EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

Fulgent COVID-19 by RT-PCR TEST
(FULGENT THERAPEUTICS)

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

INTENDED USE

The Fulgent COVID-19 by RT-PCR Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior nasal, nasopharyngeal, and oropharyngeal swab specimens from individuals suspected of COVID-19 by a healthcare provider, and anterior nasal swab specimens from individuals without symptoms or other reasons to suspect COVID-19.

This test is also for use with anterior nasal swab specimens that are either (1) self-collected using the Picture COVID-19 Home Collection Kit at home or in a healthcare setting by individuals, 18 years of age and older, including individuals without symptoms or other reasons to suspect COVID-19, when determined to be appropriate by a healthcare provider, or (2) collected using the Everlywell COVID-19 Test Home Collection Kit when used consistent with its authorization.

Testing is limited to laboratories designated by Fulgent Genetics which 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. 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 cause of disease.

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.

Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

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

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Fulgent COVID-19 by RT-PCR test is a real-time reverse transcription polymerase chain reaction test. The methods described in this application have been adapted from the "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel" document effective March 30, 2020. This test uses two SARS-CoV-2 primer and probe sets to detect regions in the N gene of SARS-CoV-2 in respiratory specimens from nasal, nasopharyngeal, or oropharyngeal swab samples. A third primer and probe set that detects human RNase P (RP) is used as an internal control. RNA is isolated from nasal, nasopharyngeal, or oropharyngeal swabs and reverse transcribed to cDNA. Amplification and detection of the SARS-CoV-2 markers and control targets are performed using the QuantStudio 6 and QuantStudio 7 Real-Time PCR System.

Zymo RNA Extraction Method

Nucleic acids (RNA) are isolated and purified from 200 µL sample input from nasal, nasopharyngeal, or oropharyngeal swabs using the Zymo *Quick*-RNA Viral Kit RNA Extraction Kits. Final extracted RNA is eluted into 50 µL of elution buffer.

The purified nucleic acid is reverse transcribed using TaqPath 1-Step RT-qPCR Master Mix, followed by the target amplification and fluorescent probe detection in the same reaction vials. Fluorescence intensity is monitored at each PCR cycle by the QuantStudio 6 or QuantStudio 7 Real-Time PCR System and the QuantStudio Real-Time PCR Software.

Apostle RNA Extraction Method

Nucleic acids (RNA) are isolated and purified from 200 µL sample input from patient specimens using the Apostle MagTouch 1000 Viral RNA Extraction System.
Final extracted RNA is eluted into 85 µL of elution buffer.

The purified nucleic acid is reverse transcribed using TaqPath 1-Step RT-qPCR Master Mix, followed by the target amplification and fluorescent probe detection in the same reaction vials. Fluorescence intensity is monitored at each PCR cycle by the QuantStudio 6 or QuantStudio 7 Real-Time PCR System and the QuantStudio Real-Time PCR Software.

Instruments

The Fulgent COVID-19 by RT-PCR test is to be used with the Zymo Quick-RNA Viral Kit RNA Extraction Kits or Apostle MagTouch 1000 Viral RNA Extraction Automation System and the QuantStudio 6 or QuantStudio 7 Real-Time PCR System.

Sample Collection Kits

This assay can be used with the Everlywell COVID-19 Test Home Collection Kit. Everlywell has granted Fulgent Genetics a right of reference to the data supporting use of this authorized home collection kit. This assay can also be used with the Picture COVID-19 Home Collection Kit manufactured by Fulgent Genetics.

Reagents

The primary reagents used in this assay, including primer and probe designs, are adapted from the "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel" document effective March 30, 2020.

Reagents used with the Fulgent COVID-19 by RT-PCR Test

Kits and Reagents	Manufacturer	Catalog #
Viral RNA Isolation Fast Kit	Apostle	A200619-6144
Zymo Quick-RNA Viral Kit RNA Extraction Kit	Zymo	R1034/R1035
TaqPath 1-Step RT-qPCR Master Mix, CG	ThermoFisher	A15300/15299
Primer: COVID-19_N1-F	IDT	10006606
Primer: COVID-19_N1-R	IDT	10006606
Probe: COVID-19_N1-P	IDT	10006606
Primer: COVID-19_N2-F	IDT	10006606
Primer: COVID-19_N2-R	IDT	10006606
Probe: COVID-19_N2-P	IDT	10006606
Primer: RP-F	IDT	10006606
Primer: RP-R	IDT	10006606
Probe: RP-P	IDT	10006606
Template: 2019-nCoV_N_Positive Control	IDT	10006625
Template: Hs_RPP30 Positive Control	IDT	10006626

Reagents are Research Use Only (RUO) reagents and are qualified by Fulgent laboratory for having acceptable performance with the Fulgent COVID-19 RT-PCR test. Fulgent laboratory will provide qualified reagents or reagent lot numbers thereof to its designated laboratories.

Picture COVID-19 Home Collection Kit

Nasal Swab Kit Components	Manufacturer	Catalog #
FedEx Pak (Poly Padded Mailer)	FedEx	139380
Custom External Shipping Box (Corrugated Fiberboard)	Imagen	Custom
Custom Instructional Inserts (Collection, Shipping, and Packaging)	Imagen	Custom
Transport Media (0.85% Saline) aliquoted into a Tube	Nest Scientific, USA or Zymo Research	Nest:202005 or A-01
Nasal Swab (Round Foam w/Polystyrene Handle, 80mm Breakpoint)	Nest Scientific, or Jiangsu Hanheng Medical Technology Company	N/A: swabs are supplied with saline tubes
Therapak Absorbent Materials (Absorbent Sheet)	Therapak 10300	22-130-039
Specimen Biohazard Bag 6x9	U-Line	S-2968
FedEx Express UN3373 Pak (with Return Shipping Label)	FedEx	163034

Sample Stability

Sample stability follows Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing from CDC (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html). Store respiratory specimens at 2-8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below.

Sample Shipping Stability with the Picture COVID-19 Home Collection Kit

A study was performed to test the stability of anterior nasal specimens collected with the Picture COVID-19 Home Collection Kit under simulated summer and winter shipping conditions. The study was conducted with 20 COVID-19 spiked samples at 2x LoD (low positives, 10 copies/µL), 5 COVID-19 spiked samples at 5x LoD (high positive, 25 copies/µL), 5 COVID-19 spiked samples at 10x LoD (high positives, 50 copies/µL), and 10 negative samples (to monitor for false positives). An aliquot was removed to test for T = 0 hr. The remaining anterior nasal samples were placed in the incubator and cycled through the Winter and Summer shipping conditions shown in the tables below. After 56 hours elapsed, the samples were removed from the incubator to room temperature, and then tested according to the Fulgent COVID-19 by RT-PCR Test SOP.

Summer Profile

Temperature	Cycle Period	Cycle Period (Hours)	Total Time (Hours)	RNA Extraction and qRT-PCR
40°C	1	8	8	-
22°C	2	4	12	-
40°C	3	2	14	-
30°C	4	36	50	-
40°C	5	6	56	Performed

Winter Profile

Temperature	Cycle Period	Cycle Period (Hours)	Total Time (Hours)	RNA Extraction and qRT-PCR
-10°C	1	8	8	-
18°C	2	4	12	-
-10°C	3	2	14	-
10°C	4	36	50	-
-10°C	5	6	56	Performed

Results shown in the table below demonstrate similar Ct values observed in the control group the summer profile and the winter profile.

Sample Stability Summary Table

	Control Group		Summer Profile		Winter Profile	
Low Positive, 2xLoD, 10 copies/µL	**N1 Ct**	**N2 Ct**	**N1 Ct**	**N2 Ct**	**N1 Ct**	**N2 Ct**
Mean Ct	36.11	38.04	36.49	38.14	36.35	37.83
SARS-CoV-2 Positive Call Rate	20/20	19/20	19/20	20/20	19/20	20/20
	100%	95%	95%	100%	95%	100%

	Control Group		Summer Profile		Winter Profile	
High Positive, 5xLoD, 25 copies/µL	**N1 Ct**	**N2 Ct**	**N1 Ct**	**N2 Ct**	**N1 Ct**	**N2 Ct**
Mean Ct	36.43	37.30	35.77	37.20	35.33	38.13
SARS-CoV-2 Positive Call Rate	5/5	5/5	5/5	5/5	5/5	5/5
	100%	100%	100%	100%	100%	100%
	Control Group		**Summer Profile**		**Winter Profile**	
High Positive, 10xLoD, 50 copies/µL	**N1 Ct**	**N2 Ct**	**N1 Ct**	**N2 Ct**	**N1 Ct**	**N2 Ct**
Mean Ct	34.05	35.53	34.22	35.31	34.29	35.22
SARS-CoV-2 Positive Call Rate	5/5	5/5	5/5	5/5	5/5	5/5
	100%	100%	100%	100%	100%	100%

Ordering of the Picture COVID-19 Home Collection Kit and Clinical Oversight

The Picture genetics testing platform is closely integrated with PWNhealth (www.pwnhealth.com), a national clinician network. While all laboratory processes are run by fulgent genetics, all medical interactions are handled by PWNhealth. This includes clinician review and approval of each test ordered, oversight of all materials produced, and contact with patients after testing is completed.

Ordering is restricted to adults (18 years and older) living in the United States. Each individual intending to order a test must first complete an eligibility screener. This screener is intended to ensure that Picture COVID-19 Home Collection Kits are provided only where testing is most needed and most responsible. In addition, the screener collects necessary information on exposure, symptoms, and risk. Screening guidelines are based on the most current CDC guidelines on COVID-19 testing and are strictly enforced. Individuals who are experiencing severe symptoms to the point of requiring medical attention are not eligible for testing but are advised to seek immediate medical assistance.

Upon confirmation of eligibility, users are directed into an order flow where they will order the test and complete additional relevant personal and demographic information which is passed to PWNhealth for review, documentation, and approval. Upon approval, kits are shipped to users using FedEx 2-day shipping.

When a patient's report is ready, they are notified via email that their report is accessible via their private Picture genetics portal. Patients may receive positive, negative, or indeterminate results. Patients with indeterminate results will be encouraged to retest. Positive and negative reports include self-quarantine/isolation guidelines as well as resources such as the CDC and WHO (supplementary: sample positive report; isolation and quarantine guide). Negative reports also include information on the risk of false negatives (supplementary: sample negative report). This content is reviewed and approved by the independent clinician network at PWNhealth. PWNhealth shares updated content requirements weekly based on the latest requirements and recommendations from the CDC. Should there be any new guidelines coming from the CDC to be implemented immediately, PWNhealth will inform picture genetics directly.

Every patient is informed of the opportunity to speak with a health professional at PWNhealth about their test results, regardless of whether their result was positive, negative, or indeterminate. PWNhealth reaches out to positive patients directly to discuss results. PWNhealth also reports relevant results and information to the appropriate public health authorities.

CONTROLS TO BE USED WITH THE FULGENT COVID-19 BY RT-PCR TEST

1. A "no template" control (NTC) serves as a negative control and is included in every assay plate to identify specimen contamination. Molecular grade, nuclease free water is used as the NTC.

2. A positive template control (2019-nCoV_N_Positive Control) is included in each assay plate to ensure the reagents and instruments are performing optimally. The positive control is a synthetic DNA plasmid containing the entire sequence of gene N of the COVID-19 virus. Two markers in gene N, as defined by the "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel" document effective March 30, 2020, will be targeted and detected by the primer and probe sets, COVID-19_N1 and COVID-19_N2.

3. An internal control (Hs_RPP30 Positive Control) targeting human RNase P mRNA (RP) is used to verify optimal RNA extraction, amplification, and the presence of nucleic acid in the samples.

4. Negative Extraction Control is a previously characterized negative patient sample. It serves both as a negative extraction control for any extraction cross contamination as well as an extraction control to validate extraction reagents and successful RNA extraction.

Sample Accessioning

A detailed laboratory protocol (SOP) has been submitted and reviewed by FDA.

Home collected samples received at the laboratory will be checked for the following criteria before entering the lab workflow. Samples with the following issues will be rejected and may require user follow-up and re-sampling:

- No sample collection tube included with the kit
- No barcode identifier attached to the sample collection tube
- Sample collection tube leaked resulting in no sample for testing
- Kit not registered/activated on the Picture platform
- Accession date is greater than 48hrs from the specimen collection date

INTERPRETATION OF RESULTS

a. Expected RT-PCR Results for Controls

Other than controls, each individual sample is examined for data quality based on raw signals and adjusted signals. The samples that pass the quality control are interpreted using rules specified below. The samples that fail the quality control are reported as assay failure.

Result Interpretation for Controls

Controls	Control Target	Expected Results
Negative	NTC	Ct Not Detected for N1, N2, and RP
Positive	2019-nCoV_N_Positive Control / N1	Ct<40
Positive	2019-nCoV_N_Positive Control / N2	Ct<40
Internal	Human RNase P (RP)	Ct≤35
Negative Extraction	Human Rnase P	N1 and N2: Ct Not Detected RP: Ct≤35

- NTC controls should not have detectable readouts for N1 or N2. False-positive readings, growth curve cycle thresholds (Ct) less than 40, indicates contamination of the assay or reagents.

- Positive controls should exhibit fluorescence growth curves for N1 and N2, respectively, that cross the threshold line and have Ct<40.

- Internal controls should exhibit fluorescence growth curves that cross the threshold line and have Ct≤35.

- Negative Extraction Control should not have detectable readouts for N1 or N2. False-positive readings, growth curve cycle thresholds (Ct) less than 40, indicates contamination of the assay or reagents.

- Any deviations from the expected results shown in the table above invalidates the entire assay. All samples from the run must be repeated starting from extracted RNA.

b. Interpretation of Clinical Samples

Assessment of clinical sample test results must be performed after the positive and negative controls have been examined and confirmed to be valid. If the controls are not valid, the customer results cannot be interpreted. Patient Results are interpreted as follows:

Result Interpretation for Clinical Samples

N1 [Ct<40]	N2 [Ct<40]	RP [Ct≤35]	Interpretation/Protocol	Report/Follow-Up
+	+	+ / –	SARS-CoV-2 detected	COVID-19 Positive
If only one of two targets are positive		+/-	- Inconclusive result - Repeat assay	Inconclusive
–	–	+	SARS-CoV-2 not detected	COVID-19 Negative

N1 [Ct<40]	N2 [Ct<40]	RP [Ct≤35]	Interpretation/Protocol	Report/Follow-Up
–	–	–	- Invalid result - Repeat assay	Invalid/QNS, request new sample

- When all controls exhibit the expected performance, a clinical specimen is considered positive for COVID-19 if the N1 and N2 marker growth curves cross the cycle threshold line and Ct<40. In this scenario, the COVID-19 positive result is still valid regardless if the RP target is or is not detected as described above.

- When all controls exhibit the expected performance, a clinical specimen is considered negative for COVID-19 if the N1 and N2 marker growth curves do not cross the threshold line. The RP target growth curve must cross the threshold line of Ct≤35 for the COVID-19 result to be valid.

- When all controls exhibit the expected performance, but the growth curves for the N1 and N2 markers and the RP target DOES NOT cross the cycle threshold line, the result is invalid. The extracted RNA from the clinical specimen must be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If a second invalid result occurs, a new specimen from the patient is needed.

- When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (N1 or N2), but not both, crosses the threshold line, the result is inconclusive for COVID-19. Re-extract RNA from residual specimen and re-test.

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) - Analytical Sensitivity

a) Zymo Extraction Method

The tentative LoD was identified by extracting and testing 10-fold serial dilutions of the control plasmid (2019-nCoV_N_Positive Control), which contains the whole sequence of the SARS-CoV-2 N gene. Serial dilutions of the positive control template were tested in triplicates. The lowest concentration at which all three replicates were positive was treated as the tentative LoD for each test.

Confirmation of the final LoD was determined using 2-fold serial dilutions of viral RNA (5 copies/µL and 2.5 copies/µL) in 20 extracted replicates. The final LoD of each test was determined to be the lowest concentration resulting in positive detection in 100% of the replicates (20/20). As shown in the summary table below (Summary of the Limit of Detection Confirmation for COVID-19 by RT-PCR Test), the final LoD determined for this test is 5 copies/µL.

Summary of Confirmatory LoD Study (Zymo Extraction Method)

Concentration (Copies/µL)	# of Valid Results	Rate of Positive Results	N1 Gene		N2 Gene	
			Detection Rate (%)	Mean Ct (SD)	Detection Rate (%)	Mean Ct (SD)
5	20	100% (20/20)	100% (20/20)	33.24	100% (20/20)	35.93
2.5	20	75% (15/20)	85% (17/20)	34.85	85% (17/20)	37.4

b) Apostle Automated Extraction Method

The tentative LoD was identified by extracting and testing 10-fold serial dilutions of inactivated virus (BEI) spiked into nasopharyngeal swab matrix. To provide comparability of the two extraction methods with the same material the preliminary LoD study was performed for the Apostle automated extraction in parallel with the previously authorized Zymo extraction method. Serial dilutions were tested in triplicates. The lowest concentrations at which all three replicates were positive for both N1 and N2 targets, 1.8-18 copies/µL, was determined to be the tentative LoD.

Summary of Preliminary LoD Study (Zymo and Apostle Extraction Method)

	Apostle					Zymo				
Concentration (Copies/µL)	Rate of Positive Results	N1 Gene		N2 Gene			N1 Gene		N2 Gene	
		Detection Rate (%)	Mean Ct (SD)	Detection Rate (%)	Mean Ct (SD)	Rate of Positive Results	Detection Rate (%)	Mean Ct (SD)	Detection Rate (%)	Mean Ct (SD)
1800	100% (3/3)	100% (3/3)	27.03	100% (3/3)	27.09	100% (3/3)	100% (3/3)	25.24	100% (3/3)	26.24
180	100% (3/3)	100% (3/3)	29.98	100% (3/3)	30.06	100% (3/3)	100% (3/3)	28.92	100% (3/3)	29.68
18	**100% (3/3)**	**100% (3/3)**	**32.2**	**100% (3/3)**	**33.09**	100% (3/3)	100% (3/3)	32.15	100% (3/3)	33.4
1.8	67% (2/3)	100% (3/3)	36.13	67% (2/3)	38.68	**100% (3/3)**	**100% (3/3)**	**35.36**	**100% (3/3)**	**37.14**
0.18	0 (0/3)	0 (0/3)	N/A	0 (0/3)	N/A	0 (0/3)	0 (0/3)	N/A	33% (1/3)	37.56
0.018	0 (0/3)	0 (0/3)	N/A	0 (0/3)	N/A	0 (0/3)	0 (0/3)	N/A	0 (0/3)	N/A

Confirmation of the final LoD was determined using serial dilutions of inactivated virus (9 copies/µL, 8 copies/µL, 7 copies/µL, 5.4 copies/µL) in 20 extracted replicates (Final LoD Study (Apostle)). The final LoD of each test was determined to be the lowest concentration resulting in positive detection in 100% of the replicates (20/20) for both targets (N1 and N2). As shown in the summary table below (Summary Final LoD Study (Apostle)), the final LoD determined for this test was 8 copies/µL.

Summary of Confirmatory LoD Study (Apostle Extraction Method)

Concentration (Copies/µL)	# of Valid Results	Rate of Positive Results	N1 Gene		N2 Gene	
			Detection Rate (%)	Mean Ct (SD)	Detection Rate (%)	Mean Ct (SD)
9	20	100% (20/20)	100% (20/20)	33.88	100% (20/20)	35.23
8	**20**	**100% (20/20)**	**100% (20/20)**	**34.28**	**100% (20/20)**	**35.75**
7	20	90% (18/20)	100% (20/20)	34.56	90% (18/20)	35.24
5.4	20	85% (17/20)	95% (19/20)	34.47	90% (18/20)	35.54

Final LoD by Extraction Method

Extraction Method	Final LoD
Apostle	8 copies/µL
Zymo	5 copies/µL

2) Inclusivity (Analytical Sensitivity):

An *in-silico* inclusivity analysis was performed by aligning each of the primer and probe sequences to all 1298 complete (>29kb), "high coverage only" hCoV-19 sequences submitted to GISAID (https://www.gisaid.org/) as of March 26, 2020 ("hCoV-19" is the name GISAID uses instead of SARS-CoV-2). All primers and probes have perfect identity to >99% of the 1298 sequences (see table below).

Identity of Primers and Probes to GISAID hCoV-19 Sequence Submissions

Primers & Probes	Sequences Aligned	Count (#) or Percentage (%) of hCoV-19 Aligned with Identity			
		# <100%	% <100%	# at 100%	% at 100%
COVID-19_N1-F	1,298	3	0.2%	1,295	99.8%
COVID-19_N1-P	1,298	10	0.8%	1,288	99.2%
COVID-19_N1-R	1,298	6	0.5%	1,292	99.5%
COVID-19_N2-F	1,298	0	0.0%	1,298	100.0%
COVID-19_N2-P	1,298	1	0.1%	1,297	99.9%
COVID-19_N2-R	1,298	2	0.2%	1,296	99.8%

No major issues were identified on the inclusivity of the primers and probes of the Fulgent COVID-19 by RT-PCR test for currently circulating virus strains as of May 2021.

No impact on the performance of the assay is expected for detecting recently emerging variants (South Africa variant 501Y.V2, lineage B.1.351; UK variant VOC202012/01, lineage B.1.1.7, and Brazilian variant B.1.1.28.1, lineage P.1) as the primers and probes do not overlap with the variant sequences.

3) Cross-Reactivity (Analytical Specificity)

The Fulgent COVID-19 by RT-PCR test uses the sequence provided by the CDC per the "2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel" document effective March 30, 2020. The *in-silico* analysis for primer and probe design has been performed by the U.S. CDC and published in the IFU for the 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. The sponsor performed a similar *in-silico* analysis and confirmed that there are no significant homologies with the human genome, other coronaviruses, or human microflora that would generate potential false positive test results.

In addition, an *in-silico* exclusivity analysis was performed by aligning each of the primer and probe sequences to all 77,943 complete genome sequences in the viral sequences division ("vrl") of GenBank as of March 27, 2020, including all human coronavirus reference genomes but excluding SARS-CoV-2 sequences. The SARS-CoV-2 N2 forward primer was found to be 100% homologous to the reference sequence for SARS-Coronavirus (ref|NC_004718.3| SARS coronavirus). However, since SARS-CoV is not circulating at this time and the reverse primer and probe are not homologous, there is no risk of false results.

4) Clinical Evaluation:

a) Orthogonal Performance Validation

A total of 94 clinical specimens, 30 positives and 64 negatives, were used to evaluate the performance of the COVID-19 RT-PCR test. Clinical samples were a mix of upper respiratory swab samples including NP, OP and mid-turbinate swabs. The concordance of positive and negative COVID-19 status was 100% for the Fulgent COVID-19 by RT-PCR test compared to a validated molecular SARS-CoV-2 assay.

b) Inter-laboratory Performance Evaluation

An inter-lab study was performed with an EUA authorized test to independently confirm Fulgent's COVID-19 results. Five COVID-19 positive and five negative samples were used in this study. Results demonstrate 100% concordance of the COVID-19 results between the two laboratories. Clinical samples were a mix or upper respiratory swab samples including NP, OP and mid-turbinate swabs). Positive and negative percent agreement to expected result was 100% for the known patient samples. Results of positive and negative clinical specimens were also confirmed by secondary testing.

Inter-laboratory Comparison Study

		EUA Authorized Comparator Test		
		Positive	**Negative**	**Total**
Fulgent (RT-PCR)	**Positive**	5	0	**5**
	Negative	0	5	**5**
	Total	5	5	**10**
Positive Percent Agreement (95% CI)		**100% (56.6%-100%)**		
Negative Percent Agreement (95% CI)		**100% (56.6%-100%)**		

c) Performance Evaluation of Specimens from Asymptomatic and Symptomatic Individuals

A prospective study was performed to evaluate the performance of the Fulgent COVID-19 by RT-PCR test in an all comer population including symptomatic subjects and subjects without symptoms or other reasons to suspect COVID-19 infection. A total of 12,644 nasal swab samples were collected in a 10-day collection period (10,835 subjects were asymptomatic, 950 subjects were symptomatic, and for 859 subjects the symptomatic status was unknown) and tested using the Fulgent COVID-19 by RT-PCR assay. Samples were tested with the Fulgent COVID-19 by RT-PCR test and results were compared with results obtained with a highly sensitive EUA authorized comparator test.

Asymptomatic Individuals

There were 10,835 samples from asymptomatic subjects (141 samples with positive Fulgent COVID-19 results and 10,694 samples with negative Fulgent COVID-19 results). Positivity rate of Fulgent COVID-19 results was 1.3% (141/10,835). Eighty-five (85) samples were randomly (blinded) selected from the positive cohort of 141 samples and 114 samples were randomly selected from the negative cohort of 10,694 samples. These samples were sent for testing with an EUA authorized highly sensitive comparator test. Nine (9) of the asymptomatic positive samples (10.6% of the total positive samples tested and 30% of the 30 samples with the highest Ct values) were low positive by the comparator test (i.e., within 3 Cts of the average Ct value at the comparator test's LoD). A summary of the results is shown in the three tables below:

Summary of Clinical Validation in Asymptomatic Population

		FDA Authorized Comparator RT-PCR		
		Positive	**Negative**	**Total**
Fulgent (RT-PCR)	**Positive**	83	2	**85**
	Negative	0	114	**114**
	Total	83	116	**199**
Positive Percent Agreement (95% CI)		**100% (95.6%-100%)**		
Negative Percent Agreement (95% CI)		**98.3% (93.9%-99.5%)**		

This validation demonstrated 100% positive percent agreement 98.3% negative percent agreement compared to the EUA-authorized comparator test for the asymptomatic population.

Two samples that were positive by the Fulgent COVID-19 RT-PCR test (EUA200156) and tested negative by the EUA comparator test had high Ct values with the Fulgent test indicative of low viral loads around the test's LoD.

Ct Values of Discordant Samples with the Fulgent COVID-19 RT-PCR test

	Fulgent (RT-PCR)		
Sample	**N1 Ct**	**N2 Ct**	**RP Ct**
1	34.57	34.17	27.54
2	34.93	33.34	31.01

Since only a fraction of the collected positive and negative samples were randomly selected and tested and the proportional fractions were different in relation to the total number of positive and negative specimens collected, the performance estimates were statistically adjusted based on the positive and negative likelihood ratios (PLR and NLR) likelihood ratios that were considered as ratios of two independent binomial proportions (StatXact):

PLR = +Infinity, 95%CI: (29.81; +Infinity)
NLR = 0.02353, 95%CI: (0.006458; 0.082)

For calculation of the confidence Intervals for PPA and NPA, positivity rate was assumed to be constant across the time the sample cohort was selected. Tables with the adjusted clinical data and performance estimates are provided below:

Adjusted Sample Number in Asymptomatic Population for Calculation of Adjusted Performance Estimates

		FDA Authorized Comparator RT-PCR		
		Positive	**Negative**	**Total**
Fulgent (RT-PCR)	**Positive**	138	3	141
	Negative	0	10,694	10,694
	Total	138	10,697	10,835

Performance Estimates of Testing Asymptomatic Subjects

	Performance Estimate	**95%CI**
PPA (adjusted)	100% (138/138)	(28.2%; 100%)*
NPA (adjusted)	99.97% (10,694/10,697)	(99.89%; 99.99%)*
PPV (not adjusted)	97.6% (83/85)	(91.8%; 99.4%)
NPV (not adjusted)	100% (114/114)	(96.7%; 100%)

*95%CI for PPA and NPA were adjusted because only 85 out 141 Fulgent (RT-PCR) test positive subjects and 114 out of 10,694 Fulgent (RT-PCR) test negative subjects had the comparator test results.

Symptomatic Individuals

There were 950 samples from symptomatic subjects (123 samples with positive Fulgent COVID-19 results and 827 samples with negative Fulgent COVID-19 results). Positivity rate of Fulgent COVID-19 results was 12.9% (123/950). Fifty-eight (58) samples were randomly (blinded) selected from the positive cohort of 123 samples and 9 samples were randomly selected from the negative cohort of 827 samples. These samples were sent for testing with an EUA authorized high sensitivity assay as the comparator test. A summary of the results is shown in the three tables below:

Summary of Clinical Validation in Symptomatic Population

		FDA Authorized Comparator RT-PCR		
		Positive	**Negative**	**Total**
Fulgent (RT-PCR)	**Positive**	58	0	**58**
	Negative	0	9	**9**
	Total	58	9	**67**
Positive Percent Agreement (95% CI)		**100% (93.8%-100%)**		
Negative Percent Agreement (95% CI)		**100% (70.1%-100%)**		

This validation demonstrated 100% positive percent agreement 100% negative percent agreement compared to the EUA-authorized comparator test for symptomatic population.

Since only a fraction of the collected positive and negative samples were randomly selected and tested and the proportional fractions were different in relation to the total number of positive and negative specimens collected, the performance estimates had to be statistically adjusted based on the positive and negative likelihood ratios (PLR and NLR) likelihood ratios as described above for the asymptomatic population. Tables with the adjusted clinical data and performance estimates for symptomatic individuals are provided below:

Adjusted Sample Number in Symptomatic Population for Calculation of Adjusted Performance Estimates

		FDA Authorized Comparator RT-PCR		
		Positive	**Negative**	**Total**
Fulgent (RT-PCR)	**Positive**	123	0	123
	Negative	0	827	827
	Total	123	827	950

Performance Estimates of Testing Symptomatic Subjects

	Performance Estimate	**95%CI**
PPA (adjusted)	100% (123/123)	(33.0%; 100%)*
NPA (adjusted)	100% (827/827)	(99.1%; 100%)*
PPV (not adjusted)	100% (58/58)	(93.8%; 100%)
NPV (not adjusted)	100% (9/9)	(70.1%; 100%)

*95%CI for PPA and NPA were adjusted because only 58 out 123 Fulgent (RT-PCR) test positive subjects and 9 out of 827 Fulgent (RT-PCR) test negative subjects had the comparator test results.

5) Picture COVID-19 Home Collection Kit Self-Collection Validation Studies

A self-collection study for nasal swab specimens was performed for 24 participants. After acknowledging consent for testing, the participants were each given 2 tubes of saline-based

transport media and 2 nasal swabs and were shown the sample collection instructional video. Each participant provided 2 self-collected samples. After sample collection, one sample from each participant was spiked with a known COVID-19 positive clinical sample in the laboratory. Each sample was then packaged and shipped back to the lab via FedEx Overnight (according to established packaging and shipping protocol). Upon arrival at the lab, the samples were unpacked and tested following the Fulgent COVID-19 by RT-PCR test. This study evaluated the users' ability to properly collect a nasal swab sample as well as the packaging/shipping protocol while samples were in possession of FedEx. The results of the self-collection validation were consistent with expected results. All positives (24/24) remained positive after shipment. All negative samples (24/24) remained negative for COVID-19. All 48 samples showed positive detection for the internal Human RNaseP control, which implies successful sample collection.

Summary of Usability Study

	Original Samples				**Inactivated COVID-19 Spiked Samples**			
Participant	**N1 (Ct)**	**N2 (Ct)**	**RP (Ct)**	**COVID-19 Status**	**N1 (Ct)**	**N2 (Ct)**	**RP (Ct)**	**COVID-19 Status**
1	-	-	29.08	Negative	33.64	34.87	26.41	Positive
2	-	-	28.67	Negative	34.72	36.63	24.96	Positive
3	-	-	25.45	Negative	33.72	34.78	24.08	Positive
4	-	-	31.20	Negative	32.98	34.85	27.22	Positive
5	-	-	34.31	Negative	32.35	33.95	28.61	Positive
6	-	-	30.25	Negative	33.91	35.96	24.85	Positive
7	-	-	28.33	Negative	32.77	35.02	26.57	Positive
8	-	-	28.98	Negative	34.01	36.50	25.17	Positive
9	-	-	31.12	Negative	33.15	34.87	27.97	Positive
10	-	-	28.95	Negative	33.82	35.64	26.46	Positive
11	-	-	28.11	Negative	34.06	35.16	26.00	Positive
12	-	-	34.08	Negative	32.73	34.24	28.05	Positive
13	-	-	32.39	Negative	32.50	33.60	27.60	Positive
14	-	-	31.31	Negative	33.56	34.96	27.79	Positive
15	-	-	31.46	Negative	34.49	35.44	25.02	Positive
16	-	-	31.89	Negative	33.24	34.72	28.14	Positive
17	-	-	34.40	Negative	32.95	34.68	27.74	Positive
18	-	-	30.21	Negative	34.40	36.52	27.31	Positive
19	-	-	30.44	Negative	35.07	37.53	24.83	Positive
20	-	-	30.61	Negative	34.85	35.73	27.03	Positive
21	-	-	30.56	Negative	35.92	38.04	27.66	Positive
22	-	-	30.57	Negative	35.00	38.90	23.32	Positive

	Original Samples				Inactivated COVID-19 Spiked Samples			
Participant	**N1 (Ct)**	**N2 (Ct)**	**RP (Ct)**	**COVID-19 Status**	**N1 (Ct)**	**N2 (Ct)**	**RP (Ct)**	**COVID-19 Status**
23	-	-	30.56	Negative	34.93	36.78	27.54	Positive
24	-	-	28.92	Negative	34.60	36.98	27.02	Positive
Mean Ct	**N/A**	**N/A**	**30.49**	**N/A**	**33.89**	**35.68**	**26.56**	**N/A**

6) 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. Extraction was completed using the Quick-RNA Viral Kit RNA Extraction Kit (Zymo Research) and RT-PCR was performed on the QuantStudio 6 (ThermoFisher Scientific). The results are summarized in the following Table.

Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provide d by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasal Swab	$3.6x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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

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.

WARNINGS:

- This product has not been FDA cleared or approved; but has been authorized by FDA under an Emergency Use Authorization (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.
- 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. § 360bbb3(b)(1), unless the declaration is terminated or authorization is revoked sooner.