University of Louisville Infectious Diseases Laboratory SARS-CoV-2 Assay EUA Summary
Updated: August 31, 2022

ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-CoV-2 real time RT-PCR test
(University of Louisville Infectious Diseases Laboratory)

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

The SARS-CoV-2 real time RT-PCR test will be performed at University of Louisville Infectious Diseases Laboratory certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Laboratory Instructions for Use reviewed by the Office of the Assistant Secretary for Health at the Department for Health and Human Services (DHHS) under this EUA.

Intended Use:

The SARS-CoV-2 assay is a real-time RT PCR (RT-PCR) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal (NP) swabs specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the University of Louisville Infectious Diseases Laboratory that is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263 a certified high complexity laboratory.

Results are for the detection 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 assay is intended for use under the U.S. Department of Health and Human Services-issued Emergency Use Authorization (HHS-EUA).

DEVICE DESCRIPTION AND TEST PRINCIPLE

The assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines. The SARS-CoV-2 real time (rt) RT-PCR test is to be used with the Luminex ARIES

system. The ARIES instrument requires extraction cassettes, ARIES PCR ReadyMix, and Luminex ARIES proprietary SYNCT Software, and the User-Defined Protocol (UDP) application. The rt RT-PCR assay primers and probes were designed to detect two different regions of the N gene found in the SARS-CoV-2 viral genome (N1 and N3). The primers/probe for RNase P is present to control for adequate extraction of nucleic acid from human cells and to monitor the presence of inhibitors in the rt RT-PCR reaction. The ARIES software generates crossing thresholds (Ct) for each sample if the target is present. Ct thresholds are set in the SARS-CoV-2 rt RT-PCR assay protocol to give an overall positive or negative result.

Control Material(s) to be Used:

The target human RNase P serves as an internal control for the extraction, amplification and detection processes, indicating human cells were present in the specimen obtained for testing.

1. The internal control RNase P must show a Ct value from 1 to 45 for each negative specimen tested in the assay.

2. A specimen is considered inhibited if the internal control Ct value is above 45. When this occurs, we repeat the assay at 1:2 or 1:4 dilutions of specimen. If the samples are still inhibited upon dilution, then the sample is reported as invalid and we request a new specimen.

Assay results and interpretation

The only acceptable results that can be reported for this test include the following:

N1 Result	N3 Result	RNase P Result	Summary Result
+	+	+/-	SARS-CoV-2 DETECTED
+	-	+/-	Presumptive SARS-CoV-2 Positive - Retest x2
-	+	+/-	Presumptive SARS-CoV-2 Positive - Retest x2
-	-	+	SARS-CoV-2 Not Detected
-	-	-	INVALID

G. PERFORMANCE EVALUATION

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

The Limit of Detection study used heat inactivated culture fluid containing SARS-CoV-2. The SARS-CoV-2 rt RT-PCR assay used by our laboratory was able to detect reliably 1.5 $TCID_{50}$/mL (24/24 replicates) of SARS-CoV-2.

Sample	Dilution	Concentration ($TDIC_{50}$/mL)
1	1:10	$1.50\ X10^{5}$
2	1:100	$1.50\ X10^{4}$
3	1:1000	$1.50\ X10^{3}$
4	1:10,000	$1.50\ X10^{2}$
5	1:100,000	$1.50\ X10^{1}$
6	1:1,000,000	1.50
7	1: 10,000,000	$1.50\ X10^{-1}$
8	1: 100,000,000	$1.50\ X10^{-2}$

2) Analytical Sensitivity and Specificity:

A combination of in silico modeling and examination of patient samples infected with other related respiratory viruses showed there was no loss in sensitivity or specificity of the assay. The sequences for each of the primers and probes used in the assay were checked against the genomes of related or similar pathogens, and no significant homology was observed. In addition, archived patient samples that were positive for other respiratory viruses that were previously tested on the BioFire Respiratory Pathogen (RP)Panel, and 11 different known respiratory virus positive samples were evaluated against SARS-CoV-2 using the SARS-CoV-2 rt RT-PCR assay. All samples were correctly identified as negative.

3) Clinical Evaluation

Method Comparison:

A total of 50 de-identified nasopharyngeal specimens (25 positives and 25 negatives) were collected and evaluated. The samples were analyzed by EUA authorized CDC SAR-CoV-2 assay. The positive agreement was 25/25 and the negative agreement was 25/25 SARS-CoV-2 rt RT- PCR assay. The assay did not detect the RNase P in 5 out of 25 positive samples. The labeling for the assay indicates that the failure of the extraction control (RNase P) does not invalidate a positive result.

WARNINGS:

- This test has not been FDA cleared or approved;

- This test has been authorized by the Office of the Assistant Secretary for Health, U.S. Department of Health and Human Services, 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
- 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.

LIMITATIONS:

- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with 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.