EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
Cuur Diagnostics SARS-CoV-2 Molecular Assay
Bandar Enterprises, LLC dba Cuur Diagnostics

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

The Cuur Diagnostics SARS-CoV-2 Molecular Assay will be performed at laboratories designated by Cuur Diagnostics, located at 8876 Spanish Ridge Avenue, Suite 203, Las Vegas, Nevada, which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests as described in the Cuur Diagnostics SARS-CoV-2 Molecular Assay Standard Operating Procedure that was reviewed by the FDA under this EUA.

INTENDED USE

1) Intended Use:
The Cuur Diagnostics SARS-CoV-2 Molecular Assay is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from the SARS-CoV2- in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, mid-turbinate, and nasal swab) from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to laboratories designated by Cuur Diagnostics, located at 8876 Spanish Ridge Avenue, Suite 203, Las Vegas, Nevada, which are certified under the Clinical Laboratory Improvements Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high-complexity tests.

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 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 Cuur Diagnostics SARS-CoV-2 Molecular 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 Cuur Diagnostics SARS-CoV-2 Molecular Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TESTPRINCIPLE

The Cuur Diagnostics SARS-CoV-2 Molecular Assay is a real-time, reverse transcription polymerase chain reaction (RT-PCR) test. The Cuur Diagnostics SARS-CoV-2 Molecular Assay is a modified version of the Thermo Fisher TaqPath COVID-19 Combo Kit, which is an authorized test.
The Cuur Diagnostics SARS-CoV-2 Molecular Assay varies from the Thermo Fisher TaqPath COVID-19 Combo Kit Methodology in three ways:

1. The platform instrumentation used to perform the RT-PCR reaction has been changed from the Applied Biosystems 7500 Fast Dx to the Applied Biosystems QuantStudio 12K Flex.

2. The plate format has been changed from a 96-well plate to a 384-well plate, which requires the total reaction volume be scaled down from 25µl to 13µl.

3. The third variation is the analysis software used to interpret qPCR data. The TaqPath COVID-19 Combo Kit EUA uses the Applied Biosystems COVID-19 Interpretive Software. This software is only formatted for use with Applied Biosystems 7500 DX Real-Time PCR data files and is not compatible with the Applied Biosystems QuantStudio 12K Flex data files. As such, the Applied Biosystems QuantStudio 12K Flex Gene Expression software, v1.4 has been validated.

The SARS-CoV-2 primer and probe set(s) are designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients recommended for testing by public health authority guidelines. The assay simultaneously detects four targets: three SARS-CoV-2 viral targets, the Nucleocapsid (N) gene, the ORF1ab gene, and the Spike protein (S) gene, and one primer/probe set detecting MS2 RNA spiked into the reaction as an extraction and process control.

Upper respiratory specimens should be collected, transported and stored according to standard procedures. Nucleic acid is extracted from patient specimens using the KingFisher Flex semi-automated purification system with the MagMax Viral/Pathogen reagent kit (CAT-A48310). Isolated viral RNA is reverse transcribed and amplified on the QuantStudio 12 K Flex instrument, and analyzed with the QuantStudio 12K Flex software, v1.4.

INSTRUMENTS USED WITH THE TEST

The Cuur Diagnostics SARS-CoV-2 Molecular Assay is to be used with the KingFisher Flex semi-automated purification system and the QuantStudio 12 K Flex instrument (software version 1.4).

REAGENTS AND MATERIALS

Equipment/Reagents/Consumables	Catalog #	Manufacturer
Copan eSwabs	480C	Thermo Fisher Scientific
BD UVT 3-mL collection kit	220531	BD
KingFisher Flex	5400630	Thermo Fisher Scientific
KingFisher Flex 96 Deep-Well Heating Block	24075430	Thermo Fisher Scientific

Equipment/Reagents/Consumables	Catalog #	Manufacturer
QuantStudio 12K Flex Real-Time PCR System, 384-well block, desktop	4471134	Thermo Fisher Scientific
ABY Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	A24736	Thermo Fisher Scientific
JUN Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	A24733	Thermo Fisher Scientific
Vortex	2215418	Fisher Scientific
Minifuge	75004081	Thermo Fisher Scientific
P10 Pipette	4641320N	Thermo Fisher Scientific
P200 Pipette	4641210N	Thermo Fisher Scientific
P1000 Pipette	461230N	Thermo Fisher Scientific
E12-10 pipette	4672020BT	Thermo Fisher Scientific
E12-200 pipette	4672070BT	Thermo Fisher Scientific
E8-1000 pipette	4671100BT	Thermo Fisher Scientific
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	A48310	Thermo Fisher Scientific
TaqPath COVID-19 Combo Kit, 1,000 rxn	A47814	Thermo Fisher Scientific
TaqPath 1-Step Multiplex Master Mix (No ROX™)	A28521, A28522, A28523	Thermo Fisher Scientific
NATtrol SARS-CoV-2 (recombinant) Stock	831042	Zeptometrix
1X PBS, pH 7.4 (Phosphate Buffered Saline, 500ml)	25-507	Genesee Scientific
Absolute Alcohol, Ethyl (200 proof, 20L)	BP2818100	Fisher Scientific
Molecular Grade PCR H2O, DIUF (Deionized Ultra Filtered)	7732-18-5	Fisher Scientific
KingFisher Deepwell 96 Plate	95040450, A48305, A48424, 95040455	Thermo Fisher Scientific
KingFisher 96 KF microplate	97002540	Thermo Fisher Scientific
KingFisher 96 tip comb for DW magnets	97002534, A48438, A48414	Thermo Fisher Scientific
MicroAmp Optical 384-well Reaction Plates	4309849	Applied Biosystems
MicroAmp Clear Adhesive Covers	4306311	Applied Biosystems
MicroAmp Optical Adhesive Covers (PCR Compatible)	4360954	Applied Biosystems
Lab Armor Thermal Beads (2L)	42370-002	Cascade Sciences
1.5ml Eppendorf Tubes	AM12450	Ambion
15ml Conical Centrifuge Tube	352196	Falcon
50ml Conical Centrifuge Tube	352098	Falcon
P10 Filter Pipette Tips	94420053	Thermo Fisher Scientific
P200 Filter Pipette Tips	94420313	Thermo Fisher Scientific
P1000 Filter Pipette Tips	94420813	Thermo Fisher Scientific
Reagent Reservoirs	8096-11	Thermo Fisher Scientific

CONTROLS TO BE USED WITH THE TEST

1. **Positive Control**: The Thermo Fisher TaqPath COVID-19 Combo Kit positive control is used and contains the SARS-CoV-2 RNA genomic regions (N, ORF1ab, and S genes) as well as the Internal Control region (MS2) targeted by the assay. The positive control monitors both the RT-PCR reaction setup and reagent integrity and is run with each batch of patient specimens.

2. **Negative (No Template) Control**: Molecular-grade, nuclease-free, non-DEPC-treated water is used to monitor non-specific amplification, cross-contamination during experimental setup, and nucleic acid contamination of reagents. The negative control is run with each batch of patient specimens.

3. **Internal Positive Control (IPC)**: The assay includes primers and probes for detection of an exogenous MS2 phage control, from the TaqPath COVID-19 Combo Kit, that is used to monitor the RNA extraction, reverse transcription, and PCR amplification processes. The internal control is run with every patient specimen.

4. **Negative Extraction Control (NEC)**: A negative extraction control consisting of 200µl naïve universal viral transport (UVT) media is run with each batch of patient specimens to monitor contamination.

5. **Positive Extraction Control (PEC)**: A positive extraction control (Zeptometrix, NAtrol SARS-CoV-2 N gene recombinant stock, catalog#0831042) is run in every exaction batch to monitor batch to batch extraction efficiency, the RT-PCR reaction setup and reagent integrity.

INTERPRETATON 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.

Table 1. Expected Results for the Cuur Diagnostics SARS-CoV-2 Molecular Assay Controls

Control	Expected Results (Expected Crt)			
	ORF1ab	**N**	**S**	**MS2 (IPC)**
Positive Control	Positive (27≤Crt≤30	Positive (27≤Crt≤30)	Positive (27≤Crt≤30	Positive (27≤Crt≤30)
Negative (No Template) Control	Negative (Undetermined)	Negative (Undetermined)	Negative (Undetermined)	Positive (27≤Crt≤30)
Negative Extraction Control	Negative (Undetermined)	Negative (Undetermined)	Negative (Undetermined)	Positive (27≤Crt≤30)
Positive Extraction Control	Negative (Undetermined)	Positive (27≤Crt≤30)	Negative (Undetermined)	Positive (27≤Crt≤30)

Assessment of clinical specimens should be performed after the positive, negative (no template), negative extraction, positive extraction and internal controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Cycle relative thresholds (Crt) for each of the three SARS-CoV-2 target genes, along with the evaluation of the MS2- IPC Crt, will be used to determine patient infected status.

The Cuur Diagnostics SARS-CoV-2 Molecular Assay uses the Relative Threshold Expression estimation algorithm (Crt), which calculates a relative threshold from a fitted efficiency model for gene quantification. This differs from traditional Baseline Threshold Expression estimation algorithm (Ct), which subtracts a baseline component and sets a fluorescent threshold in the exponential region for gene quantification. Relative Threshold analysis was used because it is automated within the QuantStudio Gene Expression software v1.4, while Baseline Threshold placement requires technician interpretation and manipulation to adjust threshold based on batch background data. In addition, the relative threshold method was used because it prevents any manipulation/interpretation of the baseline, which can impact specimens with low amplification/titers.

The interpretation and reporting of clinical specimens is summarized in **Table 2**.

Table 2. Result Interpretation for Patient Samples

ORF1ab	N	S	MS2 (IPC)	Status	Result	Action
NEG (Crt >37)	NEG (Crt >37)	NEG (Crt >37)	NEG (Crt >30)	Invalid	NA	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.
NEG (Crt >37)	NEG (Crt >37)	NEG (Crt >37)	POS (27≤Crt≤30)	Valid	SARS-CoV-2 Not Detected	Report results. Consider testing for other viruses.
Only one SARS-CoV-2 target = POS (Crt≤37)			POS or NEG	Valid	SARS-CoV-2 Inconclusive[1]	Repeat test. If the repeat result remains inconclusive, report results. Additional confirmation testing should be conducted if clinically indicated.
Two or more SARS-CoV-2 targets = POS (Crt≤37)			POS or NEG	Valid	Positive SARS-CoV-2	Report results.

[1] Samples with a result of SARS-CoV-2 Inconclusive shall be retested from extraction one time.

PERFORMANCE EVALUATION

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

The LoD was determined by spiking pooled, negative clinical nasopharyngeal matrix with SARS-CoV-2 genomic RNA. The SARS-CoV-2 genomic RNA was extracted from a positive patient specimen and quantified at 10^5 copies/µL using a standard curve. This material was used to prepare varying concentrations ranging from 10^4 copies/µL to 10 copies/µL. Twenty (20) extraction replicates were performed for each concentration included in the study. The assay LoD was determined to be 25 copies/µL (25,000 copies/mL). Results of the LoD Study are illustrated in **Table 3**.

Table 3. LoD Study Results for the Cuur Diagnostics SARS-CoV-2 Molecular Assay

Concentration (copies/µL)	N Protein		ORF1ab		S Protein		MS2 IPC		% Detection (# Detected/# Tested)
	Mean (Crt)	StDev (Crt)	Mean (Crt)	StDev (Crt)	Mean (Crt)	StDev (Crt)	Mean (Crt)	StDev (Crt)	
10000	23.438	0.356	23.325	0.487	23.613	0.482	27.822	0.446	100% (20/20)
1000	26.351	0.494	25.936	0.742	26.577	0.317	28.039	0.365	100% (20/20)
100	28.410	0.386	28.303	0.259	28.239	0.477	27.978	0.238	100% (20/20)
25	29.760	0.386	29.653	0.259	29.589	0.477	28.147	0.596	100% (20/20)
10	31.058	0.958	32.404	2.062	33.290	1.707	27.883	0.114	75% (15/20)

2) Inclusivity – Analytical Sensitivity:

The Cuur Diagnostics SARS-CoV-2 Molecular Assay utilizes identical oligonucleotide sequences as the Thermo Fisher TaqPath COVID-19 Combo Kit authorized assay. The inclusivity has been evaluated previously and therefore, additional evaluation was not necessary. Thermo Fisher has granted a right of reference to Banda Enterprises / Cuur Diagnostics for the inclusivity performance data contained in the Thermo Fisher EUA request (EUA200010).

3) Cross-reactivity – Analytical Specificity:

The Cuur Diagnostics SARS-CoV-2 Molecular Assay utilizes identical oligonucleotide sequences as the Thermo Fisher TaqPath COVID-19 Combo Kit authorized assay. The cross-reactivity has been evaluated previously and therefore, additional evaluation was not necessary. Thermo Fisher has granted a right of reference to Banda Enterprises / Cuur Diagnostics for the inclusivity performance data contained in the Thermo Fisher EUA request (EUA200010).

4) Clinical Evaluation:

Clinical Performance of the Cuur Diagnostics SARS-CoV-2 Molecular Assay was evaluated using 100 residual nasopharyngeal (NP) swabs (50 positives and 50 negatives) collected from patients suspected of COVID-19. These specimens were previously characterized at one of two external labs using different FDA emergency use authorized SARS-CoV-2 molecular tests. For the positive residual NP swab samples, the positive percent agreement between the Cuur Diagnostics SARS-CoV-2 Molecular Assay and the comparator assays was 96%

(48/50). For the 50 negative residual NP swab samples, the negative percent agreement between the and the comparator assays was 100% (50/50). Qualitative results of the clinical evaluation are shown in **Table 4**.

Table 4. Clinical Performance of the Cuur Diagnostics SARS-CoV-2 Molecular Assay

		Authorized Comparator -1		
		Positive	**Negative**	**Total**
Cuur Diagnostics SARS-CoV-2 Molecular Assay	**Positive**	19	0	19
	Negative	1	20	21
	Total	20	20	40
Positive Agreement		95% (19/20), 95% CI: (76.4, 99.1%)[1]		
Negative Agreement		100% (20/20), 95% CI: (83.9, 100.0%)		
		Authorized Comparator -2		
		Positive	**Negative**	**Total**
Cuur Diagnostics SARS-CoV-2 Molecular Assay	**Positive**	29	0	29
	Negative	1	30	31
	Total	30	30	60
Positive Agreement		96.7% (29/30), 95% CI: (83.3, 99.4%)		
Negative Agreement		100% (30/30), 95% CI: (88.7, 100.0%)		

[1] Two-sided 95% confidence intervals

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by laboratories designated by Cuur Diagnostics, located at 8876 Spanish Ridge Avenue, Suite 203, Las Vegas, Nevada, which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a and meet requirements to perform high complexity tests;
- 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 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.