EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
Guardant-19
(Guardant Health Inc.)

For in vitro diagnostic use
Rx only
For use under Emergency Use Authorization (EUA) Only

(Guardant-19 will be performed at the Guardant Health Clinical Laboratory, 505 Penobscot Drive, Redwood City, CA, 94063, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, as described in the laboratory procedures reviewed by the FDA under this EUA.)

INTENDED USE

Guardant-19 is a Reverse Transcriptase PCR (RT-PCR) and Next Generation Sequencing (NGS) test on the Illumina NextSeq 500 and NextSeq 550 Sequencing Systems, intended for the qualitative detection of SARS-CoV-2 RNA from nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, nasal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal wash/aspirates, nasal aspirates, and nasal washes from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Guardant Health Clinical Laboratory, 505 Penobscot Drive, Redwood City, CA, 94063, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Guardant-19 test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of RT-PCR, next generation sequencing using the Illumina NextSeq 500 and NextSeq 550 Sequencing Systems, next-generation sequencing workflows, and *in vitro* diagnostic procedures. The Guardant-19 test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

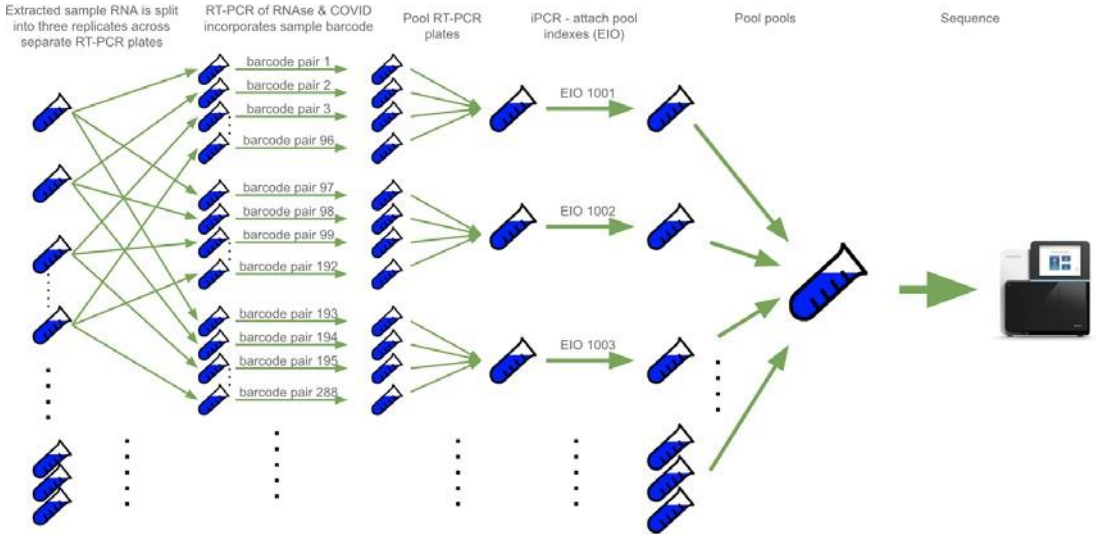
Guardant-19 is a high-throughput, automated method utilizing Reverse Transcriptase PCR (RT-PCR) followed by Next Generation Sequencing (NGS) to detect SARS-CoV-2 viral RNA in upper respiratory specimens, including nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, nasal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal wash/aspirates, nasal aspirates, and nasal washes, from patients as recommended for testing by public health authority guidelines. The test uses the same N1 (assay target) and RNase P (endogenous control) primer sequences as those described under the EUA for the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel to detect the N1 region of the SARS-CoV-2 nucleocapsid (N) gene and the human RNase P gene, respectively. These sequences are provided below.

N1 gene
NC_045512.2:28287-28358|Gene N
N1 Rev: TCTGGTTACTGCCAGTTGAATCTG
N1 Fwd: GACCCCAAAATCAGCGAAAT

RNase P gene
hg19|chr10:92631758-92631822|RNase-P
RP-F: AGATTTGGACCTGCGAGCG
RP-R: GAGCGGCTGTCTCCACAAGT

The test workflow consists of the following as illustrated in **Figure 1** below:

Figure 1. Schematic of Barcoding and Pooling in Guardant-19



SARS-CoV-2 nucleic acid is first extracted, isolated, and purified from 200 µl of upper respiratory specimens using an in-house developed silica bead-based RNA extraction method performed on Hamilton liquid handling systems. The total eluate volume is 50 µl. Subsequently, in triplicate for each sample, 10 µl of purified RNA then undergoes one-step RT-PCR using

SARS-CoV-2 primer sequences for the N1 gene and human RNase P primer sequences with attached plate- and well-specific barcodes that uniquely label each sample within a plate of 96 unique samples, respectively.

- Thermocycling conditions used in the one-step RT-PCR are as follows:
  - 25°C for 10 minutes
  - 55°C for 10 minutes
  - 95°C for 2 minutes
  - 40 cycles of:
    - 95°C for 3 seconds
    - 64°C for 30 seconds
  - 4°C ∞
  - Cover temperature is 105°C
  - Reaction volume is 25 µL

RT-PCR amplification products are then pooled in sets of 96 and a second round of PCR with universal NGS sequencing primers with attached pool-specific barcodes and NGS flow cell primer sequences for combinatorial sample barcoding and enable NGS clustering, respectively.

- Thermocycling conditions used in the second round of PCR are as follows:
  - 98°C for 30 seconds
  - 10 cycles of:
    - 98°C for 10 seconds
    - 65°C for 1 minute
  - 65°C for 3 minutes
  - 4°C for 5 minutes
  - 4°C ∞
  - Cover temperature is 105°C
  - Reaction volume is 50 µL

The set of up to 96 such sample pools is then pooled for sequencing, with up to 32 unique samples per plate x 3 RT-PCR replicates per sample x 96 total plates = 9216 barcoded samples (3072 unique samples, including controls as described below, run through triplicate RT-PCR reactions). The pools are sequenced on the Illumina NextSeq 500 or NextSeq 550 instruments, and two-level barcodes are used to uniquely identify reads originating from each of the respective samples in the pool. The first NGS read (Read 1) is 65 cycles, sequencing the 1st plate-specific barcode (cycles 1-10), and the specific amplicon (cycles 11-65). Dual Illumina index reads (i7, i5) sequence both the pool-specific barcodes. The paired-end NGS read (Read 2) is 15 cycles, sequencing the 2nd plate-specific barcode (cycles 1-10) and partial amplicon for confirmation (cycles 11-15).

The Guardant-19 Bioinformatics Pipeline Software then analyzes sequencing results from triplicate testing of RT-PCR products from each individual sample after demultiplexing. Reads are classified and several quality control checks are performed (see **Figure 2** and **Table 5** for more detail on quality control measures). A replicate-level score is calculated as the ratio of SARS-CoV-2 reads to spike-in reads observed within that replicate. The per-sample score, also

referred to as the Guardant-19 score or G-19 score, is defined as the median value of the two or three per-replicate scores. If fewer than two replicates are evaluable for a sample, the per-sample score is undefined, resulting in a No Call. A G-19 score greater than or equal to 0.01 is interpreted as SARS-CoV-2 positive.

INSTRUMENTS USED WITH THE TEST

Guardant-19 is to be used with the instruments listed in **Table 1.**

Table 1. List of Instruments used with Guardant-19

Equipment (Software) Description	Manufacturer	Catalog #
Hamilton Star (Venus Software, v4.5.0.5217)	Hamilton	17300-020/J
Hamilton Starlet Liquid Handling Systems (Venus Software, v4.5.0.5217)	Hamilton	173021
Hamilton LabElite I.D. Capper (Hamilton IDCapper Software v1.0.2)	Hamilton	193601
Applied Biosystems Veriti Thermal Cycler (Firmware v2.0.4)	Thermo Fisher	4375786
4200 Tapestation System (Tapestation Software v3.2)	Agilent	G2991AA
PlateLoc Thermal Microplate Sealer (Firmware v6.1.2)	Agilent	G5402-90001D
ALPS 3000™ Automated Microplate Heater Sealer (Firmware v2.0.2)	Thermo Fisher	AB-3000
XPeel	Brooks Life Sciences	BA-XP1
CAB SQUIX Label Printer	Cab	5977005
Illumina NextSeq 500/550 (NextSeq Software v4.0.1)	Illumina	SY-415-1001 SY-415-1002
Eppendorf 5810 Centrifuge	Eppendorf	022628185
Beckman X-30 Centrifuge	Beckman Coulter	B06314
Eppendorf centrifuge	Eppendorf	022627110; 022625501

Equipment (Software) Description	Manufacturer	Catalog #
Mini Centrifuge (minifuge)	VWR	93000-196
Rainin Pipette, various sizes	Rainin	17014382; 17014388; 17014391
Vortex Mixer	VWR	58816-121

REAGENTS AND MATERIALS

Reagents used in the Guardant-19 test are formulated into a high-throughput ready-to-use pre-plated format from the raw components listed in **Table 2**. The consumables used in the Guardant-19 test are listed in **Table 3**.

Table 2. List of Reagents used with Guardant-19

Raw Material Description	Raw Material Vendor	Raw Material Part Number
Quick-DNA/RNA Viral MagBead Kit	Zymo Research	R2141
Custom N1 Barcoded RT-PCR Primers for SARS-CoV-2 and RNase P	LGC Biosearch	Custom
Custom Synthetic RNA Spike-In	Octant Bio	Custom
Luna Universal One-Step RT-qPCR Kit	New England Biolabs	M3006
Ampure XP beads	Beckman Coulter	A63882
Custom pool index PCR primers	LGC Biosearch	Custom
NEBNext Ultra II Q5 Master Mix	New England Biolabs	M0544
TapeStation D1000 Buffer	Agilent	5067-5602
1N NaOH	VWR	EM-SX0607H-6
1M Tris-HCl	Life Technologies	AM9856
PhiX	Illumina	FC-110-3001
HT1 Buffer	Illumina	20015892
Nuclease Free Water	Thermo Fisher	AM9914G
Nextseq 75 cycle kit	Illumina	20024906

Table 3. List of Consumables used with Guardant-19

Consumable Description	Manufacturer	Catalog #
Axygen PlateMax Aluminum Sealing Foil	Axygen	PCR-AS-200/47734-817
CO-RE Tips 50 µL, 300 µL, 1000 µL (Black, Conductive, Filtered, Non-sterile)	Hamilton	235948 235903 235905
D1000 Screen tape	Agilent	5067-5582
Eppendorf 1.1mL DNA LoBind Plate	Eppendorf	951032808
PlateLoc Peelable Aluminum Heat Seal	Agilent	24210-001
EZ-Peel ALPS 3000 Heat Sealing Rolls	Thermo Fisher	AB-3738
Integra Reagent Reservoir	Integra Biosciences	6348
Bio-Rad Hard-Shell 96-Well PCR Plates	Bio-Rad Laboratories	HSP9601
Thomas Scientific Deepwell Plate (96-well)	Thomas Scientific	1149J84
MicroAmp EnduraPlate Optical 96-Well Reaction Plates with Barcode, Clear	Applied Biosystems	4483352
Micronic 2D-Coded Tubes, External Thread, Racked	Micronic	MP52706
Eppendorf Deepwell Plate (96-well)	Eppendorf	951032808
TapeStation Loading Tips, 10 pack	Agilent	5067-5599

CONTROLS TO BE USED WITH GUARDANT-19

The following control materials will be used in Guardant-19 (**Table 4**).

Table 4. Guardant-19 Control Materials

Control Type	Control Material	Control Purpose	Testing Frequency	Criteria
Negative (no template) control, NTC	Viral Transport Medium	To monitor for cross-contamination during RNA extraction, RT-PCR and NGS	Per batch of specimens	Sequenced reads for SARS-CoV-2 and RNase P below detection threshold
Positive Control	1000 copies of SARS-CoV-2 AccuPlex material (SeraCare) spiked in VTM representing 625-5000 copies/mL (5-40X LoD†)	To monitor the integrity of the reagents and process	Per batch of specimen	Control is detected as SARS-CoV-2 positive
Internal Spike Control	Synthetic RNA with SARS-CoV-2 RNA primer binding sites with a distinct internal sequence* (2000 copies/mL)	To measure efficiency of RT-PCR and NGS for each specimen	Added to each specimen, Positive and the Negative Control prior to RT-PCR	Sequenced reads for synthetic barcoded SARS-CoV-2 plus SARS-CoV-2 above QC threshold
Endogenous Control	N/A (endogenous controls take the form of barcoded RNase P primers that amplify human RNase P)	Confirm presence of human RNase P RNA, i.e. confirm presence of specimen	Endogenous within each specimen	Sequenced reads for RNase P above detection threshold

† The sponsor will validate the positive control concentration at 5X LoD for Guardant-19 in an FDA agreed upon post-authorization study.

* The sequence of the Internal Spike Control is: GACCCCAAAATCAGCGAAATGCACCCCGCATTACG**AAACCA**GGACCCTCAGATTCAACTGG CAGTAACCAGA. **Bolded** characters indicate the subsequence that differs from the SARS-CoV-2 consensus.

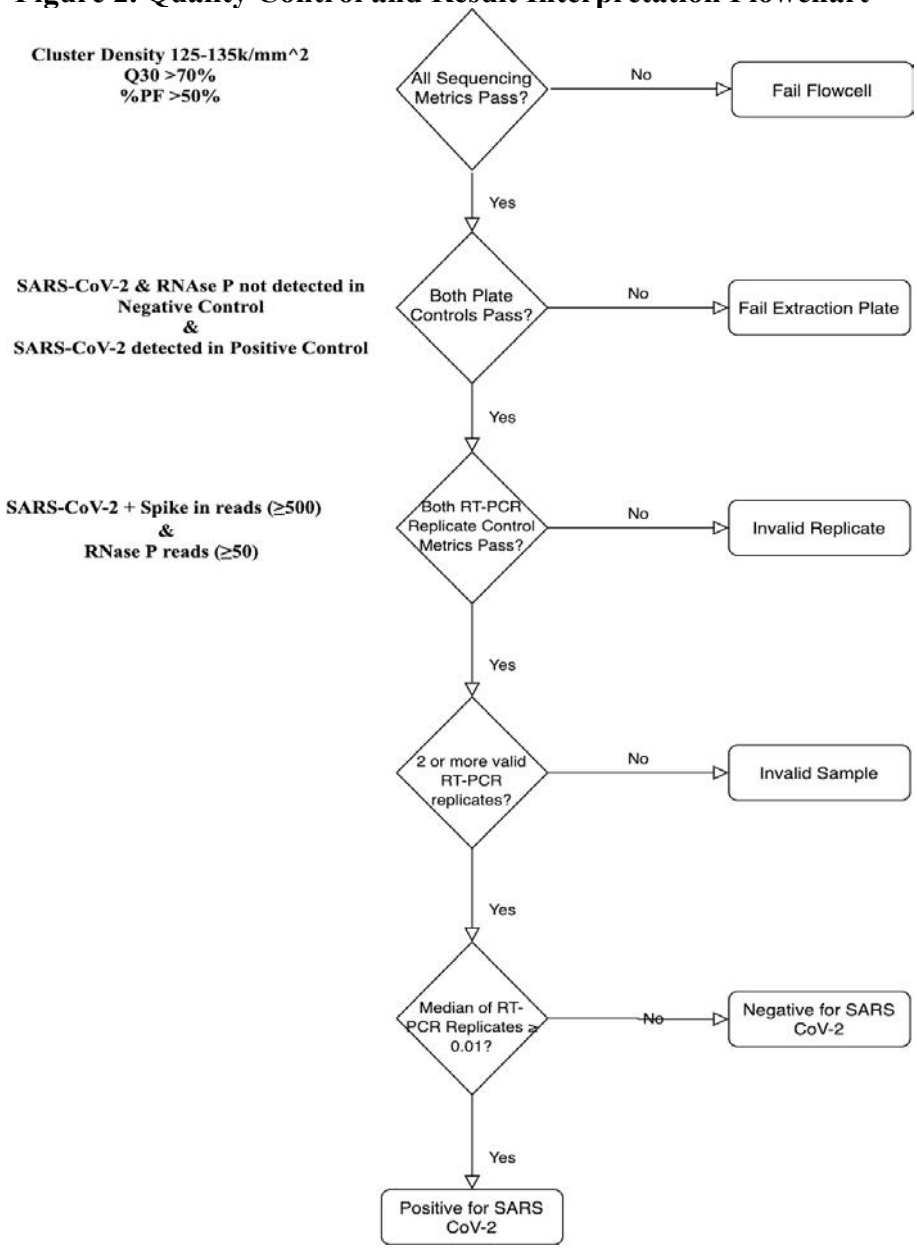
INTERPRETATION OF RESULTS

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) Guardant-19 – Negative (No Template) Control, Positive Control, Internal Spike Control, and Endogenous Control

The flowchart showing QC assessment is included in **Figure 2** below. QC metrics are described in **Table 5**.

Figure 2. Quality Control and Result Interpretation Flowchart*



*Q30 = Quality 30, a sequencing Quality Control metric; %PF = Percent Pass Filter

Table 5. Assay QC Criteria for Flow cell, Plate, and Sample Results

QC Metric	Threshold	Metric Type	Level
Cluster Density	125-335k/mm^2	Flow cell QC	Sequencing Batch
Q30	>70%	Flow cell QC	Sequencing Batch
%PF	>50%	Flow cell QC	Sequencing Batch
Positive Control	SARS-CoV-2 detected	Plate Control QC	Extraction Plate
Negative Control	SARS-CoV-2, RNase P not detected	Plate Control QC	Extraction Plate
RNase P reads	≥50	Sample QC	RT-PCR well
SARS-CoV-2 + Spike in reads	≥500	Sample QC	RT-PCR well
Number of RT PCR sample replicates passing QC	≥2	Sample QC	Patient Sample

2) Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. The Guardant-19 test result interpretation algorithm is described in **Table 6**, following the explanation of the bioinformatics analysis described in Description of Test Steps above.

Table 6. Summary of Assay Interpretation and Patient Reporting

Sample / plate / flow cell QC status	Validity	Interpretation	Action
At least 2 out of 3 RT PCR replicates pass QC; flow cell and plate controls pass QC	Valid	**Detected:** Guardant-19 sample score >= 0.01 **Not Detected:** Guardant-19 sample score < 0.01	Report results to physician, patient, and appropriate health authorities.
Either less than 2 RT PCR replicates passing QC or flow cell fails	Invalid	**No Call**	Repeat extraction and Guardant-19. If the repeated result remains invalid, report

Sample / plate / flow cell QC status	Validity	Interpretation	Action
QC or plate controls fail QC			INVALID and consider collecting a new specimen.

The final clinical laboratory report is issued through a HIPAA-compliant secure web portal.

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) -Analytical Sensitivity:

The limit of detection (LoD) is defined as the lowest concentration at which 19/20 replicates (or approximately 95% of all true positive replicates) are positively detected. The LoD of Guardant-19 was established using a dilution series of inactivated SARS-CoV-2 virus (ATCC VR-1986HK) spiked into negative NP-swab specimens. A total of 10 concentrations were tested (1500, 1250, 1000, 750, 500, 375, 250, 125, 50, and 25 copies/ml) with ≥20 replicates analyzed per level. For each concentration, the percentage of samples with a SARS-CoV-2 detected status was calculated out of the total number of replicates tested and passing QC for that viral concentration.

Out of 408 total samples analyzed, 360 (88.2%) contrived samples passed QC. Spiked virus was detected above the detection threshold of 0.01 for the Guardant-19 score in 100% of samples passing QC at all input levels greater than or equal to 25 copies per 200µl (125 copies/ml), in 27 of 32 (84%) of samples with an input level of 10 copies, and in 15 of 32 (47%) of samples with an input level of 5 copies. These results are summarized in **Table 7**. The 95% LoD was established as 25 copies per 200µl (125 copies/ml), the lowest input level tested that yielded a detection rate ≥ 95%.

Note that the QC failure rate observed in this study was not associated with system failures, but rather reflective of the usage of diluted clinical VTM negative matrix where RNase P counts were observed to be systematically lower for some donors after 1:5 dilution of the matrix. This is reflective of the study design using diluted negative matrix, rather than system failure rate. This is confirmed in the clinical validation cohort where no failures were observed.

Table 7: Summary of Limit of Detection Determination Results

SARS-CoV-2 Copies per 200 µL	Equivalent SARS-CoV-2 Copies per 1 mL	Number Tested	Detection Rate
300	1500	33	33/33 (100%)
250	1250	33	33/33 (100%)
200	1000	32	32/32 (100%)

SARS-CoV-2 Copies per 200 µL	Equivalent SARS-CoV-2 Copies per 1 mL	Number Tested	Detection Rate
150	750	29	29/29 (100%)
100	500	29	29/29 (100%)
75	375	37	37/37 (100%)
50	250	71	71/71 (100%)
25	**125**	**32**	**32/32 (100%)**
10	50	32	27/32 (84%)
5	25	32	15/32 (47%)

2) Inclusivity (Reactivity)

The Guardant-19 test utilizes identical oligonucleotide sequences for the N1 SARS-CoV-2 target genes as those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel under EUA. The inclusivity and cross-reactivity of CDC assay under an EUA has been evaluated. Nevertheless, additional evaluations were performed as the Guardant-19 primers include additional barcode sequences to identify each patient sample. An *in silico* assessment was performed of the percent identity matches against publicly available SARS-CoV-2 sequences that can be detected by the proposed molecular assay.

Each primer sequence with additional barcode sequences was examined against 1338 whole genome sequences of SARS-CoV-2 retrieved from NCBI on April 28, 2020. Please refer to **Table 8** for the NCBI taxid used to identify genome sequences used for this analysis. Each targeted primer sequence with additional barcode sequences was 100% identical in these strains and fully inclusive.

3) Exclusivity (Cross-reactivity):

The Guardant-19 test utilizes identical oligonucleotide sequences for the N1 SARS-CoV-2 target genes as those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel under EUA. The inclusivity and cross-reactivity of CDC assay under an EUA has been evaluated. Nevertheless, additional evaluations were performed as the Guardant-19 primers include additional barcode sequences to identify each patient sample. An *in silico* analysis was performed to compare the assay primers to common respiratory flora and other viral pathogens. The *in silico* cross-reactivity, as measured by sequence homology for any common targeted respiratory species, must be <80%.

For each primer sequence with additional barcode sequences used in the assay, an in silico analysis was performed using BLAST to identify all homologous matches. We used NCBI BLASTN 2.10.0+ MegaBlast to search the nt database with default parameters and inclusion

of specific taxonomic groups. The taxa groups for each taxonomic group or species in the Recommended List of Organisms queried on June 18th, 2020. No matches were found in any taxonomic groups (**Table 8**).

Table 8. Summary Results of Cross-Reactivity Study

	Pathogen/Contaminate	**NCBI taxid#**	**%Homology Forward and Reverse Primers**
1	Human coronavirus 229E	11137	no BLAST matches found
2	Human coronavirus OC43	31631	no BLAST matches found
3	Human coronavirus HKU1	290028	no BLAST matches found
4	Human coronavirus NL63	277944	no BLAST matches found
5	SARS-coronavirus	228407	no BLAST matches found
6	MERS-coronavirus	1335626	no BLAST matches found
7	Adenovirus (e.g. C1 Ad. 71)	10509	no BLAST matches found
8	Human Metapneumovirus (hMPV)	162145	no BLAST matches found
9	Parainfluenza virus 3,4	39744	no BLAST matches found
10	Parainfluenza virus 1,2	186938	no BLAST matches found
11	Influenza A	11320	no BLAST matches found
12	Influenza B	11520	no BLAST matches found
13	Enterovirus (e.g. EV68)	12059	no BLAST matches found
14	Respiratory syncytial virus	12814	no BLAST matches found
15	Rhinovirus	12058	no BLAST matches found
16	*Chlamydia pneumoniae*	83558	no BLAST matches found
17	*Haemophilus influenzae*	727	no BLAST matches found
18	*Legionella pneumophila*	446	no BLAST matches found
19	*Mycobacterium tuberculosis*	1773	no BLAST matches found

	Pathogen/Contaminate	NCBI taxid#	%Homology Forward and Reverse Primers
20	*Streptococcus pneumoniae*	1313	no BLAST matches found
21	*Streptococcus pyogenes*	1314	no BLAST matches found
22	*Bordetella pertussis*	520	no BLAST matches found
23	*Mycoplasma pneumoniae*	2104	no BLAST matches found
24	*Pneumocystis jirovecii* (PJP)	42068	no BLAST matches found
25	*Candida albicans*	5476	no BLAST matches found
26	*Pseudomonas aeruginosa*	287	no BLAST matches found
27	*Staphylococcus epidermidis*	1282	no BLAST matches found
28	*Staphylococcus salivarius*	1304	no BLAST matches found
29	*Nicotiana tabacum*	4097	no BLAST matches found
30	*Cannabis sativa*	3483	no BLAST matches found
31	Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract	N/A	N/A

4) ***Clinical Evaluation:***

A total of 127 (67 positive, 60 negative) remnant nasopharyngeal (NP) samples were processed with Guardant-19 and compared against results previously generated by a comparator assay under EUA performed at two different laboratories from two distinct counties (Cohort A and Cohort B). The same comparator EUA assay was used for both Cohort A and B. These samples were leftover de-identified remnants that were not individually identifiable and had no HIPAA identifiers.

Cohort A: 67 nasopharyngeal swabs and oropharyngeal swabs collected by healthcare providers from patients seeking SARS-CoV-2 testing over March and April 2020. This cohort contained 37 positive samples and 30 negative samples. 35 of the 37 positive results generated by Guardant-19 matched those generated by the comparator EUA assay. 30 of the 30 negative results generated by Guardant-19 matched those generated by the comparator EUA assay. There was not enough remnant specimen for further analysis of the discordant results.

Cohort B: 60 nasopharyngeal swabs collected by healthcare providers from patients seeking SARS-CoV-2 testing over March and April 2020 in (1) the given (anonymized) county and (2) from a cruise ship. The cruise ship included patients who were asymptomatic; however, the breakdown of patients by symptomatic vs asymptomatic is unavailable. This cohort contained 30 positive samples and 30 negative samples. 29 of the 30 positive results generated by Guardant-19 matched those generated by the comparator EUA assay. 29 of the 30 negative results generated by Guardant-19 matched those generated by the comparator EUA assay. There was not enough remnant specimen for further analysis of the discordant results.

Positive percent agreement (PPA) and negative percent agreement (NPA) were determined by comparing observed results generated by Guardant-19 with the results of the comparator assay under EUA (**Table 9**).

Table 9. Performance of Nasopharyngeal Swabs: Guardant-19 Compared to a Comparator Assay under EUA

		Comparator EUA Assay		
		Positive	**Negative**	**Total**
Guardant-19	**Positive**	64	1	65
	Negative	3	59	62
	Total	67	60	127
Positive Agreement		**95.52% (87.47% – 99.07%)[1]**		
Negative Agreement		**98.33% (91.06% – 99.96%)[1]**		

[1] Two-sided 95% confidence intervals, Clopper-Pearson method

LIMITATIONS

The performance of Guardant-19 was established using nasopharyngeal swab specimens in VTM. Oropharyngeal swabs, nasal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal washes/aspirates, and nasal washes/aspirates are also considered acceptable specimen types for use with Guardant-19, but the performance has not been established with these specimens.

WARNINGS:

- This test has not been FDA cleared or approved;

This test has been authorized by FDA under an EUA for use by Guardant Health Clinical Laboratory located at 505 Penobscot Drive, Redwood City, CA, 94063.

- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests 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 authorization is terminated or revoked sooner.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method used was the Quick-DNA/ RNA Viral MagBead Kit (Zymo Research). The RT-PCR was run in the Applied Biosystems Veriti Thermal Cycler. The sequencing was performed using NextSeq 550 Sequencing System. The results are summarized in the following Table.

Table 10. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Pane l

Reference Materials Provide d by FDA	Spe cimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasal Swab	$5.4x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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