EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
PerkinElmer SARS-CoV-2 RT-qPCR
Reagent Kit
(PerkinElmer Genomics)

For *in vitro* Diagnostic Use
Rx Only
For Use Under Emergency Use Authorization (EUA) Only

(The PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit assay will be performed at the PerkinElmer Genomics (PKIG) laboratory, located at 250 Industry Drive, Pittsburgh, PA 15275, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets requirements to perform high-complexity tests, as described in the Laboratory Standard Operating Procedure that was reviewed by the FDA under this EUA.)

INTENDED USE

The PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit is a real-time, RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in oropharyngeal and nasopharyngeal swab specimens from individuals suspected of COVID-19 by their healthcare provider.

This test is also for use with anterior nasal swab specimens that are collected using the Everlywell COVID-19 Test Home Collection Kit when used consistent with its authorization.

Testing is limited to PerkinElmer Genomics, 250 Industry Drive, Pittsburgh, PA (PKIG) which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in the upper respiratory specimens during the acute phase of infection. Positive results are indicative of 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. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.

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.

The assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The SARS-CoV-2 primer and probe sets are designed to detect RNA from SARS-CoV-2 in oropharyngeal and nasopharyngeal swab specimens from patients suspected of COVID-19 by their healthcare provider. This test is also for use with anterior nasal swab specimens that are collected using the Everlywell COVID-19 Test Home Collection Kit when used consistent with its authorization.

PerkinElmer has received a Right to Reference letter from Everlywell for their Everlywell COVID-19 Test Home Collection Kit. The Everlywell accessioning protocol will be used to process samples collected with the Everlywell COVID-19 Test Home Collection Kit.

INSTRUMENTS FOR USE WITH THE TEST

The PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit (P/N: 3501-0010) test is to be used with the instruments described in Table 1.

Table 1. Instruments and Software Versions

Instrument	Manufacturer	Software Version
PerkinElmer Chemagic 360 automated extraction	PerkinElmer	V6.3.0.3
BioRad CFX384, qPCR Real-Time System	BioRad	3.1 or 3.1.1517.0823

HOME COLLECTION KIT USED WITH THE TEST

This test is to be used with the Everlywell COVID-19 Test Home Collection Kit for the self-collection of anterior nasal swab specimens when used consistent with its authorization.

REAGENTS AND MATERIALS

Table 2a. Equipment to be used with the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit:

Equipment			
Instrument	**Manufacture**	**Model**	**Part No./Catalog No.**
Chemagic 360	PerkinElmer	360 or 360-D	20240056
Minicentrifuge	Any	Any	Any
Centrifuge	Any	Any	Any
Vortex Mixer	Any	Any	Any
Biosafety Cabinet	Any	Any	Any
PCR Hood	Any	Any	Any
Microcentrifuge	Any	Any	Any
Vortex mixer	Any	Any	Any
Plate Centrifuge	Any	Any	Any

Equipment			
Instrument	**Manufacture**	**Model**	**Part No./Catalog No.**
Pipettes (single and multi-channel) p10, p200, p1000	Any	Any	Any
qPCR Real-Time System (software version 3.1 or 3.1.1517.0823)	Bio-Rad	CFX384	184-5384

Table 2b. Reagents to be used with the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit:

Reagents			
Reagent	**Manufacture**	**Model**	**Part No./Catalog No**
Chemagic Viral 300 RNA/DNA Kit H96	PerkinElmer	Turku	CMG-1033-S
Molecular Biology Grade Water	Any	Any	Any
PrimeStore MTM		PrimeStore MTM	
AccuPlex SARS-CoV-2 Reference Material Kit	SeraCare	SeraCare	0505-0126

Table 2c. Supplies to be used with the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit:

Supplies **NOTE:** DNAse, RNAse and Nuclease Free consumables are used where possible.			
Reagent	**Manufacture**	**Model**	**Part No./Catalog No**
2 mL Deep Well Plate	PerkinElmer	PerkinElmer	CMG-555
96 Rod Head Disposable Tips	PerkinElmer	PerkinElmer	CMG-550
Low-well Plate	PerkinElmer	PerkinElmer	CMG-555-1
Heat Sealing Foil	Thermo Fisher Scientific	Thermo Fisher Scientific	AB0559
Reagent trough	Any	Any	Any
2 mL Deep Well Plate	PerkinElmer	PerkinElmer	CMG-555
96 Rod Head Disposable Tips	PerkinElmer	PerkinElmer	CMG-550
Low-well Plate	PerkinElmer	PerkinElmer	CMG-555-1
Heat Sealing Foil	Thermo Fisher Scientific	Thermo Fisher Scientific	AB0559
Full set of pipettes (single and multichannel + filtered tips)	Any	Any	Any
Tips (10, 200, 1,000 µL) compatible with pipettes	Any	Any	Any
384 Plates for RT-PCR	Thomas Scientific	4titude	1149R56
qPCR Seal	Thomas Scientific	4titude	1149R84
1.7 mL to 5 mL tube	Any	Any	Any

Table 2d. Materials included in the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit:

SARS-CoV-2 RT-qPCR Reagent Kit		
Component	**Abbreviation used in this text**	**96 reaction kit Volumes**
CoV2 Reagent A	Reagent A	110 µL
CoV2 Enzyme Mix	Enzyme	550 µL
CoV2 Positive Control	Positive Control	70 µL
CoV2 Negative Control	Negative Control	1000 µL

CONTROLS TO BE USED WITH THE PERKINELMER SARS-CoV-2 RT-QPCR REAGENT KIT Assay controls are run concurrently with all test samples. The test includes a negative control, positive control, and internal control (IC) as described below:

Negative Control:
The negative control is nuclease-free water provided in the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit. The negative control is added during the extraction step to every assay plate to monitor contamination during the extraction step. PCR of the Negative Control is conducted using the same PCR mix as used for sample testing in a given batch.

Positive Control:
The positive control is a plasmid containing SARS-CoV-2 target sequences. The positive control is included in each extraction plate to ensure the reagents and instruments are performing optimally. Sequences detecting the ORF1ab and N targets of SARS-CoV-2 are used as positive controls. Both targets should be detected with Ct values of ≤35 and ≤32, respectively.

Internal Control:
A primer/probe set detecting a human RNA target is included in the reaction mix as an internal control. The Internal Control is RNAse P (*RPP30)* labeled with a Cy5 fluorescent dye and is used to monitor the processes from nucleic acid extraction to fluorescence detection. RNAse P (internal control) should not be detected in the positive control or negative control.

The controls run with the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit assay are described in **Table 3a**.

Table 3a. Positive and Negative Control Requirements

Control	**Ct**		
	***N*-gene (FAM)**	***ORF1ab*-gene (HEX/VIC)**	**RNAse P (Cy5)**
Negative	Undetermined or Ct > 40	Undetermined or Ct > 40	Undetermined or Ct > 37
Positive	Ct ≤ 32	Ct ≤ 35	Undetermined or Ct > 37

INTERPRETATION OF RESULTS
Assessment of clinical specimen test results must be performed after the positive and negative controls have been examined and confirmed to be valid and acceptable. If the controls are not valid,

the patient results cannot be interpreted.

The interpretation of results for the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit are described in **Table 3b**.

Table 3b. Results Interpretation

Ct			Result Interpretation
RNAse P(Cy5)[3]	**N (FAM)**	**ORF1ab (HEX)**	
≤ 37	undetermined or > 40	undetermined or > 40	SARS-CoV-2 not detected
No requirements on the Ct value	At least one target has Ct ≤ 40 [1]		SARS-CoV-2 detected
> 37 or undetermined	Both targets undetermined or > 40		Invalid result, specimen needs to be re-tested from re-extraction or re-collected from patient for testing.[2]

[1] The detection of only one target (*N* or *ORF1ab*) is resulted as a positive result; however, if a single positive target (*N* or *ORF1ab*) does not show the expected exponential curve the result will be invalid and require re-testing.
[2] Specimens with an invalid result can be re-extracted and re-tested if there is sufficient remaining sample; otherwise, a new specimen should be collected for re-testing.
[3] RNAse P is used as the Internal Control

PERFORMANCE EVALUATION

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

LoD Range Finding: Negative nasopharyngeal and anterior nasal swabs collected in PrimeStore MTM were pooled to create a matrix into which SARS-CoV-2 genomic RNA was spiked at the following concentrations (copies/ml): 60, 120, 180, 540, 1000, 2000 and 3000 to find the preliminary LoD. Three replicates were tested per concentration. RNA was extracted using the Chemagic Viral 300 RNA/DNA Kit H96 extraction kit with the Chemagic 360 or 360-D system and tested with the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit on the BioRad CFX384 instrument. The results indicate that the preliminary LoD was 60 copies/ml for the *N* target and 120 copies/ml for the *ORF1ab* target, as shown is Table 4.

Table 4: LOD Range Finding in Pooled Negative Medium

Copies/ mL	*N*			*ORF1ab*			*RPP30*		
	Average	**±SD[3]**	**Positives**	**Average**	**±SD[3]**	**Positives**	**Average**	**±SD[3]**	**Positive**
0	N/A	N/A	0 of 3	N/A	N/A	0 of 3	22.92	0.16	3 of 3
60	38.07	0.17	3 of 3	35.55	0.32	2 of 3	22.40	0.11	3 of 3
120	38.05	2.27	3 of 3	35.26	1.00	3 of 3	22.99	0.95	3 of 3

Copies/ mL	N			ORF1ab			RPP30		
	Average	±SD[3]	Positives	Average	±SD[3]	Positives	Average	±SD[3]	Positive
180[1]	39.42	5.90	1 of 3	33.18	N/A	1 of 3	27.14	4.79	3 of 3
540[2]	37.08	1.97	2 of 3	33.64	0.12	2 of 3	23.97	0.57	2 of 3
1000	35.02	0.18	3 of 3	32.57	0.18	3 of 3	23.88	0.16	3 of 3
2000	33.70	0.49	3 of 3	31.32	0.69	3 of 3	23.57	0.31	3 of 3
3000	32.59	0.04	3 of 3	29.72	0.17	3 of 3	23.44	0.07	3 of 3

[1] Possible contrived blending error or insufficient master mix at RT-PCR to cause 2 of 3 failures
[2] One invalid sample due to potential inefficient master mix or sample addition at extraction or RT-PCR setup causing failed sample
[3] SD: Standard Deviation

LOD Confirmation: To confirm the LoD findings, negative nasopharyngeal and anterior nasal swab media were pooled to create a matrix into which SARS-CoV-2 genomic RNA was spiked at the following concentrations (copies/mL): 60, 120, 180, 540. After preparing the dilutions, RNA was extracted using the Chemagic Viral 300 RNA/DNA Kit H96 extraction kit with the Chemagic 360 or 360-D system and tested with the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit on the BioRad CFX384 instrument. The results are shown in Table 5**.** Based on this data, it is determined that the LoD is 120 copies/mL.

Table 5: LOD Confirmation in Pooled Negative Nasopharyngeal and Anterior Nasal Swab Specimens

Positive SARS-COV-2 RNA copies/ml	N			ORF1ab			RPP30		
	Average	±SD[1]	Frequency	Average	±SD[1]	Frequency	Average	±SD[1]	Frequency
60	36.41	1.19	10 of 20	36.40	2.78	13 of 20	22.68	3.59	18 of 20
120	**35.79**	**1.21**	**20 of 20**	**34.62**	**1.80**	**19 of 20**	**23.30**	**1.89**	**20 of 20**
180	34.83	0.59	20 of 20	32.89	0.82	20 of 20	22.65	1.10	20 of 20
540	33.56	0.63	20 of 20	31.42	0.65	20 of 20	22.96	1.03	20 of 20

[1] SD; Standard Deviation

2) *Inclusivity (analytical sensitivity):*

The inclusivity of the SARS-CoV-2 specific assay primers/probes have been routinely evaluated using full length genomic sequences available in the NCBI and GISAID databases. The most recent *in silico* evaluation was completed in February 2021.

More than 53,000 sequences in from the NCBI database, and more than 436,000 sequences in the GISAID database at this time were classified as full length (“complete”), and met the inclusion criteria for the analysis described below:

- Complete sequences: Genomes with >29000 bp

- 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

The criteria for primer sequences predicted to be impacted are as follows: (1) primer sequence has at least one mismatch to the genome in the last five base pairs from the primer's 3' end, (2) primer sequence has multiple mismatches to the genome with at least one mismatch landing in the 3' half of the primer, or (3) primer sequence has no match to the genome. The criteria for probe sequences predicted to be impacted are as follows: (1) probe sequence has greater than two mismatches to the genome, or (2) probe sequence has no match to the genome. For all criteria regarding impact, any mismatches caused by Ns or other ambiguous nucleotide nomenclature are ignored.

With the above criteria, the results are summarized in Tables 6a, 6b, and 6c.

Table 6a: Summary of individual oligo nucleic acid substitutions

Target	***N* gene**						***ORF1ab* gene**					
Database	**NCBI**			**GISAID**			**NCBI**			**GISAID**		
Oligo	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe
# Sequences	53188	53188	53188	436988	436988	436988	53188	53188	53188	436988	436988	436988
Sequences with mismatches	20337	2057	246	186229	99035	1368	114	270	192	863	1244	1540
1 mismatch	4036	2037	240	12930	98443	1123	108	255	100	850	1216	1387
2 mismatches	53	11	0	167	289	7	0	1	2	1	2	9
3 or more mismatches	16177	9	1	172776	301	16	0	14	5	5	2	52
Other mismatches	71	0	5	356	2	222	6	0	85	7	24	92
Sequences predicted to impact	609	312	5	2979	1412	222	10	74	85	65	160	92

For: Forward primer, Rev: Reverse primer.

Table 6b: Summary of types of nucleic acid changes among impacted sequences

Target		**N gene**				**ORF1ab gene**			
Database		**NCBI**		**GISAID**		**NCBI**		**GISAID**	
Total Failures		789	*100.00%*	3518	*100.00%*	151	*100.00%*	306	*100.00%*
For	**Multiple mms with 1 mm in 3' half**	165	*20.94%*	914	*25.98%*	0	*0.00%*	0	*0.00%*
	1 mm in last 5bps of 3' end	373	*47.34%*	1709	*48.58%*	4	*2.65%*	58	*18.95%*

Target		N gene				ORF1ab gene			
Database		NCBI		GISAID		NCBI		GISAID	
Total Failures		789	*100.00%*	3518	*100.00%*	151	*100.00%*	306	*100.00%*
	Other mismatches	71	*9.01%*	356	*10.12%*	6	*3.97%*	7	*2.29%*
Rev	**Multiple mms with 1 mm in 3' half**	17	*2.16%*	411	*11.68%*	11	*7.28%*	0	*0.00%*
	1 mm in last 5bps of 3' end	295	*37.44%*	998	*28.37%*	63	*41.72%*	136	*44.44%*
	Other mismatches	0	*0.00%*	2	*0.06%*	0	*0.00%*	24	*7.84%*
Probe	**More than 2 mms**	0	*0.00%*	0	*0.00%*	0	*0.00%*	0	*0.00%*
	Other mismatches	0	*0.63%*	222	*6.31%*	85	*56.29%*	92	*30.07%*

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

Table 6c: Prediction of impacts of detection.

Target	*N* gene				*ORF1ab* gene			
Database	NCBI		GISAID		NCBI		GISAID	
Number of Sequences	53188	*100.00%*	436988	*100.00%*	53188	*100.00%*	436988	*100.00%*
Sequences with Mismatches	21298	*40.04%*	203723	*46.62%*	562	*1.06%*	3601	*0.80%*
Predicted No Detection	788	*1.48%*	3518	*0.81%*	151	*0.28%*	306	*0.07%*

The main reason for the reduction in overall homology between the assay primer/probe sequences with strains from the NCBI and GISAID databases is the recent emergence of circulating SARS-CoV-2 strains with a three base pair substitution at the 5' end of the PerkinElmer New Coronavirus Nucleic Acid Detection Kit *N* forward primer (GGG -> AAC).

However, the performance of the kit is not expected to be impacted by the nucleotide changes. This is because this nucleotide change occurs on the 5' end of the primer and has less impact on Tm primer binding efficiency. The Tm with the GGG -> AAC nucleotide substitution is still 5 degrees higher than the annealing temperature used in the assay protocol, which is at 55 ^{o}C. Furthermore, the detection of SARS-CoV-2 is based on detection of either the *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 are expected to have minimal impact on inclusivity for SARS-CoV-2.

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 expected impact on final detection is summarized in Table 6d.

Table 6d: Summary of nucleotide change impact on final detection

Database	NCBI		GISAID	
Number of Sequences	53188	*100.00%*	436988	*100.00%*
Predicted Both Genes Detected	52249	*98.23%*	433165	*99.13%*
Predicted One Gene Detected	939	*1.77%*	3822	*0.87%*
Predicted Neither Gene Detected	0	*0.00%*	1*	*0.00%*

*The 1 failure is EPI_ISL_936056. This fails the criteria because of one mismatch in the last five base pairs on the 3' end of the reverse ORF1ab-gene primer, and all three N-gene oligos had many Ns in the N-gene target region.

Although the emerging strains with nucleic acid substitutions appear to be localized to just one of the assay targets, reference material containing the *N* forward primer GGG -> AAC nucleotide substitution was tested for impact on assay performance.

To investigate the effect of the GGG -> AAC mismatch on the *N* gene forward primer and the C -> T mismatch on the reverse primer, reference material for a strain that contains this variant (the "UK variant" B.1.1.7,) was obtained from Twist BioScience. The recently identified high-frequency UK-variant (VUI 202012/01) contains the variant of interest in the *N*-gene of the SARS-CoV-2 RNA-genome that overlaps with the *N* forward primer of the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit. In addition, this strain has a single nucleotide change (C -> T) in the reverse primer of the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit. The exact locations of these variants in the primers used in the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit are shown in **Figure 1**.

Figure 1. High frequency variations recently detected in the N-gene of the SARS-CoV-2 genome. The sequences used for the N-gene primers and probes are underlined and the variant sequences are marked in green.

CACCCGCAATCCTGCTAACAATGCTGCAATCGTGCTACAACTTCCTCAAGGAACAACATTGCCAAAAGGCTTCTACGCAGAAG
GGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCACGTAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGT
AAACGAACTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTGCTGCTTGACAGATTGAACCAGCT
TGAGAGCAAAATGTTTGGTAAAGGCCAACAACAACAAGGCCAAACTGTCACTAAGAAATCTGCTGCTGAGGCTTCTAAGAAGC
CTCGGCAAAAACGTACTGCCACTAAAGCATACAATGTAACACAAGCT

The impact of the GGG -> AAC and C ->T variants in the forward and reverse *N*-gene primer SARS-CoV-2 sequence, respectively, on the amplification efficiency of the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit was evaluated in a wet testing study. The synthetic RNA concentrations listed in Table 7 were used.

Table 7. The concentrations of the synthetic RNA control used in the wet testing study.

UK variant synthetic RNA concentration	Copies per ul (20ul reaction)	Study sample concentration
75 copies/reaction	3.75 copies / ul	3.75 x LoD

UK variant synthetic RNA concentration	Copies per ul (20ul reaction)	Study sample concentration
150 copies/reaction	7.5 copies / ul	7.5 x LoD
10 000 copies/reaction	500 copies / ul	500 x LoD (concentration equivalent to the 3501-0010 kit's CoV2 Positive Control)

The plate contained synthetic RNA at a concentration of 75, 150 and 10,000 copies/reaction. These samples were run side by side with samples containing the original SARS-CoV-2 sequence to obtain a direct comparison of Ct values. All synthetic RNA samples were tested in three replicates, and the plate also included 3 replicates of the Negative Control from the kit. The assay protocol described in the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit insert was run using the QuantStudio Dx Real-Time PCR instrument.

The Ct-values for all test samples are shown in Table 8.

Table 8. The measured Ct-values for synthetic RNA (original SARS-CoV-2 and GGG -> AAC variant), Undet. = Undetermined.

Synthetic RNA concentration (copies/reaction)	Synthetic RNA sequence	N-gene		
		Ct	Average	SD
Negative Control	**N/A**	Undet.	N/A	N/A
		Undet.		
		Undet.		
75	GGG -> AAC **variant**	31.667	31.7	0.1
		31.745		
		31.776		
150	GGG -> AAC **variant**	30.592	30.7	0.1
		30.693		
		30.675		
10 000	GGG -> AAC **variant**	24.978	24.9	0.1
		24.873		
		24.941		
75	**Wild type**	32.641	33.4	0.7
		33.892		
		33.652		
150	**Wild type**	32.974	32.5	0.5
		32.518		
		32.055		
10 000	**Wild type**	26.781	26.7	0.1
		26.761		
		26.633		

Figure 2 shows the measured mean *ORF1ab* and *N* Ct-values in the RT-qPCR analysis with the wild-type and the GGG -> AAC (UK)-variant of SARS-CoV-2.

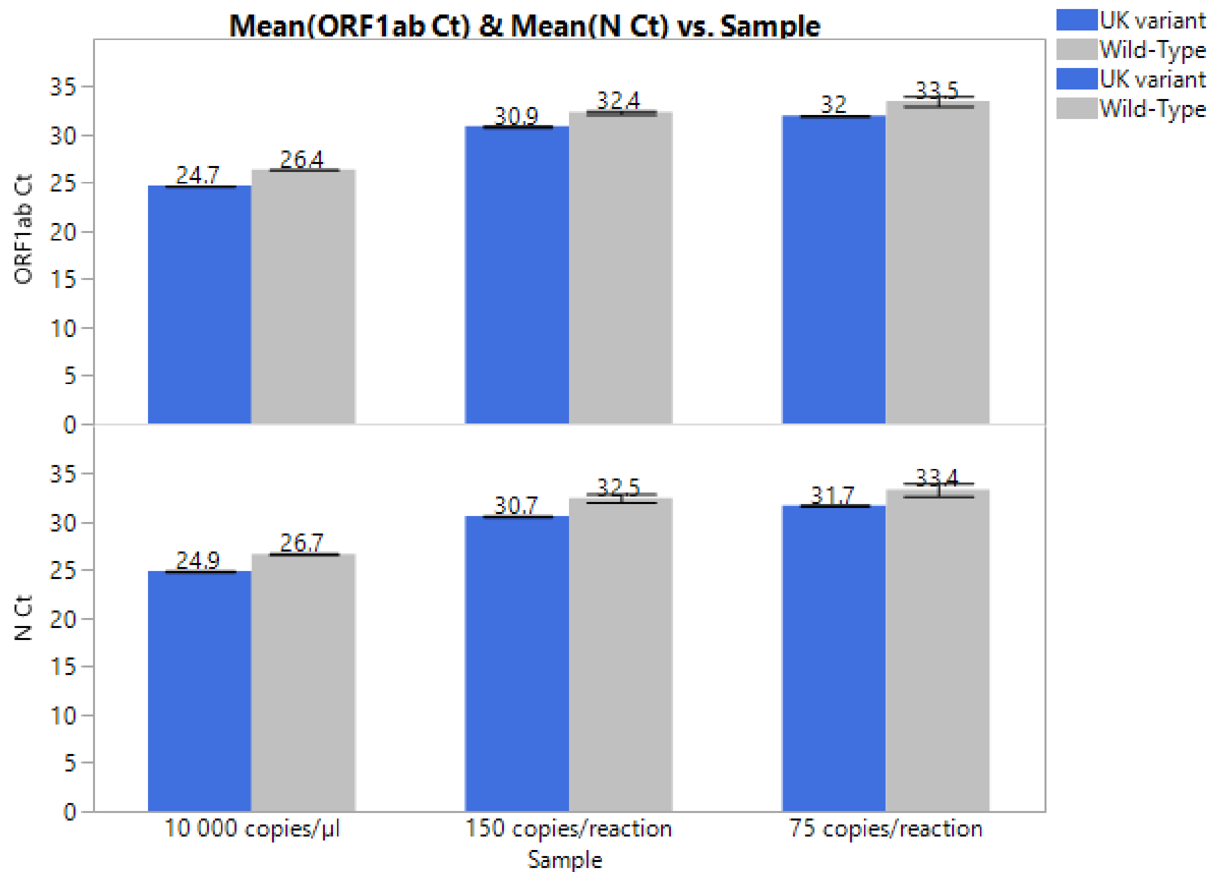

Figure 2. Mean Ct-values for ORF1ab and N vs. the sample concentrations.

The difference in the measured *N* - *ORF1ab* Ct-values between the GGG -> AAC (UK)-variant and the wild type SARS-CoV-2 were not statistically significant (p>0.05).

Comparing the Ct values in the wild-type and variant strains demonstrated that the ability of the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit to detect the UK-variant (GGG -> AAC and C ->T variants in the forward and reverse *N*-gene primer SARS-CoV-2 sequence, respectively) is comparable to the ability to detect the wild type SARS-CoV-2 sequence.

3) Cross-reactivity (Analytical Specificity):

Cross-reactivity of the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit was evaluated using *in silico* analysis.

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

The % homology of the primer and probes for each assay gene target are summarized in Table 9. Although there is ≥80% homology for the forward primer and probe for SARS-coronavirus and SARS-CoV-2 ORF1ab and N genes, there are significant differences in the reverse primers, so amplification is unlikely to occur. In addition, there are also significant differences within the probe binding site at the 5' end of SARS-coronavirus N gene. This will also reduce the risk of detection by the TaqMan assay.

In summary, none of the organisms evaluated in the in-silico analysis exhibited >80% for both primers and probe for the assay target genes.

Table 9. 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

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
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

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
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

4) Clinical Evaluation

Nasopharyngeal clinical samples that were collected in PrimeStore MTM from patients suspected of COVID-19 by their healthcare provider were tested in a single run in this clinical study with the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit. Clinical specimens had been previously tested using one of two different highly sensitive EUA-authorized comparator assays. A total of 30 comparator negative samples, 30 comparator positive samples, 1 negative control, and 1 positive control were tested in this study. The BioRad CFX384 qPCR Real-Time System was used when testing with the PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit.

Samples were first tested with the comparator assays and then tested using the PerkinElmer assay. Among the positive specimens, 46.7% (14/30) were considered to be low positives as defined by the comparator assays.

The performance of the testing can be found in Table 10a and 10b.

Table 10a. Results of Clinical Evaluation of PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit

		EUA-Authorized Comparators	
PerkinElmer SARS-CoV-2 RT-qPCR Reagent Kit		Positive	Negative
	Positive	30	0
	Negative	0	30

Table 10b. Performance Metric Evaluation

Performance Metric	Definition	Score
Positive Percentage Agreement	TP/(TP+FN)	100% (95% CI: 88.7-100%)
Negative Percentage Agreement	TN/(TN+FP)	100% (95% CI: 88.7-100%)

LIMITATIONS:

- This kit is used for qualitative detection of SARS-CoV-2 RNA from RNA extracted from a human oropharyngeal swab and nasopharyngeal and anterior nasal swab sample. The results do not directly reflect the viral load in the original specimens.
- This kit is only applicable to specimen types described in the section "INTENDED USE". Testing other types of specimen may cause inaccurate results. The specimens to be tested shall be collected, processed, stored and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
- The limit of detection (LoD) is determined based on a 95% confidence of detection. When SARS-CoV-2 presents at or above the LoD concentration in the test specimen, there is a low probability that SARS-CoV-2 is not detected. When SARS-CoV-2 presents below the LoD concentration in the test specimen, there is also a low probability that SARS-CoV-2 can be detected.
- When determining LoD of this kit, a known number of SARS-CoV-2 RNA copies were used.
- The results are only applicable to this kit, and the copy numbers defined by other methods are not necessarily equivalent.
- Primers and probes for this kit target highly conserved regions within the genome of SARS-CoV-2. Mutations occurring in these highly conserved regions, although rare, may result in RNA being undetectable.
- This kit uses an UNG/dUTP PCR products carryover prevention system that can be effective in preventing contamination caused by PCR products. However, in the actual operation process, only by strictly following the instructions of PCR laboratories can PCR contamination be avoided.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs on assay performance have not been evaluated.
- Laboratories are required to report all results to the appropriate public health authorities.
- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

WARNINGS:

- This test has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by the authorized laboratory;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.