# ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

## *SARS-CoV-2 (N gene detection) Test*

## (Exact Sciences Laboratory)

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

**(The SARS-CoV-2 RT-PCR assay will be performed at the Exact Sciences Laboratories laboratory, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a as per Laboratory Standard Operating Procedure that was reviewed by the FDA under this EUA.)**

## INTENDED USE

The *SARS-CoV-2 (N gene detection) Test* is a high-throughput real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens), from individuals suspected of COVID-19 by a healthcare professional. Testing is limited to Exact Sciences Laboratories, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

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 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.

The *SARS-CoV-2 (N gene detection) Test* is intended for use by clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR assays. The assay is intended for use under the Food and Drug Administration's Emergency Use Authorization.

## DEVICE DESCRIPTION AND TEST PRINCIPLE

The SARS-CoV-2 (N gene detection) Test is a high-throughput real-time reverse transcription polymerase chain reaction (rRT-PCR) test with a testing capacity of 40,000 tests per week. The test uses specific primer and probe sets developed by CDC to detect two regions (nCOV_N1 and nCov_N2) in the SARS-CoV-2 nucleocapsid (N) gene and one primer and probe set to detect human RNase P (RP) in a clinical sample. All targets are amplified in a separate reaction because all probes are labeled with the same reporter dye (FAM). RNA isolated from upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal or nasal swab) is reverse transcribed to cDNA and subsequently amplified using the ABI 7500 Fast Dx (Applied Biosystems) instrument with Sequence Detection Software (SDS) version 1.4.1. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye (FAM) to separate from the quencher dye (BHQ), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the ABI 7500 Fast Dx.

## INSTRUMENTS USED WITH TEST

RNA extraction based on conventional well-known bead-based technology is conducted using the non-commercial, Exact Sciences Corporation extraction procedure on the Hamilton STARlet liquid handler. RT-PCR is performed on the ABI 7500 Fast Dx (Applied Biosystems) real-time PCR instrument.

## EQUIPMENT, REAGENTS AND MATERIALS

The following equipment/reagents/materials are required to run this test:

1. Exact Sciences Corporation extraction reagents
2. Hamilton STARlet liquid handler for nucleic acid extraction
3. ABI 7500 Fast Dx (Applied Biosystems) for cDNA synthesis and PCR amplification of the target sequences.
4. GoTaq 1-Step RT-qPCR system (Promega; #A6121)
5. 2019-nCoV Kit (Primers & Probes) (IDT; #10006713)
6. Hs_RPP30 Positive Control (RP) (IDT; #10006626)
7. 2019-nCoV_N_Positive Control (N1 & N2) (IDT; #10006625)

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

- A Negative Control (NC) serves as a negative extraction control to monitor for any cross-contamination that could occur during the extraction process. The NC consists of saline and is run once for every batch of extracted specimens.

- A no template control (NTC) is used to monitor the possibility of sample contamination in the assay run and is used once on every PCR assay plate. The control is DNA suspension buffer (TE buffer).

- A Positive Control (POC) (nCoVPC) is used to verify that the assay run is performing as intended. The nCoVPC contains targets for N1, N2 and RP and consists of (IDT) Hs_RPP30 Positive Control, (IDT) 2019-nCoV_N_Positive Control and DNA suspension buffer (TE buffer). The positive control is used once on every PCR plate.

## INTERPRETATION OF RESULTS

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

### *a. Control Result Interpretation*

**Table 1: Expected Performance of Controls**

| Control Type | External Control Name | N1 | N2 | RP | Expected Ct values |
|---|---|---|---|---|---|
| Negative Control | NC | - | - | - | No Ct, or Ct ≥ 40 for all targets |
| Negative Template Control | NTC | - | - | - | No Ct, or Ct ≥ 40 for all targets |
| Positive Control | 2019-nCoV PC | + | + | + | < 40 Ct for N1, N2 and RP targets |

If any of the above controls do not exhibit the expected performance as described, the assay may have been improperly set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

### *b. Examination and Interpretation of Patient Specimen Results:*

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 2: Interpretation of Patient Results**

| N1 | N2 | RP | Result Interpretation | Patient Report Verbiage |
|---|---|---|---|---|
| + | + | +/- | All targets are valid. SARS-CoV-2 (COVID-19) RNA detected. | SARS-CoV-2 (COVID-19) RNA detected |
| If only one of the two targets is positive | | +/- | Inconclusive | This specimen did not meet the full criteria established for the detection of SARS-CoV-2 (COVID-19) RNA. The sample will be retested. If the result is inconclusive the sample will be sent to a second laboratory for alternate testing. |
| - | - | + | All targets are valid. SARS-CoV-2 (COVID-19) RNA **NOT** detected. | SARS-CoV-2 (COVID-19) RNA **NOT** detected. Negative results do not preclude SARS-CoV-2 (COVID-19) infection and should not be used as the sole basis for treatment or other patient management decisions. |
| - | - | - | Results are invalid. Repeat testing If the result is still invalid, a new specimen should be obtained. | **Invalid** – This specimen exhibited inhibition in the PCR assay or the specimen contained an inadequate amount of clinical material. Repeat testing is suggested if clinically indicated. |

## PERFORMANCE EVALUATION

### *1) Analytical Sensitivity:*

The LoD of the SARS-CoV-2 (N gene detection) Test was determined using the AccuPlex SARS-CoV-2 Reference Material Kit (SeraCare). AccuPlex recombinant materials are constructed with a replication-deficient mammalian virus producing a safe, non-infectious material. These mammalian virus-based reference materials resemble the complexity of virus targets found in true patient samples, including the viral particle protein coat and lipid bilayer.

#### a. Tentative LoD

The tentative LoD for the N1 and N2 targets within the SARS-CoV-2 genome was determined using 2-fold serial dilutions with 20 to 29 replicates per dilution using known titers (genome copies (cp)/µL) of AccuPlex SARS-CoV-2 Positive Reference Material (SeraCare) Spiked samples were tested with the SARS-CoV-2 (N gene detection) Test following extraction with the Exact Sciences Hamilton STARlet. Real-Time RT-PCR assays were performed using the GoTaq 1-Step RT-qPCR System (Promega) on the ABI 7500 Fast Dx real-time PCR instrument.

Results are summarized in Table 3. The LoD was determined as the lowest concentration where ≥ 95% of the replicates were positive. Accordingly, the N1 and N2 genes have an LoD of 2.6 copies/µL of specimen (**Table 3**).

**Table 3: SARS-CoV-2 Tentative LoD**

| Target Level | Valid results | N 1 Positive | | SARS-CoV-2 (N 1 Detection Rate | N 2 Positive | | SARS-CoV-2 (N 2) Detection Rate | RP Positive | | SARS-CoV-2 (RP) Detection Rate |
|---|---|---|---|---|---|---|---|---|---|---|
| | | n | Mean Ct | | n | Mean Ct | | n | Mean Ct | |
| 0.33 cp/µL | 29 | 25 | 37.5 | 86% | 16 | 38.9 | 55% | 29 | 34.4 | 100% |
| 0.65 cp/µL | 29 | 26 | 37.5 | 90% | 25 | 37.9 | 86% | 29 | 34.4 | 100% |
| 1.3 cp/µL | 29 | 27 | 35.4 | 93% | 25 | 36.5 | 86% | 29 | 34.2 | 100% |
| 2.6 cp/µL | 20 | 20 | 33.3 | 100% | 20 | 33.9 | 100% | 20 | 32.7 | 100% |
| **Tentative LoD: 2.6 cp/µL** [lowest target level demonstrating >95% detection rate of SARS-COV-2] | | | | | | | | | | |

### b. Confirmatory LoD

The LoD was confirmed verified using RNA samples from 20 additional replicates. Dilutions were generated using AccuPlex SARS-CoV-2 Reference Material (SeraCare) spiked into a negative patient specimen pool in Universal Transport Medium (UTM) to mimic clinical specimens. Spiked samples were tested with the SARS-CoV-2 (N gene detection) Test following extraction with the Exact Sciences Hamilton STARlet. Real-Time RT-PCR assays were performed using the GoTaq 1-Step RT-qPCR System (Promega) on the ABI 7500 Fast Dx real-time PCR instrument. Results are summarized in Table 4.

**The SARS-CoV-2 (N gene detection) Test has an LoD of 2.6 copies/µl.**

**Table 4: Confirmatory LOD**

| | N 1 gene | N-2 gene | RP (Internal Control) |
|---|---|---|---|
| **RNA concentration - 2.6 cp/µl of sample** | | | |
| **Positives** | 19/20 | 20/20 | 20/20 |
| **Mean Ct** | 33.3 | 34.1 | 27.4 |
| **Std Dev (Ct)** | 1.4 | 2.3 | 0.8 |
| **RNA concentration - 1.3 cp/µl of sample** | | | |
| **Positives** | 20/20 | 20/20 | 20/20 |
| **Mean Ct** | 34.1 | 35.4 | 25.5 |
| **Std dev** | 1.3 | 1.5 | 1.2 |

## 2) ***Analytical Inclusivity/Specificity:***

### a. Inclusivity

The SARS-CoV-2 (N gene detection) Test utilizes primer and probe sets identical to the N1 and N2 SARS-CoV-2 target genes used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. Accordingly, an inclusivity analysis was not repeated.

### b. Cross-Reactivity

#### i. *In Silico* Analysis

The SARS-CoV-2 (N gene detection) Test utilizes primer and probe sets identical to the N1 and N2 SARS-CoV-2 target genes used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. Accordingly, an *in silico* cross-reactivity analysis was not repeated.

#### ii. Wet Testing

Additionally, nucleic acid from common respiratory pathogens was extracted and tested with the SARS-CoV-2 (N gene detection) Test to further demonstrate analytical specificity and exclusivity. Nucleic acids were extracted from the NATtrol Respiratory Pathogen Panel-1 (NATRPP-1, Zeptometrix) that is formulated with purified, intact virus particles and bacterial cells that have been chemically modified to render them non-infectious. Samples were tested on the according to the Laboratory SOP using the Exact Sciences Corporation nucleic acid extraction method on the Hamilton STARlet liquid handler and the ABI 7500 Fast Dx (Applied Biosystems) real-time PCR instrument for cDNA synthesis, PCR amplification and detection. No cross-reactivity was observed for the tested organisms.

**Table 5. Organisms Tested for Cross-Reactivity**

| | Respiratory Virus/Bacteria | N1 and N2 Genes |
|---|---|---|
| 1 | Coronavirus NL63 | Not detected |
| 2 | Coronavirus OC43 | Not detected |
| 3 | Coronavirus HKU | Not detected |
| 4 | Coronavirus 229E | Not detected |
| 5 | Influenza A H3 (A/Brisbane/10/07) | Not detected |
| 6 | Influenza B (B/Florida/02/06) | Not detected |
| 7 | Respiratory Syncytial Virus B (CH93(18)-18) | Not detected |
| 8 | Parainfluenza Type 2 | Not detected |
| 9 | Parainfluenza Type 3 | Not detected |
| 10 | Human Metapneumovirus (Peru6-2003) | Not detected |
| 11 | Human Bocavirus | Not detected |
| 12 | Legionella pneumophila | Not detected |
| 13 | Chlamydophila pneumoniae (CWL-029) | Not detected |

### c. Endogenous Interference

The SARS-CoV-2 (N gene detection) Test was evaluated for susceptibility to endogenous interferents associated with upper respiratory specimens. UTM samples were spiked with AccuPlex SARS-CoV-2 Positive Reference Material at 1.8X LoD (4.7 cp/µL specimen) or with AccuPlex SARS-CoV-2 Negative Reference Material. All samples were processed in the presence or absence of blood or mucin at the concentrations indicated in the table below. Neither blood nor bovine mucin had an adverse effect on the ability of the assay to correctly detect the absence or presence of the SARS-CoV-2 target.

**Table 6: Interference Study with Mucin and Blood**

| SARC-CoV Target | Substance | Concentration | Number of replicates | Mean Ct | | | Interference |
|---|---|---|---|---|---|---|---|
| | | | | RP | N1 | N2 | |
| + | Control | N/A | 3 | 35.3 | 31.6 | 32.4 | **N/A** |
| | Blood | 5% v/v | 3 | 23.3 | 31.9 | 32.2 | **No** |
| | Control | N/A | 3 | ND | 32.8 | 33.1 | **N/A** |
| | Mucin[1] | 2.5 mg/mL | 3 | ND | 33.1 | 33.3 | **No** |
| | | 1.2 mg/mL | 3 | 36.5 | 32.3 | 34.7 | **No** |
| - | Control | N/A | 3 | 32.6 | | | **N/A** |
| | Blood | 5% v/v | 3 | 22.3 | | | **No** |
| | Control | N/A | 3 | 33.3 | | | **N/A** |
| | Mucin[1] | 2.5 mg/mL | 3 | 32.9 | | | **No** |
| | | 1.2 mg/mL | 3 | 32.5 | | | **No** |
| | | 0.6 mg/mL | 3 | 33.0 | | | **No** |

[1]Bovine submaxillary glands Type I-S (Sigma-Aldrich; Cat. No. M3895)

## *3) Clinical Evaluation:*

A total of 84 de-identified respiratory specimens were used for clinical evaluation. The specimens were a mix of nasopharyngeal (NP) and oropharyngeal (OP) clinical specimens. All samples were tested with the SARS-CoV-2 (N gene detection) Test and concordance was determined based on either results reported by the Wisconsin State Laboratory of Hygiene (WSLH) after testing specimens with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel or with Exact Sciences Laboratories SARS-CoV-2 (E, RdRP, N gene detection) Test authorized under EUA200162. Concordance was based on a Ct value < 40 for specimens considered positive and a Ct value of ≥ 40 for specimens considered negative for the presence of SARS-CoV-2 N1/ N2 genes. Two specimens were invalid when tested with the SARS-CoV-2 (N gene detection) Test and were excluded from the analysis (invalid rate of 2.4%). Out of the remaining 82 samples, two negative specimens and two positive specimens produced inconclusive results (positive of only one of the targets) with the SARS-CoV-2 (N-gene detection) Test. Results are summarized in Table 7.

**Note**: Because an inconclusive result indicates that one of the test targets is concordant with the comparator while the other target is discordant with the comparator, inconclusive results are excluded from performance calculation. The rate of inconclusive results is 4.9% (4/82).

**Table 7: Evaluation with Clinical Specimens**

| | | **CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel at WSLH (N1, N2)** | | |
|---|---|---|---|---|
| | | Positive | Inconclusive | Negative |
| **Exact Sciences Laboratories** | Positive | 17 | 1[1] | 0 |
| | Inconclusive[1] | 0 | 0 | 2[1] |
| | Negative | 0 | 0 | 32 |
| Positive Percent Agreement (PPA): 17/17 = 100% (95% CI: 81.6% - 100%)<br>Negative Percent Agreement (NPA): 32/32[1] = 100% (95% CI: 89.3% - 100%) | | | | |
| | | **SARS-CoV-2 (E, RdRP, N gene detection) Test** | | |
| **Exact Sciences Laboratories** | Positive | 21 | 0 | 0 |
| | Inconclusive[1] | 1[1] | 0 | 0 |
| | Negative | 2[2] | 0 | 6 |
| Positive Percent Agreement (PPA): 21/23[1] = 91.3% (95% CI: 73.2% - 97.6%)<br>Negative Percent Agreement (NPA): 6/6 = 100% (95% CI: 61.0% - 100%) | | | | |
| | | **Comparator** | | |
| **Exact Sciences Laboratories** | Positive | 38 | 1[1] | 0 |
| | Inconclusive[1] | 1[1] | 0 | 2[1] |
| | Negative | 2[2] | 0 | 38 |
| | **TOTAL (n=82)** | 41 | 1[1] | 40 |
| **Positive Percent Agreement (PPA): 38/40 = 95.0% (95% CI: 83.5% - 98.6%)**<br>**Negative Percent Agreement (NPA): 38/38 = 100% (95% CI: 90.8% - 100%)** | | | | |

[1] All inconclusive results were obtained from specimens with a target Ct values indicative of a concentration below the *SARS-CoV-2 (N gene detection) Test* LoD. Because one target is concordant with the comparator and one is discordant, inconclusive results are excluded from performance calculation. These results would be followed up by additional testing in order to produce a valid reportable result.

[2] The two false negative results were obtained from specimens with target Ct values indicative of concentrations below the assay LoD. Note that the established LoD for the *SARS-CoV-2 (N gene detection)* Test is 0.6 copies/µL with the following mean Ct values for RdRP =34.4 and N=35.1 (please refer to EUA200162), whereas the LoD determined for the SARS-CoV-2 (N gene detection) Test is 2.6 copies/ul.

The table above includes the first 6 positives and 5 negatives confirmed with additional testing with an FDA EUA authorized assay. The testing on these clinical specimens performed at Exact Sciences Laboratories and at the alternate testing laboratory meets the requirement for confirmatory testing for at least 5 positive and 5 negative specimens.

**Table 8: Discordant and Inconclusive Clinical Specimens**

| Sample | Test | IC* Ct | E Ct | RdRP Ct | N/N1* Ct | N2 Ct | Result |
|---|---|---|---|---|---|---|---|
| **Mean Ct at LoD** | **Comparator** | **28.1** | **30.7** | **34.4** | **35.1** | N/A | **0.6 copies/µl** |
| | **Investigational** | **34.1** | N/A | N/A | **33.3** | **27.4** | **2.6 copies/µl** |
| 1 | Comparator | 27.4 | 35.3 | 38.4 | 35 | N/A | Positive |
| | Investigational | 25.3 | N/A | N/A | N.D. | N.D. | Negative |
| 2 | Comparator | 26.6 | 33.8 | 39.1 | 32.2 | N/A | Positive |
| | Investigational | 26.5 | N/A | N/A | N.D. | N.D. | Negative |
| **Inconclusive Specimens** | | | | | | | |
| 3 | Comparator | 30.9 | 35 | 38.1 | 35.7 | N/A | Positive |
| | Investigational | 25.2 | N/A | N/A | 37.3 | 40.3 | Inconclusive |
| 4 | Comparator | N/A: Targets not part of the comparator or the investigational test | | | N.D. | N.D. | Negative |
| | Investigational | | | | 38.4 | 40.5 | Inconclusive |
| 5 | Comparator | | | | N.D. | N.D. | Negative |
| | Investigational | | | | N.D. | 39.9 | Inconclusive |

*Comparator Test Targets: E, RdRP and N (plus Internal Control [IC])
Investigational Test Targets: N1 and N2 (plus RNase P as IC)
N/A = Not a target for the test: greyed out
N.D. = Not detected

For the clinical sample testing PPA and NPA after exclusion of the two invalid specimens and the Wilson Score Method based 95% Confidence Intervals were as follows:

**PPA: 38/40 = 95.0% (95% CI: 83.5% - 98.6%)**
**NPA: 38/38 = 100% (95% CI: 90.8% - 100%)**

**WARNINGS:**

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