ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
LifeHope 2019-NCOV REAL-TIME RT-PCR DIAGNOSTIC PANEL
(LIFEHOPE LABORATORY)

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

(The LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel will be performed at LifeHope Laboratory, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, to perform high complexity tests as per the Instructions of Use that were reviewed by the FDA under this EUA.)

INTENDED USE

The LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 RNA in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens) and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to LifeHope Labs 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 and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens and bronchoalveolar lavage 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 infective 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 LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel is only 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. Testing is performed on the QuantStudio 5 (ThermoFisher) and analysis is done using the QuantStudio Design and Analysis Software version 1.5.1. The SARS-CoV-2 primer and probe set(s) provided by Integrated DNA Technologies (IDT) are designed to detect RNA from the SARS-CoV-2 in upper respiratory specimens and bronchoalveolar lavage from patients. All nucleic acid is extracted using the IndiMag Extraction Kit (Indical, SP54106 IB).

INSTRUMENTS USED WITH TEST

The LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel is to be used with the following PCR and nucleic acid extraction instrument:

- QuantStudio 5 (ThermoFisher, Software version 1.5.1)
- IndiMag Extraction Kit (Indical, SP54106 IB)

REAGENTS AND MATERIALS

The LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel has been validated using only the components referenced in this submission.

- 2019-nCoV CDC EUA Kit, 500 rxns- N1, N2, RNase P (Integrated DNA Technologies, cat # 10006606)
- 2019-nCoV_N_Positive Control (Integrated DNA Technologies, cat # 10006625)
- Hs_RPP30 Positive Control (Integrated DNA Technologies, cat # 10006626)
- Takara OneStep PrimeScript RT-PCR (Perfect Real Time) (Takara Bio Inc; cat #RR064B)
    - 2X OneStep RT-PCR Buffer III
    - TaKaRa Ex Taq HS (5 U/ μl)
    - Rox Reference Dye II
    - Molecular grade water, nuclease-free
- 200 Proof Ethanol (Fisher Healthcare, BP2818500 FH)
- Molecular Grade Isopropanol (Fisher Healthcare, BP2618500 FH)
- Hypure Molecular Grade Water (Hyclone, #SH30538.02)

CONTROLS TO BE USED WITH THE LifeHope 2019-NCOV REAL-TIME RT-PCR DIAGNOSTIC PANEL

Controls that provided with the test kit are as follows:

Positive Control (nCoVPC): The nCoVPC (Integrated DNA Technologies, #10006625) is a noninfectious positive DNA plasmid control. It is included in every run. nCoVPC should produce a positive result with each run.

Negative Control (NTC): Molecular Biology Grade Water (Nuclease-Free, Deionized, Distilled, 0.1um Sterile Filtered, Hyclone, #SH30538.02). Molecular Grade Water is used as an extraction control to ensure that there was no contamination during automated extraction. Water will be used on every extraction run.

Extraction Control: The extraction control targets the RNase P gene present in the human genome. This control is used to ensure that there was a successful recovery of the sample.

INTERPRETATION 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.

Table 1: Interpretation of Results for Quality Controls

Control Type	External Control Name	Used to Monitor	2019 nCoV_N1	2019 nCoV_N2	RP	Expected Ct Values
Positive Control	nCoVPC	Substantial reagent failure including primer and probe integrity	+	+	+	≤35.00 Ct
Negative Control	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Extraction Control	RNase P	Failure in lysis extraction procedure, potential contamination during extraction.	-	-	+	≤35.00 Ct

Table 2: Interpretation of test results for the LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel

2019 nCoV_N1	2019 nCoV_N2	RP	Result Interpretation	Report	Actions
+	+	+/-	SARS-CoV-2 detected	Positive SARS-CoV-2	Report results to Department of Public Health and physician.
If only one of the two targets is positive		+/-	Inconclusive Result	Inconclusive	Repeat testing of nucleic acid and/or re-extract and repeat rRT-PCR. If the repeated result remains inconclusive, contact physician/sender for a recollection.
-	-	+	SARS-CoV-2 not detected	Negative	Report results to physician/sender. Consider testing for other respiratory viruses.
-	-	-	Invalid Result	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen for the patient.

- Negative: A specimen is considered negative if both the N1 and N2 markers do not cross the threshold line within 35 cycles and RNaseP does cross the threshold line within 35 cycles.
- Positive: A specimen is considered positive if both the N1 and N2 markers cross the threshold line within 35 cycles and the RNaseP is either positive (crosses the threshold line within 35 cycles) or is negative.
- Invalid: A specimen is considered invalid if the N1, N2 and RNaseP markers do not cross the threshold line within 35 cycles. The specimen must be re-extracted and run again, if the same result is produced, the specimen is considered invalid, and a new specimen must be collected.
- Inconclusive: A specimen is considered inconclusive if N1 or N2 but not both markers cross the threshold line within 35 cycles. The extracted RNA must be retested. If there is residual specimen available, re-extract and re-test. If the same result is produced, the result is inconclusive and a new specimen must be obtained.
-

LIMITATIONS

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.
- Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
- The performance of LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel was established using nasopharyngeal swabs. Testing of nasal and mid-turbinate nasal swabs (self- collected at a healthcare site or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- False-negative results may arise from:
  - Improper sample collection
  - Degradation of the viral RNA during shipping/storage
  - Using unauthorized extraction or assay reagents
  - The presence of RT-PCR inhibitors
  - Mutation in the SARS-CoV-2 virus
  - Failure to follow instructions for use
- False-positive results may arise from:
  - Cross contamination during specimen handling or preparation
  - Cross contamination between patient samples
  - Specimen mix-up
  - RNA contamination during product handling
- The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not yet been evaluated.
- Please note, Negative results do not preclude infection of SARS-CoV-2 virus and should not be the sole basis of a patient management decision. A positive result indicates detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.
- Laboratories are required to report all positive results to the appropriate public health authorities.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

The Limit of Detection (LOD) study established the lowest detectable concentration of SARS-CoV-2 that can be detected by the LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel at least 95% of the time.

An initial LOD study was performed to determine the preliminary LoD of each target assay in the LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel using SARS-CoV-2 (BEI Resources, SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat Inactivated, Catalog No. NR-52286) that was spiked in SARS-CoV-2 confirmed-negative nasopharyngeal (NP) swab specimens at concentrations ranging from $3x10^5$ to 0.29 genome equivalents/µL. Nucleic acid was extracted from the contrived samples using the IndiMag Extraction Kit and the reverse transcription RT-PCR was performed using the QuantStudio 5 PCR system and tested in triplicate. The preliminary LoD was determined to be 4.5 genome equivalents/µL.

The LOD was confirmed by spiking 20 replicates of 1.5, 2.5, and 5 genome equivalents/µL of SARS-CoV-2 (BEI Resources, SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat Inactivated, Catalog No. NR-52286) into nasopharyngeal swab matrix previously confirmed to be negative for SARS-CoV-2. Nucleic acid was extracted from the contrived samples using the IndiMag Extraction Kit and the reverse transcription RT-PCR was performed using the QuantStudio 5 PCR system. Results are summarized in the Table below:

Table 3: LoD Confirmation Study Summary

Target	SARS-CoV-2 N1 gene			SARS-CoV-2 N2 gene		
Concentration (genome equivalents/µL)	1.5	**2.5**	5	1.5	**2.5**	5
Concentration (genome equivalents/rxn)	7.5	**12.5**	25	7.5	**12.5**	25
Positive/Total	17/20	**19/20**	20/20	20/20	**19/20**	20/20
Mean Ct	34.377	**33.644**	32.592	33.645	**33.691**	32.437
Standard Deviation	0.820	**0.731**	0.429	0.512	**0.521**	0.362

The LoD for the LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel was confirmed to be 2.5 genome equivalents/µL based on a positivity rate of ≥95% for 19/20 replicates.

2) ***Analytical Inclusivity:***

The LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel utilizes the identical oligonucleotide sequences for the N2 and RP genes as those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. *In silico* testing of the SARS-CoV-2 N1 and N2 assay was previously performed by CDC as part of their EUA authorized test. The inclusivity and cross-reactivity of the CDC EUA assay has been previously evaluated and therefore, additional evaluation for the N1/N2 target is not required. The CDC has granted a right of reference to the performance data contained in the CDC's EUA request (FDA submission number EUA200001) to any entity seeking an FDA EUA for a COVID-19 diagnostic device.

3) ***Cross-Reactivity:***

The LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel utilizes identical oligonucleotide sequences for the N1 and N2 SARS-CoV-2 target genes as those used in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

As reported under the CDC EUA, *in silico* analysis of the N1 probe sequence showed high sequence homology of the N1 probe with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

Wet Testing Analysis:

To confirm the cross-reactivity of the LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel in wet-testing conditions, 24 samples containing non-target organisms were prepared by spiking each organism into negative nasopharyngeal swab matrix and tested in duplicate. Nucleic acid was extracted using the IndiMag Extraction Kit and the reverse transcription RT-PCR was performed using the QuantStudio 5 PCR system. As a result, all non-target organisms were not detected.

Table 4: Organisms Assessed for Potential *in-vitro* Cross-Reactivity

Respiratory Pathogens			
Pathogen	**Initial Concentration (copies/µL)**	**Pathogen**	**Initial Concentration (copies or CFU/µL)**
SARS-coronavirus	10^6	Influenza A	250
MERS-coronavirus	10^6	Influenza B	125
Human coronavirus 229E	75	Enterovirus	125
Human coronavirus OC43	125	Respiratory Syncytial Virus (RSV)	75
Human coronavirus HKU1	125	Rhinovirus	75
Human coronavirus NL63	75	*Chlamydia pneumoniae*	19
Adenovirus	38	*Haemophilus influenzae*	75
Human Metapneumovirus (hMPV)	125	*Legionella (pneumophila & longbeachae)*	75
Parainfluenza 1	38	*Streptococcus pneumoniae*	125
Parainfluenza 2	75	*Bordetella pertussis*	10^4
Parainfluenza 3	38	*Mycoplasma pneumoniae*	125
Parainfluenza 4	250	Pooled Nasal Wash (representative of diverse microbial flora in the human respiratory tract)	unknown

4) Clinical Evaluation

Clinical Evaluation of the LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel

A clinical study was performed to evaluate the performance of the LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel. Results obtained with a total of 60 clinical NP swab specimens (30 negatives and 30 positives) tested with the CDC 2019-Novel Coronavirus (2019-nCoV) Real_Time RT-PCR Diagnostic Panel (performed as authorized) were compared to results obtained with the LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel. Samples were extracted using the IndiMag Extraction Kit and the reverse transcription RT-PCR was performed using the QuantStudio 5 PCR system. The results are summarized in the Table below.

Table 5: Clinical Evaluation of the LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel

2019-nCoV Real-Time RT-PCR Diagnostic Panel	FDA EUA Real-Time RT-PCR Test		Total	% Performance Agreement	95% CI
	Detected	Not Detected			
Detected	30	0	30	PPA 100%	88.7-100%
Not Detected	0	30	30	NPA 100%	88.7-100%
Total	30/30	30/30	60		

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 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* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.