EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
Discover Labs COVID-19 Assay
(Discover Labs)

For *in vitro* Diagnostic Use
Rx Only
For Use Under Emergency Use Authorization (EUA) Only

The Discover Labs COVID-19 Assay will be performed at Discover Labs, located at 13776 N US Highway 183 Unit 108, Austin, TX 78750, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests, as described in the Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA.

INTENDED USE

The Discover Labs COVID-19 Assay is a real-time reverse transcription PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal, nasopharyngeal, and oropharyngeal swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Discover Labs, located at 13776 N US Highway 183 Unit 108, Austin, TX 78750, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets 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 nasal, nasopharyngeal, and oropharyngeal swab 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 definitive cause of disease. Laboratories within the United States and its territories are required to report all test 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/or epidemiological information.

The Discover Labs COVID-19 Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures. The Discover Labs COVID-19 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Device Description

The Discover Labs COVID-19 Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 collected from upper respiratory specimens (nasal, nasopharyngeal and oropharyngeal swabs) in universal transport media (UTM) or viral transport media (VTM). Viral RNA is extracted from patient samples using the Applied Biosystems MagMax Viral/Pathogen Nucleic Acid Isolation Kit and the Thermo Fisher KingFisher Flex extraction instrument. Reverse transcription PCR amplification is performed using the Thermo Fisher QuantStudio 5 instrument system.

Description of Test Steps:

Samples and controls are extracted using the MagMax Viral/Pathogen II (MVPII) Nucleic Acid Isolation Kit and the KingFisher Flex extraction instrument in 96 well processing plates. Master mix and amplification reagents are prepared and added to test wells of the 96 well plates. Extracted samples including processed negative template control and positive control are then added to each well. Plates are loaded into the QuantStudio 5 instrument to begin the RT-qPCR reaction. Once the RT-qPCR run is complete, results are analyzed with the QuantStudio software.

INSTRUMENTS USED WITH THE TEST

Table 1. Instruments and Software for Use with the Discover Labs COVID-19 Assay

Instrument	Manufacturer	Software Version
QuantStudio 5 Real-Time PCR instrument	Thermo Fisher	1.3.3
KingFisher Flex extraction instrument	Thermo Fisher	1.01.0
QuantStudio Design & Analysis Software	Thermo Fisher	2.5.0
QuantStudio 5 Real-Time PCR Instrument Computer-QuantStudio Design & Analysis	Thermo Fisher	1.5.2

REAGENTS AND MATERIALS

Table 2. Reagents and Materials Used for the Discover Labs COVID-19 Assay

Reagent/Material	Manufacturer/ Supplier	Catalogue/Part Number
MagMax Viral/Pathogen II (MVPII) Nucleic Acid Isolation Kit	Thermo Fisher	A48383
TaqPath 1-Step RT-qPCR Master Mix, CG	Thermo Fisher	A6120 or A61
nCOV_N1 Forward Primer Aliquot	Integrated DNA Technologies	10006830
nCOV_N1 Reverse Primer Aliquot	Integrated DNA Technologies	1006831
nCOV_N1 Probe Aliquot	Integrated DNA Technologies	1006832
nCOV_N2 Forward Primer Aliquot	Integrated DNA Technologies	1006833
nCOV_N2 Reverse Primer Aliquot	Integrated DNA Technologies	1006834
nCOV_N2 Probe Aliquot	Integrated DNA Technologies	1006835
RNase P Forward Primer Aliquot	Integrated DNA Technologies	1006836
RNase P Reverse Primer Aliquot	Integrated DNA Technologies	1006837
RNase P (ATTO 647) Probe Aliquot	Integrated DNA Technologies	10007062
10X TE pH 7	Invitrogen	MB-006

CONTROLS

The Discover Labs COVID-19 assay includes a positive control, human specimen control and a negative control, described in **Table 3** below. Each control is inserted into the assay workflow beginning at sample extraction. Four of each control are used per 384-well plate or once for up to 93 samples evaluated.

Table 3. Assay Controls Used with the Discover Labs COVID-19 Assay

Control	Description	Manufacturer	Purpose
Positive control	2019-nCoV_N_Positive Control	Integrated DNA Technologies Catalog #10006625	Used at a concentration of 4.7 copies/µL and monitors functioning of RT-qPCR reagents
Human specimen control	Hs_RPP30 Positive Control	Integrated DNA Technologies Catalog #10006626	Verifies that the extraction process is working
Negative template control	Nuclease free water	Integrated DNA Technologies Catalog #11-05-01-04	Used to monitor for contamination during sample processing

INTERPRETATION OF RESULTS

Assay Controls

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

- Positive Control: The positive control should yield a "Detected" result for the COVID target and "Not Detected" or "Detected" for the RNase P control.
- Negative Template Control: The NTC should yield a "Not Detected" result for both the COVID and RNase P targets

Clinical Specimens

Results are interpreted according to the requirements in **Table 4** below.

Table 4. Discover Labs COVID-19 Assay Results Interpretation

Results	N1	N2	RnaseP	Action
Positive	≤37	≤37	+/-	Report positive
Inconclusive	If one of the two targets is positive		+/-	Repeat specimen from extraction. If results remain inconclusive, please notify client for recollection.
Negative	>37	>37	<36	Report negative
Invalid	>37	>37	>36	Repeat specimen from extraction. If repeated results remain invalid, consider collecting a new specimen from the patient.

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) - Analytical Sensitivity

The LoD of the Discover Labs COVID-19 Assay was determined using Heat Inactivated 2019 Novel Coronavirus (VR-1986HK) from ATCC. A preliminary LoD was determined by testing serial dilutions (1000 copies/µL - 0.39 copies/ µL) of heat-inactivated virus spiked into banked de-identified negative nasopharyngeal samples collected in UTM/VTM using five replicates at each target level. Spiked samples were tested with the Discover Labs COVID-19 Assay following

extraction. The results of the LoD preliminary study are summarized in **Table 5** below.

Table 5. Discover Labs COVID-19 Assay Preliminary LoD Results

Concentration (copies/µL)	N1 Results	N2 Results
1000.0	5/5 detected	5/5 detected
100	5/5 detected	5/5 detected
50	5/5 detected	5/5 detected
25	5/5 detected	5/5 detected
12.5	5/5 detected	5/5 detected
6.25	5/5 detected	5/5 detected
3.125	5/5 detected	5/5 detected
1.5625	5/5 detected	5/5 detected
0.7813	4/5 detected	4/5 detected
0.3906	3/5 detected	3/5 detected

Three preliminary concentrations of 3.13 copies/µL, 1.56 copies/µL and 0.78 copies/µL were chosen for confirmatory testing with 20 individual extraction replicates. The established LoD of the Discover Labs COVID-19 Assay was 1.56 copies/µL and confirmed by testing an additional 20 replicates. The results of the LoD confirmatory studies are summarized in **Tables 6** and **7** below.

Table 6. Discover Labs COVID-19 Assay Confirmation LoD Study Results – Concentrations 3.125, 1.562 and 0.781

Concentration (copies/µL)	N1 Results	N2 Results
3.125	20/20 detected	20/20 detected
1.562	20/20 detected	20/20 detected
0.781	17/20 detected	17/20 detected

Table 7. Discover Labs COVID-19 Assay Confirmation LoD Study Results – Concentration 1.56

Concentration (copies/µL)	N1 Results	N2 Results
1.562	20/20 detected	20/20 detected
Mean	35.24	35.53
STDEV	0.55	0.84
CV	1.57%	2.37%

2) Inclusivity (Analytical Reactivity):

The Discover Labs COVID-19 Assay utilizes oligonucleotide primer and probe sequences for the N1 and N2 target genes identical to those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel.

The CDC granted a right of reference to the performance data contained in the CDC's EUA request (EUA200001) to any entity seeking an FDA EUA for a COVID19 diagnostic device. Since the alignments of the CDC's primers/probes were completed in February 2020, an additional *in silico* inclusivity analysis was completed in February 2023 as shown in **Table 8** below. This *in silico* analysis assessed the impact of the recently emerged SARS-CoV-2 variants and demonstrated that none of the observed mutations would be expected to interfere with N gene detection and affect the homology between sequences. As a result, all the sequences and variants matched 100% homology between the primer sequences and the variants sequences. Discover Labs also performed an *in silico* analysis with variants over 5% prevalence as of February 2023 which includes XBB.1.5, BQ.1.1 and BQ.1. No impact to the assay from these viral mutations was determined.

Table 8. Discover Labs COVID-19 Assay Inclusivity *In Silico* Analysis

Pango Lineage		N1			N2		
	GenBank	Forward	Reverse	Probe	Forward	Reverse	Probe
B.1.1.7 and Q lineages	OV054768.1	100%	100%	100%	100%	100%	100%
B.1.351 and descendent lineages	OX003129.1	100%	100%	100%	100%	100%	100%
P.1 and descendent lineages	OX000832.1	100%	100%	100%	100%	100%	100%
B.1.617.2 and AY lineages	OW998779.1	100%	100%	100%	100%	100%	100%
B.1.427	OQ412782.1	100%	100%	100%	100%	100%	100%
B.1.429	OQ412714.1	100%	100%	100%	100%	100%	100%
B.1.525	ON192033.1	100%	100%	100%	100%	100%	100%
B.1.526	OQ312853.1	100%	100%	100%	100%	100%	100%
B.1.617.1	OQ204216	100%	100%	100%	100%	100%	100%
P.2	OQ412680.1	100%	100%	100%	100%	100%	100%
B.1.621.1	OQ225929.1	100%	100%	100%	100%	100%	100%
B.1.1.529	OQ407910	100%	100%	100%	100%	100%	100%
BA.1	OQ411325	100%	100%	100%	100%	100%	100%
BA.1.1	OQ430213	100%	100%	100%	100%	100%	100%
BA.2	OQ423385	100%	100%	100%	100%	100%	100%
BA.3	ON722983	100%	100%	100%	100%	100%	100%
BA.4	OQ423443	100%	100%	100%	100%	100%	100%
BA.5	OQ430463	100%	100%	100%	100%	100%	100%
XBB.1.5	OQ443274	100%	100%	100%	100%	100%	100%
BQ.1.1	OQ443286	100%	100%	100%	100%	100%	100%
BQ.1	OQ443273.1	100%	100%	100%	100%	100%	100%
XBB	OQ438493	100%	100%	100%	100%	100%	100%

3) Cross-Reactivity (Analytical Specificity) and Microbial Interference:

To assess for potential cross-reactivity of the Discover Labs COVID-19 Assay, an *in silico* analysis of the SARS-CoV-2 N1 and N2 primer and probe sequences was performed using BLAST analysis from NCBI to identify any homologies between the primers and probes, and target organisms. None of the pathogen sequences displayed equal to or greater than 80% homology with any of the SARS-CoV-2 N1 and N2 primers/probes.

In addition to the *in silico* analysis, several organisms were tested with the Discover Labs COVID-19 Assay using banked negative clinical nasopharyngeal swabs in UTM un-spiked to assess potential cross-reactivity, or spiked with heat-inactivated SARS-CoV-2 at 2x the LoD to evaluate potential microbial interference.

Samples were prepared as a 10-fold dilution from the manufacturer stock by pipetting 20µL of potentially cross-reactive pathogen stock into 180µL of negative nasopharyngeal swabs in UTM, or negative nasopharyngeal swabs in UTM spiked with heat-inactivated SARS-CoV-2 virus at 2X the LoD. Testing was performed following the Discover Labs COVID-19 assay protocol. No cross reactivity or microbial interference from common upper respiratory organisms was observed as shown in **Table 9** below.

Table 9. Discover Labs COVID-19 Assay Cross-reactivity and Microbial Interference Study Results

Microbial Interference Study - Positive Samples					
Organism	**Stock Concentration**	**Final Concentration**	**Ct N1**	**Ct N2**	**Ct RnaseP**
Influenza A Virus H3N2	2.30E+09	2.30E+08	30.30	29.97	25.78
			30.97	30.31	25.77
			30.98	30.59	25.93
human Rhinovirus A2	1.86E+04	1.86E+03	30.55	30.16	25.84
			31.21	30.67	25.90
			30.50	30.14	25.79
Bordetella pertussis	3.35E+09	3.35E+08	30.41	30.10	25.68
			30.71	30.03	25.65
			31.09	30.45	25.55
Bordetella holmesii	5.20E+08	5.20E+07	30.34	29.96	25.66
			30.44	29.97	26.08
			30.41	29.98	25.91
human Coronavirus HKU1	5.00E+05	5.00E+04	30.60	30.28	25.99
			30.70	30.33	25.86
			30.63	30.14	26.09
human Coronavirus 229E	1.26E+06	1.26E+05	30.65	30.08	26.22
			30.58	29.97	25.89
			30.78	30.32	25.97
human Coronavirus NL63	1.70E+05	1.70E+04	30.09	29.74	25.89
			30.75	30.15	26.10
			31.09	30.67	24.20
human Coronavirus	5.01E+05	5.01E+04	30.28	29.84	23.95

OC43			30.48	29.86	23.92
			30.71	30.34	23.87
Bocavirus	8.00E+05	8.00E+04	30.07	29.64	24.06
			30.58	29.95	25.80
			30.64	30.03	25.82
Respiratory Syncytial Virus A	1.60E+08	1.60E+07	30.10	29.72	25.67
			30.32	29.85	25.70
			30.56	30.02	25.88
Respiratory Syncytial Virus B	8.00E+06	8.00E+05	30.14	29.61	24.56
			30.79	30.14	24.66
			31.10	30.73	24.37
Haemophilus influenza	1.35E+09	1.35E+08	29.86	29.44	24.40
			30.69	30.10	24.45
			31.00	30.56	25.65
Cross Reactivity Study - Negative Samples					
Organism	**Stock Concentration**	**Final Concentration**	**Ct N1**	**Ct N2**	**Ct RnaseP**
Influenza A Virus H3N2	2.30E+09	2.30E+08	=	=	26.72
			=	=	26.85
			=	=	26.76
human Rhinovirus A2	1.86E+04	1.86E+03	=	=	26.72
			=	=	26.68
			=	=	26.92
Bordetella pertussis	3.35E+09	3.35E+08	=	=	27.02
			=	=	26.97
			=	=	26.86
Bordetella holmesii	5.20E+08	5.20E+07	=	=	26.84
			=	=	26.81
			=	=	26.77
human Coronavirus HKU1	5.00E+05	5.00E+04	=	=	26.81
			=	=	26.75
			=	=	26.61
human Coronavirus 229E	1.26E+06	1.26E+05	=	=	27.19
			=	=	27.04
			=	=	27.10
human Coronavirus NL63	1.70E+05	1.70E+04	=	=	27.07
			=	=	26.93
			=	=	27.03
human Coronavirus OC43	5.01E+05	5.01E+04	=	=	27.14
			=	=	26.97
			=	=	27.04
Bocavirus	8.00E+05	8.00E+04	=	=	26.93

			=	=	26.89
			=	=	26.90
Respiratory Syncytial Virus A	1.60E+08	1.60E+07	=	=	26.96
			=	=	27.01
			=	=	26.97
Respiratory Syncytial Virus B	8.00E+06	8.00E+05	=	=	26.92
			=	=	26.89
			=	=	26.87
Haemophilus influenza	1.35E+09	1.35E+08	=	=	26.89
			=	=	26.70
			=	=	26.75

4) Interfering Substances

Banked negative clinical nasopharyngeal swabs in UTM and negative clinical nasopharyngeal swabs in UTM spiked with heat-inactivated SARS-CoV-2 at 4.68 copies/µL (2x-3x the LoD), were tested with potential interfering substances to examine the potential effect on the Discover Labs COVID-19 Assay. No assay interference was observed with any of the negative or positive SARS-CoV-2 nasopharyngeal swabs in UTM samples in the presence of the substances at the concentrations indicated in **Tables 10** and **11** below.

Table 10. Discover Labs COVID-19 Assay Interfering Substances Study Results Using SARS-CoV-2 Positive Samples

Substance	Concentration	N1 Results	N2 Results	RnaseP Results	Interpretation
AFRIN	15%v/v	5/5	5/5	Detected	Detected
Cepacol Lozenges	3mg/mL	5/5	5/5	Detected	Detected
Chloraseptic Sore Throat Spray	5% v/v	5/5	5/5	Detected	Detected
Mouth Wash (Listerine	5%v/v	5/5	5/5	Detected	Detected
Robitussin	5% v/v	5/5	5/5	Detected	Detected
Mucin	2.5mg/mL	5/5	5/5	Detected	Detected
Tobacco	0.03mg/mL	5/5	5/5	Detected	Detected
Toothpaste	0.5% v/v	5/5	5/5	Detected	Detected

Table 11. Discover Labs COVID-19 Assay Interfering Substances Study Results Using SARS-CoV-2 Negative Samples

Substance	Concentration	N1 Results	N2 Results	RnaseP Results	Interpretation
AFRIN	15%v/v	0/5	0/5	Not Detected	Not Detected
Cepacol	3mg/mL	0/5	0/5	Not Detected	Not Detected

Substance	Concentration	N1 Results	N2 Results	RnaseP Results	Interpretation
Lozenges					
Chloraseptic Sore Throat Spray	5% v/v	0/5	0/5	Not Detected	Not Detected
Mouth Wash (Listerine	5%v/v	0/5	0/5	Not Detected	Not Detected
Robitussin	5% v/v	0/5	0/5	Not Detected	Not Detected
Mucin	2.5mg/mL	0/5	0/5	Not Detected	Not Detected
Tobacco	0.03mg/mL	0/5	0/5	Not Detected	Not Detected
Toothpaste	0.5% v/v	0/5	0/5	Not Detected	Not Detected

5) Specimen Stability:

Stability was assessed for storage of samples at 15-25°C for eight days and 0-8°C for ten days. Five random positive nasopharyngeal samples previously tested with the Discover Labs COVID-19 Assay were split into two aliquots each for storage at 15-25°C for eight days and at 0-8°C for 10 days. Samples stored at 15-25°C were tested on days 1, 2, 3, 4, 7 and 8 with all samples detected through day seven. Samples stored at 0-8°C were tested on days 1, 2, 3, 4, 7, 8, 9 and 10 with all samples detected through day ten. Therefore, the study supports stability of samples at 15-25°C for 4 days, and 9 days for samples stored at 0-8°C.

6) Clinical Evaluation:

Clinical performance of the Discover Labs COVID-19 Assay was evaluated using 150 randomly selected banked positive and negative nasopharyngeal swab specimens previously tested by Discover Labs. These 150 specimens were simultaneously tested by the Discover Labs COVID-19 Assay and the comparator assay. Results from testing on the comparator assay show that this cohort included a total of 57 positives and 92 negatives. One specimen resulted in "invalid" by the comparator assay (tested negative by the candidate test) and was excluded from the performance evaluation. Therefore, 57 positive samples, including 18 (31.5%) low positive, and 92 negative samples were used to calculate performance. Both positive and negative percent agreement (PPA and NPA) were calculated at 100% as summarized in **Table 12** below.

Table 12. Discover Labs COVID-19 Assay Clinical Evaluation Study Summary

		Comparator Assay		
		Positive	**Negative**	**Total**
Discover Labs COVID-19 Assay	**Positive**	57	0	57
	Negative	0	92	92
	Total	57	92	149
Positive Agreement		100% (93.7-100%)		
Negative Agreement		100% (96-100%)		

Limitations

- The performance of this test was established based on the evaluation of a limited number of

clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

- 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

- For in vitro diagnostic use
- Rx Only
- For use under Emergency Use Authorization (EUA) only
- This product has not been FDA cleared or approved, but has been authorized for emergency use 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.