ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
COVID-19 by RT-PCR TEST
(FULGENT THERAPEUTICS, LLC)

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 upper and lower respiratory specimens (nasal, nasopharyngeal, and oropharyngeal swabs) from individuals suspected of COVID-19 infection.

This test is also for use with nasal swab specimens that are self-collected at home or in a healthcare setting by individuals using an authorized home-collection kit when determined to be appropriate by a healthcare provider.

Testing is limited to Fulgent Genetics, a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in nasal, nasopharyngeal, or oropharyngeal swab 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. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities. Fulgent Genetics will be one of these laboratories.

Negative results do not preclude COVID-19 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. Fulgent COVID-19 by RT-PCR test final reports will state this information.

The COVID-19 by RT-PCR test is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures. The 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.

1. Nucleic acids (RNA) are isolated and purified from nasal, nasopharyngeal, or oropharyngeal swabs using the Qiagen QIAamp Viral RNA Mini Kit or Zymo *Quick*-RNA Viral Kit RNA Extraction Kits.
2. Final extracted RNA is eluted into 50 µL of elution buffer.
3. 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.
4. 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 USED WITH TEST

Instruments

The Fulgent COVID-19 by RT-PCR test is to be used with the Qiagen QIAamp Viral RNA Mini Kit or Zymo *Quick*-RNA Viral Kit RNA Extraction Kits and the QuantStudio 6 or QuantStudio 7 Real-Time PCR System.

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.

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.

Kits and Reagents	Manufacturer	Catalog #
Qiagen QIAamp Viral RNA Mini Kit	Qiagen	52906
Zymo Quick-RNA Viral Kit RNA Extraction Kit	Zymo	R1035/R1041
TaqPath 1-Step RT-qPCR Master Mix, CG	ThermoFisher	A15300/15299
Primer: COVID-19_N1-F	IDT	Custom
Primer: COVID-19_N1-R	IDT	Custom
Probe: COVID-19_N1-P	IDT	Custom
Primer: COVID-19_N2-F	IDT	Custom
Primer: COVID-19_N2-R	IDT	Custom
Probe: COVID-19_N2-P	IDT	Custom
Primer: RP-F	IDT	Custom
Primer: RP-R	IDT	Custom
Probe: RP-P	IDT	Custom
Template: 2019-nCoV_N_Positive Control	IDT	10006625
Template: Hs_RPP30 Positive Control	IDT	10006626

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

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.

INTERPRETATION OF RESULTS

Expected RT-PCR Results for Controls

Controls	Control Target	Expected Results
Negative	NTC	Ct Not Detected
Positive	2019-nCoV_N_Positive Control / N1	Ct<40
Positive	2019-nCoV_N_Positive Control / N2	Ct<40
Internal	Human RNase P (RP)	Ct<40

- NTC controls should not have detectable readouts. 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 that cross the threshold line and have Ct<40.
- Internal controls should exhibit fluorescence growth curves that cross the threshold line and have Ct<40.
- 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.

COVID-19 RT-PCR Patient Result Interpretation Table

COVID-19 Marker: N1	COVID-19 Marker: N2	RP	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
–	–	–	- 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 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:

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.

Tentative LoD: Results of triplicate testing of serial dilutions of the positive control

Target	Serial 10-Fold Dilution Factor	Concentration in Dilution Tested	Replicate 1 (Ct)	Replicate 2 (Ct)	Replicate 3 (Ct)	Call Rate	Lowest Concentration with Uniform Positivity per Analyte
N1	1:10	20,000 copies/µL	24.233	24.214	24.114	100%	20 copies/µL
	1:100	2000 copies/µL	29.198	27.553	27.743	100%	
	1:1000	200 copies/µL	34.435	31.763	33.234	100%	
	1:10,000	20 copies/µL	40.866	34.785	36.287	100%	
	1:100,000	2 copies/µL	41.856	36.609	39.250	67%	
N2	1:10	20,000 copies/µL	25.256	25.412	25.322	100%	20 copies/µL
	1:100	2000 copies/µL	28.889	31.641	29.101	100%	
	1:1000	200 copies/µL	42.698	36.083	37.153	100%	
	1:10,000	20 copies/µL	37.878	35.621	37.775	100%	
	1:100,000	2 copies/µL	43.606	Undetermined	Undetermined	33%	

Ct Values for Final LoD Confirmatory Test Results Using Viral RNA

	Marker N1 (Ct)		**Marker N2 (Ct)**	
Replicant	**5 copies/µL**	**2.5 copies/µL**	**5 copies/µL**	**2.5 copies/µL**
1	33.04	35.76	37.01	37.73
2	33.27	34.23	36.83	37.29
3	33.34	33.97	35.92	37.73
4	33.97	31.90	35.78	34.44
5	32.96	35.22	35.98	38.68
6	33.08	34.80	36.03	38.30
7	33.91	35.13	35.34	38.02
8	33.22	35.93	36.02	36.56
9	33.09	34.73	36.57	Undetermined
10	33.86	37.12	35.47	37.13
11	32.71	35.22	36.40	37.73
12	33.74	32.65	36.18	35.52
13	34.17	35.92	35.95	37.57

	Marker N1 (Ct)		Marker N2 (Ct)	
Replicant	**5 copies/µL**	**2.5 copies/µL**	**5 copies/µL**	**2.5 copies/µL**
14	32.20	34.78	34.38	37.02
15	32.14	36.06	35.86	36.89
16	32.79	Undetermined	35.14	38.89
17	33.56	34.13	35.91	38.81
18	33.87	34.89	35.40	Undetermined
19	32.67	Undetermined	36.46	Undetermined
20	33.22	Undetermined	36.03	37.51

Summary of the LoD Confirmation for COVID-19 by RT-PCR Test Using Viral RNA

	Marker N1		Marker N2	
RNA Concentration	**5 copy/µL**	**2.5 copy/µL**	**5 copy/µL**	**2.5 copy/µL**
Positive Detection/Total	20/20	17/20	20/20	17/20
Mean Ct	33.24	34.85	35.93	37.40
Standard Deviation Ct	0.57	1.26	0.60	1.14

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%

3) Cross-Reactivity (Analytical Specificity)

This 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 addressed by the U.S. CDC in the same document. We 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.

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. Only one significant (zero or one mismatch) alignment was found to reference virus genomes, but no primer pair has a significant alignment on the same sequence, so no amplicons are expected (see table below).

Significant (zero or one mismatch) alignments to virus reference genomes

Primer		Virus Sequence		Alignment Results				Primer Sequence			Virus Sequence		
qseqid	qlen	sseqid	slen	mismatch	gaps	pident	evalue	qstart	qseq	qend	sstart	sseq	send
COVID-19_N2-F	20	ref|NC_004718.3| SARS coronavirus	29,751	0	0	100.0	0.0130	1	**TTACAAACATTGGCCGCAAA**	20	29,013	**TTACAAACATTGGCCGCAAA**	29,032

4) Clinical Evaluation:

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 or 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.

Inter-laboratory Performance Evaluation

An inter-lab study was performed with Altru Dx (8562 Katy Freeway, Houston, TX 77024; CLIA #45D2128678) to independently confirm Fuglent's COVID-19. Five COVID-19 positive and five negative samples were used in this test. Results demonstrate 100% concordance of the COVID-19 results between the two labs. Clinical samples were a mix or upper respiratory swab samples including NP, OP and mid-turbinate swabs).

Specimen #	Fulgent	Altru Dx	% Concordance
1	Negative	Negative	100%
2	Positive	Positive	100%
3	Negative	Negative	100%
4	Negative	Negative	100%
5	Negative	Negative	100%
6	Negative	Negative	100%
7	Positive	Positive	100%
8	Positive	Positive	100%
9	Positive	Positive	100%
10	Positive	Positive	100%

Conclusion

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.