RPERFORMANCE EVALUATION
(Real-Q 2019-nCoV Detection Kit)

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

1. Analytical sensitivity(Limit of Detection, LOD)

To determine the LoD, RdRP in-vitro transcribed RNA and E gene in-vitro transcribed RNA was serially diluted in simulated nasal matrix. The LoD was confirmed by testing 5 panel members with target concentrations at 30, 15, 7.5, 3.75 and 0.5 copies/uL tested on AB 7500 and CFX96 instrument in 20 repetitions. Then, the 95% LOD concentration was determined through probit analysis.

The results are summarized in below.

- LOD in CFX96 real-time PCR detection system

INPUT RdRP (copies/ul)	Positive Reaction	Replicates	%
30	20	20	100
15	20	20	100
7.5	19	20	95
3.75	15	20	75
0.5	0	20	0

RdRP gene 95% LOD : 6.87 copies/ul

INPUT E gene (copies/ul)	Positive Reaction	Replicates	%
30	20	20	100
15	20	20	100
7.5	18	20	90
3.75	15	20	75
0.5	0	20	0

E gene 95% LOD : 7.99 copies/ul

- LOD in Applied Biosystems 7500 Real-Time PCR Instrument System

INPUT RdRP (copies/ul)	Positive Reaction	Replicates	%
30	20	20	100
15	20	20	100

7.5	19	20	95
3.75	16	20	80
0.5	0	20	0

<div align="center">RdRP gene 95% probit : 6.51 copies/ul</div>

INPUT E gene (copies/ul)	Positive Reaction	Replicates	%
30	20	20	100
15	20	20	100
7.5	18	20	90
3.75	16	20	80
0.5	0	20	0

<div align="center">E gene 95% probit : 7.60 copies/ul</div>

2) *Inclusivity (analytical sensitivity):*

Inclusivity was demonstrated by comparing the Real-Q 2019-nCoV Detection Kit primers and probes to an alignment of all SARS-CoV-2 sequences available in Genbank (https://www.ncbi.nlm.nih.gov/nucleotide). Multiple sequence alignment by The MUSCLE alignment software was performed all SARS-CoV-2 sequences. The inclusivity of the 2019-nCoV detection was confirmed by in silico analyzing the position of the detection probe and primer used in the Real-Q 2019-nCoV Detection Kit in the alignment data through the GeneDoC MSA program. As a result of GeneDoC multiple sequence alignment on primer and probe sequences, all of the primers and probes in the Real-Q 2019-nCoV Detection Kit test had 100% homology to all of the available circulating SARS-CoV-2 sequences.

3) *Cross-reactivity (Analytical Specificity):*

Cross reactivity performance of Real-Q 2019-nCoV Detection Kit test was evaluated by testing 64 virus and bacterial standard strains representative samples listed in below. The cross-reactivity test was repeated twice. No cross-reactivity of the Real-Q 2019-nCoV Detection Kit test duplicate was observed at the concentrations tested. The results are summarized in below.

NO.	Strains	Concentration	Result
1	Adenovirus	1×10^6copies/ul	Negative
2	Parainfluenza virus 4	1×10^6copies/ul	Negative
3	Enterovirus	1×10^6copies/ul	Negative
4	Influenza A virus	1×10^6copies/ul	Negative
5	Influenza B virus	1×10^6copies/ul	Negative
6	Coronavirus 229E	1×10^6copies/ul	Negative

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7	Coronavirus OC43	1×10^6copies/ul	Negative
8	Coronavirus NL63	1×10^6copies/ul	Negative
9	Rhinovirus	1×10^6copies/ul	Negative
10	Parainfluenza virus 3	1×10^6copies/ul	Negative
11	Parainfluenza virus 2	1×10^6copies/ul	Negative
12	Parainfluenza virus 1	1×10^6copies/ul	Negative
13	Respiratory syncytial virus A	1×10^6copies/ul	Negative
14	Respiratory syncytial virus B	1×10^6copies/ul	Negative
15	Metapneumovirus	1×10^6copies/ul	Negative
16	Bocavirus	1×10^6copies/ul	Negative
17	Mycoplasma pneumoniae	1×10^6copies/ul	Negative
18	Chlamydophila pneumoniae	1×10^6copies/ul	Negative
19	Streptococcus pneumoniae	1×10^6copies/ul	Negative
20	Haemophilus influenzae	1×10^6copies/ul	Negative
21	Legionella pneumophila	1×10^6copies/ul	Negative
22	Bordetella pertussis	1×10^6copies/ul	Negative
23	Klebsiella pneumoniae	1×10^6copies/ul	Negative
24	Haemophilus haemolyticus	1×10^6copies/ul	Negative
25	Bordetella parapertussis	1×10^6copies/ul	Negative
26	Streptococcus mitis	1×10^6copies/ul	Negative
27	Haemophilus parainfluenza	1×10^6copies/ul	Negative
28	Streptococcus Pseudopneumonia	1×10^6copies/ul	Negative
29	Mycobacterium abscessus	1×10^6copies/ul	Negative
30	Mycobacterium avium	1×10^6copies/ul	Negative
31	Mycobacterium bolletii	1×10^6copies/ul	Negative
32	Mycobacterium celatum	1×10^6copies/ul	Negative
33	Mycobacterium chelone	1×10^6copies/ul	Negative
34	Mycobacterium conceptionense	1×10^6copies/ul	Negative
35	Mycobacterium fortuitum	1×10^6copies/ul	Negative
36	Mycobacterium gordonae	1×10^6copies/ul	Negative
37	Mycobacterium intracellulare	1×10^6copies/ul	Negative
38	Mycobacterium kansasii	1×10^6copies/ul	Negative
39	Mycobacterium marinum	1×10^6copies/ul	Negative
40	Mycobacterium massiliense	1×10^6copies/ul	Negative
41	Mycobacterium scrofulaceum	1×10^6copies/ul	Negative
42	Mycobacterium szulgai	1×10^6copies/ul	Negative
43	Mycobacterium terrae	1×10^6copies/ul	Negative
44	Mycobacterium xenopi	1×10^6copies/ul	Negative

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45	Chlamydia Trachomatis	1×10^6 copies/ul	Negative
46	Ureaplasma parvum	1×10^6 copies/ul	Negative
47	Mycoplasma genitalium	1×10^6 copies/ul	Negative
48	Trichomonas vaginalis	1×10^6 copies/ul	Negative
49	Neisseria Gonorrhoeae	1×10^6 copies/ul	Negative
50	Ureaplasma urealyticum	1×10^6 copies/ul	Negative
51	Mycoplasma hominis	1×10^6 copies/ul	Negative
52	Herpes simplex virus I	1×10^6 copies/ul	Negative
53	Herpes simplex virus II	1×10^6 copies/ul	Negative
54	BK virus	1×10^6 copies/ul	Negative
55	Cytomegalovirus	1×10^6 copies/ul	Negative
56	Epstein–Barr virus	1×10^6 copies/ul	Negative
57	Salmonella bongori	1×10^6 copies/ul	Negative
58	Campylobacter coli	1×10^6 copies/ul	Negative
59	Shigella sonnei	1×10^6 copies/ul	Negative
60	Clostridium perfringens	1×10^6 copies/ul	Negative
61	Yersinia enterocolitica	1×10^6 copies/ul	Negative
62	GroupA Rotavirus	1×10^6 copies/ul	Negative
63	Astrovirus	1×10^6 copies/ul	Negative
64	Aeromonas sobria	1×10^6 copies/ul	Negative

4) *Clinical Evaluation:*

A clinical evaluation study was performed to evaluate the performance of the Real-Q 2019-nCoV Detection Kit test using nasopharyngeal swab specimens. A total of 20 contrived positive specimens at approximately 2X LOD and 10 contrived positive specimens at approximately 20x to 100x LOD were tested. Samples were contrived by spiking known concentrations of SARS-CoV-2 genomic RNA* containing SARS-CoV-2 RNA sequences into negative patient specimens. In addition to the contrived positive specimens, 30 negative specimens were tested.

There were 30 total samples tested twice at the 2X to 100X LOD level with all results valid and included in the analysis. There were 30 total samples tested for the negative level with all results valid and included in the analysis

SARS-CoV-2 Concentration	Number Tested	Positive Detected	% Detection
2X LOD**	20	20	100
20x to 100x LOD	40	40	100
Negative	30	0	0

- SARS-CoV-2 genomic RNA＊：This is RNA sample from the National Culture Collection for Pathogen (NCCP) in Republic of Korea.
- LOD**: E gene LOD (7.99 copies/ul) confirmed in CFX96 was used.

5) *Clinical Verification:*

We verified Real-Q 2019-nCoV Detection Kit test on 73 patient samples that knew the COVD-19 diagnosis result and confirmed that all the results matched.

Sample No.	COVD-19	2019-nCoV Ct (RdRP, ≤38)	Beta CoV Ct (E gene, ≤38)	IC Ct (RNaseP, ≤35)	Interpretation
1	Positive	34.2	36.0	24.7	2019-nCoV positive
2	Positive	25.6	25.7	24.6	2019-nCoV positive
3	Positive	31.4	31.7	24.5	2019-nCoV positive
4	Positive	28.4	28.6	24.5	2019-nCoV positive
5	Positive	26.9	27.2	28.5	2019-nCoV positive
6	Positive	23.3	23.9	27.9	2019-nCoV positive
7	Positive	33.5	34.4	28.6	2019-nCoV positive
8	Positive	30.2	30.7	28.7	2019-nCoV positive
9	Positive	29.4	30.0	26.1	2019-nCoV positive
10	Positive	31.0	31.7	24.0	2019-nCoV positive
11	Positive	35.9	35.0	27.8	2019-nCoV positive
12	Positive	33.1	33.3	25.1	2019-nCoV positive
13	Positive	29.9	30.5	30.8	2019-nCoV positive
14	Positive	22.9	23.7	23.4	2019-nCoV positive
15	Positive	31.6	32.4	24.7	2019-nCoV positive
16	Positive	26.4	27.1	27.9	2019-nCoV positive
17	Positive	34.7	35.1	27.1	2019-nCoV positive
18	Positive	27.2	28.0	27.5	2019-nCoV positive
19	Positive	35.4	36.0	27.2	2019-nCoV positive
20	Positive	35.8	36.1	25.9	2019-nCoV positive
21	Positive	33.7	35.1	25.5	2019-nCoV positive
22	Negative	N/A	N/A	29.0	Negative
23	Negative	N/A	N/A	28.0	Negative
24	Negative	N/A	N/A	26.1	Negative
25	Positive	34.7	36.7	26.3	2019-nCoV positive
26	Positive	34.2	35.9	26.9	2019-nCoV positive
27	Negative	N/A	N/A	27.2	Negative
28	Negative	N/A	N/A	28.2	Negative
29	Negative	N/A	N/A	28.7	Negative
30	Negative	N/A	N/A	26.8	Negative
31	Negative	N/A	N/A	27.0	Negative
32	Negative	N/A	N/A	29.3	Negative
33	Negative	N/A	N/A	26.7	Negative
34	Negative	N/A	N/A	26.3	Negative
35	Negative	N/A	N/A	25.9	Negative
36	Negative	N/A	N/A	26.9	Negative
37	Negative	N/A	N/A	26.1	Negative

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38	Negative	N/A	N/A	27.0	Negative
39	Negative	N/A	38.8	26.7	Negative
40	Negative	N/A	N/A	26.0	Negative
41	Positive	32.6	32.4	25.5	2019-nCoV positive
42	Positive	23.5	24.0	26.1	2019-nCoV positive
43	Positive	29.7	30.0	26.0	2019-nCoV positive
44	Negative	N/A	N/A	26.1	Negative
45	Positive	27.0	27.6	26.2	2019-nCoV positive
46	Positive	27.1	27.5	30.2	2019-nCoV positive
47	Negative	N/A	N/A	29.4	Negative
48	Positive	23.2	23.4	29.9	2019-nCoV positive
49	Positive	33.5	33.2	29.8	2019-nCoV positive
50	Positive	30.4	30.6	30.1	2019-nCoV positive
51	Negative	N/A	N/A	23.6	Negative
52	Positive	24.5	24.8	23.9	2019-nCoV positive
53	Positive	31.4	31.8	24.0	2019-nCoV positive
54	Negative	N/A	N/A	23.8	Negative
55	Positive	28.0	28.4	23.7	2019-nCoV positive
56	Positive	25.3	25.4	28.1	2019-nCoV positive
57	Negative	N/A	N/A	28.7	Negative
58	Positive	22.1	22.3	28.1	2019-nCoV positive
59	Positive	32.8	33.5	29.5	2019-nCoV positive
60	Positive	28.6	29.1	28.5	2019-nCoV positive
61	Positive	25.7	25.6	31.3	2019-nCoV positive
62	Positive	25.9	25.9	32.3	2019-nCoV positive
63	Positive	27.3	27.4	32.7	2019-nCoV positive
64	Negative	N/A	N/A	29.7	Negative
65	Negative	N/A	N/A	31.2	Negative
66	Positive	25.6	25.9	33.0	2019-nCoV positive
67	Positive	25.5	25.6	32.5	2019-nCoV positive
68	Positive	27.2	27.4	34.4	2019-nCoV positive
69	Negative	N/A	N/A	28.6	Negative
70	Negative	N/A	N/A	30.4	Negative
71	Positive	25.5	25.7	34.1	2019-nCoV positive
72	Positive	26.3	26.5	34.9	2019-nCoV positive
73	Positive	27.0	27.2	34.9	2019-nCoV positive

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