# 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 laboratories designated by WREN Laboratories LLC, which are also 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 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 (1) 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) and (2) saliva specimens that are collected at home or in a healthcare setting using the WREN Laboratories COVID-19 Saliva Test Collection Kit by any individuals 18 years and older (self-collected), 14 years and older (self-collected under adult supervision), or 5 years and older (collected with adult assistance) including from individuals without symptoms or other reasons to suspect COVID-19, when determined to be appropriate by a healthcare provider.

Testing 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 RNA which is generally detected in respiratory specimens and saliva 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.

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 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. Note that WREN Laboratories intends to use the pre-spotted primer plates to test extracted RNA from saliva specimens; however, the pre-spotted primer plates have also been validated for use with extracted RNA from NP swab specimens. 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

Nasopharyngeal (NP), oropharyngeal (throat) (OP), 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 System (BD Cat # 220529) and are stored in Universal Transport Medium (UTM) for evaluation. 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 collection tube provided in the WREN Laboratories COVID-19 Saliva Test Collection Kit. Collection of saliva can occur using two different approaches:

1) collected under the supervision of an HCP in the healthcare setting by any individual 18 years and older (self-collected), 14 years and older (self-collected under adult or HCP supervision), or 5 years and older (collected with adult or HCP assistance)
2) collected without HCP supervision (unsupervised) in the home setting by any individuals 18 years and older (self-collected), 14 years and older (self-collected under adult supervision), or 5 years and older (collected with adult assistance)

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 all specimen types using either the QIAamp Viral RNA Mini Kit (Qiagen, Cat # 52906) or the ZYMO Research Quick-RNA Viral 96 Kit (Cat # R1041). Note that WREN Laboratories intends to use the ZYMO kit to extract RNA from saliva specimens; however, the ZYMO kit has been validated for use with NP swab specimens. 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. To increase the throughput of the extraction procedure, WREN Laboratories has also validated the ZYMO Quick-RNA Viral 96 Kit that 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. RNA extracted by either of these methods 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). If using the liquid primers and probes protocol, the final reaction volume is 16 µL (8.8 µL master mix and 7.5 µL cDNA); when using the pre-spotted primer plate, 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 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 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.

## HOME COLLECTION KITS USED WITH THE TEST

This assay can be used with saliva collected with the WREN Laboratories COVID-19 Saliva Test Collection Kit.

## COMPONENTS AND SET-UP OF THE WREN LABORATORIES COVID-19 SALIVA TEST COLLECTION KIT

The WREN Laboratories COVID-19 Saliva Test Collection Kit includes the following components:

- Lab requisition form (optional to complete)
- Instructions for collection and shipping
- Uncapped 2 mL tube for saliva collection (contains a QR code)
- Funnel/mouthpiece to aid in saliva collection
- Sealed cap containing stabilization buffer (red color)
- Biohazard bag with absorbent pad
- Cardboard box containing a UN3373 label on the back
- Pre-paid/pre-addressed FedEx return address label that will be affixed to the front of the shipping box
-

To assist in the collection of saliva, a mouthpiece is used that attaches to the 2 mL collection tube. A red line is marked on the 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 gently for 10 seconds. The collection tube is labeled with the patient's initials, date of birth, and collection date and prepared for shipment to WREN Laboratories or an alternative designated testing laboratory.

## 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 COVID-19 Saliva Test Collection Kit for testing with the WREN Laboratories COVID-19 PCR Test:

- Patients can self-collect a saliva sample under the supervision of a trained healthcare provider in a healthcare setting. Collection can occur by any individual 18 years and older (self-collected), 14 years and older (self-collected under adult or HCP supervision), or 5 years and older (collected with adult or HCP assistance). The WREN Laboratories COVID-19 Saliva Test Collection Kit 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.

- Patients can self-collect a saliva sample unsupervised in the patient's home environment. Collection can occur by any individual 18 years and older (self-collected), 14 years and older (self-collected under adult supervision), or 5 years and older (collected with adult assistance).

The following depicts two scenarios for self-collection of saliva using the WREN Laboratories COVID-19 Saliva Test 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 eligibility for receiving the WREN Laboratories COVID-19 Saliva Test Collection Kit.
2. If the patient is determined to be eligible to receive the WREN Laboratories COVID-19 Saliva Test Collection Kit, a prescription is written and the patient will collect the sample by following the provided kit instructions under the supervision of an HCP.
3. The HCP will activate the kit by completing the paper copy of the lab requisition form.
4. The HCP will prepare the specimen for shipping to WREN Laboratories or a laboratory designated by WREN Laboratories using the provided UN3373 labeled cardboard box and prepaid FedEx return address label.
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. An HCP from WREN Laboratories performs a follow up phone call for all positive SARS-CoV-2 cases, presumptive positives, and invalid results.

**Unsupervised in the Home Setting**

**a. Call to WREN Laboratories/HCP Verbal Assessment**

1. The patient calls WREN Laboratories (203-208-3464) to request the WREN Laboratories COVID-19 Saliva Test Collection Kit. The patient will be evaluated by a healthcare provider who uses their medical expertise and a screening questionnaire to determine patient eligibility.

2. If the patient is determined to be eligible to receive the WREN Laboratories COVID-19 Saliva Test Collection Kit, the HCP writes a prescription for the collection kit and associated test. 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.
3. Upon receipt, the patient is required to activate the kit by choosing one of three methods:
    - Complete the paper copy of the lab requisition form.
    - Scan the QR code on the collection tube and provide necessary information online.
    - Access https:///www.wrencovidtesting.com/start to type in the QR code numbers and provide necessary information online.

4. Following kit activation online or completion of the lab requisition form, the patient collects the sample following the kit's included instructions and returns the specimen to WREN Laboratories or a laboratory designated by WREN Laboratories using the provided UN3373 labeled cardboard box and prepaid FedEx return address label.
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  An HCP from WREN Laboratories performs a follow up phone call for all positive SARS-CoV-2 cases, presumptive positives, and invalid results.

**b. Online Ordering Via WREN Laboratories Website**

1. The patient requests a collection kit by accessing the following web address: https://www.wrencovidtesting.com/#pricing-and-ordering. A screening questionnaire is administered, and results are evaluated in real-time by a WREN Laboratories healthcare provider to determine patient eligibility.
2. If eligibility is denied, a message will appear indicating that the prescription cannot be fulfilled at the current time. Ordering options are disabled. If deemed eligible to receive the kit, a pop-up message will appear on the screen indicating that the prescription has been approved. The patient can then add the collection kit to their cart and check-out. The WREN Laboratories COVID-19 Saliva Test Collection Kit is shipped directly to the recipient's home via next day/overnight shipping or 2-day ground shipping, depending upon what the patient selects during the check-out procedure.
3. Upon receipt, the patient is required to activate the kit by choosing one of the three methods:
    - Complete the paper copy of the lab requisition form.
    - Scan the QR code on the collection tube and provide necessary information online.
    - Access https:///www.wrencovidtesting.com/start to type in the QR code numbers and provide necessary information online.
4. Following kit activation online or completion of the lab requisition form, the patient collects the sample following the kit's included instructions and returns the specimen to WREN Laboratories or a laboratory designated by WREN Laboratories using the provided UN3373 labeled cardboard box and prepaid FedEx return address label.
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. An HCP from WREN Laboratories performs a follow up phone call for all positive SARS-CoV-2 cases presumptive positives, and invalid results.

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

Specimens collected with the WREN Laboratories COVID-19 Saliva Test Collection Kit must be checked for the following criteria upon receipt at WREN Laboratories or designated testing laboratories prior to processing as outlined in the specimen requirements section of the assay and accessioning SOP:

- Saliva sample must be collected using the collection device provided with the WREN Laboratories COVID-19 Saliva Test Collection Kit.
- Sample collection tube must be intact and not visibly damaged or leaking.
- Sample volume meets the minimum required for testing.
- Sample collection tube contains all required information (patient's initials, date of birth, and date/time of saliva collection).
- Specimen must arrive within the established stability window for testing (i.e., within 96 hours from the recorded collectiontime).
- Specimen is associated with a completed requisition form in the outer sleeve of the biohazard bag or an electronic requisition form completed during kit activation online.
- Specimen was collected using an unexpired collection kit.

## REAGENTS AND MATERIALS

| 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)<br>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

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 purified plasmid DNA that contains one copy each of the 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) **<u>WREN Laboratories COVID-19 PCR Test Controls – NTC, SARS-CoV-2 Positive Viral Control, Positive Plate Control, and Internal RNase P Control:</u>**

   - 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 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, 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 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] 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.

## PERFORMANCE EVALUATION (WREN LABORATORIES COVID-19 PCR TEST)

### 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 Liquid Primers/Probes:
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 per extraction (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 liquid primers/probes (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) | Ct 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 | 20/20 | 100% | 100% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | (60/60 wells) | (60/60 wells) | | |
| **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 kit and run on the WREN Laboratories COVID-19 PCR Test using the pre-spotted primer plate.

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) | Ct 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 | 20/20 | 100% | 100% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | (60/60 wells) | (60/60 wells) | | |
| **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 (lyophilized primers/probes) with 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 the liquid primers/probes 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/liquid primers/probes 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 bridging studies were completed as outlined in Figure 1. Each individual assay change was evaluated to determine equivalence to the original method.

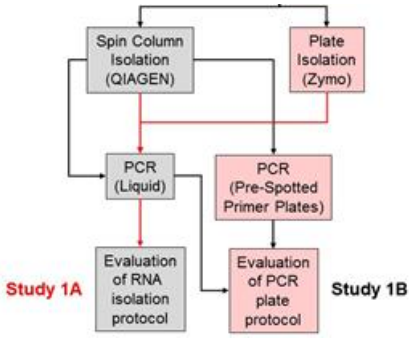


**Figure 1. Representation of the Bridging Studies Performed to Validate the New Extraction and Pre-Spotted Primer Plate Procedure**

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 (12/12 wells) | 4/4 (12/12 wells) | 100% | 100% | 33.7± 1.0 | 33.9± 1.9 | 4/4 (12/12 wells) | 4/4 (12/12 wells) | 100% | 100% |
| **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) <u>Analytical Inclusivity/Specificity:</u>**

a. <u>Inclusivity:</u>

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<br>99.8% | 17,982 | 17,932/17,982<br>99.7% |
| Reverse | | 17,968/17,982<br>99.9% | | 17,969/17,982<br>99.9% |
| Probe | | 17,908/17,982<br>99.6% | | 17,955/17,982<br>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 associated with the N1 oligonucleotides, specifically the N1 probe binding region. Of the sequences evaluated, 99.6% had 100% homology to the N1 probe while 99.8% and 99.9% had 100% homology with the N1 forward and reverse primers, respectively. Similarly, 99.8% had 100% homology to the N3 probe while 99.7% and 99.9% had 100% homology to the N3 forward and reverse primers, 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<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Human coronavirus OC43 | taxid:31631 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Human coronavirus HKU1 | taxid:290028 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Human coronavirus NL63 | taxid:277944 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| SARS-coronavirus | taxid:694009 | Fw: < 80% similarity<br>Rev: 100% similarity<br>Probe: 95% similarity | Fw: 82% similarity<br>Rev: 100% similarity<br>Probe: 96% similarity |
| MERS-coronavirus | taxid:1335626 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Adenovirus C1 | taxid:10533 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Human Metapneumovirus (hMPV) | taxid:162145 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Parainfluenza virus 1-4 | taxid:12730<br>taxid:1979160<br>taxid:11216<br>taxid:11203 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Influenza A & B | taxid:11320<br>taxid:11520 | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity | Fw: < 80% similarity<br>Rev: < 80% similarity<br>Probe: < 80% similarity |
| Enterovirus (e.g. EV68) | taxid:42789 | Fw: < 80% similarity | Fw: < 80% similarity |

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

## 3) Clinical Evaluation:

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 119 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 Saliva from Asymptomatic Subjects as a Specimen Type by Comparing Against NP Swabs:
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 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 |
| WREN Laboratories COVID-19 PCR Test (Saliva) | Positive | 21 | 23[a] | 44 |
| | 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).

## PERFORMANCE EVALUATION (WREN LABORATORIES COVID-19 SALIVA TEST COLLECTION KIT)

### 1) Simulated Shipping Study for Saliva Collected in the WREN Laboratories Saliva Stabilization Buffer:

a. 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 or a designated laboratory for processing. The shipping study was designed to simulate shipping at ambient temperature as well as extreme temperature conditions that could be experienced during the summer and winter months. See Tables 23 and 24 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 (based on the LoD determined using synthetic SARS-CoV-2 RNA from Twist Bioscience), 5 samples at 2-5X LoD , and 10 samples at 5-10X LoD . 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 using the liquid primers/probes protocol.

**Table 23. 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 24. 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 25. 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 | 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 | 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 | 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 23
[3] Testing performed at the conclusion of the thermal excursions described in Table 24

The results in Table 25 demonstrate that when tested with the WREN Laboratories COVID-19 PCR Test, SARS-CoV-2 RNA contrived positive saliva specimens are stable in the collection tube buffer when exposed to a broad range of temperature conditions. These data support the use of the WREN Laboratories COVID-19 Saliva Test Collection Kit for transport and storage of specimens following self-collection of saliva in the home or healthcare setting.

b. 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 COVID-19 Saliva Test 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 hours), Day 2 (48 hours), Day 3 (72 hours), Day 4, (96 hours) and Day 5 (120 hours) following room temperature (~22°C) incubation. Viral RNA was isolated using the QIAamp Viral RNA Mini Kit (Cat # 52906), and reactions were prepared for RT-PCR using liquid primers/probes followed by testing with the WREN Laboratories COVID-19 PCR Test. The impact on assay performance of saliva storage under room temperature conditions is summarized in Table 26. The claimed duration of saliva specimen stability is 96 hours prior to testing.

**Table 26. 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 | 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%) |

c. Pressure and Travel Stability Testing of Collection Tube:

i. Simulated Testing:

To assess the watertight seal status of the buffer cap (stabilization buffer is contained within an aluminum seal) along with the combined cap/collection tube system as a single unit (i.e., the cap applied to the tube), vacuum testing on the cap and unit was performed based on D6653/D6653M protocols (ASTM Standard Test Methods for Determining the Effects of High Altitude on Packaging Systems by Vacuum Method). Three sealed buffer caps and three cap/tube combinations were evaluated at 0, 25, 50, 75, and 95kPa of pressure for 0.5, 1, 2, 4, 8, 16, 24, and 48 hours.

Aluminum seals within the cap withstood pressures of 95kPa for up to 24 hours (3/3 met acceptance criteria at each test point). One of the three caps at 95kPa for 48 hours failed due to a bond land failure (i.e., where the aluminum foil seals to the plastic; the sealing site) and not to rupture of the PCR foil itself . This seal failure occurred under extreme conditions beyond those that the sealed cap should experience during normal air and ground transportation. All cap/tube combinations (3/3 at each test point) withstood pressure of up to 95kPa for 48 hours.

ii. Real World Pressure and Travel Stability Testing:

Since saliva collection tubes within the WREN Laboratories COVID-19 Saliva Test Collection Kit will be collected from across the United States and sent for testing to WREN Laboratories or a laboratory designated by WREN, pressure testing was evaluated by physically shipping the caps and the combined cap/collection tube units via FedEx. Common pressure conditions that could be experienced during transport include 75kPa (<8 hours) within a

cargo air jet and 64.5kPa during ground transportation. Two different transportation studies were completed.

- Study I (n=50): Pre-filled buffer caps were transported by FedEx cross-country (from the manufacture site in Phoenix, AZ) to WREN Laboratories in Branford, CT. Samples were sent from AZ on a Friday at a temperature of 73°F with delivery to WREN Laboratories on Monday afternoon following 72 hours in transit via Indianapolis, IN and East Grandby, CT. No evidence of seal leakage or any alteration of the aluminum sealing was identified (n=50) following long distance road/air transportation.

- Study II (n=40x2): In a separate study, 40 kits were shipped on two separate occasions via FedEx overnight shipping. Tubes were intentionally routed from Connecticut via Memphis, TN to New York City (48 hours transport total). No leaks were noted in the sealed buffer caps after arrival in New York City (80/80). Following saliva collection, samples (i.e., capped, sealed tubes with saliva) were returned to WREN Laboratories in Branford, CT via overnight FedEx shipping (24 hours). No leakage of clinical sample content was identified in the 80 capped saliva tubes. Overall, no leakage of contents was identified on kit send-out or on the return of clinical samples. These data demonstrated the integrity of both the foil seal on the cap and the capped tube under real-world conditions.

2) **Home Collection Kit Stability:**

The expiration date of the WREN Laboratories COVID-19 Saliva Test Collection Kit is based on the least stable component which is the stabilization buffer. Therefore, the expiration date for the WREN Laboratories COVID-19 Saliva Test Collection Kit is 12 months from the date of manufacture when stored at room temperature and this is displayed on the back of the kit's outer box. A specific accessioning criterion is to ensure that the kit's expiration date has not been exceeded.

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

Two usability studies were conducted to assess user comprehension of the WREN Laboratories COVID-19 Saliva Test Collection Kit instructions, including both collection and packaging the saliva specimen for shipment to WREN Laboratories or a designated laboratory for processing. One usability study included 122 participants from an industrial setting and the second study with 55 participants was completed in a school setting with individuals ranging from 5-65 years old. For children aged 5-13 years old, the parent/adult assisted the collection process including kit activation via the QR code or manual online entry, holding the funnel up to the child's mouth and instructing them to spit to the fill line (i.e., adult assistance), as well as packaging the specimen for shipment. Adolescents/teenagers aged 14-17 years old performed the kit activation, collection, and packaging steps under adult supervision in case they had questions on the procedure. Those individuals 18 years or older performed the entire process of saliva collection and specimen packing unsupervised.

Participants in both studies were recruited to reflect a variety of ages and education levels, including participants currently in elementary and high school, those that received a high school diploma or equivalent, and those currently enrolled in college or have received higher education (i.e., BA, BS, MA, MEd, Ph.D). Other demographics were also documented (See Tables 27 and 28). Each participant was provided with a kit (including instructions for collection, packaging and shipping) and a questionnaire. Note that individuals were given the option to use either the longer version of the collection/shipping instructions or the streamlined instructions. Based on the results of the post-collection questionnaire, all 177 individuals chose to use the streamlined collection instructions which combined the collection and shipping procedures into one document. Subjects were evaluated in-person by staff from WREN Laboratories to monitor the collection procedure and to document potential errors or difficulties with the tasks. .

At the conclusion of the two usability studies, each study site shipped the individual samples that were packaged in their own kit boxes via FedEx, as per the instructions, to WREN Laboratories for processing. All samples were processed within the claimed stability window (96 hours when shipped at ambient conditions). A total of 121 adults (and 1 individual 17 years old) completed usability study #1 (Table 27). For usability study #2 performed in a school setting, a total of 40 minors between 5 and 17 years of age along with 15 adults participated (Table 28).

**Table 27. Participant Demographics from Usability Study #1 (Industry)**

| **Characteristic** | **N / N122 (%)** |
|---|---|
| **Gender** | |
| Male | 115/122 (94.3%) |
| Female | 7/122 (5.7%) |
| **Age** | |
| 17-25 | 11/122 (9.0%) |
| 26-35 | 20/122 (16.4%) |
| 36-45 | 28/122 (23.0%) |
| 46-55 | 26/122 (21.3%) |
| 56 and older | 37/122 (30.3%) |
| **Race** | |
| White | 91/122 (74.6%) |
| Black/African American | 10/122 (8.2%) |
| Asian | 20/122 (16.4%) |
| American Indian/Asian Native | 1/122 (0.8%) |
| **Ethnicity** | |
| Hispanic/Latino | 13/122 (10.7%) |
| Non-Hispanic/Latino | 109/122 (89.3%) |
| **Marital Status** | |
| Divorced | 20/122 (16.4%) |
| Married | 82/122 (67.2%) |
| Single | 20/122 (16.4%) |
| **Education Level** | |
| In School (elementary – high school) | 1/122 (13.9%) |
| High School Diploma | 94/122 (27.8%) |

| | |
|---|---|
| Undergraduate Degree | 22/122 (33.3%) |
| Post-graduate Degree | 5/122 (25.0%) |

**Table 28. Participant Demographics from Usability Study #2 (School)**

| Characteristic | N / N55 (%) |
|---|---|
| **Gender** | |
| Male | 30/55 (54.5%) |
| Female | 25/55 (45.5%) |
| **Age** | |
| 5-13 | 20/55 (36.4%) |
| 14-17 | 20/55 (36.4%) |
| 18-35 | 8/55 (14.5%) |
| 36-55 | 3/55 (5.4%) |
| 56 and older | 4/55 (7.3%) |
| **Race** | |
| White | 32/55 (58.2%) |
| Black/African American | 17/55 (30.9%) |
| Asian | 5/55 (9.1%) |
| American Indian/Asian Native | 1/55 (1.8%) |
| **Ethnicity** | |
| Hispanic/Latino | 9/55 (16.4%) |
| Non-Hispanic/Latino | 46/55 (83.6%) |
| **Marital Status** | |
| Divorced | 1/55 (1.8%) |
| Married | 6/55 (10.9%) |
| Single | 48/55 (87.3%) |
| **Education Level** | |
| In School (elementary – high school) | 42/55 (76.4%) |
| High School Diploma | 8/55 (14.5%) |
| Undergraduate Degree | 4/55 (7.3%) |
| Post-graduate Degree | 1/55 (1.8%) |

Children 13 and younger had a parent/adult assist with the collection and shipping procedure
Children 14-17 performed the collection and shipping procedure under the supervision of a parent/adult

Of the 177 kits that were used for self-collection and shipped to WREN Laboratories for downstream testing, all (100%) were received and processed using the WREN Laboratories COVID-19 PCR Test within 48 hours of collection. No damage or leaks from the collection tubes were observed upon accessioning. Of those collection kits received at WREN Laboratories, RNase P was detected in 176/177 (99.4%) samples, indicating successful collection of human biological material that was extracted and amplified. During the post-study questionnaire, the participant who provided a sample that was negative for RNase P indicated that they had difficulty generating saliva (i.e., dry mouth) and drank water prior to providing their sample.

The results of the usability testing were analyzed qualitatively to determine if the design of the kit and/or kit instructions needs 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 discussed previously, one participant had difficulty producing saliva. Noted within the instructions is a helpful hint to rub the outside of the cheeks, just behind the back teeth while performing chewing motions to prevent dry mouth. No other difficulties were noted during the collection process which indicated the user's understanding of the collection and shipping instructions. Answers to the user 15-item questionnaire were also collected for each of the 177 sample kits. Based on the feedback received, the collection instructions were understandable, and the kit was easy to use. No changes or modifications to the new streamlined instructions needed to be made based on discussions with the participants.

The results from the usability studies performed by WREN Laboratories indicate that individuals 5-13 years of age could provide a saliva specimen with adult assistance, individuals 14-17 years of age were able to collect saliva under adult supervision, and those 18 years and older could collect saliva safely and appropriately without supervision, with sufficient human biological material for downstream molecular testing.

**LIMITATIONS:**

- 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.
- 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.
- 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:**

- 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.