

**ACCELERATED EMERGENCY USE
AUTHORIZATION (EUA) SUMMARY COVID-19
RT-PCR Test**

(Baptist Health Care System)

For *In vitro* Diagnostic Use

Rx Only

For use under Emergency Use Authorization (EUA) only

(The COVID-19 RT-PCR test will be performed at the Pathology/Laboratory Medicine lab of Baptist Hospital Miami, Florida, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The COVID-19 RT-PCR test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens including nasopharyngeal and oropharyngeal swabs collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Pathology/Laboratory Medicine of Baptist Hospital Miami that is also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper 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 patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the COVID-19 RT-PCR test is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures in CLIA certified high-complexity laboratories. The COVID-19 RT-PCR is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The COVID-19 RT-PCR Test is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The primer and probe sets used in this test are identical to those described in the CDC emergency use authorized assay and detect RNA from the SARS-CoV-2 in upper (such as nasopharyngeal or oropharyngeal swabs) from individuals suspected of COVID-19 by their healthcare provider.

SARS-CoV-2 nucleic acid from patient samples is extracted utilizing the Promega Maxwell RSC Viral Total Nucleic Acid Purification Kit and then reverse transcribed to complementary DNA (cDNA) which is then amplified during the PCR reaction using the ThermoFisher Scientific TaqPath 1-Step RT-qPCR Master Mix. During amplification, the primers and probe bind to the SARS-CoV-2 target sequences. Subsequent extension of the PCR product hydrolyzes the probe and separates the probe's fluorescent reporter from the quencher molecule. As the PCR product is amplified the fluorescent signal increases and detection is measured by the Applied Biosystems QuantStudio 7 Flex thermal cycler.

One sample tube contains primers and probe specific to human RNase P as an internal control. If negative for SARS-CoV-2 RNA, all clinical samples with successful nucleic acid extraction and the absence of inhibitors should exhibit amplification of the RNase P target.

INSTRUMENTS USED WITH TEST

The COVID-19 RT-PCR test is to be used with the Applied Biosystems QuantStudio 7 Flex using QuantStudio Real-Time PCR software. The following extraction platform can be used: Promega Maxwell® CSC/RSC Instrument.

REAGENTS/EQUIPMENT/INSTRUMENTS

1. Materials / Equipment

- a. Instrument Components
 - Maxwell CSC in RUO mode/RSC Instrument
 - Tablet PC preloaded with Maxwell® CSC /RSC User Interface
 - Maxwell CSC/RSC Deck Tray
 - Bar Code Reader
- b. Equipment:
 - Biosafety Cabinet (BSL-2)
 - Real-time PCR instrument: Applied Biosystem QuantStudio 7 Flex
 - Beckman i5 Automated Workstation
 - Calibrated single- and multi-channel pipettes
 - Centrifuge or My Spin
 - Vortexer - ThermoScientific Model No. M16715
 - Eppendorf Thermomixer C5382 or equivalent

2. Reagents, Kits, and Supplies

- i. MAXWELL RSC VIRAL TOTAL NUCLEIC ACID PURIFICATION Kit, (Promega, AS1330)
- ii. Supplies and consumables:
 - 1.5 mL- 2.0 mL Safe-Lock micro centrifuge tubes - Eppendorf DNA LoBind tube 1.5 mL -2.0 mL
 - 15 mL or 50 mL conical tube Aerosol barriers sterile, RNase-free pipet tips 10, 20, 200, 1000uL
 - Pipettes of 10, 20, 200, 1000uL
 - Heating blocks set at 56°C
 - Benchtop vortex mixer
 - Centrifuge or Quick Spin
 - RNase-/DNase-free pipet tips and tubes
 - Pipet tips, 50µL, sterile, filter, Beckman Coulter, 960 tips B85888
 - MicroAmp Optical 96-well Reaction Plate with/without barcode; Life Technologies, Cat. # 4306737 / N8010560
 - PCR strip caps, Life Technologies, Cat. # 4323032
 - Aerosol barriers sterile, RNase-free pipet tips 10, 20, 200, 1000uL
 - PLATE PCR TWIN TEC 96 SEMI SKIRTED Green Cardinal Health, Cat. #: C3514-446 or equivalent
 - 96-well cold block
 - 24-well cold block
- iii. Reagents
 - RT-qPCR primer/probe sets, IDT Cat. #: 10006606
 - 2019-nCoV_N_Pos Control, IDT Cat. #: 10006625
 - Hs_RPP30 Positive Control, IDT Cat. #: 10006626
 - SARS-CoV-2 Negative – ExactDiagnostics, COV000
 - SARS-CoV-2 Standard – ExactDiagnostics, COV019
 - AccuPlex SARS-CoV-2 Reference Material Kit – SeraCare Cat. #: 0505-0126
 - TaqPath 1-Step RT-qPCR Master Mix, CG (ThermoFisher; cat # A15299 or A15300)
 - Molecular biology grade RNase- and DNAase-free water
 - DNAZap - Life Technologies, Cat. # AM9890

CONTROLS TO BE USED WITH THE COVID-19 RT-PCR

1. The SeraCare AccuPlex SARS-CoV-2 Reference Material Kit contains positive reference material consistent with publicly available sequences. SeraCare Positive and Negative Control (RNase P) is performed from the nucleic acid extraction through RT-qPCR whole process as assay sensitivity control for each run.
 - a. The positive control (diluted with either negative control or known negative VTM) is close to LOD level of 2-4 copies/µL and contains sequences for SARS-CoV-2, including the N gene target.

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- b. The SeraCare Accuplex negative control contains sequences for the human RNase P gene target.
2. No template control (NTC; molecular grade, nuclease-free water) is included in each RT-PCR run to monitor potential reagent contamination.

INTERPRETATION OF RESULTS

Controls:

All test controls are examined prior to interpretation of patient results. If the controls are not valid, the patient results are not interpreted.

- SeraCare Accuplex SARS-CoV-2 positive control at a concentration of 4 copies/ μ L has a valid Ct value for the N2 and RP primer/probe sets.
- SeraCare Accuplex negative control has valid Ct value only for the RP primer/probe set (Ct before 38).
- NTC has no Ct call or Ct > 40 for N2 and RP;

If any control does not perform as described below, the run is considered invalid and all specimens are repeated from extraction step.

Patient Specimen Results:

There are only 4 possible results for this test.

1. **Detected** – Ct range from 12-37.527 for N2; RP has a Ct value. This positive result has to be reported to the ordering physician in a timely fashion.
2. **Not Detected** – Ct is Undetermined or more than 40 for N2, RP has a Ct value.
3. **Invalid** – no Ct value for RP. The sample needs to be repeated. If RP has no Ct, but N2 has valid Ct, then this can be result.
4. **Inconclusive** – Ct value is at the range between 37.5 to 40 for N2 because the LOD of 2 copies/ μ L is at the range of 35.021-37.527. RP has a Ct value. Inconclusive results mean that the presence or absence of 2019-Novel Coronavirus (SARS-COV-2) nucleic acids cannot be determined. A repeat specimen should be considered if clinically indicated. This borderline result may need to be communicated with the ordering physician on a timely fashion. Repeat the sample in duplicates from extraction. Its final result will take the two out of all three tested results

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

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Pooled nasal swabs were used for the clinical matrix. The preliminary LoD was established using 2 copies/μl and 1 copy/μl input viral RNA transcript tested in quadruplet for each condition. Then, LoD was confirmed with 20 replicates of 2 copies/μl concentration (from the LoD = 2 RNA copies/μl (10 copies/reaction and 500 copies/mL of original specimen)). N2 was detected in 19/20 samples resulting in 95% detection for LoD confirmation which is acceptable.

2) **Analytical Inclusivity:**

This test uses the identical N2 primers and probe sequences as the CDC EUA test and, therefore, additional *in silico* studies were not performed.

3) **Cross-Reactivity:**

This test uses the identical N2 primers and probe sequences as the CDC EUA test and, therefore, additional *in silico* studies were not performed.

4) **Clinical Evaluation:**

Sixty patient samples were tested by the candidate Baptist Hospital, Miami, FL COVID-19 RT-PCR test compared to results obtained using the Abbott RealTime SARS-CoV2 assay on the M2000 platform (EUA200023/A001). The results are shown below.

		Comparator		Result			
		Positive	Negative				
Test Result	Positive	28	1				
	Negative	2	29				

Five positive and five negative samples were confirmed using both the Baptist Hospital Miami, FL COVID-19 RT-PCR test and another EUA authorized test.

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 method and instrument used were Promega RSC/CSC AS1330 and ThermoFisher QuantStudio 7. The results are summarized in the following Table.

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

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal	1.8x10 ⁴ NDU/mL	N/A
MERS-CoV	Swab in VTM	N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not applicable

ND: Not detected