EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
Nexus High Throughput SARS-CoV-2 Assay
Nexus Medical Labs, LLC

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

The Nexus High Throughput SARS-CoV-2 Assay will be performed at Aldatu Diagnostics, 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 as described in the Nexus High Throughput SARS-CoV-2 Assay Standard Operating Procedures that were reviewed by the FDA under this EUA.

INTENDED USE

1. Intended Use

The Nexus High Throughput SARS-CoV-2 Assay is an in vitro diagnostic real-time reverse transcription polymerase chain reaction (RT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior nasal swab specimens that are collected using the Rhinostics Nasal Swab Collection Kit for Nexus High Throughput SARS-CoV-2 Assay from individuals who are suspected of COVID-19 by a healthcare provider, including specimens collected by a healthcare provider from individuals of any age and self-collected specimens that are collected at home or in a healthcare setting by individuals 18 years of age or older.

Testing is limited to Aldatu Diagnostics, located at 313 Pleasant Street, Watertown MA 02472, that 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.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA 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. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Nexus High Throughput SARS-CoV-2 Assay is intended for use by clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Nexus High Throughput SARS-CoV-2 Assay and Rhinostics Nasal

Swab Collection Kit for Nexus High Throughput SARS-CoV-2 Assay are only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Special Conditions of Use Statements

For prescription use only
For in vitro diagnostic use only
For Emergency Use only

The Rhinostics Nasal Swab Collection Kit for Nexus High Throughput SARS-CoV-2 Assay is only authorized for use by individuals 18 years of age or older and when used in conjunction with the Nexus High Throughput SARS-CoV-2 Assay, an in vitro diagnostic (IVD) test for the detection of SARS-CoV-2 that has been issued an EUA and is authorized for use with this collection device.

DEVICE DESCRIPTION AND TEST PRINCIPLE

1) Rhinostics Nasal Swab Collection Kit for Nexus High Throughput SARS-CoV-2 Assay

The Rhinostics Nasal Swab Collection Kit for Nexus High Throughput SARS-CoV-2 Assay (referred to as Rhinostics Nasal Swab Collection Kit for the Nexus Assay in this document) is the same as the COVID-19 Self-Swab Collection Kit for HUCL which was granted emergency use authorization under EUA210157. Harvard University Clinical Laboratory (HUCL) has granted Nexus Labs, LLC a right of reference to the validation data supporting use of this collection kit. The Rhinostics Nasal Swab Collection Kit for the Nexus Assay implements the same testing workflow as that authorized for the COVID-19 Self-Swab Collection Kit for HUCL under EUA210157.

The Rhinostics Nasal Swab Collection Kit for the Nexus Assay consists of a sterile packaged medical-grade hydrophobic polymer nasal swab with a threaded lid attached, a dry collection tube with a threaded end, a barcode card, instructions for use, a biohazard bag with an absorbent pad, a rigid outer safety box with UN3373 marking, and a FedEx shipping envelope with a prepaid return address label. Self-collected nasal swab samples are transported under dry conditions at ambient temperature to Aldatu Diagnostics for processing with the Nexus High Throughput SARS-CoV-2 Assay (referred to as Nexus Assay in this document).

a) Collection Kit Ordering and Processing:
Individuals may request the Rhinostics Nasal Swab Collection Kit for the Nexus Assay as part of a community-based distribution framework that is physician ordered provided in partnership with Vault Medical Services, P.A. (Vault). Healthcare providers (HCPs) who are licensed and have prescriptive authority in their respective states use a COVID-19 questionnaire that is based on current CDC testing guidelines to evaluate patient eligibility. Ordering physicians must be licensed in the state where the kits will be provided or shipped, as appropriate.

Nexus Medical Labs LLC and Aldatu Diagnostics has an agreement with Vault Health (Vault) to provide services related to the self-collection kit. Vault provides an online HIPAA-compliant portal for patient registration, pre-test kit activation, and post-test access to results. In addition, Vault assembles the collection kit for distribution. At the healthcare provider's discretion, the patient is directed to access the Vault websites which are specific to individual states and services. Patient answers questions related to patient exposure, symptoms, as well as underlying health conditions and other risk factors. This task is to document the patient's responses and link the patient with a specific kit and barcode that will be used for accessioning at the testing laboratory. Reporting for the test results is done through Vault's provider network. Results of the Nexus Assay for detection of SARS-CoV-2 are communicated to the ordering physician who follows up with the patient regarding the test results. Patients can log into Vault's HIPAA-compliant portal to access their results there. Patients can also consent to receive test results from Vault via email.

When offering testing to the Harvard University community-based distribution network, the interface for patient registration, pre-test kit activation, and post-test access to results will be provided by Color Health, Inc (Color). Kit activation and result reporting does not differ significantly when performed through the Color patient portal or the Vault portal. The steps in the patient workflow for on-site and at-home collection are described below. Color Health assembles the Rhinostics Nasal Swab Collection Kit for the Nexus Assay for distribution in the Harvard University community. Samples collected with the Rhinostics Nasal Swab Collection Kit will be shipped to Aldatu Diagnostics for processing with the Nexus Assay. For samples collected from the Harvard University community, Aldatu Diagnostics will report results to Color Health which will then communicate them to the ordering physician who will follow up with the patient. Patients can also log into Color's HIPAA-compliant patient portal to access their results.

The patient flow for on-site and at-home collection is described below.

i. Patients begin the testing process by accessing a Vault URL or Color website via smartphone, tablet or computer both when they are testing on-site and at-home. Tablets are provided on-site for those without internet access.
ii. Through this flow, patients will create an account and enter relevant medical details related to patient exposure, symptoms, as well as underlying health conditions and other risk factors.
iii. Patients will activate their kit by entering the provided barcodes that verify that they have a valid, unused Rhinostics Nasal Swab collection kit for the Nexus Assay. This also marks the system with a time stamp, which corresponds to the collection time.
iv. Patients follow detailed step-by-step instructions on completing the collection of the sample.
v. Upon completing the collection of the sample, they will either hand off the collection kit to the administrator (on-site) or following return shipping instructions provided (at-home).
vi. Results of the Nexus Assay are communicated to the ordering physician who follows up with the patient regarding the test results. Patients can log into Vault's or Color's

HIPAA-compliant portal to access their results there. Patients can also consent to receive test results from Vault via email.

b) Shipping:

Self-collected nasal swab samples are transported under dry conditions at ambient temperature to Aldatu Diagnostics for processing with the Nexus Assay.

c) Specimen Accessioning:

Specimens collected with the Rhinostics Nasal Swab Collection Kit for the Nexus Assay for the Nexus assay must be checked for the following criteria upon receipt at the testing facility prior to processing:

- Sample collection tube must be intact and not visibly damaged.
- The tube barcode label must be present and readable by a barcode scanner.
- The tube cap must be properly secured onto the tube.
- The tube must contain a swab.
- The swab should not be visibly bloody.
- Accession time is within 52 hours of the collection time.

Specimens not received in the approved collection kits, damaged vials, vials containing swabs that are received without a top properly closed, vials without swabs, and specimens not labeled with kit barcode labels are not acceptable for testing. These specimens will be rejected upon receipt. Additionally, visibly bloody specimens will not be accepted for testing. Another specimen will be requested. Specimens must be tested within 56 hours of collection. Any specimen received more than 52 hours after collection will be rejected.

Upon accessioning, the LIMS will query the Vault or Color registration database with the scanned barcode. The LIMS will record whether the barcode is in the registration database. If the barcode is in the registration database, the sample can be processed; if the barcode is not in the registration database, the sample should not be processed.

2) Nexus High Throughput SARS-CoV-2 Assay

The Nexus Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test for qualitative detection of RNA from SARS-CoV-2. It uses the same reagents and sample processing procedures as the previously authorized Quaeris SARS-CoV-2 Assay from Harvard University Clinical Laboratory (HUCL) authorized under EUA210157. The SARS-CoV-2 primer and probe set is designed to detect RNA from the SARS-CoV-2 nucleocapsid (N1) and RNA dependent RNA polymerase (RdRp) genes and from the human RNase P gene in anterior nasal swab specimens from patients. The test consists of three processes in a single tube: 1) reverse transcription of target RNA to cDNA, 2) PCR amplification of target and internal control, and 3) simultaneous detection of all three target amplicons using different fluorescent dye labeled probes. During the amplification process, the probes anneal to their respective 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 probes, causing the reporter dyes to separate from the quencher dye, generating fluorescent signal.

The Nexus Assay to be used with the Rhinostics Nasal Swab Collection Kit for the Nexus Assay employs an RNase P internal control. This control is used to determine whether the collection device has been used appropriately. Anterior nasal swab (ANS) specimens are collected and shipped in a dry collection tube. When received by the laboratory, samples are rehydrated with 300 µL phosphate buffered saline (PBS), then inactivated at 65ºC and subsequently used directly as input for the assay. There is no extraction step. Liquid handling is automated using the Hamilton Star Liquid Handling System and RT-PCR is performed on an Applied Biosystem Quantstudio 7 Flex instrument (software version 1.7).

INSTRUMENTS USED WITH THE TEST

The Nexus High Throughput SARS-CoV-2 Assay is to be used with the Applied Biosystem Quantstudio 7 Flex instrument (software version 1.7). The assay does not include a sample extraction step. Liquid handling for the assay is automated on a Hamilton Star Liquid Handling System; removal of the integrated swab and cap from the sample tube can be done manually or with the aid of a Hamilton LabElite Decapper.

REAGENTS AND MATERIALS

The following equipment/reagents/materials are required to run this test:

Table 1: Equipment / Instruments for use with the Nexus High Throughput SARS-CoV-2 Assay

Equipment	Manufacturer	Catalog Number
Quant Studio 7 Flex	ABI/Thermo Fisher	4485690
Quant Studio Real-Time PCR Software version 1.7	ABI/Thermo Fisher	N/A
RHINOrac	Rhinostics	RR-HTM0001
Hamilton Microlab STARline Liquid Handling System	Hamilton	173000
Hamilton LabElite ID DeCapper	Hamilton	193601
Eppendorf 12 channel pipettor 30-300µL	Fisher Scientific	13-690-052
Drummond Pipet-Aid	Drummond Scientific	4-000-100
15mL nuclease free conical tube	VWR	21008-216
50mL nuclease free conical tube	Genesee	28-106
1.5 mL DNA Lo-Bind Eppendorf Tubes®	VWR	80077-230
50µl Filter Tips for Automaton	Hamilton	235948
1000µl Hamilton Robotic Tip	Hamilton	235905
60mL Reagent Reservoirs	Hamilton	56694-01
96-format disposable cap holder rack	Hamilton	HST-193514
Calibrated Pipettes capable of delivering 1.0 µL to 1000 µL)	MLS*	N/A
Benchtop Microcentrifuge	MLS*	N/A
Vortex	MLS*	N/A
Laboratory Freezer, -10°C to -30°C	MLS*	N/A

*MLS: Major Laboratory Supplier; N/A: Not Applicable

Table 2: Reagents and materials for use with the Nexus High Throughput SARS-CoV-2 Assay		
Reagent/Material	**Manufacturer & Catalog Number (specified or equivalent)**	
384-well microplate (barcoded) MicroAmp Optical microplates	Thermo Fisher	4343814
Flat-Top 96-Well PCR Plate	VWR	490003-750
PCR strips	VWR	53509-304
PBS - Phosphate-Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher	AM9624
Luna Universal Probe One-Step RT-qPCR kit (No ROX) (store at -20°C)	NEB	E3007E
MicroAmp Optical Adhesive Film	Thermo Fisher	4311971
Silicone adhesive film for PCR plates	VWR	89134-428
RNaseIn Plus RNase inhibitor (store at -20C)	Promega	N2615
Nuclease-Free Water (not DEPC-Treated)	Thermo Fisher	4387936
Primers for master mix N1 (store at -20°C)	IDT	N/A
Primers for master mix RP (store at -20°C)	IDT	N/A
Primers for master mix RdRp (store at -20°C)	IDT	N/A
TE, pH 8.0, RNase-free	Fisher Scientific	648314100ML
Probes for master mix N1 (stored at -20°C)	IDT	N/A
Probes for master mix RdRp (store at -20°C)	IDT	N/A
Probes for master mix RNaseP (store at -20°C)	IDT	N/A
80% Ethanol	Fisher Scientific	T08204K7

N/A: Not Applicable

Table 3: Components of the collection kit		
Components	**On-site**	**At-home**
Sterile packaged medical grade hydrophobic polymer swab (Part Number: RH-S000001 from Rhinostics)	X	X
Sterile collection tube	X	X
Barcode card	X	X
Instructions for use	X[1]	X[2]
A biohazard bag with absorbent pad	X	X
Rigid box outer safety box with UN3373 marking	N/A	X
Overnight or Next-day FedEx soft shipping envelope with prepaid return shipping label with UN3373 marking	N/A	X

N/A: Not Applicable
[1]Instructions for On-site collection/specimen drop-off
[2]Instruction for at-home collection/return by mail

CONTROLS TO BE USED WITH THE TEST

Table 4: Controls to be used with the Nexus High Throughput SARS-CoV-2 Assay

Control Type	Description	Source/Catalog Number	Purpose	Frequency of Testing
Negative (no template NTC1)	1x PBS - Phosphate-Buffered Saline pH 7.4	VWR 75800-990	To monitor for cross-contamination	1 per 384-well assay plate
Negative (no template NTC2)	Nuclease-Free Water (not DEPC-Treated)	Thermo Fisher 4387936	To monitor for cross-contamination	1 per 384-well assay plate
SARS-CoV-2 Positive Control (DNA) (at 5x LoD each)	2019-nCoV_N_Positive Control, 2019-nCoV_RdRp_Positive Control, and 2019-nCoV_RPP30 Positive Control	IDT 10006625, IDT 10006626, and IDT 10006897	To monitor the integrity of the RT-PCR reagents and process	2 per 384-well assay plate
SARS-CoV-2 Positive Control (RNA) (at 5x LoD)	Mix of non-replicative recombinant virus containing the entire SARS-CoV-2 RNA genome and human genomic DNA	Aldatu Biosciences PR.0012.PC.384.NXS	To monitor the integrity of the RT-PCR reagents and ensure reverse transcriptase	1 per 384-well assay plate
Internal control (process control)	Primer and probe pair to detect RNase P RNA (same as the RNase P primer/probe used in the CDC 2019-nCoV assay)	From each patient sample	To ensure the sample collection was properly performed and to monitor sample integrity.	Every tested specimen

No Template Control (NTC)
A negative (no template) control must be used to monitor sample contamination during all processing steps, including sample inactivation (NTC1) and RT-PCR assay set-up (NTC2). Two additional negative controls are included on each 384-well plate. Phosphate buffered saline (pH 7.4) (NTC-1), which is added as a rehydration buffer, and molecular grade, nuclease-free water [not DEPC treated] (NTC-2) should be added to a sterile collection tube during the dry swab rehydration step and processed as if it were a clinical sample (e.g., it should be heat inactivated).

SARS-CoV-2 Positive Control (DNA)
A positive SARS-CoV-2 control is needed to verify proper assay set-up and SARS-CoV-2

reagent integrity. A positive control consists of molecular grade, nuclease-free water spiked with a mixture of control plasmids from IDT at a concentration of 5x LoD each. Two positive controls must be used on every assay plate starting at master mix addition. Positive control plasmids from IDT are given in Table 4.

SARS-CoV-2 Positive Control (RNA)
An RNA positive control is needed to monitor the reverse transcriptase step of the assay. Positive control (RNA) from Aldatu Biosciences (Table 4) consists of a mixture of non-recombinant virus containing the entire SARS-CoV-2 RNA genome and human genomic DNA. This control is diluted using phosphate buffered saline (pH 7.4) to a concentration of 5x LoD and must be used on every assay plate starting at master mix addition.

Endogenous Internal Control (Process Control)
An internal control targeting RNase P is needed to verify that nucleic acid is present in every sample and is used for every sample that processed with the assay. Detection of the RNase P gene in the patient test samples verifies proper assay setup, sample integrity, and collection of human biological material. The Nexus Assay utilizes the same primer and probe pair to detect RNase P RNA as the CDC 2019-nCoV EUA assay.

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.

a) Interpretation of Controls – Positive, Negative and Endogenous
All test controls must be examined prior to interpretation of patient results. If the N1 and RdRp Ct values for the 2 SARS-CoV-2 positive controls (DNA and RNA) are >35 or are <35 for the 2 negative template controls (NTC-1 and NTC-2), the run is considered invalid. If the controls are not valid, the patient results cannot be interpreted. The whole plate should be invalidated and all samples on the plate should be re-run.

b) Interpretation of Patient Samples

- The patient results are interpreted according to the criteria shown in Table 5. All positive and negative results must be confirmed by manually inspecting the amplification curve to ensure the automated call of Ct is not erroneous.
    - If the Ct value for either N1 or RdRp is equal to or below 30, the sample is positive.
    - If both N1 and RdRp are equal or below a Ct of 38, the sample is positive
    - If both N1 and RdRp are undetermined or above a Ct of 38 and RNase P is equal or below a Ct of 34, the sample is negative.
    - If both, N1 and RdRp are negative, and RNase P is negative or has a Ct above 34 the sample is inconclusive.

Table 5: Interpretation of Nexus High Throughput SARS-CoV-2 Assay results (expected cycle thresholds, Ct): Initial Run

N1 Target	RdRp Target	RNase P	Result Interpretation	Report	Actions
≤30	N/A	N/A	2019-nCoV detected	Positive 2019-nCoV	Report results to public health authorities and sender.
N/A	≤30	N/A			
≤38	≤38	N/A			
>38	>38	≤34	2019-nCoV not detected	Not Detected	Report results to public health authorities and sender.
>38	>38	>34	Inconclusive Result A	Inconclusive A	Perform rerun and interpret per **Inconclusive A Interpretation** (see Rerun A Table 6 below)
≤38	>38	N/A	Inconclusive Result B	Inconclusive B	Perform rerun and interpret per **Inconclusive B Interpretation** (see Rerun B Table 7 below)
>38	≤38	N/A			

N/A – Any Ct value

Samples that are inconclusive should be rerun and interpreted as shown in Tables 6 and 7 below.

- Rerun A is likely the result of either of poor sample collection (i.e., patient error) or sample processing error (e.g., clogged pipette tip).
- Rerun B is either a false positive or a low viral titer positive; these samples will have inconsistent results between the N1 and RdRp, i.e., one Ct value will be above the 38 Ct cutoff and the other will be below the 38 Ct cutoff.

Table 6: Inconclusive - Rerun A Interpretation (expected cycle thresholds, Ct)

N1 Target	RdRp Target	RNase P	Result Interpretation	Report	Actions
≤30	N/A	N/A	2019-nCoV detected	Positive 2019-nCoV	Report results to public health authorities and sender.
N/A	≤30	N/A			
≤38	≤38	N/A			
>38	>38	≤34	2019-nCoV not detected	Not Detected	Report results to sender.
>38	>38	>34	Inconclusive Result	Inconclusive	Report results to sender. Consider collecting a new specimen from patient.
≤38	>38	N/A			

Table 6: Inconclusive - Rerun A Interpretation (expected cycle thresholds, Ct)					
N1 Target	**RdRp Target**	**RNase P**	**Result Interpretation**	**Report**	**Actions**
>38	≤38	N/A			

N/A – Any Ct value

Table 7: Inconclusive – Rerun B Interpretation (expected cycle thresholds, Ct)					
N1 Target	**RdRp Target**	**RNase P**	**Result Interpretation**	**Report**	**Actions**
≤30	N/A	N/A	2019-nCoV detected	Positive 2019-nCoV	Report results to public health authorities and sender.
N/A	≤30	N/A			
≤38	≤38	N/A			
≤38	>38	N/A			
>38	≤38	N/A			
>38	>38	≤34	2019-nCoV not detected	Not Detected	Report results to sender.
>38	>38	>34	Inconclusive Result	Inconclusive	Report results to sender. Consider collecting a new specimen from patient.

N/A – Any Ct value

PERFORMANCE EVALUATION

1) Summary of Previous Performance Data

The Nexus High Throughput SARS-CoV-2 Assay and Rhinostics Nasal Swab Collection Kit for the Nexus Assay use the same reagents, consumables, specimen collection instructions and testing workflow as those authorized for the Quaeris SARS-CoV-2 Assay and COVID-19 Self-Swab Collection Kit for HUCL under EUA210157. Harvard University Clinical Laboratory (HUCL) has granted Nexus Labs, LLC a Right of Reference to the validation data supporting use of the Quaeris SARS-CoV-2 Assay and COVID-19 Self-Swab Collection Kit for HUCL. Details of the following studies, that are summarized here, are documented under EUA210157 for the Quaeris SARS-CoV-2 Assay:

a) Limit of Detection (Analytical Sensitivity)
The LoD was determined to be 2.5 copies/µL of inactivated SARS-CoV-2 in anterior nasal swab specimens.

b) Inclusivity

An *in silico* analysis of inclusivity was originally performed in February 2021. The primers and probes were determined to be able to detect circulating strains of SARS-CoV-2. Please also see the additional analysis of inclusivity below.

c) Cross-reactivity

In silico analysis and laboratory testing demonstrated no evidence of potential for cross-reaction with non-SARS-CoV-2 nucleic acids.

d) Interfering Substances Testing

Various endogenous and exogenous substances were tested to evaluate the potential to affect assay performance. The potential for interference was noted with high levels of blood (50% v/v), tobramycin (10% and 50% v/v) and oseltamivir phosphate (10% and 50% v/v). One of the accessioning criteria for specimens collected with the Rhinostics Nasal Swab Collection Kit for the Nexus Assay is that they must be checked for visible signs of blood upon receipt at the testing facility prior to processing. Any swab that are visibly bloody are rejected. A limitation has been included in the Nexus Assay labeling noting that false negative or inconclusive results may occur due to high concentrations of interfering Tobramycin and oseltamivir phosphate (Tamiflu).

e) Clinical Evaluation

Positive and negative agreement in comparison to another FDA-authorized test were 95.7% (85.8-96.8%) and 100% (90.6-100%), respectively.

f) Specimen Stability

Anterior nasal swabs collected with the COVID-19 Self-Swab Collection Kit for HUCL were shown to be stable during transport and storage for up to 56 hours at ambient temperature.

g) Usability of the COVID-19 Self-Swab Collection Kit for HUCL

The workflow and Instructions for Use for the COVID-19 Self-Swab Collection Kit for HUCL were shown to be acceptable for unsupervised self-collection by individuals ≥18 years of age.

h) Collection Kit Stability

The COVID-19 Self-Swab Collection Kit for HUCL was shown to be stable for up to 6 months under the recommended storage conditions.

2) <u>New Performance Data</u>

Inclusivity

Because the original *in silico* analysis of inclusivity to support authorization of the Quaeris SARS-CoV-2 Assay was performed in February 2021, an additional contemporary analysis was conducted for the Nexus High Throughput SARS-CoV-2 Assay using ~503,000 complete SARS-CoV-2 genomic sequences from the USA that were submitted to NCBI between 2/11/2021 and 2/3/2022. The results are presented below:

a) N1 Gene Target

The N1 primers used in the Nexus Assay are the CDC primers and have been analyzed extensively for inclusivity by other groups (2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC). Of the ~503,000 SARS-CoV-2 sequences downloaded, there were only 6569 variants with a single mismatch in the forward primer and 2112 variants with a single mismatch in the reverse primer. A C>T mismatch was seen in the Omicron variant at the third nucleotide of the N1 gene target probe. This mismatch was not seen in the Delta variant or any other preceding SARS-CoV-2 variants. Of the 502,572 sequences in the *in silico* analysis, 90,178 sequences were from the Omicron variant of which 95.98% (n = 86,535) contained the C>T mismatch. Given the location of this mismatch (mutation is a SNP at third nucleotide at the 5' terminus) and the test being a multiplex assay, it was predicted not to have a negative impact on assay sensitivity.

Data generated for sequences from 2/11/2021 to 2/3/2022

Oligonucleotide	Number of Mismatches	Count	Sequence Identity
Forward Primer	0 1	496,141 6,569	98.7%
Reverse Primer	0 1	500,460 2,112	99.6%
Probe	0 1 2	397,319 104,779 589	79.1%

b) RdRp Gene Target

Data generated for sequences from 2/11/2021 to 2/3/2022*

Oligonucleotide	Number of Mismatches	Count	Sequence Identity
Forward Primer	0 1 2	320,079 182,612 297	63.6%
Reverse Primer	0 1	502,836 337	99.9%
Probe	0 1	502,534 633	99.9%

*The RdRp reverse primer was designed with a C>A mismatch at the 5' terminus. This is the design that was fully validated and authorized in EUA210157. As such, this mismatch by design is excluded from this updated inclusivity analysis as it was in the original analysis for EUA210157.

The RdRp forward primer and probe used in the Nexus Assay are the WHO primers and have been analyzed extensively for inclusivity by other groups. In the forward primer, a G>A mismatch has arisen at the 13th nucleotide in ~36% of sequences (8th nucleotide from the 3' end of the primer). It was confirmed that this mismatch was not present in sequences prior to 2/11/2021. This mismatch appears to have arisen in the Delta variant, where it is present in ~75% of sequences. However, the G>A mismatch is not present in the Omicron variant, which is the dominant variant in the USA at the time of this submission. Given the location

of this mismatch (8th nucleotide from the 3' end of the primer), it is not predicted to impact assay sensitivity negatively.

RdRp reverse primer was a published sequence designed with a C>A mismatch at the 5' terminus. This primer design was fully validated and authorized in EUA210157. Of the ~503,000 sequences downloaded for RdRp, there were only 633 variants with a single mismatch in the probe, and 337 variants with an additional mismatch in the reverse primer.

c) Effect of N1 Probe and RdRp Forward Primer Mismatches on the Nexus Assay

The effect of N1 probe and RdRp gene forward primer mismatches was evaluated by the sponsor using RNA transcribed from a synthetic DNA construct containing a 272-nucleotide region of the N1 target gene with the N1 probe mismatch and a 282-nucleotide region of the RdRp target gene with the RdRp forward primer mismatch. Transcribed RNA was serially diluted and 16 replicates were assayed using the Nexus Assay. As a control, full genomic RNA from SARS-CoV-2, isolate USA-WA1/2020, which does not contain either mismatch was used at the same concentrations.

Mean Ct data for both the mismatch samples and controls are shown below. There was no loss in assay sensitivity with the mismatch samples despite containing mismatches to the primers/probe for both target regions.

Sample with Both N1 Gene and RdRp Gene Mismatches

Concentration	N1 Gene Target			RdRp Gene Target			Positive Results Interpretation
	n	Mean Ct	Detection	n	Mean Ct	Detection	
25,000 cp/µL	16/16	22.6	100%	16/16	23.9	100%	16/16
2,500 cp/µL	16/16	25.9	100%	16/16	27.4	100%	16/16
250 cp/µL	16/16	29.5	100%	16/16	30.9	100%	16/16
25 cp/µL	16/16	33.1	100%	16/16	34.5	100%	16/16
2.5 cp/µL	16/16	35.6	100%	10/16	37.4	63%	16/16

Control (SARS-CoV-2, Isolate USA-WA1/2020) with No N1 Gene and RdRp Gene Mismatches

Concentration	N1 Gene Target			RdRp Gene Target			Positive Results Interpretation
	n	Mean Ct	Detection	n	Mean Ct	Detection	
25,000 cp/µL	16/16	22.3	100%	16/16	24.1	100%	16/16
2,500 cp/µL	16/16	25.3	100%	16/16	27.2	100%	16/16
250 cp/µL	16/16	28.5	100%	16/16	30.4	100%	16/16
25 cp/µL	16/16	32.2	100%	16/16	34.1	100%	16/16
2.5 cp/µL	16/16	35.6	100%	14/16	37.3	88%	16/16

LIMITATIONS:

- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Results from the Nexus High Throughput SARS-CoV-2 Assay should be used as an adjunct to clinical observations and other information available to the physician. The result is only for clinical reference, and the clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and treatment responses.
- Although the detected target sequences of this kit are in conserved regions of the SARS-CoV-2 genome, rare mutations may lead to negative results.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.
- False negative or inconclusive results may occur due to high concentrations of interfering tobramycin.
- False negative or inconclusive results may occur due to high concentrations of interfering oseltamivir phosphate (Tamiflu).

WARNINGS:

- For in vitro diagnostic use.
- Rx only.
- This product has not been FDA cleared or approved, but has been authorized by FDA under an EUA for use by the authorized laboratory; use by Aldatu Diagnostics, located at 313 Pleasant Street, Watertown MA 02472, 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.
- 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 authorization is terminated or revoked sooner.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious sample.
- Do not use reagents after the expiry date
- Dispose of waste in compliance with local, state, and federal regulations.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.