

**ACCELERATED EMERGENCY USE  
AUTHORIZATION (EUA) SUMMARY SARS-CoV-  
2 RT-PCR Assay  
(Stanford Health Care Clinical Virology 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 Stanford Health Care Clinical Virology Laboratory, 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 SARS-CoV-2 assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal, nasal, and mid turbinate nasal swabs in Viral Transport Medium and bronchoalveolar lavage fluid from individuals suspected of COVID-19. Testing is limited to the Stanford Health Care Clinical Virology Laboratory, which is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory.

Results are for the detection and 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.

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

**DEVICE DESCRIPTION AND TEST PRINCIPLE**

The assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set is designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines.

SARS-CoV-2 nucleic acid from patient samples is extracted utilizing either the Qiagen EZ1 XL or QIASymphony SP/AS extraction platforms and then reverse transcribed to complementary DNA (cDNA). The cDNA is then amplified during the PCR reaction. 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 Rotor-Gene Q real-time PCR thermal cycler.

The reaction mix also contains primers and probe specific to 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 2019 novel Coronavirus (SARS-CoV-2) Real-Time, RT-PCR test is to be used with the Qiagen Rotor-Gene Q thermocycler using Rotor-Gene Q Series Software 2.3.1 (Build 49). The following extraction platforms can be used: Qiagen EZ1 XL and QIASymphony SP/AS.

### **REAGENTS AND MATERIALS**

EZ1 Virus Mini Kit v2.0

Qiagen, Catalog # 955134 (48 isolations)

PMM # 92453

Contains:

- Reagent cartridges, Virus Mini v2.0
- Disposable Tip Holders
- Disposable Filter-Tips
- Sample Tubes (2 mL)
- Elution Tubes (1.5 mL)
- Carrier RNA (cRNA)
- AVE Buffer

QIASymphony DSP Virus/Pathogen Midi Kit (96 extractions)

Qiagen Cat #937055

PMM#186996

Contains:

- Reagent Cartridge X2
- Enzyme rack X2
- Piercing Lid X2
- Buffer AVE (20 mL) X2
- Buffer AVE (2 mL) X2
- Reuse Seal Set X2

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- Carrier RNA X2
- Handbook X1

Superscript III One Step RT-PCR (Life Technologies)

Cat. No. 11732-020 Size: 100 reactions

PMM# 207751

Cat. No. 11732-088 Size: 500 reactions

PMM# 207752

Molecular-Grade, Glass-Distilled H<sub>2</sub>O

Teknova Catalog # W3335

PMM # 178379

Pooled Negative Nasopharyngeal Patient Samples

Prepared in the Virology Laboratory

SARS-CoV-2 E single-stranded DNA (ssDNA)

Prepared in the Virology Laboratory

Synthesized as 4nmol ultramers by Integrated DNA Technologies (IDT)

100X TE (Tris-EDTA) for Primer-Probes

Sigma Catalog # T9285-100 mL

PMM # 190656

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

#### SARS-CoV-2 Positive Control:

A positive control is needed to assess the integrity of the PCR run. Synthesized single stranded DNA (ssDNA) of the SARS-CoV-2 E gene is used as positive control material. One positive control is included on each run.

#### Negative/Extraction Control:

A negative control is needed to monitor for any cross-contamination that occurs during the RT-PCR process. Pooled negative nasopharyngeal samples are used as a negative control. This also serves as an extraction control. One negative control is included on each run.

#### RNase P in patient samples (Extraction Control)

An internal control is needed to verify that nucleic acid is present in every sample and is used for every sample processed. Detection of human RNase P present in patient samples serves as the internal control.

#### NTCs (No template controls):

A negative (no template) control is needed to eliminate the possibility of sample contamination on the assay run. DNase and RNase free water is used as the NTC and is included on every run.

## INTERPRETATION OF RESULTS

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

Positive control: The positive control must be Detected, showing an exponential growth curve in the SARS-CoV-2 (Green) channel. The CT must be detected within the lot specific ranges.

Negative control: The negative SARS-CoV-2 control must be Not Detected, showing no fluorescent signal above the threshold in the SARS-CoV-2 (Green) channel. The CT value for RNase P (Yellow) channel must be detected within the expected lot specific ranges.

NTC: The No Template Control must be Not Detected, showing no fluorescent signal above the threshold in the SARS-CoV-2 (Green) and RNase P IC (Yellow) channels.

If one or more controls fail, consult with the Section Head, Supervisor or Medical Director. Failure of controls will result in a failed run and the entire run may need to be repeated from extraction as soon as possible. If it can be determined that an error was made in the PCR portion of the run, repeat the run from the eluates.

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

It is necessary to look at the data for a sample's SARS-CoV-2 RNA result as well as its corresponding RNase P Internal control (IC) result to render an interpretation. The presence or absence of a fluorescent growth curve and the CT value to determine the result for each sample should be assessed.

#### 1. Positive Specimens:

Samples in which the E gene SARS-CoV-2 RNA target is detected within the first 40 cycles of amplification are considered "Detected" (the CT value will be < 40).

If the specimen is Detected (CT < 40) but the RNase P does not amplify within the expected range, the reaction is acceptable, and the specimen will be reported as SARS-CoV-2 RNA "Detected".

#### 2. Negative Specimens:

Samples in which there is no value for SARS-CoV-2 (E Gene) are considered Not Detected (the CT value will be blank). In order to accept these results as Not Detected, the RNase P Internal Control (IC) must be detected at a CT ≤ 35 cycles.

#### 3. Indeterminate Results:

If the E gene channel shows detection between CT 40-45 and the RNase P is not detected, the PCR run is repeated from extraction. If the repeated testing yields an E gene CT <45, with or without RNase P, the run is reported as detected. If the E gene CT is not detected and RNase P Ct is ≤35, the medical director/designee must be notified.

4. Invalid Results:

If SARS-CoV-2 RNA is Not Detected and the RNase P does not amplify within the expected range, the sample should be re-extracted.

Upon re-extraction, if the SARS-CoV-2 RNA is Not Detected, and the RNase P does not amplify within the expected range, the specimen may be inadequate or may have inhibitors.

Any scenarios not mentioned above or if any questionable curves are observed, must be submitted for immediate review by the Medical Director or Designee.

**Interpretation of Patient Results**

	<b>Green Channel CT:</b>	<b>Yellow Channel CT:</b>	
<b>Patient Sample</b>	<b>E Gene</b>	<b>RNase P IC</b>	<b>Conclusion</b>
Not Detected	No CT	≤ 35	Report as NDET.
1 <sup>st</sup> Invalid	No CT	> 35 or No CT	<b>Invalid, repeat from extraction.</b>
2 <sup>nd</sup> Invalid	No CT	> 35 or No CT	<b>Report as UNABLE.</b>
<b>Detected</b>	<b>&lt;40</b>	<b>N/A</b>	<b>Report as DTD</b>
Indeterminate	40-45	N/A	Repeat from Extraction
Any scenarios not mentioned or questionable curve(s).			Notify Medical Director/Designee.
<b>After Indeterminate Result is Repeated</b>			
<b>Detected</b>	<b>&lt;45</b>	<b>N/A</b>	<b>Report as DTD</b>
Review	No CT	≤ 35	Notify Medical Director/Designee.

**PERFORMANCE EVALUATION**

1) Analytical Sensitivity:

*Limit of Detection (LoD):*

One SARS-CoV-2 positive clinical nasopharyngeal swab specimen was diluted in pooled viral transport medium from clinical nasopharyngeal swab specimens negative for SARS-CoV-2. Twenty 400 µL replicates were extracted on the QIASymphony. 100% (20/20) of replicates were detected at 1000 copies/mL. The positive clinical specimen was quantitated via a standard curve generated using ssDNA obtained from IDT. The ssDNA standard curve was run in triplicate with concentrations ranging from 150 to 150,000,000 copies/mL.

The original clinical nasopharyngeal swab specimen was diluted in viral transport medium from negative pooled clinical NP swab specimens to make a 100,000 copies/mL stock. The dilutions were made according to the tables below and then tested with the SARS-CoV-2 assay:

Concentration (copies/mL)	Number of Replicates Tested	Number of Replicates Detected	Mean Ct (E target)	Mean Ct (RNase P)
10000	5	5	32.12	19.89
5000	5	5	32.85	19.74
2500	5	5	34.21	19.73
2000	20	20	34.21	19.89
1000	20	20	35.55	19.90
500	10	10	37.19	19.86

The LoD was confirmed to be 1000 copies/mL based on a positivity rate of  $\geq 95\%$  for 20 replicates.

2) **Analytical Inclusivity:**

In silico evaluation of the SARS-CoV-2 E gene primers and probes were evaluated against all publicly available SARS-CoV-2 (taxid: 2697049) sequences present in GenBank as of March 9<sup>th</sup>, 2020. Resulting primer and probe alignments demonstrated 100% match to all SARS-CoV-2 strains.

3) **Cross-Reactivity:**

In silico evaluation of the SARS-CoV-2 E gene primers and probe was carried out with the following parameters:

- The program used was the BLASTN 2.10.0 with default parameters for short input sequences.
- The database used was the Nucleotide collection (nt) which was queried March 25<sup>th</sup>, 2020.
- SARS-CoV-2 (taxid:2697049), HCoV-SARS (taxid:694009), and Bat Betacoronavirus SARS related virus (taxid:1508227) sequences were excluded.

The organisms tested are outlined in the table below:

Other high priority pathogens from the same genetic family	High priority organisms likely in circulating areas
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus (e.g. EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	<i>Chlamydia pneumoniae</i>
	<i>Haemophilus influenzae</i>
	<i>Legionella pneumophila</i>
	<i>Mycobacterium tuberculosis</i>
	<i>Streptococcus pneumoniae</i>
	<i>Streptococcus pyogenes</i>
	<i>Bordetella pertussis</i>
	<i>Mycoplasma pneumoniae</i>
	<i>Pneumocystis jirovecii</i> (PJP)
	Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract
	<i>Candida albicans</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Staphylococcus epidermis</i>
	<i>Staphylococcus salivarius</i>

The only sequences with substantial similarity (>80% homology) were pangolin and bat betacoronavirus sequences that were not appropriately assigned a taxid. Given that these viruses are not known to circulate in humans, cross-reactivity with these zoonotic sequences are not expected to impact the clinical utility of this assay. Forward and Reverse primers and probe for the SARS-CoV-2 E gene demonstrated no substantial similarities with any of the recommended organisms tested.

Wet testing with clinical specimens was also conducted to assess potential cross-reactivity. No cross-reactivity was observed with NP swab specimens positive for seasonal coronaviruses (n=50) including three mixed infections; influenza A (n=1); rhinovirus/enterovirus (n=2). In addition, no cross-reactivity was observed with NP swab specimens positive for influenza A (no subtype; n=2), influenza A 2009 H1N1 (n=2), influenza B (n=2), respiratory syncytial virus (n=5), human metapneumovirus (n=4), parainfluenza virus 1 (n=2), and parainfluenza virus 4 (n=1), as well as two mixed infections: respiratory syncytial virus/parainfluenza virus 3/rhinovirus/enterovirus and human metapneumovirus/rhinovirus/enterovirus.

**4) Clinical Evaluation:**

The clinical evaluation was conducted by testing a total of 60 nasopharyngeal swab specimens in viral transport media (30 reactive and 30 non-reactive). These specimens were collected from individuals under investigation for COVID-19 in

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Northern California. All 60 specimens were tested with the SARS-CoV-2 E gene assay as well as a validated molecular comparator assay targeting the RNA-polymerase (RdRp) sequence in the *ORF1ab* gene to confirm results. Specimens were extracted using the QIAasympyphony and EZ1 XL extraction platforms.

All 30 specimens positive by the comparator assay were also positive by the SARS-CoV-2 assay (100% PPA). All 30 specimens negative by the validated comparator assay were also negative by the SARS-CoV-2 assay (100% NPA). The table below shows the mean CT values for the SARS-CoV-2 E gene assay and the comparator test:

<b>Test</b>	<b>Number tested</b>	<b>Number positive</b>	<b>Mean Ct</b>
<b>SARS-COV-2 E gene test</b>	30	30	27.97
<b>Validated comparator test</b>	30	30	28.4

In addition, the first 5 positive and first 5 negative samples by the SARS-CoV-2 test were sent to the Santa Clara Department of Public Health Laboratory for confirmatory testing. All 10 patient specimens yielded concordant results.