PerkinElmer® New Coronavirus Nucleic Acid Detection Kit

Analytical Study Report

PerkinElmer

Outline

PerkinElmer® New Coronavirus Nucleic Acid Detection Kit

Analytical Study

A. PERFORMANCE EVALUATION OVERVIEW

This document is to describe the performance studies conducted on the New Coronavirus (SARS-CoV-2) Nucleic acid Detection Kit, the results generated from these studies are to verify that the product performance meet the design input defined in the Product Requirements Specification and that it is safe and effective for its intended use. The LoD, specificity and precision studies were conducted using three batches of kits.

1. Limit of Detection (LOD)

The assay LoD is the measured quantity value, obtained by a given measurement procedure, the LoD is calculated from a probit regression model as the measurand concentration at which, with a predefined probability (usually 95%), measurement results yield a positive classification.

1.1 Instruments and Materials

Instruments: Pre-NAT II, ABI7500, QX200™ Droplet Digital™ PCR System.

Materials: SARS-CoV-2 RNA capsulated in bacteriophage (ORF1ab and N), OneStep RT-ddPCR Advanced Kit for Probes, PerkinElmer New Coronavirus Nucleic Acid Detection Kit.

1.2 Method

1.2.1 Pre-test for a LOD range

SARS-CoV-2 N and ORF1ab RNA targets capsulated in bacteriophage MS2 were used in this study. The concentration of these encapsulated RNA (*ORF1ab* and *N*) was determined by droplet digital PCR from Bio-Rad. The RNA extraction of bacteriophage was done on Pre-NAT II using Nucleic Acid Extraction Kit of Perkinelmer at 3.584×10^4 /10^2 /10^1 /10^0 copies/ml for

ORF1ab bacteriophage and at 2.784 × 10^4 /10^2 /10^1 /10^0 copies/ml for *N* bacteriophage. Real-time RT-PCR assays were performed on ABI 7500 instrument.

1.2.2 LOD determination

According to the results from this pre-test, the RNA extraction of SARS-CoV-2 RNA capsulated in bacteriophage (ORF1ab and N) were done on Pre-NAT II using Nucleic Acid Extraction Kit of Perkinelmer at 70, 35, 17.5, 8.75, 4.38 copies/ml for both bacteriophages. Each concentration was test 24 replicates using 3 batches of kits. The LoD is calculated using a probit regression model.

1.2.3 LOD verification

A determined LoD was verified using 3 batches of reagents, with each sample being repeated 20 times to calculate the detection rate.

1.3 Acceptable Results for Verification

1.3.1 LOD determination

The LoD was calculated from a probit regression model as the measurand concentration.

1.3.2 LOD verification

The LoD was determined as the lowest concentration where ≥ 85% (17/20) of the replicates were positive (Reference: EP-17A2).

Table 1: The relationship between Total Number of Measurements and Observed Proportion Boundary (Reference: EP-17A2).

Total Number of Measurements in Study (N)	Observed Proportion Boundary
20	85%
30	87%
40	88%
50	88%
60	90%

1.4 Results

1.4.1 Pre-test for a LOD range

The concentration of the SARS-CoV-2 RNA capsulated in bacteriophage (ORF1ab and N) was determined by droplet digital PCR from Bio-Rad. The RNA extraction of bacteriophage was done on Pre-NAT using Nucleic Acid Extraction Kit of Perkinelmer at 3.584×10^4 /10^2 /10^1 /10^0 copies/ml for *ORF1ab* bacteriophage and at 2.784×10^4 /10^2 /10^1 /10^0 copies/ml for *N* bacteriophage. Results are showed in Table 2.

Table 2:LoD pre-test results.

Gene	Concentration	Positive rate
N	3.584×10^4 copies/ml	4/4
	3.584×10^2 copies/ml	4/4
	3.584×10^1 copies/ml	4/4
	3.584×10^0 copies/ml	0/4
ORF1ab	2.784×10^4 copies/ml	4/4
	2.784×10^2 copies/ml	4/4
	2.784×10^1 copies/ml	4/4
	2.784×10^0 copies/ml	0/4

According to the results shown in the above table, ≥35.84 copies/ml for *N* and ≥27.84 copies/ml for *ORF1ab* can be detected.

1.4.2 LOD determination

According to the results from pre-test, the RNA extraction of SARS-CoV-2 RNA capsulated in bacteriophage (*ORF1ab* and *N*) were done on Pre-NAT using Nucleic Acid Extraction Kit of Perkinelmer at 70, 35, 17.5, 8.75, 4.38 copies/ml. Samples at each concentrations were tested with 24 replicates using 3 batches of kits. Results are showed in Table 3 and Table 4.

Table 3: Results of target *N.*

Concentration (copies/ml)	No.	AY20200202	AY20200203	AY20200204
70	1	37.80	35.91	36.88
	2	36.61	35.68	36.13
	3	37.23	35.94	36.55
	4	37.16	35.95	38.38
	5	36.46	36.08	36.70
	6	37.26	36.01	36.86

	7	36.62	36.62	36.42
	8	36.61	35.48	35.89
	9	37.15	35.67	36.54
	10	37.02	36.62	36.92
	11	36.77	35.36	41.90
	12	36.83	36.05	36.98
	13	36.61	35.84	36.89
	14	36.16	36.01	35.90
	15	36.36	35.48	36.17
	16	36.39	35.40	37.34
	17	36.81	36.86	36.24
	18	35.60	35.48	37.29
	19	36.12	36.11	36.78
	20	35.84	35.44	36.75
	21	35.87	36.21	36.55
	22	35.69	35.26	36.30
	23	36.55	36.45	36.84
	24	35.62	37.19	36.87
35	1	37.95	36.59	39.36
	2	37.33	37.19	37.89
	3	38.24	36.63	37.89
	4	37.77	37.07	38.37
	5	38.29	36.50	37.50
	6	37.21	36.99	37.83
	7	39.67	37.55	38.99
	8	38.28	37.12	39.24
	9	38.61	37.88	37.47
	10	39.28	37.95	37.69
	11	37.94	36.67	36.49
	12	37.78	36.78	36.56
	13	37.25	36.81	37.49
	14	37.57	38.46	37.29
	15	39.23	36.11	38.43
	16	37.33	37.01	37.37
	17	37.49	36.22	37.21
	18	37.50	38.94	38.40
	19	37.08	38.53	38.47
	20	37.40	36.60	37.70
	21	37.89	37.34	39.93
	22	38.67	36.95	37.60
	23	36.26	38.21	37.55
	24	38.40	38.04	37.33

17.5	1	38.93	37.40	Undet
	2	38.03	39.06	39.26
	3	38.70	37.73	39.57
	4	38.09	Undet	39.41
	5	39.84	38.46	39.79
	6	38.62	37.29	38.18
	7	38.94	37.63	39.56
	8	38.30	39.16	39.31
	9	39.98	37.61	43.94
	10	40.00	37.34	39.36
	11	38.59	37.72	40.34
	12	38.20	37.98	38.89
	13	39.32	36.99	38.50
	14	38.42	39.05	38.10
	15	39.64	40.91	38.00
	16	38.00	37.97	39.61
	17	Undet	37.34	39.05
	18	37.63	37.95	38.03
	19	37.73	38.36	39.20
	20	39.12	38.18	38.79
	21	Undet	38.62	38.81
	22	36.89	39.15	38.04
	23	38.52	40.40	38.40
	24	38.63	Undet	39.26
8.75	1	40.89	39.29	38.72
	2	38.97	38.47	39.54
	3	42.32	37.48	39.07
	4	Undet	39.43	38.41
	5	Undet	38.13	40.43
	6	40.21	39.25	39.48
	7	39.43	40.95	38.55
	8	Undet	40.28	38.57
	9	39.01	37.82	Undet
	10	43.85	39.10	37.88
	11	38.49	39.79	37.81
	12	39.56	38.00	39.48
	13	39.56	39.43	40.07
	14	39.24	38.09	38.43
	15	39.23	39.31	39.64
	16	43.07	Undet	Undet
	17	39.46	38.49	Undet
	18	Undet	39.27	Undet

	19	39.58	37.89	Undet
	20	38.56	39.44	Undet
	21	39.53	37.76	41.94
	22	39.37	37.95	44.10
	23	38.99	Undet	39.83
	24	38.21	Undet	40.42
4.38	1	40.84	Undet	Undet
	2	42.94	38.30	Undet
	3	39.32	Undet	38.29
	4	Undet	Undet	38.87
	5	Undet	Undet	39.73
	6	40.24	37.83	39.79
	7	39.85	39.02	39.64
	8	Undet	Undet	Undet
	9	Undet	38.84	39.19
	10	40.09	Undet	Undet
	11	39.84	43.95	Undet
	12	40.14	40.03	Undet
	13	40.25	38.53	Undet
	14	38.34	Undet	Undet
	15	Undet	38.65	Undet
	16	Undet	Undet	Undet
	17	38.72	Undet	Undet
	18	39.48	Undet	Undet
	19	43.38	Undet	Undet
	20	Undet	39.24	Undet
	21	Undet	38.06	Undet
	22	39.20	39.19	39.76
	23	38.74	42.05	Undet
	24	Undet	39.60	39.94

Remark: Undet means not been detected.

Table 4: Results for target *ORF1ab.*

Concentration (copies/ml)	No.	AY20200202	AY20200203	AY20200204
70	1	36.69	36.34	34.61
	2	36.11	35.29	35.40
	3	35.55	35.48	35.51
	4	35.75	35.60	35.99
	5	35.57	36.03	35.70
	6	35.04	35.80	35.10
	7	34.61	35.65	34.90

	8	35.32	35.56	34.92
	9	34.55	35.91	35.69
	10	35.27	35.17	35.88
	11	35.27	36.09	35.45
	12	35.22	35.99	35.48
	13	35.49	36.59	34.74
	14	35.85	35.27	36.02
	15	35.34	35.33	35.66
	16	35.69	35.49	35.68
	17	35.25	35.65	36.24
	18	35.74	35.57	35.51
	19	35.46	36.02	34.96
	20	34.75	35.78	35.81
	21	36.46	35.77	35.62
	22	36.15	35.39	35.09
	23	35.08	35.65	36.39
	24	35.20	35.86	36.44
35	1	36.57	36.56	37.10
	2	36.65	36.54	36.55
	3	36.01	36.16	36.50
	4	36.21	36.76	37.07
	5	37.49	36.52	36.76
	6	37.15	36.24	36.58
	7	36.20	37.09	37.40
	8	37.84	36.88	38.06
	9	28.22	37.22	36.18
	10	36.80	36.61	37.00
	11	37.05	36.41	35.82
	12	36.05	37.14	38.47
	13	36.41	37.51	36.01
	14	36.60	36.55	37.02
	15	37.75	37.01	36.82
	16	36.45	36.62	36.93
	17	36.80	36.64	35.74
	18	36.72	37.79	36.76
	19	37.55	38.65	38.12
	20	36.07	36.55	36.91
	21	36.33	36.95	37.64
	22	38.00	37.03	36.91
	23	36.58	37.49	36.62
	24	37.14	38.65	36.30
17.5	1	38.84	38.90	37.20

	2	37.40	38.10	36.57
	3	37.71	37.05	36.40
	4	38.60	37.03	39.49
	5	37.35	37.03	36.73
	6	38.40	37.87	37.87
	7	38.00	39.25	37.78
	8	36.40	39.18	37.26
	9	38.88	38.35	37.70
	10	37.93	38.86	37.25
	11	37.29	Undet	39.18
	12	40.27	36.88	38.71
	13	38.20	39.16	37.02
	14	37.50	39.62	38.41
	15	38.58	37.96	37.61
	16	38.59	40.24	38.45
	17	Undet	38.59	38.25
	18	40.02	38.16	38.64
	19	36.63	39.54	40.49
	20	37.58	38.35	37.05
	21	37.95	36.74	38.45
	22	37.30	37.50	37.89
	23	37.28	38.09	37.85
	24	37.31	37.31	38.35
8.75	1	37.72	40.06	Undet
	2	37.88	39.06	38.04
	3	40.19	38.95	39.20
	4	39.00	37.98	38.80
	5	38.17	Undet	39.00
	6	38.38	39.20	38.61
	7	39.56	40.53	Undet
	8	38.30	39.87	Undet
	9	38.00	Undet	Undet
	10	Undet	39.11	Undet
	11	40.56	Undet	37.98
	12	Undet	37.31	Undet
	13	39.18	38.51	38.13
	14	39.18	38.23	38.07
	15	37.61	38.94	37.52
	16	40.14	Undet	Undet
	17	37.94	40.22	37.63
	18	38.15	40.18	39.66
	19	39.20	37.89	Undet

	20	37.94	38.72	Undet
	21	38.75	38.70	38.48
	22	39.39	39.45	36.76
	23	37.60	38.39	Undet
	24	37.99	39.16	41.80
4.38	1	Undet	Undet	Undet
	2	39.52	Undet	Undet
	3	40.19	38.70	38.98
	4	37.25	39.12	38.99
	5	38.46	39.51	33.12
	6	40.18	39.43	40.20
	7	39.31	Undet	40.42
	8	39.79	40.05	Undet
	9	Undet	Undet	40.36
	10	Undet	38.12	Undet
	11	39.04	Undet	Undet
	12	Undet	39.33	38.14
	13	Undet	40.32	38.93
	14	39.93	38.11	39.60
	15	Undet	Undet	38.81
	16	39.41	39.25	38.30
	17	Undet	38.71	41.92
	18	Undet	Undet	Undet
	19	38.95	44.06	39.00
	20	38.05	Undet	Undet
	21	38.90	39.94	Undet
	22	40.39	Undet	Undet
	23	37.41	Undet	Undet
	24	38.02	38.56	40.19

Remark: Undet means not been detected.

The LoD was calculated using a probit regression model as the measurand concentration, results are shown in Table 5 and Table 6.

Table 5: LOD determination for *N.*

Gene	*N*					
Concentration (copies/ml)	AY20200202		AY20200203		AY20200204	
	Mean CT	Positive rate	MEAN CT	Positive rate	MEAN CT	Positive rate
70.00	36.55	100.00%	35.96	100.00%	36.92	100.00%
35.00	37.93	100.00%	37.26	100.00%	37.92	100.00%

17.50	38.64	95.65%	38.29	91.67%	39.19	91.67%
8.75	39.88	70.83%	38.84	87.50%	39.58	70.83%
4.38	40.09	54.17%	39.48	45.83%	39.4	33.33%
Probit LOD (copies/ml)	17.337		17.342		18.276	
95% CI (copies/ml)	13.137-33.227		13.177-32.284		14.472-28.680	

Table 6: LOD determination for *ORF1ab.*

Gene	*ORF1ab*					
Concentration (copies/ml)	AY20200202		AY20200203		AY20200204	
	MEAN CT	Positive rate	MEAN CT	Positive rate	MEAN CT	Positive rate
70.00	35.48	100.00%	35.72	100.00%	35.53	100.00%
35.00	36.44	100.00%	36.98	100.00%	36.89	100.00%
17.50	38	100.00%	38.25	95.83%	37.94	100.00%
8.75	38.67	91.67%	39.02	83.33%	38.55	58.33%
4.38	39.05	66.67%	39.51	54.17%	39.07	58.33%
Probit LOD (copies/ml)	9.865		15.497		16.179	
95% CI (copies/ml)	7.530-32.614		11.755-29.917		12.443-29.535	

According to the LoD results shown in table 5 and table 6, it was determined to verify a tentative LoD of 20 copies/mL for both *N* and *ORF1ab* using 3 batches of reagents.

1.4.3 LOD verification

LOD verification was conducted by testing 20 replicates of a sample with target *N* and *ORF1ab* at 20 copies/mL, 3 batches of reagents were used for the verification. Results are showed in Table 7.

Table 7: Results of LOD verification.

Reagent batch	AY20200202		AY20200203		AY20200204	
Gene	*N*	*ORF1ab*	*N*	*ORF1ab*	*N*	*ORF1ab*
Ct	36.85	Undet	38.57	37.96	Undet	38.26

	37.74	36.78	37.62	39.23	41.23	37.64
	38.83	40.35	38.53	40.32	Undet	38.08
	39.52	39.03	38.88	38.77	38.06	39.66
	38.16	37.67	38.15	38.14	38.70	38.34
	39.74	39.17	36.94	39.59	39.74	38.40
	40.41	Undet	39.04	24.03	38.95	37.16
	38.36	38.83	Undet	38.99	39.15	37.36
	38.18	38.06	39.17	39.91	38.47	37.67
	36.94	37.79	37.77	37.43	38.53	37.68
	38.85	40.50	37.81	38.05	40.15	39.03
	38.36	Undet	38.26	38.53	38.57	38.91
	39.03	37.25	39.24	39.00	Undet	37.76
	39.23	39.34	37.18	39.43	39.17	38.34
	38.04	37.29	37.60	38.57	38.37	37.58
	39.66	37.37	37.50	Undet	37.34	36.66
	38.01	37.20	37.46	38.32	38.90	37.91
	38.77	38.80	39.71	36.93	39.97	38.53
	38.74	37.86	38.03	38.93	40.18	37.41
	39.66	39.09	37.99	37.91	38.73	38.53
Positive rate	**20/20**	**17/20**	**19/20**	**19/20**	**17/20**	**20/20**

According to the results shown in table 7, it was verified that the tentative LoD 20 copies/mL can be detected at a rate ≥ 85%.

2. Specificity (cross-reactivity and interference substances)

Cross-reactivity of the SARS-CoV-2 Nucleic Acid Detection Kit was studied by testing a range of human pathogens. The assay tolerance to interference substances was tested by spiking different endogenous and exogenous interfering substances into test samples.

2.1 Instruments and Materials

Instruments: Pre-NAT II, ABI7500.

Materials: SARS-CoV-2 RNA capsulated in bacteriophage (*ORF1ab* and *N*), cross-reactivity test panel (table 8) and interfering substances (table 9), PerkinElmer New Coronavirus Nucleic Acid Detection Kit.

Table 8: Potential cross-reactivity test panels.

Pathogen	Source	Concentration	
Coronavirus 229E	ATCC VR-740™	2.8×10^2	$TCID_{50}$/mL
Coronavirus OC43	ATCC VR-1558™	2.8×10^3	$TCID_{50}$/mL
Adenovirus type 3	ATCC VR-847™	$5.0 \times 10^{5.5}$	$TCID_{50}$/mL
Adenovirus type 2	ATCC VR-846™	5.6×10^4	$TCID_{50}$/mL
Adenovirus type 31	ATCC VR-1109™	1.6×10^6	$TCID_{50}$/mL
Adenovirus type 37	ATCC VR-929™	1.8×10^4	$TCID_{50}$/mL
Adenovirus type 51	ATCC VR-1603™	2.3×10^6	$TCID_{50}$/mL
Enterovirus A71	ATCC VR-1432™	$5.0 \times 10^{5.5}$	$TCID_{50}$/mL
Enterovirus D68	ATCC VR-1823™	1.6×10^6	$TCID_{50}$/mL
Influenza A virus (H3N2)	ATCC VR-1679™	$5.0 \times 10^{3.5}$	$TCID_{50}$/mL
Influenza B virus	ATCC VR-1807™	7.6×10^2	PFU/mL
Influenza A virus (H1N1pdm09)	ATCC VR-1736™	2.6×10^3	PFU/mL
Influenza A virus (seasonal H1N1)	ATCC VR-1520™	$5.0 \times 10^{4.5}$	$TCID_{50}$/mL
Respiratory syscytial virus	ATCC VR-1400™	$5.0 \times 10^{3.5}$	$TCID_{50}$/mL
Parainfluenza virus type 1	ATCC VR-94™	2.8×10^4	$TCID_{50}$/mL
Chlamydophila pneumoniae	ATCC 53592™	2.9×10^5	IFU/mL
Mycoplasma pneumoniae	ATCC 15531™	3.5×10^6	CFU/mL
Haemophilus influenzae	ATCC 51907D™	10	μg/mL
Streptococcus pyogenes	ATCC 700294D-5™	7	μg/mL
Streptococcus salivarius	ATCC BAA-250D-5™	5.2	μg/mL
Rhinovirus B17	ATCC VR-1663™	2.0×10^6	PFU/mL
Rhinovirus A2	ATCC VR-482™	8.9×10^4	$TCID_{50}$/mL
Measles virus	National Standard for Influenza A/B Viral Nucleic Acids Detection Kit	Unkown	
Mumps virus		Unkown	
Staphylococcus aureus		Unkown	
Influenza A virus (H7N9)		Unkown	
hepatitis B virus	WHO NIBSC 10/266	9.55E+05	IU/mL
hepatitis c virus	WHO NIBSC 14/150	1.00E+05	IU/mL
HIV-1	WHO NIBSC 16/194	1.26E+05	IU/mL

HIV-2	WHO NIBSC 08/150	1.00E+03	IU/mL
Hepatitis A virus	Symbio	1.84E+05	copies/mL
Epstein-barr virus	Symbio	1.46E+05	copies/mL
Cytomegalovirus	Symbio	1.15E+04	copies/mL
Herpes simplex virus type I	Symbio	5.71E+04	copies/mL
Herpes simplex virus type II	Symbio	9.01E+05	copies/mL
SARS (Plasmid)	BIOLIGO	1.00E+05	copies/mL
MERS (Plasmid)	BIOLIGO	1.00E+05	copies/mL

Table 9: Potential interfering substances.

Interfering substances	Tested concentration	Interfering substances	Tested concentration
Valacyclovir	3.6mg/mL	Clarithromycin	30μg/mL
Entecavir	24.6ng/mL	Ciprofloxacin	7.5μg/mL
Adefovir	90ng/mL	Telbivudine	15μg/mL
Ribavirin	5mg/mL	Efavirenz	12.2μg/mL
Acyclovir	3.6mg/mL	Tenofovirdisoproxil	1335ng/mL
Azithromycin	1.35mg/mL	Tobramycin	0.6 mg/mL
Fluticasone propionate	1 mg/mL	Oxymetazoline	15% v/v
Normal saline NS	1 mg/mL	Sulphur	0.05% v/v
Beclomethasone	22.5 μg/mL	Eschscholtzia	0.05% v/v
Dexamethasone Acetate	375 μg/mL	Benzocaine	1.25 mg/mL
Flunisolide	20 mg/mL	Menthol	5% v/v
Triamcinolone	25 μg/mL	Zanamivir	5 mg/mL
Budesonide	16.7 μg/mL	Mupirocin	0.02% w/v
Mometasone furoate	41.7 μg/mL	Hemoglobin	5mg/ml
Human genome DNA	3mg/L	Bilirubin	0.6mg/ml
Theumatoid factor	unknown	Triglyceride	25mg/ml
Autoantibody	unknown	Human Serum Albumin	60mg/mL
Antinuclear antibody	unknown		

2.2 Method

Cross-reactivity was tested on the pathogens listed in table 8 at the highest clinically relevant level or highest commercially available level. RNA extraction of samples was done on Pre-NAT II using Nucleic Acid Extraction Kit from Perkinelmer. Real-time RT-PCR assays were performed on ABI 7500 instrument. Tolerance to interfering substances was tested by spiking the substances listed in table 9 respectively at the test concentrations into SARS-CoV-2 positive and negative samples. Positive samples were

prepared with encapsulated RNA (*N* and *ORF1ab*) at 3 times of LoD (60 copies/mL). Then RNA extraction of samples was done on Pre-NAT II using Nucleic Acid Extraction Kit from Perkinelmer. Real-time RT-PCR assays were performed on ABI 7500 instrument.

2.3 Acceptable Results for Verification

Negative results for pathogens listed in table 8, and positive and negative results, respectively, for positive samples and negative samples spiked with interfering substances listed in table 9.

2.4 Results

The test results were all negative for pathogens listed in table 8, indicating that the kit does not show cross-reactivity to these pathogens.

The positive samples (3 times LoD concentration) spiked with interfering substances listed in table 9 all showed positive results, and the negative samples spiked with interfering substances listed in table 9 all showed negtive results, indicating that the kit performance is not affected by these interfering substances.

All the results are showed in Table 10.

Table 10 Results of the specificity

Lot	AY20200202				AY20200203				AY20200204			
Name	Negative (Ct)		Postive (Ct)		Negative (Ct)		Postive (Ct)		Negative (Ct)		Postive (Ct)	
	N	*ORF1ab*	*N*	*ORF1ab*	*N*	*ORF1ab*	*N*	*ORF1ab*	*N*	*ORF1ab*	*N*	*ORF1ab*
SARS (Plasmid)	Undet	Undet	37.30	37.10	Undet	Undet	37.74	37.99	Undet	Undet	37.55	36.64
MERS (Plasmid)	Undet	Undet	37.71	36.65	Undet	Undet	38.19	36.67	Undet	Undet	38.55	35.56
Coronavirus 229E	Undet	Undet	37.00	36.68	Undet	Undet	38.91	35.57	Undet	Undet	37.10	37.80
Coronavirus OC43	Undet	Undet	37.84	35.09	Undet	Undet	36.65	36.15	Undet	Undet	36.87	35.89
Adenovirus type 3	Undet	Undet	37.33	36.02	Undet	Undet	36.45	35.87	Undet	Undet	37.42	34.85
Adenovirus type 6	Undet	Undet	37.66	36.90	Undet	Undet	37.28	36.67	Undet	Undet	37.94	36.31
Adenovirus type 31	Undet	Undet	38.21	36.78	Undet	Undet	36.33	36.17	Undet	Undet	37.32	36.81
Adenovirus type 37	Undet	Undet	37.63	37.43	Undet	Undet	36.56	37.38	Undet	Undet	36.94	33.40
Adenovirus type 51	Undet	Undet	37.15	36.65	Undet	Undet	37.37	36.20	Undet	Undet	37.73	36.42
Enterovirus A	Undet	Undet	37.12	37.31	Undet	Undet	36.02	37.03	Undet	Undet	36.78	35.89
Enterovirus D	Undet	Undet	37.58	36.56	Undet	Undet	37.07	36.24	Undet	Undet	37.63	36.80
Rhinovirus A	Undet	Undet	37.27	35.61	Undet	Undet	35.76	36.97	Undet	Undet	36.72	36.78
Rhinovirus B	Undet	Undet	36.73	34.79	Undet	Undet	36.39	36.53	Undet	Undet	36.63	35.99
Influenza A virus	Undet	Undet	37.82	36.04	Undet	Undet	36.48	37.12	Undet	Undet	37.46	37.11
Influenza B virus	Undet	Undet	37.16	36.33	Undet	Undet	37.02	37.40	Undet	Undet	36.79	36.35
Influenza A (H1N1pdm09)	Undet	Undet	37.25	36.27	Undet	Undet	36.61	35.91	Undet	Undet	38.06	36.41
Influenza A (seasonal H1N1)	Undet	Undet	37.84	37.83	Undet	Undet	37.26	37.66	Undet	Undet	36.87	32.32
Respiratory syscytial virus	Undet	Undet	38.08	36.06	Undet	Undet	36.59	36.66	Undet	Undet	37.64	37.51
Parainfluenza virus	Undet	Undet	36.74	36.03	Undet	Undet	36.16	36.60	Undet	Undet	37.60	36.31
Measles virus	Undet	Undet	37.41	35.78	Undet	Undet	35.75	35.78	Undet	Undet	36.63	37.14
Mumps virus	Undet	Undet	37.02	37.18	Undet	Undet	36.36	36.43	Undet	Undet	37.43	37.74

Mycoplasma pneumoniae	Undet	Undet	38.46	35.85	Undet	Undet	36.58	36.35	Undet	Undet	38.69	36.07
Chlamydophila pneumoniae	Undet	Undet	37.35	35.97	Undet	Undet	35.97	37.37	Undet	Undet	37.60	36.83
Haemophilus influenzae	Undet	Undet	37.97	37.05	Undet	Undet	36.45	36.51	Undet	Undet	37.99	36.11
Staphylococcus aureus	Undet	Undet	37.40	36.39	Undet	Undet	36.17	36.22	Undet	Undet	36.69	36.70
Streptococcus pyogenes	Undet	Undet	38.80	36.54	Undet	Undet	36.73	36.39	Undet	Undet	36.96	36.39
Streptococcus salivarius	Undet	Undet	37.05	35.79	Undet	Undet	36.25	36.50	Undet	Undet	37.03	36.18
Hepatitis A virus	Undet	Undet	38.23	36.98	Undet	Undet	35.54	36.60	Undet	Undet	37.03	38.54
Hepatitis B virus	Undet	Undet	37.17	36.70	Undet	Undet	38.71	36.36	Undet	Undet	37.91	37.27
Hepatitis C virus	Undet	Undet	37.70	35.95	Undet	Undet	37.73	36.33	Undet	Undet	36.95	37.05
Human cytomegalovirus	Undet	Undet	36.97	37.11	Undet	Undet	36.77	35.94	Undet	Undet	37.21	37.96
Epstein-barr virus	Undet	Undet	37.15	36.07	Undet	Undet	36.24	36.88	Undet	Undet	37.07	36.77
Herpes simplex virus type I	Undet	Undet	37.25	35.19	Undet	Undet	36.23	35.75	Undet	Undet	38.36	36.87
Theumatoid factor	Undet	Undet	39.03	36.08	Undet	Undet	36.28	36.48	Undet	Undet	39.31	35.74
Autoantibody	Undet	Undet	37.58	35.81	Undet	Undet	36.10	37.58	Undet	Undet	36.51	35.37
Antinuclear antibody	Undet	Undet	38.13	37.19	Undet	Undet	35.37	35.83	Undet	Undet	37.09	37.23
Hemoglobin	Undet	Undet	37.71	36.76	Undet	Undet	35.89	36.03	Undet	Undet	37.97	36.17
Bilirubin	Undet	Undet	37.32	36.04	Undet	Undet	37.43	35.81	Undet	Undet	38.53	36.15
Human Serum Albumin	Undet	Undet	37.76	36.31	Undet	Undet	35.94	36.69	Undet	Undet	37.91	36.11
Triglyceride	Undet	Undet	36.49	35.47	Undet	Undet	36.11	36.20	Undet	Undet	36.74	36.22
Human genome DNA	Undet	Undet	37.58	36.58	Undet	Undet	36.75	36.44	Undet	Undet	38.90	36.52
Valacyclovir	Undet	Undet	37.47	36.31	Undet	Undet	36.20	37.16	Undet	Undet	37.08	36.69
Entecavir	Undet	Undet	38.15	35.78	Undet	Undet	38.54	36.73	Undet	Undet	37.48	35.68
Adefovir	Undet	Undet	37.64	36.99	Undet	Undet	36.23	35.68	Undet	Undet	37.38	35.66
Ribavirin	Undet	Undet	37.44	35.91	Undet	Undet	37.01	36.09	Undet	Undet	37.37	37.39
Acyclovir	Undet	Undet	38.14	35.55	Undet	Undet	39.34	36.12	Undet	Undet	37.15	37.10

Azithromycin	Undet	Undet	36.97	36.14	Undet	Undet	37.22	36.81	Undet	Undet	38.20	36.65
Clarithromycin	Undet	Undet	36.83	36.30	Undet	Undet	36.35	36.33	Undet	Undet	37.27	36.89
Ciprofloxacin	Undet	Undet	37.60	35.38	Undet	Undet	35.96	37.41	Undet	Undet	37.91	37.37
Telbivudine	Undet	Undet	37.98	36.63	Undet	Undet	36.62	36.18	Undet	Undet	37.31	35.71
Efavirenz	Undet	Undet	37.89	36.66	Undet	Undet	36.65	35.99	Undet	Undet	38.09	37.79
Tenofovirdisoproxil	Undet	Undet	38.17	37.80	Undet	Undet	37.30	36.27	Undet	Undet	36.73	36.69
Normal saline NS	Undet	Undet	37.80	35.59	Undet	Undet	36.69	36.19	Undet	Undet	37.45	36.74
Beclomethasone	Undet	Undet	37.23	35.56	Undet	Undet	36.29	38.17	Undet	Undet	37.01	36.65
Dexamethasone Acetate	Undet	Undet	37.24	35.97	Undet	Undet	36.82	36.47	Undet	Undet	37.02	36.79
Flunisolide	Undet	Undet	37.88	36.05	Undet	Undet	36.97	36.05	Undet	Undet	37.46	37.45
Triamcinolone	Undet	Undet	37.64	35.72	Undet	Undet	36.51	35.99	Undet	Undet	36.70	35.67
Budesonide	Undet	Undet	38.16	36.20	Undet	Undet	37.69	36.25	Undet	Undet	37.43	36.37
Mometasone furoate	Undet	Undet	37.89	36.52	Undet	Undet	36.44	36.25	Undet	Undet	38.05	37.95
Fluticasone propionate	Undet	Undet	37.69	36.57	Undet	Undet	36.53	37.24	Undet	Undet	36.89	36.65
Oxymetazoline	Undet	Undet	36.70	35.85	Undet	Undet	35.86	36.29	Undet	Undet	38.10	37.21
Sulphur	Undet	Undet	36.75	35.87	Undet	Undet	37.03	37.25	Undet	Undet	37.86	36.75
Eschscholtzia	Undet	Undet	38.01	35.49	Undet	Undet	36.54	36.81	Undet	Undet	37.03	37.01
Benzocaine	Undet	Undet	37.50	35.88	Undet	Undet	36.99	35.90	Undet	Undet	37.20	35.94
Menthol	Undet	Undet	37.64	36.81	Undet	Undet	36.46	36.13	Undet	Undet	37.02	35.89
Zanamivir	Undet	Undet	38.18	35.73	Undet	Undet	36.76	36.08	Undet	Undet	37.42	36.01
Mupirocin	Undet	Undet	37.62	36.47	Undet	Undet	37.30	36.39	Undet	Undet	36.67	36.53
Tobramycin	Undet	Undet	38.09	36.20	Undet	Undet	36.01	35.57	Undet	Undet	37.45	35.85
Herpes simplex virus type II	Undet	Undet	37.40	35.61	Undet	Undet	36.17	35.69	Undet	Undet	37.08	35.70
HIV-1	Undet	Undet	37.53	35.91	Undet	Undet	36.45	36.32	Undet	Undet	37.25	35.93
HIV-2	Undet	Undet	37.78	35.10	Undet	Undet	36.00	36.10	Undet	Undet	37.72	36.88

Influenza A virus (H7N9)	Undet	Undet	38.33	36.20	Undet	Undet	35.74	36.66	Undet	Undet	36.82	36.72

3. Precision

The aim of this study was to demonstrate the precision of the kit. The study used three batches of reagents.

3.1 Instruments and Materials

Instruments: Pre-NAT II, ABI7500.

Materials: SARS-CoV-2 RNA capsulated in bacteriophage (*ORF1ab* and *N*), PerkinElmer New Coronavirus Nucleic Acid Detection Kit.

3.2 Method

Samples with encapsulated RNA particles at 140 copies/mL (weak positive) and 1400 copies/mL (middle positive, or M positive in the following table) were used in this study. These two samples were tested in 10 experimens over 5 days, with 4 replicates of each sample being tested in each experiment. The same experiments were repeated using 3 batches of reagents.

3.3 Acceptable Resulsts for Verification

Negative results for all negative samples.

Positive results for all positive sasmples, and CV of Ct value should be ≤5%.

3.4 Results

Table 11, 12 and 13 listed the Ct values of the tested samples using three batches of reagents, and table 13 summarized the CV of Ct values from all experiments using three batches of reagents.

Table 11: Ct values of samples tested using reagent batch AY20200202.

Lot		AY20200202								
Concentration		Middle positive			Weak positive			Negative		
Days	Runs	Ct								
	Gene	IC	*N*	*ORF1ab*	IC	*N*	*ORF1ab*	IC	*N*	*ORF1ab*
Day 1	run 1	34.73	32.65	31.74	34.36	35.67	34.50	34.28	NA	NA
		34.70	32.70	31.63	34.52	35.97	35.34	34.77	NA	NA
		34.54	32.44	31.62	34.73	35.78	35.02	34.39	NA	NA
		34.50	32.39	31.57	34.59	36.66	34.91	35.62	NA	NA
	run 2	35.01	32.11	31.89	34.61	35.35	35.16	34.88	NA	NA
		34.67	31.83	31.81	34.76	35.21	35.13	34.57	NA	NA
		34.28	31.65	31.80	34.81	35.09	34.68	34.10	NA	NA
		34.26	31.37	31.57	35.02	35.27	34.84	34.15	NA	NA
Day 2	run 1	34.29	32.58	31.75	34.36	35.43	35.96	34.24	NA	NA

		33.80	31.98	31.31	34.37	35.41	34.71	34.23	NA	NA
		34.59	32.33	31.63	34.66	36.03	35.16	33.96	NA	NA
		34.71	32.53	31.69	34.51	36.38	34.83	33.85	NA	NA
	run 2	35.06	32.58	32.23	34.91	36.24	34.33	34.30	NA	NA
		34.94	32.64	31.65	34.66	35.76	35.11	34.52	NA	NA
		34.91	32.73	31.51	34.66	35.72	34.30	34.32	NA	NA
		34.99	32.70	31.66	34.43	35.82	34.79	34.50	NA	NA
Day 3	run 1	35.24	31.72	32.42	35.03	35.28	35.55	34.98	NA	NA
		35.09	31.37	32.00	35.64	35.07	36.05	34.43	NA	NA
		34.82	31.51	31.85	35.55	35.41	34.94	34.38	NA	NA
		34.69	31.38	31.80	35.74	35.23	35.99	34.30	NA	NA
	run 2	35.61	32.21	32.18	36.15	36.21	36.21	34.63	NA	NA
		35.53	32.85	32.06	35.21	35.54	35.68	34.98	NA	NA
		35.65	32.70	32.51	35.30	35.79	35.69	35.41	NA	NA
		34.93	32.08	32.44	35.04	35.63	35.41	35.32	NA	NA
Day 4	run 1	35.93	32.85	32.97	35.22	36.71	35.81	34.28	NA	NA
		35.61	32.79	32.35	35.16	36.69	36.21	34.77	NA	NA
		35.46	33.24	32.54	34.91	36.06	35.30	34.79	NA	NA
		35.86	33.37	32.69	35.10	35.69	34.76	34.97	NA	NA
	run 2	33.37	32.35	31.31	35.03	35.92	35.62	34.58	NA	NA
		35.71	33.14	32.21	34.64	36.25	36.00	34.96	NA	NA
		36.00	33.29	32.83	34.55	35.88	36.23	34.97	NA	NA
		36.29	33.34	33.05	34.50	35.80	35.42	35.54	NA	NA
Day 5	run 1	35.33	32.14	32.29	35.70	35.28	35.71	34.74	NA	NA
		35.03	31.66	32.01	35.74	35.66	36.22	34.60	NA	NA
		35.22	32.29	32.38	35.20	35.18	35.15	34.90	NA	NA
		35.31	32.29	32.46	35.89	36.15	35.86	34.63	NA	NA
	run 2	34.25	31.74	32.12	34.77	36.32	36.18	33.43	NA	NA
		34.23	31.81	32.02	34.97	35.87	35.55	33.41	NA	NA
		34.82	31.73	32.34	34.58	36.13	35.87	33.93	NA	NA
		34.13	31.52	32.12	34.39	35.83	35.09	33.88	NA	NA

Table 12: Ct values of samples tested using reagent batch AY20200202.

Lot		AY20200203								
Concentration		Middle positive			Weak positive			Negative		
Days	Runs	Ct								
	Gene	IC	*N*	*ORF1ab*	IC	*N*	*ORF1ab*	IC	*N*	*ORF1ab*

Day 1	run 1	34.99	31.69	32.14	34.37	34.41	34.83	34.52	NA	NA
		34.67	31.41	31.93	34.61	34.67	34.80	33.84	NA	NA
		34.58	31.37	31.81	34.88	34.98	35.15	34.02	NA	NA
		34.12	31.27	31.52	34.85	34.48	35.03	33.99	NA	NA
	run 2	34.64	31.69	31.97	35.50	34.76	34.97	34.83	NA	NA
		34.58	31.28	32.03	35.13	34.63	34.90	34.54	NA	NA
		34.33	31.37	31.69	35.26	34.84	35.61	34.09	NA	NA
		34.17	31.42	31.08	34.92	34.60	35.32	34.61	NA	NA
Day 2	run 1	34.39	31.58	32.18	34.57	34.44	35.10	34.46	NA	NA
		34.12	31.24	31.96	35.06	34.82	36.36	34.72	NA	NA
		34.26	31.46	31.76	34.95	34.64	35.22	34.55	NA	NA
		34.27	31.38	31.97	35.10	35.05	35.79	34.31	NA	NA
	run 2	35.38	32.28	32.70	34.02	34.56	35.96	35.03	NA	NA
		35.00	31.82	32.23	33.71	34.23	34.61	34.77	NA	NA
		34.86	31.41	31.87	36.10	35.04	36.12	34.17	NA	NA
		35.46	32.19	32.18	35.78	35.49	35.78	34.24	NA	NA
Day 3	run 1	34.19	31.67	32.30	34.58	35.20	36.06	33.44	NA	NA
		34.38	32.16	32.41	34.67	34.63	35.43	33.69	NA	NA
		34.41	32.12	32.34	34.61	34.63	35.27	33.84	NA	NA
		34.32	32.44	32.61	34.06	35.28	35.61	33.70	NA	NA
	run 2	34.17	32.16	32.35	34.54	36.04	35.54	33.85	NA	NA
		34.06	31.54	32.31	34.67	35.73	35.46	33.62	NA	NA
		33.90	31.66	32.02	34.59	36.18	36.32	33.93	NA	NA
		33.87	31.67	32.27	34.76	35.21	35.78	33.98	NA	NA
Day 4	run 1	34.43	32.06	32.32	34.94	35.92	35.89	33.39	NA	NA
		33.43	31.56	32.11	34.81	35.23	36.09	33.95	NA	NA
		33.96	31.83	32.07	34.56	35.40	36.27	34.03	NA	NA
		33.85	31.80	32.08	35.02	35.85	36.41	33.88	NA	NA
	run 2	33.19	30.89	31.59	33.92	34.65	35.30	33.56	NA	NA
		32.93	31.18	31.20	34.29	35.28	35.80	33.90	NA	NA
		33.29	31.40	31.83	33.79	34.97	35.61	33.84	NA	NA
		32.91	31.14	31.53	33.86	35.21	35.53	33.82	NA	NA
Day 5	run 1	34.68	31.55	32.07	34.55	35.90	36.25	33.44	NA	NA
		34.50	31.89	32.11	34.97	35.13	36.50	33.58	NA	NA
		34.56	32.25	32.45	34.86	35.88	36.15	33.90	NA	NA
		34.55	32.04	32.51	34.78	35.16	36.21	33.90	NA	NA

	run 2	34.40	31.63	32.14	35.01	36.19	35.64	33.76	NA	NA
		33.64	31.27	32.15	34.71	35.31	35.74	33.80	NA	NA
		34.52	32.01	32.28	34.79	34.96	36.37	34.16	NA	NA
		34.39	31.67	32.24	34.68	35.50	35.26	33.87	NA	NA

Table 13: Ct values of samples tested using reagent batch AY20200202.

Lot		AY20200204								
Concentration		Middle positive			Weak positive			Negative		
Days	Runs	Ct								
	Gene	IC	*N*	*ORF1ab*	IC	*N*	*ORF1ab*	IC	*N*	*ORF1ab*
Day 1	run 1	34.43	32.22	31.65	34.50	35.62	34.95	35.02	NA	NA
		34.15	32.24	31.86	34.85	35.41	35.03	34.80	NA	NA
		34.67	32.45	32.09	34.45	35.63	35.16	34.83	NA	NA
		34.81	32.41	31.77	34.39	35.23	34.70	34.80	NA	NA
	run 2	34.29	31.15	32.13	34.36	34.10	34.91	34.71	NA	NA
		33.88	31.51	31.94	34.67	35.11	34.80	34.46	NA	NA
		34.08	30.84	31.94	34.74	35.04	35.19	34.18	NA	NA
		33.82	30.55	31.61	34.75	34.58	35.41	34.25	NA	NA
Day 2	run 1	34.06	32.35	32.16	34.29	35.63	35.38	34.29	NA	NA
		34.22	32.13	31.90	34.42	36.04	36.03	34.44	NA	NA
		34.29	32.43	32.42	34.56	35.49	34.71	34.52	NA	NA
		34.71	32.35	31.76	34.41	35.71	35.65	34.61	NA	NA
	run 2	33.62	32.37	31.84	33.82	35.81	35.38	33.76	NA	NA
		33.85	32.53	32.14	33.88	35.48	35.48	33.68	NA	NA
		33.95	32.72	32.43	34.00	35.71	34.86	33.78	NA	NA
		34.16	32.61	31.95	33.85	36.05	35.27	33.80	NA	NA
Day 3	run 1	34.81	31.98	32.27	34.63	35.51	35.94	34.89	NA	NA
		34.62	31.92	31.68	34.86	35.46	35.66	35.08	NA	NA
		34.74	32.29	31.78	34.60	35.01	35.62	34.90	NA	NA
		34.58	32.00	31.57	34.90	35.48	35.09	34.80	NA	NA
	run 2	34.45	32.88	31.99	35.05	35.74	35.58	34.50	NA	NA
		34.28	32.70	31.81	34.37	36.24	35.46	34.58	NA	NA
		34.54	32.82	31.86	34.36	35.80	36.12	34.71	NA	NA
		34.47	32.32	31.64	34.20	35.85	35.17	34.70	NA	NA
Day 4	run 1	34.97	32.06	31.93	34.77	35.53	35.71	34.47	NA	NA
		34.82	32.47	31.83	34.69	35.35	35.72	34.81	NA	NA

		34.79	32.40	32.10	34.62	35.27	35.61	34.92	NA	NA
		34.97	32.63	32.65	34.71	35.42	36.00	34.95	NA	NA
	run 2	34.13	32.43	32.18	34.36	35.66	36.51	34.46	NA	NA
		37.18	32.09	32.06	34.44	35.96	35.77	33.98	NA	NA
		34.44	32.51	32.36	34.21	36.31	35.85	34.21	NA	NA
		34.08	32.32	32.06	34.44	36.00	35.13	34.21	NA	NA
Day 5	run 1	34.09	32.58	32.43	33.95	35.59	35.57	33.95	NA	NA
		33.54	32.66	32.31	33.84	35.89	36.57	33.77	NA	NA
		34.03	32.60	32.41	34.09	36.00	35.04	33.89	NA	NA
		33.93	32.42	32.07	33.73	35.44	34.87	34.03	NA	NA
	run 2	35.00	32.54	32.16	34.90	35.99	35.34	34.75	NA	NA
		34.71	32.47	32.19	34.99	35.31	36.05	34.71	NA	NA
		35.04	32.76	32.47	34.97	35.51	35.45	34.95	NA	NA
		34.68	32.52	32.25	34.78	35.64	36.05	34.92	NA	NA

Table 14: Summary of the precision.

Lot	AY20200202		AY20200203		AY20200204	
Concentration	CV (%)		CV (%)		CV (%)	
	N	*ORF1ab*	*N*	*ORF1ab*	*N*	*ORF1ab*
Middle positive	1.79%	1.36%	1.15%	1.08%	1.54%	0.84%
Weak positive	1.24%	1.59%	1.50%	1.43%	1.18%	1.32%

From the results shown in table 14, it is clear that the kit demonstrates very good precision with CV of Ct values less than 2% when testing in different experiments using different batches of reagents.

B. CONCLUSIONS

According to all the results showed above, the kit performance can meet design input and Product Requirement Specifications and is safe and effective for its intended use.

Part I: Inclusivity and Cross-Reactivity

Reactivity/Inclusivity:

The inclusivity of the PerkinElmer® New Coronavirus Nucleic Acid Detection Kit target genes are N and ORF1ab. The primer and probes are designed based on the recommendations from China CDC. An alignment was performed with the oligonucleotide primer and probe sequences with all publicly available nucleic acid sequences for SARS-CoV-2 in GenBank as of March 3, 2020 to demonstrate the predicted inclusivity of the PerkinElmer® New Coronavirus Nucleic Acid Detection Kit. All the alignments show 100% identity of the panel to the available SARS-CoV-2 sequences.

Evaluation of reactivity of the PerkinElmer® New Coronavirus Nucleic Acid Detection Kit is not applicable due to short of supply of strain isolates.

Result summary for N gene primers and probes (March 13th). They are all SARS-CoV-2.

GenBank Acc#	% Homology Test FP (i.e. 0-100%)	% Homology Test RP	% Homology Test Probe
LC528233.1	100%	100%	100%
LC528232.1	100%	100%	100%
MT123293.1	100%	100%	100%
MT123292.1	100%	100%	100%
MT123291.1	100%	100%	100%
MT123290.1	100%	100%	100%
MT118835.1	100%	100%	100%
MT106054.1	100%	100%	100%
MT106053.1	100%	100%	100%
MT106052.1	100%	100%	100%
MT093631.1	100%	100%	100%
MT093571.1	100%	100%	100%
MT081068.1	100%	100%	100%
MT081067.1	100%	100%	100%
MT081066.1	100%	100%	100%
MT081065.1	100%	100%	100%
MT081064.1	100%	100%	100%
MT081063.1	100%	100%	100%
MT081062.1	100%	100%	100%
MT081061.1	100%	100%	100%
MT081060.1	100%	100%	100%
MT081059.1	100%	100%	100%

MT072688.1	100%	100%	100%
MT066176.1	100%	100%	100%
MT066175.1	100%	100%	100%
MT049951.1	100%	100%	100%
MT044258.1	100%	100%	100%
MT044257.1	100%	100%	100%
MT039888.1	100%	100%	100%
MT039887.1	100%	100%	100%
MT039890.1	100%	100%	100%
MT039873.1	100%	100%	100%
LC522975.1	100%	100%	100%
LC522974.1	100%	100%	100%
LC522973.1	100%	100%	100%
LC522972.1	100%	100%	100%
MT027064.1	100%	100%	100%
MT027062.1	100%	100%	100%
MT020781.1	100%	100%	100%
MT019533.1	100%	100%	100%
MT019532.1	100%	100%	100%
MT019531.1	100%	100%	100%
MT019530.1	100%	100%	100%
MT019529.1	100%	100%	100%
LR757998.1	100%	100%	100%
LR757997.1	100%	100%	100%
LR757996.1	100%	100%	100%
LR757995.1	100%	100%	100%
MT007544.1	100%	100%	100%
MN996531.1	100%	100%	100%
MN996530.1	100%	100%	100%
MN996529.1	100%	100%	100%
MN996528.1	100%	100%	100%
MN996527.1	100%	100%	100%
MN997409.1	100%	100%	100%
MN994468.1	100%	100%	100%
MN994467.1	100%	100%	100%
MN988668.1	100%	100%	100%
MN988713.1	100%	100%	100%
MN985325.1	100%	100%	100%
MN975262.1	100%	100%	100%
MN938384.1	100%	100%	100%
NC_045512.2	100%	100%	100%

Result summary for ORF1ab gene primers and probes (March 13th). They are all SARS-CoV-2.

GenBank Acc#	% Homology Test FP (i.e. 0-100%)	% Homology Test RP	% Homology Test Probe
LC528233.1	100%	100%	100%
LC528232.1	100%	100%	100%
MT123293.1	100%	100%	100%
MT123292.1	100%	100%	100%
MT123291.1	100%	100%	100%
MT123290.1	100%	100%	100%
MT118835.1	100%	100%	100%
MT106054.1	100%	100%	100%
MT106053.1	100%	100%	100%
MT106052.1	100%	100%	100%
MT093631.1	100%	100%	100%
MT093571.1	100%	100%	100%
MT072688.1	100%	100%	100%
MT066176.1	100%	100%	100%
MT066175.1	100%	100%	100%
MT049951.1	100%	100%	100%
MT044258.1	100%	100%	100%
MT044257.1	100%	100%	100%
MT039888.1	100%	100%	100%
MT039887.1	100%	100%	100%
MT039890.1	100%	100%	100%
MT039873.1	100%	100%	100%
LC522975.1	100%	100%	100%
LC522974.1	100%	100%	100%
LC522973.1	100%	100%	100%
LC522972.1	100%	100%	100%
MT027064.1	100%	100%	100%
MT027062.1	100%	100%	100%
MT020781.1	100%	100%	100%
MT019533.1	100%	100%	100%
MT019532.1	100%	100%	100%
MT019531.1	100%	100%	100%
MT019530.1	100%	100%	100%
MT019529.1	100%	100%	100%
LR757998.1	100%	100%	100%
LR757996.1	100%	100%	100%
LR757995.1	100%	100%	100%
MT007544.1	100%	100%	100%
MN996532.1	100%	100%	100%
MN996531.1	100%	100%	100%
MN996530.1	100%	100%	100%
MN996529.1	100%	100%	100%

MN996528.1	100%	100%	100%
MN996527.1	100%	100%	100%
MN997409.1	100%	100%	100%
MN994468.1	100%	100%	100%
MN994467.1	100%	100%	100%
MN988668.1	100%	100%	100%
MN988713.1	100%	100%	100%
MN985325.1	100%	100%	100%
MN975262.1	100%	100%	100%
MN938384.1	100%	100%	100%
NC_045512.2	100%	100%	100%

Cross-Reactivity:

Cross-reactivity of the PerkinElmer® New Coronavirus Nucleic Acid Detection Kit was evaluated using both *in silico* analysis and by testing cross-reactivity test panels.

BLASTn analysis queries of the rRT -PCR assays primers and probes were performed against public domain nucleotide sequences with default settings: 1) The match and mismatch scores are 1 and -3, respectively; 2) The penalty to create and extend a gap in an alignment is 5 and 2 respectively; 3) parameters automatically adjust for short input sequences and the expect threshold is 1000.

The %Homology of primer and probes are summarized in the following table. Among the tested organisms, Streptococcus pyogenes shows slightly homology for the reverse primers of the N gene. However, the alignment of the forward sequence and reverse sequence is in the same direction. Therefore, the risk of non-specific amplification is low. Despite ≥80% homology of forward primers and probes for both genes are identified for SARS-coronavirus, there are significant differences in the reverse primers. Reverse primer binding efficiency is critical for the first step of RT-PCR. Without efficient cDNA synthesis due to the poor homologous of the reverse primers, the cross-reactivity to SARS-coronavirus is not likely to occur. There is also significant difference of the probe binding site at 5' end of N gene. It will also reduce the risks of detection by the TaqMan assay. This has been further validated by wet lab cross-reactivity study.

In summary, combining primers and probe for each target gene, there is no significant homologies (more than 80%) with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results in the following tables.

Pathogen	Strain	GenBank Acc#	% Homology Test N FP (i.e. 0-100%)	% Homology Test N RP	% Homology Test N Probe	% Homology Test ORFlabFP	% Homology ORFlab Test RP	% Homology ORFlab Test Probe

						(i.e. 0-100%)		
Human coronavirus 229E	229E	NC_002645.1	36.36	40.91	45	47.62	47.37	35.71
Human coronavirus OC43	ATCC VR-759	NC_006213.1	45.45	45.45	50	61.9	47.37	39.29
Human coronavirus HKU1	HCoV-HKU1	NC_006577.2	36.36	40.91	45	47.62	63.16	35.71
Human coronavirus NL63	NL63	NC_005831.2	40.91	45.45	50	47.62	47.37	35.71
SARS-coronavirus	NA (isolate "Tor2")	NC_004718.3	90.91	68.18	75	90.48	52.63	96.43
MERS-coronavirus	NL140455	MG987421.1	40.91	40.91	55	42.86	47.37	60.71
Adenovirus (e.g. C1 Ad. 71)	type 2	J01917.1	40.91	45.45	55	47.62	63.16	35.71
Human Metapneumovirus (hMPV)	CAN97-83	NC_039199.1	36.36	45.45	55	52.38	47.37	32.14
Parainfluenza virus 1 (Human respirovirus 1)	HPIV1/Los_Angeles/USA/CHLA36/2016	MK167043.1	40.91	45.45	40	52.38	42.11	28.57
Parainfluenza virus 2 (Human rubulavirus 2)	HPIV2/Seattle/USA/SC9949/2018	MN369034.1	40.91	59.09	50	47.62	47.37	28.57
Parainfluenza virus 3 (Human respirovirus 3)	NIV1721711	MH330335.1	36.36	36.36	45	42.86	42.11	28.57
Parainfluenza virus 4a (Human rubulavirus 4a)	4a M-25	NC_021928.1	36.36	45.45	45	42.86	52.63	32.14
Influenza A	New York/392/2004(H3N2)	NC_007373.1, NC_007372.1, NC_007371.1, NC_007366.1, NC_007369.1, NC_007368.1, NC_007367.1, NC_007370.1	40.91	40.91	50	38.1	52.63	32.14
Influenza B	B/Lee/1940	NC_002205.1, NC_002206.1, NC_002207.1, NC_002208.1, NC_002209.1, NC_002210.1, NC_002211.1, NC_002204.1	40.91	63.64	45	42.86	47.37	46.43
Enterovirus (e.g. EV68)	coxsackievirus B1	NC_001472.1	40.91	36.36	40	47.62	36.84	35.71
Respiratory syncytial virus	V13-0285	NC_030454.1	45.45	45.45	40	47.62	47.37	32.14
Rhinovirus	ATCC VR-1559	NC_038311.1	36.36	45.45	40	38.1	52.63	42.86

Chlamydia pneumonia	CWL029	NC_000922.1	63.64	59.09	65	57.14	63.16	42.86
Haemophilus influenzae	Rd KW20	NC_000907.1	54.55	54.55	65	57.14	63.16	53.57
Legionella pneumophila	Philadelphia 1	NC_002942.5	59.09	59.09	65	61.9	68.42	42.86
Mycobacterium tuberculosis	H37Rv	NC_000962.3	54.55	50	70	52.38	68.42	53.57
Streptococcus pneumoniae	R6	NC_003098.1	68.18	54.55	60	71.43	63.16	42.86
Streptococcus pyogenes	M1 GAS	NC_002737.2	54.55	59.09	85	57.14	68.42	42.86
Bordetella pertussis	Tohama I	NC_002929.2	63.64	68.18	65	52.38	68.42	57.14
Mycoplasma pneumoniae	M129	NC_000912.1	50	54.55	60	57.14	57.89	46.43
Pneumocystis jirovecii	RU7	NW_017264775.1	68.18	54.55	65	66.67	78.95	50
Candida albicans	SC5314	NC_032089.1	59.09	59.09	75	61.9	68.42	50
Pseudomonas aeruginosa	PAO1	NC_002516.2	59.09	50	65	52.38	63.16	46.43

The wet testing evaluation were performed with cross-reactivity evaluation panel (listed in the following table). The test specimens were prepared at the concentrations shown in the table below. The study was performed on 3 lots of reagent. For each lot, it was test once for each specimen. All testes results were negative (Ct values were all undetermined). There were no cross-reactivity identified for the triplicate tests on the specimen.

Pathogen	**Source**	**Concentration**	
		Evaluation	**Unit**
Coronavirus 229E	ATCC VR-740™	2.8×10^2	$TCID_{50}$/mL
Coronavirus OC43	ATCC VR-1558™	2.8×10^3	$TCID_{50}$/mL
Adenovirus type 3	ATCC VR-847™	$5.0 \times 10^{5.5}$	$TCID_{50}$/mL
Adenovirus type 2	ATCC VR-846™	5.6×10^4	$TCID_{50}$/mL
Adenovirus type 31	ATCC VR-1109™	1.6×10^6	$TCID_{50}$/mL

Adenovirus type 37	ATCC VR-929™	1.8×10^4	$TCID_{50}$/mL
Adenovirus type 51	ATCC VR-1603™	2.3×10^6	$TCID_{50}$/mL
Enterovirus A71	ATCC VR-1432™	$5.0 \times 10^{5.5}$	$TCID_{50}$/mL
Enterovirus D68	ATCC VR-1823™	1.6×10^6	$TCID_{50}$/mL
Influenza A virus (H3N2)	ATCC VR-1679™	$5.0 \times 10^{3.5}$	$TCID_{50}$/mL
Influenza B virus	ATCC VR-1807™	7.6×10^2	PFU/mL
Influenza A virus (H1N1pdm09)	ATCC VR-1736™	2.6×10^3	PFU/mL
Influenza A virus (seasonal H1N1)	ATCC VR-1520™	$5.0 \times 10^{4.5}$	$TCID_{50}$/mL
Respiratory syscytial virus	ATCC VR-1400™	$5.0 \times 10^{3.5}$	$TCID_{50}$/mL
Parainfluenza virus type 1	ATCC VR-94™	2.8×10^4	$TCID_{50}$/mL
Parainfluenza virus type 2	ATCC VR-92D™	0.303	ng/µL
Parainfluenza virus type 3	ATCC VR-93™	$5.0 \times 10^{4.5}$	$TCID_{50}$/mL
Parainfluenza virus type 4a	ATCC VR-1378™	2.8×10^4	$TCID_{50}$/mL
Parainfluenza virus type 4b	ATCC VR-1377™	1.6×10^3	$TCID_{50}$/mL
Chlamydophila pneumoniae	ATCC 53592™	2.9×10^5	IFU/mL
Mycoplasma pneumoniae	ATCC 15531™	3.5×10^6	CFU/mL
Haemophilus influenzae	ATCC 51907D™	10	µg/mL
Streptococcus pyogenes	ATCC 700294D-5™	7	µg/mL
Streptococcus salivarius	ATCC BAA-250D-5™	5.2	µg/mL
Rhinovirus B17	ATCC VR-1663™	2.0×10^6	PFU/mL
Rhinovirus A2	ATCC VR-482™	8.9×10^4	$TCID_{50}$/mL
Measles virus	National Standard for Influenza A/B Viral Nucleic Acids Detection Kit	Unknown	
Mumps virus		Unknown	
Staphylococcus aureus		Unknown	
Influenza A virus (H7N9)		Unknown	
Human cytomegalovirus	Symbio	Unkown	
Bordetella pertussis	ZeptoMetrix Panel	Unknown	
hepatitis B virus	WHO NIBSC 10/266	9.55E+05	IU/mL
hepatitis c virus	WHO NIBSC 14/150	1.00E+05	IU/mL
Human immunodeficiency virus type I (HIV-1)	WHO NIBSC 16/194	1.26E+05	IU/mL
Human immunodeficiency virus type II (HIV-2)	WHO NIBSC 08/150	1.00E+03	IU/mL
Hepatitis A virus	Symbio	1.84E+05	copies/mL
Epstein-barr virus	Symbio	1.46E+05	copies/mL

Cytomegalovirus	Symbio	1.15E+04	copies/mL

Herpes simplex virus type I	Symbio	5.71E+04	copies/mL
Herpes simplex virus type II	Symbio	9.01E+05	copies/mL
Severe acute respiratory syndrome (Plasmid)	BIOLIGO	1.00E+05	copies/mL
Middle East Respiratory Syndrome (Plasmid)	BIOLIGO	1.00E+05	copies/mL

Part II. Other questions.

- Q: Other than NP and OP, is there any plan to add other specimen type such as nasal swab or sputum? How about the transport medium? VTM, UTM, Amies, or saline?

  A: This is more related to sample extraction kit performance and capability. As long as the extraction kits has the feasible/supporting results on the sample matrix, it will be feasible for the real-time PCR kit.