

WHO Prequalification of In Vitro Diagnostics PUBLIC REPORT

Product: Alinity m HIV-1
WHO reference number: PQDx 0640-027-00

Alinity m HIV-1 with product code 08N45-090, manufactured by Abbott Molecular Inc., CE-marked regulatory version, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 31 May 2024.

Summary of WHO prequalification assessment for Alinity m HIV-1

	Date	Outcome
Prequalification listing	31 May 2024	listed
Dossier assessment	22 April 2024	MR
Site inspection of quality management system	21 September 2021	MR
Product performance evaluation	Quarter (Q) 4 2022 -Q 2 2023	MR

MR: Meets Requirements

Intended use

According to the intended use claim from Abbott Molecular Inc., *“The Alinity m HIV-1 assay is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for use with the automated Alinity m System to detect and quantitate Human Immunodeficiency Virus type 1 (HIV-1) RNA. 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 viral response to antiretroviral treatment by measuring changes in plasma HIV-1 RNA levels. Performance for quantitative results is not established with serum specimens.

The Alinity m HIV-1 assay may also be used as a diagnostic test to aid in the diagnosis of HIV-1 infection by confirming HIV-1 infection in individuals that have repeat reactive results with HIV immunoassays. The performance of the diagnostic confirmatory interpretation is established with both plasma and serum specimens.

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 to be used in screening blood, blood products, tissue or organ donors for HIV.

INTENDED USER

The intended users for the Alinity m HIV-1 assay are laboratory professionals.”

Assay description

According to the claim of the assay description from Abbott Molecular Inc., *“The Alinity m HIV-1 assay requires 3 separate assay specific kits:*

- *Alinity m HIV-1 AMP Kit (08N45-090) 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 reagents. The intended storage condition for the Alinity m HIV-1 AMP Kit is 2 to 8°C.*
- *Alinity m HIV-1 CAL Kit (08N45-070) 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 $-20 \pm 5^{\circ}\text{C}$.*
- *Alinity m HIV-1 CTRL Kit (08N45-080) 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 $-20 \pm 5^{\circ}\text{C}$.*

The Alinity m HIV-1 assay utilises 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 or serum 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 or serum 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 in plasma specimens and for HIV-1 RNA detection in serum specimens. 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. Plasma specimens may be tested for viral load determination and for diagnostic confirmatory evaluation. Serum specimens may only be tested for diagnostic confirmatory evaluation.

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.”*

Test kit contents:

Component	Number of tests and product codes
Alinity amplification kit <ul style="list-style-type: none"> • Alinity m HIV AMP TRAY 1 • Alinity m HIV AMP TRAY 2 	192 Tests, product code 08N45-090 4 trays / 48 tests each 4 trays / 48 tests each

Items required but not provided:

- 08N45-070 Alinity m HIV-1 CAL Kit,
- 08N45-080 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 I³,
- 08N45-01F (v6.00) or higher Alinity m HIV-1 Application, Specification File,
- Vortex mixer,

- Centrifuge capable of 2000g
- 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 (such as Corning Catalog No. 3820 or Eppendorf Catalog No. 022638955),
- Centrifuge with swing plate rotor capable of accommodating the plate adapter and capable of ≥ 100g,
- 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.

Storage:

The test kit should be stored at 2-8 °C.

Shelf-life upon manufacture:

24 months.

Warnings/limitations:

Refer to the current version of the manufacturer's instructions for use attached to this public report.

Prioritisation for prequalification

Based on the established criteria, the Alinity m HIV-1 was given priority for WHO prequalification assessment.

Product dossier assessment

Abbott Molecular Inc. submitted a product dossier for the Alinity m HIV-1 as per the "Instructions for compilation of a product dossier" (PQDx_018). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO. The manufacturer's responses to the nonconformities found during dossier screening and assessment findings were accepted on 22 April 2024.

Commitments for prequalification

1. The manufacturer will provide additional information regarding the precision of measurement. At the time of this public report, the information has been provided and is under evaluation.

2. The manufacturer will provide additional information regarding accelerated reagent stability and in-use stability. At the time of this public report, the information has been provided and is under evaluation.

Based on the product dossier screening and assessment findings, the product dossier for Alinity m HIV-1 meets WHO prequalification requirements.

Manufacturing site inspection

A desk review of 1300 East Touhy Avenue, 60018 Des Plaines, IL, USA, was conducted from 21 September 2021¹. At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality. Routine inspections of the Manufacturing site will be conducted with copies of the WHO Public Inspection Report (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current assessment performed at a manufacturing site for in vitro diagnostic products and summarises the assessment findings.

<https://extranet.who.int/pqweb/vitro-diagnostics/who-public-inspection-reports>

All published WHOPIRs are with the agreement of the manufacturer.

The desk assessment was accepted on 21 September 2021.

Based on the site inspection and corrective action plan review, the quality management system for Alinity m HIV-1 meets WHO prequalification requirements.

Product performance evaluation

Alinity m HIV-1 (Abbott Molecular Inc.) was evaluated at the National Health Laboratory Service (NHLS, South Africa) and the CDC (United States of America) on behalf of WHO in the 2nd quarter of 2022 at the NHLS site and the CDC site in the 4th quarter of 2022 and the first two quarters of 2023, according to protocol PQDx 215, version 7. Product code 08N45-090, with IFU version 53-608055/R5, was used for the evaluation at both sites.

Clinical performance evaluation

In this limited laboratory-based evaluation of clinical performance characteristics, a panel of 560 plasma specimens was used. The specimens were characterized using the following

¹ A desk assessment is ongoing, and the public report will be updated when the assessment is completed.

reference assay: cobas HIV-1 Quantitative nucleic acid test for use on the cobas 6800/8800 Systems (Roche Diagnostics GmbH).

Clinical performance characteristics in comparison with an agreed reference standard	
Bias	-0.27
Limits of agreement	-0.70 to 0.17
Invalid rate % (N=560)	0.7%
Sensitivity and specificity for the detection of virological failure at 1000 copies/mL	
Sensitivity % (95% CI) (N=231)	93.1 (89.1 - 95.7)
Specificity % (95% CI) (N=237)	100 (98.4 - 100)
Specificity among HIV- negative individuals	
Specificity % (95% CI) (N=90)	100 (96.0 - 100)

Analytical performance evaluation

Analytical performance characteristics	
Limit of detection (LoD)	The LoD was estimated to be 42.6 IU/mL (95% CI: 26.5 – 103.5 IU/mL), equivalent to 26.2 copies/mL (95% CI: 16.3 – 63.5 copies/mL) using the conversion factor provided by the manufacturer. The LoD claimed by the manufacturer (20 copies/mL) was verified during this performance evaluation.
Within-run precision (repeatability)	At 3.0 log ₁₀ copies /mL, CV% were < 2% At 4.0 log ₁₀ copies /mL, CV% were < 2%
Within-laboratory precision (reproducibility)	At 3.0 log ₁₀ copies /mL, CV% were < 3% At 4.0 log ₁₀ copies /mL, CV% were < 2%
Linearity	Linearity of the assay was verified for subtypes A, B, C, D, and AG over a range of viral loads from 10 ² to 10 ⁶ copies/mL.
Cross-contamination	No cross-contamination was observed when high positive and negative specimens were tested alternatively.

Operational characteristics and ease of use

This assay requires laboratory equipment and cannot be performed in laboratories with limited facilities or non-laboratory settings. The instrument is supplied with an uninterruptible power supply (UPS) and can, therefore, be used in environments with an unstable source of electricity. The Abbott Alinity m System requires a significant amount of physical space. Furthermore, training and implementation of good laboratory practice is

essential to obtaining accurate results. Adequate technical support from the manufacturer, field service engineers, or local representative is critical.

The assay was found easy to use by the operators performing the evaluation, who had received a minimum of a 3-day training by the manufacturer prior to the evaluation.

Key operational characteristics	
Specimen type(s) and volume	Plasma, 600 µL volume required for testing, minimum of 750 µL of plasma is required to be loaded on to the Alinity m System for the HIV-1 assay.
Number of steps for one specimen*	5 steps in total No steps with precision pipetting. All pipetting is automated and done by the instrument.
Number of steps for instrument management**	7 steps per day
Time to result for one test	115 minutes to the first 12 results, every 15 minutes thereafter.
Operator hands-on time for one run	30-45 minutes for specimen, reagent preparation and loading of the initial batch of specimens.
Level of automation	Fully automated following loading of sample to be tested on the Alinity m System. All specimen tubes (primary and secondary tubes) must be labelled with specimen ID barcodes or must be identified with a specimen ID and rack and position.
Quality controls	The manufacturer provides QC, and it should be purchased separately. High positive, low positive, and negative controls are purchased separately from the Alinity m HIV-1 AMP Kit.
Operating temperature	15 – 28 °C. Reagents are to be stored as directed on packaging and in the IFU.
Result display and connectivity	Results are displayed on the instrument. They may be printed using a printer. The results can be exported to the laboratory information system and other health information systems.
Power sources	Main power. The instrument is supplied with a UPS to ensure backup electricity is available.
Biosafety (<i>outside of infectious specimen handling</i>)	Operators reported no concerns about biosafety for the user. NOTE: Some Alinity m bulk reagents contain guanidine thiocyanate, which, when mixed with bleach, produces a highly toxic gas.

Waste	<p>The volume of liquid was approx—1L per run. The volume of solid waste is approximately 7kg per run (IRUs and amplified container waste). Waste disposal requires specific measures and usual laboratory biohazard waste disposal procedures. NOTE: Some Alinity m bulk reagents contain guanidine thiocyanate, which, when mixed with bleach, produces a highly toxic gas.</p>
Calibration	<p>Calibrators are provided by the manufacturer and should be purchased separately. Calibration is required at least every 6 months when one or more reagent lots is/are changed, when software is updated, or when indicated by the Alinity m System.</p>
Maintenance	<p>Weekly, monthly, and yearly maintenance is required. Daily maintenance is required when conducting testing. It is imperative that Abbott Field Service providers are available to ensure the smooth operation of the Alinity m instrument.</p>
Other specific requirements	<p>The instrument requires a stable power supply, has a considerable footprint, and requires consistent maintenance and service by Abbott-authorized engineers/service providers.</p>

Based on these results, the performance evaluation for Alinity m HIV-1 meets the WHO prequalification requirements.

Labelling

- 1. Labels**
- 2. Instructions for use**

1. Labels

Alinity m

HIV-1

1

Abbott

UNIT 1

IVD

Σ 48

AMP TRAY

2°C - 8°C

53-602038/R1

REF 8N45P



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



LOT

Colors:

■ Pantone Reflex Blue

■ Pantone 185

■ Black

Labeling: Renee

Alinity m


HIV-1 AMP Kit



HIV-1 AMP Kit

AMP TRAY 4 x Σ 48

ACT TRAY 4 x Σ 48

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

 **EC REP**
1434

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



REF 08N45-090



H302, H317, H316, H370,
H412, P260, P261, P264,
P272, P273, P280,
P301+P312, P302+P352,
P308+P311, P333+P313,
P362+P364, P501

IVD

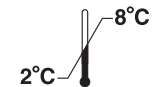
In Vitro Test

PRODUCT OF USA



www.molecular.abbott/portal

REF 08N45-090



53-602081/R3




LOT

Alinity m

HIV-1

Σ 48

53-602085/R1

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

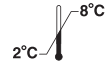
2

ACT TRAY

 **Abbott**

UNIT 1

IVD

 2°C - 8°C


REF 8N45M




LOT

Colors:

 Pantone 376 C

 Pantone 185 C

 Black

Labeling: Renee

Alinity m

HIV-1 Application Specification File

en

IVD

REF 08N45-01E

53-608203/R1

Created July 2020

For assay related information please refer to the corresponding Alinity m HIV-1 AMP Kit (List No. 08N45-090) package insert.

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 Customer service section or Technical assistance section of these instructions.

INTENDED USE





The Alinity m HIV-1 Application Specification File is intended for use with the Alinity m HIV-1 assay on the automated Alinity m System to allow for processing of assay calibrators, controls, and patient samples.

INTENDED USER

The intended users for the Alinity m HIV-1 Application Specification File are laboratory and healthcare professionals.

- This application specification contains unique information for communicating with middleware or laboratory information systems. The unique identifier for the assay in this application specification is 1006. Use this number when working with your middleware or laboratory information system provider.
- In the event that you experience error conditions during processing this application specification, refer to the Alinity m System Operations Manual for the corrective action associated with the specific message code identified.

KEY TO SYMBOLS USED

	Reference Number
	In Vitro Diagnostic Medical Device
	Authorized Representative in the European Community
	Manufacturer

Assay Specific Information	
Application Name	Version
HIV-1-01E	5.00

Specimen Type	
Host Code	Screen (Manual Order)
PLAS	Plasma
SER	Serum
LDT Specimen	LDT Specimen (Laboratory Defined Test)

Manual Dilution
1:2.5 or 1:50

Host Configuration	
Assay Name	Assay Number
HIV-1	1006

The following are important facts to know about this application specification:


- The installation of the application specification file is to be performed by Abbott Molecular Field Service.
- Upon installation by an Abbott Field Service Representative, the Alinity m HIV-1 application specification can be used for processing assay results. The Abbott Field Service Representative will ensure the application is properly installed and ready for processing assay results.
- Prior to processing results, refer to section 2 (Configure screen, Assay tab) of the Alinity m System Operations Manual for options for configuring your application specification. Refer to section 5 of the Alinity m System Operations Manual for ordering tests (Specimen, calibration, and control orders) and reviewing results (Results screen).

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.

Abbott Molecular Inc. is the legal manufacturer of the Alinity m HIV-1 Application Specification File.

The Alinity m HIV-1 Application Specification is imported into the European Union by Abbott Diagnostics GmbH, located at Max-Planck-Ring 2, 65205 Wiesbaden, Germany.

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



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

www.molecular.abbott/portal
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53-608203/R1



(01)00884999049468(240)08N45-01E(8012)5.00

2. Instructions for use²

²WHO assessed the English versions of the IFU. The manufacturer is responsible for ensuring the correct translation into other languages.

Alinity m

HIV-1 AMP Kit

en
HIV-1

REF 08N45-090
53-608055/R6

Revised June 2023

REF 08N45-090

53-608055/R6

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 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 use with the automated Alinity m System to detect and quantify Human Immunodeficiency Virus type 1 (HIV-1) RNA. 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 viral response to antiretroviral treatment by measuring changes in plasma HIV-1 RNA levels. Performance for quantitative results is not established with serum specimens.

The Alinity m HIV-1 assay may also be used as a diagnostic test to aid in the diagnosis of HIV-1 infection by confirming HIV-1 infection in individuals that have repeat reactive results with HIV immunoassays. Performance of the diagnostic confirmatory interpretation is established with both plasma and serum specimens.

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 to be used in screening blood, blood products, tissue or organ donors for HIV.

INTENDED USER

The intended users for the Alinity m HIV-1 assay are laboratory professionals.

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

The diagnosis of HIV infection utilizes testing algorithms that rely on a sequential two-step process with the initial test leveraging the presence/absence of HIV-specific antigen and antibodies (for both HIV-1 and HIV-2), which is followed by a confirmatory assay (ie, Western blot, immunofluorescence assay, or RNA assay).^{10,11}

By assessing the presence/absence of HIV-1 RNA in patient plasma and serum specimens, the Alinity m HIV-1 assay will be used to confirm HIV-1 infection in individuals that have repeat reactive results with HIV immunoassays.

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.¹²⁻¹⁷

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.¹⁸⁻²⁰

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.¹⁹ In addition, optimal viral suppression is considered when the viral load remains persistently below the lower limit of detection.^{19,20}

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.^{19,21,22} 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.²³⁻²⁵

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.²⁶ 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 (08N45-090) 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 (08N45-070) 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 $-20 \pm 5^\circ\text{C}$.
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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 or serum 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 or serum 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 in plasma specimens and for HIV-1 RNA detection in serum specimens. 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. Plasma specimens may be tested for viral load determination and for diagnostic confirmatory evaluation. Serum specimens may only be tested for diagnostic confirmatory evaluation.

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. 08N45-090


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
	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,²⁷ OSHA Standard on Bloodborne Pathogens,²⁸ CLSI Document M29-A4,²⁹ and other appropriate biosafety practices.³⁰ 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.²⁷

Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.³⁰

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



WARNING H317 Contains 2-Methyl-4-isothiazolin-3-one.
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 H302 H316 H317 H370 H412 Contains: Tetramethylammonium chloride and 2-Methyl-4-isothiazolin-3-one
Harmful if swallowed.
Causes mild skin irritation^a
May cause an allergic skin reaction.
Causes damage to organs.
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 Types

The specimen types listed below can be used with this assay on the Alinity m System. Plasma specimens may be tested for viral load determination and for diagnostic confirmatory evaluation. Serum specimens may only be tested for diagnostic confirmatory evaluation. For the Alinity m HIV-1 assay, only use the collection tubes as described in the following table for the corresponding specimen type. Alinity m HIV-1 assay performance with other specimen types or collection tubes has not been evaluated.

Specimen Types ^a	Blood Collection Tubes
Plasma	Acid Citrate Dextrose (ACD) K ₂ EDTA K ₃ EDTA Plasma Preparation Tube (PPT) ^b
Serum	Serum Serum Separator Tube (SST) ^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 and Serum Separator Tube are gel tubes.

Specimen Storage: Plasma Testing

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 25°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 25°C	1 day	
	-20°C	30 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 Storage: Serum Testing

Specimen	Temperature	Maximum Storage Time	Special Instructions
Whole Blood	2 to 8°C	2 days	Whole blood may be stored between draw and serum separation.
	15 to 25°C	12 hours	
Serum	2 to 8°C	3 days	Serum may be stored in primary or secondary tubes after separation from the clot.
	15 to 25°C	12 hours	
	-20°C	30 days	Serum may be stored frozen in primary gel tubes (SST) or secondary tubes after separation from the clot. ^a Serum from non-gel tubes must be transferred to secondary tubes prior to storage. ^a
-70°C or colder	Longer storage		

^a Avoid more than 3 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 and serum from cells or clot 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. Serum may be stored on the clot (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 or on the clot cannot be frozen without a gel.

- Plasma and serum 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, plasma and serum specimens in the secondary tubes may be stored frozen.

Frozen Specimens (Plasma and Serum): Primary Gel Tubes

- Thaw specimens at 15 to 25°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 2000g 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 (Plasma): Secondary Aliquot Tubes

- Thaw specimens at 15 to 25°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 2000g 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.

Frozen Specimens (Serum): Secondary Aliquot Tubes

- Thaw specimens at 15 to 25°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 at 2000g 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.

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

08N45-090 Alinity m HIV-1 AMP Kit

Materials Required but not Provided

- 08N45-070 Alinity m HIV-1 CAL Kit
- 08N45-080 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
- 08N45-01F (v6.00) or higher Alinity m HIV-1 Application Specification File
- Vortex mixer
- Centrifuge capable of 2000g
- 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 (such as Corning Catalog No. 3820 or Eppendorf Catalog No. 022638955)
- Centrifuge with swing plate rotor capable of accommodating the plate adapter and capable of ≥100g
- 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 (Corning Catalog No. 3820 or 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 800g 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), and then select the appropriate specimen type (plasma or serum) being tested. Failure to assign specimen type correctly may cause unintended result reporting that may require a retest.

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 or serum 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/Serum 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, clot, 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
Alinity m Transport Tube Pierceable Capped	09N49-010	1.0 mL	Uncapped ^d
Other aliquot tubes with minimum inner diameter 10.0 mm	NA	0.9 mL for tubes with 10.6 mm or less inner diameter.	Uncapped
		1.4 mL for tubes with 13.2 mm or less inner diameter.	

^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 or serum above the gel/clot/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 × ID².

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

^d Cap must be removed prior to loading.

When loading sample tubes to the Alinity m System, the Sample Rack Retention Bar is required for the following situations.

1. Calibrator, Control with pierceable caps
2. Specimen in blood collection tubes with gel separator
3. Specimen in Transport tube with pierceable cap

Clean the retention bar after each use

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

- Ensure individual specimen tubes are labeled correctly with specimen ID barcodes.
- Inspect serum and plasma 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 plasma or serum specimens with a minimum of 260 µL volume available for Alinity m HIV-1 testing can be diluted 1:2.5. Plasma or serum specimens with 50 to 259 µL volume available for Alinity m HIV-1 testing can be diluted 1:50. High-titer plasma 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 (plasma/serum)	≥ 260 µL	1 : 2.5
	50 to 259 µL	1 : 50
> ULOQ result (plasma)	≥ 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 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 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 in plasma specimens and controls and to determine HIV-1 RNA detection in serum specimens.

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 accepted 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 threshold cycle [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 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. 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.

If quality control results do not meet the acceptance criteria, refer to the Alinity m System Operations Manual, Section 10, for troubleshooting information.

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 control and Alinity m high-positive control 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 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.

Qualitative results are reported for serum specimens as "Detected" or "Not Detected." No quantitative results are reported for serum specimens.

Interpretation of Results

Plasma Specimens (Viral Load and Diagnostic Confirmatory Testing)

Alinity m HIV-1 assay results for plasma specimens can be interpreted for viral load determination and for diagnostic confirmatory evaluation. The diagnostic confirmatory interpretation is not directly reported by the Alinity m System for plasma specimens. A confirmatory interpretation is performed by the user, based on the viral load result/interpretation.

For each specimen, the Alinity m System will report a result and an interpretation as shown in the tables below. If applicable, message codes or flags will also be displayed. The last column provides the diagnostic confirmatory interpretation corresponding to each test result.

Undiluted Plasma Specimens

Alinity m System Reported			User Performed
Result	Interpretation	Flags	Confirmatory Interpretation
Not Detected	Target not detected		HIV-1 RNA not detected
< 1.30 Log Copies/mL	Detected < LLOQ		HIV-1 RNA detected
1.30 to 7.00 Log Copies/mL			HIV-1 RNA detected
> 7.00 Log Copies/mL	> ULOQ		HIV-1 RNA detected

For plasma 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 the specimen has been diluted. The quantitative results and the upper and lower limits of the quantitation range (ULOQ and LLOQ, respectively) reported represent the HIV-1 RNA concentrations in the specimens prior to dilution.

For diluted plasma specimens from which the HIV-1 signal is not detected, 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 undiluted specimens or from a newly prepared dilution.

Plasma Specimens Tested Using 1:2.5 Dilution

Alinity m System Reported			User Performed
Result	Interpretation	Flags	Confirmatory Interpretation
< 1.70 Log Copies/mL	Detected < LLOQ	DIL	HIV-1 RNA detected
1.70 to 7.40 Log Copies/mL		DIL	HIV-1 RNA detected
> 7.40 Log Copies/mL	> ULOQ	DIL	HIV-1 RNA detected

Plasma Specimens Tested Using 1 : 50 Dilution

Alinity m System Reported			User Performed
Result	Interpretation	Flags	Confirmatory Interpretation
< 3.00 Log Copies/mL	Detected < LLOQ	DIL	HIV-1 RNA detected
3.00 to 8.70 Log Copies/mL		DIL	HIV-1 RNA detected
> 8.70 Log Copies/mL	> ULOQ	DIL	HIV-1 RNA detected

NOTE: The upper and lower limits of the quantitation range (ULOQ and LLOQ, respectively) displayed for Plasma Specimens Tested Using Dilution are not the same as the ULOQ and LLOQ of the Alinity m HIV-1 assay when applied to plasma specimens tested without dilution. Their corresponding values are specified in the Result column in each table.

Serum Specimens (Diagnostic Confirmatory Testing Only)

Alinity m HIV-1 assay results for serum specimens can be interpreted for diagnostic confirmation. Quantitative viral load results are not reported for serum specimens.

As shown in the table below, the Alinity m System will display a qualitative result and its confirmatory interpretation. If applicable, message codes or flags will also be displayed.

Undiluted Serum Specimens

Alinity m System Reported		
Result	Interpretation	Flags
Not Detected	HIV-1 RNA not detected	
Detected	HIV-1 RNA detected	

For serum specimens diluted 1 : 2.5 or 1 : 50, the Alinity m System reports a qualitative result, its interpretation and a DIL flag indicating that the specimen has been diluted.

For diluted serum specimens from which the HIV-1 signal is not detected, a message code (9827) is displayed and no result is reported. These specimens cannot be interpreted as “HIV-1 RNA not detected” and should be retested with undiluted specimens or from a newly prepared dilution.

Serum Specimens Tested Using 1 : 2.5 Dilution

Alinity m System Reported		
Result	Interpretation	Flags
Detected	HIV-1 RNA detected	DIL

Serum Specimens Tested Using 1 : 50 Dilution

Alinity m System Reported		
Result	Interpretation	Flags
Detected	HIV-1 RNA detected	DIL

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 serum (including serum separator) and plasma (ACD, EDTA, and PPT) specimens may be used with the Alinity m HIV-1 assay. The use of other anticoagulants have not been evaluated.
- Debris within serum and plasma specimens (eg, clots, fibrin strands) may interfere with sample processing.
- If the HIV-1 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- 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.

SPECIFIC PERFORMANCE CHARACTERISTICS

Limit of Detection

The limit of detection (LOD) of Alinity m HIV-1 is 20 Copies/mL. The LOD was determined by testing dilutions of 3rd World Health Organization (WHO) 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. Detected	Detection Rate (%)
40.00	95	95	100.0
30.00	96	96	100.0
20.00	96	93	96.9
10.00	96	89	92.7
5.00	96	64	66.7
2.50	96	36	37.5

Probit analysis determined that the concentration of HIV-1 RNA detected with 95% probability was 13.88 Copies/mL (95% CI 11.16 to 18.98 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 cultured virus or clinical specimen to 3 different concentrations in HIV-1 negative plasma. Testing was performed with 1 lot 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 that Alinity m HIV-1 detected HIV-1 at and above 20 Copies/mL, with an upper one-sided 95% confidence interval equal to or greater than the expected rate of 95.0%. The results are summarized in Table 2.

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

Group/Subtype	HIV-1 RNA Copies/mL	No. Valid Replicates	No. Detected	Detection Rate (%)	95% CI (%) ^a
Group M, subtype A	40.00	24	24	100.0	100.0
	20.00	24	24	100.0	100.0
	10.00	23	21	91.3	97.6
Group M, subtype BF	40.00	24	24	100.0	100.0
	20.00	24	24	100.0	100.0
	10.00	24	22	91.7	97.7
Group M, subtype C	40.00	24	24	100.0	100.0
	20.00	24	24	100.0	100.0
	10.00	23	23	100.0	100.0
Group M, subtype D	40.00	24	24	100.0	100.0
	20.00	24	24	100.0	100.0
	10.00	24	19	79.2	90.8
Group M, CRF01-AE	40.00	24	24	100.0	100.0
	20.00	24	24	100.0	100.0
	10.00	24	22	91.7	97.7
Group M, subtype F	40.00	24	24	100.0	100.0
	20.00	23	23	100.0	100.0
	10.00	21	21	100.0	100.0
Group M, CRF02-AG	40.00	23	23	100.0	100.0
	20.00	24	24	100.0	100.0
	10.00	24	22	91.7	97.7
Group M, subtype G	40.00	24	24	100.0	100.0
	20.00	24	24	100.0	100.0
	10.00	24	22	91.7	97.7
Group M, subtype H	40.00	24	24	100.0	100.0
	20.00	24	24	100.0	100.0
	10.00	24	23	95.8	99.3

Group O	40.00	24	24	100.0	100.0
	20.00	24	24	100.0	100.0
	10.00	24	24	100.0	100.0
Group N	40.00	23	23	100.0	100.0
	20.00	24	24	100.0	100.0
	10.00	23	23	100.0	100.0

^a Upper One-Sided 95% Confidence Interval (%).

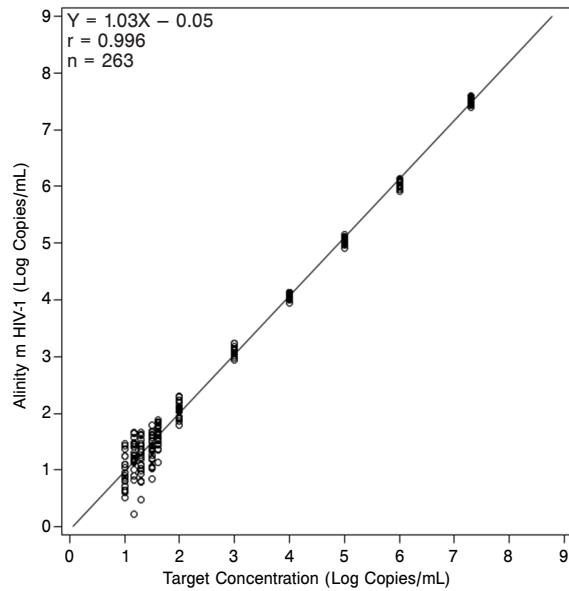
Linear Range

Linearity of Alinity m HIV-1 was assessed by testing a dilution series of a 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



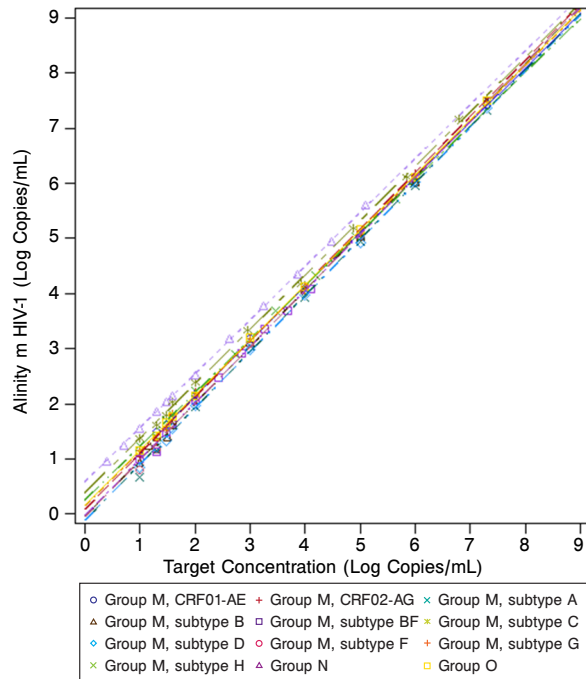
Linearity Across Groups and Subtypes

Linearity of Alinity m HIV-1 for 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 to 12 panel members for each group/subtype, prepared using clinical specimen or cultured virus in 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, BF, C, D, CRF01-AE, F, CRF02-AG, G and H), group O and group N.

Figure 2. Linearity Across Groups and Subtypes



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

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 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).

Precision of Alinity m HIV-1 was determined by analyzing a 7-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 5 days, on 3 Alinity m Systems with 3 AMP Kit lots by 3 operators, for a total of 150 replicates.

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

Table 3. Precision

Panel	N ^d	Mean Conc. (Log Copies/mL)	Within Run SD	Between Run SD	Between Day SD	Within Laboratory ^a SD	Between Instrument ^b SD	Total SD ^c
1	146	1.80	0.16	0.07	0.05	0.19	0.00	0.19
2	143	2.31	0.10	0.02	0.03	0.10	0.00	0.10
3	140	3.02	0.06	0.01	0.02	0.06	0.00	0.06
4	144	3.99	0.05	0.03	0.03	0.07	0.01	0.07
5	144	4.98	0.07	0.05	0.03	0.09	0.00	0.09
6	147	5.99	0.05	0.08	0.00	0.09	0.02	0.10
7	145	7.44	0.08	0.09	0.00	0.12	0.06	0.13

^a Within-Laboratory includes Within-Run, Between-Run and Between-Day components.

^b The Between-Instrument component consists of Alinity m System, AMP Kit lot, and operator.

^c Total includes Within-Run, Between-Run, Between-Day, and Between-Instrument components.

^d Valid replicates.

Specificity

The specificity of Alinity m HIV-1 was determined by testing HIV-1 negative plasma and serum specimens from individual donors. A total of 501 specimens were analyzed including 250 plasma and 251 serum. The specificity for plasma was 100.0% (95% CI: 98.5 to 100.0%). The specificity for serum was 100.0% (95% CI: 98.5 to 100.0%). The overall specificity was 100.0% (95% CI: 99.2 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 and positive plasma containing 200 Copies/mL HIV-1. No cross-reactivity or interference in Alinity m HIV-1 performance 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 1
Human T-Lymphotropic Virus Type 2
Influenza A
Vaccinia Virus
Varicella-Zoster Virus
Bacteria
<i>Chlamydia trachomatis</i>
<i>Mycobacterium gordonae</i>
<i>Mycobacterium smegmatis</i>
<i>Neisseria gonorrhoeae</i>
<i>Propionibacterium acnes</i>
<i>Staphylococcus aureus</i>
<i>Staphylococcus epidermidis</i>
Yeast
<i>Candida albicans</i>

Analytical Specificity – Potentially Interfering Substances

The effect of endogenous substances, the presence of autoimmune disorders and non-HIV-1 serological markers, and the presence of high levels of potentially interfering drugs, were evaluated. Potential interference on Alinity m HIV-1 performance was assessed by testing HIV-1 negative samples, and positive samples containing 60 and 200 Copies/mL HIV-1.

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).

No interference was observed for specimens collected from patients with the following disease states: systemic lupus erythematosus (SLE), anti-nuclear antibodies (ANA), rheumatoid factor (RF), HBsAg, anti-HTLV-I/II, anti-HCV, and 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	Hydrocodone
10	Biotin

Carryover

The carryover rate for Alinity m HIV-1 was determined in two studies. Study 1 evaluated the carryover rate in the Sample Input Rack and Sample Processing Unit by analyzing 362 valid replicates of HIV-1 negative samples processed from alternating positions in the sample input rack with 362 valid replicates of high concentrated HIV-1 positive samples at 1,000,000 Copies/mL, across multiple runs. HIV-1 RNA was not detected in any of the HIV-1 negative samples, resulting in a carryover rate of 0.0% (95% CI: 0.0% to 1.0%).

Study 2 evaluated the carryover rate in the AMP tray by evaluating 412 valid replicates of HIV-1 negative samples processed from alternating positions at the AMP Tray with 412 valid replicates of high concentrated HIV-1 positive samples at 1,000,000 Copies/mL across multiple runs. HIV-1 RNA was not detected in any of the HIV-1 negative samples resulting in a carryover rate of 0.0% (95% CI: 0.0% to 0.9%).

HIV-1 RNA was not detected in any of the total 774 HIV-1 negative samples resulting in an overall Alinity m HIV-1 carryover rate of 0.0% (95% CI: 0.0% to 0.5%).

Matrix Equivalency

26 HIV-1 negative plasma and serum pairs and 52 HIV-1 positive plasma and serum pairs were analyzed. All HIV-1 negative plasma and serum samples were not detected, and all HIV-1 positive plasma and serum samples were detected, resulting in an overall percent agreement between plasma and serum samples of 100.0% (95% CI: 95.3 to 100.0%).

Alinity m HIV-1 Testing Using Dilution Procedure

The 1:2.5 and 1:50 dilution procedures were evaluated by comparing quantitation of neat (undiluted) samples and samples that were tested using Alinity m HIV-1 dilution procedures. Panel members in plasma, consisting of HIV-1 concentrations within the quantitation ranges for the dilution procedures, were tested using both dilution factors. Each panel member was tested as neat or diluted in 5 replicates. The test results for neat and diluted panel members are shown in Table 6.

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

Dilution	Panel Member	Neat (Undiluted)	Diluted
		Mean Conc. (Log Copies/mL)	Mean Conc. (Log Copies/mL)
1:2.5	01	2.04	2.00
	02	3.61	3.51
	03	6.32	6.18
	04	5.22	5.11
	05	5.66	5.54
1:50	06	3.61	3.36
	07	6.32	6.09
	08	5.22	5.11
	09	5.66	5.46
	10	7.53	7.13

The 1:2.5 and 1:50 dilution procedures were also evaluated in serum samples by assessing detection of HIV-1 positive samples. HIV-1 positive samples were tested at 150 and 5,000 Copies/mL with the 1:2.5 dilution procedure, and at 3,000 and 5,000 Copies/mL with the 1:50 dilution procedure. All HIV-1 RNA positive samples were detected by Alinity m HIV-1 using the dilution procedures.

Precision of Alinity m HIV-1 Using Dilution Procedure

Precision of Alinity m HIV-1, using the dilution procedures, was determined by analyzing three 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 5 days, on 3 Alinity m Systems with 3 AMP Kit lots by 3 operators, for a total of 150 replicates.

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

Panel Member	Dilution	N ^d	Mean Conc. (Log Copies/mL)	Within Run SD	Between Run SD	Between Day SD	Within Laboratory SD ^a	Between Instrument SD ^b	Total SD ^c
1	1:2.5	146	2.73	0.08	0.05	0.06	0.11	0.00	0.11
2	1:50	147	6.46	0.05	0.03	0.01	0.06	0.03	0.06
3	1:50	147	4.50	0.06	0.00	0.03	0.07	0.01	0.07

^a Within-Laboratory includes Within-Run, Between-Run and Between-Day components.

^b The Between-Instrument component consists of Alinity m System, AMP Kit lot, and operator.

^c Total includes Within-Run, Between-Run, Between-Day and Between-Instrument components.

^d Valid replicates.

Group/Subtype Inclusivity

The ability of Alinity m HIV-1 to detect HIV-1 group M subtypes A, B, BF, C, D, CRF01-AE, F, CRF02-AG, G, H, group O and group N was evaluated. A total of 115 samples in plasma and serum were analyzed, including a minimum of 10 samples for each of the groups/subtypes (with the exception of group N, for which 2 samples were analyzed). All samples were detected.

Seroconversion Sensitivity

Sequential specimens from 12 HIV seroconversion panels, each starting with a seronegative bleed, were tested. A total of 43 early seroconversion panel members were tested across all panels. These panels were commercially available and pre-characterized for HIV infection. Alinity m HIV-1 detected HIV-1 in 62 out of 108 total number of bleeds compared with 40 that were reactive by a HIV antigen/antibody combination test (ARCHITECT HIV Ag/Ab Combo). Among the bleeds reactive by the HIV antigen/antibody combination test, 100% (40/40) were detected by Alinity m HIV-1. The first detected bleed for Alinity m HIV-1 occurred earlier than or on the same bleed date as the HIV antigen/antibody combination test in all 12 panels (median 8.5 days; mean 8.5 days). Results are presented in Table 8.

Table 8. Seroconversion Panel Data Summary

Panel ID	No. of Panel Members Analyzed	No. of Detected/Reactive Panel Members		Days to First Detected/Reactive Result		Difference in Days to First Detected/Reactive Result (Based on Bleed Date) ^c	
		Alinity m HIV-1	HIV Ag/Ab Combo ^{a,b}	Alinity m HIV-1	HIV Ag/Ab Combo		
0600-0271	8	8	6	0 ^d	7	7	
0600-0272	5	4	2	7	18	11	
0600-0238	7	5	2	7	17	10	
0600-0252	4	4	4	0 ^d	0 ^d	0	
9011	11	3	2	30	38	8	
9031	16	5	3	47	59	12	
9032	10	6	5	17	29	12	
9077	11	7	5	9	25	16	
9021	10	5	4	18	22	4	
9030	10	5	3	19	26	7	
9012	8	6	3	7	16	9	
6244	8	4	1	8	14	6	
						Median =	8.5
						Mean =	8.5
Total	108	62	40				8.5

^a Based on data from the vendor of the seroconversion panels.

^b ARCHITECT HIV Ag/Ab Combo.

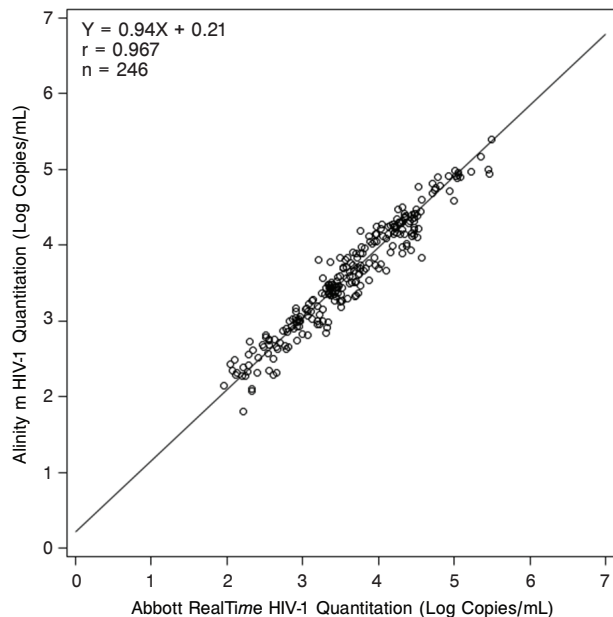
^c Days to first reactive result by HIV Ag/Ab Combo minus days to first detected result by Alinity m HIV-1.

^d All bleeds in these panels were detected or reactive. Zero was used as the "Days to First Detected/Reactive Result."

Clinical Performance – Method Correlation

The performance of Alinity m HIV-1 was compared with Abbott RealTime HIV-1 by analyzing 247 plasma specimens from HIV-1 infected patients (including group M, subtypes A, B, C, CRF01_AE, CRF02_AG, CRF06, CRF11, D, F, G and group O) that generated valid results. The Deming regression analysis was performed on 246 specimens that generated results within the quantitation range common to both assays, as shown in Figure 3. The mean bias between the two assays (Alinity m HIV-1 minus Abbott RealTime HIV-1) was -0.03 Log Copies/mL (95% CI: -0.05 to 0.00).

Figure 3 . Correlation between Alinity m HIV-1 and Abbott RealTime HIV-1



Clinical Performance – Test Agreement

To assess test agreement in support of confirmatory claim, a total of 494 specimens from HIV-1 positive and negative individuals were analyzed using Alinity m HIV-1 and a comparator CE-marked HIV-1 RNA assay and generated valid results. The overall percent agreement between the confirmatory interpretations of the two assays (Table 9) was 100.0% (494/494; 95% CI: 99.2 to 100.0%), with positive percent agreement of 100.0% (245/245; 95% CI: 98.5 to 100.0%) and negative percent agreement of 100.0% (249/249; 95% CI: 98.5 to 100.0%).

Table 9. Agreement Between Alinity m HIV-1 and Comparator HIV-1 RNA Assay

Comparator HIV-1 RNA Assay	Reactive for HIV-1 RNA	Alinity m HIV-1	
		HIV-1 RNA Detected	HIV-1 RNA Not Detected
	Reactive for HIV-1 RNA	245	0
	Non-Reactive for HIV-1 RNA	0	249 ^a

^a One specimen was initially detected by Alinity m HIV-1. Upon duplicate retests of additional aliquots, both replicates were not detected. Final interpretation was HIV-1 RNA not detected.

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KEY TO SYMBOLS

	Reference Number
	In Vitro Diagnostic Medical Device
	Lot Number
	In Vitro Test
	AMP TRAY
	ACT TRAY
	Unit
	Systemic Health Effects
	Warning
	Caution
	Consult Instructions for Use
	Temperature Limitation
	Sufficient for
	Use By
	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 HIV-1 AMP Kit.

The Alinity m HIV-1 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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53-608055/R6