ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY OF THE SANDIA NATIONAL LABORATORIES SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Test

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

(The SARS-CoV-2 Assay will be performed at the Sandia National Laboratories' laboratories located at 1515 Eubank Blvd. SE, Albuquerque, NM 87123 and 7011 East Ave., Livermore, CA 94551, which are certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as described in the laboratory procedures that were reviewed by the FDA under this EUA.)

INTENDED USE

The SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic assay is a Real-Time reverse transcription (rRT-PCR) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (including nasopharyngeal swab, anterior nasal swab, mid-turbinate nasal swab and oropharyngeal swab, nasal washes, nasal aspirates) and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Sandia National Laboratories' laboratories located at 1515 Eubank Blvd SE, Albuquerque, NM 87123 and 7011 East Ave., Livermore, CA 94551 which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements 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 upper 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.

The SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic 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 SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic test is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test based on the FDA issued EUA CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel. The test uses two primer and probe sets to detect RNA from two regions in the SARS-CoV-2 nucleocapsid (N) gene and one primer and probe set to detect RNA from human RNase P (RP) in clinical specimens from patients as recommended for testing by public health authority guidelines.

RNA is isolated from upper respiratory specimens using the Zymo Research Quick-RNA Viral kit using a manual viral RNA extraction method via vacuum manifold. Isolated RNA is reverse transcribed to cDNA and subsequently amplified using Applied Biosystems 7500 (ABI7500), Applied Biosystems Quant Studio 5, Bio-Rad CFX Connect, or Bio-Rad CFX 96 Real PCR instruments (**Table 2**).

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 measured following each PCR cycle by the PCR instrument.

The Integrated DNA Technologies, Inc. (IDT) 2019-nCoV CDC EUA kit oligonucleotide primer and probes sequences can be found in **Table 1.**

Table1: Oligonucleotide Primer and Probe Sequences (Source: CDC, Supplier: IDT)

Name	Description	Oligonucleotide Sequence (5' to 3')
SARS-CoV-2_N1-F	2019 nCoV_N1 Forward Primer	GAC CCC AAA ATC AGC GAA AT
SARS-CoV-2_N1-R	2019 nCoV_N1 Reverse Primer	TCT GGT TAC TGC CAG TTG AAT CTG
SARS-CoV-2_N1-P	2019 nCoV_N1 Probe	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1
SARS-CoV-2_N2-F	2019-nCoV_N2 Forward Primer	TTA CAA ACA TTG GCC GCA AA
SARS-CoV-2_N2-R	2019-nCoV_N2 Reverse Primer	GCG CGA CAT TCC GAA GAA
SARS-CoV-2_N2-P	2019 nCoV_N2 Probe	FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1
RP-F	Human RNAseP (RP) Forward Primer	AGA TTT GGA CCT GCG AGC G
RP-R	Human RNAseP (RP) Reverse Primer	GAG CGG CTG TCT CCA CAA GT
RP-P	Human RNaseP (RP) Probe	FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1

INSTRUMENTS USED WITH TEST

The SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic assay is to be used with the following reagents, instruments, and software versions:

Table 1: Instruments for use with SNL-NM 2019 nCoV RT-PCR Diagnostic Assay

Instrument Number	Manufacturer	Model	Software/Version
1	Applied Biosystems, Inc.	7500	7500 Software v2.3
2	Applied Biosystems, Inc.	Quant Studio 5	QuantStudio Design & Analysis Software v1.5.1
3	Bio-Rad	CFX Connect	CFX Manager v3.1
4	Bio-Rad	CFX 96	CFX Maestro 1.1 V4.1.2433.1219

Table 3: Reagents for use with SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Assay

	Manufacturer	Product Name	Catalog #*
Extraction Reagents	Zymo Research	Quick-RNA Viral kit	R1035 (200 preps) kit components: R1034-1-100: Viral RNA Buffer R1034-2-48: Viral RNA Wash Buffer (Concentrate) C1004-50: Zymo-Spin IC Columns
PCR Reagents	Integrated DNA Technologies, Inc.	2019-nCoV CDC qPCR Probe Assay CDC Emergency Use Authorization Kits	10006606
	Thermo Fisher	TaqMan Fast Virus 1-step Master Mix (4x)	4444432, 4444434, or 4444436
Positive Control	Exact Diagnostics	SARS-CoV-2-Standard	COV019

*Catalog numbers could be different for different sizes or volumes.

CONTROLS:

The following controls are utilized with the SNL-NM 2019 nCoV RT-PCR Diagnostic Assay:

No template control (NTC):

- Product: Sterile, nuclease-free water (Zymo Product #W1001-10 or ThermoFisher Ambion #AM9937)
- Preparation: Aliquot if desired, keep sterile.
- Use: to check for contamination of PCR reagents or contamination occurring during PCR plate set-up
- Frequency of Use: NTC control is run on every plate.
- Expected Results: Negative for all targets (Ct Not Detected)

Positive control (+CTRL):

- Product: Exact Diagnostics SARS-CoV-2-Standard (Product # COV019)
- Preparation: prepare aliquots of working concentration of 25 copies/µL (equivalent to 4x LOD). Store at -20°C prior to use. Avoid freeze, thaw.
- Use: Added to the plate during qPCR to monitor the integrity of the RT-PCR reagents and process. Used at a concentration of 25 copies/µL and 5 µL/well for a total of 125 copies/well.
- Frequency of Use: The positive control is run on every plate.

- Expected Results: Positive for both N1 and N2 targets (Ct ≤38), Positive for RP (Ct≤38)
  Note: The EXD SARS-CoV-2 Standard is manufactured with synthetic RNA transcripts containing 5 gene targets (E, N, S, ORF1a, and RDRP genes of SARS-CoV-2, 200,000 copies/mL each). The product is formulated in a synthetic matrix and contains human genomic DNA (75,000 copies/mL).

Negative extraction control (Human specimen control, HSC):

- Product: A549 cells prepared in-house in enough volume to be used across multiple runs, aliquoted for individual use (200 µL).
- Preparation: Thaw aliquot on ice.
- Use: Extraction control to validate a successful RNA extraction and monitor for any cross-contamination that occurs during the extraction process. HSC control sample is run through the entire process end-to-end (sample extraction and qPCR amplification).
- Frequency of Use: HSC control is run on every plate.
- Expected Results: Negative for SARS-CoV-2 targets (Ct Not Detected), positive for RNase P (RP) target (Ct ≤ 38)

Internal RNaseP Control (RP):

- RNase P mRNA is co-extracted and amplified from all patient samples as an internal control.
- Use: This internal control is used to assess the extraction efficiency and specimen quality. This also serves as an extraction control to ensure that samples resulting as negative for SARS-CoV-2 targets contained nucleic acid for testing. Detection of the RNase P gene in patient test samples verifies successful extraction of the sample, proper assay setup, sample integrity, and efficient sample collection.
- Frequency of Use: coextracted with every patient sample.
- Expected results: positive for RNase P (RP) target (Ct ≤ 38)

Table 4: SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Assay Controls

Control Type	Description	Purpose	Frequency of Testing
NTC	Sterile, nuclease-free water (Zymo Product #W1001-10 or ThermoFisher Ambion #AM9937)	To check for contamination of PCR reagents or contamination occurring during PCR plate set-up	Run on every plate
Positive Control	Exact Diagnostics SARS-CoV-2-Standard (Product # COV019), prepare aliquots of working concentration of 25 copies/µL (equivalent to 4x LOD)	Added to the plate during qPCR to monitor the integrity of the RT-PCR reagents and process	Run on every plate
Negative Extraction Control	A549 cells prepared in-house in enough volume to be used across multiple runs	Extraction control to validate a successful RNA extraction and monitor for any cross-contamination that occurs during the extraction process	Run on every plate
Internal RNaseP Control	RNase P is co-extracted and amplified from all patient samples as an internal control.	Verifies successful extraction of the sample, proper assay setup, sample integrity, and efficient sample collection	Run for every sample

INTERPRETATION OF RESULTS

Assay Controls Interpretation

All controls must be examined prior to interpretation of patient results. If the controls are not as expected, the patient results cannot be interpreted, and all patient samples must be repeated from the extraction step.
The expected Ct thresholds for the controls are shown in **Table 5**.
The results from the controls are interpreted according to the criteria shown in **Table 6.**

Table 5: Expected Cycle Thresholds (Ct) for SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Assay Controls

Control	2019 nCoV N1	2019 nCoV N2	RNAseP
Positive (+ CTRL)	≤38	≤38	≤38
Extraction	not detected	not detected	≤38
NTC	not detected	not detected	not detected
Internal Control	n/a	n/a	≤38

Table 6: Interpretation of SNL-NM 2019 nCoV RT-PCR Diagnostic Assay Controls

Control Type	External Control Name	Used to Monitor	2019 nCoV_N1	2019 nCoV_N2	RP
No Template Control	NTC	To check for contamination of PCR reagents or contamination occurring during PCR plate set-up	Not detected	Not detected	Not detected
Positive	+ CTRL	To validate the integrity of the RT-PCR reagents and process	Detected Ct ≤38	Detected Ct≤38	Detected Ct ≤38*
Negative Extraction Control	HSC[1]	To validate a successful RNA extraction and monitor for any cross-contamination that occurs during the extraction process	Not detected	Not detected	Detected Ct ≤38
Internal Control (Endogenous Control)	RP	To verify successful extraction of the sample, proper assay setup, sample integrity, and efficient sample collection	N/A	N/A	Detected Ct ≤38

*Note: The EXD SARS-CoV-2 Standard is manufactured with synthetic RNA transcripts containing 5 gene targets (E, N, S, ORF1a, and RDRP genes of SARS-CoV-2, 200,000 copies/mL each). The product is formulated in a synthetic matrix that also contains human genomic DNA (75,000 copies/mL).
[1]It also has RNaseP present

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 based on **Tables 5-6** above. Samples that fail to show detection of RP and both SARS-CoV-2 targets (N1 and N2) are considered invalid and must be repeated from the extraction step. If the

SARS-CoV-2 targets (N1 and N2) are detected (Ct ≤ 38), the lack of amplification of RP target does not invalidate a positive result. **Table 7** below lists the expected results for the SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Assay.

Table 7: Interpretation of Patient Specimen Results

SARS-CoV-2 N1	SARS-CoV-2 N2	RP	Results Interpretation	Report	Actions
+	+	+/-	SARS-CoV-2 detected	Positive SARS-CoV-2	Report results to CDC and sender
If only one of the two targets are positive		+/-	Inconclusive	Inconclusive	Repeat NA extraction and RT-PCR, if still inconclusive, contact your State Public Health Laboratory or CDC for instructions for further guidance.
-	-	+	SARS-CoV-2 not detected	SARS-CoV-2 not detected	Report results to sender. Consider testing for other respiratory viruses.
-	-	-	Invalid results	Invalid	Repeat from extraction and RT-PCR, if still invalid, report results and recommend collection of a new specimen from the patient.

G. PERFORMANCE EVALUATION

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

The limit of detection (LoD) was established by first performing a preliminary LoD study by testing 2-fold dilutions (50 copies/µL – 1.56 copies/µL) of synthetic RNA spiked into pooled nasopharyngeal swab sample matrix using 2 replicates at each target level. Pooled nasopharyngeal swab samples were previously confirmed negative by the New Mexico Department of Health using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (EUA200001). Spiked samples were extracted using the Zymo Research Quick-RNA Viral kit using manual extraction via vacuum manifold and tested with the SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic assay on the ABI 7500 PCR instrument. The preliminary LoD was determined to be between 3.125 and 6.25 copies/µL of extracted RNA (**Table 8**).

A confirmatory LoD study was performed by evaluating concentrations of 3.125 copies/µL and 6.25 copies/µL using 20 replicates at each target level. The LoD was defined as the lowest concentration of SARS-CoV-2 (genome copies/µL) that can be detected by the SNL test at least 95% of the time. The LoD for the SARS-CoV-2 Detection Test was confirmed to be 6.25 copies/µL resulting in correct identification of 19 out 20 replicates were positive for both N1 and N2 targets in 2 independent experiments (**Table 9**, Study 1 and Study 2 are two independent data sets). The pooled negative samples were generated independently for each

study and the entire workflow (RNA extraction, RT-PCR and interpretation) was performed independently for each study. The 3.125 copies/µL concentration resulted in correct identification of 17 out of 20 replicates. Two samples were falsely reported as negatives and one sample was determined to be inconclusive (N2 Ct=40.1).

Table 8: Results (Ct values) from Preliminary Limit of Detection Study (N=2)

Concentration Copies/µL	Mean N1	St dev N1	Mean N2	St dev N2	Mean RP	St dev RP
50	29.18	0.13	30.52	0.33	24.75	0.21
25	30.32	0.35	31.41	0.38	24.69	0.13
12.5	31.39	0.35	32.49	0.09	24.94	0.10
6.25	32.46	0.03	33.75	0.42	24.82	0.18
3.125	33.24	0.13	34.62	1.09	24.80	0.14
1.56	34.55	0.46	36.44	0.53	24.83	0.25
Negative	n.d.	n/a	n.d.	n/a	24.69	0.29

*Note: n.d. indicates no signal was detected above threshold prior to cycle 45.

Table 9: Limit of Detection Confirmation Results (Ct values).

		Study #1			Study #2		
Replicate	Concentration	N1	N2	RP	N1	N2	RP
1	6.25 copy/ul	30.66	31.39	24.59	34.80	35.23	27.42
2	6.25 copy/ul	30.33	31.04	24.65	33.97	35.77	27.36
3	6.25 copy/ul	29.97	31.79	24.83	33.98	34.49	27.90
4	6.25 copy/ul	29.16	30.60	24.85	33.47	34.88	27.61
5	6.25 copy/ul	32.44	33.27	25.02	34.60	34.60	27.83
6	6.25 copy/ul	29.32	30.81	24.89	34.12	36.26	27.82
7	6.25 copy/ul	31.38	32.45	24.98	33.86	35.34	27.51
8	6.25 copy/ul	29.06	30.16	24.51	34.70	n.d.	n.d.
9	6.25 copy/ul	31.05	32.42	24.83	35.51	36.09	27.37
10	6.25 copy/ul	31.75	32.59	25.02	33.98	34.70	27.77
11	6.25 copy/ul	34.07	34.79	27.81	34.12	36.20	27.79
12	6.25 copy/ul	33.97	34.18	28.35	34.96	35.88	27.63
13	6.25 copy/ul	33.93	36.19	27.97	34.22	34.76	27.76
14	6.25 copy/ul	33.64	34.56	26.97	34.89	36.31	28.01
15	6.25 copy/ul	34.31	35.11	28.14	33.09	34.93	27.25
16	6.25 copy/ul	34.17	34.09	28.25	33.88	34.96	27.31
17	6.25 copy/ul	34.74	36.66	28.35	34.91	37.58	27.51
18	6.25 copy/ul	33.39	35.55	27.37	34.35	35.22	27.17
19	6.25 copy/ul	n.d.	n.d.	27.90	34.01	36.46	27.81
20	6.25 copy/ul	33.78	34.34	27.85	33.79	34.21	27.01

*Note: n.d. indicates no signal was detected above threshold prior to cycle 45.

2) Inclusivity (Analytical Sensitivity):

The SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Assay uses the CDC primer and probe kit that targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene

that are unique to SARS-CoV-2. The sequences for the N1, N2 primers/probes used in this assay are identical to the N1, N2 primers/probes sequences used in the FDA authorized original CDC 2019-Novel Coronavirus (2019-nCoV) real time RT-PCR Diagnostic Panel.

Inclusivity was demonstrated to be acceptable in the amended CDC EUA (June 20, 2020) by mapping the primers and probes to all SARS-CoV-2 genomes available.
https://www.fda.gov/media/134922/download

3) Cross-reactivity (Analytical Specificity)

The analytical specificity of the SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Assay was demonstrated in silico under the original EUA for the 2019-nCov CDC EUA Kit from IDT. The analysis included evaluation of the primer and probe homology with the 43 organisms and viruses listed in recommend list of organisms below. Based on this analysis, significant amplification of non-target sequences that could result in cross-reaction (false-positive results) or interference (false-negative results) was considered unlikely to occur.

4) Clinical Evaluation:

Contrived Clinical Sample Evaluation:

Performance of the Sandia National Laboratories was evaluated using a randomized, blinded clinical study of 30 unique negative and 30 unique contrived positive clinical samples.

Negative nasopharyngeal swab specimens from patients previously determined to be negative for SARS-CoV-2 by the NM Department of Health using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (EUA200001) were provided frozen and stored at -80 °C until use.

Positive contrived samples were created by spiking synthetic RNA of SARS-CoV-2 (SARS-CoV-2-Standard, Exact Diagnostics) into negative samples at 1X LoD (10 samples), 2X LOD (10 samples), 4X LoD (5 samples) or 8X LoD (5 samples).

Contrived samples were extracted using the Zymo Research Quick-RNA Viral kit using manual extraction via vacuum manifold and tested with the SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic assay on the ABI 7500 PCR instrument. Results of the contrived study are summarized in **Table 10**.

Overall performance of the SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic test against the expected results for contrived clinical specimens:
Positive Percent Agreement 29/30 = 96.67% (95% CI: 82.78% - 99.92%)
Negative Percent Agreement 30/30 = 100% (95% CI: 88.43% - 100%)

Conclusion (contrived clinical specimens): The results demonstrated a 96.67% agreement against the expected results with all positive samples and 95% correct results for positive samples at 1x and 2x the LoD (one 1x LoD sample was inconclusive (N1: 33.7, N2: 40.6, RP: 24.8), and 100% agreement against the expected results for all negative samples, NTC, and positive controls.

Table 10: Summary of Contrived Sample Testing

SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Assay	Concentration	# of NP swabs	N1 Target			N2 Target			RP Internal Control		
			Pos	%	Ct avg	Pos	%	Ct avg	Pos	%	Ct avg
	1x LoD	10	10/10	100	33.67	9/10*	90	34.54	10/10	100	26.63
	2x LoD	10	10/10	100	32.21	10/10	100	33.11	10/10	100	25.51
	4x LoD	5	5/5	100	31.67	5/5	100	32.62	5/5	100	27.66
	8x LoD	5	5/5	100	31.82	5/5	100	32.79	5/5	100	28.61
	Negative	30	n.d	100	n.d	n.d	100	n.d	30/30	100	29.48

** one 1x LOD sample was inconclusive (N1: 33.7, N2: 40.6, RP: 24.8)*

Clinical Patient Samples:
In addition to the contrived clinical study, the clinical performance of the SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Test was evaluated using a randomized, blinded clinical study which consist of a total of 30 unique negative and 30 positive clinical samples (nasopharyngeal swabs) from patients previously determined to be negative or positive for SARS-CoV-2 by TriCore Reference Laboratories, Albuquerque, NM using FDA authorized tests.

The samples were randomized and assigned unique identifiers and the results kept from the personnel performing the RNA extraction and RT-PCR to preserve blinding. Samples were extracted using the Zymo Research Quick-RNA Viral kit using manual extraction via vacuum manifold and tested with the SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic assay on the ABI 7500 PCR instrument. After analysis, the samples were unblinded for comparison.

Confirmation of clinical specimens: In addition to the clinical study described above (30 positive/30 negative clinical samples), the first five presumptive positive and the first five presumptive negative clinical specimens were confirmed by the New Mexico Department of Health, Scientific Laboratory Division (SLD) using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

Nasopharyngeal swab samples were collected by clinical personnel in ~3mL of viral transport media (produced by the New Mexico Department of Health). Samples were transported cold (2-8 °C) or frozen and either stored for testing (<48 hours) at 4°C or frozen and stored at -80 °C until testing. At SNL-NM, samples were extracted using the Zymo Research Quick-RNA Viral kit using manual extraction via vacuum manifold and tested with the SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic assay on the ABI 7500 PCR instrument. At SLD, samples were tested using the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC) on an ABI 7500 Fast Dx PCR instrument. All 10 patient samples yielded concordant results**.** Performance of the SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Test compared to FDA authorized tests is presented in **Table 11**.

Table 11: Overall performance of the SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic test against FDA authorized tests

Patient NP Specimens		FDA authorized Tests		
		Positive	**Negative**	**Total**
SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic assay	**Positive**	34	0	34
	Negative	0	35	35
	Inconclusive	1*	0	1
	Total	35	35	70
Positive Agreement		100% (34/34) (95% CI: 89.9% - 100%)[1]		
Negative Agreement		100% (35/35) (95% CI: 90.10% - 100%)[1]		

[1]95% confidence interval calculated using the score method.
*There was one sample that was inconclusive (*N1: 37.4, N2: n.d., RP: 25.8)*. It was re-run as per protocol and remained inconclusive. It is not included in the agreement calculations

Conclusion (overall performance): A total of 70 clinical samples (60 from randomized blinded study + 10 from confirmation study) were compared to FDA authorized tests and 100% of positive clinical samples, and 100% of all negative clinical samples were in agreement with FDA authorized tests. One positive sample was inconclusive by SNL-NM 2019 nCoV RT-PCR twice and is not included in the statistical agreement calculations.

5) Validating performance of additional RT-qPCR Instruments:

The validation studies were performed on one qPCR instrument (ABI 7500). A bridging study to demonstrate similar analytical performance was conducted on three additional instruments, ABI QuantStudio 5, Bio-Rad CFX Connect, and Bio-Rad CFX 96. An experiment was designed utilizing contrived positive samples spanning the range of 1x LOD – 8x LOD in a clinical sample background (nasopharyngeal swab samples from asymptomatic (healthy) patients, previously determined negative for SARS-CoV-2. These contrived positive samples were independently prepared for each validation experiment and are not the same samples used in the LOD study described above. The design included three replicates at four concentrations of synthetic RNA of SARS-CoV-2 (SARS-CoV-2-Standard, Exact Diagnostics) prepared at 1x LoD, 2x LoD, 4x LoD, and 8x LoD, as well as six negative clinical samples, a no template control and a positive control. Nasopharyngeal swab samples from asymptomatic (healthy) patients were pooled and spiked with synthetic RNA prior to extraction. Negative samples received 10 µL of nuclease-free water. Samples were extracted using the Zymo Research Quick-RNA Viral kit using manual extraction via vacuum manifold. Samples were eluted into 40 µL of nuclease-free water. Two identical RT-PCR plates were prepared by adding sample from each tube to both plates. Plates were run pairwise comparing each of the instruments to the ABI7500. **Tables 12-14** highlight the results. For all the instruments, the qualitative outcome was identical for all the dilutions except for one 4x LOD RP that did not amplify. In general, the Ct values exhibited ≤1 Ct difference and ≤ 3x the standard deviation of the replicates on the ABI7500 for that dilution. A t-test for significance at each concentration revealed no significant differences for the N1 and N2 primers across instruments at 1x and 2x LODs. A slight statistical significance was observed in a few samples for the 4x and/or 8x LOD dilutions as a result of the extremely small standard deviations at these high concentrations. Importantly, the Ct values never differed by more than 2 Ct, which is the criteria used by the NM Department of Health and other laboratories to confirm the quality of new lots of reagents and the instruments post-service.

Conclusion: *Based on the results of this bridging study, these four instruments provide equivalent performance for the SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic assay.*

Table 12: Validation of ABI QuantStudio Performance

		ABI 7500	ABI QuantStudio 5	
Dilution	Primer	mean +/- stdev	mean +/- stdev	% correct
1x LOD	N1	32.42 +/- 0.34	33.28 +/- 0.41	100% (3/3)
	N2	33.52 +/- 0.46	33.72 +/- 0.28	100% (3/3)
	RP	26.55 +/- 0.23	27.36 +/- 0.25	100% (3/3)
2x LOD	N1	31.90 +/- 0.38	32.32 +/- 0.62	100% (3/3)
	N2	32.33 +/- 0.56	32.46 +/- 0.44	100% (3/3)
	RP	26.31 +/-0.39	27.05 +/- 0.05	100% (3/3)
4x LOD	N1	30.55 +/- 0.38	31.30 +/- 0.16	100% (3/3)
	N2	31.42 +/- 0.17	31.81 +/- 0.54	100% (3/3)
	RP	26.38 +/- 0.11	27.21 +/- 0.13	100% (3/3)
8x LOD	N1	29.57 +/- 0.16	30.55 +/- 0.20	100% (3/3)
	N2	30.34 +/- 0.48	30.35 +/- 0.30	100% (3/3)
	RP	26.29 +/- 0.15	27.07 +/- 0.46	100% (3/3)
Negative	N1	--	--	100% (6/6)
	N2	--	--	100% (6/6)
	RP	26.19 +/- 0.24	27.18 +/- 0.31	100% (6/6)

Table 13: Validation of Bio-Rad CFX Connect performance

		ABI 7500	Bio-Rad CFX Connect	
Dilution	Primer	mean +/- stdev	mean +/- stdev	% correct
1x LOD	N1	32.40 +/- 0.41	33.63 +/- 0.41	100% (3/3)
	N2	33.43 +/- 0.53	33.94 +/- 0.53	100% (3/3)
	RP	26.97 +/- 0.14	27.34 +/- 0.09	100% (3/3)
2x LOD	N1	31.57 +/- 0.17	32.23 +/-0.95	100% (3/3)
	N2	32.48 +/- 0.27	32.68 +/- 0.17	100% (3/3)
	RP	27.04 +/- 0.06	27.00 +/- 0.20	100% (3/3)
4x LOD	N1	30.36 +/- 0.19	31.53 +/- 0.14	100% (3/3)
	N2	31.55 +/- 0.20	31.68 +/- 0.38	100% (3/3)
	RP	26.88 +/- 0.24	32.68 +/- 9.06*	66.67% (2/3)
8x LOD	N1	28.26 +/- 0.11	28.41 +/- 0.20	100% (3/3)
	N2	29.22 +/- 0.66	29.31 +/- 0.04	100% (3/3)
	RP	26.60 +/- 0.10	27.08 +/- 0.57	100% (3/3)
Negative	N1	--	--	100% (6/6)
	N2	--	--	100% (6/6)
	RP	26.85 +/- 0.11	27.27 +/- 0.26	100% (6/6)

*One 4x sample did not show RP signal

Table 14: Validation of Bio-Rad CFX 96 performance

		ABI 7500	Bio-Rad CFX 96	
Dilution	Primer	mean +/- stdev	mean +/- stdev	% correct
1x LOD	N1	32.76 +/- 0.34	33.52 +/- 0.28	100% (3/3)
	N2	33.12 +/- 0.49	34.18 +/- 0.15	100% (3/3)
	RP	27.06 +/- 0.18	27.93 +/- 0.27	100% (3/3)
2x LOD	N1	31.85 +/- 0.34	32.37 +/- 0.49	100% (3/3)
	N2	32.32 +/- 0.42	32.72 +/- 0.16	100% (3/3)
	RP	26.89 +/- 0.40	27.62 +/- 0.05	100% (3/3)
4x LOD	N1	30.65 +/- 0.08	31.66 +/- 0.25	100% (3/3)
	N2	31.03 +/- 0.33	32.11 +/- 0.39	100% (3/3)
	RP	27.04 +/- 0.20	27.99 +/- 0.29	100% (3/3)
8x LOD	N1	29.67 +/- 0.09	30.29 +/- 0.45	100% (3/3)
	N2	30.05 +/- 0.12	30.79 +/- 0.29	100% (3/3)
	RP	26.59 +/- 0.21	27.95 +/- 0.76	100% (3/3)
Negative	N1	--	--	100% (6/6)
	N2	--	--	100% (6/6)
	RP	26.98 +/- 0.16	28.16 +/- 0.40	100% (6/6)

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the Sandia National Laboratories' laboratories located at 1515 Eubank Blvd. SE, Albuquerque, NM 87123 and 7011 East Ave., Livermore, CA 94551;
- 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.

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 used was manual viral RNA extraction method (Zymo Research Quick Viral RNA kit) with amplification and detection accomplished using Applied Biosystems 7500 running the 7500 Software v2.3. The results are summarized in the following Table.

Table 15: 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	$5.4x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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