Helix COVID-19 NGS Test

EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR HELIX COVID-19 NGS TEST
(Helix OpCo LLC (dba Helix)

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

(The Helix COVID-19 NGS Test will be performed in the Helix Laboratory located at 9875 Towne Centre Drive San Diego, CA 92121, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity test, per the laboratory procedures that were reviewed by the FDA under this EUA).

INTENDED USE

The Helix COVID-19 NGS Test is an amplicon based next generation sequencing (NGS) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 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 Helix Laboratory located at 9875 Towne Centre Dr San Diego, CA 92121, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets 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 positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Helix COVID-19 NGS Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the use of the Illumina NovaSeq 6000 Sequencing System and Next-Generation Sequencing workflows as well as real-time PCR assays and *in vitro* diagnostic procedures. The Helix COVID-19 NGS Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The assay is an amplicon-based NGS test designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health

authority guidelines. The assay simultaneously detects one SARS-CoV-2 target, the Spike protein gene (S gene), and one internal human internal control target, RPP30.

Upper respiratory specimens (nasopharyngeal swabs, oropharyngeal (throat) swab, mid-turbinate nasal swabs and anterior nasal swabs) should be collected, transported and stored according to standard procedures. The acceptable transport media for these collected upper respiratory specimen types are iSwab Microbiome collection media (ISWAB-MD-1200, Mawi DNA Technologies).

Anterior nasal swabs (502CS01, Copan FLOQSwabs) may also be self-collected under the supervision of a healthcare provider at a healthcare or testing site. The self-collected nasal swab specimens in iSwab Microbiome collection media (ISWAB-MD-1200, Mawi DNA Technologies) should be shipped and tested within 48 hours of collection. All specimens received at the clinical laboratory for testing will undergo review and accessioning prior to acceptance for testing.

RNA extraction for all specimen types is performed using the MagMax Viral/Pathogen II (MVP II) Nucleic Acid Isolation kit and semi-automated workflow on the Hamilton Microlab STAR liquid handler. The input sample volume for iSwab solution is 200µl and the elution volume is 50µL.

Reverse-transcriptase-PCR (RT-PCR) is performed using the TaqPath 1-Step Multiplex Master Mix and is run on the Bio-Rad C-1000 Touch Thermocycler.
Each well will also have the following added:

1) S primers: Designed to target the spike protein of SARS-CoV-2. Each of the barcoded primers also include Illumina p5 & p7 sequences. The barcodes are designed with unique dual indices enabling 384 samples to be multiplexed.
2) S Synthetic Control RNA spike-in control: The RNA spike-in control contains a 6 nucleotide stretch of unique sequence that differentiates it from the actual virus target during sequencing. This RNA spike-in is amplified by the same primers targeted at the virus genome, which provides an external positive control.
3) RPP30 primers: Human control that is also barcoded and includes p5 and p7 sequences (human control).

Sequencing libraries are pooled in equal volume prior to sequencing and each pool is loaded onto one of the two lanes provided on the Illumina NovaSeq 6000 SP flow cell using the XP workflow. The instruments are run under dual-indexed single-read sequencing for 26 cycles (26 bp reads). The samples are also pooled with indexed PhiX spiked at 50% to provide sufficient sequence diversity, which assists with template registration and improves run and base quality.

Sequencing data are demultiplexed using the bcl2fastq software. Sequencing reads for each sample are mapped against the expected amplicons using the Helix COVID-19 NGS Test Bioinformatics Pipeline. The number of reads that align to each amplicon are counted. Per-sample quality control filters are applied, and then the software interprets the results to generate a test result. The constant S Synthetic Control RNA spike-in enables relative quantification of the abundance of viral RNA to the spike-in. A rule-based algorithm (e.g. decision tree) is used to provide a qualitative result for COVID-19 diagnosis.

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INSTRUMENTS USED WITH THE TEST

Instruments

The Helix COVID-19 NGS Test is to be used with the Illumina NovaSeq 6000 and Illumina SP Flow Cell. The software includes Illumina NovaSeq Software v1.6, Illumina Real Time Analysis Software (RTA3 v3.4.4), Illumina bcl2fastq v2.20, and Helix COVID-19 NGS Test Bioinformatics Pipeline (AmpFinder) v0.1.0.

The Helix COVID-19 NGS Test can be used with the following liquid handling instruments:

- Hamilton Microlab STAR liquid handler with Software Venus 3 version 4.5.0.7797
- STP Labtech Mosquito HV with Software Mosquito Genomics V1.0.0.0

REAGENTS AND MATERIALS

Table 1. Reagents and materials required for use with the Helix COVID-19 NGS Test

Material ID	Vendor	Catalog No.
MagMax Viral/Pathogen II (MVP II) Nucleic Acid Isolation kit	Thermo Fisher Scientific	A48383
TaqPath 1-Step Multiplex Master Mix	Thermo Fisher Scientific	A28523
Custom Oligonucleotides (RT-PCR primers, sequencing primers)	IDT	16773623
TailorMix Dual-Indexed PhiX Control Library (Non-denatured)	SeqMatic	TM-580
NovaSeq 6000 SP Reagent Kit, 100 Cycles	Illumina	PN:20027464
XP- 2-Lane Kit	Illumina	PN:20021664
XP 2-Lane Manifold Pack	Illumina	PN:20021666

CONTROLS TO BE USED WITH THE HELIX COVID-19 NGS TEST

Batch-level Positive Control: For each 384 well plate, a positive control sample using heat inactivated SARS-CoV-2 virus (ATCC, VR-1986HK) in negative anterior nares clinical matrix in Mawi transport medium will be included to assess reagent purity, and cross contamination.

Batch-level Negative Control - molecular-grade, nuclease-free, non-DEPC-treated water

will be used to monitor non-specific amplification, cross-contamination during experimental setup, and nucleic acid contamination of reagents. One negative control will be included on each 384 well plate.

Human Positive Control (IPC) – RnaseP Primer (RPP30) provides a human and extraction positive control. This is expected in each well with clinical samples irrespective of SARS-CoV-2 presence or absence.

External positive control - S Synthetic Control RNA Spike is used to monitor for failures of reverse transcriptase, PCR and reaction conditions. This provides a negative control for each well for reverse transcription and amplification of the viral sequence.

INTERPRETATION OF RESULTS

1) Helix COVID-19 NGS Test Controls Interpretation:

All test controls should be examined prior to interpretation of patient results. The expected sequencing output for the Batch-level Positive Control is Positive and for the Batch-Level Negative Control is Not Detected. If the controls are not valid, the patient results cannot be interpreted.

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

2) Examination and Interpretation of Patient Specimen Results:

The qualitative test result is determined by using the SARS-CoV-2 status and read counts of the RPP30 human control, as shown in Table 2. The four possible qualitative results are:

1. **Positive**: Patient is positive for SARS-CoV-2. Report the result to the healthcare provider and appropriate public health authorities.
2. **Not Detected:** SARS-CoV-2 was not detected. Report result to healthcare provider.
3. **Invalid:** The sample was invalid. Repeat testing will start from extraction. If the repeat result remains invalid, consider collecting a new specimen.
4. **Inconclusive:** The result was inconclusive. Repeat testing will start from extraction. If the repeat result remains inconclusive, consider confirmation testing if clinically indicated.

Table 2. Results Interpretation Table

	SARS-CoV-2 Positive	SARS-CoV-2 Not Detected	SARS-CoV-2 Not Determined
RPP30 present (RPP30 read count ≥ 100)	Positive	Not Detected	Invalid
RPP30 not present (RPP30 read count < 100)	Inconclusive	Invalid	Invalid

PERFORMANCE EVALUATION

I. Analytical Sensitivity

Limit of Detection (LoD):
The LoD was performed by spiking in heat inactivated SARS-CoV-2 virus (ATCC, VR-1986HK) in negative anterior nares clinical matrix in Mawi iSwab Microbiome Collection Media (Mawi) using a two-fold dilution series. Three extraction replicates were performed per concentration. The preliminary LoD was defined as the lowest concentration with 3 of 3 replicates that test positive which was 125 GCE/mL.

Table 3. Preliminary LoD Determination Results

Viral Concentration (GCE/ml)	**Detection Rate (%)**
4000	3/3 (100%)
2000	3/3 (100%)
1000	3/3 (100%)
500	3/3 (100%)
250	3/3 (100%)
125	3/3 (100%)
62.5	2/3 (66.7%)
31.25	1/3 (33.3%)

LoD confirmation study was performed by spiking in heat inactivated SARS-CoV-2 virus (ATCC, VR-1986HK) in negative anterior nares clinical matrix in Mawi at the LoD previously determined. Twenty (20) extraction replicates were performed and the LoD was determined to be 125 GCE/mL for Mawi.

Table 4. Confirmatory LoD Study Results

Preservation Solution	**Viral Concentration (GCE/mL)**	**Detection Rate (%)**
Mawi	125	20/20 (100%)
Mawi	62.5	14/20 (70%)

II. Analytical specificity

Inclusivity

In silico analysis was performed on 3,715 complete SARS-CoV-2 genomes from the NCBI database as of June 10, 2020. The viral genomes with "N" characters in directly overlapping portions of the genome were removed from analysis.

Table 5. *In silico* analysis for Inclusivity of the Helix COVID-19 NGS Test

Targets	# of Complete Sequences Available	0 Mismatch	1 Mismatch	2+ Mismatch
S Target	3715	3715	0	0
S Forward Primer	3715	3706	9	0
S Reverse Primer	3715	3642	71	2

No viral genome sequences exhibited mismatches in more than one primer, and 80 of 82 had only one nucleotide mismatch. MT372482 and MT358745 are the only sequences with more than 1 mismatch, and in both cases all mismatches are on the S reverse primer; furthermore, the 8 bp on the 3' end of the primer are perfect matches. Primers are designed with melting temperatures ≥60ºC and the assay is run with annealing temperature at 60ºC to tolerate non-exact matches. Due to a combination of these factors, these mismatches are not expected to result in a significant risk of false negatives.

Cross-reactivity

In silico analysis of the following organisms recommended by FDA was performed by aligning primer sequences against their genomes and attempting to identify regions of high sequence homology.

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Recommended List of Organisms to be analyzed *in silico* or by Wet Testing

Other high priority pathogens from the same genetic family	High priority organisms likely in circulating areas
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus (e.g. EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Pooled human nasal wash – *to represent diverse microbial flora in the human respiratory tract*
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Streptococcus salivarius

The *in silico* analysis revealed no primer pairs where both primers have significant homology against any of the genomes in the cohort. The only homology is a region of the *Candida albicans* genome (NC_032095.1) that exhibits 90% homology with the S forward primer. The other primers do not have significant homology with this genome; therefore,

amplification of non-target sequences that result in cross-reactivity or potentially interfere with the detection of SARS-CoV-2 is not likely to occur. Furthermore, the genomic sequence adjacent to this homologous region is significantly divergent from our expected amplicon sequences and thus false positive detection is highly unlikely.

III. Clinical evaluation

A clinical study was performed to evaluate the performance of the Helix COVID-19 NGS Test using thirty remnant positive nasopharyngeal swab clinical samples and thirty negative nasal swab clinical samples in Mawi iSwab Collection Media.

Positive patient clinical samples (nasopharyngeal swabs) in Mawi iSwab Collection Media were previously confirmed positive by an EUA authorized comparator assay. These specimens were also confirmed positive by the EUA authorized Helix COVID-19 Test. RNA was extracted using MagMax Viral/Pathogen II (MVP II) Nucleic Acid Isolation kit and semi-automated workflow using the Hamilton Microlab STAR. Of the 30 positive patient samples, 30 (100.0%) were detected by the Helix COVD-19 NGS Test and 30/30 (100%) negative patient specimens were confirmed negative. Results are summarized in Table 6 below.

Table 6. Evaluation with Clinical Specimens in Mawi iSwab Collection Media

		EUA Authorized Comparator Assay		
		Positive	Negative	Total
Helix COVID-19 NGS Test	Positive	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive Agreement		100.0% (30/30); 88.7% - 100.0%[1]		
Negative Agreement		100.0% (30/30); 88.7% - 100.0%		

[1]Two-sided 95% score confidence intervals

Warnings:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by Helix Laboratory located at 9875 Towne Centre Drive San Diego, CA 92121;
- 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.