Validation Report for SARS-CoV-2 Dry Swab Supplemental

CA-VALRPT-LAB-021
Version number 1.0

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Approval	Lab Director	9/10/2021	1.0	Adam Rosendorff	
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1.0	Approved and Current	Initial version	9/10/2021	9/10/2021	Indefinite

Approved: 9/10/2021 by Adam Rosendorff

1. VALIDATION TYPE: SARS-CoV-2 Dry Swab Validation Supplement

2. PURPOSE

2.1 The purpose of this study is to verify Analytical Specificity and Cross Reactivity of the molecular diagnostic assay that utilizes real-time polymerase chain reaction (PCR) in order to determine the presences or absences of SARS-CoV-2 RNA in dry human nasal swabs at CDPH Branch Laboratory, Valencia, CA. These data will supplement the 28May2021 validation report SARS-CoV-2 Dry Swab (CA-VALRPT-LAB-015). Studies will be carried out according to CA-VALPLN-LAB-015.

3. SCOPE

3.1 This procedure applies to individuals performing the SARS-CoV-2 Assay to be performed at CDPH Branch Laboratory, Valencia, CA.

3.2 This validation report is for SARS-CoV-2 RT-qPCR Reagent kit (Cat # 2019-nCoV-PCR-AUS).

3.2.1 These tests are completed using contrived samples created by spiking Heat Inactivated SARS-CoV-2 virus into MTM then fully saturating the swab within the MTM heat inactivated virus solution. Pooled positive patient samples were also used in this supplemental validation. Certificate of Analysis must be obtained to determine stated copies/mL per lot, when using Heat Inactivated SARS-CoV-2 virus from ATCC, (Cat # VR-1986HK).

4. DEFINITION

4.1 IC: An exogenous internal control comprised of TE buffer and bacteriophage MS2, provided in the kit (Cat #: 2019-nCoV-PCR-AUS, tube: nCoV Positive Control)

4.2 Ct or Cq: Cycle number at which PCR response starts to become exponential

4.3 AN: Anterior Nasal

4.4 NSP: Nasopharyngeal

4.5 *ORF1ab* and *N* genes: 2 different coding areas in the SARS-CoV2 genome interrogated in this validation using RT-PCR

4.6 *RPP30 (*encodes RNAseP)- a human gene target included in some assays to verify adequacy of specimen collection, human origin of specimen, and as a second extraction control. RPP30 is labelled as Cy5 in this molecular diagnostic assay.

4.7 FAM, ROX, HEX/VIC: Abbreviations for 3 fluorescent dyes used in reporter oligonucleotides in RT-PCR assay. FAM probe reports on SARS-Cov2 *N* gene, ROX on *ORF1ab* gene, and HEX reports on the IC (bacteriophage MS2)

4.8 RT-PCR: real-time PCR

4.9 SARS-CoV-2: Severe acute respiratory syndrome-related coronavirus of the genus *Betacoronavirus*, strain 2

4.10 Molecular Transport Medium (MTM): synthetic media in which human nasal swabs are transported to testing labs. In this validation only MTM will be utilized: PrimeStore MTM (Cat# MT0501-1 & MT0501-2)

5. ROLES AND RESPONSIBILITIES

5.1 Responsibilities

5.1.1 It is the responsibility of the laboratory and delegates to establish any methods required to perform this assay. This must be properly documented in a validation plan, report, standard operating procedures, and any other required documentation.

5.1.2 All personnel that will perform this assay must understand and comply with established procedures.

5.1.3 CDPH Branch Laboratory management will provide leadership and support to ensure the development of a properly validated assay in a safe working environment.

5.1.4 The Laboratory Director will approve the validation plan and report. This approval will authorize the test for clinical use.

5.2 Key Personnel

5.2.1 Clinical laboratory staff at CDPH Branch Laboratory Valencia, CA

5.2.2 Adam Rosendorff, MD, Laboratory Director

6. BACKGROUND / CLINICAL SIGNIFICANCE

6.1 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously called 2019-nCoV) is a beta-coronavirus, a novel coronavirus belonging to a family of coronaviruses.

6.2 The PerkinElmer® New Coronavirus Nucleic Acid Detection Kit is a real-time RT-PCR *in vitro* diagnostic test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 virus in human specimens collected from individuals suspected of SARS-CoV-2 by their healthcare provider.

6.3 Results are for the identification of SARS-CoV-2 RNA, which is generally detectable in human respiratory specimens during the acute phase of infection While positive results indicate the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.[11.1]

6.4 Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

7. PROTOCOLS AND EQUIPMENT TO BE USED IN THIS VALIDATION

7.1 Controlled Document Titles and Reference ID that will be used in this validation:

7.1.1 Accessioning for SARS-CoV-2 Samples (CA-ACC-SOP-001)

7.1.2 Dry Swab Hydration and Inactivation (CA-EXT-SOP-008)

7.1.3 Sample Transfer Using the Janus G3 (CA-EXT-SOP-003)

7.1.4 Viral RNA_DNA Extraction Using the chemagic™ 360-D (CA-EXT-SOP-004)

7.1.5 SARS-CoV-2 RT-PCR Set-up with RPP30 for Dry Swabs (CA-PCR-SOP-004)

7.1.6 SARS-CoV-2 RT-PCR Using the Analytik Jena (CA-PCR-SOP-002)

7.1.7 SARS-CoV-2 Assay Data Extraction (CA-RPT-SOP-001)

7.1.8 Analysis and Reporting of SARS-CoV-2 Assay (CA-RPT-SOP-002)

7.2 Table A: Equipment and Systems with ID

Table A. Equipment and System IDs					
Equipment	**Manufacturer**	**Model**	**Part/Cat#**	**Purpose**	**Environmental Requirements Temperature/ Humidity**
chemagic™	PerkinElmer®	360 or 360-D	20240056	DNA/RNA Extraction	Temp: 18 - 35 °C Humidity: < 80 %
Biosafety Hood	Any	Any	Any	Prepare master mix	Dependent on model
JANUS G3	PerkinElmer®	Any	Any	Sample Reformatting and PCR workstation	Temp: 15 – 35 °C Humidity: 60 – 80 %
Microcentrifuge	Any	Any	Any	Collect samples at bottom of well	Dependent on model
Vortex mixer	Any	Any	Any	Mix reagents and/or samples	Dependent on model
Plate Centrifuge	Any	Any	Any	Collect samples at bottom of well	Dependent on model
Pipettes (single and multi-channel) p10, p200, p1000	Any	Any	Any	Pipette reagent and samples	Dependent on model
qPCR Real-Time System	Analytica Jena	AJ384	844-00569-4	RT-PCR	Temp: 15 - 31 °C Humidity: 10 - 85 %

7.3 Table B: Reagents, Supplies, Materials, etc.

Table B. Reagents					
Reagent	**Vendor**	**Manufacturer**	**Part/ Cat#**	**Purpose**	**Storage Conditions**
chemagic™ Viral 300 RNA/DNA Kit H96 component Magnetic Beads B 1 bottle, 250 mL Prep: Ready to use Exp: Unopened, according to the labeled Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Bind RNA/DNA	2 - 25 °C
chemagic™ Viral 300 RNA/DNA Kit H96 component Lysis Buffer 1 1 bottle, 500 mL Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Lyses cells or other DNA source to get the RNA/DNA into solution	2 - 25 °C
chemagic™ Viral 300 RNA/DNA Kit H96 component Binding Buffer 2 1 bottle, 2.5 L Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Create condition to allow the RNA/DNA to bind to the Magnetic beads	2 - 25 °C
chemagic™ Viral 300 RNA/DNA Kit H96 component Wash Buffer 3 1 bottle, 1 L Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Remove non-DNA/RNA contaminants during washing step	2 - 25 °C

Table B. Reagents					
Reagent	**Vendor**	**Manufacturer**	**Part/ Cat#**	**Purpose**	**Storage Conditions**
chemagic™ Viral300 RNA/DNA Kit H96 component Wash Buffer 4 1 bottle, 1 L Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Remove non-DNA/RNA contaminants during washing step	2 - 25 °C
chemagic™ Viral 300 RNA/DNA Kit H96 component Wash Buffer 5 1 bottle, 1 L Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Remove last traces of non-DNA/RNA contaminates	2 - 25 °C
chemagic™ Viral 300 RNA/DNA Kit H96 component Elution Buffer 6 1 bottle, 250 mL TRIS-HCl pH 7.8 - 8.4 Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Elute DNA/RNA	2 - 25 °C
chemagic™ Viral 300 RNA/DNA Kit H96 component Proteinase K 1 * 11 mL bottle Prep: Reconstituted with 11 mL molecular grade water Exp: Unopened, according to the label Opened: 14 days, 2 - 8 °C	PerkinElmer®	AUS	CMG-1033-S	Added to enhance the efficiency of the lysis step	2 - 8 °C
Molecular Biology Grade Water	Any	Any	Any	Used to reconstitute Proteinase K, NTC control	15 - 25 °C

Table B. Reagents					
Reagent	**Vendor**	**Manufacturer**	**Part/ Cat#**	**Purpose**	**Storage Conditions**
chemagic™ Viral 300 RNA/DNA Kit H96 component Poly (A) RNA 10 bottles Prep: Reconstituted with 440 µL of Poly (A) RNA Buffer Mix Exp: Unopened, according to label Opened: 4 weeks, 2 - 8 °C	PerkinElmer®	AUS	CMG-1033-S	Added to enhance the efficiency of the lysis step	2 - 8 °C
chemagic™ Viral 300 RNA/DNA Kit H96 component Poly (A) RNA Buffer Mix 10 bottle, 2 mL Exp: Unopened, according to label Opened: 30 days.	PerkinElmer®	AUS	CMG-1033-S	Added to Poly (A) RNA to activate.	15 - 25 °C
New Coronavirus Nucleic Acid Detection Kit	PerkinElmer®	PerkinElmer®	2019-nCoV-PCR-AUS	RT-PCR kit	-25 to -15 °C
PrimeStore Molecular Transport Media	PrimeStore	PrimeStore	MT0501-1 & MT0501-2	Molecular Transport Media	4 - 30°C
3M Lysis Buffer	PerkinElmer	PerkinElmer		Inactivate Virus	20 - 30°C
Reagent HC	PerkinElmer	PerkinElmer		Detection of RNaseP gene for home collection	-20 to 4°C
Swabs used for “dry swab” collection	SteriPack	SteriPack	60564RevB	Sample collection device	15-25°C
2019 Heat inactivated Novel Coronavirus; SARS-CoV-2 Virus; Strain USA-WA1/2020	ATCC	ATCC	VR-1986HK	Reference material used for contrived positive samples	-80 to -70°C

6.4 Table C: Training Requirements

Table C. Materials and Supplies					
Material and Supplies	**Vendor**	**Manufacturer**	**Part/Cat #**	**Purpose**	**Storage Conditions**
2 mL Deep Well Plate	PerkinElmer®	PerkinElmer®	CMG-555	Load sample/lysis and Elution Buffer B onto the chemagic™ 360	15 - 25 °C
96 Rod Head Disposable Tips	PerkinElmer®	PerkinElmer®	49017-0006	Protect 96 Rod Magnetic Head (on chemagic™ 360) from contamination	15 - 25 °C
Low-well Plate	PerkinElmer®	PerkinElmer®	CMG-555-1	Load Magnetic Beads B onto the chemagic™ 360	15 - 25 °C
Heat Sealing Foil	Approved vendor	Approved vendor	Approved vendor	Seal plates	N/A
Reagent trough	Any	Any	Any	Preserve reagent stock bottle and allow for multi-channel pipetting	N/A
Tips (10 µL, 200 µL, 1000 µL) compatible with pipettes	Any	Any	Any	Pipette reagent and samples	Dependent on brand
384 Plates for RT-PCR	Approved vendor	Approved vendor	Approved vendor	Hold samples and master mix	N/A

Table C. Materials and Supplies					
Material and Supplies	**Vendor**	**Manufacturer**	**Part/Cat #**	**Purpose**	**Storage Conditions**
qPCR Seal	Approved vendor	Approved vendor	Approved vendor	Seal 384 well plate	N/A; Expiration Date is written on the box
1.7 mL to 50 mL tube	Any	Any	Any	For master mix preparation	Dependent on brand

6.5 Training Requirements

6.5.1 Personnel that performing this assay will require proper training. This training will include complete review and understanding of all associated documentation and hands-on laboratory training in the performance of this assay.

7. SPECIMENS REQUIRED

7.1 Negative control: nCoV negative control from 2019-nCoV-PCR-AUS Kit, run in every extraction plate

7.2 Positive control: nCoV positive control from 2019-nCoV-PCR-AUS Kit containing 1000 copies/mL of SARS-CoV-2 RNA fragments capsulated in bacteriophage, run in every extraction plate

7.3 Pooled positive samples: A sample created by combining samples previously tested and resulted as positive to obtain sufficient volume specificity and interfering substances studies.

7.4 Contrived samples: Samples created by spiking Heat Inactivated SARS-CoV-2 virus into MTM (Certificate of Analysis must be obtained to determine stated copies/mL per lot) OR pooled negative samples as well as Heat Inactivated SARS-CoV-2 virus from ATCC (Cat # VR-1986HK).

8. VALIDATION PROTOCOL AND STUDY METHODS

8.1 The SARS-CoV-2 assay was performed according to the protocols listed in Section 7.

8.2 Study Methods

8.2.1 Interfering Substances

8.2.1.1 Interfering substances were spiked into MTM that contained human cells, with a final concentration per substance indicated in table I.

8.2.1.2 Heat Killed SARS-CoV-2 (ATCC VR-1986HK 2019; Novel Coronavirus, strain; 2019-nCoV/USA-WA1/2020.) virus at 300X the LDT LoD (~36,000 viral copies/mL) was added to the MTM media where for the positive tests.

8.2.1.3 Spun polyester dry swabs were submerged into the MTM media mixture and dried for 20 minutes. The swabs were then hydrated with an addition of 1mL of 3M Lysis buffer, and isolation of RNA was performed as per laboratory protocols.

Table I: Interfering Substances

Sample Number	Substances	Stock Concentration	Evaluation Concentration	Units
Int-1	Afrin – nasal spray	100	15	% (v/v)
Int-2 (0.1%)	Human blood	20	0.1	% (v/v)
Int-3	Chloraseptic	700	3.5	mg/mL
Int-4	Flonase	100	5	% (v/v)
Int-5	Halls Relief Cherry Flavor	800	8	mg/mL
Int-6	Nasocort Allergy 24 hour	100	5	% (v/v)
Int-7	Neo-Synephrine	100	5	% (v/v)
Int-8	Saline nasal spray	100	15	% (v/v)
Int-9	Zicam Cold Remedy	100	5	% (v/v)
Int-10	Purified mucin protein	2.5	0.06	mg/mL
Int-11	Mouthwash	100	5	% (v/v)
Int-13	Rhinocort	100	1	% (v/v)
Int-14	Oseltamivir	175	2.5	mg/mL
Int-15	Tobramycin	400	4	µg/mL
Int-16	Zanamivir	400	3.3	mg/mL
Int-17	Mupirocin	103	10	mg/mL
Int-18	Peramivir	10,000	45	ng/mL

8.2.2 Cross Reactivity

8.2.2.1 The pathogens listed in Table II were spiked into MTM that contained human cells, with a final concentration per substance indicated below. Positive samples were contrived from pooling known clinical positive samples with a Ct value less than 25. These were then spiked with the cross-reactivity pathogens to create the sample.

8.2.2.2 Spun polyester dry swabs were submerged into the MTM media mixture and dried for 20 minutes. The swabs were then hydrated with an

addition of 1mL of 3M Lysis buffer, and isolation of RNA was performed as per laboratory protocols.

Table II: Cross Reactivity Pathogens

	Name/Description	Panel code	Concentration
1	Human coronavirus 229E	Ex-1	1.6 × 10^6 TCID50 per mL
2	Human coronavirus OC43	Ex-2	104 ng/100 µL = 1.04 ng/µL
3	Human coronavirus HKU1*	Ex-3	5.4 x 10^5 genome copies/µL 103 µL/vial
4	Human coronavirus NL63	Ex-4	1.6 × 10^5 TCID50 per mL
5	SARS-coronavirus	Ex-5	1.4 x 10^6 gce/uL
6	MERS-coronavirus	Ex-6	8.9 × 10^5 TCID50 per mL
7	Adenovirus (e.g. C1 Ad. 71)	Ex-7	TCID50: 2.5 × 10^7 per mL
8	Human Metapneumovirus (hMPV)	Ex-8	25.8 ng per 100 µL
9	Human parainfluenza virus 1	Ex-9	0.41 ng/µL 102 ul in TE
10	Human parainfluenza virus 2	Ex-10	TCID50: 1 1.0 X 10^8 per mL
11	Human parainfluenza virus 3	Ex-11	0.201 ng/µL 10 ul in TE
12	Parainfluenza virus 4b	Ex-12	5.0 × 10^6TCID50 per mL
13	Enterovirus (e.g. EV68)	Ex-13	117 ng per 100 µL
14	Rhinovirus	Ex-14	TCID50: 1 × 10^6 per mL
15	Haemophilus influenzae	Ex-16	8.4 x 10^5 genome copies/µL
16	Legionella pneumophila	Ex-17	6 µg/vial
17	Mycobacterium tuberculosis	Ex-18	1.6 mg/mL
18	Streptococcus pneumoniae	Ex-19	10 µg/vial
19	Streptococcus pyogenes	Ex-20	6 µg/vial
20	Bordetella pertussis	Ex-21	6 µg/vial
21	Mycoplasma pneumoniae	Ex-22	3.1 ng/µ 217 ng/vial
22	Pneumocystis jirovecii (PJP)	Ex-23	NA
23	Candida albicans	Ex-24	5.5 µg in 79 µL per vial (70 µg/mL)
24	Pseudomonas aeruginosa	Ex-25	7 µg/vial
25	Staphylococcus epidermis	Ex-26	6 µg/vial
26	Streptococcus salivarius	Ex-27	0.9 µg in 35 µL per vial (26 µg/mL)
27	Herpes Simplex virus	Ex-28	500000 c/ml
28	Varicella-zoster virus	Ex-29	100000 c/ml
29	Epstein Barr virus	Ex-30	94 µg/mL 11 µg/vial
30	Measles Virus	Ex-31	111 ng per 100 µL

31	Mumps virus	Ex-32	Conditions: Rhesus monkey kidney TCID50:11 X 10^5 – 1.6 X 10^6 per mL Conditions: Human amnion TCID50: 5 X 10^4 per mL Conditions: 8-day chicken embryo (allantoic) TCID50: 3.2 X 10^7 per mL
32	CMV	Ex-33	$5E^6$ IU/mL
33	Corynebacterium diphtheriae	Ex-34	5 µg/vial
34	E.coli	Ex-35	5.9 µg in 26 µL per vial (227 µg/mL)
35	Lactobacillus plantarum	Ex-36	1.58 x 10^9 cfu/vial
36	Moraxella catarrhalis	Ex-37	9 µg/vial
37	Staphylococcus aureus	Ex-38	5.8 µg in 52 µL per vial (112 µg/mL)
38	Neisseria elongata	Ex-39	>10^4 cfu/vial
39	Neisseria meningitidis	Ex-40	1.0 µg in 31 µL per vial (31 µg/mL)
40	Chlamydophila pneumoniae	Ex-41	9.1 x 10^7 IFU/mL

*Samples were not available for testing

8.2.3 Samples were prepared for extraction by adding the following to a deep-well plate: 34µL master mix consisting of Proteinase K (10 0181L), poly (A) RNA (4µL) and Internal Control (20µL), followed by 300µL of sample, and lastly 300µL of Lysis Buffer.

8.2.4 RNA from all samples was extracted using the chemagic™ 360 running the "chemagic viral300 360 H96 drying prefilling VD200617.che" program and using the chemagic™ Viral 300 RNA/DNA Kit H96 kit.

8.2.5 RT-PCR Setup

8.2.5.1 SARS-CoV-2 RT-qPCR reagent Kit: 10µL of each eluate was mixed with 5µL of a real time PCR master mix prepared using nCoV Reagent A, nCoV Reagent B, nCoV Reagent HC and nCoV Enzyme Mix.

8.2.5.2 The reactions were then subjected to RT-PCR in an AnalytikJena qTower^{3}84G thermocycler.

8.2.6 Quality Control and Data Analysis

8.2.6.1 Quality control and data analysis were performed in accordance with kit instructions for the RT-PCR kit (Cat#: 2019-nCoV-PCR-AUS).

8.3 Protocol variation

8.3.1 Barcodes will be created specifically for this study.

8.3.1.1 Barcodes will not be tracked in LIMC.

8.3.1.2 No data will be transferred outside of the laboratory for reporting.

8.3.2 All protocol deviations with will be described in relevant sections of the plan.

8.3.3 Studies described in this plan may use automation or manual set-up options described the laboratory protocols.

9. VALIDATION

Section	Study	Subsection	Sample type	PCR Targets
9.1	Analytical Sensitivity (Inclusivity)	N/A	Bioinformatic data	N/A
9.2	Analytical Specificity (Cross-Reactivity)	9.2.1	Bioinformatic data	N/A
		9.2.2	Virus / pathogen panel in dry swab	Viral + EC + *RPP30*
9.3	Analytical Specificity (Interfering substances)	N/A	Various substances in dry swab	Viral + EC + *RPP30*

*Extraction control

9.1 Analytic Sensitivity (42CFR493.1253 (2) iii)

9.1.1 *In silico* Analysis (performed at PerkinElmer with Right to Reference – see Appendix A)

- SARS-CoV-2 specific primers and probes used in this assay were compared to the NCBI and GISAID databases
- Inclusion criteria for sequences used for comparison were:
  - Complete sequences: Genomes with >29000bp
  - High coverage inclusivity: Entries with <1% N base pair and <0.05% unique nucleic acid substitutions (not seen in other sequences in the database) and no indels unless verified by the submitter
  - High coverage: Entries with >5% N base pairs were excluded

9.1.2 Primers

- Criteria for primers impacted by sequence variant are:
    - Primer sequence has at least one mismatch to the genome in the last five base pairs from the primer's 3' end
    - Primer sequence has multiple mismatches to the genome with at least one mismatch landing in the 3' half of the primer
    - Primer sequence has no match to the genome

9.1.3 Probes

- Criteria for probe sequences predicted to be impacted are as follows:
    - Probe sequence has greater than two mismatches to the genome
    - Probe sequence has no match to the genome

Table D1: Summary of individual oligo nucleic acid substitutions

Target	*N* gene						*ORF1ab* gene					
Database	GISAID			NCBI			GISAID			NCBI		
Oligo	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe
# Sequences	1467673	1467673	1467673	354403	354403	354403	1467673	1467673	1467673	354403	354403	354403
Sequences with mismatches	1028210	798077	4319	227220	170272	1225	4368	2622	5372	568	1315	1147
1 mismatch	120526	793952	4160	20641	169519	1213	4333	2601	5024	538	1297	1114
2 mismatches	1843	3720	82	133	706	1	18	6	63	2	4	10
3 or more mismatches	905841	405	77	206446	47	11	17	15	285	28	14	23
Other mismatches	2061	978	978	852	829	829	59	268	372	29	1150	1227
Sequences predicted to impact	2766	2100	981	996	977	829	62	268	373	29	1150	1227

9.1.4 For all criteria regarding impact, any mismatches caused by Ns or other ambiguous nucleotide nomenclature were ignored.

9.1.5 The number of mismatched sequences are show in Table D1 and the type of nucleic acid changes are shown in Table D2.

Table D2: Summary of types of nucleic acid changes among impacted sequences		
Target	***N* gene**	***ORF1ab* gene**

Database		GISAID		NCBI		GISAID		NCBI	
Total Failures		3965	*100%*	1424	*100%*	528	*100%*	1260	*100%*
For	At Least 1 mm in Last 3bps	705	*17.78%*	144	*10.11%*	3	*0.57%*	0	*0.0%*
	No Match to Genome	2061	*51.98%*	852	*59.83%*	59	*11.17%*	29	*2.3%*
Rev	At Least 1 mm in Last 3bps	693	*17.48%*	144	*10.11%*	0	*0.0%*	0	*0.0%*
	No Match to Genome	1407	*35.49%*	833	*58.5%*	268	*50.76%*	1150	*91.27%*
Probe	At Least 3mms to Genome	3	*0.08%*	0	*0.0%*	1	*0.19%*	0	*0.0%*
	No Match to Genome	978	*24.67%*	829	*58.22%*	372	*70.45%*	1227	*97.38%*

For: Forward primer, Rev: Reverse primer, mm: mismatch, mms: mismatches.

Note: that some impacted sequences can fail multiple criteria thus counted in each criterion failed.

9.1.6 Risk for assay failure due to mismatch of primer or probe sequences was assessed.

- Impact the 5' substitutions in the *N* forward primer of the assay is expected to have on overall sensitivity of the assay
- Tm analysis of both the changed and unchanged N primer sequence was conducted and compared against the PCR annealing temperature parameters used in the assay workflow.
- The result of this analysis indicated that the likelihood of the identified substitutions at the 5' end of N forward primer sequence impacting the sensitivity of *N* target detection is low.
- The criteria used to determine a positive or negative result states that if either the *N* or *ORF1ab* target gene is detected, then the result is reported as SARS-CoV-2 detected. These predictions are summarized in Tables D3 and D4.

Table D3: Prediction of impacts of detection.

Target	*N* gene				*ORF1ab* gene			
Database	GISAID		NCBI		GISAID		NCBI	
Number of Sequences	1467673	*100%*	354403	*100%*	1467673	*100%*	354403	*100%*

Sequences with Mismatches	1089040	*74.2%*	241004	*68.0%*	12730	*0.87%*	4263	*1.2%*
Predicted No Detection	3965	*0.27%*	1424	*0.4%*	528	*0.04%*	1260	*0.36%*

- With the consideration of the decision algorithm of the kit: if either *N* or *ORF1ab* gene is detected, the SARS-CoV-2 is detected. The impact on final detection is summarized in Table D4.

Table D4: Summary of nucleotide change impact on final detection

Database	GISAID		NCBI	
Number of Sequences	1467673	*100%*	354403	*100%*
Predicted Both Genes Detected	1463222	*99.7%*	351942	*99.31%*
Predicted One Gene Detected	4409	*0.3%*	2238	*0.63%*
Predicted Neither Gene Detected	5*	*0.0%*	0	*0.0%*
Unable to Predict	37	*0.0%*	223	*0.06%*

The "Unable to Predict" category is a class of sequences that fail criteria because of sequence quality. Although, there are automatic attempts to filter poor quality sequences from NCBI and GISAID, some sequences make it through to analysis. An outcome is unable to be predicted for these sequences because (1) the sequences contain an abundance ambiguous bases like "N" or other IUPAC nomenclature around the oligo binding region, or because (2) the genes are not arranged in the typical SARS-CoV-2 order. There are 37 sequences from GISAID and 223 sequences from NCBI fit into this category. Of the 37 GISAID sequences, 36 are because of reason (1) and 1 because of reason (2). Of the 223 NCBI sequences, all are because of reason (1).

*These failures are of sequences where a bat species is the host, meaning these are non-human hosted SARS-CoV-2 samples.

In summary, based on in silico analysis, the performance of the kit is not impacted by the nucleotide changes.

9.1.7 Analysis of *N* forward primer 5' end nucleotide substitutions (5' GGG -> AAC) characteristic of the Alpha or B.1.1.7 variant are the following.

- This nucleotide change on its own should not cause assay failure as it occurs on the 5' end and it has less impact on Tm which will impact primer binding efficiency.
- The Tm with the GGG -> AAC nucleotide substitution is still 5 degree higher than the annealing temperature, which is at 55 °C. Furthermore, the detection on SARS-CoV-2 is based on *N* or *ORF1ab* gene: as long as one of the genes (*N, ORF1ab*) is amplified/detected, the target virus is detected.
- For the sequences with nucleotide changes at 5' end of *N* forward primer, the corresponding *ORF1ab* gene can be efficiently detected by the kit for more than 99.9% cases. **Therefore, the identified sequence variances have less impact on inclusivity for SARS-CoV-2.**

9.2 Analytical Specificity (42CFR493.1253 (2) iv)

9.2.1 *In silico* analysis (performed at PerkinElmer with Right to Reference – see Appendix A)

- SARS-CoV-2 specific primers and probes used in this assay were compared to the sequences of pathogens in Table D using BLASTn with default settings:
  - The match and mismatch scores are 1 and -3, respectively
  - The penalty to create and extend a gap in an alignment is 5 and 2 respectively
  - parameters automatically adjust for short input sequences and the expect threshold is 1000.
- Homology was evaluated based on % homology and orientation of primers and probes (table D).
- **Based on this *in silico* analysis, the primers and probes used in this assay are specific to SARS-CoV-2.**

Table D. Summary of % Homology of Primer and Probes

Pathogen	Strain	GenBank Acc#	% Homology To *N* FP	% Homology to *N* RP	% Homology to *N* Probe	% Homology to *ORFlab* FP	% Homology to *ORFlab* RP	% Homology to *ORFlab* Probe
Staphylococcus epidermidis	ASM609437v1	NZ_CP035288.1	54.55	54.55	55	61.9	63.16	46.43
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_01726 4775.1	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

9.2.2 Wet testing evaluation was performed with the cross-reactivity evaluation panel listed in Table E for dry swab samples

9.2.2.1 Samples required: Specimens were obtained from the Fand then spiked into MTM containing human cells.

Table E: Cross Reactivity Panel for Testing

Name/Description	Concentration	Vendor	Part No.	Lot No.
Human coronavirus 229E	1.6×10^6 $TCID_{50}$ per mL	BEI	NR-52726	70035627
Human coronavirus OC43	104 ng/100 µL = 1.04 ng/µL	ATCC	VR-1558D	70033324
Human coronavirus HKU1*	5.4×10^5 genome copies/µL	ATCC	VR-3262SD	70034816
Human coronavirus NL63	1.6×10^5 $TCID_{50}$ per mL	BEI	NR-470	70033870
SARS-coronavirus	NA	ZeptoMetrix	NATSARS-ST	324003
MERS-coronavirus	8.9×10^5 $TCID_{50}$ per mL	BEI	NR-50549	70003285
Adenovirus (e.g. C1 Ad. 71)	$TCID_{50}$: 2.5×10^7 per mL	BEI	NR-51436	NA
Human Metapneumovirus (hMPV)	25.8 ng per 100 µL	BEI	NR-49122	359
Human parainfluenza virus 1	0.41 ng/µL 102 ul in TE	ATCC	VR-94D	70021695
Human parainfluenza virus 2	$TCID_{50}$: 1.0 X 10^8 per mL	BEI	NR-3229	V-322-001-020
Human parainfluenza virus 3	0.201 ng/µL 10 ul in TE	ATCC	VR-93D	70035215
Parainfluenza virus 4b	5.0×10^6 $TCID_{50}$ per mL	BEI	NR-3238	V-324-011-010
Enterovirus (e.g. EV68)	117 ng per 100 µL	BEI	NR-4960	580661372
Rhinovirus	$TCID_{50}$: 1×10^6 per mL	BEI	NR-51453	NA
Haemophilus influenzae	8.4×10^5 genome copies/µL	ATCC	51907DQ	70014401
Legionella pneumophila	6 µg/vial	ATCC	33152D-5	70021661
Mycobacterium tuberculosis	1.6 mg/mL	BEI	NR-14867	60013987
Streptococcus pneumoniae	10 µg/vial	ATCC	6308D-5	57968184
Streptococcus pyogenes	6 µg/vial	ATCC	BAA-572D-5	57907324
Bordetella pertussis	6 µg/vial	ATCC	9797D-5	70025842
Mycoplasma pneumoniae	3.1 ng/µL	ATCC	15531D	58390290
Pneumocystis jirovecii (PJP)	NA	ZeptoMetrix	NATPJI-ERC	324266
Candida albicans	70 µg/mL	BEI	NR-50361	64362135
Pseudomonas aeruginosa	7 µg/vial	ATCC	9027D-5	70013361
Staphylococcus epidermis	6 µg/vial	ATCC	12228D-5	70006950
Streptococcus salivarius	26 µg/mL	BEI	HM-121D	62380438

Herpes Simplex virus	500000 c/ml	Thermo/AcroMetrix	954501	184432
Varicella-zoster virus	100000 c/ml	Thermo/AcroMetrix	954512	221587
Epstein Barr virus	94 µg/mL	ATCC	CRL-5957D	70030677
Measles Virus	111 ng per 100 µL	BEI	NR-44104	62819330
Mumps virus	Conditions: Rhesus monkey kidney $TCID_{50}$:11 X 10^5 – 1.6 X 10^6 per mL Conditions: Human amnion $TCID_{50}$: 5 X 10^4 per mL Conditions: 8-day chicken embryo (allantoic) $TCID_{50}$: 3.2 X 10^7 per mL	BEI	NR-3846	V-325-001-000
CMV	$5E^6$ IU/mL	WHO	09/162	?
Corynebacterium diphtheriae	5 µg/vial	ATCC	700971D-5	70033040
E.coli	227 µg/mL	BEI	NR-9281	58607113
Lactobacillus plantarum	1.58 x 109 cfu/vial	ATCC	BAA-793	70030525
Moraxella catarrhalis	9 µg/vial	ATCC	25240D-5	70027323
Staphylococcus aureus	112 µg/mL	BEI	NR-10320	58325746
Neisseria elongata	$>10^4$ cfu/vial	ATCC	25295	70032926
Neisseria meningitidis	31 µg/mL	BEI	NR-48806	62778965
Chlamydophila pneumoniae	9.1 x 10^7 IFU/mL	ATCC	VR-2282	70025701

*Samples were not available for testing

9.2.2.2 Study Design

- For negative samples the pathogens listed in Table E were spiked into MTM that contained human cells, with a final concentration per substance indicated below.

Ex-1	Human coronavirus 229E	stock concentration (TCID50/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn ($TCID_{50}$/mL)
		3.87E+00	100	900	1000	**3.87E-01**

Ex-2	Human coronavirus OC43	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		1.04	10	990	1000	**1.04E-02**

Ex-4	Human coronavirus NL63	stock concentration (TCID50/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn ($TCID_{50}$/mL)
		1.60E+05	100	900	1000	**1.60E+04**

Ex-5	SARS-coronavirus	stock concentration (TCID50/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn ($TCID_{50}$/mL)
		N/A	100	900	1000	**N/A**

Ex-6	MERS-coronavirus	stock concentration (TCID50/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn ($TCID_{50}$/mL)
		8.90E+05	100	900	1000	**8.90E+04**

Ex-7	Adenovirus (e.g. C1 Ad. 71)	stock concentration (TCID50/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn ($TCID_{50}$/mL)
		2.50E+07	50	950	1000	**1.25E+06**

Ex-8	Human Metapneumovirus (hMPV)	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		2.58E-01	5	995	1000	**1.29E-03**

Ex-9	HPV 1	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		4.10E-01	5	995	1000	**2.05E-03**

Ex-10	HPV2	stock concentration (TCID50/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn ($TCID_{50}$/mL)
		1.00E+08	50	950	1000	**5.00E+06**

Ex-11	HPV3	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		2.01E-01	1	999	1000	**2.01E-04**

Ex-12	Parainfluenza virus 4b	stock concentration (TCID50/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn ($TCID_{50}$/mL)
		5.00E+06	50	950	1000	**2.50E+05**

Ex-13	Enterovirus (e.g. EV68)	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		7.60E+05	5	995	1000	**3.80E+03**

Ex-14	Rhinovirus	stock concentration (TCID50/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn ($TCID_{50}$/mL)
		2.90E+06	100	900	1000	**2.90E+05**

Ex-16	Haemophilus influenzae	stock concentration (cp/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (cp/mL)
		8.40E+08	5	995	1000	**4.20E+06**

Ex-17	Legionella pneumophila	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		60.00	2	998	1000	**1.20E-01**

Ex-18	Mycobacterium tuberculosis	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		4.30E+05	2	998	1000	**8.60E+02**

Ex-19	Streptococcus pneumoniae	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		100	2	998	1000	**0.20**

Ex-20	Streptococcus pyogenes	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		60	2	998	1000	**0.12**

Ex-21	Bordetella pertussis	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		60	2	998	1000	**0.12**

Ex-22	Mycoplasma pneumoniae	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		3.1	4	997	1000	**0.0109**

Ex-23	Pneumocystis jirovecii (PJP)	stock concentration	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn
		N/A	200	800	1000	**N/A**

Ex-24	Candida albicans	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		70	4	996	1000	**0.28**

Ex-25	Pseudomonas aeruginosa	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		70	2	998	1000	**0.14**

Ex-26	Staphylococcus epidermis	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		60	2	998	1000	**0.12**

Ex-27	Streptococcus salivarius	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		26	2	998	1000	**0.05**

Ex-28	Herpes Simplex virus	stock concentration (c/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (c/mL)
		5.00E+05	250	750	1000	**1.25E+05**

Ex-29	Varicella-zoster virus	stock concentration (c/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (c/mL)
		1.00E+05	10	990	1000	**1.00E+03**

Ex-30	Epstein Barr virus	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		94	2	998	1000	**0.19**

Ex-31	Measles Virus	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		1.110	5	995	1000	**0.0056**

Ex-32	Mumps virus	stock concentration (TCID50/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn ($TCID_{50}$/mL)
		3.20E+07	50	950	1000	**1.60E+06**

Ex-33	CMV	stock concentration (IU/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (IU/mL)
		5.00E+06	10	990	1000	**5.00E+04**

Ex-34	Corynebacterium diphtheriae	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		50	5	995	1000	**0.25**

Ex-35	E.coli	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		227	2	998	1000	**0.45**

Ex-36	Lactobacillus plantarum	stock concentration (cfu/mL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (cfu/mL)
		1.58E+09	2	998	1000	**3.16E+06**

Ex-37	Moraxella catarrhalis	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		90	5	995	1000	**0.45**

Ex-38	Staphylococcus aureus	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		112	2.5	998	1000	**0.28**

Ex-39	Neisseria elongata	stock concentration (TCID50/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn ($CEID_{50}$/uL)
		N/A	5	995	1000	**N/A**

Ex-40	Neisseria meningitidis	stock concentration (ng/uL)	Target vol. (uL)	human cell contained-MTM (uL)	Total rxn vol. (uL)	Target conc. per 300 uL rxn (ng/uL)
		31	2	998	1000	**0.0620**

Ex-41	Chlamydophila pneumoniae	stock concentration (IFU/mL)	Target vol. (µL)	human cell contained-MTM (µL)	Total rxn vol. (µL)	Target conc. per 300 µL rxn (IFU/mL)
		9.10E+07	50	950	1000	**4.55E+06**

- Positive samples were contrived from pooling known clinical positive samples with a Ct value less than 25. These were then separated into aliquots and spiked with the cross-reactivity pathogens to create the sample. The same pathogen list and target concentrations were used for the positive and negative samples.
- Spun polyester dry swabs were submerged into the MTM media mixture, removed and then dried for 20 minutes. The swabs were then hydrated with an addition of 1mL of 3M Lysis buffer, and isolation of RNA was performed as per laboratory protocols.
- The SARS-CoV-2 assay was performed on spiked samples in triplicate.
- PCR was performed with primers for *RPP30.*

9.2.2.3 Interpretation

- Cross-reactivity was defined as amplification by the SARS-CoV-2 assay with a Ct value $\leq$ 42 for either N or Orf1ab gene target or both.

Table F: Results of Cross Reactivity Panel with human cell spiked MTM with and without SARS-CoV-2 Virus			
Sample Code	Cross-reactivty Substance	Negative Sample	Positive Sample
Ex-1	Human coronavirus 229E	0/3	3/3
Ex-2	Human coronavirus OC43	0/2	3/3
Ex-4	Human coronavirus NL63	0/3	3/3
Ex-5	SARS-coronavirus	0/3	3/3
Ex-6	MERS-coronavirus	0/3	3/3
Ex-7	Adenovirus	0/3	3/3
Ex-8	Human Metapneumovirus	0/3	3/3
Ex-9	Human Parainfluenzavirus 1	0/3	3/3
Ex-10	Human Parainfluenzavirus 2	0/3	3/3
Ex-11	Human Parainfluenzavirus 3	0/3	3/3
Ex-12	Parainfluenza virus 4b	0/3	3/3
Ex-13	Enterovirus	0/3	3/3
Ex-14	Rhinovirus	0/3	3/3
Ex-16	Haemophilus influenzae	0/3	3/3
Ex-17	Legionella pneumophila	0/3	3/3
Ex-18	Mycobacterium tuberculosis	0/3	3/3
Ex-19	Streptococcus pneumoniae	0/3	3/3
Ex-20	Streptococcus pyogenes	0/3	3/3
Ex-21	Bordetella pertussis	0/3	3/3
Ex-22	Mycoplasma pneumoniae	0/3	3/3
Ex-23	Pneumocystis jirovecii (PJP)	0/2	3/3
Ex-24	Candida albicans	0/3	3/3
Ex-25	Pseudomonas aeruginosa	0/3	3/3
Ex-26	Staphylococcus epidermis	0/3	3/3
Ex-27	Streptococcus salivarius	0/3	3/3
Ex-28	Herpes Simplex virus	0/3	3/3
Ex-29	Varicella-zoster virus	0/3	3/3
Ex-30	Epstein Barr virus	0/3	3/3
Ex-31	Measles Virus	0/3	3/3
Ex-32	Mumps virus	0/3	3/3
Ex-33	Cytomegalovirus	0/3	3/3
Ex-34	Corynebacterium diphtheriae	0/3	3/3
Ex-35	Escherichia coli	0/3	3/3
Ex-36	Lactobacillus plantarum	0/3	3/3
Ex-37	Moraxella catarrhalis	0/3	3/3
Ex-38	Staphylococcus aureus	0/3	3/3
Ex-39	Neisseria elongata	0/3	3/3
Ex-40	Neisseria meningitidis	0/3	3/3
Ex-41	Chlamydophila pneumoniae	0/3	3/3

Table F shows a summary of the data collected to establish if there is cross reactivity in the SARS-CoV-2 assay when using dry swabs as a collection media. For negative samples, human cells were mixed with MTM and then spiked with each cross-reactivity substance. The negative samples (no SARS-CoV-2 present), were extracted and run in a PCR assay with *RPP30*. For positive samples, positive clinical patient samples under Ct 25 were collected in a pool, aliquoted, then spiked with each cross-reactivity substance. The positive samples (cross-reactive pathogen added), were extracted and run in a PCR assay with *RPP30*. This data set shows that every positive sample in the cross-reactivity study was 100% concordant with the expected results. Only two samples in the negative data set presented as invalid and were removed from analysis.. In EX-2 (Human Coronavirus OC43) the Cy5 channel presented with No Ct in one of the three samples tested. The second sample that was found to be invalid was EX-23 (Pneumocystis Jirovecii). In EX-23 the Cy5 channel also presented with No Ct for one of three samples tested. Since RPP30 is not added at the start of the PCR process, it is possible that there was not enough RNaseP gene found in the elution after the extraction. Both invalid samples did have a valid internal control which, indicates that the extraction process was successful. Though the samples did have valid internal controls, the sample is considered invalid due to the absence of RPP30 and will be removed from analysis. Further testing to confirm that these microbes will not be cross reactive to the assay may be performed but is not necessary as 2 of 2 replicates that were valid performed as expected in each case.

9.3 **Analytical Specificity (42CFR493.1253 (2) iv) (Interfering substances)**

9.3.1 Samples required.

- Interfering substances to be tested are listed in Table G at the concentration indicated.

Table G: Interfering Substances Tested

Substances	Stock Concentration	Evaluation Concentration
Afrin – nasal spray	100	15
Human blood	20	1
Chloraseptic	700	3.5
Flonase	100	5
Halls Relief Cherry Flavor	800	8
Nasocort Allergy 24 hour	100	5
Neo-Synephrine	100	5
Saline nasal spray	100	15

Zicam Cold Remedy	100	5
Purified mucin protein	3.4	0.06
Mouthwash	100	5
Rhinocort	100	1
Oseltamivir	175	2.5
Tobramycin	400	4
Zanamivir	400	3.3
Mupirocin	103	10
Peramivir	10,000	45

- Interfering substances were spiked into MTM that contained human cells, with a final concentration per substance indicated in table below:

Sample Number	Substances	Stock Concentration	Evaluation Concentration	Units	**Volume of Substances (µL)**	**MTM containing Human Cells (µL)**	Total Target Volume (µL)
Int-1	Afrin – nasal spray	100	15	% (v/v)	150.0	850	1,000
Int-2 (0.1%)	Human blood	20	0.1	% (v/v)	5.0	995	1,000
Int-3	Chloraseptic	700	3.5	mg/mL	5.0	995	1,000
Int-4	Flonase	100	5	% (v/v)	50.0	950	1,000
Int-5	Halls Relief Cherry Flavor	800	8	mg/mL	10.0	990	1,000
Int-6	Nasocort Allergy 24 hour	100	5	% (v/v)	50.0	950	1,000
Int-7	Neo-Synephrine	100	5	% (v/v)	50.0	950	1,000
Int-8	Saline nasal spray	100	15	% (v/v)	150.0	850	1,000
Int-9	Zicam Cold Remedy	100	5	% (v/v)	50.0	950	1,000
Int-10	Purified mucin protein	3.4	0.06	mg/mL	17.6	982	1,000
Int-11	Mouthwash	100	5	% (v/v)	50.0	950	1,000
Int-13	Rhinocort	100	1	% (v/v)	10.0	990	1,000
Int-14	Oseltamivir	175	2.5	mg/mL	14.3	986	1,000
Int-15	Tobramycin	400	4	µg/mL	10.0	990	1,000
Int-16	Zanamivir	400	3.3	mg/mL	8.3	992	1,000
Int-17	Mupirocin	103	10	mg/mL	97.1	903	1,000
Int-18	Peramivir	10,000	45	ng/mL	4.5	996	1,000

- Positive contrived samples were created using heat Killed SARS-CoV-2 (ATCC VR-1986HK 2019; Novel Coronavirus, strain; 2019-nCoV/USA-WA1/2020.) virus at

300X the LDT LoD (~36,000 viral copies/mL) and was added to the MTM containing human cells. These positive contrived samples were used as the positive samples with a final concentration per substance indicated in table below:

Sample Number	Substances	Stock Concentration	Evaluation Concentration	Units	**Volume of Substances (µL)**	**SC-2 300XLOD Volume (µL)**	**Human cell containing VTM (µL)**	Total Target Volume (µL)
Int-1	Afrin – nasal spray	100	15	% (v/v)	150	10	840	1,000
Int-2 (1%)	Human blood	20	1	% (v/v)	50	10	940	1,000
Int-3	Chloraseptic	700	3.5	mg/mL	5	10	985	1,000
Int-4	Flonase	100	5	% (v/v)	50	10	940	1,000
Int-5	Halls Relief Cherry Flavor	800	8	mg/mL	10	10	980	1,000
Int-6	Nasocort Allergy 24 hour	100	5	% (v/v)	50	10	940	1,000
Int-7	Neo-Synephrine	100	5	% (v/v)	50	10	940	1,000
Int-8	Saline nasal spray	100	15	% (v/v)	150	10	840	1,000
Int-9	Zicam Cold Remedy	100	5	% (v/v)	50	10	940	1,000
Int-10	Purified mucin protein	2.5	0.06	mg/mL	24	10	966	1,000
Int-11	Mouthwash	100	5	% (v/v)	50	10	940	1,000
Int-13	Rhinocort	100	1	% (v/v)	10	10	980	1,000
Int-14	Oseltamivir	175	2.5	mg/mL	14	10	976	1,000
Int-15	Tobramycin	400	4	µg/mL	10	10	980	1,000
Int-16	Zanamivir	400	3.3	mg/mL	8.25	10	982	1,000
Int-17	Mupirocin	103	10	mg/mL	97	10	893	1,000
Int-18	Peramivir	10,000	45	ng/mL	5	10	986	1,000

9.3.2 Study Design

- Spun polyester dry swabs were submerged into the MTM media mixture, removed and then dried for 20 minutes. The swabs were then hydrated with an addition of 1mL of 3M Lysis buffer, and isolation of RNA was performed as per laboratory protocols.
- Samples were tested in triplicate.
- PCR was performed with primers for *RPP30*.

9.3.3 Interpretation

- Positive and negative controls must be valid.
- A substance will be deemed an interfering substance if any positive

sample is not detected.

- A substance will be deemed an interfering substance if any negative sample is detected.

Table H: Interfering Substances			
Sample Number	Substances	Positive Sample	Negative Sample
Int-1	Afrin – nasal spray	3/3	0/3
Int-2	Human blood	3/3	0/3
Int-3	Chloraseptic	3/3	0/3
Int-4	Flonase	3/3	0/3
Int-5	Halls Relief Cherry Flavor	3/3	0/3
Int-6	Nasocort Allergy 24 hour	3/3	0/3
Int-7	Neo-Synephrine	3/3	0/3
Int-8	Saline nasal spray	3/3	0/3
Int-9	Zicam Cold Remedy	3/3	0/3
Int-10	Purified mucin protein	3/3	0/3
Int-11	Mouthwash	3/3	0/3
Int-13	Rhinocort	3/3	0/3
Int-14	Oseltamivir	3/3	0/3
Int-15	Tobramycin	3/3	0/3
Int-16	Zanamivir	3/3	0/3
Int-17	Mupirocin	3/3	0/3
Int-18	Peramivir	3/3	0/3

Table H shows a summary of the data collected to establish if there is interference from commonly occurring substances found in the nasal passages of someone who is symptomatic of SARS-CoV-2. In this study human cells were mixed with MTM and then spiked with each interference substance. For the negative results, the samples were extracted and run in a PCR with RPP30. For the positive results, heat killed SARS-CoV-2 virus was added to each interference sample, extracted and run in a PCR assay with RPP30. This data set shows that none of the interfering substances inhibit the SARS-CoV-2 assay. All samples were concordant with the expected results.

9.3.4 Acceptance Criteria

- Samples with IC drop-out in a negative sample will be invalid.
- 3/3 replicates must be valid to interpret results

10. CONCLUSION

10.1 *In silico* analysis of nucleotide changes in the SARS-CoV-2 sequence determined that the performance of the kit is not impacted by the nucleotide changes.

10.2 Based on this in silico analysis and wet-testing, the primers and probes used in this assay are specific to SARS-CoV-2.

10.3 The data produced from the Cross-Reactivity Study showed that 232/232 (100%) of valid samples produced results with the expected outcome. This meets the acceptable criteria and shows that the assay is not cross-reactive with any of the pathogens listed in table E. It is still possible that the assay cross-reacts with pathogens that we did not test.

10.4 The Interference study showed that 102/102 (100%) of the samples tested were concordant with the expected outcome. This meets the acceptable criteria and shows that no common substances interfere with the detection of SARS-CoV-2.

Category	CFR	Section	Performance criteria met
Accuracy	CFR 484.1253(2)(i)	CA-VALRPT-LAB-015	Yes
Precision	CFR 484.1253(2) (ii)	CA-VALRPT-LAB-015	Yes
Analytical Sensitivity	CFR 484.1253(2) (iii)	9.1, CA-VALRPT-LAB-015	Yes
Analytical Specificity	CFR 484.1253(2) (iv)	9.2	Yes
Reportable range	CFR 484.1253(2) (v)	CA-VALRPT-LAB-015	Yes
Reference intervals	CFR 484.1253(2) (vi)	N/A	N/A

11. REFERENCES

11.1 Instructions for PerkinElmer® New Coronavirus Nucleic Acid Detection Kit v8.0

11.2 https://www.cdc.gov/coronavirus/2019-ncov/cdcresponse/about-COVID-19.html

11.3 https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

11.4 CAP Molecular Pathology Checklist current edition

11.5 CAP Immunology Checklist current edition

11.6 CAP Laboratory General Checklist current edition

11.7 CAP All Common Checklist current edition

11.8 42 CFR Part 493 Laboratory Requirements

12. APPENDIX

12.1 Appendix A: Creation of Contrived Samples

13. DOCUMENT HISTORY

Version	Summary of Changes	Date
1	New Document.	10Sep2021

Appendix A: Creation of Contrived Samples

A stock solution of LODs to be created using the following formula:

$$C1V1 = C2V2$$

Where:

C1 = Stock Concentration (5,000 copies/mL)

V1 = variable to be calculated

C2 = Desired Concentration (for example, 500 copies/mL)

V2 = 0.3 mL (volume of sample needed for extraction)

Example: For 500 copies/mL: 5,000x = 500 x 0.3 mL = 0.030 mL (or 30 µL)

- 6 µL will be added to 294 µL of Transport Medium to achieve 0.3 mL at 20 copies/mL
- The stock concentration (C1) for the SeraCare product must be obtained on a lot-by-lot basis from the SeraCare website.

Appendix B

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.perkinelmer.com

July 15, 2021

California Department of Public Health (CDPH)
Branch Laboratory
28454 Livingston Avenue
Valencia, CA 91355

RE: PerkinElmer® New Coronavirus Nucleic Acid Detection Kit (EUA200055)

Dear Dr. Adam Rosendorff,

PerkinElmer grants the right-of-reference to the following information included in the PerkinElmer® New Coronavirus Nucleic Acid Detection Kit EUA200055 application authorized by the FDA under the Emergency Use Authorization.

1. Inclusivity (analytical sensitivity)
2. Cross-reactivity (Analytical Specificity)

Sincerely,

Kelsi Schultz
Sr. Director, Quality Assurance
PerkinElmer | For the Better
Kelsi.Schultz@perkinelmer.com
Mobile: +1 920-285-0313
940 Winter Street, Waltham, MA 02451 USA
www.perkinelmer.com

Appendix C: Cross-Reactivity Data

Negative Samples					Positive Samples				
Sample name	FAM	HEX_3	ROX	Cy5	Sample name	FAM	HEX_3	ROX	Cy5
EX-1	No Ct	31.09	No Ct	35.38	EX-1	21.68	31.29	21.06	26.97
EX-1	No Ct	31.2	No Ct	35.61	EX-1	21.86	31.11	21.63	27.63
EX-1	No Ct	34.08	No Ct	38.87	EX-1	22.84	33.06	22.31	29.14
EX-2	No Ct	35.23	No Ct	No Ct	EX-2	22.08	32.03	21.73	28.58
EX-2	No Ct	32.81	No Ct	34.69	EX-2	22.1	31.71	21.55	28.06
EX-2	No Ct	32.23	No Ct	34.87	EX-2	22.56	31.74	21.58	27.51
EX-4	No Ct	31.51	No Ct	35.71	EX-4	22.68	31.9	21.81	26.99
EX-4	No Ct	32.06	No Ct	35.17	EX-4	21.39	31.93	20.51	26.4
EX-4	No Ct	32.06	No Ct	35.17	EX-4	22.96	30.93	21.81	27.17
EX-5	No Ct	33.51	No Ct	33.48	EX-5	22.87	32.3	22	27.18
EX-5	No Ct	32.97	No Ct	36.8	EX-5	22.14	30.91	21.55	27.23
EX-5	No Ct	33.43	No Ct	36.97	EX-5	22.17	31.52	21.32	27.2
EX-6	No Ct	31.95	No Ct	34.78	EX-6	22.37	31.94	21.53	26.52
EX-6	No Ct	31.41	No Ct	34.41	EX-6	22.45	32.64	21.52	26.16
EX-6	No Ct	32.87	No Ct	33.47	EX-6	22.77	30.57	21.63	26.27
EX-7	No Ct	31.26	No Ct	36.01	EX-7	22.32	31.24	21.44	27.29
EX-7	No Ct	32.27	No Ct	33.42	EX-7	22.15	31.17	21.46	26.04
EX-7	No Ct	31.23	No Ct	32.75	EX-7	22.43	31.26	22.07	27.16
EX-8	No Ct	31.46	No Ct	34.44	EX-8	23.05	31.75	22.15	27.1
EX-8	No Ct	32.48	No Ct	35.36	EX-8	22.05	31.74	21.18	26.3
EX-8	No Ct	31.08	No Ct	33.41	EX-8	23.19	32.22	22.08	27.08
EX-9	No Ct	32.85	No Ct	37.16	EX-9	23.39	32.48	22.51	27.29
EX-9	No Ct	32.58	No Ct	35.97	EX-9	22.88	31.14	22.02	26.29
EX-9	No Ct	32.37	No Ct	34.05	EX-9	23.37	31.67	22.4	26.89
EX-10	No Ct	31.74	No Ct	37.26	EX-10	21.95	32.15	20.95	26.2
EX-10	No Ct	32.69	No Ct	34.4	EX-10	22.09	31.47	21.46	26.89
EX-10	No Ct	34.85	No Ct	38.5	EX-10	22.43	31.74	21.56	26.42
EX-11	No Ct	32.74	No Ct	37.39	EX-11	23	31.24	22.12	27.91
EX-11	No Ct	31.23	No Ct	37.97	EX-11	22.24	30.8	21.7	26.49
EX-11	No Ct	32.64	No Ct	33.66	EX-11	22.4	31.37	21.49	27.01
EX-12	No Ct	31.46	No Ct	34.53	EX-12	22.63	31.6	21.77	27.22
EX-12	No Ct	30.54	No Ct	34.25	EX-12	23.05	32.24	22.15	27.28
EX-12	No Ct	30.45	No Ct	34.44	EX-12	22.01	32.1	21.01	26.66
EX-13	No Ct	32.45	No Ct	35.04	EX-13	22.88	31.33	21.64	27.12
EX-13	No Ct	33.31	No Ct	37.82	EX-13	22.1	30.26	21.22	26.65
EX-13	No Ct	32.08	No Ct	34.8	EX-13	21.75	31.06	21.1	26.26
EX-14	No Ct	32.05	No Ct	34.99	EX-14	21.94	32.83	21.04	26.77

EX-14	No Ct	33.55	No Ct	32.77	EX-14	22.41	32.23	21.64	27.09
EX-14	No Ct	31.78	No Ct	34.81	EX-14	21.46	32.3	20.73	26.02
EX-16	No Ct	32.79	No Ct	33.42	EX-16	23.02	32.49	21.62	27.23
EX-16	No Ct	32.09	No Ct	34.08	EX-16	22.77	31.89	21.55	27.02
EX-16	No Ct	31.91	No Ct	34.34	EX-16	22.4	32.68	21.37	26.34
EX-17	No Ct	30.43	No Ct	32.52	EX-17	21.79	32.58	21.22	26.68
EX-17	No Ct	31.98	No Ct	33.15	EX-17	22.49	32.54	21.55	26.45
EX-17	No Ct	31.54	No Ct	31.77	EX-17	22.23	32.75	21.33	27.05
EX-18	No Ct	29.87	No Ct	35.09	EX-18	22.73	32.85	21.52	27.07
EX-18	No Ct	31.29	No Ct	36.11	EX-18	22.12	32.18	21.23	26.36
EX-18	No Ct	31.6	No Ct	33.82	EX-18	22.32	32.03	21.23	27.07
EX-19	No Ct	31.73	No Ct	35.06	EX-19	21.85	32.63	21.1	25.48
EX-19	No Ct	31.96	No Ct	33.69	EX-19	21.33	32.05	20.48	25.31
EX-19	No Ct	31.08	No Ct	33.44	EX-19	22	32.07	21.1	26.48
EX-20	No Ct	32.32	No Ct	34.49	EX-20	22.8	32.56	21.69	27.18
EX-20	No Ct	31.56	No Ct	34.56	EX-20	22.44	32.73	21.24	26.71
EX-20	No Ct	32.94	No Ct	34.67	EX-20	23.03	32.31	21.95	26.73
EX-21	No Ct	32.21	No Ct	34.78	EX-21	21.88	32.31	21.16	26.16
EX-21	No Ct	31.54	No Ct	33.15	EX-21	22.1	32.33	21.22	26.54
EX-21	No Ct	31.02	No Ct	34	EX-21	22.59	32.2	21.57	26.53
EX-22	No Ct	30.62	No Ct	32.66	EX-22	22.16	32.59	21.29	26.64
EX-22	No Ct	30.79	No Ct	33.77	EX-22	22.16	32.52	21.2	26.51
EX-22	No Ct	32.11	No Ct	34.89	EX-22	21.84	31.48	21.05	26.14
EX-23	No Ct	31.73	No Ct	34.84	EX-23	22.24	30.61	21.39	26.61
EX-23	No Ct	33.09	No Ct	34.4	EX-23	22.37	31.93	21.59	26.45
EX-23	No Ct	33.34	No Ct	No Ct	EX-23	21.85	30.68	21.2	26.57
EX-24	No Ct	31.8	No Ct	35.21	EX-24	22.22	32.14	21.41	26.66
EX-24	No Ct	32.03	No Ct	36.11	EX-24	22.08	32.06	21.42	26.17
EX-24	No Ct	32.8	No Ct	34.54	EX-24	22.86	31.18	22.07	27.16
EX-25	No Ct	33.42	No Ct	36.88	EX-25	21.62	30.71	21.36	26.39
EX-25	No Ct	32.76	No Ct	34.24	EX-25	22.21	31.42	21.88	26.51
EX-25	No Ct	31.61	No Ct	33.65	EX-25	22.26	31.2	21.54	26.74
EX-26	No Ct	31.18	No Ct	33.78	EX-26	21.88	31.5	21.14	26.41
EX-26	No Ct	33.12	No Ct	34.08	EX-26	22.33	32	21.54	27.17
EX-26	No Ct	32.43	No Ct	33.46	EX-26	22.2	31.56	21.03	26.38
EX-27	No Ct	32.94	No Ct	36.35	EX-27	22.35	33.29	21.28	26.3
EX-27	No Ct	32.27	No Ct	34.12	EX-27	22.77	33.07	22.05	27.14
EX-27	No Ct	31.43	No Ct	33.32	EX-27	22.12	32.98	21.1	26.4
EX-28	No Ct	31.27	No Ct	35.42	EX-28	22.12	33.27	21.24	26.98
EX-28	No Ct	31.77	No Ct	32.47	EX-28	22.47	33.24	21.46	27.77
EX-28	No Ct	32.59	No Ct	33.81	EX-28	22.41	33.4	21.18	27.92

EX-29	No Ct	29.91	No Ct	36.21	EX-29	23.09	31.74	21.57	26.99
EX-29	No Ct	30.71	No Ct	33.43	EX-29	23.02	31.35	21.6	26.39
EX-29	No Ct	31.32	No Ct	34.44	EX-29	22.68	32.28	21.65	26.47
EX-30	No Ct	32.38	No Ct	33.17	EX-30	23.34	32.25	22.25	27.22
EX-30	No Ct	32.47	No Ct	30.84	EX-30	22.84	30.88	21.55	27.31
EX-30	No Ct	31.73	No Ct	30.91	EX-30	22.48	31.15	21.52	26.73
EX-31	No Ct	30.75	No Ct	30.7	EX-31	22.72	30.21	22.01	27.18
EX-31	No Ct	31.5	No Ct	31.52	EX-31	22.64	30.42	22.05	27.7
EX-31	No Ct	30.08	No Ct	31.74	EX-31	21.44	32.1	21.13	27.4
EX-32	No Ct	30.85	No Ct	32.49	EX-32	24.06	32.42	23.45	28.59
EX-32	No Ct	31.39	No Ct	31.26	EX-32	22.03	30.07	21.64	27.77
EX-32	No Ct	31.71	No Ct	32.28	EX-32	22.22	30.18	21.25	27.36
EX-33	No Ct	31.06	No Ct	31.13	EX-33	22.27	29.55	21.7	27.24
EX-33	No Ct	31.23	No Ct	31.46	EX-33	21.86	29.92	21.5	27.03
EX-33	No Ct	30.67	No Ct	30.96	EX-33	22.07	30.8	21.71	27.19
EX-34	No Ct	31.05	No Ct	30.8	EX-34	23.09	30.49	21.97	27.51
EX-34	No Ct	30.43	No Ct	31.13	EX-34	22.49	30.51	21.82	26.72
EX-34	No Ct	30.01	No Ct	31.36	EX-34	22.7	31.5	21.97	27.51
EX-35	No Ct	30.3	No Ct	30.21	EX-35	22.55	31.41	21.87	26.78
EX-35	No Ct	29.85	No Ct	30.46	EX-35	21.34	32.27	20.8	26.48
EX-35	No Ct	29.87	No Ct	30.55	EX-35	22.09	31.88	21.21	26.23
EX-36	No Ct	30.37	No Ct	30.9	EX-36	22.17	32.37	21.44	26.99
EX-36	No Ct	31.13	No Ct	31.04	EX-36	22.34	32.25	21.66	27.08
EX-36	No Ct	30.57	No Ct	30.92	EX-36	22.04	32.15	21.43	27.63
EX-37	No Ct	31.41	No Ct	31.12	EX-37	22.46	31.82	21.69	27.59
EX-37	No Ct	31.41	No Ct	31.28	EX-37	21.94	32.24	21.26	26.95
EX-37	No Ct	31.49	No Ct	30.28	EX-37	21.62	31.95	20.8	26.13
EX-38	No Ct	31.12	No Ct	31.22	EX-38	22.79	31.03	21.93	27.01
EX-38	No Ct	31.14	No Ct	30.9	EX-38	22.82	32.01	21.8	26.96
EX-38	No Ct	30.34	No Ct	31.21	EX-38	22.68	32.35	21.75	27.05
EX-39	No Ct	29.96	No Ct	30.46	EX-39	22.17	32.05	21.14	26.38
EX-39	No Ct	31.18	No Ct	31.03	EX-39	22.08	31.36	20.97	26.48
EX-39	No Ct	30.86	No Ct	31.23	EX-39	21.99	32.04	21.01	26.88
EX-40	No Ct	30.45	No Ct	30.8	EX-40	22.37	32.92	21.56	27.71
EX-40	No Ct	30.16	No Ct	30.54	EX-40	22.67	32.73	21.25	27.2
EX-40	No Ct	30.14	No Ct	30.49	EX-40	21.64	32.75	20.94	26.91
EX-41	No Ct	30.26	No Ct	30.31	EX-41	22.21	32.99	21.37	27.04
EX-41	No Ct	30.69	No Ct	30.41	EX-41	22.68	32.49	21.86	28.09
EX-41	No Ct	31.91	44.04	30.33	EX-41	22.18	32.14	21.3	27.46
nCoV NC	No Ct	30.29	No Ct	No Ct	nCoV NC	No Ct	30.89	No Ct	No Ct
nCoV PC	29.88	28.55	33.07	No Ct	nCoV NC	No Ct	31.78	No Ct	No Ct

nCoV NC	No Ct	30.62	No Ct	No Ct	nCoV NC	No Ct	29.8	No Ct	No Ct
nCoV PC	30.1	28.3	33.06	No Ct	nCoV PC	31.4	28.8	31.64	No Ct
nCoV NC	No Ct	31.33	No Ct	No Ct	nCoV PC	31.88	30.05	31.64	No Ct
nCoV PC	31.58	27.51	32.54	No Ct	nCoV PC	31.35	27.22	31.73	No Ct

Appendix D: Interfering Substances

Positive Samples						Negative Samples					
Well	Sample name	FAM	HEX_3	ROX	Cy5	Well	Sample name	FAM	HEX_3	ROX	Cy5
A23	nCoV NC	No Ct	27.01	No Ct	No Ct	A1	Int-1 Neg	No Ct	28.28	No Ct	30.99
B2	Int-1 Pos	33.73	31.22	33.73	39.26	A3	Int-1 Neg	No Ct	28.01	No Ct	30.4
B4	Int-1 Pos	34.25	29.95	33.76	35.43	A5	Int-1 Neg	No Ct	27.46	No Ct	29.25
B6	Int-1 Pos	33.9	29.21	33.99	37.04	A7	Int-2 Neg	No Ct	28.21	No Ct	29.56
B8	Int-2 Pos	33.4	30.2	33.53	35.01	A9	Int-2 Neg	No Ct	26.71	No Ct	29.09
B10	Int-2 Pos	35.01	28.9	32.72	34.71	A11	Int-2 Neg	No Ct	28.14	No Ct	29.13
B12	Int-2 Pos	34.58	30.15	32.52	31.19	A13	Int-3 Neg	No Ct	27.81	No Ct	29.19
B14	Int-3 Pos	33.09	29	31.92	32.63	A15	Int-3 Neg	No Ct	27.78	No Ct	29.23
B16	Int-3 Pos	33.21	28.56	33.25	32.14	A17	Int-3 Neg	No Ct	27.82	No Ct	28.61
B18	Int-3 Pos	34.89	29.26	33.21	31.23	A23	nCoV NC	No Ct	27.01	No Ct	No Ct
B24	nCoV NC	No Ct	29.44	No Ct	No Ct	B24	nCoV NC	No Ct	29.44	No Ct	No Ct
D2	Int-4 Pos	32.91	29.61	32.48	32.08	C1	Int-4 Neg	No Ct	28.52	No Ct	28.95
D4	Int-4 Pos	33.53	29.85	32.55	30.68	C3	Int-4 Neg	No Ct	27.83	No Ct	28.75
D6	Int-4 Pos	34.3	29.27	33.57	30.57	C5	Int-4 Neg	No Ct	27.59	No Ct	29.01
D8	Int-5 Pos	33.22	28.55	33.62	30.91	C7	Int-5 Neg	No Ct	27.75	No Ct	28.53
D10	Int-5 Pos	35.77	28.77	33.49	30.12	C9	Int-5 Neg	No Ct	28.58	No Ct	29.35
D12	Int-5 Pos	33.9	29.39	32.84	29.55	C11	Int-5 Neg	No Ct	25.93	No Ct	28.15
D14	Int-6 Pos	32.92	29.43	33.41	34.86	C13	Int-6 Neg	No Ct	27.92	No Ct	29.35
D16	Int-6 Pos	34.79	29.34	33.13	36.13	C15	Int-6 Neg	No Ct	28.32	No Ct	28.25
D18	Int-6 Pos	33.81	30.02	32.72	35.42	C17	Int-6 Neg	No Ct	27.33	No Ct	28.51
E23	nCoV PC	32.28	27.26	33.11	No Ct	E1	Int-7 Neg	No Ct	27.45	No Ct	29.46
F2	Int-7 Pos	32.99	28.01	32.04	29.25	E3	Int-7 Neg	No Ct	27.34	No Ct	29.13
F4	Int-7 Pos	32.93	28.84	32.43	29.47	E5	Int-7 Neg	No Ct	27.09	No Ct	28.49

F6	Int-7 Pos	33.07	28.65	32.3	29.39	E7	Int-8 Neg	No Ct	26.85	No Ct	27.36
F8	Int-8 Pos	33.69	29.17	32.47	30.26	E9	Int-8 Neg	No Ct	26.86	No Ct	28.87
F10	Int-8 Pos	34.19	28.79	32.4	29.33	E11	Int-8 Neg	No Ct	27.6	No Ct	28.44
F12	Int-8 Pos	33.79	29.22	34.27	29.8	E13	Int-9 Neg	No Ct	27.3	No Ct	27.82
F14	Int-9 Pos	33.36	29.07	33	29.5	E15	Int-9 Neg	No Ct	27.45	No Ct	28
F16	Int-9 Pos	33.59	28.73	33.07	29.44	E17	Int-9 Neg	No Ct	27.43	No Ct	28.1
F18	Int-9 Pos	33.64	29.26	32.47	29.41	E23	nCoV PC	32.28	27.26	33.11	No Ct
F24	nCoV PC	28.96	27.23	29.99	No Ct	F24	nCoV PC	28.96	27.23	29.99	No Ct
H2	Int-1 Pos	33.13	28.66	32.13	30.11	G1	Int-1 Neg	No Ct	27.44	No Ct	29.11
H4	Int-1 Pos	34.26	28.61	33.04	29.58	G3	Int-1 Neg	No Ct	27.76	No Ct	28.24
H6	Int-1 Pos	33.38	28.54	33.18	29.57	G5	Int-1 Neg	No Ct	26.19	No Ct	28.03
H8	Int-11 Pos	34.59	29.22	33.23	30.69	G7	Int-11 Neg	No Ct	27.23	No Ct	28.1
H10	Int-11 Pos	34.73	28.53	33.55	29.58	G9	Int-11 Neg	No Ct	26.85	No Ct	27.6
H12	Int-11 Pos	33.97	29.75	33.07	29.15	G11	Int-11 Neg	No Ct	27.96	No Ct	28.32
H14	Int-13 Pos	33.92	29.54	32.87	29.19	G13	Int-13 Neg	No Ct	27.67	No Ct	28.2
H16	Int-13 Pos	32.76	29.36	32.26	30.01	G15	Int-13 Neg	No Ct	27.06	No Ct	27.34
H18	Int-13 Pos	32.16	28.43	31.95	29.7	G17	Int-13 Neg	No Ct	27.47	No Ct	28.01
J2	INT-14	22.44	31.25	21.11	26.43	I1	Int-14 Neg	No Ct	26.18	No Ct	27.67
J4	INT-14	21.55	30.67	21.01	25.9	I3	Int-14 Neg	No Ct	27.87	No Ct	27.86
J6	INT-14	22.35	31.06	21.14	25.74	I5	Int-14 Neg	No Ct	26.93	No Ct	27.84
J8	INT-15	21.46	30.26	20.91	26.26	I7	Int-15 Neg	No Ct	26.7	No Ct	27.75
J10	INT-15	21.53	31.42	20.87	26.4	I9	Int-15 Neg	No Ct	27.9	No Ct	28.25
J12	INT-15	21.44	31.62	20.69	26.21	I11	Int-15 Neg	No Ct	26.84	No Ct	27.51
J14	INT-16	21.25	30.9	20.41	26.02	I13	Int-16 Neg	No Ct	25.8	No Ct	28.05
J16	INT-16	21.14	30.6	20.27	25.25	I15	Int-16 Neg	No Ct	26.3	No Ct	27.63

J18	INT-16	21.79	29.46	20.86	26.35	I17	Int-16 Neg	No Ct	27.57	No Ct	28.21
J20	INT-17	21.17	30.95	20.33	24.89	K1	Int-17 Neg	No Ct	27.76	No Ct	29.06
J22	INT-17	20.95	30.67	20.18	25.18	K3	Int-17 Neg	No Ct	27.57	No Ct	28.29
J24	INT-17	20.41	29.8	20.07	24.61	K5	Int-17 Neg	No Ct	28.16	No Ct	28.12
L2	INT-18	22.04	30.83	21.05	25.17	K7	Int-18 Neg	No Ct	28.85	No Ct	28.33
L4	INT-18	21.42	30.38	20.54	25.57	K9	Int-18 Neg	No Ct	28.08	No Ct	28.08
L6	INT-18	21.41	29.67	20.95	26.44	K11	Int-18 Neg	No Ct	27.98	No Ct	28.44