Alinity m

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SARS-CoV-2 AMP Kit

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NOTE: Changes Highlighted

CUSTOMER SERVICE: 1-800-553-7042

CUSTOMER SERVICE INTERNATIONAL: CALL YOUR ABBOTT REPRESENTATIVE

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 SARS-CoV-2 AMP Kit

INTENDED USE

The Alinity m SARS-CoV-2 assay is a real-time (rt) reverse transcriptase (RT) polymerase chain reaction (PCR) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal (NP) and oropharyngeal (OP) swabs collected by a healthcare provider, from patients who are suspected of COVID-19 infection.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal and oropharyngeal swabs 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.

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

The Alinity m SARS-CoV-2 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 SARS-CoV-2 AMP Kit are laboratory and healthcare professionals.

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 the SARS-CoV-2 nasopharyngeal (NP) and oropharyngeal (OP) swabs collected by a healthcare worker, from patients suspected of COVID-19 by their health care provider.

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 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 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. 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.

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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

Kit Contents

Alinity m SARS-CoV-2 AMP Kit (List No. 09N78-090)

Alinity m SARS-CoV-2 AMP Kit (List No. 09N78-090) 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.

	Quantity
Σ	192 tests
Alinity m SARS-CoV-2 AMP TRAY 1	4 trays / 48 tests each
Alinity m SARS-CoV-2 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 SARS-CoV-2 AMP TRAY 1.

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]

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.

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 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 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.
- Positive results are indicative of the presence of 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 SARS-CoV-2 assay and the Alinity m System should perform this procedure.

Handling Precautions for Specimens

- The Alinity m SARS-CoV-2 assay is only for use nasopharyngeal and oropharyngeal swabs 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.
- 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

Specimen Collection and Storage

Human nasopharyngeal and oropharyngeal swab specimens may be used with the Alinity m SARS-CoV-2 assay. Refer to the European Centre for Disease Prevention and Control at ecdc.europa.eu/en/novel-coronavirus/laboratory-support.[6]

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 potential SARS-CoV-2 specimens.

Preparation for Analysis

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

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

If needed, centrifuge specimens 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 before loading onto the Alinity m System.

IMPORTANT: If present, swab 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 IFU 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. 09N78-090)

Materials Required But Not Provided

- 09N78-080 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
- 09N78-01F (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*
- 09N49-010 Alinity m Transport Tube Pierceable Capped
- 09N49-011 Alinity m Transport Tube
- 09N49-013 Alinity m Aliquot Tube

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

- Sealable plastic bags

Procedural Precautions

- Read the instructions in this IFU 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 IFU for details. Refer to the Alinity m SARS-CoV-2 CTRL Kit IFU 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 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 (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 instrument.

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 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 – 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.

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 and Positive CTRL are recommended to be tested, at or above the minimum frequency of 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. 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 IFU. 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 a 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.

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 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 positive 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 IFU.
- 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 IFU).
- 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.
- 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 and should not be used with this assay.
- 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.

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 one of the target regions.

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 one of the target regions. 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.

Overall, these analyses predict no impact to the detection of SARS-CoV-2 strains included in the GISAID and GenBank databases.

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%).

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 3.** All positive samples were detected. All negative samples were not detected.

Table 3. 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 testing lab). A total of 104 specimens were analyzed by both Abbott RealTi*m*e SARS-CoV-2 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 4**.

Table 4. Clinical Evaluation of the Alinity m SARS-CoV-2 Assay

		Alinity m SARS-CoV-2	
		Positive	Negative
Abbott RealTi*m*e SARS-CoV-2	Positive	47	0
	Negative	2[a]	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)

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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
	Sufficient for
	Use By
EC REP	Authorized Representative in the European Community
	Manufacturer

TECHNICAL ASSISTANCE

For technical assistance, call Abbott Technical Services at 1-800-553-7042 (within the US) or +49-6122-580 (outside the US), 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. 09N78-090)

Alinity m SARS-CoV-2 CTRL Kit (List No. 09N78-080)

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

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