ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
FRL SARS-CoV-2 Test
(UAB Fungal Reference Lab)

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

(The FRL SARS-CoV-2 Test will be performed at the UAB Fungal Reference Lab Clinical Laboratory in Birmingham, Alabama, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, as per the laboratory procedures reviewed by the FDA under this EUA.)

INTENDED USE

The FRL SARS-CoV-2 Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in respiratory specimens (nasopharyngeal swab, oropharyngeal swab, nasal swab, mid-turbinate nasal swab, anterior nasal swab, nasopharyngeal wash/aspirate, nasal aspirate, and bronchoalveolar lavage specimens) collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the UAB Fungal Reference Lab in Birmingham, Alabama, 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 detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper and lower 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.

Testing with the FRL SARS-CoV-2 Test is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays and *in vitro* diagnostic procedures. The FRL SARS-CoV-2 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The FRL SARS-CoV-2 Test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe sets are used to detect RNA from the SARS-CoV-2 virus in nasopharyngeal swab, oropharyngeal swab, nasal swab, mid-turbinate nasal swab, anterior nasal swab, nasopharyngeal wash/aspirate, nasal aspirate, and bronchoalveolar lavage specimens collected from patients as recommended for testing

by public health authority guidelines. The FRL SARS-CoV-2 Test is a modification of the CDC 2019-nCoV Real-Time RT-PCR assay which utilizes the same SARS-CoV-2 N1 target and human RNAase P control primers and probes for target amplification and detection. The FRL SARS-CoV-2 Test differs from the CDC 2019-nCoV Real-Time RT-PCR assay in that it only uses the N1 assay target, it utilizes a different extraction kit (E.Z.N.A. Viral RNA Kit, Omega Scientific), and it uses a different PCR detection system (QuantStudio 5).

Nucleic acids are first isolated and purified from upper and lower respiratory specimens using the E.Z.N.A. Viral RNA Kit manual extraction system. To perform the nucleic acid extraction, 150 µL of specimen is mixed with 500 µL of a mastermix containing QVL extraction buffer and carrier RNA. The final elution volume is 50 µL. Five µL of the purified RNA is then reverse transcribed into cDNA using Thermo Fisher Scientific's TaqMan Fast Virus 1-Step Master Mix and subsequently amplified using Applied Biosystems QuantStudio 5 Real-Time PCR System with QuantStudio Real-Time PCR software v1.4.3. During real-time PCR, if the target of interest is present, the probe anneals to the 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 cleaves the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored after each PCR cycle.

INSTRUMENTS USED WITH THE TEST

The FRL SARS-CoV-2 Test is to be used with the Applied Biosystems QuantStudio 5 Real-Time PCR System, with QuantStudio Real-Time PCR software v1.4.3 (Thermo Fisher, Waltham, MA).

REAGENTS AND MATERIALS

Table 1. Reagents and Materials Required for Use with the FRL SARS-CoV-2 Test

Component	Supplier	Catalog#	Description
2019-nCoV_N Positive Control	Integrated DNA Technologies, Inc.	10006625	Positive amplification control; 200,000 copies/µL in IDTE pH 8.0; used at 620 copies/mL (~5X LOD), or 3.1 µl of a 200 copy/µl dilution
N1 and RP primer/probe sets	Integrated DNA Technologies, Inc.	10006606	Primer Final Conc.= 500nM Probe Final Conc.= 125nM
E.Z.N.A. Viral RNA Kit	Omega Scientific	R6874-02	RNA extraction kit for 200 purifications
Molecular grade water	Ambion	AM9938	Reagent; 100% H_2O
TaqMan Fast Virus 1 Step Master Mix	Thermo Fisher	4444432	RT-PCR master mix; 4X
96 well plates	Thermo Fisher	4483354	Disposable; RT-PCR

Component	Supplier	Catalog#	Description
8 well strips	Thermo Fisher	A30588	Disposable; RT-PCR
Sterile nuclease free 1.5 mL Microfuge tubes	Thermo Fisher	3451	Disposable; liquid handling
MicroAmp Optical Adhesive Film	Thermo Fisher	4360954	Disposable; RT-PCR
Disposable pipettes	Thomas Scientific	1207F56	Disposal; liquid handling; sterile; individually wrapped
100% ethanol	Sigma	E7023-500ML	Reagent preparation; 100% ethanol
Sterile nuclease-free pipette tips	Rainin	30389212 30389241 30389225 30389175	1000ul 200ul 20ul 10ul
Microcentrifuge capable of at least 13,000g	Eppendorf	5424 or equivalent	Centrifuge spin columns
Large Centrifuge	Eppendorf	5910R or equivalent	Centrifuge 96 well plates
Vortexer (Vortex Genie 2)	Scientific Industries	SI-0236	Vortex

CONTROLS TO BE USED WITH THE FRL SARS-CoV-2 Test

1) <u>No Template PCR Control (NTC):</u>
    a. RNAse free molecular grade ddH_2O (Ambion cat. # AM9938) is inoculated into 2 wells of a 96 well plate during RT-PCR setup to monitor reagent contamination and primer/probe specificity. The NTC control is included with each RT-PCR run and evaluated with the N1 and RP primer/probe sets in independent wells.
2) <u>Positive Template Control (PTC):</u>
    a. The IDT DNA 2019-nCoV Plasmid Positive Control (cat. # 10006625) contains the nucleocapsid gene targets from 2019-nCoV and is inoculated into 1 well of a 96 well plate during RT-PCR setup to monitor the performance of primers/probes and the RT-PCR master mix. The PTC control is included with each RT-PCR run and evaluated with the N1 primer/probe set.
3) <u>Internal Extraction Control (IEC):</u>
    a. The IEC validates both the RNA extraction procedure and RT-PCR assay. Primers/probes targeting the human RNAse P (RP) gene are used to detect intact RNA extracted from each patient sample. Each patient specimen is run in

2 wells using either the assay target primer/probe set for N1 or the internal control primer/probe set for RP.

4) No Template Extraction Control (NTEC):
    a. RNAse free molecular grade ddH_2O (Ambion cat. # AM9938) is used as a negative control for RNA extraction and RT-PCR. One NTEC control is processed alongside patient samples for each RNA extraction batch and split into two samples for running the RT-PCR with each the N1 and RP primer/probe sets.

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) FRL SARS-CoV-2 Test Controls – No Template PCR Control, Positive Template Control, Internal Extraction Control, No Template Extraction Control

- The positive Ct cutoff for analytes and controls is ≤ 42 cycles.
- Two No Template PCR Controls (NTC) are added to each PCR run and evaluated with the N1 and RP primer/probe sets in two distinct wells.
- One Positive Template Control (PTC) is included in each RT-PCR run and is evaluated with the N1 primer/probe set.
- Internal Extraction Controls are intrinsic to each sample and are evaluated with the RP primer/probe set.
- One Negative Template Extraction Control (NTEC) is performed with each batch of RNA extractions and evaluated with N1 and RP primer/probe sets.
- All controls must pass to interpret and report data on patient samples.
- If any of the controls in Table 2 do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred.

Table 2. Expected Performance of Controls

Control Type	External Control Name	Used to Monitor	N1	RP	Expected Ct Values	If control does not pass
Positive Template Control	PTC	RT-PCR reagent failure including primer and probe integrity	+	N/A	N1 ≤ 42 Ct	Invalidate the run & retest
Negative Template Control	NTC	Potential contamination during PCR setup	-	-	N1- No amplification Rp- No amplification	Invalidate the run & retest
Negative Template Extraction Control	NTEC	Potential contamination during RNA extraction and PCR setup	-	-	N1- No amplification Rp- No amplification	Invalidate the RNA extraction batch & retest

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 positive Ct cutoff for analytes and controls is ≤ 42 cycles. Negative indicates a Ct value of >42 cycles. If all controls pass, proceed to report patient sample results according to the following table:

Table 3. Patient Specimen Results Interpretation Guide[a, b]

N1 target	RP target	Result Interpretation	Report	Actions
+	±	2019-nCoV detected	POSITIVE	Report results to public health lab and sender
-	+	2019-nCoV not detected	NEGATIVE	Report results to sender. Consider testing for other respiratory viruses.
-	-	Invalid Result	Invalid	Repeat extraction and RT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

[a] Report diagnostic result as appropriate and in compliance with the laboratory's reporting system.

[b] Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation suggest that 2019-nCoV infection is possible, and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If 2019-nCoV infection is still suspected, re-testing should be considered in consultation with public health authorities.

PERFORMANCE EVALUATION

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

LoD Range Finding Study:

For the analytical studies, the AccuPlex SARS-CoV-2 Reference Material Kit from SeraCare (cat. # 0505-0126) was used as spiking material. This material contains synthetic RNA encoding the following viral genes: ORFIa, RdRp, S, E, and N, as well as the human RNAse P gene. It is a non-infectious, replication deficient, virus-like particle with an intact viral capsid and outer membrane mimicking an encapsulated RNA virus. Remnant nasopharyngeal swabs (n=15 total) in viral transport media were obtained from patients with respiratory symptoms in the pre-COVID era. AccuPlex positive reference material was spiked into these clinical matrices at concentrations of 1000 copies/mL, 500 copies/mL, 250 copies/mL, 125 copies/mL, and 62.5 copies/mL (each concentration tested in triplicate). Independent RNA extractions (with the E.Z.N.A. Viral RNA Kit from Omega Bio-Tek, cat. # R6874-02) and RT-PCR (with the Applied Biosystems QuantStudio 5 Real-Time PCR System from Thermo Fisher)

were performed for each of these 15 contrived samples. Based on the results, the preliminary LoD was determined to be 125 copies/mL.

Table 4. LoD Range Finding Results

Sample	Viral RNA (copies/mL)	N1 CT	RP CT	Interpretation
1	1000	31.598	24.686	POSITIVE
2	1000	30.912	24.711	POSITIVE
3	1000	31.462	24.698	POSITIVE
4	500	31.973	24.452	POSITIVE
5	500	32.277	24.460	POSITIVE
6	500	32.286	24.490	POSITIVE
7	250	34.315	24.445	POSITIVE
8	250	33.484	24.507	POSITIVE
9	250	34.619	24.454	POSITIVE
10	125	35.538	24.419	POSITIVE
11	125	33.865	24.360	POSITIVE
12	125	34.122	24.274	POSITIVE
13	62.5	37.443	25.224	POSITIVE
14	62.5	Undetermined	25.223	NEGATIVE
15	62.5	Undetermined	25.244	NEGATIVE

LoD Confirmatory Study:

The preliminary LoD of 125 copies/mL was tested as the LoD of the FRL SARS-CoV-2 Test in a confirmatory study consisting of 20 contrived samples. Contrived NP specimens were created by spiking AccuPlex SARS-CoV-2 Reference Material Kit from SeraCare (cat. # 0505-0126) into viral transport media from remnant NP swabs collected from SARS-CoV-2 negative individuals. This positive reference material was spiked at a concentration of 125 copies/mL as described for the LoD Range Finding Study above. Independent RNA extractions and RT-PCR were performed for each of these 20 contrived samples. The results show that 20/20 (100%) of the contrived samples tested at a concentration of 125 copies/mL test positive for SARS-CoV-2 (Table 5), confirming this concentration as the LoD for this assay.

Table 5. LoD Confirmatory Results

Sample	Viral RNA (copies/mL)	N1 CT	Rp CT	Interpretation
1	125	33.265	22.954	POSITIVE
2	125	34.405	23.041	POSITIVE
3	125	32.515	23.084	POSITIVE
4	125	33.871	23.018	POSITIVE
5	125	35.942	23.107	POSITIVE
6	125	32.081	23.101	POSITIVE
7	125	33.107	23.233	POSITIVE
8	125	34.410	23.062	POSITIVE
9	125	33.613	23.127	POSITIVE

Sample	Viral RNA (copies/mL)	N1 CT	Rp CT	Interpretation
10	125	33.948	23.061	POSITIVE
11	125	33.710	23.121	POSITIVE
12	125	34.721	23.061	POSITIVE
13	125	33.949	23.015	POSITIVE
14	125	35.421	24.389	POSITIVE
15	125	34.257	24.372	POSITIVE
16	125	37.682	25.381	POSITIVE
17	125	35.870	25.372	POSITIVE
18	125	34.237	25.756	POSITIVE
19	125	37.951	25.687	POSITIVE
20	125	36.516	23.110	POSITIVE

Limit of Detection: 125 copies/mL

2) Inclusivity (Reactivity) and Cross-reactivity (Analytical Specificity):

The N1 and RP primers/probe sequences were not changed compared to the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel; as such additional *in silico* analyses of cross reactivity and reactivity was not performed.

3) Clinical Evaluation:

For the clinical evaluation, thirty (30) positive and thirty (30) negative nasopharyngeal swab clinical specimens were evaluated by the FRL SARS-CoV-2 Test and an EUA approved SARS-CoV-2 real-time RT-PCR assay as the comparator. The positive percent agreement was 100% (30/30, see Table 6), and the negative percent agreement was 100% (30/30, see Table 7).

Positive Percent Agreement: 30/30 = 100% (CI: 88.7-100%)
Negative Percent Agreement: 30/30 = 100% (CI: 88.7-100%)

Table 6. Positive Specimen Concordance

		EUA approved SARS-CoV-2 real-time RT-PCR assay				FRL SARS-Cov-2 Assay		
Specimen #	Specimen Name	E Ct	N2 Ct	SPC* Ct	Interpretation	N1 Ct	RP Ct	Interpretation
1	3323	29	31	28	POS	28	24	POS
2	3312	23	26	28	POS	24	26	POS
3	3306	24	27	28	POS	27	26	POS
4	3310	15	17	28	POS	15	24	POS
5	3305	16	18	28	POS	16	27	POS
6	2086	31	35	28	POS	35	29	POS
7	2065	34	38	28	POS	38	31	POS
8	1999	36	39	27	POS	33	28	POS
9	2019	36	39	27	POS	36	27	POS
10	2054	37	38	28	POS	38	27	POS

UAB Fungal Reference Laboratory SARS-CoV-2 Test EUA Summary

		EUA approved SARS-CoV-2 real-time RT-PCR assay				FRL SARS-Cov-2 Assay		
Specimen #	Specimen Name	E Ct	N2 Ct	SPC* Ct	Interpretation	N1 Ct	RP Ct	Interpretation
11	2168	17	19	28	POS	15	27	POS
12	2180	28	30	28	POS	27	29	POS
13	2812	29	31	28	POS	34	30	POS
14	2130	18	20	28	POS	17	37	POS
15	2105	19	21	28	POS	19	29	POS
16	1825	36	38	28	POS	12	25	POS
17	1875	38	41	28	POS	38	28	POS
18	1860	25	28	27	POS	22	25	POS
19	1895	31	35	28	POS	25	29	POS
20	1835	34	38	27	POS	25	29	POS
21	602	33	37	28	POS	30	28	POS
22	618	36	38	28	POS	27	30	POS
23	6S7	34	38	28	POS	27	26	POS
24	665	31	35	28	POS	32	30	POS
25	948	35	38	28	POS	27	31	POS
26	1145	34	38	28	POS	30	23	POS
27	1149	30	33	28	POS	37	26	POS
28	1234	24	26	27	POS	25	22	POS
29	1031	0	42	28	POS	36	25	POS
30	1310	16	18	28	POS	12	21	POS

*SPC = Specimen Processing Control, serves as internal extraction control

Table 7. Negative Specimen Concordance

		EUA approved SARS-CoV-2 real-time RT-PCR assay				FRL SARS-CoV-2 Assay		
#	Specimen Name	E Ct	N2 Ct	SPC* Ct	Interpretation	N1 Ct	RP Ct	Interpretation
1	3304	no amplification	no amplification	28	NEG	no amplification	26	NEG
2	3297	no amplification	no amplification	28	NEG	no amplification	25	NEG
3	3301	no amplification	no amplification	28	NEG	no amplification	32	NEG
4	3303	no amplification	no amplification	28	NEG	no amplification	31	NEG
5	3318	no amplification	no amplification	28	NEG	no amplification	27	NEG
6	1940	no amplification	no amplification	28	NEG	no amplification	32	NEG
7	1942	no amplification	no amplification	28	NEG	no amplification	23	NEG
8	1943	no amplification	no amplification	27	NEG	no amplification	27	NEG
9	1943	no amplification	no amplification	28	NEG	no amplification	28	NEG
10	2087	no amplification	no amplification	28	NEG	no amplification	37	NEG
11	2223	no amplification	no amplification	29	NEG	no amplification	26	NEG

UAB Fungal Reference Laboratory SARS-CoV-2 Test EUA Summary

		EUA approved SARS-CoV-2 real-time RT-PCR assay				FRL SARS-CoV-2 Assay		
#	Specimen Name	E Ct	N2 Ct	SPC* Ct	Interpretation	N1 Ct	RP Ct	Interpretation
12	2073	no amplification	no amplification	28	NEG	no amplification	28	NEG
13	2074	no amplification	no amplification	28	NEG	no amplification	29	NEG
14	2075	no amplification	no amplification	28	NEG	no amplification	29	NEG
15	2076	no amplification	no amplification	28	NEG	no amplification	30	NEG
16	2077	no amplification	no amplification	27	NEG	no amplification	25	NEG
17	2942460	no amplification	no amplification	28	NEG	no amplification	27	NEG
18	2245606	no amplification	no amplification	28	NEG	no amplification	27	NEG
19	513662	no amplification	no amplification	28	NEG	no amplification	25	NEG
20	604649	no amplification	no amplification	28	NEG	no amplification	25	NEG
21	1834	no amplification	no amplification	28	NEG	no amplification	32	NEG
22	1836	no amplification	no amplification	28	NEG	no amplification	29	NEG
23	1837	no amplification	no amplification	28	NEG	no amplification	26	NEG
24	1838	no amplification	no amplification	28	NEG	no amplification	23	NEG
25	1839	no amplification	no amplification	27	NEG	no amplification	26	NEG
26	1840	no amplification	no amplification	28	NEG	no amplification	25	NEG
27	1841	no amplification	no amplification	28	NEG	no amplification	25	NEG
28	1842	no amplification	no amplification	28	NEG	no amplification	26	NEG
29	1843	no amplification	no amplification	28	NEG	no amplification	27	NEG
30	1844	no amplification	no amplification	28	NEG	no amplification	28	NEG

*SPC = Specimen Processing Control, serves as internal extraction control

Clinical Confirmation with EUA Test

The first 5 positive and the first 5 negative nasopharyngeal specimens as determined by the FRL SARS-CoV-2 Test were sent to a high complexity CLIA laboratory for confirmatory testing using a SARS-CoV-2 real-time RT-PCR assay approved under EUA. There was 100% (5/5) positive agreement and 100% (5/5) negative agreement for the specimens tested. The results are acceptable and support use of the FRL SARS-CoV-2 Test for testing clinical specimens. The results are reported below.

Table 8. Clinical Confirmation Results

	EUA approved SARS-CoV-2 real-time RT-PCR assay	FRL SARS-Cov-2 Assay	
Specimen #	**Reported Results**	**N1 Ct**	**Rp Ct**
1	NEGATIVE	No amplification	22.4
2	NEGATIVE	No amplification	24.8
3	NEGATIVE	No amplification	24.6
4	NEGATIVE	No amplification	26.8
5	NEGATIVE	No amplification	28.0
6	POSITIVE	30.2	27.1
7	POSITIVE	31.0	26.3
8	POSITIVE	18.1	27.6
9	POSITIVE	33.8	31.4
10	POSITIVE	33.9	28.5

LIMITATIONS

The performance of the FRL SARS-CoV-2 Test was established using nasopharyngeal swabs specimens in viral transport media. Oropharyngeal swabs, nasal swabs, mid-turbinate nasal swabs, anterior nasal swabs, nasopharyngeal wash/aspirates, nasal aspirates, and bronchoalveolar lavage specimens are also considered acceptable specimen types for use with the FRL SARS-CoV-2 Test 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 authorized laboratory;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.