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 laboratories designated by Viracor Eurofins Clinical Diagnostics, which are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet the requirements to perform high complexity tests, 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 nucleic acid from SARS-CoV-2 in: (1) nasopharyngeal swabs (NP), mid-turbinate swabs, anterior nasal swabs, nasopharyngeal wash, nasal wash, oropharyngeal swab (OP) and bronchoalveolar lavage from individuals suspected of COVID-19 by their healthcare provider (HCP); and (2) anterior nasal swab specimens collected from individuals without symptoms or other reasons to suspect COVID-19. Testing is limited to laboratories designated by Viracor Eurofins Clinical Diagnostics, which are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263, and meet the requirements to perform high-complexity tests.

This test is also for the qualitative detection of nucleic acid from 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.

This test is also for the qualitative detection of RNA from SARS-CoV-2 in pooled samples containing up to 10 individual human anterior nasal swabs placed in a single vial containing transport media after being collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19, when tested as part of a serial testing program including testing at least once per week.

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 Thermofisher MagMax Viral/Pathogen Nucleic acid isolation kit and GSD NovaPrimer RNA extraction kit with the ThermoFisher KingFisher FLEX for automated extraction can also be used. 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 and GSD NovaPrime RNA Extraction with ThermoFisher KingFisher FLEX for automated nucleic acid extraction and Applied Biosystems 7500 Fast Real-Time PCR System

Designated laboratories will receive an FDA accepted instrument qualification protocol included as part of the laboratory SOP and will be directed to execute the protocol prior to testing clinical samples. Designated laboratories must follow the authorized SOP, which includes the instrument qualification protocol, as per the letter of authorization.

Table 1: Reagents and Materials For Viracor 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

CONTROLS TO BE USED WITH THE VIRACOR SARS-COV-2 ASSAY

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) <u>Viracor SARS-CoV-2 Assay 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. 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 2: Interpretation of Results For Internal, No Template, Negative and Positive Control Reactions

Control	Valid result[a]	Invalid result
Internal control (MS2)	$C_T < 35$ No amplification signal detected	$C_T > 35$[b, c]
No template control		Amplification detected
RNase P control	$C_T < 40$ No amplification signal detected	$C_T > 40$[d, e]
Negative control		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 and Table 5 below:

Table 3: 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 4: 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.

Table 5: Interpretation and reporting of 10 swab pools received in a single tube

SARS-CoV-2 RT-PCR Result	10-Swab Pool reporting	Action for 10-swab Pools
Detected	Presumed Positive	Report pooled result to program administrator. [a]A new sample from all subjects in the pool should be collected and re-tested individually. Individuals should isolate until receiving a negative result when re-tested individually Individuals should not be cohorted with other individuals who have received a positive or presumptive positive result
Not Detected	Negative	Report pooled result to program administrator and appropriate public health authorities
Invalid (due to any control results)	Invalid	Repeat test on pooled sample one time. If the result is Negative, report

		pooled result to program administrator and appropriate public health authorities. If the result is Positive or Invalid, report the result as Presumed Positive to program Administrator [a]A new sample from all subjects in the pool should be collected and re-tested individually

[a]Results of follow-up testing are reported to the program administrator and appropriate public health authorities.

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 1.7×10^{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 5 below:

Table 5: 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])[2]**	**No. pos./ No. tested**	**C_T mean (SD)[2]**	**No. pos./ No. tested**	**C_T mean (SD)[2]**
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.[3]	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] Assay targets are detected in a single well by probes labeled with the same fluorophore, resulting in a single C_T value for each positive sample.
[3]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. Additionally, the GSD Extraction kit on the ThermoFisher KingFisher FLEX instrument platform was validated via a side-by-side study comparing detection of a diluted positive clinical sample at the limit of detection with the MagMax workflow:

	# positive replicates	Mean Ct of SARS-CoV-2
MagMax Extraction	20/20	35.68
GSD Extraction	20/20	35.85

*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) ***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 table below:

Table 6: 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

3) ***Analytical Inclusivity***

A total of 81,765 sequences from the GISAID database covering Viracor SARS-CoV-2 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.

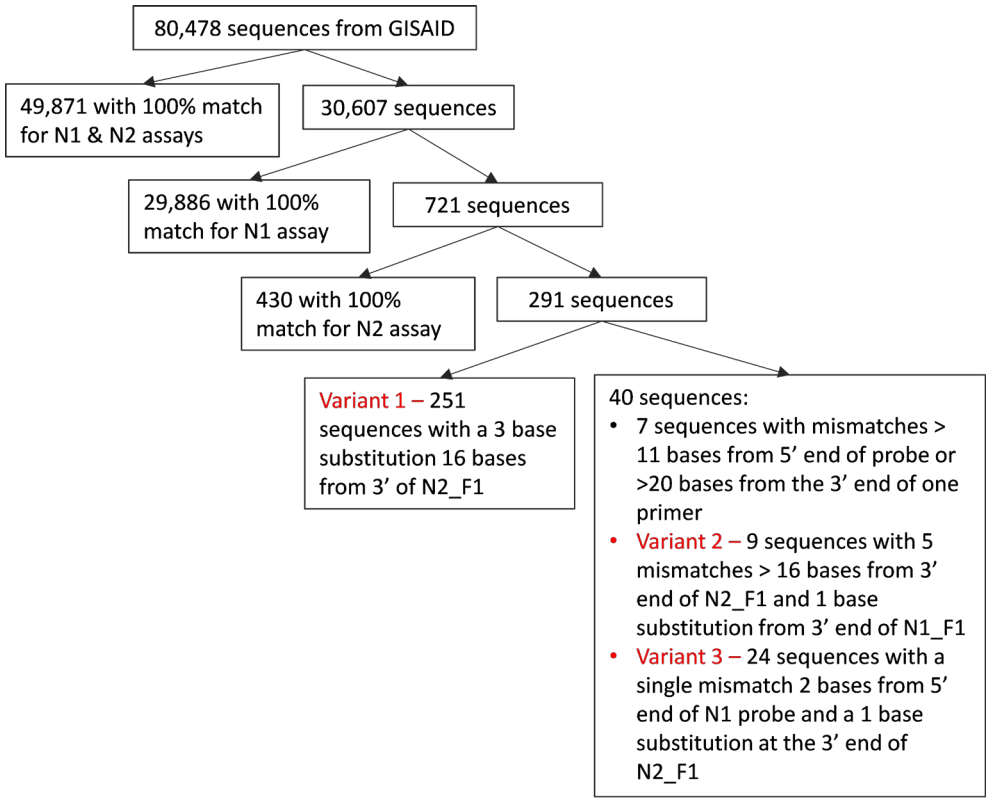

Figure 1. Summary of *in silico* analysis of Viracor SARS-CoV-2 Assay for inclusivity: Description – There were a total 80478 sequences from GISAID at the time of the analysis. Of these 80478 total sequences, 49871 had 100% match for the N1 and N2 assays, and 30607 sequences remained. Of the 30607 sequences, 29886 had 100% match for the N1 assay, and 721 sequences remained. Of the 721 sequences, 430 had 100% match for the N2 assay, and 291 sequences remained. Of the 291 sequences, 251 were sequences with a 3 base substitution 16 bases from 3' of N2_F1 (Variant 1), and 40 sequences remained. Of the 40 sequences, 7 sequences were with mismatches > 11 bases from 5' end of probe or >20 bases from the 3' end of one primer, 9 sequences were with 5 mismatches > 16 bases from 3' end of N2_F1 and 1 base substitution from 3' end of N1_F1 (Variant 2), and 24 sequences were with a single mismatch 2 bases from 5' end of N1 probe and a 1 base substitution at the 3' end of N2_F1 (Variant 3).

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 C_T 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.

In addition to the above, Viracor SARS-CoV-2 Assay N1 and N2 primer and probe sequences were aligned to emerging variants of potential public health importance. The results of these analyses are summarized in the table below.

Table 7: Summary of *in silico* analysis of Viracor's SARS-CoV-2 Assay (N1 and N2 primers/probes) for detection of SARS-CoV-2 variants of potential public health importance.

		No. sequences		
Variant	**Lineage**	**Total Identified**	**100% match for N1 and/or N2 assay[1]**	**Assay coverage for strains <100% in N1 or N2 assay**
UK	B.1.1.7 (N501Y.V1)	17,307	17,168 (99.1%)	3 base mismatch in N2 forward primer 16 bases from 3' end.
South Africa	B.1.351 (N501Y.V2)	400	393 (98.3%)	Single base mismatch in N2 forward primer 13 bases from 3' end.
California	B.1.429 (CAL.20C0)	360	355 (98.6%)	Single base mismatch in N2 forward primer 13 bases from 3' end.
Brazilian	P.1	11	11 (100%)	Not applicable

The *in silico* analysis predicts that these variants of potential public health importance would be detected by the Viracor SARS-CoV-2 Assay. For the strains that do not have 100% identity with each primer and the probe for one of the two targets (N1 and N2) of the Viracor assay, the mismatches in the impacted target are distant from the 3' end of the primer and are not likely to lead to a significant impact on detection. For the UK variant, Viracor has demonstrated experimentally that the 3-base mismatch in N2 forward primer located 16 bases from 3' end result in a minor (1.35 cycle) shift in C_T values.

4) 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 the table below**:**

Table 8: 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	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	5476	% Ident.	60.0%	N.A.	59.3%	N.A.	N.A.	65.2%

albicans		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 9: 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

5) *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. All 30 negative specimens were negative for SARS-CoV-2 and each had an internal control C_T value <35. For nasopharyngeal swabs, 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 10: 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 11: 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 12: 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.

6) Adding population screening of individuals without symptoms or other reasons to suspect COVID-19 to an authorized test

Data was provided to support testing of a screening population consisting of individuals without symptoms or other reasons to suspect COVID-19. Testing using the Viracor SARS- CoV-2 Assay was performed using the ThermoFisher KingFisher FLEX instrument with ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation kits for nucleic acid extraction, and RT-PCR amplification by the Applied Biosystems 7500 Fast Real-Time PCR Systems (SDS v1.5.1) with TaqPath 1-step

RT-qPCR master mix CG kits for nucleic acid amplification and detection. The comparator was a highly sensitive FDA EUA authorized assay.

Samples were collected from individuals not exhibiting clinical signs characteristic of SARS-CoV-2 infection (i.e. individuals being screened) or other reasons to suspect COVID-19. The samples tested were anterior nasal swab specimens collected during community screening events with initial testing by Viracor Eurofins Clinical Diagnostic Laboratories. A total of 511 samples were tested with the Viracor SARS-CoV-2 Assay. Of these 23 consecutively collected positive specimens and 102 consecutively collected negative specimens were also tested with the comparator assay. All samples were stored at -70˚C (or colder) after collection, and all samples were fully de-identified prior to re-testing with the comparator assay. A summary of results is shown in the table below.

Table 13: Results of Clinical Evaluation Individuals Without Symptoms or Other Reasons to Suspect COVID-19

		FDA EUA Authorized Assay		
		Positive	**Negative**	**Total**
Viracor SARS CoV-2 Assay	**Positive**	19	4	23
	Negative	0	102	102*
	Total	19	106	125

*102 of 488 subjects with negative Viracor SARS CoV-2 Assay results were consecutively collected and were tested by the comparator FDA EUA Authorized assay.

Because all negative samples were not tested by the comparator assay, there is verification bias. To address this, the clinical evaluation data was adjusted and then used to calculate performance estimates. Tables with the adjusted clinical data and performance estimates are provided below:

Table 14: Adjusted Results of Clinical Evaluation Individuals Without Symptoms or Other Reasons to Suspect COVID-19

		FDA EUA Authorized Assay		
		Positive	**Negative**	**Total**
Viracor SARS CoV-2 Assay	**Positive**	19	4	23
	Negative	0	488	488
	Total	19	492	513

Table 15: Performance Estimates of Clinical Evaluation Individuals Without Symptoms or Other Reasons to Suspect COVID-19

	Estimate	**95%CI**
Positive Percent Agreement (PPA)	100% (19/19)	(51.5%; 100%)*
Negative Percent Agreement (NPA)	99.2% (488/492)	(97.9%; 99.7%)*
Positive Predictive Value (PPV)	82.6% (19/23)	(62.9%; 93.0%)
Negative Predictive Value (NPV)	100% (102/102)	(96.4%; 100%)

*95%CI for PPA and NPA were adjusted to account for the fact that only 102 out of 488 samples negative by Viracor-SARS-CoV-2 Assay were tested by the comparator assay.

7) 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 C_T 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 C_T 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 C_T value for the pooled sample and X is the original C_T value for single sample testing.

This C_T 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 C_T 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.

Limitations:

- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Warnings:

- This product has not been FDA cleared or approved, but, has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories designated by Viracor Eurofins Clinical Diagnostics which are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet the requirements to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- The emergency use of this product 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 declaration is terminated or authorization is revoked sooner.