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 in at Phosphorus Diagnostics LLC, a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory, per the Standard Operating Procedures that were 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 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.

This test is also for use with saliva specimens that are self-collected at home or in a healthcare setting by patients using the Oragene Dx OGD-510 collection device when determined to be appropriate by a healthcare provider.

Testing is limited to Phosphorus Diagnostics LLC in Secaucus, NJ which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity tests.

Results are for the 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 positive results to the appropriate public health authorities.

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

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

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Phosphorus COVID-19 RT-qPCR Test is a real-time reverse transcription polymerase chain reaction test. The assay uses primers and probes that were developed and validated under the Emergency Use Authorization (EUA) for the 2019-nCoV CDC assay and are designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients suspected of COVID-19 by their healthcare provider. The purpose of this EUA request is to enable testing of upper respiratory tract specimens and bronchoalveolar lavage specimens.

Saliva specimens are self-collected at home or in the healthcare setting using the OGD-510[1] collection device. 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 this data. In addition, the posted decision summaries for K141410 and K192920 provide overall results from usability studies that were completed.

Anterior nasal swabs, mid-turbinate nasal swabs, oropharyngeal (throat) swabs, nasopharyngeal swabs, nasopharyngeal washes/aspirates or nasal washes/aspirates, and BALs should be collected, transported and stored according to standard procedures. All swab and wash/aspirate/BAL collections will 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. 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.

RNA extraction from saliva collected in the OGD-510, upper respiratory tract specimens as well as BALs have been validated for use with 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

[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.

volume, eluate, and elution volume 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	400 µL	Manufacturer Provided Elution Solution	100 µL
Promega Maxwell HT Viral TNA Kit	200 µL	Manufacturer Provided Nuclease Free Water	60 µL
Maxwell RSC TNA Viral Kit	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 the 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.

MEDICAL OVERSIGHT AND PROCESS TO BE USED

For At-Home Saliva Collection Test Ordering

1. The patient visits the Phosphorus website to request/pay for the test (https://www.phosphorus.com/covid-19-order-now/covid-19-rt-pcr-test)
2. Once payment is received, the patient will be sent a link to complete the COVID-19 Medical Questionnaire via the Phosphorus website which adheres to the CDC COVID-19 screening guidelines. A healthcare provider (HCP), via a contracted entity, authenticates the information 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 the test request has been approved, the patient will receive a notification that the at-home saliva collection kit has shipped (48 hour shipping).
4. The patient collects the sample following the kit's included instructions and returns the specimens to Phosphorus Diagnostics 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.

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, via the CDC COVID-19 screening guidelines.
2. The HCP orders the test using the test requisition form or the online portal.
3. The saliva sample is collected by the patient 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 via overnight shipping.

6. When results are available, the HCP will receive a notification and results will be sent via encrypted email, fax, or Phosphorus portal. The HCP will share the results with the patient.

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 COVID-19 RT-qPCR

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

Table 3. Phosphorus COVID-19 RT-qPCR Test Controls

Control Type	Purpose	Frequency of Testing
Negative Extraction Control	To monitor for cross-contamination during RNA extraction and RT-PCR	Once per batch of specimens
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

If the results obtained with the positive, internal, and no template controls 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. If the negative extraction control does not meet the acceptability criteria, all specimens on the batch should be re-extracted from residual clinical samples and the RT-PCR assay should be re-run.

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) ***COVID-19 RT-qPCR test Controls – Positive, Negative, and Internal:***

- **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 < 40.0 and negative results for the RP target (Ct Not detected). Negative results with either N1 or N2 primer/probe sets invalidates 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 from patient samples or carry-over of PCR inhibitors from patient samples. 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. Ct Values for Controls that Must be Observed to Obtain Valid Results

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
NTC	Not Detected	Not Detected	Not Detected
Negative Extraction Control	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 and acceptable. 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. Result Interpretation for Patient Samples

N1	N2	RNase P	Results Interpretation	Report	Actions
+	+	+/-	SARS-CoV-2 detected	Positive	Report results to sender and appropriate public health authorities.
If any 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. 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.

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 selected 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)

The LoD study was completed for both nasopharyngeal swabs and saliva.

Nasopharyngeal Swab LoD
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 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

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 Method	Mean Ct (Standard Deviation)			Detection Rate (N Detected/N Total)		
	N1	N2	RNase P	N1	N2	RNase P
MagMAX	37.71	38.50	31.39	100%	100%	100%

Viral/Pathogen Nucleic Acid Isolation Kit	(0.7)	(0.8)	(0.2)	(20/20)	(20/20)	(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)

Saliva LoD

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 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 Test. The preliminary LoD was 5 copies/µL for all of the extraction methods and was confirmed using 20 additional extraction replicates prepared with each claimed extraction kit/platform.

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 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

The preliminary LoD selected from the range finding study of 5 copies/µL for saliva was confirmed with twenty additional extraction replicates using each claimed extraction kit/platform.

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)

2) Analytical Reactivity and Specificity

Inclusivity

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. The *in silico* inclusivity analysis was completed by the CDC in February 2020, and since this time, several additional SARS-CoV-2 sequences have been deposited in publicly available databases. Phosphorus completed an additional inclusivity study to predict the inclusivity of the Phosphorus COVID-19 RT-qPCR Test. An alignment was performed with the N1 and N2 oligonucleotide primer and probe sequences designed by the CDC with all publicly available nucleic acid sequences for SARS-CoV-2 in the NCBI database (Betacoronavirus BLAST) as of May 26, 2020. There was a total of 100 sequences that consisted of complete genomes and complete coding sequences (cds). All of the alignments showed 100% identity to the CDC primers and probes to the available SARS-CoV-2 sequences, demonstrating suitability of the CDC primers in the Phosphorus RT-qPCR assay.

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

Saliva (Paired NP Swab and Saliva Clinical Study)
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 Assay 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. The NP swabs were also confirmed positive/negative using the EUA authorized assay at Rutgers Clinical Genomics Laboratory (See Tables 14-16 for qualitative NP study results). A summary of the results of the paired study is presented in Tables 10-13 below.

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 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 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 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 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 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 10. 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 11. 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]Two-sided 95% score confidence intervals

Table 12. 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]Two-sided 95% score confidence intervals

Table 13. 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

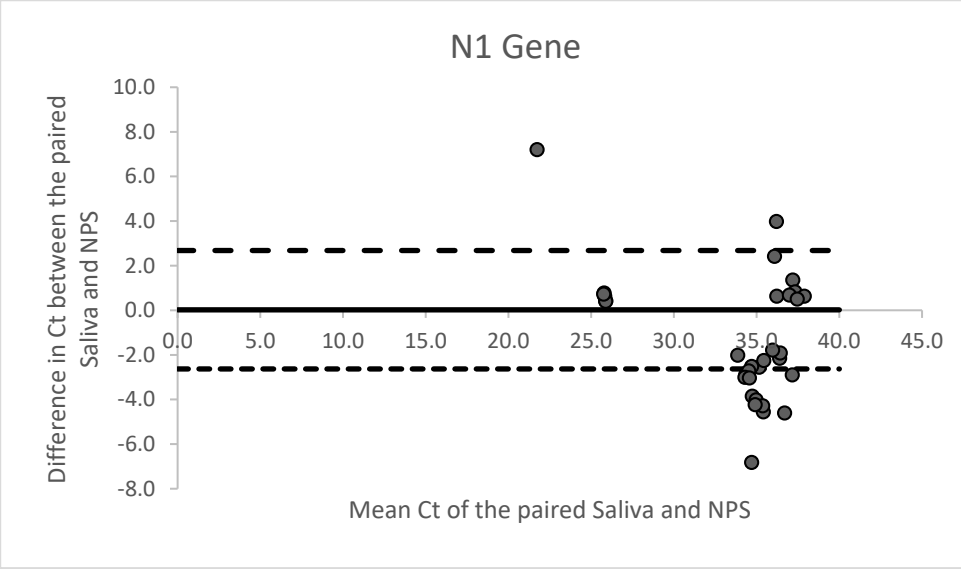
Maxwell RSC TNA Viral Kit run on Maxwell RSC 48 System		Nasopharyngeal Swab		
		Positive	Negative	Total
Saliva	Positive	33	2	35
	Negative	1	55	56
	Total	34	57	91
Positive Percent Agreement		97.1% (33/34); 85.1-99.5% [1]		
Negative Percent Agreement		96.5% (55/57); 88.1-99.7%		

[1]Two-sided 95% score confidence intervals

Evaluation of Ct Cycle Differences Among Paired Samples
The difference between Ct values from the paired samples are shown in Figures 1-3 for each extraction method. Overall mean Ct values were similar for saliva and nasopharyngeal swab and demonstrated that there was no systematic difference between testing saliva and NP swab specimens from the same patient within 15 minutes of one another. There was no trending of the Ct differences between testing saliva and NPS specimens that would indicate that either one of the sample types is superior in terms of SARS-CoV-2 RNA detectability.

Figures 4-6 shows a correlation analysis among the individual Ct values for the 31/34 paired positive NP and saliva specimens. According to the regression analyses when samples were extracted with 3 different kits, there appears to be a correlation between the Ct values obtained with the two sample types for the two assay targets. The slope of each regression line is close to 1, indicating that the Ct values between the paired NP swabs and saliva samples trend in the same direction. However, based on the R squared values for the 2 assay targets (N1 and N2 genes), it appears that a linear model does not explain all the variations between the Ct values for the two sample types. Nevertheless, the results support the use of saliva collected in the Oragene Dx OGD 510 as a specimen type for use with the Phosphorus COVID-19 RT-qPCR Test.

Figure 1. Bland Altman Plot for each SARS-CoV-2 target gene for specimens extracted with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit



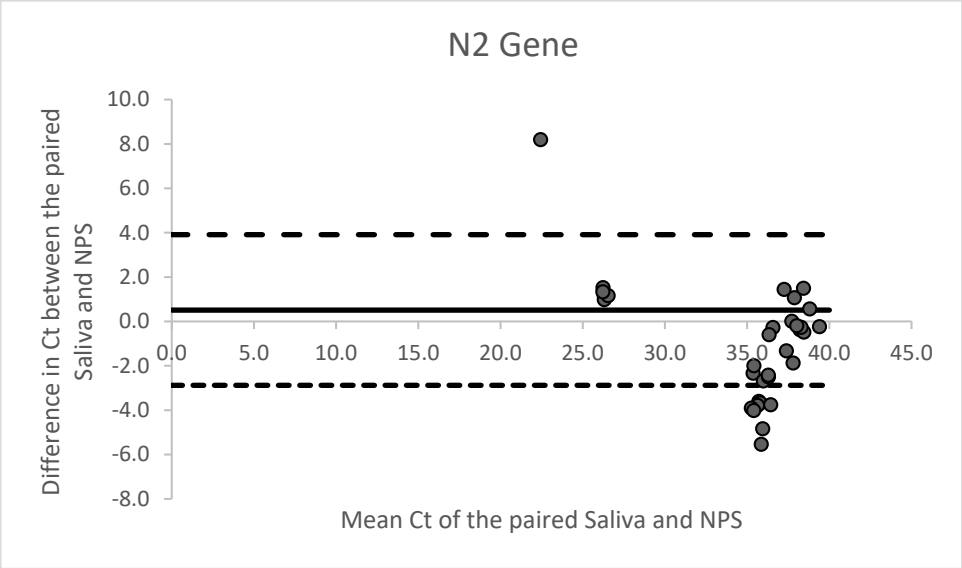
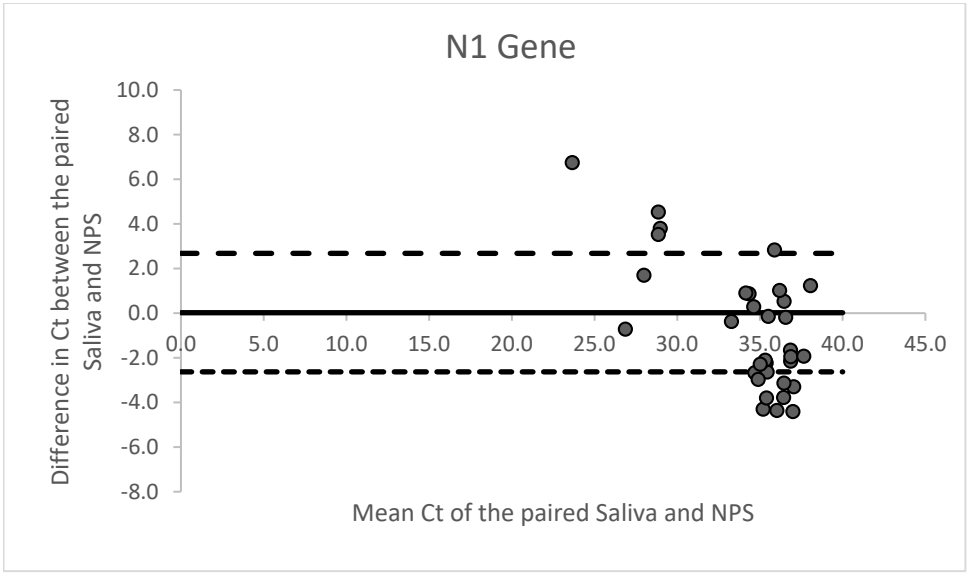


Figure 2. Bland Altman Plot for each SARS-CoV-2 target gene for specimens extracted with the Promega Maxwell HT Viral TNA Kit



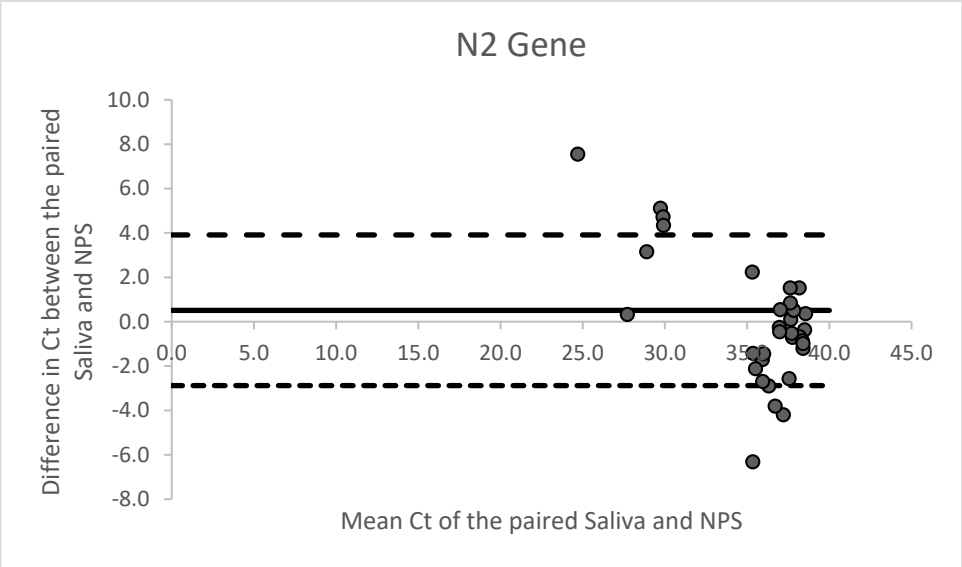
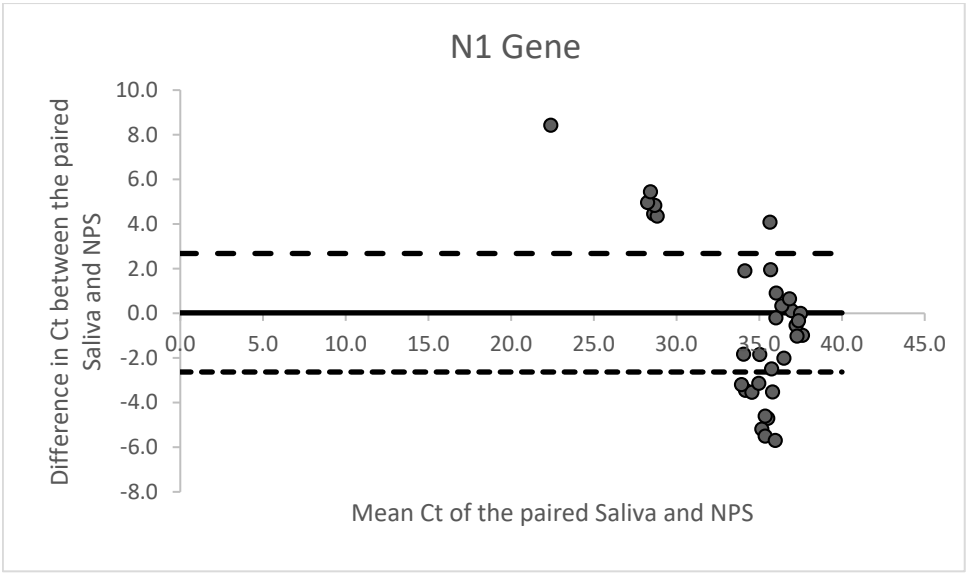


Figure 3. Bland Altman Plot for each SARS-CoV-2 target gene for specimens extracted with the Maxwell RSC TNA Viral Kit run on the Maxwell RSC 48 System



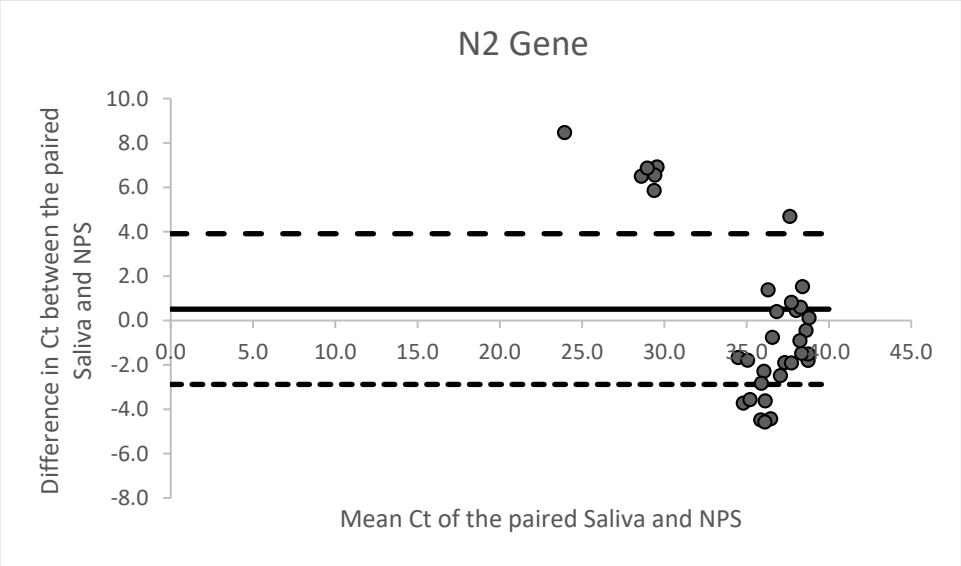
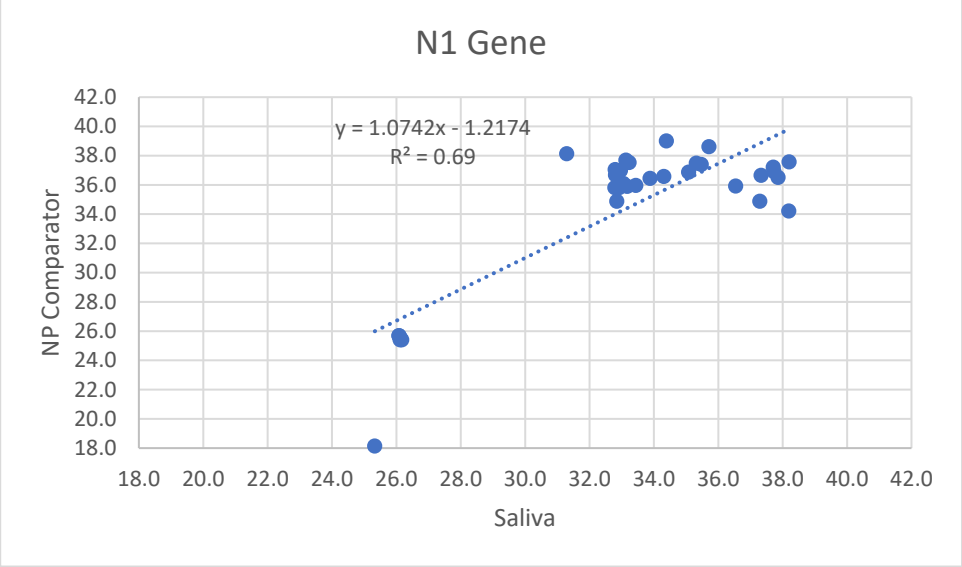


Figure 4. Regression analysis for individual Ct values for the paired NP and saliva specimens extracted using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit



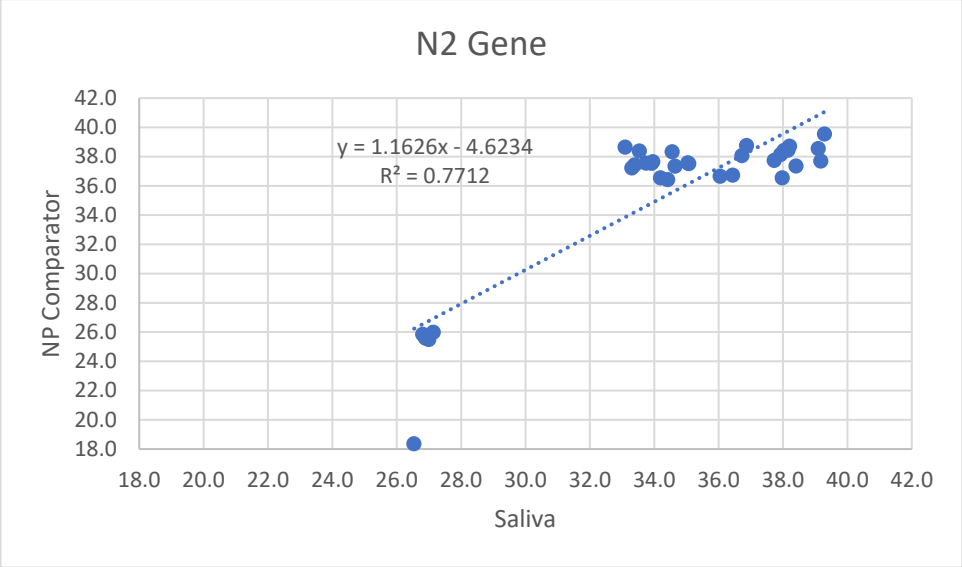
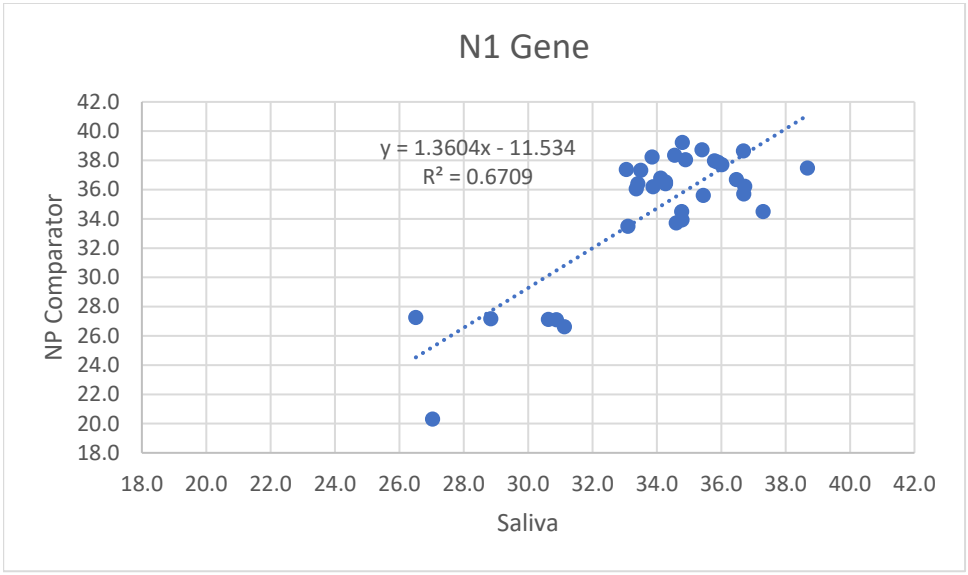


Figure 5. Regression analysis for individual Ct values for the paired NP and saliva specimens extracted using the Promega Maxwell HT Viral TNA Kit



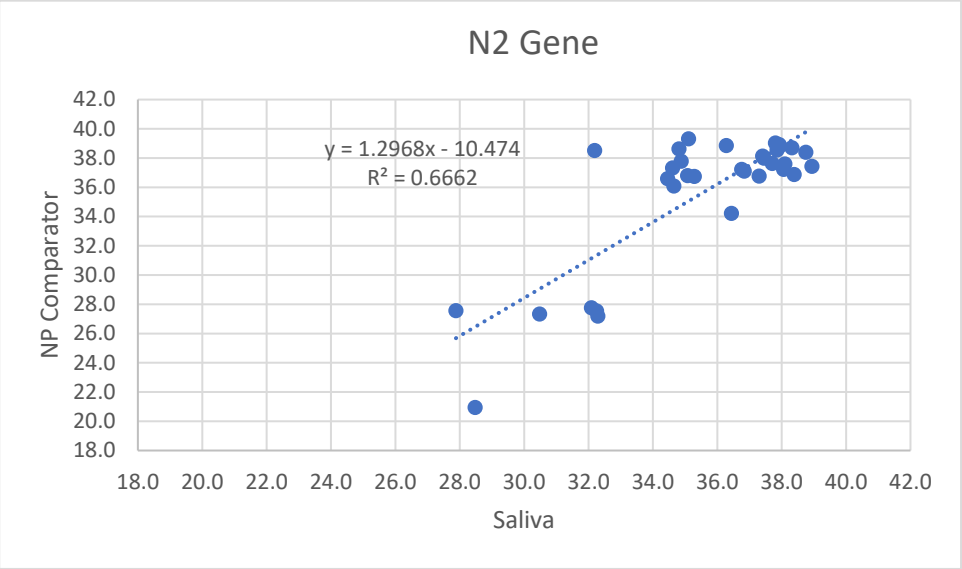
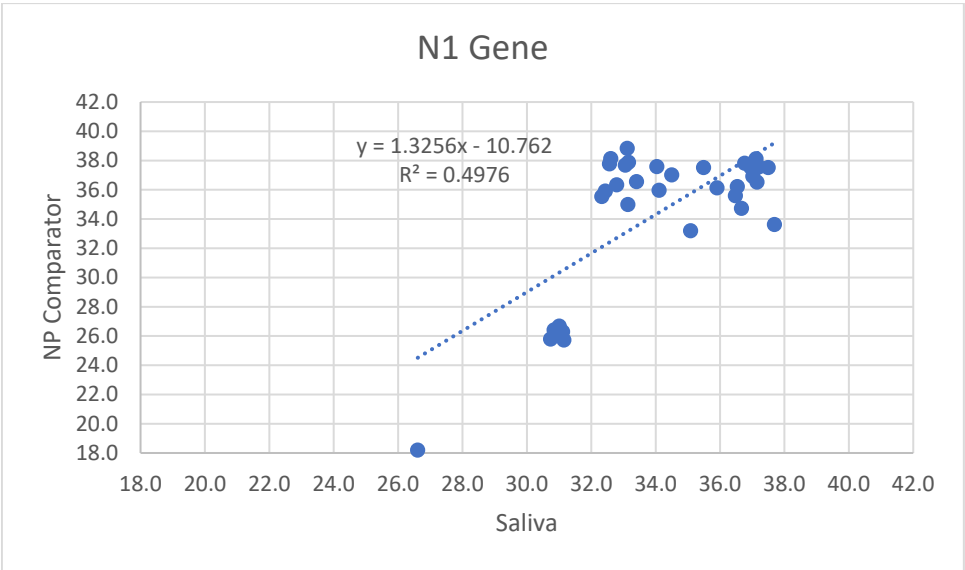
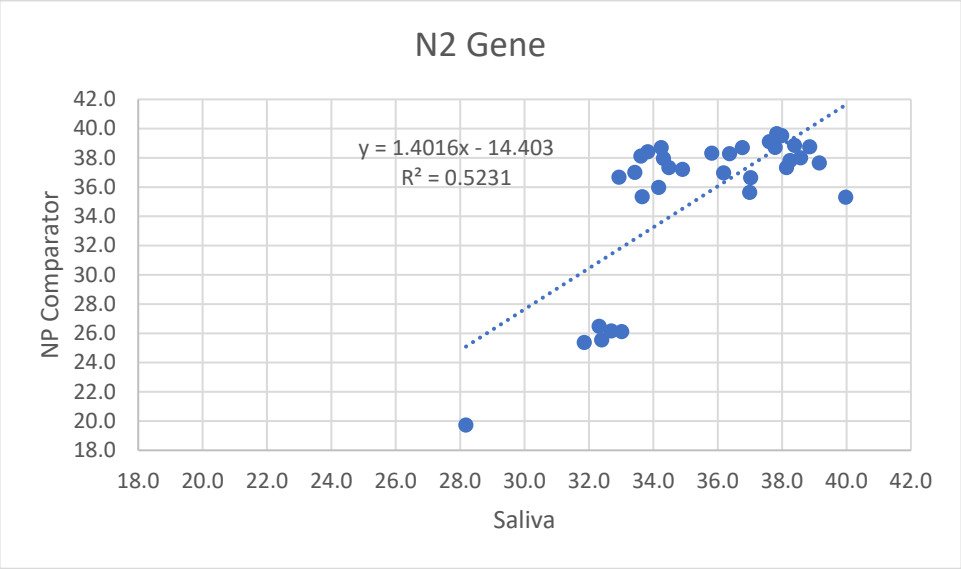


Figure 6. Regression analysis for individual Ct values for the paired NP and saliva specimens extracted using the Maxwell RSC TNA Viral Kit run on the Maxwell RSC 48 System





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 the Rutgers Clinical Genomics Laboratory EUA authorized assay. NP swab results for both assays demonstrated 100% concordance and qualitative results are shown in Tables 14-16 below.

Table 14. Nasopharyngeal swab performance when evaluated using the Rutgers EUA Authorized Assay, Extracted with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit

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

[1]Two-sided 95% score confidence intervals

Table 15. Nasopharyngeal swab performance when evaluated using the Rutgers EUA Authorized Assay, Extracted with the Promega Maxwell HT Viral TNA Kit

Promega Maxwell HT Viral TNA Kit		Nasopharyngeal Swab – Rutgers Assay Comparator		
		Positive	**Negative**	**Total**
Nasopharyngeal Swab – Phosphorus COVID-19 RT-qPCR Test	**Positive**	34	0	37
	Negative	0	57	57
	Total	34	57	91
Positive Agreement		100% (34/34); 95% CI: 89.8-100%[1]		
Negative Agreement		100% (57/57); 95% CI: 93.7-100%		

[1]Two-sided 95% score confidence intervals

Table 16. Nasopharyngeal swab performance when evaluated using the Rutgers EUA Authorized 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 – Rutgers Assay Comparator		
		Positive	**Negative**	**Total**
Nasopharyngeal Swab – Phosphorus COVID-19 RT-qPCR Test	**Positive**	34	0	37
	Negative	0	57	57
	Total	34	57	91
Positive Agreement		100% (34/34); 95% CI: 89.8-100%[1]		
Negative Agreement		100% (57/57); 95% CI: 93.7-100%		

[1]Two-sided 95% score confidence intervals

Contrived Clinical Evaluation

Nasopharyngeal Swabs

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 17.

Table 17. 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 (2X LoD)	10	Positive (%)	10/10 (100%)	10/10 (100%)	10/10 (100%)
		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)

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

To support home use of the Oragene Dx OGD-510 collection device 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 18. Summer Temperature Excursion*

Temperature	Cycle Period	Cycle Period Hours	Cumulative 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

*Shipping conditions for cycle periods 2 through 5 are modeled after ISTA 7D 2007 shipping standard (48 hour domestic freight transport) where for cycle period 3 and 5 the temperature has been increased from 35°C to 40°C. The cycle period 1 (8 hours) has been included for the time delay between collection of the sample and shipment of the sample. The remaining time (48 hours) covers the domestic shipment within the continental US. Cycle periods are sequential with the "cycle period hours" required per cycle listed in the table. After each cycle period, the "total time hours" increments by the number of hours in the cycle period.

[1] Sum of cycle periods

Shipping Study Using Contrived Saliva Samples

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, following

shipping at ambient conditions, from individuals that had tested negative on a third party assay (Accurate Diagnostics-sent to Rutgers Clinical Genomics Laboratory). The saliva specimens were also screened negative using the Phosphorus 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 18. 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 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 19-21 for each claimed extraction method.

Table 19. 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 30 copies/µL	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)
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 18
[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 20. 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 18
[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 21. 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 2X LoD 10 copies/µL	Day 0 (RT)[1]	10	34.94 (0.13)	35.50 (0.06)	21.31 (0.20)	10/10 (100)
	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 18
[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 performance of the Phosphorus COVID-19 RT-qPCR Test was not impacted when testing specimens that had underwent a summer thermal excursion compared to contrived specimens 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.

LIMITATIONS:

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

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratory;
- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.