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 (HCP).

The WREN Laboratories COVID-19 PCR Test is also for use with saliva specimens that are self-collected at home or in a healthcare setting using the WREN Laboratories Saliva Collection Kit when determined to be appropriate by an HCP.

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 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. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

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

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 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.

Specimen Collection

Nasopharyngeal, oropharyngeal (throat), anterior nasal, and mid-turbinate nasal swabs, as well as nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage (BAL) specimens should be collected, transported and stored according to standard procedures. The NP swabs that were validated for use with the WREN Laboratories COVID-19 PCR Test are included in the BD Universal Viral Transport Kit (BD Cat # 220529) and stored in UTM for evaluation. Other flocked swabs with plastic shafts may be acceptable to use with the WREN Laboratories Test pending compatibility testing. Washes/aspirates/BALs can be collected in sterile containers such as the Corning TP52C002 (ThermoFisher, Cat # 07-202-025). All NP/OP, and mid-turbinate nasal swabs as well as washes/aspirates/BALs are collected by a trained healthcare provider (HCP) in a healthcare setting.

Saliva specimens must be collected, transported, and stored using the WREN Laboratories SARS Saliva Collection Tube provided in the WREN Laboratories Saliva Collection Kit. Collection of saliva can occur using two different approaches:

1) self-collected under the supervision of an HCP in the healthcare setting
2) self-collected without HCP supervision (unsupervised) in the home setting

Saliva specimens must be transported and stored at ambient temperature and tested within 120 hours of collection (5 days).

Nucleic Acid Extraction and RT-PCR

RNA is isolated from all specimen types using the QIAamp Viral RNA Mini Kit (Qiagen, Cat # 52906). Nucleic acid is manually extracted from 140 µL of acceptable specimen and the final purified nucleic acid is eluted in a 60 µL volume. 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.

COLLECTION KITS USED WITH THE TEST

- This assay can be used with saliva collected with the WREN Laboratories Saliva Collection Kit.

COMPONENTS AND SET-UP OF THE WREN LABORATORIES SALIVA COLLECTION KIT

The WREN Laboratories Saliva Collection Kit includes the following components:

- Lab requisition form
- Instructions for collection
- Instructions for shipping
- Kit components
    - Uncapped 2 mL tube for saliva collection
    - Funnel/mouthpiece to aid in saliva collection
    - Sealed cap containing stabilization buffer (red color)
    - Biohazard bag with absorbent pad
    - Brown cardboard box with 2nd absorbent pad
    - Pre-paid FedEx return address label
    - FedEx UN3773 envelope

To assist in the collection of saliva, a mouthpiece is used that attaches to the labeled tube containing the patient's initials, date of birth, and collection date. A red line is marked on the WREN Laboratories SARS Collection Tube to indicate when sufficient volume has been collected for testing (0.5 mL). Any bubbles should be above the red line. Following collection, the mouthpiece is discarded and the tube cap containing the red-colored stabilization buffer is screwed onto the collection tube. This action pierces that pouch containing the buffer that is within the tube cap and the red liquid will automatically flow into the collection tube. Saliva is mixed with the stabilization buffer by shaking back and forth 10 times and then prepared for shipment to WREN Laboratories.

MEDICAL OVERSIGHT AND PROCESS TO BE USED FOR SALIVA COLLECTION

There are two workflows with respect to how saliva is collected with the WREN Laboratories Saliva Collection Kit for testing with the WREN Laboratories COVID-19 PCR Test:

- Patient self-collected under the supervision of a trained healthcare provider in a healthcare setting. The saliva collection kits at designated healthcare facilities will be kept stocked based upon requests/demands of each institution. On a 30-day basis, kit numbers will be re-assessed and restocked based upon the client's request.
- Unsupervised patient self-collected in the patient's home environment.

Regardless of the workflow, the patient is able to obtain the saliva collection kit if they meet one of the following eligibility criterions:

- signs and symptoms (e.g., fever, cough, difficulty breathing)
- lives in or has recently traveled to a place where transmission of COVID-19 is known to occur
- has been in close contact with an individual suspected of or confirmed to have COVID-19

The following depicts two scenarios for self-collection of saliva using the WREN Laboratories Saliva Collection Kit:

For WREN Laboratories Saliva Collection Kit (Supervised in a Healthcare Facility)

1. The patient visits a healthcare institution (walk-in clinic) or is a resident of a healthcare facility (i.e., nursing home or skilled nursing facility) and is evaluated by a healthcare provider (HCP) to determine suitability for receiving the WREN Laboratories Saliva Collection Kit. The eligibility criteria discussed previously are applied to each patient.
2. If the patient is determined to be suitable to receive the saliva collection kit, the patient will collect the sample by following the provided kit instructions under the supervision of a HCP.
3. The HCP will prepare the specimen for shipping to WREN Laboratories using the shipping instructions provided with the collection kit.

4. When results are available, the patient and the requisitioner will receive a notification via email with their test results. If a patient does not have an email address, a hard copy of the results can be sent via mail or discussed over the phone with their HCP. After viewing the emailed results, the patient will have the option to discuss with an HCP. Specifically, for a positive result, the patient is instructed to immediately contact their HCP and the HCP is instructed to contact their positive patient.

For WREN Laboratories Saliva Collection Kit Ordering (Unsupervised in the Home Setting)

1. The patient calls WREN Laboratories (203-208-3464) to request the WREN Laboratories Saliva Collection Kit. Contact information about COVID-19 testing can be found at https://www.wrenboratories.com/covid-19/covid-19-home.
2. A healthcare provider (HCP) screens the patient to determine suitability for receiving the saliva collection using the eligibility criteria mentioned previously.
3. If the patient is determined to be suitable to receive the saliva collection kit, the patient will then pay for the test kit and WREN Laboratories will ship the kit to the patient's home via next day/overnight shipping.
4. The patient collects the sample following the kit's included instructions and returns the specimen to WREN Laboratories via a prepaid FedEX return shipment pack.
5. When results are available, the patient and the requisitioner will receive a notification via email with their test results. If a patient does not have an email address, a hard copy of the results can be sent via mail or discussed over the phone with their HCP. After viewing the emailed results, the patient will have the option to discuss with an HCP. Specifically, for a positive result, the patient is instructed to immediately contact their HCP and the HCP is instructed to contact their positive patient.

INSPECTION OF SALIVA SPECIMENS RECEIVED AT WREN LABORATORIES FOR TESTING:

Specimens collected with the WREN Laboratories Saliva Collection Kit must be checked for the following criteria upon receipt at WREN Laboratories prior to processing as outlined in the accessioning section of the laboratory's SOP:

- Sample collection tube must be intact and not visibly damaged (no leakage).
- The tube must contain the appropriate information including initials and time of collection as well as the requisition form in the outer sleeve of the biohazard bag.
- Accession date is within 96 hours of the collection date/time.

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 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 *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 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 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 < 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 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 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.

Assessment of clinical specimen test results must 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 < 38)	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 and appropriate public health authorities.
+/-[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 < 38 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 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 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 for NP/OP Swab Matrix

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

To validate the use of saliva as an acceptable specimen type, a LoD study was completed using saliva collected in the WREN Laboratories Saliva Stabilization Buffer. A preliminary LoD was determined using Twist Bioscience SARS-CoV-2 RNA material spiked into negative saliva buffer at four different concentrations and tested with three replicates per concentration with the WREN Laboratories COVID-19 PCR Test (See Table 5).

Table 5. Estimated Assay LoD Using Saliva Collected in the WREN Laboratories Saliva Stabilization Buffer

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 (66%)	2/3 (66%)
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%)

The saliva assay LoD was confirmed using 40 independent extraction replicates spiked at 1.5X LoD, (15 copies/µL), 3-10X LoD (40-100 copies/µL), and 50-100X LoD (500-1000 copies/µL). 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 Positives and 10 Negatives

Concentration (copies/µL)	Average Ct Values			# Detected / Total Tested
	N1	N3	RNase P	
15 copies/µL (1.5X LoD)	36.75	36.77	30.14	24/24
40-100 copies/µL (3X-10X LoD)	36.06	36.6	29.63	10/10
100 copies/µL (10X LoD)	33.83	33.51	30.06	6/6
Negative	UD*	UD	29.4	10/10

The LoD for saliva was estimated to be 10 copies/µL, based on the preliminary range finding study data; however, the LoD was confirmed at 15 copies/µL.

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 7.

Table 7. 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 8. 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 8. *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 taxid:11520	Fw: < 80% similarity Rev: < 80% similarity Probe: < 80% similarity	Fw: < 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	Fw: < 80% similarity Rev: < 80% similarity

Pathogen Name	Tax ID	N1 Homology	N3 Homology
		Probe: < 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

2) ***Clinical Evaluation:***

a. Testing of Previously Confirmed Positive and Negative Clinical Specimens Using an EUA 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 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 Table 9.

Table 9. Summary of Qualitative Clinical Study Results Performed on the QuantStudio 7-Flex Instrument

		Authorized Assay - Comparator		
		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 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.

b. 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 9). 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.

c. Paired Nasopharyngeal Swab and Saliva Clinical Study

A prospective study was performed to evaluate the use of saliva as a specimen type compared to nasopharyngeal swab for the detection of SARS-CoV-2 in patients who were suspected of COVID-19 using the medical judgement of a healthcare provider and the 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 SARS Saliva Collection Tube included within the WREN Laboratories Saliva 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 nasopharyngeal swabs from each patient for parallel testing for SARS-CoV-2. The second nasopharyngeal swab (NP) was collected for orthogonal testing.

The NP swabs were collected using the BD Universal Viral transport Kit (BD Cat # 220529) and stored in UTM for shipment to the WREN Laboratories for testing. The NP swabs were transported on ice and the saliva specimens were shipped at ambient temperature. All paired specimens were tested within 48 hours of collection. One set of NP swabs was evaluated at WREN Laboratories using an unmodified CDC EUA test (CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel; N1 and N2 targets

only) and the second set of paired swabs was tested using another EUA authorized RT-PCR assay 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 10) when using the CDC assay as the comparator. A summary of the results of the clinical study using the CDC authorized assay as a comparator is presented in Table 10 and 11 below.

The results of the clinical evaluation with paired nasopharyngeal swabs and saliva collected using the WREN Laboratories Saliva Collection Kit were therefore considered acceptable.

Table 10. Agreement between the WREN Laboratories COVID-19 PCR Test that Evaluated Saliva and an Unmodified CDC Assay that Evaluated the Paired Nasopharyngeal Swab Samples

		CDC EUA Unmodified Assay 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 intervals

Table 11. 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

Orthogonal Validation Testing:

A second nasopharyngeal swab was collected from each patient that provided a saliva specimen with the WREN Laboratories Saliva Collection Kit. All 60 paired

nasopharyngeal swabs were tested by a different EUA authorized RT-PCR assay 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 EUA authorized comparator assay. There were four false positive samples that were found to be positive for SARS-CoV-2 RNA after discordant analysis was completed using an unmodified CDC assay (N1 and N2 targets).

Table 12. Performance of the WREN Laboratories COVID-19 PCR Test with Saliva Compared to Paired NP Swabs Tested Using Another EUA Authorized RT-PCR Assay

		EUA Authorized RT-PCR Assay 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 an unmodified CDC assay (N1 and N2 targets) and found to be positive for SARS-CoV-2 RNA
[1]Two-sided 95% score confidence intervals

Assessment of Low Positive NP Samples:
An evaluation of the number of low positives based on the NP swab samples tested by the unmodified CDC assay was completed to ensure that corresponding saliva samples were able to be 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 the CDC EUA Comparator method.

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

3) *Simulated Shipping Study for Saliva Collected in the WREN Laboratories Saliva Stabilization Buffer:*

Summer and Winter Thermal Excursions

A simulated shipping study was performed to evaluate the effect of temperature variation on the stability of SARS-CoV-2 RNA during transport of saliva specimens from the patient's home or healthcare setting to WREN Laboratories for processing. The shipping study was designed to simulate shipping at ambient temperature as well as the extreme temperature conditions that could be experienced during the summer and winter months. See Tables 13 and 14 for summer and winter thermal profiles, respectively, that were evaluated in this study.

Simulated sample stability and shipping studies were performed using a total of 38 contrived positive saliva specimens including 23 samples at 1-2X LoD (10-20 copies/µL), 5 samples at 2-5X LoD (20-50 copies/µL), and 10 samples at 5-10X LoD (50-100 copies/µL). Ten negative saliva samples collected from asymptomatic individuals and screened negative using the WREN Laboratories COVID-19 PCR Test were also included in the simulated shipping studies. After the contrived positive and negative samples underwent the thermal excursions, they were equilibrated to room temperature, extracted with the Qiagen QIAamp Viral RNA Mini Kit, and tested with the WREN Laboratories COVID-19 PCR Test.

Table 13. Summer Temperature Excursion

Temperature	Cycle Period	Cycle Period Hours	Total Hours[1]
40°C	1	8	8
22°C	2	4	12
40°C	3	2	14
30°C	4	36	50
40°C	5	6	56

[1] Sum of cycle periods

Table 14. Winter Temperature Excursion

Temperature	Cycle Period	Cycle Period Hours	Total Hours[1]
-10°C	1	8	8
18°C	2	4	12
-10°C	3	2	14
10°C	4	36	50
-10°C	5	6	56

[1] Sum of cycle periods

Table 15. Summary of Results from the Simulated Shipping Studies Using Contrived Specimens

Sample Group	Test Point	N	Mean Ct			SARS-CoV-2 Positive (%)
			N1	N3	RNase P	
Negative	Day 0 (RT)[1]	10	Und	Und	29.47	0 (0)
	Summer[2]	10	N/A	N/A	29.27	0 (0)
	Winter[3]	10	N/A	N/A	29.02	0 (0)
Low Positive 1-2X LoD 10-20 copies/µL	Day 0 (RT)	23	36.27	35.68	30.08	23/23 (100)
	Summer	23	36.51	35.81	30.04	22/23 (95.7)
	Winter	23	34.88	33.17	27.41	23/23 (100)
Moderate Positive 2-5X LoD 20-50 copies/µL	Day 0 (RT)	5	35.36	35.04	29.83	5/5 (100)
	Summer	5	36.64	35.93	29.86	5/5 (100)
	Winter	5	35.72	36.25	29.19	5/5 (100)
High Positive 5-10X LoD 50-100 copies/µL	Day 0 (RT)	10	33.61	33.09	29.22	10/10 (100)
	Summer	10	34.54	34.28	28.86	10/10 (100)
	Winter	10	34.62	34.10	28.62	10/10 (100)

[1] UND; Undetermined. Day 0 - room temperature
[2] Testing performed at the conclusion of the thermal excursions described in Table 13
[3] Testing performed at the conclusion of the thermal excursions described in Table 14

The results in Table 15 demonstrate that when tested with the WREN Laboratories COVID-19 PCR Test, SARS-CoV-2 RNA contrived positive saliva specimens are stable in the WREN Laboratories Saliva Buffer when exposed to a broad range of temperature conditions. These data support the use of the WREN Laboratories Saliva Collection Kit for transport and storage of specimens following self-collection of saliva in the home or healthcare setting.

Room Temperature Stability Studies

Twelve different donors collected 0.5 mL of saliva that was stabilized with 1 mL of stabilization buffer, as per the instructions provided with the WREN Laboratories Saliva Collection Kit. Each saliva/stabilization buffer sample was spiked with Twist Bioscience SARS-CoV-2 RNA material (Cat # MT007544.1, 1,000,000 copies/µL) at 1-2X LoD. Samples were evaluated at Day 0, Day 1 (24 hrs), Day 2 (48 hrs), Day 3 (72 hrs), Day 4, (96 hrs) and Day 5 (120 hrs) following room temperature (~22°C) incubation. Viral RNA was isolated using the QIAamp Viral RNA Mini Kit (Cat # 52906) and tested with the WREN Laboratories COVID-19 PCR Test. The impact of extended saliva storage at room temperature conditions on assay performance for each of the 5 days is summarized in Table 16.

Table 16. Room Temperature Stability Studies Up to 120 Hours (5 Days)

Sample Group	Test Point	N	Mean Ct			SARS-CoV-2 Positive (%)
			N1	N3	RNase P	
Low Positive 1-2X LoD 10-20 copies/µL	Day 0	12	35.63	36.14	29.44	12/12 100%)
	Day 1	12	35.76	36.26	29.58	12/12 100%)
	Day 2	12	35.70	36.37	29.76	12/12 100%)
	Day 3	12	35.92	36.48	29.09	12/12 100%)
	Day 4	12	36.10	36.21	29.31	12/12 100%)
	Day 5	12	36.00	36.21	29.31	12/12 100%)

Data demonstrated that saliva specimens are stable for 96 hours when transported at room temperature conditions.

4) ***Usability/Human Factors Assessment for the Saliva Collection Protocol:***

A usability study was conducted to assess user comprehension of the WREN Laboratories Saliva Collection Kit, including both collection and packaging the saliva specimen for shipment to WREN Laboratories for processing. The study inclusion criterion that was applied was internet availability in order to review the process and be observed during collection. A demographic question was administered as part of the screening questionnaire to ensure recruitment of a user cohort reflective (or as closely as feasible) to that of the 2019 US population. Participants were also recruited to reflect a variety of ages and education levels, including adolescent participants (under 18 years of age) that collected the saliva with their parent's supervision, the youngest being in second grade. In addition, participants included elementary school/high school students, as well as those that achieved a high school diploma or equivalent, undergraduate degree, and post-graduate higher education. The complete demographics of the usability study are presented in Table 17.

Thirty-six individuals were included in the usability/human factor assessment study. Each participant was provided with a kit (including instructions) and a post-collection questionnaire. Subjects were evaluated by an interviewer via video conferencing to observe the collection in the home setting. Parents supervised collection from children (n=5, 8-16 years old, male to female ratio of 2:3) and prepared the samples for shipping to WREN Laboratories.

Table 17. Demographics of the Usability Study

Characteristic	**N / N36 (%)**
Gender	
Male	16/36 (44.4%)
Female	20/36 (55.6%)
Age	
8	1/36 (2.8%)
9-18	4/36 (11.1%)
19-35	2/36 (5.6%)
36-55	12/36 (33.3%)
55 and older	17/36 (47.2%)
Race	
White	30/36 (83.3%)
Black/African American	3/36 (8.3%)
Asian	3/36 (8.3%)
North America/American Indian	0/36 (0%)
Oceanic/Pacific	0/36 (0%)
Ethnicity	
Hispanic/Latino	4/36 (11.1%)
Non-Hispanic/Latino	32/36 (88.9%)
Marital Status	
Divorced	8/36 (22.2%)
Married	17/36 (47.2%)
Single (never married)	10/36 (27.8%)
Widowed	1/36 (2.8%)
Education	
In School (elementary – high school)	5/36 (13.9%)
High School Diploma	10/36 (27.8%)
Undergraduate Degree	12/36 (33.3%)
Post-graduate Degree	9/36 (25.0%)

Of the 36 kits that were shipped to study participants for self-collection, 36/36 (100%) of the sample kits were received and processed using the WREN Laboratories COVID-19 PCR Test within 48 hours of collection. Of those collection kits received at WREN Laboratories, RNase P was detected in 36/36 (100%) samples, indicating successful collection of human biological material that was extracted and amplified. During the post-study questionnaire and post-collection interviews, three participants indicated that they had difficulty generating saliva (i.e., dry mouth); however, these subjects were able to produce a sufficient volume of saliva for testing with the WREN Laboratories COVID-19 PCR Test.

Results of the usability testing were analyzed qualitatively to determine if the design of the kit and/or kit instructions need to be modified to reduce the use-related risks to acceptable levels. Cognitive debriefing interviews were conducted following the actual-use testing to gather users' perspectives on each critical task or use scenario. As noted previously, three participants had difficulty producing sufficient saliva to the indicated fill-to line; however, these instances did not affect the ability to receive and process the samples. No other difficulties were noted during video conferencing of the collection process. Answers to the user 9-item questionnaire were also collected for the 36 sample kits. 33/36 participants successfully answered all questions (3 had a hard time generating saliva) indicating the understanding of the collection and shipping instructions. Based on the usability study data and feedback, the collection instructions were understandable, and the kit was easy to use. No changes or modifications to the current instructions needed to be made based on discussions with the participants.

5) ***Additional Requirement:***

In addition to validation studies, WREN Laboratories will submit a report to the FDA (within 30 days of authorization) summarizing any testing performed with WREN Laboratories Saliva Collection Kit including how many kits were requested, sent for home collection, or used at a collection site or institution. WREN Laboratories will also document the number of kits that were shipped and returned to the laboratory according to the instructions, how many specimens were rejected during accessioning and the reasons for rejection, and the positivity rate of the first WREN Laboratories Saliva Collection Kit lot.

LIMITATIONS:

- Testing of saliva specimens is limited to patients with symptoms of COVID-19.
- Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.
- 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.

WARNINGS:

- This product has not been FDA cleared or approved;
- This product has been authorized by FDA under an EUA for use by the authorized laboratory;
- This product has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- 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 authorization is terminated or revoked sooner.