for RNase P, all specimens in the batch must be re-extracted from residual clinical samples and the RT-PCR assay must be re-run.

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Please see the table below (Table 2) for guidance on interpretation and reporting of results using three technical replicates per assay.

**Table 2. Interpretation of Patient Results Using the Wren Laboratories COVID-19 PCR Test**

| N1 (Ct < 40) | N3 (Ct < 40) | RNase P (Ct < 34) | Interpretation | Report Result | Actions |
|---|---|---|---|---|---|
| +[a] | +[a] | +[a] | SARS-CoV-2 **Detected** | **POSITIVE** | Results reported to test requisitioner and appropriate public health authorities |
| +[a] | -[b] | +[a] | SARS-CoV-2 **Detected** | **POSITIVE** | Results reported to test requisitioner and appropriate public health authorities |
| -[b] | +[a] | +[a] | **SARS-CoV-2 Presumptive Positive** | **Presumptive Positive** | Sample is repeated once using residual extracted nucleic acid and 3 technical replicates. If the repeated result remains Presumptive Positive, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management. |
| -[b] | -[b] | +[a] | SARS-CoV-2 **Not Detected** | **NEGATIVE** | Results reported to test requisitioner |
| +/-[b] | +/-[b] | -[b] | **Invalid test** | **INVALID** | Repeat using residual extracted nucleic acid and 3 technical replicates. If results remain invalid, nucleic acid should be re-extracted from residual clinical sample and the assay must be re-run. If the internal control remains undetected/negative, the sample is reported as invalid and specimen re-collection is recommended. |

[a] At least 2 technical replicates show signal (Ct < 40 for N1/N3, Ct < 34 for RNase P), the sample is positive for the target

[b] No signal detected or if signal is detected but does not reach at least 2/3 technical replicates, the sample is negative for the target

## PERFORMANCE EVALUATION

**1) *Analytical Sensitivity:***

*Limit of Detection (LoD):*

The LoD (lowest SARS-CoV-2 viral RNA concentration that consistently yields a 95% positivity rate) of the Wren Laboratories COVID-19 PCR Test was determined using synthetic SARS-CoV-2 viral RNA from Twist Bioscience (Cat # MT007544.1). A preliminary LoD was determined by testing serial dilutions (1000 copies/µL – 10 copies/µL) of synthetic RNA spiked into pooled clinical negative, nasopharyngeal swab or oropharyngeal swab matrix using three replicates at each target level. Spiked samples were tested with the Wren Laboratories COVID-19 PCR Test following extraction with the QIAamp Viral RNA Mini Kit. Fifty microliters of extracted RNA was used for cDNA synthesis on the Nexus Gradient Mastercycler and the QuantStudio 7-Flex Real-Time PCR Instrument was used for amplification. The preliminary LoD concentration of the assay was 10 copies/µL.

**Table 3. Preliminary LoD Range Finding Study**

| Concentration (copies/µL) | Mean Ct Values (SD) | | Detection Rate (# Detected/Total Tested) | |
|---|---|---|---|---|
| | N1 | N3 | N1 | N3 |
| 1 | 41.87 (2.76) | 43.51 (0.73) | 2/3 (66%) | 2/3 (66%) |
| 10 | 36.76 (0.59) | 37.35 (1.37) | 3/3 (100%) | 3/3 (100%) |
| 20 | 37.12 (1.64) | 36.63 (1.72) | 3/3 (100%) | 3/3 (100%) |
| 60 | 36.42 (1.36) | 36.61 (1.45) | 3/3 (100%) | 3/3 (100%) |
| 100 | 36.19 (0.76) | 35.81 (1.03) | 3/3 (100%) | 3/3 (100%) |
| 1000 | 32.25 (1.48) | 32.45 (1.54) | 3/3 (100%) | 3/3 (100%) |

SD (standard deviation)

Confirmatory testing was completed using a total of 30 individual extraction replicates consisting of samples spiked at the following concentrations in clinical matrix; 15 copies/µL (1.5X LoD), 50 copies/µL (5X LoD), and 100 copies/µL (10X LoD). The confirmed LoD of the Wren Laboratories COVID-19 PCR Test was 10 copies/µL. Results of the LoD confirmatory study are summarized below.

**Table 4. LoD Verification Study Results**

| Concentration (copies/µL) | Average Ct Values | | | # Detected / Total Tested |
|---|---|---|---|---|
| | N1 | N3 | RNase P | |
| 15 copies/µL (1.5X LoD) | 36.7 | 35.8 | 30.3 | 20/20 |
| 50 copies/µL (5X LoD) | 34.4 | 33.6 | 28.8 | 5/5 |
| 100 copies/µL (10X LoD) | 33.3 | 32.4 | 29.6 | 5/5 |
| Negative | UD* | UD | 29.4 | 10/10 |

UD; Undetermined

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

*Inclusivity:*

The Wren Laboratories COVID-19 PCR Test utilizes identical oligonucleotide sequences for the N1 and N3 target genes to those used in the original CDC authorized assay, CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. The inclusivity and cross-reactivity of the CDC EUA assay has been evaluated previously and therefore, additional evaluation was not necessary. The CDC has granted a right of reference to the performance data contained in the CDC's EUA request (FDA submission number EUA200001) to any entity seeking an FDA EUA for a COVID-19 diagnostic device.

Since the alignments of the CDC's primers/probes were completed in February 2020, an additional *in silico* inclusivity analysis was completed to assess the predicted inclusivity to other deposited SARS-CoV-2 sequences in the NCBI database. An *in silico* analysis (BLAST) evaluated the N1 and N3 primers and probe sets against more than 9500 publicly available, full and partially sequenced strains of SARS-CoV-2 (both domestic and international) that were included in the NCBI Betacoronavirus database (updated 7/13/2020, filtered on SARS-CoV2 [taxid:2697049]). The results of the analysis are included in Table 5.

**Table 5. Results of BLAST Inclusivity Analysis (7/13/2020)**

| Oligonucleotide | N1 Target | N3 Target |
|---|---|---|
| Forward | 100% alignment in 9543/9557 sequences | 100% alignment in 9409/9557 sequences |
| Reverse | 100% alignment in 9524/9555 sequences | 100% alignment in 9520/9542 sequences |
| Probe | 100% alignment in 9508/9559 sequences | 100% alignment in 9532/9549 sequences |

For the N1 primer and probe set, there were 85 strains (out of 9557) that exhibited mismatches (0.9%). Of the 85 strains, 75 had a mismatch in only one sequence (forward, reverse, or probe). For the remaining 10 strains, mismatches occurred in two sequences (8 are in both the probe and reverse primer, 2 are in both the forward and reverse primers). Annealing temperature calculations indicate that these mismatches do not significantly impact the annealing temperature and therefore, all 85 strains are predicted to anneal to the N1 oligonucleotides.

For the N3 primer and probe set, there were 186 strains (out of 9557) that exhibited mismatches (1.9%). Of these 186 strains, 185 strains had a mismatch in only one sequence (forward, reverse, or probe). Only one strain had mismatches in two sequences (forward and reverse primers). Annealing temperature calculations indicate that these mismatches do not significantly impact the annealing temperature and therefore, all 186 strains are predicted to anneal to the N3 oligonucleotides. Further, 3 strains (0.03%) exhibited one mismatch in both N1 and N3 sequences; however, these mismatches are not predicted to affect the annealing of the N1 and N3 oligonucleotides.

Overall, *in silico* testing confirmed that the N1 and N3 primers and probes will bind to and amplify all available SARS-CoV-2 partial and complete genomes published by NCBI in taxid:2697049.

*Exclusivity:*

To assess for potential cross-reactivity of the Wren Laboratories COVID-19 PCR Test, an *in silico* analysis of the N1 and N3 primer and probe sequences was performed against representative RefSeq genomes of other common respiratory viral, bacterial, and yeast pathogens listed in Table 6. With the exception of SARS-CoV, none of the pathogen sequences displayed greater than 80% homology with the assay's N1 and N3 primers/probes.

**Table 6. *In Silico* Cross-Reactivity Analysis of N1 and N3 Oligonucleotides**

| Pathogen Name | Tax ID | N1 Homology | N3 Homology |
|---|---|---|---|
| Human coronavirus 229E | taxid:11137 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Human coronavirus OC43 | taxid:31631 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Human coronavirus HKU1 | taxid:290028 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Human coronavirus NL63 | taxid:277944 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| SARS-coronavirus | taxid:694009 | Fw: < 80% similarity<br>Rev: 100% similarity<br>Probe: 95% similarity | Fw: 82% similarity<br>Rev: 100% similarity<br>Probe: 96% similarity |
| MERS-coronavirus | taxid:1335626 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Adenovirus C1 | taxid:10533 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Human Metapneumovirus (hMPV) | taxid:162145 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Parainfluenza virus 1-4 | taxid:12730<br>taxid:1979160<br>taxid:11216<br>taxid:11203 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Influenza A & B | taxid:11320<br>taxid:11520 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Enterovirus (e.g. EV68) | taxid:42789 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Respiratory syncytial virus | taxid:11250 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Rhinovirus | taxid:12059 | Fw: < 80% similarity | Fw: < 80% similarity |

| Pathogen Name | Tax ID | N1 Homology | N3 Homology |
|---|---|---|---|
| | | Rev: < 80% similarity<br>Probe: < 80% similarity | Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Chlamydia pneumoniae* | taxid:83558 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Haemophilus influenzae* | taxid:727 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Legionella pneumophila* | taxid:446 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Mycobacterium tuberculosis* | taxid:1773 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Streptococcus pneumoniae* | taxid:1313 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Streptococcus pyogenes* | taxid:1314 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Bordetella pertussis* | taxid:520 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Mycoplasma pneumoniae* | taxid:2104 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Pneumocystis jirovecii* (PJP) | taxid:42068 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Candida albicans* | taxid:5476 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Pseudomonas aeruginosa* | taxid:287 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Staphylococcus epidermis* | taxid:1282 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| *Streptococcus salivarius* | taxid:1304 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |

**3) *Clinical Evaluation:***

Performance of the Wren Laboratories COVID-19 PCR Test was evaluated using clinical nasopharyngeal positive and negative swab specimens that were previously tested with an FDA EUA authorized SARS-CoV-2 molecular test.

For the positive clinical nasopharyngeal swab samples, the positive percent agreement (PPA) between the Wren Laboratories Test and the comparator assay was 100% (60/60). The Ct range for the N1 and N3 targets used in the Wren Laboratories Test for the 60 positive clinical samples was 15.66 – 38.38 and 15.51 – 38.02, respectively. For the 60 clinical negative samples that were evaluated, 57/60 tested

negative (95.00% NPA) using the Wren Laboratories COVID-19 PCR Test when run on the QuantStudio 7-Flex platform. There were three SARS-CoV-2 negative samples determined by the comparator assay that were positive by the Wren Laboratories Test. Qualitative results of the clinical evaluation are shown in Tables 7.

**Table 7. Summary of Qualitative Clinical Study Results Performed on the QuantStudio 7-Flex Instrument**

| | | Authorized Assay - Comparator | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Total** |
| **Wren Laboratories SARS-CoV-2 RT-PCR Assay** | **Positive** | 60 | 3[a] | 63 |
| | **Negative** | 0 | 57 | 57 |
| | **Total** | 60 | 60 | 120 |
| **Positive Percent Agreement** | | 100.00% (60/60); 93.98-200.00%[1] | | |
| **Negative Percent Agreement** | | 95.00% (57/60); 86.30-98.29%[1] | | |

[1]Two-sided 95% score confidence intervals

[a] Discordant analysis was performed on the 3 false positive results using a second FDA EUA authorized SARS-CoV-2 molecular test (N1, N2 and RP targets). Two out of the 3 false positives were also positive by the second comparator assay.

*Discordant Analysis:*

The discordant samples for the three false positive (FP) results generated by the Wren Laboratories COVID-19 PCR Test were investigated. These samples were evaluated by a second FDA EUA authorized SARS-CoV-2 molecular test that targets N1, N2, and RNase P. It was determined that 2/3 discordant specimens were also positive with second comparator assay as footnoted in the performance table (Table 7). The average Ct value for N1 (for 3 three technical replicates of the three FP samples) was 37.97 for the Wren Laboratories Test versus 36.97 for the second comparator assay. The average Ct for the N3 target on the Wren Laboratories Test was 36.9; the average Ct for the second comparator assay was 37.57. Both the Wren Laboratories COVID-19 PCR Test and the second comparator assay were run a second time on the three FP samples and results were confirmed; Wren 3/3 positive and second comparator 2/3 positive.

*Clinical Confirmation:*

In addition, the first 5 positive and 5 negative samples determined by the Wren Laboratories COVID-19 PCR Test were sent to an outside laboratory running the first FDA EUA authorized SARS-CoV-2 molecular test for confirmatory testing. All 10 patient specimens yielded concordant results.

**WARNINGS:**

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratory;
- 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.