ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY AVELLINOCoV2 TEST (AVELLINO LAB USA)

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

(The AvellinoCoV2 test will be performed at Avellino Lab USA, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per ALUSF-DOC-236 and ALUSF-DOC-237 laboratory instructions for use that were reviewed by the FDA under this EUA.)

INTENDED USE

The AvellinoCoV2 test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal and oropharyngeal swab specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Avellino Lab USA, Inc., that is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets the requirements to perform high complexity tests.

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

Testing with the AvellinoCoV2 test is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays. The AvellinoCoV2 test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The AvellinoCoV2 test is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The test uses two primer and probe sets to detect two regions in the

SARS-CoV-2 nucleocapsid (N) gene and one primer and probe set to detect human RNase P (RP) in a clinical sample.

RNA isolated from nasopharyngeal and oropharyngeal swab specimens using the QIAGEN QIAamp DSP Viral RNA Mini Kit is reverse transcribed to cDNA and subsequently amplified using Applied Biosystems 7500 Fast Real-Time PCR System with software version 2.3. 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 (MGBNFQ), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle.

INSTRUMENTS USED WITH TEST

The AvellinoCoV2 test is to be used with the Applied Biosystems 7500 Fast Real-Time PCR System with software version 2.3.

REAGENTS AND MATERIALS

Reagent	Manufacturer	Catalog #
QIAamp DSP Viral RNA Mini Kit (50/250)	QIAGEN	61904
	QIAGEN	52906
TaqPath™ 1-Step RT-qPCR Master Mix, CG	ThermoFisher	A15299
Water, UltraPure Distilled Water, Protease Free or Equivalent	Invitrogen	10977-023
T1 Forward-Primer, Reverse-Primer and Probe	Avellino	NA
T2-Forward-Primer, Reverse-Primer and Probe	Avellino	NA
RP Forward-Primer, Reverse-Primer and Probe	Avellino	NA
T1 and T2 Positive Control Template	Avellino	NA

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

1) A no template control (NTC) is needed to eliminate the possibility of sample contamination and is taken through the entire process from nucleic acid extraction to RT-PCR. This control is molecular grade, nuclease-free water.

2) A positive template control (PTC) is needed to verify that the assay run is performing as intended. The positive template control consists of quantified synthetic DNA oligonucleotides containing the N1 and N3 real-time RT-PCR amplicon sequences, diluted to concentrations equivalent to three times the assay LoD in nuclease-free water. The positive template control does not include RNase P target and will yield a "negative" for that marker.

3) An internal 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 the extraction control to ensure that samples resulting as negative contain nucleic acid for testing.

4) A negative extraction (EC) 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.

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

- No template controls should be negative for all targets. If any of the N1, N3 or RP NTC reactions exhibit positive fluorescence (Ct <40 N1/N3; Ct ≤ 35 RP), 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 both the N1 and N3 targets (Ct <40 N1/N3) and negative for the RP target. If the results are not as expected, the RT-PCR run is invalid. Repeat from the RT-PCR step using residual extraction material. If the repeat test result does not yield expected results, re-extract and re-test all samples.

- The extraction control (negative clinical matrix) should be negative for N1 and N3 targets, and positive for the RP target (Ct ≤ 35 RP). If positive results are obtained for N1 or N3 targets, 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 AvellinoCoV2 Test

Control Type	Control Name	Used to Monitor	Expected results and Ct Values		
			SARS-CoV-2 N1 (T1)	SARS-CoV-2 N3 (T2)	RNase P (RP)
Negative	NTC	Assay or extraction reagent contamination	Negative Ct ND*	Negative Ct ND	Negative Ct ND
Positive	SARS-CoV-2-Positive Template Control (PTC)	Improper assay setup, reagent failure including primer and probe degradation	Positive Ct < 40.0	Positive Ct < 40.0	Negative Ct ND
Extraction control	Negative Human Clinical Sample (HCS)	Cross-contamination during extraction, inefficient lysis of specimen, poor specimen collection, improper assay setup, extraction failure	Negative Ct ND	Negative Ct ND	Positive Ct ≤ 35.0

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 for guidance on interpretation and reporting of results.

Table 2: Interpretation of Patient Results Using the AvellinoCoV2 Test

SARS-CoV-2 N1 (T1) Ct value <40	SARS-CoV-2 N3 (T2) Ct value < 40	RNase P (RP) Ct value ≤ 35	Result Interpretation	Report	Actions
+	+	±	SARS-CoV-2 detected	SARS-CoV-2 Positive	Report results.
+	-	±	SARS-CoV-2 detected	SARS-CoV-2 Positive	Report results.
-	+	±	SARS detected	SARS-CoV-2 presumptive positive	Repeat RT-PCR. If repeat result is the same, report the result as presumptive positive. Additional confirmatory testing may be conducted.*
-	-	+	SARS-CoV-2 not detected	SARS-CoV-2 Negative	Report results.
-	-	-	Invalid	Invalid	Re-extract residual patient specimen. If repeat result is invalid, collect new sample and repeat test.

***If the N3 target only is positive, additional confirmatory testing may be conducted if necessary to differentiate between SARS-CoV-2 and other SARS-like viruses, for epidemiological purposes or clinical management.**

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

The LoD of the AvellinoCoV2 test was determined using quantified whole viral SARS-related coronavirus 2 (USA-WA1/2020) RNA obtained from ATCC (VR-1986D). A preliminary LoD was determined by testing 5 to10-fold serial dilutions of RNA ranging from 0.1-500 genomic copies/µL spiked into pooled nasopharyngeal and oropharyngeal iSWAB matrix in triplicate. Spiked samples were tested with the AvellinoCoV2 test following extraction with the QIAamp DSP Viral RNA Mini Kit. The lowest concentration of SARS-CoV-2 RNA that yielded a detection rate of ≥95% was 1 genomic copy/µl.

The LoD was verified by testing 20 additional extraction replicates consisting of pooled nasopharyngeal and oropharyngeal iSWAB matrix spiked at the preliminary LoD concentration of 1 copy/µl. Samples were spiked with RNA prior to extraction with the QIAamp DSP Viral RNA Mini Kit. The results of the summary are summarized below.

Table 3: LoD Verification Study Results

Target	Concentration (genomic copies/ µL)	Concentration (genomic copies/reaction)	Detection Rate	Mean Ct
N1 (T1)	1	5	100% (20/20)	35.3
N3 (T2)	1	5	100% (20/20)	35.0

2) Analytical Inclusivity/Specificity:

The AvellinoCoV2 test utilizes identical oligonucleotide sequences for the N1 and N3 SARS-CoV-2 target genes as those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel the CDC assay. The inclusivity and cross-reactivity of CDC assay under an EUA has been evaluated and therefore, additional evaluation is not required.

As reported under the CDC EUA, the *in silico* analysis for the N1 primer/probe set showed high sequence homology of the N1 probe with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there is no significant homologies with human

genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

Analysis of the forward and reverse primer and probe sequences of the N3 target showed significant homology only to human SARS coronavirus and bat SARS coronavirus. No significant homology with human genome, other coronaviruses or human microflora was observed. that would predict potential false positive rRT-PCR results.

3) Clinical Evaluation:

Contrived Specimen Testing

Performance of the AvellinoCoV2 test was evaluated using individual clinical oropharyngeal swabs and nasopharyngeal swab specimens spiked with whole viral SARS-CoV-2 RNA (USA—WA1/2020). In total, 30 negative clinical matrix samples and 30 contrived positive clinical matrix samples were tested.

Of the 30 contrived positive clinical samples, 24 were prepared with concentrations of SARS-CoV-2 RNA at the assay LoD (55 copies/µl – based on the original study). Half of the remaining six samples contained RNA at concentrations equivalent to 10X the assay LoD, while the other half contained RNA at concentrations equivalent to 100X the assay LoD.

Prepared samples were randomized and blinded, and RNA was extracted using the QIAamp DSP Viral RNA Mini Kit. Testing was performed in a total of two RT-PCR runs with one positive, one negative, and one extraction control included per run. Results of the study are summarized below.

Table 5: Contrived Clinical Evaluation Summary Data

SARS-CoV-2 concentration	Number of samples	Detection rate		Mean Ct		
		N1	N3	N1	N3	IC
1X LoD	24	24/24	24/24	33.66	33.73	25.25
10X LoD	3	3/3	3/3	32.84	31.34	25.16
100X LoD	3	3/3	3/3	29.95	29.14	25.24
Negative	30	30/30	30/30	-	-	23.85

PPA = 100% (88.65-100%)
NPA = 100% (88.65-100%)

Clinical Specimen Testing

The performance of the AvellinoCoV2 test was further evaluated and validated against a previously authorized SARS-CoV-2 RT-PCR EUA test using a total of 60 SARS-CoV-2 positive and 30 SARS-CoV-2 negative patient samples. The samples consisted of a

mixture of nasopharyngeal and oropharyngeal swabs collected from individuals suspected of COVID-19 infection by their healthcare provider. The mean Ct of the 60 SARS-CoV-2 positive samples as determined by the comparator method was 28, with exactly half having a Ct value above 30. The percent positive and negative agreement between the comparator method and the AvellinoCoV 2 test was 100%. A summary of the data is provided below.

Table 6: Clinical Specimen Evaluation Summary Data

		Comparator EUA	
		Positive	**Negative**
AvellinoCoV2	**Positive**	60	0
	Negative	0	30

PPA: 100% (94.0-100%)
NPA: 100% (88.7-100%)

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, Avellino Lab USA;
- 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 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. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

LIMITATIONS

- 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.

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. Column based extraction either manually by QIAGEN QIAamp DSP Viral RNA Mini Kit and/or with QIAGEN QIACube automated instrument were used in this study. The instrument used was Applied Biosystems 7500 Fast Real-Time PCR System with software version 2.3. The results are

summarized in Table below.

Table 7: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NP and OP	$1.8x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

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