ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

Modified Thermo Fisher TaqPath COVID-19 SARS-CoV-2 Test

(ORF1ab, N, and S gene detection)

(Biocerna)

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 Biocerna, 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 reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Biocerna LLC, certified under 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 5 real-time PCR system (Applied Biosystems) with Software version 2.3.3.

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 5.

PCR Program:

Table 1. PCR Program

Step	Temperature	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Reverse transcription	53°C	10 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	40
Anneal / extension	60°C	30 seconds	

INSTRUMENTS USED WITH TEST

RNA extraction is conducted using the MagMAX Viral/Pathogen Nucleic acid isolation kit automated on the KingFisher Flex Purification system (KingFisher).
RT-PCR including cDNA synthesis and PCR amplification of the target sequences is performed on the QuantStudio 5 real-time PCR system. The data is analyzed and interpreted by Applied Biosystems Design and Analysis Software version 2.3.3.

EQUIPMENT, REAGENTS AND MATERIALS

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

1. Magmax Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher #42352)
2. KingFisher Flex Purification system (KingFisher)
3. TaqPath RT-PCR COVID-19 Kit (Thermo Fisher, #A47814)
4. TaqPath COVID-19 Control Kit (Thermo Fisher, #A47816)
5. TaqPath 1-Step Multiplex Master Mix (Thermo Fisher, #A28525)
6. QuantStudio 5
7. OraCollect-RNA (OR-100) swabs (Genotek), Copan eSwab (Copan), or other swabs systems found to be equivalent per FDA's FAQ document (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2)

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) serves as an amplification control for the Orf1ab, N gene and S gene amplicon sequences. 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. The positive control is used to monitor for failures of RT-PCR reagents and reaction conditions. This control is only included in the RT-PCR reaction.

- 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 during the nucleic acid extraction. This acts as a spiked internal control to monitor the RNA extraction, reverse transcription and amplification process.

- A negative extraction control (molecular-grade, nuclease-free water) is added to each KingFisher extraction run and carried through to RT-PCR. This control monitors for contamination during the extraction and/or the PCR steps.

- A negative/no template control is molecular-grade, nuclease-free, water and is used to monitor non-specific amplification, cross-contamination during PCR setup, and nucleic acid contamination of PCR reagents. This control is only included in the RT-PCR reaction.

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) in a sample 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 on patient specimens.
- TaqPath COVID-19 Control Kit (External Positive Control) must be positive for all three SARS-CoV-2 targets, i.e., the ORF1ab, the N Protein, and the S Protein genes and amplification must have a Ct <37 in order for the test result to be valid. The Positive control does not contain MS2.
- Nuclease-Free Water (Negative Control) must be negative in order for the test result to be valid.
- A negative Extraction Control is processed with each batch of samples. The negative Extraction Control should only show MS2 amplification curve with a Ct of <37 but must be negative for all SARS-CoV-2 targets (undetermined).

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 37 as a cutoff. Any target with a Ct ≤37 is positive and any target with a Ct >37 is negative.

Table 2. Interpretation of Patient Samples

ORF1ab	N gene	S gene	MS2	Controls Status	Result	Action
NEG	NEG	NEG	NEG	Invalid	NA	Repeat test. If the repeat result remains invalid, consider collecting a new specimen
NEG	NEG	NEG	POS	Valid	SARS-CoV-2 Not Detected	Report result to healthcare provider. Consider testing for other viruses
Only one SARS-CoV-2 target = POS			POS or NEG	Valid	SARS-CoV-2 Inconclusive	Repeat test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.
Two or more SARS-CoV-2 targets = POS			POS or NEG	Valid	Positive SARS-CoV-2	Report result to healthcare provider and appropriate public health authorities.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Anterior nares swabs collected with the OR-100 sample collection device and NP samples collected with the Copan eSwab were pooled and spiked with the SARS-CoV-2 genomic RNA from the TaqPath COVID-19 Control Kit and processed according to the Biocerna SOP. The preliminary LoD was established by using varying quantities of SARS-CoV-2 RNA which resulted in preliminary LoD estimate of 250 GCE/mL for the Genotek OR-100 and 375 GCE/mL for the Copan eSwab. These results were confirmed by testing 20 replicates for each of the sample types.

The final LoD was determined to be 250 GCE/mL for the Genotek OR-100 collection device and 375 GCE/mL for the Copan eSwab.

Table 3. SARS-CoV-2 LoD[a]

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		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
Genotek OR-100													
250 GEC/mL	20	20	30.5	100%	19	32.2	95%	17	30.5	85%	20	25.4	100%
Copan eSwab													
375 GEC/mL	20	20	32.5	100%	18	29.6	100%	20	30.2	100%	20	27.3	100%

[a] Result Interpretation of a positive result is based on positivity of a minimum of 2 targets

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

a. Inclusivity

Inclusivity studies for the assays have been performed by ThermoFisher (www.fda.gov/media/136122/download) and the information has been provided in the FDA-EUA granted to this manufacturer.

b. Cross-Reactivity

Inclusivity studies for the assays have been performed by ThermoFisher (*www.fda.gov/media/136122/download*) and the information has been provided in the FDA-EUA granted to this manufacturer.

3) ***Clinical Evaluation:***

A total of sixty samples were assessed as part of this evaluation; thirty (30) contrived positive and thirty negative nasal swab samples (collected from the anterior nares) were tested, all collected with the RNA Genotek OR-100 swab. Samples were contrived by spiking known concentrations of extracted SARS-CoV-2 viral genomic RNA (BEI, Manassas,VA), relative to the LoD, into the negative nasal swab eluant from individuals who were determined to be negative by the TaqPath RT-PCR COVID-19 Kit prior to spiking in the RNA. Samples were extracted using the KingFisher Flex Purification system using the MagMAX Viral/Pathogen Nucleic Acid Extraction Reagents as per the manufacturer's procedures. All negative samples yielded negative results (Table 6).

Table 4. **Evaluation of Contrived Clinical Samples**

Final RNA Concentration in Samples	Number of Positives	Mean Ct Values			
		S Gene	ORF1ab Gene	N Gene	MS2
2x LoD	20 of 20	30.0	29.6	30.0	
3x LoD	5 of 5	28.8	29.0	28.9	
5x LoD	5 of 5	27.7	27.8	28.8	
Negative Clinical Samples	0 of 30	UND*	UND*	UND*	

*UND = Undetermined

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 use of this assay as an in vitro diagnostic under the FDA Emergency Use Authorization (EAU) is limited to Biocerna laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 9 CLIA), 42 U.S.C. 263a, to perform high complexity tests.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified method listed in this procedure. Other extraction approaches and processing systems have not been elevated.
- The impacts of vaccines, antiviral therapeutics, antibiotics, and chemotherapeutic or immunosuppressant drugs have not ben evaluated. The test cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
- Laboratories are required to report all positive results to the appropriate public health authorities**.**