Alinity m

HIV-1 AMP Kit

en
HIV-1
REF 08N45-095
53-608158/R1

Created July 2020

Rx ONLY

REF 08N45-095
53-608158/R1

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.

NAME

Alinity m HIV-1 AMP Kit

INTENDED USE

The Alinity m HIV-1 assay is an *in vitro* reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantification of Human Immunodeficiency Virus type 1 (HIV-1) RNA on the automated Alinity m System in human plasma from HIV-1 infected individuals. The Alinity m HIV-1 assay is intended for use in the clinical management of HIV-1 infected individuals in conjunction with clinical presentation and other laboratory markers.

The Alinity m HIV-1 assay may be used to monitor disease prognosis by measuring the baseline plasma HIV-1 RNA level and to assess response to antiretroviral treatment by measuring changes in plasma HIV-1 RNA levels.

The results from the Alinity m HIV-1 assay must be interpreted within the context of all relevant clinical and laboratory findings.

This assay is not intended for use in screening blood, blood products, tissue or organ donors for HIV.

The assay is not intended as an aid in diagnosis or to confirm HIV-1 infection.

SUMMARY AND EXPLANATION OF THE TEST

Human Immunodeficiency Virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS).[1–3] It can be transmitted through sexual contact, exposure to infected blood or blood products, or from an infected mother to the fetus.[4] Acute HIV syndrome, characterized by flu-like symptoms, develops three to five weeks after initial infection and is associated with high levels of viremia.[5,6] Within four to six weeks of the onset of symptoms, HIV specific immune response is detectable.[7,8] After seroconversion, viral load in peripheral blood declines and most patients enter an asymptomatic phase that can last for years.[9]

Quantitative measurement of HIV-1 RNA levels in plasma has been shown to be an essential parameter in prognosis and management of HIV-1 infected individuals.[10–15] Viral load monitoring of HIV-1 levels is considered the most reliable indicator of initial and sustained response to anti-retroviral therapy (ART) and should be obtained at the entry into care, at initiation and during therapy.[16–18]

Decisions regarding changes in antiretroviral therapy are guided by monitoring changes in plasma HIV-1 viral load levels over time. The minimal change in viral load considered to be reflective of a significant change associated with antiretroviral therapy within the first 2 to 8 weeks is equal to 0.5 Log Copies/mL reduction.[17] In addition, optimal viral suppression is considered when the viral load remains persistently below the lower limit of detection.[17,18]

Virological response failure, which is suggestive of resistance to current antiretroviral therapies, is considered to occur when there is a persistently elevated HIV-1 viral load according to guidelines.[17,19,20] If resistance is confirmed, the ART is revised to use higher-tiered drugs. HIV-1 RNA levels in plasma can be quantitated by nucleic acid amplification.[21–23]

The Alinity m HIV-1 assay will be used to measure the levels of HIV-1 RNA isolated from patient plasma and to determine changes in viral load, which, in conjunction with clinical presentation and other laboratory markers, is indicative of the effectiveness of antiviral therapy.

The RNA genome of HIV-1 exhibits a high degree of genetic variability.[24] High-frequency occurrence of natural polymorphisms within primer/probe binding sites can result in inefficient hybridization and lead to under-quantitation or lack of detection for a nucleic acid test method based on the PCR technology. Therefore, to ensure assay robustness, the Alinity m HIV-1 assay is designed to target two highly conserved sequences within the HIV-1 genome.

In addition to the HIV-1 primer/probe sets, the Alinity m HIV-1 assay utilizes an internal control (IC) primer/probe set for amplification and detection of an IC target sequence, which is not related to HIV-1. The IC probe is labeled with a different fluorophore than the HIV-1 probes. This allows for simultaneous detection and discrimination of both the HIV-1 and IC amplified products within the same reaction vessel.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Alinity m HIV-1 assay requires 3 separate assay specific kits:

- Alinity m HIV-1 AMP Kit (08N45-095) consisting of 2 types of multi-well assay trays. The amplification trays (AMP Trays) contain lyophilized, unit-dose RT-PCR amplification/detection reagents and lyophilized, unit-dose IC in separate wells, and the activation trays (ACT Trays) contain liquid activation reagent. The intended storage condition for the Alinity m HIV-1 AMP Kit is 2 to 8°C.
- Alinity m HIV-1 CAL Kit (08N45-075) consisting of two calibrator levels, each supplied as liquid in single-use tubes. The intended storage condition for the Alinity m HIV-1 CAL Kit is −25 to −15°C.
- Alinity m HIV-1 CTRL Kit (08N45-085) consisting of negative controls, low-positive controls and high-positive controls, each supplied as liquid in single-use tubes. The intended storage condition for the Alinity m HIV-1 CTRL Kit is −25 to −15°C.

The Alinity m HIV-1 assay utilizes real-time reverse transcription polymerase chain reaction (RT-PCR) to amplify and detect HIV-1 RNA genomic sequences that have been extracted from human plasma specimens. The steps of the Alinity m HIV-1 assay consist of sample preparation, RT-PCR assembly, amplification/detection, and result calculation and reporting. All steps of the Alinity m HIV-1 assay procedure are executed automatically by the Alinity m System. Manual dilutions may be performed for low-volume specimens to meet the minimum volume requirement, and for high-titer specimens above the upper limit of quantitation (ULoQ).

The Alinity m System is designed to be a random access analyzer that can perform the Alinity m HIV-1 assay in parallel with other Alinity m assays on the same instrument.

HIV-1 RNA from human plasma is extracted using the Alinity m Sample Prep Kit 2, Alinity m Lysis Solution, and Alinity m Diluent Solution. The Alinity m System employs magnetic microparticle technology to facilitate nucleic acid capture, wash, and elution. The resulting purified RNA is then combined with liquid unit-dose Alinity m HIV-1 activation reagent and lyophilized unit-dose Alinity m HIV-1 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 of HIV-1.

At the beginning of the Alinity m HIV-1 sample preparation process, a lyophilized unit-dose IC on the AMP Tray is rehydrated by the Alinity m System and delivered into each sample preparation reaction. The IC is then processed through the entire sample preparation and RT-PCR procedure along with the specimens, calibrators and controls to demonstrate proper sample processing and validity.

The Alinity m HIV-1 amplification/detection reagents consist of enzymes, primers, probes and activation reagents that enable reverse transcription, polymerization, and detection. The Alinity m HIV-1 amplification/detection reagent also contains Uracil-DNA Glycosylase (UDG) as a contamination control for amplicons containing uracil, which may be present in molecular laboratories.

An HIV-1 calibration curve is required for determination of HIV-1 RNA concentration. Two levels of calibrators are processed through sample preparation and RT-PCR to generate the calibration curve. The concentration of HIV-1 RNA in controls and concentration/detection of HIV-1 RNA in specimen is then determined from the stored calibration curve.

Assay controls are tested at or above an established minimum frequency to help ensure that instrument and reagent performance remains satisfactory. During each control event, a negative control, a low-positive control, and a high-positive control are processed through sample preparation and RT-PCR procedures that are identical to those used for specimens. 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 HIV-1 AMP Kit List No. 08N45-095

The Alinity m HIV-1 AMP Kit is comprised of 2 types of multi-well trays: Alinity m HIV-1 AMP TRAY 1 and Alinity m HIV-1 ACT TRAY 2.

Each Alinity m HIV-1 AMP TRAY 1 (individually packed in a foil pouch with a desiccant bag) contains 48 unit-dose lyophilized amplification reagent wells and 48 unit-dose lyophilized IC wells. One well of each is used per test.

- Amplification reagent wells consist of synthetic oligonucleotides, DNA Polymerase, Reverse Transcriptase, Uracil-DNA Glycosylase, excipient, dNTPs, and 0.1019% ProClin® 950 in a buffered solution with a reference dye.
- Internal control (IC) wells consist of noninfectious Armored RNA® with IC sequences and excipient 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.

Each Alinity m HIV-1 ACT TRAY 2 (individually packed in a foil pouch without a desiccant bag) contains 48 unit-dose liquid activation reagent wells. One reagent well is used per test.

- Activation reagent wells consist of magnesium chloride, potassium chloride, and tetramethyl ammonium chloride. Preservative: 0.15% ProClin 950.

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

WARNINGS AND PRECAUTIONS

IVD

- **For In Vitro Diagnostic Use**

Safety Precautions

The following warnings and precautions apply to:
Alinity m HIV-1 AMP TRAY 1.

CAUTION: This preparation contains human-sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. Components sourced from human blood have been tested and found to be non-reactive by appropriate FDA-licensed, approved, or cleared tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, HBsAg, HIV-1 antigen, 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 safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[25] OSHA Standard on Bloodborne Pathogens,[26] CLSI Document M29-A4,[27] and other appropriate biosafety practices.[28] 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.[25]

Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[28]

Use only supplied or specified required consumables to ensure optimal test performance.

The following warnings and precautions apply to:
Alinity m HIV-1 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.

The following warnings and precautions apply to:
Alinity m HIV-1 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 29CFR1910.1200 (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 HIV-1 AMP Kit	On dry ice

Reagent Storage

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

	Storage Temperature	Maximum Storage Time
Unopened	2 to 8°C	Until expiration date
Onboard	System Temperature	30 days (not to exceed expiration date)

Reagent Handling

- Do not use reagents that have been damaged.
- 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 instrument. 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.
- For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity m System Operations Manual, Section 8.

Indications of Reagent Deterioration

- Deterioration of the reagents may be indicated when a calibration or control error occurs or controls are repeatedly out of the specified ranges.
- Reagents are shipped on dry ice and are stored at 2 to 8°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 HIV-1 assay application specification file must be installed on the Alinity m System prior to performing the assay.

For detailed information on viewing and editing the customizable assay parameters, refer to the Alinity m System Operations Manual, Section 2.

For information on printing assay parameters, refer to the Alinity m System Operations Manual, Section 5.

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

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Type

The specimen type listed below can be used with this assay on the Alinity m System. For the Alinity m HIV-1 assay, only use the collection tubes as described in the following table. Alinity m HIV-1 assay performance with other specimen types or collection tubes has not been evaluated.

Specimen Type[a]	Blood Collection Tubes
Plasma	Acid Citrate Dextrose (ACD) K_2 EDTA K_3 EDTA Plasma Preparation Tube (PPT)[b]

[a] The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to use the correct specimen types in the assay.

[b] The Plasma Preparation Tube is a gel tube.

Specimen Storage

Specimen	Temperature	Maximum Storage Time	Special Instructions
Whole Blood	2 to 8°C	2 days	Whole blood may be stored between draw and plasma separation.
	15 to 30°C	1 day	
Plasma	2 to 8°C	3 days	Plasma may be stored in primary or secondary tubes after separation from blood cells.
	15 to 30°C	1 day	
	−20°C	60 days	Plasma may be stored frozen in primary gel tubes (PPT) or secondary tubes after separation from blood cells.[a] Plasma from non-gel tubes must be transferred to secondary tubes prior to storage.[a]
	−70°C or colder	Longer storage	

[a] Avoid more than 2 freeze-thaw cycles.

Specimen Shipping

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.

Preparation for Analysis

Freshly Drawn Whole Blood Specimens:

- Follow the specimen collection tube manufacturer instructions for blood collection and centrifugation. Separate plasma from cells by centrifugation.
- After centrifugation, plasma may be stored on the blood cells (in tube with or without gel) prior to being loaded onto the Alinity m System or used for dilution.

  NOTE: Specimens stored on the blood cells cannot be frozen without a gel.
- Plasma specimens may also be transferred to a secondary tube for storage prior to being loaded onto the Alinity m System or used for dilution. If longer storage is required, specimens in the secondary tubes may be stored frozen.

Frozen Specimens: Primary Gel Tubes

- Thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours if not processed immediately.
- Vortex each specimen 3 times for 2 to 3 seconds.
- Centrifuge specimens stored in gel tubes at 2000*g* for 5 minutes before loading onto the Alinity m System or before preparing a specimen dilution. If any clot or debris is observed, transfer the supernatant of the specimen into a new tube. Avoid transferring any debris or clot into the new tube.

Frozen Specimens: Secondary Aliquot Tubes

- Thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours if not processed immediately.
- Vortex each specimen 3 times for 2 to 3 seconds. If any debris is observed, transfer the supernatant of the specimen into a new tube. Avoid transferring any debris into the new tube.
- Alternatively, vortex each specimen 3 times for 2 to 3 seconds, then centrifuge specimens at 2000*g* for 5 minutes, before loading onto the Alinity m System or before preparing a specimen dilution. If any debris is observed, transfer the supernatant of the specimen into a new tube. Avoid transferring any debris into the new tube.

All specimen tubes (primary and secondary tubes) must be labeled with specimen ID barcodes, or must be identified with a specimen ID and rack and position. Refer to the **Assay Procedure** section of this package insert or the Alinity m System Operations Manual, Section 4, for tube sizes. Avoid touching the inside of the cap when opening tubes.

PROCEDURE

Materials Provided

08N45-095 Alinity m HIV-1 AMP Kit

Materials Required but not Provided

- 08N45-075 Alinity m HIV-1 CAL Kit
- 08N45-085 Alinity m HIV-1 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
- 09N50-001 Alinity m Specimen Dilution Kit I[a]
- Alinity m HIV-1 Application Specification File
- Vortex mixer
- Centrifuge capable of 2000*g*
- 09N49-001 Alinity m LRV Tube[a]
- Calibrated pipettes capable of delivering 10 to 1000 µL[a]
- Aerosol barrier pipette tips for 10 to 1000 µL pipettes[a]
- 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
- 09N49-012 Alinity m Pierceable Cap
- 09N49-013 Alinity m Aliquot Tube

[a] These items are used in the **Specimen Dilution Procedure** if dilution is required.

For information on materials required for operation of the instrument, 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.

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 HIV-1 AMP TRAY 1 is tapped prior to loading on the Alinity m System per instructions in the **Assay Procedure** section.
- Ensure the Alinity m HIV-1 ACT TRAY 2 is centrifuged prior to loading on the Alinity m System per instructions in the **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 calibrator, a control, or a reagent. Always use powder-free gloves.
- The use of the Alinity m HIV-1 CAL and CTRL Kits is integral to the performance of the Alinity m HIV-1 assay. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details. Refer to the Alinity m HIV-1 CAL Kit package insert and/or Alinity m HIV-1 CTRL Kit package insert for preparation and usage.
- The Alinity m HIV-1 calibrator and control reagents are contained in single-use tubes with pierceable caps. Avoid contamination or damage to the caps after removal from their original packaging. Discard tubes after use.

Assay Procedure

Prior to loading on the Alinity m System, hold the AMP TRAY 1 by the edges with the label facing up and tap 3 times on the bench.

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

1. Load the ACT TRAY 2 onto the plate adapter (eg, Eppendorf Catalog No. 022638955).
2. Load the plate adapter (with the ACT TRAY 2) 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 ACT TRAY 2 to the Alinity m Assay Tray Carriers. Take care to minimize disturbance to the ACT TRAY 2. Load the tray carriers per the Alinity m System Operations Manual, Section 5.
4. If disturbance occurs during transfer that could potentially introduce bubbles (eg, dropping, bumping, inversion of the ACT TRAY 2), re-centrifuge the ACT TRAY 2.
5. Proceed with the **Reagent and sample inventory management** procedure 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 calibration and control status. If recalibration or control testing is required, refer to the **QUALITY CONTROL PROCEDURES** section. Calibrators and/or controls may be tested separately or with specimens.

From the Specimen tab on the Create Order screen, enter the specimen ID (SID), select the assay (HIV-1).

The Alinity m System will track the onboard storage time of amplification reagents, calibrators, controls, and specimens while on the instrument. The Alinity m System will not allow the use of amplification reagents, calibrators, controls, or process specimens that have exceeded the allowable onboard storage time.

Specimen tubes need to meet the requirements for minimum sample volume and the use of caps when loaded on the Alinity m System. Blood collection tubes with separated plasma and specimen aliquot tubes may be placed on the Alinity m Universal Sample Rack (sample rack) onboard the system for up to 4 hours prior to processing.

Tube Type[a]	List No.	Minimum Plasma Volume Required	Cap Requirement on Instrument
Blood Collection Tube (Primary Tube)			
Blood collection tubes with minimum inner diameter 10.0 mm	NA	11.0 mm[b] above the gel or blood cells	Uncapped
Specimen Aliquot Tube (Secondary Tube)			
Alinity m Aliquot Tube	09N49-013	0.75 mL	Capped[c] or uncapped
Alinity m Transport Tube	09N49-011	1.0 mL	Uncapped
		0.75 mL	Capped[c]
Alinity m Transport Tube Pierceable Capped	09N49-010	1.0 mL	Uncapped
		0.75 mL	Capped
Other aliquot tubes with minimum inner diameter 10.0 mm	NA	0.9 mL for tubes with 10.6 mm or less inner diameter. 1.4 mL for tubes with 13.2 mm or less inner diameter.	Uncapped

[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] Represents requirement for minimum column height of plasma above the gel/blood cells in the primary tube. The minimum volume in milliliters can be calculated using the inner diameter (ID in mm) of the tube in the formula: Minimum Volume = 0.00864 x ID^2.

[c] Alinity m Pierceable Cap, List No. 09N49-012, is the only type of cap that can be used when loaded on the Alinity m System.

Prior to loading the specimen tubes on the Alinity m System:

- Ensure individual specimen tubes are labeled correctly with specimen ID barcodes.
- Inspect specimens for bubbles and foam. Specimens should be free of bubbles and foam. If found, remove them with a new sterile pipette tip for each tube to prevent cross-contamination.

Specimen Dilution Procedure (Optional)

Specimens may be diluted manually for testing on the Alinity m System using the Alinity m Specimen Dilution Kit I per the table below.

Low volume specimens with a minimum of 260 µL to 749 µL volume available for Alinity m HIV-1 testing can be diluted 1 : 2.5 in a total volume of 0.65 mL, which is the minimum volume required (0.65 mL) in the Alinity m LRV tube. Specimens with 50 to 259 µL volume available for Alinity m HIV-1 testing can be diluted 1 : 50 in the Alinity m Specimen Diluent Tube to a final volume of 2.5 mL (i.e., > 0.75 mL, the minimum volume required for this tube type). High-titer specimens above the upper limit of quantitation (> ULoQ) can also be diluted 1:50 before testing.

Specimen Dilution Scenario	Available Specimen Volume	Dilution Factor
Low volume	≥ 260 µL to ≤ 749 µL	1 : 2.5
	50 to 259 µL	1 : 50
> ULoQ result	≥ 50 µL	1 : 50

The operator must select the dilution factor in the Specimen tab of the Create Order screen of the Alinity m System software. The system will use the selected dilution factor to automatically calculate and report the result of the neat specimen.

NOTE: Upon dilution, the specimen must be loaded onto the system within 2 hours.

Specimens are diluted with a dilution factor of 2.5, using Specimen Dilution Kit I as follows:

1. Apply a barcode label for the designated specimen ID to an Alinity m LRV Tube.
2. Open a fresh Alinity m Specimen Diluent Tube and transfer 390 µL of Specimen Diluent into the Alinity m LRV Tube.
3. Add 260 µL of the patient specimen into the Alinity m LRV Tube.
4. Cap the tube, vortex 3 times for 2 to 3 seconds, and tap upright on the bench to bring liquid to the bottom of the tube.
5. Remove the cap from the Alinity m LRV Tube. Inspect the fluid in the tube and remove any bubbles if found.
6. Place the Alinity m LRV Tube in the sample rack.

Specimens are diluted with a dilution factor of 50, using Specimen Dilution Kit I as follows:

1. Apply a barcode label for the designated specimen ID to an unused Alinity m Specimen Diluent Tube. Remove the cap from the Alinity m Specimen Diluent Tube. Save the cap for later use.
2. Add 50 µL of the patient specimen to the Alinity m Specimen Diluent Tube.
3. Cap the tube, vortex 3 times for 2 to 3 seconds, and tap upright on the bench to bring liquid to the bottom of the tube.
4. Load the tube directly onto the sample rack. The cap may remain on the tube.

NOTE: Do not use an Alinity m Specimen Diluent Tube that has crystals or liquid on the outside of the tube because this may be evidence of leakage.

QUALITY CONTROL PROCEDURES

Assay Calibration

For instructions on performing an assay calibration, refer to the Alinity m System Operations Manual, Section 6.

Lot-specific concentration values for assay calibrators and controls are available via: Abbott Mail, the Abbott Molecular customer portal www.molecular.abbott/portal, and from your Abbott Representative.

When an assay calibration is being performed:

- Lot-specific concentration values can be automatically imported to the Alinity m System via Abbott Mail upon scanning the calibrators (HIV-1 CAL A and HIV-1 CAL B) or controls (HIV-1 NEG CTRL, HIV-1 LOW POS CTRL, and HIV-1 HIGH POS CTRL) tube barcodes.
- Lot-specific concentration values can also be obtained from the Abbott Molecular customer portal or provided by your Abbott Representative and imported via a USB drive.

For instructions on creating a test order for calibration and loading calibrators on the instrument, refer to the Alinity m System Operations Manual, Section 5.

A calibration curve is required to quantitate the HIV-1 RNA concentration. At a minimum, 1 Alinity m HIV-1 CAL A tube and 1 Alinity m HIV-1 CAL B tube from the Alinity m HIV-1 CAL Kit are required for performing an assay calibration on the Alinity m System. The Alinity m System will process 3 replicates from each calibrator tube. The output data of the 2 calibrators will be used to generate a calibration curve (lot-specific HIV-1 concentration versus the threshold cycle [C_t] at which a reactive level of fluorescent signal is detected). The calibration curve slope and intercept are calculated and stored on the instrument.

Once an assay calibration is valid and stored, all subsequent samples may be tested without further calibration unless any of the following situations occur:

- An Alinity m HIV-1 AMP Kit with a new lot number is used.
- An Alinity m Sample Prep Kit 2 or Alinity m Lysis Solution with a new lot number is used.
- The assay calibration has expired.
- A new version of the Alinity m HIV-1 Application Specification File is installed.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed. Contact your Abbott Representative for further instructions.

Detection of Inhibition

An IC C_t assay validity parameter is established during a calibration run.

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

The median IC C_t value from calibrator samples establishes an IC C_t validity range for subsequently processed specimens and controls.

A Message Code is assigned to a specimen or control when its IC C_t value is outside of the IC C_t validity range. When the IC C_t value exceeds the upper limit of the IC C_t validity range, abnormal assay conditions, such as inhibition, are indicated.

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

Negative and Positive Controls

An Alinity m HIV-1 Negative CTRL, Low Positive CTRL, and High Positive 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. The assay controls are also tested following calibrators and valid results for controls are required to establish a new calibration curve.

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 HIV-1 must not be detected in the negative control. HIV-1 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 a set of assay controls are being processed, the lot-specific concentration values of the Alinity m HIV-1 Low Positive CTRL and Alinity m High Positive CTRL can be:

- Automatically imported to the Alinity m System via Abbott Mail upon scanning the barcode labels on control tubes (HIV-1 LOW POS CTRL and HIV-1 HIGH POS CTRL).
- Obtained from the Abbott Molecular customer portal or provided by your Abbott Representative and imported to the Alinity m System via a USB drive.

RESULTS

Calculation

Quantitative viral load results are reported for plasma specimens with HIV-1 viral concentrations within the assay's quantitation range. The concentration of HIV-1 RNA in a plasma specimen is calculated from the calibration curve by the system software. The Alinity m System reports the results in Copies/mL, Log [Copies/mL], IU/mL or Log [IU/mL].

1 International Unit (IU) = 0.61 Copies for HIV-1. 1 Copy = 1.63 IUs.

Refer to the Alinity m System Operations Manual for configuration of result units.

For plasma specimens tested with the Specimen Dilution Procedures, the Alinity m System calculates and reports the neat concentration (ie, prior to dilution), by using the dilution factor selected by the user.

Interpretation of Results

Undiluted Specimens

The Alinity m System will report a Result and an Interpretation for each specimen. If applicable, message codes or flags will also be displayed.

Diluted Specimens

For specimens diluted 1:2.5 or 1:50, the Alinity m System reports a viral load result, a viral load interpretation (if applicable), and a DIL flag indicating that the specimen has been diluted. The quantitative results represent the HIV-1 RNA concentration in the specimen prior to dilution.

For diluted specimens with analyte concentration below the detection limit, no result is reported, and a message code (9827) is displayed. These specimens cannot be interpreted as "Target not detected" and should be retested with a new neat specimen. For diluted specimens with a result of < LLoQ: it is recommended to collect and test another neat specimen.

Note: The LLoQ of Alinity m HIV-1 is 20 Copies/mL (1.30 Log Copies/mL) for specimens tested without dilution. Therefore, the lowest HIV-1 RNA concentration that can be reported for a specimen that is tested diluted is 50 Copies/mL (1.70 Log Copies/mL) for the 1:2.5 dilution procedure, and 1000 Copies/mL (3.00 Log Copies/mL) for the 1:50 dilution procedure.

The ULoQ of Alinity m HIV-1 is 10,000,000 Copies/mL (7.00 Log Copies/mL) for specimens tested without dilution. Therefore, the HIV-1 RNA concentration of a specimen that is tested diluted and returns a result of > ULoQ is > 25,000,000 Copies/mL (7.40 Log Copies/mL) for the 1:2.5 dilution procedure, and > 500,000,000 Copies/mL (8.70 Log Copies/mL) for the 1:50 dilution procedure.

Result and Interpretation

Result	Interpretation
Not Detected	Target not detected
< LLoQ	Detected < LLoQ
20 Copies/mL to ≤ ULoQ (1.30 Log Copies/mL to ≤ ULoQ)	Detected and quantified
> ULoQ	> ULoQ[a]

[a] Specimens tested neat or with 1:2.5 dilution procedure that have >ULoQ interpretation may be retested using the 1:50 dilution procedure to determine a result within the quantitation range.

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

- Optimal performance of this test requires appropriate specimen collection and handling (refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert.)
- Human plasma (ACD, K_2 EDTA, K_3 EDTA, and PPT) specimens may be used with the Alinity m HIV-1 assay. The use of other anticoagulants have not been evaluated.
- Performance has not been established with serum specimens.
- Debris within plasma specimens (eg, clots, fibrin strands) may interfere with sample processing.
- Diluted specimens must be tested within 2 hours after dilution and should not be frozen.
- If the HIV-1 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- HIV-1 RNA concentration (i.e., number of virus particles present in the samples) may be affected by patient factors (age, presence of symptoms) and/or stage of infection.
- Though rare, mutations within the highly conserved regions of a viral genome detected by Alinity m HIV-1 may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus. To ensure assay robustness, the Alinity m HIV-1 assay is designed to target two highly conserved sequences within the HIV-1 genome.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to evaluate technology differences. Users should follow their own specific policies/procedures.
- The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the calibrators, positive controls, or specimens must be controlled by good laboratory practice and careful adherence to the procedures specified in this package insert.
- Assay linearity for HIV-1 Group M subtype BF, subtype H and Group N quantification was tested up to 10,000 Copies/mL for subtype BF, 300,000 Copies/mL for subtype H, and 1,000,000 Copies/mL for Group N, respectively.

SPECIFIC PERFORMANCE CHARACTERISTICS

Limit of Detection

The limit of detection (LOD) was determined by testing dilutions of World Health Organization (WHO) 3rd HIV-1 International Standard (NIBSC code: 10/152; group M subtype B) prepared in HIV-1 negative human plasma. Testing for each HIV-1 RNA concentration was performed with 4 lots of amplification reagents across multiple days. The results, representative of the analytical sensitivity performance of Alinity m HIV-1, are summarized in **Table 1**.

Table 1. Alinity m HIV-1 Limit of Detection (LOD)

HIV-1 RNA (Copies/mL)	No. of Valid Replicates	No. of Detected Replicates	Detection Rate (%)
40.00	94	94	100.0
20.00	90	87	96.7
15.00	90	87	96.7
12.50	90	85	94.4
10.00	91	80	87.9
7.50	87	69	79.3
5.00	91	63	69.2

Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95% probability (LOD by Probit) was 13.99 Copies/mL (95% CI 11.69 Copies/mL to 19.22 Copies/mL).

The LOD of Alinity m HIV-1 is 20 Copies/mL (1.30 Log Copies/mL).

Limit of Detection Across Groups and Subtypes

HIV-1 group M (subtypes A, BF, C, D, CRF01-AE, F, CRF02-AG, G and H), group O and group N panels were prepared by diluting HIV-1 viral stock or HIV-1 positive clinical specimen to 3 different concentrations in HIV-1 negative human plasma. Testing for each HIV-1 RNA concentration was performed with 4 lots of amplification reagents across multiple days. The results, representative of the analytical sensitivity performance of Alinity m HIV-1 for group M subtypes, group O and group N, are summarized in **Table 2**. These results demonstrate the ability of Alinity m HIV-1 to detect HIV-1 group M (subtypes A, BF, C, D, CRF01-AE, F, CRF02-AG, G and H), group O and group N at and above claimed LOD (20 Copies/mL) with a detection rate of 95.0% or greater.

Table 2. Alinity m HIV-1 Limit of Detection (LOD) Across Groups and Subtypes

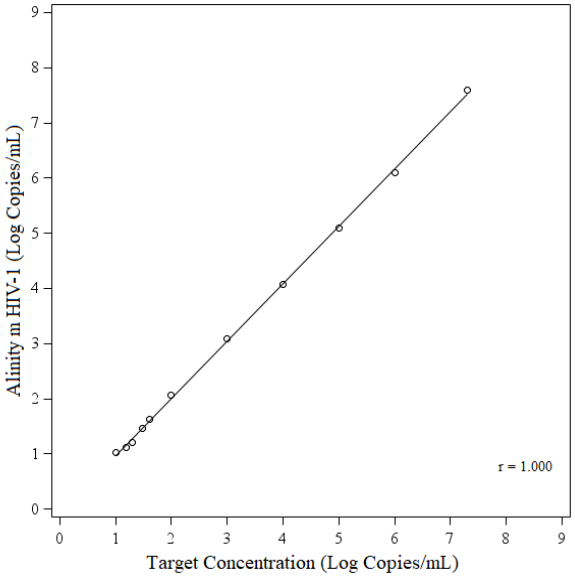
Group/ Subtype	HIV-1 RNA (Copies/mL)	No. of Valid Replicates	No. of Detected Replicates	Detection Rate (%)
Group M, subtype A	40	93	93	100.0
	20	95	94	98.9
	10	94	88	93.6
Group M, subtype BF	40	94	94	100.0
	20	95	95	100.0
	10	96	82	85.4
Group M, subtype C	40	95	95	100.0
	20	95	95	100.0
	10	94	92	97.9
Group M, subtype D	40	95	95	100.0
	20	95	94	98.9
	10	96	86	89.6
Group M, CRF01-AE	40	93	93	100.0
	20	96	96	100.0
	10	94	89	94.7
Group M, subtype F	40	94	94	100.0
	20	96	95	99.0
	10	93	88	94.6
Group M, CRF02-AG	40	93	93	100.0
	20	94	94	100.0
	10	94	90	95.7
Group M, subtype G	40	96	96	100.0
	20	93	93	100.0
	10	91	84	92.3
Group M, subtype H	40	92	92	100.0
	20	95	95	100.0
	10	91	89	97.8
Group O	40	90	90	100.0
	20	92	92	100.0
	10	92	91	98.9
Group N	40	96	96	100.0
	20	92	92	100.0
	10	95	95	100.0

Linear Range

Linearity of Alinity m HIV-1 was assessed by testing a dilution series of an HIV-1 viral stock representing group M subtype B in negative human plasma, consisting of 11 panel members spanning from 10 Copies/mL to 20,000,000 Copies/mL.

Representative results for Alinity m HIV-1 linearity performance are shown in **Figure 1**. Alinity m HIV-1 was linear across the range of HIV-1 RNA concentrations tested (from 10 Copies/mL to 20,000,000 Copies/mL).

Figure 1. Linearity[a]

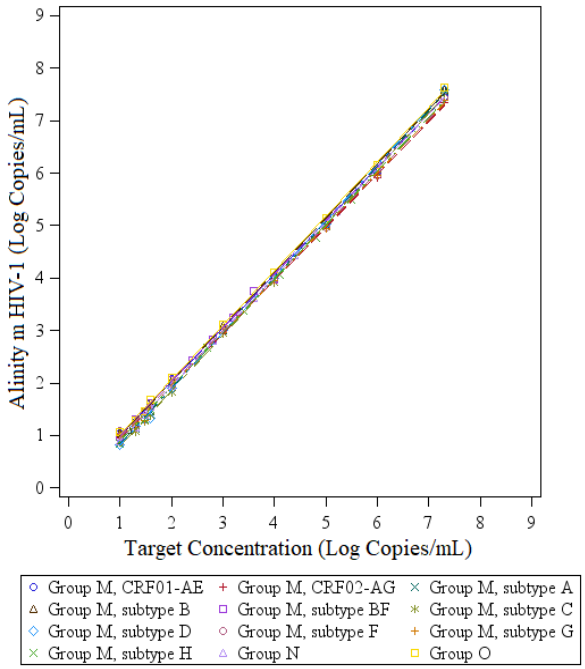


[a]The markers in the plot represent the mean Alinity m HIV-1 concentration (in Log Copies/mL) for each panel member.

Linearity Across Groups and Subtypes

Linearity of Alinity m HIV-1 for HIV-1 group M (subtypes A, BF, C, D, CRF01-AE, F, CRF02-AG, G and H), group O and group N was confirmed by testing a dilution series consisting of 10 panel members for each group/subtype, prepared using HIV-1 cultured virus or HIV-1 positive clinical specimen in HIV-1 negative human plasma. Representative results for Alinity m HIV-1 linearity performance for group M (subtypes A, BF, C, D, CRF01-AE, F, CRF02-AG, G and H), group O and group N, along with results for group M subtype B (see Linear Range section), are shown in **Figure 2**. Alinity m HIV-1 was linear across the range of HIV-1 RNA concentrations tested for group M (subtypes A, B, BF, C, D, CRF01-AE, F, CRF02-AG, G and H), group O and group N (r value ranging from 0.999 to 1.000)

Figure 2. Linearity across Groups and Subtypes.



Precision

Alinity m HIV-1 was designed to achieve a within-laboratory standard deviation (SD) of less than or equal to 0.25 Log Copies/mL of HIV-1 RNA from 2.3 to 7.0 Log Copies/mL (200 to 10,000,000 Copies/mL), and less than or equal to 0.46 Log Copies/mL at three times the lower limit of quantitation (LLoQ) or lower.

Precision of Alinity m HIV-1 was determined by analyzing an 8-member plasma panel, which was prepared by diluting an HIV-1 viral stock into HIV-1 negative human plasma. Each panel member was tested in 5 replicates, twice each day for 12 days, on 3 Alinity m Systems with 3 Alinity m HIV-1 AMP Kit lots by 3 operators.

The results, representative of the precision of Alinity m HIV-1, are summarized in **Table 3**.

Table 3. Precision

Panel Member	N[a]	Mean Conc (Log Copies/mL)	Within-Run Component		Between-Run Component		Between-Day Component		Within-Laboratory[b]		Between-Instrument Component		Total[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
8	349	7.34	0.04	0.5	0.01	0.2	0.00	0.1	0.04	0.6	0.08	1.1	0.09	1.2
7	347	6.02	0.04	0.7	0.00	0.0	0.01	0.2	0.04	0.7	0.05	0.8	0.07	1.1
6	348	5.04	0.04	0.8	0.02	0.3	0.01	0.2	0.04	0.9	0.02	0.5	0.05	1.0
5	353	4.04	0.05	1.3	0.00	0.0	0.01	0.3	0.05	1.3	0.03	0.8	0.06	1.5
4	353	3.11	0.05	1.5	0.01	0.5	0.01	0.2	0.05	1.6	0.04	1.2	0.06	2.0
3	353	2.40	0.09	3.6	0.02	1.0	0.00	0.0	0.09	3.7	0.04	1.5	0.10	4.0
2	352	1.87	0.15	8.2	0.00	0.0	0.03	1.5	0.16	8.4	0.04	1.9	0.16	8.6
1	353	1.34	0.27	19.8	0.02	1.7	0.00	0.0	0.27	19.9	0.05	3.4	0.27	20.2

[a] Number of valid replicates.
[b] Within-Laboratory includes Within-Run, Between-Run, and Between-Day components.
[c] Total includes Within-Run, Between-Run, Between-Day, and Between-Instrument components.

Performance with HIV-1 negative specimens

The specificity of Alinity m HIV-1 was determined by testing 250 HIV-1 negative plasma specimens from individual donors. HIV-1 RNA was not detected in any of the specimens tested (specificity 100.0%; 95% CI: 98.5 to 100.0%).

Analytical Specificity – Potential Cross-Reactants

The analytical specificity of Alinity m HIV-1 was evaluated with a panel of microorganisms (**Table 4**) in HIV-1 negative plasma, positive plasma containing 60 Copies/mL HIV-1 RNA and positive plasma containing 200 Copies/mL HIV-1 RNA. No cross-reactivity or interference in the performance of Alinity m HIV-1 was observed in the presence of the tested microorganisms.

Table 4. Microorganisms

Viruses
Adenovirus Type 5
BK Polyomavirus
Cytomegalovirus
Dengue Virus 1
Dengue Virus 2
Dengue Virus 3
Dengue Virus 4
Epstein-Barr Virus
GB Virus C/Hepatitis G Virus
Hepatitis A Virus
Hepatitis B Virus
Hepatitis C Virus
Herpes Simplex Virus 1
Herpes Simplex Virus 2
Human Herpesvirus 6B
Human Herpesvirus 8
Human Immunodeficiency Virus 2
Human Papilloma Virus 16
Human Papilloma Virus 18
Human T-Lymphotropic Virus Type 2
Human T-Lymphotropic Virus Type 1
Influenza A
Vaccinia Virus
Varicella-Zoster Virus

Table 4. Microorganisms

Bacteria
Chlamydia trachomatis
Mycobacterium gordonae
Mycobacterium smegmatis
Neisseria gonorrhoeae
Propionibacterium acnes
Staphylococcus aureus
Staphylococcus epidermidis
Yeast
Candida albicans

Analytical Specificity - Potentially Interfering Substances

The effects of endogenous substances, the presence of autoimmune disorders and non-HIV serological disorders, and the presence of high levels of therapeutic drugs commonly prescribed for the treatment of HIV-1 and related diseases were evaluated. Potential interference on Alinity m HIV-1 performance was assessed by testing HIV-1 negative samples, and HIV-1 positive samples containing 60 Copies/mL HIV-1 RNA and/or HIV-1 positive samples containing 200 Copies/mL HIV-1 RNA.

No interference was observed in the presence of albumin (60 mg/mL), hemoglobin (2 mg/mL), triglycerides (37 mM), conjugated bilirubin (0.342 mM), unconjugated bilirubin (0.342 mM) or human genomic DNA (2 mg/L) that were introduced in the sample. In addition, no interference was observed in specimens collected from individual donors containing the naturally elevated interfering substances, ie, albumin (>5.1 g/dL), bilirubin (>2 mg/dL), hemoglobin (>2 g/L) or triglycerides (> 325 mg/dL).

No interference was observed for specimens collected from patients with the following autoimmune disorders and non-HIV serological disorders: Systemic Lupus Erythematosus (SLE), Antinuclear antibodies (ANA), Rheumatoid factor (RF), Hepatitis B surface antigen (HBsAg), anti-Human T-lymphotropic virus I/II (anti-HTLV-I/II), anti-Hepatitis C virus (anti-HCV), anti-Human immunodeficiency virus-2 (anti-HIV-2).

No interference was observed in the presence of drug compounds tested in pools that are listed in **Table 5**, at a concentration of 3 times the reported C_{max} or higher.

Table 5. Drug Compounds

Pools Tested	Drug Compounds
1	Abacavir sulfate, Acetaminophen, Acyclovir, Adefovir, Amitriptyline, Amlodipine, Aspirin, Atazanavir, Atenolol, Atorvastatin, Azithromycin, Celecoxib, Cidofovir, Clarithromycin, Clopidogrel
2	Didanosine, Efavirenz, Entecavir, Fluconazole, Fluoxetine, Ibuprofen, Indinavir, Kaletra (Lopinavir and Ritonavir), Lamivudine, Levofloxacin, Maraviroc, Nelfinavir, Nevirapine, Paroxetine
3	Prednisone, Raltegravir, Ribavirin, Rifamate (Rifampin and Isoniazid), Saquinavir, Sertraline, Stavudine, Stribild (Elvitegravir, Cobicistat, Emtricitabine, and Tenofovir), Bactrim (Sulfamethoxazole and Trimethoprim)
4	Darunavir, Ethambutol, Etravirine, Flucytosine, Fluticasone propionate, Furosemide, Hydrochlorothiazide, Levothyroxine, Rifabutin, Rilpivirine, Salmeterol xinafoate, Simeprevir, Sofosbuvir, Telaprevir, Tenofovir alafenamide, Trazodone, Warfarin, Zalcitabine
5	Fosamprenavir, Keflex (Cephalexin), Metformin, Naproxen, Pyrazinamide
6	Tipranavir
7	Ceftriaxone, Ciprofloxacin, Foscarnet, Lisinopril, Peginterferon alfa-2a, Enfuvirtide, Imipramine
8	Cyclosporine, Telbivudine, Valacyclovir, Valganciclovir, Zidovudine, Amphotericin B, Ganciclovir
9	Acetaminophen, Hydrocodone
10	Biotin

Carryover

The carryover rate for Alinity m HIV-1 was determined by analyzing 360 replicates of HIV-1 negative samples processed from alternating positions with high concentration HIV-1 positive samples at 1,000,000 Copies/mL, across a total of 15 runs. HIV-1 RNA was not detected in any of the HIV-1 negative samples, resulting in an overall carryover rate of 0.0% (95% CI: 0.0 to 1.0%).

Alinity m HIV-1 Testing Using Dilution Procedure

The 1:2.5 and 1:50 dilution procedures were evaluated by comparing quantitation of neat samples and samples tested using the Alinity m HIV-1 dilution procedure. Panel members in plasma consisted of HIV-1 RNA concentrations within the quantitation ranges for the dilution procedures. Each panel member was tested, neat or using the dilution procedures, in multiple replicates. The test results for samples tested neat and using the dilution procedures are shown in **Table 6**.

Table 6. Alinity m HIV-1 Results for Plasma Samples Tested Using Dilution Procedure

	Neat	Dilution Procedure
Dilution	Mean Conc. (Log Copies/mL)	Mean Conc. (Log Copies/mL)
1:2.5	2.20	2.11
	3.06	2.98
	3.56	3.50
	3.89	3.84
	4.19	4.15
	5.10	4.97
	5.21	5.16
	5.57	5.46
	5.76	5.60
	5.84	5.74
1:50	3.56	3.30
	3.89	3.66
	4.19	4.02
	5.10	4.89
	5.21	4.97
	5.57	5.32
	5.76	5.43
	5.84	5.58
	6.81	6.63
	7.58	7.17

Precision of Alinity m HIV-1 Using Dilution Procedures

Precision of Alinity m HIV-1, using the 1:2.5 and 1:50 dilution procedures, was determined by analyzing 3 panel members prepared by spiking HIV-1 viral stock in HIV-1 negative human plasma. Each panel member was tested in 5 replicates, twice each day for 12 days, on 3 Alinity m Systems with 3 Alinity m HIV-1 AMP Kit lots and 3 Alinity m HIV-1 Specimen Dilution Kit I lots by 3 operators.

The results, representative of the precision of Alinity m HIV-1 using dilution procedures, are summarized in **Table 7**.

Table 7. Precision of Alinity m HIV-1 Using Dilution Procedures for Plasma

				Within-Run Component		Between-Run Component		Between-Day Component		Within-Laboratory[b]		Between-Instrument Component		Total[c]	
Panel Member	Dilution Factor	N[a]	Mean Conc. (Log Copies/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
3	1:50	341	4.60	0.06	1.3	0.01	0.3	0.02	0.4	0.06	1.3	0.01	0.2	0.06	1.4
2	1:50	352	6.32	0.05	0.7	0.00	0.0	0.02	0.3	0.05	0.8	0.03	0.5	0.06	0.9
1	1:2.5	340	2.82	0.08	2.9	0.04	1.3	0.00	0.0	0.09	3.2	0.02	0.6	0.09	3.2

[a] Number of valid replicates.
[b] Within-Laboratory includes Within-Run, Between-Run, and Between-Day components
[c] Total includes Within-Run, Between-Run, Between-Day, and Between-Instrument components.

CLINICAL PERFORMANCE

Clinical Specificity Study

Retrospectively collected plasma specimens from a total of 596 HIV-1 negative volunteer whole blood donors were included in the evaluation for Alinity m HIV-1 specificity. The HIV-1 negative specimens were tested at 3 clinical testing sites with 2 Alinity m HIV-1 reagent kit lots. Clinical specificity was calculated as the percentage of HIV-1 negative specimens with the results of “Not Detected”. HIV-1 RNA was not detected in any of the specimens. Specificity was 100.0% (596/596, 95% CI: 99.4% to 100.0%).

Validation of Viral Load Quantitation

The performance of Alinity m HIV-1 was compared to that of the FDA-approved Abbott RealTi*m*e HIV-1 assay in a representative study. Prospectively and retrospectively collected plasma samples from a total of 326 HIV-infected individuals were included in the evaluation. The Alinity m HIV-1 assay testing was performed at 3 clinical testing sites with 4 Alinity m HIV-1 reagent kit lots. Demographic characteristics of the subjects are shown in **Table 8**.

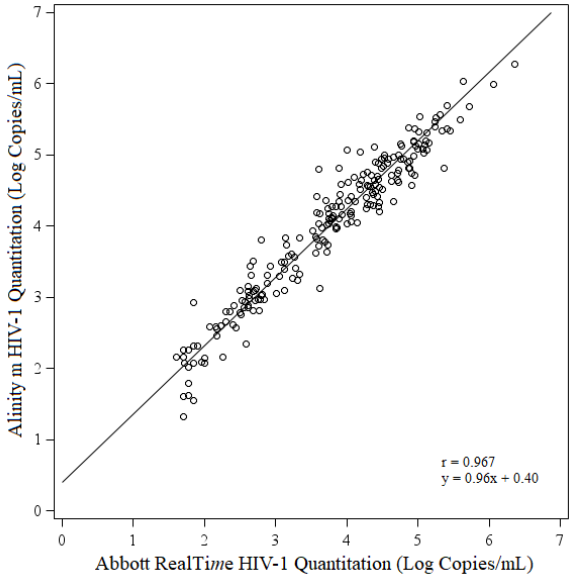
Table 8. Summary of Demographic Characteristics

Demographic Characteristic	Statistics (N = 326)	Demographic Characteristic	Statistics (N = 326)
Age (years)		**Ethnicity**	**n (%)**
Mean	41.2	African American	149 (45.7%)
SD	15.3	Hispanic	79 (24.2%)
Median	44	White	65 (19.9%)
Range	5 to 75	Other	33 (10.1%)
Age Group	**n (%)**	**Gender**	**n (%)**
Pediatric (≤12 years)	24 (7.4%)	Male	231 (70.9%)
Non-Pediatric (>12 years)	302 (92.6%)	Female	94 (28.8%)
CD4+ Cell Count (cells/ µL)	**n (%)**	Unknown	1 (0.3%)
<200	78 (23.9%)	**Anitviral Medication**	**n (%)**
200 to 500	113 (34.7%)	Yes	217 (66.6%)
>500	110 (33.7%)	No	109 (33.4%)
N/A	25 (7.7%)		

N/A = Not Available

Regression analysis included a total of 216 subjects with results that fell within the common quantitative range of Alinity m HIV-1 and Abbott RealTi*m*e HIV-1. **Figure 3** shows the results of the Deming regression analysis with a correlation coefficient of 0.967. The mean bias between Alinity m HIV-1 and Abbott RealTi*m*e HIV-1 is 0.25 Log Copies/mL with a 95% CI of (0.21, 0.28).

Figure 3. Deming Regression Analysis



Reproducibility

Reproducibility performance of Alinity m HIV-1 was evaluated by testing a 10-member reproducibility panel. All panel members were prepared using HIV-1 virus diluted in negative human plasma. The concentration levels targeted for the reproducibility panels spanned the linear quantitation range of the assay. A total of 3 Alinity m HIV-1 AMP Kit lots were used. Each of the 3 clinical sites tested 2 Alinity m HIV-1 AMP Kit lots, on 5 non-consecutive days for each lot. Five replicates of each panel member were tested on each of 5 days. The reproducibility results are summarized in **Table 9**.

Table 9. Reproducibility of Alinity m HIV-1

Panel Member	N[a]	Mean Conc. (Log Copies/mL)	Within-Run Component		Between-Run Component		Within-Laboratory[b]		Between-Lot Component		Between-Site Component		Total[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
10	149	7.35	0.06	0.8	0.04	0.5	0.07	1.0	0.05	0.7	0.00	0.0	0.09	1.2
9	150	6.47	0.06	1.0	0.05	0.7	0.08	1.2	0.03	0.5	0.01	0.2	0.09	1.4
8	149	5.81	0.09	1.5	0.05	0.9	0.10	1.8	0.00	0.0	0.02	0.4	0.11	1.8
7	150	5.14	0.07	1.3	0.06	1.2	0.09	1.7	0.00	0.0	0.03	0.6	0.09	1.8
6	150	4.45	0.08	1.8	0.05	1.1	0.09	2.1	0.01	0.2	0.01	0.2	0.09	2.1
5	149	3.78	0.08	2.0	0.04	1.1	0.09	2.3	0.02	0.5	0.00	0.0	0.09	2.4
4	150	3.09	0.08	2.7	0.05	1.7	0.10	3.1	0.03	1.0	0.02	0.6	0.10	3.4
3	149	2.43	0.10	4.1	0.06	2.3	0.11	4.7	0.06	2.4	0.00	0.0	0.13	5.3
2	150	1.85	0.16	8.6	0.07	3.6	0.17	9.3	0.05	2.5	0.04	2.0	0.18	9.8
1	149	1.38	0.31	22.3	0.10	7.1	0.32	23.4	0.05	3.7	0.00	0.0	0.33	23.7

[a] Number of valid replicates with detectable viral load.
[b] Within-Laboratory includes Within-Run and Between-Run components.
[c] Total includes Within-Run, Between-Run, Between-Lot, and Between-Site components.

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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
For In Vitro Diagnostic Use	For In Vitro Diagnostic Use
AMP TRAY	AMP TRAY
ACT TRAY	ACT TRAY
UNIT	Unit
Rx ONLY	For Prescription Use Only
	Systemic Health Effects
	Warning
	Caution
	Consult Instructions for Use
	Temperature Limitation
	Contains sufficient for <*n*> tests
	Use By
	Manufacturer

TECHNICAL ASSISTANCE

For technical assistance, call Abbott Molecular Technical Services at 1-800-553-7042 (within the US) or +49-6122-580 (outside the US), or visit the Abbott Molecular website at www.molecular.abbott/portal.

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