EMERGENCY USE AUTHORIZATION (EUA)
SUMMARY Viracor SARS-CoV-2 assay
(Viracor Eurofins Clinical Diagnostics)

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

(The Viracor SARS-CoV-2 assay will be performed at Viracor Eurofins Clinical Diagnostics, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a as described in the laboratory procedures reviewed by 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 by their healthcare provider (HCP). Testing is limited to Viracor Eurofins Clinical Diagnostics, located at 1001 NW Technology Dr., Lee's Summit, MO, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets the requirements to perform high-complexity tests.

This test is also authorized for use with the EmpowerDX At-Home COVID-19 PCR Test Kit for individuals to self-collect nasal swabs at home, when determined by an HCP to be appropriate based on the results of an online COVID-19 questionnaire.

This test is also for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled samples containing up to five individual nasopharyngeal swab specimens that are collected by an HCP using individual vials containing transport media, from individuals suspected of COVID-19 by their HCP. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

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 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 Viracor SARS-CoV-2 assay is only intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR assays and in vitro diagnostic procedures. 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 following instruments:

1. 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.
2. Thermofisher MagMax Viral/Pathogen Nucleic acid isolation kit with ThermoFisher KingFisher FLEX for automated nucleic acid extraction and Applied Biosystems 7500 Fast Real-Time PCR System

Table 1: Reagents and Materials For SARS-CoV-2 Assay

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

Table 2: Reagents and Materials for EmpowerDX At-Home COVID- 9 PCR Test Kit

Name	**Description**	**Quantity**	**Material Supplier**
Instructions	Kit registration, sample collection and shipping instructions	1	EmpowerDX
Label	bar-coded label with individuals name and line for addition of date of birth	1	Path-Tec
Nasal Swab	Polyester tipped, plastic shaft nasal swab	1	Path-Tec
Collection tube	Screw-capped collection tube containing 3 mL 0.9% buffered saline solution	1	Path-Tec
Biohazard bag	Sealing (zip-lock) biohazard bag containing an absorbent pad	1	Path-Tec
Box	Cardboard box for material shipping to individual and return of sample to the laboratory	1	Path-Tec
FedEx shipping envelop	Addressed, pre-paid FedEx shipping envelop, UN 3373 Biological Substance Category B labeled	1	FedEx

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. Additionally, a primer/probe set detecting human RNaseP is included to ensure an adequate biological specimen is present in home-collected specimens.

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

- 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. The expected C_T value for the RNaseP control is <40. In samples with no SARS-CoV-2 target detected, a C_T value less than or equal to these values for MS2 and RNaseP 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 3: 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
RNase P control	$C_T \leq 40$	$C_T > 40$[d, e]
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.

[d]In clinical specimens with SARS-CoV-2 target "Not detected" (i.e. $C_T > 38$), results are invalid when RNase P control $C_T > 40$.
[e]In clinical specimens with SARS-CoV-2 target "Detected" (i.e. $C_T \leq 38$), RNase P control 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 of individually tested specimens are reported as shown in Table 3 below; when all control values are valid as stated above, results of pooled specimens are reported as shown in Table 4 below:

Table 4: Interpretation of Patient Results from Individually Tested Specimens

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"

Table 5: Interpretation of Results from Pooled Specimens

Real-time RT-PCR result	Reported result
$C_T > 40$	SARS-CoV-2 RNA "Not Detected"
$C_T \leq 40$	SARS-CoV-2 RNA "Detected"[1]

[1]For pools with SARS-CoV-2 "Detected", the samples making up that pool will be tested individually to identify the positive sample(s). The results interpretation for individual testing will follow Table 3 above.

MEDICAL OVERSIGHT FOR HOME COLLECTION:

Viracor Laboratories will be testing samples collected using the EmpowerDx At-Home COVID-19 PCR Test Kit. Medical oversight for the home collection process will be provided by Clinical Enterprises/Empower Dx, as they have physician review and will be responsible for approval of prescription orders. Viracor will provide results to EmpowerDx, who will then communicate results back to the patients.

PERFORMANCE EVALUATION

1) ***Analytical Sensitivity:***

Limit of Detection (LoD):

The analytical sensitivity of Viracor 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 x 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. Samples were extracted using the bioMerieux NucliSENS easyMAG and bioMerieux eMAG for nucleic acid extraction and tested using the ABI 7500 Real-Time PCR thermocycler. Results are shown in Table 6 below:

Table 6: 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 LoD for each of the three matrices, therefore, is 73 copies/mL.

The LoD (73 copies/mL) was also confirmed when nucleic acid was extracted using the ThermoFisher MagMax Viral/Pathogen Nucleic Acid Isolation kit on the ThermoFisher KingFisher FLEX instrument platform and then tested on the Applied Biosystems 7500 Fast Real-Tim PCR System.

*The LoD study was conducted in March 2020 and initially published at the time of the original authorization on April 6, 2020, when SARS-CoV-2 virus isolates or clinical samples were not widely available.

2) ***Analytical Inclusivity***

A total of 81,765 sequences from the GISAID database covering Viracor SARS-CoV-2 rRT-PCR assay were aligned to the N1 and N2 assay primer and probe sequences. Alignments in which the SARS-CoV-2 sequences contained either an N or a degeneracy (e.g. R, M) were eliminated from analysis. All sequences covering only part of the full Viracor assays (N1 and N2) were also eliminated for analysis. A total of 80,478 sequences remained and were then subjected to analysis for mismatches relative to Viracor SARS-CoV-2 N1 and N2 primer and probe sequences. Figure 1 summarizes the results.

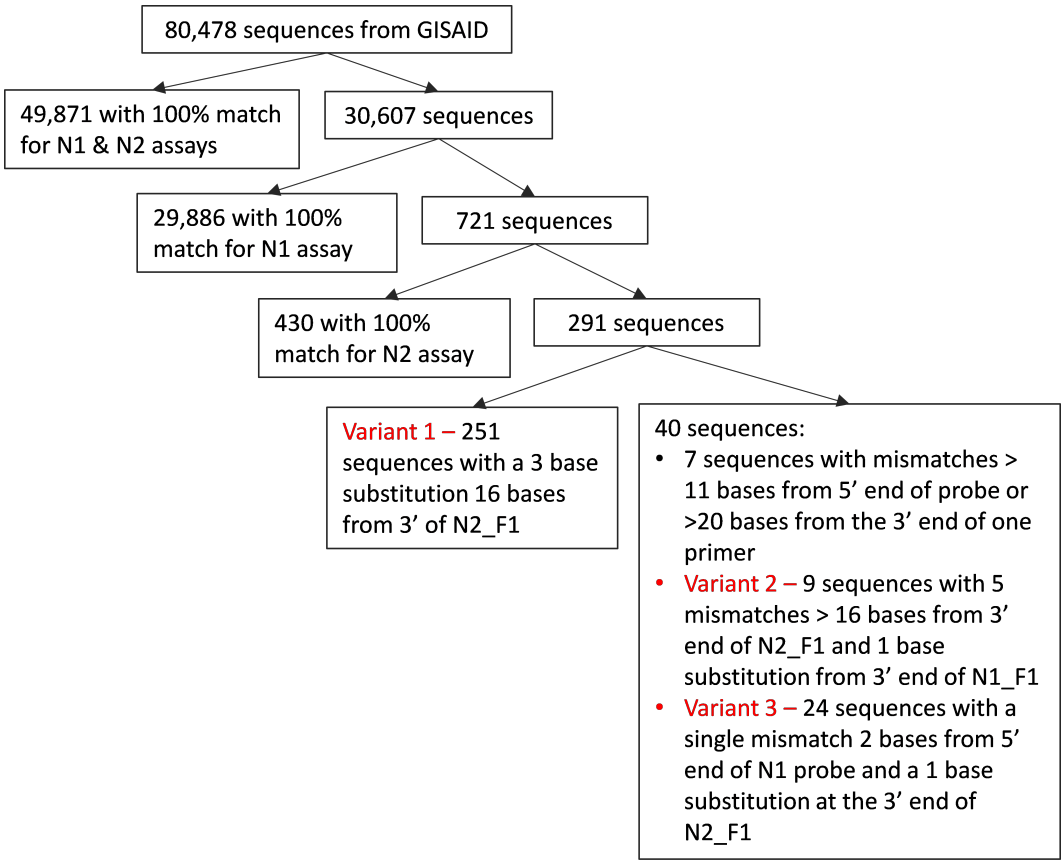

Figure 1. Summary of *in silico* analysis of Viracor SARS-CoV-2 rRT-PCR assay for inclusivity

A total of 80,187 (99.64%) of GISAID sequences as of September 20, 2020 demonstrated 100% identity to both forward and reverse primers and the probe for at least one of the two Viracor assay primer/probe sets (N1 and N2), and it was concluded that these sequences would be effectively detected by the Viracor assay. A total of 291 sequences (0.36%) were subjected to further analysis. A total of 251 sequences (variant 1) shared a common mutation, which consists of a 3 base substitution 16 bases upstream of the 3' end of the N2 forward primer (N2_F1), and did not have 100% identity for each of the N1 assay primers and probes. To investigate the impact of the 3-base mismatch in N2_F1, a synthetic DNA molecule

representing this variant was synthesized (Integrated DNA Technologies) along with a separate wild-type (100% match to N2 primers and probe) DNA molecule. rRT-PCR testing was performed using both the variant and wild-type template. On average the C_T values increased 1.35 cycles with the variant template, with higher Ct shifts noted at lower template concentrations. Importantly, the lowest concentrations of template tested were at 1 – 2 x the LoD of the assay (75 copies/mL) and 4 of 4 samples tested were positive with C_T values below the cutoff ($C_T \leq 38$). Of the remaining 40 (0.05%) sequences, 7 had single mismatches more than 11 bases from the 5' end of the probe or more than 20 bases from the 3' end of one primer and these 7 sequences were not considered to significantly impact inclusivity. For variant 2, a total of 9 sequences had the same 3 base substitution as variant 1 plus an additional 2 bases at the 5' end of N2_F1. To determine the impact of this mutation, a synthetic DNA molecule with the variant 2 sequence will be compared to wild-type template. For variant 3, a total of 24 sequences had a single mismatch 2 bases from 5' end of N1_P1 probe. All of the variant 3 sequences also had a 1 base substitution at the 3' end of N2_F1 which would likely prevent the N1 assay from detecting SARS-CoV-2. To determine the impact of this N1 probe mutation, a synthetic DNA molecule with the variant 3 sequence will be compared to wild-type template.

In summary, 99.64% of sequences analyzed had 100% identity to both primers and probes of at least one of the two primer/probe sets in Viracor SARS-CoV-2 rRT-PCR assay. Experimental rRT-PCR results demonstrate that an additional 0.31% of sequences would be detected with a minor (1.35 cycle) shift in C_T values. The locations of single base mismatches strongly suggest no impact on detection for 7 sequences (0.01%) analyzed. Experiments are underway to determine the impact of mismatches identified in two variants representing 33 (0.04%) of the sequences analyzed.

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 are shown in Table 7 below**:**

Table 7: 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	277944	% Ident.	N.A.	N.A.	66.7%	N.A.	N.A.	N.A.

coronavirus NL63		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	1335626	% 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	1907210	% 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 metapneumovirus	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	1979160	% 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	1979161	% 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 8: 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 9 and 10). All 30 negative specimens were negative for SARS-CoV-2 and each had an internal control C_T value <35. For nasopharyngeal swabs (Table 11**)**, 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 9. 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

*The clinical study was conducted in March 2020 and initially published at the time of the original authorization on April 6, 2020, when SARS-CoV-2 virus isolates or clinical samples were not widely available.

Table 10. 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 11. 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.

5) Sample pooling:

Pooling Validation:

A study was performed to assess the impact of sample pooling on assay sensitivity. This study used archived, de-identified residual SARS-CoV-2 positive and negative clinical

samples randomly selected from Viracor's archive of healthcare provider collected nasopharyngeal samples. Samples used for this analysis were evenly divided among three US geographic regions (Midwest – 6 samples, Northeast – 7 samples, and Southeast – 7 samples).

To assess the impact of pooling, aliquots of 20 individual positive samples were combined with equal volume aliquots of 4 negative samples to create pooles of 5 samples. Each of the 20 positive pools was constructed with 4 unique negative samples. A total of 25% of the positive samples used to construct the pools had Ct values near the LoD of the assay. Additionally, each pool of 4 unique negative samples was combined with one additional unique negative sample to form a total of 20 pools of 5 negative samples per pool.

Nucleic acid extraction of the original (undiluted) positive samples and pooled samples was performed using the ThermoFisher KingFisher FLEX instrument with ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation kits for nucleic acid extraction, and rRT-PCR amplification was performed using Applied Biosystems 7500 Fast Real-Time PCR Systems (SDS v1.5.1) with TaqPath 1-step RT-qPCR master mix CG kits using the "fast" rRT-PCR amplification protocol in a 30 µL reaction volume.

For the 5 sample pools with a single positive sample, 20 of 20 pools remained positive (100% positive agreement, 95% CI 83.16% – 100%). For the 5 negative sample pools, 20 of 20 pools remained negative (100% negative agreement, 95% confidence interval 83.16 – 100%).

In Silico Sensitivity:

An *in silico* analysis was performed to evaluate the clinical sensitivity of 5 sample pooling on a historical dataset of samples tested at Viracor. A Passing-Bablok regression analysis was performed using the pooling validation dataset (described above) to calculate the Ct shift between pooled and individual samples at the cutoff of the assay. The equation from the regression analysis was as follows:

$Y = 0.929X + 3.639$, where Y is the shifted Ct value for the pooled sample and X is the original Ct value for single sample testing.

This Ct shift was then applied to a historical dataset consisting of 97 consecutive samples that were previously positive by the Viracor assay when tested individually. The Ct shift was applied to these samples to determine the proportion of samples that would remain positive if 5-sample pooling took place. These data indicated that the *in silico* sensitivity of the pooled assay was 100% (97/97, 95% CI 96.19%; 100%), indicating no samples would have been missed with 5-sample pooling.

6) Home Collection Validation

Shipping Stability for the EmpowerDx At-Home COVID-19 PCR Kit:

The EmpowerDX At-Home COVID-19 PCR Test Kit uses wrapped polyester nasal swabs transported in 0.9% saline and therefore references the COVID-19 swab stability studies conducted by Quantigen Biosciences through the right of reference granted to any sponsor wishing to pursue an EUA request.

Two SARS-CoV-2-positive pools (2xLoD and 10xLoD) were contrived by combining SARS- CoV-2- negative human/porcine matrix with previously confirmed, high-positive patient samples. The 2xLoD and 10xLoD pools were added directly to swabs through a procedure that mimics a nasal swabbing action: swabs were submerged into a reservoir of either 2xLoD or 10xLoD mixture and "abraded" against the side of the (Eppendorf style) tube while the viral solution absorbs into the swab (whether foam or polyester). The 20 low-positive samples and the 10 intermediate-positive samples used with each test condition did not come from individual patients. Rather, for each of the two concentrations, a single preparation of virus + media or virus + matrix was prepared, from which technical replicates were prepared.

The human/porcine negative matrix swabs were prepared by spiking them into negative porcine nasal mucous using the same procedure described above. Swabs were then placed into 1 mL saline.

Samples were tested using an EUA authorized assay at times 0, 30 hours, and 54 hours post incubation. Samples were held at 40℃ for 12 hours, then 32℃ for 18 or 42 hours, respectively. Samples were equilibrated to room temperature for 2 hours before testing.

The acceptance criteria laid out for the study was a 95% agreement or greater for positive samples. Both time points met this criteria and supported sample shipping stability, using a drop box, with over-night or 48-hour shipping.

Table 12: Average C_T values for each time point for both sample dilutions

Swab	Time point	N	Internal Control	Target 1	Target 2	Target 3
2xLoD swab in saline	0 h	5	23.74	32.23	30.03	31.80
10xLoD swab in saline	0 h	5	23.27	29.46	27.58	28.67
2xLoD swab in saline	30 h	20	26.00	32.69	31.33	34.59
10xLoD swab in saline	30 h	10	26.19	29.54	28.37	28.69
2xLoD swab in saline	54 h	20	25.70	32.03	31.09	32.10
10xLoD swab in saline	54 h	10	26.11	28.73	27.25	25.09

Human Usability Studies for the EmpowerDX At-Home COVID-19 PCR Kit:

Testing was performed with 30 participants and took place in the actual use environment (participant's home). None of the 30 participants had medical or laboratory training or prior experience with self-collection. The ages and educational levels of the participants varied and is shown in the table below with the results of testing.

The entire workflow was performed by each individual participant and included registration of the kit, sample collection, packaging of the sample, and mailing to the laboratory with pre- prepared FedEx envelop. Sample collection of each participant was observed by remote visual monitoring and no difficulties were noted.

The following criteria were used to assess the sample collection procedure (a summary of results is provided after each criterion in italics):

1. Did participant read the instructions? *30 out of 30 participants indicated they had read the instructions.*
2. Did participant wash their hands before opening the kit? *30 out of 30 participants washed their hands prior to opening the kit.*
3. Did participant open the swab without touching the tip to hands/ surfaces? *30 out of 30 participants opened the swab without touching the tip to hands/surfaces.*
4. Did participant properly collect both nasal swabs? *29 out of 30 participants properly collected both nostrils; one participant properly collected a single nostril.*
5. Did participant place swab in collection tube? *30 out of 30 participants placed the swab in the collection tube with the soft (collection) end contacting the liquid.*
6. Did participant spill any liquid from collection tube? *None of the 30 participants spilled any liquid from the collection tube.*
7. Did participant place vial in biohazard bag? *30 out of 30 participants placed the collection tube in the biohazard bag.*
8. Did participant place biohazard bag in the box? *30 out of 30 participants placed the biohazard bag in the box.*
9. Did participant place box in shipping envelop and seal? *30 out of 30 participants placed the box in the shipping envelop and sealed.*
10. Did any injury occur during the procedure? *None out of 30 participants experienced any injury in the procedure.*
11. Were there any deviations from the instructions? *One out of 30 participants deviated from the instructions (noted above in no. 4 in which a single nostril was collected).*

Laboratory personnel inspected the packaging and samples upon receipt. No packaging errors were noted. Each sample from the 30 participants was acceptable for testing. The following parameters were evaluated during packaging inspection (a summary of results is provided after each criterion in italics):

1. Is the sample tube in the biohazard bag? *30 out of 30 sample tubes were in a biohazard bag*
2. Is the sample tube labeled with name/barcode sticker? *29 out of 30 sample tubes were correctly labeled with the name/barcode sticker; for one sample the barcode sticker was on the tube but did not have a name. Based on the barcode, this participant was contacted to verify the correct name of the participant.*

3. Is the DOB on the name/barcode sticker? *28 out of 30 participants correctly printed their DOB on the name/barcode sticker; for the two stickers that that did not have a DOB, follow-up contact confirmed this information.*
4. Is the biohazard bag sealed? *30 out of 30 biohazard bags were sealed.*
5. Is the absorbent pad in the biohazard bag? *30 out of 30 biohazard bags contained the absorbent pad.*
6. Is a swab present in the tube? *30 out of 30 sample tubes had a swab present.*
7. Is the volume of liquid in the tube 2 - 3 mL? *30 out of 30 sample tubes had 2 – 3 mL of liquid.*
8. Is there evidence of leaking from the tube? *None of the 30 sample tubes showed evidence of leaking in the biohazard bag.*
9. Is the biohazard bag with the tube in the box? *30 out of 30 biohazard bags (containing sample tubes) were in the box.*
10. Is the consent form in the box or external package? *30 out of 30 consent forms were in the box or external packaging.*

The samples were tested for specimen adequacy using a primer/probe set that detects RNAse P gene. Pre-defined acceptance criteria for sample adequacy were RNAse P CT value <40 and MS2 (exogenous internal control) CT value <35. Study participants ranged in age from 19 to 58 years old and in educational level from a high school degree to a doctoral level degree. Results of testing for each of the 30 participants are shown in the following table:

Table 13: Participant age, educational level and Viracor SARS-CoV-2 rRT-PCR results

Subject ID	**Age (yrs)**	**Education level**	**MS2 CT**	**RNAse P CT**
1	35	BS/BA[1]	26.80	25.35
2	56	BS/BA	26.40	26.10
3	34	MS[3]	26.18	31.83
4	31	BS/BA	26.54	26.15
5	58	MS	26.40	26.65
6	55	Doctoral[4]	26.15	31.42
7	27	Doctoral	26.30	30.55
8	55	MS	27.03	23.58
9	35	BS/BA	26.22	27.12
10	34	MS	26.36	29.25
12	46	MS	26.47	29.29
13	24	HS[5]	26.33	29.36
14	34	MS	26.38	29.39
15	54	MS	26.74	24.37
16	50	BS/BA	26.43	31.89
17	57	BS/BA	26.83	25.23
18	56	BS/BA	27.17	22.56
19	22	BS/BA	26.33	27.28

20	54	Associates[6]	26.41	27.43
21	57	BS/BA	27.16	24.17
22	30	BS/BA	26.59	25.23
23	27	Associates	26.24	28.71
24	35	BS/BA	26.67	23.52
25	31	Associates	27.46	25.30
26	21	Some college	26.23	30.71
27	48	BS/BA	26.50	25.80
28	51	BS/BA	26.54	25.22
29	22	Associates	27.31	21.03
30	19	Some college	26.28	26.48

[1]4-year bachelors level degree
[2]Not detected (negative)
[3]Master's level degree
[4]Doctoral level degree
[5]High school degree
[6]2-year associates level degree

The results from the usability indicate users are able to safely and appropriately collect a nasal swab specimen with sufficient human biological material.

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: Viracor Eurofins Clinical Diagnostics laboratory located at 1001 NW Technology Dr., Lee's Summit, MO, that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements to perform high complexity tests;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-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. Nucleic acid extraction was performed by the ThermoFisher KingFisher FLEX instrument using ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation kits for nucleic acid extraction. The amplification was run on the Applied Biosystems 7500 Real-Time PCR Systems (SDS v1.5.1) for nucleic acid amplification and detection. The results are summarized in the following Table.

Table 14: 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 Swabs	$1.8x10^2$ NDU/mL	N/A
MERS-CoV		N/A	ND

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