Alinity m

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Resp-4-Plex AMP Kit

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NOTE: Changes Highlighted

CUSTOMER SERVICE: 1-800-553-7042

CUSTOMER SERVICE INTERNATIONAL: CALL YOUR ABBOTT REPRESENTATIVE

INTRODUCTION

Instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from these instructions.

NOTICE TO USER

If a serious incident occurs in relation to this device, the incident should be reported to the manufacturer and to the appropriate competent authority of the member state in which the user and/or the patient is established. To report to the manufacturer, see the contact information provided in the technical assistance section of these instructions.

NAME

Alinity m Resp-4-Plex AMP Kit

INTENDED USE

The Alinity m Resp-4-Plex assay is a multiplex real-time reverse transcription (RT) polymerase chain reaction (PCR) test intended for the qualitative detection and differentiation of RNA from influenza A virus (flu A), influenza B virus (flu B), Respiratory Syncytial Virus (RSV), and SARS-CoV-2 in nasopharyngeal (NP) swab specimens collected by a healthcare provider, from individuals with signs and symptoms of respiratory tract infection.

Results are for the identification and differentiation of RNA from flu A, flu B, RSV, and SARS-CoV-2. Flu A, flu B, RSV, and SARS-CoV-2 RNA are generally detectable in nasopharyngeal swab specimens during the acute phase of infection. Positive results are indicative of the presence of flu A, flu B, RSV, or 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.

The Alinity m Resp-4-Plex assay is not intended to detect influenza C virus infections.

Negative results do not preclude flu A, flu B, RSV, or 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 Alinity m Resp-4-Plex 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.

INTENDED USER

The intended users for the Alinity m Resp-4-Plex assay are laboratory and healthcare professionals.

SUMMARY AND EXPLANATION OF THE TEST

The Alinity m Resp-4-Plex assay is a multiplex real-time reverse transcription (RT) polymerase chain reaction (PCR) test for use with the automated Alinity m System for the qualitative detection and differentiation of RNA from flu A, flu B, RSV, and SARS-CoV-2 in nasopharyngeal swab specimens from individuals with signs and symptoms of respiratory tract infection.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Alinity m Resp-4-Plex assay consists of 2 reagent kits:

- Alinity m Resp-4-Plex AMP Kit
- Alinity m Resp-4-Plex CTRL Kit

Flu A, flu B, RSV, and internal control (IC) are amplified and detected by the Alinity m Resp-4-Plex assay using separate primer/probes sets (1 primer/probe set per target). SARS-CoV-2 is amplified and detected using 2 primer/probe sets, each targeting a different gene 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. The 2 SARS-CoV-2-specific probes are labeled with the same fluorophore and the flu A, flu B, RSV, and IC-specific probes are each labeled with distinct fluorophores, thus allowing for simultaneous detection and differentiation of amplified products of all 4 viruses and IC in a single reaction vessel.

An RNA sequence that is unrelated to the flu A, flu B, RSV, and SARS-CoV-2 sequences is introduced into each sample at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR and serves as an IC to demonstrate that the process has proceeded correctly for each sample.

The Alinity m Resp-4-Plex assay is to be used with the Alinity m System, which performs sample preparation, RT-PCR assembly, amplification, detection, result analysis and reporting. All testing steps of the Alinity m Resp-4-Plex assay procedure are executed automatically by the Alinity m System.

The Alinity m System is 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.

Application parameters specific to Alinity m Resp-4-Plex assay are contained in an assay-specific application specification file, which is distributed electronically and loaded onto the Alinity m System.

Sample Preparation

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 purpose of sample preparation is to extract and concentrate the target RNA molecules to make the target accessible for amplification and to remove potential inhibitors of amplification from the extract. 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.

During the sample preparation protocol, flu A, flu B, RSV, and/or SARS-CoV-2 virions are disrupted by lysis reagent, nucleic acids are captured on the magnetic microparticles, and inhibitors and unbound sample components are removed by washing steps within the Integrated Reaction Unit (IRU).

The resulting purified RNA is then 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 processed in the same manner and tested at least once every 48 hours to help confirm that instrument and reagent performance remain satisfactory.

Amplification

During the amplification reaction, the target RNA is converted to cDNA by the reverse transcriptase. The flu A, flu B, RSV, and SARS-CoV-2 and IC reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded cDNA:RNA product, a second primer anneals to the cDNA strand and is extended by the DNA polymerase to create a double-stranded DNA product.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences. Amplification of the 6 targets (flu A, flu B, RSV, SARS-CoV-2 RdRp gene, SARS-CoV-2 N gene, and IC) takes place simultaneously in the same reaction mixture.

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

- the 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

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The selected target sequences are highly conserved and also specific to the target viruses.

The IC target sequence is derived from the hydroxypyruvate reductase gene from the pumpkin plant, *Cucurbita pepo*, and is delivered in an Armored RNA® particle that has been diluted in negative human plasma. A gene from the pumpkin plant was selected for the IC so that it is not competitive with any microorganism or human sequence of interest that may be in the specimen.

Detection

Fluorescent detection of amplification products occurs as the flu A, flu B, RSV, SARS-CoV-2, and IC probes anneal to their targets (real-time fluorescence detection). The probes have a fluorescent moiety that is covalently linked to the 5′ end and have a quencher molecule at the 3′ end. In the absence of target sequences, probe fluorescence is quenched. In the presence of target sequences, hybridization to complementary sequences separates the fluorophore and the quencher and allows fluorescent emission and detection.

The Alinity m Resp-4-Plex assay detects the flu A, flu B, RSV, SARS-CoV-2 and IC target sequences through the use of target-specific fluorescent-labeled oligonucleotide probes. The probes do not generate a detectable signal unless they are specifically bound to the amplified product. The 2 SARS-CoV-2-specific probes are labeled with the same fluorophore and the flu A, flu B, RSV, and IC-specific probes are each labeled with distinct fluorophores, thus allowing for simultaneous detection and differentiation of all 4 viruses and IC amplified products in a single reaction vessel.

PREVENTION OF NUCLEIC ACID CONTAMINATION

The possibility of nucleic acid contamination on the Alinity m System is minimized because:

- Aerosol barrier pipette tips are used for all pipetting. The pipette tips are discarded after use.
- PCR amplification and detection are carried out automatically in a sealed reaction vessel.
- Disposal of the reaction vessel into the waste container is performed automatically by the Alinity m System.

For additional information on system and assay technology, refer to the Alinity m System Operations Manual, Section 3.

REAGENTS

Kit Contents

Alinity m Resp-4-Plex AMP Kit (List No. 09N79-090)

Alinity m Resp-4-Plex AMP Kit (List No. 09N79-090) is comprised of 2 types of multi-well trays: Alinity m Resp-4-Plex AMP TRAY 1 and Alinity m Resp-4-Plex ACT TRAY 2.

- Each Alinity m Resp-4-Plex AMP TRAY 1 (individually packed in a foil pouch) contains 48 unit-dose liquid amplification reagent wells and 48 unit-dose liquid IC wells. One well of each is used per test. Amplification reagent wells consist of synthetic oligonucleotides, DNA polymerase, reverse transcriptase, dNTPs and 0.15 % ProClin® 950 in a buffered solution. IC wells consist of noninfectious Armored RNA® with unrelated IC sequences in negative human plasma. Negative human plasma was tested and found to be nonreactive for HBsAg, HIV-1 antigen, Syphilis, HIV-1 RNA, HCV RNA, HBV DNA, antibody to HCV, antibody to HIV-1, and antibody to HIV-2.
  Preservative: 0.15% ProClin 950.
- Each Alinity m Resp-4-Plex ACT TRAY 2 (individually packed in a foil pouch) contains 48 unit-dose liquid activation reagent wells. One reagent well is used per test. Activation reagent wells consist of magnesium chloride and tetramethylammonium chloride.
  Preservative: 0.15% ProClin 950.

	Quantity
Σ	192 tests
Alinity m Resp-4-Plex AMP TRAY 1	4 trays / 48 tests each
Alinity m Resp-4-Plex ACT TRAY 2	4 trays / 48 tests each

WARNINGS AND PRECAUTIONS

IVD

- **For In Vitro Diagnostic Use.**
- Do not use beyond expiration date.

Safety Precautions

The following warnings and precautions apply to:
Alinity m Resp-4-Plex AMP TRAY 1.

WARNING	Contains 2-Methyl-4-isothiazolin-3-one
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapors / spray
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

CAUTION: This preparation contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive by appropriate FDA-licensed, approved, or cleared tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, HIV-1 Ag, HBsAg, and Syphilis. The material is also tested and found to be negative by appropriate FDA-licensed, approved, or cleared PCR methods for HIV-1 RNA, HCV RNA, and HBV DNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using laboratory safety procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[1] OSHA Standards on Bloodborne Pathogens,[2] CLSI Document M29-A4,[3] and other appropriate biosafety practices.[4] Therefore all human sourced materials should be considered infectious.

These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[1]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[4]

The following warnings and precautions apply to:
Alinity m Resp-4-Plex ACT TRAY 2.

DANGER	Contains Tetramethylammonium chloride, and 2-Methyl-4-isothiazolin-3-one
H302	Harmful if swallowed.
H316	Causes mild skin irritation.[a]
H317	May cause an allergic skin reaction.
H370	Causes damage to organs.
H412	Harmful to aquatic life with long lasting effects.
Prevention	
P260	Do not breathe mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P272	Contaminated work clothing should not be allowed out of the workplace.
P273	Avoid release to the environment.
P280	Wear protective gloves / protective clothing / eye protection.

Response	
P301+P312	IF SWALLOWED: Call a POISON CENTER / doctor if you feel unwell.
P302+P352	IF ON SKIN: Wash with plenty of water.
P308+P311	IF exposed or concerned: Call a POISON CENTER / doctor.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

[a] Not applicable where regulation EC 1272/2008 (CLP) or OSHA Hazard Communication 29 CFR 1910.1200 (HCS) 2012 have been implemented.

Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet.

Safety Data Sheets are available from your Abbott Representative.

For a detailed discussion of safety precautions during system operation, refer to the Alinity m System Operations Manual, Section 7 and Section 8.

Reagent Shipment

	Shipment Condition
Alinity m Resp-4-Plex AMP Kit	On dry ice

If you receive reagents that are in a condition contrary to label recommendation, or that are damaged, contact your Abbott Representative.

Reagent Storage

In order to minimize damage to foil pouches, it is recommended that the Alinity m Resp-4-Plex AMP TRAY 1 (AMP TRAY 1) and Alinity m Resp-4-Plex ACT TRAY 2 (ACT TRAY 2) are stored in the original kit packaging. Thaw reagent trays and open the foil pouch for the reagent trays just prior to loading on the Alinity m System. Onboard storage time begins when reagents are thawed and immediately loaded on the Alinity m System.

	Storage Temperature	Maximum Storage Time
Unopened	−25 to −15°C	Until expiration date
Onboard	System Temperature	96 hours (not to exceed expiration date)

Reagent Handling

- Do not use reagents that have been damaged.
- IMPORTANT: Immediately prior to use on the Alinity m System, thaw amplification reagents at 15 to 30°C or at 2 to 8°C. Onboard storage time begins immediately after thaw. See **ASSAY PROCEDURE** section for additional instructions.
- Minimize contact with the surface of reagent trays during handling.
- Up to 2 lots of assay trays (AMP TRAY 1 and ACT TRAY 2) can be loaded on each Alinity m Assay Tray Carrier, as long as the AMP TRAY 1 and ACT TRAY 2 from the same AMP kit lot are included together as a set.
- The Alinity m System will track the onboard storage time of the AMP TRAY 1 and ACT TRAY 2 while on the Alinity m System. The Alinity m System will not allow the use of AMP TRAY 1 and ACT TRAY 2 if the maximum onboard storage time has been exceeded. **IMPORTANT: The maximal allowable onboard storage for the Alinity m Resp-4-Plex AMP TRAY 1 and ACT TRAY 2 is 96 hours from thaw/onboarding.**
- For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity m System Operations Manual, Section 8.

SPECIAL PRECAUTIONS

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use.
- Positive results are indicative of the presence of flu A, flu B, RSV, and/or SARS-CoV-2 RNA.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories[1] and in the CLSI Document M29-A4.[3] Only personnel proficient in handling infectious materials and the use of the Alinity m Resp-4-Plex assay and the Alinity m System should perform this procedure.

Handling Precautions for Specimens

- The Alinity m Resp-4-Plex assay is only for use with nasopharyngeal swab specimens that have been handled and stored as described in the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section.
- Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. Refer to CLSI MM13-A[5] as an appropriate resource.
- During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of ribonucleases (RNases) into samples during and after the extraction procedure.
- To achieve SARS-CoV-2 viral inactivation prior to testing, specimens can be treated at 65°C for 30 minutes (https://www.beiresources.org/Catalog/antigen/NR-52286.aspx).
- Proper aseptic technique should always be used when working with nucleic acid amplification.
- Amplification technologies, such as PCR, are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the reagents used become contaminated by accidental introduction of even a small amount of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in the handling of contaminated waste in compliance with good laboratory practices.

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

- Deterioration of the reagents may be indicated when a control error occurs or controls are repeatedly out of the specified ranges.
- Reagents are shipped on dry ice and are stored at −25 to −15°C upon arrival. If reagents arrive in a condition contrary to this recommendation or are damaged, immediately contact your Abbott Representative.
- For troubleshooting information, refer to the Alinity m System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The Alinity m Resp-4-Plex application specification file must be installed on the Alinity m System prior to performing the assay.

For a detailed description of system operating instructions, refer to the Alinity m System Operations Manual, Section 5.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Specimen Collection and Storage

Human nasopharyngeal swab specimens can be used with the Alinity m Resp-4-Plex assay on the Alinity m System. Refer to the European Centre for Disease Prevention and Control (ECDC) at ecdc.europa.eu/en/novel-coronavirus/laboratory-support,[6] the US Centers for Disease Control and Prevention (CDC) at https://www.cdc.gov/flu/pdf/professionals/flu-specimen-collection-poster.pdf,[7] and the World Health Organization at https://www.who.int/influenza/rsv/rsv_collection_transport_storage_samples/en/.[8]

Specimen Transport

For domestic and international shipments, specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potentially infectious specimens.

Preparation for Analysis

Thaw frozen specimens at 15 to 30°C or at 2 to 8°C.

Prior to processing, vortex each specimen 3 times for 2 to 3 seconds.

If needed, centrifuge specimens at 2000 *g* for 5 minutes before loading on the Alinity m System. Specimens can be transferred into an Alinity m Transport Tube or an Alinity m Aliquot Tube before loading onto the Alinity m System.

IMPORTANT: If present, swab and cap should be removed from the specimens before loading onto the Alinity m System.

All specimen tubes must be labeled with specimen ID barcodes or must be identified with a specimen ID, rack ID, and position in the rack. Refer to the **ASSAY PROCEDURE** section of this package insert for tube sizes and requirements for minimum sample volume and use of caps. Avoid touching the inside of the cap when opening tubes.

PROCEDURE

Materials Provided

- Alinity m Resp-4-Plex AMP Kit (List No. 09N79-090)

Materials Required But Not Provided

- 08N53-002 Alinity m System with software version 1.5.2 or higher
- 09N79-080 Alinity m Resp-4-Plex CTRL Kit
- 09N12-001 Alinity m Sample Prep Kit 2
- 09N20-001 Alinity m Lysis Solution
- 09N20-003 Alinity m Diluent Solution
- 09N20-004 Alinity m Vapor Barrier Solution
- 09N79-01A (or higher) Alinity m Resp-4-Plex Application Specification File
- Vortex mixer
- Centrifuge capable of 2000 *g*
- Plate adapter for 384 well plates (eg, Eppendorf Catalog No. 022638955)
- Centrifuge with swing plate rotor capable of accommodating the plate adapter and capable of ≥ 100 *g*

For information on materials required for operation of the Alinity m System, refer to the Alinity m System Operations Manual, Section 1.

For general operating procedures, refer to the Alinity m System Operations Manual, Section 5.

For optimal performance, it is important to perform routine maintenance as described in the Alinity m System Operations Manual, Section 9.

Other Optional Materials

- 09N49-010 Alinity m Transport Tube Pierceable Capped
- 09N49-011 Alinity m Transport Tube
- 09N49-013 Alinity m Aliquot Tube
- Sealable plastic bags

Procedural Precautions

- Read the instructions in this package insert carefully before processing samples.
- Use aerosol barrier pipette tips or disposable pipettes only one time when pipetting specimens. To prevent contamination to the pipette barrel while pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Work area and instrument platforms must be considered potential sources of contamination.
- Ensure the Alinity m Resp-4-Plex AMP TRAY 1 and ACT TRAY 2 are centrifuged prior to loading on the Alinity m System per instructions in Assay Procedure section.
- Monitoring procedures for the presence of amplification target contamination can be found in the Alinity m System Operations Manual, Section 9.
- To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.
- To prevent contamination, change to new gloves before handling the Alinity m Sample Prep Kit 2, assay trays, system solutions, Integrated Reaction Unit (IRU) sleeves, and pipette tips. Also change to new gloves whenever they are contaminated by a specimen, a control, or a reagent. Always use powder-free gloves.
- The use of the Alinity m Resp-4-Plex CTRL Kit is integral to the performance of the Alinity m Resp-4-Plex assay. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details. Refer to the Alinity m Resp-4-Plex CTRL Kit package insert for preparation and usage.
- The Alinity m Resp-4-Plex control reagents are contained in single-use tubes with solid caps. Remove caps from the tube prior to use. Discard tubes after use.

ASSAY PROCEDURE

Thaw AMP TRAY 1 and ACT TRAY 2 at 15 to 30°C or at 2 to 8°C immediately prior to use.

Prior to loading on the Alinity m System, the AMP TRAY 1 and ACT TRAY 2 must be centrifuged as follows:

1. Load the trays onto the plate adapter (eg, Eppendorf Catalog No. 022638955).
2. Load the plate adapter (with the trays) on a swing plate centrifuge capable of accommodating the plate adapter. Spin at 100 to 800 *g* for 1 to 5 minutes to ensure reagents remain at the bottom of the well and to remove potential bubbles.
3. Immediately following centrifugation, carefully transfer the trays to the Alinity m Assay Tray Carriers. Take care to minimize disturbance to the trays. Load the tray carriers per the Alinity m System Operations Manual, Section 5.
4. If disturbance occurs during the transfer that could potentially introduce bubbles or displace reagents from the bottom of the wells (eg, dropping, bumping, inversion of the trays), re-centrifuge the trays.
5. Proceed with **Reagent and sample management** per the Alinity m System Operations Manual, Section 5.

For a detailed description of how to run an assay, refer to the Alinity m System Operations Manual, Section 5. Prior to testing specimens, check the control status. If control testing is required, refer to the **QUALITY CONTROL PROCEDURES** section. Controls may be tested separately or with specimens.

One PCR reaction can detect one or more pathogens with the Alinity m Resp-4-Plex assay. Therefore, only one patient specimen aliquot is required for detection of the selected assay(s).

To create a test order, select any combination of the test target names to match the requested tests for each patient specimen. Refer to the following table.

Assay Name	Test Target
FLUA_4PCE	Flu A
FLUB_4PCE	Flu B
RSV_4PCE	RSV
COV2_4PCE	SARS-CoV-2

The testing results will be reported only for those test targets selected for the specimen in the test order.

A test result for an assay, that was not originally selected for testing in the test order, may still be obtained without repeat testing, within a user-configured period of time. Refer to Alinity m System Operations Manual, section 5 Operating Procedures, Stored Result Retrieval subsection.

The Alinity m System will track the onboard storage time of AMP TRAY 1, ACT TRAY 2, controls, and specimens while on the Alinity m System. The Alinity m System will not allow the use of AMP TRAY 1, ACT TRAY 2, controls, or process specimens that have exceeded the allowable onboard storage time setting by the system.

IMPORTANT: The maximal allowable onboard storage for Alinity m Resp-4-Plex AMP TRAY 1 and ACT TRAY 2 is 96 hours from thaw/onboarding.

Specimen tubes need to meet the requirements below for sample volumes and use of caps when loaded on the Alinity m System.

Tube Type[a]	List No.	Minimum Volume Required	Maximum Volume	Cap Requirement on Instrument
Alinity m Aliquot Tube	09N49-013	0.8 mL	3.5 mL	Uncapped[b]
Alinity m Transport Tube	09N49-011	1.0 mL	3.5 mL	Uncapped[b]
Alinity m Transport Tube Pierceable Capped	09N49-010	1.0 mL	3.5 mL	Uncapped[b]
Tube with 11.5 to 14.0 mm diameter		1.3 mL	2.5 mL	Uncapped[b]
Tube with 14.5 to 16.0 mm diameter		1.4 mL	3.5 mL	Uncapped[b]

[a] Refer to the Alinity m System Operations Manual, Section 4, for sample tube specifications and requirements and Section 5 for sample rack loading instructions.

[b] Avoid touching the inside of the cap when opening the tubes.

Place the uncapped positive and negative controls, if applicable, and patient specimens into the sample rack. If used, bar codes on tube labels must face the correct orientation for scanning.

QUALITY CONTROL PROCEDURES

Detection of Inhibition

A defined, consistent quantity of IC is introduced into each specimen and control at the beginning of sample preparation and measured on the Alinity m System to demonstrate proper specimen processing and assay validity.

A Message Code is displayed for the control when the IC Cycle Number (CN) value exceeds the established range.

A Flag or Message Code is displayed for the sample when the IC Cycle Number (CN) value falls outside of the established range:

- For Positive Specimens: If the IC CN is out of range, and any analyte(s) (flu A, flu B, RSV or SARS-CoV-2) in that specimen is detected, the specimen will yield a Positive interpretation for the detected analyte(s). An IC Flag will be reported next to the detected analyte(s).
- For Negative Specimens: If the IC CN is out of range and any of the analyte(s) (flu A, flu B, RSV or SARS-CoV-2) in that specimen is not detected, no result will be reported for that analyte(s) and a Message Code will be generated.
- For Negative and Positive Controls: If the IC CN is out of range, a Message Code will be generated for all the analytes in the Controls.
- Note that for a specimen, each analyte test is treated independently. For example, a specimen may be reported Positive for flu A with an IC flag, but may get a Message Code indicating IC failure for each of the other 3 analytes if they are not detected.

Refer to the Alinity m System Operations Manual, Section 5 for an explanation of the Flags.

Refer to the Alinity m System Operations Manual, Section 10 for an explanation of the corrective actions for Message Codes.

Negative and Positive Controls

A set of Alinity m Resp-4-Plex Negative Control and Positive Control are required to be tested at least once every 48 hours, to monitor the performance of the assay and Alinity m System. Valid results for all control levels must be obtained before specimen results are reported.

Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

A Flag is displayed for specimens when a control result is invalid. If the controls for a given target analyte (flu A, flu B, RSV, or SARS-CoV-2) are invalid, all of the specimens for that target analyte processed under the same conditions following an invalid assay control must be retested. If control results are invalid, refer to the Alinity m System Operations Manual, Section 5 for a description of quality control flags, and Section 10 for troubleshooting information.

The presence of flu A, flu B, RSV, or SARS-CoV-2 must not be detected in the negative control. Flu A, flu B, RSV, or SARS-CoV-2 detected in the negative control is indicative of contamination by other samples or by amplified product. If contamination is suspected, clean the Alinity m System and repeat sample processing for controls and specimens following the Procedural Precautions in this package insert. Cleaning and monitoring procedures for the presence of amplification target contamination can be found in the Alinity m System Operations Manual, Section 9.

If negative controls are persistently reactive, contact your Abbott Representative at www.molecular.abbott/portal.

INTERPRETATION OF RESULTS

The Alinity m System will report a Result and an Interpretation for each specimen. If applicable, message codes or flags will also be displayed. A clinical interpretation can be performed by the user, based on the Result, according to the table below:

SID	Assay	Result	Interpretation	Flags	Result Codes
Resp-4-Plex POS CTRL	FLUA_4PCE				9198[a]
Resp-4-Plex POS CTRL	FLUB_4PCE	XX.XX CN			
Resp-4-Plex POS CTRL	RSV_4PCE	XX.XX CN			
Resp-4-Plex POS CTRL	COV2_4PCE	XX.XX CN			
Resp-4-Plex NEG CTRL	FLUA_4PCE	Not Detected			
Resp-4-Plex NEG CTRL	FLUB_4PCE				9193[b]
Resp-4-Plex NEG CTRL	RSV_4PCE	Not Detected			
Resp-4-Plex NEG CTRL	COV2_4PCE	Not Detected			
Sample 1	FLUA_4PCE	Not Detected	Flu A Negative	FPC[c]	
Sample 1	FLUB_4PCE	Not Detected	Flu B Negative	FNC[c]	
Sample 1	RSV_4PCE	Not Detected	RSV Negative		
Sample 1	COV2_4PCE	Not Detected	SARS-CoV-2 Negative		
Sample 2	FLUA_4PCE				9186[d]
Sample 2	FLUB_4PCE				9186[d]
Sample 2	RSV_4PCE	XX.XX CN	RSV Positive	IC[e]	
Sample 2	COV2_4PCE				9186[d]
Sample 3	FLUA_4PCE	XX.XX CN	Flu A Positive	FPC[c], IC[e]	
Sample 3	FLUB_4PCE				9186[d]
Sample 3	RSV_4PCE				9186[d]
Sample 3	COV2_4PCE				9186[d]
Resp-4-Plex POS CTRL	FLUA_4PCE	XX.XX CN			
Resp-4-Plex POS CTRL	FLUB_4PCE	XX.XX CN			
Resp-4-Plex POS CTRL	RSV_4PCE	XX.XX CN			
Resp-4-Plex POS CTRL	COV2_4PCE	XX.XX CN			
Resp-4-Plex NEG CTRL	FLUA_4PCE	Not Detected			
Resp-4-Plex NEG CTRL	FLUB_4PCE	Not Detected			
Resp-4-Plex NEG CTRL	RSV_4PCE	Not Detected			
Resp-4-Plex NEG CTRL	COV2_4PCE	Not Detected			
Sample 4	FLUA_4PCE	XX.XX CN	Flu A Positive		
Sample 4	FLUB_4PCE	Not Detected	Flu B Negative		
Sample 4	RSV_4PCE	Not Detected	RSV Negative		
Sample 4	COV2_4PCE	Not Detected	SARS-CoV-2 Negative		
Sample 5	FLUA_4PCE	XX.XX CN	Flu A Positive		
Sample 5	FLUB_4PCE	Not Detected	Flu B Negative		
Sample 5	RSV_4PCE	Not Detected	RSV Negative		
Sample 5	COV2_4PCE	XX.XX CN	SARS-CoV-2 Positive		
Sample 6	FLUA_4PCE	Not Detected	Flu A Negative		
Sample 6	FLUB_4PCE	XX.XX CN	Flu B Positive		
Sample 6	RSV_4PCE	Not Detected	RSV Negative		
Sample 6	COV2_4PCE	Not Detected	SARS-CoV-2 Negative		

[a] Error code generated due to positive control failure.

[b] Error code generated due to negative control failure.

[c] Indicates failed control. All of the specimens processed following an invalid assay control must be retested.

[d] Error code generated due to no amplification of target and internal control failure.

[e] Patient sample with positive amplification of target but failed internal control will produce valid result with a flag for internal control failure.

Flags, Results Codes, and Message Codes

Some results may contain information in the Flags and Codes fields. For a description of the flags and result codes that may appear in these fields, refer to the Alinity m System Operations Manual, Section 5. For a description of message codes refer to the Alinity m System Operations Manual, Section 10.

LIMITATIONS OF THE PROCEDURE

- This assay is for in vitro diagnostic use.
- Use of the Alinity m Resp-4-Plex assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and the Alinity m System.
- Report SARS-CoV-2 test results to healthcare providers and relevant public health authorities, as required.
- The instrument and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive controls or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this package insert.
- Optimal performance of this test requires appropriate specimen collection, storage, and transport to the test site (refer to the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section of this package insert).
- Detection of flu A, flu B, RSV, or SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (eg, presence of symptoms), and/or stage of infection.
- False-negative results may arise from degradation of the viral RNA during storage and transport of the specimens.
- As with any molecular test, mutations within the target regions of Alinity m Resp-4-Plex assay could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform comparison studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated.
- Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
- Negative results do not preclude infection with flu A, flu B, RSV, or SARS-CoV-2 and should not be the sole basis of a patient treatment/management or public health decision. Follow up testing should be performed according to the current ECDC recommendations.
- The performance of this device has not been assessed in a population vaccinated against COVID-19

SPECIFIC PERFORMANCE CHARACTERISTICS

Limit of Detection (Analytical Sensitivity)

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

The LoD was determined by testing dilutions of 7 cultured viruses, including 1 SARS-CoV-2 strain, 2 flu A strains (H1N1 and H3N2), 2 flu B strains (Victoria and Yamagata lineages), and 2 RSV strains (RSV A and RSV B), spiked in pooled negative clinical nasopharyngeal swab specimens. For each virus, the preliminary LoD was determined by testing a minimum of 3 levels, each in 3 replicates. The final LoD was confirmed by testing 3 to 4 panel members with target concentrations bracketing the preliminary LoD, each panel member in replicates of 21. LoD is defined as the lowest concentration at which greater than or equal to 95% of all replicates tested positive, as summarized in **Table 1**.

Table 1. Limit of Detection

Virus	Strain	LoD
SARS-CoV-2	Isolate USA-WA1/2020, gamma irradiated (Cat No NR-52287 Lot 70033322[a])	0.005 $TCID_{50}$/mL (30 GE/mL)[b]
Flu A	A/Brisbane/59/2007 (H1N1) (Cat No 0810244CF Lot 323919)	0.002 $TCID_{50}$/mL
	A/Switzerland/9715293/13 (H3N2) (Cat No 0810511CF Lot 322440)	0.015 $TCID_{50}$/mL
Flu B	B/Brisbane/33/08 (Victoria lineage) (Cat No 0810253CF Lot 316752)	0.020 $TCID_{50}$/mL
	B/Massachusetts/2/12 (Yamagata lineage) (Cat No 0810239CF Lot 324519)	0.050 $TCID_{50}$/mL
RSV	RSVA/Long/MD/56 (Cat No VR-26PQ Lot 70024412)	0.300 $TCID_{50}$/mL
	RSVB/WestVirginia/14617/85 (Cat No VR-1400 Lot 70013461)	0.100 $TCID_{50}$/mL

[a] Based on the information provided in the Certificate of Analysis from the vendor, 1 $TCID_{50}$/mL is equal to 6,071 genome equivalents (GE) by ddPCR.

[b] GE/mL = Genome Equivalent/mL.

Inclusivity

The inclusivity of Alinity m Resp-4-Plex for the detection of SARS-CoV-2, flu A, flu B, and RSV was evaluated by testing 6 isolates of SARS-CoV-2, 15 strains of flu A virus (including H1N1, H3N2, H5N1, and H7N2), 7 strains of flu B virus (including Victoria and Yamagata lineages), and 5 strains of RSV (including RSV A and B). Each individual virus isolate or strain (cultured virus or viral RNA) was tested in simulated nasal matrix, in a minimum of 3 replicates. The Alinity m Resp-4-Plex assay detected all replicates of all strains at the concentrations tested (see **Table 2**).

Table 2. Inclusivity

Viral Target	Strain	Catalog Number	Lot Number	Test Concentration
SARS-CoV-2	SARS-CoV-2/USA-IL1/2020	NR-52503	70035254	100 GE/mL[a]
	SARS-CoV-2/Germany/BavPat1/2020	NR-52502	70036181	100 GE/mL[a]
	SARS-CoV-2/USA-AZ1/2020	NR-52505	70035256	100 GE/mL[a]
	SARS-CoV-2/USA-CA3/2020	NR-52507	70035258	100 GE/mL[a]
	SARS-CoV-2/Italy-INMI1	NR-52498	70035261	100 GE/mL[a]
	SARS-CoV-2/Hong Kong/VM20001061/2020	NR-52388	70034679	100 GE/mL[a]
Flu A	A/NewCaledonia/20/1999 (H1N1)	0810036CF	324516	0.006 $TCID_{50}$/mL
	A/Brisbane/59/2007 (H1N1)	0810244CF	323919	0.006 $TCID_{50}$/mL
	A/HongKong/8/1968 (H3N2)	0810250CF	323534	0.045 $TCID_{50}$/mL
	A/Perth/16/2009 (H3N2)	0810251CF	313219	0.045 $TCID_{50}$/mL
	A/Wisconsin/67/2005 (H3N2)	0810252CF	324078	0.045 $TCID_{50}$/mL
	A/California/07/2009 (H1N1)	ATCC-VR-1894	70014833	10 $CEID_{50}$/mL
	A/FortMonmouth/1/1947 (H1N1)	ATCC-VR-1754	59523491	10 $CEID_{50}$/mL
	A/NewJersey/8/1976 (H1N1)	ATCC-VR-897	58810588	10 $CEID_{50}$/mL
	A/Victoria/3/1975 (H3N2)	ATCC-VR-822	61834480	10 $CEID_{50}$/mL
	A/PuertoRico/8/1934 (H1N1)	ATCC-VR-1469	70020665	0.067 PFU/mL
	A/Aichi/2/1968 (H3N2)	NR-9534	58007006	1.077 pg/µL
	A/Vietnam/1203/2004 (H5N1)	NR-12148	VNV1F009A	9.0×10^{-5} mcg/mL[c]
	A/equine/Prague/1/1956 (HA) x A/Aichi/2/1968 (NA) x A/Puerto Rico/8/1934 (H7N2), Reassortant X-33, NA Deficient Influenza A virus (H7N2)	NR-10082	58406990	12.5 pg/µL

Table 2. Inclusivity

Viral Target	Strain	Catalog Number	Lot Number	Test Concentration
Flu A	CVV:A/H1N1/ Guangdong_Maonan/ 1536/2019 (H1N1)	NA[b]	3001293900	5.12 x 10^{-4} HA[d]
	CVV:A/H3N2/ HongKong/2671/2019 (H3N2)	NA[b]	3001292400	2.56 x10^{-4} HA[d]
Flu B	B/Brisbane/60/2008 (Victoria lineage)	0810254CF	313375	0.06 $TCID_{50}$/mL
	B/Malaysia/2506/04 (Victoria lineage)	0810258CF	324158	0.06 $TCID_{50}$/mL
	B/Lee/40	0810257CF	315896	0.06 $TCID_{50}$/mL
	B/Allen/1945	ATCC-VR-102	70021278	10 $CEID_{50}$/mL
	B/GL/1739/1954 (Yamagata lineage)	ATCC-VR-103	64295716	10 $CEID_{50}$/mL
	CVV: B/Washington/ 02/2019-like virus (Victoria lineage)	NA[b]	3026019537	5.12 x 10^{-5} HA[d]
	CVV: B/Phuket/3073/ 2013-like virus (Yamagata lineage)	NA[b]	2014768619	6.4 x 10^{-5} HA[d]
RSV	RSVA/2/Australia/61	VR-1540	70021273	0.9 PFU/mL
	RSVB/Washington/ 18537	VR-1580PQ	70025292	0.3 $TCID_{50}$/mL
	RSVA/ 1/2015 Isolate 1	0810466CF	318843	0.9 $TCID_{50}$/mL
	RSVA/ 12/2014 Isolate 12	0810462CF	318841	0.9 $TCID_{50}$/mL
	RSVA/ 2014 Isolate 341	0810290CF	315911	0.9 $TCID_{50}$/mL

[a] GE/mL = Genome Equivalent/mL.

[b] CVV = Candidate Vaccine Virus, catalog number is not applicable.

[c] Inactivated H5N1 influenza Vaccine (non-adjuvanted). Unit of measurement is based on hemagglutinin antigen concentration.

[d] HA = Hemagglutination titer, indicates Hemagglutinin levels.

In addition, *in silico* analyses were performed in which the sequence of each of the Alinity m Resp-4-Plex analyte primers and probes was analyzed for homology with sequences available in GISAID and/or NCBI Virus Sequences for Discovery (GenBank) databases. A total of 104,885 full-length SARS-CoV-2 sequences available in the GISAID database as of September 28, 2020 and 17,216 full-length SARS-CoV-2 sequences available in the NCBI database as of October 4, 2020 were analyzed for SARS-CoV-2 inclusivity. A total of 67,426 full-length target gene sequences (consisting of 27,550 H1 strains, 39,162 H3 strains, and 714 H7 strains) available in the GISAID database as of May 20, 2020 were analyzed for flu A inclusivity. A total of 20,917 full-length target gene sequences (consisting of 10,934 Victoria strains, 9,967 Yamagata strains, and 16 unclassified strains) available in the GISAID database as of May 22, 2020 were analyzed for flu B inclusivity. A total of 460 full-length sequences (consisting of 316 RSV A strains and 144 RSV B strains) available in the NCBI database as of August 3, 2020 were analyzed for RSV inclusivity.

Overall, the results of these *in silico* analyses and inclusivity testing predict no impact to the detection of SARS-CoV-2, flu A, flu B, and RSV.

Precision

Alinity m Resp-4-Plex assay within-laboratory precision was evaluated using a 9-member panel composed of SARS-CoV-2, flu A, flu B, and RSV each at 2 target concentrations in simulated nasal matrix, as well as a negative panel member in simulated nasal matrix. Each panel member was tested with a target of 6 replicates in a run on each of 5 days (see **Table 3**).

Table 3. Precision

					Within Run/Day Component		Between Run/Day Component		Total[c]	
Panel Target Concentration	N[a]	n[b]	Agreement (%)	Mean CN	SD	% CV	SD	% CV	SD	% CV
SARS-CoV-2[d] (0.015 $TCID_{50}$/mL)	30	30	100%	35.56	0.592	1.7	0.000	0.0	0.592	1.7
SARS-CoV-2[d] (0.025 $TCID_{50}$/mL)	29	29	100%	34.95	0.762	2.2	0.338	1.0	0.833	2.4
Flu A[e] (0.045 $TCID_{50}$/mL)	29	29	100%	33.54	0.523	1.6	0.000	0.0	0.523	1.6
Flu A[e] (0.075 $TCID_{50}$/mL)	30	30	100%	32.68	0.312	1.0	0.126	0.4	0.337	1.0
Flu B[f] (0.06 $TCID_{50}$/mL)	30	30	100%	33.94	0.354	1.0	0.338	1.0	0.489	1.4
Flu B[f] (0.10 $TCID_{50}$/mL)	29	29	100%	33.20	0.534	1.6	0.069	0.2	0.539	1.6
RSV[g] (0.30 $TCID_{50}$/mL)	30	30	100%	32.97	0.447	1.4	0.401	1.2	0.601	1.8
RSV[g] (0.50 $TCID_{50}$/mL)	28	28	100%	32.09	0.387	1.2	0.276	0.9	0.475	1.5
Negative	29	29	100%	--	--	--	--	--	--	--

[a] Number of valid replicates.

[b] Number of positive replicates for SARS-CoV-2, flu A, flu B, and RSV panel members and number of negative replicates for the negative panel member.

[c] Total includes Within-Run/Day Component and Between-Run/Day Component.

[d] Strain USA-WA1/2020, Catalog NR-52287, lot 70033322.

[e] Strain A/Switzerland/9715293/13 (H3N2), Catalog 0810511CF, lot 322440.

[f] Strain B/Brisbane/33/08 (Victoria lineage), Catalog 0810253CF, lot 316752.

[g] Strain RSVB/WestVirginia/14617/85, Catalog VR-1400, lot 70013461.

Analytical Specificity

A total of 55 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 to assess analytical specificity. The microorganisms were tested at 10^5 Units/mL for viruses and 10^6 Units/mL for bacteria and fungi, where these concentrations were available. The unit of measure was specific to each microorganism. Bacteria and fungi were tested as whole microorganisms. Viruses were tested as viral particles or viral lysate unless noted otherwise. No cross reactivity was observed in the presence of these potential cross-reactants (see **Table 4**).

Table 4. Potential Cross-Reactants

Potential Cross-Reactant	Test Concentration	Potential Cross-Reactant	Test Concentration
Adenovirus type 1	1.00E+05 $TCID_{50}$/mL	*Legionella pneumophila*	1.00E+06 CFU/mL
Adenovirus Type 5	1.00E+05 $TCID_{50}$/mL	Measles	1.00E+05 $TCID_{50}$/mL
Adenovirus type 7A	1.00E+05 $TCID_{50}$/mL	MERS-coronavirus[b]	1.00E+05 Copies/mL
Bordetella pertussis	1.00E+06 CFU/mL	Moraxella catarrhalis	1.00E+06 CFU/mL
Candida albicans	1.00E+06 CFU/mL	Mumps	1.00E+05 $TCID_{50}$/mL
Chlamydia pneumoniae	1.00E+06 IFU/mL	*Mycobacterium tuberculosis*	1.00E+06 CFU/mL
Corynebacterium diptheriae	1.00E+06 CFU/mL	*Mycoplasma pneumoniae*	1.00E+06 CFU/mL
Coxsackievirus	1.00E+05 $TCID_{50}$/mL	*Neisseria elongata*	1.00E+06 CFU/mL
Cutibacterium acnes[a]	1.00E+06 CFU/mL	*Neisseria meningitidis*	1.00E+06 CFU/mL
Cytomegalovirus	1.00E+05 IU/mL	Parainfluenza virus 1	1.00E+05 $TCID_{50}$/mL
EBV	1.00E+05 Copies/mL	Parainfluenza virus 2[b]	1.00E+05 Copies/mL
Echovirus	1.00E+05 $TCID_{50}$/mL	Parainfluenza virus 3	1.00E+05 $TCID_{50}$/mL
Enterococcus faecalis	1.00E+06 CFU/mL	Parainfluenza virus 4	1.00E+05 $TCID_{50}$/mL
Enterovirus (EV68)	1.00E+05 $TCID_{50}$/mL	*Pneumocystis jirovecii* (PJP)	N/A[f]
Escherichia coli	1.00E+06 CFU/mL	Pooled Human Nasal Wash	N/A[g]
Haemophilus influenzae	1.00E+06 CFU/mL	*Proteus mirabilis*	1.00E+06 CFU/mL
Herpes Simplex virus	1.00E+05 $TCID_{50}$/mL	*Pseudomonas aeruginosa*	1.00E+06 CFU/mL
Human coronavirus 229E	1.00E+05 Copies/mL	Rhinovirus	1.00E+05 Copies/mL
Human coronavirus HKU1[b]	1.00E+05 Copies/mL	RSV A[e]	1.00E+05 Copies/mL
Human coronavirus NL63	1.00E+05 Copies/mL	RSV B[e]	1.00E+05 Copies/mL
Human coronavirus OC43[b]	1.00E+05 Copies/mL	SARS-coronavirus[b]	1.00E+05 Copies/mL
Human Metapneumovirus[b]	1.00E+05 Copies/mL	*Staphylococcus aureus*	1.00E+06 CFU/mL
Influenza A (H1N1)[c]	1.00E+05 Copies /mL	*Staphylococcus epidermis*	1.00E+06 CFU/mL
Influenza A (H3N2)[c]	1.00E+05 Copies /mL	*Streptococcus pneumoniae*	1.00E+06 CFU/mL
Influenza B[d]	1.00E+05 Copies /mL	*Streptococcus pyogenes*	1.00E+06 CFU/mL
Influenza C	1.00E+05 $CEID_{50}$/mL	*Streptococcus salivarius*	1.00E+06 CFU/mL
Klebsiella pneumoniae	1.00E+06 CFU/mL	Varicella-zoster virus	1.00E+05 Copies/mL
Lactobacillus	1.00E+06 CFU/mL		

[a] Also known as *Propionibacterium acnes*.

[b] Tested as viral RNA.

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

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

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

f Concentration not available; tested as a neat sample. Certificate of Analysis expressed concentration in Ct Range of 23 to 25.

[g] Concentration not available; individual nasal wash samples were pooled and tested neat.

Cross-reactivity of Alinity m Resp-4-Plex for the same potential cross-reacting microorganisms was evaluated *in silico* to identify the % homology between the probe/primer sequences and the sequences present in the potential cross-reacting microorganisms.

Overall, this *in silico* analysis predicts no cross-reactivity or microbial interference for the detection of SARS-CoV-2, flu A, flu B, and RSV by Alinity m Resp-4-Plex.

On-Panel Cross-Reactivity

The cross-reactivity of each signal channel of the Alinity m Resp-4-Plex assay for on-panel viruses was evaluated by testing representative strains, at 10^5 Units/mL. No cross reactivity was observed for on-panel viruses (see **Table 5**).

Table 5. On-Panel Cross Reactivity

Viral Target	Strain	Test Concentration	Results (minimum n = 3 replicates)[b]			
			SARS-CoV-2	Flu A	Flu B	RSV
SARS-CoV-2	Isolate USA-IL1/2020	10^5 GE/mL[a]	positive	negative	negative	negative
Flu A	A/Denver/1/57 (H1N1)	10^5 $CEID_{50}$/mL	negative	positive	negative	negative
	A/PortChalmers/1/1972 (H3N2)	10^5 $CEID_{50}$/mL	negative	positive	negative	negative
Flu B	B/GL/1739/1954 (Yamagata)	10^5 $CEID_{50}$/mL	negative	negative	positive	negative
	B/Malaysia/2506/04 (Victoria)	10^5 $TCID_{50}$/mL	negative	negative	positive	negative
RSV	RSVA/Long/MD/56	10^5 $TCID_{50}$/mL	negative	negative	negative	positive
	RSVB/WestVirginia/14617/85	10^5 $TCID_{50}$/mL	negative	negative	negative	positive

[a] GE/mL = Genome Equivalent/mL

[b] All replicates had the same result for each viral target.

Co-Infection (Competitive Interference)

To assess potential competitive interference between SARS-CoV-2, flu A, flu B, and RSV, samples containing low concentrations of 3 analyte targets were mixed with a high concentration of the fourth analyte target and tested in 21 or more replicates. None of the analyte targets present at the high concentration interfered with the detection of low levels of the other 3 analyte targets.

Matrix Equivalency

Equivalence between multiple respiratory specimen collection media [Universal Viral Transport Medium (UVT), Universal Transport Medium (UTM), and saline] was evaluated by testing 1 strain each for SARS-CoV-2 (USA-WA1/2020), flu A [A/Switzerland/9715293/13 (H3N2)], flu B [B/Brisbane/33/08 (Victoria lineage)], and RSV (RSVB/WestVirginia/14617/85) diluted in pooled negative clinical nasopharyngeal swab specimens at low concentration for each virus strain and collection media, in 21 or more replicates. All replicates tested were positive in all matrices for SARS-CoV-2, flu A, flu B, and RSV.

Interfering Substances

Potentially interfering substances that may be encountered in respiratory specimens were evaluated by testing 1 strain of SARS-CoV-2 (USA-WA1/2020), and 2 strains each of flu A [A/Switzerland/9715293/13 (H3N2) and A/Brisbane/59/2007 (H1N1)], flu B [B/Brisbane/33/08 (Victoria lineage) and B/Massachusetts/02/2012 (Yamagata lineage)], and RSV [RSVB/WestVirginia/14617/85 and RSVA/Long/MD/56 (RSV A)] at low concentration. No interference resulting in a negative result was observed in the presence of any of the substances at the concentrations shown in **Table 6**.

Table 6. Potentially Interfering Endogenous and Exogenous Substances

Substance	Active Ingredient(s)	Tested Concentration
Blood	Blood (human)	10% (v/v)
Throat Lozenges, Oral Anesthetic and Analgesic - Cepacol®	Benzocaine, Menthol	0.63 mg/mL
Mucin	Purified mucin protein	1060 µg/mL
Antibiotic, Nasal Ointment - Bactroban®	Mupirocin	5 mg/mL
Nasal Spray-Afrin®	Oxymetazoline	5% (v/v)
Anti-Viral Drug - Relenza	Zanamivir	3.3 mg/mL
Anti-Viral Drug - Remdesivir	Remdesivir	13.26 µg/mL
Antibacterial, systemic	Tobramycin	4 µg/mL
Nasal Gel /Homeopathic Allergy Relief Medicine - Zicam®	Galphimia glauca, Histaminum hydrochloricum, Luffa operculata, Sulfur	5% (v/v)
FluMist©	Live intranasal influenza virus	6.7% (v/v)
Nasal Corticosteroid-Flonase® Sensimist	Fluticasone Furoate	5% (v/v)
BD Universal viral transport (UVT) medium with swab	Transport medium	100% (v/v)

Clinical Performance Evaluation

The performance of Alinity m Resp-4-Plex was evaluated by testing individual banked clinical nasopharyngeal (NP) swab specimens in viral transport medium.

A total of 114 specimens were analyzed for SARS-CoV-2 detection by both Alinity m Resp-4-Plex and a comparator SARS-CoV-2 assay. The positive percent agreement (PPA) between the 2 assays was 100% (55/55) and the negative percent agreement (NPA) was 96.6% (57/59).

A total of 113 specimens were analyzed for flu A detection by both Alinity m Resp-4-Plex and a comparator FDA-cleared assay for flu A, flu B, and RSV. The positive percent agreement (PPA) between the 2 assays was 100% (63/63) and the negative percent agreement (NPA) was 100% (50/50).

A total of 113 specimens were analyzed for flu B detection by both Alinity m Resp-4-Plex and a comparator FDA-cleared assay for flu A, flu B, and RSV. The positive percent agreement (PPA) between the 2 assays was 100% (63/63) and the negative percent agreement (NPA) was 100% (50/50).

A total of 114 specimens were analyzed for RSV detection by both Alinity m Resp-4-Plex and a comparator FDA-cleared assay for flu A, flu B, and RSV. The positive percent agreement (PPA) between the 2 assays was 100% (64/64) and the negative percent agreement (NPA) was 100% (50/50).

The results are summarized in **Table 7** through **Table 11.**

Table 7. SARS-CoV-2 Detection

		Comparator Assay for SARS-CoV-2	
		Positive	Negative
Alinity m Resp-4-Plex	Positive	55	2[a]
	Negative	0	57

[a] These specimens had an Alinity m Resp-4-Plex CN > 39.0.

Table 8. Flu A Detection

		Comparator Assay for Flu A, Flu B, and RSV	
		Positive	Negative
Alinity m Resp-4-Plex	Positive	63	0
	Negative	0	50

Table 9. Flu B Detection

		Comparator Assay for Flu A, Flu B, and RSV	
		Positive	Negative
Alinity m Resp-4-Plex	Positive	63	0
	Negative	0	50

Table 10. RSV Detection

		Comparator Assay for Flu A, Flu B, and RSV	
		Positive	Negative
Alinity m Resp-4-Plex	Positive	64	0
	Negative	0	50

Table 11. Agreement between Alinity m Resp-4-Plex and Comparators

	PPA		NPA	
Viral Target	**Estimate (%) (95% Exact CI)**	**n/N**	**Estimate (%) (95% Exact CI)**	**n/N**
SARS-CoV-2	100 (93.5, 100)	55/55	96.6 (88.3, 99.6)	57/59
Flu A	100 (94.3, 100)	63/63	100 (92.9, 100)	50/50
Flu B	100 (94.3, 100)	63/63	100 (92.9, 100)	50/50
RSV	100 (94.4, 100)	64/64	100 (92.9, 100)	50/50

PPA – Positive Percent Agreement

NPA – Negative Percent Agreement

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KEY TO SYMBOLS

REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
In Vitro Test	In Vitro Test
AMP TRAY	AMP Tray
ACT TRAY	ACT Tray
	Warning
	Systemic Health Effects
	Caution
	Consult Instructions for Use
	Temperature Limitation
	Contains sufficient for <*n*> tests
	Use By
EC REP	Authorized Representative in the European Community
	Manufacturer

TECHNICAL ASSISTANCE

For technical assistance, call Abbott Molecular Technical Services at 1-800-553-7042 in the US and from outside the US at +49-6122-580, email molecularsupport@abbott.com, or visit the Abbott Molecular website at www.molecular.abbott/portal.

Abbott Molecular Inc. is the legal manufacturer of the Alinity m Resp-4-Plex AMP Kit (List No. 09N79-090)

The Alinity m Resp-4-Plex AMP Kit is imported into the European Union by Abbott Diagnostics GmbH, located at Max-Planck-Ring 2, 65205 Wiesbaden, Germany.

Abbott Molecular Inc.
1300 East Touhy Avenue
Des Plaines, IL 60018 USA

CE

EC REP

Abbott GmbH
Max-Planck-Ring 2
65205 Wiesbaden, Germany

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