EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
THE GRAVITY DIAGNOSTICS COVID-19 ASSAY
(GRAVITY DIAGNOSTICS, LLC)

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

(The Gravity Diagnostics COVID-19 ASSAY will be performed at Gravity Diagnostics, LLC, Covington, KY certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a to perform high complexity tests as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The Gravity Diagnostics COVID-19 Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal, nasopharyngeal (NP) and oropharyngeal (OP) swab and Bronchoalveolar Lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider.

This test is also for use with nasal swab specimens that are self-collected at home or in a healthcare setting by individuals using an authorized home-collection kit when determined to be appropriate by a healthcare provider.

Testing is limited to Gravity Diagnostics, LLC certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

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

The Gravity Diagnostics COVID-19 Assay is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time

PCR and in vitro diagnostic procedures. The Gravity Diagnostics COVID-19 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization**.**

SPECIAL CONDITIONS FOR USE

For Emergency Use Authorization (EUA) only
For prescription use only
For in vitro diagnostic use

This assay can be used with the Everlywell COVID-19 test home collection kit. Everlywell has granted Gravity Diagnostics, LLC a right of reference to the data supporting use of this collection kit.

This assay can be used with the Kroger Health COVID-19 Test Home Collection Kit. The Kroger Co. has granted Gravity Diagnostics, LLC a right of reference to the data supporting use of this collection kit.

INSTRUMENTS USED WITH TEST

The Gravity Diagnostics COVID-19 Assay is to be used with the King Fisher Flex with 96 Deep Well Head instrument using the Magmax Pathogen Kit RNA/DNA for nucleic acid extraction and the Applied Biosystems QuantStudio7 Flex instrument with software version 1.3 or Applied Biosystems QuantStudio12 Flex instrument with software version 1.2.2 for real-time PCR Detection.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Gravity Diagnostics COVID-19 Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The test uses two primer and probe sets, which are identical to those described in the CDC emergency use authorized assay, the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel, to detect two regions in the SARS-CoV-2 nucleocapsid (N) gene and one primer and probe set to detect human RNase P (RP) in a clinical sample. The primers/probes, used to detect the CDC N2 target and RP target, are combined in the same reaction well. RNA isolated from nasal, NP and OP swabs and BAL is reverse transcribed to cDNA and subsequently amplified using Applied Biosystems QuantStudio7 Flex instrument (QS7) with software version 1.3 or QuantStudio12 Flex (QS12) instrument with software version 1.2.2. 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 (FAM) to separate from the quencher dye (BHQ1), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by QS7 or QS12.

REAGENTS AND MATERIALS

Provided with the Gravity Diagnostics COVID-19 ASSAY

Reagent	**Manufacturer**	**Catalog #**
Magmax Pathogen Kit RNA/DNA	Life Technologies	4462359

TaqPath 1-Step RT-PCR Master Mix, GC (no ROX)	ThermoFisher	A28522
COVID-19_N1-F Primer	IDT	Custom
COVID-19_N1-R Primer	IDT	Custom
COVID-19_N1-P Probe	IDT	Custom
COVID-19_N2-F Primer	IDT	Custom
COVID-19_N2-R Primer	IDT	Custom
COVID-19_N2-P Probe	IDT	Custom
RP-F Primer	IDT	Custom
RP-R Primer	IDT	Custom
RP-P Probe	IDT	Custom
COVID-19_N_Positive Control	IDT	Custom

Required but Not Provided with the Gravity Diagnostics COVID-19 ASSAY

Item	**Manufacturer**	**Catalog #**
King Fisher Flex Purification System	Fisher	711-2727
QuantStudio 7 Flex	Applied Biosystems	4485701
Quant Studio 12 Flex	Applied Biosystems	4471086
Applied Biosystems Optical Reaction Plate	ThermoFisher	N801-560
Magmax 96 standard well plates	Life Technologies	97002540
Magmax 96 deep well plates	Life Technologies	95040460
384 well PCR plates	Fisher	4309849
Sorvall T1 centrifuge	Thermo/Fisher	750002382
Mini vortex		

CONTROLS TO BE USED WITH THE COVID-19 RT-PCR

a) A positive template control (PTC) is needed to verify that the assay run is performing as intended and is used on every assay plate starting at master mix addition at a concentration of 1250 cp/µL. The positive control is the 2019-nCoV_N_Positive Control (Integrated DNA Technologies, CAT#: 10006625) containing a DNA sequence of the nCoV nucleocapsid gene which is the target of the three CDC designed assays. A separate LoD study was performed in order to determine the optimal concentration for the control plasmid (EUA200001).

b) An internal control (RP) targeting RNase P is needed to verify that nucleic acid is present in every sample and is used for every sample processed. Because control *(a)* is a DNA template, this serves as a control to ensure that the reverse transcription step is proceeding as intended. This also serves as the extraction positive control to ensure that samples resulting as negative contain nucleic acid for testing.

a) A no template control (NTC) is needed to verify that there is no contamination in the assay run and is used on every assay plate starting at master mix. The NTC consists of no-sample elution buffer from the MagMax Pathogen RNA/DNA Kit.

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.

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

   PC – positive for all SARS-CoV-2 targets (Ct < 40)
   RP – negative for SARS-CoV-2 targets (Ct Not Detected), positive for RNase P (RP) target (Ct < 40)
   NTC – negative for SARS-CoV-2 targets (Ct Not Detected) and RP (Ct < 40)

   If any control does not perform as described above, run is considered invalid and all specimens are repeated from extraction step.

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

   Note on RP internal control: all clinical samples should yield positive results for RP target at < 40 Ct. Samples that fail to show detection of RP within this range and both SARS-CoV-2 targets should be repeated from extraction step. If the sample detects any of the SARS-CoV-2 targets, the lack of amplification of RP target can still be valid.

N1 Target (positive for Ct < 40)	N2 Target (positive for Ct < 35)	RP Target (positive for Ct < 40)	Results Interpretation	Action
If only one or both of targets positive		±	POSITIVE	Report result to sender and appropriate health authorities
-	-	+	NEGATIVE	Report result to sender
-	-	-	INVALID	Repeat extraction and RT-PCR. If additional clinical sample is unavailable, report INVALID

VALIDATION OF A COMPARATOR ASSAY

In order to evaluate the clinical performance of the Gravity Diagnostics COVID-19 assay, the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel was first validated for use with the Magmax Pathogen Kit RNA/DNA and the QS7 and QS12 instruments, so that it could be used as the comparator assay. This comparator assay is referred to as the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-

Time RT-PCR Diagnostics Panel for the remainder of this document. The results of this validation are described below.

1) Analytical Sensitivity:

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies(cp)/µL) that can be detected by the s modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel at least 95% of the time. The LoD study was performed on both the QS7 and the QS12. The preliminary LoD was established by testing 10-fold dilutions of Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WA1/2020 (BEI Resources Cat#: NR-52285, Lot#: 70033320) in liquid Amies media, in quintuplicate.

The preliminary LoD was confirmed by testing 20 replicates of 2-fold dilutions (4.8 cp/µL and 2.4 cp/µL). The samples of 2-fold dilutions were prepared by spiking the Genomic RNA into nasopharyngeal (NP) clinical matrix collected in Liquid Amies media, presumed negative. By applying the decision algorithm, that only one out of three targets need to test positive for a positive SARS-CoV-2 call to the results in Table 2, an LoD for the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel of 2.4 cp/µL, summarized in Table 3, is determined for both the QS7 (19/20 positive) and the QS12 (20/20 positive).

Table 2. Result of the Analytical Sensitivity for the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel, Stratified by Target

	QuantStudio 7		QuantStudio 12 Flex	
	4.8 cp/µL	2.4 cp/µL	4.8 cp/µL	2.4 cp/ µL
N1	20/20	19/20	20/20	18/20
N2	20/20	18/20	20/20	20/20
N3	20/20	20/20	20/20	19/20

Table 3. Result of the Analytical Sensitivity for the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel

	QuantStudio 7		QuantStudio 12 Flex	
	4.8 cp/µL	2.4 cp/µL	4.8 cp/µL	2.4 cp/µL
SARS-CoV-2 RNA detected	20/20	19/20	20/20	20/20

2) Cross-reactivity:

The primer/probe set for the N1, N2, and N3 SARS-CoV-2 targets were designed by the CDC which conducted cross-reactivity testing. The data from this analysis is available in the FDA EUA "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel"

Cross-reactivity was also assessed by testing 60 nasopharyngeal swab patient specimens, positive for the organisms or viruses listed in Table 4. Organisms and viruses were identified by Real-Time RT-PCR analysis in Gravity Diagnostics

Laboratory Developed Test for Upper Respiratory Pathogen Panel. Each sample was then assayed using the Gravity Diagnostics COVID-19 Assay. All 60 clinical specimens tested negative for the N1, N2, and N3 targets, while positive for the RNase P assay.

Table 4. Organisms present in clinical samples tested with the Gravity Diagnostics COVID-19 Assay.

Organism Present in Clinical Sample	# of Positive Clinical Samples tested
Adenovirus	1
H1N1 Influenza A	3
Haemophilus influenzae	13
Human coronavirus 229E	5
Human coronavirus NL63	7
Human coronavirus OC43	1
Human coronavirus HKU1	2
Human Metapneumovirus (hMPV)	5
Influenza B	2
Klebsiella pneumoniae	1
Moraxella catarrhalis	14
Negative	5
Parainfluenza virus 4	2
Respiratory syncytial virus	5
Rhinovirus	11
Staphylococcus aureus	27
Streptococcus pneumoniae	12

3) Clinical Evaluation:

A contrived clinical study was performed to evaluate the performance of the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel. A total of 60 individual nasopharyngeal specimens, collected in Liquid Amies Media, presumed negative for SARS-CoV-2, were used in this study. Sixty nasopharyngeal swab clinical samples were tested using the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel on the QuantStudio 12K Flex. All specimens tested negative for SARS-CoV-2 RNA. Viral genomic RNA, at a concentration of 5X LoD (12 cp/µL) and 2X LoD (4.8 cp/µL), was spiked into 10 and 20, respectively, of the confirmed-negative NP swab specimens and then tested using the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel on the QuantStudio 12K Flex. The remining 30 negative clinical sample and the 30 contrived samples were tested on the QS12 and QS7 using the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel. The positive and negative percent agreements between the Gravity Diagnostics COVID-19 Assay and the expected results in NP swabs are shown below for each instrument:

Table 5. Clinical Performance of the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel on The QS12, using contrived specimens:

SARS-CoV-2 concentration	Number of NP swabs	N1 target % Positive (95% CIs)	N2 target % Positive (95% CIs)	N3 target % Positive (95% CIs)
2x LoD	20	20/20 100% (83.9 – 100)	20/20 100% (83.9 – 100)	20/20 100% (83.9 – 100)
5x LoD	10	10/10 100% (72.3 – 100)	10/10 100% (72.3 – 100)	7/10[b] 70% (39.7 – 89.2)
Negative	60	0/60 (NA)	0/60 (NA)	0/60 (NA)

NA = Not available

[b]three of the ten samples failed detection of N3 target. Since the N1 and N2 targets are detected, the overall result for this sample was "POSITIVE". The ten samples, prepared at 5x LoD, were made by spiking genomic viral RNA directly into nasopharyngeal matrix, rather than into matrix mixed with lysis buffer, as was done for the 2x LoD samples. The missed N3 targets may, therefore, be due to the RNA instability in matrix without lysis buffer.

Performance of the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel on the QS12 against the expected results are:

Positive Percent Agreement 30/30 = 100% (95% CI: 88.6% - 100%)
Negative Percent Agreement 60/60 = 100% (95% CI: 94.0% - 100%)

Table 6. Clinical Performance of the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel on the QS7:

SARS-CoV-2 concentration	Number of NP swabs	N1 target % Positive (95% CIs)	N2 target % Positive (95% CIs)	N3 target % Positive (95% CIs)
2x LoD	20	20/20 100% (83.9 – 100)	20/20 100% (83.9 – 100)	20/20 100% (83.9 – 100)
5x LoD	10	10/10 100% (72.3 – 100)	10/10 100% (72.3 – 100)	7/10[c] 70% (39.7 – 89.2)
Negative	30	0/30 (NA)	0/30 (NA)	0/30 (NA)

NA = Not available

[c] three of the ten samples failed detection of N3 target. Since the N1 and N2 targets are detected, the overall result for this sample was "POSITIVE". The ten samples, prepared at 5x LoD, were made by spiking genomic viral RNA directly into nasopharyngeal matrix, rather than into matrix mixed with lysis buffer, as was done for the 2x LoD samples. The missed N3 targets may, therefore, be due to the RNA instability in matrix without lysis buffer.

Performance of the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel on QS7 against the expected results are:

Positive Percent Agreement 30/30 = 100% (95% CI: 88.6% - 100%)
Negative Percent Agreement 30/30 = 100% (95% CI: 88.6% - 100%)

Additionally, five positive and five negative patient samples were sent to the Kentucky Public Health Lab (KYPHL) and tested on the CDC assay under an EUA. All results were concordant, except for sample 10. The Ct values for the N1, N2, and N3 targets, determined for sample 10 by the Gravity Diagnostics COVID-19 Assay, were 36.1, 38.0, and 35.8, respectively. The nucleic acid extracted using the Magmax Pathogen Kit RNA/DNA by Gravity Diagnostics, LLC, from sample 10, was then sent to the KYPHL and tested positive for SARS-CoV-2.

Sample	Gravity Test Date	Result	KYPHL Test Date	Result
1	3/16/2020	Negative	3/18/2020	Negative
2	3/16/2020	Negative	3/18/2020	Negative
3	3/16/2020	Negative	3/18/2020	Negative
4	3/17/2020	Negative	3/20/2020	Negative
5	3/17/2020	Negative	3/20/2020	Negative
6	3/18/2020	Positive	3/20/2020	Positive
7	3/17/2020	Positive	3/20/2020	Positive
8	3/19/2020	Positive	3/23/2020	Positive
9	3/25/2020	Positive	3/27/2020	Positive
10	3/25/2020	Positive	3/27/2020	Negative[d]

[d]extracted nucleic acid was sent to the Kentucky Public Health Lab on 3/31/2020 and tested positive for SARS-CoV-2.

PERFORMANCE EVALUATION OF THE GRAVITY DIAGNOSTICS COVID-19 ASSAY

1) Comparative Analytical Sensitivity Study:

An analytical sensitivity study was performed in order to demonstrate equivalency between the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel and the Gravity Diagnostics COVID-19 Assay. Three three-fold dilutions of viral genomic RNA (BGI) were spiked, in triplicate, into liquid Amies Media. Samples were extracted using the Magmax Pathogen Kit RNA/DNA on the KingFisher extraction system and run in parallel on the QS12. The study results, summarized in tables 9, 10, and 11 below, demonstrate similar analytical sensitivity for the N2 target in both assays. The results of the N1 target, the procedure for which does not differ between both assays, are shown in Table 9. The risk of low-titer patient positive samples testing analytically-negative by setting the N2 target

cutoff at a Ct of 35 is mitigated by continuing to assay the N1 target, in a separate reaction well, with a cutoff at a Ct of 40.

Table 9. Results for the N1 target Using Both Assays.

Targets	N1 Target				
RNA Concentration (cp/µL)	46	15	5.1	2.6	1.7
Positives (n/3)	3/3	3/3	3/3	3/3	3/3
Mean Ct	29.1	31.2	32.5	34.1	33.8
Standard Deviation (Ct)	0.4	0.2	0.6	0.7	1.1

Table 10. Results for Analytical Sensitivity of the N2 and RP Targets Using the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel.

Targets	N2 Target					RP target		
RNA Concentration (cp/µL)	46	15	5.1	2.6	1.7	5.1	2.6	1.7
Positives (n/3)	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Mean Ct	31.3	33.4	34.1	35.2	36.2	26.6	27.8	27.4
Standard Deviation (Ct)	0.2	0.3	0.6	0.5	0.5	1.0	0.5	0.2

Table 11. Results for Analytical Sensitivity of the N2 and RP Targets Using the Gravity Diagnostics COVID-19 Assay.

Targets	N2 Target					RP target		
RNA Concentration (cp/µL)	46	15	5.1	2.6	1.7	5.1	2.6	1.7
Positives (n/3)	3/3	3/3	0/3[a]	0/3[a]	0/3[a]	3/3	3/3	3/3
Mean Ct	32.3	34.6	36.4	37.8	38.1	31.2	31.0	31.2
Standard Deviation (Ct)	0.4	0.6	0.6	0.7	0.2	0.5	0.3	0.2

[a]N2 target is reported as a qualitative negative at Ct > 35 in the Gravity Diagnostics COVID-19 Assays

2) Inclusivity:

The primer/probe sets were designed by the CDC, which conducted the *in silico* inclusivity analysis on known sequences of SARS-CoV-2. The data from this analysis is available in the FDA EUA "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel"

3) Cross-reactivity:

The Cross-reactivity for the Gravity Diagnostics COVID-19 ASSAY primers and probes was evaluated in "COMPARATOR ASSAY VALIDATION", section (2) of this summary.

4) Clinical Performance

Ninety-six NP patient samples, collected in Liquid Amies media, were processed with the Gravity Diagnostics COVID-19 Assay, using the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel as the comparator,

on a combination of the QS7 and QS12 instruments. The positive and negative percent agreements between the Gravity Diagnostics COVID-19 Assay and the comparator assay are shown below.

Clinical Performance of the Gravity Diagnostics COVID-19 Assay as Compared with the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel:

		Comparator	
		Positive	Negative
Gravity Diagnostics COVID-19 Assay	Positive	30	0
	Negative	1*	65

*positive by the comparator for the N2 target only, at a Ct of 39.0.

Performance of the Gravity Diagnostics COVID-19 Assay against the modified CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel:

Positive Percent Agreement 30/31 = 96.8% (95% CI: 83.8% - 99.4%)
Negative Percent Agreement 65/65 = 100% (95% CI: 94.2% - 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 was the Magmax Pathogen Kit RNA/DNA on the KingFisher Flex. The RT-PCR occurred on a QS12 instrument. The results are summarized in Table 12.

Table 12: 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 and Nasal Swabs	$1.8x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

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

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.