Updated: December 28, 2020

EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
The Gravity Diagnostics SARS-CoV-2 RT-PCR assay
Gravity Diagnostics, LLC

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

The Gravity Diagnostics SARS-CoV-2 RT-PCR assay will be performed at Gravity Diagnostics, LLC, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, as described in the Gravity Diagnostics SARS-CoV-2 RT-PCR assay Standard Operating Procedure that was reviewed by the FDA under this EUA.

INTENDED USE

1) Intended Use

The Gravity Diagnostics SARS-CoV-2 RT-PCR assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal, nasopharyngeal (NP), and oropharyngeal (OP) swab specimens collected by a healthcare provider (HCP) from any individual, including individuals without symptoms or other reasons to suspect COVID-19 infection.

This test is also for use with nasal swab specimens that are self-collected at home or in a healthcare setting using the Everlywell COVID-19 Test Home Collection Kit or the Kroger Health COVID-19 Test Home Collection Kit, when determined by an HCP to be appropriate based on results of a COVID-19 questionnaire. In addition, this test is also for use with saliva specimens collected in a healthcare setting using the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device from individuals suspected of COVID-19 by an HCP due to symptoms.

Testing is limited to Gravity Diagnostics, LLC, located at 632 Russell Street, Covington, KY 41011 and 812 Russell Street, Covington, KY 41011, which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable 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 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.

Testing with the Gravity Diagnostics SARS-CoV-2 RT-PCR assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

2) Special Conditions of Use Statements

For prescription use only
For in vitro diagnostic use only
For Emergency Use only

Testing of saliva specimens is limited to patients with symptoms of COVID-19 using the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device.

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.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Gravity Diagnostics SARS-CoV-2 RT-PCR assay is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The test uses three primer and probe sets to detect one region in the N gene, one region in the ORF1ab, and one region in the S gene, as well as one additional primer and probe set to detect an MS2 internal control, added to each clinical sample before extraction. It also contains one primer and probe set to detect human RNase P (RP) in a clinical sample. The primers/probes, used to detect all targets, are combined in the same reaction well. RNA is isolated using the Thermo Fisher MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit from nasal, NP, and OP swabs and saliva and is reverse transcribed to cDNA and subsequently amplified using an Applied Biosystems QuantStudio7 Flex instrument (QS7) with software version 1.3 or a QuantStudio12 Flex (QS12) instrument with software version 1.2.2. During the amplification process, each 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[1] to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the QS7 or QS12 instruments.

[1] Target-reporter dye pairs are as follows: (1) ORF1ab-FAM, (2) N gene-VIC, (3) S gene-ABY, (4) MS2-JUN, (5) RP-Cy5

INSTRUMENTS USED WITH THE TEST

The Gravity Diagnostics SARS-CoV-2 assay is to be used with the Thermo Fisher KingFisher Flex with software version 2.5 and the Hamilton Microlab STAR with software version 1.01 automated nucleic acid isolation instruments. The RT-PCR occurs on the Thermo Fisher QuantStudio 12K Flex and QuantStudio 7. The software version on the QuantStudio 12K Flex is V1.2.2 from Applied Biosystems and on the QuantStudio 7 is version 1.3 from Applied Biosystems.

REAGENTS AND MATERIALS

1) Included with the Assay

Equipment/Reagents/Consumables	Catalog #	Manufacturer
TaqPath™ 1 Step Multiplex Master Mix (No ROX™) (4X)	A28522	ThermoFisher
TaqPath™ COVID-19 Combo Kit	A47814	Applied Biosystems™
Thermo Fisher MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit	A48383	ThermoFisher
RNase P Primer F	custom	IDT/Eurofins
RNase P Primer R	custom	IDT/Eurofins
RNase P Primer Probe	custom	IDT/Eurofins

2) Not Included with the Assay

Equipment/Reagents/Consumables	Catalog #	Manufacturer
King Fisher Flex Purification System	24074421	ThermoFisher
Hamilton Microlab STAR	A42352	Hamilton Robotics
Fisher accuSpin Micro17	75002461	ThermoFisher
Sorvall T1 centrifuge	750002382	ThermoFisher
MiniVortex	NA	NA
Quant Studio 12 Flex	4471086	Applied Biosystems
Quant Studio 7 Flex	4485701	Applied Biosystems
Magmax 96 standard well plates 200ul	97002540	Life Technologies
Magmax tip comb	97002534	Life Technologies
Magmax 96 deep well plates	95040460	Life Technologies
50mL conical tube	12565271	Fisher
15mL conical tube	1495953A	Fisher
384 well PCR plates	4309849	Fisher
Adhesive PCR film	4311971	Fisher
Tips ranging from 1.0ul to 1000ul	NA	Integra and USA Scientific
10mL serological pipettes	1071-0810	Fisher
0.2 strip tubes, 8 well	AB2000	Fisher

CONTROLS TO BE USED WITH THE TEST

1. **Positive Control (PC)**: The positive control is run on every plate. The positive control is a diluted mix of Thermo Fisher TaqPath COVID-19 Control (25 copies/uL) with Hs-RPP30 Positive Control (Integrated DNA Technologies, CAT#: 10006626; 2,000 copies/uL). The TaqPath COVID-19 control contains the sequence for the three SARS-CoV-2 assays, while the Hs-RPP30 control contains the sequence for the RNase P assay. This control monitors amplification and signal production and ensures the integrity of the PCR reagents.
2. **Negative (No Template) Control (NTC)**: The negative control is run on every plate. The negative control is blank extraction reagents without target nucleic acid that are extracted and processed in the real time RT-PCR along with the patient samples. This control monitors for contamination during the extraction process and in the real time RT-PCR reagents.
3. **Internal Control 1 (MS2)**: The MS2 internal control RNA is added to each sample prior to nucleic acid isolation and is run for every sample. The MS2 is a bacteriophage RNA target included in the TaqPath COVID-19 Combo Kit (Applied Biosystems, CAT #: A47814). This control monitors for nucleic acid extraction, reverse transcription, amplification and signal production in each sample.
4. **Internal Control 2 (RNAse P):** This internal control is human RNAse P gene and is run with every sample. This control monitors for nucleic extraction and ensures sample integrity of the human specimen collected for testing.

INTERPRETATON OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Table 1. Expected Results for the Gravity Diagnostics SARS-CoV-2 RT-PCR assay Controls

Control	Expected Results (Expected Ct)				
	N target	Orf1ab target	S Target	MS2	RNAse P
Positive Control	Positive (Ct < 38)	Positive (Ct < 38)	Positive (Ct < 38)	Negative (Ct ≥ 38)	Positive (Ct < 38)
No Template Control	Negative (Ct ≥ 38)	Negative (Ct ≥ 38)	Negative (Ct ≥ 38)	Negative (Ct ≥ 38)	Negative (Ct ≥ 38)

Assessment of clinical specimens must be performed after the positive, negative (no template), and internal controls (RNAse P and MS2) have been examined and determined to be valid. If the positive or negative controls are not valid, the patient results cannot be interpreted.

Note on the internal controls: Samples that test positive for SARS-CoV-2 targets do not require amplification of the internal controls to be valid. However, amplification of the RNase P internal control is required for a negative SARS-CoV-2 sample to be valid.

The interpretation and reporting of clinical specimens are summarized in Table 2.

Table 2. Result Interpretation for Patient Samples

Orf1ab	N Gene	S Gene	MS2	RNAse P	Result	Action
NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	Invalid	Repeat test. If the repeat result remains invalid, report "invalid" and request an additional specimen be collected for testing.
NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	POS (Ct < 38)	NEG (Ct ≥ 38)	Invalid	Report "invalid" and request an additional specimen be collected for testing.
NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	POS or NEG	POS (Ct < 38)	SARS-CoV-2 Not Detected	Report "not detected" to healthcare provider and appropriate public health authorities.
Only one SARS-CoV-2 target POS (Ct < 38)			POS or NEG	POS or NEG	SARS-CoV-2 Inconclusive	Repeat test. If the repeat result remains inconclusive, report "inconclusive" or "indeterminate" and request an additional specimen be collected for testing.
Two or more SARS-CoV-2 targets = POS (Ct < 38)			POS or NEG	POS or NEG	SARS-CoV-2 Positive	Report "detected" to healthcare provider and appropriate public health authorities.

PERFORMANCE EVALUATION

1) Analytical Sensitivity

1a) Limit of Detection (LoD)

The LoD study established the lowest concentration of SARS-CoV-2 that can be detected by the Gravity Diagnostics SARS-CoV-2 RT-PCR assay at least 95% of the time. Contrived samples were prepared by spiking heat inactivated SARS-CoV-2 virus (SARS-CoV-2 USA-WA1/2020, BEI Resources, cat# NR-52286) into pooled SARS-CoV-2 negative nasopharyngeal (NP) matrix.

The LoD was established for both the KingFisher and Hamilton extraction platforms by testing serial dilutions comprising four panels of heat inactivated virus described in Tables 3 and 4. All testing was carried out on the QS12 RT-PCR instrument. The results, summarized in Table 3, established an LoD of 1,000 GE/mL for the Gravity Diagnostics SARS-CoV-2 RT-PCR assay using the KingFisher extraction platform, with 20 out of 20 replicates testing positive. The results, summarized in Table 4, established an LoD of 500 GE/mL for the Gravity Diagnostics SARS-CoV-2 RT-PCR assay using the Hamilton extraction platform, with 19 out of 19 replicates testing positive (one inconclusive replicate was excluded from the calculation). The KingFisher and Hamilton extraction systems show LoD's within two-fold of each other, demonstrating the functional equivalence of both systems.

Table 3. Results of the LoD Study for the KingFisher Extraction Platform.

Concentration (GE/mL)		5×10^3	2.5×10^3	1×10^3	500
Overall Result[a]	Positive replicates	10/10	10/10	20/20	4/10
ORF1ab Target	Positive replicates	10/10	10/10	19/20	3/10[b]
	mean Ct	33.11	34.24	35.11	37.22
	Standard Deviation	0.61	0.65	1.38	1.91
N Target	Positive replicates	10/10	10/10	18/20	6/10[b]
	mean Ct	34.18	35.68	37.02	37.93
	Standard Deviation	0.39	0.65	1.38	1.24
S Target	Positive replicates	10/10	10/10	20/20	6/10[b]
	mean Ct	33.05	34.11	35.10	36.39
	Standard Deviation	0.66	0.77	1.07	0.79
MS2 IC	Positive replicates	10/10	10/10	20/20	10/10[b]
	mean Ct	27.21	0.27	26.55	26.61
	Standard Deviation	0.39	0.27	0.41	0.47
RNAse P IC	Positive replicates	10/10	10/10	20/20	10/10[b]
	mean Ct	26.32	26.84	26.72	27.00
	Standard Deviation	0.38	0.36	0.24	0.50

[a]per the results interpretation in Table 2.

[b]Gravity's analysis suggested an error in nucleic acid extraction, as the 10 samples which failed amplification were all on the same row. These samples were not repeated and therefore 1×10^3 was determined as the LoD.

Table 4. Results of the LoD Study for the Hamilton Extraction Platform.

Concentration (GE/mL)		5×10^3	2.5×10^3	1×10^3	500
Overall Result[a]	Positive replicates	10/10	10/10	18/18[b]	19/19[b]
ORF1ab Target	Positive replicates	10/10	10/10	20/20	20/20
	mean Ct	31.74	32.40	34.29	34.38
	Standard Deviation	0.45	1.04	1.74	1.11
N Target	Positive replicates	10/10	10/10	6/20	5/20

Concentration (GE/mL)		5×10^3	2.5×10^3	1×10^3	500
	mean Ct	33.99	35.35	37.10	38.00
	Standard Deviation	0.32	1.05	1.74	1.26
S Target	Positive replicates	10/10	10/10	18/20	19/20
	mean Ct	32.06	32.54	35.08	34.91
	Standard Deviation	0.45	1.02	1.69	1.07
MS2 IC	Positive replicates	10/10	10/10	20/20	20/20
	mean Ct	31.09	1.48	28.57	29.54
	Standard Deviation	3.17	1.48	1.37	0.96
RNAse P IC	Positive replicates	10/10	10/10	20/20	20/20
	mean Ct	27.93	27.93	28.09	27.52
	Standard Deviation	0.36	0.36	0.45	0.48

[a]per the results interpretation in Table 2.
[b]inconclusive results are excluded from the final performance calculation.

1b) Inclusivity

The primer/probe set for the ORF1ab, N, and S SARS-CoV-2 targets were designed by ThermoFisher, which conducted the *in silico* inclusivity analysis. The data from this analysis is available in the FDA EUA "TaqPath COVID-19 Combo Kit". A right of reference letter was obtained by Gravity Diagnostics for these data.

2) Analytical Specificity - Cross-reactivity

The primer/probe set for the ORF1ab, N, and S SARS-CoV-2 targets were designed by ThermoFisher, which conducted the cross-reactivity studies. The data from this analysis is available in the FDA EUA "TaqPath COVID-19 Combo Kit". A right of reference letter was obtained by Gravity Diagnostics for these data.

3) Clinical Evaluation

A clinical study was conducted to evaluate performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR assay compared to an EUA-authorized RT-PCR SARS-CoV-2 assay. The study evaluated 118 Nasopharyngeal Swab (NP) and 49 nasal swab specimens, collected in either VTM, liquid Amies, or PrimeStore MTM, by a health care provider (HCP) and three nasal swabs self-collected in saline under observation of a healthcare provider for a total of 170 upper respiratory swab specimens. All testing was carried out using the KingFisher extraction platform and on the QS12 RT-PCR instrument

Of the 45 HCP-collected NP specimens that tested positive by the comparator assay, 43 tested positive using the Gravity Diagnostics SARS-CoV-2 RT-PCR assay. All 73 of the HCP-collected NP specimens that tested negative by the comparator assay also tested

negative by the Gravity Diagnostics SARS-CoV-2 RT-PCR assay. The results for the Gravity Diagnostics SARS-CoV-2 RT-PCR assay clinical performance using HCP-collected NP swab specimens are summarized in Table 5.

All nine of the HCP-collected nasal swab specimens that tested positive by the comparator assay also tested positive by the Gravity Diagnostics SARS-CoV-2 RT-PCR assay. All 40 of the HCP-collected nasal swab specimens that tested negative by the comparator assay also tested negative by the Gravity Diagnostics SARS-CoV-2 RT-PCR assay. The results for the Gravity Diagnostics SARS-CoV-2 RT-PCR assay clinical performance using HCP-collected nasal swab specimens are summarized in Table 6.

One self-collected nasal swab specimen that tested positive by the comparator assay also tested positive by the Gravity Diagnostics SARS-CoV-2 RT-PCR assay. Two self-collected nasal swab specimens that tested negative by the comparator assay also tested negative by the Gravity Diagnostics SARS-CoV-2 RT-PCR assay.

An analysis revealed that 28% (12/43) of all the positive samples were weak positive[2] by the comparator. All weak positive samples were detected by the Gravity Diagnostics SARS-CoV-2 RT-PCR assay.

The combined results for the Gravity Diagnostics SARS-CoV-2 RT-PCR assay clinical performance are summarized in Table 7.

Table 5. Clinical Performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR assay with HCP-collected NP swabs.

		EUA-authorized RT-PCR Assay Comparator		
		Positive	**Negative**	**Total**
Gravity Diagnostics SARS-CoV-2 RT-PCR assay	**Positive**	43	0	43
	Negative	2	73	75
	Total	45	73	118
Positive Agreement		95.6% (43/45) (95% CI 85.2 – 98.8)		
Negative Agreement		100.0% (73/73) (95% CI 95.0 – 100.0)		

Table 6. Clinical Performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR assay with HCP-collected Nasal swabs.

		EUA-authorized RT-PCR Assay Comparator		
		Positive	**Negative**	**Total**
Gravity Diagnostics SARS-CoV-2 RT-PCR assay	**Positive**	9	0	9
	Negative	0	40	40
	Total	9	40	49
Positive Agreement		100% (9/9) (95% CI 70.1 –99.0)		
Negative Agreement		100.0% (40/40) (95% CI 91.2– 100.0)		

[2] Weak positive samples are defined here as samples with at least one target within 3 Ct of the mean Ct at the LoD of the comparator assay for that target.

Table 7. Combined Clinical Performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR assay with All Specimen Types.

		EUA-authorized RT-PCR Assay Comparator		
		Positive	**Negative**	**Total**
Gravity Diagnostics SARS-CoV-2 RT-PCR assay	**Positive**	53	0	53
	Negative	2	115	117
	Total	55	115	170
Positive Agreement		96.4% (53/55) (95% CI 87.7 –99.0)		
Negative Agreement		100.0% (115/115) (95% CI 96.8 – 100.0)		

4) Validation for the Saliva Specimen Type

4a) Analytical Sensitivity:

A bridging study was performed between saliva matrix and NP matrix. Contrived samples were prepared by spiking heat inactivated SARS-CoV-2 virus (ATCC, Cat #: VR-1986HK; Lot #: 70036071) into pooled SARS-CoV-2 negative nasopharyngeal (NP) matrix collected in VTM or pooled negative saliva collected in the Spectrum SDNA-1000 (Spectrum Solutions) device. All testing was carried out using the KingFisher extraction platform and on the QS12 RT-PCR instrument.

The bridging study tested serial dilutions comprising three panels of heat-inactivated virus, as described in Tables 8 and 9. The results showed three out of three positive replicates for each target concentration and similar mean Ct values, for each target, between samples from pooled NP matrix and samples from saliva matrix.

Table 8. Results from the Comparative LoD Study for NP matrix

NP Matrix	Overall result	ORF1ab Target			N Target			S Target		
Concentration (GE/mL)	Positive replicates	Positive replicates	mean Ct	Standard Deviation	Positive replicates	mean Ct	Standard Deviation	Positive replicates	mean Ct	Standard Deviation
9×10^3	3/3	3/3	29.77	0.37	3/3	30.01	0.20	3/3	29.89	0.12
3×10^3	3/3	3/3	30.88	0.24	3/3	31.79	0.27	3/3	31.14	0.42
1×10^3	3/3	3/3	31.89	0.06	3/3	33.46	0.33	3/3	32.05	0.27

Table 9. Results from the Comparative LoD Study for Saliva matrix

Saliva Matrix	Overall result	ORF1ab Target			N Target			S Target		
Concentration (GE/mL)	Positive replicates	Positive replicates	mean Ct	Standard Deviation	Positive replicates	mean Ct	Standard Deviation	Positive replicates	mean Ct	Standard Deviation
9×10^3	3/3	3/3	29.60	0.09	3/3	29.64	0.35	3/3	29.82	0.08
3×10^3	3/3	3/3	30.71	0.41	3/3	31.41	0.34	3/3	30.80	0.24
1×10^3	3/3	3/3	31.93	0.39	3/3	32.71	0.48	3/3	32.14	0.09

4b) Clinical Evaluation:
A clinical study, using 209 paired nasal swab specimens and saliva specimens, collected using the Spectrum SDNA-1000 (Spectrum Solutions) device, was conducted to evaluate the performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR assay with the saliva specimens. All testing was carried out using the KingFisher extraction platform and on the QS12 RT-PCR instrument.

Seven of the 209 paired specimens returned negative results for the nasal swab specimens and returned invalid results for the saliva specimens, due to failed internal controls. These samples were not included in the performance analysis, leaving 202 samples with valid results.

One of the 202 paired specimens returned a positive result for the nasal swab specimen and returned an inconclusive result for the corresponding saliva specimen and was not included in the final performance calculation. Two of the 202 paired specimens returned positive results for the nasal swab specimen and negative results for the saliva specimens. Thirty-eight of the 202 paired specimens returned positive results for both saliva and nasal swabs. One hundred and sixty-one of the 202 paired specimens returned negative results for both saliva and nasal swabs. The results for the Gravity Diagnostics SARS-CoV-2 RT-PCR assay clinical performance with saliva specimens are summarized in Table 10 and support the use of the saliva specimens with the Gravity Diagnostics SARS-CoV-2 RT-PCR.

An analysis revealed 8% (3/38) of all the positive saliva specimens were weak positive[3] and 8% (3/38) of all the positive nasal swab specimens were weak positive[3]. Two of the three weak positive nasal swab specimen results had a positive paired saliva specimen result. One of the three weak positive nasal swab specimen results had an inconclusive paired saliva specimen result.

Table 10. Clinical Performance of Gravity Diagnostics SARS-CoV-2 RT-PCR assay from saliva samples against paired NP samples.

		Results from Nasal Swab Specimens		
		Positive	**Negative**	**Total**
Results from Saliva Specimens	**Positive**	38	0	38
	Inconclusive[a]	1[b]	0	1
	Negative	2[c]	161	163
	Total	41	161	202
Positive Agreement		95.0% (38/40) (95% CI 83.5 – 98.6)		
Negative Agreement		100.0% (82/82) (95% CI 97.7 – 100.0)		

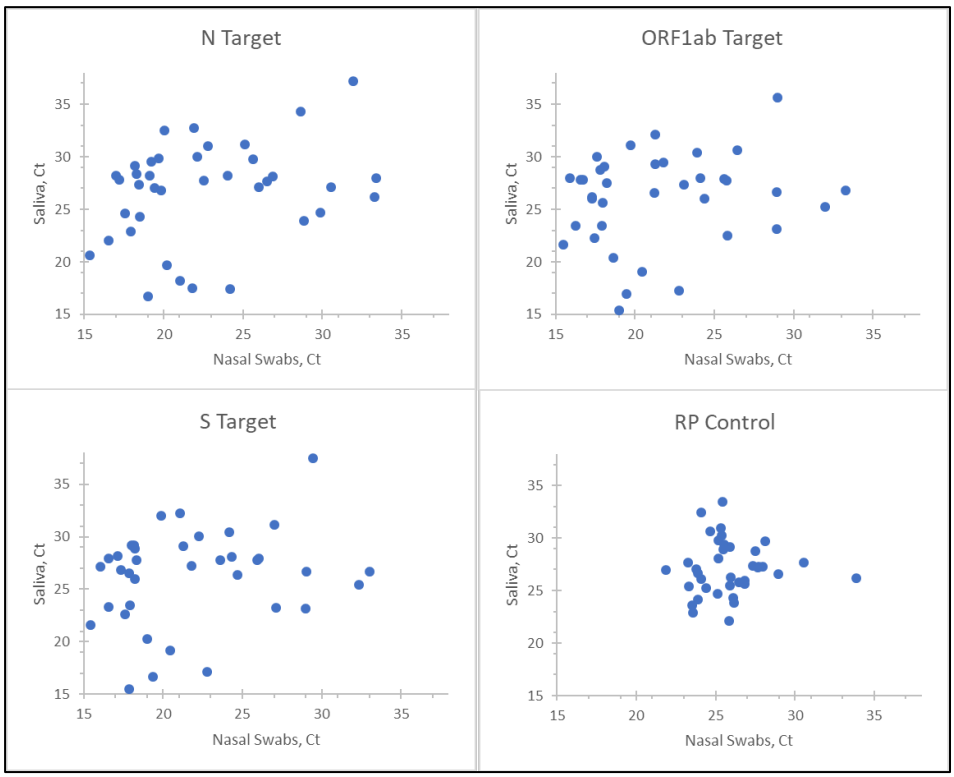
[a]Inconclusive results are excluded from the final performance calculation.
[b]The nasal swab specimen produced a Ct values of 32.79, 32.73, and 33.40 for the N, ORF1ab, and S targets, respectively. The saliva specimen produced a Ct value of 36.28 for the ORF1ab target only but was not retested despite the indication to retest in the standard operating procedure. This specimen was therefore excluded from the analysis of performance. Had resting been performed, PPA would have ranged from 38/41 (92.7%; 80.6-97.5%) to 39/41 (95.1%; 83.9-98.7%), depending on the outcome.

[3]Weak positive samples are defined here as having at least one target within 3 Ct of the mean Ct at the LoD for that target as determined on the Kingfisher extraction platform at 1000 GE/mL.

[c]The nasal swab specimens produced a Ct values of 32.47 (N), 31.29 (ORF1ab), and 31.86 (S); and 30.97 (N), 30.22 (ORF1ab), and 30.81 (S).

In addition, to analyze for a correlation between nasal swab specimens and saliva specimens, the Ct values for positive paired samples were plotted with nasal swab results on the X-axis and saliva results on the Y-axis. The data, summarized in Figure 1, show that the positive samples covered a broad range of Ct values, but do not indicate a strong linear correlation between the Ct values from nasal swab samples and saliva samples.

Figure 1. Scatter Plot of Ct Values from Positive Paired Nasal Swab Samples and Saliva Samples.



5) Performance among individuals without symptoms or other reasons to suspect COVID-19

In order to evaluate performance among individuals without symptoms or other reasons to suspect COVID-19, Gravity Diagnostics retained either nasal or nasopharyngeal swab specimens from the first 20 consecutive positive samples out of a total of 327 asymptomatic patient samples[4] processed for testing on September 23rd, 2020. The first 100 consecutive negative patient samples from this group were also retained. These 120 deidentified specimens (87 nasal swab specimens and 33 NP specimens) were then sent to Preferred Lab

[4]The asymptomatic individuals tested using the Gravity Diagnostics SARS-CoV-2 RT-PCR assay included pre-operative patients, long term-care employees, and college students.

Partners (CLIA: 18D2148030) and were tested on September 25th, 2020 using an EUA-authorized RT-PCR SARS-CoV-2 assay that has authorization to test upper respiratory specimens from individuals without symptoms or other reasons to suspect COVID-19 infection. The results, stratified by specimen type in Tables 11 and 12, showed 100% concordance between the Gravity Diagnostics SARS-CoV-2 RT-PCR assay and the comparator assay. The combined results are presented in Table 13. These data support the use of the Gravity Diagnostics assay for screening individuals without symptoms or other reasons to suspect COVID-19 using either NP, OP, or nasal swabs specimen types.

Table 11. Clinical Performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR assay among Asymptomatic Individuals with Nasal Swabs.

		EUA-authorized RT-PCR Assay Comparator		
		Positive	**Negative**	**Total**
Gravity Diagnostics SARS-CoV-2 RT-PCR assay	**Positive**	9	0	9
	Negative	0	78	78
	Total	9	78	87
Positive Agreement		100.0% (9/9) (100% CI 70.1 – 100.0)		
Negative Agreement		100.0% (78/78) (95% CI 95.3 – 100.0)		

Table 12. Clinical Performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR assay among Asymptomatic Individuals with NP Swabs.

		EUA-authorized RT-PCR Assay Comparator		
		Positive	**Negative**	**Total**
Gravity Diagnostics SARS-CoV-2 RT-PCR assay	**Positive**	11	0	11
	Negative	0	22	22
	Total	11	22	33
Positive Agreement		100.0% (11/11) (100% CI 74.1 – 100.0)		
Negative Agreement		100.0% (22/22) (95% CI 85.1 – 100.0)		

Table 13. Combined Clinical Performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR assay among Asymptomatic Individuals with Both Specimen Types.

		EUA-authorized RT-PCR Assay Comparator		
		Positive	**Negative**	**Total**
Gravity Diagnostics SARS-CoV-2 RT-PCR assay	**Positive**	20	0	20
	Negative	0	100	100
	Total	20	100	120
Positive Agreement		100.0% (20/20) (100% CI 83.9 – 100.0)		
Negative Agreement		100.0% (100/100) (95% CI 96.3 – 100.0)		

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 13.

Table 13: 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

LIMITATIONS:

- Testing of saliva specimens is limited to patients with symptoms of COVID-19.
- Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.
- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by Gravity Diagnostics, LLC, located at 632 Russell Street, Covington, KY 41011 and 812 Russell Street, Covington, KY 41011, which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-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 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 authorization is terminated or revoked sooner.