EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
TEXAS DEPARTMENT OF STATE HEALTH SERVICES (DSHS) SARS-CoV-2 ASSAY
(TEXAS DEPARTMENT OF STATE HEALTH SERVICES, LABORATORY SERVICES SECTION)

Texas Department of State Health Services (DSHS) SARS-CoV-2 Assay

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

(The Texas Department of State Health Services (DSHS) SARS-CoV-2 Assay will be performed at the Texas Department of State Health Services, Laboratory Services Section, located at 1100 W. 49th Street, Austin, TX 78756, that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets the requirements to perform high complexity tests, as described in the Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA).

INTENDED USE

The Texas Department of State Health Services (DSHS) SARS-CoV-2 Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes and bronchoalveolar lavage (BAL) fluid specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Texas Department of State Health Services, Laboratory Services Section located at 1100 W. 49th Street, Austin, TX 78756, which 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 infected 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 positive 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 Texas Department of State Health Services (DSHS) 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 Texas Department of State Health Services (DSHS) SARS-CoV-2 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Texas DSHS SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The assay uses primers and probes that were developed and validated under the Emergency Use Authorization (EUA) for the PerkinElmer New Coronavirus Nucleic Acid Detection Kit and which are designed to detect RNA from the SARS-CoV-2 nucleocapsid (N) gene and ORF1ab. The assay is for use with respiratory specimens from patients suspected of COVID-19 by their healthcare provider.

INSTRUMENTS USED WITH THE TEST

The Texas DSHS SARS-CoV-2 Assay is to be usedwith the PerkinElmer Chemagic 360 (software version 6.3.0.3) and ThermoFisher Applied Biosystems 7500 Fast Dx Real-Time PCR System (software version 1.4). Optionally, the PerkinElmer Janus G3 Automated Workstation (Janus Application Assistant software version 5.5.48.0) can be used to automate the process of aliquoting prepared PCR mix to a 96-well PCR plate and then adding extracted nucleic acid into the same plate containing PCR mix.

REAGENTS AND MATERIALS

Reagent	Catalogue Number	Storage Temperature
Chemagic Viral DNA/RNA 300 Kit H96	PerkinElmer CMG-1033-S or equivalent	15 to 25 °C
New Coronavirus Nucleic Acid Detection Kit	PerkinElmer 2019-nCoV-PCR-AUS or equivalent	-15 to -25 °C
70% ethanol	--	--
RNase Away or equivalent	--	--
Milli-Q Type I Ultrapure Water	--	15 to 25 °C

Equipment and Supplies
Perkin Elmer Chemagic 360 (equipped with software V6.3.0.3)
Biosafety Cabinet (hood)
Sterile pipettes
3 mL Conical Tube
Sharps container
Biohazard waste box (UN3291)
Micropipettors (0.5-10 µL, 10-100 µL, 50-200 µL, and 100-1000 µL)
Multichannel micropipettes (10-100 µL, 30-300 µL, 50-1200 µL)
Troughs
Water bath
Disposable powder-free gloves, surgical gowns
Vortex mixer
Nuclease-free pipette tips
RNase/DNase-free 1.5 ml polypropylene microcentrifuge tubes
Chemagic Viral DNA/RNA 300 Kit required plastic consumables:

Equipment and Supplies
• Chemagic Disposable Tip plate • Chemagic Low-well plate • Chemagic Deep-well plate
Cold Block/Tray with ice bath
Racks for 1.5 mL, 0.5 mL microcentrifuge tubes
Reagent cold blocks
Rack for sample vials
Benchtop Microcentrifuge
Personal Protective Equipment (PPE)
Sealable isolator carrying case
Disposable/reusable plate seals
-70 °C and -20 °C Freezers
4 °C refrigerator
96-well Plate Centrifuge, refrigerated
96-well cold blocks
Water bath
PerkinElmer Janus G3 Workstation equipped with Janus Application Assistant software V5.5.48.0
175 µL conductive filter tips, sterile (PerkinElmer Cat. #6000687)
1.5/2 mL Cooling Tube Holder Set (PerkinElmer Cat. #6001310)
Janus 96-Well Magnet (PerkinElmer Cat. #7002416)
Applied Biosystems 7500 Fast Dx Real-time PCR Instrument with SDSSoftware V1.4
Applied Biosystems MicroAmp Fast Optical 96-Well reaction Plate with Barcode, 0.1 mL (Thermo Fisher Cat. #4346906 or #4366932)
Applied Biosystems Microamp Optical Adhesive Film Covers (Thermo Fisher Cat. #4311971)
Applied Biosystems MicroAmp Optical 8-cap Strip (Thermo Fisher Cat. #4323032)
Sealable, leak-proof carry case

CONTROLS

The PerkinElmer New Coronavirus Nucleic Acid Detection Kit includes the following control materials:

a) nCoV Positive Control:
Comprised of SARS-CoV-2 RNA fragments capsulated in a bacteriophage coat.
Used to monitor the adequacy of nucleic acid extraction andintegrity of the RT-PCR reagents and process. Tested in parallel with every batch of patient samples.

b) nCoV Negative Control:
Comprised of Tris-EDTA (TE) buffer.
Used to monitor for cross-contamination during the nucleic acid extraction and RT-PCR process. Tested in parallel with every batch of patient samples.

c) nCov Internal Control:
Comprised of bacteriophage MS2 in TE buffer.
Used to monitor nucleic acid extraction, reverse transcription, PCR amplification and fluorescence detection. Added to each patient sample and control prior to nucleic acid extraction.

In addition to the controls provided with the assay kit, a No Template Control containing none of the SARS-CoV-2 targets or the Internal Control is included in every PCR run.

INTERPRETATION OF RESULTS

The results from the controls are interpreted according to the criteria shown in **Table 1**. If the results obtained with the Positive, Negative and No Template Controls do not meet the criteria shown, the results from the entire batch of samples are considered invalid and repeat testing must be performed.

Table 1. Acceptable RT-PCR results for controls

Control Type	Acceptable Ct Value		
	N Gene (FAM)	ORF1ab (ROX)	Internal Control (VIC)
Negative	Undetermined or > 42	Undetermined or > 42	Ct ≤ 40
Positive	≤ 35	≤ 35	~ [1]
No Template	Undetermined or > 42	Undetermined or > 42	Undetermined or > 40

[1] No requirements on Ct value

The results from testing of patient samples are interpreted according to the criteria described in **Table 2**.

Table 2. Summary of results interpretation for patient samples

Ct Value			Interpretation
N Gene (FAM)	ORF1ab (ROX)	Internal Control (VIC)	
Both Undetermined or > 42		≤ 40	SARS-CoV-2 not detected
Either or both targets ≤ 42		~ [1]	SARS-CoV-2 detected
Both Undetermined or > 42		> 40 or Undetermined	Invalid [2]

[1] No requirements on Ct value
[2] Specimen needs to be re-tested by re-extracting nucleic acid or collection of a new sample

PERFORMANCE EVALUATION

All PCR plates for the validation studies were set up using PerkinElmer Janus G3 Automated Workstation.

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

The Limit of Detection (LoD) of the Texas DSHS SARS-CoV-2 Assay was determined by testing dilutions of the PerkinElmer nCoV Positive Control bacteriophage that is provided within the assay kit. The LoD was initially estimated by testing three different concentrations of the control in Viral Transport Medium (VTM), Liquid Amies Medium and sterile saline (**Table 3**). [1] The lowest concentration at which all three replicates produced positive results for both the N gene and ORF1ab was 20 copies/mL. This estimated LoD was then confirmed by testing a further 20 replicates in each transport medium. The LoD of the Texas DSHS SARS-CoV-2 Assay was confirmed to be 20 copies of the Positive Control bacteriophage/mL medium. No difference in analytical sensitivity was observed between the different types of transport medium.

Table 3. Estimation of the Texas DSHS SARS-CoV-2 Assay LoD

Transport Medium	Copies/mL	N Gene		ORF1ab	
		Positive (%)	Mean Ct (SD)	Positive (%)	Mean Ct (SD)
VTM	7	3/3 (100)	35.8 (0.36)	2/3 (67) [1]	36.7 (0.74)
	20	3/3 (100)	34.9 (0.50)	3/3 (100)	35.8 (0.28)
	60	3/3 (100)	33.5 (0.27)	3/3 (100)	35.1 (0.19)
Liquid Amies Medium	7	3/3 (100)	35.6 (0.55)	3/3 (100)	33.5 (0.39)
	20	3/3 (100)	34.7 (0.63)	3/3 (100)	32.8 (0.80)
	60	3/3 (100)	33.1 (0.07)	3/3 (100)	31.7 (0.35)
Sterile Saline	7	2/3 (67) [1]	37.2 (1.45)	3/3 (100)	34.2 (0.43)
	20	3/3 (100)	35.0 (0.10)	3/3 (100)	33.2 (0.17)
	60	3/3 (100)	33.2 (0.41)	3/3 (100)	32.1 (0.36)

SD: Standard Deviation; VTM: Viral Transport Medium

[1] 1/3 samples gave a result of "Undetermined" for this analyte; however, based on the result algorithm and detection of the other SARS-CoV-2 target, the sample was still reported as "Positive for SARS-CoV-2"

Table 4. Confirmation of the Texas DSHS SARS-CoV-2 Assay LoD

Transport Medium	Copies/mL	N Gene		ORF1ab	
		Positive (%)	Mean Ct (SD)	Positive (%)	Mean Ct (SD)
VTM	20	20/20 (100)	34.6 (0.68)	20/20 (100)	32.9 (0.62)
Liquid Amies Medium	20	20/20 (100)	34.6 (0.47)	20/20 (100)	32.6 (0.66)
Sterile Saline	20	20/20 (100)	35.1 (1.29)	20/20 (100)	33.5 (1.17)

[1] The study was performed using sterile transport medium in the absence of clinical matrix. This approach was sanctioned in an email from FDA (CDRH-EUA-Templates) to Texas DSHS dated 03/30/2020.

2) Inclusivity (Analytical Sensitivity):

The Texas DSHS SARS CoV-2 Assay is a modification of the previously authorized PerkinElmer New Coronavirus Nucleic Acid Detection Kit. The assay targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene and ORF1ab region. The inclusivity of the assay was demonstrated by PerkinElmer under the original EUA request using BLAST (Basic Local Alignment of Search Tool) analysis against the available SARS-CoV-2 nucleic acid sequences. All the alignments showed 100% identity to the PerkinElmer SARS-CoV-2 primers and probes.

Texas DSHS has obtained a Right of Reference from PerkinElmer to the analytical sensitivity and specificity data and associated summaries included in the original EUA request for the New Coronavirus Nucleic Acid Detection Kit. As a result, Texas DSHS did not perform any additional analysis of the inclusivity or specificity of the New Coronavirus Nucleic Acid Detection Kit primers and probes.

3) Cross-reactivity (Analytical Specificity)

As noted above, the Texas DSHS SARS CoV-2 Assay is a modification of the previously authorized PerkinElmer New Coronavirus Nucleic Acid Detection Kit (EUA200055), and Texas DSHS has obtained a Right of Reference to the analysis of specificity performed in support of PerkinElmer's original EUA request. The specificity of the PerkinElmer assay was demonstrated through a combination of *in silico* analysis of nucleic acid sequences for common respiratory pathogens/flora and laboratory testing. No cross-reactivity with the human genome, other coronaviruses, or human microbial flora was predicted or observed.

4) Clinical Evaluation:

The performance of the Texas DSHS SARS-CoV-2 Assay was evaluated using a combination of contrived positive samples and previously characterized clinical specimens.

Evaluation of Contrived Samples

Contrived SARS-CoV-2 positive clinical samples were prepared by spiking the PerkinElmer nCoV Positive Control bacteriophage-packed RNA into individual SARS-CoV-2 negative clinical specimens. A total of 24 contrived samples were tested at concentrations equivalent to 1 or 2X LoD (20 or 40 copies/mL). All 24 samples (100%) produced the expected result for both the N gene and ORF1ab (**Table 5**). Similar Ct values were observed with nasopharyngeal, oropharyngeal and nasal swabs specimens, and with different transport media (liquid Amies, UTM, VTM).

Table 5. Results from testing contrived SARS-CoV-2 positive clinical specimens with the Texas DSHS SARS-CoV-2 Assay

Copies/mL	Swab Type	Medium	Number (%)		
			Positive	Negative	Invalid
20	Nasopharyngeal	Amies	2 [1]	0	0
		UTM	1	0	0
		VTM	2	0	0
	Oropharyngeal	UTM	2	0	0
		VTM	4	0	0
	Nasal	VTM	1	0	0
	Sub-Total (%)	**All Media**	**12 (100) [2]**	**0 (0)**	**0 (0)**
40	Nasopharyngeal	Amies	2	0	0
		UTM	2	0	0
		VTM	3	0	0
	Oropharyngeal	UTM	4	0	0
		VTM	1	0	0
	Sub-Total (%)	**All Media**	**12 (100) [3]**	**0**	**0**
Total (%)		**All Media**	**24 (100)**	**0 (0)**	**0 (0)**

Amies: Liquid Amies Medium; UTM: Universal Transport Medium; VTM: Viral Transport Medium

[1] 1 sample only gave a positive result for the N gene (Ct = 41.8) and was negative for ORF1ab

[2] Mean Ct values (standard deviation): N Gene = 36.3 (0.92); ORF1a = 36.8 (1.68)

[3] Mean Ct values (standard deviation): N Gene = 35.4 (0.69); ORF1a = 34.9 (0.89)

Evaluation of Known Positive/Negative Clinical Specimens

Additional testing was performed using a total of 47 natural clinical specimens of known SARS-CoV-2 status, as determined using the Centers for Disease Control and Prevention (CDC) 2019-Novel Coronavirus (2019-nCoV) Diagnostic Panel (**Table 6**). There was 100% positive and negative percent agreement between the two assays. In **Table 7**, the results of the study are stratified by specimen type and transport medium. A comparison of the Ct values obtained for the N gene using the Texas DSHS and CDC assays is presented in **Figure 1** and shows a high degree of correlation ($R^2 > 0.90$) over a broad range of Ct values (~16-33).

Table 6. Evaluation of the Texas DSHS SARS-CoV-2 Assay with natural clinical specimens

		Comparator		
		Positive	**Negative**	**Total**
Texas DSHS SARS-CoV-2 Assay	**Positive**	11 [1]	0	**11**
	Negative	0	36	**36**
	Total	**11**	**36**	**47**
Positive Agreement		**100% (11/11); 74.1-100% [2]**		
Negative Agreement		**100% (36/36); 90.4-100%**		

[1] Ct values for the comparator CDC assay ranged from 16.8 to 35.4 for N1 and 17.0 to 34.1 for N2; 3/11 samples (27.3%) had Ct values for both N1 and N2 >33 (**Figure 1**)

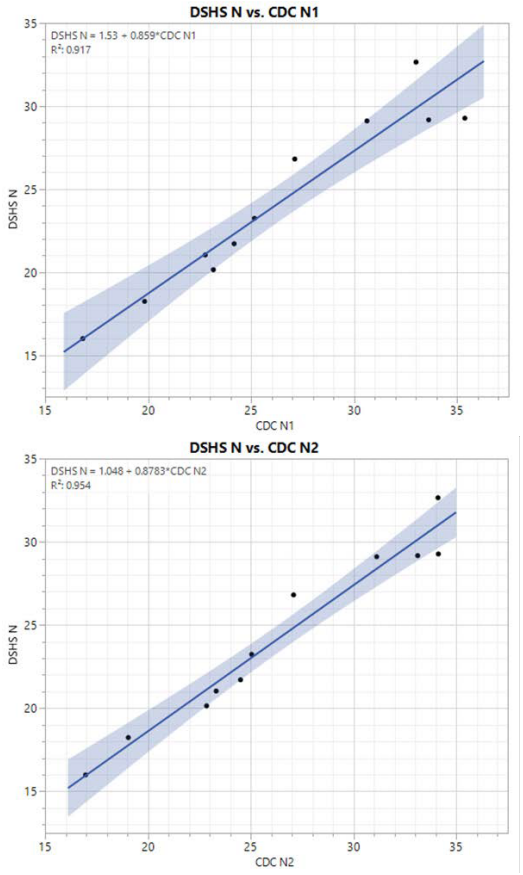
[2] Two-sided 95% score confidence interval

Table 7. Evaluation of the Texas DSHS SARS-CoV-2 Assay with natural clinical specimens, stratified by specimen type and transport medium

Swab Type	Transport Medium	Agreement with Comparator	
		Positive	Negative
Nasal	VTM	0	1/1
Nasopharyngeal	Amies	0	5/5
	UTM	3/3	8/8
	VTM	4/4	8/8
Oropharyngeal	UTM	1/1	8/8
	VTM	2/2	5/5
Nasopharyngeal/Oropharyngeal	VTM	1/1	1/1
Total (%)		**11/11 (100)**	**36/36 (100)**

Amies: Liquid Amies Medium; UTM: Universal Transport Medium; VTM: Viral Transport Medium

Figure 1. Comparison of Ct values obtained with the Texas DSHS SARS-CoV-2 Assay and the CDC 2019-nCoV RT-PCR Diagnostic Panel



Ct cut-offs: Texas DSHS = 42; CDC = 40
3/11 samples (27.3%) exhibited Ct values >33 for both N1 and N2 using the CDC assay
Similar analysis of the Ct values for the ORF1ab target was not performed because the CDC assay does not detect this region of the SARS-CoV-2 genome

Clinical Confirmation

The first 5 positive and first 5 negative nasopharyngeal specimens as determined by the Texas DSHS SARS-CoV-2 Assay were also tested using the previously FDA-authorized CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. There was 100% (5/5) positive and negative agreement for the specimens tested (**Table 8**). These results are acceptable and support use of the by Texas DSHS SARS-CoV-2 Assay for testing clinical specimens.

Table 8. Results obtained in the Clinical Confirmation Study with nasopharyngeal swabs using the Texas DSHS SAR-CoV-2 Assay in comparison to the FDA-authorized CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel

Sample	Transport Medium	Texas DSHS				Comparator (CDC 2019-nCoV)			
		N Gene	ORF1ab	IC	Result	N1	N2	RNase P	Result
1	Amies	25.2	27.5	37.5	Positive	27.6	29.1	22.8	Positive
2	Amies	24.2	23.1	37.3	Positive	26.9	27.9	20.8	Positive
3	Amies	28.7	28.5	37.6	Positive	32.5	33.1	23.0	Positive
4	VTM	14.1	16.0	35.1	Positive	17.3	17.0	23.3	Positive
5	VTM	16.3	15.8	36.0	Positive	17.3	17.1	25.5	Positive
6	VTM	Und.	Und.	37.4	Negative	Und.	Und.	21.1	Negative
7	VTM	Und.	Und.	36.6	Negative	Und.	Und.	21.5	Negative
8	Amies	Und.	Und.	37.2	Negative	Und.	Und.	22.1	Negative
9	Amies	Und.	Und.	36.2	Negative	Und.	Und.	22.3	Negative
10	VTM	Und.	Und.	36.8	Negative	Und.	Und.	28.2	Negative

Amies: Liquid Amies Medium; VTM: Viral Transport Medium; IC: Internal Control; Und.: Undetermined

Table 9 summarizes the overall positive and negative agreement observed by combining the results from retrospective testing of known SARS-CoV-2 positive and negative specimens and prospective testing of the first 5 positive and 5 negative specimens following the completion of assay validation. These results are acceptable.

Table 9. Cumulative results from testing prospectively collected and retrospective natural clinical specimens with the Texas DSHS SARS-CoV-2 Assay

		Comparator		
		Positive	**Negative**	**Total**
Texas DSHS SARS-CoV-2 Assay	**Positive**	16 [1]	0	**16**
	Negative	0	41	**41**
	Total	**16**	**41**	**57**
Positive Agreement		**100% (16/16); 80.6-100% [2]**		
Negative Agreement		**100% (41/41); 91.4-100%**		

[1] 4/16 (25.0%) samples exhibited Ct values >33 for the N1 and/or N2 targets in the comparator CDC assay (**Figure 1** and **Table 8**)

[2] Two-sided 95% score confidence interval

The sponsor will perform an additional evaluation of the clinical performance of the Texas DSHS SARS-CoV-2 Assay in an agreed upon post authorization study.

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 the Texas Department of State Health Services, Laboratory Services Section, located at 1100 W. 49th Street, Austin, TX 78756.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-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 diagnostics 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.

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 was automated using the PerkinElmer Chemagic 360 (software version 6.3.0.3) and the amplification was run on the ThermoFisher Applied Biosystems 7500 Fast Dx Real-Time PCR System. The results are summarized in the following Table.

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

Reference Materials Provide d by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swabs in VTM	$1.8x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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