

**ACCELERATED EMERGENCY USE  
AUTHORIZATION (EUA) SUMMARY**

**SARS-CoV-2 Assay  
(Integrity Laboratories)**

For *In vitro* Diagnostic Use  
Rx Only

For use under Emergency Use Authorization (EUA) only

**(The SARS-CoV-2 Assay will be performed at the Integrity Laboratories, 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 Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasal, nasopharyngeal and oropharyngeal swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Integrity Laboratories, which is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory.

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 patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

This assay is intended for use under the Food and Drug Administration’s Emergency Use Authorization.

**DEVICE DESCRIPTION AND TEST PRINCIPLE**

The TaqMan Probe-based chemistry uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR. An oligonucleotide probe is constructed with a fluorescent reporter dye (i.e., 6-FAM) bound to the 5’ end and a quencher (i.e., BHQ) on the 3’ end. While the probe is intact, the proximity of the

quencher greatly reduces the fluorescence emitted by the reporter dye through a process called fluorescence resonance energy transfer (FRET).

If the target sequence is present, the probe anneals between primer sites and is cleaved by the 5' nuclease activity of Taq polymerase enzyme during primer extension. This cleavage of the probe separates the reporter dye from the quencher, increasing the reporter dye signal. It also removes the probe from the target strand, allowing primer extension to continue to the end of the template strand. Thus, inclusion of the probe does not inhibit the overall PCR process.

Additional reporter dye molecules are cleaved from their respective probes with each cycle, resulting in an increase in fluorescence intensity proportional to the amount of amplicon produced. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed.

The Integrity Laboratories SARS-CoV-2 RT-PCR assay for SARS-CoV-2 (originally called 2019 Novel Coronavirus or 2019 nCoV) testing utilizes the CDC-developed assay that targets the Nucleocapsid gene of this virus. Two gene targets, N1 and N2, are used to detect cases of COVID-19. During testing of clinical samples, both N1 and N2 nucleocapsid genes must be detected in order for the sample to be determined as a positive. Positivity of only one SARS-CoV-2 target results in an inconclusive call that triggers repeat testing.

## **INSTRUMENTS USED WITH TEST**

RNA extraction is conducted using the MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit on the MagMax Express 96.

The SARS-CoV-2 RT-PCR test is to be used with the TaqPath 1-step RT-qPCR Master Mix (ThermoFisher Scientific) on the Applied Biosystems QuantStudio 12K Flex Real-Time PCR System. Results are analyzed with the QuantStudio 12K Flex Software v1.3.

## **REAGENTS AND MATERIALS**

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

- TWIST Biosciences TWIST Synthetic SARS-CoV-2 RNA controls (Cat No. 102019)
- Primers and Probes: 2019-nCoV CDC EUA Kit, 500 rxn (IDT #10006606)
- 2019-nCoV\_N\_Positive Control (IDT #10006625)
- Hs\_RPP30 Positive Control (IDT #10006626)
- TaqPath 1-Step RT-qPCR Master Mix, CG (ThermoFisher; cat # A15299 or A15300)
- MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Applied Biosystems)
- MagMAX Express 96 instrument (Applied Biosystems)

- KingFisher 96 tip comb for DW magnets, 10 x 10 pcs/box (Cat No. 97002534) used for *MagMAX Express 96* instruments
- KingFisher Deepwell 96 Plate, V-bottom, polypropylene (Cat No. 95040450) used for *MagMAX Express 96* instruments
- KingFisher 96 KF microplate (200 $\mu$ L) (Cat No. 97002540) used for *MagMAX Express 96* instruments
- QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems) with the QuantStudio 12K Flex Software v1.3.
- MicroAmp Optical 384-Well Reaction Plate with Barcode (Cat No. 4309849)
- MicroAmp Optical Adhesive Film (Cat No. 4311971)
- Pipettors
- Fisherbrand SureOne Micropoint Graduated Pipet Tips (Cat No. 02-707-401)
- ClipTip 384 125, Filter, Sterile (Cat No. 94420053, 94420813, 94420153)
- TE Buffer
- Molecular grade water, nuclease-free
- Vortex mixer
- Microcentrifuge
- Surface disinfectant (10% bleach wipes and alcohol wipes)

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

### SARS-CoV-2 Negative Control:

A negative extraction control using TE buffer is taken through the entire extraction and PCR procedure.

### NTCs (No template controls):

NTC should be run on each plate. The NTC consists of reaction master mix, primer/probe mix, and nuclease-free water and is included into the PCR reaction only.

This control is negative for N1, N2, and RNaseP2 and is included in each PCR run once for every patient sample run (i.e., in every 384-well plate).

### SARS-CoV-2 Positive Controls:

One positive control each for RNase P and for the N1/N2 targets is included in each PCR run once for every patient sample run (i.e., in every 384-well plate). This control is designed to assess the integrity of the PCR run. The positive controls are plasmid-based controls separate for RNase P and the nucleocapsid (N) gene (N1 and N2 target) from IDT. Positive controls run at 150 copies/uL and are only included in the PCR.

### Internal Control (RNase P)

The internal RNaseP gene is co-extracted and amplified from all patient samples as an internal control. The presence of RNase P in clinical samples assesses specimen quality. All clinical samples should exhibit fluorescence growth curves in the RNase P reaction.

It is possible that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of SARS-CoV-2 virus RNA in a clinical specimen.

## INTERPRETATION OF RESULTS

The result Interpretation follows the one from the CDC 2019-nCoV rRT-PCR Diagnostic Panel including any applicable cutoff.

### 1) **SARS-CoV-2 RT-PCR test Controls – Positive, Negative, and Internal:**

**Table 2.** Expected performance of controls

<b>Control Type</b>	<b>Step controlled</b>	<b>Used to Monitor</b>	<b>N1</b>	<b>N2</b>	<b>RP</b>
Positive	PCR	Substantial reagent failure including primer and probe integrity	+	+	+
Negative	PCR	Reagent and/or environmental contamination	–	–	–
Negative	Extraction & PCR	Reagent and/or environmental contamination	–	–	–
Internal	Extraction & PCR	Failure in lysis and extraction procedure, potential contamination during extraction	–	–	+

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.

### 2) **Examination and Interpretation of Patient Specimen Results:**

1. **Positive Specimens:**  
Specimens with Ct values of <40 in both N1 and N2 targets, with or without an acceptable RNase P, are reported as “Detected” for SARS-CoV-2 RNA.
2. **Negative Specimens:**  
Specimens with undetectable Ct values (i.e. Ct  $\geq$ 40.0) for the N1 and N2 targets but with an acceptable RNase P (Ct <40) are reported as “Not Detected” for SARS-CoV-2 RNA.
3. **Inconclusive Results:**  
When all controls exhibit the expected performance and the cycle threshold growth curve for any one of the two SARS-CoV-2 markers (N1 or N2) but not both crosses the threshold line within 40 cycles (<40 Ct) the result is

“inconclusive”. Inconclusive results should be repeated since inconsistent detection of one target may still represent a true positive result.

4. Invalid Results:

Samples without any growth curves in the SARS-CoV-2 targets AND the RNase P (RP) are invalid. With initially invalid results, the sample will be re-extracted and repeated. If the result remains invalid, consider collecting a new specimen from the patient.

**Interpretation of Patient Results**

2019 nCoV_N1	2019 nCoV_N2	RP	Result Interpretation	Report	Actions
+	+	±	2019-nCoV detected	Positive 2019-nCoV	Report results in LIS and to State DPH
+	-	±	Inconclusive Result	Inconclusive	This is an inconclusive result Repeat extraction and RT-PCR. If the repeated result remains indeterminant, contact CDC for instructions for transfer of the specimen to CDC for additional testing and further guidance.
-	+				
-	-	+	2019-nCoV not detected	Not Detected	Report results. Consider testing for other respiratory viruses.
-	-	-	Invalid Result	Invalid	Repeat extraction and rRT-PCR. If the result remains invalid, consider collecting a new specimen from the patient.

**PERFORMANCE EVALUATION**

**1) Analytical Sensitivity:**

***Limit of Detection (LoD):***

An LoD study was conducted by spiking in SARS-CoV-2 RNA (Twist) into Nasopharyngeal Swab matrix. The SARS-CoV-2 material was spiked at concentrations of 250, 100, 50, and 12.5 copies of RNA per reaction (i.e., 50, 20, 10 and 2.5 copies/μl) and tested according to the SOP. The tentative LoD was identified at 10 copies/μl.

SARS-CoV-2 - Tentative LoD Nasopharyngeal swab										
Target Level	Valid results	SARS-CoV-2 (N1) Positive		SARS-CoV-2 (N1) Detection Rate	SARS-CoV-2 (N2) Positive		SARS-CoV-2 (N2) Detection Rate	RNase P Positive		RNase P Detection Rate
		n	Mean Ct		n	Mean Ct		n	Mean Ct	
50 cp/uL	10	10	29	100%	10	26	100%	10	24	100%
20 cp/uL	10	10	30	100%	10	29	100%	10	24	100%
10 cp/uL	10	10	32	100%	10	32	100%	10	23	100%
5 cp/ul	10	7	33	70%	9	28	90%	10	24	100%
2.5 cp/ul	10	8	31	80%	9	30	90%	10	24	100%
Negative	10	n/a		n/a	n/a		n/a	10	25	100%
<b>Tentative LoD: 10 cp/uL</b> [lowest target level demonstrating ≥95% detection rate of both targets]										

The tentative LoD was then confirmed with 20 replicates at 10 copies/μl for both targets, N1 and N2.

SARS-CoV-2 - Confirmatory LoD Nasopharyngeal Swab										
Target Level	Valid results	SARS-CoV-2 (N1) Positive		SARS-CoV-2 (N1) Detection Rate	SARS-CoV-2 (N2) Positive		SARS-CoV-2 (N2) Detection Rate	RNase P Positive		RNase P Detection Rate
		n	Mean Ct		n	Mean Ct		n	Mean Ct	
10 cp/μL	20	19	32	95%	20	31	100%	20	23	100%
<b>Final LoD: 10 cp/μL</b> [lowest target level demonstrating ≥95% detection rate of both targets]										

***Bridging Study (Aimes – VTM):***

A bridging study was performed at 2x LoD, confirming equal performance of the test when swabs in VTM and Aimes medium are tested.

Bridging Study – VTM/Aimes										
Target Level	Valid results	SARS-CoV-2 (N1) Positive		SARS-CoV-2 (N1) Detection Rate	SARS-CoV-2 (N2) Positive		SARS-CoV-2 (N2) Detection Rate	RNase P Positive		RNase P Detection Rate
		n	Mean Ct		n	Mean Ct		n	Mean Ct	
<b>Aimes-Medium</b>										
20 cp/μL	20	20	32	100%	19	32	95%	20	32	100%
<b>VTM</b>										
20 cp/μL	20	19	32	95%	20	32	100%	20	32	100%

**2) Analytical Inclusivity/Specificity:**

The Integrity Laboratories SARS-CoV-2 PCR assay test utilizes identical oligonucleotide sequences for the N1 and N2 SARS-CoV-2 target genes as those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel the CDC assay. The inclusivity and cross-reactivity of the primers and probes have been evaluated under the EUA for the 2019-nCoV rRT-PCR Diagnostic Panel from CDC and therefore, additional evaluation was not performed for inclusivity.

For cross reactivity wet testing was performed with 5 replicates each for the following organisms; no cross reactivity has been observed for the tested organisms and the tested concentration:

Human Coronavirus HKU1, Human Coronavirus NL63, Human Coronavirus 229E, Human Coronavirus OC43, Human Metapneumovirus (hMPV), Human Rhinovirus 1/2, Human Rhinovirus 2/2, Human Enterovirus (pan assay), Influenza A, Influenza B, Influenza C, Influenza A/H1-2009, Human Parainfluenza virus 1, Human Parainfluenza virus 2, Human Parainfluenza virus 3, Human Parainfluenza virus 4 Human Respiratory Syncytial Virus A (RSVA), Human Respiratory Syncytial Virus B (RSVB), Adenovirus 1/2, Adenovirus 2/2, Human Bocavirus, Human Parechovirus.

**3) Clinical Evaluation:**

***a. Clinical Specimen Testing***

Clinical nasopharyngeal swab specimens in Aimes or Viral Transport Media have been tested from patients at Integrity Laboratories for whom the patient infected status has been determined in parallel by other laboratories using EUA authorized tests. The testing was performed prospectively as routing diagnostic evaluation for

COVID-19. Thirteen (13) individual presumptive positive and 65 negative patients were included. For the negative samples, samples were split in aliquots of which one was sent to Integrity Laboratories and one aliquot was sent to a different laboratory for confirmation. The positive samples that were tested by Integrity Laboratories and by the reference laboratory were different from each patient, but the samples were obtained within a few days of one another. Therefore, the following tables list the patient infected status (PIS) as comparator and not the individual reference laboratory/comparator assay. Specimens were collected at 4 different institutions from Tennessee.

		Patient Infected Status	
		Presumptive positive	Negative
Integrity Laboratories	Presumptive positive	13	0
	Negative	0	65

**Positive Percent Agreement (PPA): 100% (95% CI: 77.3% - 100%)**  
**Negative Percent Agreement (NPA): 100% (95% CI: 94.4% - 100%)**

The testing on these clinical specimens performed at Integrity Laboratories and at the alternate testing laboratories fulfills the requirement for confirmatory testing for at least 5 positive and 5 negative specimens.

***b. Contrived Specimen Testing***

For contrived specimens TWIST Synthetic SARS-CoV-2 RNA was spiked into Aimes (which was validated by a bridging study, see LoD section above).

Contrived Specimen Study							
Sample Concentration	n	N1 Target		N2Target		RNase P	
		positive (n)	Mean Ct	positive (n)	Mean Ct	Positive (n)	Mean Ct
10 copies/uL 1 X LoD	10	10	32	10	31	10	23
20 copies/uL 2 X LoD	10	10	27	10	27	10	23
30 copies/uL 3 X LoD	10	10	27	10	25	10	23
Negative	30	0	n/a	0	n/a	30	34

**Performance against the expected results are:**  
**Positive Percent Agreement (PPA): 30/30 = 100% (95% CI: 88.7% - 100%)**  
**Negative Percent Agreement (NPA): 30/30 = 100% (95% CI: 88.7% - 100%)**

**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.

#### **WARNINGS AND PRECAUTIONS**

- This product has not been FDA cleared or approved by FDA, but has been authorized by FDA under an EUA for use by authorized laboratories;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics 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 declaration is terminated or the authorization is revoked sooner.