ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
Helix COVID-19 Test
(Helix OpCo, LLC)

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

(The Helix COVID-19 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, as described in the Standard Operating Procedures that were reviewed by the FDA under this EUA).

INTENDED USE

The Helix COVID-19 Test is a real-time RT-PCR 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 who are suspected of COVID-19 by their healthcare provider. Testing is limited to the 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.

In addition, the Helix COVID-19 Test is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in self-collected anterior nasal swab specimens (supervised specimens collected in transport media or unsupervised specimens collected in saline) from any individual, including individuals without symptoms or other reasons to suspect COVID-19 using the Helix Self-Collection Kit when directly ordered and provided by a HCP. Specimens collected using the Helix Self-Collection Kit will be dropped off at the designated location and transported via courier for testing at the Helix Laboratory.

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. Nasal swab specimens that are self-collected will not be tested with an internal control to confirm that

the specimen was properly collected. Self-collected specimens from SARS-CoV-2 positive individuals may yield negative results if the specimen was not collected properly.

Use of the Helix COVID-19 Test in a general, asymptomatic screening population is intended to be used as part of an infection control plan, that may include additional preventative measures, such as a predefined serial testing plan or directed testing of high-risk individuals. Negative results should be considered presumptive and do not preclude current or future infection obtained through community transmission or other exposures. Negative results must be considered in the context of an individual's recent exposures, history, presence of clinical signs and symptoms consistent with COVID-19.

The Helix COVID-19 Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays and *in vitro* diagnostic procedures. The Helix COVID-19 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Helix Self-Collection Kit

The Helix Self-Collection Kit is intended to be provided as part of a community-based distribution framework (e.g., drive-throughs, college campuses, employer sponsored collection sites, etc...) that is physician ordered. Healthcare providers (HCP) at specific institutions, who are licensed and have prescriptive authority in their respective states determine eligibility based on current CDC testing guidelines. Ordering physicians must be licensed in the state where the kits will be provided or shipped. The Helix Self-Collection Kit can be provided at a designated on-site collection location that is part of a centrally coordinated program. Helix does not accept requests for kits from patients directly. Patient results are communicated directly to the ordering physician.

The Helix Self-Collection Kit consists of a swab and collection tube. Instructions provided with the Helix Self-Collection Kit guide users on how to appropriately collect the nasal swab specimen unsupervised. The self-collection instructions have been reviewed and based on the usability study results deemed acceptable. A site administrator will register the patient into the collection site system and apply a barcode label to the tube. Following collection, the swab tip is broken off and inserted into a collection tube pre-filled with saline. The collected specimen is sealed in the biohazard bag and placed into a designated, secure collection bin or handed directly to on-site staff. The collection sponsor (site) is instructed to bulk ship the collected samples to the Helix Laboratory via overnight shipping or same day shipping via a courier at ambient conditions on the same day the specimens are collected. All contracts for testing of specimens collected without observation will include language that requires specimens be promptly collected onsite or will be rejected and overnight shipping or daily courier of specimens to occur on the same day as specimen collection. Specimens received for testing at Helix Laboratory will undergo a thorough review and accessioning prior to acceptance for testing with the Helix COVID-19 Test.

Helix COVID-19 Test

The assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set(s) is 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 four targets: three SARS-CoV-2 viral targets, the Nucleocapsid gene (N gene), the ORF1ab gene and the Spike protein gene (S gene), and one primer/probe set detecting MS2 RNA spiked into the reaction 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 standard procedures. The acceptable transport media for these collected upper respiratory specimen types are VTM, saline and 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 or self-collected unsupervised. The self-collected nasal swab specimens in iSwab Microbiome collection media (ISWAB-MD-1200, Mawi DNA Technologies), saline or VTM (ThermoFisher cat. #R125500 or equivalent) should be shipped on the same day as collection 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 semi-automated on the Hamilton Microlab STAR liquid handler. The input sample volume for Saline and iSwab solution is 200ul, while the input sample volume for VTM is 400 µL, the elution volume is 50 µL in both cases.

Reverse-transcriptase-PCR (RT-PCR) is performed using the Applied Biosystems TaqPath COVID-19 Combo kit and plating of 384-well plates with STP Labtech Mosquito HV and used with the QuantStudio 7 Flex Quantitative Real Time PCR Instrument.

INSTRUMENTS USED WITH THE TEST

Instruments

The Helix COVID-19 Test is to be used with the QuantStudio 7 Flex Quantitative Real Time PCR Instrument. All results are interpreted using QuantStudio RealTime PCR Software v2.4.

The Helix COVID-19 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. Materials included in the Helix Self-Collection Kit

Name	Description	Quantity	Material Supplier
Nasal Swab (Copan 502CS01)	Synthetic nylon flocked anterior nares swab	1	Affordable IHC Solutions
Saline (XHLX001)	5 mL tubes with 1 mL sterile 0.85% saline	1	Thomas Scientific
Specimen Biohazard Bag (40007)	Includes an absorbent pad	1	Therapack
Helix IFU	Unsupervised self-collection Instructions for Use	1	Helix

Table 2. Reagents and materials required for use with the Helix COVID-19 Test

Material ID	Vendor	Catalog #
MagMax Viral/Pathogen II (MVP II) Nucleic Acid Isolation kit	Applied Biosystems	A48383
TaqPath COVID-19 Combo Kit	Applied Biosystems	A47814
TaqPath 1-Step Multiplex Master Mix	Applied Biosystems	A28523

COLLECTION KITS USED WITH THIS TEST

The Helix COVID-19 Test can be used with synthetic nasal swabs collected in saline using the Helix Self-Collection Kit.

MEDICAL OVERSIGHT AND PROCESS TO BE USED FOR UNMONITORED NASAL SWAB COLLECTION

On-Site Unsupervised Collection Workflow

1. Healthcare providers (HCP) at specific institutions, who are licensed and have prescriptive authority in their respective states determine eligibility based on current CDC testing guidelines or for purposes of an infection control plan.
2. The patients collect their own nasal samples following the instructions provided with the Helix Self-Collection Kit and place the sample into a designated, secure collection bin or handed directly to on-site staff.

3. The collection sponsor is instructed to bulk ship the collected samples to the Helix Laboratory via overnight shipping (or same day shipping via a courier for those collections completed on-site) at ambient conditions.
4. Patient results are communicated to the ordering physician via electronic transfer. The HCP relays the results to the patient. If requested by the HCP, Helix can provide an electronic copy of the patient report to the patient as well.
5. Results are automatically shared with local Department of Public Health registries.

INSPECTION OF SPECIMENS

Sample Acceptance Criteria for Nasal Swabs Collected without Supervision

Specimens received at Helix Laboratory for testing with the Helix COVID-19 Test undergo the following accessioning prior to acceptance for testing:

- Date of shipment received must be less than or equal to 48 hr from the date of samples collected.
- Tube must contain a swab
- The specimen tube must be intact and contain at least 200 µl of media to be accepted for processing
- Tubes that are open or damaged with leaked media, and empty tubes are rejected
- Tubes with missing or damaged identifiers are rejected
- Non-Helix tubes and swabs used for unsupervised self-collection are rejected

CONTROLS TO BE USED WITH THE HELIX COVID-19 TEST

- **Internal Positive Control (IPC) –** MS2 phage control which is required as an extraction positive control. This is spiked into every well prior to extraction.

- **External positive control -** TaqPath COVID-19 Control contains the SARS-CoV-2 RNA genomic regions targeted by the kit. The positive control is used to monitor for failures of rRT-PCR reagents and reaction conditions. One positive control will be included with each 384 well plate.

- **Negative Control -** molecular-grade, nuclease-free, non-DEPC-treated water 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.

INTERPRETATION OF RESULTS

1) TaqPath SARS-CoV-2 RT-PCR Test Controls Interpretation:

All control wells must pass for the patient results to be considered valid and acceptable. A positive result for a target is defined as a Cq < 37 based on the TaqPath COVID-19 Combo Kit IFU. Refer to Table 3 for a summary of control results.

Table 3. Cq Values for Controls that Must Be Observed to Obtain Valid Results

	Cq Value			
	N gene	S gene	Orf 1ab	MS2 Phage
Negative Extraction Control	Undetermined >37	Undetermined >37	Undetermined >37	<37
Positive Control	<37	<37	<37	Undetermined[1] >37
NTC	Undetermined >37	Undetermined >37	Undetermined >37	Undetermined[1] >37
MS2 Internal Control	Any	Any	Any	<37

Undetermined/Negative (Cq > 37 or No Detectable Cq)
[1]MS2 Internal Control is not added to the Positive Control or No Template Control and no signal should be generated

2) ***Examination and Interpretation of Patient Specimen Results:***

The assay interpretation and reporting of results is shown in Table 4. Assessment of patient specimen test results should be performed after the positive, extraction and NTC controls have been examined and determined to be valid and acceptable.

Table 4. Result Interpretation for Patient Samples

Orf1ab gene	**N gene**	**S gene**	**MS2**	**Status**	**Result**	**Action**
NEG	NEG	NEG	NEG	Invalid	Invalid	Repeat test. If repeat test is also invalid, consider collecting a new sample
NEG	NEG	NEG	POS	Valid	SARS-CoV-2 not detected	Report results to healthcare provider and appropriate public health authorities. Consider testing for other viruses.
Only one SARS-CoV-2 target = POS			POS or NEG	Valid	SARS-Cov-2 Inconclusive	Repeat test. If repeat result is inconclusive, consider additional confirmation testing if clinically indicated.
Two or more SARS-CoV-2 targets = POS			POS or NEG	Valid	Positive for SARS-Cov-2	Report results to healthcare provider and appropriate public health authorities

PERFORMANCE EVALUATION

I. Analytical Sensitivity

The LoD was performed by spiking in heat inactivated SARS-CoV-2 virus (ATCC, VR-1986HK) in negative anterior nares clinical matrix in VTM, and 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 1000 GCE/mL (Table 5).

Table 5. Preliminary LoD Determination Results

Viral Concentration (GCE/ml)	Detection Rate (%) VTM	Detection Rate (%) Mawi
2000	3/3 (100%)	3/3 (100%)
1000	3/3 (100%)	3/3 (100%)
500	2/3 (66.7%)	3/3 (100%)

A LoD confirmation study was performed by spiking in heat inactivated SARS-CoV-2 virus (ATCC, VR-1986HK) in negative anterior nares clinical matrix in VTM and Mawi at the LoD previously determined. Twenty (20) extraction replicates were performed for each media type. The LoD was determined to be 1000 GCE/mL for VTM and Mawi (Table 6).

Table 6. Confirmatory LoD Study Results for Clinical Samples in Different Media Types

Preservation Solution	Viral Concentration (GCE/mL)	Detection Rate (%)
VTM	1000	20/20 (100%)
Mawi	1000	19/20 (95%)

II. Analytical specificity

Inclusivity

The Helix COVID-19 Test utilizes the identical oligonucleotide sequences for the spike (S), nucleocapsid (N) and ORF 1ab regions as those used in the TaqPath COVID-19 Combo Kit. *In silico* testing was previously performed by Thermo Fisher Scientific as part of their EUA (EUA200010) and this information has been provided in the FDA authorized EUA granted to this manufacturer. Helix Laboratory obtained a right of reference from Thermo Fisher Scientific to use the *in silico* data.

Cross-reactivity

Helix Laboratory obtained a right of reference from Thermo Fisher Scientific to incorporate the *in silico* cross reactivity analysis findings. As part of Thermo Fisher Scientific's EUA, they performed an *in silico* analysis of potentially cross-reactive organisms and determined that there was low risk of non-specific amplification.

III. Clinical evaluation

A clinical study was performed to evaluate the performance of the Helix COVID-19 Test using sixty remnant positive upper respiratory clinical samples, and sixty negative upper respiratory clinical samples in two collection media types.

A total of 30 positive patient clinical samples (nasopharyngeal and oropharyngeal swabs) in Mawi iSwab Collection Media were previously tested using an EUA authorized comparator assay. 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 test and 30/30 (100%) negative patient specimens were confirmed negative. Results are summarized in Table 7.

Table 7. Evaluation with Clinical Specimens in Mawi iSwab Collection Media

		EUA Authorized Comparator Assay		
		Positive	Negative	Total
Helix COVID-19 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

Additional 30 positive patient clinical samples (nasopharyngeal swabs, oropharyngeal swabs and anterior nasal swabs) in UTM were previously tested by an EUA authorized comparator assay. RNA was extracted using MagMax Viral/Pathogen II (MVP II) Nucleic Acid Isolation kit and semi-automated workflow using the Hamilton Microlab STAR. The results are summarized in Table 8, below. The positive agreement (29/30) was 96.7% and negative agreement (30/30) was 100.0%.

Table 8. Evaluation with Clinical Specimens in VTM

		EUA Authorized Comparator Assay		
		Positive	Negative	Total
Helix COVID-19 Test	Positive	29	0	29
	Negative	1*	30	31
	Total	30	30	60
Positive Agreement		96.7% (95% CI: 83.3% - 99.4%)		
Negative Agreement		100.0% (95% CI: 88.7% - 100.0%)		

*One result was invalid without enough clinical specimen for retesting

IV. Specimen stability

Mawi iSwab Collection Media Sample stability

A simulated transport stability study was conducted to evaluate conditions that could affect SARS-CoV-2 RNA integrity of anterior nasal samples. Contrived samples were created by spiking in heat inactivated SARS-CoV-2 virus (ATCC, VR-1986HK) in negative anterior nares clinical matrix in Mawi collection media at the levels described in Table 9 below and subject to the conditions described in Tables 10 and 11.

Table 9. Contrived Samples for Stability Study

Titer	Replicates for each Profile
2x LoD	20
5-10x LoD	10
Negative	10
Total	**40**

Table 10. Summer Profile Conditions for Stability Study

Temperature	Cycle Period	Cycle Period Hours	Cumulative Time Hours
40°C	1	8	8
22°C	2	4	12
40°C	3	2	14
30°C	4	36	50
40°C	5	6	56

Table 11. Winter Profile Conditions for Stability Study

Temperature	Cycle Period	Cycle Period Hours	Cumulative Time Hours
-20°C	1	8	8
18°C	2	4	12
-20°C	3	2	14
10°C	4	36	50
-20°C	5	6	56

Summary of Results

All positive samples were detected after being subjected to summer and winter profile stability conditions and the findings are summarized in Table 12.

Table 12. Summary of Sample Stability Results

Titer	Summer Profile	Winter Profile
2x LOD	20/20	20/20
5-10x LOD	10/10	10/10
Negative	10/10	10/10
PPA	100% (88.6-100.0%)	100% (88.6-100.0%)
NPA	100% (75.1-100.0%)	100% (75.1-100.0%)
Interpretation	PASS	PASS

Baseline "no treatment" controls, in triplicate, were tested at time zero (t_0) for each virus concentration. Mean Cq values were compared to the mean Cq values for each season profile (summer and winter). The mean Cq values are less than one (1) Cq different when comparing baseline to the treatments for each of the three (3) SARS-CoV-2 targets in the rRT-PCR assay. The mean Cq values for all concentrations of viral particles are shown below in Table 13. No degradation was observed in RNA after subjecting the samples to Summer and Winter profiles.

Table 13. Mean Ct Values from Stability Samples Subjected to Summer/Winter Profiles

Profile	# Viral Copies (GCE/mL)	# Samples Tested	N Gene Mean Ct	Orf1ab Gene Mean Ct	S Gene Mean Ct
ΔCT (t_0 - Summer)	2x LOD	20	-0.16	0.35	0.30
	5x LOD	5	0.25	0.12	-0.17
	10x LOD	5	0.36	0.56	0.43
ΔCT (t_0 - Winter)	2x LOD	20	0.10	0.27	0.32
	5x LOD	5	0.44	0.61	0.50
	10x LOD	5	-0.31	0.11	-0.02

Helix Self-Collection Kit Sample stability

The Helix Self-Collection Kit include synthetic nasal FLOQswabs transported in tubes containing sterile 0.9% saline. FDA has reviewed analytical validation data from a swab stability study conducted by Quantigen Biosciences, with support from The Gates Foundation and UnitedHealth Group, that can be used, in conjunction with other data from the sponsor, to support sample stability of foam or polyester nasal swabs shipped dry (in an empty tube) or in saline (0.9%) for testing with authorized SARS-CoV-2 molecular diagnostic assays. Quantigen Biosciences has granted a right of reference to any sponsor wishing to pursue an EUA to leverage their COVID-19 swab stability data as part of that sponsor's EUA request.

V. Usability

Helix performed a usability study for unsupervised collection using the Helix Self-Collection Kit for transport to Helix Laboratory for testing. Participants who consented to the study were provided the Helix Self-Collection Kit and asked to follow the instructions for use and self-collect while the study coordinator observed and filled out an observation form. Once the sample was collected by the participant, it was returned to the study coordinator. Of the 43 study participants, one participant was classified as 'research observation not captured' and another participant was classified as 'questionnaire not captured'. Study samples were shipped in bulk to the Helix Laboratory by the study coordinator via overnight shipping. Samples were evaluated according to accessioning criteria per laboratory SOP and were tested for presence of RPP30, human internal control.

Results of the usability testing were analyzed qualitatively to determine if the design of the kit and/or kit instructions need to be modified to reduce the use-related risks to acceptable levels.

Successful sample collection was measured by the presence of RPP30 in the sample. All samples were acceptable for testing and RPP30 was detected in all samples (41/41).

Warnings:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratory;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

Limitations:

- Specimens that are self-collected will not be tested with an internal control to confirm that the specimen was properly collected. Self-collected specimens from SARS-CoV-2 positive individuals may yield negative results if the specimen was not collected properly;
- The requirement to run a sample adequacy control for all samples that were self-collected will be waived provided that the following disclosure has been acknowledged by the entity utilizing authorized unsupervised self-collection swab materials and a statement is included in the test reports for specific patients who self-collected a specimen:

Acknowledgement

(Insert Client name) acknowledges it has received the disclosure below:

Specimens that are self-collected will not be tested with an internal control to confirm that the specimen was properly collected. Self-collected specimens from SARS-CoV-2 positive individuals may yield negative results if the specimen was not collected properly.

Test Report Limitation

Specimens that are self-collected were not tested with an internal control to confirm that the specimen was properly collected. As such, unsupervised self-collected specimens from SARS-CoV-2 positive individuals may yield negative results if the specimen was not collected properly.

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. The extraction method and instrument used were the MagMax Viral/Pathogen II (MVP II) Nucleic Acid Isolation kit semi-automated on the Hamilton Microlab STAR liquid handler and the QuantStudio 7 Flex Quantitative Real Time PCR Instrument. The results are summarized in the following Table.

Table 14. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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