Alinity m

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REF 09N78-095
53-608191/R11

SARS-CoV-2 AMP Kit

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REF 09N78-095
53-608191/R11

For Use Under an Emergency Use Authorization (EUA) Only.
For Prescription Use Only.

Rx ONLY

IVD

NOTE: Changes Highlighted

CUSTOMER SERVICE: 1-800-553-7042

CUSTOMER SERVICE INTERNATIONAL: CALL YOUR ABBOTT REPRESENTATIVE

INTRODUCTION

This Emergency Use Authorization (EUA) package insert must be read carefully prior to use. EUA package insert instructions must be followed accordingly. Reliability of EUA assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NAME

Alinity m SARS-CoV-2

INTENDED USE

The Alinity m SARS-CoV-2 assay is a real-time reverse transcriptase (RT) polymerase chain reaction (PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in mid-turbinate nasal, anterior nasal, nasopharyngeal (NP) and oropharyngeal (OP) swabs, and bronchoalveolar lavage (BAL) specimens collected from individuals suspected of COVID-19 by their healthcare provider (HCP), as well as mid-turbinate nasal, anterior nasal, NP and OP swabs collected from any individual, including individuals without symptoms or other reasons to suspect COVID-19. Testing of non-pooled specimens is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263a, that meet requirements to perform moderate or high complexity tests.

This test is also for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled samples containing up to 5 individual upper respiratory specimens (i.e., mid-turbinate nasal, anterior nasal, NP, and OP swabs) that are collected by an HCP using individual vials containing transport media. Testing of pooled specimens is limited to laboratories certified under CLIA, 42 U.S.C §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result and if results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

The Alinity m SARS-CoV-2 assay is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Alinity m SARS-CoV-2 assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION OF THE TEST

The Alinity m SARS-CoV-2 assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in mid-turbinate nasal, anterior nasal, nasopharyngeal (NP) and oropharyngeal (OP) swabs and bronchoalveolar lavage (BAL) specimens collected from individuals suspected of COVID-19 by their healthcare provider (HCP), as well as mid-turbinate nasal, anterior nasal, NP and OP swabs collected from any individual, including individuals without symptoms or other reasons to suspect COVID-19 infection.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Alinity m SARS-CoV-2 assay consists of 2 reagent kits:

- Alinity m SARS-CoV-2 AMP Kit
- Alinity m SARS-CoV-2 CTRL Kit

The Alinity m SARS-CoV-2 assay is a dual target assay for the RdRp and N genes.

An RNA sequence that is unrelated to the SARS-CoV-2 sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample.

The Alinity m SARS-CoV-2 assay detects the SARS-CoV-2 virus and IC target sequences through the use of target-specific fluorescent-labeled oligonucleotide probes. The probes do not generate a signal unless they are specifically bound to the amplified product. The two SARS-CoV-2-specific probes are labeled with the same fluorophore and the IC-specific probe is labeled with a different fluorophore, thus allowing for simultaneous detection of both SARS-CoV-2 and IC amplified products in the same reaction vessel.

The Alinity m SARS-CoV-2 assay is to be used with the Alinity m System which performs sample preparation, RT-PCR assembly, amplification, detection, and result calculation and reporting. All steps of the Alinity m SARS-CoV-2 assay procedure are executed automatically by the Alinity m System.

The Alinity m System is a random access analyzer that can perform the Alinity m SARS-CoV-2 assay in parallel with other Alinity m assays on the same instrument.

Application parameters specific to Alinity m SARS-CoV-2 assay are contained on an assay-specific application specification file, that will be distributed electronically, and loaded onto the Alinity m System.

Sample Preparation

The Alinity m System provides 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 nucleic acid 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 Internal Control (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, SARS-CoV-2 virions are disrupted by guanidine isothiocyanate, 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 combined with liquid unit-dose Alinity m SARS-CoV-2 activation reagent and liquid unit-dose Alinity m SARS-CoV-2 amplification/detection reagents and transferred into a reaction vessel. Alinity m Vapor Barrier Solution is then added to the reaction vessel which is

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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 included at or above an established minimum frequency of once every 24 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. First, the 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 three targets (SARS-CoV-2 RdRp, SARS-CoV-2 N and IC) takes place simultaneously in the same reaction.

The target sequences for the Alinity m SARS-CoV-2 assay are in the SARS-CoV-2 RdRp and N genes of the SARS-CoV-2 genome. The selected target sequences are highly conserved and also specific to this strain of coronavirus.

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 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 has a quencher molecule at its 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 SARS-CoV-2 probes are labeled with a different fluorophore from the IC probe, thus allowing for simultaneous detection of both SARS-CoV-2 and IC amplified products.

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 is carried out automatically in a sealed reaction vessel.
- Disposal of the reaction vessel 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

Alinity m SARS-CoV-2 AMP Kit (List No. 09N78-095)

Alinity m SARS-CoV-2 AMP Kit (List No. 09N78-095) is comprised of 2 types of multi-well trays: Alinity m SARS-CoV-2 AMP TRAY 1 and Alinity m SARS-CoV-2 ACT TRAY 2.

- Each Alinity m SARS-CoV-2 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, and dNTPs in a buffered solution with a reference dye. Internal control (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, anti-HIV-1/HIV-2, and anti-HCV. Preservative: 0.15% ProClin® 950.
- Each Alinity m SARS-CoV-2 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 tetramethyl ammonium chloride. Preservative: 0.15% ProClin 950.

WARNINGS AND PRECAUTIONS

IVD

- **For In Vitro Diagnostic Use Under the FDA Emergency Use Authorization**
- For use under an Emergency Use Authorization
- Do not use beyond expiration date
- For Prescription Use Only
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
- This product has been authorized by FDA under an EUA for use by laboratories certified under CLIA, to perform moderate or high complexity tests;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

Safety Precautions

The following warnings and precautions apply to:
Alinity m SARS-CoV-2 AMP TRAY 1.

WARNING	Contains 2-Methyl-4-isothiazolin-3-one
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapours / 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 SARS-CoV-2 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 / vapours / 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 EU 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 SARS-CoV-2 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 SARS-CoV-2 AMP TRAY 1 (AMP TRAY 1) and Alinity m SARS-CoV-2 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 PROTOCOL section for additional instructions.
- Minimize contact with the surface of reagent trays during handling.
- Only load AMP TRAY 1 and ACT TRAY 2 from the same AMP Kit lot on the same Alinity m Assay Tray Carrier. Do not load AMP TRAY 1 and ACT TRAY 2 from different AMP Kit lots on the same Alinity m Assay Tray Carrier.
- The Alinity m System will track the onboard storage time of 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 SARS-CoV-2 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 under Emergency Use Authorization only.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- 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 SARS-CoV-2 assay and the Alinity m System should perform this procedure.
- Always follow best laboratory practices to monitor for potential contamination and unexpected positive results.

Handling Precautions for Specimens

- The Alinity m SARS-CoV-2 assay is only for use with mid-turbinate nasal, anterior nasal, nasopharyngeal and oropharyngeal swabs or bronchoalveolar lavage fluid (BAL) 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.
- Testing of pooled specimens may impact the detection capability of the Alinity m SARS-CoV-2 Assay and decrease sensitivity.
- 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.
- Proper aseptic technique should always be used when working with RNA.
- 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 few molecules 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 SARS-CoV-2 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

Human mid-turbinate nasal, anterior nasal, nasopharyngeal and oropharyngeal swab or bronchoalveolar lavage fluid (BAL) specimens can be used with the Alinity m SARS-CoV-2 assay on the Alinity m System. Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19)[6] (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html) or the FDA FAQs on Diagnostic Testing for SARS-CoV-2 (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-testing-sars-cov-2).

An Abbott *m*ulti-Collect Specimen Collection Kit (List No. 09K12-03 or 09K12-04) or the Abbott Universal Collection Kit (List No. 09N77-055) can be used for the transport of nasopharyngeal swab specimens or the collection and transport of mid-turbinate nasal, anterior nasal and oropharyngeal swab specimens from the collection site to the testing laboratory. Abbott *m*ulti-Collect Specimen Collection Kit List No. 09K12-04 utilizes pierceable caps. Neither the swab (contained in both the Abbott *m*ulti-Collect Specimen Collection Kit and the Abbott Universal Collection Kit) nor the transfer pipette (contained in the Abbott multi-Collect Specimen Collection Kit) are authorized for nasopharyngeal specimen collection. The transfer pipette (contained in the Abbott *m*ulti-Collect Specimen Collection Kit) is not authorized for mid-turbinate nasal, anterior nasal or oropharyngeal specimen collection. The Transport Tube contains Specimen Transport Buffer which is used to stabilize nucleic acid until sample testing. Transport and store transport tube at 2 to 25°C for up to 48 hours. If delivery and processing exceed 48 hours, specimens should be transported on dry ice and once in laboratory frozen at −70°C or colder for a maximum of 7 days prior to testing.

Ship specimens according to the recommended storage temperature and time listed in the Specimen Storage section. Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical, diagnostic, or biological specimens.

Specimen Collection Procedure for Mid-Turbinate Nasal, Anterior Nasal and Oropharyngeal Swabs:

1. Discard disposable transfer pipette (if present); it is not required for mid-turbinate nasal, anterior nasal or oropharyngeal swab specimen collection.
2. Remove the sterile swab from the wrapper, taking care not to touch swab tip or lay it down on any surface. Do not pre-wet swab.
3. Collect patient specimen per CDC guidelines.[6]
4. Handle the cap and tube carefully to avoid contamination, including the outside of the transport tube and cap. If necessary, change gloves.
5. Unscrew the transport tube cap and immediately place the specimen collection swab into the transport tube so that the white tip is down.
6. Carefully break the swab at the scored line on the shaft; use care to avoid splashing of contents.
7. Recap the transport tube. Ensure the cap seals tightly. The cap must be tight or leakage may occur.
8. Label the transport tube with sample identification information, including date of collection using an adhesive label. It is recommended that each tube be placed in an individual, sealable bag prior to transport.

Specimen Transport of Nasopharyngeal Swabs:

1. Discard disposable transfer pipette (if present) and the swab; they are not authorized for nasopharyngeal swab specimen collection.
2. Collect patient specimen per CDC guidelines.[6]
3. Handle the cap and tube carefully to avoid contamination, including the outside of the transport tube and cap. If necessary, change gloves.
4. Unscrew the transport tube cap and immediately place the specimen collection swab into the transport tube so that the swab tip is down.
5. If necessary, carefully break any swab shaft that protrudes out of the tube; use care to avoid splashing of contents.
6. Recap the transport tube. Ensure the cap seals tightly. The cap must be tight or leakage may occur.
7. Label the transport tube with sample identification information, including date of collection using an adhesive label. It is recommended that each tube be placed in an individual, sealable bag prior to transport.

See the package insert within the Abbott Universal Collection Kit (List No. 09N77-055) for additional instructions for its use.

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 potential SARS-CoV-2 specimens.

Specimen Pooling - Determining Appropriate Strategy for Implementation and Monitoring

When considering specimen pooling, laboratories should evaluate the appropriateness of a pooling strategy based on the positivity rate in the testing population and the efficiency of the pooling workflow. Refer to Appendix A of this package insert for additional information prior to implementation of specimen pooling.

Preparation for Analysis

Frozen specimen is thawed at 15 to 30°C or at 2 to 8°C.

If specimen pooling is performed:

1. Establish a process that ensures traceability between individual specimen IDs and pool IDs.
2. Determine the appropriate volume required from each individual specimen based on the pool size being implemented and tube type used. Volume requirements are listed in the Assay Procedure section below. Use the same volume from each specimen. For example, if a pool size of 5 specimens is being utilized with an Alinity m Transport Tube, 200 µL of each individual specimen (1.0 mL total) is required. Enough specimen should remain to retest individual specimens should the pool be positive.
3. Uncap the individual specimen collection container and retain the cap. Ensure appropriate specimen handling technique to reduce the risk of cross contamination of pools and the original patient specimens.
4. Carefully transfer the determined volume of each individual specimen from the specimen collection container to the tube being used for the pool.

Prior to processing, each specimen/pool is vortexed 3 times for 2 to 3 seconds.

If needed, centrifuge specimens/pools at 2000 *g* for 5 minutes before loading on the Alinity m System. Specimen can be transferred into an Alinity m Transport Tube or an Alinity m Aliquot Tube and/or recapped with an Alinity m Pierceable Cap before loading onto the Alinity m System.

IMPORTANT: Except where pierceable caps are used, 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 SARS-CoV-2 AMP Kit (List No. 09N78-095)

Materials Required But Not Provided

- 08N53-002 Alinity m System with software version 1.5.2 or higher
- 09N78-085 Alinity m SARS-CoV-2 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
- 09N78-03F (version 6.0 or higher) Alinity m SARS-CoV-2 Application Specification File
- Vortex mixer
- 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.

Other Optional Materials

- Abbott *m*ulti-Collect Specimen Collection Kit (List No. 09K12-03 or 09K12-04)
  NOTE: List No. 09K12-04 utilizes a Pierceable Cap. 09K12-03 utilizes a solid cap.
- Abbott Universal Collection Kit (List No. 09N77-055)
- Sealable plastic bags
- 09N49-010 Alinity m Transport Tube Pierceable Capped
- 09N49-011 Alinity m Transport Tube
- 09N49-012 Alinity m Pierceable Caps
- 09N49-013 Alinity m Aliquot Tube

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 SARS-CoV-2 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 product 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% (v/v) 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 SARS-CoV-2 CTRL Kit is integral to the performance of the Alinity m SARS-CoV-2 assay. Refer to the QUALITY CONTROL PROCEDURES section of this package insert for details. Refer to the Alinity m SARS-CoV-2 CTRL Kit package insert for preparation and usage.
- The Alinity m SARS-CoV-2 control reagents are contained in single-use tubes with solid caps. Remove caps from the tube prior to use. Discard tubes after use.

ASSAY PROTOCOL

Prior to loading on the Alinity m System, thaw AMP TRAY 1 and ACT TRAY 2 at 15 to 30°C or at 2 to 8°C immediately prior to use on the Alinity m System.

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.

From the Create Order screen, select the assay (SARS-CoV-2) being tested.

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 SARS-CoV-2 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 Number(s)	Minimum Volume Required	Maximum Volume Allowed	Cap Requirement on Instrument
Abbott *m*ulti-Collect Specimen Collection Tube with pierceable cap	09K12-04	1.0 mL	3.5 mL	Uncapped[b] / Capped[c]
Abbott *m*ulti-Collect Specimen Collection Tube with solid cap	09K12-03	1.0 mL	3.5mL	Uncapped[b]
Abbott Universal Collection Tube	09N77-055	1.0 mL	3.5 mL	Uncapped[b]
Abbott Universal Collection Tube where solid cap is replaced with a pierceable cap (09N49-012)	09N77-055, 09N49-012	1.0 mL	3.5 mL	Capped[c]
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 where pierceable cap (09N49-012) is added	09N49-011, 09N49-012	1.0 mL	3.5 mL	Capped[c]
Alinity m Transport Tube Pierceable Capped	09N49-010	1.0 mL	3.5 mL	Uncapped[b] / Capped[c]
Tube with 11.5 – 14.0 mm diameter		1.3 mL	2.5 mL	Uncapped[b]
Tube with 14.5 – 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.

[c] Avoid touching inside of the cap when replacing or adding a new cap. Avoid touching the septum when handling the sample 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:

- If the IC CN is out of range, but the SARS-CoV-2 is detected, the sample will yield a Positive interpretation. An IC Flag will be reported.
- If the IC CN is out of range and the SARS-CoV-2 is not detected, no result/interpretation will be reported for the sample and a Message Code will be generated.

Refer to the Alinity m System Operations Manual, Section 5 for an explanation of the corrective actions for 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 SARS-CoV-2 Negative CTRL (CTRL –) and Positive CTRL (CTRL +) are recommended to be tested, at or above the minimum frequency of once every 24 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. All of the specimens processed 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 SARS-CoV-2 must not be detected in the negative control. SARS-CoV-2 detected in the negative control is indicative of contamination by other samples or by amplified product. To avoid contamination, clean the Alinity m System and repeat sample processing for controls and specimens following the Procedural Precautions in this package insert. Monitoring procedures for the presence of amplification product can be found in the Alinity m System Operations Manual, Section 9.

If negative controls are persistently reactive, contact your Abbott Representative.

When the Alinity m SARS-CoV-2 is being used on the Alinity m System, the target CN value of the Alinity m SARS-CoV-2 Positive CTRL can be:

- Automatically imported to the Alinity m System via Abbott Mail.
- Obtained from the Abbott customer portal or provided by your Abbott Representative and imported to the Alinity m System via a USB drive.

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
SARS-CoV-2 NEG CTRL	SARSCoV2				9186[a]
SARS-CoV-2 POS CTRL	SARSCoV2				9198[b]
Sample 1	SARSCoV2	Not Detected	Negative	FPC, FNC[c]	
Sample 2	SARSCoV2	XX.XX CN	Positive	FPC, FNC[c]	
SARS-CoV-2 NEG CTRL	SARSCoV2	Not Detected			
SARS-CoV-2 POS CTRL	SARSCoV2	XX.XX CN			
Sample 3	SARSCoV2	XX.XX CN	Positive		
Sample 4	SARSCoV2	Not Detected	Negative		
Sample 5	SARSCoV2	XX.XX CN	Positive	IC[d]	
Sample 6	SARSCoV2				9186[e]

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

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

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

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

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

Interpretation of Results for Pooled Samples

- If the result of the pool is negative, then each sample is reported as negative. Negative results from pooled sample testing should not be treated as definitive. If the patient's clinical signs and symptoms are inconsistent with a negative result and if results are necessary for patient management, then the patient should be considered for individual testing. The utilization of sample pooling should be indicated for any specimens with reported negative results.
- Specimens with a positive or invalid sample pool result must be tested individually, and the individual result reported. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.
- Flags and Result Code interpretation is not changed by pooling.

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

For use under an Emergency Use Authorization only.

- This assay is for in vitro diagnostic use under FDA Emergency Use Authorization only.
- Use of the Alinity m SARS-CoV-2 assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and the Alinity m System.
- Laboratories are required to report all results to the appropriate public health authorities.
- 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).
- Use of Pierceable Caps without the removal of swabs has only been validated using the swabs provided in the Abbott *m*ulti-Collect Specimen Collection Kit and Abbott Universal Collection Kit.
- Detection of 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.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.
- As with any molecular test, mutations within the target regions of Alinity m SARS-CoV-2 assay could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- The clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- 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.
- The Alinity m SARS-CoV-2 assay was validated with nasopharyngeal swabs. Mid-turbinate nasal, anterior nasal (self-collected under healthcare provider (HCP) supervision or HCP-collected) and oropharyngeal swab specimens as well as bronchoalveolar lavage specimens are also considered acceptable specimen types, but performance has not been established.
- 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 the SARS-CoV-2 virus 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 CDC recommendations.
- Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.
- Use of the Alinity m SARS-CoV-2 assay in a general asymptomatic screening population is intended to be used as part of an infection control plan, that may include additional preventative measures, such as a predefined serial testing plan or directed testing of high-risk individuals. Negative results should be considered presumptive and do not preclude current or future infection obtained through community transmission or other exposures. Negative results must be considered in the context of an individual's recent exposures, history, and presence of clinical signs and symptoms consistent with COVID-19.
- Asymptomatic individuals infected with COVID-19 may not shed enough virus to reach the limit of detection of the test, giving a false negative result.
- Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.
- Sample pooling has only been validated using nasopharyngeal swab specimens.

CONDITIONS OF AUTHORIZATION FOR LABORATORIES

The Alinity m SARS-CoV-2 assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories using the Alinity m SARS-CoV-2 assay ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product must include with test result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories[1] using specimen pooling strategies when testing patient specimens with the authorized test must include with test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that "Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing."

C. Authorized laboratories[1] using your product must use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

D. Authorized laboratories[1] implementing pooling strategies for testing patient specimens must use the "Specimen Pooling Implementation and Monitoring Guidelines" provided in the authorized tests' Instructions for Use/Package Insert to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.

E. Authorized laboratories[1] that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

F. Authorized laboratories[1] using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

G. Authorized laboratories[1] must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Abbott (email: molecularsupport@abbott.com; 1-800-553-7042) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

H. All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.

I. Abbott, authorized distributor(s), and authorized laboratories[1] using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

J. Authorized laboratories[1] must keep records of specimen pooling strategies implemented including type of strategy, date implemented, and quantities tested, and test result data generated as part of the Protocol for Monitoring of Specimen Pooling Strategies. For the first 12 months from the date of their creation, such records will be made available to FDA within 48 business hours for inspection upon request, and will be made available within a reasonable time after 12 months from the date of their creation.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform moderate or high complexity tests" as "authorized laboratories." Testing of pooled specimens is limited to authorized laboratories certified under CLIA that meet the requirements to perform high complexity tests.

SPECIFIC PERFORMANCE CHARACTERISTICS

Limit of Detection (Analytical Sensitivity)

Limit of Detection (LOD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater than or equal to 95% of all (true positive) replicates test positive.

To determine the LOD, a recombinant virus containing SARS-CoV-2 RNA (SeraCare, AccuPlex COVID-19, 1.3E + 07 Copies/mL as determined by digital PCR) was diluted in simulated nasal matrix (SNM). The initial LOD was determined by testing 5 levels at target concentrations of 800, 400, 200, 100, and 50 Copies/mL. Each panel member was tested in replicates of 12.

The final LOD was confirmed by testing 4 panel members with target concentrations at 400, 300, 200, and 100 Copies/mL in replicates of 21. The results are summarized in **Table 1.** The lowest concentration level with observed positive rates ≥ 95% was 100 virus Copies/mL.

Table 1. LOD Determination Using Recombinant Virus Containing SARS-CoV-2

Virus Copies/mL	Total Valid Replicates	Positive Replicates	Positive Rate (%)
400	21	21	100
300	21	21	100
200	21	21	100
100	21	21	100

LOD was further evaluated by testing dilutions of inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources; NR-52287) in SNM, in a minimum of 20 replicates at each dilution level. LOD estimated from probit analysis was 0.0037 $TCID_{50}$/mL (95% CI: 0.0022 – 0.0099). Refer to **Table 2**.

Table 2. Summary of Detection Rate

Panel	Target Concentration ($TCID_{50}$/mL)	Number of Replicates Tested	Number of Replicates Detected	Detection Rate (%)
01	0.028	23[a]	23	100.0
02	0.009	24	24	100.0
03	0.003	24	22	91.7
04	0.001	20	13	65.0
05	0.0003	24	6	25.0

[a] One sample was invalid and resulted in exception 9186 (Internal Control failed). It was excluded from the analysis.

Inclusivity

Inclusivity was demonstrated by analyzing the sequences of the RdRp and N primer/probe sets for homology with 8,634,788 full-length sequences available in the GISAID database (http://www.gisaid.org) as of March 16, 2022. 8,585,868 sequences (99.4%) either have no mismatches in the assay target regions or have mismatches in one of the target regions. Among 48,920 sequences (0.6%) containing at least one mismatch in both target regions, 48,857 were predicted unlikely to impact the detection of SARS-CoV-2. Among 7,565,966 isolates with variant designation (including 1,139,842 Alpha, 40,183 Beta, 4,086,797 Delta, 116,655 Gamma, 2,033,303 Omicron, 64,677 Epsilon, 7,480 Eta, 41,807 Iota, 7,173 Kappa, 7,420 Lambda, 14,876 Mu, 618 Theta, and 5,135 Zeta), 7,519,355 sequences (99.4%) either have no mismatches in the assay target regions or have mismatches in only one of the target regions and are therefore predicted to be detected.

An additional analysis was also performed using 989,930 full-length SARS-CoV-2 sequences available in the NCBI database (https://www.ncbi.nlm.nih.gov/datasets/coronavirus/genomes/) as of April 14, 2022. 987,119 sequences (99.7%) either have no mismatches in the assay target regions or have mismatches in only one of the target regions and are therefore predicted to be detected. Among 2,811 sequences (0.3%) containing at least one mismatch in both target regions, 2,806 were predicted unlikely to impact the detection of SARS-CoV-2.

Precision

Alinity m SARS-CoV-2 assay within-laboratory precision was evaluated using a 3-member panel: 2 positive panel members 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 3 replicates in a run, 2 runs on each of 5 days, on 3 Alinity m instruments for a total of 90 replicates of each panel (see **Table 3**).

Table 3. Precision

Target Concentration	N[a]	n[b]	Agreement[c]	Mean (CN)	Within-Run Component		Between-Run Component		Between-Day Component		Within-Lab Component.[d]		Between-Inst. Component		Total[e]	
					SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
1-2X LoD[f]	90	90	100.0%	35.48	0.74	2.1	0.14	0.4	0.23	0.6	0.79	2.2	0.32	0.9	0.85	2.4
5X LoD[f]	90	90	100.0%	33.74	0.55	1.6	0.22	0.7	0.00	0.0	0.59	1.8	0.00	0.0	0.59	1.8
Negative	90	90	100.0%	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] N: Total number of valid replicates.

[b] n: Replicates with detected analyte for positive panels, not detected for negative panel.

[c] Agreement = n/N.

[d] Within-laboratory Includes Within-Run, Between-Run and Between-Day Components.

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

[f] Isolate USA-WA1/2020, Catalog NR-52287, lot 70039068 (2x LoD= 110 GE/mL, 5xLoD= 275 GE/mL).

Cross-reactivity

In Silico Analysis

Related pathogens, high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimen have been evaluated in silico to identify the % homology between the selected probe/primer sequences and the sequence present in the microorganism.

The conclusion of this analysis is that there is limited opportunity for cross-reactivity to allow for false-positive reporting or affect performance of SARS-CoV-2 virus detection based upon the following:

- For many organisms, only one primer (forward or reverse) has > 80% homology, making an amplified product unlikely.
- The probe is unlikely to bind for any of the hits (< 80% homology).
- Mismatches in the 3′ end of primers makes extension unlikely.
- For the N amplicon, two organisms with forward and reverse primers having > 80% homology (LS483366.1, CP040804.1) have both primer binding sites on the same plus-sense strand and will not result in amplification.
- For the N amplicon, the remaining two organisms that may potentially give rise to amplicons due to both forward and reverse primers having > 80% homology on opposite strands (CP000262.1, CP002888.1) have primer binding sites separated by > 100,000 nucleotides in the bacterial chromosome, making amplification unlikely.

Overall, the results of this analysis predict no significant cross-reactivity or microbial interference.

Carryover

The carryover rate for Alinity m SARS-CoV-2 assay using application specification version 6.0 was determined by testing alternating replicates of SARS-CoV-2 high positive samples and SARS-CoV-2 negative samples across multiple runs. The high positive samples were prepared by diluting SARS-CoV-2 synthetic target (plasmid DNA) in Simulated Nasal Matrix targeting final concentration of 2.0E+09 copies/mL. SARS-CoV-2 negative Simulated Nasal Matrix served as negative sample. Out of the 361 negative valid samples, 0 samples were positive (“detected”) for SARS-CoV-2. The sample carryover rate was 0.0% (0/361, 95% CI: 0.0% to 1.1%).

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed on the Alinity m SARS-CoV-2 assay using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LOD. Blinded sample testing was used to establish specificity and to confirm the LOD. The results are summarized in **Table 4**.

Table 4. Summary of FDA SARS-CoV-2 Reference Panel Results

Reference Materials Provided by FDA	Specimen Type	Product LOD	Cross-Reactivity
SARS-CoV-2	NP Swabs in VTM	600 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not applicable

ND Not detected

Clinical Performance Evaluation

A clinical evaluation study was performed to evaluate the performance of the Alinity m SARS-CoV-2 assay using nasopharyngeal swab specimens. A total of 40 contrived positive specimens at approximately 1X to 2X LOD and 20x LOD were tested. Samples were contrived by spiking known concentrations of recombinant virus containing SARS-CoV-2 RNA sequences into individual negative patient specimens. In addition to the contrived positive specimens, 31 individual negative specimens were tested.

There were 20 total samples tested at the 1X to 2X LOD level with 20 results valid and included in the analysis. There were 20 total samples tested at 20X LOD with 20 results valid and included in the analysis. There were 31 total samples tested for the negative level with 31 results valid and included in the analysis.

The results are summarized in **Table 5**. All positive samples were detected. All negative samples were not detected.

Table 5. Clinical Evaluation of the Alinity m SARS-CoV-2 Assay

SARS-CoV-2 Concentration	Number Tested	Number Detected	% Detection
1X to 2X LOD	20	20	100 (N=20/20)
20X LOD	20	20	100 (N=20/20)
Negative	31	0	0 (N=0/31)

	N	Agreement	Exact 95% CI
PPA	40	100%	(91.2, 100.0)
NPA	31	100%	(88.8, 100.0)

PPA – Positive Percent Agreement.

NPA – Negative Percent Agreement.

An additional study was performed to evaluate the performance of the Alinity m SARS-CoV-2 assay testing individual nasopharyngeal swab specimens (banked and acquired from a clinical lab). A total of 104 specimens were analyzed by both a comparator EUA RT-PCR and Alinity m SARS-CoV-2 assays. Specimens acquired from the clinical lab were treated for viral inactivation at 65°C for 30 minutes prior to analysis. The positive percent agreement (PPA) between the 2 assays was 100% (47/47) and the negative percent agreement (NPA) was 96.5% (55/57). The results are summarized in **Table 6**.

Table 6. Clinical Evaluation of the Alinity m SARS-CoV-2 Assay

		Comparator EUA RT-PCR	
		Positive	Negative
Alinity m SARS-CoV-2	Positive	47	2[a]
	Negative	0	55

a These samples had an Alinity m SARS-CoV-2 CN >40.

	N	Agreement	Exact 95% CI
PPA	47	100%	(92.5, 100.0)
NPA	57	96.5%	(87.9, 99.6)

Clinical Performance with Specimens from Asymptomatic Individuals

Nasopharyngeal (NP) swab specimens were prospectively collected in viral transport media by healthcare providers from asymptomatic individuals and were tested on the Alinity m SARS-CoV-2 and a comparator EUA RT-PCR assay. Results from 19 consecutive individuals who were positive and 125 consecutive individuals who were negative were included in the analysis. The positive percent agreement (PPA) between the 2 assays was 100% (19/19) and the negative percent agreement (NPA) was 100% (125/125). The results are summarized in **Table 7**.

Table 7. Clinical Performance with Specimens from Asymptomatic Individuals

		Comparator EUA RT-PCR	
		Positive	Negative
Alinity m SARS-CoV-2	Positive	19	0
	Negative[a]	0	125

[a] One additional specimen not included in the analysis was negative for Alinity m SARS-CoV-2 and inconclusive for comparator.

	N	Agreement	Exact 95% CI
PPA	19	100%	(82.4, 100)
NPA	125	100%	(97.1, 100.0)

Clinical Performance of Specimen Pooling

The clinical performance of the Alinity m SARS-CoV-2 assay was evaluated in pools consisting of 5 specimens. For the study, 70 positive and 55 negative specimen pools were evaluated in a pool size of 5 specimens. Each positive pool consisted of one positive specimen and 4 negative specimens. Each negative pool consisted of 5 negative specimens. The positive specimens used in the study covered the detectable range of the assay and included 30% (21/70) weak positive specimens (i.e., having a CN value within 3 cycles of the CN value of the LOD target level). Both the pooled and individual specimens were evaluated with the Alinity m SARS-CoV-2 assay.

The positive percent agreement (PPA) in relation to the individual result was 98.6% (69/70) and the negative percent agreement (NPA) was 100% (55/55). The results are summarized in **Table 8**.

Table 8. Clinical Evaluation of Specimen Pooling

		Individual Results	
		Positive	Negative
Pooled Results	Positive	69	0
	Negative	1[a]	55

[a] This individual specimen had CNs less than 1 CN from the LOD

	N	Agreement	Exact 95% CI
PPA	70	98.6%	(92.3, 100.0)
NPA	55	100%	(93.5, 100.0)

A linear relationship was observed between individual and pooled specimen with an expected shift in CN. When using application specification file 09N78-03A, the slope and y-intercept from the Passing-Bablok linear regression model were calculated to be 1.02 and 2.09, respectively. When using application specification file 09N78-03B or higher, the slope and y-intercept from the Passing-Bablok linear regression model were calculated to be 0.98 and 2.13, respectively.

In Silico Estimated Performance in Pooled Specimens

A model based on observed data in the specimen pooling validation study above was used to estimate SARS-CoV-2 detection in pooled specimens using historical CN values from consecutive positive individual specimens. CN shifts between individual and pooled specimens were estimated based on Passing-Bablok regression analysis for specimens tested using application specification file version 1 (09N78-03A) and version 2 (09N78-03B or higher). In addition, three CN intervals were identified where an individual specimen with a CN value in one of these intervals, when combined into a pool of five specimen containing one positive specimen is expected to be detected 100% of the time (Zone 3), <100% of the time (Zone 2), or 0% of the time (Zone 1). Refer to **Table 9**.

Table 9. In Silico Performance Estimation Rules

Application Specification File Version	CN Shift (at the Cut-off)	Zone 3[a]	Percent Detection	Zone 2[b]	Percent Detection	Zone 1[c]	Percent Detection
1[d]	2.91	<35.51	100%	35.51 to <39.09	24%	39.09 to 42	0%
2	1.27	<35.99	100%	35.99 to <40.73	81%	40.73 to 42	0%

[a] Zone 3 represents the CN range that has the upper bound bordering Zone 2 and the lower bound at 0.

[b] Zone 2 represents the CN range that has the upper bound bordering Zone 1 and the lower bound at the highest CN value of positive individual specimens tested in the specimen pooling validation study.

[c] Zone 1 represents the CN range that has the upper bound at the assay cutoff cycle and the lower bound at the assay cutoff cycle minus the CN shift caused by specimen pooling.

[d] Application specification file version 1 is being replaced by specification file version 2.

Application of the in silico performance estimation rules to historical data from individual positive results from 3 geographically diverse sites in the US is presented in **Table 10**.

Table 10. Results of In Silico Performance Estimation from 3 Geographically Diverse Sites in the U.S.

Site	Total Number of Specimens	Percentage of Specimens in Zone 3	Percentage of Specimens in Zone 2	Percentage of Specimens in Zone 1	Estimated 5-Sample Pooling PPA
1[a]	40	75.0%	20.0%	5.0%	91.2%
2[a]	40	92.5%	5.0%	2.5%	96.6%
3[b]	40	90.0%	2.5%	7.5%	90.6%

[a] Application specification file version 2.

[b] Application specification file version 1.

Example of an application of the in silico performance estimation using historical data from Site 1 where the percentages of specimens in the three CN intervals are: 75.0% in Zone 3, 20.0% in Zone 2 and 5.0% in Zone 1. The PPA is (100.0% X 75.0%) + (81.0% X 20.0%) + (0% X 5.0%) = 75.0% + 16.2% + 0% = 91.2%

Some positive samples may not be detected when tested in pools due to the dilution effect by the pooling. Performance estimations above may underestimate the loss of detection from testing in pools. Laboratories should also consider the assay's limit of detection when evaluating testing in pools.

BIBLIOGRAPHY

1. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. *Type*> www.cdc.gov, *search*> BMBL> *look up* sections III and IV.]
2. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
3. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Fourth Edition*. CLSI Document M29-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
4. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.
5. Clinical and Laboratory Standards Institute. *Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline.* CLSI Document MM13-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
6. Centers for Disease Control and Prevention (CDC). *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19)*. Available online at: https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

TECHNICAL ASSISTANCE

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

Abbott Molecular Inc. is the legal manufacturer of the:

Alinity m SARS-CoV-2 AMP Kit (List No. 09N78-095)

Alinity m SARS-CoV-2 CTRL Kit (List No. 09N78-085)

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53-608191/R11

KEY TO SYMBOLS

REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
In Vitro Test	In Vitro Test
For In Vitro Diagnostic Use	For In Vitro Diagnostic Use
AMP TRAY	AMP Tray
ACT TRAY	ACT Tray
Rx ONLY	For Prescription Use Only
	Warning
	Systemic Health Effects
	Caution
	Consult Instructions for Use
	Temperature Limitation
	Sufficient for
	Use By
	Manufacturer

APPENDIX A: SPECIMEN POOLING IMPLEMENTATION AND MONITORING GUIDELINES

Before Implementation of Pooling: Determine Appropriate Pool Size

Before a pooling strategy is implemented, a laboratory should determine the appropriate pool size based on percent positivity rate and desired testing efficiency. The Alinity m SARS-CoV-2 assay has been validated for n-sample pool sizes up to five samples per pool.

If historical laboratory data for individual specimens is available:

- If historical data for individual specimens from the previous 7-10* days is available, estimate the percent positivity rate ($P_{individual}$) based on individual results. ($P_{individual}$) = (Number of positive specimens over chosen date range ÷ Total number of specimens tested over chosen date range)x100.
- Using the calculated $P_{individual}$ and **Table 10**, identify the appropriate *n* number of samples to pool.
  - If $P_{individual}$ is less than 5%, the maximum pool size validated, (n=5), should be selected to maximize the efficiency of specimen pooling. Pooling with greater than 5 samples has not been validated and should not be performed.
  - If $P_{individual}$ is greater than 25%, Dorfman pooling of patient specimens is not efficient and should not be implemented.

If historical laboratory data for individual specimens is unavailable:

- If historical data from the previous 7-10* days is unavailable, 5, 4, or 3-specimen pooling may still be implemented as the Alinity m SARS-CoV-2 assay has been validated for 5-specimen pooling.
- Note: without calculating $P_{individual}$ the pooling size implemented may not maximize pooling efficiency.

Table 10. Result Interpretation

P, percent of positive subjects in the tested population	$n_{maxefficiency}$ (n corresponding to the maximal efficiency)	Efficiency of n-sample pooling (a maximum increase in the number of tested patients when Dorfman n-pooling strategy used)
5% - 6%	5	2.15 - 2.35
7% - 12%	4	1.54 - 1.99
13% - 25%	3	1.10 - 1.48

To calculate the efficiency of n-sample pooling, using Pindividual, apply the formula F=1/(1+1/n-(1- Pindividual)n) where F is the efficiency and n is the pool size. For example, when Pindividual is 5%, the efficiency, F, is 2.35 for n=5. This means that 1,000 tests can cover testing of 2,350 patients on average.

Implementation of Pooling

See above sections titled *Specimen Pooling* and *Preparation Analysis* and perform pooling procedure as outlined.

After Implementation of Pooling: Ongoing Monitoring of Pooling Strategy

If historical laboratory data for individual specimens is available:

- After implementing a pooling strategy, evaluate the performance of pooled testing by comparing the percent positivity rate of pooled testing to that of individual testing.
- Calculate the percent positivity rate among patient specimens during specimen pooling (P_{pools}) on a daily basis using a moving average of the data from the previous 7-10* days of testing.

  (P_{pools}) = (Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools over chosen date range ÷ Total number of patient specimens tested in pools over chosen date range) X 100
- Compare P_{pools} to $P_{individual}$. If P_{pools} is less than 85% of $P_{individual}$, ($P_{pools} < 0.85 \times P_{individual}$), it is recommended that the pool size be reassessed and adjusted to maximize pooling efficiency (if necessary), according to the criteria in Table 10.
- To ensure maximum pooling efficiency, it is recommended that $n_{maxefficiency}$ be reassessed periodically while sample pooling is implemented by the laboratory.

If historical laboratory data for individual specimens is unavailable:

- After initiating a pooling strategy, evaluate the performance of pooled testing by calculating the initial percent positivity rate for pooled specimens ($P_{pools\text{-}initial}$). ($P_{pools\text{-}initial}$) is the percent positivity rate for pooled specimens for the first 7-10* days of pooled testing.
- Calculate the initial percent positivity rate for individual specimens from pool testing ($P_{pools\text{-}initial}$) from the first 7-10* days of testing. $P_{pools\text{-}initial}$ = (Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools in first 7-10* days ÷ Total number of patient specimens tested in pools in the first 7-10* days) X 100.
  - If $P_{pools\text{-}initial}$ is greater than 25%, pooling of patient specimens is not efficient and should be discontinued until the percent positivity rate decreases.
  - If $P_{pools\text{-}initial}$ is less than or equal to 25%, pooling of patient specimens can be continued.
- Continue to monitor pooling strategy by calculating the percent positivity rate among patient specimens during specimen pooling ($P_{pools\text{-}x}$) for subsequent 7-10* day periods. ($P_{pools\text{-}x}$) should be updated daily using a moving average.
- Compare $P_{pools\text{-}x}$ to $P_{pools\text{-}initial}$. If $P_{pools\text{-}x}$ is less than 90% of $P_{pools\text{-}initial}$ ($P_{pools\text{-}x} < 0.90 \times P_{pools\text{-}initial}$), it is recommended that the pool size be reassessed and potentially adjusted to maximize pooling efficiency.
- To ensure maximum pooling efficiency, it is recommended that $n_{maxefficiency}$ be reassessed periodically while sample pooling is implemented by the laboratory.

* 7-10 days is recommended for calculating $P_{individual}$, P_{pools}, $P_{pools\text{-}initial}$, and $P_{pools\text{-}x}$. Laboratories should determine if 7-10 days is appropriate by taking into consideration laboratory testing volume and percent positivity. If the number of individual or pooled positive results collected during a given time frame is less than 10, $P_{individual}$, P_{pools}, $P_{pools\text{-}initial}$, and $P_{pools\text{-}x}$ may not be representative of the percent positivity in the testing population. Consider extending the data collection time period to increase the number of positives evaluated.