EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
Global Direct RT-PCR Test
(Access Medical Laboratories, Inc.)

For in vitro Diagnostic Use
Rx Only
For Use Under Emergency Use Authorization (EUA) Only

The Global Direct RT-PCR Test will be performed at Access Medical Laboratories, Inc., located at 5151 Corporate Way, Jupiter, FL 33458, 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, as described in the Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA.

INTENDED USE

The Global Direct RT-PCR Test is an in vitro real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal and anterior nasal swab specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to Access Medical Laboratories, Inc., located at 5151 Corporate Way, Jupiter, FL 33458, 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 and anterior nasal swab 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 definitive cause of disease. Laboratories within the United States and its territories are required to report all test 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/or epidemiological information.

The Global Direct RT-PCR Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures. The Global Direct RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Device Description

a. Global Direct RT-PCR Test

The Global Direct RT-PCR Test is an extraction-free, dual plex real-time reverse transcription polymerase chain reaction (RT-PCR) test. It is based on amplification of specific RNA from the SARS-CoV-2 in collected anterior nasal swab (ANS) and nasopharyngeal swab (NPS) specimens from patients, as recommended for testing by public health authority guidelines. The test evaluates samples collected with dry swabs, and thus does not require specialized transport tubes containing any viral transport media, additives, or preservatives, nor does it require specialized equipment or reagents for nucleic acid extraction. The SARS-CoV-2 primers and probe sets are specifically designed to detect one region (N1) in the SARS-CoV-2 nucleocapsid gene, and human RNaseP (RP).

b. Specimen Transport and Storage

Upper respiratory swab specimens including nasopharyngeal and anterior nasal swabs are collected on a sterile flocked or polyester swab and placed in a sterile container. There is no transport media employed with this test.

c. Specimen Testing

The Global Direct RT-PCR Test is a modified Luna Universal Probe One-Step RT-qPCR Kit where the RNase P (RP) was modified with a different (5') fluorophore dye to allow primer/probe sets to be combined in a dual-plex assay. The Global Direct RT-PCR Test includes primers and probes for the detection of SARS-CoV-2 from a select region of the virus nucleocapsid protein (N1) gene. The assay also includes a primer and probe set to detect the endogenous human RNase P gene in clinical specimens for specimen adequacy and process integrity.

Crude samples are eluted in TE buffer prior to RT-PCR. Nucleic acid is reverse transcribed into cDNA which is then subsequently amplified. The probe anneals to a specific target sequence located between the forward and reverse primers. 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 by the ABI-7500 Fast Dx thermal cycler/reader.

d. Result Reporting

All test results are reported to the requesting healthcare provider via the authorized distributor's Electronic Health Record (EHR) system and public health authorities in accordance with local, state, and federal requirements.

INSTRUMENTS USED WITH THE TEST

Table 1: Instruments and Software

Item	Manufacturer	Version
7500 fast DX Real-time PCR instrument	Applied Biosystems	Cat. Number: 4406984
7500 Fast Dx Real-Time PCR Instrument Sequence Detection Software	Applied Biosystems	1.4.1

REAGENTS AND MATERIALS

Table 2: RT-PCR Reaction Reagents

Item	Manufacturer	Catalog Number
Luna Universal Probe One-Step RT-qPCR Kit	New England Biolabs (NEB)	E3006S, E3006L, E3006X, E3006E
TE Buffer Low EDTA	G Biosciences	786-152

Table 3: Primers and Probes

Item	Manufacturer	Catalog Number
nCOV_N1 Forward Primer	Integrated DNA Technologies	10006830
nCOV_N1 Reverse Primer	Integrated DNA Technologies	10006831
nCOV_N1 Probe	Integrated DNA Technologies	10006832
RNase P Forward Primer	Integrated DNA Technologies	10006836
RNase P Reverse Primer	Integrated DNA Technologies	10006837
RNase P (ATTO 647) Probe Or RPNase P (Cy5) Custom Probe	Integrated DNA Technologies	10007062 N/A

Table 4: Controls

Control	Item	Manufacturer	Catalog Number
Positive	Twist Synthetic SARS-CoV-2 RNA Control MN908947.3	Twist Biosciences	102024
Negative	Nuclease-free (NF) water	New England Biolabs	B1500S

Table 5: Consumables

Reagents and Materials	Manufacturer	Serial Number or Cat. Number
Sterile swabs, nasopharyngeal (NP)	Jiangsu Medical	Lot 20200210JZ
Deep well plates (96-well)	Eppendorf	M1311
Optical adhesive film PCR plate covers	Applied Biosystems	4311971
PCR plates, MicroAmp, Fast Optical	Applied Biosystems	4346906

Reagents and Materials	Manufacturer	Serial Number or Cat. Number
SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat Inactivated	BEI Resources	NR-52286
Nuclease-free (NF) water	New England Biolabs	B1500S

Acceptable Transport Medium:
N/A

CONTROLS

Table 6: Control Materials

Control Type	Description	Purpose	Expected Results
Negative Template Control (NTC)	Nuclease-free water (5 µL added to a specific well of the PCR plate)	To monitor for possible contamination of PCR reagents	SARS-CoV-2 Negative, RP Negative
Negative Elution Control (NEC)	Buffer (400 µL added to a specific well of the PCR plate)	To monitor the full buffer/heat inactivation process for process contamination and nucleic acid degradation due to buffer or plasticware	SARS-CoV-2 Negative, RP Negative
Positive Control (POS) (CT ≤ 37)	Twist Synthetic SARS-CoV-2 RNA control (~100 cp/µL)	To monitor the functional reactivity of RT-PCR reagents	SARS-CoV-2 Positive, RP Negative
Internal Process Control (IPC)	Set of 2 (two) primers and 1 (one) probe detecting RNaseP	To ensure that a specimen of proper biological origin, sufficient quality and quantity was processed and tested	Ct < 35

INTERPRETATION OF RESULTS

Assay Controls
The criteria for interpretation of the results obtained with the assay controls are shown in **Table 7**. All controls must produce the expected results to enable interpretation of the results from testing of patient samples. The Internal Process Control is applied to each patient sample and the Negative Template Control, Negative Elution Control, and Positive Control are used with each plate.

Table 7: Expected Results of Test Controls

Control Name	SARS-CoV-2 Ct ≤ 37	IPC Ct < 35
Positive Control	+	-
Negative Template Control	-	-
Negative Elution Control	-	-

Clinical Specimens

The criteria for interpretation of clinical specimen test results are shown in Table 8 below.

Table 8: Interpretation of Patient Results

N1 Result	RP Result	Interpretation	Action
Detected (Ct ≤ 37)	+/-	SARS-CoV-2 Positive	Release results to sender and report to Public Health authorities.
Not Detected (Ct ≥ 37)	Detected (Ct < 35)	SARS-CoV-2 Negative	Release results to sender and report to Public Health authorities.
Not Detected (Ct ≥ 37)	Not Detected (Ct ≥ 35)	Invalid Specimen or Preparation	Repeat testing from eluted swab solution. If invalid again, request a repeat specimen.

PERFORMANCE EVALUATION

Limit of Detection (LoD) - Analytical Sensitivity:

The LoD of the Global Direct RT-PCR Test was determined by using quantified, SARS-Related Coronavirus 2, Isolate USAWA1/2020, Heat-Inactivated material (BEI Resources, NR-52286). To estimate the LoD, ten (10) random clinical-matrix nasopharyngeal swabs were immersed in 4 mL nuclease-free water to create a clinical matrix solution. This solution was then spiked with quantified, SARS-Related Coronavirus 2, Isolate USAWA1/2020, Heat-Inactivated material, diluted 1:2 to create 9 different concentrations. The dilutions were run in triplicate and the lowest concentration at which all three replicates produced positive result was determined to be the preliminary LoD (**Table 9**). The preliminary LoD was then confirmed by testing an additional 20 replicates at the estimated LoD concentration (**Table 10**). The confirmed LoD of the Global Direct RT-PCR Test was 4 copies/µL of starting sample.

Table 9: Preliminary LoD Determination

Instrument	Concentration (cp/µL)								
	525	263	131	66	33	16	8	**4**	2
7500 fast DX Real-time PCR instrument	3/3	3/3	3/3	3/3	3/3	3/3	3/3	**3/3**	2/3

The LoD was confirmed using a total of 20 replicates with samples at the preliminary LoD concentration 4 cp/µL and additional dilution of 3 cp/µL. Quantified, SARS-Related Coronavirus 2, Isolate USAWA1/2020, Heat-Inactivated material (BEI Resources, NR-52286) was spiked to both concentrations in the negative clinical matrix as described above to make 20 individual samples. There was 95% agreement at 4 cp/µL and 60% agreement at 3 cp/µL. Therefore, the confirmed LoD of the Global Direct RT-PCR Test was 4 copies/µL.

Table 10: Confirmation of LoD

Day	Concentration (cp/µL)	
	4 cp/µL	3 cp/µL
Positive	19/20 (95%)	12/20 (60%)
SD	0.82	0.82
CV%	2.31	2.24

Dry Swab Resuspension Study

To demonstrate that nasopharyngeal dry swabs used are an acceptable specimen type for testing with Global Direct, the performance of the assay was evaluated using dry swabs resuspended in TE buffer elution mix. Samples consisted of natural, respiratory samples collected on sterile nasopharyngeal swabs, via nasopharyngeal collection (one per nostril, two per individual) from volunteers who had previously tested negative for SARS-CoV-2. Fifteen (15) random clinical-matrix swabs were immersed in 6 mL TE Buffer in a sterile polypropylene tube and subjected to ten minutes of orbital shaking (500 rpm) at room temperature. The swabs were subsequently removed, and the resulting solution was spiked with viral material (NR-52286, certified as 3.75×10^8 cp/mL) diluted to approximately 5,250 cp/µL as the starting dilution. 4 total dilutions were created by diluting the starting concentration by 1:4 for dilution 1 and 2 and then 1:2 for dilution 3 and 4. 40 µL of each dilution was placed on a blank sterile nasopharyngeal swab, then air dried within a laminar flow biosafety cabinet for approximately 12 hours at room temperature. Swabs were then tested per the Global Direct RT-PCR Test workflow. Each dilution was run in triplicate and the correlation between the determined LoD, and the resuspended contrived samples was consistent at 2x, 4x, 8x, and 32x LoD (**Table 11**). Therefore, dry swabs are considered an equivalent specimen type for the Global Direct RT-PCR method.

Table 11: Dry Swab Resuspension Study

Level	Viral Load	Confirmed LoD	Resuspended Preparation	
	cp/µL	Ct (N1)	Ct (N1)	Ct (RP)
32 x LoD	131	3/3	3/3	3/3
8 x LoD	33	3/3	3/3	3/3
4 x LoD	16	3/3	3/3	3/3
2 x LoD	8	3/3	3/3	3/3

Inclusivity (analytical reactivity):

N/A - The sponsor relies on the right of reference from LGC Biosearch UltraDX SARS-CoV-2 N1/N2/RP assay for the inclusivity data of their assay.

Cross Reactivity (analytical specificity)

N/A - The sponsor relies on the right of reference from CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time Reverse Transcriptase (RT)-PCR Diagnostic Panel for the cross-reactivity data of their assay.

Microbial Interference:

N/A - The sponsor relies on the right of reference from CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time Reverse Transcriptase (RT)-PCR Diagnostic Panel for the microbial interference data of their assay.

Endogenous/Exogenous Interference Evaluation:

The potential impact of interfering substances on the Global Direct RT-PCR Test was evaluated. Weak positive specimens at 2×LOD and negative specimens without inactivated virus were spiked with plausible interference substances and tested in 3 replicates. The eight (8) substances evaluated were selected from the Diagnostic Molecular Template. The study showed no false negative or false positive results (**Table 12**).

Table 12: Interference Testing

Substance	Concentration	SARS-CoV-2 Concentration	Positive Sample Results (#Pos/Total)	Negative Sample Results (#Pos/Total)
Flonase Nasal Spray	15% v/v	2 x LoD	3/3	0/3
Afrin Nasal Spray	15% v/v	2 x LoD	3/3	0/3
Sore Throat/Cough Lozenges	3mg/mL	2 x LoD	3/3	0/3
Sore Throat Spray	5% v/v	2 x LoD	3/3	0/3
Cough Syrup	5% v/v	2 x LoD	3/3	0/3
Mucin (type I-S, bovine)	2.5 mg/mL	2 x LoD	3/3	0/3
Nicotine	0.03 mg/mL	2 x LoD	3/3	0/3
Cocaine	0.03 mg/mL	2 x LoD	3/3	0/3

Sample Stability:

Sample stability of the Global Direct RT-PCR Test was evaluated with ambient temperatures expected during typical storage and shipping conditions. The transport temperature profile was designed to represent the worst-case scenario for shipping conditions, considering summer weather and storage time followed by 2-day shipping to Access Medical Laboratories (**Table 13**). Dry swab samples were tested per the current Global Direct RT-PCR SARS-CoV-2 workflow and the stability of the specimens following their exposure to typical handling and shipping conditions over the course of 56 hours resulted in 100% concurrence with the initial test results and has been determined to be satisfactory (**Tables 14**). Based on this specimen stability data, dry swab specimens for use with this test (nasopharyngeal or anterior nasal swab specimens) may be stored for up to 48 hours at room temperature.

Table 13: Transport Temperature Profile

Cycle Number	Cycle Temperature (°C)	Cycle Time (Hours)	Total Time (Hours)
1	40	8	8
2	23 (Room Temp)	4	12
3	40	2	14
4	30	36	50
5	40	6	56

Table 14: Sample Stability Study

Sample	Initial Results		56 hrs. Results	
	Pos	Neg	Pos	Neg
NEG	0/10	10/10	0/10	10/10
WP	30/30	0/30	30/30	0/30
SP	10/10	0/10	10/10	0/10

Clinical Evaluation for Patients Suspected of COVID-19:

Clinical performance of the Global Direct RT-PCR Test was evaluated by testing a total of 102 nasopharyngeal dry swab patient samples collected from patients suspected of COVID-19 by a healthcare provider and by a highly sensitive FDA-authorized Molecular SARS-CoV-2 RT-PCR Assay. Among these specimens, 32 were positive and 70 were negative as determined by the comparator method. After testing, the positive percent agreement was 100% (32/32) and the negative percent agreement was 100% (70/70). The results of this study support the use of the Global Direct RT-PCR Test for SARS-CoV-2 testing for individuals suspected of COVID infection and are presented in **Table 15**.

Table 15: Clinical evaluation results for patients suspected of COVID-19

		EUA Authorized Comparator Test	
		Positive	Negative
Global Direct RT-PCR Test	**Positive**	31	0
	Negative	0	71
Positive Agreement		100% (CI: 89.0%, 100%)	
Negative Agreement		100% (CI: 94.9%, 100%)	

WARNINGS

- For use under Emergency Use Authorization (EUA) only.
- For *in vitro* diagnostic use.
- For prescription use only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by the authorized laboratory.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- The emergency use of this product 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 Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

LIMITATIONS

- Laboratories are required to report all negative and positive results to the appropriate public health authorities.
- Primers and probes for the Global Direct RT-PCR Test target a highly conserved region within the genome of SARS-CoV-2. Mutations rarely occur in these highly conserved regions, but if a mutation did occur in these regions, SARS-CoV-2 RNA could become undetectable.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- The performance of the Global Direct RT-PCR Test was established using nasopharyngeal swab specimens only. Anterior nasal swab specimens are considered acceptable specimen types for use with the Global Direct RT-PCR Test but performance has not been established.