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

(Viracor Eurofins Clinical Diagnostics)

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

(The SARS-CoV-2 RT-PCR test will be performed at Viracor Eurofins Clinical Diagnostics, 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 Viracor SARS-CoV-2 assay is a real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 viral RNA in nasopharyngeal swab, nasal swab, nasopharyngeal wash, nasal wash, oropharyngeal swab and bronchoalveolar lavage from individuals suspected of COVID-19. Testing is limited to Viracor Eurofins Clinical Diagnostics, 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 viral RNA. 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 management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The assay is intended for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe sets are designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines.

Nucleic acid extractions are performed using a bioMerieux NucliSENS easyMAG or eMAG instrument with bioMerieux NucliSENS nucleic acid extraction reagents. The SARS-CoV-2 nucleic acid amplification assay is a real-time (TaqPath) reverse transcription polymerase chain reaction assay for the amplification and detection of SARS-CoV-2 genomic RNA. Oligonucleotide primers

hybridize to specific nucleotide sequences of the SARS-CoV-2 N gene. RNA is reverse transcribed and then amplified in the presence of thermostable DNA polymerase (Taq) enzyme and deoxy nucleotide triphosphates (dNTPs). A dual-labeled oligonucleotide probe that is complementary to an internal sequence of the amplification product is also present in the RT-PCR reaction mixture. The 5' exonucleolytic activity of Taq cleaves the fluorescent molecule (FAM) at the 5' end of the dual-labeled probe, thus releasing it from the effects of a fluorescence-quenching molecule (e.g. Black Hole Quencher 1) at the 3' end of the probe.

Additionally, oligonucleotide primers and a TaqMan probe for PCR detection of an internal extraction and amplification control are also present in the SARS-CoV-2 RT-PCR reaction mix. This allows for the simultaneous detection of internal extraction/amplification control DNA in a multiplex reaction for each sample. Fluorescence intensity for both SARS-CoV-2 amplification and internal control amplification is measured in individual wells during each of the 40 amplification cycles. A sample is considered positive when the signal intensity exceeds a predetermined baseline threshold value. The cycle number at which this occurs is referred to as the cycle threshold C_T. Detection of SARS-CoV-2 RNA in a sample is determined by the C_T value.

INSTRUMENTS USED WITH TEST

The Viracor SARS-CoV-2 assay is to be used with the bioMérieux NUCLISENS® easyMAG® and bioMérieux EMAG® for automated nucleic acid extraction and the Applied Biosystems™ 7500 Real-Time PCR Systems (SDS v1.5.1) for nucleic acid amplification and detection.

Table 1: REAGENTS AND MATERIALS

Reagent	Manufacturer	Catalog #
TaqPath 1-step RT-qPCR master mix, CG	Life Technologies	A15299 or A15300
COV2 Assay Oligo Mix	Viracor Eurofins	25 700143
RNAse Free H20	Fisher Scientific or equivalent	BP561-1 or equivalent
MS2 RNA bacteriophage (internal control)	ATCC	15597
SARS-CoV-2 Low positive control	Viracor	25 000414
SARS-CoV-2 High Positive Control	Viracor	25 000415
Plasma Negative Extraction Control	Viracor	25 000003
COV2 Curve Control 1 (S2)	Viracor	25850048
COV2 Curve Control 2 (S4)	Viracor	25 850048

CONTROLS TO BE USED WITH THE SARS-COV-2 RT-PCR

1. A negative (no template) control is needed to eliminate the possibility of sample contamination on the assay run and is used on every assay plate. This control has no extracted nucleic acid added to the rRT-PCR reaction. This control reaction contains RNase-, DNase-free water, the oligonucleotide primers and probes for SARS-CoV-2, as well as the internal control primers and probes.

2. A positive template control is needed to verify that the assay run is performing as intended and is included in each testing run. The positive control material is cloned plasmid DNA representing the N gene of SARS-CoV-2 (GenBank accession NC_045512.2).

3. Two SARS-CoV-2 positive amplification curve controls (low and high) are included on each amplification plate to ensure that SARS-CoV-2 RNA can be detected by the rRT-PCR test and demonstrate that the anticipated level of sensitivity has been achieved. This control material is *in vitro* transcribed RNA.

4. An internal control is needed to verify that nucleic acid is present in every sample and is used for every sample processed. MS2 (an RNA bacteriophage) is an internal lysis, extraction and amplification control that is added to each clinical specimen as the first step of nucleic acid extraction. Oligonucleotide primers and a TaqMan probe for detection of MS2 are included in primer/probe mixtures in combination with SARS-CoV-2 primers and probes.

5. A negative control is needed to monitor for any cross-contamination that occurs during the RT-PCR process. This control consists of known negative phosphate buffered saline that has previously been tested for SARS-CoV-2 by rRT-PCR. This control goes through the entire extraction and amplification process with every set of samples. The resulting eluted nucleic acid from this control is added to the rRT-PCR reaction as the negative control well. This control reaction contains all oligonucleotide primers and probes for the SARS-CoV-2, as well as the internal control target.

INTERPRETATION OF RESULTS

The test is run as a multiplex reaction with SARS-CoV-2 N1, SARS-CoV-2 N2 and MS2 internal control assays combined in a single tube. Since both SARS-CoV-2 N1 and SARS-CoV-2 N2 assays use probes with the same fluorophore (FAM), a single SARS-CoV-2 C_T value is generated and interpreted for each rRT-PCR reaction. The MS2 fluorescence signal is differentiated from SARS-CoV-2 since different fluorophore (Cy5) is used for the MS2 probe. All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted, and results cannot be reported.

1) ***<u>SARS-CoV-2 RT-PCR test Controls – Positive, Negative, and Internal</u>:***

- Negative (no template control) – the no template control should be negative for all targets detected (C_T Not Detected)

- Positive controls – Each lot of working concentration positive control is analyzed to generate lot specific C_T acceptance ranges. A C_T value within established ranges ensures that the reproducibility and repeatability of the test is consistent between days, equipment and analysts.

- Internal control – The expected C_T value for MS2 is ≤ 35. In samples with no SARS-CoV-2 target detected, a C_T value less than or equal to this value for MS2 RNA demonstrates that effective nucleic acid extraction and rRT-PCR amplification has been achieved.

- Negative control– this control should be negative for the SARS-CoV-2 assay but positive for the MS2 internal control

If any control does not perform as described above, the run is considered invalid and all specimens are repeated from extraction.

Table 2: Interpretation of results for internal, no template, negative and positive control reactions

Control	Valid result[a]	Invalid result
Internal control (MS2)	$C_T \leq 35$	$C_T > 35$[b, c]
No template control	No amplification signal detected	Amplification detected
Negative control	No amplification signal detected	Amplification detected
Positive amplification curve control (low)	C_T 23 – 27	$C_T < 23$ or > 27
Positive amplification curve control (high)	C_T 9.7 – 13.7	$C_T < 9.7$ or > 13.7
Positive control (low)	C_T 26.79 – 29.73	$C_T < 26.79$ or > 29.73
Positive control (high)	C_T 20.21 – 23.03	$C_T < 20.21$ or > 23.03

[a]Patient results can only be interpreted if all control reactions generate valid results.
[b]In clinical specimens with SARS-CoV-2 target "Not detected" (i.e. $C_T > 38$), results are invalid when internal control MS2 C_T values are > 35.
[c]In clinical specimens with SARS-CoV-2 target "Detected" (i.e. $C_T \leq 38$), internal control MS2 C_T values are not interpreted.

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. When all control values are valid as stated above, results are reported as shown in Table 3 below:

Table 3: Interpretation of Patient Results

Real-time RT-PCR result	Reported result
$C_T > 38$	SARS-CoV-2 RNA "Not Detected"
$C_T \leq 38$	SARS-CoV-2 RNA "Detected"

PERFORMANCE EVALUATION

1) ***Analytical Sensitivity:***

Limit of Detection (LoD):

The analytical sensitivity of Viracor's SARS-CoV-2 RT-PCR test was determined in Limit of Detection (LOD) studies. Since no quantified viral isolates of SARS-CoV-2 were available for testing, stocks of 1.5 kb *in vitro* transcribed (IVT) RNA of the SARS-CoV-2 N gene (positions 28061 – 29533 of GenBank accession NC_045512.2) were used for spiking into clinical samples negative for SARS-CoV-2. These samples included BAL, nasal wash, and nasopharyngeal swab matrices. The number of RNA copies/µL of the stock IVT RNA was determined to be 1.7×10^{12}

copies/μL by Qubit Broad Range (BR) RNA reagents and associated fluorometer. To determine the preliminary LOD, range finding experiments were performed on three spiked extraction/amplification replicates using 2-fold dilutions of IVT in BAL, nasal wash and nasopharyngeal swab matrices. Results are shown in Table 4 below:

Table 4: Summary of Limit of Detection Range Finding Results Using SARS-CoV-2 N Gene IVT

	Bronchoalveolar lavage		**Nasal wash**		**Nasopharyngeal swabs**	
RNA concentration (copies/mL)	**No. pos./ No. tested**	**C_T mean (SD[1])**	**No. pos./ No. tested**	**C_T mean (SD)**	**No. pos./ No. tested**	**C_T mean (SD)**
292	3/3	33.40 (0.42)	3/3	33.82 (0.31)	3/3	33.30 (0.58)
146	3/3	34.64 (0.41)	3/3	34.88 (0.35)	3/3	33.76 (0.33)
73	3/3	37.68 (2.75)	3/3	35.58 (0.82)	3/3	35.55 (1.19)
37	3/3	36.84 (1.39)	3/3	37.02 (0.51)	3/3	36.47 (0.52)
18	1/3	N.A.[2]	2/3	N.A.	3/3	36.46 (0.84)
9	1/3	N.A.	0/3	N.A.	0/3	N.A.

[1]Standard deviation
[2]Not applicable

The provisional LOD was 37 – 73 copies/mL. To confirm the final LOD, 20 extraction/amplification replicates for each sample matrix at the provisional LOD values identified in the range finding experiment were tested. These results demonstrated detection rates of ≥95% at 73 copies/mL for each of the three sample matrices tested. The LODs for each of the three matrices, therefore, is 73 copies/mL.

2) ***Analytical Inclusivity***

Inclusivity was evaluated by *in silico* analysis using all publicly available SARS-CoV-2 (taxonomy ID 2697049) sequences available on 19 March 2020. A total of 119 SARS-CoV-2 N gene sequences covering the regions of the assay were included in the alignment. Sporadic mismatches were identified for primers N2 F1, N1 F1 and N1 R1 and in probe N2 P1. However, each mismatch is >7 bases from the 3' end of primers and >15 bases from the 5' end of the probe and therefore unlikely to result in false negative results. Furthermore, each identified mismatch involved only a single primer or probe for that identified GenBank accession number. Therefore, in silico analysis of the SARS-CoV-2 test predicts 100% inclusivity.

3) Cross reactivity

Cross-reactivity (analytical specificity) was evaluated by *in silico* analysis against normal flora, pathogens that cause similar symptoms, and pathogens related to SARS-COV-2. The pathogens evaluated by *in silico* analysis by taxon identification (taxon ID) and the accession with the highest percent identity for each primer is shown in Table 5 below**:**

Table 5: In Silico Analysis for Cross Reactivity

			Oligonucleotide primer or Taqman probe					
Pathogen	**taxon ID**		**N1 forward**	**N1 probe**	**N1 reverse**	**N2 forward**	**N2 probe**	**N2 reverse**
Human coronavirus 229E	11137	% Ident.	65.0%	51.9%	59.3%	N.A.	50.0%	56.5%
		Acc. No.	KT253264.1	KT253271.1	KT253270.1	N.A.	KT253271.1	KT253272.1
Human coronavirus OC43	31631	% Ident.	N.A.	63.0%	N.A.	N.A.	N.A.	N.A.
		Acc. No.	N.A.	AY903460.1	N.A.	N.A.	N.A.	N.A.
Human coronavirus HKU1	290028	% Ident.	N.A.	51.9%	59.3%	N.A.	N.A.	N.A.
		Acc. No.	N.A.	DQ339101.1	AY884001.1	N.A.	N.A.	N.A.
Human coronavirus NL63	277944	% Ident.	N.A.	N.A.	66.7%	N.A.	N.A.	N.A.
		Acc. No.	N.A.	N.A.	MK334045.1	N.A.	N.A.	N.A.
SARS coronavirus	694009	% Ident.	100%	59.3%	89.9%	91.7%	N.A.	95.7%
		Acc. No.	AY297028.1	KJ473811.1	KY352407.1	AY297028.q	N.A.	AY297028.1
MERS coronavirus	133562 6	% Ident.	75.0%	N.A.	59.3%	N.A.	N.A.	N.A.
		Acc. No.	KJ473821.1	N.A.	MG923469.1	N.A.	N.A.	N.A.
Human adenovirus	190721 0	% Ident.	70.0%	N.A.	59.3%	62.5%	72.7%	60.9%
		Acc. No.	LC215429.1	N.A.	MK570618.1	LC215429.1	KY002683.1	MK241690.1
Human metapneumoviru s	162145	% Ident.	65.0%	55.6%	59.3%	N.A.	N.A.	56.5%
		Acc. No.	KJ627397.1	AY525843.1	KJ627383.1	N.A.	N.A.	AF371337.2
Parainfluenza virus 1	12730	% Ident.	70.0%	44.4%	81.5%	66.7%	N.A.	56.5%
		Acc. No.	M14887.1	AF457102.1	KF687307.1	AF457102.1	N.A.	KX639498.1
Parainfluenza virus 2	197916 0	% Ident.	65.0%	N.A.	59.%	58.3%	N.A.	60.9%
		Acc. No.	NC_003443.1	N.A.	AF533011.1	KM190939.1	N.A.	NC_003443.1
Parainfluenza virus 3	11216	% Ident.	60.0%	N.A.	66.7%	N.A.	N.A.	69.6%
		Acc. No.	KM190938.1	N.A.	KY973556.1	N.A.	N.A.	MH678682.1
Parainfluenza virus 4	197916 1	% Ident.	60.0%	44.4%	66.7%	N.A.	N.A.	47.8%
		Acc. No.	NC_021928.1	MH892407.1	KY460515.1	N.A.	N.A.	KF483663.1
Influenza A virus	11320	% Ident.	65.0%	51.9%	62.9%	70.8%	63.6%	69.6%
		Acc. No.	AB827993.1	AB818499.1	NC_007367.1	HE589468.1	AB822988.1	NC_007371.1
Influenza B virus	11520	% Ident.	65.0%	59.3%	59.3%	58.3%	59.1%	60.9%
		Acc. No.	NC_002206.1	NC_002211.1	NC_002205.1	NC_002207.1	NC_002205.1	NC_002211.1
Enterovirus	12059	% Ident.	85.0%	51.8%	74.1%	N.A.	72.7%	82.6%
		Acc. No.	KP202389.1	MK593172.1	FJ445142.1	N.A.	FJ445125.1	AB647318.1
Respiratory syncytial virus	11250	% Ident.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
		Acc. No.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

Rhinovirus	433730	% Ident.	85.0%	62.9%	74.1%	75%	72.7%	65.2%
		Acc. No.	MG950178.1	DQ473499.1	FJ445142.1	FJ445174.1	FJ445125.1	FJ445147.1
Chlamydia pneumoniae	83558	% Ident.	N.A.	N.A.	51.9%	70.8%	68.2%	N.A.
		Acc. No.	N.A.	N.A.	CP001713.1	AE009440.1	AE009440.1	N.A.
Haemophilus influenza	727	% Ident.	N.A.	N.A.	74.1%	N.A.	86.4%	N.A.
		Acc. No.	N.A.	N.A.	CP031689.1	N.A.	NC_000907.1	N.A.
Legionella pneumophila	446	% Ident.	85.0%	N.A.	77.8%	N.A.	N.A.	N.A.
		Acc. No.	CP041668.1	N.A.	CP025491.2	N.A.	N.A.	N.A.
Mycobacterium tuberculosis	1773	% Ident.	N.A.	N.A.	N.A.	N.A.	81.8%	N.A.
		Acc. No.	N.A.	N.A.	N.A.	N.A.	CP000717.1	N.A.
Streptococcus pneumoniae	1313	% Ident.	80.0%	N.A.	N.A.	N.A.	72.7%	N.A.
		Acc. No.	CP007593.1	N.A.	N.A.	N.A.	CP001845.1	N.A.
Streptococcus pyogenes	1314	% Ident.	N.A.	N.A.	N.A.	N.A.	81.8%	N.A.
		Acc. No.	N.A.	N.A.	N.A.	N.A.	AE009949.1	N.A.
Bordetella pertussis	520	% Ident.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
		Acc. No.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Mycoplasma pneumoniae	2104	% Ident.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
		Acc. No.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Pneumocystis jirovecii	42068	% Ident.	50.0%	N.A.	66.7%	54.2%	N.A.	78.3%
		Acc. No.	AY685194.1	N.A.	AY127566.1	AY130996.1	N.A.	JX499143.1
Candida albicans	5476	% Ident.	60.0%	N.A.	59.3%	N.A.	N.A.	65.2%
		Acc. No.	NC_002653.1	N.A.	NC_002653.1	N.A.	N.A.	NC_002653.1
Pseudomonas aeruginosa	287	% Ident.	80.0%	66.7%	N.A.	N.A.	81.9%	N.A.
		Acc. No.	NZ_CP040684.1	NZ_CP027174.1	N.A.	N.A.	NZ_CP007147.1	N.A.
Staphylococcus epidermidis	1282	% Ident.	N.A.	N.A.	N.A.	66.7%	N.A.	N.A.
		Acc. No.	N.A.	N.A.	N.A.	NZ_CP018842.1	N.A.	N.A.
Streptococcus salivarius	1304	% Ident.	70.0%	62.9%	N.A.	62.5%	77.3%	82.6%
		Acc. No.	NZ_CP040804.1	NZ_CP018187.1	N.A.	NZ_CP018189.1	NZ_CP020451.2	NZ_CP020451.2

A number of individual primers or probes had > 80% identity. However, potential cross-reactivity was not identified in full primer/probe sets. To confirm that cross-reactivity does not occur, amplification of these pathogens with the SARS-CoV-2 assay was performed. Additionally, the common respiratory coronaviruses (strains 229E, NL63, and OC43) and DNA templates corresponding to the N gene sequence of SARS (position 29034 – 29233 and 28669 – 28868 of NC_004718.3) were tested. None of the pathogens tested by the SARS-CoV-2 RT-PCR assay generated detectable amplification signals.

Table 6: Wet testing for cross reactivity

Pathogen	Source	Concentration	SARS-CoV-2 rRT-PCR C_T	Internal Control C_T
Coronavirus 229E	Zeptometrix	$1x10^{4.10}$ $TCID_{50}$/mL	N.D.[2]	29.47
Coronavirus NL63	Zeptometrix	$1x10^{3.75}$ $TCID_{50}$/mL	N.D.	30.39
Coronavirus OC43	Zeptometrix	$1x10^{4.10}$ $TCID_{50}$/mL	N.D.	28.83
SARS NC_004718	IDT	$5x10^4$ copies/mL	N.D.	N.A.[3]
Parainfluenza virus 1	Zeptometrix	$1x10^4$ PFU/mL	N.D.	29.56
Enterovirus	Zeptometrix	$5x10^4$ copies/mL	N.D.	30.07
Rhinovirus	Zeptometrix	$1x10^4$ PFU/mL	N.D.	29.80
Haemophilus influenza	Zeptometrix	$5x10^4$ CFU/mL	N.D.	29.61
Legionella pneumophila	Zeptometrix	$5x10^4$ CFU/mL	N.D.	29.71
Mycobacterium tuberculosis	ATCC	$5x10^4$ GEq/mL	N.D.	N.A.
Streptococcus pneumoniae	Zeptometrix	$5x10^4$ CFU/mL	N.D.	32.70
Streptococcus pyogenes	Zeptometrix	$5x10^4$ CFU/mL	N.D.	29.82
Pseudomonas aeruginosa	Zeptometrix	$5x10^4$ CFU/mL	N.D.	29.67
Streptococcus salivarius	Zeptometrix	$5x10^4$ CFU/mL	N.D.	29.77
Pooled human nasal wash	De-identified residual	N.A.[1]	N.D.	30.57
Pooled human NP swab (UTM)	De-identified residual	N.A.	N.D.	32.17
Pooled human BAL	De-identified residual	N.A.	N.D.	32.02

[1]Not applicable
[2]Not detected
[3]Obtained as a genomic DNA sample therefore extraction was not performed

4) *Clinical Evaluation:*

Clinical evaluation of the SARS-CoV-2 RT-PCR assay was performed by spiking IVT into known negative samples at concentrations ranging from 2x LOD through the range of the assay. The negative samples consisted of three different matrices: BAL, nasal wash, and nasopharyngeal swabs. For BAL and nasal wash, 100% agreement was achieved for 62 samples spiked at 7 concentrations, ranging from 2x LOD to 800,000x LOD (Table 7 and 8). All 30 negative specimens were negative for SARS-CoV-2 and each had an internal control C_T value <35. For nasopharyngeal swabs (Table 9**)**, a signal was detected for all samples at each spiking concentration. However, one sample at 2x LOD yielded a C_T of 38.2 which is above the C_T cutoff for the assay, yielding 95% (19 of 20) positive agreement at 2x LOD. An agreement of 100% was achieved for all 42 spiked nasopharyngeal swabs samples at higher concentrations. For nasopharyngeal swabs all 30 negative specimens were negative for SARS-CoV-2 and each had an internal control C_T value <35.

Table 7. Clinical performance of the SARS-CoV-2 RT-PCR test in bronchoalveolar lavage

Fold of LOD	RNA concentration (copies/mL)	No. pos./ No. tested	Agreement	SARS-CoV-2 Mean C_T (SD^2)	Internal Control Mean C_T (SD)
Negative	N.A.[1]	30/30	100%	N.D.[3]	30.39 (0.17)
2x	146	20/20	100%	34.57 (0.85)	N.A.

8x	585	6/6	100%	32.46 (0.43)	N.A.
80x	5,850	6/6	100%	29.16 (0.08)	N.A.
800x	5.8×10^4	9/9	100%	25.89 (0.33)	N.A.
8000x	5.8×10^5	6/6	100%	22.48 (0.06)	N.A.
80,000x	5.8×10^6	9/9	100%	19.15 (0.34)	N.A.
800,000x	5.8×10^7	6/6	100%	15.78 (0.16)	N.A.

[1]Not applicable
[2]Standard deviation
[3]Not detected

Table 8. Clinical performance of the SARS-CoV-2 RT-PCR test in nasal wash

Fold of LOD	RNA concentration (copies/mL)	No. pos./ No. tested	Agreement	SARS-CoV-2 Mean C_T (SD[2])	Internal Control Mean C_T (SD)
Negative	N.A.[1]	30/30	100%	N.D.[3]	28.58 (0.22)
2x	146	20/20	100%	34.99 (0.94)	N.A.
8x	585	6/6	100%	32.95 (0.27)	N.A.
80x	5,850	6/6	100%	29.25 (0.14)	N.A.
800x	5.8×10^4	9/9	100%	26.04 (0.30)	N.A.
8000x	5.8×10^5	6/6	100%	22.56 (0.08)	N.A.
80,000x	5.8×10^6	9/9	100%	19.34 (0.32)	N.A.
800,000x	5.8×10^7	6/6	100%	15.97 (0.08)	N.A.

[1]Not applicable
[2]Standard deviation
[3]Not detected

Table 9. Clinical performance of the SARS-CoV-2 RT-PCR test in nasopharyngeal swab

Fold of LOD	RNA concentration (copies/mL)	No. pos./ No. tested	Agreement	SARS-CoV-2 Mean C_T (SD[2])	Internal Control Mean C_T (SD)
Negative	N.A.[1]	30/30	100%	N.D.[3]	31.15 (1.06)
2x	146	19/20	95%	34.92 (0.71)	N.A.
8x	585	6/6	100%	32.75 (0.35)	N.A.
80x	5,850	6/6	100%	29.44 (0.33)	N.A.
800x	5.8×10^4	9/9	100%	25.96 (0.47)	N.A.
8000x	5.8×10^5	6/6	100%	22.77 (0.30)	N.A.
80,000x	5.8×10^6	9/9	100%	19.26 (0.34)	N.A.
800,000x	5.8×10^7	6/6	100%	16.03 (0.29)	N.A.

[1]Not applicable
[2]Standard deviation
[3]Not detected

[It should be noted that the internal control value is displayed as N.A. because a signal was detected in the SARS-CoV-2 channel.]

In addition, the first 5 positive and first 5 negative results from patient specimens tested with this assay were sent to the Missouri Department of Health and Senior Services for confirmation testing. All 10 specimens yielded concordant results.