EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
LILLY SARS-CoV-2 ASSAY

(The Lilly SARS-CoV-2 Assay will be performed at the Eli Lilly Clinical Diagnostics Laboratory, located at located at Lilly Corporate Center 98A/3C, Indianapolis, IN 46285, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a that meets requirements to perform high-complexity tests, per the Laboratory Standard Operating Procedure that was reviewed by the FDA under this EUA).

INTENDED USE

The Lilly SARS-CoV-2 Assay is a real-time *in vitro* diagnostic RT-PCR test intended for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes and bronchoalveolar lavage (BAL) fluid from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Eli Lilly and Company Clinical Diagnostics Laboratory, located at located at Lilly Corporate Center 98A/3C, Indianapolis, IN 46285, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity tests.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in 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 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 Lilly SARS-CoV-2 Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays and *in vitro* diagnostic procedures. The Lilly SARS-CoV-2 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Lilly SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The SARS-CoV-2 primer and probe set is intended to detect SARS-CoV-2 RNA in upper respiratory specimens and BAL fluid from patients suspected of COVID-19 by their healthcare provider. The assay is designed to amplify and detect two different regions of the SARS-CoV-2 nucleocapsid gene (N1 and N2),

together with endogenous human RNase P nucleic acid as an internal control. Nucleic acid extraction is performed using the QIAGEN QIAamp 96 Virus QIAcube HT kit.

INSTRUMENTS FOR USE WITH THE TEST

PCR Instruments	Applied Biosystems QuantStudio 7 Flex Real-Time PCR System (software v1.3)
	Applied Biosystems QuantStudio Dx Real-Time PCR System (software v1.0.3)
	Applied Biosystems QuantStudio 12K Flex Real-Time PCR System (software v1.3)
Sample Processing	QIAGEN QIAcube HT (software v1.1.0.39)
	Hamilton MicroLab STARlet (software v4.5)
Liquid Handling	Hamilton MicroLab STARlet (software v4.5)

REAGENTS AND MATERIALS

The Lilly SARS-CoV-2 Assay uses the CDC N1 and N2 primer and probe sets targeting the nucleocapsid "N" gene of SARS-CoV-2, and the endogenous human RNase P gene as a control to monitor for specimen adequacy and nucleic acid recovery (**Table 1**). A list of all the reagents and materials required to perform the test is provided in **Table 2**.

Table 1. Primer and probe sequences used in the Lilly SARS-CoV-2 Assay

Target	Designation	5'-3' Sequence
N1	Forward	GACCCCAAAATCAGCGAAAT
	Reverse	TCTGGTTACTGCCAGTTGAATCTG
	Probe	**FAM**-ACCCCGCATTACGTTTGGTGGACC-**BHQ1**
N2	Forward	TTACAAACATTGGCCGCAAA
	Reverse	GCGCGACATTCCGAAGAA
	Probe	**FAM**-ACAATTTGCCCCCAGCGCTTCAG-**BHQ1**
RNase P	Forward	AGATTTGGACCTGCGAGCG
	Reverse	GAGCGGCTGTCTCCACCAGT
	Probe	**FAM**-TTCTGACCTGAAGGCTCTGCGCG-**BHQ1**

Table 2. Reagents and materials required to perform the Lilly SARS-CoV-2 Assay

Reagent Name	Vendor	Catalog# or ID#
QIAamp 96 Virus QIAcube HT kit	QIAGEN	57731
QIAcube HT Plasticware	QIAGEN	950067
Isopropanol	Example: Thermo Scientific	Example: A416-500
Ethanol 96-100%; 200-proof absolute	Sigma-Aldrich	E7023-500ML
Ethanol 70%	Example: Decon Labs	Example: 2401
Cell Line: HEL 92.1.7 25,000 cell/mL	ATCC	N/A
Decon QUAT 100	Example: Veltek Associates, Inc	Example: DQ100-06-16Z-01
CO-RE Tips	Hamilton	235940
Sterile Tube Caps	Example: Corning	Example: 14-959-53A

Reagent Name	Vendor	Catalog# or ID#
120 mL reagent reservoir	QIAGEN	194052
60 mL reagent reservoir	QIAGEN	194051
96 groove reservoir	Example: Thermo Scientific	Example: 1064-15-8
Costar 96 Well Lid	Corning	3931
DEPC-Treated Water	Applied Biosystems / Ambion	AM9906 (may substitute)
TaqPath 1-step RT-qPCR Master Mix	Thermo Fisher	A15299 or A15300
2019-nCoV CDC qPCR Probe Assay	IDT	10006606
nCoVPC	IDT	10006625
Pipette tips - PCR clean/filter	Eppendorf	Example: 22491229
Optical Adhesive Film, PCR compatible, DNA/RNA/RNase-free	Applied Biosystems	Example: 4311971
1.5 mL or 5 mL Centrifuge tubes - DNA LoBind	Eppendorf	Example: 22431021
96 well plate	Nunc	24994
384 well reaction plate with RNA barcode	Applied Biosystems	4343814
Hamilton STARlet 1 mL tips	Hamilton	235904
Hamilton STARlet 384 50 µL tips	Hamilton	235447

CONTROLS

Assay controls are run concurrently with all test samples.

Positive Template Control:
The Positive Template Control is comprised of 2019-nCoV_N_Positive Control plasmid from Integrated DNA Technologies (Cat. #10006625) that contains a cloned copy of the nucleocapsid ("N") gene targeted by the N1/N2 primer/probe sets. The control plasmid is used at a concentration of 5×10^5 copies/reaction.

Negative Template Control:
The Negative Template Control is molecular grade water that is added to one well during the RT-PCR reaction set up.

Extraction Control:
The Extraction Control is comprised of human cell line HEL 92.1.7 (ATCC TIB-180) and is extracted concurrently with test samples. The Extraction Control contains 2,500 cells/mL in RNAlater Stabilization Solution.

Internal Control:
The assay includes primers and a probe for detection of the endogenous human RNase P gene to monitor for specimen adequacy and nucleic acid recovery.

INTERPRETATION OF RESULTS

Table 3 summarizes the expected results for the assay external controls. The Positive, Negative and Extraction Controls must produce the expected results in order to

interpret the results from patient samples. A summary of the method of result interpretation for patient samples is show in **Table 4**.

Table 3. Expected results for the external controls for the Lilly SARS-CoV-2 Assay

Control	N1	N2	RNase P
Positive Template Control	Positive Ct < 45	Positive Ct < 45	Negative No Ct
Negative Template Control	Negative No Ct	Negative No Ct	Negative No Ct
Extraction Control	Negative No Ct	Negative No Ct	Positive Ct < 45

Table 4. Summary of result interpretation for the Lilly SARS-CoV-2 Assay [1]

N1	N2	RNase P	Interpretation
Positive Ct < 45	Positive Ct < 45	Positive/Negative Ct < 45 or No Ct	*Positive for SARS-CoV-2.* RT-PCR has detected the presence of SARS-CoV-2 RNA. This does not rule out co-infection with other infectious pathogens. Clinical correlation is necessary to put this finding into the appropriate diagnostic and therapeutic context.
Positive Ct < 45	Negative No Ct	Positive/Negative Ct < 45 or No Ct	
Negative No Ct	Positive Ct < 45	Positive/Negative Ct < 45 or No Ct	
Negative No Ct	Negative No Ct	Positive Ct < 45	*Negative for SARS-CoV-2*. RT-PCR has not detected the presence of the SARS-CoV-2 RNA in the patient's specimen. 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, epidemiological information, and any other diagnostic testing to place this finding into the appropriate diagnostic and therapeutic context.
Negative No Ct	Negative No Ct	Negative No Ct	*Invalid.* RT-PCR for SARS-CoV-2 RNA could not be evaluated. Common causes may include inhibitors of PCR present in the specimen, inadequate sampling, invalid specimen type, or other causes. If clinical suspicion for SARS-CoV-2 infection remains, another specimen should be obtained and submitted for this patient.

[1] All PCR amplification curves are reviewed for clear evidence of amplification prior to reporting results

PERFORMANCE EVALUATION

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

Estimation and Confirmation of the LoD

The limit of detection (LoD) of the Lilly SARS-CoV-2 Assay was initially determined using Positive Control plasmid DNA that was added to viral transport medium at concentrations of 10^6, 5 x 10^5, 10^5 and 5 x 10^4 copies/mL and tested in triplicate. The lowest concentration at which all three replicates produced positive results was 10^5 copies/mL. The plasmid LoD was confirmed by testing an additional 20 replicates at the estimated LoD concentration, all of which produced positive results.

Because plasmid DNA is not representative of viral RNA and does not require reverse transcription prior to PCR amplification, the LoD of the Lilly SARS-CoV-2 Assay was redetermined using packaged SARS-CoV-2 RNA (SeraCare Milford, MA) that was diluted to concentrations of 10^5, 10^4, and 10^3 copies/mL in viral transport medium and tested in triplicate. The lowest concentration at which all three replicates produced a positive result with both the N1 and N2 primer/probe sets was 10^3 copies/mL. The estimated LoD with packaged viral RNA was then confirmed by testing an additional 20 replicates at the estimated LoD concentration. All 20 replicates (100%) were reported positive for both the N1 and N2 targets, with mean Ct values of 34.9 and 36.4 for the N1 and N2 targets, respectively. The LoD was therefore confirmed to be 10^3 copies of packaged viral RNA/mL of viral transport medium.

Nucleic Acid Extraction Bridging Studies

Testing was performed to demonstrate that the Lilly SARS-CoV-2 Assay would produce similar results with RNA extracted from clinical specimens on different QIAGEN QIAcube HT instruments and using a Hamilton Microlab STARlet. for automation of the QIAamp 96 Virus QIAcube HT Kit workflow. Five known SARS-CoV-2 positive and five known SARS-CoV-2 negative nasopharyngeal swab specimens were processed using each instrument system and tested, together with appropriate controls. Included in the study were four QIAcube HT instruments and three Hamilton STARlet robots.

In general, there was a good correlation between the Ct values observed for each of the targets (N1, N2 and RNase P) across the different extraction instruments (**Figure 1**; **Table 5**). However, Sample #4 was positive for N1 and N2 from only 2/4 extractions performed with the QIAcube and gave positive results for N2 from only 1/3 extractions performed with the Hamilton STARlet. and none (0/3) of the extractions was positive for N1. The mean Ct values obtained with Sample #4 with the QIAcube were 36.1 and 37.9 for N1 and N2, respectively, indicating the presence of a low concentration of viral RNA, which is the likely explanation for the variable performance with this sample. Overall, the results from the bridging study with alternative automated extraction instruments were determined to be acceptable.

Figure 1. Correlation of mean Ct values obtained with RNA extracted using the QIAGEN QIAcube HT and Hamilton STARlet instruments

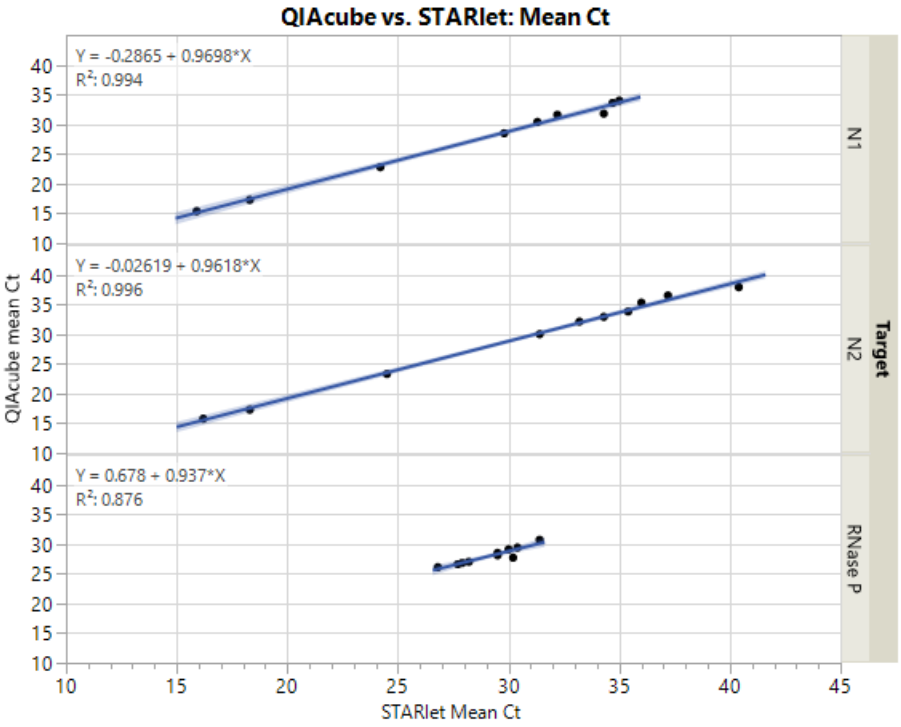


Table 5. Summary of results from bridging studies with different automated instruments for nucleic acid extraction using the QIAamp 96 Virus QIAcube HT Kit

N1 Target						
Sample	**QIAGEN QIAcube HT**		**Hamilton STARlet**		**All Instruments Combined**	
	Positive	**Mean (SD)**	**Positive**	**Mean (SD)**	**Positive**	**Mean (SD)**
1	4/4	15.4 (0.76)	3/3	15.9 (0.98)	7/7	15.6 (0.83)
2	4/4	31.6 (0.49)	3/3	32.2 (0.55)	7/7	31.8 (0.57)
3	4/4	28.5 (0.52)	3/3	29.8 (0.98)	7/7	29.0 (0.96)
4	2/4	36.1 (0.17)	0/3	N/A	2/7	36.1 (0.17)
5	4/4	33.6 (0.71)	3/3	34.7 (0.52)	7/7	34.1 (0.85)
6	4/4	22.8 (0.85)	3/3	24.2 (1.15)	7/7	23.4 (1.17)
7	4/4	17.3 (0.81)	3/3	18.3 (0.97)	7/7	17.7 (0.96)
8	4/4	31.8 (1.10)	3/3	34.3 (2.73)	7/7	32.9 (2.23)
9	4/4	34.0 (0.70)	3/3	35.0 (0.98)	7/7	34.4 (0.89)
10	4/4	30.4 (0.61)	3/3	31.3 (0.87)	7/7	30.8 (0.80)
N2 Target						
Sample	**QIAGEN QIAcube HT**		**Hamilton STARlet**		**All Instruments Combined**	
	Positive	**Mean (SD)**	**Positive**	**Mean (SD)**	**Positive**	**Mean (SD)**
1	4/4	15.8 (0.37)	3/3	16.2 (0.93)	7/7	15.9 (0.63)
2	4/4	32.9 (0.57)	3/3	34.3 (0.74)	7/7	33.5 (0.96)
3	4/4	30.0 (0.39)	3/3	31.4 (0.81)	7/7	30.6 (0.90)
4	2/4	37.9 (0.09)	1/3	40.4 (N/A)	4/7	38.7 (1.47)
5	4/4	35.3 (1.00)	3/3	36.0 (1.15)	7/7	35.6 (1.05)
6	4/4	23.3 (0.94)	3/3	24.5 (0.98)	7/7	23.8 (1.09)
7	4/4	17.3 (0.60)	3/3	18.3 (1.09)	7/7	17.8 (0.92)
8	4/4	33.8 (0.84)	2/3	35.4 (1.82)	6/7	34.3 (1.33)
9	4/4	36.5 (0.49)	3/3	37.2 (1.06)	7/7	36.8 (0.80)
10	4/4	32.1 (0.19)	3/3	33.2 (1.07)	7/7	32.6 (0.89)
RNase P Target						
Sample	**QIAGEN QIAcube HT**		**Hamilton STARlet**		**All Instruments Combined**	
	Positive	**Mean (SD)**	**Positive**	**Mean (SD)**	**Positive**	**Mean (SD)**
1	4/4	26.8 (2.01)	3/3	27.9 (1.23)	7/7	27.3 (1.69)
2	4/4	28.5 (0.66)	3/3	29.5 (0.31)	7/7	28.9 (0.70)
3	4/4	27.0 (0.62)	3/3	28.2 (0.65)	7/7	27.5 (0.86)
4	4/4	28.1 (1.30)	3/3	29.5 (0.65)	7/7	28.7 (1.25)
5	4/4	26.6 (0.63)	3/3	27.7 (0.35)	7/7	27.1 (0.76)
6	4/4	26.1 (0.88)	3/3	26.8 (1.75)	7/7	26.4 (1.25)
7	4/4	29.1 (1.00)	3/3	30.0 (0.94)	7/7	29.5 (1.00)
8	4/4	27.7 (0.87)	3/3	30.2 (1.31)	7/7	28.8 (1.61)
9	4/4	29.4 (1.16)	3/3	30.4 (0.58)	7/7	29.9 (1.03)
10	4/4	30.7 (0.71)	3/3	31.4 (0.81)	7/7	31.0 (0.79)

N/A: Not Applicable; SD: Standard Deviation

PCR Instrument Bridging Studies

Studies were conducted to demonstrate that the Lilly SARS-CoV-2 Assay performs equivalently with alternative PCR instrument systems. A total of 70 contrived samples containing different levels of SARS-CoV-2 RNA (SeraCare) were tested on

each system. The results are summarized in **Table 6** and show that similar Ct values were obtained at each target level with each PCR instrument.

Table 6. Summary of results from bridging studies with alternative PCR instrument systems using contrived specimens

Sample		QuantStudio Instrument					
		Dx		12K Flex		7 Flex	
		N1	N2	N1	N2	N1	N2
1X LoD	Positive (%)	19/20 (95.0)	20/20 (100)	20/20 (100)	20/20 (100)	20/20 (100)	19/20 (95.0)
	Mean Ct (SD)	34.8 (0.96)	36.4 (1.39)	34.7 (1.03)	35.8 (0.78)	34.8 (0.82)	35.9 (0.81)
5X LoD	Positive (%)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)
	Mean Ct (SD)	32.3 (0.14)	33.0 (1.00)	32.0 (0.08)	33.6 (0.41)	32.0 (0.09)	33.0 (0.09)
10X LoD	Positive (%)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)
	Mean Ct (SD)	30.6 (0.13)	31.6 (0.05)	30.6 (0.44)	31.9 (0.36)	31.0 (0.04)	31.6 (0.18)
25X LoD	Positive (%)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)
	Mean Ct (SD)	30.1 (0.14)	30.8 (0.23)	29.9 (0.08)	31.0 (0.40)	30.2 (0.31)	31.1 (0.32)
50X LoD	Positive (%)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)
	Mean Ct (SD)	28.8 (0.21)	29.7 (0.64)	29.0 (0.01)	29.9 (0.08)	29.2 (0.17)	30.1 (0.40)
100X LoD	Positive (%)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)
	Mean Ct (SD)	27.6 (0.14)	28.4 (0.12)	27.7 (0.01)	28.5 (0.10)	27.8 (0.03)	28.6 (0.12)
Negative	Positive (%)	0/30 (0)	0/30 (0)	0/30 (0)	0/30 (0)	0/30 (0)	0/30 (0)
	Mean Ct (SD)	N/A	N/A	N/A	N/A	N/A	N/A

SD: Standard Deviation

In addition to the contrived specimens, 5 known SARS-CoV-2 positive and 5 known SARS-CoV-2 negative specimens were also tested using each PCR instrument system (QuantStudio Dx, 12K Flex and 7 Flex). The mean and standard deviation of the Ct values obtained for the N1, N2 and RNase P targets across the three PCR instruments are listed in **Table 7**. The results show that there was negligible difference in Ct values between the different systems.

Table 7. Summary of results from bridging studies with alternative PCR instrument systems using natural clinical specimens

Clinical Specimen	Mean Ct Value (SD) [1]		
	N1	N2	RNase P
1	20.7 (0.12)	20.7 (0.37)	27.5 (0.14)
2	35.5 (0.90)	35.3 (0.73)	27.0 (0.08)
3	26.6 (0.08)	27.2 (0.16)	25.4 (0.11)
4	25.0 (0.11)	24.7 (0.27)	22.6 (0.03)
5	21.1 (0.04)	20.9 (0.20)	25.5 (0.04)
6	N/A	N/A	23.1 (0.15)
7	N/A	N/A	24.2 (0.11)
8	N/A	N/A	23.4 (0.04)
9	N/A	N/A	22.4 (0.09)
10	N/A	N/A	27.3 (0.14)

SD: Standard Deviation; N/A: Not applicable

[1] Each specimen was tested once on each PCR instrument system (Applied Biosystems QuantStudio Dx, 12K Flex and 7 Flex)

Note: The same specimens using were also tested using a QuantStudio Dx Real-Time PCR System as part of the Clinical Evaluation

The PCR instrument bridging study showed that the Lilly SARS-CoV-2 Assay can be performed on each of the Applied Biosystems QuantStudio Real-Time PCR Systems that was evaluated without detriment to performance.

2) Inclusivity (Analytical Sensitivity):

The target sequences for the Lilly SARS-CoV-2 Assay are the N1 and N2 regions of the viral nucleocapsid gene and the endogenous RNase P internal control from the CDC 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel.

The CDC has granted a Right of Reference to the performance data contained in the CDC's EUA request to any entity seeking an FDA EUA for a COVID-19 diagnostic device. *In silico* analysis of primer and probe inclusivity was performed by CDC using all the publicly available nucleic acid sequences for 2019-nCoV that were deposited in GenBank as of February 1, 2020. All the alignments showed 100% homology to the available 2019-nCoV sequences except for one nucleotide mismatch with the N1 forward primer in one deposited sequence.

3) Cross-reactivity (Analytical Specificity):

In silico BLASTn analysis of primer and probe specificity was performed by CDC, Details are provided in the Package Insert for the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. No significant homology was identified with the human genome, other coronaviruses or human microbial flora that is predicted to cause false positive results with the Lilly SARS-CoV-2 Assay. Additional analysis conducted by Eli Lilly in March 2020, confirmed CDC's original conclusions.

As noted above, the CDC has granted a right of reference to the performance data contained in the CDC's EUA request to any entity seeking an FDA EUA for a COVID-19 diagnostic device.

4) Clinical Evaluation:

All testing described in this section was performed using an Applied Biosystems QuantStudio Dx Real-Time PCR System.

Contrived Samples

The performance of the Lilly SARS-CoV-2 Assay was initially evaluated using contrived samples consisting of packaged viral RNA (Seracare) that was diluted in viral transport medium (**Table 8**). An additional 30 SARS-CoV-2 contrived negative samples containing 5,000 cultured erythroblasts/mL (HEL 92.1.7 ATCC TIB-180) were also tested. All samples containing SARS-CoV-2 RNA at ≥1,000 copies/mL were reported as positive for both the N1 and N2 targets whereas all contrived negative samples were reported as negative for SARS-CoV-2 (mean RNase P Ct = 24.5; standard deviation = 0.06).

Table 8. Summary of results from testing contrived specimens using the Lilly SARS-CoV-2 Assay on the Applied Biosystems QuantStudio Dx Real-Time PCR System

Target Level (copies/mL)	Positive/Tested	Mean Ct (SD)	
		N1	N2
1,000 [1]	20/20 (100)	34.9 (0.72)	36.4 (0.98)
5,000	2/2 (100)	31.7 (0.19)	33.2 (0.03)
10,000	2/2 (100)	30.9 (0.00)	31.7 (0.02)
25,000	2/2 (100)	30.1 (0.02)	31.3 (0.47)
50,000	2/2 (100)	28.9 (0.19)	30.4 (0.18)
100,000	2/2 (100)	27.9 (0.04)	28.9 (0.03)

SD: Standard Deviation

[1] The same samples were used to confirm the estimated LoD as described above

Clinical Specimens

The performance of the Lilly SARS-CoV-2 Assay was evaluated using a total of 29 clinical nasopharyngeal swab specimens. Included were 5 SARS-CoV-2 positive and 4 SARS-CoV-2 negative specimens provided by the Indiana State Health Department (ISDH) that were characterized using the FDA-authorized CDC assay, as well as 10 SARS-CoV-2 positive and 10 SARS-CoV-2 negative specimens that were identified by Eli Lilly and sent to ISDH for confirmatory testing. A summary of the qualitative test results is presented in **Table 9** and a comparison of the Ct values between the Eli Lilly and ISDH assay is shown in **Figure 2**. Positive agreement for the Lilly assay in comparison to the results obtained by ISDH was 100% (14/14); negative agreement was 93.3% (14/15).

The one discrepancy observed between the Lilly and ISDH assays was with a sample that was initially identified by Eli Lilly as positive for the N1 target with a Ct value of 39.7 and negative for N2 (Ct cut-off is 45). Subsequent retesting with the Lilly assay

gave Ct values of 33.7 and 36.9 for N1 and N2, respectively. The sample was sent to ISDH for confirmation and was reported as SARS-CoV-2 negative. Based on the Ct values observed with the Lilly assay, it appears that this sample may have contained low concentrations of viral RNA at or below the limit of detection of both the Lilly and comparator assays. Freezing and thawing of the sample between testing at Eli Lilly and with the comparator assay may also have contributed to degradation of the target nucleic acid.

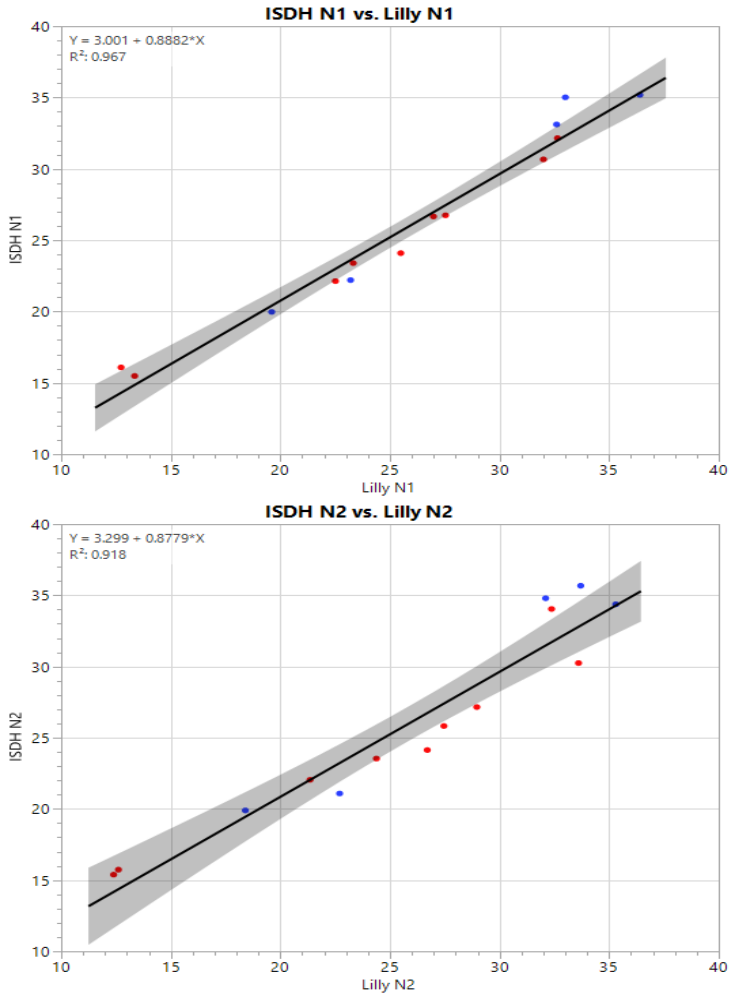
Table 9. Clinical performance of the Lilly SARS-CoV-2 Assay

		Comparator		
		Positive	**Negative**	**Total**
Lilly SARS-CoV-2 Assay	**Positive**	14	1 [1]	**15**
	Negative	0	14	**14**
	Total	**14**	**15**	**29**
Positive Agreement		**100% (14/14); 78.5-100% [2]**		
Negative Agreement		**93.3% (14/15); 70.2-98.8%**		

[1] This sample had no Ct value for N1 and a Ct value of 39.7 for N2 on initial testing with the Lilly assay. Upon repeat, Ct values of 33.7 and 36.9 were obtained for N1 and N2, respectively, suggesting the presence of low levels of SARS-CoV-2 RNA. Both these results imply the presence of low levels of SARS-CoV-2 RNA, close to the LoD of the assay. An additional freeze-thaw cycle prior to testing may have also degraded the target RNA, leading to negative result with the comparator assay.

[2] Two-sided 95% score confidence interval

Figure 2. Comparison of Ct values obtained with the ISDH and Lilly SARS-CoV-2 Assays for the N1 and N2 targets



Red dots: ISDH (Indiana State Health Department) known positive samples tested at Eli Lilly
Blue dots: Samples originally identified as positive by Eli Lilly, confirmed by ISDH

Ten of the known positive/negative specimens from the Clinical Evaluation were retested as part of the bridging studies to validate alternative PCR instrument systems. The same qualitative PCR results and similar Ct values were obtained for each analyte (N1, N2 and RNase P) on each of the three different PCR instrument systems on which the bridging studies were performed.

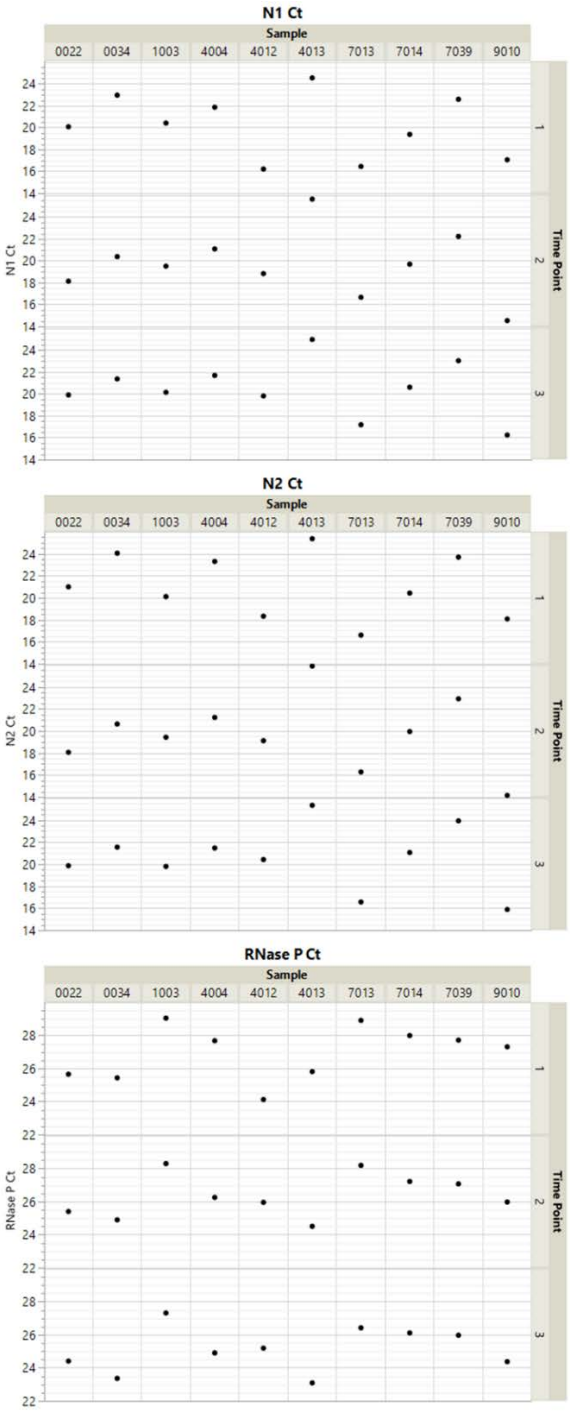
Post -authorization Clinical Evaluation
To supplement the Clinical Evaluation described above, additional characterization of test performance was performed following FDA-authorization of the Lilly SARS-CoV-2 Assay. Thirty (30) residual nasopharyngeal specimens that had previously been characterized with the Lilly SARS-CoV-2 Assay were submitted for confirmatory testing using an FDA-authorized comparator method. Included were 15 SARS-CoV-2 positive and 15 SARS-CoV-2 negative nasopharyngeal swab specimens, as determined using the

Lilly SARS-CoV-2 Assay. The SARS-CoV-2 positive specimens had Ct values for the N1 and N2 targets ranging from 13.7 to 30.1 and 13.5 to 31.3, respectively. All 30 specimens produced the expected results when tested using the comparator method (i.e., 100% positive and negative agreement with two-sided 95% score confidence intervals of 79.6-100%).

Specimen Stability

Due to a shortage of commercially available viral transport medium, Eli Lilly validated the use of an in-house formula of transport medium comprised of Hanks Balanced Salt Solution containing approximately 2% fetal bovine serum and 0.001 µg/mL amphotericin and 0.2 µg/mL gentamycin. To validate the performance of the medium, 10 known positive clinical nasopharyngeal specimens were tested using the Lilly SARS-CoV-2 Assay after storage at 4°C for different periods of time. Each specimen was reported as positive for the N1, N2 and RNase P targets at each test point, with similar Ct values (**Figure 3**). The results of the study demonstrate the stability of clinical nasopharyngeal swab specimens in Eli Lilly viral transport medium for up to 11 days at 4°C.

Figure 3. Stability of SARS-CoV-2 RNA and RNase P nucleic acid in nasopharyngeal swab specimens collected and stored in Eli Lilly viral transport medium



Time points: **1:** day of receipt or +1 day; **2:** +7 to +9 days; **3:** +9 to +11 days
All samples were stored at 4°C for the duration of the study

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA.

The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method used was the Lilly SARS- CoV-2 (Covid-19) Qualitative Detection by RT-PCR Method (described in document 6-EUA200041 Eli Lilly PCR-Extraction Combined SOP 07-27-2020.FINAL) performed on the Hamilton Microlab STARLet (software v4.5), while the RT-PCR was performed on an Applied Biosystems QuantStudio DX Real-Time PCR System (software v1.0.3). The results are summarized in Table 10.

Table 10. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	1.8×10^4 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

WARNINGS

- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- For *in vitro* diagnostic use.
- This test has not been FDA cleared or approved.
- This test has been authorized by FDA under an EUA for use by Eli Lilly and Company Clinical Diagnostics Laboratory, located at Lilly Corporate Center 98A/3C, Indianapolis, IN 46285.
- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.