ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY CURATIVE SARS-COV-2 ASSAY (KorvaLabs Inc. Clinical Laboratory)

For in vitro diagnostic use
Rx only
For use under Emergency Use Authorization (EUA) Only

(The Curative SARS-Cov-2 Assay will be performed in the KorvaLabs Inc. Clinical Laboratory, a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory, per the Instructions for Use that were reviewed by the FDA under this EUA).

INTENDED USE

The Curative SARS-Cov-2 Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in oropharyngeal (throat) swab, nasopharyngeal swab, nasal swab, and oral fluid specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the single site of KorvaLabs, Inc. San Dimas, CA, that is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory.

Collection of nasal swabs and oral fluid specimens is limited to symptomatic individuals within 14 days of COVID-19 symptom onset. Specimen collection must be directly observed and directed during the sample collection process by a trained healthcare worker at the specimen collection site. Negative results for SARS-CoV-2 RNA from oral fluid specimens should be confirmed by testing of another specimen type authorized for use with this test if clinically indicated.

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.

Curative SARS-Cov-2 Assay

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Curative SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The assay uses primers and probes that were developed and validated under the Emergency Use Authorization (EUA) for the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel and are designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines. The purpose of this EUA request is to enable testing of additional specimen types, including oral fluid specimens and use of alternative nucleic acid extraction and amplification systems available in KorvaLabs, Inc.

Oropharyngeal (throat) swab, nasopharyngeal swab, and nasal swabs should be collected, transported and stored according to standard procedures. Nasal swabs and oral fluid specimens must be collected using a swab (Curative Inc., #K00023) into the sample collection tube (Curative Inc., #K00016) containing DNA/RNA Shield (Zymo Research, #R1100). Nasal swabs and oral fluid specimens must be transported and stored at ambient temperature and tested within 4 days of collection.

RNA Extraction for all specimen types is performed using Total RNA Purification 96-Well Kit (Norgen Biotek Corporation) manually or using Tecan Resolvex A200. The input sample volume is 300µl, the elution volume is 60µl.

Reverse transcriptase-PCR (RT-PCR) is performed using Applied Biosystems TaqPath™ 1-Step Multiplex Master Mix with 5µl of the extracted sample.

INSTRUMENTS USED WITH THE TEST

The Curative SARS-CoV-2 Assay is for use with the BioRad CFX 96 Touch, BioRad CFX Connect Real-Time PCR systems and Roche LightCycler 480 II Real-Time PCR systems. RT-PCR processing and data analysis is being performed by BioRad CFX Maestro V1.1 and Roche LightCycler Software V1.5.

REAGENTS AND MATERIALS

Table 1. Reagents and materials required for use with Curative SARS-CoV-2 Assay

Material ID	Vendor	Catalog #
Ultrapure water	RX Bioscience	P01-UPW01-500
N1 Primers/Probes	IDT Biosearch Technologies	10006606 KIT-NCOV-PP1-1000
N2 Primers/Probes	IDT Biosearch Technologies	10006606 KIT-NCOV-PP1-1000
RP Primers/Probes	IDT Biosearch Technologies	10006606 KIT-NCOV-PP1-1000
TaqPath™ 1-Step Multiplex Master Mix	Applied Biosystems™	A28527

LIMITATIONS

The performance of the Curative SARS-CoV-2 Assay was established using oral fluid specimen. Nasopharyngeal swabs, oropharyngeal swabs and nasal swabs are considered acceptable specimen types for use with the Curative SARS-CoV-2 Test but performance has not been established.

Testing of nasal swabs and oral fluid specimens (self-collected while directly observed and directed by a trained healthcare worker at the site of sample collection or collected by a trained healthcare worker) is limited to symptomatic individuals within 14-days of COVID-19 symptom onset.

CONTROLS

Negative Process Control: The negative process control consists of uninfected collection media, and two replicates are processed with every extraction batch run.
SARS-CoV-2 and RP Positive Process Control: This control monitors the success of the RNA extraction and confirms the performance of the RT-PCR master mix. The positive process control consists of uninfected collection media spiked with quantified nCoV plasmid and Hs_RPP30 gene.
RP Positive Extraction Control: Detection of RNase P RNA in extracted nucleic acid serves as a positive extraction control for each sample. It also confirms the absence of PCR inhibitors in each eluted RNA sample.

Table 2. Controls performed with Curative SARS-CoV-2 Assay

Control Type	Used to Monitor	Frequency of Testing	
		SARS-CoV-2 N	RP
Negative extraction control	Cross-contamination during extraction	Once per run of RT-PCR	Once per run of RT-PCR
SARS-CoV-2/ RP Positive Process control	Inefficient lysis of specimen, poor specimen collection, improper assay setup, extraction failure, PCR inhibition, reagent failure including primer and probe degradation	Once per run of RT-PCR	Once per run of RT-PCR
RP Positive extraction control	Inefficient lysis of specimen, poor specimen collection, improper assay setup, extraction failure, PCR inhibition, reagent failure including primer and probe degradation	Once per run of RT-PCR	Once per run of RT-PCR

If the SARS-CoV-2 N assay is positive with a negative RP result, consider the sample valid and proceed with the next steps.
If the SARS-CoV-2 N assay is negative in conjunction with a negative RP, the specimen results are considered invalid and should be repeated. If the residual specimen is available, re-extract the specimen and perform testing again. If results remain invalid, a new specimen should be collected.

Table 3. Ct values of controls for validation of results

Control Type	Used to Monitor	Expected results and Ct Values	
		SARS-CoV-2 N	RP
Negative extraction control	Cross-contamination during extraction	Negative Ct not detected or Ct>40	Negative Ct not detected or Ct >35
SARS-CoV-2/ RP Positive Process control	Inefficient lysis of specimen, poor specimen collection, improper assay setup, extraction failure, PCR inhibition, reagent failure including primer and probe degradation	Positive $0 < Ct \leq 40$	Positive $0 < Ct \leq 35$
RP Positive extraction control	Inefficient lysis of specimen, poor specimen collection, improper assay setup, extraction failure, PCR inhibition, reagent failure including primer and probe degradation	Negative Ct not detected	Positive $0 < Ct \leq 35$

INTERPRETATION OF RESULTS

The results from testing of patient samples are interpreted according to the criteria described in **Table 4.**

Table 4. Interpretation of Patient Samples

SARS-CoV-2 N	RP	Result Interpretation	Report	Action
Ct detected in the range $0 < Ct \leq 35$	±	SARS-CoV-2 detected	Presumptive Positive SARS-CoV-2	Report result to sender and local or state department of health.
No Ct or Ct>40	$0 < Ct \leq 35$	SARS-CoV-2 not detected	Negative SARS-CoV-2	Report results to submitter.
Ct detected in the range $35< Ct\leq 40$	±	Mandatory repeat	Hold reporting till confirmatory testing	Repeat extraction from the sample
No Ct or Ct>40	No Ct or Ct>35	Invalid Result	Invalid	Repeat extraction from the sample. If the repeated result remains invalid, report the result to the sender and local or state department of health, and recommend the collection of a new

				specimen from the patient.

PERFORMANCE EVALUATION

I. Analytical Sensitivity

The LoD was determined using viral genomic RNA (BEI Resources Catalog No. NR-52285 (Lot # 70333700) that were diluted in SARS-CoV-2 negative oral fluid specimens from volunteer donors into DNA/RNA Shield solution (Zymo Research, #R1100). An initial estimate of the LoD with both the Bio-Rad CFX and Roche LightCycler 480 II was obtained by testing four replicates at each of four different target levels: 1600, 800, 400 and 200 copies/mL. At all analyte levels, four out of four replicates passed. Based on these results, two analyte concentration ranges (200 copies/mL and 100 copies/mL) were selected for confirmation with 20 replicates each. For both automated and manual extraction methods and both PCR systems, all 20 replicates at 200 copies/mL produced the expected results for the SARS-CoV-2 target, and the LoD was therefore confirmed to be 200 copies/mL.

Table 5: Summary of Analytical Sensitivity Results for Curative SARS-CoV-2 Assay

	Manual Extraction		**Automated Extraction**	
Concentration of Viral RNA	BioRad CFX	Roche LightCycler 480	BioRad CFX	Roche LightCycler 480
200 copies/mL	20/20	20/20	20/20	20/20
100 copies/mL	15/20	20/20	19/20	17/20

II. Analytical specificity

Inclusivity

The Curative SARS-CoV-2 Assay comprises only primers and probes designed by CDC from the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel without any changes. Inclusivity of the CDC Diagnostic panel has been previously established.

Cross-reactivity

The Curative SARS-CoV-2 Assay comprises only primers and probes designed by CDC from the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel without any changes. Cross-reactivity of the CDC Diagnostic panel has been previously established.

III. Clinical evaluation

A clinical study was performed to evaluate the use of oral fluid specimens as a specimen type for detection of SARS-CoV-2 in patients who are suspected of COVID-19. People

who were previously tested at a drive through location in Los Angeles where testing was performed by Curative Inc. were contacted for their willingness to participate in this study.

Tables 7-10 summarize performance of the Curative SARS-CoV-2 test for self-collected oral fluid swabs, non-healthcare worker (HCW) observed or directed and put into Zymo DNA/RNA Shield RNA preservative compared to clinician collected nasopharyngeal swab put into Zymo DNA/RNA Shield RNA preservative:

Table 7: All Individuals (Symptomatic and Asymptomatic)

		Clinician Collected Nasopharyngeal Swab (in RNA preservative)		
		Positive	**Negative**	**Total**
Self-Collected Oral Fluid Swab (Non-HCW observed/directed) in RNA preservative	**Positive**	15	4	19
	Negative	8	17	25
	Total	23	21	44
Positive Agreement		65.2% (95% CI 44.9-81.2%)		
Negative Agreement		81.0% (95% CI 60.0-92.3%)		

*1 oral fluid swab sample was QNS (quantity not sufficient) and was excluded from analysis

When the test is limited to individuals symptomatic at the time of collection, within 21 days of symptom onset, but not observed and directed by HCW during collection, the test performance is as follows:

Table 8: Symptomatic at time of collection

		Clinician Collected Nasopharyngeal Swab (in RNA preservative)		
		Positive	**Negative**	**Total**
Self-Collected Oral Fluid Swab (Non-HCW observed/directed) in RNA preservative	**Positive**	15	1	16
	Negative	5	3	8
	Total	20	4	24
Positive Agreement		75.0% (95% CI 53.1-88.8%)		
Negative Agreement		75.0 % (95% CI 30.1-95.5%)		

When the test is limited to individuals symptomatic at time of collection and less than14 days since symptom onset, but not observed and directed by HCW during collection, the test performance is as follows:

Table 9: Symptomatic at time of collection, >14 days since symptom onset

		Clinician Collected Nasopharyngeal Swab (in RNA preservative)		
		Positive	**Negative**	**Total**
Self-Collected Oral Fluid Swab (Non-HCW observed/directed) in RNA preservative	**Positive**	4	1	5
	Negative	3	0	3
	Total	7	1	8
Positive Agreement		57.1 % (95% CI 25.0-84.2%)		
Negative Agreement		0/1		

When testing is limited to asymptomatic individuals at time of collection, but not observed and directed by HCW during collection, the test performance is as follows:

Table 10: Asymptomatic at time of collection

		Clinician Collected Nasopharyngeal Swab (in RNA preservative)		
		Positive	**Negative**	**Total**
Self-Collected Oral Fluid Swab (Non-HCW observed/directed) in RNA preservative	**Positive**	0	3	3
	Negative	3	14	17
	Total	3	17	20
Positive Agreement		0/3		
Negative Agreement		82.4 % (95% CI 59.0-93.8%)		

*1 oral fluid swab sample was QNS (quantity not sufficient) and was excluded from analysis

In the table above, all three false negatives were from individuals tested as asymptomatic at time of collection, but previously symptomatic, within 17 days of symptom onset (7, 14, and 17 days).

The data above does not support use of the Curative SARS-CoV-2, for any individuals for self-collected oral fluid samples when not directly observed and directed by a trained healthcare worker during collection and at the site of collection. A modification to the original clinical study acceptance criteria was made. Subjects more than 14 days from symptom onset at time of collection, and subjects who were no longer symptomatic, were excluded from the final clinical study. A total of 52 subjects were enrolled. Upon obtaining consent, a clinician drove to the subjects' home with a testing kit, the written research study information form, and the sample collection materials. Testing kits included components for collecting 4 different sample types and all samples were collected into DNA/RNA Shield (Zymo Research).

Subjects were instructed to complete self-collection of the oral fluid specimen and nasal specimen while observed and directed by the study clinician and package the sample into the collection bag. Then the clinician collected a nasopharyngeal sample from the subject. All three specimens were collected in the same visit within a 30-minute window.

There was 100% positive and negative agreement between the results obtained from testing of clinician observed and directed, self-collected oral fluid swabs put into Zymo

DNA/RNA Shield and those obtained from clinician collected nasopharyngeal swabs put into Zymo DNA/RNA Shield. There was 97% positive agreement and 100% negative agreement between the results obtained from testing of clinician observed and directed, self-collected nasal swabs put into Zymo DNA/RNA Shield and those obtained from clinician collected nasopharyngeal swabs put into Zymo DNA/RNA Shield. A summary of the results of the study is presented in **Table 11**.

Table 11. **Summary of qualitative results obtained from parallel testing of nasopharyngeal swabs, nasal swabs and oral fluid specimens.**

		Nasopharyngeal Swab (In RNA Preservative)		
		Positive	**Negative**	**Total**
Oral Fluid Swab (observed and directed) in RNA preservative	**Positive**	34	0	34
	Negative	0	18	18
	Total	34	18	52
Positive Agreement		100 % (34/34)		
Negative Agreement		100 % (18/18)		

		Nasopharyngeal Swab (In RNA Preservative)		
		Positive	**Negative**	**Total**
Nasal Swab (observed and directed) in RNA preservative	**Positive**	32*	0	32
	Negative	1	18	19
	Total	33	18	51*
Positive Agreement		97.0 % (32/33)		
Negative Agreement		100 % (18/18)		

*one nasal swab sample was QNS (quantity not sufficient) and is excluded

A modified second study was performed to evaluate the use of oral fluid specimens as a specimen type for detection of SARS-CoV-2 in patients who are suspected of COVID-19. The study was modified to collect the nasopharyngeal swabs into Viral Transport Media which was sent to another laboratory (CMB Laboratories, Cypress, CA) and run on the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel which has been authorized by the FDA under an Emergency Use Authorization and validated by CMB Laboratories. Oral fluid swab and nasal swab samples were collected as before and processed at Curative Inc. CDC Sample Handling Guidelines were followed for collection, transport and processing of nasopharyngeal swabs samples in viral transport media. A further 28 participants were enrolled in this modified study.

There was 100% positive and negative agreement between the results obtained from testing of oral fluid specimens or nasal swabs and those obtained from nasopharyngeal swabs. A summary of the results of the study is presented in **Table 12**.

Table 12. **Summary of qualitative results obtained from parallel testing of clinician collected nasopharyngeal swabs (following CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel) compared to nasal swabs and oral fluid specimens self-collected while observed and directed by a clinician.**

Curative SARS-Cov-2 Assay

		Nasopharyngeal Swab (In VTM)		
		Positive	**Negative**	**Total**
Oral Fluid Swab (observed and directed) in RNA preservative	**Positive**	16	0	16
	Negative	0	12	12
	Total	16	12	28
Positive Agreement		100 % (16/16)		
Negative Agreement		100 % (12/12)		

		Nasopharyngeal Swab (In VTM)		
		Positive	**Negative**	**Total**
Nasal Swab (observed and directed) in RNA preservative	**Positive**	16	0	16
	Negative	0	12	12
	Total	16	12	28
Positive Agreement		100 % (16/16)		
Negative Agreement		100 % (12/12)		

Clinical Confirmation

The first 5 positive and the first 5 negative oral fluid specimens as determined by the Curative SARS-CoV-2 Assay were sent to the UCLA Clinical Microbiology Laboratory and processed using the Thermo Fisher Scientific, Inc. TaqPath COVID-19 Combo Kit. There was 100% (5/5) positive and 100% (5/5) negative agreement for the specimens tested. The results are acceptable and support use of the Curative SARS-CoV-2 Assay for testing clinical specimens only according to the intended use, limitations, and only with the reagents, instruments, and processes described herein

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.