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, and meet the requirements to perform high complexity tests, as described in the laboratory procedures that were 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) collected from individuals suspected of COVID-19 by their healthcare provider (HCP), as well as upper respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, nasal swabs, or mid-turbinate swabs) collected from any individual, including for testing of individuals without symptoms or other reasons to suspect COVID-19 infection.

This test is also for use with individual nasal swab specimens that are self-collected by individuals using the LabCorp At Home COVID-19 test home collection kit when directly ordered by a HCP.

The COVID-19 RT-PCR test is also for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled samples, using a matrix pooling strategy (i.e., group pooling strategy), containing up to five individual upper respiratory swab specimens (nasopharyngeal, mid-turbinate, nasal or oropharyngeal swabs collected using individual vials containing transport media) per pool and 25 specimens per matrix. Nasal swab specimens are collected in individual vials containing transport media either under observation by a HCP or self-collected using a home collection kit authorized for use with this test.[1] Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools where the positive sample cannot be

[1] Home collection kits currently authorized for use with this test include the LabCorp At Home COVID-19 test home collection kit and the Pixel by LabCorp COVID-19 Test Home Collection Kit. Please note that LabCorp, authorized laboratories, and authorized distributors must follow the terms and conditions set forth in EUA 203057 concerning specimens collected using the Pixel by LabCorp COVID-19 Test Home Collection Kit.

identified using the matrix must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

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, and meet the requirements 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 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 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 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 the 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.

DESCRIPTION OF POOLING

Traditionally, pooling employs a 2-stage approach where samples are tested as pools and then any positive pools are retested at a later time to determine which individual was positive. While this approach saves reagents, it is not practical to implement in a high throughput testing environment where many thousands of samples would need to be pulled and retested every day. Matrix based pooling strategies allow the lab to test samples as pools while preventing the need to retest individual samples as long as the expected (and observed) number of positive samples per matrix is less than or equal to 1 (Table 5). To combat the retest problem, LabCorp will employ a matrix pooling strategy where samples will be tested twice in pools of 4 samples which increases lab efficiency by a factor of 2 if the tested population prevalence remains < 6% (Table below).

	Expected Positives			
Prevalence (%)	**3x3**	**4x4**	**5x5**	**10x10**
0.1	0.009	0.016	0.025	0.1
0.5	0.045	0.08	0.125	0.5
1	0.09	0.16	0.25	1
3	0.27	0.48	0.75	3
5	0.45	0.8	1.25	5
10	0.9	1.6	2.5	10
15	1.35	2.4	3.75	15
Throughput	**1.5**	**2**	**2.5**	**5**

Matrix Based Pooling Strategies Increase Throughput Without Requiring Retesting. Green - <1 positive per matrix at indicated prevalence, red - >1 positive per matrix at indicated prevalence

Matrix based pooling as described below will be performed in the laboratory using liquid handling robots and will not be performed or analyzed by hand. Using a 4x4 matrix as an example (Figure A), 16 samples are arranged in a 4x4 grid. Each sample is then combined into horizontal (rows) and vertical (columns) pools to create X and Y positional information for each sample. As long as no more than 1 sample per matrix is

positive, an individual positive can be ascertained without retesting any of the pools (Figure B). If there are 2 positives in a matrix, 12.5% of the time (1/8), both positives can be ascertained if they fall in either the same row or the same column (Figure C) while 87.5% of the time they will result in an equivocal result (Figure D). If 4 or more pools (2 per row or column set) return positive, all samples in each equivocal pool must be retested to determine which are positive (Figure D). If one or more row or column pools returns positive without a corresponding row or column pool returning positive (No X/Y intersection), then all samples within the positive pools must be retested as individuals (Figure E).

Figure A: 4x4 Matrix Example. Arrows – Indicate pooling direction. Boxes outside matrix grid represent the final pools

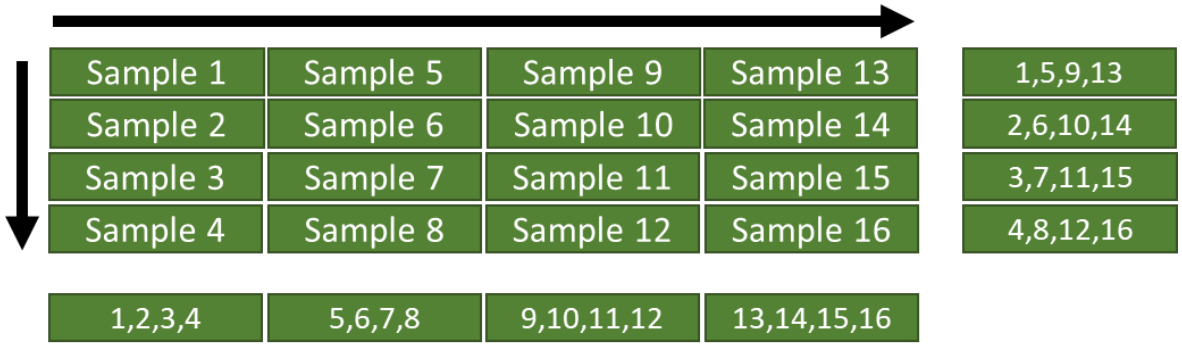


Figure B: Unequivocal Identification in a 4x4 matrix. Red – positive sample or pool

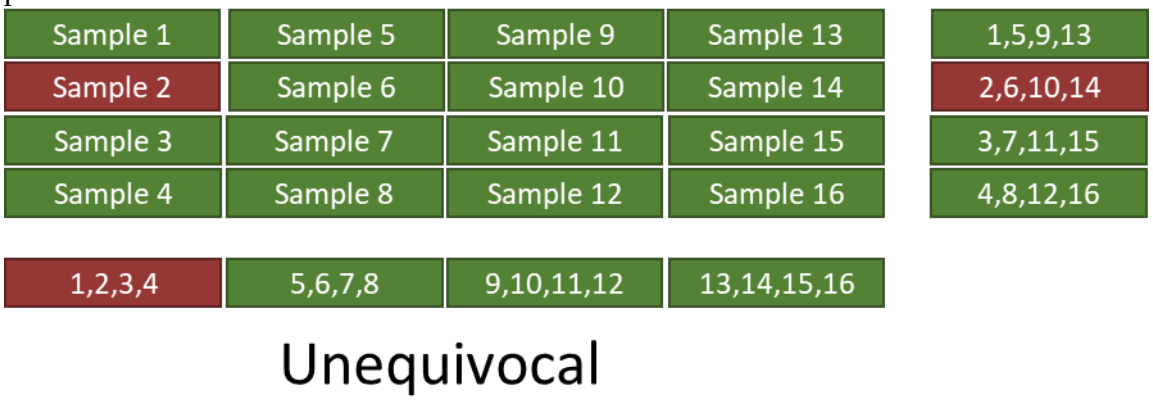


Figure C: Unequivocal identification in a 4x4 matrix when 2 samples are positive. Red – positive sample or pool

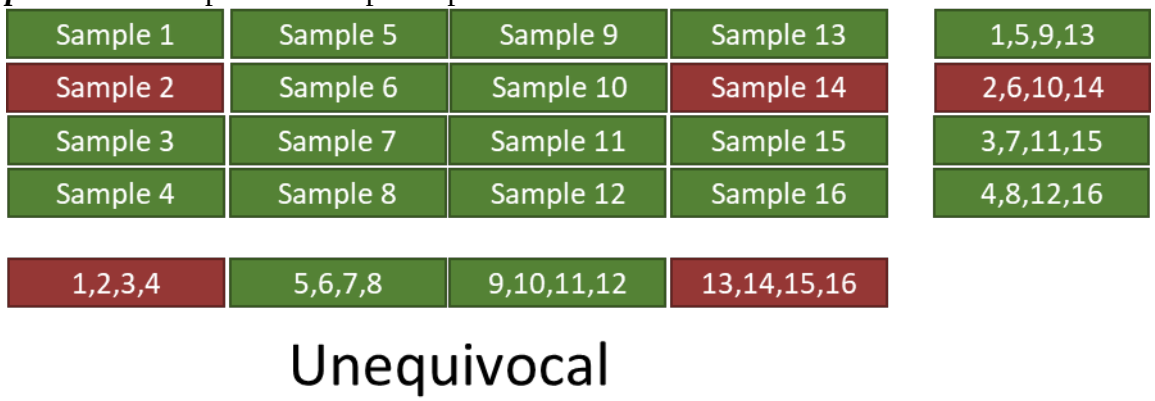


Figure D: Equivocal identification in a 4x4 matrix when 2 samples are positive. Red – positive sample or pool. Question Marks – Row and column pools are equivocal, and must be repeated as individuals

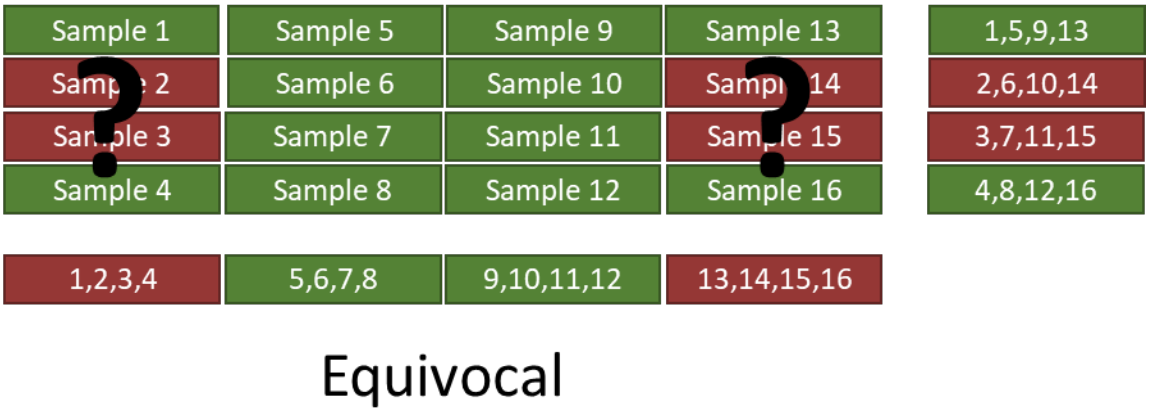
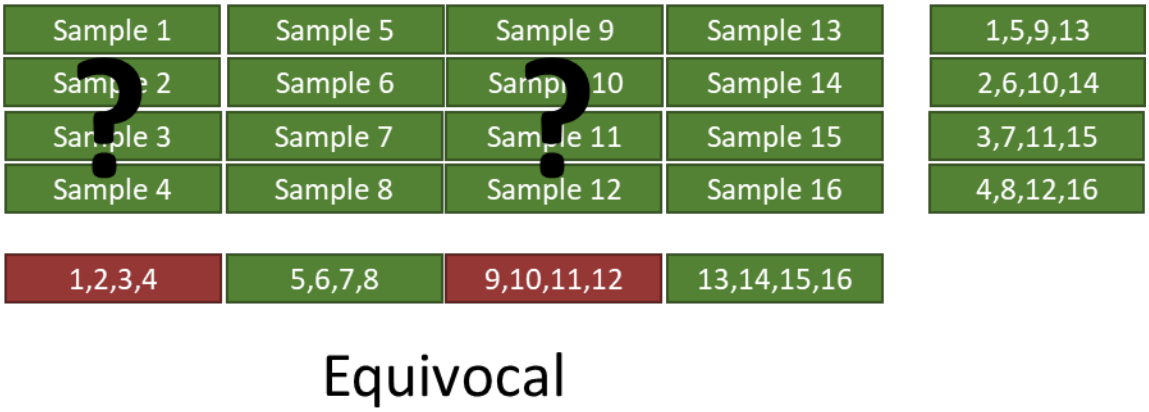


Figure E: Equivocal identification in a 4x4 matrix when no samples are positive. Occurs when 1 or 2 pools are positive without a corresponding row or column resulting

positive. Red – positive sample or pool, Question Marks – Column pools are equivocal, and must be repeated as individuals



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. The COVID-19 RT-PCR test can also be used with the CERES Nanosciences Nanotrap Virus Capture Kit.

Pooling is conducted on the Tecan Evo 200 liquid handling instrument. All pooling of samples is performed before sample extraction.

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 LabCorp At Home COVID-19 test home collection kit to self-collect nasal swab specimens at home or in a healthcare setting when ordered by a HCP. Also refer to footnote 1 above.

REAGENTS AND MATERIALS

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:

Home self-collection with the LabCorp At Home COVID-19 Test Home Collection Kit is intended for individuals 18 years and older when directly ordered by a HCP.

INSPECTION OF SPECIMENS:

Applies to specimens received from patients using home collection kit
Specimen received through the 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 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/µL. The positive template control does not include RNase P target and will result as "undetermined" for that marker.

3) Optional: An internal (Hs_RPP30) control targeting RNase P detects that nucleic acid is present in the sample. This also serves as an extraction control to ensure that samples resulting as negative contain nucleic acid for testing. (This control does not need to be run for pooled samples.)

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)
+	+	+/-	SARS-CoV-2 Detected	DETECTED	Report results to sender and 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.
-	-	+	SARS-CoV-2 Not Detected	NOT DETECTED	Report results to sender.
-	-	-	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.

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)
+	+	+	+/-	SARS-CoV-2 Detected	DETECTED	Report results to sender and 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.
-	-	-	+	SARS-CoV-2 Not Detected	NOT DETECTED	Report results to sender.
-	-	-	-	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.

PERFORMANCE EVALUATION

Some of the validation studies summarized in this EUA Summary to support use of home collection kits with the LabCorp COVID-19 RT-PCR Test were performed with the Pixel COVID-19 Test Home Collection Kit, which is subject of a separate EUA. The Pixel COVID-19 Test Home Collection Kit has the same components and instructions as the LabCorp At Home COVID-19 Test Home Collection Kit, therefore the studies are supportive of both LabCorp home collection kits.

***1) <u>Analytical Sensitivity</u>*:**

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*:**

This test has Right of Reference to the 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC) and the information on in silico inclusivity analysis are contained in that assay EUA summary.

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) <u>Clinical Evaluation</u>:

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)
	1x LoD	10	100%	100%	100%

			(72.25 – 100)	(72.25 – 100)	(72.25 – 100)
COVID-19 RT-PCR test	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

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)
	1x LoD	10	100% (72.25 – 100)	100% (72.25 – 100)	90%* (59.59 – 98.22)
COVID-19 RT-PCR test	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. Six (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/µL) to 1e3cp/µL 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/µL (<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:***

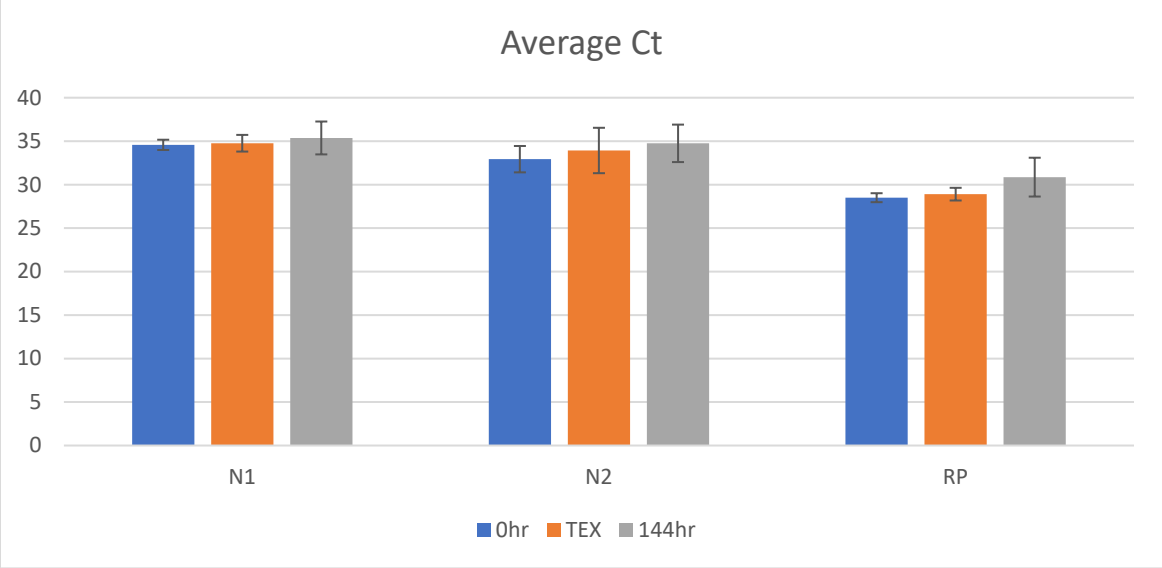
A total of 40 samples, were collected by participants who swabbed their anterior nasal cavity according to the Pixel kit instructions, 20 participants were given the Azer Foam swab and 20 participants were given a Purtian Foam swab (swab type used in Quantigen study). 20 positive samples (10 for each 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 ℃ for an additional 94 hrs (total of 144 hrs). The average Ct values for N1, N2 and RP did not deviate substantially throughout the stability experiment.

Average Ct during stability. Error Bars are standard deviation.



9) Clinical Concordance Validation for Asymptomatic Positive and Negative Samples: Two cohorts of samples were used in the validation of asymptomatic testing. 20 positive results from Pixel screening where the patient indicated that they were asymptomatic, but potentially exposed, are included in this analysis. The samples are dated from June 16th and June 25th. They were collected by reviewing the questionnaires from the Pixel samples received for testing and identified themselves as asymptomatic and also reported as positive. 113 random negative samples were selected from the employee screening initiative as the negative cohort. All samples were run on both the LabCorp COVID-19 RT-PCR Test and the Authorized SARS-CoV-2 Test to compare concordance within asymptomatic testing populations.

The Ct values between symptomatic and asymptomatic samples received at LabCorp for COVID-19 testing were analyzed and the average Ct values for asymptomatic patients were similar to those of a similarly sized population of symptomatic patients (N=39). Positive symptomatic results, 36 symptomatic positives and 57 symptomatic negatives, were evaluated clinically and produced average Cts that were approximately 2 cycles later for asymptomatic patients that tested positive, but low positive samples with high Ct values were still detected (See Table 1 and Figure 1). For each target Ct values were within the standard deviation of the average between symptomatic and asymptomatic positives (See Figure 1). Interestingly, analysis of the RNaseP internal control demonstrated that there was also an observable 2Ct increase in symptomatic individuals (See Figure 1).

Finally, all 133 asymptomatic samples (20 positive and 113 negative) were run on a second comparator assay, an Authorized SARS-CoV-2 Test. Unlike the LabCorp COVID-19 RT-PCR Test, the Authorized test uses a synthetic spike in for its internal control so RNaseP signals could not be compared between test methods. However, all results were 100% concordant with 113 asymptomatic negatives returning

negative on the Roche Cobas SARS-CoV-2 Test and all 20 positives positive by the Authorized SARS-CoV-2 Test (See Table 2).

Table 1. LabCorp COVID-19 RT-PCR Test result for 20 asymptomatic positive samples.

Samples	N1	N2	RP	Samples	N1	N2	RP
Pos1	38.824	38.778	29.768	Pos11	25.403	23.225	30.216
Pos2	22.065	20.943	28.909	Pos12	22.652	20.842	26.835
Pos3	26.897	24.808	27.344	Pos13	23.186	21.647	26.575
Pos4	23.109	21.189	26.814	Pos14	31.352	30.314	26.487
Pos5	27.646	25.852	30.747	Pos15	21.612	19.993	24.893
Pos6	18.524	16.661	36.827	Pos16	22.703	20.972	31.34
Pos7	36.38	35.819	28.054	Pos17	37.678	36.006	30.164
Pos8	18.443	16.849	27.495	Pos18	32.261	31.185	28.707
Pos9	20.893	19.362	24.909	Pos19	19.673	18.251	33.285
Pos10	20.982	18.691	24.01	Pos20	25.016	23.305	23.957

Average Ct for Asymptomatic patients using the LabCorp SARS-CoV-2 RT-PCR Test, N1= 25.76, N2= 24.23 and RP=28.37.

Figure 1: Asymptomatic vs Symptomatic Average Cts. Asym – Asymptomatic, Sym – Symptomatic, RP-RNaseP. Error bars are standard deviation

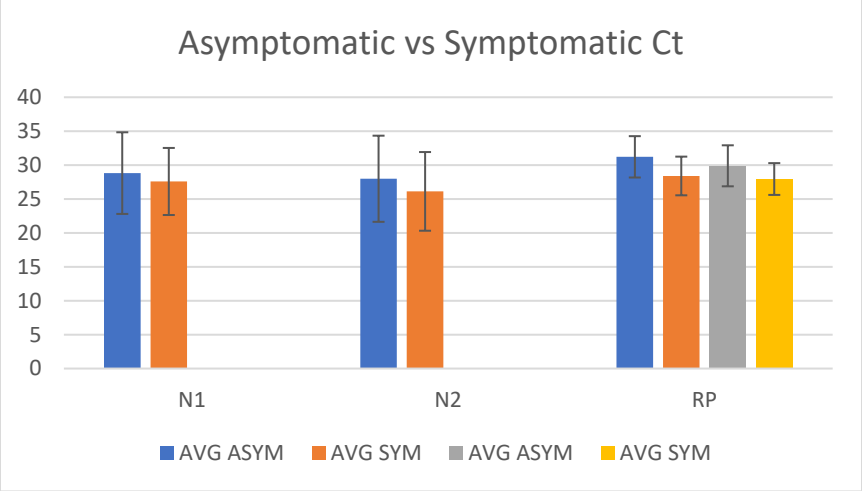


Table 2: Comparison of Authorized SARS-COV-2 test to the LabCorp COVID-19 RT-PCR Test

Asymptomatic Patients		Comparator EUA Assay for SARS-COV-2	
		Positive	Negative
LabCorp COVID-19 RT-PCR Test	Positive	20	0
	Negative	0	113

10) Sample Pooling – Limit of Detection Validation:

To determine the limit of detection when samples are pooled, a well characterized positive sample (2e5 cp/µL) was diluted into negative sample matrix (Saline - 0.9% NaCl) to concentrations of 500, 250, 125, 62.5, and 31.25 cp/RXN. 50uL of each dilution was combined with 20 pools of negative matrix sample for the N=4 pools and 40uL was combined with 20 pools of negative matrix for the N=5 pools. Negative pools were created by combining 145 negative samples together individually into pools of 4 or 5. Pooled samples were then processed using the LabCorp COVID-19 RT-PCR Test. The results of the limit of detection validation produced a limit of detection of 62.5 cp/RXN for both pools of N=4 and N=5 (Table 1). The Limit of Detection of the LabCorp COVID-19 RT-PCR Test on individual samples is 15.625 cp/RXN representing a 4x loss of sensitivity when samples are combined for testing in pools of N=4 or N=5. No difference is observed in the LoD between pools of 4 and 5 because of the limited dilutions tested. Based on the dilution the difference in LoD between pools of 4 and 5 would be less than 16 cp/RXN. Titers with such small differences were not evaluated in this study, resulting in the LoD from n=4 and n=5 being the same, 62.5 cp/RXN.

Table 3. Results of LoD Validation

Copies	**500**	**250**	**125**	**62.5**	**30.125**
N=4	20/20	20/20	20/20	**20/20**	18/20
N=5	20/20	20/20	20/20	**19/20**	13/20

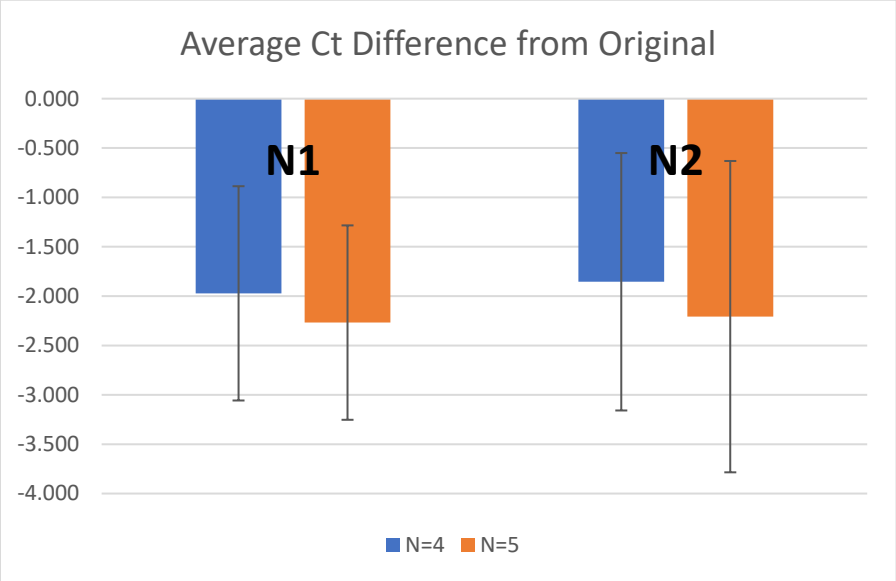
11) Sample Pooling – Clinical Concordance Evaluation of Pooled Samples:

To assess sample pooling with clinical samples, randomly chosen known positives were combined with either 3 or 4 negative samples to create sample pools of N=4 or N=5. The negative sample matrix was created by individually pooling 145 negative clinical samples into 49 pools of N=4 or N=3 before combination with either a single positive or an additional negative to create the final testing pools of N=4 and N=5. 39 positive samples were used for pooling along with 20 negative samples.

For both N=4 and N=5 pools, 38/39 positive pools were positive and 20/20 negative pools were negative. The average difference between the original Ct and the pooled Ct for N=4 was -1.972 for N1 and -1.855 for N2, and for N=5 the average difference

was -2.268 for N1 and -2.208 for N2 (See Figure 1) confirming a slight loss of assay sensitivity due to sample dilution within the pools. The observed Ct difference range for N=4 was -0.461 - 6.063 for N1 and -0.359 - -4.179 for N2. The observed Ct difference range for N=5 was -.0.823 - -4.086 for N1 and 0.959 - -5.634 for N2. For the samples that resulted as indeterminate by pooling, each was positive for N1 but Undetermined for N2. All samples in these pools would be repeated as individuals according to the decision matrix in the LabCorp Sample Pooling SOP.

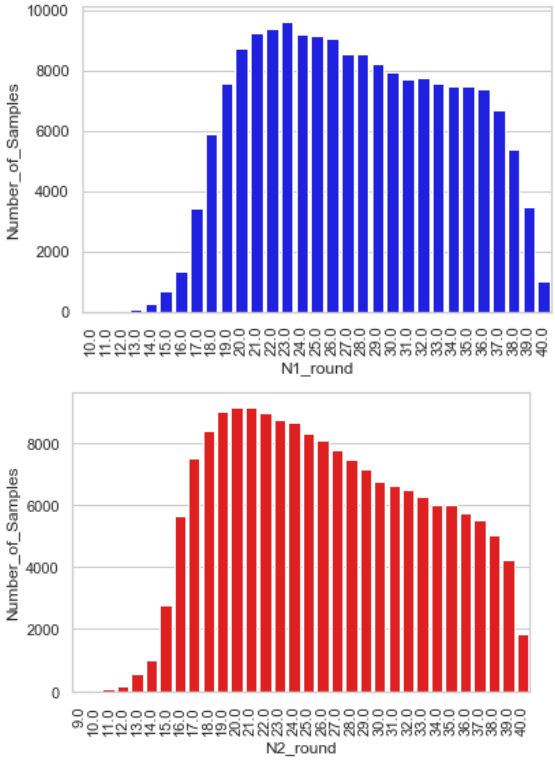
Figure 1: Average Ct Difference. The Average difference from the original N1/N2 Ct and the pooled N1/N2 Ct was calculated for both N=4 and N=5 pools. Error bars are standard deviation.



***In Silico* Retrospective Analysis of Pooled Samples (based on clinical data ΔCt)**

Once the average Ct Difference between pooled and individual samples was determined using clinical samples, an analysis of a large database of regionally diverse clinical positive samples within the LabCorp COVID-19 RT-PCR testing dataset was assayed to calculate what percentage of positive samples might be missed due to sample Ct dilution by combining samples into pools of N=4 or N=5. The average N1 and N2 Ct difference (2) from the clinical sample evaluation was added to 178,952 positive sample results (Figure 2) and the number of samples that then had both N1 and N2 Ct >40 was calculated. 4,175 samples resulted in a Ct >40 after this addition, indicating that 2.3% of samples within the dataset would be missed using an N=4 or N=5 pooling strategy.

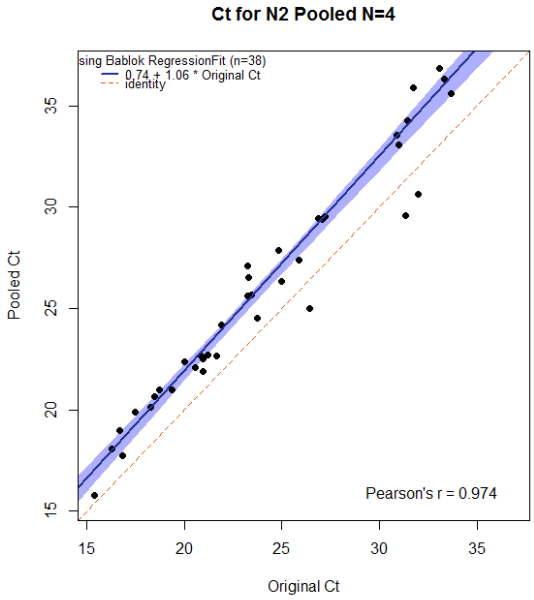
Figure 2: Histogram of N1 and N2 Cts for 148,550 clinical samples. N1 - Blue, N2 – Red.



Regression Analysis using Passing-Bablock Analysis

To rule out any assay bias due to pooling, particularly in low viral concentration samples, a Passing-Bablock regression analysis was performed to compare individual Cts to Pooled Cts for N=4 (Figure 3) and N=5 (Figure 4) pools. Results of this analysis can be seen in table 4; however, the regression slope for N1 and N2 in either N=4 or N=5 pooling strategies is approximately 1 with R2 between 0.96 and 0.98, indicating that there is a linear relationship but Ct change may increase as the Ct value increases, this is observed by the trendline (black, highlighted in purple) from clinical data being drawn above the example dotted red line with a slope of 1.

Figure 3: N=4 Passing-Bablock Analysis



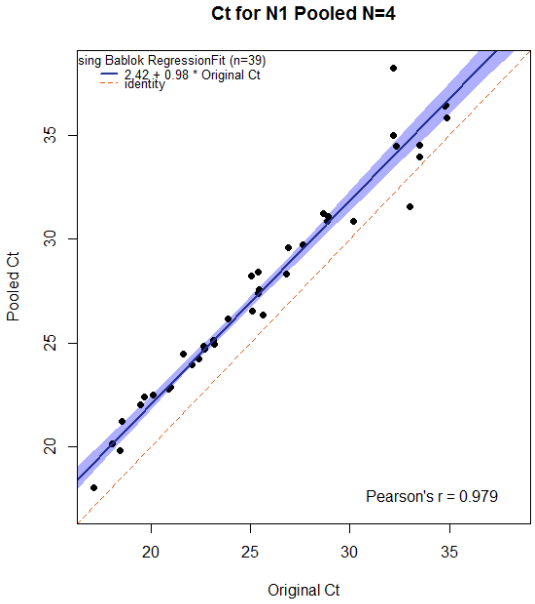
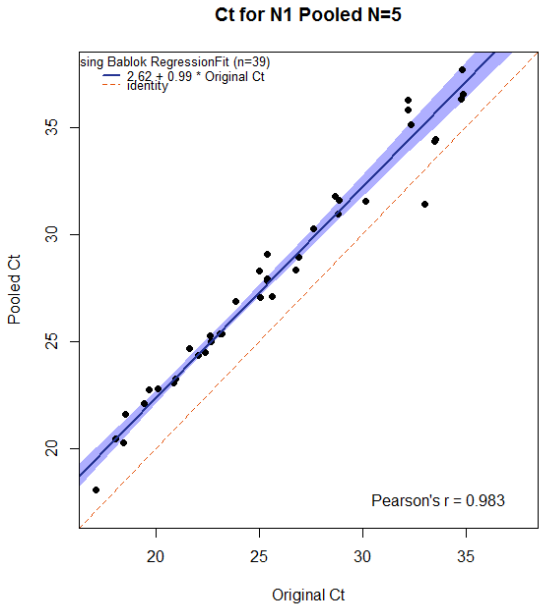


Figure 4: N=5 Passing-Bablock Analysis



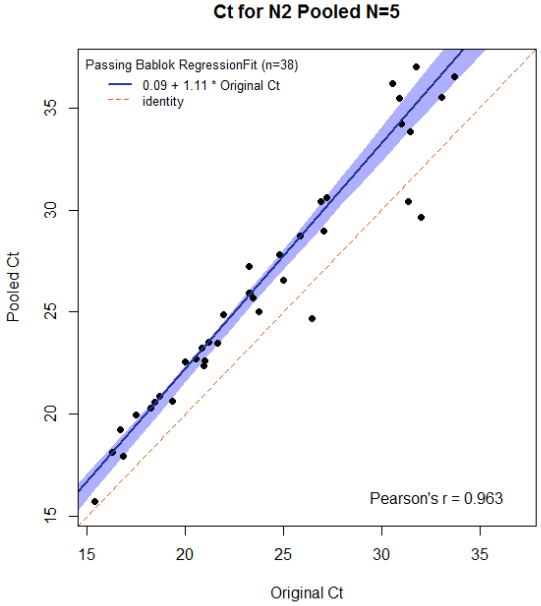


Table 4: Summary of Passing-Bablock Analysis. LCI and UCI are estimates of upper and lower 95% confidence intervals for the slope and Intercept. The R2 value is a Pearson's r estimated from the model.

Target	Pool Size	Intercept	Intercept LCI	Intercept UCI	Slope	Slope LCI	Slope UCI	R2
N1	**4**	2.423	0.914	3.779	0.981	0.928	1.046	0.979
N2	**4**	0.745	-0.805	2.130	1.061	0.999	1.122	0.974
N1	**5**	2.620	1.018	4.273	0.986	0.921	1.057	0.983
N2	**5**	0.095	-2.130	1.619	1.107	1.027	1.203	0.963

***In Silico* Retrospective Analysis of Pooled Samples (based on Passing-Bablock Analysis ΔCt)**

Once the regression analysis was performed, an analysis of a large database of regionally diverse clinical positive samples within the LabCorp COVID-19 RT-PCR testing dataset was assayed to calculate what percentage of positive samples might be missed due to sample Ct dilution by combining samples into pools of N=4 or N=5. To determine how many positive samples might be missed due to sample pooling dilution, the regression equations from the Passing-Bablock analysis were used to calculate how many of 178,952 positive sample results would return false positive if pooled. The calculation below was derived from the slope of the regression line and used to determine the change in Ct to be used to evaluate the positive samples *in silico*.

Intervals [X*, 40]
N=4 for N1: 40=0.981X+2.423 => X*=38.3 ΔCt =1.7
For N2: 40=1.061X+0.745 => X*=37.0 ΔCt =3.0
N=5 for N1: 40=0.986X+2.620 => X*=37.9 ΔCt =2.1
For N2: 40=1.107X+0.095 => X*=36.0 ΔCt =4.0

For N=4 pools, 4,568, or 2.55% of samples, would result as negative and for N=5, 7020, or 3.92%, of samples, would result as negative. This results in a **PPA=97.4%** (4451/4568); 95%CI: (96.9%; 97.9%) in Pools of 4 and **PPA=96.1%** (6745/7020); 95% CI: (95.6%; 96.5%) in Pools of 5 when compared to individual samples.

12) Support for Pooling Unobserved Self-Collected Samples:

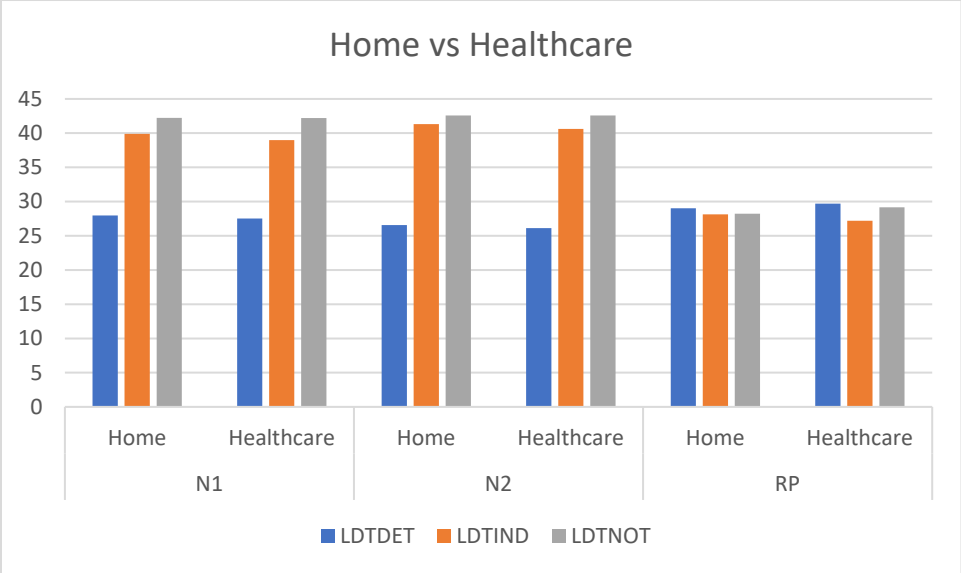
This summary is intended to provide data to document the ability of users to self-collect at home an adequate sample. During pooling, the sample control RNaseP, cannot be evaluated to demonstrate that each sample in the pool was collected adequately. The data presented here demonstrates previous experience at LabCorp that home collected samples are reliable and suitable for use in a sample pooling strategy.

LabCorp has tested approximately 60,000 home collected samples to date. This home collection kit is based on the use of a foam swab and sampling performed in the absence of a healthcare provider. 60,624 home collected samples were analyzed for their result status and RnaseP collection status and compared with a random sampling of 61,999 healthcare collected samples that used the same collection device in the presence of a healthcare worker (Table 5). The mean N1, N2 and RP signals for samples collected in both settings is similar (Figure 1). Most importantly, the number of samples that result as indeterminate or as sample failures are nearly identical between home collected and healthcare collected samples (Home Indeterminate ("LDTIND") – 46 (0.08%), Healthcare Indeterminate ("LDTIND") 55 (0.09%) and Home Failed ("PCRINH")– 23 (0.04%), Healthcare Failed ("PCRINH") 24 (0.04%). This data shows that home collected samples do not have an increase in collection failure and that adequate sample is collected in the absence of observation by a healthcare provider.

Table 5: Results of Home Collected Vs Healthcare Collected sample results. LDTDET – Detected Result, LDTIND – Indeterminate Result, LDTNOT – Not Detected, PCRINH – Sample Failure

	Home Collected				Healthcare Collected			
	Count	**N1**	**N2**	**RP**	**Count**	**N1**	**N2**	**RP**
LDTDET	3910	27.96	26.57	29.03	5315	27.52	26.13	29.7
LDTIND	46 (0.08%)	39.9	41.32	28.14	55 (0.09%)	38.98	40.62	27.2
LDTNOT	56645	42.22	42.58	28.23	56605	42.2	42.58	29.16
PCRINH	23 (0.04%)				24 (0.04%)			41.32

Figure 5: Comparison of Mean Ct value of Home Collected Vs Healthcare Collected samples. LDTDET – Detected Result, LDTIND – Indeterminate Result, LDTNOT – Not Detected.



13) **Validation of New Extraction Method (CERES Nanosciences Nanotrap Virus Capture Kit)**
To determine the limit of detection using CERES Nanotraps, a well characterized positive sample (ATCC NR-52350, qPCR extraction control from SARS-CoV 2, USA-WA1/2020, 3.4e5 cp/µL) was diluted into negative sample matrix (Saline, UTM and BAL) to concentrations of 25, 12.5, 6.25 and 3.125 cp/µL. Each contrived sample was then extracted using the CERES Nanosciences Nanotrap Virus Capture Kit method.

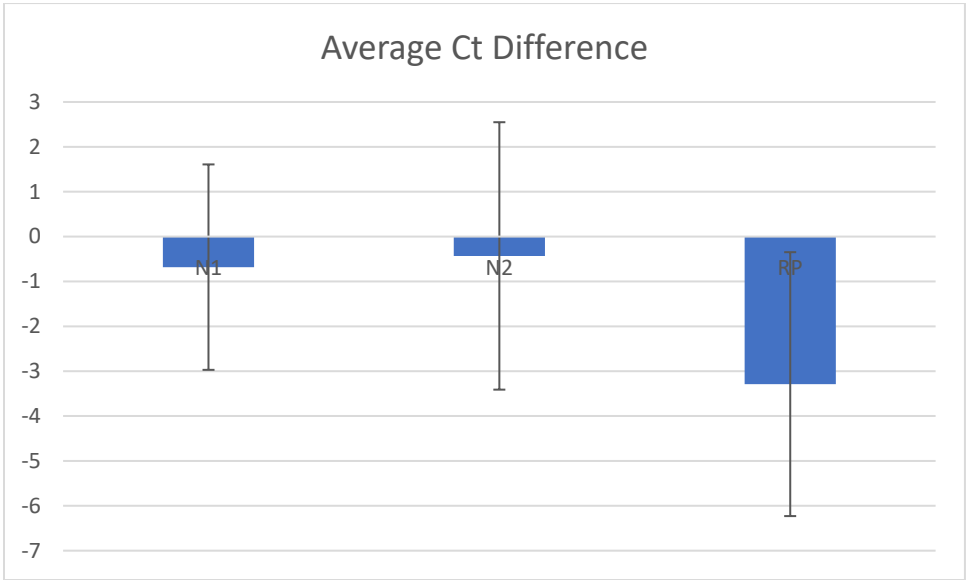
The results of the Limit of Detection Validation produced a limit of detection of 3.125 cp/µL for Saline and UTM and 6.25 cp/µL for BAL.

Table 6: Results of LOD Validation

cp/µL	25	12.5	**6.25**	**3.125**
NP	10/10	10/10	**10/10**	**10/10**
SAL	10/10	10/10	**10/10**	**9/10**
BAL	10/10	10/10	**9/10**	**9/10**

To further validate this extraction method clinical samples were tested using the new extraction method and results were compared to the standard extraction method authorized with the LabCorp COVID-19 RT-PCR test. A total of 93 samples were re-run using the new extraction method, including 11 positives. The results of the clinical comparison were 100% concordant for positives and 100% for negatives; 11/11 positives were called along with 82/82 negatives. The average Ct Difference for N1 was -0.68, for N2 was -0.43 and for RP was -3.28 (Figure 6).

Figure 6: Average Ct Difference. Error bars are standard deviation



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 the Center for Esoteric Testing in 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, and meet the requirements to perform high complexity tests;
- 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.