EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
ALIMETRIX SARS-COV-2 RT-PCR ASSAY
(ALIMETRIX, INC.)

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

(The Alimetrix SARS-CoV-2 RT-PCR Assay will be performed at Alimetrix, Inc., located at 800 Hudson Way, Suite 2200, Huntsville, AL 35806, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity tests, as per the Standard Operating Procedure that was reviewed by the FDA under this EUA.)

INTENDED USE

The Alimetrix SARS-CoV-2 RT-PCR Assay is a reverse transcription polymerase chain reaction (RT-PCR) test with microarray detection intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate nasal swab specimens, as well as nasopharyngeal washes/aspirates or nasal aspirates and bronchoalveolar lavage (BALs) specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to Alimetrix, Inc. located at 800 Hudson Way, Suite 2200, Huntsville, AL 35806 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 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.

Testing with the Alimetrix SARS-CoV-2 RT-PCR Assay is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of reverse transcription PCR, hybridization, and in vitro diagnostic procedures. The Alimetrix SARS-CoV-2 RT-PCR Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Alimetrix SARS-CoV-2 RT-PCR Assay is a reverse transcription polymerase chain reaction (RT-PCR) assay with microarray hybridization for the qualitative detection of nucleic acid from SARS-CoV-2. The process uses a master mix containing primer pairs for reverse transcription and selective amplification of SARS-CoV-2 viral RNA 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 N1 (nucleocapsid) and N2 genes. The assay also includes a primer set to detect RNase P as an endogenous specimen control as well as the MS2 phage internal control that is spiked into the external positive and negative controls as well as all clinical samples prior to extraction.

RNA is isolated from acceptable upper respiratory specimens collected in 1X DNA/RNA Shield media (Zymo Research, Cat # R1100) using one of the following methods:

1) Zymo Research Quick-DNA/RNA Viral MagBead Extraction Kit (Cat # 2141) performed on the MagMAX Express 96 instrument (with software version 1.2)
2) Qiagen QIAamp 96 Virus QIAcube HT (manual) (Cat # 57731) processed on a QiaVac manifold

When using the Zymo extraction kit automated on the MagMAX Express 96 instrument, 300 µL of patient specimen/positive control/negative control that has been spiked with 5 µL of MS2 phage internal control is the starting volume for processing. Purified nucleic acid is eluted in 50 µL of RNA/DNA free water. For the manual Qiagen QIAamp 96 Virus QIAcube HT Kit (manual), 200 µL of patient specimen is processed on a QiaVac manifold and eluted in 140 µL of Buffer AVE. The manifold provides vacuum filtration for RNA extraction.

Purified RNA from the patient sample (5 µL from the Zymo Research extraction, 9.75 µL from the Qiagen kit extraction) is reverse-transcribed into cDNA, which is then amplified using the Qiagen QuantiFast Multiplex RT-PCR +R kit (Cat # 204956) with primers specific to each gene target. Each amplicon is modified with a biotin tag which enables detection via a fluorescent label applied after hybridization on a microarray. The PCR products of each patient are transferred by multichannel pipette into individual microarray wells, each containing probes designed to detect the gene targets of interest. Negative wells are randomly utilized on the array to monitor for cross-contamination.

Hybridization of amplicons to specific capture probes on the microarray occurs via a proprietary, environmentally-controlled hybridization robot. Unbound amplicons are removed from each well using a robotic flushing method. Bound amplicons are then labeled with fluorescent Streptavidin Phycoerythrin (SAPE), washed, dried and imaged on a fluorescence array scanner. Results are analyzed using a custom built FAAS program (Flair Automated Analysis Software version 3.0)

INSTRUMENTS USED WITH TEST

The Alimetrix SARS-CoV-2 RT-PCR Assay is used with the MagMAX Express 96 Instrument for automated nucleic acid extraction as well as the QiaVac manifold for manual extraction. The Applied Biosystems GeneAmp 9700 Thermal Cycler with software version 3.12 is used for reverse transcription and PCR amplification. The Sensovation Sensopot microarray scanner with ArrayReader software version 3.1.0.15245 measures hybridization intensity levels for each gene probe in relative fluorescence units (RFU).

REAGENTS AND MATERIALS

REAGENTS/CONSUMABLES	SUPPLIER	CATALOG #
Nucleic Acid Extraction		
Quick DNA/RNA Viral MagBead Kit	Zymo Research	2141
MS2 Internal Control (2×10^3 copies/µL)	Zeptometrix	810274
Extraction (QiaVac method only) QIAamp 96 Virus QIAcube HT Kit	Qiagen	57731
KingFisher Deep Well 96 plates	ThermoFisher Scientific	95040460
DeepWell Corning Costar plates	Corning	3960
KingFisher 96well 200 µL plates	ThermoFisher	97002540
96 Well Microplates MP	Qiagen	1031656
KingFisher Tip Comb DW plate	ThermoFisher	97002534
Large 96- Rod Covers	Qiagen	1031668
RT-PCR and Hybridization		
QuantiFast Multiplex RT-PCR+R Kit	Qiagen	204956
Alimetrix SARS-CoV-2 RT-PCR Assay Primer Pool	Integrated DNA Technologies	Custom order; primers designed and pooled by Alimetrix
Hybridization Buffer (Hybridization of biotinylated amplicons to microarray printed probes)	Alimetrix	Proprietary formulation
Wash buffer A (medium stringency buffer to remove residual dye and non-specific binding)	Alimetrix	Proprietary formulation
Wash Buffer B (high stringency buffer to remove all non-specific probe binding)	Alimetrix	Proprietary formulation
Wash Buffer C (dye stabilizing buffer)	Alimetrix	Proprietary formulation
18 MΩ Purified Water	Elga Veolia	PURELAB Flex 3
SAPE Solution	ThermoFisher Scientific	C29532
COVID19-RTPCR Panel 96-well microarray assay plates spotted in triplicate with controls	Microarrays Inc.	Custom Order
Instruments		
MagMax Express 96 Deep Well Magnetic Particle Processor	Applied Biosystems	4400077
QiaVac 96 manifold connected to pressure gauge and vacuum pump capable of reaching 35kPA or 10.3 in Hg	Qiagen	19504
GeneAmp 9700 Thermal Cycler	Applied Biosystems	GeneAmp 9700

Environmentally-controlled assay hybridization robot	Alimetrix	Proprietary, Custom Built
SensoSpot Microarray Scanner	Sensovation	SensoSpot Fluorescence

CONTROLS TO BE USED WITH THE ALIMETRIX SARS-COV-2 RT-PCR ASSAY

Table 1. Assay Controls, Functions, and Frequency of Testing

Control Type	Purpose	Source	Frequency of Testing
External Negative Control (no template)	Monitor for cross-contamination during RNA extraction, RT-PCR, and hybridization	1X DNA/RNA Shield Solution (Zymo Research)	At least one per run of RT-PCR
External Positive Control	Monitor the integrity of extraction and amplification of SARS-CoV-2	Heat Inactivated SARS-CoV-2 Culture Fluid (Zeptometrix) prepared to a concentration 1 log dilution above LoD*	One per run of RT-PCR
Specimen Collection Control	Endogenous specimen collection indicator	RNase P from human cellular specimen	Within each clinical specimen**
Internal Control	Monitor integrity of each step of the assay from extraction through hybridization	MS2 bacteriophage (Zeptometrix)	Added to each specimen and external control prior to extraction

*500 copies/mL (Zymo extraction) and 2000 copies/mL (Qiagen extraction)
**This is an internal/endogenous marker that should be detected within every clinical specimen to indicate if biological material was successfully collected.

External Negative Control (NTC)

- The extraction control monitors for any potential cross-contamination that could occur during the nucleic acid extraction process or RT-PCR assay setup, or hybridization. This control consists of 1X DNA/RNA Shield with a spike-in of MS2 control that is processed through nucleic acid extraction and added to at least one well of the RT-PCR assay plate.

External Positive Control

- 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 and prepared to a final concentration of either 500 copies/mL or 2000 copies/mL when samples are extracted with the Zymo or Qiagen kits, respectively.

Specimen Collection Control (RNase P)

- Human RNase P is an endogenous control that functions to ensure that sufficient human biological material was collected for testing.

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 controls contain sufficient RNA to be used in the RT-PCR assay. The MS2 control is spiked into all clinical samples and the external negative and positive controls 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 – External Negative Control, External Positive Control, RNase P, and Internal MS2 Phage Control:***

- **External Negative Control (NTC);** The external negative control is processed with each batch of extraction samples. The NTC must show detection of MS2, with no detection of RNase P or SARS-CoV-2 targets. The external negative control is invalid if there is detection of SARS-CoV-2 and/or RNase P. An invalid external negative control could indicate cross-contamination or contamination of the 1X DNA/RNA shield media.
    - If SARS-CoV-2 is detected in the external negative control, all positive patient specimens must be repeated using new extracted RNA from residual clinical specimens. All valid negative patient results may be released since they are known to be free of SARS-CoV-2 contamination.
    - If RNase P is detected in the external negative control, all specimens must be repeated as it cannot be determined if the RNase P detected in the clinical wells is from the patient or from the contamination event.

- **External Positive Control;** The positive control must show detection of at least one SARS-CoV-2 target; N1, N2, or ORF1ab of any combination of these targets for the test result to be valid. The positive control must show detection of MS2 but lack detection of RNase P. If there is no detection of N1, N2, or ORF1ab, the test is invalid and may reflect pipetting error, degradation of control material, failure of nucleic acid extraction, amplification, or hybridization of the target due to reagent degradation, or error in preparation of the reaction mix.
    - All negative patient specimens must be repeated using new extracted RNA from residual clinical samples.
    - All valid positive patient results can be released since they are free of the error causing non-detection of SARS-CoV-2.

- **RNase P Control;** RNase P RNA from human cellular material is endogenous to human respiratory samples and serves as the specimen adequacy control. RNase P can also be used to indicate extraction, amplification, hybridization and

detection were successfully performed. Detection of RNase P demonstrates adequate collection of human biological material. If RNase P is not detected in a clinical specimen, this could indicate insufficient human cellular material due to poor collection or there could have been a loss in specimen integrity, resulting in an invalid specimen result. The assay is re-run using new extracted nucleic acid from residual clinical sample. If RNase P is not detected upon repeat testing of the specimen, the Medical Director must be consulted for further guidance.

- **Internal Control (MS2 Phage);** Detection of the MS2 internal control in a patient sample indicates proper extraction, amplification, hybridization, and detection have been performed. If MS2 is not detected, but SARS-CoV-2 and/or RNase P are detected results may still be valid and patient results that are released are dependent on interpretation of the RNase P control as stated previously. Therefore, MS2 may or may not be detected in a valid test on patient specimens.

  If MS2 is not detected, and all targets are not detected (N1, N2, ORF1ab, and RNase P), either extraction, amplification, or hybridization were unsuccessful. Invalid results could be due to improper extraction of nucleic acid, error in assay set-up, reagent or equipment malfunction, or RT-PCR inhibitors in the specimen. Nucleic acid from the original clinical specimen is re-extracted and re-tested. If results are still invalid upon repeat testing, the sender is notified of the invalid test and recollection should be considered.

Table 2. Expected Results of Controls Used in the Alimetrix SARS-CoV-2 RT-PCR Assay

Control	N1	N2	ORF1ab	RNase P	MS2 Phage Internal
External Negative Control (NTC)	-	-	-	-	+
External Positive Control*	+/-	+/-	+/-	-	+/-**
RNase P Endogenous Control	NA	NA	NA	+/-	NA
MS2 Phage Internal Control	NA	NA	NA	NA	+/-

*At least one of the SARS-CoV-2 targets must be detected for a valid External Positive Control result. The MS2 Phage internal control is added to the External Negative and Positive Controls so there should be detection.

**The External Positive Control should be positive for the MS2 control; however, on rare occasions, a PCR with a high positive sample can deplete the dNTPs in the reaction leading to minimal to no signal of the lower-copy MS2 target.

If any of the above controls do not exhibit the expected performance as described, the assay may have been improperly set-up and/or executed improperly, or reagent or equipment malfunction could have occurred. If the results obtained with the External Positive or Negative Controls 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.

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. Results are reported as detected (positive) or not detected (negative), following assessment of all assay controls.

- If one or more of the SARS-CoV-2 specific targets (N1, N2, and ORF1ab, N, and S) are positive (detected), and the RNase P endogenous control is positive (detected), irrespective of the MS2 control results, the patient sample is reported as positive for SARS-CoV-2 RNA.
- If all three SARS-CoV-2 specific targets (N1, N2 and ORF1ab) are negative (not detected) and the RNase P endogenous control is positive (detected), irrespective of the MS2 control results, the patient sample is reported as negative for SARS-CoV-2 RNA.
- If all three SARS-CoV-2 specific targets (N1, N2, and ORF1ab) are negative (not detected) and the RNase P endogenous control is also negative (not detected), irrespective of the MS2 control results, the assay run is invalid. The sample must be re-tested including RT-PCR and hybridization using new extracted material from residual clinical specimen. If the repeat result remains invalid, the medical director must be consulted, and a new specimen should be collected from the patient.

Table 3. Interpretation of Patient Results Using the Alimetrix SARS-CoV-2 RT-PCR Assay

SARS-CoV-2 N1	SARS-CoV-2 N2	SARS-CoV-2 ORF1ab	RNase P	MS2	Result Interpretation	Report	Actions
+	±	±	+	±	SARS-CoV-2 Detected (Positive)	SARS-CoV-2 Detected	Report results to sender and to appropriate State Health Department.
±	+	±	+	±	SARS-CoV-2 Detected (Positive)	SARS-CoV-2 Detected	Report results to sender and to appropriate State Health Department.
±	±	+	+	±	SARS-CoV-2 Detected (Positive)	SARS-CoV-2 Detected	Report results to sender and to appropriate State Health Department.
-	-	-	+	±	SARS-CoV-2 Not Detected (Negative)	SARS-CoV-2 Not Detected	Report results to sender and to appropriate State Health Department.
-	-	-	-	+	Invalid Result	Invalid	Repeat extraction, RT-PCR, and Hybridization. If the repeated result remains invalid, consult the Medical Director and consider collecting a new specimen from the patient.

-	-	-	-	-	Invalid Result	Invalid	Repeat extraction, RT-PCR, and Hybridization. If the repeated result remains invalid, consult the Medical Director and consider collecting a new specimen from the patient.

± Indicates either detected (+) or not detected (-)
Note: Alimetrix internal thresholding value for all probe detections (SARS-CoV-2 targets, RNase P, and MS2) is ≥2000 net signal relative fluorescence units (RFU). The final report of results does not contain numerical values. Reported result is detected (+) or not detected (-).

PERFORMANCE EVALUATION

1) ***Analytical Sensitivity:***

Limit of Detection (LoD):
The LoD (lowest SARS-CoV-2 viral RNA concentration that consistently yields at least a 95% minimum positivity rate) of the Alimetrix SARS-CoV-2 RT-PCR Assay was determined using quantitated, inactivated whole SARS-CoV-2 virus (isolate USA-WA1/2020) from Zeptometrix (Cat # NATSARS(COV2)-ST). A preliminary LoD was determined by testing two replicates at each of five different target levels (5000, 1000, 500, 250, and 100 copies/µL) using inactivated virus spiked into pooled clinical negative mid-turbinate swab matrix in DNA/RNA Shield. The clinical matrix used in LoD studies was screened negative using the Alimetrix SARS-CoV-2 RT-PCR Assay. Spiked samples were tested with the Alimetrix SARS-CoV-2 RT-PCR Assay following extraction with the claimed methods including the Quick-DNA/RNA Viral MagBead Kit on the MagMAX Express 96 instrument and the QIAamp 96 Virus QIAcube HT Kit on the QiaVac 96 manifold. Replicates were run on the GeneAmp 9700 Thermal Cycler followed by hybridization and detection with the Sensospot Microarray Scanner.

The preliminary LoD study results for both extraction methods were 250 copies/mL and 500 copies/mL for the Zymo and Qiagen kits, respectively. Results are summarized in Table 4 below.

Table 4. Preliminary LoD Range Finding Study Results

Target	Concentration (copies/mL)	Extraction Method					
		Zymo (Automated Method)			Qiagen (Manual Method)		
		Replicates Detected (Detection Rate)	Assay Targets	Lowest Probe Net Signal Detected (RFU)	Replicates Detected (Detection Rate)	Assay Targets	Lowest Probe Net Signal Detected (RFU)
SARS-CoV-2	5000	100% (2/2)	N1	63,204	100% (2/2)	N1	62,859
		100% (2/2)	N2	63,339	100% (2/2)	N2	63,113
		100% (2/2)	ORF1ab	63,154	100% (2/2)	ORF1ab	62,860
	1000	100% (2/2)	N1	63,078	100% (2/2)	N1	56,132
		100% (2/2)	N2	63,304	100% (2/2)	N2	60,580
		100% (2/2)	ORF1ab	60,837	100% (2/2)	ORF1ab	49,874
	500	100% (2/2)	N1	49,447	100% (2/2)	N1	50,203
		100% (2/2)	N2	63,384	50% (1/2)	N2	56,281

		100% (2/2)	ORF1ab	52,719	50% (1/2)	ORF1ab	59,712
	250	100% (2/2)	N1	63,708	0% (0/2)	N1	Not Detected
		100% (2/2)	N2	63,498	0% (0/2)	N2	Not Detected
		50% (1/2)	ORF1ab	53,234	0% (0/2)	ORF1ab	Not Detected
	100	0% (0/2)	N1	Not Detected	0% (0/2)	N1	Not Detected
		50% (1/2)	N2	62,128	0% (0/2)	N2	Not Detected
		50% (1/2)	ORF1ab	27,857	0% (0/2)	ORF1ab	Not Detected

RFU; Relative Fluorescence Units

Confirmatory LoD testing was completed using a total of 20 individual extraction replicates consisting of specimens that were prepared at one dilution above, one dilution below, and at the prescreened LoD concentration. The confirmed LoD of the Alimetrix SARS-CoV-2 RT-PCR Assay was 250 copies/mL and 1000 copies/mL for the Zymo and Qiagen extraction methods, respectively. Results of the LoD confirmatory study are summarized in Table 5 and 6, respectively.

Table 5. LoD Verification Study Results using Replicates Extracted with the Zymo Research Quick-DNA/RNA Viral MagBead Extraction (Automated)

Assay Target	Concentration Tested (copies/mL)	Average SNR* (20 wells)	Lowest Probe Net Signal (RFU) (20 wells)	Replicates (# Detected / # Tested)
N1	1,000	36.7	63,164	20/20
N2		36	56,228	20/20
ORF1ab		39.7	38,993	20/20
N1	500	39.8	62,914	19/20
N2		38.9	60,965	19/20
ORF1ab		37.2	41,460	19/20
N1	250	41.8	40,055	19/20
N2		40.7	51,753	19/20
ORF1ab		33.9	3,271	19/20

*SNR – Signal to noise ratio
Probe Net Signal is measured in Relative Fluorescence Units (RFU)

Table 6. LoD Verification Study Results using Replicates Extracted with the Qiagen QIAamp 96 Virus QIAcube HT Kit (Manual)

Assay Target	Concentration Tested (copies/mL)	Average SNR* (20 wells)	Lowest Probe Net Signal (RFU) (20 wells)	Replicates (# Detected / # Tested)
N1	2,500	33	63,396	20/20
N2		32.5	63,326	20/20
ORF1ab		35.8	44,247	20/20
N1	1,000	37.5	62,105	20/20
N2		35.6	63,438	20/20
ORF1ab		41.1	59,411	20/20
N1	500	37.3	59,537	16/20
N2		37.8	61,460	16/20
ORF1ab		33.1	22,977	16/20

*SNR – Signal to noise ratio
Probe Net Signal is measured in Relative Fluorescence Units (RFU)

Note that there is a 4-fold difference in assay LoD between samples extracted with the automated Zymo kit and the manual QIAamp kit (250 copies/mL and 1000 copies/mL, respectively). The clinical comparison testing data described in Section 3 below contained a sufficient number of low positive samples that were extracted using both methods. Clinical performance demonstrated that both extraction methods perform similarly with the Alimetrix SARS-CoV-2 RT-PCR Assay (PPA of 100% and 97.67% for Zymo and Qiagen, respectively and NPA of 93.33% for both methods). In addition, the manual extraction kit was only validated for use as a backup method and serves the preparedness role for dealing with supply chain issues.

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

In silico Inclusivity Analysis:
The inclusivity of the Alimetrix SARS-CoV-2 RT-PCR Assay was evaluated by *in silico* analysis of published sequences using the Alimetrix assay primers and probes. BLASTn analysis queries of the Alimetrix SARS-CoV-2 RT-PCR assay primers and probes were performed against publicly available nucleotide sequences found in the NCBI database. The Betacoronavirus Genbank database was queried and contained 15,964 SARS-CoV-2 whole genome sequences. The following search parameters were applied:

- The search parameters automatically adjust for short input sequences and the expected threshold is 10,000.
- The match and mismatch scores are 2 and -3, respectively.
- The penalty to create and extend a gap in an alignment is 5 and 2, respectively.

The Alimetrix SARS-CoV-2 RT-PCR Assay primers and probes show homology with all available SARS-CoV-2 sequences in the NCBI Betacoronavirus database as of June 2020, and significant homology (≥ 80%) to SARS coronavirus, pangolin coronavirus, and bat SARS-like/SARS-related coronavirus, for all primers and probes for the N1, N2, and ORF1ab gene targets. Both SARS-CoV and SARS-CoV-2 are Sarbecoviruses and share a 79.6% homology between the genomes and are also highly-related to coronaviruses found in bats and pangolins. One primer, the ORF1ab reverse primer, showed 84% homology to a single strain of Human coronavirus HKU1. However, neither the ORF1ab forward primer nor ORF1ab probe showed any homology to Human coronavirus HKU1 and therefore, it is unlikely that any amplification or detection would occur in the presence of this template. Wet testing of Human coronavirus HKU1 was also conducted and the assay did not detect this virus.

In Silico Analysis of Primer and Probe Cross-Reactivity:
A combination of laboratory wet testing and *in silico* analyses of exclusivity were conducted to assess the analytical specificity of the primers and probes used in the Alimetrix SARS-CoV-2 RT-PCR Assay. *In silico* analysis was conducted for the following respiratory pathogens in Tables 7-8. BLASTn analysis queries of the Alimetrix SARS-CoV-2 RT-PCR Assay primers and probes were performed against

publicly available nucleotide sequences found in the NCBI database. The database search parameters on June 23, 2020 were as follows:

- The nucleotide collection (nr/nt) consists of GenBank + EMBL +DDBJ +PDB +RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb.
- The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry.
- The search parameters automatically adjust for short input sequences and the expected threshold is 1000.
- The match and mismatch scores are 1 and -3, respectively.
- The penalty to create and extend a gap in an alignment is 5 and 2, respectively.

Cross-reactivity was defined as greater than 80% homology between one of the primers/probes and any sequence present in the targeted microorganism. Expected homology with the SARS-CoV target was observed in the *in silico* analysis with the N1, N2, and ORF1ab gene targets. Aside from the expected homology with SARS-CoV, results showed one instance of ≥ 80% homology of the RNase P forward primer with *Mycobacterium tuberculosis*. However, the absence of reverse primer and probe homology to *M. tuberculosis* indicates the unlikely detection of a false positive associated with *M. tuberculosis* in a specimen.

Table 7. *In silico* Results for Select Organisms for the N1, N2, and ORF1ab Primer and Probe Sets

Pathogen	Strain	GenBank Accession #	SARS-CoV-2 N1			SARS-CoV-2 N2			SARS-CoV-2 ORF1ab		
			N1 F	N1 R	N1 probe	N2 F	N2 R	N2 probe	ORF1ab F	ORF1ab R	ORF1ab probe
SARS-Coronavirus	Tor2	NC_004718.3	76.7	92.0	88.0	91.7	87.0	84.0	79.2	65.4	82.9
Legionella pneumophilia	Philadelphia_1_CDC	CP015928.1	50.0	52.0	28.0	54.1	56.5	34.0	50.0	53.8	34.0
Mycobacterium tuberculosis	H37Rv	NC_000962.3	0.0	0.0	30.0	50.0	56.5	34.0	50.0	46.1	34.0
Pneumocystis jirovecii (PJP)	RU7	NW_017264775.1	42.8	0.0	22.0	46.0	0.0	30.0	45.8	46.1	29.8

Table 8. *In silico* Results for Select Organisms for the RNase P and MS2 Primer and Probe Sets

Pathogen	Strain	GenBank Accession #	Specimen Collection Control (RNase P)			Internal Assay Control (MS2)		
			RP F	RP R	RP probe	MS2 F	MS2 R	MS2 probe
SARS-Coronavirus	Tor2	NC_004718.3	50.0	45.0	20.5	46.4	35.7	21.3
Legionella pneumophilia	Philadelphia_1_CDC	CP015928.1	65.0	60.0	34.1	42.9	53.6	31.9
Mycobacterium tuberculosis	H37Rv	NC_000962.3	80.0	60.0	36.4	50.0	42.8	31.9
Pneumocystis jirovecii (PJP)	RU7	NW_017264775.1	60.0	60.0	27.2	0.0	40.0	25.5

Exclusivity Wet Testing

Specimens for exclusivity testing were prepared to a final concentration as detailed in the table below. All specimens were prepared in DNA/RNA Shield transport medium to simulate clinical specimen collection. A single replicate of each target organism suspension was extracted by both the Zymo and Qiagen methods. The qualitative samples from Zeptometrix are assay verification control suspensions that are not quantitated. With the exception of SARS-Coronavirus and SARS-Coronavirus control plasmid, no assay cross-reactivity with any of the organisms in Table 9 was observed. Since there are no known circulating SARS-Coronavirus strains in the human population, cross-reactivity (false positive) is not expected with the Alimetrix SARS-CoV-2 RT-PCR Assay.

Table 9. Wet Tested Organisms to Evaluate Potential Assay Cross-Reactivity

Pathogen	Strain or Source	Concentration
SARS-Coronavirus	2003-00592	NATSARS-ST: Qualitative
SARS-Coronavirus control plasmid	N2 gene only	2 x 10^5 copies/mL
MERS-coronavirus	Florida/USA-2_Saudi Arabia_2014, heat- inactivated	0.7 x 10^5 PFU/mL
Coronavirus	NL63	0.7 x 10^5 PFU/mL
Coronavirus	229E	0.7 x 10^5 PFU/mL
Coronavirus	OC43	0.7 x 10^5 PFU/mL
Coronavirus	HKU-1	NATRVP2-BIO: Qualitative
Influenza A H1N1	A/New Caledonia/20/99	1x10^5 U/mL
Influenza B	B/Florida/02/06	NATRVP2-BIO: Qualitative
Human Metapneumovirus 8	Peru6-2003	NATRVP2-BIO: Qualitative
Respiratory Syncytial Virus A	n/a	NATRVP2-BIO: Qualitative
Rhinovirus 1A	n/a	NATRVP2-BIO: Qualitative
Parainfluenza virus Type 1	n/a	NATRVP2-BIO: Qualitative
Parainfluenza virus Type 2	n/a	NATRVP2-BIO: Qualitative
Parainfluenza virus Type 3	n/a	NATRVP2-BIO: Qualitative
Parainfluenza virus Type 4	n/a	NATRVP2-BIO: Qualitative
Adenovirus Type 3	n/a	NATRVP2-BIO: Qualitative
Adenovirus Type 31	n/a	NATRVP2-BIO: Qualitative
Adenovirus Type 1	n/a	NATRVP2-BIO: Qualitative
Mycoplasma pneumoniae	ATCC 15531	NATRVP2-BIO: Qualitative
Haemophilus influenzae	type b; Eagan	1 x 10^6 CFU/mL
Streptococcus pyogenes (A)	Z018, M58, Lancefield's group A	1 x 10^6 CFU/mL
Streptococcus pneumoniae	ATCC 49619	1.7 x 10^4 CFU/mL
Staphylococcus epidermidis	ATCC 12228	1 x 10^6 CFU/mL
Pseudomonas aeruginosa	ATCC 27853	1 x 10^6 CFU/mL
Enterovirus 68	2014 Isolate 1	1 x 10^5 U/mL
Candida albicans	Z006	1 x 10^6 CFU/mL
Streptococcus salivarius	Z127	1 x 10^6 CFU/mL
Chlamydia pneumoniae	CWL-029	NATRVP2-BIO: Qualitative
Bordetella pertussis	A639	NATRVP2-BIO: Qualitative
Bordetella parapertussis	A747	NATRVP2-BIO: Qualitative

Influenza A H3	A/Brisbane/10/07	NATRVP2-BIO: Qualitative
Influenza A 2009 H1N1 pdm (pandemic swine flu)	A/NY/02/09	NATRVP2-BIO: Qualitative
Pooled human nasal swab specimens to represent diverse microbial flora in the human respiratory tract	Healthy donors, collected in 1X DNA/RNA Shield	N/A
Pooled human saliva to represent diverse microbial flora in the human respiratory tract	Healthy donors, collected in 2X DNA/RNA Shield	N/A

N/A; Not Applicable

3) *Carry-Over/Cross-Contamination:*

To assess the potential for cross-contamination between patient samples within the 96-well testing platform the following test was performed. Highly positive samples (10X LoD) along with a series of negative controls including DNA/RNA Shield media were processed through extraction to hybridization and data analysis. During processing the positive and negative samples were ultimately interlaced into a checker-board pattern on 96-well plates. Forty-two negatives were interspersed within a lattice of 108 highly positive samples. The study was designed to detect cross-contamination occurring at any point within the testing process. The data demonstrated 108/108 positive results and 42/42 negative results, thus indicating that no cross-contamination occurred.

4) *Clinical Evaluation:*

Performance of the Alimetrix SARS-CoV-2 RT-PCR Assay was evaluated using leftover, clinical nasopharyngeal swab specimens (BBL SARS-CoV-2 Validation Panel) that were purchased from BocaBiolistics Biobanx (Product # C0040-0001) as well as leftover NP swab samples obtained from an outside laboratory. All clinical samples were previously tested with one of two different EUA authorized molecular RT-PCR assays.

One milliliter of specimen was aliquoted into 1 mL of 2X DNA/RNA Shield solution to simulate patient swab collection. The specimens were tested following extraction by both the Zymo and Qiagen extraction methods.

For the positive clinical nasopharyngeal swab samples tested with the EUA authorized comparator #1, the positive percent agreement (PPA) between the Alimetrix SARS-CoV-2 RT-PCR Assay and the comparator assay was 100% (30/30) when samples were extracted using the Zymo Quick-DNA/RNA Viral MagBead Extraction Method (automated). When using samples extracted with the Qiagen QIAamp 96 Virus QIAcube HT Kit (manual), one sample was negative by the Alimetrix assay but positive by the comparator method (96.67% PPA).

For the 30 clinical negative samples tested with the EUA authorized comparator #1, two samples were positive by the Alimetrix SARS-CoV-2 RT-PCR Assay but negative by the comparator method when the clinical samples were extracted using both methods. Therefore, the NPA of the Alimetrix SARS-CoV-2 RT-PCR Assay

with both validated extraction methods was 93.33%. The two discordant specimens were re-tested from the original clinical sample with the Alimetrix assay and the results remained positive for SARS-CoV-2. Additional discordant analysis was completed on the two false positive results using a different EUA authorized molecular assay. The specimens were shipped to an outside laboratory and tested in duplicate. Results of the discordant analysis demonstrated that the samples were positive for SARS-CoV-2. Qualitative results of the clinical evaluation using both validated extraction methods and comparator assay #1 are shown in Table 10 and 11 below for the Zymo and Qiagen kits, respectively.

For the additional clinical samples that were tested by the EUA authorized comparator #2 (6 positives and 12 negatives), the PPA and NPA using samples extracted with both the Zymo and Qiagen kits were both 100%. Clinical study results using comparator #2 are shown in Tables 12 and 13. Overall combined performance tables for all samples tested using either comparator method are displayed in Tables 14 and 15 for the Zymo and Qiagen extraction kits, respectively.

Table 10. Summary of Qualitative Clinical Study Results Using Specimens Extracted with the Quick-DNA/RNA Viral MagBead Extraction Method (Automated) and Tested with EUA Authorized Comparator #1

		EUA Authorized Comparator #1		
		Positive	**Negative**	**Total**
Alimetrix SARS-CoV-2 RT-PCR Assay	**Positive**	30	2[1]	**32**
	Negative	0	28	**28**
	Total	30	30	**60**
Positive Percent Agreement		100.00% (30/30); 88.65-100.00%[2]		
Negative Percent Agreement		93.33% (28/30); 78.68-98.15%[2]		

[1] 2 discordant samples were re-tested using another EUA authorized molecular assay and were found to be positive for SARS-CoV-2 RNA
[2] Two-sided 95% score confidence intervals

Table 11. Summary of Qualitative Clinical Study Results Using Specimens Extracted with the Qiagen QIAamp 96 Virus QIAcube HT Extraction Method (Manual) and Tested with EUA Authorized Comparator #1

		EUA Authorized Comparator #1		
		Positive	**Negative**	**Total**
Alimetrix SARS-CoV-2 RT-PCR Assay	**Positive**	29	2[1]	**31**
	Negative	1	28	**29**
	Total	30	30	**60**
Positive Percent Agreement		96.67% (29/30); 85.83-99.51%[2]		
Negative Percent Agreement		93.33% (28/30); 78.68-98.15%[2]		

[1] 2 discordant samples were re-tested using another EUA authorized molecular assay and were found to be positive for SARS-CoV-2 RNA
[2] Two-sided 95% score confidence intervals

Table 12. Summary of Qualitative Clinical Study Results Using Specimens Extracted with the Quick-DNA/RNA Viral MagBead Extraction Method (Automated) and Tested with EUA Authorized Comparator #2

		EUA Authorized Comparator #2		
		Positive	**Negative**	**Total**
Alimetrix SARS-CoV-2 RT-PCR Assay	**Positive**	6	0	**6**
	Negative	0	12	**12**
	Total	6	0	**18**
Positive Percent Agreement		100% (6/6); 60.97-100.00%[1]		
Negative Percent Agreement		100% (12/12.); 75.76-100.00%[1]		

[1] Two-sided 95% score confidence intervals

Table 13. Summary of Qualitative Clinical Study Results Using Specimens Extracted with the Qiagen QIAamp 96 Virus QIAcube HT Extraction Method (Manual) and Tested with EUA Authorized Comparator #2

		EUA Authorized Comparator #2		
		Positive	**Negative**	**Total**
Alimetrix SARS-CoV-2 RT-PCR Assay	**Positive**	6	0	**6**
	Negative	0	12	**12**
	Total	6	0	**18**
Positive Percent Agreement		100% (6/6); 60.97-100.00%[1]		
Negative Percent Agreement		100% (12/12.); 75.76-100.00%[1]		

[1] Two-sided 95% score confidence intervals

Table 14. Combined Summary of Qualitative Clinical Study Results Using Specimens Extracted with Quick-DNA/RNA Viral MagBead Extraction Method (Automated)

		EUA Authorized Comparators Combined		
		Positive	**Negative**	**Total**
Alimetrix SARS-CoV-2 RT-PCR Assay	**Positive**	36	2	**38**
	Negative	0	40	**40**
	Total	36	42	**78**
Positive Percent Agreement		100% (36/36); 90.36-100.00%[1]		
Negative Percent Agreement		95.24% (40/42); 84.21-98.69%[1]		

[1] Two-sided 95% score confidence intervals

Table 15. Combined Summary of Qualitative Clinical Study Results Using Specimens Extracted with the Qiagen QIAamp 96 Virus QIAcube HT Extraction Method (Manual)

		EUA Authorized Comparators Combined		
		Positive	**Negative**	**Total**
Alimetrix SARS-CoV-2 RT-PCR Assay	**Positive**	35	2	**37**
	Negative	1	40	**41**
	Total	36	42	**78**
Positive Percent Agreement		97.22% (35/36); 85.83-99.51%[1]		
Negative Percent Agreement		95.24% (40/42); 84.21-98.69%[1]		

[1] Two-sided 95% score confidence intervals

Clinical Confirmation:
The first five positive and five negative samples determined by the Alimetrix SARS-CoV-2 RT-PCR 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 the authorized laboratory;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-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.