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ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

SARS-CoV-2 (E, N and RdRP gene detection) Test

(AIT Laboratories)

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 AIT 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 Test is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab) and lower respiratory specimens (such as sputum and BAL) from individuals suspected of COVID-19 by a healthcare professional. Testing is limited to AIT 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 assay is intended for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The SARS-CoV-2 Test uses the ThermoFisher Scientific TaqPath COVID-19 Combo Kit that was FDA authorized for emergency use (EUA) on March 13, 2020. The ThermoFisher assay is a real-time reverse transcription polymerase chain reaction assay. The primer and probe sets used with the test are designed to amplify and detect three regions of the SARS-CoV-2 single stranded RNA genome: the Orf1ab, N gene and S gene.

All probes are labeled with unique fluorophores that are detected and distinguished within the same reaction. RNA isolated from respiratory specimens is reverse transcribed to cDNA and subsequently amplified using the QuantStudio 12K Flex real-time PCR system (Applied Biosystems) with Software version 1.5.1.

During the amplification process, the probe anneals to the three specific target sequences 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 dyes 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. Fluorescence intensity is monitored at each PCR cycle by the QuantStudio 12K.

INSTRUMENTS USED WITH TEST

RNA extraction is conducted using the MagBind Universal Pathogen Kit (Omega Biotek, Georgia, USA) automated on the Hamilton Nimbus liquid handling system for high throughput extraction and the QuantStudio 12K Flex real-time PCR system for cDNA synthesis and PCR amplification of the target sequences.

EQUIPMENT, REAGENTS AND MATERIALS

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

1. MagBind Universal Pathogen Kit (Omega Biotek, Georgia, USA)
2. Hamilton Nimbus liquid handling system
3. TaqPath RT-PCR COVID-19 Kit (Thermo Fisher, #A47817)
4. TaqPath COVID-19 Control Kit (Thermo Fisher, #A47816)
5. TaqPath 1-Step Multiplex Master Mix (Thermo Fisher, #A28525)
6. QuantStudio 12K Flex
7. Nuclease - free Water
8. Ethanol - molecular biology grade
9. CO-RE Filter tips-1000µl Hamilton
10. 96 Deep well plate-2ml (Fisher Scientific, #12566121)
11. Single well reagent trough (Hamilton, #56669-01)
12. Elution plate (Plate One, #1833-9610)
13. Clip Tip 12.5 µl and 125 µl Filter (Thermo Fisher)

14. MicroAmp 96 Fast Optical plate (Thermo Fisher, #4346906)
15. MicroAmp Clear film (Thermo Fisher, #4306311)
16. Alumaseal 96 sealing film (Sigma Aldrich, #Z721549)
17. Pipettes and Filter tips
18. Mini Plate Centrifuge
19. Biosafety cabinet
20. AirClean PCR Workstation

CONTROLS TO BE USED WITH THE SARS-CoV-2 RT-PCR

- The positive control included in the Thermo Fisher Scientific TaqPath COVID-19 Combo Kit (www.fda.gov/media/136112/download) is an RNA sequence containing the Orf1ab, N gene and S gene amplicon sequences provided at a concentration of 10^4copies/µl. The dilution and reverse transcription of the positive control is performed according to the kit instructions and a single positive control is included in every PCR run. This also acts as a reaction setup and reagent integrity control.

- The extraction/reverse transcription control is provided in the form of intact MS2 Phage. An aliquot of the MS2 Phage particle is included in every sample prior to nucleic acid extraction. This acts as a spiked internal control to monitor the RNA extraction process.

- A negative/no template control is included in every PCR run and is used to monitor non-specific amplification, cross-contamination during experimental setup, and nucleic acid contamination of reagents. NTC is included in the PCR reaction only.

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. Interpretation of TaqPath RT-PCR COVID-19 Controls – Internal Positive, Positive and Negative Controls

- MS2 (Internal Positive Control) indicates that PCR amplification occurred in the well. The presence of MS2 and no detectable SARS-CoV-2 during the analysis indicates that proper RNA extraction and amplification occurred, however, no SARS-CoV-2 is present. If SARS-CoV-2 is present in the specimen, amplification of the target RNA may reduce or abrogate MS2 amplification. In this case, the amplified SARS-CoV-2 indicates proper RNA extraction and amplification. Therefore, MS2 may or may not be detectable in a valid test.

- TaqPath COVID-19 Control Kit (External Positive Control) must be positive in order for the test result to be valid.
- Nuclease-Free Water (Negative Control) must be negative in order for the test result to be valid.

  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. Assessment of the clinical specimen test results are performed in accordance with the parameters defined in the ThermoFisher Scientific TaqPath COVID-19 Combo Kit using a Ct 35 as a cutoff. Any target with a Ct ≤35 is positive and any target with a Ct >35 is negative.

Table 1: Interpretation of Patient Samples

ORF1ab	N gene	S gene	MS2	Controls Status	Result
NEG	NEG	NEG	NEG	Invalid	NA
NEG	NEG	NEG	POS	Valid	SARS-CoV-2 Not Detected
Only one SARS-CoV-2 target = POS			POS or NEG	Valid	SARS-CoV-2 Inconclusive
Two or more SARS-CoV-2 targets = POS			POS or NEG	Valid	Positive SARS-CoV-2

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Sputum samples previously collected from patients were pooled and spiked with the SARS-CoV-2 RNA from the TaqPath COVID-19 Control Kit and processed as described in the test steps section above. A ten-fold dilution series ranging from 10^4 copies/µl to 1.0 copies/µl of the RNA was used. A total of 20 samples were tested for each concentration of the positive control.

The final LoD of the test is 10 cp/µL.

Table 2: SARS-CoV-2 Tentative LoD

Target Level*	Valid results	SARS-CoV-2 N-Gene Positive			SARS-CoV-2 Orf1ab-Gene Positive			SARS-CoV-2 S-Gene Positive			Internal Control MS2 Positive			Detection Rate Per Result Interpretation*
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	
10^4 cp/µL	20	20	25.4	100%	20	25.2	100%	20	24.8	100%	20	27.7	100%	100%
10^3 cp/µL	20	20	29.3	100%	20	29.3	100%	20	29.1	100%	20	28.0	100%	100%
10^2 cp/µL	20	20	32.8	100%	20	32.7	100%	20	32.7	100%	20	27.6	100%	100%
10^1 cp/µL	**20**	**20**	**34.3**	**100%**	**20**	**34.4**	**100%**	**12**	**34.1**	**60%***	**20**	**25.4**	**100%**	**100%**
1.0 cp/µL	20	0	N/A	100%	2	33.9	10%	1	34.7	5%	20	27.1	100%	0%*
0.25 cp/µL	10	0	N/A	0%	0	N/A	0%	0	N/A	0%	10	24.2	50%	0%
Negative	20	Not detected			Not detected			Not detected			20	26.1	100%	0%

* Result Interpretation of a positive result is based on positivity of a minimum of 2 targets. Inconclusive results are counted as negative in this column

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

a. Inclusivity

Inclusivity studies for the assays has been performed by ThermoFisher (https://www.fda.gov/media/136112/download) and the information has been provided in the FDA-EUA granted to this manufacturer.

b. Cross-Reactivity

i. *In Silico* Analysis

Inclusivity studies for the assays have been performed by ThermoFisher (https://www.fda.gov/media/136112/download) and the information has been provided in the FDA-EUA granted to this manufacturer.

ii. Wet Testing

Thirty (30) patient samples positive for the presence of other respiratory bacterial and/or viral pathogens were tested and previously published (Singh et al., 2019). These samples were randomly selected and tested for cross reactivity with the ThermoFisher-COVID-19 EUA assays. There was no cross reactivity for the ThermoFisher (Orf1ab, N gene, S gene) EUA assays in patient samples positive for the viral and bacterial respiratory pathogens listed below. Note that some samples were positive for multiple analytes.

Table 3. Organisms Tested for Cross-Reactivity

Organism	No. Samples	Result (E, RdRP, N)
RSV	4	Negative
Influenza A/B (pooled)	3	Negative
Human metapneumovirus	5	Negative
Parainfluenza virus	1	Negative
Rhinovirus	7	Negative
Coronavirus (pooled 229E, NL63, OC43, HKU1 strains)	5	Negative
Streptococcus pneumoniae	10	Negative
H. Influenza	4	Negative
M. catarrhalis	3	Negative
S. aureus	1	Negative

3) Clinical Evaluation:

A total of 100 specimens (50 positive and 50 negative) were collected and tested at AIT laboratory with the AIT SARS-CoV-2 assay in a blinded manner. These 100 specimens were confirmed at AIT laboratory using the CDC assay as validated at the testing site. The specimens were a mix of nasal and oropharyngeal swab specimens and sputum. Results are shown in Table 4.

Table 4: Evaluation with Clinical Specimens

		CDC PCR	
		Sputum	
		PRESUMPTIVE POSITIVE	NEGATIVE
AIT Laboratory	POSITIVE	29	0
	NEGATIVE	0	27
		Nasal	
		PRESUMPTIVE POSITIVE	NEGATIVE
AIT Laboratory	POSITIVE	13	0
	NEGATIVE	0	13
		Oropharyngeal (Throat)	
		PRESUMPTIVE POSITIVE	NEGATIVE
AIT Laboratory	POSITIVE	8	0
	NEGATIVE	0	10
		Total	
		PRESUMPTIVE POSITIVE	NEGATIVE
AIT Laboratory	POSITIVE	50	0
	NEGATIVE	0	50

Positive Percent Agreement (PPA): 50/50 = 100% (95% CI: 92.9% -100%)
Negative Percent Agreement (NPA): 50/50 = 100% (95% CI: 92.9% -100%)

In addition, the first 5 positive and negative samples (all sputum) were confirmed by another laboratory also using the ThermoFisher EUA test (see Table 5).

Table 5: Confirmed Sputum Specimens

		Confirming Laboratory	
		Sputum	
		POSITIVE	NEGATIVE
AIT Laboratory	POSITIVE	5	0
	NEGATIVE	0	5

Correlation with the comparator result was 100% for both positive and negative samples.

The results of 5 positive and 5 negative specimens tested with the AIT SARS-CoV-2 assay were confirmed at an alternate laboratory and fulfills the requirement for confirmatory testing of at least five positive and five negative specimens.

Limitations

The performance of AIT Labs SARS-CoV-2 Assay was established using nasopharyngeal and oropharyngeal swabs and sputum. Nasal and mid-turbinate swabs and BAL are also considered acceptable specimen types for use with the SARS-CoV-2 Assay but performance has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected at a healthcare site or collected by a healthcare provider) is limited to patients with symptoms of COVID-19.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were MagBind Universal Pathogen Kit (Omega Biotek, Georgia, USA) automated on the Hamilton Nimbus liquid handling system for high throughput extraction and the QuantStudio 12K Flex real-time PCR system for cDNA synthesis and PCR amplification of the target sequences. The results are summarized in Table 6.

Table 6: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	18,000 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

WARNINGS:

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