EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
ALPHADx SARS-COV-2 RT-PCR Test
(Alphadera Labs, LLC)

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

The ALPHADx SARS-COV-2 RT-PCR Test will be performed at Alphadera Labs, LLC, located at 15355 W. Vantage Pkwy., Suite 195, Houston, TX 77032, 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 ALPHADx SARS-COV-2 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 mid-turbinate nasal swab specimens collected from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to Alphadera Labs, LLC, located at 15355 W. Vantage Pkwy., Suite 195, Houston, TX 77032 which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that 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 mid-turbinate nasal swabs 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 ALPHADx SARS-COV-2 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 ALPHADx SARS-COV-2 RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Device Description

The ALPHADx SARS-COV-2 RT-PCR Test is a RT-PCR Test Kit designed to detect RNA from SARS-CoV-2 in nasopharyngeal and mid-turbinate nasal swabs specimens collected in viral transport media (VTM) by a healthcare provider.

The ALPHADx SARS-COV-2 RT-PCR Test utilizes three primer and probe sets to detect the E-gene, N-gene and RdRP gene targets for specific detection of SARS-CoV-2 viral RNA utilizing individual reporter dyes. It also utilizes one primer and probe to detect a full process Internal Control (RP-V IC) composed of MS2 phage genome. RNA from swab specimens is reverse transcribed to cDNA and subsequently amplified using the Bio-Rad CFX96 Touch instrument. During the RT-PCR reaction the probe anneals to a specific target sequence between the forward and reverse primers, the 5' exonuclease activity of Taq polymerase degrades the bound probe during the extension phase of the PCR cycle, which causes the 5' labeled reporter to dye separate from the quencher generating a fluorescent signal. During the PCR amplification, fluorescence generated by degradation of the target-specific probe is monitored by the Bio-Rad CFX96 Touch instrument

Description of Test Steps:

1. Specimen Transport and Storage

Nasopharyngeal and mid-turbinate nasal specimens are collected on nasopharyngeal swabs, placed in VTM. Specimens are acceptable for use up to 48 hours when stored at 4°C. The temperature of collected specimens in temporary storage at the collection site should be maintained at 2-8°C and transported on wet ice or refrigerant gel packs.

2. Specimen Testing

Prior to performing RT-PCR, reagents are added to crude samples in an incubation tube and incubated to create a lysate sample. The lysate samples are manually pipetted into the RT-PCR plate and extraction is automated by the Maxwell 16 Instrument. The master mix is manually pipetted into the prepared PCR plate and RT-PCR amplification is performed in a 96-well format using CFX96 Touch with CFX Maestro software version 5.3.022.1030. Data interpretation and analysis are performed by Seegene Viewer Software version 3.00.01.006.

3. 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

Equipment	Manufacturer	Serial Number or Software Version
CFX96 Touch	Bio-Rad	CT053899
CFX Maestro Software	Bio-Rad	V: 5.3.022.1030

Equipment	Manufacturer	Serial Number or Software Version
Seegene Viewer Software	Seegene Technologies	V: 3.00.01.006
Maxwell 16 Instrument	Promega	SN: 23629503
Maxwell 16 Software	Promega	V: 4.60

REAGENTS AND MATERIALS

Reagents should not be used past their expiration date.

Table 2: Assay Reagents

Reagent	Manufacturer	Catalog Number
VTM	Biopathogenix	CS-10101
Maxwell RSC PureFood GMO and Authentication Kit	Promega	AS1600
Allplex 2019-nCoV assay kit	Seegene Technologies	RP10244Y or RP10243X
5x Real-time One-step Buffer	Seegene Technologies	VB-CA02
Real-time One-step Enzyme	Seegene Technologies	VE-CA02
2019-nCoV Primer/Probe Set (MOM)	Seegene Technologies	R-RP10243W MOM-CA03
RP-V Internal Control	Seegene Technologies	R-RPVX IC-CA03
2019-nCoV Positive Control	Seegene Technologies	R-RP10243W PC-CA03
RNase-free Water	Seegene Technologies	RW-CA01

CONTROLS

The ALPHADx SARS-COV-2 RT-PCR Test utilizes an Internal Control (IC), Negative Control, Negative Extraction Control, and Positive Control. (**Table 3**). The Internal Control is applied to each patient sample prior to extraction. One Negative Extraction Control is included in every extraction batch and the Negative and Positive Control are used on every PCR plate.

Table 3: Control Materials

Control Type	Description	Expected Results	Purpose
Internal Control (IC)	Seegene RP-V IC	Ct < 40	Monitors specimen quality and demonstrates that nucleic acid was generated by the extraction process
Negative Control	Nuclease free water	SAR-S CoV-2 and IC negative	Monitors non-specific amplification, cross-contamination, or contamination of RT-PCR reagents
Negative Extraction Control	Blank viral transport media spiked with internal control	SARS-CoV-2 negative, IC positive	Monitors for cross contamination and validates the amplification reaction

Control Type	Description	Expected Results	Purpose
Positive Control	Positive control from Seegene assay kit (238 copies/µL)	SARS-CoV-2 and IC positive	Control for amplification and E/N/RdRP primer-probe reagent integrity.

Table 4: Interpretation of Controls

Control Type	IC	E	RdRP	N	Expected Ct Value
Negative Control (NC)	NA	NA	NA	NA	No Ct, or Ct ≥ 40 for all targets
Negative Extraction Control (NEC)	+	-	-	-	Ct < 40 for IC target. No Ct or Ct ≥ 40 for E, RdRP and N genes.
Positive Control (PC)	+	+	+	+	Ct < 40 for all targets. Ct < 40 for IC target.

INTERPRETATION OF RESULTS

Assessment of clinical specimen test results are performed after positive and negative controls have been examined and determined to be valid and acceptable (**Table 5**). If the controls are not valid, patient results cannot be interpreted. Any patient specimen that produces an invalid result is retested and collection of a new specimen if the repeat result is invalid.

Table 5: Interpretation of Patient Results

Scenario #	IC (HEX)	E (FAM)	RdRP (CalRed 610)	N (Quasar 670)	Results	Interpretation
1	+/−	+	+	+	Detected	All targets are valid. nCoV-19 is detected.
2	+/−	+	−	+	Detected	All targets are valid. nCoV-19 is detected. Negative target results suggest that: 1. Target concentration is near of below limit of detection of the test 2. Mutation in the corresponding target region, or 3. Other factors. 4. Confirm Case 5 or Case 6 positives with a repeat run.
3	+/−	+	+	−		
4	+/−	−	+	+		
5	+/−	−	−	+		
6	+/−	−	+	−		
7	+/−	+	−	−	Presumptive Positive	All targets are valid. nCoV-19 is detected. Negative target results suggest that: 1. Target concentration is near of below limit of detection of the test 2. Mutation in the corresponding target region, or 3. Other factors. ***Repeat test with more nucleic acids.*** If result is repeated, other confirmatory testing may be conducted to confirm and differentiate from other Corona viruses/Sarbecovirus currently unknown to infect humans.
8	+	−	−	−	Negative	All targets are valid. nCoV-19 is not detected.
9	−	−	−	−	Invalid	Result is invalid. Repeat test. Collect new specimen if repeat result is invalid.

PERFORMANCE EVALUATION

Limit of Detection (LoD) - Analytical Sensitivity:

The LoD of the ALPHADx SARS-COV-2 RT-PCR Test was determined using quantified, heat inactivated SARS-CoV-2 isolate obtained from BEI Resources (Cat# NR_56495) at a starting concentration of 1.19 x 10^9 copies/mL. To estimate the LoD, three replicate samples were contrived at 6 different concentrations using negative, clinical matrix consisting of known, negative nasopharyngeal sample matrix in VTM. Samples were extracted using the Maxwell RSC PureFood GMO and Authentication Kit (Cat# AS1600) then amplified on the Bio-Rad CFX96 Touch 1000 Real-Time PCR system. The lowest concentration at which all three replicates produced positive results was defined as the preliminary LoD (**Table 6**). The preliminary LoD was then confirmed by testing an additional 20 replicates at the estimated LoD concentration (**Table 7**). The confirmed LoD of the ALPHADx SARS-COV-2 RT-PCR Test was 1190 copies/mL of starting sample.

Table 6: Preliminary LoD Determination

Bio-Rad CFX96 Touch 1000 Real-Time PCR	Concentration (cp/mL)					
	1,190,000	119,000	11,900	**1,190**	119	11.9
E gene	3/3	3/3	3/3	**3/3**	3/3	2/3
RdRP gene	3/3	3/3	3/3	**2/3**	0/3	0/3
N gene	3/3	3/3	2/3	**3/3**	1/3	0/3
IC	3/3	3/3	3/3	**3/3**	1/3	0/3

Table 7: Confirmation of LoD

Bio-Rad CFX96 Touch 1000 Real-Time PCR	E gene	RdRP gene	N gene	IC
1,190 Copies/mL	20/20	20/20	20/20	20/20
Mean	33.00	35.74	37.40	36.49
SD	0.26	0.52	0.39	0.89
CV%	0.80	1.45	1.03	2.45

Inclusivity (analytical reactivity):

N/A - The sponsor relies on the right of reference from the Seegene Technologies for the inclusivity data of their assay.

Cross Reactivity (analytical specificity)

N/A - The sponsor relies on the right of reference from the Seegene Technologies for the inclusivity data of their assay.

Microbial Interference:

N/A - The sponsor relies on the right of reference from the Seegene Technologies for the inclusivity data of their assay.

Endogenous/Exogenous Interference Evaluation:

The impact of potential interfering substances on the ALPHADx SARS-COV-2 RT-PCR Test was evaluated via spiking potential interfering substances into five (5) positive and six (6) negative specimens. Positive specimens were created via spiking inactivated virus to create two (2) samples at 1x LoD, two (2) samples at 2x LoD and one (1) sample at 3x LoD. Each interfering substance was tested with 5 positive samples and 6 negative samples. No false negative or false positive results occurred during the study (**Table 8**).

Table 8: Interference Testing

Substance	Brand	Concentration	SARS-CoV-2 Concentration	Positive Sample Results (#Pos/Total)	Negative Sample Results (#Pos/Total)
Afrin Original Nasal Spray	Afrin	15% v/v	1 x LoD	2/2	0/6
	Afrin	15% v/v	2 x LoD	2/2	
	Afrin	15% v/v	3 x LoD	1/1	
Bovine Submaxillary Gland, Type I-S	Sigma Aldrich (M3895)	2.5 mg/mL	1 x LoD	2/2	0/6
			2 x LoD	2/2	
			3 x LoD	1/1	
Nicotine Nasal Spray	Nicorette	0.3 mg/mL	2 x LoD	2/2	0/6
			1 x LoD	2/2	
			2 x LoD	1/1	

Sample Stability:

N/A - Specimens are collected, stored, and handled according to CDC guidelines and manufacture's protocol.

Clinical Evaluation for Patients Suspected of COVID-19:

Clinical performance of the ALPHADx SARS-COV-2 RT-PCR Test was evaluated by testing a total of 60 clinical nasopharyngeal swabs specimens collected in VTM 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, 30 were positive and 30 were negative as determined by the comparator method. The positive percent agreement was 100% (30/30) and the negative percent agreement was 100% (30/30). Based on the Ct values obtained with the comparator method, at least 20% of positive samples have Ct values within 3 cycles of the average Ct at the LoD of the comparator assay and were considered "weak positive". The results of this study support the use of the ALPHADx SARS-COV-2 RT-PCR Test for SARS-CoV-2 testing for individuals suspected of COVID infection and are presented in **Table 9**.

Table 9: Clinical evaluation results for patients suspected of COVID-19

		EUA Authorized Comparator Test	
		Positive	**Negative**
ALPHADx SARS-COV-2 RT-PCR Test	**Positive**	30	0
	Negative	0	30
Positive Agreement		100% (CI: 88.7, 100%)	
Negative Agreement		100% (CI: 88.7, 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

- The ALPHADx SARS-COV-2 RT-PCR Test is intended and validated for use only with nasopharyngeal and mid-turbinate nasal swabs. Testing of other sample types may result in inaccurate results.
- Primers and probes for the ALPHADx SARS-COV-2 RT-PCR Test target highly conserved regions 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.
- Negative results in the ALPHADx SARS-COV-2 RT-PCR Test do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported, or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- If the virus mutates in the regions targeted by the RT-PCR assay, SARS-CoV-2 may not be detected or may be detected less predictably.
- A false positive result may occur if there is cross-contamination by target organisms, their nucleic acids or amplified product.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- 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.