EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
PHOSPHORUS COVID-19 RT-QPCR TEST
(PHOSPHORUS DIAGNOSTICS LLC)

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

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

INTENDED USE

The Phosphorus COVID-19 RT-qPCR 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) oropharyngeal (throat) swabs, nasopharyngeal swabs, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates as well as bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider (HCP) and (2) saliva specimens that are self-collected at home or in a healthcare setting using the Pinpoint by Phosphorus COVID-19 Test Home Collection Kit by any individuals 18 years or older, 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 Phosphorus Diagnostics 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 detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

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

DEVICE DESCRIPTION AND TEST PRINCIPLE

Overview of RT-qPCR Test

The Phosphorus COVID-19 RT-qPCR Test is a real-time reverse transcription polymerase chain reaction test that is performed at Phosphorus Diagnostics in Secaucus, NJ or at laboratories designated by Phosphorus Diagnostics. The assay uses two primer and probe sets to detect two SARS-CoV-2 specific regions of the nucleocapsid (N) gene (the N1 and N2 targets). The Phosphorus COVID-19 RT-qPCR Test also includes a primer and probe set to detect the human RNase P (RP) gene. A separate master mix is prepared for each target to perform the Phosphorus COVID-19 RT-qPCR Test.

Specimen Collection

Saliva specimens are self-collected at home or in the healthcare setting using the OGD-510[1] collection device that is part of the Pinpoint by Phosphorus COVID-19 Test Home Collection Kit. Saliva specimens must be collected in the OGD-510 device, transported, and stored at ambient temperature and tested within 56 hours of sample collection. The collection device manufacturer (DNA Genotek) previously completed human factors and user comprehension studies for the FDA cleared OGD-510 saliva collection device and provided Phosphorus Diagnostics a right of reference to these data. In addition, the posted decision summaries for K141410 and K192920 provide overall results from the usability studies that were completed by lay users in the home environment to support an over-the-counter (OTC) use.

Anterior nasal swabs, mid-turbinate nasal swabs, oropharyngeal (throat) swabs, nasopharyngeal swabs, nasopharyngeal washes/aspirates or nasal washes/aspirates, and BALs must be collected, transported and stored according to standard procedures. All swab and wash/aspirate/BAL collections must be performed directly by a trained healthcare provider in a healthcare setting. Acceptable swabs for the Phosphorus COVID-19 RT-qPCR Test include sterile flocked swabs (minitip/regular) with a flexible plastic shaft transported in VTM, UTM, liquid Amies, sterile phosphate buffered saline (PBS), or normal saline solution. If shipment of samples will occur within 72 hours of collection, specimens can be stored at 2-8°C if necessary. If a delay in shipment is expected to exceed 72 hours, the collected specimens can be stored at -70°C prior to shipment to Phosphorus Diagnostics or designated laboratories. When samples are ready to be shipped, shipment should occur on dry ice or frozen ice packs. Once swab/wash/aspirate/BAL samples are received in the laboratory, specimens may be refrigerated (2-8°C) for an additional 3 days before they are extracted and processed if specimens cannot be processed immediately upon receipt.

All specimens received at the clinical laboratory for testing will undergo review and accessioning prior to acceptance for testing.

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[1] The Oragene Dx OGD-510 collection device is cleared for certain uses under K141410, K152556, and K192920, The Oragene Dx OGD-510 collection device is used in combination with the Phosphorus COVID-19 RT-qPCR Test for an unapproved/uncleared use, i.e., collection of RNA in saliva.

Nucleic Acid Extraction and RT-PCR

RNA extraction from saliva collected in the OGD-510 device as well as nasopharyngeal swab specimens has been validated using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher Scientific), Promega Maxwell HT Viral TNA Kit (Promega), and the Maxwell RSC TNA Viral Kit performed on the Maxwell RSC 48 System. The sample input and elution volumes for the three extraction kits are shown in Table 1 and are applicable to the previously mentioned, validated specimen types.

Table 1. Input Volume, Eluate, and Elution Volume for Validated Extraction Kits

Extraction Method	Sample Input Volume	Eluate	Elution Volume
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Manual)	400 µL	Manufacturer Provided Elution Solution	100 µL
Promega Maxwell HT Viral TNA Kit (Manual)	200 µL	Manufacturer Provided Nuclease Free Water	60 µL
Maxwell RSC TNA Viral Kit (Automated)	200 µL	Manufacturer Provided Nuclease Free Water	50 µL

Reverse transcription-PCR (RT-PCR) is performed using the ThermoFisher Scientific TaqPath 1-Step Multiplex Master Mix (No ROX) with 5 µL of extracted sample.

REAL-TIME PCR INSTRUMENT USED WITH THE TEST

The Phosphorus COVID-19 RT-qPCR Test is for use with the CFX384 Touch Real-Time PCR Detection System with Bio-Rad CFX Manager software version 3.1.

COMPONENTS OF THE PINPOINT BY PHOSPHORUS COVID-19 TEST HOME COLLECTION KIT

Collected saliva specimens will be sent to either Phosphorus Diagnostics LLC in Secaucus, NJ or laboratories designated by Phosphorus to perform the downstream testing. The Phosphorus COVID-19 RT-qPCR Test will be performed as authorized at each designated location under Phosphorus' EUA.

The saliva collection kit includes the following components:

- Phosphorus Kit Box
- Prepaid FedEx Return Shipment Pack
- Biohazard Bag with Absorbent Pouch
- DNA Genotek OGD-510 Saliva Collection Device and Instructions
- Pre-populated Test Requisition Form with Label Stickers
  At-Home Step-By-Step Instructions

MEDICAL OVERSIGHT AND PROCESS TO BE USED

For At-Home Saliva Collection Kit Ordering

1. The patient (18 years or older) visits the Phosphorus Diagnostics, to request/pay for the collection kit (https://www.phosphorus-c19-pcr.com/order-now/p/covid-19-rt-qpcr-test)
2. Once payment is received, the patient will be sent a link to complete a COVID-19 Medical Questionnaire. A healthcare provider (HCP), via a contracted entity,

authenticates the information using their medical expertise and determines patient suitability for saliva collection. If the patient is deemed inappropriate, the patient will receive a refund to the test kit; however, a provider fee is still applied.

3. Once assessed and determined to be appropriate for the collection kit, the patient will receive an email notification that the at-home saliva collection kit has shipped (48 hour shipping).
4. The patient (18 years or older) collects the sample following the kit's included instructions and returns the specimens to Phosphorus Diagnostics or a designated testing laboratory via a prepaid FedEx Return Shipment Pack.
5. When results are available, the patient and HCP will receive a notification via email with instructions for viewing test results on the Phosphorus online portal. Patients are given the option of talking with an HCP about their results.

For In-Clinic Saliva Collection

1. The patient visits the clinic and the healthcare provider (HCP) evaluates the patient for acceptability for the saliva kit using their medical expertise.
2. The HCP orders the test using the test requisition form or the Phosphorus online portal.
3. The saliva sample is collected by the patient (18 years or older) in the clinical setting according to the device's included instructions.
4. The HCP attaches the identifying label to the collected sample and places the sample in a biohazard bag with the patient's requisition form.
5. The HCP ships all samples collected from the day to Phosphorus Diagnostics or a designated testing laboratory via overnight shipping.
6. When results are available, the HCP will receive a notification and results will be sent via encrypted email, fax, or via the Phosphorus online portal. The HCP will share the results with the patient.

INSPECTION OF SALIVA SPECIMENS RECEIVED AT PHOSPHORUS DIAGNOSTICS ORDESIGNATED TESTING LABORATORIES:

Saliva specimens collected with Pinpoint by Phosphorus COVID-19 Test Home Collection Kit must be checked for the following criteria upon receipt at Phosphorus Diagnostics or designated testing laboratories prior to processing as outlined in the "COVID-19 Sample Accessioning for Phosphorus COVID-19 RT-qPCR Test" accessioning SOP:

- Saliva must be specifically collected in the OGD-510 Oragene Dx collection device.
- Sample collection tube must be intact and not visibly damaged or leaking.
- Sample volume meets the minimum required for testing.
- Specimen should not appear turbid or show signs of apparent bacterial contamination.
- Specimen must arrive within the established stability window for testing (i.e., within 56 hours from collection time).
- Specimen was collected using an unexpired collection kit.
- Specimen must bear patient's unique identifier (name, date of birth) along with the date/time of specimen collection on the provided label stickers (e.g., attached at the bottom of the pre-populated test requisition form).

REAGENTS AND MATERIALS

Table 2. Reagents and Materials Required for Use with the Phosphorus COVID-19 RT-qPCR Test

Reagent	Manufacturer	Catalogue #
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	ThermoFisher Scientific	A42352, A48310
Promega Maxwell HT Viral TNA Kit	Promega	AX2340
Promega Maxwell RSC Viral TNA Kit	Promega	AS1330
TaqPath 1-Step Multiplex Master Mix (No ROX)	ThermoFisher Scientific	A28523, A28522, A28521
2019-nCoV CDC EUA Kit, 500 rxn	Integrated DNA Technologies	10006606
2019-nCoV_N_Positive Control	Integrated DNA Technologies	10006625
Hs_RPP30 Positive Control	Integrated DNA Technologies	10006626
384-Well PCR plate	BioRad	HSP3865
BioRad microseal C optical adhesive film	BioRad	MSC1001
96-well MicroAmp reaction plate	ThermoFisher Scientific	N8010560
Pure Ethyl Alcohol, 200 Proof	Sigma Aldrich	E7023-500ML
2-Propanol, 99.5%	Sigma Aldrich	I9516-500ML
Growcells Nuclease Free Water	Fisher Scientific	50-103-4778

CONTROLS TO BE USED WITH THE PHOSPHORUS COVID-19 RT-qPCR TEST

The following controls described in Table 3 are used with the Phosphorus COVID-19 RT-qPCR Test.

Table 3. Function and Testing Frequency of the Phosphorus COVID-19 RT-qPCR Test Controls

Control Type	Purpose	Frequency of Testing
Positive Control (2019-nCoV_N_Positive Control)	To monitor the integrity of the RT-PCR reagents and process	Once per run of RT-qPCR
Internal Control (Hs_RPP30)	To monitor the integrity of nucleic acid extraction and RT-PCR for each specimen	Added to each specimen and the Negative Control prior to extraction; also run on its own in every RT-qPCR plate
No Template Control (NTC)	To monitor for contamination of extraction and assay reagents	Once per run of RT-qPCR
Negative Extraction Control (NEC)	To monitor for cross-contamination during RNA extraction and RT-PCR	Once per batch of specimens

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 4 for a summary of control results).

1) Phosphorus COVID-19 RT-qPCR Test Controls – Positive, Internal, Negative, and Extraction:

- **External Positive Control (2019-nCoV_N_Positive Control);** Positive control reactions for the N1 and N2 assays must yield positive results with a Ct value < 40 and negative results for the RP target (Ct Not detected). Negative results with either N1 or N2 primer/probe sets invalidate the run and suggests the assay may have been set up incorrectly, or the integrity of the primers/probes is compromised. The RT-PCR run is invalid. Repeat from the RT-PCR step using residual extraction material. If the repeat test result is negative for SARS-CoV-2 targets, re-extract and re-test all samples.

- **Internal Control (Hs_RPP30);** RNase P (RP) reactions must yield negative results with the N1 and N2 assays, and a positive result for the RP target with a Ct value < 40. Detection of RP serves as a positive extraction control for each patient test sample in the run. Failure of a patient sample to yield an RP Ct value < 40 may indicate improper extraction of nucleic acid or carry-over of PCR inhibitors. If the internal control does not meet acceptability criteria, the user is instructed to repeat the RT-PCR using residual extracted nucleic acid.

- **No Template Control (NTC);** The negative control is molecular grade, nuclease-free water and must be negative (Not Detected) for all SARS-CoV-2 specific targets and the RP control for the test result to be valid.

- **Negative Extraction Control (NEC);** NEC reactions must yield negative results with the N1 and N2 targets, and a positive result with the RP target with a Ct value < 40. If positive results occur in the N1 or N2 reaction wells with the NEC control, contamination of nucleic acid extraction reagents or cross-contamination of samples may have occurred. The extraction run and the RT-PCR run are invalid and should be repeated using residual patient sample.

Table 4. Expected Results of Controls Used in the Phosphorus COVID-19 RT-qPCR Test

Control	Expected N1 Result	Expected N2 Result	Expected RP Result
2019-nCoV_N_Positive Control	Ct < 40	Ct < 40	Not Detected
Internal Control (Hs_RPP30)	Not Detected	Not Detected	Ct < 40
No Template Control (NTC)	Not Detected	Not Detected	Not Detected
Negative Extraction Control (NEC)	Not Detected	Not Detected	Ct < 40

2) Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive, negative, extraction, and internal 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 5) for guidance on interpretation and reporting of results.

Table 5. Interpretation for Patient Results Using the Phosphorus COVID-19 RT-qPCR Test

N1	N2	RNase P	Results Interpretation	Report	Actions
+	+	+/-	SARS-CoV-2 detected	Positive	Report results to sender and appropriate public health authorities.
If only one of the two targets is positive		+/-	Inconclusive results	Inconclusive	Repeat once with residual extracted material. If the repeated result remains inconclusive, report as inconclusive, and recommend resubmission of a new sample, if there is still clinical indication.
-	-	+	SARS-CoV-2 not detected	Negative	Report results to sender and appropriate public health authorities. Consider testing for other respiratory viruses.
-	-	-	Invalid results	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

+ (positive); Ct < 40
− (negative); Ct Not Detected

PERFORMANCE EVALUATION

1) Analytical Sensitivity

The analytical sensitivity of the Phosphorus COVID-19 RT-qPCR Test was evaluated through a preliminary range finding determination of the assay's LoD, followed by confirmation of the preliminary LoD using 20 extraction replicates for each RNA extraction methodology including:

- MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher Scientific)
- Promega Maxwell HT Viral TNA Kit (Promega)
- Maxwell RSC TNA Viral Kit performed on the Maxwell RSC 48 System (Promega)

LoD studies were completed for both nasopharyngeal swabs and saliva collected in the OGD-510 device using two different sources of SARS-CoV-2 target, as described below.

a. Nasopharyngeal Swab LoD Determined Using Synthetic SARS-CoV-2 RNA

Leftover, pooled negative nasopharyngeal swab matrix was spiked with Twist Bioscience synthetic SARS-CoV-2 RNA (MT007544.1) (Twist Bioscience, Cat #102019). For the preliminary determination of the LoD, the Twist viral synthetic RNA was diluted to a starting concentration of 10,000 copies/µL and then spiked into NP swab matrix at the following concentrations, in copies/µL: 1000, 500, 200, 100, 50, 10, 5 and 2.5. Prepared samples at varying dilutions were extracted using each of the three extraction kits mentioned previously and tested in triplicate with Phosphorus COVID-19 RT-qPCR Test. The preliminary LoD was 5 copies/µL for all of the extraction methods based on the triplicate performance at each concentration level including standard deviation and mean

Ct (Table 6).

Table 6. Preliminary LoD Determination Results for Nasopharyngeal Swabs Using 3 Extraction Kits

Concentration (copies/µL)	N1			N2			RNase P		
	Detection Rate (%)	Mean Ct	SD	Detection Rate (%)	Mean Ct	SD	Detection Rate (%)	Mean Ct	SD
MagMAX Viral/Pathogen Nucleic Acid isolation Kit (Manual)									
1000	3/3 (100%)	30.40	0.1	3/3 (100%)	31.19	0.1	3/3 (100%)	26.69	0.1
500	3/3 (100%)	31.37	0.2	3/3 (100%)	32.27	0.04	3/3 (100%)	26.66	0.05
200	3/3 (100%)	33.22	0.5	3/3 (100%)	33.87	0.5	3/3 (100%)	29.16	0.23
100	3/3 (100%)	34.01	0.3	3/3 (100%)	34.84	0.2	3/3 (100%)	29.65	0.2
50	3/3 (100%)	35.37	0.3	3/3 (100%)	35.73	0.2	3/3 (100%)	29.96	0.1
10	3/3 (100%)	36.24	1.3	3/3 (100%)	38.08	0.3	3/3 (100%)	31.16	0.02
5	3/3 (100%)	37.82	0.6	3/3 (100%)	38.45	0.7	3/3 (100%)	31.52	0.2
2.5	1/3 (33.3%)	38.21	NA	0/3 (0%)	NA	NA	3/3 (100%)	29.51	0.1
Concentration (copies/µL)	**N1**			**N2**			**RNase P**		
	Detection Rate (%)	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**
Promega Maxwell HT Viral TNA Kit (Manual)									
1000	3/3 (100%)	31.55	0.1	3/3 (100%)	36.24	0.2	3/3 (100%)	27.65	0.2
500	3/3 (100%)	32.80	0.2	3/3 (100%)	33.55	0.2	3/3 (100%)	27.85	0.1
200	3/3 (100%)	33.80	0.3	3/3 (100%)	34.85	0.4	3/3 (100%)	29.81	0.1
100	3/3 (100%)	34.38	0.1	3/3 (100%)	35.62	0.4	3/3 (100%)	30.34	0.1
50	3/3 (100%)	35.60	0.6	3/3 (100%)	37.19	0.8	3/3 (100%)	30.45	0.1
10	3/3 (100%)	37.40	0.4	3/3 (100%)	39.10	0.8	3/3 (100%)	31.83	0.1
5	3/3 (100%)	38.14	0.1	3/3 (100%)	38.94	0.1	3/3 (100%)	31.50	0.1
2.5	1/3 (33.3%)	38.13	NA	0/3 (0%)	NA	NA	3/3 (100%)	30.21	0.1
Concentration (copies/µL)	**N1**			**N2**			**RNase P**		
	Detection Rate (%)	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**
Maxwell RSC TNA Viral Kit (Automated - Maxwell RSC 48 System)									
500	3/3 (100%)	31.03	0.1	3/3 (100%)	32.09	0.2	3/3 (100%)	26.50	0.2
200	3/3 (100%)	32.49	0.2	3/3 (100%)	33.58	0.04	3/3 (100%)	28.06	0.2
100	3/3 (100%)	33.15	0.3	3/3 (100%)	34.66	0.5	3/3 (100%)	28.47	0.3
50	3/3 (100%)	33.79	0.7	3/3 (100%)	34.99	0.6	3/3 (100%)	28.48	0.2
10	3/3 (100%)	36.85	0.6	3/3 (100%)	37.90	0.3	3/3 (100%)	29.83	0.2
5	3/3 (100%)	36.77	0.8	3/3 (100%)	37.53	0.5	3/3 (100%)	30.32	0.2
2.5	1/3 (33.3%)	38.24	NA	2/3 (66.7%)	39.34	NA	3/3 (100%)	28.24	0.02

SD; Standard Deviation of Ct values
NA; Not Applicable

The preliminary LoD of 5 copies/µL for nasopharyngeal swabs was confirmed with twenty individual extraction replicates using each claimed extraction kit/platform (Table 7).

Table 7. Confirmatory LoD study for Nasopharyngeal Swab Specimens Using 5 copies/µL of RNA, Stratified by Each RNA Extraction Method

RNA Extraction	Mean Ct	Detection Rate

Method	(Standard Deviation)			(N Detected/N Total)		
	N1	**N2**	**RNase P**	**N1**	**N2**	**RNase P**
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	37.71 (0.7)	38.50 (0.8)	31.39 (0.2)	100% (20/20)	100% (20/20)	100% (20/20)
Promega Maxwell HT Viral TNA Kit	37.95 (0.6)	38.56 (0.7)	31.77 (0.4)	100% (20/20)	100% (20/20)	100% (20/20)
Maxwell RSC TNA Viral Kit run on Maxwell RSC 48 System	38.03 (1.0)	38.69 (0.9)	30.20 (0.2)	100% (20/20)	100% (20/20)	100% (20/20)

b. Saliva LoD Determined Using Synthetic SARS-CoV-2 RNA

To validate the use of saliva as an acceptable specimen type, an LoD study was completed using pooled SARS-CoV-2 negative saliva that was self-collected without supervision in the OGD-510 device following the Oragene Dx collection instructions. All donor saliva samples were screened negative for SARS-CoV-2 using the Phosphorus COVID-19 RT-qPCR Test. Twist Bioscience synthetic SARS-CoV-2 RNA was spiked into negative human saliva at the same concentrations tested for NP swabs (1000-2.5 copies/µL). Prepared samples at varying dilutions were extracted using each of the three extraction kits mentioned previously and tested in triplicate with the Phosphorus COVID-19 RT-qPCR Test. The preliminary LoD was 5 copies/µL for all of the extraction methods (See Table 8) and was confirmed using 20 additional extraction replicates prepared with each claimed extraction kit/platform (See Table 9).

Results showed that manual RNA extractions using both the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher Scientific) and the Maxwell HT Viral TNA Kit (Promega) yielded 100% detection (20/20) for both N1 and N2 targets, whereas the automated RNA extraction using Maxwell RSC TNA Viral Kit (Promega) run on Maxwell RSC 48 System (Promega) yielded 95% detection (19/20) for N1 and 100% detection for the N2 target (Table 8). The data demonstrated that the LoDs for NP swabs and saliva were equivalent.

Table 8. Preliminary LoD Determination Results for Saliva in OGD-510 Stabilization Buffer Using 3 Extraction Kits

Concentration (copies/µL)	N1			N2			RNase P		
	Detection Rate (%)	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**
MagMAX Viral/Pathogen Nucleic Acid isolation Kit (Manual)									
1000	3/3 (100%)	26.38	0.01	3/3 (100%)	27.08	0.02	3/3 (100%)	22.98	0.08
500	3/3 (100%)	27.16	0.06	3/3 (100%)	27.96	0.08	3/3 (100%)	24.89	0.03
200	3/3 (100%)	28.85	0.09	3/3 (100%)	29.86	0.16	3/3 (100%)	23.21	0.05
100	3/3 (100%)	29.49	0.12	3/3 (100%)	30.64	0.19	3/3 (100%)	23.55	0.22
50	3/3 (100%)	30.78	0.09	3/3 (100%)	31.94	0.24	3/3 (100%)	22.96	0.13
10	3/3 (100%)	32.97	0.09	3/3 (100%)	33.74	0.12	3/3 (100%)	23.25	0.08
5	3/3 (100%)	33.47	0.26	3/3 (100%)	34.46	0.46	3/3 (100%)	24.91	0.07
2.5	2/3 (66.7%)	36.52	0.45	1/3 (33.3%)	37.98	NA	3/3 (100%)	21.78	0.04
Concentration (copies/µL)	**N1**			**N2**			**RNase P**		
	Detection Rate (%)	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**

Promega Maxwell HT Viral TNA Kit (Manual)									
1000	3/3 (100%)	26.97	0.09	3/3 (100%)	28.08	0.09	3/3 (100%)	23.27	0.09
500	3/3 (100%)	27.84	0.15	3/3 (100%)	28.38	0.20	3/3 (100%)	25.16	0.07
200	3/3 (100%)	29.51	0.15	3/3 (100%)	30.43	0.20	3/3 (100%)	23.35	0.11
100	3/3 (100%)	30.31	0.03	3/3 (100%)	31.06	0.16	3/3 (100%)	23.91	0.14
50	3/3 (100%)	31.52	0.13	3/3 (100%)	32.65	0.23	3/3 (100%)	22.79	0.18
10	3/3 (100%)	33.46	0.08	3/3 (100%)	34.79	0.40	3/3 (100%)	23.35	0.14
5	3/3 (100%)	33.92	0.53	3/3 (100%)	34.66	0.38	3/3 (100%)	25.13	0.03
2.5	2/3 (66.7%)	37.77	0.50	1/3 (33.3%)	38.55	NA	3/3 (100%)	24.10	0.04
Concentration (copies/µL)	**N1**			**N2**			**RNase P**		
	Detection Rate (%)	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**
Maxwell RSC TNA Viral Kit (Automated - Maxwell RSC 48 System)									
500	3/3 (100%)	29.30	0.08	3/3 (100%)	29.74	0.09	3/3 (100%)	22.11	0.12
200	3/3 (100%)	30.57	0.42	3/3 (100%)	31.31	0.38	3/3 (100%)	22.27	0.26
100	3/3 (100%)	30.87	0.21	3/3 (100%)	31.53	0.28	3/3 (100%)	21.86	0.20
50	3/3 (100%)	32.04	0.39	3/3 (100%)	32.42	0.30	3/3 (100%)	21.83	0.22
10	3/3 (100%)	34.94	0.13	3/3 (100%)	35.50	0.06	3/3 (100%)	21.31	0.20
5	3/3 (100%)	34.97	0.29	3/3 (100%)	35.30	0.43	3/3 (100%)	20.98	0.06
2.5	1/3 (33.3%)	37.23	0.56	1/3 (33.3%)	37.89	NA	3/3 (100%)	21.38	0.10

SD; Standard Deviation of Ct values
NA; Not Applicable

Table 9. Confirmatory LoD study for Saliva Specimens Using 5 copies/µL of RNA, Stratified by RNA Extraction Method

RNA Extraction Method	**Mean Ct (Standard Deviation)**			**Detection Rate (N Detected/N Total)**		
	N1	**N2**	**RNase P**	**N1**	**N2**	**RNase P**
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	34.87 (0.7)	35.50 (0.7)	23.40 (0.5)	100% (20/20)	100% (20/20)	100% (20/20)
Promega Maxwell HT Viral TNA Kit	35.36 (0.9)	36.89 (0.9)	22.52 (0.4)	100% (20/20)	100% (20/20)	100% (20/20)
Maxwell RSC TNA Viral Kit run on Maxwell RSC 48 System	35.57 (1)	36.95 (1.2)	22.58 (0.7)	95% (19/20)	100% (20/20)	100% (20/20)

c. Re-Establishment of Assay LoD Using Whole Inactivated SARS-CoV-2 in Negative NP Swab and Saliva Clinical Matrices

The LoD for the Phosphorus COVID-19 RT-qPCR Test was re-established using a dilution series of heat-inactivated SARS-CoV-2 (2019-nCoV/USA-WA1/2020 [ATCC, Cat #VR-1986HK]) spiked into either pooled, SARS-CoV-2 negative NP swab clinical matrix or saliva that was self-collected in the OGD-510 device. For the preliminary LoD determination, heat-inactivated whole SARS-CoV-2 was diluted to a starting concentration of 10,000 copies/µL and then spiked into negative NP swab and saliva clinical matrices at the following concentrations: 1000, 500, 200, 100, 50, 10, 5, 2.5, 2, 1, 0.5, and 0.25 copies/µL with three replicates per concentration. Each spiked replicate was processed through the entire assay, beginning with RNA extraction using each of the previously validated extraction kits shown in Table 10 (e.g., MagMax Viral/Pathogen Nucleic Acid Isolation kit [manual], Promega Maxwell HT Viral TNA Kit [manual], and

Maxwell RSC Viral TNA Kit [automated]) followed by testing with the Phosphorus COVID-19 RT-qPCR Test.

The preliminary LoD was defined as the lowest concentration at which 3/3 replicates produced positive results in accordance with the result reporting algorithm described in Table 5. The preliminary LoD for NP swab clinical matrix was 2.5 copies/µL for the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, and 1 copy/µL for both the Promega Maxwell HT Viral TNA Kit and Maxwell RSC TNA Viral Kit (See Table 10). The preliminary LoD for saliva clinical matrix was 1.0 copy/µL for all three extraction kits (See Table 11).

Table 10. Preliminary LoD Range Finding Study with NP Swab Clinical Matrix and Inactivated Whole SARS-CoV-2

Concentration (copies/µL)	N1			N2			RNase P		
	Detection Rate (%)	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**
MagMAX Viral/Pathogen Nucleic Acid isolation Kit (Manual)									
1000	3/3 (100%)	23.76	0.4	3/3 (100%)	23.50	0.3	3/3 (100%)	22.52	0.3
500	3/3 (100%)	25.86	0.3	3/3 (100%)	25.71	0.4	3/3 (100%)	23.22	0.3
200	3/3 (100%)	26.58	0.4	3/3 (100%)	26.46	0.5	3/3 (100%)	22.59	0.4
100	3/3 (100%)	27.33	0.1	3/3 (100%)	27.14	0.1	3/3 (100%)	22.29	0.1
50	3/3 (100%)	27.80	0.3	3/3 (100%)	27.56	0.3	3/3 (100%)	22.01	0.2
10	3/3 (100%)	30.29	0.1	3/3 (100%)	30.36	0.1	3/3 (100%)	22.30	0.2
5	3/3 (100%)	31.38	0.2	3/3 (100%)	30.93	0.2	3/3 (100%)	22.26	0.2
2.5	3/3 (100%)	31.33	0.5	3/3 (100%)	31.18	0.3	3/3 (100%)	22.08	0.04
2.0	1/3 (33.3%)	37.25	0.00	1/3 (33.3%)	36.74	0.0	3/3 (100%)	22.03	0.3
1.0	0/3 (0%)	NA	NA	1/3 (33.3%)	36.91	0.0	3/3 (100%)	22.35	0.2
0.5	0/3 (0%)	NA	NA	0/3 (0%)	NA	NA	3/3 (100%)	22.61	0.3
0.0	0/3 (0%)	NA	NA	0/3 (0%)	NA	NA	3/3 (100%)	22.03	0.2
Concentration (copies/µL)	**N1**			**N2**			**RNase P**		
	Detection Rate (%)	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**
Promega Maxwell HT Viral TNA Kit (Manual)									
1000	3/3 (100%)	22.93	0.1	3/3 (100%)	22.87	0.1	3/3 (100%)	24.30	0.2
500	3/3 (100%)	23.75	0.1	3/3 (100%)	23.66	0.1	3/3 (100%)	23.70	0.2
200	3/3 (100%)	25.15	0.2	3/3 (100%)	25.20	0.1	3/3 (100%)	23.58	0.4
100	3/3 (100%)	26.48	0.5	3/3 (100%)	26.47	0.6	3/3 (100%)	23.98	0.4
50	3/3 (100%)	28.35	0.2	3/3 (100%)	28.31	0.1	3/3 (100%)	24.42	0.1
10	3/3 (100%)	30.67	0.3	3/3 (100%)	30.59	0.4	3/3 (100%)	24.23	0.2
5	3/3 (100%)	30.22	0.4	3/3 (100%)	30.51	0.3	3/3 (100%)	24.24	0.1
2.5	3/3 (100%)	31.28	0.2	3/3 (100%)	31.24	0.1	3/3 (100%)	24.45	0.02
2.0	3/3 (100%)	33.92	0.4	3/3 (100%)	33.95	0.1	3/3 (100%)	24.21	0.1
1.0	3/3 (100%)	34.65	0.8	3/3 (100%)	35.77	0.5	3/3 (100%)	24.19	0.1
0.5	2/3 (66.7%)	35.48	0.5	3/3 (100%)	36.71	0.7	3/3 (100%)	23.37	0.1
0.0	0/3 (0%)	NA	NA	0/3 (0%)	N/A	NA	3/3 (100%)	23.38	0.1
Concentration (copies/µL)	**N1**			**N2**			**RNase P**		
	Detection Rate (%)	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**
Maxwell RSC TNA Viral Kit (Automated - Maxwell RSC 48 System)									
1000	3/3 (100%)	22.33	0.1	3/3 (100%)	22.36	0.2	3/3 (100%)	23.49	0.1

500	3/3 (100%)	23.52	0.1	3/3 (100%)	23.73	0.03	3/3 (100%)	24.32	1.6
200	3/3 (100%)	24.67	0.1	3/3 (100%)	24.68	0.1	3/3 (100%)	23.13	0.1
100	3/3 (100%)	25.56	0.1	3/3 (100%)	25.80	0.2	3/3 (100%)	23.26	0.1
50	3/3 (100%)	26.75	0.4	3/3 (100%)	26.71	0.2	3/3 (100%)	23.09	0.1
10	3/3 (100%)	28.84	0.2	3/3 (100%)	28.91	0.1	3/3 (100%)	23.02	0.1
5	3/3 (100%)	28.69	0.1	3/3 (100%)	28.82	0.3	3/3 (100%)	22.96	0.05
2.5	3/3 (100%)	29.72	0.1	3/3 (100%)	29.75	0.3	3/3 (100%)	22.95	0.1
2.0	3/3 (100%)	34.21	0.1	3/3 (100%)	34.27	0.2	3/3 (100%)	24.60	0.1
1.0	3/3 (100%)	35.88	0.8	3/3 (100%)	35.08	0.2	3/3 (100%)	24.57	0.04
0.5	3/3 (100%)	36.67	0.6	2/3 (66.7%)	37.82	2.0	3/3 (100%)	23.95	0.1
0.25	0/3 (0%)	NA	NA	0/3 (0%)	N/A	NA	3/3 (100%)	22.43	0.1
0.0	0/3 (0%)	NA	NA	0/3 (0%)	N/A	NA	3/3 (100%)	23.91	0.1

SD; Standard Deviation of Ct Values
NA; Not Applicable

Table 11. Preliminary LoD Range Finding Study With Saliva in OGD-510 Stabilization Buffer and Inactivated SARS-CoV-2

Concentration (copies/μL)	**N1**			**N2**			**RNase P**		
	Detection Rate (%)	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**
MagMAX Viral/Pathogen Nucleic Acid isolation Kit (Manual)									
1000	3/3 (100%)	22.54	0.1	3/3 (100%)	23.08	0.1	3/3 (100%)	20.55	0.1
500	3/3 (100%)	23.72	0.3	3/3 (100%)	23.83	0.1	3/3 (100%)	20.06	0.02
200	3/3 (100%)	24.83	0.2	3/3 (100%)	24.96	0.1	3/3 (100%)	19.95	0.03
100	3/3 (100%)	25.38	0.1	3/3 (100%)	25.71	0.04	3/3 (100%)	19.94	0.1
50	3/3 (100%)	26.65	0.1	3/3 (100%)	27.19	0.1	3/3 (100%)	20.17	0.02
10	3/3 (100%)	29.48	0.3	3/3 (100%)	29.64	0.1	3/3 (100%)	20.25	0.1
5	3/3 (100%)	29.97	0.1	3/3 (100%)	30.17	0.2	3/3 (100%)	20.04	0.02
2.5	3/3 (100%)	30.41	0.5	3/3 (100%)	30.90	0.6	3/3 (100%)	20.73	0.9
2.0	3/3 (100%)	32.71	0.2	3/3 (100%)	33.54	0.2	3/3 (100%)	20.26	0.1
1.0	3/3 (100%)	33.83	0.4	3/3 (100%)	34.21	0.5	3/3 (100%)	20.21	0.1
0.5	2/3 (100%)	37.63	2.0	3/3 (100%)	35.42	1.1	3/3 (100%)	20.82	0.9
0.25	3/3 (100%)	35.78	0.7	2/3 (66.7%)	36.55	0.3	3/3 (100%)	20.33	0.2
0.0	0/3 (0%)	NA	NA	0/3 (0%)	NA	NA	3/3 (100%)	19.72	0.3
Concentration (copies/μL)	**N1**			**N2**			**RNase P**		
	Detection Rate (%)	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**	**Detection Rate (%)**	**Mean Ct**	**SD**
Promega Maxwell HT Viral TNA Kit (Manual)									
1000	3/3 (100%)	22.92	0.3	3/3 (100%)	23.58	0.4	3/3 (100%)	20.06	0.2
500	3/3 (100%)	23.92	0.3	3/3 (100%)	24.49	0.3	3/3 (100%)	20.34	0.3
200	3/3 (100%)	24.55	0.2	3/3 (100%)	25.14	0.2	3/3 (100%)	20.91	0.3
100	3/3 (100%)	25.94	0.1	3/3 (100%)	26.74	0.1	3/3 (100%)	20.86	0.2
50	3/3 (100%)	26.43	0.04	3/3 (100%)	27.09	0.01	3/3 (100%)	21.05	0.02
10	3/3 (100%)	28.78	0.1	3/3 (100%)	29.34	0.02	3/3 (100%)	21.04	0.02
5	3/3 (100%)	29.12	0.1	3/3 (100%)	29.68	0.2	3/3 (100%)	21.03	0.1
2.5	3/3 (100%)	29.83	0.1	3/3 (100%)	30.53	0.3	3/3 (100%)	21.34	0.3
2.0	3/3 (100%)	33.56	0.2	3/3 (100%)	34.23	0.3	3/3 (100%)	22.26	0.1
1.0	3/3 (100%)	34.49	0.5	3/3 (100%)	34.84	0.4	3/3 (100%)	22.16	0.1
0.5	3/3 (100%)	35.68	0.2	2/3 (66.7%)	38.93	1.4	3/3 (100%)	21.30	0.1
0.25	2/3 (66.7%)	38.44	0.4	0/3 (0%)	43.08	0.3	3/3 (100%)	22.86	0.1
0.0	0/3 (0%)	NA	NA	0/3 (0%)	NA	NA	3/3 (100%)	20.12	0.04

Concentration (copies/µL)	N1			N2			RNase P		
	Detection Rate (%)	Mean Ct	SD	Detection Rate (%)	Mean Ct	SD	Detection Rate (%)	Mean Ct	SD
Maxwell RSC TNA Viral Kit (Automated - Maxwell RSC 48 System)									
1000	3/3 (100%)	26.08	0.9	3/3 (100%)	26.12	1.0	3/3 (100%)	22.64	0.1
500	3/3 (100%0	24.27	0.1	3/3 (100%)	24.43	0.2	3/3 (100%)	21.46	0.2
200	3/3 (100%)	25.35	0.1	3/3 (100%)	25.47	0.2	3/3 (100%)	21.29	0.1
100	3/3 (100%)	26.39	0.1	3/3 (100%)	26.68	0.1	3/3 (100%)	21.40	0.1
50	3/3 (100%)	27.35	0.1	3/3 (100%)	27.76	0.02	3/3 (100%)	21.29	0.1
10	3/3 (100%)	29.35	0.2	3/3 (100%)	29.59	0.1	3/3 (100%)	21.12	0.02
5	3/3 (100%)	31.02	0.2	3/3 (100%)	30.95	0.1	3/3 (100%)	21.36	0.1
2.5	3/3 (100%)	32.37	0.3	3/3 (100%)	32.48	0.3	3/3 (100%)	21.64	0.1
2.0	3/3 (100%)	34.50	0.1	3/3 (100%)	35.83	0.2	3/3 (100%)	23.40	0.1
1.0	3/3 (100%)	35.39	0.2	3/3 (100%)	36.41	0.6	3/3 (100%)	23.37	0.1
0.5	3/3 (100%)	36.94	0.5	1/3 (33.3%)	39.51	1.6	3/3 (100%)	21.97	0.1
0.0	0/3 (0%)	NA	NA	0/3 (0%)	NA	NA	3/3 (100%)	22.74	0.6

SD; Standard Deviation of Ct Values
NA; Not Applicable

A confirmatory LoD study was performed with both NP swab clinical matrix and saliva collected in the OGD-510 device by testing 20 independent replicates that were extracted with each of the three previously validated methods. Samples consisted of heat-inactivated whole SARS-CoV-2 spiked into negative NP swab or saliva matrix based on the preliminary LoD target levels. The Phosphorus COVID-19 RT-qPCR Test was shown to detect >95% of replicates at 5 copies/µL of heat-inactivated whole SARS-CoV-2 in NP swab matrix when extracted with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit and 1.0 copy/µL when extracted with both the Promega Maxwell GT Viral TNA Kit and Maxwell RSC TNA Viral Kit (See Table 12). The LoD of the Phosphorus COVID-19 RT-qPCR Test in saliva was determined to be 1.0 copy/µL for all three claimed extraction methods (See Table 13).

Table 12. Confirmatory LoD Study Results for NP Swab Matrix

RNA Extraction Method	Concentration (copies/µL)	Mean Ct (Standard Deviation)			Detection Rate (N Detected/N Total)		
		N1	N2	RNase P	N1	N2	RNase P
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	5.0	32.39 (0.62)	33.46 (0.59)	25.40 (0.33)	100% (20/20)	100% (20/20)	100% (20/20)
Promega Maxwell HT Viral TNA Kit	1.0	33.56 (0.45)	35.68 (1.18)	25.89 (0.21)	100% (20/20)	100% (20/20)	100% (20/20)
Maxwell RSC TNA Viral Kit run on Maxwell RSC 48 System	1.0	34.26 (0.33)	35.58 (0.56)	26.36 (0.13)	100% (20/20)	100% (20/20)	100% (20/20)

Table 13. Confirmatory LoD Study Results for Saliva Matrix in OGD-510 Stabilization Buffer

RNA Extraction Method	Concentration (copies/µL)	Mean Ct (Standard Deviation)			Detection Rate (N Detected/N Total)		
		N1	N2	RNase P	N1	N2	RNase P
MagMAX	1.0	34.98	35.47	22.70	100%	100%	100%

Viral/Pathogen Nucleic Acid Isolation Kit		(0.58)	(0.39)	(0.33)	(20/20)	(20/20)	(20/20)
Promega Maxwell HT Viral TNA Kit	1.0	33.24 (0.60)	33.67 (0.42)	21.46 (0.12)	100% (20/20)	100% (20/20)	100% (20/20)
Maxwell RSC TNA Viral Kit run on Maxwell RSC 48 System	1.0	34.39 (0.57)	35.12 (0.51)	19.98 (0.09)	100% (20/20)	100% (20/20)	100% (20/20)

Although the LoD of the Phosphorus COVID-19 RT-qPCR Test as determined using inactivated virus differed between the different extraction methods, clinical evaluation with NP swabs in comparison to another FDA-authorized test among patients suspected of COVID-19 described in Section 3(a), below, demonstrated acceptable performance using all three methods. Similar performance with all three extraction methods was also demonstrated with saliva samples collected from patients suspected of COVID-19 (Section 3(a)) and asymptomatic subjects (See Section 4 below). The three extraction methods may therefore be used interchangeably with the Phosphorus COVID-19 RT-qPCR Test.

2) Analytical Reactivity and Specificity

a. *In silico* Inclusivity Assessment

The Phosphorus COVID-19 RT-qPCR Test is a modification of the previously authorized CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. The inclusivity and cross-reactivity of the CDC EUA was previously evaluated (EUA200001). CDC has provided a right of reference to utilize the *in silico* analytical reactivity and specificity study data. Phosphorus completed an additional *in silico* inclusivity study on April 26, 2021by aligning the N1 and N2 primer/probe sequences against SARS-CoV-2 sequences available at GISAID. Of the 1,192,087 GISAID sequences, 1,153,368 (96.75%) exhibited 100% identity to both the N1 and N2 oligonucleotides. Of the remaining 38,719 mismatched sequences 38,247 (98.8%) exhibited 100% homology with either the N2 or N1 oligonucleotides and are thus likely to be detected. The remaining 472 sequences (0.04% of the total) were subjected to additional analysis to assess their potential effect on assay performance.

A total of 154 sequences (0.013%) shared common mutations which consisted of 1 base substitution 8 bp from the 5′ end of the N1 reverse primer and 7 bp from the 5′ end of the N2 probe. Additionally, the remaining 318 sequences all showed single nucleotide mismatches within the N1 reverse primer and/or the N1 and N2 probes. A summary of the single nucleotide mismatches including the location and frequency of the mismatches is shown in Table 14.

Table 14. Location and Frequency of Single Nucleotide Mismatches

Variant ID #	Number of Sequences	Location	Variant ID #	Number of Sequences	Location
1	154	N1 reverse 8bp from 5’ end	32	1	N1 probe 4 bp from 5’ end
		N2 probe 7 bp from 5’ end			N2 probe 7 bp from 5’ end
2	90	N1 reverse 10 bp from 5’ end	33	1	N1 probe 5 bp from 3’ end
		N2 probe 7 bp from 5’ end			N2 probe 10 bp from 5’ end
3	21	N2 reverse 2 bp from 3’ end	34	2	N1 probe 5 bp from 5’ end

Variant ID #	Number of Sequences	Location	Variant ID #	Number of Sequences	Location
		N2 probe 7 bp from 5’ end			N2 probe 10 bp from 5’ end
4	17	N1 reverse 9 bp from 3’ end	35	2	N1 probe 5 bp from 5’ end
		N2 probe 10 bp from 5’ end			N2 probe 13 bp from 5’ end
5	14	N1 reverse 3 bp from 5’ end	36	1	N1 probe 5 bp from 5’ end
		N2 probe 10 bp from 5’ end			N2 probe 7 bp from 5’ end
6	14	N1 probe 3 bp from 5’ end	37	1	N1 probe 6 bp from 5’ end
		N2 probe on first bp on 5’ end			N2 probe 10 bp from 5’ end
7	12	N1 probe 4 bp from 3’ end	38	2	N1 probe 7 bp from 3’ end
		N2 probe 10 bp from 5’ end			N2 probe 10 bp from 5’ end
8	1	N1 reverse on first bp on 5’ end	39	2	N1 probe 7 bp from 3’ end
		N2 probe 10 bp from 5’ end			N2 probe 13 bp from 5’ end
9	3	N1 probe 12 bp from 5’ end	40	2	N1 probe 7 bp from 5’ end
		N2 probe 10 bp from 5’ end			N2 probe 10 bp from 5’ end
10	1	N1 probe 12 bp from 5’ end	41	1	N1 probe 7 bp from 5’ end
		N2 probe 13 bp from 5’ end			N2 probe 8 bp from 5’ end
11	11	N1 probe 12 bp from 5’ end	42	1	N1 probe 7 bp from 5’ end
		N2 probe 7 bp from 5’ end			N2 probe on first bp on 5’ end
12	6	N1 probe 13 bp from 5’ end	43	3	N1 reverse 10 bp from 5’ end
		N2 probe 10 bp from 5’ end			N2 probe 10 bp from 5’ end
13	10	N1 probe 13 bp from 5’ end	44	6	N1 reverse 10 bp from 5’ end
		N2 probe 13 bp from 5’ end			N2 probe 3 bp from 5’ end
14	1	N1 probe 13 bp from 5’ end	45	1	N1 reverse 10 bp from 5’ end
		N2 probe on first bp on 3’ end			N2 probe 9 bp from 3’ end
15	8	N1 probe 2 bp from 5’ end	46	1	N1 reverse 11 bp from 5’ end
		N2 probe 10 bp from 5’ end			N2 probe 10 bp from 5’ end
16	2	N1 probe 2 bp from 5’ end	47	5	N1 reverse 12 bp from 5’ end
		N2 probe 13 bp from 5’ end			N2 probe 10 bp from 5’ end
17	1	N1 probe 2 bp from 5’ end	48	1	N1 reverse 12 bp from 5’ end
		N2 probe 2 bp from 3’ end			N2 probe 8 bp from 5’ end
18	3	N1 probe 2 bp from 5’ end	49	1	N1 reverse 13 bp from 5’ end
		N2 probe 7 bp from 5’ end			N2 probe 10 bp from 5’ end
19	3	N1 probe 2 bp from 5’ end	50	1	N1 reverse 14 bp from 5’ end
		N2 probe 8 bp from 5’ end			N2 probe 10 bp from 5’ end
20	4	N1 probe 2 bp from 5’ end	51	1	N1 reverse 14 bp from 5’ end
		N2 probe on first bp on 5’ end			N2 probe 13 bp from 5’ end
21	1	N1 probe 3 bp from 5’ end	52	3	N1 reverse 2 bp from 3’ end
		N2 probe 7 bp from 5’ end			N2 probe 10 bp from 5’ end
22	1	N1 probe 3 bp from 5’ end	53	2	N1 reverse 2 bp from 5’ end
		N2 probe 10 bp from 3’ end			N2 probe 13 bp from 5’ end
23	6	N1 probe 3 bp from 5’ end	54	1	N1 reverse 2 bp from 5’ end
		N2 probe 10 bp from 5’ end			N2 probe 7 bp from 5’ end
24	8	N1 probe 3 bp from 5’ end	55	1	N1 reverse 3 bp from 5’ end
		N2 probe 13 bp from 5’ end			N2 probe 13 bp from 5’ end
25	4	N1 probe 3 bp from 5’ end	56	3	N1 reverse 4 bp from 5’ end
		N2 probe 3 bp from 5’ end			N2 probe 10 bp from 5’ end
26	2	N1 probe 3 bp from 5’ end	57	1	N1 reverse 4 bp from 5’ end
		N2 probe 5 bp from 3’ end			N2 probe 13 bp from 5’ end
27	4	N1 probe 3 bp from 5’ end	58	2	N1 reverse 6 bp from 5’ end
		N2 probe 7 bp from 5’ end			N2 probe 10 bp from 5’ end
28	1	N1 probe 3 bp from 5’ end	59	1	N1 reverse 8 bp from 5’ end
		N2 probe 8 bp from 5’ end			N2 probe 10 bp from 5’ end
29	1	N1 probe 4 bp from 3’ end	60	2	N1 reverse 9 bp from 3’ end
		N2 probe 7 bp from 5’ end			N2 probe 13 bp from 5’ end
30	10	N1 probe 4 bp from 5’ end	61	2	N1 reverse on first bp on 3’ end
		N2 probe 10 bp from 5’ end			N2 probe 10 bp from 5’ end
31	2	N1 probe 4 bp from 5’ end			
		N2 probe 13 bp from 5’ end			

To evaluate the impact that the mismatches could have on primer binding, melt temperature (Tm) analyses of the mismatched bases were completed using thermodynamic modeling tools such as MFEprimer (mfeprimer3.igenetech.com) and OligoAnalyzer Tool (www.idtdna.com). None of the mismatched sequences are predicted to impact hybridization of the assay's primers and probes. However, there was an A to G substitution on the first base pair of the 5′ end of the N2 probe for 14 sequences (0.0011% of all sequences) that may quench the 5' fluorophore, resulting in reduced analytical sensitivity. Considering the very low frequency of this mismatch, it is not expected to affect clinical sensitivity.

An analysis against the main SARS-CoV-2 variants of concern was also performed on April 26, 2021. Included in the analysis were variants with sequences available in the NCBI and GISAID databases: B.1.1.7 (450,534 sequences), P.1 (6,839 sequences), B.1.351 (12,002 sequences), B.1.427 and B.1.429 (32,721 sequences). A summary of the predicted assay inclusivity for the prominent SARS-CoV-2 variants that were in circulation at the time of the analysis is shown in Table 15. *In silico* analyses demonstrated that the majority of the variants of potential public health importance are predicted to be detected by the Phosphorus COVID-19 RT-qPCR Test. The N1 and N2 primers and probes exhibited 100% homology with 99.98% of the B.1.427 and B.1.429 variant sequences.

Table 15. Alignment of Assay Oligonucleotides Against Circulating SARS-CoV-2 Variants

Oligo	**Variant**	**N1 Target**		**N2 Target**		**Mismatches Identified in N1 and/or N2 Assays**
		# of Sequences Analyzed	**Inclusivity %***	**# of Sequences Analyzed**	**Inclusivity %***	
Forward	B.1.1.7	450,534	450,534/450,534 100%	450,534	450,534/450,534 100%	None
	P.1	6,839	6,839/6,839 100%	6,839	6,839/6,839 100%	None
	B.1.351	12,002	12,002/12,002 100%	12,002	12,002/12,002 100%	None
	B.1.427 &.1.429	32,721	32,721/32,721 100%	32,721	32,721/32,721 100%	None
Reverse	B.1.1.7	450,534	450,534/450,534 100%	450,534	450,534/450,534 100%	None
	P.1	6,839	6,839/6,839 100%	6,839	6,839/6,839 100%	None
	B.1.351	12,002	12,002/12,002 100%	12,002	12,002/12,002 100%	None
	B.1.427 &.1.429	32,721	32,721/32,721 100%	32,721	32,721/32,721 100%	None
Probe	B.1.1.7	450,534	450,534/450,534 100%	450,534	450,534/450,534 100%	None
	P.1	6,839	6,839/6,839 100%	6,839	6,839/6,839 100%	None
	B.1.351	12,002	12,002/12,002 100%	12,002	12,002/12,002 100%	None
	B.1.427 &.1.429	32,721	32,715/32,721 99.98%	32,721	32,715/32,721 99.98%	• N1 probe - 4 strains had a

						1 bp mismatch 2 bp from 5' end AND the N2 probe had a 1 bp mismatch on the first bp on the 3' end • N1 probe - 2 strains had a 1 bp mismatch 4 bp from 5' end AND the N2 probe had a 1 bp mismatch 13 bp from the 5' end

*Inclusivity is defined as 100% homology

b. Cross-Reactivity Wet Testing

The analytical specificity of the Phosphorus COVID-19 RT-qPCR Test was demonstrated *in silico* under the original EUA for the CDC authorized test. As stated previously, CDC has provided a right of reference for their *in silico* exclusivity data and therefore, additional *in silico* analyses to assess the potential for assay cross-reactivity were not necessary. Wet bench testing of the MERS (MERS_CoV control, Cat # 10006623) and SARS plasmid controls (SARS-CoV control, Cat # 10006624) from Integrated DNA Technologies was completed. The controls were spiked at 200 copies/µL and tested in triplicate using the Phosphorus Test. All results were negative and no cross-reactivity occurred.

3) Clinical Evaluation

a. Saliva (Paired NP Swab and Saliva Clinical Study from Patients Suspected of COVID-19)

A study was performed to evaluate the use of saliva as a specimen type for detection of SARS-CoV-2 in patients who were suspected of COVID-19. The study was conducted prospectively with patients presenting with signs and symptoms of COVID-19 at two different physician offices. Symptomatic patients at each site were each provided with instructions for self-collection of saliva using the Oragene Dx (OGD-510) collection device from DNA Genotek. Upon consenting and enrollment of the patient into the study, the healthcare provider collected the nasopharyngeal swab first. Within 15 minutes of NP swab collection, the patient independently self-collected the saliva sample under the observation of a healthcare provider, but without intervention, for parallel testing for SARS-CoV-2. Patients were not given the option for healthcare provider assistance. The swabs were collected and shipped to the testing laboratory using the BD Universal Viral Transport Standard Kit (Cat #220221). Both the saliva and swabs were transported at ambient temperature and tested using the Phosphorus COVID-19 RT-qPCR Test within

48 hours of collection. A total of 91 paired study samples (34 NP positive and 57 NP negative) were evaluated to establish the clinical performance of the assay when using self-collected saliva. A summary of the results of the paired study is presented in Tables 16-19 below. The NP swabs were also confirmed positive/negative using an FDA authorized molecular assay (See Tables 20-22 for qualitative NP study results).

There was 97.1% positive percent agreement (PPA) and 96.5% negative percent agreement (NPA), respectively between the results obtained from testing of saliva and those obtained from nasopharyngeal swab when extracted with the MagMAX kit. Of the 34 confirmed positive NP swab samples, 33 paired NP and saliva specimens produced positive results for the N1 and N2 genes (33/34; 97.1%); however, there was one false negative where the NP swab showed positive amplification (Ct < 40) but the saliva sample was negative. For the 57 confirmed NPS negatives, the Phosphorus COVID-19 RT-qPCR Test did generate two saliva positive results (both N1 and N2 targets had Ct values < 40). According to the testing algorithm described in Table 5 above, a sample is considered positive for SARS-CoV-2 RNA when amplification is detected with both the N1 and N2 targets. No discordant analyses or a root cause determination were completed.

When the paired clinical samples were extracted with the Promega Maxwell HT Viral TNA Kit (manual extraction), the PPA and NPA for the Phosphorus COVID-19 RT-qPCR Test was 97.1% and 98.2%, respectively. Of the 34 confirmed positive NP swab samples, 33 paired NP and saliva specimens produced positive results for the N1 and N2 targets (33/34; 97.1%); however, there was one negative where the NP swab showed positive amplification (Ct < 40) but the saliva sample was negative. For the 57 confirmed NPS negatives extracted with the Promega Maxwell kit, the Phosphorus COVID-19 RT-qPCR Test had one saliva positive result (both N1 and N2 had Ct < 40).

When the paired clinical samples were extracted with the automated Promega kit on the Maxwell RSC 48 System, 34/34 (100%) NP samples were positive for N1 and N2 but only 33/34 corresponding, paired saliva samples were positive with the Phosphorus COVID-19 RT-qPCR Test. There was one negative where the NP swab showed positive amplification (Ct < 40) but the saliva sample was negative. For the 57 confirmed NPS negatives, the Phosphorus COVID-19 RT-qPCR Test did generate two saliva positives (both N1 and N2 targets had Ct values <40).

Overall, the results of the clinical evaluation with paired nasopharyngeal swabs and saliva using the three extraction methods was considered acceptable. The same patient samples generated either 2 or 3 discordant results when comparing saliva to NP swab performance using the three validated extraction methods. Specimens extracted with the MagMAX kit and the MaxWell RSC automated system generated the same 3 discordant results; the Promega MaxWell HT Kit generated 2 of the 3 discordant results mentioned previously. Regardless, clinical performance of the saliva specimens appears to be comparable to NP swabs specimens in regard to detecting SARS-CoV-2 RNA.

Table 16. Summary of Results Obtained from Parallel Testing of Nasopharyngeal Swab Samples and Saliva from Patients Suspected of COVID-19, Stratified by Measurand and RNA Extraction Method

Number of Patients	Sample Type	Analysis	Target		
			N1	N2	RNase P
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit					
34 NP Positive	NP swab	Positive (%)	34 (100)	34 (100)	34 (100)
		Mean Ct (SD)	34.5 (5.0)	35.4 (5.2)	22.6 (2.0)
	Saliva	Positive (%)	33 (97.1)	33 (97.1)	33 (97.1)
		Mean Ct (SD)	33.2 (3.9)	34.4 (4.0)	23.1 (2.5)
57 NP Negative	NP swab	Positive (%)	0 (0)	0 (0)	57 (100)
		Mean Ct (SD)	N/A	N/A	26.5 (2.8)
	Saliva	Positive (%)	2 (3.5)	2 (3.5)	55 (96.5)
		Mean Ct (SD)	34.5 (3.5)	35.3 (2.6)	21.7 (1.0)
Promega Maxwell HT Viral TNA Kit					
34 NP Positive	NP swab	Positive (%)	34 (100)	34 (100)	34 (100)
		Mean Ct (SD)	34.8 (4.5)	35.7 (4.6)	24.0 (1.6)
	Saliva	Positive (%)	33 (97.1)	33 (97.1)	33 (97.1)
		Mean Ct (SD)	34.0 (2.7)	35.5 (2.9)	23.6 (1.9)
57 NP Negative	NP swab	Positive (%)	0 (0)	0 (0)	57 (100)
		Mean Ct (SD)	N/A	N/A	27.9 (2.6)
	Saliva	Positive (%)	1 (1.8)	1 (1.8)	56 (98.2)
		Mean Ct (SD)	32.6 (N/A)	33.6 (N/A)	23.0 (1.0)
Maxwell RSC TNA Viral Kit run on the Maxwell RSC 48 System					
34 NP Positive	NP swab	Positive (%)	34 (100)	34 (100)	34 (100)
		Mean Ct (SD)	34.6 (4.9)	35.5 (5.1)	22.8 (1.5)
	Saliva	Positive (%)	33 (97.1)	33 (97.1)	33 (97.1)
		Mean Ct (SD)	34.1 (2.6)	35.5 (2.7)	23.1 (1.9)
57 NP Negative	NP swab	Positive (%)	0 (0)	0 (0)	57 (100)
		Mean Ct (SD)	N/A	N/A	26.7 (2.5)
	Saliva	Positive (%)	2 (3.5)	2 (3.5)	55 (96.5)
		Mean Ct (SD)	35.0 (2.7)	36.7 (2.7)	22.1 (1.1)

NP: Nasopharyngeal swab; N/A: Not applicable

Table 17. Summary of Qualitative Results Obtained from Parallel Testing of Nasopharyngeal Swab Samples and Saliva from Patients Suspected of COVID-19 Using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit

MagMAX Viral/Pathogen Nucleic Acid Isolation Kit		Nasopharyngeal Swab		
		Positive	Negative	Total
Saliva	**Positive**	33	2	35
	Negative	1	55	56
	Total	34	57	91
Positive Percent Agreement		97.1% (33/34); 85.1-99.5%[1]		
Negative Percent Agreement		96.5% (55/57); 88.1-99.0%[1]		

[1] Two-sided 95% score confidence interval

Table 18. Summary of Qualitative Results Obtained from Parallel Testing of Nasopharyngeal Swab Samples and Saliva from Patients Suspected of COVID-19 Using the Promega Maxwell HT Viral TNA Kit

Promega Maxwell HT Viral TNA Kit		Nasopharyngeal Swab		
		Positive	Negative	Total
Saliva	Positive	33	1	34
	Negative	1	56	57
	Total	34	57	91
Positive Percent Agreement		97.1% (33/34); 85.1-99.5%[1]		
Negative Percent Agreement		98.2% (56/57); 90.7-99.7%[1]		

[1]Two-sided 95% score confidence interval

Table 19. Summary of Qualitative Results Obtained from Parallel Testing of Nasopharyngeal Swab Samples and Saliva from Patients Suspected of COVID-19 Using the Maxwell RSC TNA Viral Kit Run on the Maxwell RSC 48 System

Maxwell RSC TNA Viral Kit run on Maxwell RSC 48 System		Nasopharyngeal Swab		
		Positive	Negative	Total
Saliva	Positive	33	2	35
	Negative	1	55	56
	Total	34	57	91
Positive Percent Agreement		97.1% (33/34); 85.1-99.5%[1]		
Negative Percent Agreement		96.5% (55/57); 88.1-99.7%[1]		

[1]Two-sided 95% score confidence interval

To support the upper respiratory specimen claim, the NP swabs that were collected as part of the paired clinical study with saliva, were tested by both the Phosphorus COVID-19 RT-qPCR Test and an EUA authorized molecular assay. NP swab results for both assays demonstrated 100% concordance and qualitative results are shown in Tables 14-16 below.

Table 20. Nasopharyngeal Swab Performance When Evaluated Using an EUA Authorized Molecular Assay, Extracted with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit

MagMAX Viral/Pathogen Nucleic Acid Isolation Kit		Nasopharyngeal Swab – FDA Authorized Comparator Assay		
		Positive	Negative	Total
Nasopharyngeal Swab – Phosphorus COVID-19 RT-qPCR Test	Positive	34	0	37
	Negative	0	57	57
	Total	34	57	91
Positive Percent Agreement		100% (34/34); 95% CI: 89.8-100%[1]		
Negative Percent Agreement		100% (57/57); 95% CI: 93.7-100%[1]		

[1]Two-sided 95% score confidence interval

Table 21. Nasopharyngeal Swab Performance When Evaluated Using an EUA Authorized Molecular Assay, Extracted with the Promega Maxwell HT Viral TNA Kit

Promega Maxwell HT Viral TNA Kit	Nasopharyngeal Swab – FDA Authorized Comparator Assay

		Positive	Negative	Total
Nasopharyngeal Swab – Phosphorus COVID-19 RT-qPCR Test	**Positive**	34	0	37
	Negative	0	57	57
	Total	34	57	91
Positive Percent Agreement		100% (34/34); 95% CI: 89.8-100%[1]		
Negative Percent Agreement		100% (57/57); 95% CI: 93.7-100%[1]		

[1] Two-sided 95% score confidence interval

Table 22. Nasopharyngeal Swab Performance When Evaluated using an EUA Authorized Molecular Assay, Extracted with the Maxwell RSC TNA Viral Kit on the Maxwell RSC 48 System

Maxwell RSC TNA Viral Kit run on Maxwell RSC 48 System		**Nasopharyngeal Swab – FDA Authorized Comparator Assay**		
		Positive	**Negative**	**Total**
Nasopharyngeal Swab – Phosphorus COVID-19 RT-qPCR Test	**Positive**	34	0	37
	Negative	0	57	57
	Total	34	57	91
Positive Percent Agreement		100% (34/34); 95% CI: 89.8-100%[1]		
Negative Percent Agreement		100% (57/57); 95% CI: 93.7-100%[1]		

[1] Two-sided 95% score confidence interval

b. Contrived Clinical Evaluation (Nasopharyngeal Swabs Only)

The performance of the Phosphorus COVID-19 RT-qPCR Test with nasopharyngeal swabs (NPS) was further evaluated using contrived specimens composed of leftover, unique nasopharyngeal swab samples spiked with Twist Bioscience synthetic SARS-CoV-2 RNA at various concentrations (X LoD). A total of 37 contrived positive and 30 negative NP swab samples were blinded and randomized for testing with the Phosphorus Test. All 30 contrived negative specimens were non-reactive for N1 and N2 targets but showed amplification with RNase P, as expected. Of the 37 contrived positive samples prepared in individual (not pooled) NP swab matrix, all 37 yielded positive results for the SARS-CoV-2 assay targets (N1 and N2) and RNase P. A summary of the contrived clinical study results for NP swabs is presented in Table 23.

Table 23. Summary of Results from the Contrived Specimen Study with Nasopharyngeal Swabs, Stratified by Target Level, Measurand, and Extraction Method

Copies/µL	**Number Tested (N)**	**Analysis**	**Detected Target**		
			N1	**N2**	**RNase P**
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit					
1000 (1000X LoD)	3	Positive (%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
		Mean Ct (SD)	30.40 (0.1)	31.19 (0.1)	26.69 (0.1)
500 (500X LoD)	3	Positive (%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
		Mean Ct (SD)	31.37 (0.2)	32.22 (0.04)	26.72 (0.05)
200 (200X LoD)	3	Positive (%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
		Mean Ct (SD)	33.22 (0.5)	33.87 (0.6)	29.16 (0.1)
100 (100X LoD)	4	Positive (%)	4/4 (100%)	4/4 (100%)	4/4 (100%)
		Mean Ct (SD)	33.83 (0.4)	34.66 (0.4)	29.64 (0.1)
50 (10X LoD)	4	Positive (%)	4/4 (100%)	4/4 (100%)	4/4 (100%)
		Mean Ct (SD)	35.24 (0.3)	35.80 (0.2)	29.96 (0.1)
10	10	Positive (%)	10/10 (100%)	10/10 (100%)	10/10 (100%)

(2X LoD)		Mean Ct (SD)	36.76 (1.0)	38.23 (0.9)	31.10 (0.1)
5 (1X LoD)	10	Positive (%)	10/10 (100%)	10/10 (100%)	10/10 (100%)
		Mean Ct (SD)	37.65 (0.5)	38.51 (0.7)	31.46 (0.2)
Negatives	30	Positive (%)	0 (0)	0 (0)	30/30 (100%)
		Mean Ct (SD)	N/A	N/A	24.07 (0.1)
Promega Maxwell HT Viral TNA Kit					
1000 (1000X LoD)	3	Positive (%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
		Mean Ct (SD)	31.55 (0.1)	32.24 (0.2)	27.65 (0.2)
500 (500X LoD)	3	Positive (%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
		Mean Ct (SD)	32.80 (0.2)	33.55 (0.3)	27.85 (0.1)
200 (200X LoD)	3	Positive (%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
		Mean Ct (SD)	33.80 (0.3)	34.85 (0.4)	29.81 (0.1)
100 (100X LoD)	4	Positive (%)	4/4 (100%)	4/4 (100%)	4/4 (100%)
		Mean Ct (SD)	34.55 (0.3)	35.45 (0.5)	30.32 (0.1)
50 (10X LoD)	4	Positive (%)	4/4 (100%)	4/4 (100%)	4/4 (100%)
		Mean Ct (SD)	35.98 (0.8)	37.11 (0.7)	30.45 (0.1)
10 (2X LoD)	10	Positive (%)	10/10 (100%)	10/10 (100%)	10/10 (100%)
		Mean Ct (SD)	37.52 (0.4)	39.29 (0.6)	31.58 (0.3)
5 (1X LoD)	10	Positive (%)	10/10 (100%)	10/10 (100%)	10/10 (100%)
		Mean Ct (SD)	38.02 (0.7)	38.56 (0.6)	31.90 (0.5)
Negatives	30	Positive (%)	0 (0)	0 (0)	30/30 (100%)
		Mean Ct (SD)	N/A	N/A	24.95 (0.7)
Maxwell RSC TNA Viral Kit run on the Maxwell RSC 48 System					
1000 (1000X LoD)	3	Positive (%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
		Mean Ct (SD)	29.43 (0.2)	30.64 (0.2)	26.2 (0.1)
500 (500X LoD)	3	Positive (%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
		Mean Ct (SD)	31.03 (0.1)	32.09 (0.2)	26.50 (0.2)
200 (200X LoD)	3	Positive (%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
		Mean Ct (SD)	32.49 (0.2)	33.58 (0.04)	28.06 (0.2)
100 (100X LoD)	4	Positive (%)	4/4 (100%)	4/4 (100%)	4/4 (100%)
		Mean Ct (SD)	33.17 (0.2)	34.82 (0.5)	28.55 (0.3)
50 (10X LoD)	4	Positive (%)	4/4 (100%)	4/4 (100%)	4/4 (100%)
		Mean Ct (SD)	33.94 (0.7)	34.87 (0.6)	28.58 (0.2)
10 (2X LoD)	10	Positive (%)	10/10 (100%)	10/10 (100%)	10/10 (100%)
		Mean Ct (SD)	36.34 (0.7)	37.81 (0.9)	30.10 (0.3)
5 (1X LoD)	10	Positive (%)	10/10 (100%)	10/10 (100%)	10/10 (100%)
		Mean Ct (SD)	38.44 (1.1)	38.72 (0.6)	30.28 (0.2)
Negatives	30	Positive (%)	0 (0)	0 (0)	30/30 (100%)
		Mean Ct (SD)	N/A	N/A	24.11 (0.7)

N/A; Not Detected or Negative; No detectable Ct value

4) Clinical Validation for Testing of Asymptomatic Saliva Samples

To validate testing of self-collected saliva samples from asymptomatic individuals, a prospective clinical study was completed using paired NP swab and saliva samples collected at two different physician offices in New York City that see a diverse patient population. This was an all-comers study that recruited symptomatic and asymptomatic individuals.

Paired NP swabs and saliva collected using the Pinpoint by Phosphorus COVID-19 Test Home Collection Kit (Rx) were taken from all study participants. Upon consent and enrollment of the patient into the study, the healthcare provider collected the NP swab first. Within 15 minutes of NP swab collection, the patient self-collected saliva using the Oragene Dx (OGD-510) collection device and the instructions for use included with the Pinpoint by Phosphorus COVID-19 Test Home Collection Kit. The paired NP swabs and saliva specimens were transported at ambient temperature and tested using the authorized Phosphorus COVID-19 RT-qPCR Test within 48 hours of collection. RNA was isolated from each of the paired samples using each of the three previously authorized extraction methods including the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, Promega Maxwell HT Viral TNA Kit, and Maxwell RSC TNA Viral Kit run on the Maxwell RSC 48 System. Extracted RNA was reverse transcribed using the ThermoFisher Scientific TaqPath 1-Step Multiplex Master Mix (No ROX) with 5 µL of extracted sample and the Phosphorus COVID-19 RT-qPCR Test was run on CFX384 Touch Real-Time PCR Detection System.

A total of 147 patients participated in this study. Included were 20 consecutively collected paired samples from asymptomatic individuals with positive NP swab results and 127 paired samples from the same population with negative results for the NP swab comparator. There was 95.00% positive percent agreement (PPA) and 99.21% negative percent agreement (NPA) between the results obtained from testing saliva samples in comparison to the paired NP swabs using each of the three manual and automated extraction kits. See Table 24-27 below for a stratification of the data based on extraction method. Of the 20 confirmed positive NP swab samples, 19 paired NP and saliva specimens produced positive results for the N1 and N2 targets (19/20; 95.00%); however, there was one false negative result where the NP swab showed positive amplification (Ct < 40) but the paired saliva sample was negative (not detected). For the 127 comparator assay NP swab negative samples, one paired saliva sample yielded a positive SARS-CoV-2 result (both N1 and N2 targets had Ct values < 40) and was therefore considered a false positive. No discordant analyses or a root cause determination were completed on the false positive and false negative samples.

Overall, the results of the clinical evaluation with paired NP swabs and saliva samples that were collected from an asymptomatic patient population and tested in parallel with the Phosphorus COVID-19 RT-qPCR Test using each of the three validated extraction methods were considered acceptable. The same two patient samples generated discordant results (one false negative and one false positive) with each extraction method. Regardless, the clinical performance of the Phosphorus COVID-19 RT-qPCR Test with saliva specimens for detection of SARS-CoV-2 RNA in individuals without symptoms or other reasons to suspect COVID-19 (asymptomatic patients) appears to be comparable to that of NP swab specimens.

Table 24. Summary of Results Obtained from Parallel Testing of NP Swabs and Saliva from Asymptomatic and Mildly Symptomatic Individuals, Stratified by Measurand and RNA Extraction Method

Number of Individuals	Sample Type	Analysis	Target		
			N1	N2	RNase P
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit					
20 NP Positive	NP swab	Positive (%)	20 (100.00)	20 (100.00)	20 (100.00)
		Mean Ct (SD)	28.92 (4.59)	28.81 (4.76)	28.99 (2.05)

	Saliva	Positive (%)	19 (95.00)	19 (95.00)	20 (100.00)
		Mean Ct (SD)	26.87 (3.97)	28.36 (4.47)	22.02 (2.10)
127 NP Negative	NP swab	Positive (%)	0 (0)	0 (0)	127 (100.00)
		Mean Ct (SD)	N/A	N/A	28.74 (6.06)
	Saliva	Positive (%)	1 (0.79)	1 (0.79)	127 (100.00)
		Mean Ct (SD)	34.06 (0)	36.75 (0)	21.64 (2.31)
Promega Maxwell HT Viral TNA Kit					
20 NP Positive	NP swab	Positive (%)	20 (100.00)	20 (100.00)	20 (100.00)
		Mean Ct (SD)	26.91 (5.17)	27.48 (5.19)	29.94 (2.19)
	Saliva	Positive (%)	19 (95.00)	19 (95.00)	20 (100.00)
		Mean Ct (SD)	27.77 (3.92)	30.26 (4.82)	22.60 (2.37)
127 NP Negative	NP swab	Positive (%)	0 (0)	0 (0)	127 (100.00)
		Mean Ct (SD)	N/A	N/A	29.41 (2.61)
	Saliva	Positive (%)	1 (0.79)	1 (0.79)	127 (100.00)
		Mean Ct (SD)	33.09 (0)	35.59 (0)	22.35 (2.12)
Maxwell RSC TNA Viral Kit run on the Maxwell RSC 48 System					
20 NP Positive	NP swab	Positive (%)	20 (100.00)	20 (100.00)	20 (100.00)
		Mean Ct (SD)	26.10 (5.00)	26.36 (5.49)	28.57 (1.92)
	Saliva	Positive (%)	19 (95.00)	19 (95.00)	20 (100.00)
		Mean Ct (SD)	27.38 (3.90)	29.43 (4.50)	22.26 (2.20)
127 NP Negative	NP swab	Positive (%)	0 (0)	0 (0)	127 (100.00)
		Mean Ct (SD)	N/A	N/A	28.26 (2.35)
	Saliva	Positive (%)	1 (0.79)	1 (0.79)	127 (100.00)
		Mean Ct (SD)	33.23 (0)	33.72 (0)	21.95 (1.71)

N/A; Not Detected or Negative; No detectable Ct value
SD; Standard Deviation of Ct values

Table 25. Performance of NP Swabs Tested with the Phosphorus COVID-19 RT-qPCR Test Against Paired Saliva Samples from an Asymptomatic/Pre-Symptomatic Patient Population Using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit

MagMAX Viral/Pathogen Nucleic Acid Isolation Kit		**FDA Authorized Comparator - Phosphorus COVID-19 RT-qPCR Test (Nasopharyngeal Swab)**		
		Positive	**Negative**	**Total**
Phosphorus COVID-19 RT-qPCR Test (Saliva)	**Positive**	19	1	20
	Negative	1	126	127
	Total	20	127	147
Positive Percent Agreement		19/20; 95.00% (76.39% - 99.11%)[1]		
Negative Percent Agreement		126/127; 99.21% (95.68% - 99.86%)[1]		

[1] Two-sided 95% confidence interval

Table 26. Performance of NP Swabs Tested with the Phosphorus COVID-19 RT-qPCR Test Against Paired Saliva Samples from an Asymptomatic/Pre-Symptomatic Patient Population Using the Promega Maxwell HT Viral TNA Kit

Promega Maxwell HT Viral TNA Kit		**FDA Authorized Comparator - Phosphorus COVID-19 RT-qPCR Test (Nasopharyngeal Swab)**		
		Positive	**Negative**	**Total**

Phosphorus COVID-19 RT-qPCR Test (Saliva)	**Positive**	19	1	20
	Negative	1	126	127
	Total	20	127	147
Positive Percent Agreement		19/20; 95.00% (76.39% - 99.11%)[1]		
Negative Percent Agreement		126/127; 99.21% (95.68% - 99.86%)[1]		

[1] Two-sided 95% confidence interval

Table 27. Performance of NP Swabs Tested with the Phosphorus COVID-19 RT-qPCR Test Against Paired Saliva Samples from an Asymptomatic/Pre-Symptomatic Patient Population Using the Promega Maxwell HT Viral TNA Kit

Maxwell RSC TNA Viral Kit Run on the Maxwell RSC 48 System		**FDA Authorized Comparator - Phosphorus COVID-19 RT-qPCR Test (Nasopharyngeal Swab)**		
		Positive	**Negative**	**Total**
Phosphorus COVID-19 RT-qPCR Test (Saliva)	**Positive**	19	1	20
	Negative	1	126	127
	Total	20	127	147
Positive Percent Agreement		19/20; 95.00% (76.39% - 99.11%)[1]		
Negative Percent Agreement		126/127; 99.21% (95.68% - 99.86%)[1]		

[1] Two-sided 95% confidence interval

5) Simulated Shipping Study with the Oragene Dx OGD-510 Saliva Collection Device

To support home use of the Oragene Dx OGD-510 collection device that is part of the Pinpoint by Phosphorus COVID-19 Test Home Collection Kit as well as shipping conditions from healthcare professional locations, a simulated shipping study was performed that was designed to evaluate the effect of temperature variation on the stability of SARS-CoV-2 RNA during transport of saliva specimens. The shipping study was designed to simulate shipping at room temperature as well as the extreme temperature conditions that could be experienced during the summer months. See Table 18 for the summer thermal profile that was evaluated in this study.

Simulated sample stability and shipping studies were performed using contrived positive saliva specimens at 2X (low positive) and 5-10X LoD (high positive) concentrations. After the samples underwent the thermal excursions, they were incubated at 50°C for 1 hour and then equilibrated to room temperature, extracted, and tested with the Phosphorus COVID-19 RT-qPCR Test.

Table 28. Summer Temperature Excursion

Temperature	Cycle Period	Cycle Period Hours	Total Time 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

Contrived samples were prepared using pooled known negative patient saliva matrix and spiking with Twist Bioscience synthetic SARS-CoV-2 RNA to establish 20 low positive samples of 2X LoD (LoD previously established as 10 copies/µL) and 10 moderate to high positive saliva samples between 5-10X LoD. Ten negative saliva specimens were also evaluated in the shipping study. For the spiked specimens, saliva was collected in the OGD-510 device and pooled. Saliva specimens were received by Phosphorus, from individuals that had tested negative using a third party FDA authorized molecular assay, following shipment at ambient conditions. The saliva specimens were also screened negative using the Phosphorus COVID-19 RT-qPCR Test within 56 hours of collection.

The contrived positive and negative saliva samples were stored for the duration of the simulated shipping study as shown in Table 28. These temperature range conditions are intended to replicate worst case scenario shipping conditions (for spring/summer) for an 8-hour wait at the customer’s house/healthcare location before shipping and then a subsequent 48 hour shipping cycle. At the conclusion of the summer thermal profile, the samples were treated as if they were actual clinical specimens received at the laboratory for processing. The contrived samples were first incubated at 50°C for 1 hour to inactivate any RNases in the collected saliva, followed by equilibration to ambient temperature. Specimens were then extracted using the three extraction kits (MagMAX, Maxwell HT, and Maxwell RSC) and retested with the Phosphorus COVID-19 RT-qPCR Test. Results were compared to those reported upon initial testing when specimens were received and spiked with various concentrations at time 0 (day 0, room temperature).

Ten out of 10 (100%) low positive samples (2X LoD) and 10/10 moderate to high positive contrived samples (100%) ranging from 5-10X LoD were reported as positive after exposure to the summer temperature cycles. The mean and standard deviation of the Ct values for each gene target were similar before and after each simulated shipping scenario (within ~3 Cts), with no evidence of significant degradation of the SARS-CoV-2 RNA. All SARS-CoV-2 negative specimens were reported as negative after enduring the summer temperature excursion (no amplification of N1 or N2 genes).

A summary of the mean Ct values observed for each SARS-CoV-2 specific target gene is provided in Tables 29-31 for each claimed extraction method.

Table 29. Summary of Results from the Simulated Shipping Study Using Contrived Samples Extracted Using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit

Sample Group	Test Point	N	Mean Ct (Standard Deviation)			Positive[4] (%)
			N1	N2	RNase P	
Negative	Day 0 (RT)[1]	10	N/A[3]	N/A	23.15 (0.7)	0 (0)
	Summer[2]	10	N/A	N/A	23.60 (0.4)	0 (0)
Low Positive 2X LoD 10 copies/µL	Day 0 (RT)[1]	10	32.97 (0.09)	33.74 (0.12)	23.25 (0.08)	10/10 (100)
	Summer[2]	10	34.84 (0.6)	36.22 (1.2)	23.56 (0.9)	10/10 (100)
High Positive 5X LoD 25 copies/µL	Day 0 (RT)[1]	3	34.37 (0.4)	35.03 (0.7)	24.16 (0.3)	3/3 (100)
	Summer[2]	2	33.46 (0.2)	34.93 (0.1)	24.55 (0.0)	2/2 (100)
High Positive 6X LoD	Day 0 (RT)[1]	2	34.50 (0.2)	35.13 (0.7)	23.70 (0.5)	3/3 (100)
	Summer[2]	2	33.12 (0.1)	34.87 (0.3)	24.59 (0.1)	2/2 (100)

30 copies/µL						
High Positive 7.5X LoD 37.5 copies/µL	Day 0 (RT)[1]	2	34.70 (0.8)	35.37 (0.2)	24.15 (0.1)	3/3 (100)
	Summer[2]	2	32.65 (0.0)	33.87 (0.2)	24.55 (0.0)	2/2 (100)
High Positive 9X LoD 45 copies/µL	Day 0 (RT)[1]	3	33.25 (0.5)	33.95 (0.7)	22.32 (0.3)	3/3 (100)
	Summer[2]	2	32.93 (0.10)	34.13 (0.2)	24.36 (0.0)	2/2 (100)
High Positive 10X LoD 50 copies/µL	Day 0 (RT)[1]	3	30.78 (0.09)	31.94 (0.24)	22.96 (0.13)	3/3 (100)
	Summer[2]	2	32.35 (0.2)	33.22 (0.0)	24.26 (0.1)	2/2 (100)

[1] Day 0 (RT) = within 56 hours of collection at room temperature shipping conditions
[2] Testing performed at the conclusion of the thermal excursions described in Table 28
[3] N/A = No detectable Ct value
[4] Positive; Number of replicates positive for SARS-CoV-2 targets only (N1 and N2), not RNase P target

Table 30. Summary of Results from the Simulated Shipping Study Using Contrived Samples Extracted Using the Maxwell HT Viral TNA Kit

Sample Group	Test Point	N	Mean Ct (Standard Deviation)			Positive[4] (%)
			N 1	N2	RNase P	
Negative	Day 0 (RT)[1]	10	N/A[3]	N/A	22.87 (0.4)	0 (0)
	Summer[2]	10	N/A	N/A	23.30 (0.2)	0 (0)
Low Positive 2X LoD 10 copies/µL	Day 0 (RT)[1]	10	33.46 (0.08)	34.79 (0.40)	23.35 (0.14)	10/10 (100)
	Summer[2]	10	35.00 (0.6)	36.58 (1.0)	23.35 (0.5)	10/10 (100)
High Positive 5X LoD 25 copies/µL	Day 0 (RT)[1]	3	34.09 (0.5)	35.48 (0.5)	24.21(0.3)	3/3 (100)
	Summer[2]	2	33.35 (0.3)	34.85 (0.1)	25.13 (0.1)	2/2 (100)
High Positive 6X LoD 30 copies/µL	Day 0 (RT)[1]	3	34.88 (0.6)	35.77 (0.8)	24.20 (0.3)	3/3 (100)
	Summer[2]	2	34.13 (0.1)	35.39 (0.2)	24.67 (0.1)	2/2 (100)
High Positive 7.5X LoD 37.5 copies/µL	Day 0 (RT)[1]	3	34.10 (0.7)	35.51 (0.7)	24.11 (0.2)	3/3 (100)
	Summer[2]	2	33.47 (0.5)	34.91 (0.0)	24.66 (0.3)	2/2 (100)
High Positive 9X LoD 45 copies/µL	Day 0 (RT)[1]	3	34.24 (0.3)	35.88 (0.6)	23.09 (0.3)	3/3 (100)
	Summer[2]	2	32.46 (0.1)	33.19 (0.0)	24.72 (0.3)	2/2 (100)
High Positive 10X LoD 50 copies/µL	Day 0 (RT)[1]	3	31.52 (0.13)	32.65 (0.23)	22.79 (0.18)	3/3 (100)
	Summer[2]	2	32.75 (0.2)	33.38 (0.1)	24.82 (0.0)	2/2 (100)

[1] Day 0 (RT) = within 56 hours of collection at room temperature shipping conditions
[2] Testing performed at the conclusion of the thermal excursions described in Table 28
[3] N/A = No detectable Ct value
[4] Positive; Number of replicates positive for SARS-CoV-2 targets only (N1 and N2), not RNase P target

Table 31. Summary of Results from the Simulated Shipping Study Using Contrived Samples Extracted Using the Maxwell RSC TNA Viral Kit on the Maxwell RSC 48 System

Sample Group	Test Point	N	Mean Ct (Standard Deviation)			Positive[4] (%)
			N 1	N2	RNase P	
Negative	Day 0 (RT)[1]	10	N/A[3]	N/A	21.82 (0.5)	0 (0)
	Summer[2]	10	N/A	N/A	22.0 (0.3)	0 (0)
Low Positive	Day 0 (RT)[1]	10	34.94 (0.13)	35.50 (0.06)	21.31 (0.20)	10/10 (100)

2X LoD 10 copies/µL	Summer[2]	10	35.16 (0.9)	36.04 (1.2)	22.04 (0.3)	10/10 (100)
High Positive 5X LoD 25 copies/µL	Day 0 (RT)[1]	2	35.24 (0.2)	34.10 (0.5)	23.65 (0.2)	3/3 (100)
	Summer[2]	2	32.78 (0.0	33.36 (0.3)	23.49 (0.3)	2/2 (100)
High Positive 6X LoD 30 copies/µL	Day 0 (RT)[1]	2	34.34 (0.4)	35.25 (0.5)	23.43 (0.2)	3/3 (100)
	Summer[2]	2	33.63 (0.1)	33.99 (0.4)	23.52 (0.0)	2/2 (100)
High Positive 7.5X LoD 37.5 copies/µL	Day 0 (RT)[1]	2	33.75 (0.7)	34.64 (0.3)	23.68 (0.6)	3/3 (100)
	Summer[2]	2	32.36 (0.3)	32.70 (0.1)	23.64 (0.1)	2/2 (100)
High Positive 9X LoD 45 copies/µL	Day 0 (RT)[1]	2	34.09 (0.5)	34.55 (0.4)	22.21 (0.2)	3/3 (100)
	Summer[2]	2	32.07 (0.0)	32.67 (0.1)	23.69 (0.0)	2/2 (100)
High Positive 10X LoD 50 copies/µL	Day 0 (RT)[1]	3	32.04 (0.39)	32.42 (0.30)	21.83 (0.22)	3/3 (100)
	Summer[2]	2	32.37 (0.4)	33.05 (0.3)	23.76 (0.0)	2/2 (100)

[1] Day 0 (RT) = within 56 hours of collection at room temperature shipping conditions

[2] Testing performed at the conclusion of the thermal excursions described in Table 28

[3] N/A = No detectable Ct value

[4] Positive; Number of replicates positive for SARS-CoV-2 targets only (N1 and N2), not RNase P target

These results demonstrate that SARS-CoV-2 RNA positive saliva specimens are stable in the Oragene Dx OGD-510 collection device when exposed to a broad range of temperature conditions. The results obtained with the Phosphorus COVID-19 RT-qPCR Test with contrived specimens that had undergone a summer thermal excursion were the same as those obtained when the specimens were tested at time zero. These data support the use of the Oragene Dx OGD-510 for transport and storage of specimens following self-collection of saliva in the home or healthcare setting.

Phosphorus Diagnostics will conduct a post-authorization study to verify the stability of SARS-CoV-2 RNA in specimens collected using the Pinpoint by Phosphorus COVID-19 Test Home Collection Kit that are transported under low ambient temperature conditions, including multiple freeze-thaw cycles.

6) Collection Device Reagent Stability

The expiration date of the Pinpoint by Phosphorus COVID-19 Test Home Collection Kit is based on the least stable component. Therefore, the established expiration for the home collection kit is 30 months when stored at room temperature. The expiration date is displayed on the OGD-510 collection device.

7) FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were the manual RNA extraction by MagMAX Viral/Pathogen Nucleic Acid Isolation Kit and the CFX384 Touch Real-Time PCR

Detection System with Bio-Rad CFX Manager software version 3.1. The results are summarized in the following tables.

Table 32a. Summary of LoD Confirmation Result for Saliva Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Saliva	1.8 x 10^4 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

Table 32b. Summary of LoD Confirmation Result for Nasopharyngeal Swab Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	1.8 x 10^4 NDU/mL	N/A
MERS-CoV		N/A	D

NDU/mL = RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

LIMITATIONS:

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

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.