

**ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
ONE HEALTH LABORATORIES SARS-CoV-2 REAL-TIME RT-PCR TEST
(ONE HEALTH LABORATORIES, LLC)**

For *In vitro* Diagnostic Use

Rx Only

For use under Emergency Use Authorization (EUA) only

The One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test will be performed at One Health Laboratories, LLC in Philadelphia, Pennsylvania, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, as per the One Health Labs SARS-CoV-2 Real-Time RT-PCR Test Laboratory Instructions for Use (SOP-OHL-SARS-CoV-2 Assay Instructions) that was reviewed by the FDA under this EUA.

INTENDED USE

The One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens, and nasopharyngeal wash/aspirate or nasal aspirate specimens) collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to One Health Laboratories, LLC certified under 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. 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 One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays. The One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test is a real-time RT-PCR test that uses two primer and probe sets to detect specific regions in the SARS-CoV-2 genome, including a target sequence in the SARS-CoV-2 Open Reading Frame 1 (ORF1ab) and another in the SARS-CoV-2 Spike (S) gene. The third primer and probe set included the assay is designed to detect the MS2 bacteriophage RNA internal control which is added to each control and patient sample prior to nucleic acid extraction.

The test consists of reagents purchased from Biomeme, Inc. The nucleic acid extraction kit and PCR instrument required to perform the test are also manufactured by Biomeme. The nasopharyngeal and oropharyngeal swabs used to collect specimens for validation of this test were purchased from Becton, Dickinson and Company (BD). Nasal swabs, mid-turbinate swabs, nasopharyngeal wash/aspirates and nasal aspirates were not included in the validation studies.

The Biomeme RUOs used for this assay include:

- SARS-CoV-2 Go Strips (REF 3000555)
- SARS-CoV-2 Go-Plates (REF 3000562)
- RNA, MS2 extraction control (REF 3000149)

The Biomeme extraction kit used for this assay includes:

- M1 Sample Prep Cartridge for RNA 2.0 (REF 300134)

The Biomeme PCR instrument used for this assay includes:

- Biomeme Franklin three9 Real-Time PCR Thermocycler equipped with Franklin Firmware version v6.3.1 and Franklin Software version v1.4.5.

The BD specimen collection kits used in validation studies:

- Universal Viral Collection Kit BD 3ML Vial (REF 220527/220531)

RNA is purified from patient samples using the Biomeme RNA extraction kit and is reverse transcribed and amplified in the Biomeme Go-Strip (3-well format) on the Biomeme Franklin three9 Real-Time PCR Thermocycler. Each Biomeme Go-Strip reaction well contains three primer and probe sets for the detection of SARS-CoV-2 ORF1ab (FAM), SARS-CoV-2 S (ATTO647N), and the MS2 bacteriophage control (Texas Red-X). During the amplification process, specific probes anneal to each of the amplified sequences located between the forward and reverse primers for each target. During the extension phase of the PCR cycle, the 5' nuclease activity of the polymerase degrades the bound probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity.

The Biomeme Franklin three9 Real-Time PCR Thermocycler is controlled using the Biomeme Go Android App. Upon launching the app, the user can scan in the Go-Strips by taking a picture of the QR code printed on the strip with the camera phone. The user

then checks that the PCR settings are correct, selects the quantity of Go-Strips included in the run, and enters each of the sample IDs manually or by scanning. Programmed runs are then saved into a folder. Once the phone is connected to the PCR thermocycler either by Bluetooth or USB connection, the run can be started. Results are displayed via the App when the run is completed.

- If the ORF1ab target is detected, the corresponding wells are depicted in green.
- If the S target is detected, the corresponding wells are depicted in red.
- If the internal control target is detected, the corresponding wells are depicted in amber/yellow.

*Ct values for all detected targets are also shown as part of the display.

Brief Description of Test Steps Including Reagent/Sample Volumes:

- Nucleic Acid Extraction
 - Kit: Biomeme M1 Sample Prep Cartridge for RNA 2.0
 - Protocol: Manufacturer’s Sample Extraction Protocol
 - Recommendation(s): Add 200 µL of sample to pre-aliquoted tube of RCP Buffer. Add 20 µl of MS2 suspension before proceeding with the extraction protocol. Elution volume is 850 µl.
- rRT-PCR
 - 20 µL extracted RNA from patient or control samples is added to one well of the Biomeme Go-Strip (3-well format).
 - The Go-Strips are capped and the following PCR parameters are run on the Biomeme Franklin Real-Time PCR Thermocycler (SW v1.4.5):

Step	Cycles	Temp	Time
RT Incubation	1	55°C	2 min
Enzyme Deactivation	1	95°C	1 min
PCR Amplification	45	95°C	3 sec
		60°C	30 sec

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

No Template Control (NTC)

- The NTC consists of nuclease-free water and is used to monitor for potential reagent and/or environmental contamination. One NTC reaction must be included in every PCR run. The NTC should be negative for all targets.

SARS-CoV-2 Positive Control (PC)

- The PC is used to monitor the integrity of the reverse transcription (RT) and PCR reagents, and successful RT and PCR processes. The PC is prepared by spiking SARS-CoV-2 genomic RNA into negative clinical nasopharyngeal swab matrix at 2X the assay LoD with the MS2 extraction control. The PC is processed with the Biomeme RNA extraction kit and stored in single use aliquots at -80°C. One PC reaction must be included in every PCR run. The PC should be positive for all

targets. PC values are tracked over time and monitored in the monthly QA review and assessed for possible trends.

Extraction Control

- The extraction control consists of a lyophilized MS2 bacteriophage pellet, resuspended in Biomeme RPC Buffer, that is added to each test sample prior to extraction with the Biomeme M1 Sample Prep Cartridge for RNA 2.0. The MS2 RNA serves as a control for nucleic acid extraction, reverse transcriptase, assay set-up, PCR reagent integrity, and monitors for the presence of PCR inhibitors. All patient samples and positive control samples should be positive for the MS2 target.

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.

1) **One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test Controls –No Sample Control, Positive Control and Extraction Control**

Before the results can be determined for each clinical specimen, the PCR run must be determined to be valid. For a run to be valid, the controls must yield the expected results:

Table 1: Assay Control Results Interpretation

Control Type	Control Name	Used to Monitor	2019 nCoV ORF1ab	2019 nCoV S	MS2 Extraction Control	Expected Ct values (all targets)
Negative	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Positive	PC	Improper assay set-up, reagent failure including RT, PCR, and primer/probe integrity	+	+	+	< 40

If the controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Repeat the PCR test. If the controls still exhibit unexpected test results, re-extract the entire batch of samples and repeat the test.

2) **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.

SARS-CoV-2 Markers (ORF1ab and S)

- When both SARS-CoV-2 targets are negative and MS2 is positive (Ct < 40), the result should be considered as negative.
- When both SARS-CoV-2 targets are positive (Ct < 40) and MS2 is positive (Ct < 40) or negative, the result should be considered as valid and positive.
- If only one SARS-CoV-2 target is positive (Ct < 40) and MS2 is positive (Ct < 40) or negative, the result is inconclusive. Re-extract the sample and repeat the test. If the same result is obtained, report as inconclusive.
- When both SARS-CoV-2 targets are negative and MS2 is negative, the result is invalid. Repeat the RNA extraction and RT-PCR processes using residual sample. If the repeat result is the same, report as invalid.

Table 2. Clinical Sample Results Interpretation

2019 nCoV ORF1ab	2019 nCoV S	MS2	Result Interpretation	Report	Actions
-	-	+	SARS-CoV-2 RNA not detected	Negative	N/A
+	+	±	SARS-CoV-2 RNA detected	Positive	Report result to state and/or local health department.
If only one target is positive		±	Inconclusive	Inconclusive	Re-extract the sample and repeat the test. If the same result is obtained, report as inconclusive.
-	-	-	Invalid	Invalid	Re-extract the sample and repeat the test. If the same result is obtained, report as invalid.

The assay reports Ct values for each individual target from which the user will need to interpret independently. For all targets, a Ct value < 40 indicates a positive result. Only qualitative results (positive/negative/inconclusive/invalid) will be reported to the physician/outside of the organization.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD)

A preliminary LoD was established for each SARS-CoV-2 target in the One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test using nasopharyngeal swab specimens spiked with various dilutions of known concentrations of genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI Resources). As stated in the instructions for use, sample matrix was pre-mixed with Biomeme Lysis Buffer (BLB) prior to addition of the SARS-CoV-2 RNA. The MS2 control was also added to the mix as described in the Biomeme M1 Sample Prep Cartridge for RNA 2.0 user manual.

The preliminary LoD was then confirmed by testing 40 individual nasopharyngeal swab specimens spiked with 1.8 genomic copies/ μ l of SARS CoV-2 RNA (BEI Resources). The observed positivity rate among the 40 samples was 95% and 95.7% for the ORF1ab and S targets, respectively..

2) Analytical Inclusivity/Specificity:

Inclusivity in silico Analysis

Inclusivity *in silico* analysis of the SARS-CoV-2 ORF1ab and S oligonucleotide primer and probe sequences was performed using 7,874 publicly available nucleic acid sequences for SARS-CoV-2 on March 24, 2020. All alignments showed 100% identity of the One Health Laboratories SARS-CoV-2 primers and probes to the available SARS-CoV-2 sequences.

Specificity/Exclusivity in silico Analysis

BLASTn analysis queries of the 2019-nCoV rRT-PCR assays primers and probes were performed against public domain nucleotide sequences. The database search parameters were as follows:

- The nucleotide collection consists of all GenBank + EMBL + DDBJ + PDB sequences, excluding sequences from PAT, EST, STS, GSS, WGS, TSA and phase 0, 1 or 2 HTGS sequences.
- Non-redundant, records with identical sequences collapsed into a single entry.
- The search parameters automatically adjust for short input sequences and the expected threshold is 1000.
- The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment is 5 and 2 respectively.

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ORF1ab: The forward and reverse primer sequences for SARS-CoV-2 ORF1ab showed high sequence homology (F = 91%, R= 95%) to Bat SARS-like coronaviruses, while the ORF1ab probe was homologous (81%) to only one Bat SARS-like coronavirus isolate. The reverse primer sequence for SARS-CoV-2 ORF1ab also exhibited high sequence homology to human SARS coronaviruses and *Candida albicans* (85%). However, when combining primers and probe, no significant homologies with the human genome, other coronaviruses, common respiratory flora, human microflora and other viral pathogens that would predict potential false positives rRT-PCR results.

S: The forward primer sequence for SARS CoV-2 S showed sequence homology (82%) to Bat SARS-like coronavirus. The reverse primer and probe sequence showed no significant homologies with the human genome, other coronaviruses, common respiratory flora, human microflora and other viral pathogens that would predict potential false positives rRT-PCR results.

In summary, the One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test, designed for the specific detection of SARS-CoV-2, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive rRT-PCR results.

3) *Interfering Substances*

The following substances were tested for inhibitory effects on RT-PCR using respiratory specimens positive for RNA viruses processed with the Biomeme M1 Sample Prep for RNA 2.0. No interference with any of the substances was observed.

Table 3: List of Endogenous/Exogenous Substances Tested

Interfering Substance	Active Ingredient	Concentration Tested
Blood	Human blood	2% (v/v)
Throat lozenges	Benzocaine, menthol	0.15 mg/mL
Saline Nasal Spray	Sodium chloride	0.026 mg/mL
No Drip Nasal Mist	Oxymetazoline hydrochloride (0.05%)	10% (v/v)
Extra Strength Nose Drops	Phenylephrine hydrochloride (1%)	10% (v/v)
Saline Nasal Spray with Aloe	Sodium chloride (0.65%)	10% (v/v)
Zicam, Nasal Swab Gel	Luffa operculata, Galphimia glauca, histaminum hydrochloricum, sulfur	10% (w/v)
Flonase/Nasal corticosteroid	Fluticasone propionate (50mcg/ spray)	10% (v/v)

A separate study was conducted to test the effect of mucin on the One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test. In this study, three replicates of VTM containing SARS-CoV-2 RNA at 3X the LoD with or without 2% mucin were

extracted using the Biomeme M1 Sample Prep Cartridge for RNA 2.0. All replicates were positive for SARS-CoV-2 with the One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test.

4) Clinical Evaluation:

The performance of the One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test was evaluated using contrived clinical samples. A total of 60 contrived positive samples were prepared for testing by spiking individual negative clinical nasopharyngeal swab (NP) specimen matrix with known concentrations of genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI NR-522285, Lot: 70033320, Mftg Date: 11FEB2020). Prior to the addition of SARS-CoV-2 RNA, sample matrix was pre-mixed with Biomeme Lysis Buffer (BLB) from the Biomeme M1 Sample Prep Cartridge for RNA 2.0 nucleic acid extraction kit and the MS2 control as described in the manufacturer’s instructions.

Of the 60 contrived positive samples, 40 contained SARS-CoV-2 RNA at 1X the LoD (same samples as those in the LoD confirmation study), 10 contained SARS-CoV-2 RNA at 2X the LoD, and the remaining 10 contained SARS-CoV-2 RNA between 3X and 5X the LoD. An additional 30 individual negative nasopharyngeal swab specimens were also included in the study. RNA from each sample was manually extracted using the Biomeme M1 Sample Prep Cartridge for RNA 2.0 with a total elution volume of 850µl. The results of the One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test are shown in Table 7 below.

Table 4. Clinical Evaluation with Contrived Nasopharyngeal Swab Specimens

RNA Concentration (relative to LoD)	RNA Concentration (GE/µl)	Number of Positives	Mean Ct	
			ORF1ab	S
1X	1.8	39/40 ^a	34.75	33.51
2X	3.6	10/10	33.19	31.14
3X	5.4	5/5	33.46	32.36
4X	7.2	3/3	30.96	29.71
5X	9	2/2	31.24	30.05
Negative	0	30/30	NA	NA

^a Samples prepared at 1X LoD are the same as those tested in the LoD confirmation study.

PPA at 1-2X = 97.5% (87.1%-99.6%)

PPA at 3-5X = 100% (72.5-100%)

NPA= 100% (88.65-100%)

In addition to the data above, One Health Laboratories sent a total of 16 samples to the Pennsylvania Department of Health for confirmatory testing. Based on the One Health Laboratories result interpretation algorithm, 10 of these samples tested negative, 5 tested positive for SARS-CoV-2, and 1 yielded an inconclusive result. Of the 10 negative samples, nine had concordant results with those obtained by the state lab. Additionally,

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the 5 positive samples were all confirmed to be positive, and the inconclusive sample was confirmed to be negative.

Upon further investigation of the false negative result, the Ct values obtained by the state lab for the discordant sample suggest that the sample was below the One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test limit of detection. Upon repeat testing of the sample, both SARS-CoV-2 ORF1ab and S targets exhibited low level amplification (38.14 and 36.13 Ct, respectively) with the One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test.

To further evaluate the performance of the device with real patient samples, an additional 30 SARS-CoV-2 positive and 30 SARS-CoV-2 negative samples were tested with the One Health Laboratories SARS-CoV-2 Real-Time RT-PCR Test and a comparator method. All 60 samples produced concordant results. This testing fulfills the requirement for evaluation of clinical specimens.