EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
Aeon Global Health SARS-CoV-2 assay
(Aeon Global Health)

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

(The Aeon Global Health SARS-CoV-2 assay will be performed at the Aeon Global Health laboratory, located at 2225 Centennial Drive, Gainesville, GA 30504, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets the requirements to perform high complexity tests as described in the Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA.)

INTENDED USE

The Aeon Global Health SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens, nasopharyngeal wash/aspirate or nasal aspirate specimens, and bronchoalveolar lavage specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Aeon Global Health laboratory located at 2225 Centennial Drive, Gainesville, GA 30504 which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements to perform high-complexity tests.

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 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 Aeon Global Health SARS-CoV-2 Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The Aeon Global Health SARS-CoV-2 Assay is only for use under the Food and Drug Administration’s Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Aeon Global Health SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) multiplex test which includes three primer and probe sets designed to detect RNA from the SARS-CoV-2 nucleocapsid (N) gene, spike (S) gene and open reading frame 1ab (ORF1ab) region in nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens, nasopharyngeal wash/aspirate or nasal aspirate specimens, and bronchoalveolar lavage samples collected from individuals suspected of COVID-19 by their healthcare provider. In addition, a primer/probe set is used to detect bacteriophage MS2 as an internal process control for nucleic acid extraction. The Aeon Global Health SARS-CoV-2 Assay uses primers and probes that were developed and validated under the Emergency Use Authorization (EUA) for the Thermo Fisher Scientific TaqPath COVID-19 Combo Kit (EUA200010).

RNA isolated from respiratory specimens is reverse transcribed to cDNA and subsequently amplified using the Applied Biosystems QuantStudio 12K Flex Real-Time PCR System. 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 (VIC, ABY and FAM) to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the thermocycler.

Samples are handled and stored following the CDC guidelines (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html) for collection by a health care provider.

INSTRUMENTS USED WITH THE TEST

The Aeon Global Health SARS-CoV-2 Assay is to be used with the Applied Biosystems QuantStudio 12K Flex Real-Time PCR System (Cat. No. 4471134) with the 384-well block. The QuantStudio 12K Software (v1.3) is used for primary analysis; secondary analysis is performed using Applied Biosystems Design and Analysis Software (DA2 v2.4.0).

Automated RNA extraction for all specimen types is performed on the MagMax Express-96 Magnetic Particle Processor (Catalog #: 44079) using the Omega Bio-Tek Mag-Bind Viral DNA/RNA 96 kit (Catalog # M6246-03).

REAGENTS AND MATERIALS

The following reagents and materials are required to run the test in addition to general lab consumables for the extraction and PCR:

Reagents	**Manufacturer**	**Catalog #**
Omega Mag-Bind Viral DNA/RNA 96 Kit	Omega Bio-Tek	M6246-03
TaqPath COVID-19 Combo Kit	Thermo Fisher Scientific	A47814
TaqPath 1-Step Multiplex Master Mix	Thermo Fisher Scientific	A28523
Nuclease Free Water	Ambion	4387936
Ethanol (100%)	Fisher Chemical	A409-4
2-Propanol	Fisher Chemical	A416-4
Instruments	**Manufacturer**	**Catalog #**
MagMAX Express-96 Magnetic Particle Processor	Thermo Fisher Applied Biosystems	44079
QuantStudio 12K Flex Real-Time PCR System	Thermo Fisher Applied Biosystems	4471134
Consumables	**Manufacturer**	**Catalog #**
KingFisher Deep Well 96 Plates	Thermo Fisher Scientific	95040450
KingFisher 96 Plate 200 ul (Elution)	Thermo Fisher Scientific	97002540
MicroAmp Optical 384 well PCR plate	Thermo Fisher Scientific	4309849
MicroAmp Optical Adhesive Film	Thermo Fisher Scientific	4311971

CONTROL MATERIALS TO BE USED

The controls supplied with the Thermo Fisher Scientific TaqPath COVID-19 Combo Kit are described in the table below.

Control Type	**Purpose**	**Frequency of Testing**
Internal (MS2 Phage)	To monitor the integrity of the nucleic acid extraction and RT-PCR for each specimen	Added to each specimen and the Negative Extraction Control (NEC) prior to extraction
Positive*	To monitor the integrity of the RT-PCR reagents and process	Once per run of RT-PCR

*****TaqPath COVID-19 Control $1x10^4$ copies/ul (Cat. No. A48003) diluted to 10 copies/µl using TaqPath COVID-19 Control Dilution Buffer (Cat. No. A48002)

In addition to the controls listed above, a Negative Extraction Control (NEC) and No Template Control (NTC) are also run per batch of specimens to monitor for cross-contamination during RNA extraction and RT-PCR. This Negative Extraction Control consists of negative nasopharyngeal swab media spiked with 10 µl of Internal (MS2 Phage) control. The No Template Control consists of Nuclease free water (DNase free, Molecular biology grade, RNase free).

The results from the controls are interpreted according to the criteria shown in Table 1. If the results obtained with the Positive, Internal, Negative and No Template Controls do not meet the criteria shown, the results from the entire batch of samples are considered invalid and repeat testing must be performed using residual extracted nucleic acid. If re-testing using residual extracted nucleic acid does not meet criteria, then testing must be performed using residual original specimen.

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. Refer to Table 1 below for a summary of expected control results.

Table 1. Expected Performance of Controls

Control	Ct Value (Optical Channel)			
	N Gene (VIC)	**S Gene (ABY)**	**ORF1ab (FAM)**	**MS2 Phage (JUN)**
Positive	≤37	≤37	≤37	Undetermined
Negative	Undetermined	Undetermined	Undetermined	≤ 30
No Template Control	Undetermined	Undetermined	Undetermined	Undetermined

Assessment of clinical specimen test results should be performed after the controls have been examined and determined to be valid. If the controls are not valid, the patient results cannot be interpreted. Refer to Table 2 below for interpretation and reporting of patient results. A sample with an MS2 Ct ≤ 30 and a Ct undetermined or Ct > 37 for all three assays (ORF1ab, N Protein and S Protein) will be considered negative. Positive results have any numerical value for MS2 and a Ct ≤ 37 for at least two out of three assays (ORF1ab, N, and S genes).

Table 2. Result interpretation for patient samples

Test Sequence	ORF1ab	N gene	S gene	MS2	Status	Result	Action
Initial	NEG	NEG	NEG	> 30	INVALID	Invalid	Repeat test by re-extracting the original sample and repeating RT-PCR.
	NEG	NEG	NEG	≤30	VALID	SARS-CoV-2 Not Detected	Report results. Consider testing for other viruses.
	Only one of three targets ≤37			Any Value	VALID	SARS-CoV-2 Inconclusive	Repeat test one time. Re-test from residual extracted sample and repeat RT-PCR.
	Two or more SARS-CoV-2 targets ≤37			Any Value	VALID	SARS-CoV-2 Detected	Report results.
Repeat Test	NEG	NEG	NEG	> 30	INVALID	Invalid	Report results. Consider collecting a new specimen.
	NEG	NEG	NEG	≤ 30	VALID	SARS-CoV-2 Not Detected	Report results. Consider testing for other viruses.
	Only one Target ORF1ab or S gene ≤37. NEG for N gene.			Any Value	VALID	SARS-CoV-2 Inconclusive	Report results. Additional confirmation testing should be conducted if clinically indicated.
	NEG	≤37	NEG	Any Value	VALID	SARS-CoV-2 Detected	Report results.
	Two or more SARS-CoV-2 targets ≤37			Any Value	VALID	SARS-CoV-2 Detected	Report results.

G. PERFORMANCE EVALUATION

1) ***Limit of Detection (LoD) - Analytical Sensitivity:***

The LoD is defined from this study as the lowest concentration at which 19/20 replicates (at least 95%) are positive. The samples for the LoD study were prepared by spiking viral genomic RNA (MN985325.1 Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-WA1/2020, complete genome; ATCC Cat. No. VR-1986-D) into individual negative nasopharyngeal clinical specimens. Sample extraction was automated on a MagMAX Express-96 Magnetic Particle Processor using the Mag-Bind Viral DNA/RNA 96 Kit (Omega Biotek; Cat. No. M6246-03). rRT-PCR occurred on Applied Biosystems QuantStudio 12K Flex Real-Time PCR System (Cat. No. 4471134). The preliminary estimate of LoD included a 2-3 fold dilution series, testing three replicates per concentration. The results from the preliminary LoD testing are represented in Table 4. The preliminary LoD was determined to be 0.25 copies/µl.

Table 4. Preliminary LoD Results

Target Level	Valid tested replicates	SARS-CoV-2 N Positive			SARS-CoV-2 S Positive			SARS-CoV-2 ORF1ab Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
2.5 cp/µL	3	3	28.31	100%	3	28.03	100%	3	27.53	100%
1.25 cp/µL	3	3	29.30	100%	3	29.03	100%	3	28.53	100%
0.5 cp/µL	3	3	30.39	100%	3	29.28	100%	3	29.04	100%
0.25 cp/µL	3	3	31.46	100%	3	30.75	100%	3	29.86	100%
0.0825 cp/µL	3	3	33.25	100%	1	33.28	33%	2	32.02	66%
0.0275 cp/µL	3	1	35.44	33%	0	-	0%	1	32.33	33%

The preliminary LoD was confirmed by testing an additional 20 replicates at the determined target level of 0.25 copies/µl. Twenty replicates were also tested for each 2-3 fold dilution series above (0.5 copies/µl) and below (0.0825 copies/ul) the target level. All 20 replicates produced the expected results for each SARS-CoV-2 target, and the LoD was therefore determined to be 0.25 copies/µl (Table 5).

Table 5. Confirmatory LoD results

Target Level	Valid tested replicates	SARS-CoV-2 N Positive			SARS-CoV-2 S Positive			SARS-CoV-2 ORF1ab Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
0.5 cp/µL	20	20	30.35	100%	20	29.83	100%	20	29.43	100%
0.25 cp/µL	20	20	31.51	100%	20	31.02	100%	20	30.34	100%
0.0825 cp/µL	20	20	33.43	100%	9	34.44	45%	15	33.70	75%

2) *Inclusivity (Analytical Sensitivity)*

The Aeon Global Health SARS-CoV-2 Assay is a modification of the previously authorized Thermo Fisher Scientific TaqPath COVID-19 Combo Kit. The assay targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene, spike (S) gene, and ORF1ab region. Inclusivity was demonstrated under the original Thermo Fisher Scientific EUA and a right of reference to use their inclusivity data was provided to Aeon Global Health. Briefly, the primers and probes were mapped to 25,998 complete SARS-CoV-2 genomes of human host that were available in the GenBank and GISAID (Global Initiative on Sharing All Influenza Data) databases as of June 3, 2020. For all primers and probes, there was 100% homology to >99.99% of known SARS-CoV-2 isolates in GISAID and 100% of known isolates in GenBank databases.

3) Cross-reactivity (Analytical Specificity)

The analytical specificity of the Aeon Global Health SARS-CoV-2 Assay was demonstrated *in silico* under the original EUA for the Thermo Fisher Scientific TaqPath COVID-19 Combo Kit. As stated previously, a right of reference to use Thermo Fisher's exclusivity data was given to Aeon Global Health. The analysis included evaluation of the primer and probe homology with the organisms and viruses listed in Table 6. Based on this analysis, significant amplification of non-target sequences that could result in cross-reaction (false-positive results) were considered unlikely to occur.

Table 6. Organisms and viruses evaluated for potential cross-reaction and/or interference with the Thermo Fisher Scientific TaqPath COVID-19 Combo Kit

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
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	***Pneumocystis jirovecii* (PJP)**
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Staphylococcus salivarius

4) <u>Clinical Evaluation</u>

A total of 70 nasopharyngeal specimens (35 positive and 35 negative for SARS-CoV-2) were tested with the Aeon Global Health SARS-CoV-2 Assay and the results were compared to results obtained by 2 different FDA-authorized tests. The results are summarized in Table 7. Samples were extracted on the MagMAX Express-96 Magnetic Particle Processor using the Omega Bio-Tek Mag-Bind Viral DNA/RNA 96 Kit and the reverse transcription RT-PCR was performed using the Applied Biosystems QuantStudio 12K Flex Real-Time PCR instrument.

Table 7. Clinical performance of the Aeon Global Health SARS-CoV-2 Assay in nasopharyngeal swab specimens

		FDA EUA Tests		
		Positive Patient Specimen	Negative Patient Specimen	**Total**
Aeon Global Health SARS-CoV-2 Assay	Positive Patient Specimen	**35**	**1**	**36**
	Negative Patient Specimen	**0**	**34**	**34**
	Total	**35**	**35**	

Results: The positive and negative percent agreements between the Aeon Global Health SARS-CoV-2 Assay and FDA EUA tests are:

PPA: 35/35 = 100% (95% C.I. = 90.11% - 100%)
NPA: 34/35 = 97.14% (95% C.I. = 85.47% - 99.5%)

WARNINGS

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by Aeon Global Health laboratory, located at 2225 Centennial Drive, Gainesville, GA 30504;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-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* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

LIMITATIONS

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to Aeon Global Health laboratory which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a and meets requirements to perform high complexity tests.

- The Aeon Global Health SARS-CoV-2 Assay was established using nasopharyngeal swab specimens. Nasal, mid-turbinate and oropharyngeal swab specimens, nasopharyngeal wash/aspirate or nasal aspirate specimens, and bronchoalveolar lavage samples are also considered acceptable specimen types for use with the Aeon Global Health SARS-CoV-2 Assay but performance has not been established.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- False negative results can arise from:
    - Specimen collection conducted prior to symptom onset.
    - Failure to follow the authorized assay procedures.
    - Failure to use authorized extraction kit and platform.
- There is a risk of false negative values due to the presence of sequence variants in the viral targets of the assay.
- A false positive result may arise from cross contamination during specimen handling or preparation, or between patient samples.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.