EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE WREN LABORATORIES COVID-19 PCR TEST DTC

(WREN Laboratories LLC)

For *In vitro* Diagnostic Use
For Use Under Emergency Use Authorization (EUA) Only

(The WREN Laboratories COVID-19 PCR Test DTC will be performed at laboratories designated by WREN Laboratories LLC that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet requirements to perform high-complexity tests, as described in the laboratory procedures that were reviewed by the FDA under this EUA.)

INTENDED USE

The WREN Laboratories COVID-19 PCR Test DTC is a direct to consumer product for testing of saliva specimens collected at home (which includes in a community-based setting), using the WREN Laboratories COVID-19 Saliva Test Collection Kit DTC when used consistent with its authorization, by any individual, including individuals without symptoms or other reasons to suspect COVID-19.

Testing of collected saliva specimens is limited to laboratories designated by WREN Laboratories LLC that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a., and meet requirements to perform high-complexity tests.

Results are for the identification of SARS-CoV-2 viral RNA. SARS-CoV-2 RNA is generally detectable in saliva specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with medical history and other diagnostic information is necessary to determine 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 results to the appropriate public health authorities. Negative results do not preclude SARS-CoV-2 infection.

The WREN Laboratories COVID-19 PCR Test DTC is not a substitute for visits to a healthcare provider. The information provided by this product should not be used to start, stop, or change any course of treatment unless advised by your healthcare provider.

The WREN Laboratories COVID-19 PCR Test DTC is only 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 assay is intended for use under the Food and Drug Administration's Emergency Use Authorization.

SPECIAL CONDITIONS FOR USE STATEMENTS

For Emergency Use Authorization (EUA) Only

For *In vitro* Diagnostic Use

The WREN Laboratories COVID-19 PCR Test DTC is a direct to consumer product for testing of saliva specimens collected at home or at a community-based distribution site using the WREN Laboratories COVID-19 Saliva Test Collection Kit DTC when used consistent with its authorization by any individual, including individuals without symptoms or other reasons to suspect COVID-19.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Overview of RT-PCR Test

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 are identical to those used in the authorized CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel. 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 include SARS-CoV-2. The WREN Laboratories COVID-19 PCR 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. The assay can be run in two different formats including a standard approach with liquid primers/probes that are dispensed at the time of use or using custom-made pre-spotted, lyophilized primers/probes that are resuspended upon the addition of the master mix reagents and cDNA template. In both assay formats, for each sample or control, three separate reactions are performed for each of the three analytes (N1, N3 and the RNase P control).

Specimen Collection

Saliva specimens must be collected using the collection tube provided in the WREN Laboratories COVID-19 Saliva Test Collection Kit DTC. Saliva specimens must be transported and stored at ambient temperature and tested within 96 hours of collection (4 days).

Nucleic Acid Extraction and RT-PCR

RNA is isolated from saliva specimens using either the QIAamp Viral RNA Mini Kit (Qiagen, Cat # 52906) or the ZYMO Research Quick-RNA Viral 96 Kit (Cat # R1041). With the QIAamp spin-column based workflow, nucleic acid is manually extracted from 140 µL of acceptable specimen and the final purified nucleic acid is eluted in a 60 µL volume. The ZYMO Quick-RNA Viral 96 Kit uses a spin plate based workflow (i.e., microspin technology) whereby nucleic acid is manually extracted from 150 µL of clinical sample and eluted in a 60 µL final volume. Extracted 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.

The RT-PCR plate can either be set-up using (1) a traditional master mix containing all

reagents needed for amplification (i.e., liquid primers and probes protocol) followed by the addition of cDNA or (2) a pre-spotted 384-well primer plate can be used which contains lyophilized oligonucleotides that are resuspended upon addition of the master mix/cDNA mixture into the wells. The pre-spotted primer plates are custom-made by ThermoFisher Scientific. All reactions remain as singleplex reactions with the N1, N3, and RNase P primers/probes in separate wells and a plate layout that is designed to run three technical replicates per assay (a total of 9 reactions for 1 clinical sample). The final reaction volume is 10 µL (2.5 µL master mix and 7.5 µL cDNA). During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. 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.

INSTRUMENTS USED WITH TEST

The WREN Laboratories COVID-19 PCR Test DTC 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

Reagents Used to Perform the WREN Laboratories COVID-19 PCR Test DTC

Reagent Manufacturer and Description	Catalog #	Manufacturer
QIAamp Viral RNA Mini Kit	52906	Qiagen
ZYMO Research Quick-RNA Viral 96 Kit	R1041	ZYMO Research
DEPC-Treated Water	02123	American Biochem
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
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
Spotted 384-well PCR plate (Taqman Primer)	Custom made	ThermoFisher Scientific
E1-ClipTip 384 1-125µl, 8-channel pipette and tips	4672060BT (pipette) 94410153 (tips)	ThermoFisher Scientific
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 DTC

1) A no template control (NTC) is needed to check for contamination of the extraction process and 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 per extraction batch and 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 purified plasmid DNA that contains one copy each of N1 and N3 targets.

3) A positive plate control is used to evaluate the 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, 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) **WREN Laboratories COVID-19 PCR Test DTC Controls – NTC, SARS-CoV-2 Positive Viral Control, Positive Plate Control, and Internal RNase P Control:**

   - The no template controls (NTC) must be negative (Ct Not Detected or Ct ≥ 38) 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 < 38 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 ≥ 38) 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 must 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 < 38). 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 is 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 RNase P Result
2019-nCoV_N_ Positive Control (N1, N3 template)	Ct < 38	Ct < 38	Not Detected; Ct ≥ 38
No Template Control (NTC)	Not Detected; Ct ≥ 38	Not Detected; Ct ≥ 38	Not Detected; Ct ≥ 38
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 < 38

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.

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

Assessment of clinical specimen test results must be performed after the positive and negative controls have been examined and determined to be valid. If the controls are not valid, the patient results cannot be interpreted. Please see the table below (Table 3) for guidance on interpretation and reporting of results using three technical replicates per assay.

Table 3. Interpretation of Patient Results Using the WREN Laboratories COVID-19 PCR Test DTC

N1 (Ct < 40)	N3 (Ct < 40)	RNase P (Ct < 38)	Interpretation	Report Result	Actions
+[a]	+[a]	+[a]	SARS-CoV-2 **Detected**	**POSITIVE**	Results reported to appropriate public health authorities and individual.*
+[a]	-[b]	+[a]	SARS-CoV-2 **Detected**	**POSITIVE**	Results reported to appropriate public health authorities and individual.*
-[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, the sample is considered positive for SARS-CoV-2 RNA. If the repeated result is negative (no detectable N1 or N3 signal), the sample is considered negative for SARS-CoV-2 RNA. 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 appropriate public health authorities and individual.*
+/-[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 a new specimen from the individual is requested.

[a] If at least 2 technical replicates show signal for the specified target (Ct < 40 for N1/N3, Ct < 38 for RNase P), the sample is positive for that 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.

* For at home collection or collection from a community-based distribution site, reporting will be done via an encrypted email service. Individuals with positive or presumptive positive SARS-CoV-2 results or an invalid test result will receive a follow-up phone call from an HCP. For details on this process, please refer to the EUA summary for the WREN Laboratories Saliva Collection Kit DTC.

INSPECTION OF SALIVA SPECIMENS RECEIVED AT WREN LABORATORIES OR DESIGNATED LABORATORIES FOR TESTING

Specimens received at the clinical laboratory for testing with the WREN Laboratories COVID-19 PCR Test DTC and which are collected with the WREN Laboratories COVID-19 Saliva Test Collection Kit DTC will undergo sample accessioning according

to the specimen requirements section of the assay and accessioning SOP prior to acceptance for testing.

PERFORMANCE EVALUATION
(The WREN Laboratories COVID-19 PCR Test DTC is the same RT-PCR assay as the EUA authorized prescription use only WREN Laboratories COVID-19 PCR Test. The performance evaluation of the WREN Laboratories COVID-19 PCR Test DTC described below are the same data used to support the authorization of the prescription use only WREN Laboratories COVID-19 PCR Test. For clarity the "WREN Laboratories COVID-19 PCR Test" name is maintained in the summary of the performed studies).

Analytical and Clinical Performance of the WREN Laboratories COVID-19 PCR Test DTC

1) **Analytical Sensitivity:**
   a. Limit of Detection (LoD) Using Synthetic SARS-CoV-2 RNA:
   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 using liquid primers/probes 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 Using Negative NP/OP Swab Matrix

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 samples spiked at the following concentrations in clinical matrix: 15 copies/µL, 50 copies/µL, or 100 copies/µL. The LoD for NP/OP swabs was estimated to be 10 copies/µL, based on the preliminary range finding study data; however, the LoD of the

WREN Laboratories COVID-19 PCR Test was confirmed to be 15 copies/µL of NP/OP swab matrix using the liquid primers and probes protocol. Results of the LoD confirmatory study are summarized below.

Table 4. LoD Verification Study Results for NP/OP Swab Matrix Using Synthetic SARS-CoV-2 RNA and the Liquid Primers and Probes Protocol

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

UD; Undetermined

To validate the use of saliva as an acceptable specimen type, an LoD study was completed using saliva collected with the WREN Laboratories COVID-19 Saliva Test Collection Kit. A preliminary LoD was determined using Twist Bioscience SARS-CoV-2 RNA material spiked into negative saliva matrix (i.e., saliva with stabilization buffer) at four different concentrations that were tested with three replicates per concentration using the WREN Laboratories COVID-19 PCR Test with liquid primers and probes (See Table 5).

Table 5. Preliminary Assay LoD Using Saliva Collected with the WREN Laboratories COVID-19 Saliva Test Collection Kit

Concentration (copies/µL)	Mean Ct Values (SD)		Detection Rate (# Detected/Total Tested)	
	N1	N3	N1	N3
1	39.27 (0.97)	38.73 (0.22)	2/3 (67%)	2/3 (67%)
10	36.76 (0.59)	37.34 (0.74)	3/3 (100%)	3/3 (100%)
100	36.18 (0.21)	35.80 (0.17)	3/3 (100%)	3/3 (100%)
1000	32.24 (0.28)	32.45 (0.46)	3/3 (100%)	3/3 (100%)

SD (standard deviation)

The LoD in clinical saliva matrix was confirmed using a total of 40 samples at either 15 copies/µL, 40-100 copies/µL or 500-1000 copies/µL that were extracted independently. Ten negative saliva samples screened with the WREN Laboratories COVID-19 PCR Test were also tested in the confirmatory LoD study. All contrived positive and negative samples generated the expected results (See Table 6).

Table 6. Confirmatory LoD Data Summary of 40 Contrived Saliva Positive Samples Using Synthetic SARS-CoV-2 RNA and 10 Negatives with the Liquid Primers and Probes Protocol

Concentration (copies/µL)	Average Ct Values			# Detected / Total Tested
	N1	N3	RNase P	
15	36.75	36.77	30.14	24/24
40-100	36.06	36.6	29.63	10/10
500-1000	33.83	33.51	30.06	6/6

Negative	UD	UD	29.4	10/10

UD; Undetermined

The LoD for saliva was estimated to be 10 copies/µL, based on the preliminary range finding study data; however, the LoD was confirmed to be 15 copies/µL using the liquid primers and probes protocol.

b. Limit of Detection (LoD) Using Gamma-Irradiated Whole SARS-CoV-2 and the QIAamp Viral RNA Mini Kit with a Traditional RT-PCR Plate:
The LoD for the Wren Laboratories COVID-19 PCR Test was also established using a dilution series of gamma-irradiated SARS-CoV-2 (BEI Resources Cat # NR-52287: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, 10,000,000 copies/µL) spiked into SARS-CoV-2 negative NP swab clinical matrix and saliva (collected with the WREN Laboratories COVID-19 Saliva Test Collection Kit). The preliminary LoD study involved testing at 2000, 1000, 500, 250, 100, 50, 25, 10, and 1 copies/µL with three extraction replicates per concentration. Each spiked sample was processed through the entire assay, beginning with RNA extraction by the Qiagen QIAamp Viral RNA Mini Kit followed by testing with the WREN Laboratories COVID-19 PCR Test with three technical replicates per extraction replicate per concentration.

The preliminary LoD for NP swab matrix and saliva was estimated to be 1 copy/µL and 10 copies/µL, respectively which was the lowest concentration of SARS-CoV-2 at which at least 2/3 technical replicates were detected for both the N1 and N3 targets. A confirmatory LoD study was performed using six different target concentrations in each matrix, with 20 independent extraction replicates per concentration (Qiagen extraction kit) and three technical replicates each (total of 60 assay replicates per concentration). The confirmatory LoD wet testing studies demonstrated an analytical sensitivity of 6 copies/µL and 4 copies/µL in NP swab and saliva matrix, respectively when using the Qiagen extraction kit and the liquid primers and probes protocol (See Table 7).

Table 7. Confirmatory LoD Study Results for NP Swab and Saliva Clinical Matrices Using Inactivated Whole SARS-CoV-2 and the QIAamp Extraction Method with Liquid Primers/Probes

Concentration (copies/µL)	C_T Values				Detected Replicates [1]		Detection Rate	
	N1		N3		N1	N3	N1	N3
	Mean	SD	Mean	SD				
NP Swab								
2	37.91	0.92	37.86	0.94	12/20 (36/60 wells)	14/20 (42/60 wells)	60%	70%
4	36.74	0.69	36.60	0.94	13/20 (39/60 wells)	15/20 (45/60 wells)	65%	75%
6	35.83	1.14	34.93	0.73	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
8	34.79	0.58	33.63	0.42	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
10	34.33	0.92	33.45	0.50	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%

12	33.67	0.57	32.60	0.57	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
Saliva								
2	36.59	0.69	35.98	0.81	16/20 (48/60 wells)	18/20 (54/60 wells)	80%	90%
4	35.47	1.08	35.24	0.81	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
6	36.01	0.76	35.34	1.20	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
8	35.19	0.85	34.48	0.84	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
10	34.97	0.53	33.90	0.83	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
12	34.45	1.12	33.67	0.69	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%

20 extraction replicates per concentration with 3 technical replicates per target = 60 wells per tested concentration per target
SD; Standard Deviation of Ct values
[1] Number of samples reported positive for the analyte (i.e., with ≥ 2/3 positive technical replicates)

c. Limit of Detection (LoD) Using Gamma-Irradiated Whole SARS-CoV-2 and the ZYMO extraction method with a Pre-Spotted Primer RT-PCR Plate (Lyophilized Primers/Probes):
The LoD for the WREN Laboratories COVID-19 PCR Test was further evaluated using a dilution series of gamma-irradiated SARS-CoV-2 from BEI Resources spiked into negative NP swab and saliva clinical matrices that were extracted using the ZYMO Research Quick-RNA Viral 96 Kit and run on the WREN Laboratories COVID-19 PCR Test using the pre-spotted primer plate protocol.

The estimated LoD for both NP swab and saliva was 1 copy/µL which was the lowest concentration of SARS-CoV-2 at which at least 2/3 technical replicates were detected for both the N1 and N3 targets. A confirmatory LoD study was performed using 20 independent extraction replicates at six different target concentrations (ZYMO extraction kit) with three technical replicates each (total of 60 assay replicates per concentration). The confirmatory LoD studies demonstrated an analytical sensitivity of 6 copies/µL and 4 copies/µL in NP swab and saliva matrix, respectively when using the ZYMO extraction kit and the lyophilized primers/probes (See Table 8).

Table 8. Confirmatory LoD Study Results for NP Swab and Saliva Clinical Matrices Using Inactivated Whole SARS-CoV-2 Extracted with the ZYMO Method and Run Using the Pre-Spotted Primer Plate Protocol (Lyophilized Primers/Probes)

Concentration (copies/µL)	C_T Values				Detected Replicates [1]		Detection Rate	
	N1		N3		N1	N3	N1	N3
	Mean	SD	Mean	SD				
NP Swab								
2	37.93	0.80	38.01	1.02	13/20 (39/60 wells)	15/20 (45/60 wells)	65%	75%
4	36.53	0.80	36.73	0.89	15/20 (45/60 wells)	16/20 (48/60 wells)	75%	80%

6	35.58	1.28	34.83	0.79	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
8	34.30	0.70	33.15	0.36	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
10	33.85	0.68	33.28	0.70	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
12	33.56	0.69	32.87	0.70	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
Saliva								
2	36.28	1.21	35.38	1.03	17/20 (51/60 wells)	19/20 (57/60 wells)	85%	95%
4	35.70	0.92	35.06	1.06	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
6	35.59	1.06	34.46	0.72	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
8	35.77	0.91	34.81	0.60	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
10	35.46	1.28	34.44	0.88	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
12	34.97	0.61	34.16	0.61	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%

20 extraction replicates per concentration with 3 technical replicates per target = 60 wells per tested concentration per target
SD; Standard Deviation of Ct values
[1] Number of samples reported positive for the analyte (i.e., with ≥ 2/3 positive technical replicates)

A summary of the LoD study data using the authorized and updated assay protocols is shown in Table 9.

Table 9. Summary of Assay LoD Data Using Inactivated Whole SARS-CoV-2 with the Authorized and Updated Protocols

Matrix	Preliminary LoD (copies/µL)*	Confirmed LoD (copies/µL)**
Authorized Protocol – Qiagen Spin Column and Liquid Primers/Probes		
NP swab	1	6
Saliva	10	4
Updated Protocol – ZYMO extraction and Pre-Spotted RT-PCR Primer Plate (Lyophilized Primers/Probes)		
NP swab	1	6
Saliva	1	4

*Based on wet testing n=3 replicates
**Based on wet testing n=20 replicates

Data from the LoD studies demonstrated that the ZYMO plate extraction approach coupled with the pre-spotted primer plate set-up was equivalent to the Qiagen spin column extraction and the liquid primers and probes protocol.

d. Bridging Studies:
   i. Clinical Saliva Samples Tested Side-by-Side with the Qiagen Extraction/Liquid Primers and Probes Protocol Versus the ZYMO Extraction/Pre-Spotted Primer Plate:

To further evaluate the ZYMO extraction/pre-spotted primer plate protocol for the WREN Laboratories COVID-19 PCR Test, clinical samples previously characterized using an authorized molecular assay were evaluated in parallel using the updated procedure and the original QIAamp kit and traditional master mix set-up protocol. A total of 65 clinical saliva samples including 33 known SARS-CoV-2 positives and 32 known negatives were tested. Assay results were compared between the Qiagen spin column isolation/traditional master mix composition including primers and the ZYMO plate extraction/pre-spotted PCR plates. All 33 known positive samples (33/33; 100%) were positive by both methods, with no noticeable difference in Ct values of the N1 and N3 targets between the assay protocols. All known negative samples were negative for both the N1 and N3 targets by both methods, as expected. Results of the head-to-head study are summarized in Table 10 and demonstrated acceptable performance of the modified assay procedure with clinical saliva specimens.

Table 10. Performance of Saliva Samples Comparing the Authorized WREN Laboratories COVID-19 PCR Test to the Updated Assay Protocol

WREN Laboratories COVID-19 PCR Test (Clinical Saliva)		**Qiagen Spin Column Isolation/Liquid Primers and Probes Protocol**		
		Positive	**Negative**	**Total**
ZYMO Plate Isolation/Pre-Spotted Primer Plate Protocol	**Positive**	33	0	33
	Negative	0	32	32
	Total	33	32	65
Positive Percent Agreement		100.00% (33/33); 89.42-100.0%[1]		
Negative Percent Agreement		100.00% (32/32); 89.11-100.0%[1]		

[1] Two-sided 95% score confidence interval

ii. Contrived Saliva Specimens:

To demonstrate equivalence between the Qiagen and ZYMO extraction methods with the liquid primers and probes approach as well as the Qiagen extraction when used with both the liquid primers and probes as well as the pre-spotted RT-PCR plate (lyophilized primers and probes), a series of

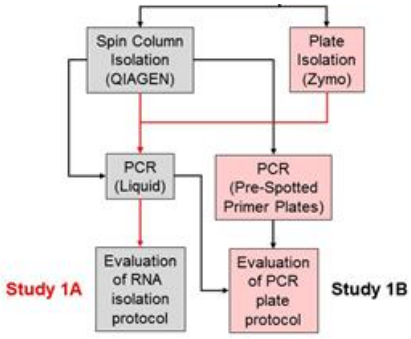

Figure 1. Representation of the Bridging Studies Performed to Validate the New Extraction and Pre-Spotted Primer Plate Procedure

bridging studies were completed as outlined in Figure 1. Each individual assay change was evaluated to determine equivalence to the original method.

a. RNA Isolation Approaches for Saliva (Study 1A in Figure 1)
The performance of the ZYMO Quick-RNA Viral 96 Kit was compared against the Qiagen spin column approach using 55 contrived positive and 10 negative samples evaluated using the liquid primers/probes protocol. Samples were prepared at multiple concentrations in saliva matrix spiked with 3-50 copies/µL of whole inactivated SARS-CoV-2 (0.75X-12.5X LoD based on the LoD established with saliva using whole inactivated virus) and extracted in parallel using both the ZYMO and Qiagen extraction methods.

All 55 contrived positive samples were positive for both N1 and N3 targets using samples that were extracted with both methods. There were no noticeable differences in Ct values among the spiked samples tested with each extraction procedure. Results (See Table 11) demonstrated concordance between the plate (ZYMO) and spin column (Qiagen) based RNA extraction methodologies run with the authorized PCR protocol.

Table 11. Summary Data Comparing the Qiagen and ZYMO RNA Isolation Methods Using 55 Contrived Positive Samples (Liquid Primers/Probes)

Replicates		Spin Column Isolation Protocol (Qiagen)						Plate-Based Isolation Protocol (ZYMO)					
		Mean Ct Values (SD)		Detected Replicates		Detection Rate		Mean Ct Values (SD)		Detected Replicates		Detection Rate	
Concentration (copies/µL)	# Tested	N1	N3	N1	N3	N1	N3	N1	N3	N1	N3	N1	N3
3 (0.75X LoD)	20	37.3± 0.7	36.8± 0.7	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%	36.8± 0.6	35.7± 0.8	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
4 (1X LoD)	3	37.8± 0.7	35.9± 0.2	3/3 (9/9 wells)	3/3 (9/9 wells)	100%	100%	38.2± 0.7	35.8± 0.8	3/3 (9/9 wells)	3/3 (9/9 wells)	100%	100%
6 (1.5X LoD)	19	36.0± 0.9	35.7± 1.5	19/19 (57/57 wells)	19/19 (57/57 wells)	100%	100%	35.6± 0.7	35.9± 1.5	19/19 (57/57 wells)	19/19 (57/57 wells)	100%	100%
9 (2.25X LoD)	5	35.3± 0.7	35.3± 1.0	5/5 (15/15 wells)	5/5 (15/15 wells)	100%	100%	35.6± 0.8	35.7± 1.0	5/5 (15/15 wells)	5/5 (15/15 wells)	100%	100%
12 (3X LoD)	4	34.5± 1.0	34.4± 1.4	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%	34.6± 1.0	34.6± 1.7	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%
50 (12.5X LoD)	4	33.1± 0.7	32.9± 1.4	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%	33.0± 1.0	32.7± 1.9	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%

SD; Standard Deviation of Ct values

The ZYMO plate isolation approach was repeated on a different day by a different technician to demonstrate that the results of the new method could be reproduced. The same 55 contrived positive and 10 negative samples were re-extracted and results are shown in Table 12. The ZYMO extraction procedure

generated reproducible results with the WREN Laboratories COVID-19 PCR Test.

Table 12. Evaluation of the ZYMO RNA Isolation Method Using 55 Contrived Positive Samples (Liquid Primers/ Probes) Extracted on a Different Day by a Different Operator

Replicates		ZYMO Isolation Protocol Run #1 [1]						ZYMO Isolation Protocol Run #2					
		Mean Ct Values (SD)		Detected Replicates		Detection Rate		Mean Ct Values (SD)		Detected Replicates		Detection Rate	
Concentration (copies/µL)	# Tested	N1	N3	N1	N3	N1	N3	N1	N3	N1	N3	N1	N3
3 (0.75X LoD)	20	36.8± 0.6	35.7± 0.8	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%	36.9± 0.7	35.8± 0.7	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
4 (1X LoD)	3	38.2± 0.7	35.8± 0.8	3/3 (9/9 wells)	3/3 (9/9 wells)	100%	100%	37.4± 0.7	35.4± 1.0	3/3 (9/9 wells)	3/3 (9/9 wells)	100%	100%
6 (1.5X LoD)	19	35.6± 0.7	35.9± 1.5	19/19 (57/57 wells)	19/19 (57/57 wells)	100%	100%	35.6± 1.1	35.3± 1.5	19/19 (57/57 wells)	19/19 (57/57 wells)	100%	100%
9 (2.25X LoD)	5	35.6± 0.8	35.7± 1.0	5/5 (15/15 wells)	5/5 (15/15 wells)	100%	100%	34.9± 0.8	35.2± 1.3	5/5 (15/15 wells)	5/5 (15/15 wells)	100%	100%
12 (3X LoD)	4	34.6± 1.0	34.6± 1.7	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%	34.2± 1.1	34.0± 1.6	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%
50 (12.5X LoD)	4	33.0± 1.0	32.7± 1.9	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%	32.4± 0.9	32.4± 2.3	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%

SD; Standard Deviation of Ct values
[1] Same data as presented in Table 11

b. PCR Plate Approaches for Saliva (Study 1B in Figure 1)

The performance of the custom-made pre-spotted primer RT-PCR plate was compared against the liquid primers/probes protocol using the same 55 contrived positive and 10 negative samples mentioned in section 2.a.i. Samples were prepared at multiple concentrations in saliva matrix spiked with 3-50 copies/µL of whole inactivated SARS-CoV-2 (0.75X-12.5X LoD based on the LoD established with saliva using whole inactivated virus), extracted using the authorized QIAamp Viral RNA Mini Kit. All samples were then tested with the WREN Laboratories COVID-19 PCR Test using the standard RT-PCR plate protocol or the pre-spotted primer plate approach.

All 55 contrived positive samples were positive for both the N1 and N3 targets using samples that were extracted with the QIAamp spin columns and run using liquid primers/probes and the pre-spotted primer plate protocol. Results (See Table 13) are summarized below and demonstrated concordance between the liquid primers/probes protocol and the pre-spotted primer plate protocol when samples were extracted using the QIAamp Viral RNA Mini Kit.

Table 13. Summary Data Comparing the Liquid Primers/Probes and the Pre-Spotted Primer Plate PCR Protocol with Samples Extracted with the Qiagen QIAamp Method

Replicates		Liquid Primers/Probes PCR Protocol [1]						Pre-Spotted Primer Plate PCR Protocol					
		Mean Ct Values (SD)		Detected Replicates		Detection Rate		Mean Ct Values (SD)		Detected Replicates		Detection Rate	
Concentration (copies/µL)	# Tested	N1	N3	N1	N3	N1	N3	N1	N3	N1	N3	N1	N3
3 (0.75X LoD)	20	37.3± 0.7	36.8± 0.7	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%	36.9± 0.7	36.4± 0.7	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
4 (1X LoD)	3	37.8± 0.7	35.9± 0.2	3/3 (9/9 wells)	3/3 (9/9 wells)	100%	100%	36.5± 0.4	36.2± 0.8	3/3 (9/9 wells)	3/3 (9/9 wells)	100%	100%
6 (1.5X LoD)	19	36.0± 0.9	35.7± 1.5	19/19 (57/57 wells)	19/19 (57/57 wells)	100%	100%	35.4± 1.6	35.5± 0.7	19/19 (57/57 wells)	19/19 (57/57 wells)	100%	100%
9 (2.25X LoD)	5	35.3± 0.7	35.3± 1.0	5/5 (15/15 wells)	5/5 (15/15 wells)	100%	100%	35.2± 1.6	34.7± 1.1	5/5 (15/15 wells)	5/5 (15/15 wells)	100%	100%
12 (3X LoD)	4	34.5± 1.0	34.4± 1.4	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%	33.4± 1.0	33.9± 1.7	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%
50 (12.5X LoD)	4	33.1± 0.7	32.9± 1.4	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%	32.3± 1.0	32.3± 1.3	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%

SD; Standard Deviation of Ct values
[1] Same data as presented in Table 11

Running of the pre-spotted primer plates was repeated on a different day by a different technician to demonstrate that the results of the new RT-PCR plate format could be reproduced. The same 55 contrived positive and 10 negative samples were re-extracted, and the data are presented in Table 14. The pre-spotted primer plates produced reproducible results with the WREN Laboratories COVID-19 PCR Test.

Table 14. Evaluation of the Pre-Spotted Primer PCR Plate Method Using 55 Contrived Positive Samples Extracted with the Qiagen QIAamp Method on a Different Day by a Different Operator

Replicates		Pre-Spotted Primer Plate PCR Run #1 [1]						Pre-Spotted Primer Plate PCR Run #2					
		Mean Ct Values (SD)		Detected Replicates		Detection Rate		Mean Ct Values (SD)		Detected Replicates		Detection Rate	
Concentration (copies/µL)	# Tested	N1	N3	N1	N3	N1	N3	N1	N3	N1	N3	N1	N3
3 (0.75X LoD)	20	36.9± 0.4	36.4± 0.8	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%	36.7± 0.7	36.2± 0.8	20/20 (60/60 wells)	20/20 (60/60 wells)	100%	100%
4 (1X LoD)	3	36.5± 0.4	36.2± 0.8	3/3 (9/9 wells)	3/3 (9/9 wells)	100%	100%	37.5± 0.6	36.7± 2.2	3/3 (9/9 wells)	3/3 (9/9 wells)	100%	100%
6 (1.5X LoD)	19	35.4± 1.6	35.5± 0.7	19/19 (57/57 wells)	19/19 (57/57 wells)	100%	100%	35.8± 0.8	35.4± 0.6	19/19 (57/57 wells)	19/19 (57/57 wells)	100%	100%
9 (2.25X LoD)	5	35.2± 1.6	34.7± 1.1	5/5 (15/15 wells)	5/5 (15/15 wells)	100%	100%	35.2± 1.1	35.1± 1.3	5/5 (15/15 wells)	5/5 (15/15 wells)	100%	100%
12 (3X LoD)	4	33.4± 1.0	33.9± 1.7	4/4	4/4	100%	100%	33.7± 1.0	33.9± 1.9	4/4	4/4	100%	100%

				(12/12 wells)	(12/12 wells)					(12/12 wells)	(12/12 wells)		
50 (12.5X LoD)	4	32.3± 1.0	32.3± 1.3	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%	32.6± 1.0	32.5± 1.4	4/4 (12/12 wells)	4/4 (12/12 wells)	100%	100%

SD; Standard Deviation of Ct values
[1] Same data as presented in Table 13

2) Analytical Inclusivity/Specificity:

a. Inclusivity:

To assess the predicted inclusivity of the WREN Laboratories COVID-19 PCR Test, an *in silico* analysis was completed by aligning the assay's oligonucleotide sequences against SARS-CoV-2 sequences found in the GISAID and NCBI databases. The Betacoronavirus database within NCBI was filtered on taxid: 2697049 (SARS-CoV-2 specific) and the assay's primers/probes were queried against this database. A total of 8,659 sequences published between July 1 and September 15, 2020 were evaluated for assay inclusivity using a primer blast allowing for 1% or less mismatches of sequences. Using the same approach, WREN Laboratories analyzed SARS-CoV-2 sequences available at GISAID collected and published from the United States as of March 26, 2021 (n=9,323). Only complete SARS-CoV-2 genomes of high quality were used in this analysis. A summary of the predicted inclusivity is shown below in Table 15.

Table 15. Inclusivity Results Using High-Quality, Complete SARS-CoV-2 Genomes from NCBI and GISAID

	N1 Target		N3 Target	
	# of Sequences Analyzed	**% Inclusivity**	**# of Sequences Analyzed**	**% Inclusivity**
Forward	17,982	17,867/17,982 99.8%	17,982	17,932/17,982 99.7%
Reverse		17,968/17,982 99.9%		17,969/17,982 99.9%
Probe		17,908/17,982 99.6%		17,955/17,982 99.8%

Inclusivity is defined as 100% homology (percent identity)
Single nucleotide mismatches for N1: Forward primer; n=115; Reverse primer; n=14; Probe; n=74
Single nucleotide mismatches for N3: Forward primer; n=50, Reverse primer; n=13; Probe; n=27

Results of the analysis demonstrated that the majority of mismatches were observed in the N1 forward primer binding region. At least 99.6% and 99.7% of analyzed sequences showed 100% homology with the N1 and N3 oligonucleotides, respectively. The mismatches for the N1 and N3 oligonucleotides presented in Table 15 were all single nucleotide mismatches and were not predicted to impact binding and subsequently N1 and N3 target detection.

An additional analysis was completed against the main circulating SARS-CoV-2 escape variants or variants of concern using sequences available in the GISAID database: B.1.1.7 (5,715 sequences), P.1 (3 sequences), B1.351 (99 sequences)

and B1426_1429 (9,951 sequences). High quality, complete SARS-CoV-2 genomes were evaluated which were defined as genomes >29,000 base pairs in length with <1% "ambiguous bases ("N's"). A summary of the predicted assay inclusivity to the prominent and currently circulating SARS-CoV-2 variants is shown in Table 16 below.

Table 16. Alignment of Assay Oligonucleotides Against Circulating SARS-CoV-2 Variants

Primer	Variant	N1 target		N3 target	
		# of Sequences Analyzed	Inclusivity %	# of Sequences Analyzed	Inclusivity %
Forward	B.1.1.7	5,715	5,656/5,715 98.93%	5,715	5,713/5,715 99.96%
	P.1	45	45/45 100.00%	45	45/45 100.00%
	B.1.351	99	99/99 100.00%	99	99/99 100.00%
	B1426_1429	9,951	9,938/9,951 99.87%	9,951	9,945/9,951 99.94%
Reverse	B.1.1.7	5,715	5,712/5,715 99.91%	5,715	5,708/5,715 99.88%
	P.1	45	45/45 100.00%	45	45/45 100.00%
	B.1.351	99	98/99 98.99%	99	99/99 100.00%
	B1426_1429	9,951	9,938/9,951 99.87%	9,951	9,948/9,951 99.97%
Probe	B.1.1.7	5,715	5,688/5,715 99.49%	5,715	5,697/5,715 99.69%
	P.1	45	45/45 100.00%	45	45/45 100.00%
	B.1.351	99	98/99 98.99%	99	95/99 95.96%
	B1426_1429	9,951	9,897/9,951 99.46%	9,951	9,937/9,951 99.86%

Inclusivity is defined as 100% homology (percent identity)

To identify the exact locations of the mismatches with the variant SARS-CoV-2 sequences, the FASTA sequences containing mismatches for either the forward primer, reverse primer or probe were downloaded from the GISAID database and subjected to sequence alignment with the assay's primer/probe sequences using the standard/default settings of Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). In the majority of cases (94%), mismatches were due to single nucleotide differences over the length of each individual oligonucleotide. All mismatched bases associated with the N1 oligonucleotides were single nucleotide mismatches. For the N3 oligonucleotides, 41/54 (76%) mismatched sequences exhibited a single nucleotide mismatch and 13/54 (24%) sequences had two nucleotide mismatches with the N3 probe. Based

on differences in melting temperature (Tm) for the mismatched bases, these SNPs are not predicted to impact detection of the N1 and N3 targets.

Further evaluation identified that 15 variant sequences, specifically B.1.1.7 and B1426_1429, exhibited single nucleotide mismatches in both the N3 forward primer and probe (15/15,810; 0.09%). Even if viral templates exhibiting a mismatch(es) to the N3 forward primer/probe would result in a reduced efficiency of detection or non-detection, the SARS-CoV-2-specific N1 gene target is still expected to be detected by the WREN Laboratories COVID-19 PCR Test. The primer sets used in the WREN Laboratories COVID-19 PCR Test are therefore predicted to detect all currently circulating SARS-CoV-2 variants.

b. 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 17. 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 17. *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 Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Human coronavirus OC43	taxid:31631	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Human coronavirus HKU1	taxid:290028	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Human coronavirus NL63	taxid:277944	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
SARS-coronavirus	taxid:694009	Fw: < 80% similarity Rev: 100% similarity Probe: 95% similarity	Fw: 82% similarity Rev: 100% similarity Probe: 96% similarity
MERS-coronavirus	taxid:1335626	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Adenovirus C1	taxid:10533	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Human Metapneumovirus (hMPV)	taxid:162145	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Parainfluenza virus 1-4	taxid:12730 taxid:1979160 taxid:11216 taxid:11203	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Influenza A & B	taxid:11320	Fw: < 80% similarity	Fw: < 80% similarity

Pathogen Name	Tax ID	N1 Homology	N3 Homology
	taxid:11520	Rev: < 80% similarity Probe: < 80% similarity	Rev: < 80% similarity Probe: < 80% similarity
Enterovirus (e.g. EV68)	taxid:42789	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Respiratory syncytial virus	taxid:11250	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Rhinovirus	taxid:12059	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Chlamydia pneumoniae	taxid:83558	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Haemophilus influenzae	taxid:727	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Legionella pneumophila	taxid:446	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Mycobacterium tuberculosis	taxid:1773	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Streptococcus pneumoniae	taxid:1313	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Streptococcus pyogenes	taxid:1314	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Bordetella pertussis	taxid:520	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Mycoplasma pneumoniae	taxid:2104	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Pneumocystis jirovecii (PJP)	taxid:42068	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Candida albicans	taxid:5476	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Pseudomonas aeruginosa	taxid:287	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Staphylococcus epidermis	taxid:1282	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity
Streptococcus salivarius	taxid:1304	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity

3) Clinical Evaluation:

a. Testing of Previously Confirmed Positive and Negative Clinical Specimens Using an FDA Authorized Molecular RT-PCR Assay:

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 authorized SARS-CoV-2 molecular test. Specimens were extracted using the Qiagen QIAamp Viral RNA Mini Kit and prepared for RT-PCR using a traditional master mix set-up containing liquid primers/probes.

For the positive clinical nasopharyngeal swab samples, the positive percent agreement (PPA) between the WREN Laboratories COVID-19 PCR Test and the comparator assay was 100% (60/60). The Ct range for the N1 and N3 targets used in the WREN Laboratories COVID-19 PCR 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. There were three SARS-CoV-2 negative samples determined by the comparator assay that were positive by the WREN Laboratories COVID-19 PCR Test. Qualitative results of the clinical evaluation are shown in Table 18.

Table 18. Summary of Qualitative Clinical Study Results for Nasopharyngeal Swabs

Nasopharyngeal Swabs		FDA Authorized Molecular RT-PCR Comparator Assay		
		Positive	**Negative**	**Total**
WREN Laboratories COVID-19 PCR Test	**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 interval

[a] Discordant analysis was performed on the 3 false positive results using a second FDA authorized SARS-CoV-2 molecular test. Two out of the 3 false positives were also positive by the second comparator assay.

b. Discordant Analysis:

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 authorized SARS-CoV-2 molecular test that targeted the nucleocapsid and RNase P genes. It was determined that 2/3 discordant specimens were also positive by the second comparator assay as footnoted in the performance table (Table 18). Both the WREN Laboratories COVID-19 PCR Test and the second FDA authorized comparator assay were run a second time on the three FP samples and results were confirmed as originally reported.

c. Paired Nasopharyngeal Swab and Saliva Clinical Study From Patients Suspected of COVID-19:

A prospective study was performed to evaluate the use of saliva as a specimen type compared to nasopharyngeal (NP) swabs for the detection of SARS-CoV-2 in patients who were suspected of COVID-19 using the medical judgement of a healthcare provider and a screening questionnaire. The study was conducted with symptomatic patients at two facilities, including one ambulatory care center and one tertiary medical school (in-patient setting). Patients were each provided instructions for self-collection of saliva using the WREN Laboratories COVID-19 Saliva Test Collection Kit. Self-collection of saliva samples was performed under the observation of a healthcare provider, without intervention, who subsequently (within 15 minutes) also collected two NP swabs from each patient for parallel testing for SARS-CoV-2. The second NP swab was collected for orthogonal testing.

The NP swabs were collected using the BD Universal Viral Transport Kit (BD Cat # 220529) and stored in Universal Transport Medium for shipment to WREN Laboratories for testing. The NP swabs were transported on ice and the saliva specimens were shipped at ambient temperature. All paired specimens were processed and tested within 48 hours of collection using the Qiagen QIAamp Viral RNA Mini Kit for extraction and a traditional master mix set-up containing liquid primers/probes. One set of NP swabs was evaluated at WREN Laboratories using an FDA authorized molecular RT-PCR assay (#1) and the second set of paired swabs was tested using a different FDA authorized RT-PCR assay (#2) as an orthogonal validation method. Paired saliva samples were evaluated with the WREN Laboratories COVID-19 PCR Test. Results demonstrated 100% concordance between the simultaneously collected NP swabs and saliva (See Table 19) when using the FDA authorized molecular assay #1 as the comparator. A summary of the clinical study results using authorized assay #1 as a comparator is presented in Table 19 and 20 below.

The results of the clinical evaluation with paired NP swabs and saliva collected using the WREN Laboratories COVID-19 Saliva Test Collection Kit were therefore considered acceptable.

Table 19. Agreement Between the WREN Laboratories COVID-19 PCR Test that Evaluated Saliva and an FDA Authorized Molecular RT-PCR Assay (#1) that Evaluated the Paired Nasopharyngeal Swab Samples

		FDA Authorized Molecular RT-PCR Assay #1 Comparator (Nasopharyngeal Swab)		
		Positive	**Negative**	**Total**
WREN Laboratories COVID-19 PCR Test (Saliva)	**Positive**	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive Percent Agreement		100% (30/30); 88.43-100.00%[1]		
Negative Percent Agreement		100% (30/30); 88.43-100.00%[1]		

[1]Two-sided 95% score confidence interval

Table 20. Summary of Results Obtained from Parallel Testing of Nasopharyngeal Swab Samples and Saliva from Patients Suspected of COVID-19, Stratified by Measurand

Number of Patients	Sample Type	Analysis	Assay Target			
			N1	N2	N3	RNase P
30 NP positive	NP swab	Positive (%)	30/30 (100)	30/30 (100)	N/A	30/30 (100)
		Mean Ct	33.14	32.45		30.46
	Saliva	Positive (%)	30/30 (100)	N/A	30/30 (100)	30/30 (100)
		Mean Ct	33.41	N/A	33.05	30.86
30 NP negative	NP swab	Positive (%)	0 (0)	0 (0)	0 (0)	30/30 (100)
		Mean Ct	N/A	N/A	N/A	
	Saliva	Positive (%)	0 (0)	0 (0)	0 (0)	30/30 (100)
		Mean Ct	N/A	N/A	N/A	

NP: Nasopharyngeal; N/A: Not applicable

d. Orthogonal Validation Testing:

A second nasopharyngeal swab was collected from each patient that provided a saliva specimen with the WREN Laboratories COVID-19 Saliva Test Collection Kit. All 60 paired nasopharyngeal swabs were tested by a different FDA authorized molecular RT-PCR assay (assay #2) as an orthogonal method of validation. Results demonstrated 100% PPA and 88.24% NPA between the paired saliva and NP swab samples when using the orthogonal FDA authorized comparator assay (See Table 21). There were four patients who were reported positive by the WREN Laboratories COVID-19 PCR Test and negative by the paired NP swab. All four NP swabs were reported positive for SARS-CoV-2 RNA using alternative authorized assay #1 for discordant analysis.

Table 21. Performance of the WREN Laboratories COVID-19 PCR Test with Saliva Compared to Paired NP Swabs Tested Using Another FDA Authorized Molecular RT-PCR Assay (#2)

		FDA Authorized Molecular RT-PCR Assay #2 Comparator (Nasopharyngeal Swab)		
		Positive	Negative	Total
WREN Laboratories COVID-19 PCR Test (Saliva)	Positive	26	4[a]	30
	Negative	0	30	30
	Total	26	34	60
Positive Percent Agreement		100.00% (26/26); 87.13-100.00%[1]		
Negative Percent Agreement		88.24% (30/34); 73.38-95.33%[1]		

[a] Discordant NP samples were tested using FDA authorized molecular assay #1 and found to be positive for SARS-CoV-2 RNA.

[1]Two-sided 95% score confidence interval

e. Assessment of Low Positive NP Samples:
An evaluation of the number of low positives based on the NP swab samples tested by the FDA authorized molecular RT-PCR assay #1 was completed to ensure that corresponding saliva samples were detected by the WREN Laboratories COVID-19 PCR Test. A total of 21 low positive NP swabs (70%) were identified in the data set. The WREN Laboratories COVID-19 PCR Test detected SARS-CoV-2 from the paired saliva samples that were determined to be low positive samples using FDA authorized assay #1 as the comparator method.

f. 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 an FDA authorized SARS-CoV-2 molecular test for confirmatory testing. All 10 patient specimens yielded concordant results.

g. Validation of Asymptomatic Testing of Saliva by Comparing Against NP Swabs Tested with the WREN Laboratories COVID-19 PCR Test:
A prospective clinical study was conducted at one clinical site in Connecticut over the course of 50 days. This was an all-comers study that recruited symptomatic and asymptomatic individuals.

Paired NP swabs and saliva collected using the WREN Laboratories COVID-19 Saliva Test Collection Kit were obtained from all study participants. First, patients self-collected saliva following the instructions for use of the WREN Laboratories COVID-19 Saliva Test Collection Kit. Immediately following saliva collection (within 15 minutes), NP swabs were collected by a healthcare provider for comparator testing. At the conclusion of each day, all paired samples were bulk shipped to WREN Laboratories and tested within the validated stability window for saliva and NP swabs. RNA was isolated from saliva samples using the Qiagen QIAamp Viral RNA Mini Kit and prepared for RT-PCR using a traditional master mix set-up with liquid oligonucleotides. Extracted RNA was reverse transcribed using the High Capacity cDNA kit (ThermoFisher Scientific) and the WREN Laboratories COVID-19 PCR Test was run on a QuantStudio 7 Flex (QS7) instrument. The NP swabs were tested using an FDA authorized molecular assay in accordance with the authorized protocol. Performance with the paired saliva specimens in comparison to that obtained with NP swabs collected from asymptomatic individuals is displayed in Table 22.

Table 22. Performance of NP Swabs with the WREN Laboratories COVID-19 PCR Test Against Paired Saliva Samples from an Asymptomatic Population

Asymptomatic Samples		FDA Authorized Molecular RT-PCR Assay Comparator (Nasopharyngeal Swab)		
		Positive	Negative	Total
	Positive	21	23[a]	44

WREN Laboratories COVID-19 PCR Test (Saliva)	**Negative**	0	121	121
	Total	21	144	165
Positive Percent Agreement		21/21; 100.00% (84.54% - 100.00%)[1]		
Negative Percent Agreement		121/144; 84.03% (77.17% - 89.12%)[1]		

[1] Two-sided 95% confidence interval

[a] A follow up FDA authorized targeted, whole genome sequencing assay was performed on the 23 false positive saliva samples and 3 randomly selected negative NP swab samples. Complete SARS-CoV-2 genomes with 100% coverage (1 million reads) were generated for the 23 false positive saliva samples. No SARS-CoV-2 sequences were amplified in the 3 negative NP swabs. Further evaluation of the sequencing data showed variation among the sequences which indicated that SARS-CoV-2 sequences were not an artifact of contamination.

There was 100% positive percent agreement (PPA) and 84.03% negative percent agreement (NPA), between the results obtained from testing saliva specimens with the WREN Laboratories COVID-19 PCR Test in comparison to paired NP swabs. Of the 44 paired NP swab and saliva samples, 21 NP swabs were confirmed positive for N1 and N3; however, there were 23 false positive results where the NP swab was negative but the saliva showed positive amplification (Ct < 40). A root cause analysis was completed on the 23 false positive saliva samples. The presence of SARS-CoV-2 was confirmed with an FDA authorized whole genome sequencing method performed by a third party (See footnote to Table 22). For the 121 comparator assay negative NP swab samples, all corresponding paired saliva samples were negative (both N1 and N3 targets not detected).

LIMITATIONS:

- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.
- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- In the absence of symptoms, it is difficult to determine if asymptomatic individuals have been tested too late or too early. Therefore, negative results in asymptomatic individuals may include individuals who were tested too early and may become positive later, individuals who were tested too late and may have serological evidence of infection, or individuals who were never infected.
- Performance with specimens collected from individuals 18 years and older by an adult in the home has not been evaluated.

WARNINGS:

- For In Vitro Diagnostic Use
- For Use Under an Emergency Use Authorization (EUA) Only

- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and,
- The emergency use of this product 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 declaration is terminated or authorization is revoked sooner.