ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
CIRRUSDX SARS-COV-2
(CIRRUSDX LABORATORY)

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

(The CirrusDx SARS-CoV-2 assay will be performed at CirrusDx Laboratory certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Instructions of Use that were reviewed by the FDA under this EUA.)

INTENDED USE

The CirrusDx SARS-CoV-2 assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, and BAL specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the CirrusDx Laboratory, which is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, certified high-complexity laboratory.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper 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 infective status. Positive results do not rule out bacterial 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 CirrusDx SARS-CoV-2 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 CirrusDx SARS-CoV-2 is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Cirrus Dx SARS-CoV-2 is a qualitative real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The test uses primer and probe sets to detect three regions in the SARS-CoV-2. These regions are the Envelope Protein (E) gene, Nucleocapsid Protein (N) gene, and RNA-dependent RNA Polymerase (RdRP) gene.

RNA from nasal swabs, nasopharyngeal swabs, oropharyngeal swabs and BAL specimens extracted using the Qiagen QIAamp Viral Mini Kit or the KingFisher Flex automated extraction is reverse transcribed to cDNA and subsequently amplified using the QuantStudio™ 5 or 7. 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 to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle.

INSTRUMENTS USED WITH TEST

The CirrusDx SARS-CoV-2 test is to be used with the following PCR instruments:

- QuantStudio™ 7 (software version 1.3)
- QuantStudio™ 5 (software version 1.4.1)

The test is intended to be used with the KingFisher FlexTM automated extraction or Manual Extraction can be performed with the Qiagen QIAamp Viral Mini Kit.

The assay has been validated using QuantStudio 7 Real-Time PCR Instrument.

REAGENTS AND MATERIALS

The CirrusDx SARS-CoV-2 assay has been validated using only the components referenced in this submission.

Table 1: CirrusDx SARS-CoV-2 Reagents

Reagent	Manufacturer	Catalog #
Allplex 2019 nCoV Assay	Seegene Technologies, Inc.	RP 10243X
MagMAX Viral / Pathogen Nucleic Acid Isolation Kit	ThermoFisher Scientific	A42352
QIAamp Viral Mini Kit	Qiagen	52906

CONTROLS TO BE USED WITH THE CIRRUSDX SARS-COV-2

Six controls are included in the CirrusDx SARS-CoV-2 test.

1) **Internal Control (IC):** For the extraction, an Internal Control is added to each sample and simultaneously extracted with the nucleic acids. This ensures that each sample was extracted properly. The IC is The IC is MS-2 exogenous RNA and will not amplify without extraction. Therefore, if the extraction did not occur properly, the IC will be negative.

2) **Extraction Control (EC):** The extraction control is included with each extraction. This control contains Universal Transport Media (UTM) and the MS-2 exogenous RNA for IC. The Extraction Control controls the extraction process (in the same manner as the IC) but also serves as a contamination control as the E, N and RdRP genes are not in this sample and thus must be negative in the RT-PCR. The EC must be tested with each extraction and typically occurs on each RT-PCR plate.

3) **High Positive Control (HPC):** The HPC is at a high titer which generates early cycle threshold (Ct) values for the N, E and RdRP gene on the RT-PCR.

4) **Positive Control (PC):** The PC is at a lower titer generating later Ct values (~3-5x Limit of Detection) for the N, E and RdRP gene on the RT-PCR.

5) **Negative Control (NC):** The NC is at a level below the Ct cutoff on the RT-PCR.

6) **No Template Control (NTC):** The NTC contains no nucleic acid and must be negative in the RT-PCR. If the NTC is positive, it is an indication of contamination in the RT-PCR assay. This control is tested on every RT-PCR plate.

INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted (Refer to Table 2 for a summary of control results).

Table 2: Assessment of the CirrusDx SARS-CoV-2 Controls

Control Name	Expected Targets in Control	Ct Value			
		IC	N Gene	E Gene	RdRP Gene
No Template Control (NTC)	None	≥ 40	≥ 40	≥ 40	≥ 40

Control Name	Expected Targets in Control	Ct Value			
		IC	N Gene	E Gene	RdRP Gene
Extraction Control (EC)	IC	< 35	≥ 40	≥ 40	≥ 40
Positive Control (PC)	N, E and RdRP gene at ~3-5x LOD	Not Evaluated	30-40	30-40	30-40
High Positive Control (HPC)	N, E and RdRP gene at high titer	Not Evaluated	15-30	15-30	15-30
Negative Control (NC)	N, E and RdRP gene below cutoff	≥ 40	≥ 40	≥ 40	≥ 40

Table 3: Interpretation of Patient Specimen Results

Interpretation		Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Gene Targets	IC	+/-	+/-	+/-	+/-	+/-	+	-
	E gene	+	+/-	-	+/-	+	-	-
	RdRP gene	+	-	+	+	-	-	-
	N gene	+	+	+	-	-	-	-
Result Interpretation		SARS-CoV-2 Detected	Presumptive Result*			SARS-CoV-2 Not Detected	SARS-CoV-2 Not Detected	Invalid

*Presumptive Positive Results will be repeated. If the repeat result remains Presumptive Positive, additional confirmatory testing may be conducted.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

The Limit of Detection (LOD) was determined for the SARS-CoV-2 assay. The Limit of Detection is the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all replicates test positive. Nasopharyngeal swabs were collected from individuals negative for SARS-CoV-2 and placed into Universal Viral Transport Media. SARS-Related Coronavirus 2 RNA, Isolate USA-WA1/2020 obtained from BEI Resources (catalog NR-52285) was diluted into the sample matrix at the lowest level detected (levels tested were 6250, 625, 312.5, 156 and 78 copies). Each concentration was tested with three replicates. The samples were extracted on the KingFisher Flex using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation protocol and then RT-PCR was performed on the QuantStudio 7.

To confirm the Limit of Detection, 20 replicates at the lowest level detected were tested: 78 copies/reaction. Nasopharyngeal swabs were collected from individuals negative for SARS-CoV-2 and placed into Universal Viral Transport Media (sample matrix). SARS-Related Coronavirus 2, Isolate USA-WA1/2020 obtained from BEI Resources (catalog NR-52285) was diluted into the sample matrix at the lowest level detected above. The samples were extracted on the KingFisher Flex using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation protocol and then RT-PCR was performed on the QuantStudio 7.

Table 4: LOD Confirmation

LOD Confirmation				
Replicate	**E gene**	**N Gene**	**RdRP gene**	**IC**
1	33.10	31.09	32.62	28.28
2	32.37	31.87	33.42	27.96
3	33.17	31.76	33.93	27.49
4	33.09	32.62	33.83	28.76
5	31.55	31.26	33.38	28.61
6	32.98	32.02	34.47	28.85
7	31.92	31.46	32.76	27.61
8	32.43	31.70	33.35	28.73
9	32.56	31.71	32.75	26.80
10	32.19	31.34	33.23	28.24
11	32.45	32.94	32.96	28.70
12	32.19	31.92	33.26	28.92
13	32.46	31.10	32.73	27.73

LOD Confirmation				
Replicate	**E gene**	**N Gene**	**RdRP gene**	**IC**
14	32.46	32.10	33.44	28.21
15	32.07	31.49	34.48	29.28
16	32.23	30.81	32.70	26.99
17	31.46	30.85	32.47	28.81
18	40.00	32.21	38.14	28.77
19	31.42	31.64	33.98	29.54
20	33.94	31.52	34.31	27.66
Average	32.80	31.67	33.61	28.30
Standard Deviation	1.80	0.55	1.24	0.74
CV	5.5%	1.7%	3.7%	2.6%
# Detected	**19**	**20**	**20**	**20**
% Detected	**95%**	**100%**	**100%**	**100%**

The LoD was confirmed to be 78 copies/reaction based on a positivity rate of ≥95% for 20 replicates.

2) ***Analytical Inclusivity:***

In Silico Analysis of Primer and Probe Inclusivity:

In silico analysis was conducted for SARS-CoV-2 strains. Inclusivity is defined as 100% homology between 'primer set' and any sequence present in the targeted microorganism. The gene amplicons for each target (E, N and RdRP) were tested on the Basic Local Alignment Search Tool (BLAST) located on the National Center for Biotechnology Information (NCBI) a division of the National Institutes of Health (NIH) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) All three gene targets had 100% detection with all SARS-CoV-2 Strains.

Table 5: *In silico* analysis

	N Gene	**E Gene**	**RdRP**
Number of NCBI Strains Evaluated	96	95	96

3) ***Analytical Specificity:***

In Silico Analysis of Primer and Probe Exclusivity:

In silico analysis was conducted by the kit manufacturer, Seegene. (Seegene is the kit manufacture, Cirrus Dx has modified the assay for use on the QuantStudio). Seegene defines *in silico* cross-reactivity as greater than 80% homology between 'primer set' and any sequence present in the targeted microorganism. The conditions under which cross-reaction can occur are at least established as being capable of producing an amplicon (<500 bp) and are limited to more than 80% homology of all the oligos that bind to the microorganism.

Table 6: *In-silico* Analysis

Microorganism	RdRP gene	E gene	N gene	Complex*
Human coronavirus 229E	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Human coronavirus OC43	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Human coronavirus HKU1	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Human coronavirus NL63	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
SARS-coronavirus	Amp. Mis.#	**100% Match**	Amp. Mis.#	Amp. Mis.#
MERS-coronavirus	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Adenovirus	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Human Metapneumovirus	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Parainfluenza virus 1	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Parainfluenza virus 2	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Parainfluenza virus 3	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Parainfluenza virus 4	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Influenza A virus	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Influenza B virus	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Enterovirus	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Respiratory syncytial virus	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Rhinovirus	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Chlamydia pneumoniae	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#

Microorganism	RdRP gene	E gene	N gene	Complex*
Haemophilus influenzae	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Legionella pneumophila	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Mycobacterium tuberculosis	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Streptococcus pneumoniae	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Streptococcus pyogenes	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Bordetella pertussis	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Mycoplasma pneumoniae	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Pneumocystis jirovecii (PJP)	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Candida albicans	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Pseudomonas aeruginosa	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Staphylococcus epidermis	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#
Streptococcus salivarius	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#	Amp. Mis.#

***Complex** means forming amplicon in the oligo combination 500 bp or less, it can be judged that there is a possibility of cross reactivity *in silico*.
#**Amp.Mis.** means Amplicon mismatch. It means that the combination of oligos that match 80% or more within 500bp is not produced in sequence of each microorganism.

In silico analysis demonstrated that there were no cross-reactivity organisms except sequences E gene targeting SARS-coronavirus matched. Therefor the following corresponding organism were additionally wet-tested to confirm the cross-reactivity of the CirrusDx *SARS-CoV-2*. 61 non-target organisms were prepared by spiking each standard organism (concentration of > 10^6 CFU/mL or 10^5 PFU/mL or 10^6 genome copies/rxn) into negative sample matrix. Nucleic acids were extracted and Real-time PCR for Allplex™ 2019-nCoV Assay was carried out on CFX96™ (Bio-Rad). Testing was performed in triplicate, under the same conditions. As a result, all 61 non-target samples were not detected.

Table 7: Cross-reactivity (Analytical Specificity)

#	Organism	Source	Isolate No	Conc.	E gene	RdRP gene	N gene	IC
1	human coronavirus HKU1	Korean isolate		$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
2	human coronavirus OC43	ATCC	VR-1558	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
3	human coronavirus NL63	Korean isolate		$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
4	human coronavirus 229E	ATCC	VR-740	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
5	human Severe Acute Respiratory Syndrom, SARS*	Korean isolate		$>10^5$ pfu/mL	3/3	0/3	0/3	3/3
6	human Middle East Respiratory Syndrome Coronavirus : MERS-CoV	Korean isolate		$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
7	influenza A virus (H1N1)	ATCC	VR-95	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
8	Influenza A virus (H3N2)	ATCC	VR-547	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
9	influenza B virus	ATCC	VR-523	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
10	Human Rhinovirus 1	KBPV	VR-81	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
11	Rhinovirus 21	KBPV	VR-40	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
12	Human rhinovirus type 90	ATCC	VR-1291	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
13	Human rhinovirus type 16	ATCC	VR-283	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
14	Human rhinovirus type 42	ATCC	VR-338	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
15	Human rhinovirus type 8	ATCC	VR-488	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
16	Human rhinovirus type 14	ATCC	VR-284	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
17	Human enterovirus type 68	ATCC	VR-1826	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
18	Human enterovirus type 70	ATCC	VR-836	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3

#	Organism	Source	Isolate No	Conc.	E gene	RdRP gene	N gene	IC
19	Human enterovirus type 71	ATCC	VR-784	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
20	human respiratory syncytial virus A	ATCC	VR-26	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
21	human respiratory syncytial virus B	ATCC	VR-955	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
22	Parainfluenza 1 virus	ATCC	VR-1380	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
23	Human parainfluenza virus 2	ATCC	VR-92	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
24	Human parainfluenza virus 3	ATCC	VR-93	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
25	human parainfluenza 4 virus 4a	ATCC	VR-1378	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
26	Human parainfluenza virus 4b	ATCC	VR-1377	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
27	Human Metapneumovirus (MPV)	KBPV	VR-87	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
28	Human adenovirus 1	ATCC	VR-1	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
29	Human adenovirus 11	KBPV	VR-63	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
30	Human adenovirus 18	ATCC	VR-1095	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
31	Human adenovirus 23	ATCC	VR-1101	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
32	Human adenovirus 3	ATCC	VR-3	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
33	Human adenovirus 4	ATCC	VR-1572	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
34	Human adenovirus 8	ATCC	VR-1368	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
35	Human adenovirus type 31	ATCC	VR-1109	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3

#	Organism	Source	Isolate No	Conc.	E gene	RdRP gene	N gene	IC
36	Human adenovirus type 40	ATCC	VR-931	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
37	Human adenovirus type 5	KBPV	VR-61	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
38	Human adenovirus type 35	ATCC	VR-718	$>10^5$ pfu/mL	0/3	0/3	0/3	3/3
39	*Legionella pneumophila* Serotype 2	ATCC	33154	$>10^6$CFU/mL	0/3	0/3	0/3	3/3
40	*Legionella pneumophila* subsp. *fraseri* Serotype 4	ATCC	33156	$>10^6$CFU/mL	0/3	0/3	0/3	3/3
41	*Legionella pneumophila* Serotype 7	ATCC	33823	$>10^6$CFU/mL	0/3	0/3	0/3	3/3
42	*Legionella pneumophila* Serotype 10	ATCC	43283	$>10^6$CFU/mL	0/3	0/3	0/3	3/3
43	*Legionella pneumophila* Serotype 11	ATCC	43130	$>10^6$CFU/mL	0/3	0/3	0/3	3/3
44	*Legionella pneumophila* Serotype 12	ATCC	43290	$>10^6$CFU/mL	0/3	0/3	0/3	3/3
45	Legionella pneumophila Serotype 13	ATCC	43736	$>10^6$CFU/mL	0/3	0/3	0/3	3/3
46	Legionella pneumophila Serotype 14	ATCC	43703	$>10^6$CFU/mL	0/3	0/3	0/3	3/3
47	*Legionella pneumophila* subsp. *fraseri* Serotype 15	ATCC	35251	$>10^6$CFU/mL	0/3	0/3	0/3	3/3
48	*Mycoplasma pneumoniae*	ATCC	15293	$>10^6$CFU/mL	0/3	0/3	0/3	3/3
49	*Mycoplasma pneumoniae* M129-B7	ATCC	29342	$>10^6$CFU/mL	0/3	0/3	0/3	3/3

#	Organism	Source	Isolate No	Conc.	E gene	RdRP gene	N gene	IC
50	*Chlamydophila pneumoniae*	ATCC	53592	>10^6CFU/mL	0/3	0/3	0/3	3/3
51	*Bordetella pertussis*	ATCC	BAA-589	>10^6CFU/mL	0/3	0/3	0/3	3/3
52	*Pseudomonas aeruginosa* (Z139; VIM-1)	Zeptometrix	801908	>10^6CFU/mL	0/3	0/3	0/3	3/3
53	*Mycobacterium tuberculosis*	ATCC	25177	>10^6CFU/mL	0/3	0/3	0/3	3/3
54	*Haemophilus influenzae*	ATCC	51907	>10^6CFU/mL	0/3	0/3	0/3	3/3
55	*Streptococcus pneumoniae*	KCCM	40410	>10^6CFU/mL	0/3	0/3	0/3	3/3
56	*Streptococcus pyogenes*	ATCC	19615	>10^6CFU/mL	0/3	0/3	0/3	3/3
57	*Staphylococcus epidermidis*	KCCM	40416	>10^6CFU/mL	0/3	0/3	0/3	3/3
58	*Candida albicans*	KCCM	11282	>10^6CFU/mL	0/3	0/3	0/3	3/3
59	*Pneumocystis pneumonia jirovecii* (PJP)	Korean isolate		> 10^6 CFU/mL	>10^6CFU/mL	0/3	0/3	3/3
60	*Staphylococcus salivarius*	Korean isolate		> 10^6 CFU/mL	>10^6CFU/mL	0/3	0/3	3/3
61	Pooled human nasal wash	Clinical sample		N/A	0/3	0/3	0/3	3/3

*No.5 – This Cross-reactivity organism were only detected for E gene because it was beta-coronavirus not 2019-nCoV.

*4) **Clinical Evaluation***

The performance of the SARS-CoV-2 assay was evaluated with blind contrived samples. All samples were prepared with nasopharyngeal swabs from individuals negative for SARS-CoV-2 collected in Universal Viral Transport Media. Positive samples were contrived by adding SARS-Related Coronavirus 2 RNA, Isolate USA-WA1/2020 RNA obtained from BEI Resources (catalog NR-52285) at various concentrations. The samples were extracted on the

KingFisher Flex using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation protocol and then RT-PCR was performed on the QuantStudio 7. A total of 60 samples were tested including 30 negative samples and 30 positive samples spanning various SARS-CoV-2 RNA concentrations. A summary of results is provided in the Table below.

Table 8: Clinical Evaluation Analyzed Results

Sample	Conc. (RNA copies / Reaction)	Number of Samples	Number Positive^	% Performance Agreement	95 % CI
Negative	0	30	0	100%	88.6-100%
LOD	78	20	19	95%	76.4-99.1%
4x LOD	312.5	6	6	100%	70-100%
8x LOD	625	3	3	100%	43.9-100%
308x	24,000	1	1	100%	20.6-100%

^All three gene targets must be positive.
*Standard Deviation, CV and 95%CI were not calculated as only 1 replicate was tested.

The results of 18 positive and six negative specimens tested with the CirrusDx SARS-CoV-2 Assay were confirmed using an alternative assay (Simplexa COVID-19 Direct assay) and fulfills the requirement for confirmatory testing of at least five positive and five negative specimens.

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 and instrument used were KingFisher Flex and the QuantStudio 7 respectively. The results are summarized in Table 9.

Table 9: 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 Swab	$1.8x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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