EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
EXPRESS GENE 2019-NCOV RT-PCR DIAGNOSTIC PANEL
(EXPRESS GENE LLC, DBA: EXPRESS GENE MOLECULAR DIAGNOSTICS LABORATORY)

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

(The Express Gene 2019-nCoV RT-PCR Diagnostic Panel will be performed at laboratories designated by Express Gene LLC, DBA: Express Gene Molecular Diagnostics Laboratory, 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 Standard Operating Procedures that were reviewed by the FDA under this EUA.)

INTENDED USE

The Express Gene 2019-nCoV RT-PCR Diagnostic Panel is a real-time reverse transcription polymerase chain reaction test for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal aspirates/washes or nasal aspirates, and bronchoalveolar lavage (BAL) specimens collected from individuals suspected of COVID-19 by their healthcare provider (HCP).

This test is also for use with saliva specimens that are collected with the assistance of a HCP in a healthcare setting, by individuals suspected of COVID-19 using the mLife True Oral Fluid/Viral Collection Kit.

Testing is limited to laboratories designated by Express Gene LLC, DBA: Express Gene Molecular Diagnostics Laboratory 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.

Results are for the identification of SARS-CoV-2 RNA which is generally detected in respiratory and saliva 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 treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological

information. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

The Express Gene 2019-nCoV RT-PCR Diagnostic Panel is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic assays. The Express Gene 2019-nCoV RT-PCR Diagnostic Panel is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

mLife True Oral Fluid/Viral Collection Kit

mLife Diagnostics LLC is the manufacturer of the mLife True Oral Fluid/Viral Collection Kit. The main components of the collection kit include an oral swab, compression tube, and a glass vial containing a viral deactivation/RNA preservation transport medium. The plastic components and stabilization buffer are manufactured by third parties. The volume of the glass vial is 3 mL and includes approximately 1 mL of buffer and 1 mL of collected saliva.

The mLife collection device is an oral swab that the patient uses to swab all surfaces of the mouth and tongue followed by pooling saliva and saturating the swab. The HCP observes this entire process. This approach differs from a more traditional saliva collection that commonly instructs the patient to spit into a collection device. Therefore, the saliva sample that is collected with the mLife collection kit is most likely a mixture of oral fluid and saliva which is referred to as "saliva" throughout this summary.

A sufficient saliva sample has been collected when the indicator strip on the oral swab turns red. The patient hands the saturated swab with the collected specimen to the HCP who compresses the saliva on the swab into the stabilization buffer using the compression tube. The HCP can visually see the collected saliva enter the medium. When the collection instructions are abided, the amount of saliva that can be released from the swab can range from ~0.8 mL to 1.2 mL. The HCP caps the collected specimen and packages/ships the specimen to a laboratory designated by Express Gene LLC, DBA: Express Gene Molecular Diagnostics Laboratory for processing with the Express Gene 2019-nCoV RT-PCR Diagnostic Panel.

Express Gene 2019-nCoV RT-PCR Diagnostic Panel

The Express Gene 2019-nCoV RT-PCR Diagnostic Panel is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The test detects three specific regions of the SARS-CoV-2 genome including the ORF1ab region and the N (nucleocapsid) and S (Spike protein) genes. The assay also includes a primer and probe set to detect the MS2 phage internal control in both the negative extraction control and clinical samples.

RNA is isolated from upper respiratory specimens including nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates as well as BAL specimens using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Cat # A42352) performed on the KingFisher

Flex automated instrument with software version 1.01. RNA is reverse transcribed to cDNA using the TaqPath 1-Step Multiplex Master Mix and subsequently amplified using the QuantStudio 12K Flex Real-Time PCR System with software version 2.2.3. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5′ nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye (VIC, ABY, and FAM for the N, S, and ORF1ab targets, respectively) to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the QuantStudio 12K Flex platform.

INSTRUMENTS USED WITH TEST

The Express Gene 2019-nCoV RT-PCR Diagnostic Panel is to be used with the following instrumentation:

- RNA extraction: KingFisher Flex automated DNA extraction instrument with software version 1.01
- RT-PCR platform: ThermoFisher Scientific QuantStudio 12K Flex with design and analysis software

REAGENTS AND MATERIALS

Materials Included with the mLife True Oral Fluid/Viral Collection Kit

- Lab requisition form
- Collection/shipping instructions
- Oral swab
- Compression tube
- Capped glass vial with deactivation/stabilization buffer
- 2 labels
- Biohazard bag
- Absorbent sheet
- Corrugated shipping box (cardboard box)
- FedEx return overpack with pre-paid shipping label

Reagents Used to Perform the Express Gene 2019-nCoV RT-PCR Diagnostic Panel

REAGENTS/CONSUMABLES	SUPPLIER	CATALOG #
TaqPath COVID-19 Combo Kit	ThermoFisher Scientific	A47813 (100 rxn); A47814 (1,000 rxn)
ABY Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well	ThermoFisher Scientific	A24734
JUN Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well	ThermoFisher Scientific	A24735
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	ThermoFisher Scientific	A42352
TaqPath 1-Step Multiplex Master Mix (No ROX)	ThermoFisher Scientific	A28521, A28522, A28523
KingFisher Deepwell 96 Plate	ThermoFisher Scientific	95040450
KingFisher 96 KF microplate	ThermoFisher Scientific	97002540
KingFisher 96 tip comb for DW magnets	ThermoFisher Scientific	97002534

REAGENTS/CONSUMABLES	SUPPLIER	CATALOG #
Optical 96-Well Fast Clear Reaction Plates with Barcode	ThermoFisher Scientific	4483485 (20 plates); 4483494 (500 plates)
MicroAmp Clear Adhesive Film	ThermoFisher Scientific	4306311
MicroAmp Optical Adhesive Film	ThermoFisher Scientific	4311971 (100 pack); 4360954 (25 pack)

COLLECTION KIT USED WITH THE TEST

This assay can be used with the mLife True Oral Fluid/Viral Collection Kit that includes an oral swab that is used collect the patient's saliva. The healthcare provider (HCP) assists the patient with saliva collection, applies the swab to a compression tube to release the saliva into the deactivation buffer vial. The HCP packages and ships the specimen to laboratories designated by Express Gene LLC, DBA: Express Gene Molecular Diagnostics Laboratory.

MEDICAL OVERSIGHT AND PROCESS TO BE USED FOR SALIVA COLLECTION

Saliva is collected by the patient with assistance from a trained healthcare provider (HCP) in a healthcare setting using the mLife True Oral Fluid/Viral Collection Kit for testing with the Express Gene 2019-nCoV RT-PCR Diagnostic Panel.

1. The patient visits a healthcare facility for evaluation by a healthcare provider.
2. If the patient is determined to be eligible to provide the saliva sample (i.e., suspected of COVID-19), a prescription is written for the mLife True Oral Fluid/Viral Collection Kit.
3. With gloved hands, the HCP prepares the saliva collection device by uncapping the vial containing the stabilization/deactivation buffer. The vial is attached to the bottom of the compression tube.
4. The HCP follows the mLife True Oral Fluid/Viral Collection Kit instructions card by giving the patient the oral swab for saliva collection and instructing them to swab all surfaces in the mouth for approximately 30 seconds followed by pooling the saliva in the mouth to saturate the swab. A sufficient sample has been collected for testing when the indicator strip on the collection swab turns red.
5. The patient is instructed to hand the collected specimen to the HCP who will place the swab into the compression tube and release the collected saliva into the vial containing the stabilization buffer. The HCP will visually see the saliva enter the buffer.
6. The compression tube with the swab is removed and placed in the biohazard waste. The HCP caps the glass vial that houses the collected specimen.
7. The HCP prepares the specimen for return shipping to a laboratory designated by Express Gene LLC, DBA: Express Gene Molecular Diagnostics Laboratory by following the specific shipping instructions included with the mLife collection kit.
8. Saliva samples are shipped at ambient conditions using either overnight or 2-day courier services to Express Gene for molecular detection of SARS-CoV-2.

INSPECTION OF SPECIMENS

All specimens received at the clinical laboratory for testing will undergo review and accessioning prior to acceptance for testing.

CONTROLS TO BE USED WITH THE EXPRESS GENE 2019-NCOV RT-PCR DIAGNOSTIC PANEL

Table 1. Assay Controls that Must be Run with Each Test

Control Type	Purpose	Frequency of Testing
Negative Extraction Control (NEC)	To monitor for cross-contamination during RNA extraction and RT-PCR	Once per batch of specimens
Positive Control	To monitor the integrity of the RT-PCR reagents and process	Once per run of RT-PCR
Internal Control (MS2 Phage)	To monitor the integrity of nucleic acid extraction and RT-PCR for each specimen	Added to each specimen and the Negative Control prior to extraction
No Template Control (NTC)	To monitor for contamination of extraction and assay reagents	Once per run of RT-PCR

No Template Control (NTC)

- A "no template" (negative) control (NTC) is needed to check for contamination of RT-PCR assay reagents. Molecular grade, nuclease-free water is used in place of sample nucleic acid for this control. The NTC is used in one well on every assay plate.

External Positive Control

- A positive control is used to verify proper assay set-up and SARS-CoV-2 reagent integrity. The positive control contains in vitro transcribed (IVT) RNA specific to the N, S, and ORF1ab regions of SARS-CoV-2. The positive control is used in one well on every assay plate.

Negative Extraction Control (NEC)

- The extraction control monitors for any potential cross-contamination that could occur during the nucleic acid extraction process or RT-PCR assay. This control is not included in the TaqPath COVID-19 Combo Kit; however, Express Gene uses RNase/DNase free water with a spike-in of MS2 control that is processed through nucleic acid extraction and added to one well of the assay plate.

MS2 Phage Internal Control

- The MS2 internal control serves as an internal process control for nucleic acid extraction to ensure that clinical samples and controls contain RNA of sufficient quality to be used in the RT-PCR reactions. The MS2 control is spiked into all clinical samples and the negative extraction control prior to performing nucleic acid extraction.

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. Refer to Table 2 for a summary of control results.

COVID-19 RT-PCR Test Controls – Positive, Negative, Extraction, and Internal:

- **Negative Extraction Control (NEC);** The negative extraction control is processed with each batch of samples. The NEC should only show an amplification curve for MS2 with a Ct of less than 33 but must be negative for all SARS-CoV-2 targets (Ct undetermined).

- **External Positive Control;** The positive control must be positive for all three SARS-CoV-2 targets, i.e., the ORF1ab, the N Protein, and the S Protein genes and amplification must have a Ct < 37 for each target in order for the test result to be valid. The positive control does not contain MS2 and no Ct value should be obtained for this target.

- **Nuclease-Free Water (Negative Control; NTC);** The negative control must be negative (undetermined; no detectable Ct value) for all assay targets for the test result to be valid.

- **MS2 (Internal Positive Control);** MS2 in a patient sample indicates that PCR amplification occurred in the well. The presence of MS2 and no detectable SARS-CoV-2 during the analysis indicates that proper RNA extraction and amplification occurred, however, no SARS-CoV-2 is present. If SARS-CoV-2 is present in the specimen, amplification of the target RNA may reduce or abrogate MS2 amplification. In this case, the amplified SARS-CoV-2 indicates proper RNA extraction and amplification. Therefore, MS2 may or may not be detectable in a valid test on patient specimens.

Table 2. Expected Results of Controls Used in the Express Gene Panel

Control	Ct Value (Optical Channel)			
	N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 Phage (JUN)
Negative Extraction Control	Undetermined*	Undetermined	Undetermined	Ct < 33
Positive Control	Ct < 37	Ct < 37	Ct < 37	Undetermined [1]
No Template Control	Undetermined	Undetermined	Undetermined	Undetermined [1]
MS2 Internal Control	Any	Any	Any	Ct < 33

[1] The MS2 Phage Internal Control is not added to the Positive Control or No Template Control and no signal should be obtained.

* Undetermined (Not detectable Ct; negative)

If any of the above controls do not exhibit the expected performance as described, the assay may have been improperly set up and/or executed improperly, or reagent or equipment malfunction could have occurred. If the results obtained with the NTC and Positive Control do not meet the criteria shown, the results from the entire batch of samples are considered invalid and repeat testing must be performed using residual

extracted nucleic acid. If the results obtained with the negative extraction control and MS2 control do not meet the criteria shown, the run is invalid and repeat testing must be performed using new extracted RNA from residual patient sample.

Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results must 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. Please see the table below (Table 3) for guidance on interpretation and reporting of results.

- If all three SARS-CoV-2 specific targets (ORF1ab, N, S) are negative (undetermined) and the MS2 control is also negative (undetermined), the result is invalid. The extracted RNA from the patient specimen should be re-tested. If the repeat result is invalid (negative for all markers), collection of a new patient sample should be considered.
- If all three SARS-CoV-2 specific targets (ORF1ab, N, S) are negative (undetermined) and the MS2 control is positive (Ct < 33), the patient sample is reported as negative for SARS-CoV-2 RNA.
- If any one or two SARS-CoV-2 specific targets is/are positive (Ct < 37), and the MS2 control is positive (Ct < 33) or negative (undetermined), the patient sample is reported as positive for SARS-CoV-2 RNA.
- If all three SARS-CoV-2 specific targets (ORF1ab, N, and S) are positive (Ct < 37), and the MS2 control is positive (Ct < 33) or negative (undetermined), the patient sample is reported as positive for SARS-CoV-2 RNA.

Table 3. Interpretation of Patient Results Using the Express Gene 2019-nCoV RT-PCR Diagnostic Panel

ORF1ab	N gene	S gene	MS2 control	Status	Result	Action
NEG	NEG	NEG	NEG	Invalid	NA	Repeat test from residual extracted material. If the result remains invalid, consider collecting a new specimen from the patient, if clinically indicated.
NEG	NEG	NEG	POS Ct < 33	Valid	SARS-CoV-2 Not Detected	Report results to healthcare provider and appropriate public health authorities.
Any 1 or 2 SARS-CoV-2 target(s) = POS Ct < 37			POS or NEG	Valid	SARS-CoV-2 Detected	Report results to healthcare provider and appropriate public health authorities.
POS Ct < 37	POS Ct < 37	POS Ct < 37	POS or NEG	Valid	SARS-CoV-2 Detected	Report results to healthcare provider and appropriate public health authorities.

NEG; Negative; Ct is undetermined or Ct = 40

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

The LoD of the Express Gene 2019-nCoV RT-PCR Diagnostic Panel was determined using quantified, whole viral SARS-related coronavirus 2 (USA-WA1/2020, Heat

Inactivated) material obtained from BEI Resources (NR-52286). The isolate USA-WA1/2020 was inactivated by heating to 65°C for 30 minutes. The preparation included heat inactivated cell lysate and supernatant from Vero E6 cells infected with SARS-CoV-2. A preliminary LoD was determined by testing six concentrations of a 10-fold dilution series (8,000,000 copies/mL to 80 copies/mL) of spiked BEI material (cell lysate) into pooled clinical negative, nasopharyngeal swab matrix, extracted with the MagMAX kit, and tested in triplicate with the Express Gene 2019-nCoV RT-PCR Diagnostic Panel on the QuantStudio 12K Flex instrument.

The initial LoD determination of the Express Gene Panel was 800 copies/mL. The LoD was verified by testing 20 additional extraction replicates consisting of pooled clinical, negative nasopharyngeal swab matrix spiked at 4000, 1600, 800, 320, and 160 copies/mL. Samples were spiked with cell lysate prior to extraction with the MagMAX kit on the KingFisher Flex platform. The LoD of the Express Gene assay was confirmed at 800 copies/mL for the S gene, 320 copies/mL for the ORF1ab region, and 160 copies/mL for the N gene. Results are summarized in Table 4 below.

Table 4. LoD Confirmatory Results Using Nasopharyngeal Swab Matrix

Genome copies/mL	N Gene			ORF1ab Region			S Gene		
	Positives/ Total	Mean Ct	SD	Positives/ Total	Mean Ct	SD	Positives/ Total	Mean Ct	SD
4000	20/20	25.45	1.24	20/20	24.30	2.31	20/20	23.09	2.82
1600	20/20	26.73	1.98	20/20	24.59	3.58	20/20	25.07	3.44
800	20/20	26.68	2.42	20/20	23.98	2.42	19/20	22.48	3.24
320	20/20	30.30	0.76	19/20	29.24	1.73	0/20	UND	NA
160	20/20	31.78	1.22	13/20	30.80	1.72	0/20	UND	NA

UND; Undetermined
NA; Not Applicable

To validate that saliva was an acceptable specimen type for testing with the Express Gene 2019-nCoV RT-PCR Diagnostic Panel, a confirmatory LoD study was completed using saliva that was collected with healthcare provider assistance into the mLife True Oral Fluid/Viral Collection Kit. This study used pooled, negative saliva matrix collected in the mLife collection device that was spiked with heat inactivated BEI material at 4000, 1600, 800, 320, and 160 copies/mL. This material was also used to establish the LoD of nasopharyngeal swab specimens in the original authorization of the Express Gene assay. Twenty individual extraction replicates at the previously mentioned concentrations were run using the Express Gene 2019-nCoV RT-PCR Diagnostic Panel. The data demonstrated that the LoDs for NP swabs and saliva were similar for the Express Gene assay targets. For both NP swabs and saliva specimen types, the LoD of the Express Gene assay was confirmed at 800 copies/mL and 320 copies/mL for the S gene and ORF1ab regions, respectively. There was a slight difference in LoD for the N gene target between NP swabs and saliva. The saliva specimen type had a higher LoD of 320 copies/mL compared to 160 copies/mL for the NP swab for the N gene target specifically.

Table 5. LoD Confirmatory Study Results Using Saliva Matrix Collected with the mLife True Oral Fluid/Viral Collection Kit

Genome copies/mL	N Gene			ORF1ab Region			S Gene		
	Positives/ Total	Mean Ct	SD	Positives/ Total	Mean Ct	SD	Positives/ Total	Mean Ct	SD
4000	20/20	21.15	2.96	20/20	22.45	3.43	20/20	19.79	4.34
1600	20/20	22.34	2.34	20/20	24.34	2.34	20/20	21.23	19.79
800	20/20	24.57	3.57	20/20	26.78	4.56	20/20	23.54	2.34
320	19/20	27.85	2.35	19/20	26.47	3.44	0/20	UND	NA
160	17/20	29.38	3.81	11/20	27.98	3.87	0/20	UND	NA

UND; Undetermined
N/A; Not Applicable

2) **Analytical Inclusivity/Specificity:**

In Silico Analysis of Primer and Probe Inclusivity:

The Express Gene 2019-nCoV RT-PCR Diagnostic Panel utilizes the identical oligonucleotide sequences for the N and S genes and ORF1ab region as those used in the ThermoFisher TaqPath COVID-19 Combo Kit. *In silico* testing of the SARS-CoV-2 assay was previously performed by ThermoFisher as part of their EUA authorization (EUA200010) and this information has been provided in the FDA authorized EUA granted to this manufacturer. Express Gene obtained a right of reference from ThermoFisher to use the *in silico* inclusivity data. Internal *in silico* analysis and *in silico* information provided by ThermoFisher support the original determination that the risk of a false negative result due to primer/probe mismatch is low due to the design of the primers/probes and the PCR cycling conditions.

In Silico Analysis of Primer and Probe Cross-Reactivity:

As stated previously, Express Gene obtained a right of reference from ThermoFisher to incorporate the *in silico* cross reactivity analysis findings. As part of ThermoFisher's EUA, they performed an *in silico* analysis of 42 potentially cross-reactive organisms and determined that there was low risk of non-specific amplification.

In addition to ThermoFisher's cross-reactivity testing, Express Gene performed wet lab testing of closely related respiratory viruses (i.e., MERS, SARS) as well as bacterial organisms that could cause respiratory symptoms. The NATtrol Respiratory Panel 2 (RP2) Controls were purchased from ZeptoMetrix (Cat #NATRPC2-BIO) and spiked into both negative clinical NP swab matrix and negative saliva collected with the mLife kit at 10,000 viral genome copies/mL and tested in triplicate. The Express Gene assay targets showed no cross-reactivity to any of the wet tested respiratory pathogens in either clinical matrix.

3) **Clinical Evaluation:**

Performance of the Express Gene 2019-nCoV RT-PCR Diagnostic Panel was evaluated using 30 previously confirmed positive nasopharyngeal samples and 30 negative nasopharyngeal samples. All clinical samples were previously tested using an EUA authorized RT-PCR molecular assay. Samples were blinded and randomized prior to receiving at Express Gene LLC, DBA: Express Gene Molecular Diagnostics Laboratory. RNA from the clinical

specimens was extracted using the MagMAX Kit and specimens were run on the Express Gene 2019-nCoV RT-PCR Diagnostic Panel. Both positive percent agreement (PPA) and negative percent agreement (NPA) between the 2 assays was 100% (PPA 30/30, NPA 30/30). Results are summarized in Table 6.

Table 6. Performance of Clinical Nasopharyngeal Swabs When Compared to an EUA Authorized RT-PCR Molecular Assay

Nasopharyngeal Swabs		Comparator Assay – EUA Authorized RT-PCR Assay		
		Positive	**Negative**	**Total**
Express Gene 2019-nCoV RT-PCR Diagnostic Panel	**Positive**	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive Percent Agreement		100.0% (30/30); 88.65% - 100.00%*		
Negative Percent Agreement		100.0% (30/30); 88.65% - 100.00%*		

*Two-sided 95% score confidence intervals

Saliva (Paired NP Swab and Saliva Clinical Study):
A study was performed to evaluate the use of saliva as a specimen type for detection of SARS-CoV-2 in patients who were suspected of COVID-19. The study was conducted prospectively with symptomatic patients from one ambulatory care center who provided informed consent and voluntarily participated in the study. The healthcare provider collected a nasopharyngeal swab from each patient and subsequently assisted the patient with collection of the saliva specimen into the mLife True device for parallel testing for SARS-CoV-2. The nasopharyngeal swabs were placed in viral transport medium for shipment to the testing laboratory. Both the saliva and swabs were transported via FedEx overnight shipping at ambient temperature and tested using the Express Gene 2019-nCoV RT-PCR Diagnostic Panel upon receipt at the laboratory (within 48 hours of collection). A summary of the results of the study is presented in Tables 7-8 below.

There was 93.88% positive agreement between the results obtained from testing of saliva and those obtained from the nasopharyngeal swab when using the Express Gene assay. There were three saliva samples that were negative by the Express Gene assay but positive with the paired NP swab samples.

For the positive NP swab and saliva samples that were analyzed, 48/49 (98.0%) NP swab samples and 40/46 (87.0%) saliva samples produced positive results for the N gene. For the S gene target, 44/49 (89.8%) NP samples were positive and 42/46 (91.3%) corresponding saliva samples tested positive. For the ORF1ab target, 46/49 (93.9%) NP samples were positive and 42/46 (91.3%) corresponding saliva samples were positive. According to the result algorithm described in Table 3 above, a sample is considered positive for SARS-CoV-2 RNA if amplification is detected with at least one of the three SARS-CoV-2-specific target sequences.

There was 95.10% negative percent agreement for the paired negative NP swab and

saliva samples that were tested with the Express Gene 2019-nCoV RT-PCR Diagnostic Panel. There were five NP swab samples that tested negative with the Express Gene assay but were positive by their paired saliva. Overall, the results of the prospective clinical evaluation with paired NP swabs and saliva were considered acceptable.

Table 7. Summary of Results Obtained From Parallel Testing of Nasopharyngeal Swab Samples and Saliva from Patients Suspected of COVID-19, Stratified by Measurand

Number of Patients	Sample Type	Analysis	Target (Optical Channel)			
			N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 (JUN)
49 NP positive	NP swab	Positive (%)	48/49 (98.0)	44/49 (89.8)	46/49 (93.9)	49/49 (100)
		Mean Ct	21.29	19.92	20.08	27.57
	Saliva	Positive (%)	40/46 (87.0)	42/46 (91.3)	42/46 (91.3)	46/46 (100)
		Mean Ct	30.09	29.31	29.13	27.60
102 NP negative*	NP swab	Positive (%)	0 (0)	0 (0)	0 (0)	97/97 (100)
		Mean Ct	N/A	N/A	N/A	27.00
	Saliva	Positive (%)	3/5 (60.0)	3/5 (60.0)	2/5 (40.0)	102/102 (100)
		Mean Ct	N/A	N/A	N/A	27.39

*During the prospective clinical studies, there were 5 paired samples that were negative by NP but positive by saliva.
N/A; Not Applicable

Table 8. Combined Results for Paired NP Swab and Saliva Clinical Evaluation

Express Gene 2019-nCoV RT-PCR Diagnostic Panel		Nasopharyngeal Swab		
		Positive	Negative	Total
Saliva	Positive	46	5	**51**
	Negative	3	97	**100**
	Total	49	102	**151**
Positive Percent Agreement		93.88% 46/49); 83.48%-97.90%[1]		
Negative Percent Agreement		95.10% (97/102); 89.0397.89%[1]		

[1]Two-sided 95% score confidence intervals

Clinical Confirmation
The first 5 positive and first 5 negative nasopharyngeal specimens as determined by Express Gene DBA Molecular Diagnostics LLC using the Express Gene 2019-nCoV RT-PCR Diagnostic Panel were also tested by Capstone Healthcare. There was 100% (5/5) positive and negative agreement for the specimens tested. These results are acceptable and support use of the by Express Gene 2019-nCoV RT-PCR Diagnostic Panel for testing clinical specimens.

4) <u>Simulated Shipping Study for Saliva Collected with the mLife True Oral Fluid/Viral Collection Kit:</u>

A simulated shipping study was performed that was designed to evaluate the effect of temperature variation on the stability of SARS-CoV-2 RNA during transport of saliva specimens in the mLife True Oral Fluid/Viral Collection Kit. The shipping study was designed to simulate shipping at extreme temperature conditions that could be experienced during the summer months. See Table 9 for the summer thermal profile that was evaluated in this study.

Table 9. Summer Temperature Excursion

Temperature	Cycle Period	Cycle Period Hours	Total Hours[1]
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

[1] Sum of cycle periods

Simulated shipping studies were performed using contrived samples prepared using pooled known negative patient sample matrix that was spiked with quantified heat inactivated BEI viral material to establish low positives at 2X LoD (LoD previously established at 320 copies/mL) and high positive samples at 5X LoD. For the spiked saliva, samples were received from asymptomatic patients that voluntarily provided saliva samples for the simulated shipping study. Saliva was screened negative using the Express Gene 2019-nCoV RT-PCR Diagnostic Panel and then pooled for use in this experiment.

At the conclusion of the summer thermal profile, samples were equilibrated to room temperature, extracted using the MagMAX kit, and tested with the Express Gene 2019-nCoV RT-PCR Diagnostic Panel. Results were compared to those reported upon initial testing when specimens were prepared at time 0 (day 0, room temperature).

Twenty out of 20 low positive (2X) samples (100%) and 10/10 high positive (5X) contrived samples (100%) were reported as positive after exposure to the summer temperature cycles. The mean and standard deviation of the Ct values for each gene target were similar before and after the simulated shipping scenario (within ~2 Cts), with no evidence of significant degradation of the SARS-CoV-2 RNA. All SARS-CoV-2 negative specimens were reported as negative (non-reactive) after enduring the summer thermal excursion (no amplification of N, ORF1ab, or S genes). Table 10 shows a summary of study results which demonstrated acceptable saliva specimen stability under simulated shipping conditions that could be experienced during the summer.

Table 10. Summary of Results From the Simulated Shipping Study Using Contrived Saliva Samples

Sample Group	Test Point	N	Mean Ct (Standard Deviation)			Positive (%)
			N Gene	ORF1ab	S Gene	
Negative	Day 0 (RT)	10	UND	UND	UND	0 (0)
	Summer	10	UND	UND	UND	0 (0)

Low Positive 2X LoD (640 copies/mL)	Day 0 (RT)	20	28.37 (1.64)	29.35 (1.32)	29.65 1.97	20/20 (100)
	Summer	20	27.67 (2.87)	28.37 1.38	28.23 0.964	20/20 (100)
High Positive 5X LoD (1600 copies/mL)	Day 0 (RT)	10	24.37 (1.42)	21.39 (2.25)	19.55 (1.83)	10/10 (100)
	Summer	10	25.56 (0.97)	22.35 (1.63)	19.68 (2.14)	10/10 (100)

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 RT-PCR instrument used were the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit performed on the KingFisher Flex and the QuantStudio 12K Flex Real-Time PCR System. The results are summarized in the following tables.

Table 11. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Pane l - Nasopharyngeal Swab

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^5$ NDU/mL	N/A
MERS-CoV		N/A	ND

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

Table 12. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Pane l - Saliva

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Saliva	$1.8x10^5$ NDU/mL	N/A
MERS-CoV		N/A	ND

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

LIMITATIONS

- Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

WARNINGS

- This product has not been FDA cleared or approved, but has been authorized by FDA under an EUA for use by the authorized laboratory;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and

- 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 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 declaration is terminated or authorization is revoked sooner.