ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY ORIG3N 2019 NOVEL CORONAVIRUS (COVID-19) Test (ORIG3N, INC.)

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

(The Orig3n 2019 Novel Coronavirus (COVID-19) Test will be performed at Orig3n, Inc. certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Instructions of Use that were reviewed by the FDA under this EUA.)

INTENDED USE

The Orig3n 2019 Novel Coronavirus (COVID-19) Test is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in oropharyngeal, nasopharyngeal, anterior nasal, and mid-turbinate nasal swab specimens from individuals suspected of COVID-19. Testing is limited to the Orig3n, Inc. laboratory located in Boston, MA, which is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, certified high-complexity laboratory.

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 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 treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the Orig3n 2019 Novel Coronavirus (COVID-19) Test is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays. The Orig3n 2019 Novel Coronavirus (COVID-19) Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Testing of self-collected or healthcare provider-collected anterior and mid-turbinate nasal swabs is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Orig3n 2019 Novel Coronavirus (COVID-19) Test is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The test uses two primer and probe sets to detect two regions in the SARS-CoV-2 nucleocapsid (N) gene (N1 and N2), one primer and probe set for the universal detection of SARS-like coronaviruses (N3), and one primer and probe set to detect human RNase P (RP) in a clinical sample.

RNA is isolated from upper respiratory specimens including oropharyngeal, nasopharyngeal, anterior nasal, and mid-turbinate nasal swab specimens using the Omega Bio-Tek Mag-Bind Viral DNA/RNA 96 bead kit and is reverse transcribed to cDNA and subsequently amplified using the Applied Biosystems QuantStudio7 Flex (QS7) instrument with QuantStudio Real-Time PCR software version 1.3. During the amplification process, the probe anneals to a 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 degrades the bound probe, causing the reporter dye (FAM) to separate from the quencher dye (BHQ-1), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle.

INSTRUMENTS USED WITH TEST

The Orig3n 2019 Novel Coronavirus (COVID-19) Test is to be used with the Applied Biosystems QuantStudio7 Flex (QS7) instrument with QuantStudio Real-Time PCR software version 1.3.

REAGENTS AND MATERIALS

Reagent Manufacturer and Description	Catalog #	Manufacturer
Mag-Bind Viral DNA/RNA 96 kit	M6246-01 (1x96 preps), M6246-02 (4x96 preps), M6246-03 (12x96 preps)	Omega Bio-Tek
KiCqStart One-Step Probe RT-qPCR ReadyMix	KCQS07, KCQS08, KCQS09	Sigma-Aldrich
COVID-19_N1-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N1-R Primer (reverse primer)	10006606	Integrated DNA Technologies
COVID-19_N1-P Probe (N1 probe)	10006606	Integrated DNA Technologies
COVID-19_N2-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N2-R Primer (reverse primer)	10006606	Integrated DNA Technologies
COVID-19_N2-P Probe (N2 probe)	10006606	Integrated DNA Technologies
COVID-19_N3-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N3-R Primer (reverse primer)	10006606	Integrated DNA Technologies
COVID-19_N3-P Probe (N3 probe)	10006606	Integrated DNA Technologies
RP-F Primer (forward primer)	10006606	Integrated DNA Technologies
RP-R Primer (reverse primer)	10006606	Integrated DNA Technologies
RP-P Probe (RNase P probe)	10006606	Integrated DNA Technologies
COVID-19_N_Positive Control Hs_RPP30_Internal Extraction Control	10006626	Integrated DNA Technologies
2019-nCoV_N_Positive Control	10006625	Integrated DNA Technologies
Nuclease-Free Water	P119E	Promega

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

1) A no template control (NTC) is needed to check for contamination of extraction and assay reagents. Molecular grade, nuclease-free water is used in place of sample nucleic acid for this control. The NTC is used on every assay plate.

2) A positive (COVID-19_N_Positive) control is needed to verify proper assay set-up and SARS-CoV-2 reagent integrity. The positive control is used on every assay plate starting at master mix addition at a concentration of 50 copies/µL. The positive control is commercially supplied from IDT and is made of *in vitro* transcribed and purified viral RNA target that contains one copy each of N1, N2, and N3.

3) An internal control internal (Hs_RPP30) control targeting RNase P is needed to verify that nucleic acid is present in every sample and is used for every sample processed. This also serves as a positive extraction control to ensure that samples resulting as negative contain nucleic acid for testing. Detection of the RP gene in patient test samples verifies successful extraction of the sample, proper assay setup, sample integrity, and efficient sample collection.

4) A negative extraction (NEC) control is a previously characterized negative patient sample. It serves both as a negative extraction control to monitor for any cross-contamination that occurs during the extraction process, as well as an extraction control to validate extraction reagents and successful RNA extraction. A NEC is used on each extraction plate.

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 (Refer to Table 1 for a summary of control results).

1) COVID-19 RT-PCR test Controls – Positive, Negative, and Internal:

- No template controls should be negative (Ct Not Detected) for all targets. If any of the N1, N2, N3, or RP NTC reactions exhibit positive fluorescence above the threshold, it is possible that contamination occurred, or that the assay was setup improperly. The RT-PCR run is invalid. Repeat from the RT-PCR step using residual extraction material. If the repeat test result is positive, re-extract and re-test all samples.

- Positive template controls should be positive for the N1, N2, and N3 targets (Ct < 40) and negative for the RP target (Ct Not Detected). Negative results with either N1, N2, or N3 primer/probe sets invalidates the run and suggests the assay may have been set up incorrectly, or the integrity of the primers/probes is

compromised. The RT-PCR run is invalid. Repeat from the RT-PCR step using residual extraction material. If the repeat test result is negative for SARS-CoV-2 targets, re-extract and re-test all samples.

Because the positive control is diluted in nuclease-free water, results for the RP assay should be negative, with no amplification curves crossing the PCR cycle threshold of < 40. If RP is positive, it is possible contamination occurred during reaction plate setup. The run is invalid. Repeat from the RT-PCR step using residual extraction material. If the repeat test result is positive, re-extract and re-test all samples.

- The positive extraction control (Hs_RPP30) should be negative for N1, N2 and N3 targets (Ct Not Detected), and positive for the RP target (Ct < 40). Failure of a patient sample to yield an RP Ct value < 40 may indicate improper extraction of nucleic acid from patient samples, carry-over of PCR inhibitors from patient samples, or absence of sufficient human cellular material. Re-extract the residual sample and re-test the sample.

  The negative extraction control (negative clinical matrix) should be negative for N1, N2 and N3 targets (Ct Not Detected), and positive for the RP target (Ct < 40). If positive results are obtained for N1, N2, or N3 targets, contamination of nucleic acid extraction reagents or cross-contamination of samples may have occurred. The extraction run and the RT-PCR run are invalid and should be repeated using residual patient sample.

Table 1: Expected Results of Controls Used in the Orig3n 2019 Novel Coronavirus (COVID-19) Test

Control Type	Control Name	Used to Monitor	Expected Results and Ct Values							
			SARS-CoV-2 N1		SARS-CoV-2 N2		SARS-CoV-2 N3		RNase P (RP)	
			Call	Ct	Call	Ct	Call	Ct	Call	Ct
Negative	NTC	Extraction/ Amplification	Negative	UND*	Negative	UND	Negative	UND	Negative	UND
Positive	COVID-19_N_Positive Template Control (PTC)	Amplification	Positive	< 40	Positive	< 40	Positive	< 40	Negative	UND
Extraction	Negative Extraction Control (NEC); Negative Clinical Sample	Extraction/ Amplification	Negative	UND	Negative	UND	Negative	UND	Positive	< 40
	Internal HS_RPP30 Control Targeting RNase P	Amplification	Negative	UND	Negative	UND	Negative	UND	Positive	< 40

*UND = Undetermined

2) Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Please see the table below (Table 2) for guidance on interpretation and reporting of results.

Table 2: Interpretation of Patient Results Using the Orig3n Novel Coronavirus (COVID-19) Test

SARS-CoV-2 N1 (Ct < 40)	SARS-CoV-2 N2 (Ct < 40)	SARS-CoV-2 N3 (Ct < 40)	RNase P (CT < 40)	Interpretation	Report Result	Actions
+	+	+	+/-	SARS-CoV-2 **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities.
If one or both of these are positive		+/-	+/-	SARS-CoV-2 **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities.
-	-	+	+/-	SARS-CoV-2 is **Presumed Positive**	**PRESUMED POSITIVE**	Sample is repeated once. If the repeated result remains "PRESUMPTIVE POSITIVE", additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management.
-	-	-	+	SARS-CoV-2 **Not Detected**	**NEGATIVE**	Reported to sender
-	-	-	-	**Invalid test**	**INVALID**	Sample is repeated once. I f a second failure occurs, it is reported to sender as invalid and recommend recollection if patient is still clinically indicated.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

The LoD of the Orig3n Test was determined using quantified whole viral SARS-related coronavirus 2 (USA-WA1/2020) RNA obtained from BEI Resources (NR-52285). A preliminary LoD was determined by testing serial dilutions (1000 cp/µL - 5.0 cp/µL) of RNA spiked into pooled clinical negative, nasopharyngeal swab matrix in quadruplicate. Spiked samples were tested with the Orig3n test following extraction with the Mag-Bind Viral DNA/RNA 96 Kit. The initial LoD was 5.0 genomic copies/µL.

The LoD was verified by testing 20 additional extraction replicates consisting of pooled negative clinical nasopharyngeal swab matrix spiked at each 12.5 copies/µL and 10 copies/µL (See Table 3). Samples were spiked with RNA prior to extraction with the Mag-Bind Viral DNA/RNA 96 kit. Controls for the LoD confirmation study were diluted 1:200 in Nuclease Free Water. The results of the study are summarized below.

Table 3: LoD Verification Study Results

Concentration (copies/µL)	Concentration (copies/reaction)	Average Ct Values				SARS-CoV-2 N1, N2, N3 Detection Rate		
		N1	N2	N3	hRP	N1	N2	N3
12.5	60	23.6	25.6	25.1	25.8	20/20	20/20	20/20
10.0	50	27.7	26.7	28.6	26.2	20/20	20/20	20/20
Negative control (NTC)	NA	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Positive control COVID-19_N_P	NA	Pos	Pos	Pos	Neg	Pos	Pos	Pos
Extraction control Hs_RPP30	No copies	Neg	Neg	Neg	Pos	Neg	Neg	Neg

2) <u>Analytical Inclusivity/Specificity:</u>

The Orig3n test utilizes identical oligonucleotide sequences for the N1, N2, and N3 target genes as those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. The inclusivity and cross-reactivity of the CDC EUA assay has been previously evaluated and therefore, additional evaluation is not required. The CDC has granted a right of reference to the performance data contained in the CDC's EUA request (FDA submission number EUA200001) to any entity seeking an FDA EUA for a COVID-19 diagnostic device.

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

Performance of the Orig3n 2019 Novel Coronavirus (COVID-19) test was evaluated using individual clinical nasopharyngeal swab specimens spiked with SARS-CoV-2 genomic RNA (BEI Resources cat # 52285). In total, 30 negative clinical matrix samples and 90 contrived positive clinical matrix samples were tested.

Of the 90 contrived positive clinical samples, 30 were prepared with concentrations of SARS-CoV-2 RNA at the assay LoD (1X, 10 copies/µL). Thirty additional samples contained RNA at concentrations equivalent to 1.25X the assay LoD, while the remaining 30 samples contained RNA at concentrations equivalent to 10X the assay LoD.

Prepared samples were randomized and blinded, and RNA was extracted using the Mag-Bind Viral DNA/RNA 96 kit. Testing was performed in one RT-PCR run with one positive, one negative, and one extraction control included per plate. Each sample also contained its own internal extraction control. Results of the study are summarized below (Table 4).

Table 4: Contrived Clinical Evaluation Summary Data

SARS-CoV-2 concentration (copies/µL)	Number of samples	Average Ct				Detection Rate			
		N1	N2	N3	hRP	N1	N2	N3	hRP

1X LoD (10.0 copies/µL)	30	25.1	26.4	26.7	28.5	30/30	30/30	30/30	30/30
1.25X LoD (12.5 copies/µL)	30	24.5	26.7	28.1	27.9	29/30	29/30	30/30	30/30
10X LoD (100.0 copies/µL)	30	22.1	23.1	23.7	27.9	30/30	30/30	30/30	30/30
Negative Clinical Matrix (no copies)	30	Neg	Neg	Neg	Pos	0/30	0/30	0/30	30/30

The results at all tested levels demonstrated greater than 95% agreement and all negative samples were non-reactive.

In addition, the first 5 positive and first 5 negative samples by the Orig3n COVID-19 test were sent to a laboratory running the CDC EUA test for confirmatory testing. All 10 patient specimens yielded concordant results.