EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
BAYCARE SARS-COV-2 RT PCR Assay
(BayCare Laboratories, LLC)

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

(The BayCare SARS-CoV-2 RT PCR Assay will be performed at BayCare Laboratories, LLC located at 5455 West Waters Ave. Suite 208, Tampa, FL 33634, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity tests, as described in the Standard Operating Procedures that were reviewed by the FDA under this EUA).

INTENDED USE

The BayCare SARS-CoV-2 RT PCR Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal swab specimens collected from individuals suspected of COVID-19 by their healthcare provider (HCP). Testing is limited to BayCare Laboratories, LLC located at 5455 West Waters Ave. Suite 208, Tampa, FL 33634, 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 two individual nasopharyngeal swab specimens that are collected by a HCP using individual vials containing transport media, from individuals suspected of COVID-19 by their HCP. 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

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

DEVICE DESCRIPTION AND TEST PRINCIPLE

The BayCare SARS-CoV-2 RT PCR Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test for pooled samples containing up to two individual nasopharyngeal 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 additional information.

INSTRUMENTS USED WITH THE TEST

The BayCare SARS-CoV-2 RT PCR 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 BayCare SARS-CoV-2 RT PCR

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 BAYCARE SARS-COV-2 RT PCR

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) Assay Result and Interpretation:

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

Table 2: BayCare SARS-CoV-2 RT PCR 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

Target 1*	Target 2*	Interpretation
		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 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 ORF1 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 Presumptive 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

1) Analytical Sensitivity:

Roche Molecular Systems, Inc. (RMS) has granted BayCare Laboratories 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.

2) Analytical Inclusivity:

Roche Molecular Systems, Inc. (RMS) has granted BayCare Laboratories 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.

3) Analytical Specificity:

Inclusivity

Roche Molecular Systems, Inc. (RMS) has granted BayCare Laboratories 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 for pooling specimens prior to loading on the cobas 6800/8800 Systems.

4) Clinical Evaluation:

BayCare Laboratories conducted the following studies to validate sample pooling:

1. Pooling Validation/Sensitivity for Pools with One Positive Sample and One Negative Sample

BayCare Laboratories evaluated the sensitivity of samples collected from a patient population with positivity rate of 0.16% based on 14,598 samples (based on single sample testing from May 4th to June 30th). Twenty individual positive and thirty (30) randomly selected individual negative nasopharyngeal specimens tested with the cobas SARS-CoV-2 test were tested individually and then re-tested after each SARS-CoV-2 positive sample was pooled with an equal volume of a previously tested negative sample. Each pool was tested, and agreement with the individual sample result was calculated.

To challenge the pool sampling method with samples containing viral amounts close to the LoD of the assay, thirty (30) positive samples with Ct values greater than 30 were selected and re-run after sample pooling (addition of equal volume of a negative sample). For 23 of the 30 samples a positive result was obtained, for 1 sample a presumptive positive result (T2 only detected) was obtained and for 6 samples a negative result was obtained; this indicates a Positive Percentage Agreement of 80% (24/30) in this sample subset.

The overall combined performance, as well as performance stratified by normal positive pools vs. near LoD positive pools are summarized in the Tables below.

Table 3: Sensitivity for Sample Pools with One Positive Sample and One Negative Sample Overall Combined Samples

Pooled Specimen Result	N	Single Test Positive	Single Test Negative	% Positive Percent Agreement, 95%CI*
Positive Pool	44	44	0	88% (44/50), (76.2-94.4%)
Negative Pool	6	6	30	100% (30/30), (88.7-100%)

* 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 2-sample pooling

Table 4: Sensitivity for 2-Sample Pools with One Negative Sample with One Random Positive Sample

Pooled Specimen Result	N	Single Test Positive	Single Test Negative	% Positive Percent Agreement, 95%CI
Positive Pool	20	20	0	100 % (20/20), (83.9-100%)
Negative Pool	30	0	30	100% (30/30), (88.7-100%)

Table 5: Sensitivity for 2-Sample Pools with One Negative Sample with Positive Sample Near LoD

Pooled Specimen Result	N	Single Test Positive	Single Test Negative	% Positive Percent Agreement, 95% CI
Positive Pool	24*	24	0	80% (24/30), (62.7-90.5%)
Negative Pool	6	6	0	-

* 1 sample was determined to be presumptive positive

In silico Sensitivity

To estimate the impact of the 2-sample pooling method, the most recent 100 positive samples were analyzed; of these 11% had Ct values indicating they were close to the LoD of the assay (T1 Ct >34.0 and/or T2 Ct >38.0). The Ct shift was applied to the T1 and T2 Ct values based on the following:

The Ct shift was determined by Passing-Bablok Regression Scatter Plot analysis using the intercept and the slope for each target, the formulas are presented below:

- T1: y = 0.9941 + 0.9787 x , where y is the shifted value for the Ct of T1 and x is the original Ct value of T1
- T2: y = 0.4981 + 1.002 x, where y is the shifted value for the Ct of T2 and x is the original Ct value of T2

Among 100 positive samples, there were 96 samples positive in 2-sample pools.
The estimated PPA for the *in silico* analysis is 96.0% (96/100) with 95% CI: (88.8%; 97.8%).

Summary of Clinical Evaluation

In summary, for the assessment of the 2-sample pooling:

1) Of the total 50 positive samples tested in the clinical study, 60% (30 out of 50) of them had viral load near the LoD of the assay. The PPA for 20 randomly selected samples was 100% and PPA for 30 samples with viral load near the LoD was 80%. The total PPA calculated for both data sets was 88% (44/50) (95% CI: 76.2% - 94.4%).

2) The calculated NPA is 100% (30/30) (95% CI: 88.7% - 100%).

3) *In silico* analysis of the 100 positive samples of the historical data demonstrated PPA of 96.0% (96/100), 95%CI: (88.8%; 97.8%).

Warnings:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by BayCare Laboratories LLC located at 5455 West Waters Ave. Suite 208, Tampa, FL 33634;
- 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 Federal Food, Drug and Cosmetic 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 and amplification steps were performed onboard the Roche Cobas 6800. The results are summarized in the following Table.

Table 6: 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	$1.8x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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