EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
ALPHA GENOMIX TAQPATH SARS-COV-2 COMBO ASSAY
(ALPHA GENOMIX LABORATORIES)

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

The Alpha Genomix TaqPath SARS-CoV-2 Combo Assay will be performed at Alpha Genomix Laboratories, located at 333 Research Ct, Peachtree Corners, GA 30092, 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 Standard Operating Procedure that was reviewed by the FDA under this EUA.

INTENDED USE

The Alpha Genomix TaqPath SARS-CoV-2 Combo Assay is a real-time, reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal (throat), anterior nasal, and mid-turbinate nasal swab specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to Alpha Genomix Laboratories located at 333 Research Ct, Peachtree Corners, GA 30092 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 which is generally detected 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 Alpha Genomix TaqPath SARS-CoV-2 Combo Assay is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Alpha Genomix TaqPath SARS-CoV-2 Combo Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Alpha Genomics TaqPath SARS-CoV-2 Combo Assay is a real-time, reverse transcription polymerase chain reaction test (rRT-PCR). The assay uses the ThermoFisher Scientific TaqPath COVID-19 Combo Kit that was developed and validated under EUA200010 and is designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients suspected of COVID-19 by their healthcare provider. The test detects three specific regions of the SARS-CoV-2 genome including the ORF1ab region as well as the N (nucleocapsid) and S (Spike protein) genes. The assay also includes a primer and probe set to detect the MS2 phage internal control that is spiked into both the negative control and clinical samples.

RNA is isolated from respiratory specimens including nasopharyngeal, oropharyngeal (throat), anterior nasal, and mid-turbinate nasal swabs using the MagNA Pure 96 DNA and Viral Nucleic Acid Small Volume Kit (Cat # 06543588001) on the Roche MagNA Pure 96 platform (Cat # 06541089001). Nucleic acid is extracted from 200 µL of acceptable specimen and negative control that has been spiked with 5 µL of MS2 phage internal control. Purified material is eluted in a 60 µL final volume using a low salt (Tris-HCl) buffer included with the MagNA Pure 96 Kit. RNA is reverse transcribed to cDNA using the TaqPath 1-Step Multiplex Master Mix (No ROX) and subsequently amplified using the QuantStudio 12K Flex Real-Time PCR System with software version 2.4.0. During the amplification process, 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 bound probe, causing the reporter dye (VIC, ABY, and FAM for the N, S, and ORF1ab targets, respectively) to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle.

INSTRUMENTS USED WITH TEST

The Alpha Genomix TaqPath SARS-CoV-2 Combo Assay is to be used with the Roche MagNA Pure 96 Instrument for automated nucleic acid extraction. The Applied Biosystems QuantStudio 12K Flex Real-Time PCR Instrument with QuantStudio Real-Time PCR Design and Analysis software version 2.4.0 is used for reverse transcription and PCR amplification.

REAGENTS AND MATERIALS

REAGENTS/CONSUMABLES	SUPPLIER	CATALOG #
Nucleic Acid Extraction		
MS2 (included in the TaqPath RT-PCR COVID-19 Kit)	ThermoFisher Scientific	A47814
MagNA Pure 96 DNA and Viral Nucleic Acid Small Volume Kit	Roche	06543588001
MagNA Pure 96 System Fluid	Roche	06640729001
MagNA Pure Tip 1000µL	Roche	06241620001
MagNA Pure 96 Processing Cartridge	Roche	06241603001
MagNA Pure 96 Output Plate	Roche	06241603002

RT-PCR		
TaqPath RT-PCR COVID-19 Kit, 1000 reactions	ThermoFisher Scientific	A47814
TaqPath 1-Step Multiplex Master Mix (No ROX)	ThermoFisher Scientific	A28521, A28522, A28523
TaqPath COVID-19 Control Kit	ThermoFisher Scientific	A47816
TaqPath COVID-19 Control Dilution Buffer	ThermoFisher Scientific	A48002
MicroAmp Optical 384-Well Reaction Plate with Barcode	ThermoFisher Scientific	4309849
Instruments		
MagNA Pure 96 Instrument	Roche	06541089001D
QuantStudio 12K Flex Real-Time PCR System	ThermoFisher Scientific	4472380

CONTROLS TO BE USED WITH THE ALPHA GENOMIX LABORATORIES TAQPATH SARS-COV-2 COMBO ASSAY

Table 1. Assay Controls, Function, and Testing Frequency

Control Type	Purpose	Frequency of Testing
Negative Control (NC)	To monitor for cross-contamination during RNA extraction and RT-PCR reaction setup	Once per extraction batch of specimens
Positive Control (PC)	To monitor the integrity of the RT-PCR reagents and process	Once per run of RT-PCR
No Template Control (NTC)	To monitor for contamination during RT-PCR reaction setup	Once per run of RT-PCR
Internal Control (MS2 Phage)	To monitor the integrity of nucleic acid extraction and RT-PCR for each specimen	Added to each specimen and the Negative Control (NC) prior to extraction

Negative Control (NC)

- The extraction control monitors for any potential cross-contamination that could occur during the nucleic acid extraction process or RT-PCR assay setup. This control is not included in the TaqPath COVID-19 Combo Kit; however, Alpha Genomix Laboratories uses RNase/DNase free water with a spike-in of MS2 control that is processed through nucleic acid extraction and added to one well of the RT-PCR assay plate.

Positive Control (PC)

- A positive control is used to verify proper assay set-up and SARS-CoV-2 reagent integrity. The positive control contains in vitro transcribed (IVT) RNA specific to the N, S, and ORF1ab regions of SARS-CoV-2. The positive control is used in one well on every RT-PCR assay plate.

No Template Control (NTC)

- A no template control (NTC) is needed to check for contamination of RT-PCR assay reagents. Molecular grade, nuclease-free water is used in place of sample nucleic acid for this control. The NTC is used in one well on every RT-PCR assay plate.

Internal Control (MS2 Phage)

- The MS2 internal control serves as an internal process control for nucleic acid extraction to ensure that clinical samples and the negative control contain sufficient RNA to be used in the RT-PCR assay. The MS2 control is spiked into all clinical samples and the negative control prior to performing nucleic acid extraction.

INTERPRETATION OF RESULTS

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted (Refer to Table 2 for a summary of control results).

1) ***COVID-19 RT-PCR Test Controls – Negative Control, Positive Control, NTC, and Internal Control:***

- **Negative Control (NC);** The negative control is processed with each batch of extraction samples. The negative control must only show an amplification curve for MS2 with a Ct of less than 37 but must be negative for all SARS-CoV-2 targets (Ct undetermined or Ct $\geq$ 37).

- **Positive Control (PC);** The positive control must be positive for all three SARS-CoV-2 targets, i.e., the ORF1ab, the N Protein, and the S Protein genes and amplification must have a Ct < 37 in order for the test result to be valid. The positive control does not contain MS2.

- **No Template Control (NTC);** The no template control must be negative (undetermined; no detectable Ct value) for all SARS-CoV-2 assay targets and the MS2 internal control for the test result to be valid.

- **Internal Control (MS2 Phage);** MS2 in a patient sample indicates that PCR amplification occurred in the well. The presence of MS2 and no detectable SARS-CoV-2 during the analysis indicates that proper RNA extraction and amplification occurred; however, no SARS-CoV-2 RNA is present. If SARS-CoV-2 is present in the specimen, amplification of the target RNA may reduce or abrogate MS2 amplification. In this case, the amplified SARS-CoV-2 indicates proper RNA extraction and amplification. Therefore, MS2 may or may not be detectable in a valid test on patient specimens.

Table 2. Expected Results of Controls Used in the Alpha Genomix TaqPath Assay

Control	Ct Value (Optical Channel)			
	N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 Phage (JUN)
Negative Control (NC)	Undetermined	Undetermined	Undetermined	Ct < 37
Positive Control (PC)	Ct < 37	Ct < 37	Ct < 37	Undetermined[1]
No Template Control (NTC)	Undetermined	Undetermined	Undetermined	Undetermined[1]
MS2 Internal Control	Any	Any	Any	Ct < 37

[1]The MS2 Phage internal control is not added to the Positive Control or No Template Control and no signal should be obtained.
*Undetermined (Not detectable Ct or Ct ≥ 37)

If any of the above controls do not exhibit the expected performance as described, the assay may have been improperly set up and/or improperly executed, or reagent or equipment malfunction could have occurred. If the results obtained with the Negative Control do not meet the criteria shown, the results from the entire batch of samples are considered invalid and repeat testing must be performed using new extracted nucleic acid from residual clinical samples. If the Positive Control or NTC does not perform as expected, the RT-PCR assay is repeated using residual extracted nucleic acid for all clinical samples on the plate.

2) Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Please see the table below (Table 3) for guidance on interpretation and reporting of results.

- If all three SARS-CoV-2 specific targets (ORF1ab, N, S) are negative (undetermined, Ct ≥ 37) and the MS2 control is positive (Ct < 37), the patient sample is reported as negative for SARS-CoV-2 RNA. The recommended action is to consider testing for other viruses.
- If two or more of the SARS-CoV-2 specific targets (ORF1ab, N, and S) are positive (Ct < 37), and the MS2 control is positive (Ct < 37) or negative (undetermined, Ct ≥ 37), the patient sample is reported as positive for SARS-CoV-2 RNA. Result are reported to the healthcare provide and the appropriate public health authorities.
- If any one of the three SARS-CoV-2 specific targets is positive (Ct < 37), and the MS2 control is positive (Ct < 37) or negative (undetermined, Ct ≥ 37), the patient sample is inconclusive and must be re-tested using new extracted material from residual clinical specimen. If the repeat result is the same as the original, the test result should be reported as inconclusive. Additional confirmation testing should be conducted if clinically indicated.
- If all three SARS-CoV-2 specific targets (ORF1ab, N, S) are negative (undetermined, Ct ≥ 37) and the MS2 control is also negative (undetermined, Ct ≥ 37), the result is invalid. The sample must be re-tested using new extracted material from residual clinical specimen. If the repeat result remains

invalid, the test result should be reported as inconclusive and a new specimen should be collected.

Table 3. Interpretation of Patient Results Using the Alpha Genomix TaqPath SARS-CoV-2 Combo Assay

Ct Value (Optical Channel)				Status	Result Interpretation
N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 Phage (JUN)		
Undetermined	Undetermined	Undetermined	<37	Valid	Negative
Two or three targets Ct < 37			Any value	Valid	Positive
One of three targets Ct < 37			Any Value	Inconclusive	Re-test[1]
Undetermined	Undetermined	Undetermined	Undetermined	Invalid	Re-test[1]

[1] Re-test is completed using new extracted RNA from the original clinical sample if sufficient volume is available; if the re-test result is the same as the original then report result as inconclusive.
Any value – indicates that the MS2 Phage internal control could be positive (Ct < 37) or negative (Undetermined, Ct ≥ 37)

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

The LoD (lowest SARS-CoV-2 viral RNA concentration that consistently yields a 95% positivity rate) of the Alpha Genomix TaqPath SARS-CoV-2 Combo Assay was determined using synthetic SARS-CoV-2 viral RNA from Twist Bioscience (Control 1 MT007544.1; Cat # 102019). A preliminary LoD was determined by testing six replicates at each of seven different target levels (10,000, 1000, 100, 50, 20, 4, and 2 copies/µL) using synthetic RNA spiked into pooled clinical negative nasopharyngeal swab matrix. Spiked samples were tested with the Alpha Genomix TaqPath SARS-CoV-2 Combo Assay following extraction with the MagNA Pure 96 DNA and Viral Nucleic Acid Small Volume Kit (Cat # 6543588001) performed on the Roche MagNA Pure 96 platform. Replicates were run on the QuantStudio 12K Flex Real-Time PCR Instrument. The preliminary LoD study results are shown in Table 4 below.

Table 4. Preliminary LoD Range Finding Study Results

Concentration (copies/µL)	Target	Mean Ct Values (Standard Deviation)	Detection Rate (# Detected/Total Tested)
10,000	ORF1ab	20.14 (0.40)	100% (6/6)
	N Gene	21.43 (0.27)	100% (6/6)
	S Gene	19.89 (0.83)	100% (6/6)
	MS2	27.16 (0.46)	100% (6/6)
1000	ORF1ab	22.65 (0.46)	100% (6/6)
	N Gene	24.88 (0.21)	100% (6/6)
	S Gene	22.45 (1.09)	100% (6/6)
	MS2	26.92 (0.28)	100% (6/6)
100	ORF1ab	25.95 (0.88)	100% (6/6)
	N Gene	27.94 (0.25)	100% (6/6)

Concentration (copies/µL)	Target	Mean Ct Values (Standard Deviation)	Detection Rate (# Detected/Total Tested)
	S Gene	25.18 (1.11)	100% (6/6)
	MS2	26.60 (0.24)	100% (6/6)
40	ORF1ab	28.03 (0.67)	100% (6/6)
	N Gene	29.10 (0.19)	100% (6/6)
	S Gene	27.81 (0.44)	100% (6/6)
	MS2	26.77 (0.70)	100% (6/6)
20	ORF1ab	29.59 (0.28)	100% (6/6)
	N Gene	30.38 (0.21)	100% (6/6)
	S Gene	28.62 (1.41)	100% (6/6)
	MS2	26.85 (0.22)	100% (6/6)
4	ORF1ab	31.73 (0.49)	100% (6/6)
	N Gene	32.69 (0.32)	100% (6/6)
	S Gene	31.30 (1.32)	100% (6/6)
	MS2	27.27 (0.70)	100% (6/6)
2	ORF1ab	31.90 (0.95)	83.3% (5/6)
	N Gene	34.44 (0.84)	83.3% (5/6)
	S Gene	32.70 (2.09)	100% (6/6)
	MS2	26.97 (0.29)	100% (6/6)

Confirmatory testing was completed using a total of 20 individual extraction replicates consisting of samples spiked at 20 copies/µL and 4 copies/µL in clinical matrix. The confirmed LoD of the Alpha Genomix TaqPath SARS-CoV-2 Combo Assay was 4 copies/µL. Results of the LoD confirmatory study are summarized in Table 5.

Table 5. LoD Verification Study Results

Concentration (copies/µL)	Target	Mean Ct Values (Standard Deviation)	# Detected/Total Tested Detection Rate
20	ORF1ab	27.55 (0.37)	20/20 (100%)
	N Gene	29.13 (0.21)	20/20 (100%)
	S Gene	26.54 (0.76)	20/20 (100%)
	MS2	26.07 (0.49)	20/20 (100%)
4	ORF1ab	26.48 (2.21)	20/20 (100%)
	N Gene	29.87 (0.33)	20/20 (100%)
	S Gene	23.5 (3.60)	20/20 (100%)
	MS2	25.13 (0.71)	20/20 (100%)

2) ***Analytical Inclusivity/Specificity:***

Inclusivity:

The Alpha Genomix TaqPath SARS-CoV-2 Combo Assay utilizes the identical oligonucleotide sequences for the N and S genes and ORF1ab region as those used in the ThermoFisher TaqPath COVID-19 Combo Kit. *In silico* testing of the SARS-CoV-2 assay was previously performed by ThermoFisher as part of their EUA authorization (EUA200010) and this information has been provided in the FDA authorized EUA granted to this manufacturer. Alpha Genomix Laboratories obtained a right of reference from ThermoFisher to use the *in silico* inclusivity data.

In Silico Analysis of Primer and Probe Cross-Reactivity:
As stated previously, Alpha Genomix Laboratories obtained a right of reference from ThermoFisher to incorporate the *in silico* cross reactivity analysis findings. As part of ThermoFisher's EUA, they performed an *in silico* analysis of 42 potentially cross-reactive organisms and determined that there was low risk of non-specific amplification.

3) Clinical Evaluation:

Performance of the Alpha Genomix TaqPath SARS-CoV-2 Combo Assay was evaluated using leftover, clinical nasopharyngeal swab specimens that were previously tested with an FDA EUA authorized SARS-CoV-2 molecular test. A total of 55 previously tested specimens (30 negatives and 25 positives) were evaluated by the Alpha Genomix TaqPath SARS-CoV-2 Assay. In addition, the first 5 positive and 5 negative samples were confirmed to be 100% concordant with the CDC EUA authorized assay tested by an outside laboratory. These results have been combined in the performance table below to reach the minimum number of tested samples recommended for a clinical study.

For the positive clinical nasopharyngeal swab samples, the positive percent agreement (PPA) between the Alpha Genomix TaqPath SARS-CoV-2 Combo Assay and the comparator assay was 96.7% (29/30). Upon initial testing, there was one sample that was positive for only one SARS-CoV-2 target (inconclusive result). Using the Alpha Genomix reporting strategy, the sample was re-tested using new extracted material from residual clinical specimen and the sample generated positive calls for all 3 assay targets (this testing scenario is represented as a footnote to Table 6). In addition, there was one sample that was positive by the comparator assay but negative by the Alpha Genomix TaqPath SARS-CoV-2 Combo Assay. The laboratory running the comparator assay confirmed that the original Ct values for the N2 and N3 targets were 35.5 and 33.5, respectively for this discordant sample. This clinical specimen was also collected on April 1st and was one month old when tested by Alpha Genomix Laboratories; therefore, the discordance could be attributed to the age of the specimen and RNA degradation. The Ct range for the ORF1ab, N, and S targets generated by the Alpha Genomix Assay for the previously tested positive clinical samples was 12.08 – 35.03, 12.61 – 36.31, and 12.20 – 36.10, respectively.

All 30 clinical negative samples were non-reactive using the Alpha Genomix TaqPath SARS-CoV-2 Combo Assay and the first 5 negative samples were also confirmed to be negative for SARS-CoV-2 using an outside laboratory running an EUA authorized assay; therefore, the NPA of the Alpha Genomix TaqPath SARS-CoV-2 Combo Assay was 100%. Qualitative results of the clinical evaluation are shown in Table 6.

Table 6. Summary of Qualitative Clinical Study Results

		Authorized Assay - Comparator		
		Positive	**Negative**	**Total**
Alpha Genomix TaqPath SARS-CoV-2 Combo Assay	**Positive**	29[1]	0	29
	Negative	1	35	36
	Total	30	35	65
Positive Percent Agreement		96.67% (29/30); 83.33-99.41%[2]		
Negative Percent Agreement		100.00% (35/35); 90.11-100.00%[2]		

[1]One sample was initially only positive for one SARS-CoV-2 target (inconclusive) but upon repeat testing, the sample yielded positive calls for all three assay targets.
[2]Two-sided 95% score confidence intervals

Clinical Confirmation:
As described above in the Clinical Evaluation section, the first five positive and five negative samples determined by the Alpha Genomix TaqPath SARS-CoV-2 Combo Assay were sent to an outside laboratory that is running an FDA EUA authorized SARS-CoV-2 molecular test for confirmatory testing. All ten patient specimens yielded concordant results.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by Alpha Genomix Laboratories located at 333 Research Ct, Peachtree Corners, GA 30092;
- 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.

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. Roche MagNA Pure 96 was used for extraction.
QuantStudio 12K Flex with software version 2.4.0 was set up for COVID RT-PCR detection according to the SOP. The results are summarized in the following Table.

Table 7. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provide d by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Oropharyngeal Swabs	$1.8x10^5$ NDU/mL	N/A
MERS-CoV		N/A	ND

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