ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
UCSD RC SARS-CoV-2 Assay
(University of California San Diego Health)

For in vitro diagnostic use
Rx only
For use under Emergency Use Authorization (EUA) Only

(The UCSD RC SARS-CoV-2 Assay will be performed at the University of California San Diego Health located at Center for Advanced Laboratory Medicine 10300 Campus Point Drive, Suite 150, San Diego, CA 92121, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity test, per the Instructions for Use that were reviewed by the FDA under this EUA).

INTENDED USE

The UCSD RC 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 clinician-instructed self-collected nasal swab specimens (collected on site), and clinician-collected upper respiratory specimens (nasopharyngeal, mid-turbinate, anterior nares or oropharyngeal swab specimens) collected from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to University of California San Diego Health located at Center for Advanced Laboratory Medicine 10300 Campus Point Drive, Suite 150, San Diego, CA 92121, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets requirements to perform high-complexity tests.

This test is also for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled samples containing up to five individual upper respiratory swab specimens (nasopharyngeal, mid-turbinate, anterior nares or oropharyngeal swab specimens) that are collected under observation using individual vials containing transport media from individuals suspected of COVID-19 by their healthcare provider. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive, presumptive positive, or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Results are for the 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 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 treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information

Testing with the UCSD RC SARS-CoV-2 Assay is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR assays and *in vitro* diagnostic procedures. The UCSD RC SARS-CoV-2 Assay is only for use under a Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The UCSD RC SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test for pooled samples containing up to five individual upper respiratory swab specimens. The assay is the cobas SARS-CoV-2 which has Emergency Use Authorization (EUA) and is designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines.

Please refer to cobas SARS-CoV-2 IFU for more information.

INSTRUMENTS USED WITH THE TEST

The UCSD RC SARS-CoV-2 Assay is to be used with the cobas 6800/8800 Systems with cobas 6800/8800 Systems Software v1.2 (ASAP v10.1.0) and v1.3 (ASAP v11.1.0).

REAGENTS AND MATERIALS

Please refer to cobas SARS-CoV-2 IFU.

Table 1. Reagents and Materials Required for the UCSD RC SARS-CoV-2 Assay

Reagent	Manufacturer	Catalog #
cobas SARS-CoV-2	Roche Molecular Diagnostics	P/N 09175431190
cobas SARS-CoV-2 Control Kit	Roche Molecular Diagnostics	P/N 09175440190
Cobas Buffer Negative Control Kit	Roche Molecular Diagnostics	P/N 07002238190

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

Please refer to cobas SARS-CoV-2 IFU.

One cobas Buffer Negative Control and one cobas SARS-CoV-2 Positive Control are processed with each batch. The RNA Internal Control is introduced into each specimen during sample processing and is used to monitor the entire sample preparation and

PCR amplification process.

In the cobas 6800/8800 software and/or report, flags and their associated results are checked to ensure batch validity.

The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch is required.

Validation of results is performed automatically by the cobas 6800/8800 software based on negative and positive control performance.

INTERPRETATION OF RESULTS

1) ***Examination and Interpretation of Patient Specimen Results:***

Individual Sample results:
Please refer to cobas SARS-CoV-2 IFU for interpretation of individual patient specimen results.

Table 2: UCSD RC SARS-CoV-2 Assay/cobas SARS-CoV-2 Results Interpretation

Target 1*	Target 2*	Interpretation
Positive	**Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Positive	**Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected. A positive Target 1 result and a negative Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 2, target region, or 3) other factors.
Negative	**Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. A negative Target 1 result and a positive Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 1 target region in the oligo binding sites, or 3) infection with some other Sarbecovirus (e.g., SARS-CoV or some other Sarbecovirus previously unknown to infect humans), or 4) other factors. Sample should be retested. For samples with a repeated 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.
Negative	**Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Not Detected.
Positive	**Invalid**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Invalid	**Positive**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. Sample should be retested. For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is

Target 1*	Target 2*	Interpretation
		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.
Negative	**Invalid**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Negative**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Invalid**	All Target Results were invalid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.

* Target 1, SARS-CoV-2 ORF1a/b gene; Target 2 pan-Sarbecovirus, E gene

Pooled Sample results:

Negative Result: If samples were pooled and the result is negative, then all samples in that pool should be reported as Negative.

Not Negative Result: If samples were pooled and the result is not negative (e.g., positive, presumptive positive, invalid, etc.), then all samples in that pool should be tested individually prior to reporting. The results of the individually tested samples should be reported.

PERFORMANCE EVALUATION

I. Analytical Sensitivity

Limit of Detection (LoD):

Roche Molecular Systems, Inc. (RMS) has granted University of California San Diego Health a right of reference to leverage the performance data from the cobas SARS-CoV-2 EUA submission (EUA200009) for use on the cobas 6800/8800 Systems.

II. Analytical specificity

Inclusivity

Roche Molecular Systems, Inc. (RMS) has granted University of California San Diego Health a right of reference to leverage the performance data from the cobas SARS-CoV-2 EUA submission (EUA200009) for use on the cobas 6800/8800 Systems.

Cross-reactivity

Roche Molecular Systems, Inc. (RMS) has granted University of California San Diego Health a right of reference to leverage the performance data from the cobas SARS-CoV-2 EUA submission (EUA200009) for use on the cobas 6800/8800 Systems.

III. Clinical evaluation

UCSD RC SARS-CoV-2 Assay

University of California San Diego Health conducted the following studies to validate sample pooling:

1. **Pooling Validation/Sensitivity for Pools with One Positive Sample and Nine Negative Samples (n = 44)**

University of California San Diego Health evaluated the sensitivity of samples collected from a patient population with a SARS-CoV-2 positivity rate of 3.2% based on 440 samples. The samples were sequentially selected from de-identified nasopharyngeal specimen remnants that had been previously tested individually using the cobas SARS-CoV-2 Test. Sample pools were made by combining one positive sample and nine negative samples; University of California San Diego Health tested 30 positive pools from retrospective positive samples and 14 positive pools from prospectively collected positive samples. Each pool was tested, and agreement with the individual sample result was calculated. Since any pool that does not yield negative results is re-tested individually, the positive percent agreement includes all pools that were not negative (i.e., positive, presumptive positive, and invalid). Of the 44 pools, 97.7% (43/44, 95% CI 88.2 – 99.6%) were not negative (37/44 were positive, 6/44 presumptive positive, 1/44 was false negative).

Table 3. Sensitivity for Sample Pools with One Positive Sample and Nine Negative Samples

		Results of Pooled Specimens				% Positive Percent Agreement*
Sample Type	N	Negative	Invalid	Presumptive Positive	Positive	
Retrospective	30	1	0	3	26	96.7% (29/30) 95% CI: (83.3 – 99.4%)
Prospective	14	0	0	3	11	100% (14/14) 95% C: (78.5 - 100%)
Total	44	1	0	6	37	97.7% (43/44) 95% CI: (88.2 – 99.6%

* Since any pool that is not negative is re-tested as individual samples, the % agreement includes all pools that were not negative (i.e., positive, presumptive positive, and invalid).

While performance was demonstrated with pools of 10 samples, to mitigate the risk of missing low positive samples and taking into consideration the current prevalence rate, sample pooling for only up to five specimens will be performed.

2. Pooling Validation/Efficiency with Pooled Negative Specimens (n = 60)

University of California San Diego Health evaluated efficiency of sample pooling using negative samples that had been previously tested individually using the cobas SARS-CoV-2 test. The samples were selected sequentially from de-identified specimen remnants that had been previously tested individually using the cobas SARS-CoV-2 Test. Each 10-sample pool contained ten negative samples. University of California San Diego Health tested 30 negative pools from retrospective samples and 30 negative pools from prospectively collected samples. Each pool was tested and the percent of negative results for these pools was calculated. Of the 60 10-sample pools, 98.3% (59/60, 95% CI 91.2-99.7%) were negative (59/60 negative and 1/60 presumptive positive). One pool would have had to be subsequently deconvoluted, with each sample being tested individually.

Table 4. Efficiency with Pooled Negative Specimens (n = 60)

		Results of Pooled Specimens				% Negative Percent Agreement*
Sample Type	n	Negative	Invalid	Presumptive Positive	Positive	
Retrospective	30	29	0	1	0	96.7% (29/30) 95% CI: (83.3 – 99.4%)
Prospective	30	30	0	0	0	100% (30/30) 95% C: (88.7 - 100%)
Total	60	59	0	1	0	98.3% (59/60) 95% CI: (91.2 – 99.7%

* Since any pool that is not negative (i.e., positive, presumptive positive and invalid) is re-tested as an individual sample, the parameter NPA affects the efficiency of 5-sample pooling

3. Pooling Validation – *In silico* Sensitivity

USCD conducted an *in silico* analysis to evaluate the effect of 10-sample pooling on the clinical sensitivity of the assay using the Ct shift calculated based on Passing-Bablok regression analyses from the "Pooling Validation / Sensitivity for Pools with One Positive Sample and Nine Negative Samples (n = 44)" where X-axis are individual Ct values for positive samples and Y-axis are Ct values for corresponding pools with one positive sample and 9 negative samples. Using regression analysis equation $Y=A+B\cdot X$, an interval of Ct individual values [X*, 40] was calculated where individual samples with Ct values within this interval will be negative in 10-sample pools (1 positive and 9 negative) as $40=A+B\cdot X^*$ (Ct shift is 40-X*). For target 1, an equation of Passing-Bablok regression for 44 samples was $Y=4.49+0.914X$ and the interval of Ct individual values that would be negative in 10-sample pools is [38.8; 40]. For target 2, an equation of Passing-Bablok regression for 44 samples was $Y=4.32+0.944X$ and the interval of Ct individual values that would be negative in 10-sample pools is [37.8; 40]. The de-identified data of 125 individually positive samples were selected from sequentially tested positives based on the cobas SARS-CoV-2 test. Then percent of samples with

individual Ct values from [X*, 40] was calculated (these positive samples can be missed using the 10-sample pooling strategy). Results are presented in Table 4 below:

Table 5: Summary of Passing-Bablok Regression Analyses

N	125
Interval [X*, 40] for Target 1	[38.8; 40]
Number of samples with Target 1 Ct values in the interval [38.8; 40]	0
Interval [X*, 40] for Target 2	[37.8; 40]
Number of samples with Target 2 Ct values in the interval [37.8; 40]	1
Number above shifted thresholds for both targets	0
Positive	124
Invalid	1
Negative	0
Positive Percent Agreement[#] (%)	100% (125/125), 95% CI: (97.0%; 100%)

[#]Since any pool that is not negative (i.e., positives, presumptive positives and invalid) is re-tested as individual samples, Positive Percent Agreement includes all pools that were not negative.

Overall the *in silico* analysis results demonstrated that none of the 125 positive samples, if pooled, would have been incorrectly determined to be negative, PPA=100% (125/125), 95% CI: (97.0%; 100%).

Warnings:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by University of California San Diego Health located at Center for Advanced Laboratory Medicine 10300 Campus Point Drive, Suite 150, San Diego, CA 92121;
- 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 Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.