ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-CoV-2 RT-PCR Assay

(Cormeum Laboratory Services)

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

(The SARS-CoV-2 RT-PCR assay will be performed at Cormeum Laboratory Services, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The SARS-CoV-2 assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Cormeum Laboratory Services certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 42 U.S.C. §263a to perform high complexity tests.

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

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

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

DEVICE DESCRIPTION AND TEST PRINCIPLE

This SARS-CoV-2 RNA assay is a real-time reverse transcription polymerase chain reaction (RT -PCR) test. The SARS-CoV-2 primer and probe set designed to amplify

and detect three regions of the SARS-CoV-2 single stranded RNA genome: the Nucleocapsid (N), Spike (S) and Open Reading Frame 1ab (Orf1ab) in respiratory specimens from patients as recommended for testing by public health authority guidelines. RNA is isolated from patient nasopharyngeal specimens, subsequently transcribed to cDNA through reverse transcription and amplified through real-time polymerase chain reaction (RT-PCR) for detection of SARS-Cov-2 targets. Through RT-PCR, the probe anneals to a specific target sequence located between the forward and reverse primers. A fluorescent signal is generated as the reporter dye is separated from the quencher dye during the extension phase. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. This fluorescence intensity is monitored each replication cycle to estimate the initial quantity of SARS-Cov-2 RNA present in the patient specimen.

INSTRUMENTS USED WITH TEST

Table 1

Process	Instrument / Manufacturer
RNA Extraction	• MagMax Express 96 Fast - MagMax Viral/Pathogen Isolation Kit A42352
Real-Time PCR	• Quantstudio 12K Flex Real-Time PCR
Software	• Applied Biosystems QuantStudio 12K Flex Software V.1.1.2

REAGENTS AND MATERIALS

Table 2

Reagent/Material	Catalog Number
TaqPath COVID-19 Combo Kit Assay	ThermoFisher, A47814
MagMax Viral/Pathogen Isolation Kit	ThermoFisher, A42352
Water (Ultrapure)	ThermoFisher, 10977023
Ethanol, 200 proof	VWR Part # EM8.18760.100
T-PCR Grade Water	ThermoFisher AM9935
TaqPath 1-step Multiplex Master Mix	ThermoFisher A28523
MagMax Purification System	ThermoFisher 5400620
Integra 96/384 ViaFlo	Integra 6001
Integra 12 Channel Voyager	Integra 473
Quant Studio 12K Flex	ThermoFisher 4471134
Eppendorf 5804 Centrifuge	VWR part no 89210-972
Vortex-Genie 2	Scientific Industries # G560
MicroAmp Optical Plate	ThermoFisher 4309849
Optical Film	ThermoFisher 4311971

Reagent/Material	Catalog Number
Plate Foils	ThermoFisherAB0626
KF Deep Well Plates	ThermoFisher9504050
KF Tip Combs	ThermoFisher 97002534
Kingfisher Standard Plates	ThermoFisher 97002540
100 mL Basins	VWR no 89094-656

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

SARS-CoV-2 Positive Control:
One positive control is run with each assay on each plate. This control is the TaqPath COVID-19 RNA control (external) which contains targets specific to the SARS-CoV-2 genomic regions targeted by the assays. The dilution and reverse transcription of the positive control is performed according to the kit instructions. The positive control is designed to monitor the RT-PCR setup and reagent integrity.

Internal Extraction Control:
The internal control amplifies MS2 phage. The MS2 control is added to each sample and the negative control during the extraction step. This control is used to monitor the RNA extraction, reverse transcription and amplification process.

NTCs (No template controls):
The negative control is no-template control. A negative no template control (molecular grade water, nuclease-free water) is added to each extraction run and carried through the RT-PCR process. The negative control verifies that no contamination of sample or reagents occurred during the extraction or PCR steps.

INTERPRETATION OF RESULTS

1) SARS-CoV-2 RT-PCR test Controls – Positive, Negative, and Internal:

All test controls should be examined prior to interpretation of patient results. If the positive and negative controls are not valid, the patient results cannot be interpreted and the assay run must be repeated.

Positive control: TaqPath COVID-19 external positive control must be positive for all three of the SARS-CoV-2 targets and amplification must have a Ct<33 in order for the results to be valid.

Internal control: The presence of MS2 during the analysis indicates proper RNA extraction. The MS2 internal control must be “detected” in order to report a negative SARS-CoV-2 result.

Negative no template control (nuclease free water): The NTC must be negative for all targets (N gene, S gene, Orf1ab gene) but positive for MS2 in order for the test to be valid.

Table 3: Control results interpretation

	N, S, Orf1ab	MS2	Status	Result
No template control (NTC)	All target genes negative	Positive	Valid	PASSED
TaqPath Covid-19 RNA Positive Controls	All target genes positive (Ct<33)	Negative	Valid	PASSED

2) ***Examination and Interpretation of Patient Specimen Results:***

The interpretation of positive and negative results is based on performance of the Positive and Negative controls, as well as the CT, Cq, and AMP score of each patient sample.

1. Positive Specimens: At least two SARS-CoV-2 targets must meet or exceed the following thresholds to be interpreted as POSITIVE: CT<33.00, Cq>0.3, AMP Score >1.5.

2. Negative Specimens: No detection of any SARS-CoV-2 target is considered a negative result. Detection of the MS2 internal control target must be detected to report a negative result when no SARS-CoV-2 target is detected. Additionally, if two or more targets have Ct values >36, then the result is considered negative.

3. Inconclusive Results: If two or more SARS-CoV-2 targets have a CT>33.00 and CT<36.00, as well as pass the thresholds for AMP score and Cq score, then the sample will be marked as INCONCLUSIVE and retested. Upon retest, if the INCONCLUSIVE sample has two or more targets with result CT>33, the sample will be classified as NEGATIVE. Additionally, the laboratory will reach out to the provider and suggest that if the patient has had an exposure or is exhibiting clinical symptoms that they be retested. If upon retest the INCONCLUSIVE result has at least two targets CT<33, Cq>0.3, and AMP score >1.5, the sample will be classified as POSITIVE.

4. Invalid Results: No detection of any SARS-CoV-2 target coupled with no detection of MS2 is considered an invalid result. Invalid results will be processed again the following day following notification to the patient's healthcare provider. A second invalid result will result in a request for sample recollection from the healthcare provider.

Table 4: Patient Results interpretation

ORF1ab	N gene	S gene	MS2	Status	Result	Action
NEG	NEG	NEG	NEG	Invalid	NA	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	POS	Valid	SARS-CoV-2 Not Detected	Report results to healthcare provider. Consider testing for other viruses.
2 targets CT >33 but CT<36			POS or NEG	Valid	SARS-CoV-2 Inconclusive	Repeat test. If the repeat result remains inconclusive, sample will be classified as negative. Additionally, we will contact the provider and suggest a retest if clinical symptoms are present or if an exposure has occurred.
2 targets CT>36			POS or NEG	Valid	SARS-CoV-2 Not Detected	Sample will be classified as negative. Report results to healthcare provider. Consider testing for other viruses.
Two or more SARS-CoV-2 targets = POS (CT<33)			POS or NEG	Valid	Positive SARS-CoV-2	Report results to healthcare provider and appropriate public health authorities.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

The LoD was conducted using a 10-fold dilution series of three extraction replicates per concentration using SARS-COV-2 genomic RNA template at concentrations ranging from 0.19 - 100 copies/µl. The RNA spiked into a clinical matrix of nasopharyngeal swabs. An additional 20 replicates were tested using the concentrations outlined in Table 5 below to confirm the LoD. The LoD is defined as the lowest concentration at which 19/20 replicates are positive for at least two of the

SARS-CoV-2 targets. Based on the result interpretation of two positive targets, the LoD of the assay is 2 copies/µl.

Table 5: LoD confirmation

Targets	**ORF1ab**					**S Gene**					**N Gene**				
RNA Concentration	4.0	2.0	1.0	0.5	0.25	4.0	2.0	1.0	0.5	0.25	4.0	2.0	1.0	0.5	0.25
Positives Detected (Total = 20)	20	20	14	10	0	20	19	10	8	0	17	11	5	0	0
Mean CrT	30.12	30.62	32.54	31.70	33.71	31.05	32.1	32.94	32.84	34.1	32.03	32.92	33.75	34.9	35.16
Std. Deviation (CrT)	0.74	0.48	1.1	0.42	0.94	0.55	0.65	0.86	0.71	1.34	0.47	0.59	1.16	0.75	2.11

2) <u>Analytical Inclusivity/Cross Reactivity</u>

For the analytical inclusivity and specificity (cross-reactivity) of the Probe and Primer set used with this assay, please refer to the approved EUA200010/A001, submitted on 03/13/20 by ThermoFisher Scientific for the use of their TaqPath COVID19 Combo Kit. There are no concerns with the primer/probes as of 04/11/2022.

3) <u>Clinical Evaluation:</u>

<u>Comparison against an EUA authorized Test:</u>
A total of 174 nasopharyngeal clinical specimens were tested using the Cormeum modified ThermoFisher assay and the EUA authorized ThermoFisher (TF) assay at a separate CLIA and CAP accredited laboratory. Out of these 174 clinical specimens, 37 were positive and 137 were negative by the EUA authorized test. All specimens were tested at Cormeum Laboratories in a randomized and blinded fashion. Results from Cormeum laboratory were 100% concordant for both the positive and negative specimens.

Table 6: Assay agreement with clinical specimens

		EUA Authorized TF Assay	
		Pos	Neg
Cormeum Modified TF Assay	Pos	37	0
	Neg	0	137
	Total	37	137

PPA: 100% (37/37)
NPA: 100% (147/147)

The mean Ct values for each target detected by the modified TF assay and EUA authorized assay are outlined below:

Table 7: Mean Ct for 37 Detected Samples

	ORF1ab	S Gene	N Gene	MS2
Cormeum	25.043	25.57	24.53	26.54
EUA authorized	25.09	25.1	24.75	26.23

Comparison against expected results:

Additionally, assay performance was further determined through the development of 30 contrived reactive specimens and 30 non-reactive specimens. The 30 reactive specimens were developed by spiking 100µl from known positive patient clinical specimens into 300µl leftover individual negative clinical samples representing individual patients. Non-reactive specimens consisted of known negative clinical patient samples. Samples were tested in a randomized blinded fashion. Of the 30 positive contrived samples, 28 resulted as positive by the Cormeum assay (PPA = 93%). For the two contrived samples that did not result as positive, one sample had no detection in any of the 4 targets (indicating an invalid result), and one sample had detection in MS2 but no detection in the SARS-CoV-2 targets (indicating a negative result). Of the 30 negative contrived samples, all 30 resulted as negative (100%). Below is a summary of the mean Ct values for each of the targets:

Table 8: 30 Reactive Samples

	ORF1ab	N	S	MS2
Mean Ct value	29.97	28.98	29.58	26.31

Table 9: 30 Non-Reactive Samples

	Orf1ab	N	S	MS2
Mean Ct value	N/A	N/A	N/A	26.79

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 and instrument used were the MagMax Purification System and Quantstudio 12K Flex Real-Time PC respectively. The results are summarized in the following Table.

Table 10. 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	Nasopharyngeal Swab	$1.8x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratory;
- 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

LIMITATION:

- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with 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.