EMERGENCY USE AUTHORIZATION (EUA) TEST SUMMARY FOR APPLIED DNA CLINICAL LABS, LLC– LINEA 2.0 COVID-19 ASSAY

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

The Linea 2.0 COVID-19 Assay will be performed at Applied DNA Clinical Labs, LLC located at 25 Health Sciences Drive, Stony Brook, NY 11790, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements to perform high complexity tests.

INTENDED USE

The Linea 2.0 COVID-19 Assay is intended for the *in vitro* qualitative detection of RNA from SARS-CoV-2 in individual human anterior nasal swabs or pooled samples containing aliquots of media from up to 5 individual human anterior nasal swab specimens that were collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals, including individuals without symptoms or other reasons to suspect COVID-19 and placed in individual vials, when tested at least once per week.

Testing is limited to Applied DNA Clinical Labs, LLC laboratory located at 25 Health Sciences Drive, Stony Brook, NY 11790, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements to perform high-complexity testing.

The Linea 2.0 COVID-19 Assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Linea 2.0 COVID-19 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 nucleic acid is generally detectable in 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 definite cause of disease.

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.

Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs

and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be reported as presumptive positive or tested individually prior to reporting a result. Individuals included in a pool that returns a positive or invalid result should be treated as a presumptive positive unless or until they receive a negative result when re-tested individually. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, they should isolate until receiving a negative result when re-tested individually and should not be cohorted with other individuals who have received a positive or presumptive positive result. Specimens with low viral loads may not be detected with pooled testing due to decreased sensitivity or increased interference from pooled testing.

For serial testing programs, additional confirmatory testing for negative results may be necessary, if there is a high likelihood of COVID-19, such as an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection. Additional confirmatory testing for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposure to COVID-19 or residing in communities with low prevalence of infection.

1. ***Special Conditions for Use Statements:***

For use under Emergency Use Authorization (EUA) only
For prescription use only
For *in vitro* diagnostic use only

This test is authorized under the Umbrella EUA for SARS-CoV-2 Molecular Diagnostic Tests for Serial Testing (https://www.fda.gov/media/154111/download) for use in Applied DNA Clinical Labs, LLC, that is certified under CLIA and meets requirements to perform high complexity tests, in which it was developed, for the *in vitro* qualitative detection of RNA from SARS-CoV-2 in individual human anterior nasal swabs or pooled samples containing aliquots of media from up to 5 individual human anterior nasal swab specimens that were collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals, including individuals without symptoms or other reasons to suspect COVID-19 and placed in individual vials, when tested at least once per week using the test procedures validated in accordance with the requirements of the Umbrella EUA for SARS-CoV-2 Molecular Diagnostic Tests for Serial Testing.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Linea 2.0 COVID-19 Assay is a reverse transcription polymerase chain reaction (RT-PCR) test. The SARS-CoV-2 primer and probe sets are designed to detect RNA from the SARS-CoV-2 envelope (E) and nucleocapsid (N) genes in anterior nasal swab specimens that were collected from individuals, including individuals without symptoms or other reasons to suspect COVID-19.

For individual sample testing, RNA from anterior nasal swab is extracted using an authorized nucleic acid extraction kit automated on the Thermo Fisher KingFisher Flex Purification System and is reverse transcribed to cDNA and subsequently amplified via the polymerase chain reaction (PCR) using an authorized RT-PCR instrument. During the PCR amplification process, the probes anneal to specific target sequences 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 to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity signal is monitored at each PCR cycle by the RT-PCR instrument.

For pooled testing, media from up to 5 individual anterior nasal swab specimens collected in individual vials is pooled. After pooling, RNA from the pooled sample is extracted using an authorized nucleic acid extraction kit automated on the Thermo Fisher KingFisher Flex Purification System and is reverse transcribed to cDNA and subsequently amplified via the polymerase chain reaction (PCR) using an authorized RT-PCR instrument. During the PCR amplification process, the probes anneal to specific target sequences 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 to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity signal is monitored at each PCR cycle by the RT-PCR instrument.

A sample is considered positive when the fluorescence intensity signal exceeds a predetermined baseline threshold value. The cycle number at which this occurs is referred to as the cycle threshold Ct. Detection of SARS-CoV-2 RNA in a sample is determined by the Ct value. For pooled testing, if a pooled sample is positive, individual reflex (disambiguation) testing with the Linea 2.0 COVID-19 Assay is performed on each individual sample comprising the positive pool to detect the one or more individual positive samples.

The Linea 2.0 COVID-19 Assay is designed to operate as a high-throughput assay run on 96-well plates, with 92-wells being available for patient specimen testing per 96-well plate. Four wells per 96-well plate are used for controls. In pooled testing, up to 460 patent specimens can be run on a 96-well plate.

INSTRUMENTS USED WITH TEST

Instruments

The Linea 2.0 COVID-19 Assay, a real-time RT-PCR test, is to be used with the following extraction methods: i) the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated on the Thermo Fisher KingFisher™ Flex Purification System; or (ii) Applied Biosystems MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit automated on the Thermo Fisher KingFisher™ Flex Purification System performed according to manufacturer instructions and the following RT-PCR instruments: (i) Thermo Fisher Scientific (Applied Biosystems) QuantStudio™ Dx Real-Time PCR system equipped with software v1.0.3; (ii) Thermo Fisher Scientific (Applied Biosystems) QuantStudio™ 5 Real-Time PCR system equipped with QuantStudio Design and Analysis software v1.4; or (iii) Applied Biosystems™ 7500 Fast Dx Real-Time PCR system equipped with Applied Biosystems Software v1.4.1.

Reagents

The primary reagents used in Linea 2.0 COVID-19 assay:

Table 11: Reagents Included with the Linea 2.0 COVID-19 Assay Kit

Component Description	Catalog #	Manufacture
SARS-CoV-2 N-Gene Primer (forward)	Custom Oligo	Life Technologies
SARS-CoV-2 N-Gene Primer (reverse)	Custom Oligo	Life Technologies
SARS-CoV-2 N-Gene (Probe)	Custom Oligo	Life Technologies
SARS-CoV-2 E-Gene Primer (forward)	Custom Oligo	Life Technologies
SARS-CoV-2 E-Gene Primer (reverse)	Custom Oligo	Life Technologies
SARS-CoV-2 E-Gene (Probe)	Custom Oligo	Life Technologies
Positive rRT-PCR Control (Synthetic RNA)	102024	Twist Bioscience
TaqMan™ Fast Virus 1-Step Master Mix	4444432	Applied Biosystems
TaqMan™ RNase P Assay, ABY dye/QSY probe	4485714	Applied Biosystems
Nuclease Free Water	AM9938	Thermo Fisher
Positive Lysis/Extraction Control (inactivated SARS-CoV-2 virus)	VR-1986HK	ATCC

CONTROL MATERTIAL(s) TO BE USED WITH LINEA 2.0 COVID-19 ASSAY*:*

The Linea 2.0 Assay uses multiple controls that accomplish various functions within the assay. The Linea 2.0 Assay includes the controls listed in the below Table 12. Up to all 4 controls are used on each 96 well plate.

Table 12: Linea 2.0 Assay Control Information

Control Type	Material	Use	Concentration As Used	Point in Workflow
Positive rRT-PCR Control	Synthetic SARS-CoV-2 RNA containing the E-Gene and N-Gene target from Twist Bioscience	Confirm rRT-PCR	10 cp/µL	After extraction and during RT-PCR plate setup
Positive Lysis/Extraction Control	Heat inactivated quantified SARS-CoV-2 Virus from ATCC	Assay Process Control	10 cp/µL	Prior to extraction. For pooling, the control is added after automated pooling step
Negative Lysis/Extraction Control	Sample Transport Media	Assay Process Control	N/A	Prior to extraction. For pooling, the control is added after automated pooling step
No Template Control	Nuclease Free Water	Reagent Contamination Control	N/A	After extraction and during RT-PCR plate setup

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. Appropriate control interpretation criteria and result interpretation criteria are described here.

1. **Examination and Interpretation of Control Results**

- The NTC reaction should be negative for E and N targets and negative for the RNase P target. If any of the NTC reactions exhibit positive fluorescence above the threshold for the E, N or RNase P targets in Table 13 below, it is possible that contamination occurred, or that the assay was setup improperly. The RT-PCR run is invalid. Repeat from the RT-PCR step using residual extraction material. If the repeat NTC result is positive, re-extract and re-test all samples.

- The positive rRT-PCR control reaction should yield positive results for the E and N targets and negative for the RNase P target. Negative results for E or N targets or a positive result

for RNase P target invalidates the run and suggests the assay may have been set up incorrectly, contaminated, or the integrity of the primers/probes is compromised. Repeat from the RT-PCR step using residual extraction material.

- The positive lysis/extraction control reaction should yield positive results for the E and N targets and a negative or positive result for the RNase P target. Negative results for E or N targets invalidates the run and suggests the assay may have been set up incorrectly, extraction and/or lysis was not successful, contamination, or the integrity of the primers/probes is compromised. Repeat from the RT-PCR step using residual sample.

- The negative lysis/extraction control reaction should yield negative results for the E and N targets and a negative or positive result for the RNase P target. Positive results for E or N targets invalidates the run and suggests the assay may have been set up incorrectly, contamination, or the integrity of the primers/probes is compromised. Repeat from the RT-PCR step using residual sample.

A summary of expected results for the Linea 2.0 COVID-19 Assay Kit controls is show in the below table.

Table 13: Interpretation of results for no template and positive control reactions.

Control	E (Ct ≤38)	N (Ct ≤38)	RNase P (Ct <35)
No Template Control	-	-	-
Positive rRT-PCR Control	+	+	-
Positive Lysis/Extraction Control	+	+	-/+
Negative Lysis/Extraction Control	-	-	-/+

2. Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results must be performed after the controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

The table below shows the interpretation of individual patient specimens run on the Linea 2.0 COVID-19 Assay. The user should repeat testing on any sample with questionable interpretation, as suggested in the results interpretation table.

Table 14: Interpretation of Patient Specimen Results

E Ct ≤38 (VIC)	N Ct ≤38 (FAM)	RNase P Ct <35 (ABY)	Interpretation	Report Result	Actions
+	+	+	SARS-CoV-2 **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities
-	+	+	SARS-CoV-2 is **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities
+	-	+	SARS-CoV-2 is **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities
-	-	+	SARS-CoV-2 **Not Detected**	**NEGATIVE**	Reported to sender and appropriate public health authorities
+/-	+/-	-	**Invalid Result**	**INVALID**	Repeat extraction and RT-PCR. If the repeated result remains INVALID, consider collecting a new specimen from the patient, if clinically indicated.

Interpretation of pooled testing results follows the results interpretation in Table 15, below.

Table 15: Interpretation of Pooled Patient Specimen Results

E Ct ≤38 (VIC)	N Ct ≤38 (FAM)	RNase P Ct <35 (ABY)	Interpretation	Report Result	Actions[c]
+	+	+	SARS-CoV-2 **Detected**	**PRESUMPTIVE POSITIVE**[a,b]	Report pooled result to program administrator with notification that subjects in the positive pool should isolate until receiving a negative result when re-tested individually. Individual should not be cohorted with other individuals who have received a
-	+	+	SARS-CoV-2 is **Detected**	**PRESUMPTIVE POSITIVE**[a,b]	
+	-	+	SARS-CoV-2 is **Detected**	**PRESUMPTIVE POSITIVE**[a,b]	

					positive or presumptive positive result. Individual samples from all subjects in the pool should be re-tested individually.
-	-	+	SARS-CoV-2 **Not Detected**	**NEGATIVE[a,d]**	Report pooled result to sender and appropriate public health authorities.
+/-	+/-	-	**Invalid Result**	**INVALID[b]**	Repeat extraction and RT-PCR. If the result is NEGATIVE, report pooled result to program administrator and appropriate public health authorities. If the result is POSITIVE or INVALID, report the result as PRESUMED POSITIVE to program administrator. A new sample from all subjects in the pool should be run and re-tested individually.

[a]Presumptive Positive" and "Negative" are the specific result interpretations for samples tested as part of the 5-media pooling procedure.
[b]Individuals included in a pool that returns a positive or invalid result should be treated as a presumptive positive unless or until they receive a negative result when re-tested individually. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, they should isolate until receiving a negative result when re-tested individually and should not be cohorted with other individuals who have received a positive or presumptive positive result. For serial testing programs, additional confirmatory testing for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposure to COVID-19 or residing in communities with low prevalence of infection.
[c]Results of follow-up testing are reported to the program administrator and appropriate public health authorities.
[d]Specimens with low viral loads may not be detected with pooled testing due to decreased sensitivity or increased interference from pooled testing. For serial testing programs, additional confirmatory testing for negative results may be necessary, if there is a high likelihood of COVID-19, such as an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection.

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) -Analytical Sensitivity:

Limit of detection (LoD) studies determine the lowest detectable concentration of the SARS-CoV-2 virus at which approximately 95% of all true positive replicates test positive for both the E-gene and N-gene assay targets. Three identical LoD studies using the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated on the King Fisher Flex were conducted for the three RT-PCR instruments (QuantStudio Dx, QuantStudio and ABI 7500) authorized for use with the Linea 2.0 COVID-19 Assay Kit.

For each study, the LoD was determined using quantified heat inactivated SARS-CoV-2 virus obtained from ATCC, Manassas, VA (VR-1986HK, strain designation 2019-nCoV/USA-WA1/2020) per the validation requirements of Appendix A of the Umbrella EUA for SARS-CoV-2 Molecular Diagnostic Tests for Serial Testing. The quantified heat inactivated SARS-CoV-2 virus was spiked into pooled negative clinical anterior nasal matrix. For each study a preliminary range finding experiment was conducted wherein the quantified heat inactivated reference material was 10-fold serially diluted with the same pooled negative clinical matrix. Six extraction replicates at each dilution were obtained with the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated on the King Fisher flex. The extracted nucleic acids were run on the Linea 2.0 Assay using the applicable RT-PCR instrument to obtain the LoD range for each RT-PCR instrument.

Once the initial LoD range was established, confirmation of the final LoD was determined for each RT-PCR instrument using 2-fold serial dilutions of inactivated whole SARS-CoV-2 virus (VR-1986HK) spiked into pooled negative clinical anterior nasal matrix. 20 extraction replicates at each dilution were obtained using the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated on the King Fisher flex and run on the applicable RT-PCR instrument to determine the final LoD for each RT-PCR instrument.

As shown in the Tables 16-18 below, the final LoD of the Linea 2.0 COVID-19 Assay Kit using the using the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated on the King Fisher Flex ranged from 1.25 copies per reaction (0.313 copies/µL) to 0.625 copies per reaction (0.156 copies/µL) depending on the RT-PCR instrument utilized.

Table 16: LoD Results of the Linea 2.0 Assay Using Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated on King Fisher flex on the QuantStudio Dx Real-Time PCR system

Copies per Reaction	Copies per µL	E Target		N Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
20	5	20/20	32.1	20/20	32.0
10	2.5	20/20	33.0	20/20	33.0
5	1.25	20/20	33.9	20/20	34.1
2.5	0.625	20/20	34.8	20/20	34.9
1.25	**0.313**	**20/20**	35.8	**20/20**	35.8
0.625	0.156	18/20	37.0	18/20	37.0

Table 17: LoD Results of the Linea 2.0 Assay Using the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit Automated on the KingFisher Flex on the QuantStudio 5 Real-Time PCR system

Copies per Reaction	Copies per µL	E Target		N Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
20	5	20/20	31.4	20/20	32.2
10	2.5	20/20	32.3	20/20	33.1
5	1.25	20/20	33.3	20/20	34.1
2.5	0.625	20/20	34.0	20/20	34.9
1.25	**0.313**	**20/20**	35.0	**20/20**	35.7
0.625	0.156	18/20	36.5	19/20	37.0

Table 18: LoD Results of the Linea 2.0 Assay Using the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit Automated on the KingFisher Flex Purification System on the Applied Biosystems 7500Dx Real-Time PCR system

Copies per Reaction	Copies per µL	E Target		N Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
20	5	20/20	32.2	20/20	32.5
10	2.5	20/20	33.1	20/20	33.4
5	1.25	20/20	34.1	20/20	34.4
2.5	0.625	20/20	34.9	20/20	35.2
1.25	**0.313**	**20/20**	**35.7**	**19/20**	**36.3**
0.625	0.156	15/20	37.4	15/20	37.5

Three additional identical LoD studies using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit automated on the King Fisher Flex were also conducted for the three RT-PCR instruments (QuantStudio Dx, QuantStudio and ABI 7500 Dx) authorized for use with the Linea 2.0 COVID-19 Assay Kit. These additional LoD studies were identical in study design as the above LoD studies.

As shown in the Tables 19-21 below, the final LoD of the Linea 2.0 COVID-19 Assay Kit using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit automated on the King Fisher Flex was 1.25 copies per reaction (0.313 copies/µL) for all authorized RT-PCR instruments.

Table 19: LoD Results of the Linea 2.0 Assay Using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit Automated on King Fisher Flex on the QuantStudio Dx Real-Time PCR system

Copies per Reaction	Copies per µL	E Target		N Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
20	5	20/20	32.1	20/20	31.8
10	2.5	20/20	33.1	20/20	32.7
5	1.25	20/20	34.1	20/20	34.1
2.5	**0.625**	**20/20**	**35.2**	**20/20**	**35.0**
1.25	0.313	15/20	36.9	20/20	35.8

Table 20: LoD Results of the Linea 2.0 Assay Using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit Automated on the KingFisher Flex on the QuantStudio 5 Real-Time PCR system

Copies per Reaction	Copies per µL	E Target		N Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
20	5	20/20	31.9	20/20	32.6
10	2.5	20/20	32.9	20/20	33.5
5	1.25	20/20	33.8	20/20	34.4
2.5	0.625	20/20	35.0	20/20	35.7
1.25	**0.313**	**19/20**	35.7	**20/20**	**36.0**
0.625	0.156	17/20	36.6	13/20	37.3

Table 21: LoD Results of the Linea 2.0 Assay Using the Using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit Automated on the KingFisher Flex Purification System on the Applied Biosystems 7500Dx Real-Time PCR system

Copies per Reaction	Copies per µL	E Target		N Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
20	5	20/20	31.9	20/20	31.7
10	2.5	20/20	32.9	20/20	32.7
5	1.25	20/20	33.8	20/20	33.6
2.5	0.625	20/20	35.3	20/20	34.8
1.25	**0.313**	**19/20**	**35.9**	**20/20**	**35.2**
0.625	0.156	14/20	36.5	16/20	36.9

A summary of each authorized RT-PCR instruments using either the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit or MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit is provided below.

Table 22: Summary of LoD Results on Authorized RT-PCR Instruments

RT-PCR Instrument	Limit of Detection (Copies per µL)	
	Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit	MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit
QuantStudio Dx	0.313	0.625
QuantStudio 5	0.313	0.313
ABI 7500 Dx	0.313	0.313

2) Inclusivity (Analytical Reactivity):

In silico evaluation of the E and N target primers and probes was performed on January 5, 2022 using 1,382,040 complete SARS-CoV-2 genomes from the GISAID database. The *in silico* analysis through results of significant sequences returned, generated the following data:

Table 23: Inclusivity Results for the Linea 2.0 COVID-19 Assay

Target	**Primer/Probe**	**% of Sequences showing 100% Homology**
E	Forward Primer	99.49
	Reverse Primer	99.90
	Probe	99.88
N	Forward Primer	99.66
	Reverse Primer	99.02
	Probe	99.53

An addition *in silico* evaluation of the E and N assay target sequences was performed on January 6, 2022 against the WHO designated Variants of Concern. All sequences were downloaded from the GISAID database between approximately June 1, 2021 and January 5, 2022.

Table 24. Percentages of WHO Variant of Concern Sequences showing 100% Homology to Assay Target Sequences

WHO Label	Pango Lineage	E-Gene Target	N-Gene Target	# of Sequence analyzed
Alpha	B.1.1.7	99.8	98.9	8814
Beta	B.1.351	99.7	98.9	15809
Gamma	P.1	99.5	99.0	27588
Delta	B.1.617.2	99.7	99.2	61500
Omicron	B.1.1.529	99.9	99.8	18897

3) Cross-reactivity (Analytical Specificity):

In silico analysis was conducted for the Linea 2.0 COVID-19 Assay Kit Assay E-gene and N-gene primers and probes utilizing the NIH NCBI BLAST sequence alignment system. The pairwise sequence alignment verifies the number of sequence matches between the primers and probes of the Linea 2.0 COVID-19 Assay and the target organism listed in Table 25. Anything less than an 80% match of the primers and probe is categorized as non-cross-reactive. The organism tested and results of the analysis are outlined in table below

Table 25: Results of *in silico* Cross-Reactivity Study

Sample Name	**E gene Target 1**	**N gene Target 2**	**Notes**
Adenovirus 11	None	None	
Adenovirus 5	None	None	
Bordetella pertussis	None	None	
Chlamydophila pneumoniae	None	None	
Enterovirus 68	None	None	
Haemophilus influenzae	None	None	
Human coronavirus 229E	None	None	
Human coronavirus OC43	None	None	
Human coronavirus HKU1	None	None	
Human coronavirus NL63	None	None	
Human metapneumovirus	None	None	

Sample Name	E gene Target 1	N gene Target 2	Notes
Human parainfluenza virus 1	None	None	
Human parainfluenza virus 2	None	None	
Human parainfluenza virus	None	None	
Human parainfluenza virus 4b	None	None	
Human respiratory syncytial virus	None	None	
Human rhinovirus 61	None	None	
Influenza A	None	None	
Influenza B	None	None	
Legionella pneumophila	None	None	
Middle East Respiratory Syndrome coronavirus	None	None	
Mycobacterium tuberculosis	None	None	
Mycoplasma pneumoniae	None	None	
Severe Acute Respiratory Syndrome coronavirus (SARS-1)	**	**	E primers and probe has >80% homology. N probe showed >80% homology
Streptococcus pneumoniae	None	None	
Streptococcus pyogenes	None	None	
Pneumocystis jirovecii	None	None	
Candida albicans	None	None	
Pseudomonas aeruginosa	None	None	
Staphylococcus epidermis	None	None	
Streptococcus salivarius	None	None	

**Result returned >80 homology

As noted in Table 25 above, the only sequence with substantial similarity (>80% homology) was SARS-1 with the E-gene target primers and probe, and N-gene target probe only. The *in silico* analysis found no substantial similarity between the N-gene target forward and reverse primers and the SARS-1 genome, and thus no amplification of the SARS-1 genome will occur for the N-gene target. In addition, there are no known current infections of SARS-1 in the human population. Historical infections have been limited only to Asia. As such, it is not

expected that the E-gene target primers/probe and/or N-gene target probe's substantial similarity with the SARS-1 genome will impact the clinical utility of the Linea 2.0 COVID-19 Assay.

4) Clinical Evaluation:

Performance of the Linea 2.0 COVID-19 Assay was evaluated using 84 clinical positive (of which 63 were weak positives with Ct>30) and 46 clinical negative retrospective deidentified individual anterior nasal swab specimens that were tested on the Linea 2.0 COVID-19 Assay and a high sensitivity EUA authorized molecular assay comparator (TaqPath COVID-19 RNase P Combo Kit 2.0; EUA210403). The TaqPath COVID-19 RNase P Combo Kit 2.0 molecular comparator assay was performed in accordance with its instructions for use.

RT-PCR was performed on the QuantStudio Dx Real-Time PCR system for the Linea 2.0 COVID-19 Assay Kit and on the QuantStudio 5 Real-Time PCR system for the TaqPath COVID-19 RNaseP Combo kit 2.0 (as required by the TaqPath IFU). Extraction for the Linea 2.0 COVID-19 Assay Kit was performed on the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit Automated on the KingFisher Flex Purification System. All samples were blinded and randomized for testing.

For both positive and negative patient specimens, 100% concordance was observed. The summary results of the clinical evaluation are shown in Table 26.

Table 26: Results of Linea 2.0 Assay Versus EUA-Authorized Comparator Assay with ANS Specimens.

		EUA Comparator Assay		
Linea 2.0 Assay		**Positive**	**Negative**	**Total**
	Positive	84	0	**84**
	Negative	0	46	**46**
	Total	**84**	**46**	**130**
Positive Percent Agreement		**100%* (84/84)**		
Negative Percent Agreement		**100%** (46/46)**		

*95% Confidence Interval: 95.70% - 100.00%
**95% Confidence Interval: 92.29% - 100.00%

An additional clinical evaluation of the Linea 2.0 COVID-19 Assay was performed on 32 deidentified anterior nasal swan samples confirmed to be the Omicron variant. The 32 clinical positive samples were sequenced via the Illumina MiSeq System. Whole genome sequencing confirmed that all 32 samples where the BA.1 sub-lineage of the B.1.1529 lineage (Omicron). These 32 samples were tested on the Linea 2.0 COVID-19 Assay and the TaqPath COVID-19

RNase P Combo Kit 2.0 comparator assay. The TaqPath COVID-19 RNase P Combo Kit 2.0 molecular comparator assay was performed in accordance with its instructions for use.

All 32 Omicron variant samples tested positive on both the Linea 2.0 COVID-19 Assay and the comparator TaqPath COVID-19 RNase P Combo Kit 2.0.

5) Additional Validation for *Media Pooling up to n=5 with validation option 2*:

To validate up to 5-sample anterior nasal swab media pooling with the Linea 2.0 COVID-19 Assay Kit, a detection study comparing the results of 5-sample pooled samples and individual samples was performed to establish the accurate detection of pooled positive and negative clinical anterior nasal swabs samples with the Linea 2.0 COVID-19 Assay. The performance of the assay in conjunction with 5-sample anterior nasal swab media pooling was evaluated using the following:

- 35 positive 5-sample pools. Each positive pool was comprised of media from 1 positive clinical ANS sample and 4 negative clinical anterior nasal swab samples.
- 25 negative 5-sample pools. Each negative pool was comprised of media from 5 randomly selected negative clinical anterior nasal swab samples.

All positive clinical anterior nasal swab samples used in the study were tested individually on the Linea 2.0 COVI-19 Assay and a comparator EUA-authorized molecular assay (TaqPath COVID-19 RNase P Combo Kit 2.0) prior to pooling. PPA for the individual positive anterior nasal swab samples was 100%. The individual sample Ct values were representative of the Ct values seen in clinical COVID-19 samples tested, and 7/35 individual positive samples were classified as low positives. In addition, media from 265 negative clinical anterior nasal swab samples, as determined by the Linea 2.0 COVD-19 Assay Kit and the comparator assay were utilized.

To create the 5-sample positive pools, 40 µL of transport media from 1 positive anterior nasal swab sample was pooled with 40 µL of transport media from each of 4 negative anterior nasal swab samples. To create the negative 5-sample pools, 40 µL of transport media from each of 5 negative anterior nasal swab samples were pooled. All pools were created utilizing the automated workflow detailed herein. After automated pooling, the total of 60, 5-sample pools (35 positive and 25 negative pools) were tested on the Linea 2.0 COVID-19 Assay utilizing the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated on the Thermo Fisher KingFisher Flex System and the QuantStudio Dx Real-Time PCR system. Based on the pooled sample results, agreement with individual sample results were calculated. In addition, the delta Ct value for all assay targets was calculated for individual samples versus 5-sample pooling.

As shown in Tables 27 and 28, below, 34/35 individual positive samples remained positive when tested in 5-sample pools. All 25 pools expected to be negative, remained negative.

Table 27: Clinical Performance of 5-Sample Pooling with Linea 2.0 COVID-19 Assay Versus Individual Sample Testing on Linea 2.0 COVID-19 Assay

		Individual Samples (Lines 2.0 COVID-19 Assay Kit)		
5-Sample Pooling		**Positive**	**Negative**	**Total**
	Positive	34	0	**34**
	Negative	1	25	**26**
	Total	**35**	**25**	**60**
Positive Percent Agreement (PPA)		**97.14%* (34/35)**		
Negative Percent Agreement (NPA)		**100%** (25/25)**		

*95% Confidence Interval: 85.08.% - 99.93%
**95% Confidence Interval: 86.3% - 100%

Table 28: Clinical Performance of 5-Sample Pooling with Linea 2.0 COVID-19 Assay Versus Individual Sample Testing on EUA Comparator Assay

		Individual Samples (EUA Comparator Assay)		
5-Sample Pooling		**Positive**	**Negative**	**Total**
	Positive	34	0	**34**
	Negative	1	25	**26**
	Total	**35**	**25**	**60**
Positive Percent Agreement (PPA)		**97.14%* (34/35)**		
Negative Percent Agreement (NPA)		**100%** (25/25)**		

*95% Confidence Interval: 85.08% - 99.93%
**95% Confidence Interval: 86.3% - 100%

In addition, the Ct values for all individual positive samples as tested on the Linea 2.0 COVID-19 Assay were compared to the Ct values obtained from the Linea 2.0 COVID-19 Assay for the 5-sample pool containing said individual sample. Summary results are shown in Table 29.

Table 29: Δ Ct for the Linea 2.0 COVID-19 Assay Targets (individual v. 5-sample pooled)

E-Gene Target		N-Gene Target		RNase P Target (IC)	
Average Δ Ct (individual v. pooled)	Std Dev	Average Δ Ct (individual v. pooled)	Std Dev	Average Δ Ct (pool as detected v. pooled sample average)	Std Dev
-2.8	0.57	-2.9	0.7	-0.50	0.75

LIMITATIONS

- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between August 2021 and December 2021 in New York State. The clinical performance of this test has not been established in all circulating variants but is anticipated to be reflective of the variants in circulation at the time and location(s) of the clinical evaluation. As such, 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.

- Clinical performance has been established in specimens collected from subjects suspected of COVID-19 by a healthcare provider. Performance of specimens collected from individuals without symptoms or other reasons to suspect COVID-19 has not been established. A study to determine the performance in individuals without symptoms or other reasons to suspect COVID-19 will be completed.

WARNINGS:

- This product has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by the laboratory that developed the test and which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements to perform high complexity tests.

- 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 Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.