EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
UCLA SwabSeq COVID-19 Diagnostic Platform
University of California, Los Angeles (UCLA)

For in vitro diagnostic use
Rx only
For use under Emergency Use Authorization (EUA) Only

(The UCLA SwabSeq COVID-19 Diagnostic Platform will be used only in the UCLA Clinical Microbiology Laboratory located at Brentwood Annex, 11633 San Vicente Blvd Los Angeles, CA 90049 and UCLA SwabSeq COVID-19 Testing Laboratory located at 650 Charles E Young Dr South, CHS Building, South Tower, 87-300 Los Angeles CA, 90095, which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests, as described in the laboratory procedures that were reviewed by the FDA under this EUA).

INTENDED USE

The UCLA SwabSeq COVID-19 Diagnostic Platform is a Next-Generation Sequencing (NGS) in vitro diagnostic test intended for the qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens (nasopharyngeal swabs, oropharyngeal (throat) swab, mid-turbinate nasal swabs and anterior nasal swabs) from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to UCLA Clinical Microbiology Laboratory located at Brentwood Annex, 11633 San Vicente Blvd Los Angeles, CA 90049 and UCLA SwabSeq COVID-19 Testing Laboratory located at 650 Charles E Young Dr South, CHS Building, South Tower, 87-300 Los Angeles CA, 90095, which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests.

Results are for the detection of SARS-CoV-2 RNA. The 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 UCLA SwabSeq COVID-19 Diagnostic Platform is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the use of MiSeq Sequencing System, NextSeq Sequencing System and Next-Generation Sequencing

workflows and in vitro diagnostic procedures. The UCLA SwabSeq COVID-19 Diagnostic Platform is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The assay is a next generation sequencing (NGS) test. The SARS-CoV-2 primer and amplicon set is designed to detect RNA from the SARS-CoV-2 in upper respiratory specimens from patients suspected of COVID-19 by their healthcare provider. The assay simultaneously detects three targets: the S2 Spike gene (S2 gene), a synthetic internal control S2 gene which differs from the target S2 gene by 6-nucleotides and a human internal control RPP30 gene used as an extraction and process control.

Upper respiratory specimens (nasopharyngeal swabs, oropharyngeal (throat) swab, mid-turbinate nasal swabs and anterior nasal swabs) should be collected, transported and stored according to CDC recommendations. Specimens are stored and transported at 4°C up to 72 hours until RNA extraction is performed.

RNA extraction for all specimen types is performed using the Thermo Fisher Kingfisher Instrument and MagMAX Viral/Pathogen Nucleic Acid Isolation Kit. The input sample volume is 400 µL for all transport media types and the elution volume is 100 µL.

DNA complementary to the extracted RNA are generated by reverse transcription with two sets of indexed primers targeting the S2 gene of SARS-CoV-2 and human control gene, RPP30. Reverse transcription and targeted PCR amplification is performed using the TaqPath 1-Step RT-qPCR Master Mix with the Veriti 384-Well Thermal Cycler. BenchSmart96 20 µL BST-96-20 with 20 µL head and BenchSmart96 200 µL BST-96-200 with 200 µL head were used for plating.

After PCR amplification, samples from each well in each plate containing uniquely barcoded amplicons are pooled using Integra Viaflow and purified using magnetic bead purification with AmpureXP beads.

The pooled library product is quantified using a fluorescent dye with concentrations determined by comparison to a DNA standard curve. Initial 10-fold dilution concentration is determined using High Sensitivity DNA Qubit and the final 7nM dilution concentration is measured with Agilent Technologies d1000 High Sensitivity Screentape.

Pooled libraries are clustered onto a flow cell, and then sequenced using sequencing by synthesis (SBS) chemistry on the Illumina MiSeq sequencer using the Illumina MiSeq Reagent Kit V3 flow- cell workflow or the Illumina NextSeq sequencer using the Illumina NextSeq500/550 High Output Kit v2 (150 cycles). The sequencing instruments are run using dual-indexed single-read sequencing for 26 cycles (26 bp reads). The samples are also pooled with indexed PhiX spiked at 35% to provide sufficient sequence diversity, which assists with template registration and improves run and base quality.

Primary analysis is performed by the Real Time Analysis (RTA) software (Illumina Inc., 'For Research Use Only') and consists of base calling of each cluster at each cycle. In

addition to base calling, RTA assigns an analytical quality score (Q-score) to each base call. Calculations of Q-scores are based on the ratio of the signal intensity of the highest base in a given cluster during a given cycle to the signal intensity of the three other bases. The quality score Q is calculated as - 10 log10 P, where P is the probability that base call is incorrect. A minimum of 80% of base calls must meet the Q30 threshold to proceed to data analysis. If fewer than 80% of base calls meet the Q30 threshold the entire run is discarded. The resulting output is a BCL file.

Illumina BCL files are downloaded and converted into FASTQ sequencing files using Illumina's bcl2fastq software. Each amplicon sequence consists of three segments: one 26 base pair read (read1) that identifies the amplicon (S2, S2- spike, or RPP30) and two 10 base pair index reads (index1 and index2) that together uniquely identify the sample. Sequences are assigned to samples using the two index reads and the amplicon is identified by allowing for at most 1 nucleotide mismatch. The total number of sequence matching to each amplicon in each sample is obtained. Quality control and detection of SARS-CoV- 2 in a sample are based on the count of sequences observed for each amplicon within each sample. The decision tree for sample QC and SARS-CoV-2 detection is shown in figure 1.

Figure 1. Data Analysis Decision Tree

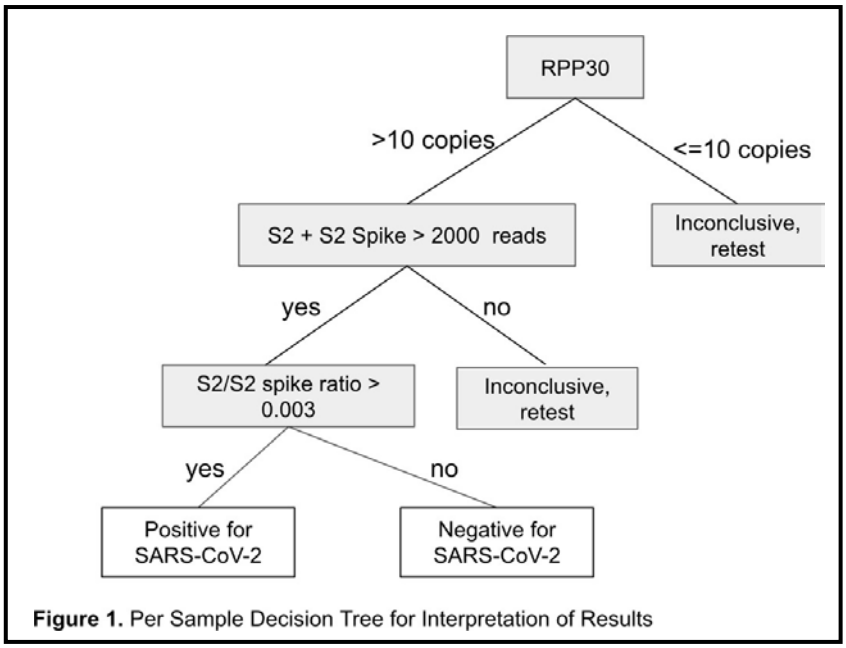

Figure 1. Per Sample Decision Tree for Interpretation of Results

INSTRUMENTS USED WITH THE TEST

Instruments

The UCLA SwabSeq COVID-19 Diagnostic Platform is used with the Illumina MiSeq or the Illumina NextSeq 500/550 sequencing systems. All results are interpreted using the UCLA Amplicon Quantification Software V1.0.

The UCLA SwabSeq COVID-19 Diagnostic Platform is used with the Thermo Fisher Veriti 384-Well Thermal Cycler, Thermo Fisher KingFisher Flex extraction system and the following semi-automated pipetting systems:

- BenchSmart96 20ul BST-96-20
- BenchSmart96 200ul BST-96-200
- Integra Viaflow

REAGENTS AND MATERIALS

Table 1. Reagents and materials required for use with the UCLA SwabSeq COVID-19 Diagnostic Platform

Material ID	Vendor	Catalog #
TaqPath 1-Step RT-qPCR Master Mix	ThermoFisher	A15300
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	ThermoFisher	A48310
MiSeq Reagent Kit v3 (150-cycle)	Illumina	MS-102-3001
NextSeq 500/550 High Output Kit v2 150 cycles	Illumina	20024907
Qubit RNA HS Assay Kit	ThermoFisher	Q32855
Qubit RNA BR Assay Kit	ThermoFisher	Q10211
PhiX Control v3	Illumina	FC-110-3001
Illumina Free Adapter Blocking Reagent (48 reactions)	Illumina	20024145
Synthetic S2 Spike	Custom Control	N/A
Custom Sequencing and PCR Primers	IDT	N/A

Material ID	Vendor	Catalog #
Heat-inactivated SARS-CoV-2	ATCC	VR-1986HK

CONTROLS TO BE USED WITH THE UCLA SWABSEQ COVID-19 DIAGNOSTIC PLATFORM

- **Internal Extraction Positive Control (RPP30) –** The RPP30 primers developed by the U.S. Center for Disease Control (CDC) were used as an extraction positive control. This is included in every well prior to RT-PCR.

- **Synthetic S2 Spike-In Control (S2 Spike) –** The S2 Spike is a control for reverse transcription and amplification of the virus sequence in each RT-PCR well.

  The S2 Spike is designed to have the same characteristics of the S2 amplicon, but has an inverted set of nucleotides that allows this amplicon to be differentiated from the true viral S2 amplicon by sequencing. The S2 primer pair will amplify both S2 amplicon and the S2 spike in, at equivalent efficiency.

  This is spiked-in every well prior to RT-PCR and is not subjected to the extraction process.

- **External positive control –** The external positive control consists of remnant clinical specimens that previously tested negative for COVID-19 spiked with Heat-inactivated SARS-CoV-2 (ATCC VR- 1986HK), where viral copy is quantified by ATCC using ddPCR. The concentration of the external positive control is 1,250 GCE/ml. The positive control is used to monitor for failures of extraction, rRT-PCR reagents and sequencing conditions. One positive control will be included with each 96 well plate. Each 384-well plate will be filled using a 96-pipet liquid handler which allows each quadrant containing 96-samples to be added separately. For each 384-well plate, there are 4 positive controls.

- **Negative Control -** The negative control is made up of elution buffer from the RNA extraction protocol. During RNA extraction, one well is left empty in the RNA extraction plate. After RNA extraction this empty well is filled with the elution buffer from the RNA purification process. One negative control will be included with each 96 well plate. Each 384-well plate will be filled using a 96-pipet liquid handler which allows each quadrant containing 96-samples to be added separately. For each 384-well plate, there are 4 negative controls.

INTERPRETATION OF RESULTS

1) The UCLA SwabSeq COVID-19 Diagnostic Platform Controls Interpretation:

All control wells must pass for the patient results to be considered valid and acceptable. If a failed negative or positive control is observed, the 96-well plate that the control was associated with would be rerun with a new negative and positive control. Refer to Table 2 below for a summary of expected control results.

Table 2 . Results for External Controls that Must Be Observed to Obtain Valid Results

	Total S2 + S2 Spike Read Count	S2/S2_Spike Ratio
Negative Control	>2000	<0.003
External Positive Control	>2000	>0.003

2) Examination and Interpretation of Patient Specimen Results:

The assay interpretation and reporting of results is shown in Table 3 below. Assessment of patient specimen test results should be performed after the external positive and negative controls have been examined and determined to be valid and acceptable.

Table 3. Result Interpretation for Patient Samples

Well-controls			**Results**			
Total S2 + S2 Spike	**RPP30 read count**		**S2/S2 spike ratio**	**Result**	**Interpretation**	**Action**
>2000 reads	>10		> 0.003	SARS- CoV-2 Detected	Positive for SARS-CoV-2 for the Sample ID.	Report results to physician, patient, and appropriate public health authorities.
>2000 reads	>10		< 0.003	SARS- CoV-2 Not Detected	Negative for SARS-CoV-2 for the Sample ID.	Report results to physician, patient, and appropriate public health authorities.
<2000 reads	>10		-	Inconclusive	Invalid for the Sample ID.	Quality control for the Sample ID is FAIL. Repeat sample or Recollect sample
>2000 reads	< 10		-	Inconclusive	Invalid for the Sample ID.	Quality control for the Sample ID is FAIL. Repeat sample or Recollect sample
<2000 reads	< 10		-	Inconclusive	Invalid for the Sample ID.	Quality control for the Sample ID is FAIL. Repeat sample or Recollect sample.

PERFORMANCE EVALUATION

I. Analytical Sensitivity

The LoD study was performed by spiking in heat inactivated SARS-CoV-2 virus (ATCC, VR-1986HK) in negative nasopharyngeal swab specimens in VTM using a two-fold dilution series. Six or seven extraction replicates were tested per concentration. The preliminary LoD was defined as the lowest concentration with 7 of 7 replicates that test positive. The LoD determination was carried out independently for the NextSeq instrumentation and MiSeq Instrumentation. For both sequencers, the preliminary LoD was determined to be 250 GCE/ml.

Table 4. Preliminary LoD Determination Results

Viral Concentration (GCE/mL)	Detection Rate (%) NextSeq	Detection Rate (%) MiSeq
8000	6/6 (100%)	7/7 (100%)
2000	6/6 (100%)	7/7 (100%)
1000	7/7 (100%)	7/7 (100%)
500	7/7 (100%)	7/7 (100%)
250	7/7 (100%)	7/7 (100%)
125	6/7 (85%)	6/7 (85%)

LoD confirmation study for both the NextSeq instrumentation and the MiSeq Instrumentation was performed by spiking in heat inactivated SARS-CoV-2 virus (ATCC, VR-1986HK) in negative nasopharyngeal swab specimens in VTM at 250 GCE/ml. Twenty (20) extraction replicates were tested for each concentration. The LoD was determined to be 250 GCE/mL for both NextSeq and MiSeq.

Table 5. Confirmatory LoD Study Results for Clinical Samples using Different Sequencing Instruments

Viral Concentration (GCE/ml)	Detection Rate (%) NextSeq	Detection Rate (%) MiSeq
250	19/20 (95%)	20/20 (100%)

II. Analytical specificity

Inclusivity

In silico analysis of the S2 primer sets and S2 amplicon sequence were performed to evaluate the inclusivity of the UCLA SwabSeq COVID-19 Diagnostic Platform. For the primer analysis, SARS- CoV-2 sequences available on GISAID (27,491 sequences) were evaluated. A BLASTn (NCBI) analysis was performed to quantify the level of primer homology across these sequences by querying each of the two SARS-CoV-2 primer sequences against the downloaded SARS-CoV-2 sequences. The analysis showed that 99.4% of all sequenced strains have 100% homology to both primer sequences and 176 strains (or 0.64%) of the 27,491 complete genomes have less than 100% homology. to a primer. For the forward S2 primers, 27,473 (99.934%) GISAID genomes have 100% homology. For the reverse S2 primers, 27,353 (99.498%) GISAID genomes have 100% homology. The S2 amplicon is 26 base pairs and 27,471 (99.993%) GISAID genomes have 100% homology.

Cross-reactivity

In silico analysis was performed to evaluate the cross-reactivity of the UCLA SwabSeq COVID-19 Diagnostic Platform with representative common respiratory pathogens. For the primer analysis, 38 non-SARS-CoV-2 consensus genomes were downloaded from NCBI as the negative sample cohort. A BLASTn (NCBI) analysis was then performed to quantify the number of primer pairs with more than 80% homology with each of the genomes in the cohort. None of the pathogens exhibit greater than 80% homology with any of the primers.

III. Clinical evaluation

A clinical study was performed to evaluate the performance of the UCLA SwabSeq COVID-19 Diagnostic Platform using 31 remnant positive upper respiratory clinical samples (nasopharyngeal swabs), and 35 negative upper respiratory clinical samples (nasopharyngeal swabs) with the MiSeq sequencer.

Positive patient clinical samples (nasopharyngeal swabs) in either VTM, Liquid Amies normal or normal saline were previously confirmed positive by one of three FDA-authorized comparator assays at the UCLA Clinical Microbiology Laboratory (Los Angeles, CA). All assays were verified in the UCLA Clinical Microbiology Laboratory and are run in accordance with manufacturer’s specifications. Results are summarized in Table 6 The positive agreement (31/31) was 100.0% and negative agreement (35/35) was 100.0%.

Table 6. Evaluation with Clinical Specimens Using MiSeq Sequencer

		FDA-Authorized Comparator Assay		
		Positive	Negative	Total
UCLA SwabSeq COVID-19 Diagnostic Platform - MiSeq	Positive	31	0	31
	Negative	0	35	35
	Total	31	35	66
Positive Agreement		100.0% (31/31); 88.8% - 100.0%[1]		
Negative Agreement		100.0% (35/35); 90.0% - 100.0%		

[1]Two-sided 95% score confidence intervals

A clinical study was performed to evaluate the performance of the UCLA SwabSeq COVID-19 Diagnostic Platform using fifty one remnant positive upper respiratory clinical samples (nasopharyngeal swabs), and fifty three negative upper respiratory clinical samples (nasopharyngeal swabs) with the NextSeq sequencer.

Positive patient clinical samples (nasopharyngeal swabs) in either VTM, Liquid Amies media or normal saline were previously confirmed positive by one of three FDA-authorized comparator assays at the UCLA Clinical Microbiology Laboratory (Los Angeles, CA). All assays were verified in the UCLA Clinical Microbiology Laboratory and are run in accordance with manufacturer's specifications. Results are summarized in Table 7. The positive agreement (51/51) was 100.0% and negative agreement (53/53) was 100.0%.

Table 7. Evaluation with Clinical Specimens Using NextSeq Sequencer

		FDA-Authorized Comparator Assay		
		Positive	Negative	Total
UCLA SwabSeq COVID-19 Diagnostic Platform - NextSeq	Positive	51	0	51
	Negative	0	53	53
	Total	51	53	104
Positive Agreement		100.0% (51/51); 93.0% - 100.0%[1]		
Negative Agreement		100.0% (53/53); 93.2% - 100.0%		

[1]Two-sided 95% score confidence intervals

Warnings:

- For in vitro diagnostic use;
- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratories; UCLA Clinical Microbiology Laboratory located at Brentwood Annex, 11633 San Vicente Blvd Los Angeles, CA 90049 and UCLA SwabSeq COVID-19 Testing Laboratory located at 650 Charles E Young Dr South, CHS Building, South Tower, 87-300 Los Angeles CA, 90095.
- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.