ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-CoV-2 RT-PCR Assay

(Acupath Laboratories, Inc.)

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

(The Acupath COVID-19 RT-PCR Assay will be performed at Acupath Laboratories, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The Acupath COVID-19 Real-Time (RT-PCR) 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 nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Acupath Laboratories 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.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) 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 Acupath COVID-19 Real-Time (RT-PCR) Assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Acupath COVID-19 Real-Time (RT-PCR) Assay is only for use under the Food and Drug Administration’s Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

RNA isolated and purified from respiratory specimens is first reverse transcribed into cDNA. The cDNA is then amplified using the TaqPath RT-PCR COVID-19 Kit and multiplexed on the QuantStudio 12K Flex instrument. In the process, the probes anneal to three (3) specific SARS-CoV-2 target sequences located between three (3) unique forward and reverse primers for the following genes: 1. ORF1ab, 2. N Protein and 3. S Protein. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the 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. Fluorescence intensity is monitored at each PCR cycle using the Applied Biosystems™ QuantStudio 12K Flex Instrument.

INSTRUMENTS USED WITH TEST

Process	Instrument / Manufacturer
RNA Extraction	• MagMAX Viral/Pathogen Nucleic Acid Isolation Kit using the KingFisher Flex Purification System
Real-Time PCR	• Applied Biosystems QuantStudio 12K Flex Real-Time PCR using QuantStudio software version 1.4.
Software for Analysis	• Applied Biosystems ExpressionSuite Software version 1.3

REAGENTS AND MATERIALS

Instrument	Serial/Catalog Number	Model Number	Manufacturer
Applied Biosystems QuantStudio 12K Flex	285881401	QS12K	ThermoFisher
Applied Biosystems 384-well block		4453553	ThermoFisher
Sorvall ST 16 centrifuge	42385424	N/A	ThermoFisher
Vortex 120V	300240334	1321	ThermoFisher
Micropipettes (P10, P100, P1000)			ThermoFisher
Multi-channel pipettors	PH71356, PH69975	4672070BT, 4671100BT	ThermoFisher
Consumables	**Serial/Catalog Number**	**Model Number**	**Manufacturer**
Reagent Reservoir	42385424	N/A	ThermoFisher
RNase-Free Microcentrifuge Tubes	108-01633	10-180	ThermoFisher
MicroAmp Clear Adhesive Film	IAKT23016	N/A	ThermoFisher
DNase/RNase-free sterile tips	300240334	1321	ThermoFisher
DNase/RNase free sterile tips	14 387-	N/A	ThermoFisher

Instrument	Serial/Catalog Number	Model Number	Manufacturer
	993/983		
KingFisher Flex Magnetic Particle Processor with 96 Deep-Well Head	n/a	5400630	ThermoFisher
KingFisher Flex 96 Deep-Well Heating Block	n/a	24075430	ThermoFisher
KingFisher Deepwell 96 Plate	n/a	95040450	ThermoFisher
KingFisher 96 KF microplate	n/a	97002540	ThermoFisher
KingFisher 96 tip comb for DW magnets	n/a	97002534	ThermoFisher

Kits and reagents	Serial/Catalog Number	Model Number	Manufacturer
MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit	n/a	A48383	ThermoFisher
TaqPath 1-Step Multiplex Master Mix (No ROX)	n/a	A28521, A28522, A28523	ThermoFisher
100% ethanol, ACS reagent grade or equivalent	n/a	n/a	Fisher Scientific
Nuclease-free Water (not DEPC-Treated)	n/a	n/a	Fisher Scientific
Tubes, plates, and other consumables	**Serial/Catalog Number**	**Model Number**	**Manufacturer**
ABY Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	n/a	n/a	A24734
JUN Dye Spectral Calibration Plate for Mutliplex qPCR, Fast 384-well	n/a	n/a	A24735
MicroAmp Fast Optical 384-Well Reaction Plate with Barcode	n/a	n/a	4309849
MicroAmp Optical Adhesive Film	n/a	n/a	4311971 and 4360954
MicroAmp Adhesive Film Applicator	n/a	n/a	4333183
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	n/a	n/a	thermofisher.com/plastics
Conical Tubes (50 mL)	n/a	n/a	AM12501
Control	**Used to monitor**	**Assays**	
Positive Control (TaqPath COVID-19 Control Kit)	RT-PCR reaction setup and reagent integrity	All three SARS-CoV-2 assays	
MS2 Phage Control	RNA extraction	MS2 assay	
Negative Control	Cross-contamination during RNA extraction and reaction setup	All three SARS-CoV-2 assays / MS2 assay	

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

SARS-CoV-2 Positive Control:

One positive control is included in each run. This control is the TaqPath COVID-19 RNA control (external), which contains targets specific to the SARS-CoV-2 genomic regions targeted by the assay. The positive control is designed to monitor RT-PCR setup and reagent integrity.

Internal Extraction Control:
The internal control amplifies MS2 phage. The MS2 control is added to each sample and the negative extraction control during the extraction step. This control is used to monitor the RNA extraction procedure.

Negative Extraction Control (NEC):
The negative control consists of nuclease-free water that is added to each extraction run. This control is carried through the RT-PCR process. The negative control monitors cross-contamination of sample or reagents during the extraction and PCR processes.

No Template Control (NTC)
The no template control consists of nuclease-free water and is added to each RT-PCR run. This control monitors for cross-contamination in the PCR step.

INTERPRETATION OF RESULTS

1) SARS-CoV-2 RT-PCR test Controls – Positive, Negative, and Internal:

All test controls should be examined prior to interpretation of patient results. If the positive and negative controls are not valid, the patient results cannot be interpreted, and the assay run must be repeated.

Positive control: The TaqPath COVID-19 external positive control must be positive for all three of the SARS-CoV-2 targets in order for the results to be valid. If the positive control fails to display amplification for all three targets, the entire run will be repeated.

Internal control: Detection of MS2 during the analysis indicates proper RNA extraction. The MS2 internal control must have a Ct<40 for a sample result to be valid. If the MS2 Ct value is not <40, the sample result will be invalid, and the extraction and PCR should be repeated.

Negative Extraction Control: The negative extraction control must be negative for all SARS-CoV-2 targets for the run to be valid. If the negative extraction control shows amplification for any of the three gene targets, cross-contamination during RNA extraction and/or reaction setup occurred, and the extraction and PCR runs should be repeated.

No Template Control: The no template control must be negative for the SARS-CoV-2 targets and MS2 internal control. If amplification is detected, then cross-contamination during the PCR setup has occurred, and the PCR should be repeated with a fresh vial of nuclease-free water as the No Template Control.

2) Examination and Interpretation of Patient Specimen Results:

1. Positive Specimens: Any two of the SARS-CoV-2 gene targets with a Ct<35 and an MS2 Ct of any value is considered a positive result.

2. Negative Specimens: No detection of at least two SARS-CoV-2 gene targets is considered a negative result. The MS2 internal control must be detected with Ct ≤30 to report a negative result.

3. Inconclusive Results: Any two of the three assay gene targets having Ct values between 35-40 will be interpreted as inconclusive. Additionally, any two targets where one target has Ct $35 \leq Ct < 40$ and the other target has Ct undetermined or 40 will be interpreted as inconclusive. Inconclusive results should be repeated from the extraction phase one time.

4. Invalid Results: If the Ct value for the MS2 internal control is undetermined or equals 40, then the result is invalid. Both the extraction and PCR should be repeated for invalid results.

Interpretation of Patient Results

ORF1ab (6-FAM dye)	N Protein assay (VIC dye)	S Protein assay (ABY dye)	MS2 IPC assay (JUN dye)	COVID-19 Interpretations
Any two gene targets with $C_t < 35$			Ct < 40	Positive.
Any two gene targets with $35 \leq C_t < 40$ OR Any two targets where one has $35 \leq C_t < 40$ and the other has Ct undetermined or 40			Ct < 40	Inconclusive. Inconclusive results shall be repeated from extraction one time.
At least two targets with C_t = Undetermined or $C_t = 40$			Ct < 40	Negative.
Any result			Ct = Undetermined or $C_t = 40$	Invalid. Re-purify the nucleic acid from the sample, then repeat the test.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

The limit of detection of the assay was evaluated using samples prepared by spiking in the TaqPath RNA positive control into pooled negative nasopharyngeal clinical specimens. A total of 6 different dilutions were prepared at the following concentrations:

Tube #	Gene copies/µl
1	1000
2	100
3	50

Tube #	Gene copies/µl
4	25
5	12.5
6	6.25

Each of the dilutions were extracted using the MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit on the KingFisher Flex Purification System and tested on the QuantStudio 12K Flex instrument. A total of 20 replicates were tested per dilution. These results indicated a preliminary LoD of 25 copies/ul based on the positivity rates for each dilution. This LoD was then confirmed by testing 20 additional extraction replicates at a concentration of 25 copies/ul. All 20 replicates generated positive assay results.

LoD Confirmation Study Mean Cts

Gene Target	ORF1ab	N	S
Mean Ct	29.02	30.88	28.29

2) Analytical Inclusivity

The Acupath COVID-19 RT-PCR Assay uses the primers/probes included in the Thermo Fisher TaqPath COVID-19 combo kit. Please refer to EUA200010 for analytical inclusivity analysis.

3) Cross-Reactivity

The Acupath COVID-19 RT-PCR Assay uses the primers/probes included in the Thermo Fisher TaqPath COVID-19 combo kit. Please refer to EUA200010 for the in silico cross reactivity analysis.

Additionally, wet testing was conducted at Acupath Laboratories using a panel of four pathogens likely to be found in respiratory specimens that could cause similar disease. The panel included Parainfluenza (Types 1, 2, 3 and 4B), Influenza A/B, AdV/hMPV/HRV, and RSV. These pathogens were spiked into individual negative nasopharyngeal clinical specimens and extracted using the MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit on the KingFisher Flex Purification System. The RNA was reverse transcribed and tested with the Acupath COVID-19 assay on the QuantStudio 12 K Flex system. None of the samples tested produced a positive result for any of the SARS-CoV-2 gene targets.

4) Clinical Evaluation:

The clinical performance of the Acupath COVID-19 RT-PCR Assay was evaluated by testing a total of 30 known negative and 30 known positive nasopharyngeal clinical specimens. These 60 clinical specimens were previously confirmed using the EUA-authorized Cepheid Xpert Xpress SARS-CoV-2 assay.

Specimens were extracted using the MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit on the KingFisher Purification System. The isolated RNA was

reverse transcribed to cDNA and subsequently amplified on the Applied Biosystems QuantStudio 12K Flex system using the QuantStudio Software version 1.4. The data were analyzed using the Applied Biosystems ExpressionSuite Software version 1.3.

All 30 negative clinical specimens tested negative using the Acupath COVID-19 RT-PCR Assay, generating an NPA of 100%. All 30 positive specimens tested positive using the Acupath Assay, generating a PPA of 100%. The Ct values for each of the assay gene targets in the positive specimens are listed below:

REPLICATES	Ct value				INTERPRETATION	% Positive
	ORF1ab	N	S	MS2		
ALL-72	14.99	14.13	14.50	28.58	COVID-19 Detected	100%
P-1	31.29	33.16	33.00	25.84	COVID-19 Detected	
P-3	19.09	19.51	18.77	25.61	COVID-19 Detected	
P-4	Undetermined	34.01	34.09	26.08	COVID-19 Detected	
P-6	25.18	26.44	23.85	26.14	COVID-19 Detected	
P-7	30.01	29.78	28.85	26.20	COVID-19 Detected	
P-10	25.56	26.88	25.45	26.73	COVID-19 Detected	
SAMPLE-1	29.58	28.34	29.80	28.48	COVID-19 Detected	
SAMPLE-2	22.98	22.44	23.56	28.91	COVID-19 Detected	
SAMPLE-3	19.47	18.97	19.92	29.19	COVID-19 Detected	
SAMPLE-4	21.16	20.20	21.67	28.69	COVID-19 Detected	
SAMPLE-5	14.90	14.10	15.16	32.54	COVID-19 Detected	
SAMPLE-6	28.96	27.83	28.95	27.10	COVID-19 Detected	
SAMPLE-7	18.78	17.37	19.35	28.74	COVID-19 Detected	
SAMPLE-8	33.34	30.99	36.69	26.18	COVID-19 Detected	
SAMPLE-9	23.55	22.86	24.56	26.91	COVID-19 Detected	
SAMPLE-10	16.00	15.26	16.45	30.75	COVID-19 Detected	
SAMPLE-11	17.92	17.15	18.60	28.00	COVID-19 Detected	
SAMPLE-12	27.86	27.55	29.04	27.23	COVID-19 Detected	
SAMPLE-13	21.23	20.25	21.97	27.20	COVID-19 Detected	
SAMPLE-14	34.44	32.50	39.29	26.67	COVID-19 Detected	
SAMPLE-15	21.88	21.32	22.73	26.96	COVID-19 Detected	
SAMPLE-16	27.12	26.72	27.59	24.52	COVID-19 Detected	
SAMPLE-17	22.60	22.11	23.59	24.11	COVID-19 Detected	
SAMPLE-18	25.83	25.05	26.36	24.73	COVID-19 Detected	
SAMPLE-19	25.33	26.59	28.55	24.36	COVID-19 Detected	
SAMPLE-20	19.44	18.59	19.99	28.30	COVID-19 Detected	
SAMPLE-22-5-3-20	31.45	31.94	32.12	25.82	COVID-19 Detected	
SAMPLE-4-5-8-20	31.07	33.69	32.41	26.84	COVID-19 Detected	
SAMPLE-8-5-8-20	32.91	31.58	33.17	27.09	COVID-19 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.

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 the MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit on the KingFisher Flex Purification System and tthe QuantStudio 12K Flex instrument respectively. The results are summarized in the following Table.

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 in VTM	$1.8x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

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