

**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 (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 (nasopharyngeal or oropharyngeal swabs, anterior nasal swabs, or mid-turbinate swabs) collected from any individual, including individuals without symptoms or other reasons to suspect COVID-19.

This test is also for use with individual anterior nasal swab specimens from individuals age 18 years and older (self-collected), 14 years and older (self-collected under adult supervision), or 2 years and older (collected with adult assistance) using the Labcorp At Home COVID-19 Test Home Collection Kit when directly ordered by an 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, anterior nasal or oropharyngeal swabs collected using individual vials containing transport media) per pool and 25 specimens per matrix. Anterior nasal swab specimens are collected in individual vials containing transport media either under observation by an HCP or self-collected using a home collection kit authorized for use with this test.¹ Negative results from pooled testing should not 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.

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 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 Test 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 with absorbent pad. 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 for each of the three SARS-CoV-2 targets) or multiplexed into a single reaction (containing all 3 SARS-CoV-2 targets) 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 as indicated in the table below.. 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).

Prevalence (%)	Expected Positives			
	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

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 collect nasal swab specimens at home or in a healthcare setting when ordered by an HCP.

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

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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
MagMAX Viral/Pathogen Extraction Kit (MVP II)	Thermo Fisher	A48383
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

Reagent	Manufacturer	Catalog #
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 collection with the Labcorp At Home COVID-19 Test Home Collection Kit is intended for the collection of anterior nasal swabs from individuals age 18 years and older (self-collected), 14 years and older (self-collected under adult supervision), or 2 years and older (collected with adult assistance) when directly ordered by an HCP.

INSPECTION OF SPECIMENS

Applies to specimens received from patients using home collection kit

Specimen received from 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
- 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:**

In the table below, N1 and N2 are considered positive (+) when the Ct < 38 and RP is considered positive (+) when the Ct < 40.

Control Type	External Control Name	N1	N2	RNase P	Interpretation
Negative Control	NTC	-	-	-	Valid Result
Positive Control	COVID-19_N_P	+	+	+	Valid Result
Negative Extraction Control	NEC	-	-	+	Valid Result

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:**

Only if a test run is valid can the results for individual patient samples be interpreted and reported. N1, N2 and RP are considered positive (+) in the table below when the Ct < 40.

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.

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MULTIPLEX					
SARS-CoV-2 N1 (FAM)	SARS-CoV-2 N2 (YY)	RNase P (Cy5)	Result Interpretation	Report	Actions (specimens from clinical sites)
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 and appropriate public health authorities.
-	-	-	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 and appropriate public health authorities.
-	-	-	-	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) Analytical Sensitivity

COVID-19 RT-PCR test (in a singleplex format): Limit of Detection (LoD) for 200 μ L sample added to extraction with the Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies(cp)/ μ L) 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/ μ L, 25 cp/ μ L, 12.5 cp/ μ L, 6.25 cp/ μ L, 3.125 cp/ μ L, and 1.25 cp/ μ L). 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). Replicates were extracted using the Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit on the Roche MagNA Pure-96 (MP96) Instrument. The study results showed in the tables below that the LoD of the COVID-19 RT-PCR test (in a singleplex format) is 6.25 cp/ μ L (19/20 positive).

Positive replicates/total replicates tested at different concentrations of SARS-CoV-2

	50 cp/ μ L	25 cp/ μ L	12.5 cp/ μ L	6.25 cp/ μ L	3.125 cp/ μ L	1.25 cp/ μ L
NP	10/10	10/10	10/10	10/10	3/8	3/11
BAL	10/10	10/10	10/10	9/10	2/8	2/11
Total	20/20	20/20	20/20	19/20	5/16	5/22

Mean Ct at N1 and N2 at different concentrations of SARS-CoV-2

	50 cp/ μ L		25 cp/ μ L		12.5 cp/ μ L		6.25 cp/ μ L		3.125 cp/ μ L		1.25 cp/ μ L	
	N1	N2	N1	N2	N1	N2	N1	N2	N1	N2	N1	N2
NP	31.08	31.28	31.58	31.66	32.86	33.53	37.30	37.65	36.38	37.49	36.66	37.07
BAL	29.90	30.63	31.71	32.20	32.27	32.82	35.72	36.19	37.26	38.82	36.81	39.00

COVID-19 RT-PCR test (in a multiplex format): Limit of Detection (LoD) for 400 μ L sample added to extraction with Thermo Fisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit and 200 μ L sample added to extraction with the Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit

To determine the LoD of the multiplex COVID-19 RT-PCR Test, quantified live SARS-CoV-2 virus (from a well characterized positive sample (2e5 genome copies (cp)/ μ L) was

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diluted into negative clinical matrix (BAL and NP) to concentrations of 25, 12.5, 6.25, and 3.125 cp/μL. 10 replicates of each concentration were then extracted using extraction methods: 1) Thermo Fisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit on the Thermo Fisher KingFisher Flex instrument and 2) Roche 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.

Positive replicates/total replicates tested at different concentrations of SARS-CoV-2 with NP swab

NP swabs				
	25 cp/ μL	12.5 cp/ μL	6.25 cp/ μL	3.125 cp/ μL
KingFisher Flex	10/10	10/10	10/10	10/10
MagNA Pure-96	10/10	10/10	10/10	7/10
Total	20/20	20/20	20/20	17/20

Positive replicates/total replicates tested at different concentrations of SARS-CoV-2 with BAL

BAL				
	25 cp/ μL	12.5 cp/ μL	6.25 cp/ μL	3.125 cp/ μL
KingFisher Flex	10/10	10/10	5/10	4/10
MagNA Pure-96	10/10	10/10	3/10	1/10
Total	20/20	20/20	8/20	5/20

KingFisher Flex - Mean Ct at N1 and N2 at different concentrations of SARS-CoV-2

	25 cp/μL		12.5 cp/μL		6.25 cp/μL		3.125 cp/μL	
	N1	N2	N1	N2	N1	N2	N1	N2
NP	31.76	31.36	32.46	32.09	33.84	33.99	34.91	35.34
BAL	31.96	34.89	33.03	36.59	34.02	39.64	34.86	39.78

MagNA Pure-96 - Mean Ct at N1 and N2 at different concentrations of SARS-CoV-2

	25 cp/μL		12.5 cp/μL		6.25 cp/μL		3.125 cp/μL	
	N1	N2	N1	N2	N1	N2	N1	N2
NP	33.16	32.91	34.26	34.43	35.38	36.31	36.61	38.17
BAL	34.13	35.85	35.29	37.26	36.85	40.87	38.50	43.03

COVID-19 RT-PCR test (in a multiplex format): Limit of Detection (LoD) for 500 μL sample added to extraction with the CERES Nanosciences Nanotrap Virus Capture Kit

To determine the limit of detection using the CERES Nanosciences Nanotrap Virus Capture Kit, 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 (UTM, Saline 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. The results produced a limit of detection of 3.125cp/μL for UTM, 6.25 cp/μL for saline and 12.5 cp/μL for BAL.

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Positive replicates/total replicates tested at different concentrations of SARS-CoV-2

	25 cp/μL	12.5 cp/μL	6.25 cp/μL	3.125 cp/μL
UTM	10/10	10/10	10/10	10/10
Saline	10/10	10/10	10/10	9/10
BAL	10/10	10/10	9/10	9/10

Mean Ct at N1 and N2 at different concentrations of SARS-CoV-2

	25 cp/μL		12.5 cp/μL		6.25 cp/μL		3.125 cp/μL	
	N1	N2	N1	N2	N1	N2	N1	N2
UTM	32.24	32.19	33.58	33.27	34.64	33.70	35.51	34.00
Saline	33.29	33.95	34.59	35.03	35.44	36.53	36.54	37.07
BAL	34.09	32.32	34.09	32.32	36.58	36.90	36.87	37.15

COVID-19 RT-PCR test (in a multiplex format): Limit of Detection (LoD) for 200μL sample added to extraction with Thermo Fisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit

To determine the limit of detection, a well characterized positive sample (2×10^5 copies/μL) was diluted into negative sample matrix (BAL and UTM – NP swab) to concentrations of 25, 12.5, 6.25, and 3.125 copies/uL. Each contrived sample was then extracted with the ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit using the Low Volume MagMax procedure. The results produced a limit of detection of 3.125 copies/uL for BAL and UTM.

Positive replicates/total replicates tested at different concentrations of SARS-CoV-2

Low Volume MagMax	25 cp/uL	12.5 cp/uL	6.25 cp/uL	3.125 cp/uL
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

Low Volume MagMax - Mean Ct at N1 and N2 at different concentrations of SARS-CoV-2

	25 cp/uL		12.5 cp/uL		6.25 cp/uL		3.125 cp/uL	
	N1	N2	N1	N2	N1	N2	N1	N2
BAL	33.88	32.62	35.24	34.44	35.78	35.23	36.21	35.87
UTM	33.03	32.27	33.53	33.16	35.64	36.28	36.65	37.32

COVID-19 RT-PCR test (in a multiplex format): Limit of Detection (LoD) for 200μL sample added to extraction with ThermoFisher MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit using the Hamilton MicroLab STAR and ThermoFisher KingFisher Flex instruments:

To determine the LoD of the multiplex COVID-19 RT-PCR Test, a well characterized positive sample (2×10^5 copies/μL) was diluted into negative clinical matrix from UTM (NP swab), BAL, and saline (anterior nasal swab) and to concentrations of 25, 12.5, 6.25, and 3.125 cp/uL. 6 replicates of each concentration were then extracted with the

ThermoFisher MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit using the Low Volume MagMax procedure (200µL sample added to extraction) on the Hamilton MicroLab STAR and ThermoFisher KingFisher Flex instruments. With either the Hamilton MicroLab STAR and ThermoFisher KingFisher Flex instrument, the ThermoFisher MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit generated the same LoD of 6.25 cp/µL or 31.25 copies/reaction for UTM, BAL and Saline. The results are summarized in the tables below.

Hamilton MicroLab STAR instrument: Positive replicates/total replicates tested at different concentrations of SARS-CoV-2

	25 cp/µL	12.5 cp/µL	6.25 cp/µL	3.125 cp/µL
UTM	6/6	6/6	6/6	5/6
BAL	6/6	6/6	6/6	3/6
Saline	6/6	6/6	6/6	5/6
Total	18/18	18/18	18/18	13/18

Hamilton MicroLab STAR instrument: Mean Ct at N1 and N2 at different concentrations of SARS-CoV-2

	25 cp/µL		12.5 cp/µL		6.25 cp/µL		3.125 cp/µL	
	N1	N2	N1	N2	N1	N2	N1	N2
UTM	34.69	33.78	35.56	35.09	36.50	36.64	37.87	37.95
BAL	34.67	33.48	36.60	35.15	37.53	36.39	38.21	39.20
Saline	35.23	33.85	35.65	34.59	37.54	37.25	38.41	38.82

ThermoFisher KingFisher Flex instrument: Positive replicates/total replicates tested at different concentrations of SARS-CoV-2

	25 cp/µL	12.5 cp/µL	6.25 cp/µL	3.125 cp/µL
UTM	6/6	6/6	6/6	6/6
BAL	6/6	6/6	6/6	4/6
Saline	6/6	6/6	6/6	3/6
Total	18/18	18/18	18/18	13/18

ThermoFisher KingFisher Flex instrument: Mean Ct at N1 and N2 at different concentrations of SARS-CoV-2

	25 cp/µL		12.5 cp/µL		6.25 cp/µL		3.125 cp/µL	
	N1	N2	N1	N2	N1	N2	N1	N2
UTM	33.23	32.32	33.89	33.05	35.69	35.23	36.69	37.24
BAL	34.54	33.33	35.60	34.00	36.22	35.26	38.47	38.81
Saline	33.95	32.52	35.83	34.65	36.87	36.21	38.64	39.69

2) Analytical Sensitivity

This test is using the primers and probes published by CDC and used in the CDC COVID-19 Diagnostic Panel without sequence modifications. 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. Based on publicly available information there are no significant concerns that reactivity of the CDC reagents have been impacted by the emergence of SARS-CoV-2 genetic variants.

3) **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)
<i>Bordetella pertussis</i>	Not detected	Not detected	Not detected	Patient Sample (1e ⁵)
<i>Chlamydomphila pneumoniae</i>	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)
<i>Haemophilus influenzae</i>	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)

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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)
<i>Legionella pneumophila</i>	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)
<i>Mycobacterium tuberculosis</i>	Not detected	Not detected	Not detected	ATCC 25177; H37Ra*
<i>Mycoplasma pneumoniae</i>	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*
<i>Streptococcus pneumoniae</i>	Not detected	Not detected	Not detected	ATCC 33400D-5 (3e ⁶)
<i>Streptococcus pyogenes</i>	Not detected	Not detected	Not detected	ATCC 12344D-5; T1 (3e ⁶)

* Concentration unknown

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
<i>Candida albicans</i>	All	All	0	0	0
<i>Neisseria meningitidis</i>	All	All	0	0	0

<i>Pseudomonas aeruginosa</i>	All	All	0	0	0
<i>Staphylococcus aureus</i>	All	All	0	0	0

4) **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)	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 BAL specimens	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	Labcorp 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

A clinical evaluation of the Labcorp COVID-19 RT-PCR test was performed with 635 positive upper respiratory specimens with results shown in the table below. Comparator results for the clinical specimens was obtained with a highly sensitive FDA EUA RT-PCR test.

		FDA EUA RT-PCR Test			Total
		Positive	Indeterminate	Negative	
Labcorp COVID-19 RT-PCR test	Positive	612	0	0	612
	Indeterminate	11	0	0	11
	Negative	12	0	0	12
	Total	635	0	0	

Positive Percent Agreement = $612/624 = 98.08\%$ (95% CI: 96.67% - 98.90%)

Indeterminate Rate = $11/635 = 1.7\%$

Number of low positive specimens = $23/624 = 3.69\%$

Note: There was insufficient material remaining to re-test the samples with indeterminate results. Indeterminate sample are excluded from the PPA calculation

A clinical evaluation of the Labcorp COVID-19 RT-PCR test was performed with 30 positive and 30 negative upper respiratory specimens with results shown in the table below. Comparator results for the clinical specimens was obtained with a highly sensitive FDA EUA RT-PCR test.

		FDA EUA RT-PCR Test		Total
		Positive	Negative	
Labcorp COVID-19 RT-PCR test	Positive	30	0	0
	Negative	0	30	30
	Total	30	30	

Positive Percent Agreement = $30/30 = 100\%$ (95% CI: 88.65% - 100%)

Negative Percent Agreement = $30/30 = 100\%$ (95% CI: 88.65% - 100%)

Number of low positive samples = $8/30 = 26.7\%$

5) 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.

6) **Validation of the ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit using the Low Volume MagMax procedure (200µL Extraction Volume)**

Refer to “Analytical Sensitivity” for the LoD study performed with the ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit using the Low Volume MagMax procedure (200 µL extraction volume).

Clinical performance of the 200µL versus the 400µL extraction procedure was demonstrated with a set of clinical samples extracted with both methods. One extraction plate for each of the extraction methods was run for clinical concordance. A total of 93 samples were on the plate, including 36 positives, 7 of which were low positive. The results of the clinical comparison were 100% concordant. 36/36 positives were detected as positive and 57/57 negatives were not detected. 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.

7) **Usability Study - Self-Collection Validation (Labcorp At Home COVID-19 Test Home Collection Kit OR Pixel by LabCorp COVID-19 Test Home Collection Kit)**

Adults age 18 years or older: 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. Participants were not observed during the study. 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 the qualitative result for either RNaseP or COVID-19 targets.

Children ages 2 years-17 years: 30 subjects were enrolled in a home-collection study, children 13 years and younger (n=14) had an adult parent or guardian collect the sample and children 14 years and older (n=16) self-collected their sample. Study

subjects and/or their guardians were consented for collection. Consent only covered the reporting of the RnaseP signal from the assay to ensure patient privacy. Samples were collected according to the Labcorp At Home COVID-19 Test Home Collection Kit IFU and then shipped overnight to the lab via FedEx. Samples were then tested for the presence of the endogenous control signal using the Labcorp COVID-19 RT-PCR Test to determine whether specimens collected from patients under the age of 18 can provide adequate sample for testing.

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).

The results of the self-collection validation were consistent with expected results. All 30 samples had strong Human RNaseP signals indicating all participants were successful in collecting human biological material.

8) **Sample Shipping Stability Study (Summer and Winter Profiles) - Labcorp At Home COVID-19 Test Home Collection Kit**

Sample stability studies were conducted using the summer profile in the table below 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.

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

20 contrived low positive samples (2x LoD of the Labcorp COVID-19 RT-PCR Test) and 10 contrived high positive samples (10x LoD of the Labcorp COVID-19 RT-PCR Test) were created by diluting a well characterized positive sample in saline. These samples as well as 10 negative samples were tested for the duration of the summer profile testing.

At time point 0hr and 50 hr, 20 of the 20 contrived low positive samples tested positive for SARS-CoV-2 with the Labcorp COVID-19 RT-PCR Test. At time point 0hr and 50hr, 10 of the 10 (100%) contrived high positive samples tested positive for SARS-CoV-2 with the Labcorp COVID-19 RT-PCR Test. At time point 0hr and 50 hr, all 10 negative samples tested negative for SARS-CoV-2 with the Labcorp COVID-19 RT-PCR Test.

An additional sample stability study was conducted to extend the sample stability from 2 days to 4 days for anterior nasal swab specimens collected using the Labcorp At Home COVID-19 Test Home Collection Kit. 20 contrived low positive samples (2x LoD of the Labcorp COVID-19 RT-PCR Test) and 10 contrived high positive

samples (10x LoD of the Labcorp COVID-19 RT-PCR Test) were created by diluting a well characterized positive sample in saline. These samples as well as 10 negative samples were tested for stability at 0hr and after a temperature excursion of 40C for 108hrs (4 days plus 12 hours).

At time point 0hr and 108hr, 20 of the 20 (100%) contrived low positive samples tested positive for SARS-CoV-2 with the Labcorp COVID-19 RT-PCR Test. At time point 0hr, 9 of the 10 (90%) contrived high positive samples tested positive for SARS-CoV-2 with the Labcorp COVID-19 RT-PCR Test. At time point 108hr, 10 of the 10 (100%) contrived high positive samples tested positive for SARS-CoV-2 with the Labcorp COVID-19 RT-PCR Test. At time point 0hr and 108hr, all 10 negative samples tested negative for SARS-CoV-2 with the Labcorp COVID-19 RT-PCR Test. The average Ct difference post-temperature excursion at 40C demonstrated that the Ct of samples at 0hr (baseline) did not differ by more than 3 Ct to samples exposed to 40C for 108 hours.

Sample stability studies were conducted using the winter profile in the table below 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.

Temperature	Cycle Period	Cycle Period Hours	Total Time Hours
-10C	1	8	8
18C	2	4	12
-10C	3	2	14
10C	4	36	50
-10C	5	6	56

30 contrived low positive samples (2x LoD of the Labcorp COVID-19 RT-PCR Test) and 10 contrived high positive samples (10x LoD of the Labcorp COVID-19 RT-PCR Test) were created by diluting a well characterized positive sample in saline. These samples as well as 10 negative samples were tested for the duration of the winter profile testing.

At time point 0hr, 30 of the 30 contrived low positive samples tested positive for SARS-CoV-2 with the Labcorp COVID-19 RT-PCR Test. At time point 56 hr, 29 of the 30 (96.67%) contrived low positive samples tested positive for SARS-CoV-2 with the Labcorp COVID-19 RT-PCR Test. At time point 0hr and 56hr, 10 of the 10 (100%) contrived high positive samples tested positive for SARS-CoV-2 with the Labcorp COVID-19 RT-PCR Test. At time point 0hr and 56 hr, all 10 negative samples tested negative for SARS-CoV-2 with the Labcorp COVID-19 RT-PCR Test.

In summary, the shipping stability study data supports a sample shipping stability claim up to 4 days in summer conditions and up to 56 hours in winter conditions. An extended winter shipping stability study will be conducted to further support a claim up to 4 days in winter conditions..

9) **Validation of New Foam Swabs for Shipping Stability**

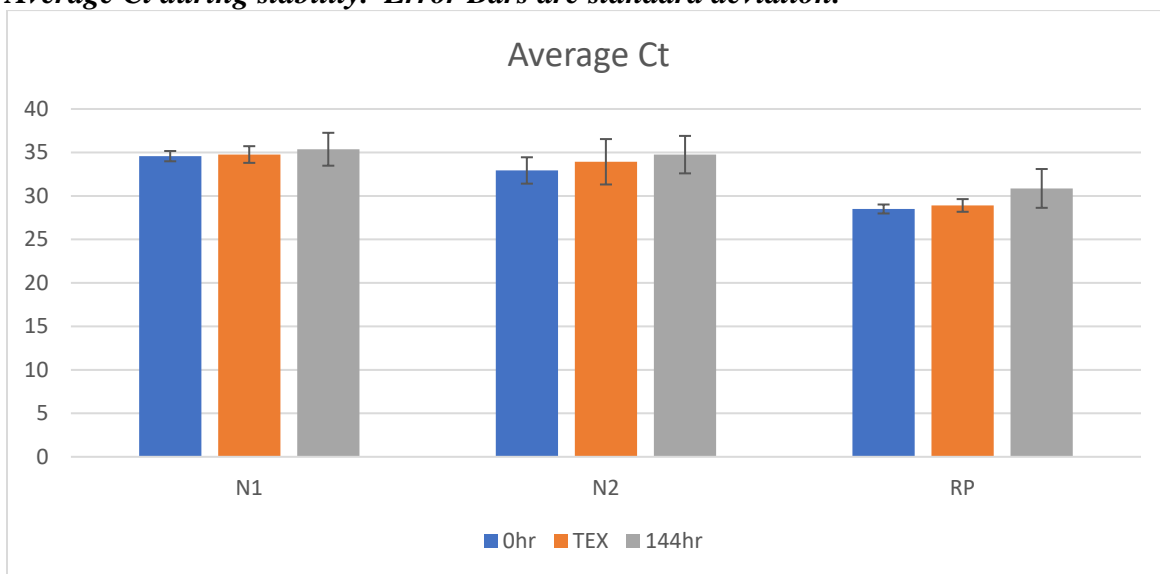
A total of 40 samples, were collected by participants who swabbed their anterior nasal cavity according to the Pixel kit instructions, 20 participants were given the Azer Foam swab and 20 participants were given a Puritan Foam swab (swab type used in Quantigen study). 20 positive samples (10 for each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

Average Ct during stability. Error Bars are standard deviation.



10) 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 a separate authorized SARS-CoV-2 Test, made by a different manufacturer, 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 value for asymptomatic patients was similar to that 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 (N1, N2 and RP) 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).

Finally, all 133 asymptomatic samples (20 positive and 113 negative) were run on a second comparator assay, an authorized SARS-CoV-2 comparator 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 results on the authorized SARS-COV-2 comparator test and all 20 positives returning positive results by the authorized SARS-CoV-2 comparator test (See Table 2).

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

Samples	N1	N2	RP
Pos1	38.824	38.778	29.768
Pos2	22.065	20.943	28.909
Pos3	26.897	24.808	27.344
Pos4	23.109	21.189	26.814
Pos5	27.646	25.852	30.747
Pos6	18.524	16.661	36.827
Pos7	36.38	35.819	28.054
Pos8	18.443	16.849	27.495
Pos9	20.893	19.362	24.909
Pos10	20.982	18.691	24.01

Samples	N1	N2	RP
Pos11	25.403	23.225	30.216
Pos12	22.652	20.842	26.835
Pos13	23.186	21.647	26.575
Pos14	31.352	30.314	26.487
Pos15	21.612	19.993	24.893
Pos16	22.703	20.972	31.34
Pos17	37.678	36.006	30.164
Pos18	32.261	31.185	28.707
Pos19	19.673	18.251	33.285
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

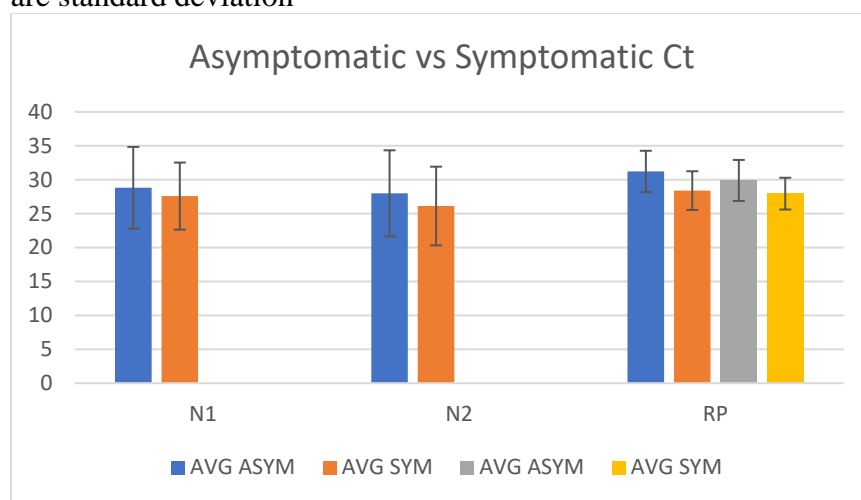


Table 2: Comparison of FDA Authorized SARS-COV-2 Comparator Test to the Labcorp COVID-19 RT-PCR Test in Asymptomatic Individuals

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

11) 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 3). 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

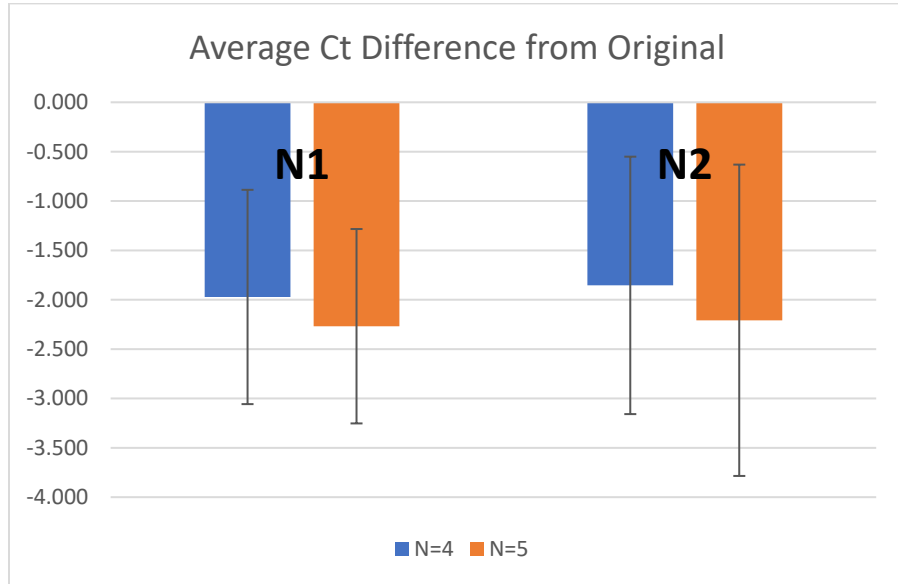
Copies	500	250	125	62.5	30.125
N=4	20/20	20/20	20/20	20/20	18/20
N=5	20/20	20/20	20/20	19/20	13/20

12) 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 2) 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.

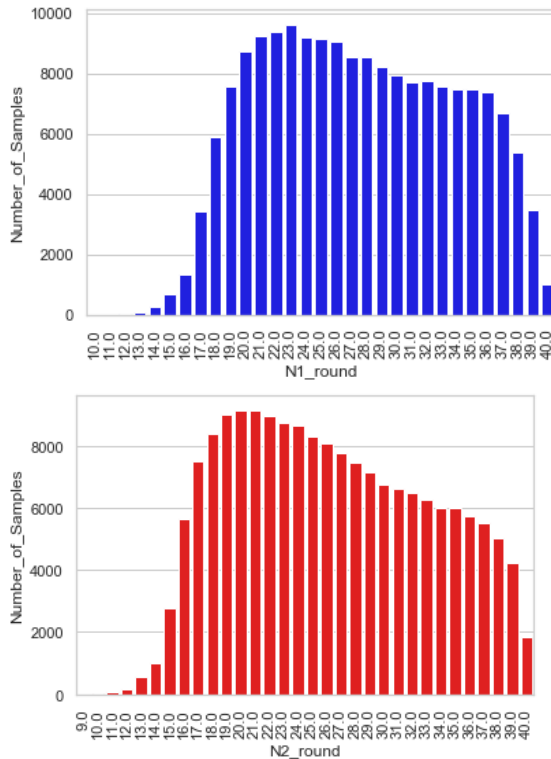
Figure 2: Average Ct Difference. The Average difference from the original N1/N2 Ct and the pooled N1/N2 Ct was calculated for both N=4 and N=5 pools. Error bars are standard deviation.



In Silico Retrospective Analysis of Pooled Samples (based on clinical data Δ Ct)

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. In Figure 3, a histogram of the Ct results for N1 and N2 is presented.

Figure 3: Histogram of N1 and N2 Cts for 148,550 clinical samples. N1 - Blue, N2 – Red.



Regression Analysis using Passing-Bablok Analysis

To rule out any assay bias due to pooling, particularly in low viral concentration samples, a Passing-Bablok regression analysis was performed to compare individual Cts to Pooled Cts for N=4 (Figure 4) and N=5 (Figure 5) 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 4: N=4 Pooling - Passing-Bablok Analysis

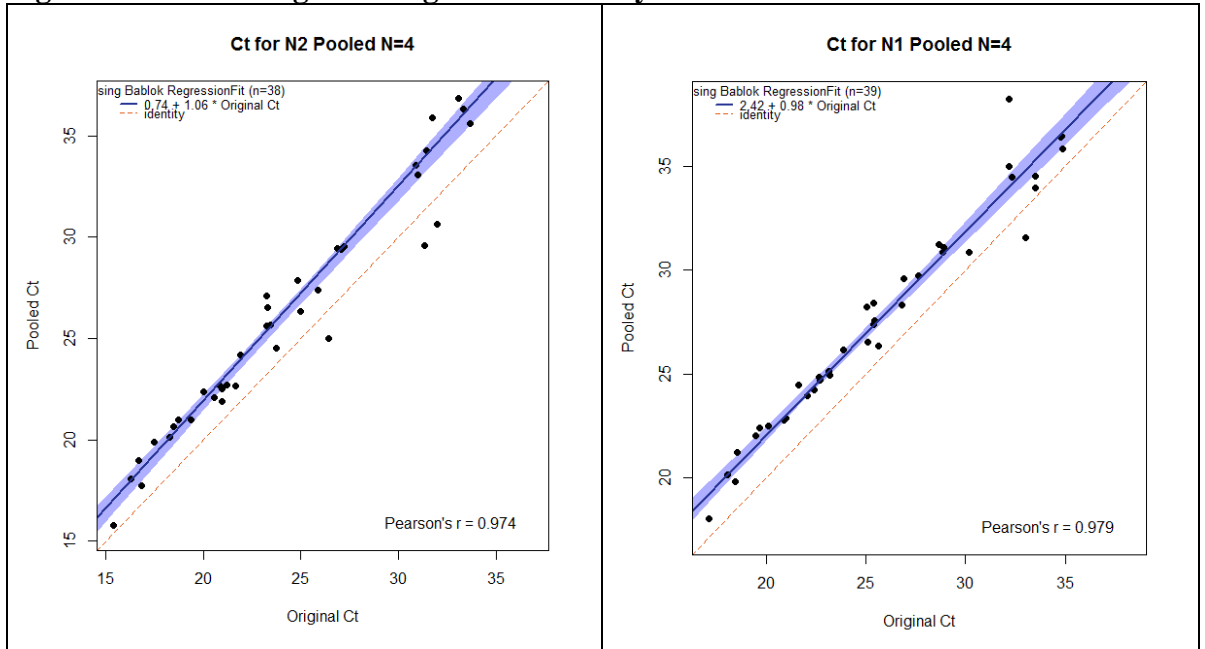


Figure 5: N=5 Pooling - Passing-Bablok Analysis

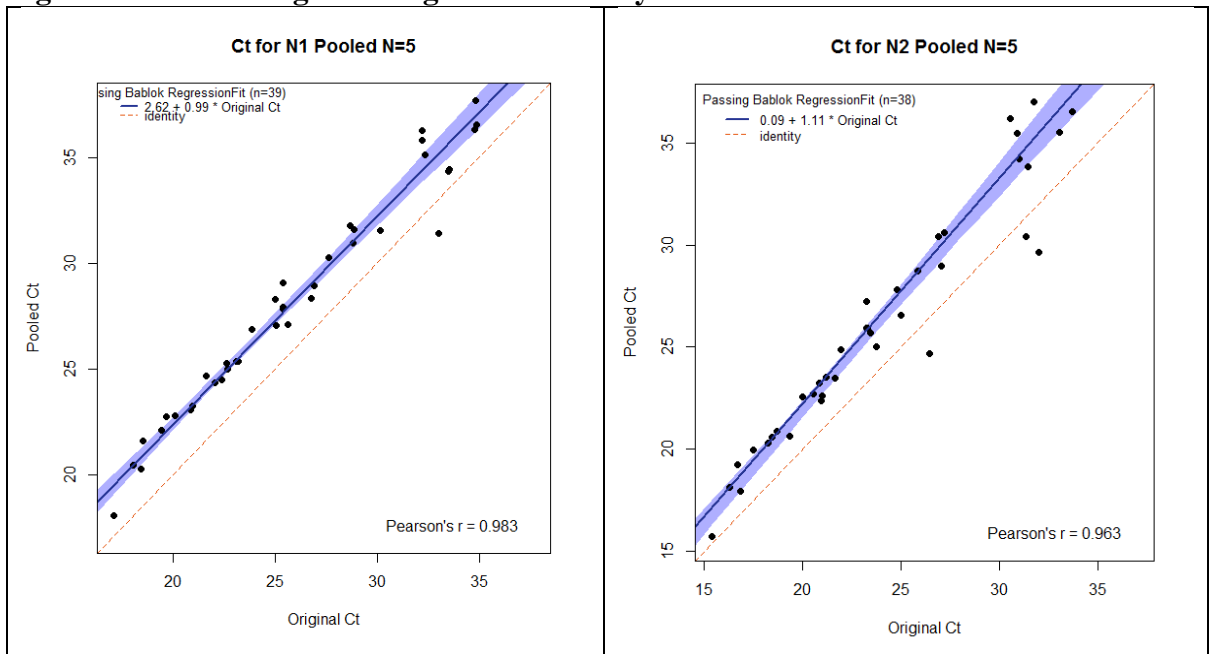


Table 4: Summary of Passing-Bablok Analysis. LCI and UCI are estimates of upper and lower 95% confidence intervals for the slope and Intercept. The R2 value is a Pearson's r estimated from the model.

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-Bablok 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-Bablok 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: $40=0.981X+2.423 \Rightarrow X^*=38.3 \quad \Delta C_t = 1.7$

For N2: $40=1.061X+0.745 \Rightarrow X^*=37.0 \quad \Delta C_t = 3.0$

N=5 for N1: $40=0.986X+2.620 \Rightarrow X^*=37.9 \quad \Delta C_t = 2.1$

For N2: $40=1.107X+0.095 \Rightarrow X^*=36.0 \quad \Delta C_t = 4.0$

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.

13) 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.

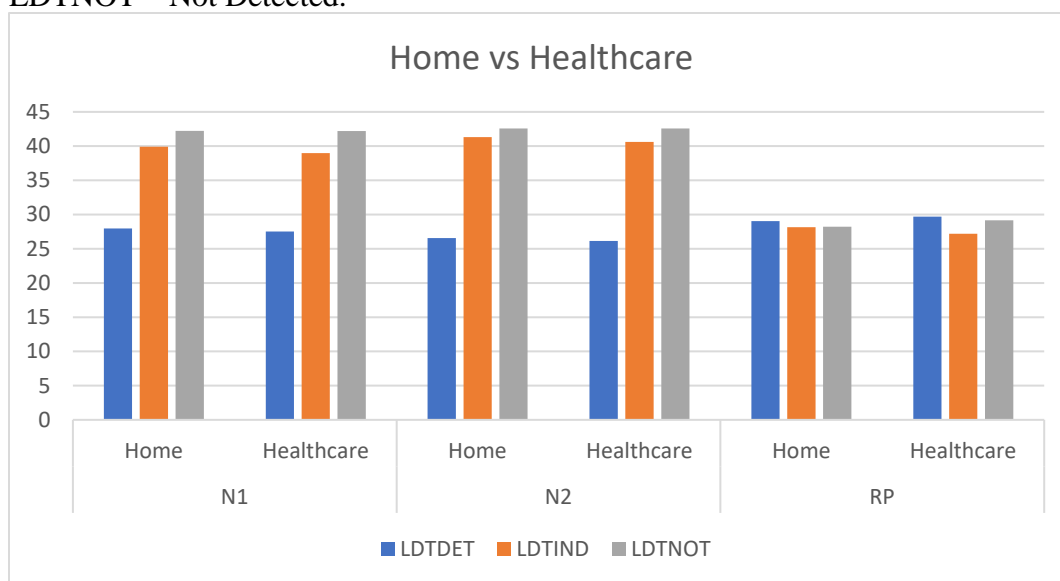
Labcorp has tested approximately 60,000 home collected samples to date. This home collection kit is based on the use of a foam swab and sampling performed in the absence of a healthcare provider. 60,624 home collected samples were analyzed for their result status and RnaseP collection status and compared 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 6). 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

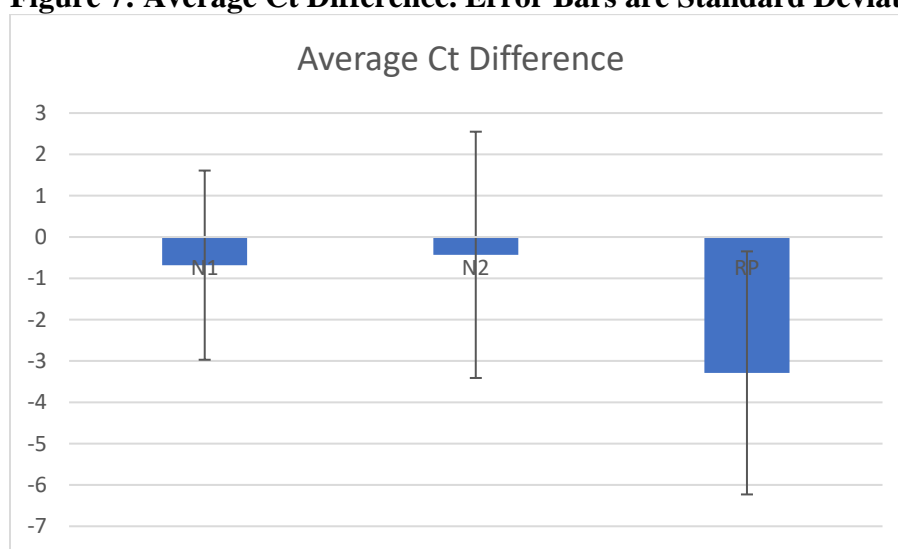
Figure 6: Comparison of Mean Ct value of Home Collected vs. Healthcare Collected Samples. LDTDET – Detected Result, LDTIND – Indeterminate Result, LDTNOT – Not Detected.



14) Validation of New Extraction Method (CERES Nanosciences Nanotrap Virus Capture Kit)

Refer to “Analytical Sensitivity” for the LoD study performed using the CERES Nanosciences Nanotrap Virus Capture Kit. To further validate the CERES Nanosciences Nanotrap Virus Capture Kit, 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 7).

Figure 7: Average Ct Difference. Error Bars are Standard Deviation



15) Validation of New Extraction Method (ThermoFisher MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit using the Hamilton MicroLab STAR and ThermoFisher KingFisher Flex instruments)

Refer to “Analytical Sensitivity” for the LoD study performed using the ThermoFisher MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit using the Hamilton MicroLab STAR and ThermoFisher KingFisher Flex instruments.

A clinical concordance evaluation was conducted comparing the performance of the previously authorized ThermoFisher MagMAX Viral/Pathogen (MVP I) Nucleic Acid Isolation Kit using the Low Volume MagMax procedure (200µL sample added to extraction) to the ThermoFisher MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit using the Low Volume MagMax procedure (200µL sample added to extraction) on the Hamilton MicroLab STAR and ThermoFisher KingFisher Flex instruments. 2 different sets of 84 clinical samples were evaluated on the Hamilton MicroLab STAR and ThermoFisher KingFisher Flex instruments with the results summarized in the tables below.

Table 6: MVP I vs. MVP II on the Hamilton MicroLab STAR instrument

		MVP II		Total
		Positive	Negative	
MVP I	Positive	7	0	7
	Negative	0	77	77
	Total	7	77	

The results of the clinical comparison were 100% concordant for positives and 100% for negatives. The average Ct Difference for N1 was 1.72, for N2 was 1.97 and for RP was -0.63.

Table 7: MVP I vs. MVP II on the ThermoFisher KingFisher Flex instrument

		MVP II		Total
		Positive	Negative	
MVP I	Positive	5	0	5
	Negative	0	79	79
	Total	5	79	

The results of the clinical comparison were 100% concordant for positives and 100% for negatives. The average Ct Difference for N1 was -0.40, for N2 was -0.69 and for RP was -0.48.

WARNINGS

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

LIMITATIONS

- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- Performance with specimens collected from individuals 18 years and older by an adult in the home has not been evaluated.