February 14, 2025

Abbott Molecular Inc
Stacy Ferguson
Director Regulatory Affairs
1300 E Touhy Ave
Des Plaines, Illinois 60018

Re: K241573
Trade/Device Name: Alinity m Resp-4-Plex
Regulation Number: 21 CFR 866.3981
Regulation Name: Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test
Regulatory Class: Class II
Product Code: QOF
Dated: May 31, 2024
Received: June 3, 2024

Dear Stacy Ferguson:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-

assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Anna M. Mielech -S

Anna Mielech, PhD.
Deputy Branch Chief (Acting)
Viral Respiratory and HPV Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K241573

Device Name

Alinity m Resp-4-Plex

Indications for Use *(Describe)*

Alinity m Resp-4-Plex is a multiplexed real-time in vitro reverse transcription polymerase chain reaction (RT-PCR) assay for use with the automated Alinity m System for the qualitative detection and differentiation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), influenza A virus, influenza B virus and Respiratory Syncytial Virus (RSV) in nasopharyngeal swab specimens collected from patients with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2, influenza A, influenza B, and RSV can be similar.

The Alinity m Resp-4-Plex assay is intended for use in the differential detection of SARS-CoV-2, influenza A, influenza B and/or RSV RNA and aids in the diagnosis of COVID-19, influenza and/or RSV infections if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A, influenza B and RSV viral RNA are generally detectable in nasopharyngeal swab specimens during the acute phase of infection. This test is not intended to detect influenza C virus infections.

Positive results are indication of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. The agent(s) detected by the Alinity m Resp-4-Plex assay may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2, influenza A, influenza B and/or RSV infections and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

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510(k) Summary

This 510(k) summary of safety and effectiveness information is being submitted in accordance with the requirement of 21 CFR Section 807.92(c).

1.0 Submitter

Applicants Name and Address:	Abbott Molecular Inc. 1300 E. Touhy Ave Des Plaines, IL 60018
Contact Person:	Stacy Ferguson Director Regulatory Affairs Abbott Molecular, Inc. 1300 E. Touhy Avenue Des Plaines, IL 60018 Phone: 224-361-7449
Date Prepared:	February 12, 2025

1.1 Device Information

Device Trade Name	Regulation Name	Product Code	Regulation Number	Class
Alinity m Resp-4-Plex	Multi-Target Respiratory Specimen Nucleic Acid Test Including Sars-Cov-2 And Other Microbial Agents	QOF	21 CFR 866.3981	II

1.2 Predicate Device

Predicate Device	510(k)	Date Cleared
Hologic™ Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay	K222736	May 16, 2023

1.3 Intended Use

Alinity m Resp-4-Plex is a multiplexed real-time *in vitro* reverse transcription polymerase chain reaction (RT-PCR) assay for use with the automated Alinity m System for the qualitative detection and differentiation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), influenza A virus, influenza B virus and Respiratory Syncytial Virus (RSV) in nasopharyngeal swab specimens collected from patients with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2, influenza A, influenza B, and RSV can be similar.

The Alinity m Resp-4-Plex assay is intended for use in the differential detection of SARS-CoV-2, influenza A, influenza B and/or RSV RNA and aids in the diagnosis of COVID-19, influenza and/or RSV infections if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A, influenza B and RSV viral RNA are generally detectable in nasopharyngeal swab specimens during the acute phase of infection. This test is not intended to detect influenza C virus infections.

Positive results are indication of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. The agent(s) detected by the Alinity m Resp-4-Plex assay may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2, influenza A, influenza B and/or RSV infections and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

2.0 Device Description

The Alinity m Resp-4-Plex assay requires two separate assay-specific kits as follows:

- **Alinity m Resp-4-Plex AMP Kit; 09N79-095**, consists of 2 types of multi-well assay trays. The amplification trays (AMP TRAY 1) contain unit-dose liquid PCR amplification/detection reagents and unit-dose liquid Internal Control (IC) in separate wells. The activation trays (ACT TRAY 2) contain liquid unit-dose activation reagent. The intended storage condition for the Alinity m Resp-4-Plex AMP Kit is −25°C to −15°C.
- **Alinity m Resp-4-Plex CTRL Kit**; **09N79-085**, consists of negative controls and positive controls, each supplied as liquid in single-use tubes. The Alinity m Resp-4-Plex controls are used for validity determination of the Alinity m Resp-4-Plex on the automated Alinity m System. The intended storage condition for the Alinity m Resp-4-Plex CTRL Kit is −25°C to −15°C.

The Alinity m Resp-4-Plex assay utilizes real-time PCR to amplify and detect genomic RNA sequences of influenza A (flu A), influenza B (flu B), RSV, and/or SARS-CoV-2 from nasopharyngeal (NP) swab specimens. The Alinity m Resp-4-Plex detects assay analytes and internal control (IC) separately, using analyte specific probes. The assay targets 2 different genes within the SARS-CoV-2 genome. The fluorescently labeled probes do not generate a detectable signal unless they are specifically bound to the amplified product. All probes are labeled with analyte specific fluorophores, thus allowing for simultaneous detection and differentiation of amplified products of all 4 viruses and IC in a single reaction vessel.

The steps of the Alinity m Resp-4-Plex assay consist of sample preparation, PCR assembly, amplification/ detection, and result calculation and reporting. All steps of the Alinity m Resp-4-Plex assay procedure are executed automatically by the Alinity m System. The Alinity m System is designed to be a continuous random-access analyzer that can perform the Alinity m Resp-4-Plex assay in parallel with other Alinity m assays on the same instrument. One PCR reaction can detect 1 or more pathogens with the Alinity m Resp-4-Plex assay. Therefore, only 1 patient specimen aliquot is required for detection of the selected assay(s).

The Alinity m System performs automated sample preparation using the Alinity m Sample Prep Kit 2, Alinity m Lysis Solution, and Alinity m Diluent Solution. The Alinity m System employs magnetic microparticle technology to facilitate nucleic acid capture, wash, and

elution. The IC is introduced into each specimen at the beginning of the sample preparation process to demonstrate that the process was completed correctly for each specimen and control sample.

The resulting purified RNA is combined with liquid unit-dose Alinity m Resp-4-Plex activation reagent and liquid unit-dose Alinity m Resp-4-Plex amplification/detection reagent and transferred into a reaction vessel. Alinity m Vapor Barrier Solution is then added to the reaction vessel which is then transferred to an amplification/detection unit for reverse transcription, PCR amplification, and real-time fluorescence detection.

A positive control and a negative control are tested at or above an established minimum frequency to help ensure that instrument and reagent performance remain satisfactory. During each control event, a negative control and positive control are processed through sample preparation and PCR procedures that are identical to those used for specimens.

The target sequences for the Alinity m Resp-4-Plex assay are:

- RdRp and N genes of the SARS-CoV-2 genome
- the Matrix gene of the flu A genome
- the Nonstructural 1 gene of the flu B genome
- the Matrix gene of the RSV genome

Patient results are automatically reported on the Alinity m instrument. The Alinity m Resp-4-Plex application parameters will be contained in an assay application specification file.

The Alinity m Resp-4-Plex assay also utilizes the following:

- Alinity m Resp-4-Plex Assay Application Specification File, List No. 09N79-03A
  The Alinity m Resp-4-Plex application specification file is intended for use with the Alinity m Resp-4-Plex assay on the automated Alinity m System to allow for processing of assay controls and patient samples.
- Alinity m System and System Software, List No. 08N53-002[a]
- Alinity m Sample Prep Kit 2, List No. 09N12-001[a]
- Alinity m Tubes and Caps, List No. 09N49[a]
  - Alinity m Transport Tubes Pierceable Capped, List No. 09N49-010
  - Alinity m Transport Tube, List No. 09N49-011
  - Alinity m Aliquot Tube, List No. 09N49-013
- Alinity m System Solutions, List No. 09N20
  - Alinity m Lysis Solution, List No. 09N20-001[a]
  - Alinity m Diluent Solution, List No. 09N20-003[a]
  - Alinity m Vapor Barrier Solution, List No. 09N20-004a

[a] Items previously approved by FDA under PMA P190025.

3.0 Similarities and Differences to the Predicate Device

The legally marketed predicate device chosen for the current submission is the Hologic™ Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay (for use on the Panther Fusion system). The Alinity m Resp-4-Plex assay is substantially equivalent to the legally marketed predicate device, Hologic™ Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay (for use on the Panther Fusion system), intended for the qualitative detection and differentiation of SARS-CoV-2, flu A virus, flu B virus, and RSV. The primary similarities between Alinity m Resp-4-Plex assay and the predicate device are presented in **Table 1**. The primary differences between Alinity m Resp-4-Plex and the predicate device are shown in **Table 2**. Both the Alinity m Resp-4-Plex assay and the predicate have the same intended use. Any technological differences that exist between Alinity m Resp-4-Plex assay and the predicate device do not raise new types of safety or effectiveness questions.

Table 1. *Similarities* Between Alinity m Resp-4-Plex Assay and Nucleic Acid Amplification Tests Predicate Device		
Feature	**Current Submission**	**Predicate Device**
Device Trade Name	**Alinity m Resp-4-Plex**	**Hologic™ Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay (K222736)**
Intended Use	Alinity m Resp-4-Plex is a multiplexed real-time *in vitro* reverse transcription polymerase chain reaction (RT-PCR) assay for use with the automated Alinity m System for the qualitative detection and differentiation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) influenza A virus, influenza B virus and Respiratory Syncytial Virus (RSV), in nasopharyngeal swab specimens collected from patients with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2, influenza A, influenza B, and RSV can be similar. The Alinity m Resp-4-Plex assay is intended for use in the differential detection of SARS-CoV-2, influenza A, influenza B and/or RSV RNA and aids in the diagnosis of COVID-19, influenza and/or RSV infections if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A, influenza B and RSV viral RNA are generally detectable in nasopharyngeal swab specimens during the acute phase of infection. This test is not intended to detect influenza C virus infections. Positive results are indication of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. The agent(s) detected by the Alinity m Resp-4-Plex assay may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A, influenza B, and/or RSV infections and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.	The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay is a fully automated multiplexed real-time polymerase chain reaction (RT-PCR) in vitro diagnostic test intended for the qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus (Flu A), influenza B virus (Flu B), and respiratory syncytial virus (RSV). Nucleic acids are isolated and purified from nasopharyngeal (NP) specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar. This assay is intended to aid in the differential diagnosis of SARS-CoV-2, Flu A, Flu B, and RSV infections in humans and is not intended to detect influenza C virus infections. Nucleic acids from the viral organisms identified by this test are generally detectable in NP specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory tract infection are indicative of the presence of the identified virus and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms. The organism(s) detected by the Panther Fusion SARS-CoV-2/ Flu A/B/RSV assay may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, or RSV infections. This assay is designed for use on the Panther Fusion system.

Table 1. *Similarities* Between Alinity m Resp-4-Plex Assay and Nucleic Acid Amplification Tests Predicate Device		
Feature	**Current Submission**	**Predicate Device**
Device Trade Name	**Alinity m Resp-4-Plex**	**Hologic™ Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay (K222736)**
Conditions for Use	For prescription use For *in vitro* diagnostic use only	Same
Organisms Detected	• influenza A (flu A) • influenza B (flu B) • Respiratory Syncytial Virus (RSV) • SARS-CoV-2	Same
Platform	All steps of this qualitative assay procedure are executed automatically by the Alinity m System. Uses Alinity m System for all steps including nucleic acid extraction, amplification, detection, and result processing. No intermediate processing or transfer steps are performed by the user.	All steps of this qualitative assay procedure are executed automatically by the Panther Fusion System. Uses Panther Fusion System for all steps including nucleic acid extraction, amplification, detection, and result processing. No intermediate processing or transfer steps are performed by the user.
Specimen Types	Nasopharyngeal (NP) swab specimens	Same
Assay Controls	• Negative Control • Positive Control • Internal Control (IC)	Same

Table 2. *Differences* Between Alinity m Resp-4-Plex Assay and Nucleic Acid Amplification Tests Predicate Device		
Feature	**Current Submission**	**Predicate Device**
Device Trade Name	**Alinity m Resp-4-Plex**	**Hologic™ Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay (K222736)**
Specimen Collection and Transport	• BD™ UVT • Copan UTM®	• Same • Same • *Remel MicroTest M4RT, M5, or M6 formulations* • *Hardy Diagnostics Viral Transport Medium* • *Hologic RespDirect Collection Kit*

4.0 Performance Data

The following performance data were provided in support of the substantial equivalence determination.

4.1 Specific Performance Characteristics – Analytical Studies

4.1.1 Limit of Detection (Analytical Sensitivity)

Limit of Detection (LoD) studies determine the lowest concentration of Influenza A (flu A), Influenza B (flu B), RSV, and SARS-CoV-2 at which greater than or equal to 95% of replicates test positive.

The LoD was determined by testing 10 cultured viruses, including 5 flu A strains (H1N1 and H3N2 subtypes), 2 flu B strains (Victoria and Yamagata lineages), 2 RSV strains (RSV A and RSV B), and 1 SARS-CoV-2 strain diluted in pooled negative NP clinical specimens collected in viral transport media.

For each virus, the preliminary LoD was determined by testing a minimum of 3 levels, each in a minimum of 3 replicates. The final LoD was confirmed by testing a minimum of 3 panel members with target concentrations bracketing the preliminary LoD, each panel member in a minimum of 20 replicates. For each strain, LoD was defined as the lowest concentration at which greater than or equal to 95% of all replicates tested positive (positive rate greater or equal to 95%), as summarized in **Table 3**.

Table 3. Limit of Detection

Virus	Strain (Source)	LoD	Mean CN at LoD[a]
Influenza A	A/H1N1/Brisbane/59/2007 (Cat No 0810244CF, Lot 323919)	0.002 $TCID_{50}$/mL	36.29
	A/H1N1/ Michigan/45/2015 (Cat No 0810538CF, Lot 326537)	0.004 $TCID_{50}$/mL	35.75
	A/H3N2/Switzerland/9715293/13 (Cat No 0810511CF, Lot 322440)	0.015 $TCID_{50}$/mL	35.71
	A/H3N2/Wisconsin/04/2018 (Cat No FR-1692, Lot 70030518)	0.06 $TCID_{50}$/mL	35.15
	A/H3N2/ Darwin/9/21 (Cat No 0810650CF, Lot 331100)	0.004 $TCID_{50}$/mL	36.02
Influenza B	B/Victoria lineage/Brisbane/33/08 (Cat No 0810253CF, Lot 316752)	0.02 $TCID_{50}$/mL	36.23
	B/Yamagata lineage/Massachusetts/2/12 (Cat No 0810239CF, Lot 324519)	0.05 $TCID_{50}$/mL	36.13
RSV	RSVA/Long/MD/56 (Cat No VR-26PQ, Lot 70024412)	0.3 $TCID_{50}$/mL	34.92
	RSVB/West Virginia/14617/85 (Cat No VR-1400, Lot 70013461)	0.1 $TCID_{50}$/mL	35.62
SARS-CoV-2	Isolate USA-WA1/2020, gamma irradiated (Cat No NR-52287, Lot 70048202)	30 Genome Copies/mL	35.93

[a] Mean cycle number (CN) was calculated for all valid replicates with a positive result in the confirmation of LoD testing.

$TCID_{50}$/mL = Median Tissue Culture Infectious Dose/mL.

4.1.2 Inclusivity

In addition to strains tested in LoD study, the inclusivity of Alinity m Resp-4-Plex assay for the detection of flu A, flu B, RSV, and SARS-CoV-2 was also evaluated by testing 16 strains of flu A virus (including H1N1, H3N2, and H5N15), 9 strains of flu B virus (including Victoria and Yamagata lineages), 6 strains of RSV (including RSV A and B), and 9 strains of SARS-CoV-2 (including A, B, BA.1 and B.1.1.7 lineages) in pooled negative clinical NP swab matrix. Each individual virus isolate or strain (cultured virus or viral RNA) was tested in replicates of 5 at the level equal to or lower than 3 × LoD (**Table 4**). For strains with reported units of measure different than units listed in LoD study, dilution series were prepared and tested. The lowest concentration tested that resulted in 100% positive results is included in **Table 4**.

Table 4. Inclusivity

Virus	Strain/Isolate	Catalog Number[a]	Lot Number	Concentration Tested[b]
Influenza A	A/H1N1/Brisbane/ 02/2018	0810585CFHI	325433	0.006 $TCID_{50}$/mL
	A/H1N1/Fort Monmouth/1/1947	VR-1754	59523491	1.67E+00 $CEID_{50}$/mL
	A/H1N1/California/ 07/2009	VR-1894	70014833	3.33E+00 $CEID_{50}$/mL
	A/H1N1/Puerto Rico/8/1934	NR-348	70013122	3.33E+00 $CEID_{50}$/mL
	A/H1N1/New Jersey/8/1976	VR-897	58810588	1.00E+01 $CEID_{50}$/mL
	A/H1N1/Wisconsin/ 588/2019 – Candidate Vaccine Virus	N/A	3026054333	1.37E-06 HA
	A/H1N1/Guangdong-Maonan/1536/2019 – Candidate Vaccine Virus	N/A	3001293900	8.53E-06 HA
	A/H1N1/Victoria/2570/2019 – Candidate Vaccine Virus	N/A	3000827519	5.12E-05 HA
	A/H3N2/Tasmania/503/2020 – Candidate Vaccine Virus	N/A	3000824430	2.37E-07 GP
	A/H3N2/Hong Kong/45/2019 – Candidate Vaccine Virus	N/A	3001584404	2.56E-06 GP

Table 4. Inclusivity

Virus	Strain/Isolate	Catalog Number[a]	Lot Number	Concentration Tested[b]
	A/H3N2/Hong Kong/2671/2019 – Candidate Vaccine Virus	N/A	3001292400	4.27E-05 GP
	A/H3N2/Singapore/ INFIMH-16-0019/ 2016	0810574CF	327298	0.045 $TCID_{50}$/mL
	A/H3N2/Brisbane/10/2007	0810138CF	324694	0.045 $TCID_{50}$/mL
	A/H3N2/Perth/16/2009	0810251CF	313219	0.045 $TCID_{50}$/mL
	A/H3N2/Delaware/01/2021	FR-1748	70048306	3.64E-01 FFU/mL
	A/H5N1/Vietnam/1203/2004	NR-12145	VNV1F014A	9.00E-05 µg/mL
Influenza B	B/Victoria lineage/Brisbane/60/2008	0810254CF	325527	0.06 $TCID_{50}$/mL
	B/Victoria lineage/Colorado/6/2017	0810573CF	325651	0.02 $TCID_{50}$/mL
	B/Victoria lineage/Alabama/2/2017	0810572CF	325540	0.02 $TCID_{50}$/mL
	B/Victoria lineage/Malaysia/2506/2004	0810258CF	327609	0.006 $TCID_{50}$/mL
	B/Victoria lineage/Washington/02/2019 – Candidate Vaccine Virus	N/A	3026019537	1.42E-06 HA
	B/Yamagata lineage/Phuket/3073/2013 – Candidate Vaccine Virus	N/A	2014768619	2.96E-07 HA
	B/Yamagata lineage/Great Lakes/1739/1954	VR-103	64295716	1.67E+00 $CEID_{50}$/mL
	Lee/1940	0810257CF	313259	0.06 $TCID_{50}$/mL
	Allen/1945	VR-102	70021278	2.78E-02 $CEID_{50}$/mL

Table 4. Inclusivity

Virus	Strain/Isolate	Catalog Number[a]	Lot Number	Concentration Tested[b]
RSV	RSVB/3/2015 Isolate 1	0810479CF	322741	0.1 $TCID_{50}$/mL
	RSVB/12/2014 Isolate 1	0810450CF	318798	0.03 $TCID_{50}$/mL
	RSVB/11/2014 Isolate 2	0810451CF	318796	0.03 $TCID_{50}$/mL
	RSVA/12/2014 Isolate 12	0810462CF	318841	0.9 $TCID_{50}$/mL
	RSVA/1/2015 Isolate 1	0810466CF	318843	0.09 $TCID_{50}$/mL
	RSVA/2014 Isolate 341	0810290CF	315911	0.09 $TCID_{50}$/mL
SARS-CoV-2	USA/GA-EHC-2811C/2021 (Lineage BA.1; Omicron Variant)	NR-56496	70050694	90 GC/mL
	Hong Kong/VM20001061/2020 (Lineage A)[c]	NR-52388	70034679	90 GE/mL
	USA-AZ1/2020 (Lineage A)[c]	NR-52505	70035256	90 GE/mL
	USA-IL1/2020 (Lineage A)[c]	NR-52503	70035254	90 GE/mL
	USA-CA3/2020 (Lineage B)[c]	NR-52507	70035258	90 GE/mL
	USA-WI1/2020 (Lineage B)[c]	NR-52506	70035257	90 GE/mL
	Germany/BavPat1/2020 (Lineage B.1)[c]	NR-52502	70036181	90 GE/mL
	USA/CA_CDC_5574/2020 (Lineage B.1.1.7; Alpha Variant)[c]	NR-55244	70042560	90 GE/mL
	Italy-INMI1[c]	NR-52498	70035261	90 GE/mL

[a] N/A indicates Catalog Number is not applicable.

[b] $TCID_{50}$/mL = Median Tissue Culture Infectious Dose/mL; $CEID_{50}$/mL = median chicken embryo infectious dose/mL; HA = Hemagglutination titer, indicates Hemagglutinin levels; FFU = focus-forming units/mL; GC/mL = Genome Copies/mL; GE/mL = Genome Equivalent/mL; GP = Glycoprotein titer, indicates Glycoprotein levels.

[c] Genomic RNA

In addition, *in silico* analyses were performed in which the sequence of each of the SARS-CoV-2 analyte primers and probes was analyzed for homology with sequences from human isolates available in GISAID and NCBI databases.

For SARS-CoV-2, *in silico* analysis of the primer/probe set for homology with all 14,818,776 virus sequences available in the GISAID database as of October 3, 2023 and all 7,590,332 virus

sequences available in the NCBI database as of October 9, 2023 showed that greater than or equal to 99.99% of the sequences are predicted to be detected by the Alinity m Resp-4-Plex assay.

4.1.3 Precision

Alinity m Resp-4-Plex assay within-laboratory precision was evaluated by testing 5 panel members: 1 flu A/flu B/RSV/SARS-CoV-2 negative panel member, 2 positive panel members containing flu A and RSV (low positive targeted at 2 × LoD and moderate positive targeted at 5 × LoD for both viruses), and 2 positive panel members containing flu B and SARS-CoV-2 (low positive targeted at 2 × LoD and moderate positive targeted at 5 × LoD for both viruses). All panel members were prepared in simulated nasal matrix (SNM). Each panel member was tested with 4 replicates twice each day for 5 days, on 3 Alinity m Systems operated by 3 operators using 3 reagent lots (1 reagent lot and 1 operator per system). The results for each analyte at each target level are summarized in **Table 5**.

Table 5. Precision

						Within-Run Component		Between-Run Component		Between-Day Component		Within-Laboratory[c]		Between-Instrument Component[d]		Total[e]	
Analyte	**Target Level**	**N[a]**	**n[b]**	**Agreement (n/N)**	**Mean CN**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**
flu A	Moderate Positive	120	120	100.0%	31.70	0.501	1.6	0.109	0.3	0.115	0.4	0.526	1.7	0.223	0.7	0.571	1.8
	Low Positive	117	117	100.0%	33.04	0.512	1.5	0.000	0.0	0.000	0.0	0.512	1.5	0.231	0.7	0.562	1.7
	Negative[f]	360	360	100.0%	-	-	-	-	-	-	-	-	-	-	-	-	-
flu B	Moderate Positive	120	120	100.0%	28.93	0.179	0.6	0.058	0.2	0.000	0.0	0.188	0.6	0.254	0.9	0.316	1.1
	Low Positive	120	120	100.0%	30.22	0.163	0.5	0.049	0.2	0.058	0.2	0.180	0.6	0.256	0.8	0.313	1.0
	Negative[f]	357	357	100.0%	-	-	-	-	-	-	-	-	-	-	-	-	-
RSV	Moderate Positive	120	120	100.0%	29.91	0.554	1.9	0.205	0.7	0.086	0.3	0.597	2.0	0.136	0.5	0.612	2.0
	Low Positive	117	117	100.0%	31.08	0.622	2.0	0.000	0.0	0.253	0.8	0.671	2.2	0.196	0.6	0.699	2.2
	Negative[f]	360	360	100.0%	-	-	-	-	-	-	-	-	-	-	-	-	-
SARS-CoV-2	Moderate Positive	120	120	100.0%	32.80	0.291	0.9	0.000	0.0	0.105	0.3	0.309	0.9	0.216	0.7	0.377	1.1
	Low Positive	120	120	100.0%	34.23	0.392	1.1	0.000	0.0	0.114	0.3	0.409	1.2	0.275	0.8	0.493	1.4
	Negative[f]	357	357	100.0%	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] N = Total number valid replicates.

[b] n = Number of replicates with positive result interpretation for positive panel members and number of replicates with negative result interpretation for negative panel member; the number of replicates used in the Mean Cycle Number (CN) and SD calculations for the positive panel members.

[c] Within-laboratory includes Within-Run, Between-Run, and Between-Day Components.

[d] Alinity m System, Alinity m Resp-4-Plex AMP Kit lot, and Operator are confounded, and the confounding effect is represented by Instrument.

[e] Total includes Within-Run, Between-Run, Between-Day, and Between-Instrument Components.

[f] The negative results included 3 panel members negative for the respective analyte.

4.1.4 Reproducibility

Alinity m Resp-4-Plex assay reproducibility was evaluated at 3 external clinical testing sites. A total of 5 panel members were tested: 1 flu A/flu B/RSV/SARS-CoV-2 negative panel member, 2 positive panel members containing flu A and RSV (low positive targeted at 2 × LoD and moderate positive targeted at 5 × LoD for both viruses), and 2 positive panel members containing flu B and SARS-CoV-2 (low positive targeted at 2 × LoD and moderate positive targeted at 5 × LoD for both viruses). All panel members were prepared in SNM. A total of 3 Alinity m Resp-4-Plex AMP Kit lots were used. Each of the 3 external sites tested 2 Alinity m Resp-4-Plex AMP Kit lots, on 5 non-consecutive days for each lot. Four replicates of each panel member were tested on each of 5 days. Each of the 3 external sites used different lots of Alinity m Resp-4-Plex CTRL Kits and Alinity m Sample Prep Kit 2. The reproducibility results are summarized in **Table 6.**

Table 6. Reproducibility

						Within-Run/Day Component		Between-Run/Day Component		Within-Laboratory[c]		Between-Lot Component		Between-Site/Instrument Component		Total[d]	
Analyte	**Target Level**	**N[a]**	**n[b]**	**Agreement (n/N)**	**Mean CN**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**
flu A	Moderate Positive	120	120	100.0%	30.92	0.568	1.8	0.149	0.5	0.587	1.9	0.207	0.7	0.000	0.0	0.623	2.0
	Low Positive	119	119	100.0%	32.18	0.592	1.8	0.208	0.6	0.628	2.0	0.000	0.0	0.065	0.2	0.631	2.0
	Negative[e]	360	360	100.0%	-	-	-	-	-	-	-	-	-	-	-	-	-
flu B	Moderate Positive	120	120	100.0%	28.88	0.220	0.8	0.119	0.4	0.250	0.9	0.081	0.3	0.081	0.3	0.275	1.0
	Low Positive	120	120	100.0%	30.08	0.590	2.0	0.123	0.4	0.603	2.0	0.000	0.0	0.188	0.6	0.631	2.1
	Negative[e]	359	359	100.0%	-	-	-	-	-	-	-	-	-	-	-	-	-
RSV	Moderate Positive	120	120	100.0%	29.09	0.736	2.5	0.314	1.1	0.800	2.8	0.000	0.0	0.000	0.0	0.800	2.8
	Low Positive	119	119	100.0%	30.35	0.665	2.2	0.000	0.0	0.665	2.2	0.120	0.4	0.155	0.5	0.693	2.3
	Negative[e]	360	360	100.0%	-	-	-	-	-	-	-	-	-	-	-	-	-
SARS-CoV-2	Moderate Positive	120	120	100.0%	32.56	0.325	1.0	0.091	0.3	0.337	1.0	0.056	0.2	0.029	0.1	0.343	1.1
	Low Positive	120	117	97.5%	34.00	0.776	2.3	0.278	0.8	0.824	2.4	0.000	0.0	0.175	0.5	0.843	2.5
	Negative[e]	360	359	99.7%	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] N = Total number valid replicates.

[b] Number of replicates with positive result interpretation for positive panel members and number of replicates with negative result interpretation for negative panel member; the number of replicates used in the Mean Cycle Number (CN) and SD calculations for the positive panel members.

[c] Within-laboratory includes Within-Run/Day and Between-Run/Day Components.

[d] Total includes Within-Run/Day, Between-Run/Day, Between-Lot, and Between-Site/Instrument Variance Components.

[e] The negative results included 3 panel members negative for the respective analyte.

4.1.5 Analytical Specificity: Potentially Interfering Substances

A total of 34 endogenous and exogenous substances that may be encountered in respiratory specimens were assessed as potentially interfering substances for Alinity m Resp-4-Plex assay. Each of the potentially interfering substances were evaluated in 2 different positive panel members (PM) each containing multiple analytes at 3 × LoD in pooled negative clinical NP swab matrix.

- PM 1: influenza A (H3N2), influenza B (Victoria lineage), RSV B, and SARS-CoV-2
- PM 2: influenza A (H31N1), influenza B (Yamagata lineage), and RSV A

All positive samples reported positive results for flu A, flu B, RSV, and SARS-CoV-2 in the presence of the potentially interfering substances. No interference in the performance of Alinity m Resp-4-Plex assay was observed for the tested substances at the concentrations listed in **Table 7**.

Table 7. Potentially Interfering Substances

Substance	Active Ingredient(s)	Tested Concentration
Antibacterial, Systemic	Tobramycin	4 µg/mL
Antibiotic, Nasal Ointment - Mupirocin	Mupirocin	5 mg/mL
Anti-Viral Drug - Relenza™	Zanamivir	5 mg/mL
Anti-Viral Drug - Remdesivir	Remdesivir	27.0 µM
Blood	N/A	10% (v/v)
Cold Eeze® (Throat lozenges)	Zincum gluconicum 2X	2.5% (w/v)
Cough Syrup – Wal-Tussin®	Dextromethorphan	5% (v/v)
FluMist®[a]	Live intranasal influenza virus	10% (v/v)
Human Genomic DNA	N/A	20 ng/uL
Leukocytes	Leukocytes	1.1E6 cells/mL
Liposomal NUMB520 Spray	Lidocaine and Phenylephrine	2.68 mg/mL
Mucin-Bovine	Purified mucin protein	5 mg/mL
Mucin-Porcine	Purified mucin protein	5 mg/mL
Nasal Corticosteroid - Budesonide	Budesonide	2% (v/v)

Table 7. Potentially Interfering Substances

Substance	Active Ingredient(s)	Tested Concentration
Nasal Corticosteroid - Dexamethasone	Dexamethasone	0.2 mg/mL
Nasal Corticosteroid - Flonase® Sensimist™	Fluticasone Furoate	10% (v/v)
Nasal Corticosteroid - Flunisolide	Flunisolide	2% (v/v)
Nasal Corticosteroid - Mometasone	Mometasone	2% (v/v)
Nasal Corticosteroid - QVAR®	Beclomethasone	2% (v/v)
Nasal Corticosteroid - Triamcinolone	Triamcinolone	2% (v/v)
Nasal Decongestant - Phenylephrine	Phenylephrine	2% (v/v)
Nasal Gel / Homeopathic Allergy Relief Medicine - Zicam®	Galphimia glauca, Histaminum, hydrochloricum, Luffa operculata, Sulfur	10% (v/v)
Nasal Spray - Perrigo Oxymetazoline HCl	Oxymetazoline	15% (v/v)
Nicotine Pouch	Nicotine	0.1% (w/v)
Oral rinse- Listerine® Cool Mint®	Ethanol, essential oil	10% (v/v)
Saline Nasal Mist	Sodium chloride	2% (v/v)
Saliva	N/A	10% (v/v)
Tamiflu®	Oseltamivir	3.3 mg/mL
Throat Lozenges, Oral Anesthetic and Analgesic - Cepacol®	Benzocaine, Menthol	5 mg/mL
Throat Spray - Chloraseptic®	Phenol	5% (v/v)
Tobacco Pouch	Nicotine	0.1% (w/v)
Toothpaste	Fluoride	1% (w/v)
Vaseline®	Petroleum Jelly	1% (w/v)
Vicks® VapoRub™	Camphor-synthetic, eucalyptus oil and menthol ointment	1% (w/v)

[a] FluMist® was not tested with negative panel but is predicted to produce positive results for flu A and flu B due to the presence of attenuated viruses. Refer to FluMist package insert for further information.

4.1.6 Analytical Specificity: Potential Cross-Reactants

A total of 74 potential cross-reacting microorganisms (viruses, bacteria, and fungi) that are phylogenetically related to the analytes of the assay or that are commonly found in respiratory tract and pooled human nasal wash were tested with Alinity m Resp-4-Plex assay to assess analytical specificity (cross-reactivity) and microbial interference. The microorganisms were tested at 1.00E+5 units/mL for viruses and fungi and 1.00E+6 units/mL for bacteria or the highest titer available (as indicated in **Table 8**). The unit of measure was specific to each microorganism. The microorganisms are listed in **Table 8**.

Cross-reactivity was evaluated by adding each of the potential cross reactants in pooled flu A/flu B/RSV/SARS-CoV-2 negative clinical NP swab matrix.

No cross-reactivity was observed with the potential cross-reactants at the concentrations tested.

In addition, the impact of the cross-reactants listed in **Table 8** on the detection of flu A, flu B, RSV, and SARS-CoV-2 was evaluated by testing them at 1.00E+5 units/mL for viruses and fungi and 1.00E+6 units/mL for bacteria or the highest titer available (as indicated in **Table 8**) in flu A/flu B/RSV/ SARS-CoV-2 positive samples targeted to 3 × LoD in pooled negative clinical NP swab matrix. All positive samples reported positive results for flu A, flu B, RSV, and SARS-CoV-2 in the presence of these potential cross-reactants at the concentrations tested.

Table 8. Potential Cross-Reactants

Bacteria		**Viruses**	
Bordetella pertussis	*Legionella pneumophila*	Adenovirus Type 01[g]	Human Metapneumovirus 9 Type 1A[g]
Chlamydia pneumoniae	*Moraxella catarrhalis*	Adenovirus Type 02[g]	Human Metapneumovirus 18 Type 2B[a, g]
Chlamydophila psittaci	*Mycobacterium tuberculosis*	Adenovirus Type 03[g]	Influenza A (H1N1)[b, g, h]
Corynebacterium diphtheria	*Mycoplasma pneumoniae*	Adenovirus Type 04[g]	Influenza A (H1N1) pdm09[b, g]
Coxiella burnetti	*Neisseria elongata*	Adenovirus Type 05[g]	Influenza A (H1N3)[b, g]
Cutibacterium acnes	*Neisseria meningitidis*	Adenovirus Type 07A[g]	Influenza A (H3N2)[b, g, h]
Enterococcus faecalis	*Proteus mirabilis*	Adenovirus Type 11[g]	Influenza B[c, g]
Escherichia coli	*Pseudomonas aeruginosa*	Adenovirus Type 14[a, g]	Influenza C
Haemophilus influenzae	*Staphylococcus aureus*	Adenovirus Type 31[g]	Measles[g]
Klebsiella pneumoniae	*Staphylococcus epidermis*	Adenovirus Type 34[g]	MERS-Coronavirus[g]
Lactobacillus gasseri	*Streptococcus pneumoniae*	Bocavirus[j]	Mumps[g]
Lactobacillus plantarum	*Streptococcus pyogenes*	Coxsackievirus[g]	Parainfluenza Virus Type 1[g]
Legionella longbeachae	*Streptococcus salivarius*	Cytomegalovirus[g]	Parainfluenza Virus Type 2[g]
Fungi		Echovirus[g]	Parainfluenza Virus Type 3[g]
Aspergillus fumigatus		Enterovirus Type 68[g]	Parainfluenza Virus Type 4[g]
Candida albicans[d]		Enterovirus E[g]	Parechovirus Type 3[g]
Pneumocystis jirovecii (PJP)[e]		Epstein-Barr Virus[g]	Rhinovirus 1A[g, h]
Other		Herpes Simplex Virus Type 1[g]	Rhinovirus B42[g]
Pooled Human Nasal Wash (10%)		Human Coronavirus 229E[g]	RSV A[f, g]
		Human Coronavirus HKU1[i]	RSV B[f, g, h]
		Human Coronavirus NL63[g]	SARS-Coronavirus[g]
		Human Coronavirus OC43[g]	Varicella-Zoster Virus[g]
		Human Metapneumovirus 5 Type B1[g]	

[a] Tested at 1.00E+04 units/mL.

[b] Only the cross-reactivity of the non-flu A signals were evaluated.

[c] Only the cross-reactivity of the non-flu B signals were evaluated.

[d] Tested at 1.00E+06 units/mL

[e] *Pneumocystis jirovecii* was not measured in terms of copy number. Instead, it was measured in cycle threshold (Ct). This specimen was tested neat.

[f] Only the cross-reactivity of the non-RSV signals were evaluated.

[g] Viral lysate.

[h] Viral particles.

[I] Viral RNA.

[j] Viral DNA.

4.1.7 On-Panel Cross-Reactivity

The cross-reactivity of each signal channel of the Alinity m Resp-4-Plex assay for on-panel viruses (flu A, flu B, RSV, and SARS-CoV-2) was evaluated by testing 2 different flu A strains (H1N1 and H3N2), 2 different flu B strains (Yamagata and Victoria lineage), 2 different RSV strains (RSV A and RSV B), and 1 SARS-CoV-2 strain. Individual viral strains were diluted in SNM at 1.00E+05 units/mL in the absence of other assay analytes. No cross-reactivity was observed for on-panel viruses at the concentrations tested.

4.1.8 Competitive Interference

Potential competitive interference between flu A, flu B, RSV, and SARS-CoV-2 analytes was evaluated by testing the 4 panel members each containing 3 viruses at low concentrations in the presence of the fourth virus at a high concentration:

- PM 1: flu A, flu B, and RSV at < 3 × LoD (Low) and SARS-CoV-2 at >1000 × LoD (High)
- PM 2: flu A, flu B, and SARS-CoV-2 at ≤ 3 × LoD (Low) and RSV at >1000 × LoD (High)
- PM 3: flu A, RSV, and SARS-CoV-2 at ≤ 3 × LoD (Low) and flu B at >1000 × LoD (High)
- PM 4: flu B, RSV, and SARS-CoV-2 at ≤ 3 × LoD (Low) and flu A at >1000 × LoD (High)

All valid replicates of the analytes tested at the low concentrations reported positive results. None of the analytes present at the high concentrations interfered with the detection of the other analytes.

4.1.9 Carryover

The carryover rate for Alinity m Resp-4-Plex assay was evaluated by testing negative and high positive samples containing 2.00E+09 Copies/mL SARS-CoV-2 (as the representative analyte) in alternating positions, across 3 Alinity m Systems. SARS-CoV-2 RNA was not detected in any of the 360 negative samples, resulting in an overall carryover rate of 0.0% (0/360, 95% CI: 0.0%,1.1%).

4.2 Clinical Performance

4.2.1 Performance with Clinical Specimens Prospective Clinical Study

The performance of the Alinity m Resp-4-Plex assay was evaluated in a multicenter study using prospectively collected nasopharyngeal swab specimens in UVT or UTM from individuals presenting with signs and symptoms of respiratory tract infection. A total of 4 US clinical sites tested the specimens with the Alinity m Resp-4-Plex assay. All 4 sites tested specimens for flu A, flu B, and RSV performance, and 3 of the 4 sites tested specimens for SARS-CoV-2 performance.

For the evaluation of flu A, flu B, and RSV performance, the Alinity m Resp-4-Plex results were compared to a composite comparator (CC) established using 2 to 3 FDA cleared assays for flu A, flu B, and RSV. For the evaluation of SARS-CoV-2 performance, the Alinity m Resp-4-Plex results were compared to CC established using 2 to 3 highly sensitive EUA SARS-CoV-2 molecular assays. A specimen was categorized as CC positive if a minimum of 2 comparator positive results were reported. A specimen was categorized as CC negative if a minimum of 2 comparator negative results were reported. A specimen was categorized CC indeterminate if a CC could not be determined due to missing results from the comparator assays.

The specimens for flu A, flu B, and RSV performance evaluation were collected during the 2021-2022 flu season at 7 geographically distributed locations in the US and during the 2020 flu season at 1 location in the Southern Hemisphere. Valid Alinity m Resp-4- Plex results included 2,753 each for flu A and flu B analytes (from 2,453 US specimens and 300 specimens from outside the US), and 2,745 for RSV analyte (from 2,445 US specimens and 300 specimens from outside the US). Of these, 2,504 flu A analyte results, 2,710 flu B analyte results, and 2,700 RSV analyte results of the Alinity m Resp-4-Plex assay were used in the analysis. CC could not be determined for 249 specimens for flu A, 43 specimens for flu B, and 45 specimens for RSV.

The specimens for SARS-CoV-2 performance evaluation were collected at 10 geographically distributed locations in the US over 2 time periods; ie, between December 2020 and February 2021 and in May 2023. A total of 826 valid SARS-CoV-2 analyte results of the Alinity m Resp-4-Plex assay were obtained. Of these, 698 SARS-CoV-2 analyte results were used in the analysis, while 128 specimens did not have CC.

Demographic characteristics for the prospective population that were included in the performance analysis are shown in **Table 9** for the evaluation of flu A, flu B, and RSV analytes and **Table 10** for SARS-CoV-2 analyte.

The performance of the Alinity m Resp-4-Plex assay with prospective specimens is summarized in **Table 11**. Alinity m Resp-4-Plex results were compared to CC by the analysis of positive percent agreement (PPA) and negative percent agreement (NPA). Alinity m Resp-4-Plex yielded PPA of 100.0% and NPA of 99.6% for the flu A analyte, NPA of 100.0% for the flu B analyte, PPA of 98.0% and NPA of 99.7% for the RSV analyte, and PPA of 95.3% and NPA of 96.0% for the SARS-CoV-2 analyte. PPA was not calculated for the flu B analyte because there was no CC positive specimen in the prospective study.

Table 9. Summary of Demographic Characteristics for Subjects Included in the Analysis - Prospective Population Evaluation of flu A, flu B, and RSV Analytes

Characteristic	Statistic
Age	
n	2,710
Mean	37.0
Median	35
SD	28.02
Minimum	0
Maximum	103
Age Group	**n (%)**
Birth to 5 years	600 (22.1%)
6 to 21 years	359 (13.2%)
22 to 59 years	1051 (38.8%)
≥ 60 years	700 (25.8%)
Gender	**n (%)**
Female	1438 (53.1%)
Male	1271 (46.9%)
Unknown	1 (0.0%)
Ethnicity	**n (%)**
Hispanic or Latino	1104 (40.7%)
Not Hispanic or Latino	1442 (53.2%)
Unknown	164 (6.1%)
Race	**n (%)**
White	1070 (39.5%)
Other[a]	672 (24.8%)
Black or African American	378 (13.9%)
Asian	136 (5.0%)
American Indian or Alaska Native	2 (0.1%)
Native Hawaiian or Other Pacific Islander	2 (0.1%)
Unknown	450 (16.6%)

[a] Documentation of races other than the categories listed were grouped under "Other".

Table 10. Summary of Demographic Characteristics for Subjects Included in the Analysis - Prospective Population Evaluation of SARS-CoV-2 Analyte

Characteristic	**Statistic**
Age	
n	698
Mean	41.3
Median	41
SD	16.88
Minimum	2
Maximum	87
Age Group	**n (%)**
Birth to 5 years	5 (0.7%)
6 to 21 years	80 (11.5%)
22 to 59 years	497 (71.2%)
≥ 60 years	116 (16.6%)
Gender	**n (%)**
Female	405 (58.0%)
Male	293 (42.0%)
Ethnicity	**n (%)**
Hispanic or Latino	261 (37.4%)
Not Hispanic or Latino	413 (59.2%)
Unknown	24 (3.4%)
Race	**n (%)**
White	508 (72.8%)
Black or African American	163 (23.4%)
Asian	10 (1.4%)
Native Hawaiian or Other Pacific Islander	2 (0.3%)
American Indian or Alaska Native	1 (0.1%)
Unknown	14 (2.0%)

Table 11. Alinity m Resp-4-Plex Assay Performance with Prospectively Collected Specimens

Analyte	Sample Type	N	CC+ Alinity+	CC+ Alinity−	CC− Alinity−	CC− Alinity+	PPA (%) Estimate (95% CI)	PPA (%) n/N	NPA (%) Estimate (95% CI)	NPA (%) n/N
flu A	Fresh	2191	94	0	2088	9	100.0 (96.1, 100.0)	94/94	99.6 (99.2, 99.8)	2088/2097
	Frozen	313	4	0	309	0	100.0 (51.0, 100.0)	4/4	100.0 (98.8, 100.0)	309/309
	Overall	2504	98	0	2397	9	100.0 (96.2, 100.0)	98/98	99.6 (99.3, 99.8)	2397/2406
flu B	Fresh	2384	0	0	2383	1	N/A[a]	N/A	100.0 (99.8, 100.0)	2383/2384
	Frozen	326	0	0	326	0	N/A[a]	N/A	100.0 (98.8, 100.0)	326/326
	Overall	2710	0	0	2709	1	N/A[a]	N/A	100.0 (99.8, 100.0)	2709/2710
RSV	Fresh	2374	48	1	2318	7	98.0 (89.3, 99.6)	48/49	99.7 (99.4, 99.9)	2318/2325
	Frozen	326	0	0	326	0	N/A[a]	N/A	100.0 (98.8, 100.0)	326/326
	Overall	2700	48	1	2644	7	98.0 (89.3, 99.6)	48/49	99.7 (99.5, 99.9)	2644/2651
SARS-CoV-2	Fresh	651	182	9	443	17	95.3 (91.3, 97.5)	182/191	96.3 (94.2, 97.7)	443/460
	Frozen	47	2	0	42	3	100.0 (34.2, 100.0)	2/2	93.3 (82.1, 97.7)	42/45
	Overall	698	184	9	485	20	95.3 (91.4, 97.5)	184/193	96.0 (94.0, 97.4)	485/505

[a] PPA was not calculated because there was no CC positive specimen in this study.

4.2.2 Retrospective Clinical Study

Flu B prevalence was very low during the prospective clinical study; there was no comparator positive specimen to evaluate the performance of the Alinity m Resp-4-Plex assay. To supplement the results of the prospective specimen population, retrospective specimen testing was performed using preselected archived flu B positive NP swab specimens in UVT or UTM collected during the 2017-2018 and 2019-2020 flu seasons, which were randomly mixed with known negative specimens to target 10% flu B prevalence. The specimens were tested with both Alinity m Resp-4-Plex and FDA cleared comparators for flu A, flu B, and RSV. Five hundred fifteen valid Alinity m Resp-4-Plex results were obtained for each of the flu A, flu B, and RSV analytes. Of these, 506 flu A analyte results, 504 of flu B analyte results, and 505 RSV analyte results were used in the analysis. CC could not be determined for 9 specimens for flu A, 11 specimens for flu B, and 10 specimens for RSV.

Demographic characteristics for the subjects included in the analysis from the retrospective population are shown in **Table 12**.

The performance of the Alinity m Resp-4-Plex assay with retrospective specimens is summarized in **Table 13**. Alinity m Resp-4-Plex yielded NPA of 99.4% for the flu A analyte, PPA of 100.0% and NPA of 98.5% for the flu B analyte, and NPA of 100.0% for the RSV analyte. PPA was not calculated for flu A or RSV because there was no CC positive for flu A and only 1 CC positive for RSV in the retrospective study.

Table 12. Summary of Demographic Characteristics for Subjects Included in the Final Analysis – Retrospective Population

Characteristic	Statistic
Age	
n	506
Mean	22.1
Median	9
SD	24.86
Minimum	0
Maximum	93
Age Group	**n (%)**
Birth to 5 years	219 (43.3%)
6 to 21 years	99 (19.6%)
22 to 59 years	139 (27.5%)
≥ 60 years	49 (9.7%)
Gender	**n (%)**
Female	246 (48.6%)
Male	260 (51.4%)
Ethnicity	**n (%)**
Hispanic or Latino	257 (50.8%)
Not Hispanic or Latino	78 (15.4%)
Unknown	171 (33.8%)
Race	**n (%)**
Other[a]	173 (34.2%)
White	109 (21.5%)
Black or African American	36 (7.1%)
Asian	9 (1.8%)
American Indian or Alaska Native	1 (0.2%)
Unknown	178 (35.2%)

[a] Documentation of races other than the categories listed were grouped under "Other".

Table 13. Alinity m Resp-4-Plex Assay Performance with Retrospective Archived Specimens

Analyte	N	CC+ Alinity+	CC+ Alinity−	CC− Alinity−	CC− Alinity+	Positive Percent Agreement (%)		Negative Percent Agreement (%)	
						Estimate (95% CI)	n/N	Estimate (95% CI)	n/N
flu A	506	0	0	503	3	N/A[a]	N/A	99.4 (98.3, 99.8)	503/506
flu B	504	50	0	447	7	100.0 (92.9, 100.0)	50/50	98.5 (96.9, 99.3)	447/454
RSV	505	1	0	504	0	N/A[a]	N/A	100.0 (99.2, 100.0)	504/504

[a] PPA was not calculated because there was no CC positive for flu A and only 1 CC positive for RSV in this study.

4.2.3 Expected Values

The Alinity m Resp-4-Plex assay included a total of 874 prospectively collected NPS specimens that were tested for SARS-CoV-2, of which 698 results were evaluable. Influenza A, influenza B, and RSV included 2,753 each for flu A and flu B analytes and 2,745 for RSV. Of these, 2,504 flu A analyte results, 2,710 flu B analyte results, and 2,700 RSV analyte results of the Alinity m Resp-4-Plex assay were evaluable. Positivity, as determined by the Alinity m Resp-4-Plex AMP Kit, are presented in Tables 14 – 16 for SARS-CoV-2 stratified by collection site and collection period and influenza A, influenza B, and RSV stratified by collection site.

Table 14: Alinity m Resp-4-Plex Expected Results by Specimen Collection Site for Specimens Collected from December 2020 to February 2021 for SARS-CoV-2

Collection Site	N	Expected Value
Site 1 (Florida)	171	38.6% (66/171)
Site 2 (Georgia)	61	21.3% (13/61)
Site 3 (Tennessee)	108	20.4% (22/108)
Site 4 (Florida)	25	48.0% (12/25)
Site 5 (Ohio)	64	37.5% (24/64)
Site 6 (California)	34	67.6% (23/34)
Site 7 (Ohio)	56	41.1% (23/56)
Site 8 (Texas)	77	27.3% (21/77)
Overall	596	34.2% (204/596)

Table 15: Alinity m Resp-4-Plex Expected Results by Specimen Collection Site for Specimens Collected in May 2023 for SARS-CoV-2

Collection Site	N	Expected Value
Site 1 (Texas)	65	0.0% (0/65)
Site 2 (Florida)	37	0.0% (0/37)
Overall	102	0.0% (0/102)

Table 16: Alinity m Resp-4-Plex Expected Results by Specimen Collection Site for Influenza A, Influenza B, and RSV

Collection Site	Influenza A		Influenza B		RSV	
	N	Expected Values	N	Expected Values	N	Expected Values
Site 1 (Florida)	300	0.0% (0/300)	301	0.0% (0/301)	301	0.0% (0/301)
Site 2 (IllInois)	1185	2.2 (26/1185)	1222	0.1% (1/1222)	1221	2.4% (29/1221)
Site 3 (California)	211	6.2% (13/211)	309	0.0% (0/309)	307	5.2% (16/307)
Site 4 (New York)	349	16.0% (56/349)	404	0.0% (0/404)	401	1.7% (7/401)
Site 5 (New Jersey)	97	9.3% (9/97)	97	0.0% (0/97)	93	2.2% (2/93)
Site 6 (Florida)	15	0.0% (0/15)	15	0.0% (0/15)	15	0.0% (0/15)
Site 7 (Georgia)	56	5.4% (3/56)	62	0.0% (0/62)	62	1.6% (1/62)
Site 8[a]	291	0.0% (0/291)	300	0.0% (0/300)	300	0.0% (0/300)
Overall	2504	4.3% (107/2504)	2710	0.0% (1/2710)	2700	2.0% (55/2700)

[a](OUS, Southern Hemisphere)

5.0 Conclusion Drawn from the Studies

The analytical and clinical study results demonstrate that the Alinity m Resp-4-Plex assay on the Alinity m System performs comparably to the predicate device in qualitative detection and differentiation of influenza A virus (flu A), influenza B virus (flu B), Respiratory Syncytial Virus (RSV), and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2); the results support a substantial equivalence decision for the Alinity m Resp-4-Plex assay.