Product Performance Evaluation Report

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PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay

Performance Evaluation

This document is to describe the performance studies conducted on the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay, including assay analytical sensitivity, specificity, tolerance to interfering substances and assay precision. 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.

1. Quantification of inactivated cultured virus

A SARS-CoV-2 virus (Isolate 2/231/human/2020/CHN) which was isolated from clinical specimen, cultured and inactivated was used for the performance evaluation study. The inactivated virus stock was quantified using droplet digital PCR (ddPCR). Briefly, the original viral stock was diluted 10 times in virus transport medium (VTM), 400µl of which was extracted in three replicates using PerkinElmer® Nucleic Acid Extraction Kit (KN0212) and PerkinElmer® Pre-NAT II Automated Workstation. The RNA was eluted into 60µl of Elution buffer, and 5µl of the elute was taken for ddPCR quantification in a 20µl reaction volume. ddPCR quantification was performed by using the BioRad One-Step RT-ddPCR Advanced Kit for Probes (Cat. No. 1864021) and the QX200 system. The reaction and thermocycling conditions were setup according to manufacturer's instructions.

For target N, the ddPCR output results are 171, 200 and 203 copies/µl for three replicates (table 1), in average 191.3 copies/µl. This gives to 3826.7 copies for the 20µl reaction, and 3826.7 copies for the 5µl RNA elute that was input into ddPCR. This means for 400µl sample, the N target yields in 60µl RNA elute is 45920 copies.

For target ORF1ab, the ddPCR output results are 68, 69 and 76 copies/µl for three replicates (table 1), in average 71 copies/ µl. This gives to 1420 copies for the 20µl reaction, and 1420 copies for the 5µl RNA elute that was input into ddPCR. This means for 400µl sample, the ORF1ab target yields in 60µl RNA elute is 17040 copies.

Table 1: ddPCR results and calculation of RNA elute concentration.

Target	ddPCR (copies/µl)	Average	Copies/ 20µl ddPCR reaction	Copies in 5µl RNA elute	Copies in 60µl RNA elute
N	171	191.333	3826.7	3826.7	45920
	200				
	203				
ORF1ab	68	71	1420	1420	17040
	69				

	76				

To estimate the concentration of RNA in the original cultured virus stock, extraction efficiency was estimated by spiking in 20µl of the RNA elute from the above-mentioned experiment into 380µl of VTM and RNA was extracted using the same extraction reagents and protocol. The eluted RNA was quantified using ddPCR as described as above, and the output results are listed in table 2.

Table 2: ddPCR results and calculation of RNA elute concentration.

Target	ddPCR (copies/µl)	Average	Copies/ 20µl ddPCR reaction	Copies in 5µl RNA elute	Copies in 60µl RNA elute
N	26.1	26.7	534	534	6408
	28.4				
	25.6				
ORF1ab	12.1	12.05	241	241	2892
	12				
	No call*				

*one of the replicates was no called due to droplet number below low limit.

From the two steps of extraction and ddPCR quantification results shown in table 1 and 2, extraction efficiency for target N was calculated to be 6408 × (60/20)/ 45920 =41.9% and extraction efficiency for target ORF1ab was calculated to be 2892 × (60/20)/ 17040 =50.9%.

From ddPCR results on the RNA sample from the first step extraction, and the extraction efficiency, the target concentrations in the original virus stock are:

N: 10 (dilution fold) × 45920 copies/ 41.9%/ 0.4ml = 2.74×10^6 copies/ml.

ORF1ab: 10 (dilution fold) × 17040 copies/ 50.9%/ 0.4ml = 8.37×10^5 copies/ml.

It was expected that target N exists at higher copy numbers than target ORF1ab, as target N locates at the 3' end of the coronavirus genome and target ORF1ab locates at the 5' end. The coronavirus transcription initiates at the 3' end of the genome, where target N locates, so it exists in either short or long cDNA fragments, while target ORF1ab is only transcribed when a long cDNA fragment is generated.

2. Analytical Sensitivity Study

According to the definition in CLSI guideline EP17-A2 [1], limit of detection (LoD) was determined as the lowest concentration of SARS-CoV-2 that the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay gives a ≥95% detection rate. Considering that the COVID-19 pandemic urgently requires disgnostic tests and the SARS-CoV-2 isolates was not readily available due to its high biosafety level, the LoD study protocol was modified from EP17-A2 [1] by taking a few recommendations from the FDA EUA guideline [2] for molecular-based tests for SARS-CoV-2. The procedure is briefly described below.

1) 10-fold dilutions of the SARS-CoV-2 isolate was tested to identify the concentration range for LoD (≥ 4 replicates per dilution);
2) Within the identified LoD concentration range, 2-fold dilutions of SARS-CoV-2 were tested (≥ 5 replicates per dilution) and detection rate will be analyzed using Probit regression to predict a concentration that gives 95% detection rate.
3) At least 20 replicates of the predicted LoD (95% detection rate) was then tested to confirm whether the concentration can result in ≥ 95% detection rate. If the predicted LoD failed to give ≥ 95% detection rate, 1.5x or 2x of the predicted LoD would be tested to verify the actual LoD for the assay.

Two extraction systems were tested in analytical sensitivity study:

1) PerkinElmer® Pre-NAT II Automated Workstation and PerkinElmer® Nucleic Acid Extraction Kit (KN0212);
2) chemagic™ 360 and chemagic™ Viral DNA/RNA 300 Kit special H96 (CMG-1033/CMG-1033-S).

2.1 Instruments and Materials

Instruments: PerkinElmer® Pre-NAT II Automated Workstation, chemagic™ 360, ABI7500, Applied Biosystems 7500 Fast / QuantStudio 3 / QuantStudio 5 Real-Time PCR Systems and Analytik Jena qTOWER^3 / qTower^3 84 Real-Time PCR system, Omni bead mill homogenizer, Hard Tissue Homogenizing Mix.

Materials: SARS-CoV-2 virus (Isolate 2/231/human/2020/CHN), SeraCare RNA reference material containing the entire SARS-CoV-2 viral genome (0505-0159), PerkinElmer® Nucleic Acid Extraction Kit (KN0212), chemagic™ Viral DNA/RNA 300 Kit special H96 (CMG-1033/CMG-1033-S), PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay, Negative clinical oropharyngeal swab, nasopharyngeal swab and saliva specimens.

2.2 Acceptable Results for Verification

2.2.1 LOD determination

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

2.2.2 LOD verification

The LoD was determined as the lowest concentration where ≥ 95% of the replicates were positive.

2.3 Results and Conclusion

2.3.1 LOD determination on Pre-NAT II

Limit of detection (LoD) was determined as the lowest concentration of SARS-CoV-2 that at which the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay can detect at a ≥95% positive rate. Samples were prepared by spiking in different concentrations of SARS-CoV-2 into negative clinical oropharyngeal swab specimen matrix. Oropharyngeal swabs were collected from healthy individuals (no COVID-19

infection history, no COVID-19 symptoms and had no contact with SARS-CoV-2 infected patients within in 14 days) and was tested using PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay and confirmed to be negative. The negative oropharyngeal swabs were mixed to form a pool for LoD study. The LoD study was conducted in two steps, in the first step of the study, a total of six 10-fold dilutions of known concentrations of inactivated SARS-CoV-2 virus (Isolate 2/231/human/2020/CHN) were prepared in negative clinical matrix and processed using the PerkinElmer® Nucleic Acid Extraction kit on the PreNAT II Automated Workstation. Four PCR replicates per concentration were tested. The results are summarized in table 3.

Table 3: LoD study results from 10-fold dilution of inactivated SARS-CoV-2 (Lot 1: AY20200202).

Dilution fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detection rate	Conc. (copies/ml)	Detection rate	N	ORF1ab	IC
1.0E+04	274	4/4	83.7	4/4	34.88	34.29	33.17
1.0E+05	27.4	4/4	8.37	3/4	38.74	37.67	33.27
1.0E+06	2.74	2/4	0.837	2/4	39.57	38.71	33.11
1.0E+07	0.274	1/4	0.0837	1/4	40.11	38.75	33.44
1.0E+08	0.0274	0/4	0.00837	0/4	/	/	32.68
1.0E+09	0.00274	0/4	0.000837	0/4	/	/	33.02
Negative	0	0/4	0.00	0/4	/	/	32.83

From the results shown in table 3, it suggests that the assay LoD is between dilution fold 1.0E+04 and 1.0E+06. The next step of LoD study was conducted on 2-fold dilutions of the virus stock covering the 10^4 to 10^6 dilutions of the stock, and the concentration is from 274 to 2.14 copies/ml. The results are summarized in table 4.

Table 4: LoD study results from 2-fold dilution of inactivated SARS-CoV-2 (Lot 1: AY20200202).

Dilution fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detection rate	Conc. (copies/ml)	Detection rate	N	ORF1ab	IC
1.0E+04	274	20/20	83.7	20/20	34.95	35.48	31.55
2.0E+04	137	20/20	41.85	20/20	35.93	36.23	31.65
4.0E+04	68.5	20/20	20.93	20/20	36.91	37.10	31.70
8.0E+04	34.25	19/20	10.46	19/20	38.15	38.64	31.61
1.6E+05	17.13	18/20	5.23	13/20	38.80	39.48	31.60

3.2E+05	8.56	11/20	2.62	11/20	39.44	39.93	31.28
6.4E+05	4.28	8/20	1.31	7/20	40.26	40.44	31.41
1.28E+06	2.14	5/20	0.65	3/20	40.10	40.65	31.16
negative	0	0/20	0	0/20	/	/	31.15

Based on the detection rate of different dilutions using reagent Lot 1 (AY20200202), six 2-fold SARS-CoV-2 dilutions of known concentrations were prepared in negative clinical matrix for LoD study using another two batches of reagents, Lot2 (AY20200203) and Lot 3 (AY20200204). Twenty individual replicates per dilution were tested from extraction. The results are summarized in table 5 and 6.

Table 5: LoD study results from 2-fold dilution of inactivated SARS-CoV-2 (Lot 2: AY20200203).

Dilution fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detection rate	Conc. (copies/ml)	Detection rate	N	ORF1ab	IC
2.0E+04	137	20/20	41.85	20/20	35.43	34.74	30.29
4.0E+04	68.5	20/20	20.93	20/20	36.30	35.63	30.36
8.0E+04	34.25	20/20	10.46	20/20	37.41	36.88	30.39
1.6E+05	17.13	19/20	5.23	18/20	38.38	37.82	30.03
3.2E+05	8.56	20/20	2.62	16/20	39.22	38.98	30.24
6.4E+05	4.28	12/20	1.31	12/20	39.87	39.61	30.44
negative	0	0/20	0	0/20	/	/	30.67

Table 6: LoD study results from 2-fold dilution of inactivated SARS-CoV-2 (Lot 3: AY20200204).

Dilution fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detection rate	Conc. (copies/ml)	Detection rate	N	ORF1ab	IC
2.0E+04	137	20/20	41.85	20/20	35.58	36.17	31.24
4.0E+04	68.5	20/20	20.93	20/20	36.22	36.42	31.23
8.0E+04	34.25	20/20	10.46	20/20	37.26	37.84	31.23
1.6E+05	17.13	18/20	5.23	14/20	38.05	38.72	31.16
3.2E+05	8.56	16/20	2.62	17/20	38.49	39.46	30.93
6.4E+05	4.28	14/20	1.31	11/20	38.97	39.02	30.72
negative	0	0/20	0	0/20	/	/	30.61

Probit analysis predicted 95% detection rate is presented in table 7.

Table 7: Probit predicted cocnetrations for 95% detection rate using inactivated SARS-CoV-2.

Lot	Probit predicted concentrations for 95% detection rate (copies/mL)	
	N	ORF1ab
Lot 1 (AY20200202)	24.884 (95% CI: 17.032 – 57.917)	9.307 (95% CI: 7.428 – 13.003)
Lot 2 (AY20200203)	13.111 (95% CI: 9.324-39.812)	6.078 (95% CI: 4.306-16.957)
Lot 3 (AY20200204)	20.669 (95% CI: 14.064-80.886)	8.958 (95% CI: 6.267-23.042)

Based on the probit analysis predicted concentrations giving 95% detection rate using three lots of reagents, Lot 1 (AY20200202) reagents give the highest concentrations for both N and ORF1ab. From the ddPCR quantification data described in section 1, in the virus stock, when N = 24.884 copies/ml, ORF1ab is 7.6 copies/ml, this is below probit predicted ORF1ab LoD 9.3 copies/ml, which means there is risk that ORF1ab can not reach 95% detection rate. When ORF1ab =9.3 copies/ml, N is 30.47 copies/ml, higher than probit predicted N LoD 24.884 copies/ml. As a confirmed positive result requires both targets to be detected, so the tentative LoD: ORF1ab =9.307 copies/ml and N= 30.467 copies/ml was furthere verified by testing 20 individual replicates using three lots of reagents. A verified LoD requires to achieve ≥95% detection rate for both targets.

2.3.2 LoD verification on Pre-NAT II

Inactivated SARS-CoV-2 virus was diluted into negative oropharyngeal swabs matrix at tentative LoD concentration (ORF1ab =9.307 copies/ml and N= 30.467 copies/ml). 20 replicates of the sample were extracted using Nucleic Acid Extraction Kit (KN0212) on Pre-NAT II and tested using the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay. The results are summarized in table 8.

Table 8: LoD verification results.

Lot	Concentration (copies/ml)		Detection rate	
	N	ORF1ab	N	ORF1ab
Lot 1 (AY20200202)	30.467	9.307	100% (20/20)	95% (19/20)
Lot 2 (AY20200203)	30.467	9.307	100% (20/20)	100% (20/20)
Lot 3 (AY20200204)	30.467	9.307	100% (20/20)	100% (20/20)

2.3.3 Sample Type Equivalency Study on Pre-NAT II using Contrived Clinical Sample

In order to evaluate whether the assay has similar detection sensitivity for oropharyngeal swab and nasopharyngeal swab specimens, these two types of swabs were collected from 141 healthy individuals (no COVID-19 infection history, no COVID-19 symptoms and had no contact with SARS-CoV-2 infected patients within in 14 days) by trained personnel. Oropharyngeal swabs were collected using Viral Transport Medium from YOCON and nasopharyngeal swabs were collected using Universal Transport Medium from COPAN. Samples were immediately tested using the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay and confirmed to be negative. A cultured SARS-CoV-2 (Isolate 2/231/human/2020/CHN) was spiked into 47 of the oropharyngeal swabs and 47 of the nasopharyngeal swabs at a range of concentrations (2×LoD, 4×LoD, 10×LoD, 20×LoD, 50×LoD, 100×LoD, 200×LoD, 250×LoD and 500×LoD, according to the LoD on Pre-NAT II) to prepare contrived positive clinical samples with low and medium viral load. 20 replicates were tested at 2×LoD, 20 replicates were tested at 4×LoD, and 1 sample were tested at 10×LoD, 20×LoD, 50×LoD, 100×LoD, 200×LoD, 250×LoD and 500×LoD. The rest of 94 oropharyngeal swabs and 94 nasopharyngeal swabs were tested as negative clinical samples directly. The 141 oropharyngeal samples and 141 nasopharyngeal samples were tested in a blinded fashion (samples were prepared and capped, then all the tubes were mixed in a box and extracted using the PerkinElmer® Nucleic Acid Extraction Kit (KN0212) and Pre-NAT II in a random order). The 282 extracted samples were tested using the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay and results are shown in the tables 9 and 10.

Table 9: Clinical evaluation with oropharyngeal samples.

SARS-CoV-2 concentration	Number of samples	Detection rate		Mean Ct		
		N	ORF1ab	N	ORF1ab	IC
2×LoD	20	20/20	20/20	37.05	37.03	31.90
4×LoD	20	20/20	20/20	35.48	35.56	32.58
10×LoD	1	1/1	1/1	34.93	35.58	33.98
20×LoD	1	1/1	1/1	34.94	34.38	30.72
50×LoD	1	1/1	1/1	34.53	34.17	34.44
100×LoD	1	1/1	1/1	32.17	31.48	31.33
200×LoD	1	1/1	1/1	33.38	32.33	34.94
250×LoD	1	1/1	1/1	32.15	31.44	34.73
500×LoD	1	1/1	1/1	30.32	30.27	33.38
Negative	94	0/94	0/94	/	/	32.63

Table 10: Clinical evaluation with nasopharyngeal samples.

SARS-CoV-2 concentration	Number of samples	Detection rate		Mean Ct		
		N	ORF1ab	N	ORF1ab	IC

2×LoD	20	20/20	20/20	38.01	37.77	31.98
4×LoD	20	20/20	20/20	37.12	36.32	32.11
10×LoD	1	1/1	1/1	35.46	34.72	31.64
20×LoD	1	1/1	1/1	35.46	34.23	32.13
50×LoD	1	1/1	1/1	33.27	32.92	29.86
100×LoD	1	1/1	1/1	31.78	31.43	30.46
200×LoD	1	1/1	1/1	32.95	31.49	32.08
250×LoD	1	1/1	1/1	31.85	30.49	32.04
500×LoD	1	1/1	1/1	30.40	29.73	30.24
Negative	94	0/94	0/94	/	/	31.78

All positive samples at 2×LoD, 4×LoD, 10×LoD, 20×LoD, 50×LoD, 100×LoD, 200×LoD, 250×LoD and 500×LoD were positive and all negative samples were negative in the background of individual oropharyngeal swab and nasopharyngeal swab matrix from the same voluenteer, suggesting that the the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay detection sensitivity is comparable with oropharyngeal swab and nasopharyngeal swab specimens.

2.3.4 LOD determination on chemagic™ 360

Samples were prepared using pooled clinical oropharyngeal swabs or nasopharyngeal swabs specimen matrix. The pooled matrix was tested using PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay and confirmed to be negative before using as LoD study matrix.

A total of six 2-fold dilutions of known concentrations of inactivated SARS-CoV-2 virus (Isolate 2/231/human/2020/CHN) were prepared in the negative clinical matrix and processed using chemagic™ Viral DNA/RNA 300 Kit special H96 (CMG-1033) on chemagic™ 360 instrument, the extracted RNA was then tested using the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay. Six individual extraction replicates per dilution were tested. The detection rate at each dilution and probit predicted LoD at 95% detection rate are summarized in tables 11-14.

Table 11: Preliminary LoD study using oropharyngeal swabs on chemagic™ 360.

Oropharyngeal swab							
Dilution fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detection rate	Conc. (copies/ml)	Detection rate	N	ORF1ab	IC
2.0E+04	137	6/6	41.85	6/6	36.48	36.82	32.18
4.0E+04	68.5	6/6	20.93	6/6	37.04	37.98	32.14
8.0E+04	34.25	6/6	10.46	6/6	39.10	38.88	32.21
1.6E+05	17.13	5/6	5.23	4/6	38.89	39.77	32.35
3.2E+05	8.56	3/6	2.62	2/6	39.35	39.85	32.28

6.4E+05	4.28	0/6	1.31	0/6	/	/	32.41
Negative	0	0/6	0	0/6	/	/	32.23

Table 12: Probit predicted 95% detection rate using oropharyngeal swabs spiked with SARS-CoV-2 on chemagic™ 360.

Probit predicted 95% detection rate (copies/mL)	
N	ORF1ab
19.077 (95% CI: 14.498 – 37.122)	7.142 (95% CI: 5.341 – 23.998)

Table 13: Preliminary LoD study using nasopharyngeal swabs on chemagic™ 360.

Nasopharyngeal swab							
Dilution fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detection rate	Conc. (copies/ml)	Detection rate	N	ORF1ab	IC
2.0E+04	137	6/6	41.85	6/6	36.65	36.55	32.32
4.0E+04	68.5	6/6	20.93	6/6	38.17	36.78	32.38
8.0E+04	34.25	6/6	10.46	6/6	38.55	38.24	32.60
1.6E+05	17.13	4/6	5.23	6/6	39.40	40.50	32.59
3.2E+05	8.56	2/6	2.62	1/6	39.59	40.53	32.86
6.4E+05	4.28	2/6	1.31	2/6	39.50	39.70	32.28
Negative	0	0/6	0	0/6	/	/	32.33

Table 14: Probit predicted 95% detection rate using nasopharyngeal swabs spiked with SARS-CoV-2 on chemagic™ 360.

Probit predicted 95% detection rate (copies/mL)	
N	ORF1ab
26.44 (95% CI: 18.338 – 69.511)	8.323 (95% CI: 5.833 – 20.685)

From the ddPCR quantification data described in section 1, for oropharyngeal swab sample, when N = 19.077 copies/ml, ORF1ab is 5.83 copies/ml, this is below probit predicted ORF1ab LoD 7.142 copies/ml, which means there is risk that ORF1ab can not reach 95% detection rate. When ORF1ab =7.142 copies/ml, N is 23.38 copies/ml, higher than the probit predicted N LoD; for nasopharyngeal swab sample, when N = 26.44 copies/ml, ORF1ab is 8.08 copies/ml, this is below probit predicted ORF1ab LoD 8.323 copies/ml, which means there is risk that ORF1ab can not reach 95% detection rate. When ORF1ab =8.323 copies/ml, N is 27.25 copies/ml, higher than probit predicted N LoD 26.44 copies/ml.

As a confirmed positive result requires both targets to be detected, so the tentative LoD: ORF1ab =7.142 copies/ml and N= 23.38 copies/ml was further verified using oropharyngeal swab matrix and ORF1ab =8.323 copies/ml and N= 27.25 copies/ml was further verified using nasopharyngeal swab matrix. A verified LoD requires to achieve ≥95% detection rate for both targets.

2.3.5 LoD verification on chemagic™ 360

Pooled negative oropharyngeal swab matrix and pooled negative nasopharyngeal swab matrix was prepared for the LoD verification study. Inactivated SARS-CoV-2 virus was spiked into oropharyngeal swab matrix at 1x and 1.5x probit predicted LoD (ORF1ab =7.142 copies/ml and N= 23.38 copies/ml) and into nasopharyngeal swab matrix at 1x and 1.5x probit predicted LoD (ORF1ab =8.323 copies/ml and N= 27.25 copies/ml) to verify the actual LoD in these two types of matrix. Twenty replicates per concentration were prepared and extracted using the chemagic™ Viral DNA/RNA 300 Kit special H96 (CMG-1033) on the chemagic™ 360 instrument and tested using the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay. The results are summarized in tables 15 and 16. For both sample types tested at 1× 95% probit LoD, one replicate was negative for the N target and one replicate was negative for the ORF1ab target, giving a both-target detection rate of 90% (18/20). At 1.5× 95% probit LoD, both sample types gave a detection rate of 100% for both targets, therefore, the actual LoDs were verified as ORF1ab =10.713 copies/ml and N= 35.07 copies/ml for oropharyngeal swab and ORF1ab =12.485 copies/ml and N= 40.871 copies/ml for nasopharyngeal swab

Table 15: chemagic™ 360 LoD verification results for oropharyngeal swab.

Concentration (copies/ml)			Detection rate		Mean Ct		
LoD	N	ORF1ab	N	ORF1ab	N	ORF1ab	IC
1X	23.380	7.142	95% (19/20)	95% (19/20)	38.44	38.76	33.13
1.5X	35.070	10.713	100% (20/20)	100% (20/20)	38.74	38.11	33.09

Table 16: chemagic™ 360 LoD verification results for nasopharyngeal swab.

Concentration (copies/ml)			Detection rate		Mean Ct		
LoD	N	ORF1ab	N	ORF1ab	N	ORF1ab	IC
1X	27.246	8.323	95% (19/20)	95% (19/20)	38.53	38.44	32.81
1.5X	40.871	12.485	100% (20/20)	100% (20/20)	38.50	37.79	32.72

The LoDs on Pre-NAT II and chemagic™ 360 are ranging from 9.3 copies/ml to 12.5 copies/ml for ORF1ab and 30.5 copies/ml to 40.9 copies/ml for N, these results show that the two extraction methods exhibited very comparable detection sensitivity when used with PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay.

2.3.6 LoD Verification Using chemagic™ 360 and Alternative PCR Systems (Equivalency of PCR Systems)

To expand the use of the PerkinElmer SARS-CoV-2 Real-time RT-PCR Assay for use with the Applied Biosystems 7500 Fast / QuantStudio 3 / QuantStudio 5 Real-Time PCR Systems and Analytik Jena qTOWER^3 / qTower^3 84 Real-Time PCR system, a study was conducted using contrived clinical nasopharyngeal swab specimens. Pooled negative nasopharyngeal swab specimens were spiked with two or three known concentrations of SeraCare RNA reference material containing the entire SARS-CoV-2 viral genome (0505-0159). Nucleic acids were extracted using the chemagic Viral DNA/RNA 300 Kit special H96 (CMG-1033) on chemagic™ 360 instrument and up to 20 individual extraction replicates were tested on each PCR instrument platforms according to the instructions for use. Testing on the original Applied Biosystems 7500 PCR System was included in this study for equivalency comparison. The results are summarized in the following tables. The LoD was confirmed to be 20 copies/mL for ABI7500, ABI 7500 Fast Dx, QuantStuido 3, QuantStudio 5 and qTower^3 84, and 10 copies/ml for qTower^3. The detection sensitivity of all six instruments is considered equivalent.

Table 17: LoD verification on alternate PCR platforms.

Instrument	Concentration (copies/mL)	Target Gene	Mean Ct	Detection Rate for Target Gene	Overall Detection Rate for Algorithm
ABI 7500	6.7	N	40.2	80% (16/20)	90% (18/20)
		ORF	39.4	75% (15/20)	
	20	N	37.8	95% (19/20)	100% (20/20)
		ORF	37.5	95% (19/20)	
ABI 7500 Fast Dx	6.7	N	38.1	45% (9/20)	90% (18/20)
		ORF	39.0	85% (17/20)	
	20	N	37.7	75% (15/20)	100% (20/20)
		ORF	37.5	100% (20/20)	
QS3	12	N	ND	0% (0/3)	67% (2/3)
		ORF	34.1	67% (2/3)	
	20	N	35.7	30% (6/20)	100% (20/20)
		ORF	35.3	95% (19/20)	
	60	N	35.8	45% (9/20)	95% (19/20)
		ORF	33.0	95% (19/20)	
QS5	12	N	ND	0% (0/3)	0% (0/3)
		ORF	ND	0% (0/3)	
	20	N	35.8	25% (5/20)	95% (19/20)
		ORF	37.0	95% (19/20)	
	60	N	36.3	55% (11/20)	100% (20/20)
		ORF	35.1	100% (20/20)	
qTower^3	6.7	N	39.3	30% (6/20)	75% (15/20)
		ORF	39.7	65% (13/20)	
	10	N	38.2	65% (13/20)	100% (20/20)
		ORF	37.8	95% (19/20)	
	20	N	38.5	75% (15/20)	100% (20/20)
		ORF	36.9	100% (20/20)	
	40	N	37.9	95% (19/20)	100% (20/20)

		ORF	36.1	100% (20/20)	
qTower^3 84	10	N	38.5	35% (7/20)	90% (18/20)
		ORF	38.4	80% (16/20)	
	20	N	39.0	55% (11/20)	95% (19/20)
		ORF	37.3	85% (17/20)	
	40	N	38.0	80% (16/20)	100% (20/20)
		ORF	36.7	100% (20/20)	

2.3.7 LOD Verification in Saliva Matrix Background

The LoD (20 copies/ml) determined on QuantStudio 5 in the nasopharyngeal swab matrix background (described in section above) was further verified in saliva matrix background using the same instrument. Briefly, SARS-CoV-2 reference control material was spiked into negative saliva matrix to prepare positive samples at 20 copies/ml. In total 20 extraction replicates of this positive sample were extracted on chemagic™ 360 and amplified on QuantStudio 5. The results are summarized in the following table and LoD 20 copies/ml was verified by a 20/20 detection rate in the saliva matrix background.

Table 18: chemagic™ 360 LoD verification results for saliva.

Concentration (copies/ml)	Detection rate		Mean Ct		
	N	ORF1ab	IC	N	ORF1ab
20	20/20	20/20	30.7	35.53	35.14

2.3.8 Equivalency of CMG1033 and CMG-1033-S

The chemagic™ Viral DNA/RNA 300 Kit special H96 has two versions: CMG-1033 and CMG-1033-S. The entire nucleic acid extraction process using these two versions of kit is the same, the only difference is that beads are removed once from the final elute for CMG-1033 and twice for CMG-1033-S.

CMG-1033: after elution, magnetic rods remove beads from elution buffer, the elute is used for PCR.

CMG-1033-S: after elution, magnetic rods collect beads from elution buffer and dispose the beads in a deep-well plate containing MilliQ water, then move back to elution buffer to collect any possible carryover beads, finally the elute is used for PCR.

Removing beads from elute one more time is to reduce the risk of beads carryover in elution buffer, theoretically this step would not affect the extraction as it was conducted after extraction process is done.

2.3.9 LOB sudy

According to the results which were showed in tables 3~6, 9~11 and 13, data from total 264 negative samples was analyzed referring to “Assign LoB = Zero and Confirm” approach described in CLSI guideline EP17-A2 [1]. All the negative samples showed no Ct with 45 cycles of amplification. The percentage of false-positive results was 0%. LoB = zero is confirmed for PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay.

3. Specificity

3.1 Instruments and Materials

Instruments: PerkinElmer® Pre-NAT II Automated Workstation, ABI7500.

Materials: Encapsulated SARS-CoV-2 ORF1ab RNA, Encapsulated SARS-CoV-2 N RNA, PerkinElmer® Nucleic Acid Extraction Kit (KN0212), PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay, Negative clinical oropharyngeal swab and nasopharyngeal swab specimens, potential cross-reactivity panel and potential interfering substances.

3.2 Acceptable Results for Verification

Positive samples and negative samples were expected to show corresponding positive and negative results in the presence of potential cross-reactivity and interfering substances.

3.3 Results and Conclusion

3.3.1 Analytical Reactivity (Inclusivity)

The primers and probes for targets N and ORF1ab used in the PerkinElmer® SARS-CoV-2 Real-time RT PCR Assay were designed at the beginning of the outbreak of COVID-19, at which time there was limited number of SARS-CoV-2 sequences. As the spreading of the disease, more and more SARS-CoV-2 sequences are publicly available. To ensure that the assay can successfully detect SARS-CoV-2 strains evolved thereafter and inform customers potential false negative results caused by virus mutation at primer/probe target regions, PerkinElmer has kept close monitoring of published sequences every 1-2 weeks, and conducted *in silico* analysis by aligning the primer and probe sequences to all available SARS-CoV-2 sequences till a date. Such sequence monitoring and *in silico* analysis will be continuingly conducted and updated after the product launch.

BLASTn analysis queries alignments (*in silico* analysis) were conducted with the SARS-CoV-2 ORF1ab and N primer and probe oligonucleotide sequences with all publicly available SARS-CoV-2 nucleic acid sequences in GenBank by the date June 15th 2020 to demonstrate the predicted inclusivity of the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay. The analysis results are shown in table 19.

Table 19: *In silico* analysis of the primer and probe inclusivity.

Target	Name	Total sequence aligned	Sequence with mismatches	Mismatch description	Effect of mismatches (theoretical analysis)
N	Forward primer	3748	153 (4.08%)	The 1st to 3rd bp from the 5'end	5' end mismatches normally do not affect PCR amplification
			14 (0.37%)	The 7th bp from the 5'end	The current assay thermal cycling condition with annealing

			21 (0.56%)	The 16th bp from the 5'end	temperature at 55 °C should be able to tolerate one mismatch within the primer
	Reverse primer	3748	1 (0.03%)	The 11th bp from the 5'end	
	Probe	3749	2 (0.05%)	The 15th bp from the 5'end	1-2 base pairs mismatches should not affect TaqMan probe PCR
ORF1ab	Forward primer	4446	3 (0.07%)	The 15th bp from the 5'end	The current assay thermal cycling condition with annealing temperature at 55 °C should be able to tolerate one mismatch within the primer
	Reverse primer	3728	5 (0.13%)	The 1st or 18 th bp at the 5' end	
	Probe	4321	5 (0.12%)	The 1st and/or 2nd or 3rd bp at the 5' end	1-2 base pairs mismatches at the 5' end of probe should not affect TaqMan probe PCR

There were 1~3 base-pair of mismatches at the 5' end of N forward primer, ORF1ab reverse primer and ORF1ab probe for a few newly released sequences. As PCR amplification mainly depends on the affinity between primer 3' end and target sequence, 5' end mismatches or overhangs normally do not affect PCR amplification. For this reason, the 5' end of primers are often modified different from the target sequence in a lot of molecular technologies without affecting the amplification efficiency. These mismatches are not expected to affect assay performance since they are located at the 5' end of the primer sequence.

For mismatch close to the 5' end of the sequences, experience has shown that a proper design of the primers and probes with melting temperatures > 60 °C and PCR run conditions of the assay with annealing temperature at 55°C could tolerate one to two mismatches. The PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay uses a traditional 3-step thermal cycling program with annealing temperature set at 55°C to tolerate potential mismatches like these single base pair mutations without affecting the assay performance.

Overall, the *in silico* analysis showed that these oligoes can still cover almost all strains and isolates identified and sequenced by the time May 10th 2020, and the assay should generate a positive result if any SARS-CoV-2 virus is present at a detectable level.

3.3.2 Analytical Specificity (Cross-reactivity)

Cross-reactivity of the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay was evaluated using both *in silico* analysis and wet testing against normal and pathogenic organisms found in the respiratory tract. BLASTn analysis queries of the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay primers and probes were performed against public domain nucleotide sequences with default settings. The database search parameters were as follow:

- The match and mismatch scores were 1 and -3, respectively.
- The penalty to create and extend a gap in an alignment was 5 and 2, respectively.
- The search parameters automatically adjusted for short input sequences and the expected threshold was 1000.

In summary no organisms, including other related SARS-coronaviruses, exhibited >80% homology to the forward primer, reverse primer, and probe for either the ORF1ab or N target except SARS-coronavirus and Bat coronavirus. For SARS-coronavirus and Bat coronavirus, only one primer (forward or reverse) or proble has >80% homology, the combining use of primers and probe for each target gene is unlikely to result in amplification or fluorescence. Other than that, at present no other SARS-like coronaviruses are circulating in the human population, making it even low chance to catch other SARS-like coronaviruses.

The %Homology of N and ORF1ab region primer and probes is summarized in tables 20 and 21. Overall, the results of this analysis predict no significant cross-reactivity or microbial interference.

Table 20: The %Homology of N primers and probe.

Pathogen	Strain	GenBank Acc#	%Homology of N forward primer	%Homology of N reverse primer	%Homology of N probe
Human coronavirus 229E	229E	NC_002645.1	36.36	40.91	45
Human coronavirus OC43	ATCC VR-759	NC_006213.1	45.45	45.45	50
Human coronavirus HKU1	HCoV-HKU1	NC_006577.2	36.36	40.91	45
Human coronavirus NL63	NL63	NC_005831.2	40.91	45.45	50
SARS-coronavirus	NA (isolate “Tor2”)	NC_004718.3	90.91	68.18	75
Bat coronavirus	bat-SL-CoVZC45	MG772933.1	100.00	63.64	65
MERS-coronavirus	NL140455	MG987421.1	40.91	40.91	55
Adenovirus (e.g. C1 Ad. 71)	type 2	J01917.1	40.91	45.45	55
Human Metapneumovirus (hMPV)	CAN97-83	NC_039199.1	36.36	45.45	55

Parainfluenza virus 1 (Human respirovirus 1)	HPIV1/Los_Angeles/USA/CHLA36/2016	MK167043.1	40.91	45.45	40
Parainfluenza virus 2 (Human rubulavirus 2)	HPIV2/Seattle/USA/SC9949/2018	MN369034.1	40.91	59.09	50
Parainfluenza virus 3 (Human respirovirus 3)	NIV1721711	MH330335.1	36.36	36.36	45
Parainfluenza virus 4a (Human rubulavirus 4a)	4a M-25	NC_021928.1	36.36	45.45	45
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
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
Enterovirus (e.g. EV68)	coxsackievirus B1	NC_001472.1	40.91	36.36	40
Respiratory syncytial virus	V13-0285	NC_030454.1	45.45	45.45	40
Rhinovirus	ATCC VR-1559	NC_038311.1	36.36	45.45	40
Chlamydia pneumonia	CWL029	NC_000922.1	63.64	59.09	65

Haemophilus influenzae	Rd KW20	NC_000907.1	54.55	54.55	65
Legionella pneumophila	Philadelphia 1	NC_002942.5	59.09	59.09	65
Mycobacterium tuberculosis	H37Rv	NC_000962.3	54.55	50.00	70
Streptococcus pneumoniae	R6	NC_003098.1	68.18	54.55	60
Streptococcus pyogenes	M1 GAS	NC_002737.2	54.55	59.09	85
Bordetella pertussis	Tohama I	NC_002929.2	63.64	68.18	65
Mycoplasma pneumoniae	M129	NC_000912.1	50.00	54.55	60
Pneumocystis jirovecii	RU7	NW_017264775.1	68.18	54.55	65
Candida albicans	SC5314	NC_032089.1	59.09	59.09	75
Pseudomonas aeruginosa	PAO1	NC_002516.2	59.09	50	65
Influenza C	C/Ann Arbor/1/50	NC_006312.2, NC_006310.2, NC_006309.2, NC_006307.2, NC_006308.2, NC_006306.2, NC_006311.1	40.91	45.46	40.00
Parechovirus	echovirus 22 EV22	NC_038319.1	45.46	45.46	50.00
Corynebacterium diphtheriae	NCTC11397	NZ_LN831026.1	54.55	59.10	75.00
Legionella micdadei	ATCC 33218	NZ_LN614830.1	54.55	59.10	65.00
Bacillus anthracosis	Ames Ancestor	NC_007530.2	59.10	59.10	65.00
Moraxella cararrhalis	BBH18	NC_014147.1	54.55	59.10	65.00

Neisseria elongate and miningitidis	ATCC 29315	NZ_CP007726.1	54.55	54.55	60.00
Staphylococcus epidermis	ATCC 12228	NC_004461.1	54.55	54.55	55.00
Leptospirosis	serovar Lai str. 56601	NC_004342.2 NC_004343.2	63.64	59.10	65.00
Chlamydia psittaci	6BC	NC_017287.1	54.55	50.00	60.00
Coxiella burneti (QFever).	Dugway 5J108-111	NC_009727.1	54.55	54.55	65.00

Table 21: The %Homology of ORF1ab primers and probe.

Pathogen	Strain	GenBank Acc#	%Homology of ORFlab forward primer	%Homology of ORFlab reverse primer	%Homology of ORFlab probe
Human coronavirus 229E	229E	NC_002645.1	47.62	47.37	35.71
Human coronavirus OC43	ATCC VR-759	NC_006213.1	61.90	47.37	39.29
Human coronavirus HKU1	HCoV-HKU1	NC_006577.2	47.62	63.16	35.71
Human coronavirus NL63	NL63	NC_005831.2	47.62	47.37	35.71
SARS-coronavirus	NA (isolate “Tor2”)	NC_004718.3	90.48	52.63	96.43
Bat coronavirus	bat-SL-CoVZC45	MG772933.1	94.44	57.89	96.43
MERS-coronavirus	NL140455	MG987421.1	42.86	47.37	60.71
Adenovirus (e.g. C1 Ad. 71)	type 2	J01917.1	47.62	63.16	35.71
Human Metapneumovirus (hMPV)	CAN97-83	NC_039199.1	52.38	47.37	32.14
Parainfluenza virus 1 (Human respirovirus 1)	HPIV1/Los_Angeles/USA/CHLA36/2016	MK167043.1	52.38	42.11	28.57

Parainfluenza virus 2 (Human rubulavirus 2)	HPIV2/Seattle/USA/SC9949/2018	MN369034.1	47.62	47.37	28.57
Parainfluenza virus 3 (Human respirovirus 3)	NIV1721711	MH330335.1	42.86	42.11	28.57
Parainfluenza virus 4a (Human rubulavirus 4a)	4a M-25	NC_021928.1	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	38.10	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	42.86	47.37	46.43
Enterovirus (e.g. EV68)	coxsackievirus B1	NC_001472.1	47.62	36.84	35.71
Respiratory syncytial virus	V13-0285	NC_030454.1	47.62	47.37	32.14
Rhinovirus	ATCC VR-1559	NC_038311.1	38.10	52.63	42.86
Chlamydia pneumonia	CWL029	NC_000922.1	57.14	63.16	42.86
Haemophilus influenzae	Rd KW20	NC_000907.1	57.14	63.16	53.57
Legionella pneumophila	Philadelphia 1	NC_002942.5	61.9	68.42	42.86

Mycobacterium tuberculosis	H37Rv	NC_000962.3	52.38	68.42	53.57
Streptococcus pneumoniae	R6	NC_003098.1	71.43	63.16	42.86
Streptococcus pyogenes	M1 GAS	NC_002737.2	57.14	68.42	42.86
Bordetella pertussis	Tohama I	NC_002929.2	52.38	68.42	57.14
Mycoplasma pneumoniae	M129	NC_000912.1	57.14	57.89	46.43
Pneumocystis jirovecii	RU7	NW_017264775.1	66.67	78.95	50.00
Candida albicans	SC5314	NC_032089.1	61.9	68.42	50.00
Pseudomonas aeruginosa	PAO1	NC_002516.2	52.38	63.16	46.43
Influenza C	C/Ann Arbor/1/50	NC_006312.2, NC_006310.2, NC_006309.2, NC_006307.2, NC_006308.2, NC_006306.2, NC_006311.1	47.62	47.37	35.71
Parechovirus	echovirus 22 EV22	NC_038319.1	38.10	42.11	28.57
Corynebacterium diphtheriae	NCTC11397	NZ_LN831026.1	57.14	68.42	46.43
Legionella micdadei	ATCC 33218	NZ_LN614830.1	57.14	68.42	46.43
Bacillus anthracosis	Ames Ancestor	NC_007530.2	61.90	68.42	46.43
Moraxella cararrhalis	BBH18	NC_014147.1	66.67	68.42	39.29
Neisseria elongate and miningitidis	ATCC 29315	NZ_CP007726.1	57.14	63.16	46.43
Staphylococcus epidermis	ATCC 12228	NC_004461.1	61.90	63.16	46.43

Leptospirosis	serovar Lai str. 56601	NC_004342.2 NC_004343.2	66.67	63.16	50.00
Chlamydia psittaci	6BC	NC_017287.1	61.90	68.42	42.86
Coxiella burneti (QFever).	Dugway 5J108-111	NC_009727.1	57.14	63.16	53.57

Wet testing against normal and pathogenic organisms of the respiratory tract was performed to confirm the results of the *in silico* analysis.

To test the effect of microorganisms on the assay result, each organism listed in table 22 was spiked into SARS-CoV-2 positive and negative samples at concentrations indicated in table 22. The concentrations of microorganisms are at clinically relevant concentrations or the highest available concentrations in the lab. SARS-CoV-2 positive samples contain 60 copies/ml of encapsulated SARS-CoV-2 ORF1ab and N RNA to represent clinically low positive samples (clinical sample viral loads range from 10^2 copies/ml to 10^9 copies/ml) [5, 6]. The samples were extracted using PerkinElmer® Nucleic Acid Extraction Kit (KN0212) on Pre-NAT II and tested using the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay. Cross-reactivity study was conducted according to CLSI guideline EP07[3] with slight modifications. Three lots of reagent were used in these tests to better evaluate the assay specificity, and one sample for each condition was tested using each lot of reagent to reduce the runs and generate data to rapidly support combating the pandemic. Results are summarized in table 23.

Table 22: Potential cross-reactivity panel tested.

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	Clinical specimen	1.84E+05	copies/mL
Epstein-barr virus	Clinical specimen	1.46E+05	copies/mL
Cytomegalovirus	Clinical specimen	1.15E+04	copies/mL
Herpes simplex virus type I	Pasteur	5.71E+04	copies/mL
Herpes simplex virus type II	Zeptometrix	9.01E+05	copies/mL
SARS (Plasmid)	BIOLIGO	1.00E+05	copies/mL
MERS (Plasmid)	BIOLIGO	1.00E+05	copies/mL

Table 23: Results of potential cross-reactivity study.

Lot Date	Lot1 (AY20200202) 07/Feb/2020				Lot2 (AY20200203) 09/Feb/2020				Lot3 (AY20200204) 11/Feb/2020			
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 2	Undet	Undet	37.33	36.02	Undet	Undet	36.45	35.87	Undet	Undet	37.42	34.85
Adenovirus type 3	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
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
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

From the cross-reactivity results summarized in table 23, it is verified that the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay does not exhibit cross-reactivity to the microorganisms listed in table 22. It is expected to have cross-reactivity study also conducted on human coronavirus HKU1, human coronavirus HL63, alpha coronaviruses, fungi, mollicutes, human metapneumovirus (HMPV), protozoa, respiratory actinomycetes and other rhinoviruses, however, it may depend on the availability of these microorganisms. Nevertheless, the *in silico* analysis results showed in 19 and 20 suggest that theoretically there is low chance of cross-reactivity between the assay and these microorganisms.

3.3.3 Interfering Substances Studies

To test the effect of potential endogenous and exogenous interfering substances on the assay result, the common interfering substances listed in table 24 was added into SARS-CoV-2 positive and negative samples at concentrations indicated in table 24. The drug interfering substances were supplemented at concentrations that are three folds of the highest concentration observed during therapeutic drug treatment, and the other interfering substances were supplemented at clinically relevant concentrations. SARS-CoV-2 positive samples contain 60 copies/ml of encapsulated SARS-CoV-2 ORF1ab and N RNA to represent clinically low positive samples (clinical sample viral loads range from 10^2 copies/ml to 10^9 copies/ml) [5, 6]. The samples were extracted using PerkinElmer® Nucleic Acid Extraction Kit (KN0212) on Pre-NAT II and tested using the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay. Interfering substance study was conducted according to CLSI guideline EP07 [3] with slight modifications. Three lots of reagent were used in these tests to better evaluate the assay specificity, and one sample for each condition was tested using each lot of reagent to reduce the runs and generate data to rapidly support combating the pandemic. Results are summarized in table 25.

Table 24: Potential interfering substances tested.

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 hydrochloride	15% v/v
Normal saline NS	1 mg/mL	Sulphur	0.05% v/v
Beclomethasone dipropionate	22.5 μg/mL	Pharyngitis lozenges	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	Relenza (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	Sputum	5% V/V
Mucin	0.1mg/ml	Blood	1% V/V
Tamiflu (oseltamivir)	1.7 μg/mL	Alpivab (peramivir)	45ng/ml
Amantadine	75ng/ml		

Table 25: Results of interfering substance study.

Lot Date	Lot1 (AY20200202) 07/Feb/2020				Lot2 (AY20200203) 09/Feb/2020				Lot3 (AY20200204) 11/Feb/2020			
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*
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 dipropionate	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 hydrochloride	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
Pharyngitis lozenges	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
Relenza (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
Sputum*	Undet	Undet	36.34	36.17	Undet	Undet	36.32	36.60	Undet	Undet	36.51	36.68
Mucin*	Undet	Undet	36.13	35.67	Undet	Undet	37.03	35.43	Undet	Undet	37.14	35.71
Blood*	Undet	Undet	37.64	35.34	Undet	Undet	37.14	36.39	Undet	Undet	36.47	35.73
Tamiflu (oseltamivir) *	Undet	Undet	36.21	37.00	Undet	Undet	37.32	36.58	Undet	Undet	36.53	36.15
Alpivab (peramivir) *	Undet	Undet	37.30	36.03	Undet	Undet	37.29	37.80	Undet	Undet	36.86	36.43
Amantadine*	Undet	Undet	37.50	36.21	Undet	Undet	36.60	36.32	Undet	Undet	36.99	36.52

*: The results were got after 1st Technical Documentation (TD) gap review by Emergo.

From the interfering substance study results summarized in table 25, the positive samples spiked with interfering substances listed in table 24 all showed positive results, and the negative samples spiked with interfering substances listed in table 24 all showed negtive results, indicating that the performance of the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay is not affected by these interfering substances.

4. Precision

4.1 Precision Study on PreNat II

4.1.1 Precision Experimental Design

The Precision study was designed according to CLSI guideline EP05-A3 [4]. Considering the urgent needs for COVID-19 assay, a 5-day precision study protocol was used and designed specifically as described in figure 1.

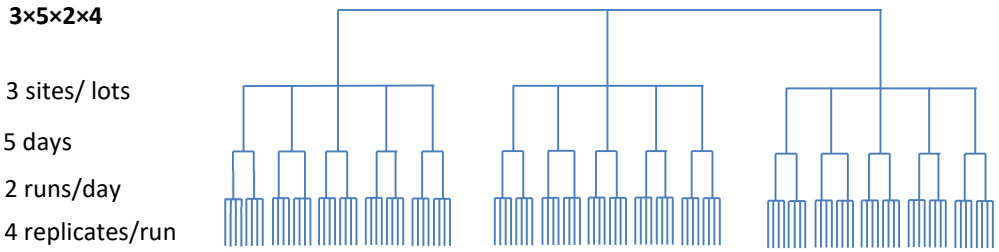

Figure 1: Diagrams of precision study design.

4.1.2 Instruments and Reagents

RNA extraction:

- PerkinElmer® Nucleic Acid Extraction Kit (KN0212)
- PerkinElmer® Pre-NAT II Automated Workstation

PCR:

- PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay (2019-nCoV-PCR-AUS)
- Applied Biosystems™ 7500 Real-Time PCR System

Note: for equipment ID #s, reagent batch lot information, test dates and personnel information, please see table 26.

4.1.3 Testing Sample

According to the recommendation in EP05-A3 [4], three samples, one negative and two positive samples representing low and moderated clinical specimens are tested for assay precision evaluation. At the time this precision study was conducted in early February, the clinical viral loads of SARS-CoV-2 was unknown, and droplet digital PCR (ddPCR) quantification data on the encapsulated SARS-CoV-2 RNAs was also not available at that moment. To urgently generate performance data for customer's reference (during the COVID-19 epidemic in China), a dilution of encapsulated SARS-CoV-2 RNAs close to the assay LoD concentration was used to represent low positive clinical specimen and 10x of this concentration was used to represent moderate positive clinical specimen. The concentrations were subsequently determined using ddPCR to be 140 copies/ml and 1400 copies/ml.

As the publishing of studies on SARS-CoV-2 viral loads in clinical upper respiratory track specimens in the past a few months, it was known that SARS-CoV-2 exists in patient specimens ranging from 10^2 copies/ml to 10^9 copies/ml [5, 6]. This verified that the concentrations used in the precision study can well represent clinical low and moderate positive specimens.

- Negative sample was prepared by pooling negative oropharyngeal swabs in virus transport medium (VTM);
- Low positive sample was prepared by spiking encapsulated RNA (SARS-CoV-2 N and ORF1ab) into negative oropharyngeal swab matrix at 140 copies/ml (approximately 5x LoD concentration according to LoD determined using SARS-CoV-2 isolate which was generated after this precision study);
- Moderate positive sample was prepared by spiking encapsulated RNA (SARS-CoV-2 N and ORF1ab) into negative oropharyngeal swab matrix at 1400 copies/ml (approximately 50x LoD concentration according to LoD determined using SARS-CoV-2);

4.1.4 Acceptance Criteria

a. Negative samples should all show negative results.
b. Positive samples should all show positive results and the Coefficient of Variance (CV) of positive samples' Ct values should be ≤5%.

4.1.5 Testing Plan

The detailed testing plan including instruments and labs is listed in table 26.

Table 26: Testing plan for precision study on Pre-NAT II.

Testing personnel: Chunhua Zhu											
Lot AY20200202 tested at R&D Testing Lab 1				Lot AY20200203 tested at R&D Testing Lab 2				Lot AY20200204 tested at R&D MDx Lab			
(Date, Run, Equip. Desc., Equipment ID#)				(Date, Run, Equip. Desc., Equipment ID#)				(Date, Run, Equip. Desc., Equipment ID#)			
06/Feb /2020	Run1	Pre-NAT II	SB-RD391	08/Feb /2020	Run1	Pre-NAT II	SB-RD478	10/Feb /2020	Run1	Pre-NAT II	SB-RD391
		ABI7500	SB-RD387			ABI7500	SB-RD403			ABI7500	SB-RD403
	Run2	Pre-NAT II	SB-RD391		Run2	Pre-NAT II	SB-RD478		Run2	Pre-NAT II	SB-RD391
		ABI7500	SB-RD403			ABI7500	SB-RD403			ABI7500	SB-RD403
07/Feb /2020	Run1	Pre-NAT II	SB-RD391	09/Feb /2020	Run1	Pre-NAT II	SB-RD478	11/Feb /2020	Run1	Pre-NAT II	SB-RD391
		ABI7500	SB-RD387			ABI7500	SB-RD403			ABI7500	SB-RD387
	Run2	Pre-NAT II	SB-RD391		Run2	Pre-NAT II	SB-RD478		Run2	Pre-NAT II	SB-RD391
		ABI7500	SB-RD387			ABI7500	SB-RD403			ABI7500	SB-RD387
08/Feb /2020	Run1	Pre-NAT II	SB-RD391	10/Feb /2020	Run1	Pre-NAT II	SB-RD478	12/Feb /2020	Run1	Pre-NAT II	SB-RD391
		ABI7500	SB-RD387			ABI7500	SB-RD403			ABI7500	SB-RD387
	Run2	Pre-NAT II	SB-RD391		Run2	Pre-NAT II	SB-RD478		Run2	Pre-NAT II	SB-RD391
		ABI7500	SB-RD387			ABI7500	SB-RD403			ABI7500	SB-RD387
09/Feb /2020	Run1	Pre-NAT II	SB-RD391	11/Feb /2020	Run1	Pre-NAT II	SB-RD478	13/Feb /2020	Run1	Pre-NAT II	SB-RD391
		ABI7500	SB-RD387			ABI7500	SB-RD403			ABI7500	SB-RD387
	Run2	Pre-NAT II	SB-RD391		Run2	Pre-NAT II	SB-RD478		Run2	Pre-NAT II	SB-RD391
		ABI7500	SB-RD387			ABI7500	SB-RD403			ABI7500	SB-RD387
10/Feb	Run1	Pre-NAT II	SB-RD391	12/Feb /2020	Run1	Pre-NAT II	SB-RD478	14/Feb /2020	Run1	Pre-NAT II	SB-RD391
		ABI7500	SB-RD403			ABI7500	SB-RD403			ABI7500	SB-RD387

/2020	Run2	Pre-NAT II	SB-RD391		Run2	Pre-NAT II	SB-RD478		Run2	Pre-NAT II	SB-RD391
		ABI7500	SB-RD387			ABI7500	SB-RD403			ABI7500	SB-RD387

Remarks:

1) As Pre-NAT II instrument conducts nucleic acid extraction and PCR mix preparation automatically, there is only very limited manual handling in the whole assay procedure, testing personal is a relatively less important impact factor for assay precision than the instruments, therefore only one testing personal is involved in the precision study.

2) all PCR instruments are in the same PCR lab for the purpose of preventing PCR amplicon contamination.

3) Pre-NAT II SB-RD391 was moved from R&D Testing Lab 1 into R&D MDx Lab for reagent lot AY20200204 testing.

4.1.6 Testing Results

The test results (Ct values) from all samples are summarized in table 27-29.

Table 27: Ct values of samples tested using reagent lot AY20200202 on Pre-NAT II.

Note: see table 26 for equipment, dates and personnel information.

Lot		AY20200202								
Concentration		Moderate positive			Low positive			Negative		
Days	Runs	Ct								
	Target	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 28: Ct values of samples tested using reagent lot AY20200203 on Pre-NAT II.

Note: see table 26 for equipment, dates and personnel information.

Lot		AY20200203								
Concentration		Moderate positive			Low positive			Negative		
Days	Runs	Ct								
	Target	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 29: Ct values of samples tested using reagent lot AY20200204 on Pre-NAT II.

Note: see table 26 for equipment, dates and personnel information.

Lot		AY20200204								
Concentration		Moderate positive			Low positive			Negative		
Days	Runs	Ct								
	Target	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

4.1.7 Data analysis

Data analysis was conducted using Analyse-it for Microsoft Excel 5.50.

The generated CVs of repeatability and reproducibility shown in table 30.

Table 30: Summary of the assay repeatability and reproducibility.

Target	N	Repeatability			Reproducibility		
		CV	95% CI		CV	95% CI	
N_Low	120	0.9%	0.8%	to 1.1%	1.6%	1.4%	to 3.3%
N_Moderate	120	0.8%	0.7%	to 0.9%	1.8%	1.6%	to 6.0%
ORF1ab_Low	120	1.2%	1.0%	to 1.4%	1.5%	1.4%	to 3.3%
ORF1ab_Moderate	120	0.8%	0.7%	to 1.0%	1.1%	1.0%	to 2.6%

From the results shown in table 30, the assay and instruments demonstrated very good repeatability and reproducibility with CV of Ct values less than 5% when testing at different labs among different days using different batches of reagents.

4.2 Precision Study on chemagic™ 360

4.2.1 Instruments and Reagents

RNA extraction:

- chemagic™ Viral DNA/RNA 300 Kit special H96 (CMG-1033-S)
- chemagic™ 360

PCR:

- PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay (2019-nCoV-PCR-AUS)
- Applied Biosystems™ 7500 Real-Time PCR System

  Note: for equipment ID #s, reagent batch lot information, and personnel information, please see table 31.

4.2.2 Testing Sample

According to the recommendation in EP05-A3 [4], three samples, one negative and two positive samples representing low and moderated clinical specimens are tested for assay precision evaluation.

By the time the precision study on chemagic™ 360 was conducted, Sym-Bio has obtained an inactivated cultured SARS-CoV-2 isolate from clinic (Isolate 2/231/human/2020/CHN), therefore this isolate instead of the encapsulated RNAs was used in this precision study to better minic clinical specimen. Taking into consideration the assay detection sensitivity and the SARS-CoV-2 viral loads in clinical upper respiratory track specimens (10^2 copies/ml to 10^9 copies/ml as reported by a few literatures [5, 6]), a sample at 105 copies/ml which is at the low end of clinical specimens and approximately 3x of the assay LoD determined on chemagic™ 360 was used as low positive sample for precision study, and a sample at 1050 copies/ml was used as moderate positive sample for the study.

- Negative sample was prepared by pooling negative oropharyngeal swabs in virus transport medium (VTM);
- Low positive sample was prepared by spiking SARS-CoV-2 virus (Isolate 2/231/human/2020/CHN) into negative oropharyngeal swab matrix at 105 copies/ml (3x LoD);
- Moderate positive sample was prepared by spiking SARS-CoV-2 virus (Isolate 2/231/human/2020/CHN) into negative oropharyngeal swab matrix at 1050 copies/ml (30x LoD).

4.2.3 Acceptance Criteria

a. Negative samples should all show negative results.
b. Positive samples should all show positive results and the Coefficient of Variance (CV) of positive samples' Ct values should be ≤5%.

4.2.4 Testing Plan

The detailed testing plan including instruments and labs is listed in table 31.

Table 31: Testing plan for precision study on chemagic™ 360.

Testing personnel: Chunhua Zhu			
Lot AY20200202 tested at R&D Testing Lab 1			
(Date, Run, Equip. Desc., Equipment ID#)			
07/Apr/2020	Run1	chemagic™ 360	SB-QC-M059
		ABI7500	SB-RD538
	Run2	chemagic™ 360	SB-QC-M059
		ABI7500	SB-RD387
08/Apr/2020	Run1	chemagic™ 360	SB-QC-M059
		ABI7500	SB-RD483
	Run2	chemagic™ 360	SB-QC-M059
		ABI7500	SB-RD387
09/Apr/2020	Run1	chemagic™ 360	SB-QC-M059

		ABI7500	SB-RD387
	Run2	chemagic™ 360	SB-QC-M059
		ABI7500	SB-RD387
10/Apr/2020	Run1	chemagic™ 360	SB-QC-M059
		ABI7500	SB-RD387
	Run2	chemagic™ 360	SB-QC-M059
		ABI7500	SB-RD403
13/Apr/2020	Run1	chemagic™ 360	SB-QC-M059
		ABI7500	SB-RD403
	Run2	chemagic™ 360	SB-QC-M059
		ABI7500	SB-RD403

*By the time the report was submitted to Emergo on the 25th May 2020, precision study using chemagic™ 360 was conducted on only one lot of reagent.

4.2.5 Testing Results

The test results (Ct values) from all samples are summarized in table 32.

Table 32: Ct values of samples tested using reagent lot AY20200202 on chemagic™ 360.

Note: see table 31 for equipment, dates and personnel information.

Lot		AY20200202								
Concentration		Moderate positive			Low positive			Negative		
Days	Runs	Ct								
	Target	IC	*N*	*ORF1ab*	IC	*N*	*ORF1ab*	IC	*N*	*ORF1ab*
Day 1	run 1	31.59	33.01	32.77	32.85	36.46	37.48	33.06	NA	NA
		32.07	33.83	33.61	32.93	37.09	36.26	33.15	NA	NA
		32.00	33.70	33.37	33.09	37.29	37.04	33.31	NA	NA
		32.32	33.98	33.65	33.02	37.26	37.76	32.97	NA	NA
	run 2	31.63	34.60	34.12	32.33	38.23	37.60	32.55	NA	NA
		31.42	34.35	34.16	32.53	37.71	37.96	32.54	NA	NA
		31.45	34.48	34.07	32.36	37.26	37.67	32.80	NA	NA
		31.39	34.35	33.54	32.67	37.15	37.37	32.54	NA	NA
Day 2	run 1	32.49	34.15	33.93	33.38	40.15	38.29	33.30	NA	NA
		32.62	34.26	33.86	33.59	37.79	36.76	33.57	NA	NA
		32.84	34.88	33.99	33.55	37.77	36.97	33.26	NA	NA
		32.56	34.32	33.98	30.04	37.50	29.90	33.10	NA	NA
	run 2	31.58	34.42	33.97	32.79	38.84	38.11	32.76	NA	NA
		31.81	34.55	34.24	32.37	37.45	37.76	33.03	NA	NA
		31.71	34.88	34.15	32.69	38.17	36.52	32.86	NA	NA
		31.95	34.76	34.34	29.95	37.27	30.30	33.10	NA	NA
Day 3	run 1	31.66	34.87	33.83	32.40	37.48	37.37	32.23	NA	NA
		31.70	34.73	34.22	32.54	37.68	37.72	32.22	NA	NA
		31.88	34.62	34.11	32.38	38.44	36.87	32.10	NA	NA
		31.77	34.37	34.00	32.72	37.31	38.23	32.51	NA	NA
		31.70	34.57	34.29	32.38	38.98	37.13	32.07	NA	NA

	run 2	31.87	34.56	34.22	32.31	39.44	38.42	32.88	NA	NA
		31.68	34.50	34.15	32.21	38.83	38.17	32.34	NA	NA
		31.79	33.99	34.20	32.38	37.43	38.19	32.64	NA	NA
Day 4	run 1	31.52	34.28	33.97	32.75	38.30	38.55	32.86	NA	NA
		31.96	34.73	33.99	32.45	38.95	36.73	32.79	NA	NA
		31.84	34.72	34.20	32.52	37.76	37.20	32.59	NA	NA
		31.89	34.66	34.27	32.86	37.53	37.71	33.06	NA	NA
	run 2	32.74	33.96	34.69	33.27	37.08	38.30	33.45	NA	NA
		32.99	33.89	34.72	33.49	37.71	37.43	33.75	NA	NA
		33.29	34.62	34.69	33.77	38.12	37.13	34.00	NA	NA
		32.94	33.90	34.53	33.69	36.88	37.50	33.99	NA	NA
Day 5	run 1	31.74	34.20	34.78	32.61	37.53	37.95	32.78	NA	NA
		32.26	34.35	34.97	32.92	37.86	37.55	32.46	NA	NA
		31.89	34.70	34.95	32.85	38.43	37.90	32.64	NA	NA
		32.29	34.19	34.99	33.23	38.23	38.45	32.72	NA	NA
	run 2	31.51	33.77	34.40	32.21	37.32	37.42	32.66	NA	NA
		31.76	34.42	34.51	32.26	37.15	37.48	32.63	NA	NA
		31.51	34.04	34.74	32.89	38.29	37.44	32.89	NA	NA
		32.02	34.32	34.78	32.89	37.65	37.86	32.95	NA	NA

4.2.6 Data analysis

Data analysis was conducted using Analyse-it for Microsoft Excel 5.50.

The generated CVs of repeatability and reproducibility shown in table 33.

Table 33: Summary of the assay repeatability and reproducibility on chemagic™ 360.

Target	N	Repeatability			Reproducibility		
		CV	95% CI		CV	95% CI	
N_Low	40	1.8%	1.4%	to 2.4%	2.0%	1.7%	to 3.4%
N_Moderate	40	0.8%	0.7%	to 1.1%	1.2%	1.0%	to 2.2%
ORF1ab_Low	38*	1.5%	1.2%	to 2.1%	1.5%	1.3%	to 2.2%
ORF1ab_Moderate	40	0.6%	0.5%	to 0.8%	1.4%	1.0%	to 3.5%

*Two of the low positive sample tested on the 8th April 2020 showed significantly deviated ORF1ab amplification as showed in Figure 2 (red symbol), they are clearly outliers comparing to the majority of low positive sample through statistical analysis (Ct ~30 vs mean Ct 37.6 of the majority). It was found that these two samples were from the same tube prepared in the beginning of the day and used in the two runs on that day. This gives a speculation that the tube of sample may be contaminated with ORF1ab target probably from the other activities in the lab on that day. Given the significant deviation of these two samples, and highly possibly the samples tested were not as they designed to be, these two samples were excluded from the calculation of assay repeatability and reproducibility.

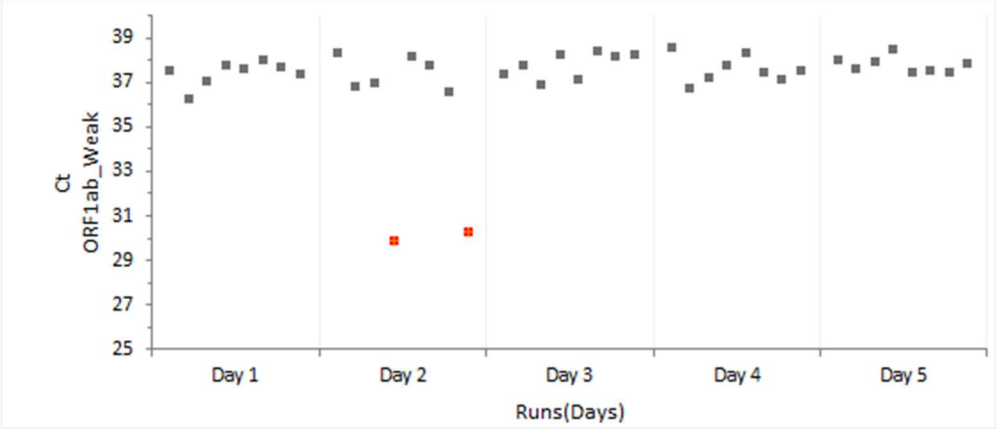

Figure 2: Ct of ORF1ab low positive sample.

From the results shown in table 33, the assay and instruments demonstrated good repeatability and reproducibility with CV of Ct values less than 5%.

5. Validation of the assay procedure

The assay procedure described in the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay kit insert was validated internally and externally.

The assay procedure involving Pre-NAT II extraction system was validated using contrived clinical oropharyngeal swabs and nasopharyngeal swabs. The study described in section 2.3.3 of this document involved the whole procedure, including sample collection (both oropharyngeal swab and nasopharyngeal swab), handling, nucleic acid extraction, PCR amplification and results analysis. The study also tested oropharyngeal swab and nasopharyngeal swab LoD samples, which validated that the assay procedure is well designed and meets the product requirement and intended use.

The assay procedure involving chemagic™ 360 extraction system was also validated at a third-party's lab using clinical specimens. The study described in the Clinical Validation Report (ID: DHF-50-2009741) involved the whole procedure, including oropharyngeal swab, nasopharyngeal swab and saliva specimenscollection, handling, pooling, nucleic acid extraction, PCR amplification and results analysis. The study results well validated that the assay procedure meets the product requirement and intended use.

References

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