ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY COVID-19 RT-PCR TEST (LABORATORY CORPORATION OF AMERICA)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

(The COVID-19 RT-PCR test (LabCorp Laboratory Test Number: 139900) will be performed at the Center for Esoteric Testing in Burlington, North Carolina, or other laboratories designated by LabCorp that are also certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The COVID-19 RT-PCR test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate, nasal aspirate, nasal swabs, or mid-turbinate swabs) collected from individuals suspected of COVID-19 by their healthcare provider.

This test is also for use with the Pixel by LabCorp COVID-19 test home collection kit to self-collect nasal swab specimens at home by individuals when determined by a healthcare provider to be appropriate based on results of a COVID-19 questionnaire and the LabCorp At Home COVID-19 test home collection kit to self-collect nasal swab specimens at home when directly ordered by a healthcare provider.

Testing is limited to the Center for Esoteric Testing, Burlington, NC, or other laboratories designated by LabCorp that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the 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.

Testing with the COVID-19 RT-*PCR* test is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time

PCR and *in vitro* diagnostic procedures. The COVID-19 RT-PCR is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Pixel by LabCorp COVID-19 Test Home Collection Kit will only be dispensed to patients meeting the inclusion criteria based on the information provided through the Pixel website COVID-19 questionnaire and reviewed by the Physician Wellness Network (PWN). The PWN will determine test eligibility and write prescriptions for testing. PWN will also follow up all positive and inconclusive test results by contacting the patients. Negative patients will be notified by email, phone message and through the website portal.

The LabCorp At Home COVID-19 Test Home Collection Kit will be dispensed to patients when prescribed by their physician using the LabCorp provider interface to order diagnostic tests. Once the physician order is placed, LabCorp will mail the home collection kit to the patient, who will perform the sample collection and mail it back to LabCorp. LabCorp will then report test results back to the ordering physician and to the patient via the LabCorp patient portal.

The Pixel by LabCorp COVID-19 Test Home Collection Kit and the LabCorp At Home COVID-19 Test Home Collection Kit is composed of a shipping box, pre-labeled return envelope, directions, specimen collection materials (nasal swab and saline tube), , and specimen biohazard bag. Instructions are included in the kit to direct the home users on how to appropriately collect the nasal swab specimen and place it in the saline transport tube, how to properly package the specimen and how to mail the specimen back to the laboratory using the pre-labeled FedEx return envelope.

The COVID-19 RT-PCR Test is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The test can be run in a singleplex format (three individual assays) or multiplexed into a single reaction and amplification set up. In a singleplex format, the test uses three primer and probe sets to detect three regions in the SARS-CoV-2 nucleocapsid (N) gene and one primer and probe set to detect human RNase P (RP) in a clinical sample. When multiplexed into a single reaction, the test uses two primer and probe sets to detect two regions in the SARS-CoV-2 N gene and one primer and probe set to detect RP. RNA isolated from upper and lower respiratory specimens (such as nasal, nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) is reverse transcribed to cDNA and subsequently amplified using Applied Biosystems QuantStudio7 Flex (QS7) instrument with software version 1.3. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by QS7.

INSTRUMENTS USED WITH TEST

The COVID-19 RT-PCR test is to be used with the Roche MagNA Pure-96 (MP96) using MagNA Pure 96 DNA and Viral NA Small Volume Kit and Applied Biosystems QuantStudio7 Flex (QS7) instrument with software version 1.3 in a singleplex format.

When the COVID-19 RT-PCR test is multiplexed into a single reaction, it is automated on the Hamilton Microlab star liquid handler and uses two extraction methods: 1) Thermo Fisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit on the Thermo Fisher KingFisher Flex instrument; 2) MagNA Pure 96 DNA and Viral NA Small Volume Kit on the Roche MagNA Pure-96 (MP6), and Applied Biosystems QuantStudio7 Flex (QS7) instrument with software version 1.3.

COLLECTION KITS USED WITH THE TEST

- This test can be used with the Pixel by LabCorp COVID-19 Test Home Collection Kit to self-collect nasal swab specimens at home.

- This test can be used with the LabCorp At Home COVID-19 test home collection kit to self-collect nasal swab specimens at home or in a healthcare setting.

REAGENTS AND MATERIALS

Pixel by LabCorp COVID-19 Test Home Collection Kit and LabCorp At Home COVID-19 Test Home Collection Kit

Reagent	**Manufacturer**	**Catalog #**
Shipping box	Therapak	23586G
Return envelope	FedEx	163034
Specimen biohazard bag	Therapak	16019G
Nasal swab	Super Brush	59-1187-BULK
Saline and tube	Sarstedt	51.550.123

COVID-19 RT-PCR test

Reagent	**Manufacturer**	**Catalog #**
DNA and Viral Small Volume Kit (3x192 purifications)	Roche	06543588001
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	Thermo Fisher	A42352 or A48310
TaqPath™ 1-Step Multiplex Master Mix (No ROX)	Thermo Fisher	A28523
COVID-19_N1-F Primer	IDT	Custom
COVID-19_N1-R Primer	IDT	Custom
COVID-19_N1-P Probe	IDT	Custom

COVID-19_N2-F Primer	IDT	Custom
COVID-19_N2-R Primer	IDT	Custom
COVID-19_N2-P Probe	IDT	Custom
COVID-19_N3-F Primer	IDT	Custom
COVID-19_N3-R Primer	IDT	Custom
COVID-19_N3-P Probe	IDT	Custom
RP-F Primer	IDT	Custom
RP-R Primer	IDT	Custom
RP-P Probe	IDT	Custom
COVID-19_N_Positive Control	IDT	Custom
Hs_RPP30_Internal Extraction Control	IDT	Custom

PATIENT INCLUSION/EXCLUSION CRITERIA:
Applies to patients using Pixel product for home collection
These criteria are based on current CDC testing guidelines (criteria below in use as of 4/17/20)
Exclusion:

- Patients with no symptoms and no exposures
- Individuals with severe symptoms (will be directed to seek immediate care)

Inclusion:

- All patients with "mild" symptoms
- Patients with exposure risks and no symptoms

INSPECTION OF SPECIMENS:
Applies to specimens received from patients using home collection kit
Specimen received through the Pixel by LabCorp Home Collection Kit and LabCorp At Home COVID-19 Test Home Collection Kit should be checked for the following criteria before entering the work flow:

- No saline collection tube included within the Pixel kit or LabCorp At Home kit
- No swab included within saline collection tube
- No registration code attached to the saline collection tube
- Saline collection tube leaked resulting in no sample for testing
- Kit not registered on Pixel platform (would have to freeze the sample until Pixel customer service contacts customer)
- Accession date is greater than 1 calendar day from the specimen collection date

CONTROLS TO BE USED WITH THE COVID-19 RT-PCR

1) A negative (no template) control is needed to eliminate the possibility of sample contamination on the assay run and is used on every assay plate. This control is molecular grade, nuclease-free water.

2) A positive template (COVID-19_N_P) control is needed to verify that the assay run is performing as intended and is used on every assay plate starting at master mix addition at a concentration of 50 copies/uL. The positive template control does not include RNase P target and will result as "undetermined" for that marker.

3) An internal (Hs_RPP30) control targeting RNase P is needed to verify that nucleic acid is present in every sample and is used for every sample processed. This also serves as the extraction control to ensure that samples resulting as negative contain nucleic acid for testing.

4) A negative extraction (NEC) control is a previously characterized negative patient sample. It serves both as a negative extraction control to monitor for any cross-contamination that occurs during the extraction process, as well as an extraction control to validate extraction reagents and successful RNA extraction.

INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

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

Negative (no template control) – negative for all targets detected (Ct Not Detected)
Positive (COVID-19_N_P) – positive for all targets detected (Ct < 38)
Internal extraction (Hs_RPP30) – negative for SARS-CoV-2 targets (Ct Not Detected), positive for RNase P (RP) target (Ct < 40)
Negative extraction (NEC) – negative for SARS-CoV-2 targets (Ct Not Detected), positive for RNase P (RP) target (Ct < 40)

If any control does not perform as described above, run is considered invalid and all specimens are repeated from extraction step.

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

RP – all clinical samples should yield positive results for RP target at < 40 Ct. Samples that fail to show detection of RP and all three SARS-CoV-2 targets within this range should be repeated from extraction step. If sample detects any of the SARS-CoV-2 targets, the lack of amplification of RP target can be valid.

MULTIPLEX						
SARS-CoV-2 N1 (FAM)	**SARS-CoV-2 N2 (YY)**	**RNase P (Cy5)**	**Result Interpretation**	**Report**	**Actions (specimens from clinical sites)**	**Actions (specimens from Pixel Home Collection Kit)**
+	+	+/-	SARS-CoV-2 Detected	DETECTED	Report results to sender and appropriate public health authorities.	Report results to PWN Health who will call the patient. Report the result to the Pixel portal.

						Report the result to the appropriate public health authorities.
If only one target is positive		+/-	SARS-CoV-2 Indeterminate	INDETERMINATE	Sample is repeated once. If results remain the same, it is reported to sender as indeterminate and recommend recollection if patient is still clinically indicated.	Sample is repeated once. If results remain the same, it is reported to sender as indeterminate to PWN Health who will call the patient. Report the result to the Pixel Portal.
-	-	+	SARS-CoV-2 Not Detected	NOT DETECTED	Report results to sender.	Report results to PWN Health and the Pixel Portal.
-	-	-	Invalid Result	INVALID	Sample is repeated once. If a second failure occurs, it is reported to sender as invalid and recommend recollection if patient is still clinically indicated.	Sample is repeated once. If a second failure occurs, it is reported to PWN Health. Pixel customer service will contact patient to discuss options. Report the result to the Pixel Portal.

If Multiplex reagents are not available singleplex testing will be performed and can be interpreted as described below.

COVID-19 RT-PCR test results interpretation

SINGLEPLEX							
SARS-CoV-2 N1 (FAM)	**SARS-CoV-2 N2 (FAM)**	**SARS-CoV-2 N3 (FAM)**	**RNase P (FAM)**	**Result Interpretation**	**Report**	**Actions (specimens from clinical sites)**	**Actions (specimens from Pixel Home Collection Kit)**
+	+	+	+/-	SARS-CoV-2 Detected	DETECTED	Report results to sender and appropriate public health authorities.	Report results to PWN Health who will call the patient. Report the result to the Pixel portal. Report the result

							to the appropriate public health authorities.
If only one target is positive		+/-	+/-	SARS-CoV-2 Indeterminate	INDETERMINATE	Sample is repeated once. If results remain the same, it is reported to sender as indeterminate and recommend recollection if patient is still clinically indicated.	Sample is repeated once. If results remain the same, it is reported to sender as indeterminate to PWN Health who will call the patient. Report the result to the Pixel Portal.
-	-	-	+	SARS-CoV-2 Not Detected	NOT DETECTED	Report results to sender.	Report results to PWN Health and the Pixel Portal.
-	-	-	-	Invalid Result	INVALID	Sample is repeated once. If a second failure occurs, it is reported to sender as invalid and recommend recollection if patient is still clinically indicated.	Sample is repeated once. If a second failure occurs, it is reported to PWN Health. Pixel customer service will contact patient to discuss options. Report the result to the Pixel Portal.

PERFORMANCE EVALUATION

***1) Analytical Sensitivity*:**

Limit of Detection (LoD) for 400µL added to extraction:

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies(cp)/µL) that can be detected by the COVID-19 RT-PCR test at least 95% of the time. The preliminary LoD was established by testing 10-fold dilutions of SARS-CoV-2 synthetic RNA. The preliminary LoD was confirmed by testing 20 replicates of 2-fold dilutions (50 cp/µL, 25 cp/µL, 12.5 cp/µL, 6.25 cp/µL, 3.125 cp/µL, and 1.25 cp/µL). The samples of 2-fold dilutions were prepared by spiking the quantified live SARS-CoV-2 into negative respiratory clinical matrices (NP swabs and BAL). The study results showed that the LoD of the COVID-19 RT-PCR test is 6.25 cp/µL

(19/20 positive). Additionally, LoD of the COVID-19 RT-PCR test (in a multiplex format) was evaluated using both extraction methods: 1) Thermo Fisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit on the Thermo Fisher KingFisher Flex instrument and 2) MagNA Pure 96 DNA and Viral NA Small Volume Kit on the Roche MagNA Pure-96 (MP6). Both extracted methods (in a multiplex format) generated the same LoD of 6.25 cp/µL or 31.25 copies/reaction for NP swabs and 12.5 cp/µL or 62.5 copies/reaction for BAL.

Limit of Detection (LoD) for 200µL added to extraction:
To determine the limit of detection, a well characterized positive sample ($2x10^5$ copies/µL) was diluted into negative sample matrix (BAL and UTM – NP swab) to concentrations of 125, 62.5, 31.25, and 15.625 copies/reaction. Each contrived sample was then extracted using the Low Volume MagMax method. The results of the Limit of Detection Validation produced a limit of detection of 15.625 copies/reaction for BAL and UTM.

Low Volume MagMax	125 cp/rxn	62.5 cp/rxn	31.25 cp/rxn	15.625 cp/rxn
BAL	10/10	10/10	10/10	10/10
UTM	10/10	10/10	10/10	10/10
Total	**20/20**	**20/20**	**20/20**	**20/20**

2) ***Analytical Specificity*:**

Cross-reactivity of the COVID-19 RT-PCR test was evaluated using both *in silico* analysis and by testing whole organisms or purified nucleic acid from a panel of organisms listed in the table below.

The empirical testing showed that all targets were negative for all tested microorganisms except for the SARS coronavirus which is expected to react with N3 target (target for the universal detection of SARS-like viruses) of the COVID-19 RT-PCR test.

Cross-reactivity test results:

Sample Name	N1 CT	N2 CT	N3 CT	Source (Concentration)
Adenovirus 11	Not detected	Not detected	Not detected	ATCC VR-12D (1e^6)
Adenovirus 5	Not detected	Not detected	Not detected	ATCC VR-5D; Adenoid 75 (1.5e^6)
Bordetella pertussis	Not detected	Not detected	Not detected	Patient Sample (1e^5)
Chlamydophila pneumoniae	Not detected	Not detected	Not detected	ATCC 53592D; AR-39 (5e^6)

Enterovirus 70	Not detected	Not detected	Not detected	ATCC VR-836; J670-71 (1e^6)
Haemophilus influenzae	Not detected	Not detected	Not detected	ATCC 51907D (1e^6)
Human coronavirus	Not detected	Not detected	Not detected	ATCC VR-740; 229E (1e^6)
Human coronavirus	Not detected	Not detected	Not detected	ATCC VR-3263SD; NL63 (7e^5)
Human coronavirus	Not detected	Not detected	Not detected	ATCC VR-3262SD; HKU1 (6e^5)
Human coronavirus	Not detected	Not detected	Not detected	Patient Sample; OC43 (1e^5)
Human metapneumovirus	Not detected	Not detected	Not detected	ATCC VR-3250SD (6e^5)
Human parainfluenza virus 1	Not detected	Not detected	Not detected	ATCC VR-94D; C35 (2e^7)
Human parainfluenza virus 2	Not detected	Not detected	Not detected	ATCC VR-92D; Greer (2e^7)
Human parainfluenza virus 3	Not detected	Not detected	Not detected	ATCC VR-1782; ATCC-2011-5
Human parainfluenza virus 4b	Not detected	Not detected	Not detected	ATCC VR-1377; CH 19503
Human respiratory syncytial virus	Not detected	Not detected	Not detected	ATCC VR-1580; 18537
Human rhinovirus 61	Not detected	Not detected	Not detected	ATCC VR-1171; 6669-CV39
Influenza A	Not detected	Not detected	Not detected	ATCC VR-1679D; H3N2, A/Hong Kong/8/68 (2e^6)
Influenza B	Not detected	Not detected	Not detected	ATCC VR-1735D; B/Taiwan/2/62 (3e^6)
Legionella pneumophila	Not detected	Not detected	Not detected	ATCC 33152D-5; Philadelphia-1 (1.5e^6)
Middle East Respiratory Syndrome coronavirus	Not detected	Not detected	Not detected	ATCC VR-3248SD; MERS (6e^5)
Mycobacterium tuberculosis	Not detected	Not detected	Not detected	ATCC 25177; H37Ra
Mycoplasma pneumoniae	Not detected	Not detected	Not detected	ATCC 15531D; FH of Eaton Agent (3e^6)
Severe Acute Respiratory Syndrome coronavirus	Not detected	Not detected	30.768	BEI NR-3882; SARS
Streptococcus pneumoniae	Not detected	Not detected	Not detected	ATCC 33400D-5 (3e^6)
Streptococcus pyogenes	Not detected	Not detected	Not detected	ATCC 12344D-5; T1 (3e^6)

BLAST analysis showed no homology with primers and probes of the COVID-19 RT-PCR test for the organisms listed in the table below.

***In silico* analysis:**

Pathogen	Strain	GenBank Acc#	% Homology Test Forward Primer	% Homology Test Reverse Primer	% Homology Test Probe
Candida albicans	All	All	0	0	0
Neisseria meningitidis	All	All	0	0	0
Pseudomonas aeruginosa	All	All	0	0	0
Staphylococcus aureus	All	All	0	0	0

***3) Clinical Evaluation*:**

A contrived clinical study was performed to evaluate the performance of the COVID-19 RT-PCR test. A total of 100 individual clinical respiratory samples, 50 NP swabs and 50 BALs, were used in this study. 100 negatives and 80 contrived positives were tested. Negative samples include 50 NP swabs and 50 BALs. Positive samples were comprised of 40 NP swabs and 40 BALs spiked with quantitated live SARS-CoV-2. 10 samples each were spiked at 8x, 4x, 2x, and 1X LoD. In one contrived BAL sample, prepared at LoD, N3 target was not determined. The positive and negative percent agreements between the COVID-19 RT-PCR test and the expected results in NP swabs and BALs are shown below:

Clinical performance of the COVID-19 RT-PCR test with NP swabs:

	SARS-CoV-2 concentration	Number of NP swabs	N1 target % Positive (95% CIs)	N2 target % Positive (95% CIs)	N3 target % Positive (95% CIs)
COVID-19 RT-PCR test	1x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	2x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	4x LoD	10	100% (72.25 –	100% (72.25 –	100% (72.25 –

			100)	100)	100)
	8x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	Negative	50	0 (NA)	0 (NA)	0 (NA)

NA = Not available

Performance of the COVID-19 RT-PCR test against the expected results are:

Positive Percent Agreement 40/40 = 100% (95% CI: 91.24% - 100%)
Negative Percent Agreement 50/50 = 100% (95% CI: 92.87% - 100%)

Clinical performance of the COVID-19 RT-PCR test with BAL specimens:

	SARS-CoV-2 concentration	Number of NP swabs	N1 target % Positive (95% CIs)	N2 target % Positive (95% CIs)	N3 target % Positive (95% CIs)
COVID-19 RT-PCR test	1x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	90%* (59.59 – 98.22)
	2x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	4x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	8x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	100% (72.25 – 100)
	Negative	50	0 (NA)	0 (NA)	0 (NA)

NA = Not available
*One BAL sample had failed detection of N3 target. Since the SARS-CoV-2 specific targets, N1 and N2 were detected, the overall result for this sample was “POSITIVE”.

Performance of the COVID-19 RT-PCR test against the expected results are:

Positive Percent Agreement 40/40 = 100% (95% CI: 91.24% - 100%)
Negative Percent Agreement 50/50 = 100% (95% CI: 92.87% - 100%)

Additionally, five positive and five negative patient samples were sent to the North Carolina Department of Health (NCDOH) and tested on the CDC assay under an EUA. All results were concordant.

Sample	COVID-19 RT-PCR test	NCSLPH Result CDC assay under and EUA

1	Not detected	Not detected
2	Not detected	Not detected
3	Positive	Presumptive Positive
4	Not detected	Not detected
5	Positive	Presumptive Positive
6	Positive	Presumptive Positive
7	Not detected	Not detected
8	Positive	Presumptive Positive
9	Not detected	Not detected
10	Positive	Presumptive Positive

4) ***Comparison between Singleplex and Multiplex COVID-19 RT-PCR test*:**

A total of 93 clinical nasopharyngeal (NP) samples were evaluated in this comparison study. Each patient sample was tested by the COVID-19 RT-PCR test using both singleplex and multiplex formats and the results of the two were compared. This comparison showed 100% concordance between singleplex and multiplex test results for a clinical sample. Of the 93 clinical samples, 16 generated positive results and 77 generated negative results by the COVID-19 RT-PCR test using both singleplex and multiplex formats.

5) ***Clinical Validation for 200µL extraction volume:***

One plate was run for clinical concordance. A total of 93 samples were on the plate, including 36 positives. The results of the clinical comparison were 100% concordant. 36/36 positives were called along with 57/57 negatives. An observed Ct shift of 1 Ct is observed with the reduced extraction volume but did not affect clinical sample results, even at samples near the limit of detection.

6) ***Self-Collection Validation*:**

30 participants were enrolled in a self-collection study. After signing a consent form, the participants were presented with 2 saline tubes, 2 cotton swabs and the instructions provided in the Pixel COVID-19 Test Home Collection Kit. 6 participants were given an additional tube for a total of 3 tubes to test the effect of shipping samples without a gel pack. After sample collection, 1 of the 2 (or 2 of the 3) collection tubes were spiked with a known COVID-19 positive sample in the laboratory after clinical matrix was collected from participants. These samples were then packaged up as described above and shipped back to the lab via FedEx (transit time, 72hr) where they were unpacked and tested using the FDA Authorized LabCorp COVID-19 RT-PCR Test, non-multiplexed version.

This study evaluated the users' ability to properly collect a swab, and shipping stability while the sample is in possession of FedEx (not while in the drop box). This

study also evaluated if swab material (cotton) could cause any false positive or false negative reactions or if it could impact the assay's internal controls.

The results of the self-collection validation were consistent with expected results. All positives (36/36) remained positive 72 hours post shipping. No false positives were detected (30/30). All samples had strong Human RnaseP signals indicating all participants were successful in collecting human biological material. Samples shipped without a gel pack (a worst-case shipping condition) showed no change in result for either RnaseP or COVID-19 targets.

7) ***Sample shipping stability study:***

Sample stability studies were conducted at high temperatures to confirm that samples shipped using a FedEx drop box will not generate false results and there will be minimal to no loss of signal for positive specimens.

Positive sample matrix was created by diluting a well characterized positive sample (2e5cp/uL) to 1e3cp/uL into negative clinical matrix. [The well characterized sample was tittered using a known concentration synthetic target to generate a standard curve and then comparing the clinical sample to the standard curve to calculate a titer.]

Negative clinical matrix was generated by participants who swabbed each nostril with the Pixel Kit cotton swab according to the Pixel Kit directions. Individual samples for testing were generated by placing the 'used' cotton swab into 3mL of saline, positive samples were spiked with the well characterized positive sample to reach a final concentration of 10cp/uL (<2xLoD). Twenty positive specimens (samples 1-20) and twenty negative specimens (samples 21-40) were included in the study.

The temperature excursion was performed in an oven according to the table below:

Temperature	Cycle	Cycle Time (time at temperature)	Total Experimental Time Elapsed
40°C	1	6 hours	6 hours
22°C	2	16 hours	22 hours
40°C	3	2 hours	24 hours
35°C	4	22 hours	46 hours
40°C	5	4 hours	50 hours

Testing was performed using the multiplexed version of the assay. The results of the temperature excursion validation were as expected. 20/20 contrived positives were detected after 50hrs of cycling in and out of high temperature. Similarly, 20/20 negatives were negative for both targets at all time points with RnaseP. Ct values for RnaseP were <40 indicating good sample collection by lay participants. No apparent degradation of signal was observed over the temperature excursion time course as observed by no increase in Ct value at 50 hours.

8) ***Validation of New Foam Swabs for Shipping Stability:***

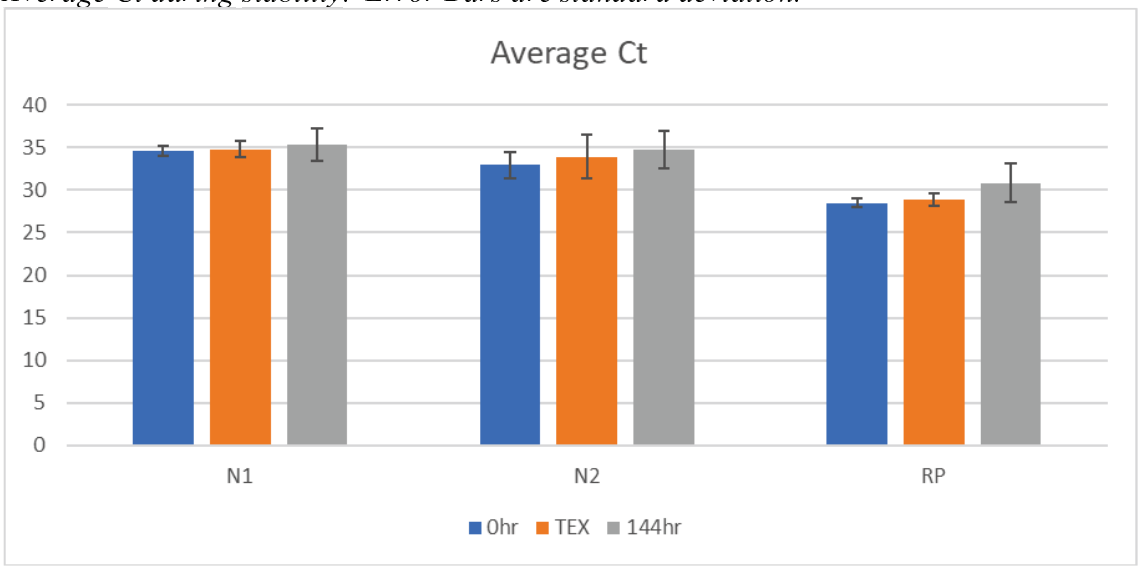
A total of 40 samples, were collected by participants who swabbed their anterior nasal cavity according to the Pixel kit instructions, 20 participants were given the Azer Foam swab and 20 participants were given a Purtian Foam swab (swab type used in Quantigen study). 20 positive samples (10 for each swab manufacturer) were then contrived by pipetting positive sample matrix (30 µL) into the tube followed by vortexing. The positive sample matrix was created by the dilution of a well characterized positive sample (2e5 cp/µL) to 1e3 cp/µL. Because the tubes contained approximately 3 mL of saline, this results in a final concentration of approximately 50 copies/reaction which is <2x the 31.25 copies/reaction LOD of the LabCorp COVID-19 test.

Temperature Excursion Conditions

Temperature	Cycle Period	Cycle Period Hours	Total Time Hours
40°C	1	6	6
22°C	2	16	22
40°C	3	2	24
35°C	4	22	46
40°C	5	4	50

The 20/20 negatives remained negative throughout the time-course for both swabs with Ct values for the RP internal control <40 indicating good sample collection. The 20/20 positives were positive at time 0, after the temperature excursion, and after sitting at 4 °C for an additional 94 hrs (total of 144 hrs). The average Ct valuess for N1, N2 and RP did not deviate substantially throughout the stability experiment.

Average Ct during stability. Error Bars are standard deviation.



WARNINGS:

- This home collection kit has not been FDA cleared or approved.
- This home collection kit has been authorized by FDA under an EUA. This home collection kit has been authorized only for the home collection and maintenance of nasal swab specimens as an aid in detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This home collection kit in combination with the authorized test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests 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