ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY OF THE GENE BY GENE SARS-CoV-2 DETECTION TEST

For *In vitro* Diagnostic Use

Rx Only

For use under Emergency Use Authorization (EUA) only

(The SARS-CoV-2 Assay will be performed at the Gene By Gene's laboratory located at 1445 North Loop, West Suite 760, Houston, TX 77008, which is certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the laboratory procedures that were reviewed by the FDA under this EUA.)

INTENDED USE

The Gene By Gene SARS-CoV-2 Detection Test is a Real-Time RT-PCR test *(rRT-PCR)* intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasal swab and nasopharyngeal swab specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Gene By Gene's laboratory located at 1445 North Loop West Suite 760, Houston, TX 77008, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263 and meets requirements to perform high complexity tests.

Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory tract specimens during the acute phase of infection. Positive results are indicative of the presence of the SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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

The Gene By Gene SARS-CoV-2 Detection Test is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Gene By Gene SARS-CoV-2 Detection Test 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 based on the EUA FDA issued for the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel. The SARS-CoV-2 primer and probe sets are designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines.

Nasal swab specimens are collected in RNAlater Stabilization Solution. Nucleic acid is extracted on the QIAGEN QIAcube HT using the miRNeasy Serum/Plasma Advanced. Real time RT-PCR is performed using Bio-Rad's Reliance 1-Step Multiplex Supermix on Bio-Rad's CFX384 Touch Real-Time PCR Detection System and data analyzed using the Bio-Rad CFX Maestro Software for CFX Real-Time PCR Instruments (version 4.1.2433.1219).

The Gene By Gene SARS-CoV-2 Detection Test uses the same primer and probe sequences as those in the FDA-authorized CDC 2019-Novel Coronavirus (2019- nCoV) Real-Time RT-PCR Diagnostic Panel (EUA200001) and is intended to amplify and detect two different regions of the SARS-CoV-2 nucleocapsid gene (N1 and N2). **Table 1** provides details of the primer and probe sequences.

Table 1. Target sequences for Detection of SARS-CoV-2

Name	Description	Oligonucleotide Sequence (5' to 3')
SARS-CoV-2_N1-F	2019 nCoV_N1 Forward Primer	GAC CCC AAA ATC AGC GAA AT
SARS-CoV-2_N1-R	2019 nCoV_N1 Reverse Primer	TCT GGT TAC TGC CAG TTG AAT CTG
SARS-CoV-2_N1-P	2019 nCoV_N1 Probe	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1
SARS-CoV-2_N2-F	2019-nCoV_N2 Forward Primer	TTA CAA ACA TTG GCC GCA AA
SARS-CoV-2_N2-R	2019-nCoV_N2 Reverse Primer	GCG CGA CAT TCC GAA GAA
SARS-CoV-2_N2-P	2019 nCoV_N2 Probe	FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1
RP-F	Human RNAseP (RP) Forward Primer	AGA TTT GGA CCT GCG AGC G
RP-R	Human RNAseP (RP) Reverse Primer	GAG CGG CTG TCT CCA CAA GT
RP-P	Human RNaseP (RP) Probe	FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1

INSTRUMENTS USED WITH TEST

The SARS-CoV-2 Detection Test is to be used with the:

- Qiagen QIAcube HT/QIAxtractorExtraction workcell
- Tecan Evo 8span Liquid Handler
- Beckman NX 8span Liquid Handler
- Beckman FX 96head Liquid Handler
- Bio-Rad CFX384 Touch Real-Time PCR Detection System
- Bio-Rad CFX Maestro Software for CFX Real-Time PCR Instruments (version 4.1.2433.1219)

REAGENTS AND MATERIALS

Table 2. Reagents and Materials used for Gene By Gene SARS-CoV-2 Detection Test

Reagent	Manufacturer	Catalogue#
RNAlater Stabilization Solution	ThermoFisher Scientific (Invitrogen)	AM7021
miRNeasy(Serum/Plasma Advanced Kit (50)	Qiagen	217204
RNA Lysis Buffer (RLT buffer)	Qiagen	79216
Isopropyl Alcohol 4L	VWR	EM-PX1835-7
Reliance 1-Step Multiplex Supermix, 5x1 mL	Bio-Rad	12010220
2019-nCoV N Positive Control	IDT	1000 6625
Human Specimen Control	---	----
2019-nCoV N1 Forward primer	IDT	10006606
2019-nCoV N1 Reverse primer	IDT	10006606
2019-nCoV Nl Probe (FAM/BHQ)	IDT	10006606
2019-nCoV N2 Forward primer	IDT	10006606
2019-nCoV N2 Reverse primer	IDT	10006606
2019-nCoV N2 Probe (FAM/BHQ)	IDT	10006606
Human RNase P Forward Primer	IDT	10006606
Human RNase P Reverse Primer	IDT	10006606
Human RNase P Probe (FAM/BHQ)	IDT	10006606
384 well PCR plate	Bio-Rad	HSP3805
MicroSeal 'B' Film	Bio-Rad	MSBI00l

CONTROLS TO BE USED WITH THE SARS-CoV-2 DETECTION TEST

1. **Negative Template Control (NTC):** Nuclease-free water is used starting from the extraction process to the rRT-PCR assay to ensure no nucleic acid contamination has occurred to reagents through the whole pipeline.

2. **Human Specimen Control (Extraction Control):** A negative human specimen control (HSC) will be used as an RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. The HSC was generated from a pool of negative samples. The HSC RNA contains human Ribonuclease P (RNase P) DNA/RNA that is detected by the rRT-PCR assay. The HSC monitors for nucleic acid extraction, amplification and detection, including improper assay setup and execution, as well as reagent and equipment malfunction.

3. **Internal Process Control**: The human RNAse P assay will detect the endogenous RNAse P gene to monitor specimen adequacy and integrity.

4. **Positive Control:** 2019-nCoV_N Positive Control plasmid [IDT, (10006625)] which contains a cloned copy of the N gene of SARS-CoV-2 is dispensed in the designated positive control wells during assay set up and should be detected by the SARS_CoV_N1 and SARS_CoV_N2 primers and probes. The Positive Control is intended to monitor the integrity of the PCR reagents and process.

INTERPRETATION OF RESULTS

1) SARS-CoV2- Assay-Controls – Positive (s), Negative, and Internal

Controls must be evaluated prior to reviewing patient results as a quality control measure. If the controls are not valid, the patient results cannot be interpreted. The thresholds to determine a positive and negative signal for each of the targets are presented in **Table 3.**

The following results for the controls are expected and failure for any of these controls would result in repeating the whole assay (extraction and the Real-Time PCR) for all samples as shown in **Table 3A**.

1. **Negative Template Control (NTC):** There should be no fluorescence detected as any signal would be indicative of sample contamination. No Cq value (Not a Number - NaN) should be observed.

2. **Positive Control:** SARS-CoV-2_N1 and SARS-CoV-2_N2 should both be positive and within 45 cycles (ca. 28 Cq). A negative signal could indicate an issue with the control reagent, primer/probe or rRT-PCR reagents (i.e., master mix).

3. **Extraction Control/Internal Process Control:** The HSC should be positive for RP and detected within 45 cycles (ca. 30 Cq). A negative signal could indicate a problem with the nucleic acid extraction process, or issues with RP primer and probe or rRT-PCR reagents.

Table 3. Quantification Cycle (Cq) Thresholds for SARS-CoV-2 Detection Test

Target	Negative Cq[1] (-)	Positive Cq (+)
SARS-CoV-2_N1	NaN[2]	< 45
SARS-CoV-2_N2	NaN	< 45
RP	NaN	< 40

[1]Quantification cycle (Cq),
[2]No Cq value obtained (Not a Number – NaN)

Table 3A. Interpretation of SARS-CoV2 Detection Test Controls

Control Type	Control Name	Used to Monitor	SARS-CoV-2_N1	SARS-CoV-2_N2	RP	Expected Cq Values
Positive	Positive Control	Reagent integrity	+	+	-	<45
Negative	NTC	Reagent and or environmental contamination	-	-	-	Not Detected (NaN)[1]
Extraction	HSC	Problems/failure in NA extraction procedure	-	-	+	<40

[1]No Cq value obtained (Not a Number – NaN)

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. **Table 4** below lists the expected results for the SARS-CoV2 Assay.

Table 4. Interpretation of SARS-CoV-2 Detection Test Results

SARS-CoV-2_N1	SARS-CoV-2_N2	RP	Result Interpretation	Report	Action
+	+	+ or -	SARS-CoV-2 Detected	Positive 2019-nCoV	Report as Positive and send results to client* and CDC
-	-	+	SARS-CoV-2 Not Detected	Negative	Report as Negative and send results to client*. Consider testing for other respiratory viruses
+	-	+ or -	Indeterminate	Indeterminate	Repeat testing of nucleic acid and/or re-extract and repeat rRT-PCR. If the repeated result remains inconclusive, contact your State Public Health Laboratory or CDC for instructions for transfer of the specimen or further guidance.
-	+	+ or -	Indeterminate	Indeterminate	Repeat testing of nucleic acid and/or re-extract and repeat rRT-PCR. If the repeated result remains inconclusive, contact your State Public Health Laboratory or CDC for instructions for transfer of the specimen or further guidance.
-	-	-	Invalid Result	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

*The reporting will be performed as specified by the individual public health authorities

G. PERFORMANCE EVALUATION

1) Limit of Detection (LoD) -Analytical Sensitivity:

The Limit of Detection (LoD) was determined by first performing a preliminary study using 30 independent negative nasal swab specimens collected in RNAlater. Each sample was spiked with diluted synthetic SARS-CoV-2 RNA (Twist Bioscience, MN908947.3 (SKU 102024))**.** Three independent samples were used for each dilution and extracted using the QIAcube HT and miRNeasy (Serum/Plasma Advanced Kit). Data acquisition was performed with the Bio-Rad CFX384 Touch Real-Time PCR Detection System and data analysis using the Bio-Rad CFX Maestro Software for CFX Real-Time PCR Instruments (version 4.1.2433.1219). The preliminary LoD was 6.25 copies/ µL.

A confirmatory LoD study was performed using nasal swab specimens containing 6.25, 3.13, 1.56, or 1.56 copies/µL of synthetic SARS-CoV-2 RNA in 20 replicates **(Table 5).** The LoD was defined as the lowest concentration of SARS-CoV-2 (genome copies/µL) that can be detected by the COVID-19 RT-PCR test at least 95% of the time. The LoD for the SARS-CoV-2 Detection Test was 6.25 copies/µL (20/20 positive).

Table 5. Limit of Detection Confirmation for SARS-CoV-2 Detection Test

Targets	SARS-CoV-2_N1				SARS-CoV-2_N2				RP			
SARS-CoV-2 RNA Copies/µL	6.25	3.13	1.56	0.78	6.25	3.13	1.56	0.78	6.25	3.13	1.56	0.78
Positive/Total	20/20	15/20	11/20	8/20	20/20	19/20	12/20	9/20	20/20	20/20	20/20	20/20
Mean Cq	37.6	38.5	38.5	38.6	36.5	37.4	37.9	38.9	30.4	31.0	32.2	33.1
SD	0.9	0.6	0.8	0.6	0.9	0.8	1.0	0.9	0.8	0.4	0.4	0.7

2) Inclusivity:

The sequences for the N1, N2 primers/probes used in this assay are identical to the N1, N2 primers/probes sequences used in the FDA authorized original CDC 2019-Novel Coronavirus (2019-nCoV) real time RT-PCR Diagnostic Panel.

Given the dramatic increase in available genomic information pertaining to SARS-CoV-2 in publicly available databases, an *in-silico* inclusivity analysis was performed, aimed at determining the potential alignment capability of the CDC-defined primers and probes relative to the numerous publicly available SARS-CoV-2 sequences.

NCBI's Blastn tool was used to evaluate the SARS-CoV-2 primer and probes using the Betacoronvirus database on May 20, 2020 with the search details and parameters shown in **Table 6A.**

Table 6A. Blastn settings

Database	Betacoronavirus nucleotide sequence data
Update date	2020/05/20
Number of sequences	11768
Max target sequences	5000
Expect threshold	1000
Match and mismatch scores	1, -3, respectively
Gap cost	Existence: 5, Cost: 2

Results were further filtered using: Severe acute respiratory syndrome coronavirus 2 (taxid:2697049) to only look at SARS-CoV-2 sequences. The total number of sequences aligned for each of the primer/probes which did not have 100% match were further investigated. Mismatches that were due to unidentified bases were excluded from the analysis. The primers and probes with sequence mismatches are outlined in **Table 6B** below. The locations of mismatches are not near the 3' end region of the primers and are in most cases internal; thus it was determined that the potential impact on assay performance is minimal.

Table 6B. Primers and Probes with sequence mismatches

Target	Accession	% Alignment	# of Mismatch	Region	Position
N1 Forward	MT350243.1	95	1	28281-28300	28294
N1 Reverse	MT293178.1	95.8	1	28327-28304	28309
N1 Probe	MT326026.1	95.8	1	27954-27977	27983
N2 Forward	MT496984.1	95.0	1	29116-29135	29129
N2 Reverse	MT451875.1	94.4	1	29189-29172	29177
N2 Reverse	MT385471.1	94.4	1	29218-29201	29206
N2 Reverse	MT385481.1	94.4	1	29214-29197	29202
N2 Reverse	MT385487.1	94.4	1	29214-29197	29202
N2 Reverse	MT385495.1	94.4	1	29209-29192	29197
N2 Probe	MT326031.1	95.7	1	29059-29081	29065
N2 Probe	MT412284.1	95.7	1	29158-29180	29169

3) Cross-reactivity (Analytical Specificity)

The analytic specificity of the Gene By Gene SARS-CoV2 Detected Test was demonstrated *in silico* under the original EUA for the CDC 2019-Novel Coronavirus (2019-nCov) Real-Time RT-PCR Diagnostic Panel. The data demonstrated that the expected results were obtained for each organism when tested. Refer to the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel FDA Emergency Use authorization.

4) <u>Clinical Evaluation</u>:

Clinical performance was evaluated using 35 positive and 36 negative nasopharyngeal swab specimens from patients suspected of COVID-19. Frozen samples were collected in Viral Transport Media (VTM) or PBS and provided by the Houston Health Department (HHD) which initially tested these specimens using the CDC assay (EUA200001/A002).

All positive and negative samples tested with the SARS-CoV-2 Detection Test were in 100% agreement to results obtained by HHD (**Table 7**).

Table 7. Performance of the SARS-CoV-2 Detection Test vs. the FDA EUA CDC Test

Nasopharyngeal Swabs		CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC)		
		Positive	**Negative**	**Total**
Gene BY Gene SARS-CoV-2 Detection Test	**Positive**	35	0	**35**
	Negative	0	36	**36**
	Total	**35**	**36**	**71**
Positive Agreement		100% (35/35), 95%CI: (90.1-100%)[1]		
Negative Agreement		100% (36/36); 95%CI: (90.3-100%)		

[1] Two-sided 95% score confidence intervals

<u>Contrived Testing:</u>

Performance of the Gene By Gene SARS-CoV2 Assay also was evaluated using clinical nasal swab specimens (obtained from the Meir hospital, Kfar Saba, Israel) in RNAlater that were spiked with synthetic SARS-CoV-2 RNA (Twist Bioscience) .

In total 31 negative clinical matrix and 34 positive contrived were tested. Of the 34 contrived positive samples, 24 were prepared at 1.5X LoD (9.38 copies//µL), five at 500 copies/µL and five at 50 copies/µl.

The results at all tested levels for spiked positives in clinical matrix demonstrated 100% agreement with expected results and all negative samples were non-reactive. A summary of the results of the study is provided in **Table 7A**.

Table 7A. Summary of contrived nasal swab study of SARS-CoV2 Assay

Nasal Swabs		**Contrived Nasal Swab Specimen**		
		Positive	**Negative**	**Total**
Gene By Gene SARS-CoV2 Detection Test	**Positive**	34[2]	0	**34**
	Negative	0	31	**31**
	Total	**34**	**31**	**65**
Positive Agreement		100 (34/34), 95% CI (89.8%-100%)[1]		
Negative Agreement		100 (31/31), 95% CI (88.9%-100%)[1]		

[1] Two-sided 95% score confidence intervals

[2]One sample did not have RP detected, but as both SARS-CoV-N1 and SARS-CoV-N2 are positive, the results is considered acceptable based on patient interpretation guidelines (Table 4)

5) Transport Media Equivalency Study:

To leverage the clinical testing data that was performed using NP swabs specimen collected in VTM, a transport media equivalency study was performed comparing negative nasal swab specimens collected in RNAlater Stabilization Solution (RNAlater) and a simulated negative swab specimen in Viral Transport Media (VTM).

A previously SARS-CoV-2 positive specimen was used to generate contrived positive samples. Viral concentration was first quantified through the standard curve method. The positive sample was diluted in 10mM Tris to a working stock of 10,000 copies/µL. Specimens were spiked at 25, 12.5, 6.25 (established LoD), 3.125 and 1.56 copies/µL SARS-CoV-2. To do this, 1,995 µL of a negative specimen in RNAlater or VTM was first spiked with 5 µL of the SARS-CoV-2 working stock virus to generate a 25 copies/µL positive sample. A 2x serial dilution was subsequently performed by taking 1 mL of the contrived positive and diluting with an equal volume of a negative sample in the respective medium. A sample volume of 150 µL was taken from each sample for extraction.

Samples were extracted using the Qiagen QIAcube HT system with miRNeasy serum/plasma advanced kit in 5 replicates followed by rRT-PCR using the Bio-Rad CFX384 Touch Real-Time PCR detection system and analyzed with Bio-Rad CFX Maestro™ Software (version 4.1.2433.1219).

The results of this study showed that both RNAlater and VTM samples spiked at 2X LoD (12.5 copies/µL) had 100% positivity (5/5) (**Table 8A, 8B**). Because the LoD with VTM was shown to be similar to that with RNAlater (i.e., <3-fold higher), the clinical performance observed with samples in VTM (see Section G(4) above) is considered

representative of that which can be expected with samples collected in RNAlater. These results therefore support the use of samples that are transported and stored in RNAlater with the Gene By Gene SARS-CoV-2 Detection Test.

Table 8A: Results for Nasal Specimens Collected in RNAlater

	SARS-CoV-2 N1		SARS-CoV-2 N2		RP		
SARS-CoV-2 copies/µL	**Avg Cq**	**SD**	**Avg Cq**	**SD**	**Avg Cq**	**SD**	**Positive**
25	35.1	0.3	34.7	0.5	28.5	0.2	5/5
12.5	36.7	0.8	35.9	0.7	28.4	0.2	5/5
6.25	37.5	0.7	37.3	0.9	28.4	0.1	5/5
3.125	38.1	0.6	37.9	0.8	28.4	0.2	4/5
1.56	39.1	--	38.4	1.0	28.3	0.1	1/5

Table 8B: Results for Nasal Specimen Collected in VTM

	SARS-CoV-2 N1		SARS-CoV-2 N2		RP		
SARS-CoV-2 copies/µL	**Avg Cq**	**SD**	**Avg Cq**	**SD**	**Avg Cq**	**SD**	**Positive**
25	36.0	0.8	35.0	0.3	28.6	0.2	5/5
12.5	36.8	0.8	36.6	0.9	28.8	0.2	5/5
6.25	37.9	0.3	37.6	0.6	28.6	0.3	3/5
3.125	38.8	0.5	38.1	1.1	28.5	0.3	4/5
1.56	39.2	0.16	38.9	0.6	28.6	0.3	4/5

Stability Study for Specimens in RNAlater Stabilization Solution

A sample stability study was conducted to confirm that positive nasal swabs specimens collected in RNAlater and shipped at room temperature (25°C) for testing after 24h, will have minimal to no loss of signal.

Negative nasal swab specimens were spiked with 2x LoD (12.5 copies/µL) SARS-CoV-2 at different time points (48h, 32h and 24h) and held at room temperature prior to processing. Each sample was extracted using the Qiagen QIAcube HT system with miRNeasy serum/plasma advanced kit in five replicates followed by rRT-PCR using the Bio-Rad CFX384 Touch Real-Time PCR detection system and analyzed with Bio-Rad CFX Maestro Software (version 4.1.2433.1219).

There was 100% agreement with expected results as all samples remained positive after 24, 32 and 48 hours of incubation at room temperature (**Table 9**).

This study demonstrates that samples shipped in RNAlater remained stable for up to 48h at room temperature with no apparent degradation of SARS-CoV-2 detection.

Table 9: SARS-CoV-2 Specimen Stability in RNAlater

	SARS-CoV-2 N1		SARS-CoV-2 N2		RP		
Time (hr)	**Avg Cq**	**SD**	**Avg Cq**	**SD**	**Avg Cq**	**SD**	**% Agreement**
24	37.1	0.9	37.2	0.8	31.8	0.5	100, (5/5), 95% CI (47.8.4-100)[1]
32	37.9	0.8	38.2	0.8	31.8	0.9	100, (5/5), 95% CI (47.8-100)
48	37.3	0.6	36.7	0.4	31.5	0.1	100, (5/5), 95% CI (47.8-100)

[1] Clopper-Pearson exact

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.