ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY EXPRESS GENE 2019-NCOV RT-PCR DIAGNOSTIC PANEL (EXPRESS GENE DBA 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 Express Gene LLC dba Molecular Diagnostics Laboratory, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Standard Operating Procedure that was 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 respiratory tract specimens including nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal aspirate/wash or nasal aspirates, and bronchoalveolar lavage (BAL) specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Express Gene LLC (dba Molecular Diagnostics Laboratory) in Palmetto Bay, FL which is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, certified high-complexity laboratory.

Results are for the detection and identification of SARS-CoV-2 RNA. 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 treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with 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

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 one 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 wash/aspirate 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 instrument 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 2.2.3

REAGENTS AND MATERIALS

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
Optical 96-Well Fast Clear Reaction Plates with Barcode	ThermoFisher Scientific	4483485 (20 plates); 4483494 (500 plates)

REAGENTS/CONSUMABLES	SUPPLIER	CATALOG #
MicroAmp Clear Adhesive Film	ThermoFisher Scientific	4306311
MicroAmp Optical Adhesive Film	ThermoFisher Scientific	4311971 (100 pack); 4360954 (25 pack)

CONTROLS TO BE USED WITH THE EXPRESS GENE COVID-19 PANEL

Table 1. Assay Controls 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 (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

The results from the controls are interpreted according to the criteria shown in Table 2. If the results obtained with the Positive, Negative, Internal, and No Template Controls 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 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. Invalidate the run and re-test.

No Template Control (NTC)

- A “no template” (negative) control (NTC) is needed to check for contamination of extraction and assay reagents. Molecular grade, nuclease-free water is used in place of sample nucleic acid for this control. The NTC is used 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.

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 H_2O with the MS2 control spiked into one well of the plate prior to performing each batch of extractions.

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 sufficient and quality RNA to be used in the RT-PCR reactions.

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).

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

- **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.

- **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 in order for the test result to be valid. The positive control does not contain MS2.

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

- **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).

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	<33
Positive Control	<37	<37	<37	Undetermined [1]
No Template Control	Undetermined	Undetermined	Undetermined	Undetermined [1]
MS2 Internal Control	Any	Any	Any	<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 control does not perform as described above, the run is considered invalid and all specimens in the invalid assay are repeated using residual extracted specimen RNA.

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

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. 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.
- 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.
- 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.

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.
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

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

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.

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 negative clinical NP swab matrix 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.

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 at CapStone Healthcare which uses the CDC authorized EUA assay (2019-nCoV Real-Time RT-PCR Diagnostic Panel). Samples were blinded and randomized prior to receiving at Express Gene. 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 5.

Table 5. Performance of Clinical Nasopharyngeal Swabs when Compared to the CDC EUA Authorized Assay

Nasopharyngeal Swabs		Comparator Assay (CDC EUA) - CapStone		
		Positive	**Negative**	**Total**
Express Gene Assay Result	**Positive**	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive Agreement		100.0% (30/30); 88.65% - 100.00%*		
Negative Agreement		100.0% (30/30); 88.65% - 100.00%*		

*2-sided 95% 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 authorized laboratories;
- 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.