ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
SARS COV-2 Test
(LABORATORIO CLINICO TOLEDO)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

(The SARS CoV-2 Assay will be performed at Laboratorio Clinico Toledo, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the laboratory procedures that were reviewed by the FDA under this EUA.)

INTENDED USE

The Laboratorio Clinico Toledo SARS-CoV-2 Assay for the diagnostic qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens) and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing location is limited to Laboratorio Clinico Toledo 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 qualitative detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens and bronchoalveolar lavage 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 infective status.

Positive results do not rule out bacterial 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 Laboratorio Clinico Toledo SARS-CoV-2 Assay is intended for use qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Laboratorio Clinico Toledo SARS-CoV-2 Assay is only for use under the Food and Drug

Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The SARS CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The test uses one primer and probe to detect one region in the SARS CoV-2 nucleocapsid N gene, one primer and probe for the detection of SARS like coronavirus E gene and one primer and probe to detect RNAase P (RdRP).

INSTRUMENTS USED WITH TEST

The SARS-CoV-2 assay test is to be performed using following equipment:

- Roche Magna Pure 96 system (Software version 3.1.1) for the extraction process
- Roche Light Cycler 480 (software version LCS480 1.5.16.2 SP@-UDF v 2.0) for the RT-PCR amplification process.

REAGENTS AND MATERIALS

The SARS-CoV-2 assay has been validated using only the components referenced in this submission.

Roche LightMix Modular SARS and Wuhan CoV E- Gene (Catalog #09155368001)
Roche LightMix Modular Wuhan CoV RdRP-gene (Catalog #09155376001)
Roche LightMix Modular SARS and Wuhan CoV N-gene (Catalog #09155350001)
Roche LightMix Modular EAV RNA Extraction Control (Catalog # 66090996)
LightCycler Multiplex RNAVirus Master (Catalog # 0675415500)

Table 1: SARS-CoV-2 Assay Reagents

Item Description	Reference Number	Quantity
SARS-CoV (COVID-19) N-gene	09155350001	96 test
SARS-CoV (COVID-19) E-gene	09155368001	96 test
SARS-CoV-2 (COVID-19) RdRP	09155376001	96 test
EAV Extraction CTRL	07374330001	96 test
Light Cycler Multiplex RNA Virus Master	06754155001	200 reaction s
MagNA Pure 96 DNA and Viral NA Small volume kit	06543522001	3x192 test
CC Hexaplex Plus	06296971001	192 tests
Magna Pure 96 System Fluid (Internal Container)	06430112001	2x1825ml

Item Description	Reference Number	Quantity
MagNA Pure 96 output plate	06241611001	60 plates
Magna Pure Processing cartridge	06441603001	36 cartridges

Table 2: Components required but not included with the test:

Item Description	Reference Number (Roche Diagnostics)
Filter tips	06241620001
Microwell plate and sealing film	05232724001

Testing Capabilities: Sample collection will take approximately 15 minutes per patient. After the sample is taken it takes up to 5 hours to be processed and reported, approximately 4 runs of 96 test can he processed by 8 hours day shift

CONTROLS TO BE USED WITH THE SARS-COV-2 ASSAY

Controls that will be provided with the test kit include:

- A no template (negative) control (NTC): is needed to check contamination of extraction and reagents. Molecular or PCR grade water is used in place of sample nucleic acid for this control. The NTC is used on every run.

- Positive template control: The SARS-CoV-2 (COVID-19) positive control detects for the N, E and RdRp Gene, the positive control should be used in each run.

- Modular EAV RNA Extraction control: The *in-vitro* transcribed EAV (Equine Arteritis Virus) RNA extraction control serves as a control to monitor the SARS CoV-2 E gene and is used in each batch of extraction. It serves as a negative extraction control to monitor any cross contamination that occurs during the extraction process, as well as to validate extraction reagents and successful RNA extraction.

INTERPRETATION OF RESULTS

The SARS-CoV-2 Assay indicates that if any of the controls do not exhibit the expected performance as stated , the assay set up and/or extraction protocol executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and retest.

Table 3: Performance Expectations for Controls

Control Type	External Control Name	Used to Monitor	SARS CoV-2 N Gene	E Gene	RdRP	Expected Ct Values
Positive	Positive Control	Primer and Probe integrity	Positive	Positive	Positive	<37 Ct N <36 Ct E <39 Ct RdRP
Negative	NTC	Reagent or Environmental Contamination	Negative	Negative	Negative	Not Detected
Extraction	Modular EAV RNA Extraction	Improper Extraction of Nucleic acid or cross contamination	Negative	Positive	Negative	<39 Ct

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Table 4: SARS-like CoV E Gene Results

Channel 530 (E Gene Target Reaction)	Channel 660 (Positive Control Reaction)	Channel 530 (NTC Control)	Result
No Amplification	Detected	Negative	Not detected
Amplification Cp<36	Detected	Negative	Sarbecovirus Positive
Amplification Cp<36	Detected	Positive	Contamination – Repeat
No Amplification	Not Detectable	N/A	PCR failure – Repeat

Table 5: SARS-like CoV N Gene Results:

Channel 530 (N Gene Target Reaction)	Channel 660 (Positive Control Reaction)	Channel 530 (NTC Control)	Result
No Amplification	Detected	Negative	Not detected
Amplification Cp<37	Detected	Negative	Sarbecovirus Positive
Amplification Cp<37	Detected	Positive	Contamination – Repeat
No Amplification	Not Detectable	N/A	PCR failure - Repeat

Table 6: 2019-nCoV RdRP Gene Results

Channel 530 (RdRP Target Reaction)	Channel 660 (Positive Control Reaction)	Channel 530 (NTC Control)	Result
No Amplification	Detected	Negative	Not detected
Amplification Cp<39	Detected	Negative	2019-nCoV Positive
Amplification Cp<39	Detected	Positive	Contamination – Repeat
No Amplification	Not Detectable	N/A	PCR failure - Repeat

Table 7: Interpretation of Clinical Samples and Result Reporting

Interpretation		Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 6
Gene Targets	SARS-like CoV E Gene[1]	+	+/-	-	+	+	-	-
	SARS-like CoV N Gene[1]	+	-	+/-	+	-	+	-
	2019-nCoV RdRP[1]	+	+	+	-	-	-	-
Result Interpretation		SARS-CoV-2 Detected			Presumptive Positive Result[2]			SARS-CoV-2 Not Detected

[1]Result determination determined by Tables 3-4 above

[2]Result for SARS-CoV-2 RNA is Presumptive Positive. For samples with a Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.

LIMITATIONS

Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

The performance of SARS-CoV-2 Assay was established using nasopharyngeal swabs. Nasal swabs, mid-turbinate nasal swabs, oropharyngeal swabs and BAL specimens are also considered acceptable specimen types for use with the SARS-CoV-2 Assay but performance has not been established. Testing of nasal and mid-turbinate nasal swabs (self- collected at a healthcare site or collected by a healthcare provider) is limited to individuals suspected of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information. Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences. Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

- False-negative results may arise from:
- Improper sample collection
- Degradation of the viral RNA during shipping/storage
- Using unauthorized extraction or assay reagents
- The presence of RT-PCR inhibitors
- Mutation in the SARS-CoV-2 virus
    - Failure to follow instructions for use
- False-positive results may arise from:
- Cross contamination during specimen handling or preparation
- Cross contamination between patient samples
- Specimen mix-up
- RNA contamination during product handling

The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not yet been evaluated.

Please note, Negative results do not preclude infection of SARS-CoV-2 virus and should not be the sole basis of a patient management decision. A positive result indicates detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable. Laboratories are required to report all positive results to the appropriate public health authorities

Warnings:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratory;

- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

To determine the Limit of Detection (LoD) and analytical sensitivity of the SARS-CoV-2 Assay, studies were performed using serial dilutions of analyte and the LoD was determined to be the lowest concentration of template that could reliably be detected with 95% of all tested positive.

LoD of each target assay in the SARS-CoV-2 Assay was conducted and verified using SARS-CoV-2 RNA (Sera Care Control Catalog #0505-0126). SARS-CoV-2 RNA was spiked at various concentrations (4,162 copies (cp)/mL to 68 cp/mL) in nasopharyngeal swab specimens that were confirmed negative for SARS-CoV-2. Nucleic acid was extracted from the nasopharyngeal swab specimens on the Magna Pure 96 automated system and the reverse transcription and subsequent PCR was performed using Roche Light Cycler 480 System.

Table 8: Determination of Tentative LoD

Concentration	SARS-like CoV E Gene		SARS-like CoV N Gene		2019-nCoV RdRP	
	Mean Ct	Detection Rate	Mean Ct	Detection Rate	Mean Ct	Detection Rate
4,162 cp/mL	31.6	3/3	38.96	2/3	36.31	3/3
1,833 cp/mL	32.9	3/3	38.35	2/3	37.5	3/3
611 cp/mL	34.1	3/3	40	2/3	39.8	3/3
203 cp/mL	**36.2**	**3/3**	**40**	**1/3**	**40.0**	**3/3**
68 cp/mL	39.2	1/3	UND	0/3	40	2/3

The tentative LoD of the SARS-like CoV E gene and the 2019-nCoV RdRP gene was determined to be 203 cp/mL. Given the inability to obtain 100% (3/3) detection rate of the SARS-like CoV N gene at the maximum dilution level of 4,162 cp/mL of the Sera Care Control, serial dilutions of a previously characterized positive nasopharyngeal patient sample for SARS-CoV-2 analyte was used to serve as a standard for LOD determination of the N gene. The patient specimen concentration was determined to be $2.1x10^7$ cp/mL by comparing against the previously generated standard curve. The results are presented in the Table below:

Table 9: Tentative LoD for SARS-CoV-2 N target Determination

Concentration	SARS-like CoV N Gene	
	Mean Ct	Detection Rate
$2.1x10^7$ cp/mL	26.7	3/3
$2.1x10^6$ cp/mL	28.9	3/3
$2.1x10^5$ cp/mL	31.5	3/3
$2.1x10^4$ cp/mL	33.85	3/3
$2.1x10^3$ cp/mL	37.7	1/3
$2.1x10^2$ cp/mL	UND	0/3
$2.1x10^1$ cp/mL	UND	0/3
2.1 cp/mL	UND	0/3

The LOD of the SARS-like CoV E gene and 2019-nCoV RdRP assay targets was confirmed by spiking the lowest detected SARS-CoV-2 RNA (Sera Care Control Catalog #0505-0126) in nasopharyngeal swab specimens negative for SARS-CoV-2. The LoD of the SARS-like CoV N gene assay target was confirmed by spiking quantified SARS-CoV-2 patient sample in nasopharyngeal swab specimens negative for SARS-CoV-2. Nucleic acid was extracted using Magna Pure 96 automated system and the real-time RT-PCR was performed on Roche Light Cycler 480 System. N and RdRP sequences were detected on all 20/20 spiked replicates, E gene was detected 19/20 spiked replicates.

Table 10: LoD Confirmation

Targets	SARS-like CoV E gene	SARS-like CoV N gene	2019-nCoV RdRP
LoD	203 cp/mL	21,000 cp/mL	203 cp/mL
Positive total	19/20	20/20	20/20
Mean Ct	31.78	35.78	39.76
Standard deviation	0.96	1.92	1.07
CV %	3.02	5.37	2.69

2) ***Analytical Specificity:***

a) *Inclusivity*:

The SARS-CoV-2 assay has been designed to detect all publicly available SARS-CoV-2 viral RNA sequences. Target sequences were retrieved from Genbank and other publicly available databases and were aligned to identify conserved regions and also specific regions of the SARS-CoV-2 genome. All the alignments show 100% identity of the assay to the top available SARS-CoV-2 sequences with the 2019-nCoV RdRP gene.

b) *Cross-reactivity*:

Cross-reactivity wet-testing was performed to assess the cross-reactivity of the SARS-CoV-2 assay. Testing was performed with known positive clinical samples with respiratory viruses and bacteria. Each sample was extracted from the on Magna Pure 96 automated system and the reverse transcription RT-PCR was performed 480 Light Cycler Real Time PCR system. The tested organisms all show negative for the three targeted genes of SARS-CoV-2.

Table 11: Organisms Analyzed by Wet Laboratory Testing for Cross Reactivity

Respiratory Pathogens	# Clinical Samples Evaluated	Respiratory Pathogens	# Clinical Samples Evaluated
Human coronavirus 229E	18	Rhinovirus/enterovirus	31
Human coronavirus OC43	16	Respiratory Syncytial Virus (A/B)	33
Human coronavirus HKUl	14	Parainfluenza 1 Virus	12
Human coronavirus NL63	14	Parainfluenza 2 Virus	11
MERS-coronavirus	5	Parainfluenza 3 Virus	14
Influenza A (H1N1)	17	Parainfluenza 4 Virus	11
Influenza A (H3N2)	16	Human metapneumovirus	16
Influenza A (untyped)	11	Adenovirus	13
Influenza A (H5N1)	1	Human bocavirus	6
Influenza A (H7N9)	1	*Legionell*a spp.	3
Influenza B	31	*Mycoplasma pneumoniae*	4

No cross reactivity was observed with respiratory panel organisms

3) Clinical Evaluation:

Evaluation of SARS-CoV-2 Assay Using Contrived Samples

The performance evaluation of SARS-CoV-2 Assay was tested using contrived samples. 20 positive samples at 2x LoD were prepared by spiking SARS-CoV-2 RNA Control (Sera Care Control Catalog #0505-0126) sample into negative NP specimens confirmed negative for SARS-CoV-2 for the SARS-like CoV E gene and 2019-nCoV RdRP assay targets. A quantified SARS-CoV-2 patient sample was used to contrived samples at 2x LoD for SARS-like CoV N gene assay target. Samples were extracted on the Magna Pure 96 automated system and the reverse transcription RT-PCR was performed 480 Light Cycler Real Time PCR system. The results from the SARS-CoV-2 assay are shown in the Table below.

Table 12: Summary of Contrived Clinical Sample Evaluation

Specimen Type	SARS-CoV-2			Performance Agreement	95% CI
	N +	E +	RP +		
Positive Samples 2x LOD Contrived swabs	20/20	19/20	20/20	95%	76.4-100%

Clinical Evaluation of the SARS-CoV-2 Assay

A clinical study was performed to evaluate the performance of the SARS-CoV-2 Assay. A total of 61 clinical specimens (31 negatives and 30 positives) from three laboratories (Memorial Regional Hospital, LabCorp and Yale University) were compared with the TaqPath COVID-19 Combo Kit (EUA200010), the Lab Corp COVID-19 RT PCR test (EUA200010) and the Roche Cobas SARS-COV-2 Assay (EUA 2000094). Samples were extracted on the Magna Pure 96 automated system and the reverse transcription RT-PCR was performed 480 Light Cycler Real Time PCR system. The results are summarized in the Tables below.

Table 13: Clinical Evaluation of the SARS-CoV-2 Assay

SARS-CoV-2 Assay	Comparator Method[1]		Total	% Performance Agreement	95% CI
	Detected	Not Detected			
Detected	30	0	30	PPA 100%	88.6-100%
Not Detected	0	31	31	NPA 100%	89-100%
Total	30/30	31/31	61		

[1]Originally tested in three labs with three different FDA-authorized assays.

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. TRoche Magna Pure 96 system (Software version 3.1.1) for the extraction process and Roche Light Cycler 480 (software version LCS480 1.5.16.2 SP@-UDF v 2.0) for the RT-PCR amplification process were used in this study. The results are summarized in the following Table.

Table 14: 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 Swab	$1.8x10^5$ NDU/mL	N/A
MERS-CoV		N/A	ND

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