EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
Labcorp VirSeq SARS-Cov-2 NGS Test
LabCorp

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

The Labcorp VirSeq SARS-CoV-2 NGS Test will be performed at laboratories designated by Labcorp that are 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 Labcorp VirSeq SARS-Cov-2 NGS Test Standard Operating Procedure that was reviewed by the FDA under this EUA.

INTENDED USE

The Labcorp VirSeq SARS-CoV-2 NGS Test is a next generation sequencing (NGS) test on the PacBio Sequel II sequencing system intended for the identification and differentiation of SARS-CoV-2 Phylogenetic Assignment of Named Global Outbreak (PANGO) lineages, when clinically indicated, from SARS-CoV-2-positive samples identified using Labcorp's COVID-19 RT-PCR Test or Labcorp SARS-CoV-2 & Influenza A/B Assay. Testing is limited to laboratories designated by Labcorp that are certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests

The Labcorp VirSeq SARS-CoV-2 NGS Test is intended to be used in conjunction with patient history and other diagnostic information, when clinically indicated, i.e., in situations where results may aid in determining appropriate clinical management. Results of this test are intended to be interpreted by the ordering health care professional. The test is not intended for use as an aid in the primary diagnosis of infection with SARS-CoV-2 or to confirm the presence of SARS-CoV-2 infection, and it is not intended for identification of specific SARS-CoV-2 genomic mutations. Results should not be used as the sole basis for treatment or other patient management decisions.

The Labcorp VirSeq SARS-CoV-2 NGS Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the operation of the PacBio Sequel II sequencing system and next generation sequencing workflows as well as in vitro diagnostic procedures. The Labcorp VirSeq SARS-CoV-2 NGS Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

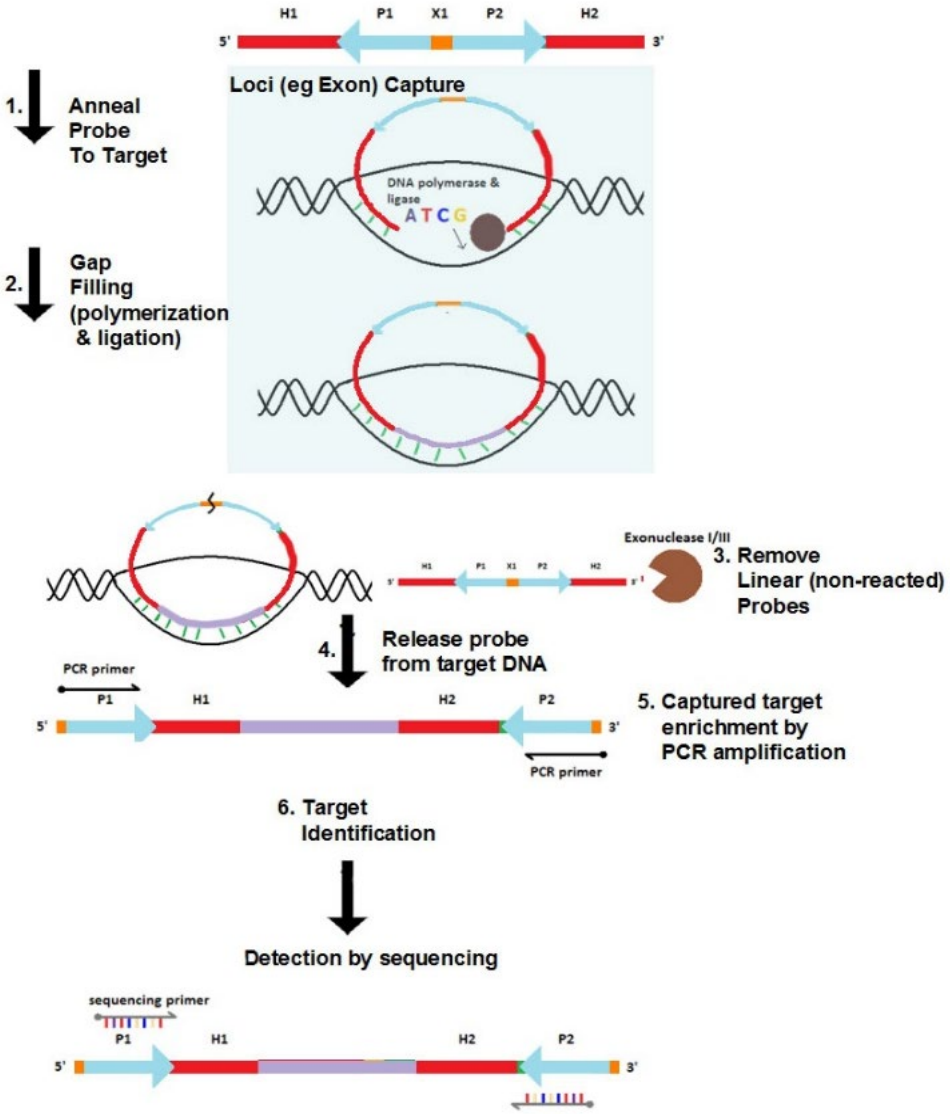
The Labcorp VirSeq SARS-CoV-2 NGS Test is a PacBio Sequel II-based whole genome sequencing assay used for the determination of PANGO lineage from extracted RNA of SARS-CoV-2 positive samples identified using Labcorp's COVID-19 RT-PCR Test or Labcorp SARS-CoV-2 & Influenza A/B Assay. The SARS-CoV-2 probe set used in this assay contains ~1000

tiled Molecular Loop Inversion Probes (MIPS) designed to amplify RNA that has been reverse transcribed to cDNA from 99.6% of the SARS-CoV-2 genome with most bases covered by 22 MIPs. The product synthesized in-between the MIPS is enriched and has sample specific molecular barcodes added via amplification followed by sequencing.

Residual total nucleic acid extract from SARS-CoV-2 positive RT-PCR diagnostic testing samples with N1 target Cycle Threshold (Ct) values < 31 are tested with the Labcorp VirSeq SARS-CoV-2 NGS Test. Residual nucleic acid extract can be stored at -20°C for up to 30 days through up to 2 freeze-thaw cycles. Residual total nucleic acid extract is transferred into a 96 well plate containing only positive RT-PCR diagnostic testing samples using Hamilton Microlab STAR. Samples are then aliquoted into a sequencing run plate of 94 samples with one water non-template control (NTC) and one positive control. Eight plates, or 752 specimens, are processed in one production batch.

A custom Molecular Loop SARS-CoV-2 Capture Kit is used to prepare samples to be sequenced on the PacBio Sequel II instrument. First, a reverse transcriptase enzyme provided by Thermo Fisher synthesizes cDNA from RNA. SARS-CoV-2 cDNA is then used as a target for hybridization of molecular loop probes (Figure 1, Step 1). Molecular loop probes are tiled across 99.6% of the 30 kb SARS-CoV-2 viral genome and consist of two binding sites 600 bp apart. After binding, the 600 bp region in-between the two probes are synthesized with DNA polymerase and ligated to form a closed molecule (Step 2). Non-binding or incomplete loops remain linear molecules and are removed with exonuclease digestion (Step 3). Circular molecules are then released from the template cDNA (Step 4) and enriched via amplification with a 3’ molecular loop specific M13 universal sequence and 5’ sample specific barcodes (Step 5). Samples are then pooled by equal volume and library prepped for PacBio sequencing. Library preparation entails DNA damage repair, ligation of sequencing adapters, non-ligated product removal by enzymatic digestion, and bead purification. Libraries are then sequenced on a PacBio Sequel II with 15 hour movies (Step 6).

Figure 1: Molecular Loop Sequencing Description.



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After sequencing, PacBio SMRT LINK software and custom molecular loop processing scripts are used to generate the FASTQ files for each sample. FASTQs are analyzed using a genome analysis pipeline implemented in the CLC genomics server version 9.1.1. This workflow starts with a sample-level fastq file, trims primers, and uses Minimap2 to align to the SARS-CoV-2 reference genome ("NC_045512v2") to generate a bam file of the alignment. A consensus sequence for each sample is generated using VCFcons (v8.5.0). When VCFcons calls a nucleotide sequence for genome construction it must have at least 4 circular consensus sequencing (CCS) reads covering that base pair and an alternate allele frequency compared to the reference of greater than 50%. If a nucleotide is covered by less than 4 reads it is reported as ambiguous (N) in the consensus sequence. The lineages for individual samples are then assigned

using the consensus sequence as input to the PANGOLIN (v3.1.20) analysis package. Lineage results are released for samples with at least 90% genome coverage and whose overall genomic coverage is greater than 10 CCS reads. The overall genomic coverage of a sample is defined as the mean of median read coverage across 29 (~1kb length) consecutive regions which spans the whole viral genome.

INSTRUMENTS USED WITH THE TEST

The Labcorp VirSeq SARS-CoV-2 NGS Test is to be used with the Pacific Bioscience Sequel II sequencing instrument, the Mantis liquid handler and the Labcorp VIRSEQ Analysis Pipeline for sequence analysis and lineage determination. The instruments and reagents required in order to perform the Labcorp VirSeq SARS-CoV-2 NGS Test are presented in Table 1.

Table 1. Reagent and Special Instrument Requirements

SPECIAL INSTRUMENTS		
Equipment	**Vendor**	**Catalog #**
ProFlex PCR System	Thermo	4483636
10, 20, 200 and 1000 µL pipettors	Rainin	BT-10, BT-2003100
Quantus	Promega	E6150
DynaMag™ Magnet	Life Technologies	12331D
Microcentrifuge	Thermo Fisher	540394
Vortex	Thermo Fisher	various
Sequel II	Pacific Bioscience	N/A
Mantis	Formulatrix	N/A
Liquidator	Rainin	17010335
REAGENTS		
Reagent	**Vendor**	**Catalog #**
AMPure PB Beads	Pacific Bioscience	100-262-900
SMRT CELL 8M TRAY EA	Pacific Bioscience	101-389-001
Sequel II Sequencing Plate 2.0	Pacific Bioscience	101-826-100
SMRTBELL ENZYME CLEAN UP KIT	Pacific Bioscience	101-746-400

SuperScript™ VILO™ Master Mix	Thermofisher	11755500
SARS-COV2 CAPTURE KIT CUST	Molecular Loop	ML5902-CP-LCPB1
Asymmetric Barcodes	IDT	14046396
200 proof ethanol	Pharmco-Aaper	111000200
Molecular Biology Grade Water	Krackler	45-W4502-1L
SMRTbell Express Template Prep kit 2.0	Pacific Biosciences	100-938-900
Sequel II Binding Kit 2.1	Pacific Biosciences	100-820-500
Sequel II DNA Internal Control Complex 1.0	Pacific Biosciences	101-717-600
Quantifluor dsDNA System	Promega	E2670
Sequel Smart Cell Oil	Pacific Biosciences	100-621-300
Twist Synthetic RNA Controls	Twist	102019,102862,103909,104043, 104044,104338,104529, 104530,104532,104533
SOFTWARE		
Software name	**Developer**	**Version**
Smrtlink	Pacific Bioscience	v9.0
Anaconda	Anaconda Inc.	v2019.03 (conda v4.11.0)
Python	Python Software Foundation	v3.7.6, v3.9
Lima	Pacific Bioscience	v1.11.0
Pbmm2	Pacific Bioscience	v1.2.1
BamTools	MIT	v2.4.1
CLC Genomics Grid Worker	Qiagen	9.1.1
CLS-plugin		1.3.5
Miniconda2	Anaconda Inc.	conda v4.8.3
Pangolin	COVID-19 Genomics UK Consortium	v3.1.20
Nextclade	NextStrain project	v1.3.0
Seqtk	MIT	v1.2
Minimap2	Dana-Farber Cancer Institute	v2.17-r941
Samtools	MIT	v1.3.1
VCFCons		v8.5.0
Hamilton Venus	Hamilton Company	v4.0

Designated laboratories will receive an FDA accepted instrument qualification protocol included as part of the Labcorp VirSeq SARS-CoV-2 NGS Test Standard Operating Procedure (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.

CONTROLS TO BE USED WITH THE TEST

1. **External Positive Control:** An external positive control will be added to each plate of 94 patient samples. The control consists of one of ten Twist synthetic SARS-CoV-2 RNA controls with predetermined PANGO Lineage designation. The lineage identification result of the external positive control will be compared to its known PANGO Lineage designation.

2. **External Negative Control:** An external non-template control (NTC) is needed to ensure master mix contamination events are not present on the given amplification plate. The control consists of molecular grade water added to the A1 position of every 96 well plate before sample addition. The NTC is then transferred along with positive samples to the sequencing run plate and taken through sequencing and quality control (QC) analysis.

3. **Other Controls:** Lineage identification results are only released for samples with at least 90% genome coverage and whose overall genomic coverage is greater than 10 CCS reads.

INTERPRETATON OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

1) Labcorp VirSeq SARS-CoV-2 NGS Test Controls – Positive:

After sequencing, the lineage designation of a given plate's positive control will be compared to the known lineage designation of the positive control. A positive control sample will be considered a failure if its assay determined lineage differs from its known lineage. Samples on plates with failed positive external control are re-analyzed one additional time with the assay using material from the original total nucleic acid extract. If a sample's residual total nucleic acid is depleted, it is reported as a failed sample.

2) Labcorp VirSeq SARS-CoV-2 NGS Test Controls – NTC:

After sequencing, the overall genomic coverage is calculated for the NTC samples. A NTC sample will be considered a failure if it has overall genomic coverage greater than 10 CCS reads. Samples on plates with failed negative external control are re-analyzed one additional time with the assay using the original total nucleic acid extract. If a sample's residual total nucleic acid is depleted, it is reported as a failed sample.

Table 2. Expected Results for the Labcorp VirSeq SARS-CoV-2 NGS Test External Controls

External Control Type	Passing Criteria	Action For Control Failure
Positive External Control	Assay determined lineage designation is concordant with known lineage designation.	Plates with failed positive external controls are re-analyzed one additional time with the assay using the original total nucleic acid extract. If a sample's residual total nucleic acid is depleted, it is reported as a failed sample.
Negative External Control	Overall genomic coverage is greater than 10 CCS reads.	Plates with failed negative external controls are re-analyzed one additional time with the assay using the original total nucleic acid extract. If a sample's residual total nucleic acid is depleted, it is reported as a failed sample.

3) Examination and Interpretation of Patient Sample Results:
Assessment of Labcorp VirSeq SARS-CoV-2 NGS Test results are performed after external control analysis and removal of any samples on a plate with a failed external control. The overall genomic coverage and percent genome coverage is calculated for each patient sample. Lineage results are released for samples with at least 90% genome coverage and overall genomic coverage greater than 10 CCS reads.

The interpretation and reporting of clinical specimens are summarized in Table 3.

Table 3. Result Interpretation for Patient Samples

SAMPLE LEVEL QC RESULTS	EXTERNAL CONTROLS RESULTS	PANGOLEARN OUTPUT	ACTION
Pass	**Pass**	**Lineage call**	**Report results to sender and appropriate public health authorities.**

		None	**Sample is repeated**. If no residual sample, it will be reported as failed*.**
	Fail	**Not Applicable**	**Plate is repeated. If no residual sample, it will be reported as failed*.**
		Not Applicable	**Plate is repeated. If no residual sample, it will be reported as failed*.**
Fail	**Pass**	**Not Applicable**	**Sample is reported as failed*.**
	Fail	**Not Applicable**	**Plate is repeated. If no residual sample, it will be reported as failed*.**
*** Failed samples will be reported as: "No lineage was able to be determined. SARS-CoV-2 virus detected, but genome sequencing was unsuccessful. No lineage information can be reported." **Individual failing samples will only be repeated once and after a second failure will be reported as described above for a failed sample.**			

PERFORMANCE EVALUATION

1. Device Tolerance:

Residual nucleic acid extracts from 8,815 respiratory specimens which tested positive for SARS-CoV-2 by the EUA200011 authorized Labcorp COVID-19 Test across 3 sites were sequenced with the Labcorp VirSeq SARS-CoV-2 NGS Test. The number of samples that produced genomic sequences that passed both of the following quality control criteria were determined for different N1 CT value categories.

- Greater than 90% genome coverage when compared to the SARS-CoV-2 reference genome ("NC_045512v2")
- Overall genomic coverage is greater than 10 CCS reads.

Table 4. Tolerance Study Result

Cycle Threshold (CT)	Number of Specimen Tested	Number of Specimen Passing Both QC Criteria	Percent (%) of Specimen Passing Both QC Criteria (95% CI)
<16	26	23	88.5% (69.8%--97.6%)
16-17	39	36	92.3% (79.1%--98.4%)
17-18	123	111	90.2% (83.6%--94.9%)
18-19	318	291	91.5% (87.9%--94.3%)
19-20	415	383	92.3% (89.3%--94.7%)
20-21	661	611	92.4% (90.1%--94.3%)
21-22	814	757	93.0% (91.0%--94.7%)
22-23	834	767	92.0% (89.9%--93.7%)
23-24	913	831	91.0% (89.0%--92.8%)
24-25	876	759	86.6% (84.2%--88.8%)
25-26	800	625	78.1% (75.1%--80.9%)
26-27	685	437	63.8% (60.1%--67.4%)
27-28	682	330	48.4% (44.6%--52.2%)
28-29	582	160	27.5% (23.9%--31.3%)
29-30	518	87	16.8% (13.7%--20.3%)
30-31	529	40	7.6% (5.5%--10.2%)

2. Precision (Repeatability): Intra-Assay

Intra-assay repeatability was assessed by testing 11 nucleic acid samples in triplicate. The intra-assay repeatability study assessed the ability of the assay to accurately detect lineages on the replicates of the same samples during one assay run. The N1 CT value of the samples tested ranges from 19.2 to 22.96.

Lineage designation of all 11 samples were concordant across the three replicates with all replicates meeting QC criteria.

3. Precision (Reproducibility): Inter-Assay

Inter-assay reproducibility was assessed by testing the same nucleic acid samples assessed in the repeatability study in triplicate over three assay runs. One of the 11 samples tested in the repeatability study was unintentionally excluded from the final run. Sequencing runs were performed by 6 different technologists using 3 different lots of SMRT cells and 2 lots of sequencing reagents. The third run was 3 weeks from the first run and was multiplexed at ~4X lower sample concentration than the previous 2 runs.

Table 5. Reproducibility Study Description

Run ID	Time	Personnel	Sequencing Reagent Lots
Run 1	7/7/2021	Technologist 1, 2, 3 and 4	1
Run 2	7/14/2021	Technologist 2, 4 and 5	2
Run 3	7/28/2021	Technologist 5 and 6	2

Lineage designations for 9 out of 10 samples were concordant across all 3 runs. The discordant sample did not pass the overall genome coverage QC criteria in Run 2 and Run 3.

4. Sample Stability (Freeze-Thaw)

Stability of patient samples under recommended storage conditions was assessed in the sample stability study. After Nucleic Acid Amplification (NAA) diagnostic testing with Labcorp COVID-19 RT-PCR TEST, extracted nucleic acid was shipped on dry ice to the testing laboratory and stored at -20°C before sequencing.

Twelve samples comprising of Alpha, Beta and Delta Variants of Concerns (VOCs) were sequenced initially. These samples were re-sequenced after 5 weeks of -20°C storage that entailed 3 freeze-thaw cycles. 11 out of the 12 samples produced concordant results between initial, and repeat, testing. The only discordant result was determined to be due to mechanical errors and not related to sample stability. The result of the study supports the stability of the Labcorp VirSeq SARS-CoV-2 NGS Test for up to 2 freeze-thaw cycles when samples are stored at 20°C for up to 30 days.

5. Concordance

Lineage designation results produced by the Labcorp VirSeq SARS-CoV-2 NGS Test (PacBio Molecular Loop Sequencing) were directly compared to lineage designation results generated by Illumina COVIDSeq (RUO-surveillance protocolwith v3 primer pool) as well as PacBio Amplicon Sequencing. The analysis included the following samples.

- Illumina COVIDSeq (RUO) Samples
  - 93 Negative (NAA) samples
  - 72 SARS-CoV-2 samples sequenced in Winter 2020
  - 29 samples previously sequenced at Center for Molecular Biology and Pathology (CMBP)
  - 50 samples previously sequenced at DNA Identification
- PacBio Amplicon Sequenced Samples

- 122 SARS-CoV-2 samples that were amplicon sequenced at 90% coverage.

Illumina COVIDSeq (RUO) Comparison:

93 samples previously determined to be negative by Labcorp COVID-19 RT-PCR diagnostic tests were sequenced in duplicate using the Labcorp VirSeq SARS-CoV-2 NGS Test and Illumina COVIDSeq (RUO). Out of the 93 samples tested, one sample resulted in reportable SARS-CoV-2 genomes using Illumina COVIDSeq (RUO) alone, and another sample resulted in reportable SARS-CoV-2 genomes for both Illumina COVIDSeq (RUO) and the Labcorp VirSeq SARS-Cov-2 NGS Test. Further investigation revealed both samples were positive for SARS-CoV-2 by the Labcorp COVID-19 RT-PCR Test and mistakenly included in the validation. The final concordance between Illumina COVIDSeq (RUO) and Labcorp VirSeq SARS-CoV-2 NGS Test for the 91 true negative samples was 100%.

From the 72 samples sequenced in winter 2020 using Illumina COVIDSeq (RUO), 51 samples produced reportable results when using the Labcorp VirSeq SARS-CoV-2 NGS Test. Out of the 51 reportable results, all lineage designation results were 100% concordant with the Illumina COVIDSeq (RUO) output.

Table 6. Comparison of PANGO Lineage Designation between Illumina COVIDSeq (RUO) and Labcorp VirSeq SARS-CoV-2 NGS Test for Winter 2020 circulating lineages.

Samples Tested	Reportable Result	Concordant Reportable Result	Reportable Result Concordant %
77	51	51	100%

A total of 7 of the 79 samples were lost in transit or did not produce a valid result with Illumina **COVIDSeq (RUO)**. 72 of the 79 samples collected at CMBP and DNA Identification were successfully sequenced with both Illumina **COVIDSeq (RUO)** and the Labcorp VirSeq SARS-CoV-2 NGS Test. All 66 out of these 72 samples that passed sequences QC criteria for the Labcorp VirSeq SARS-CoV-2 NGS Test have concordant lineage designation result with Illumina **COVIDSeq (RUO)**.

Table 7. Comparison of PANGO lineage designation between Illumina COVIDSeq (RUO) and Labcorp VirSeq SARS-CoV-2 NGS Test for Summer 2021 circulating lineages.

Samples Tested	Reportable Result	Concordant Reportable Result	Reportable Result Concordant %
72	66	66	100%

PacBio Amplicon-Based Sequencing Comparison:

122 samples originally sequenced using a PacBio amplicon-based approach were reprocessed using the Labcorp VirSeq SARS-CoV-2 NGS Test in duplicate. Each of the 122 samples with >90% coverage via amplicon sequencing, were tested in duplicate with the Labcorp VirSeq SARS-CoV-2 NGS Test; therefore, a total of 244 results were produced. Of the 244 Labcorp VirSeq SARS-CoV-2 NGS Test lineage determination results, 234 results produced genomes that passed both QC metrics. Among these 234 results, 225 were concordant with the original PacBio amplicon assay lineage designation. When compared to the PacBio amplicon sequencing lineage designation result, 96% samples produced concordant lineage identification results.

Table 8. Comparison of PANGO lineage designation between PacBio Amplicon Sequencing and Labcorp VirSeq SARS-CoV-2 NGS Test

Number of Results	Reportable Result	Concordant Reportable Result	Reportable Result Concordant %
244	234	225	96%

6. Reference Sample Testing

Heat-inactivated SARS-CoV-2 samples from B.1.1.7 (VR-3326HK), Hong Kong/VM20001061 and Italy-INMI1 lineages, characterized by ATCC, were used in this evaluation. Sequencing error associated with the Labcorp VirSeq SARS-CoV-2 NGS Test was evaluated by comparing all mutations identified in the consensus sequences produced by the Labcorp VirSeq SARS-CoV-2 NGS Test analysis pipeline to the published ATCC reference sequences. The results are shown in the following table:

Table 9. Number of nucleotide mismatches between ATCC reference sequences and Labcorp VirSeq SARS-CoV-2 NGS Test generated sequences

ATCC strain*	# Mutation Observed in Reference Sequence (Reference Mutation)	# Reference Mutations Identified in the Virseq Consensus Sequence	#Additional Mutations Identified in the Virseq Consensus Sequence	Nucleotide Positions Sequenced	Percent (%)Mismatch Between VirSeq Consensus Sequence and Reference Genome
b1117	35	33	2	29683	0.013%
HK	15	12	0	29780	0.010%
ITLY	5	3	1	29979	0.010%
Total	**55**	**48**	**3**	**89442**	0.012%

*b1117, B.1.1.7 (VR-3326HK); HK, Hong Kong/ VM20001061; ITLY, Italy-INMI1

Overall, an average of 0.012 % sequence differences were observed between the reference sequence and the consensus sequence produced by the Labcorp VirSeq SARS-CoV-2 NGS Test.

7. Simulation Study

A simulation study was conducted to assess the performance of the Labcorp VirSeq SARS-CoV-2 NGS Test to identify samples with PANGO lineages not tested in the concordance and analytical studies.

A sequencing error model that simulates how sequencing error and ambiguous nucleotides are randomly introduced by the Labcorp VirSeq SARS-CoV-2 NGS Test into the sequenced genome, was estimated based on the sequencing results of 760 clinical samples. The model estimated that the Labcorp VirSeq SARS-Cov-2 NGS Test, on average, results in 1 sequencing error per 33 SARS-CoV-2 genomes sequenced and 3,369 ambiguous nucleotides per SARS-CoV-2 genome sequenced.

Variant of Concern/Variant of Interest Simulation

A total of 23,400 reference sequences were downloaded from GISAID (https://www.gisaid.org/) on February 25th with known Pango-lineage designation representing 234 lineages (100 reference sequence per lineage). The sequencing error model was used to introduce sequencing errors into these 23,400 reference sequences to simulate the hypothetical sequence output of the assay for these genomes. Each of these 23,400 sequences were used to produce multiple simulated sequences. The PANGO Lineages of these simulated sequences are identified with the lineage identification software (PANGOLIN v3.1.20) used in the Labcorp VirSeq SARS-CoV-2 NGS Test. The lineage identification results of each simulated sequence were compared to the known PANGO Lineage Designation of the reference sequence used to produce the simulated sequence. The concordance results of simulated sequences with genome coverage of 90%, 95% and 99% are shown in the following table:

Table 10. *In silico* performance of Labcorp VirSeq SARS-CoV-2 NGS Test for 234 VOC/VOI lineages.

Genome Coverage	#Sequences Tested	# Concordance Sequence	% Concordance Sequence (95% CI)
90%	17538	16762	95.58% (95.26% - 95.86%)
95%	16274	15739	96.71% (96.42% - 96.97%)
99%	11169	10929	97.85% (97.56% - 98.10%)

100% (95% CI 99.55% - 100.00%) of the 770 simulated Omicron sequences were accurately identified on a sub-lineage level (BA.1, BA.2 and BA.3).

Time Period Simulation

A total of 10,000 high quality SARS-CoV-2 sequences were randomly sampled from GISAID for each of the 14 months before March 2022. A total of 140,000 reference sequences were downloaded from GISAID (https://www.gisaid.org/) on February 25th, 2022 with known PANGO lineage designation. The sequencing error model was used to introduce sequencing errors into these 140,000 reference sequences to simulate hypothetical sequence output of the assay for these genomes. Each of the 140,000 sequences were used to produce multiple simulated sequences. The PANGO lineages of these simulated sequences are identified with the lineage identification software (PANGOLIN v3.1.20) used in the Labcorp VirSeq SARS-CoV-2 NGS Test. The simulated lineage identification results are compared to the known PANGO lineage designation of the reference sequence used to produce the simulated sequence. The concordance results of simulated reads with genome coverage of 90%, 95% and 99% are shown in the following table:

Table 11. *In silico* performance of Labcorp VirSeq SARS-CoV-2 NGS Test for 140,000 sequences submitted to GISAID.

Genome Coverage	#Sequences Tested	# Concordance Sequence	% Concordance Sequence (95% CI)
90%	105930	102270	96.54% (96.43% - 96.65%)
95%	96333	94222	97.81% (97.71% - 97.89%)
99%	45929	45182	98.37% (98.25% - 98.48%)

All of the 43,328 simulated Omicron sequences were accurately identified as Omicron sequences. The sub-lineage concordance rates for the simulated Omicron sequences are shown in the following table:

Table 12. *In silico* performance of Labcorp VirSeq SARS-CoV-2 NGS Test for 43,328 simulated Omicron sequences.

Sub Lineage	Genome Coverage	# Sequences Tested	# Concordance Sequence	Percent (%) Concordance Sequence (95% CI)
BA.1	90%	7466	7465	99.98% (99.92% - 99.99%)
	95%	6791	6791	100.00 % (99.94% - 100.00%)
	99%	3421	3421	100.00 % (99.88% - 99.99%)
BA.1.1	90%	7167	7029	98.07% (97.73% - 98.36%)
	95%	6520	6479	99.37% (99.14% - 99.53%)
	99%	3523	3523	100.00 % (99.89% - 100.00%)
BA.2	90%	3429	3429	100.00 % (99.88% - 100.00%)
	95%	3161	3161	100.00 % (99.87% - 100.00%)
	99%	1833	1833	100.00 % (99.79% - 100.00%)
BA.3	90%	7	7	100.00 % (64.56% - 100.00%)
	95%	5	5	100.00 % (56.55% - 100.00%)
	99%	5	5	100.00 % (56.55% - 100.00%)

WARNINGS AND PRECAUTIONS:

- For *in vitro* diagnostic use.
- For use under FDA Emergency Use Authorization only.
- For prescription use only
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA for use by authorized laboratories.
- This product has been authorized only for the identification of SARS-CoV-2 PANGO Lineages, not for any other viruses or pathogens.
- This product has been authorized only for the identification of PANGO lineage of positive sampled identified using Labcorp's EUA authorized COVID-19 RT-PCR tests (COVID-19 RT-PCR TEST and Labcorp SARS-CoV-2 & Influenza A/B Assay)
- 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 diagnostic tests 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. § 360bbb3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

- All specimens should be handled in accordance with Biosafety Level 2 practices as described in the CDC/NIH Publication, Biosafety in Microbiological and Biomedical Laboratories or other equivalent guidelines.

- Always wear gloves when performing this procedure and treat all specimens and used devices as potentially infectious.

LIMITATIONS

1. Use of the Labcorp VirSeq SARS-CoV-2 NGS Test is limited to laboratory personnel specifically instructed and trained in the operation of the PacBio instrumentation and Next Generation Sequencing workflows as well as in vitro diagnostic procedures.
2. Performance has only been established with samples positive by Labcorp's EUA authorized COVID-19 RT-PCR diagnostic tests (COVID-19 RT-PCR Test and Labcorp SARS-CoV-2 & Influenza A/B Assay). Positive specimens from other assays have not been evaluated and should not be used with this test.
3. The performance of this test was established based on the evaluation of a limited number of clinical specimens. The clinical performance has not been established in 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.
4. This test does not identify specific SARS-CoV-2 mutations.
5. This test was not specifically evaluated for use in conjunction with any specific mAb or antiviral product.
6. Failed results do not preclude presence of specific SARS-CoV-2 PANGO Lineage and should not be used as the sole basis for patient management decisions.
7. PANGO Lineage results from this test should not be used as the sole basis for patient management decisions.
8. Failed result can occur for samples with greater than 90% genome coverage and whose mean of median read coverage across the whole genome is >10 CCS reads.
9. Use of this assay is limited to residual nucleic acid extracts of positive samples identified using Labcorp's COVID-19 RT-PCR Test and Labcorp SARS-CoV-2 & Influenza A/B Assay.
10. Lineage determination result is specific to the version of PANGO software used at time of testing and may very when PANGO software is updated.