

Performance Evaluation of 1copy™ COVID19/Flu A/Flu B/RSV qPCR kit

LoD Test

MR-M25-LoD-2012-001

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1. Purpose

The limits of detection and quantitation are critical because detecting extremely small amounts of an analyte can be necessary to define disease states, screen for disease, identify significant exposure, or to reveal the presence or absence of toxins, pollutants, carcinogens, contaminants, infectious agents, and illicit drugs.

The report is intended to evaluate the minimum detection level of 1copy™ COVID-19/Flu A/Flu B/RSV qPCR Kit System.

2. Study Design

LoD test was assessed by RNA using genetic information based on CDC and WHO guidelines. We evaluated LoD using standard materials through specific primer/probe sets of target pathogens. Each of the primer/probe sets was designed for detection of influenza A virus, influenza B virus, respiratory syncytial virus, and SARS-CoV-2(N, RdRp gene) specific sequences according to CDC and WHO guidelines.

The sequences of target pathogens are as below.

Flu A ¹⁾ (GQ132148.1, 156-261, 106bp)

gaccaactctgtcacctctgactaaggggaatttaggattgtgttcacgctcaccgtgccagtgagcggaggactgcagcgtagacgctttgccaaaatgccct

Flu B ¹⁾ (MW251816.1, 747-849, 103bp)

tcctcaactcactcttcgagcgtctcaatgaaggacattcaaagccaattcgagcagctgaaactcggtgggagtctatcccaatttggtcaagagcaccga

RSV ²⁾ (MK989761.1, 1128-1244, 117bp)

gctcttagcaaagtcgaagtgaatgatacattaaataaggatcagctgctgcatccagcaaatacactattcaacgtagtacaggagataatattgacactcc
caattatgatgtg

SARS-CoV-2 RdRp gene ³⁾ (NC_045512, 13921-14026, 106bp)

agacaaggaactgattacaaacattggccgcaaattgcacaatttgccccagcgttcagcgttcttcggaatgtcgcgcattggcatggaagtacacac

SARS-CoV-2 N gene ⁴⁾ (NC_045512, 29150-29251, 102bp)

gactggtatgattttgtagaaaaccagatatattacggtatagcgaacttaggtgaacgtgtacgccaagctttgttaaaaacagtacaattctgtgatgcc

The RNA reference materials for the experiment were AMPLIRUN Influenza A virus (Vircell, Cat. No. MBC029), AMPLIRUN Influenza B (Vircell, Cat.No. MBC030), AMPLIRUN Respiratory syncytial virus (Vircell, Cat.No. MBC041) and AccuPlex™ SARS-CoV-2 Verification Panel - Full Genome (Seracare, Cat. No. 0505-0168).

Standard material applies to clinical matrix sample (Nasopharyngeal swab) taken according to U.S. CDC guidelines.

https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fguidelines-clinical-specimens.html

Clinical matrix sample collection methods introduced by U.S. CDC guidelines : Nasopharyngeal swab
Insert a swab into nostril parallel to the palate. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.

For samples without spiking of reference material RNA, use it as a negative control.

For processed samples, RNA prep is according to the U.S. CDC guide (FDA) below.

<https://www.fda.gov/media/134922/download>

The RNA prep kits used in the LoD experiment are shown below.

QIAamp Viral RNA Mini Kit 50 extractions (QIAGEN, Cat no.52904)

LoD shall be determined by measuring the concentration of at least 5 dilution concentrations and then confirm the concentration with 20 replicates. The concentrations of reference material RNA were 4000~160 copies/ml and measured 20 times of each primer/probe set.

We defined LoD as the lowest concentration at which over 95% replicates are positive.

[Reference]

- 1) Selvaraju, Suresh B., and Rangaraj Selvarangan. "Evaluation of three influenza A and B real-time reverse transcription-PCR assays and a new 2009 H1N1 assay for detection of influenza viruses." *Journal of clinical microbiology* 48.11 (2010): 3870-3875.
- 2) Aamir, Uzma Bashir, et al. "Molecular characterization of circulating respiratory syncytial virus (RSV) genotypes in Gilgit Baltistan Province of Pakistan during 2011-2012 winter season." *PLoS One* 8.9 (2013): e74018.
- 3) Corman, Victor, et al. "Diagnostic detection of 2019-nCoV by real-time RT-PCR." World Health Organization, Jan 17 (2020).
- 4) Centers for Disease Control and Prevention, Respiratory Viruses Branch, Division of Viral Diseases. "2019-novel coronavirus (2019-nCoV) real-time rRT-PCR panel primers and probes." DEPARTMENT OF HEALTH & HUMAN SERVICES 24 (2020).

2-1. Limit of Detection test

To determine the LoD, RNA of SARS-CoV-2, Flu A, Flu B and RSV was measured respectively. The concentrations of reference material RNA were 4000~160 copies/ml and measured 20 times of each primer/probe set.

We defined LoD as the lowest concentration (copy number/reaction) at which over 95% replicates are positive.

* 3200, 1600, 800, 400, 200 copies/ml for Flu A, Flu B and RSV and 4000, 1600, 800, 400, 160 copies/ml for SARS-CoV-2

2-2. Cut-off value

Ct values above 40 for FAM, Texas Red, VIC, Cy5, Quabsar705 and TAMRA signals may be the result of unspecific amplification (false positive). Based on the above cut-off values, the Ct value shown for each equipment shall be determined by a positive signal only if Ct value is not more than 40. (Ct ≤ 40)

Ct Value	Result
≤40	Valid (+)
>40 or N/A	Invalid (-)

The settings for checking Cut off are as follows.

Target	Baseline				
	CFX96	ABI 7500	ABI Quantstudio5	Begin	End
Flu A; FAM	500	50,000	15,000	3	15
Flu B; VIC	500	50,000	15,000		
SARS-CoV-2; Tex RED	500	50,000	15,000		
RSV; Cy5	500	50,000	15,000		
IPC; Quasar705	500	-	-		
IPC; TAMRA	-	25,000	15,000		

3. Materials and Equipment

- 1) Test Date: 2020.12.7~11
- 2) Test Site: Laboratory (Temperature Range: 23.0°C to 25.0°C, Humidity: 30.0 ~ 50.0% RH)
- 3) Test Operator: Seongsoo Hong, R&D department
- 4) Lot No: M250L0100_Exp. Date: 2021.11.30

5) Real-time PCR amplification and detection instrument

Model Name	CFX96™ Real-time PCR Detection System
Manufacturer	Bio-Rad
S/N	787BR00450

Model Name	Applied Biosystems® Quantastudio5
Manufacturer	Thermo Fisher SCIENTIFIC

Model Name	Applied Biosystems® 7500
Manufacturer	Thermo Fisher SCIENTIFIC
S/N	275006638

6) Micropipette

Asset No.	Volume	Calibration valid date
I-C2-004	1,000 μ l	2021-09-22
I-C2-003	100 μ l	2021-09-22
I-C2-002	10 μ l	2021-09-22

7) Digital thermo-hygrometer

Model Name	Testo 622
Asset No.	I-A2-010
Calibration valid data	2021-11-12

4. Procedure

- 1) Prepare the dilute each standard genetic material to suit the set concentration.
- 2) Prep each RNA according to the QIAamp Viral RNA Mini Kit User Guide.
- 3) Prepare Master mix, Primer probe mix.
- 4) Prepare a 96-well plate and follow the table below for the composition of the experiment.

Reagent	volume.
Master mix	10 ul
Primer probe mix	1 ul
sample	5 ul (each concentration per test)
DEPC-DW	Up to total 20 ul
Total	20 ul

- 5) Once the amount of mixture required for the experiment has been prepared, dispense 20 ul into 96 well plate.
- 6) Sealing the plate and spin down the sample.
- 7) Set up the software real-time PCR instrument
- 8) PCR reaction condition followed the table below.

Step	temperature	Time	Cycle
RT	55 °C	5 min	1
Incubation	95 °C	3 min	1
Amplification	95 °C	5 sec	40
	62°C*	12 sec	

* Measure fluorescence at 62 ° C.

* For ABI 7500, set the amplification time for 62°C as 28 sec.

- 9) Check the channel of Fluorescence

* For CFX96, check FAM, TexasRed, Cy5, VIC and Quasar705

* For ABI7500 and Quanta studio 5, check FAM, TexasRed, Cy5, VIC and TAMRA

- 10) Start run of real-time PCR instrument.

5. Data Analysis

Table 1. CFX96 LoD Test result

Copies/ml	CFX96 (Flu A)				
	3200 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	200 cps/ml
Rep 1	34.49	35.19	36.33	N/A	N/A
Rep 2	34.40	35.31	35.40	N/A	39.11
Rep 3	34.23	35.20	35.93	N/A	38.95
Rep 4	34.31	35.29	36.84	38.13	38.63
Rep 5	34.34	35.34	36.29	39.54	38.25
Rep 6	34.18	35.22	37.04	38.57	N/A
Rep 7	33.99	35.14	35.72	37.54	N/A
Rep 8	34.30	35.33	35.60	38.77	39.75
Rep 9	34.24	34.96	35.97	37.11	N/A
Rep 10	33.99	35.98	35.76	37.95	38.70
Rep 11	33.90	35.99	36.42	38.91	N/A
Rep 12	34.01	35.25	35.99	38.07	38.31
Rep 13	34.23	35.25	35.58	38.21	N/A
Rep 14	33.92	35.96	37.31	N/A	38.11
Rep 15	34.05	35.26	36.23	N/A	38.66
Rep 16	34.08	35.70	36.03	38.10	39.07
Rep 17	34.01	35.55	35.88	38.15	38.78
Rep 18	34.21	34.61	35.40	39.54	N/A
Rep 19	35.04	35.00	36.47	37.35	N/A
Rep 20	34.18	35.32	36.84	39.06	39.15
AVG	34.21	35.34	36.15	38.33	38.79
%CV	0.75	0.99	1.50	1.91	1.18
Detection rate	100%	100%	100%	75%	60%

Copies/ml	CFX96 (Flu B)				
	3200 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	200 cps/ml
Rep 1	36.13	37.19	39.44	37.92	N/A
Rep 2	36.22	36.73	38.55	36.96	N/A
Rep 3	35.77	36.40	37.01	37.99	N/A
Rep 4	35.78	38.73	38.14	38.96	38.43
Rep 5	36.08	37.02	38.66	38.91	N/A
Rep 6	36.75	37.36	38.63	38.01	N/A
Rep 7	35.60	36.88	38.65	38.06	N/A
Rep 8	36.11	38.06	39.11	37.89	N/A
Rep 9	35.90	37.47	38.56	37.36	39.17
Rep 10	36.40	37.16	38.99	39.86	38.96
Rep 11	35.78	37.66	37.27	37.92	38.00
Rep 12	36.49	36.29	37.42	36.96	38.52
Rep 13	35.60	36.55	38.63	37.91	39.04
Rep 14	35.37	37.48	37.33	N/A	N/A
Rep 15	35.70	36.91	38.47	36.10	N/A
Rep 16	35.73	37.86	38.57	38.29	N/A
Rep 17	35.94	37.15	38.74	N/A	N/A
Rep 18	35.87	37.15	38.67	39.01	N/A
Rep 19	36.03	37.98	37.49	37.96	39.14
Rep 20	35.90	37.24	37.25	39.04	N/A
AVG	35.96	37.26	38.28	38.06	38.75
%CV	0.92	1.60	1.86	2.35	1.14
Detection rate	100%	100%	100%	90%	35%

Copies/ml	CFX96 (RSV)				
	3200 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	200 cps/ml
Rep 1	36.98	37.76	38.32	N/A	N/A
Rep 2	36.36	37.79	39.59	39.92	N/A
Rep 3	37.25	37.56	39.25	39.21	N/A
Rep 4	36.60	37.39	38.54	N/A	N/A
Rep 5	37.13	37.99	38.54	39.66	N/A
Rep 6	37.02	38.29	38.72	39.77	N/A
Rep 7	36.54	37.33	38.40	N/A	N/A
Rep 8	36.88	37.91	38.60	39.40	N/A
Rep 9	36.58	37.83	38.56	N/A	N/A
Rep 10	36.84	37.34	38.66	39.90	N/A
Rep 11	36.47	37.18	38.85	39.04	N/A
Rep 12	36.52	37.80	38.66	39.42	N/A
Rep 13	36.55	37.24	39.06	39.62	N/A
Rep 14	36.78	37.28	39.59	39.45	N/A
Rep 15	37.18	38.07	39.67	39.66	N/A
Rep 16	36.46	37.98	39.10	39.39	N/A
Rep 17	36.55	37.37	39.23	N/A	N/A
Rep 18	36.70	37.60	39.21	39.45	N/A
Rep 19	36.80	37.82	38.94	N/A	N/A
Rep 20	36.94	38.10	38.29	39.89	N/A
AVG	36.76	37.68	38.89	39.56	-
%CV	0.72	0.87	1.11	0.67	-
Detection rate	100%	100%	100%	70%	0%

Copies/ml	CFX96 (SARS-CoV-2)				
	4000 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	160 cps/ml
Rep 1	32.15	33.11	34.99	36.22	38.01
Rep 2	32.55	33.94	35.03	35.12	35.24
Rep 3	32.58	33.69	35.84	34.89	39.04
Rep 4	32.29	33.68	34.48	34.51	36.14
Rep 5	31.96	33.52	35.45	34.35	N/A
Rep 6	32.03	33.87	34.84	35.30	37.00
Rep 7	32.43	34.03	34.52	34.18	N/A
Rep 8	31.87	33.65	35.14	35.13	37.34
Rep 9	32.10	33.64	34.64	35.29	38.97
Rep 10	31.97	33.86	34.79	34.29	36.78
Rep 11	32.04	33.55	33.53	36.23	36.41
Rep 12	32.47	33.76	34.40	35.15	36.89
Rep 13	32.14	33.28	34.21	34.94	37.56
Rep 14	32.09	34.22	34.48	37.04	35.54
Rep 15	32.16	33.39	35.83	36.02	36.55
Rep 16	31.77	33.28	34.33	34.33	38.73
Rep 17	32.25	33.75	33.84	36.01	39.24
Rep 18	32.13	33.76	33.93	34.93	36.94
Rep 19	32.22	33.60	34.49	35.49	36.06
Rep 20	31.82	33.85	34.57	35.83	37.10
AVG	32.15	33.67	34.67	35.26	37.20
%CV	0.71	0.80	1.74	2.19	3.21
Detection rate	100%	100%	100%	100%	90%

IPC	Flu A	Flu B	RSV	Covid-19
	3200 copies/ml			4000 cps/ml
Rep 1	33.69	34.57	34.17	32.18
Rep 2	34.67	33.38	33.86	32.10
Rep 3	33.73	34.49	33.71	32.12
Rep 4	34.17	34.62	32.80	32.60
Rep 5	33.90	33.52	34.13	33.75
Rep 6	34.03	34.00	33.57	33.76
Rep 7	34.82	33.40	33.13	33.44
Rep 8	33.43	34.76	33.83	32.93
Rep 9	34.40	33.66	33.45	32.82
Rep 10	35.08	34.30	33.24	33.27
Rep 11	33.46	34.24	33.46	32.53
Rep 12	34.57	33.05	33.40	32.85
Rep 13	34.90	33.60	33.52	32.67
Rep 14	33.36	33.39	32.15	32.88
Rep 15	33.54	34.62	32.79	32.74
Rep 16	34.12	34.47	32.11	32.50
Rep 17	34.19	33.64	32.18	33.88
Rep 18	34.78	33.26	33.90	33.49
Rep 19	34.26	33.79	32.25	33.78
Rep 20	34.80	34.32	34.13	32.98
AVG	34.20	33.95	33.29	32.96
%CV	1.59	1.60	2.08	1.72
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	1600 copies/ml			
Rep 1	33.72	33.98	34.10	32.18
Rep 2	34.51	33.50	32.24	33.67
Rep 3	34.71	34.78	32.22	32.43
Rep 4	33.34	35.00	33.92	33.83
Rep 5	34.91	34.79	33.41	32.60
Rep 6	34.35	34.64	33.03	33.21
Rep 7	35.19	33.74	33.28	32.26
Rep 8	34.56	33.27	32.34	32.32
Rep 9	34.98	34.86	32.45	32.97
Rep 10	33.29	33.68	33.16	33.72
Rep 11	34.50	33.17	32.10	32.69
Rep 12	35.19	33.32	32.98	33.48
Rep 13	34.66	34.75	33.47	33.11
Rep 14	34.40	33.53	33.62	32.89
Rep 15	33.94	33.29	32.10	32.12
Rep 16	34.18	34.02	32.37	32.98
Rep 17	34.45	34.93	34.14	33.67
Rep 18	34.82	34.33	33.68	32.47
Rep 19	33.52	34.09	33.22	32.74
Rep 20	33.80	34.95	32.59	33.86
AVG	34.35	34.13	33.02	32.96
%CV	1.69	1.94	2.07	1.77
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	800 copies/ml			
Rep 1	34.13	33.72	32.31	33.64
Rep 2	34.36	34.16	33.08	32.98
Rep 3	34.29	34.63	33.80	32.83
Rep 4	33.37	34.31	33.22	32.41
Rep 5	33.99	33.14	34.16	33.19
Rep 6	35.03	34.91	33.71	33.42
Rep 7	34.61	33.56	33.79	33.02
Rep 8	34.27	34.00	32.16	32.65
Rep 9	34.85	34.76	32.34	33.62
Rep 10	34.02	33.46	33.43	33.97
Rep 11	34.83	34.30	32.68	33.79
Rep 12	33.80	33.27	33.98	33.99
Rep 13	34.57	34.94	33.70	32.97
Rep 14	34.95	33.41	33.14	32.11
Rep 15	35.12	34.57	32.84	32.97
Rep 16	33.57	33.22	34.09	33.09
Rep 17	33.84	33.05	33.28	32.13
Rep 18	35.05	33.93	32.12	32.19
Rep 19	33.87	34.96	32.88	33.15
Rep 20	34.58	34.64	34.08	32.90
AVG	34.36	34.05	33.24	33.05
%CV	1.52	1.93	2.03	1.73
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	400 copies/ml			
Rep 1	33.85	33.63	32.67	32.42
Rep 2	34.63	34.40	32.66	32.73
Rep 3	33.72	33.17	32.87	33.49
Rep 4	33.29	33.67	33.34	32.83
Rep 5	33.69	33.46	32.87	33.14
Rep 6	33.65	34.58	32.18	33.28
Rep 7	34.87	33.83	32.45	32.60
Rep 8	34.57	33.67	33.10	32.88
Rep 9	33.49	33.03	32.69	32.71
Rep 10	33.37	33.18	32.26	33.51
Rep 11	34.11	33.54	33.61	33.87
Rep 12	34.51	33.35	33.66	33.21
Rep 13	34.44	33.10	32.58	33.71
Rep 14	34.69	33.75	32.55	33.00
Rep 15	34.03	33.31	33.95	33.82
Rep 16	33.45	34.58	32.48	33.38
Rep 17	34.00	34.43	33.59	33.71
Rep 18	33.69	34.15	32.82	32.18
Rep 19	34.51	34.82	32.21	33.57
Rep 20	33.49	34.10	33.01	32.22
AVG	34.00	33.79	32.88	33.11
%CV	1.48	1.63	1.57	1.59
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	200 copies/ml			160 copies/ml
Rep 1	33.61	33.39	32.67	32.44
Rep 2	34.62	33.05	32.88	33.55
Rep 3	33.58	34.94	33.25	32.79
Rep 4	34.53	34.74	32.56	33.59
Rep 5	33.25	34.21	32.18	32.52
Rep 6	33.33	34.16	33.34	32.39
Rep 7	34.10	34.81	33.83	32.13
Rep 8	34.68	33.13	32.82	33.11
Rep 9	34.79	34.42	34.22	33.55
Rep 10	34.13	33.95	33.84	32.51
Rep 11	34.56	34.81	33.54	32.79
Rep 12	34.41	33.15	32.21	32.92
Rep 13	34.80	33.25	32.41	32.63
Rep 14	34.88	33.05	32.63	32.64
Rep 15	33.51	34.17	33.85	32.91
Rep 16	33.44	34.72	33.76	32.48
Rep 17	34.89	34.75	32.82	32.45
Rep 18	33.67	33.31	32.86	33.28
Rep 19	34.25	34.66	33.52	32.86
Rep 20	33.76	34.54	33.64	32.49
AVG	34.14	34.06	33.14	32.80
%CV	1.67	2.07	1.85	1.29
Detection rate	100%	100%	100%	100%

Table 2. 7500 LoD Test result

Copies/ml	ABI7500 (Flu A)				
	3200 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	200 cps/ml
Rep 1	34.26	36.95	37.26	38.23	ND
Rep 2	35.04	36.99	37.09	37.16	39.39
Rep 3	34.58	36.14	36.96	37.08	38.32
Rep 4	34.88	36.08	38.02	37.06	ND
Rep 5	35.08	35.87	37.48	37.51	ND
Rep 6	34.93	36.93	36.48	38.67	39.12
Rep 7	34.54	37.06	38.26	38.02	ND
Rep 8	34.91	35.93	38.20	ND	39.68
Rep 9	34.48	36.55	36.65	38.34	ND
Rep 10	34.36	36.90	36.25	36.98	ND
Rep 11	35.59	36.78	37.60	38.14	ND
Rep 12	34.53	38.65	38.29	37.46	ND
Rep 13	35.14	35.75	37.16	38.06	ND
Rep 14	34.80	36.44	37.03	39.16	ND
Rep 15	35.23	36.98	39.40	37.93	ND
Rep 16	34.80	36.12	37.94	38.34	ND
Rep 17	35.33	36.28	37.33	38.85	ND
Rep 18	34.92	36.75	37.86	ND	ND
Rep 19	34.57	36.86	36.78	39.16	ND
Rep 20	34.81	36.76	ND	37.69	39.10
AVG	34.84	36.64	37.48	37.99	39.12
%CV	1.00	1.74	2.05	1.84	1.29
Detection rate	100%	100%	95%	90%	25%

	ABI7500 (Flu B)				
Copies/ml	3200 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	200 cps/ml
Rep 1	35.11	35.89	38.73	39.31	39.29
Rep 2	35.60	36.24	37.18	38.32	ND
Rep 3	35.03	38.37	38.11	39.24	37.92
Rep 4	35.53	35.63	37.23	38.12	38.40
Rep 5	35.47	37.10	37.35	36.86	ND
Rep 6	35.02	36.95	37.05	37.05	ND
Rep 7	34.83	36.11	38.07	38.08	38.35
Rep 8	34.60	37.22	37.73	37.54	ND
Rep 9	34.45	36.20	37.20	37.72	38.61
Rep 10	35.36	36.84	38.10	39.52	37.47
Rep 11	35.92	36.24	39.53	37.07	ND
Rep 12	34.77	35.39	37.99	ND	39.59
Rep 13	34.90	35.95	37.58	38.16	ND
Rep 14	34.69	36.82	38.05	38.19	38.58
Rep 15	35.59	37.43	38.24	38.13	39.83
Rep 16	35.53	36.47	36.70	ND	ND
Rep 17	35.52	36.29	37.20	38.04	38.13
Rep 18	35.02	36.30	38.02	38.09	ND
Rep 19	34.82	36.45	38.04	36.94	37.65
Rep 20	34.80	36.08	37.40	38.04	ND
AVG	35.13	36.50	37.77	38.02	38.53
%CV	1.16	1.88	1.73	2.04	1.99
Detection rate	100%	100%	100%	90%	55%

	ABI7500 (RSV)				
Copies/ml	3200 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	200 cps/ml
Rep 1	35.16	35.92	37.08	38.32	39.82
Rep 2	34.91	35.61	37.36	37.63	ND
Rep 3	34.82	36.29	36.96	38.57	37.89
Rep 4	34.88	35.55	36.86	38.88	39.08
Rep 5	35.06	35.97	37.23	37.85	38.83
Rep 6	34.62	35.97	37.21	37.16	ND
Rep 7	34.29	35.31	37.29	37.21	38.91
Rep 8	34.73	35.99	37.15	ND	ND
Rep 9	34.60	35.51	37.50	38.64	ND
Rep 10	34.38	36.68	37.52	37.69	ND
Rep 11	35.10	36.58	36.13	37.57	ND
Rep 12	34.46	35.21	36.54	38.30	38.66
Rep 13	35.41	35.64	36.47	ND	38.50
Rep 14	34.81	35.56	36.74	38.01	ND
Rep 15	34.54	36.17	37.03	37.63	ND
Rep 16	34.78	35.82	37.37	38.15	39.43
Rep 17	34.64	35.30	36.60	38.43	38.87
Rep 18	35.02	36.58	36.58	38.01	39.75
Rep 19	34.96	35.42	37.44	37.52	ND
Rep 20	35.05	35.62	37.07	36.83	ND
AVG	34.81	35.84	37.01	37.91	38.97
%CV	0.81	1.24	1.05	1.47	1.50
Detection rate	100%	100%	100%	90%	50%

Copies/ml	ABI7500 (SARS-CoV-2)				
	4000 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	160 cps/ml
Rep 1	32.47	33.59	34.99	35.57	38.90
Rep 2	32.56	34.34	35.32	ND	37.43
Rep 3	32.81	33.79	35.16	35.68	37.43
Rep 4	32.66	34.21	34.72	35.78	36.64
Rep 5	32.31	33.72	34.98	36.00	39.43
Rep 6	32.49	34.71	34.66	35.95	ND
Rep 7	32.63	33.91	34.52	36.43	ND
Rep 8	32.40	34.46	34.64	35.69	36.30
Rep 9	32.06	33.93	34.33	36.92	ND
Rep 10	32.18	34.25	34.72	35.99	37.52
Rep 11	32.88	33.56	34.05	35.06	39.46
Rep 12	32.48	33.40	34.40	35.41	ND
Rep 13	32.38	34.13	34.89	35.26	37.01
Rep 14	32.30	33.98	35.28	36.18	37.04
Rep 15	32.55	33.52	35.85	35.27	ND
Rep 16	32.49	33.99	34.33	35.31	ND
Rep 17	32.27	33.53	34.86	35.43	ND
Rep 18	32.25	34.12	34.77	36.16	36.75
Rep 19	32.51	34.18	34.61	35.56	38.11
Rep 20	32.08	33.91	34.78	35.18	38.36
AVG	32.44	33.96	34.79	35.73	37.72
%CV	0.67	1.02	1.17	1.34	2.78
Detection rate	100%	100%	100%	95%	65%

IPC	Flu A	Flu B	RSV	Covid-19
	3600 copies/ml			4000 copies/ml
Rep 1	32.65	32.82	32.60	34.90
Rep 2	32.42	32.64	32.21	35.09
Rep 3	32.50	33.04	32.21	34.81
Rep 4	32.47	32.90	32.21	34.83
Rep 5	32.48	32.87	32.63	34.62
Rep 6	32.60	32.64	32.57	34.81
Rep 7	32.73	32.74	32.21	34.68
Rep 8	32.67	32.48	32.41	34.69
Rep 9	32.64	32.33	32.31	34.72
Rep 10	32.43	32.70	32.57	34.44
Rep 11	32.23	33.00	32.67	34.42
Rep 12	32.49	32.58	32.52	34.50
Rep 13	32.46	32.66	32.60	34.85
Rep 14	32.64	32.59	32.61	34.73
Rep 15	32.83	32.47	32.45	34.67
Rep 16	32.73	32.45	32.40	34.72
Rep 17	32.64	32.39	32.43	34.55
Rep 18	32.53	32.45	32.59	34.50
Rep 19	32.56	32.55	32.24	34.48
Rep 20	32.68	32.59	33.01	32.54
AVG	32.57	32.64	32.47	34.58
%CV	0.43	0.61	0.63	1.47
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	1600 copies/ml			
Rep 1	33.97	33.88	33.76	34.85
Rep 2	33.97	33.54	33.58	34.95
Rep 3	33.83	33.63	33.93	35.26
Rep 4	33.67	33.46	33.87	34.77
Rep 5	33.60	33.56	33.79	35.00
Rep 6	33.91	33.62	33.65	34.79
Rep 7	33.93	33.77	33.79	35.08
Rep 8	33.86	33.77	33.58	35.09
Rep 9	33.69	33.66	33.28	35.03
Rep 10	33.55	33.61	33.41	35.06
Rep 11	33.74	33.34	34.00	34.46
Rep 12	33.57	33.51	33.60	34.82
Rep 13	33.85	33.55	33.61	34.73
Rep 14	33.67	33.73	33.55	35.01
Rep 15	33.92	33.93	33.40	35.13
Rep 16	33.87	33.72	33.36	35.08
Rep 17	33.45	33.82	33.46	35.39
Rep 18	33.53	33.69	33.49	35.06
Rep 19	33.61	33.74	33.60	34.92
Rep 20	33.77	33.25	33.57	35.19
AVG	33.75	33.64	33.61	34.98
%CV	0.48	0.51	0.58	0.59
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	800 copies/ml			
Rep 1	33.72	33.71	33.89	34.90
Rep 2	33.73	33.46	33.52	34.80
Rep 3	33.60	33.63	33.90	34.73
Rep 4	33.44	33.43	33.82	34.63
Rep 5	33.47	33.36	33.84	34.62
Rep 6	33.65	33.59	33.65	34.76
Rep 7	33.47	33.76	33.68	34.72
Rep 8	33.54	33.56	33.39	34.72
Rep 9	33.36	33.48	33.38	34.54
Rep 10	33.31	33.54	33.69	34.64
Rep 11	33.42	33.35	33.91	34.80
Rep 12	33.42	33.42	33.51	34.51
Rep 13	33.52	33.38	33.64	34.80
Rep 14	33.39	33.60	33.63	34.51
Rep 15	33.57	33.75	33.51	34.70
Rep 16	33.67	33.77	33.37	34.70
Rep 17	33.37	33.70	33.37	34.54
Rep 18	33.34	33.59	33.64	34.45
Rep 19	33.44	33.48	33.50	34.67
Rep 20	33.52	33.19	33.60	34.56
AVG	33.50	33.54	33.62	34.67
%CV	0.38	0.47	0.54	0.35
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	400 copies/ml			
Rep 1	32.90	33.12	33.19	34.99
Rep 2	32.62	33.05	33.03	34.21
Rep 3	32.75	33.05	33.18	34.39
Rep 4	32.79	32.85	32.95	34.66
Rep 5	33.11	32.93	33.08	35.04
Rep 6	32.99	33.03	33.17	34.61
Rep 7	32.84	33.02	33.27	34.69
Rep 8	32.89	33.08	33.18	34.57
Rep 9	32.88	32.95	33.13	34.65
Rep 10	33.01	32.90	33.14	35.11
Rep 11	33.25	33.02	33.00	34.70
Rep 12	32.96	32.84	33.01	34.97
Rep 13	33.09	33.08	33.06	34.88
Rep 14	32.99	33.02	33.17	35.02
Rep 15	32.81	33.17	33.41	34.79
Rep 16	32.99	33.18	33.30	34.76
Rep 17	32.95	33.04	33.26	34.88
Rep 18	32.94	33.00	33.14	34.91
Rep 19	32.87	33.03	33.21	35.12
Rep 20	32.88	33.03	32.84	34.78
AVG	32.93	33.02	33.14	34.79
%CV	0.42	0.28	0.39	0.68
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	200 copies/ml			160 copies/ml
Rep 1	34.20	34.04	34.53	34.87
Rep 2	34.00	33.59	34.38	34.97
Rep 3	34.86	33.47	34.32	35.27
Rep 4	34.47	33.67	34.18	35.02
Rep 5	34.57	34.29	34.20	35.35
Rep 6	34.31	33.80	34.35	35.05
Rep 7	34.23	33.77	34.24	34.87
Rep 8	34.24	33.78	34.14	34.81
Rep 9	33.94	33.92	34.16	34.88
Rep 10	34.05	34.31	34.15	34.80
Rep 11	34.69	34.22	34.08	35.40
Rep 12	33.99	34.10	34.22	34.86
Rep 13	34.47	34.04	34.08	34.96
Rep 14	34.19	34.26	34.21	34.84
Rep 15	34.25	34.05	34.12	34.84
Rep 16	33.99	34.00	34.35	34.64
Rep 17	33.92	34.19	33.82	34.58
Rep 18	34.06	34.07	34.10	34.74
Rep 19	33.92	34.13	34.06	34.78
Rep 20	34.00	34.04	34.21	34.62
AVG	34.22	33.99	34.20	34.91
%CV	0.80	0.70	0.43	0.64
Detection rate	100%	100%	100%	100%

Table 3. Quantstudio5 LoD Test result

Copies/ml	Quanta5 (Flu A)				
	3200 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	200 cps/ml
Rep 1	34.35	35.59	36.75	ND	ND
Rep 2	34.83	34.71	35.45	39.93	38.19
Rep 3	34.56	35.22	37.11	39.07	ND
Rep 4	34.50	34.79	37.46	ND	ND
Rep 5	34.72	34.35	37.12	ND	ND
Rep 6	34.77	34.88	36.11	ND	37.27
Rep 7	34.19	35.17	35.43	ND	ND
Rep 8	34.37	35.37	37.71	ND	39.32
Rep 9	34.38	35.29	35.77	37.60	ND
Rep 10	34.18	35.06	35.93	39.03	ND
Rep 11	34.77	35.21	35.79	ND	ND
Rep 12	34.47	35.17	36.48	37.66	37.80
Rep 13	34.23	34.31	35.84	ND	38.46
Rep 14	34.13	34.81	36.07	38.12	ND
Rep 15	34.30	35.58	35.83	38.28	ND
Rep 16	34.25	35.57	36.33	39.90	ND
Rep 17	33.96	35.79	36.70	ND	ND
Rep 18	33.97	35.17	37.63	37.63	ND
Rep 19	34.16	35.34	36.15	ND	ND
Rep 20	34.64	34.99	35.79	38.79	37.74
AVG	34.37	35.12	36.37	38.60	38.13
%CV	0.77	1.12	1.96	2.29	1.87
Detection rate	100%	100%	100%	50%	30%

Copies/ml	Quanta5 (Flu B)				
	3200 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	200 cps/ml
Rep 1	34.84	35.67	38.08	37.63	38.09
Rep 2	34.57	35.52	36.16	39.08	ND
Rep 3	34.61	35.41	36.23	38.51	38.54
Rep 4	34.57	36.18	36.98	37.67	38.74
Rep 5	34.82	35.75	36.13	37.30	38.87
Rep 6	35.08	35.94	36.75	37.80	36.72
Rep 7	35.47	35.50	38.03	37.30	ND
Rep 8	35.41	35.34	36.40	39.37	ND
Rep 9	35.08	36.39	37.01	ND	39.07
Rep 10	34.77	36.20	36.32	ND	38.76
Rep 11	34.59	35.46	36.48	37.29	38.05
Rep 12	34.49	35.95	37.37	36.86	37.49
Rep 13	35.24	35.79	36.79	37.08	38.69
Rep 14	34.65	35.54	37.74	36.75	ND
Rep 15	34.54	35.33	36.73	36.91	ND
Rep 16	34.98	35.37	36.64	37.12	ND
Rep 17	34.54	35.45	36.84	37.68	ND
Rep 18	34.69	35.28	37.48	37.79	39.45
Rep 19	35.21	36.31	36.62	37.18	ND
Rep 20	34.74	37.75	37.26	38.95	ND
AVG	34.85	35.81	36.90	37.68	38.41
%CV	0.90	1.61	1.60	2.10	2.02
Detection rate	100%	100%	100%	90%	55%

Copies/ml	Quanta5 (RSV)				
	3200 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	200 cps/ml
Rep 1	34.91	35.66	36.57	ND	ND
Rep 2	34.90	34.94	37.26	36.41	ND
Rep 3	34.69	35.13	36.61	37.94	ND
Rep 4	34.43	35.18	36.65	38.13	ND
Rep 5	34.75	35.72	36.97	38.65	39.77
Rep 6	34.57	35.17	36.73	ND	ND
Rep 7	34.66	35.66	36.33	37.53	ND
Rep 8	34.27	35.37	37.78	39.65	ND
Rep 9	34.96	35.61	36.99	37.90	38.10
Rep 10	35.00	35.37	36.56	37.59	39.36
Rep 11	34.60	35.76	36.95	38.20	ND
Rep 12	33.94	35.72	36.48	37.53	39.30
Rep 13	34.98	34.95	36.34	38.85	ND
Rep 14	34.79	36.08	36.92	37.48	ND
Rep 15	34.82	35.80	37.11	37.64	ND
Rep 16	34.50	36.46	36.61	37.29	38.55
Rep 17	34.76	35.40	36.26	37.34	39.16
Rep 18	34.72	35.96	37.06	37.03	39.27
Rep 19	35.01	35.99	36.59	38.39	38.39
Rep 20	34.79	35.55	37.61	36.97	39.18
AVG	34.70	35.57	36.82	37.81	39.01
%CV	0.77	1.10	1.11	2.02	1.38
Detection rate	100%	100%	100%	90%	45%

Copies/ml	Quanta5 (SARS-CoV-2)				
	4000 cps/ml	1600 cps/ml	800 cps/ml	400 cps/ml	160 cps/ml
Rep 1	32.03	33.60	33.67	35.61	ND
Rep 2	32.03	32.94	34.92	35.97	38.09
Rep 3	31.72	33.77	34.47	35.48	39.15
Rep 4	31.97	33.40	35.16	36.01	35.65
Rep 5	32.02	33.99	35.23	35.29	36.92
Rep 6	32.19	34.07	34.57	34.71	36.42
Rep 7	32.09	33.44	35.35	35.65	37.74
Rep 8	31.73	33.58	33.71	34.43	35.97
Rep 9	31.86	33.23	35.54	34.50	39.11
Rep 10	31.60	33.80	33.61	35.74	ND
Rep 11	32.09	33.72	33.94	35.70	36.11
Rep 12	32.22	32.96	34.72	35.54	38.01
Rep 13	32.10	33.60	33.77	36.60	37.77
Rep 14	32.03	33.66	34.01	34.96	37.24
Rep 15	32.33	33.22	34.26	35.08	36.12
Rep 16	31.81	33.53	34.34	35.97	36.97
Rep 17	31.93	33.28	34.73	36.39	37.74
Rep 18	32.41	32.98	34.71	35.31	38.36
Rep 19	31.84	33.62	34.18	35.38	37.86
Rep 20	31.70	33.49	35.00	35.99	36.69
AVG	31.99	33.49	34.49	35.52	37.33
%CV	0.67	0.95	1.73	1.63	2.80
Detection rate	100%	100%	100%	100%	90%

IPC	Flu A	Flu B	RSV	Covid-19
	3200 copies/ml			4000 copies/ml
Rep 1	35.13	34.29	33.41	33.71
Rep 2	34.57	34.62	32.68	32.51
Rep 3	34.04	33.62	32.76	32.11
Rep 4	34.03	33.60	32.13	33.58
Rep 5	35.03	34.68	33.80	32.27
Rep 6	33.55	34.11	33.43	33.89
Rep 7	33.58	33.26	33.19	32.84
Rep 8	34.32	34.98	33.23	33.72
Rep 9	34.23	33.53	32.30	33.41
Rep 10	33.96	34.94	32.66	32.66
Rep 11	33.30	34.82	33.19	32.99
Rep 12	33.72	34.41	33.77	32.35
Rep 13	33.32	33.73	32.40	33.38
Rep 14	34.55	34.68	33.00	33.74
Rep 15	34.55	34.31	33.27	32.24
Rep 16	33.23	33.21	34.05	33.04
Rep 17	34.28	34.64	32.66	32.23
Rep 18	34.43	34.38	33.03	33.05
Rep 19	35.10	33.59	33.00	33.20
Rep 20	34.70	33.52	33.25	32.21
AVG	34.15	34.18	33.06	32.96
%CV	1.75	1.70	1.53	1.83
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	1600 copies/ml			
Rep 1	32.51	32.21	31.94	33.79
Rep 2	32.17	32.00	31.96	33.56
Rep 3	32.34	32.30	31.90	33.64
Rep 4	32.49	32.39	31.59	33.75
Rep 5	32.57	32.24	32.25	33.77
Rep 6	32.82	32.07	32.10	33.83
Rep 7	32.46	32.05	32.15	33.45
Rep 8	32.62	32.29	31.93	33.70
Rep 9	32.73	32.44	32.18	33.79
Rep 10	32.18	32.17	32.09	33.58
Rep 11	31.97	32.15	32.05	33.22
Rep 12	32.14	32.31	32.48	33.50
Rep 13	32.15	32.25	32.12	33.64
Rep 14	32.05	32.26	32.04	33.46
Rep 15	32.17	32.09	32.31	33.48
Rep 16	32.30	32.12	32.10	33.82
Rep 17	32.51	32.45	32.46	33.63
Rep 18	32.32	32.43	32.74	33.40
Rep 19	32.01	32.02	32.50	33.04
Rep 20	31.85	32.30	32.11	33.08
AVG	32.32	32.23	32.15	33.56
%CV	0.82	0.44	0.80	0.70
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	800 copies/ml			
Rep 1	31.98	32.63	32.21	33.77
Rep 2	31.94	32.26	32.41	33.78
Rep 3	32.32	32.26	32.40	34.07
Rep 4	32.38	32.60	32.48	33.92
Rep 5	32.04	32.27	32.50	34.31
Rep 6	32.08	33.07	32.10	33.30
Rep 7	32.14	32.38	32.00	33.58
Rep 8	32.29	32.42	32.17	33.66
Rep 9	32.69	32.65	32.41	34.12
Rep 10	32.47	32.21	32.37	33.83
Rep 11	32.23	31.98	32.26	34.24
Rep 12	31.84	32.05	32.33	33.53
Rep 13	31.93	32.17	32.49	33.65
Rep 14	32.48	32.05	32.20	33.44
Rep 15	32.32	32.32	32.30	33.79
Rep 16	32.15	32.41	32.34	33.69
Rep 17	32.29	32.49	32.49	33.90
Rep 18	32.37	32.20	32.59	34.03
Rep 19	32.32	32.00	32.49	34.11
Rep 20	31.89	31.91	32.43	33.70
AVG	32.21	32.32	32.35	33.82
%CV	0.70	0.87	0.47	0.79
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	400 copies/ml			
Rep 1	34.19	34.43	33.39	32.26
Rep 2	35.11	33.97	32.68	32.76
Rep 3	34.33	34.19	32.94	32.57
Rep 4	35.05	33.34	33.28	33.49
Rep 5	33.56	34.97	33.77	33.83
Rep 6	34.38	33.80	33.99	33.27
Rep 7	33.22	33.67	34.00	32.63
Rep 8	33.97	33.21	34.20	32.63
Rep 9	33.88	34.01	34.12	32.81
Rep 10	34.38	33.54	32.60	33.37
Rep 11	34.44	34.76	32.37	32.62
Rep 12	34.15	33.90	33.92	32.67
Rep 13	34.66	33.98	33.96	33.63
Rep 14	35.16	34.15	33.76	33.83
Rep 15	33.70	33.95	34.12	32.76
Rep 16	34.48	33.12	32.88	33.37
Rep 17	34.27	34.68	33.79	32.70
Rep 18	35.18	33.39	34.01	33.35
Rep 19	33.33	34.57	32.16	33.64
Rep 20	34.37	33.79	33.98	33.28
AVG	34.29	33.97	33.50	33.07
%CV	1.67	1.53	1.96	1.45
Detection rate	100%	100%	100%	100%

IPC	Flu A	Flu B	RSV	Covid-19
	200 copies/ml			160 copies/ml
Rep 1	33.27	32.06	32.22	32.90
Rep 2	32.76	32.25	32.07	32.83
Rep 3	32.76	32.20	32.46	33.09
Rep 4	33.06	32.00	32.22	33.30
Rep 5	32.87	32.35	32.27	33.25
Rep 6	33.34	32.33	32.41	32.77
Rep 7	32.82	32.61	32.39	32.75
Rep 8	33.11	32.08	32.70	33.21
Rep 9	32.94	32.26	32.91	32.98
Rep 10	32.76	32.16	33.00	33.18
Rep 11	32.40	32.24	32.56	33.21
Rep 12	32.61	32.68	32.45	33.14
Rep 13	32.80	32.28	32.41	33.22
Rep 14	32.41	32.08	32.46	32.97
Rep 15	32.74	32.39	32.53	33.14
Rep 16	32.76	32.30	32.42	33.28
Rep 17	33.14	32.54	32.47	33.17
Rep 18	32.48	33.22	32.61	33.17
Rep 19	32.53	32.59	32.52	33.25
Rep 20	32.10	31.98	32.20	33.30
AVG	32.78	32.33	32.46	33.11
%CV	0.95	0.90	0.69	0.53
Detection rate	100%	100%	100%	100%

6. Summary Table

Limit of Detection	CFX96	7500	Quantstudio5
Flu A	800 copies/mL	800 copies/mL	800 copies/mL
Flu B	800 copies/mL	800 copies/mL	800 copies/mL
RSV	800 copies/mL	800 copies/mL	800 copies/mL
SARS-CoV-2	400 copies/mL	400 copies/mL	400 copies/mL

7. Conclusion

According to the above results, we claimed that LoD concentration is 800 copies/ml for Flu A, 800 copies/ml for Flu B, 800 copies/ml for RSV and 400 copies/ml for SARS-CoV-2.

문서의 끝 (End of the document.)

제정 및 개정 이력 (History of Establishment & Revision)

개정번호 Rev. No.	시행 일자 Implementation Date	변경사항 Summary of Changes	변경관리번호 CC No.	작성자 Prepared by
00	2020-12-30	신규 제정 (Initial Release)	N/A	홍성수