# 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 the quality management system** | 21 September 2021 | MR |
| **Product performance evaluation** | Quarter (Q) 4 2022 -Q 2 2023 | MR |

MR: Meets Requirements

**Report amendments and/or product changes**

This public report has since been amended. Amendments may have arisen due to changes to the prequalified product for which the WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

| Version | Summary of amendments | Date of report amendment |
|---|---|---|
| 2.0 | Extended the claim and implemented the use of the DBS sample type for an HIV-1 viral load (VL) assay that can measure HIV-1 RNA levels in dried blood spot samples. | 10 June 2025 |

**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 Use claim for DBS Supplement:*

*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 in whole blood spotted on cards as dried blood spots (DBS) (ie, obtained via venipuncture or capillary blood). 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 testing DBS may be used to monitor disease prognosis by measuring baseline HIV-1 RNA level and to assess viral response to antiretroviral treatment by measuring changes in 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 to be used in screening blood, blood products, tissue or organ donors for HIV or as a diagnostic test to confirm the presence of HIV-1 infection.*

*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 ± 5°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 ± 5°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<br>• Alinity m HIV AMP TRAY 1<br>• Alinity m HIV AMP TRAY 2 | 192 Tests, product code 08N45-090<br>4 trays / 48 tests each<br>4 trays / 48 tests each |
| Alinity m Dried Blood Spot (DBS) Supplement v2.0 to the Alinity m HIV-1 Assay | Product code 08N45-040<br>Supplement |

**Items required but not provided:**

- 08N45-070 Alinity m HIV-1 CAL Kit,
- 08N45-080 Alinity m HIV-1 CTRL Kit,
- 09N66-001 Alinity m DBS Buffer 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,
- 08N45-04A (v1.0) or higher Alinity m HIV-1 Application Specification File for DBS Supplement v2.0,
- Vortex mixer,
- Centrifuge capable of 2000g
- 09N49-001 Alinity m LRV Tube[a],
- 15.8 mm well diameter Heat Block capable of reaching 55°C. If using DBS
- 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.
Alinity m DBS Buffer Kit should be stored at 15-30°C

**Shelf-life upon manufacture:**
24 months.
Alinity m DBS Buffer Kit is 18 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 discrepancies found during dossier screening and assessment findings were accepted on 22 April 2024.

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

## Manufacturing site inspection

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.

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 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<br>Limits of agreement | -0.27<br>-0.70 to 0.17 |
| Invalid rate %<br>(N=560) | 0.7% |
| **Sensitivity and specificity for the detection of virological failure at 1000 copies/mL** | |
| Sensitivity % (95% CI)<br>(N=231) | 93.1 (89.1 - 95.7) |
| Specificity % (95% CI)<br>(N=237) | 100 (98.4 - 100) |
| **Specificity among HIV- negative individuals** | |
| Specificity % (95% CI)<br>(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.<br>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%<br>At 4.0 $\log_{10}$ copies /mL, CV% were < 2% |
| Within-laboratory precision (reproducibility) | At 3.0 $\log_{10}$ copies /mL, CV% were < 3%<br>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<br>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.<br>The instrument is supplied with a UPS to ensure backup electricity is available. |
| Biosafety *(outside of infectious specimen handling)* | Operators reported no concerns about biosafety for the user.<br>**NOTE:** Some Alinity m bulk reagents contain guanidine thiocyanate, which, when mixed with bleach, produces a highly toxic gas. |
| Waste | The volume of liquid was approx—1L per run.<br>The volume of solid waste is approximately 7kg per run (IRUs and amplified container waste).<br>Waste disposal requires specific measures and usual laboratory biohazard waste disposal procedures.<br>**NOTE:** Some Alinity m bulk reagents contain guanidine thiocyanate, which, when mixed with bleach, produces a highly toxic gas. |

| | |
|---|---|
| Calibration | Calibrators are provided by the manufacturer and should be purchased separately.<br>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. |
| Maintenance | 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. |
| Other specific requirements | The instrument requires a stable power supply, has a considerable footprint, and requires consistent maintenance and service by Abbott-authorized engineers/service providers. |

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

2°C 8°C

Σ 48

AMP TRAY

REF 8N45P

53-602038/R1

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

LOT

Alinity m

HIV-1 AMP Kit

Abbott

# HIV-1 AMP Kit

REF 08N45-090

**AMP TRAY** 4 x Σ 48

**ACT TRAY** 4 x Σ 48

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

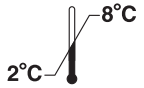
IVD

In Vitro Test

PRODUCT OF USA

www.molecular.abbott/portal

REF 08N45-090




53-602081/R3

LOT

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

CE 1434

EC REP

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

Abbott

Alinity m

# HIV-1

2

Abbott

UNIT 1

IVD

Σ 48

8°C
2°C

ACT TRAY

REF 8N45M

53-602085/R1

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

LOT

## 2. Instructions for use[1]

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

# 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.

| 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).
- 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

| | |
|---|---|
| REF | Reference Number |
| IVD | In Vitro Diagnostic Medical Device |
| EC REP | Authorized Representative in the European Community |
| | 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.

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

The Alinity m HIV-1 Applification 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

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

CE

www.molecular.abbott/portal



53-608203/R1

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



Abbott

# 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 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. 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±5°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±5°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 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.



Abbott

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 |
|---|---|
| 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,[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** | 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 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)<br>$K_2$ EDTA<br>$K_3$ EDTA<br>Plasma Preparation Tube (PPT)[b] |
| Serum | Serum<br>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 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.

**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 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 (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 ≥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 (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 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), 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.<br>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 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 x $ID^2$.

[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 |

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

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

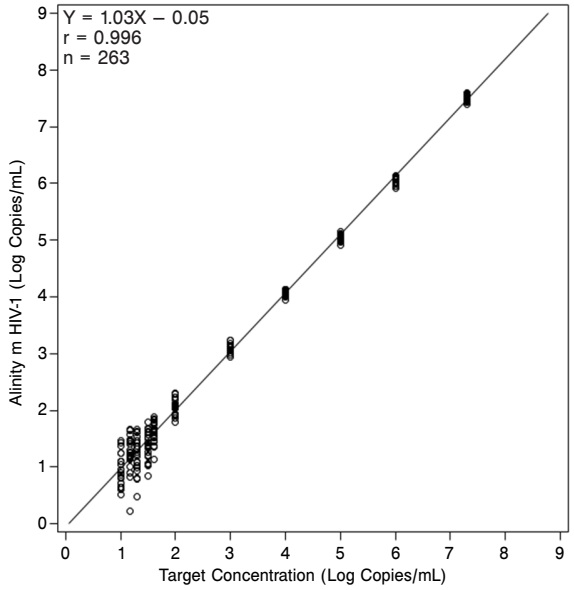
## Linear Range

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



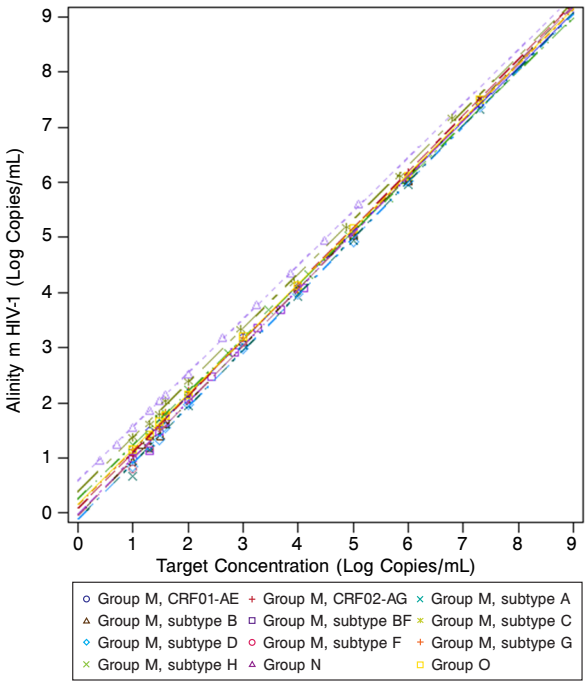

## Linearity Across Groups and Subtypes

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

## 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

| | | Neat (Undiluted) | Diluted |
|---|---|---|---|
| Dilution | Panel Member | 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

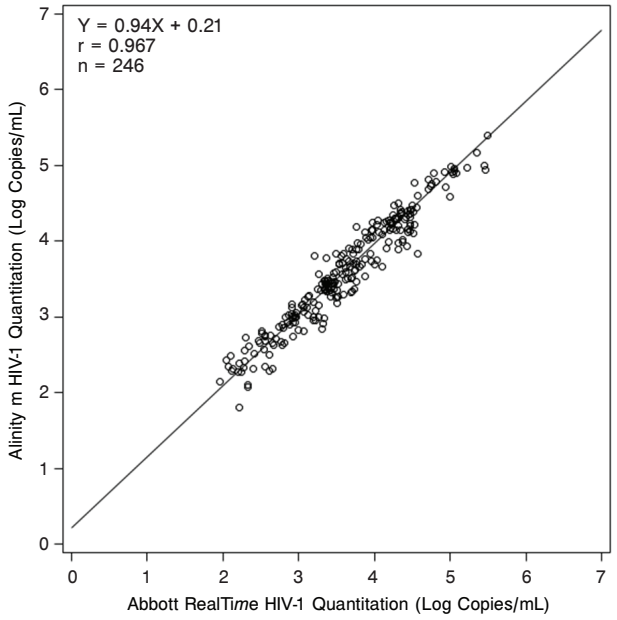
| | | No. of Detected/Reactive Panel Members | | Days to First Detected/ Reactive Result | | Difference in Days to First Detected/ Reactive Result (Based on Bleed Date)[c] |
|---|---|---|---|---|---|---|
| Panel ID | No. of Panel Members Analyzed | Alinity m HIV-1 | HIV Ag/Ab Combo[a,b] | Alinity m HIV-1 | 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 |
| Total | 108 | 62 | 40 | | Mean = | 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 RealTi*m*e 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 RealTi*m*e 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 RealTi*m*e 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

| | | Alinity m HIV-1 | |
|---|---|---|---|
| | | HIV-1 RNA Detected | HIV-1 RNA Not Detected |
| Comparator HIV-1 RNA Assay | 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.

## BIBLIOGRAPHY


1. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983;220:868-71.
2. Popovic M, Sarngadharan MG, Read E, et al. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-I) from patients with AIDS and pre-AIDS. *Science* 1984;224:497-500.
3. Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 1984;224:500-3.
4. Curran JW, Jaffe HW, Hardy AM, et al. Epidemiology of HIV infection and AIDS in the United States. *Science* 1988;239:610-16.
5. Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N Engl J Med* 1991;324:961-4.
6. Clark SJ, Saag MS, Decker WD, et al. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *N Engl J Med* 1991;324:954-60.
7. Albert J, Abrahamsson B, Nagy K, et al. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. *AIDS* 1990;4:107-12.
8. Horsburgh CR Jr, Ou CY, Jason J, et al. Duration of human immunodeficiency virus infection before detection of antibody. *Lancet* 1989;2(8664):637-40.
9. Pantaleo G, Graziosi C, Fauci AS. New concepts in the immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med* 1993;328:327-35.
10. Centers for Disease Control and Prevention and Association of Public Health Laboratories. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations. Available at http://stacks.cdc.gov/view/cdc/23447. Published June 27, 2014. Accessed July 17, 2014.
11. Gökengin D, Geretti AM, Begovac J, et al. 2014 European guideline on HIV testing. Int J STD AIDS 2014;25:695-704.
12. Ho DD, Neumann AU, Perelson AS, et al. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995;373:123-6.
13. Wei X, Ghosh SK, Taylor ME, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 1995;373:117-22.
14. Mellors JW, Rinaldo CR J, Gupta P, et al. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 1996;272:1167-70.
15. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997;126(12):946-54.
16. Chene G, Sterne JA, May M, et al. Prognostic importance of initial response in HIV-1infected patients starting potent antiretroviral therapy: analysis of prospective studies. *Lancet* 2003;362:679-86.
17. Egger M, May M, Chene G, et al. Prognosis of HIV-1 infected drug patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet* 2002;360:119-29.
18. Wood E, Hogg RS, Yip B, et al. Higher baseline levels of plasma human immunodeficiency virus type 1 RNA are associated with increased mortality after initiation of triple-drug antiretroviral therapy. *J Infect Dis* 2003;188:1421-5.
19. US Department of Health and Human Services. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. Available at: http://AIDSinfo.nih.gov/guidelines.
20. Perelson AS, Essunger P, Cao Y, et al. Decay characteristics of HIV-1 infected compartments during combination therapy. *Nature* 1997;387(6629):188-91.
21. European AIDS Clinical Society (EACS). Guidelines Version 8 November, 2014. Available at http://www.eacsociety.org.
22. The World Health Organization (WHO) Consolidated Guidelines on the Use of Antiviral Drugs for Treating and Preventing HIV Infection (2013). Available at http://www.who.int/hiv/pub/guidelines/arv2013/en.
23. Mulder J, McKinney N, Christopherson C, et al. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. *J Clin Microbiol* 1994;32:292-300.
24. Dewar RL, Highbarger HC, Sarmiento MD, et al. Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. *J Inf Diseases* 1994;170:1172-9.
25. Van Gemen B, Kievits T, Schukkink R, et al. Quantification of HIV-1 RNA in plasma using NASBA™ during HIV-1 primary infection. *J Virol Methods* 1993;43:177-87.
26. Taylor BS, Sobieszczyk ME, McCutchan FE, et al. The challenge of HIV-1 subtype diversity. *N Engl J Med* 2008;358:1590-1602.
27. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. URL:https://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf].
28. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, *Bloodborne pathogens*.
29. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. CLSI Document M29-A4. Wayne, PA: CLSI; 2014.
30. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.


## KEY TO SYMBOLS

| | |
|---|---|
| REF | Reference Number |
| IVD | In Vitro Diagnostic Medical Device |
| LOT | Lot Number |
| In Vitro Test | In Vitro Test |
| AMP TRAY | AMP TRAY |
| ACT TRAY | ACT TRAY |
| UNIT | Unit |
|  | Systemic Health Effects |
|  | Warning |
|  | Caution |
|  | Consult Instructions for Use |
|  | Temperature Limitation |
|  | Sufficient for |
|  | Use By |
| EC REP | Authorized Representative in the European Community |
|  | Manufacturer |

## TECHNICAL ASSISTANCE

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

Abbott Molecular Inc. is the legal manufacturer of the Alinity m 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.

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

CE 1434

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


53-608055/R6



Abbott

# Alinity m

# Dried Blood Spot (DBS) Supplement v2.0 to the Alinity m HIV-1 Assay

en

IVD

REF 08N45-040

53-608536/R1

Created December 2024

REF 08N45-040

53-608536/R1

## 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.

This Instructions For Use (IFU) is a supplement to the Alinity m HIV-1 assay (List No. 08N45-090) Package Insert, specifically for dried blood spot (DBS) sample type.

## 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

## 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 quantitate Human Immunodeficiency Virus type 1 (HIV-1) RNA in whole blood spotted on cards as dried blood spots (DBS) (ie, obtained via venipuncture or capillary blood). 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 testing DBS may be used to monitor disease prognosis by measuring baseline HIV-1 RNA level and to assess viral response to antiretroviral treatment by measuring changes in 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 to be used in screening blood, blood products, tissue or organ donors for HIV or as a diagnostic test to confirm the presence of HIV-1 infection.

## 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]

Quantitative measurement of HIV-1 RNA levels in plasma or dried blood spot (DBS) specimens has been shown to be an essential parameter in prognosis and management of HIV-1 infected individuals.[10–16] Viral load monitoring of HIV-1 levels is considered the most reliable indicator of initial and sustained response to antiretroviral therapy (ART) and should be obtained at the entry into care, at initiation and during therapy.[17–20]

Decisions regarding changes in ART are guided by monitoring changes in HIV-1 viral load levels over time.[10] The minimal change in viral load considered to be reflective of a significant change associated with ART within the first 2 to 8 weeks is equal to 0.5 Log Copies/mL reduction.[18] In addition, optimal viral suppression is considered when the viral load remains persistently below the lower limit of detection.[18,20]

Virological response failure, which is suggestive of resistance to current ARTs, is considered to occur when there is a persistently elevated HIV-1 viral load according to guidelines.[10,18,21] If resistance is confirmed, the ART is revised to use higher-tiered drugs. HIV-1 RNA levels can be quantitated by nucleic acid amplification.[10,22–24]

The Alinity m HIV-1 assay can be used to measure the levels of HIV-1 RNA isolated from patient DBS specimens 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.[25] 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: the Integrase and the Long Terminal Repeat regions.

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 three 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°C 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 −25°C to −15°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 −25°C to −15°C.

The Alinity m HIV-1 assay utilizes RT-PCR to amplify and detect HIV-1 RNA genomic sequences that have been extracted from human DBS specimens. The steps of the Alinity m HIV-1 assay consist of pre-analytical sample elution from the DBS card, sample preparation, RT-PCR assembly, amplification/detection, and result calculation and reporting. Following the pre-analytical DBS sample elution, all steps of the Alinity m HIV-1 assay procedure are executed automatically by the Alinity m System.

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

HIV-1 RNA from human DBS samples is extracted using the Alinity m DBS Buffer Kit during the pre-analytical DBS specimen elution step and using the Alinity m Sample Prep Kit 2, Alinity m Lysis Solution, and Alinity m Diluent Solution during sample preparation on the Alinity m System. 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 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.



Abbott

At the beginning of the Alinity m HIV-1 sample preparation process on Alinity m System, 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 DBS samples. Two levels of calibrators are processed through sample preparation and RT-PCR to generate the HIV-1 DBS assay-specific calibration curve. The concentration of HIV-1 RNA in controls and concentration/detection of HIV-1 RNA in specimens 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.

For information regarding REAGENTS, WARNINGS AND PRECAUTIONS, Safety Precautions, Reagent Shipment and Storage, Reagent Shipment and Handling, and Indications of Reagent Deterioration, refer to the Alinity m HIV-1 AMP Kit Package Insert (08N45-090).

## STANDARDIZATION

Concentrations were standardized against an HIV-1 viral standard from the Virology Quality Assurance (VQA) laboratory of the AIDS Clinical Trial Group.

## INSTRUMENT PROCEDURE

The Alinity m HIV-1 Application Specification File for DBS Supplement v2.0 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

DBS specimens can be prepared using either capillary blood or venipuncture whole blood collected in an EDTA tube. If whole blood needs to be shipped or stored before spotting, the whole blood sample should be maintained under controlled temperature conditions (as specified in the **Specimen Storage** section). Before spotting whole blood collected in a tube, mix the blood gently (ie, by inversions). Spot the blood onto the one-half-inch (12 millimeters) circles of an Ahlstrom-Munktell TFN card (or equivalent such as Whatman 903), ensuring that the entire circle is covered by spotting at least 70 microliters of blood (approximately 3 to 5 blood drops) on each circle.

Air dry DBS cards at ambient temperature (15°C to 30°C) for at least 3 hours. High humidity may require longer drying time.

DBS specimen can be stored under the conditions as specified in the **Specimen Storage** section prior to assay processing.

### Specimen Storage

| Specimen | Condition | Maximum Storage Time | Special Instructions |
|---|---|---|---|
| Whole Blood | 2°C to 8°C | 2 days | Whole blood may be stored between draw and DBS preparation. |
| | 15°C to 30°C | 1 day | |
| DBS | 2°C to 8°C | 12 weeks | Each DBS card should be packaged in a sealable bag with desiccant packs.[26] |
| | 15°C to 30°C Ambient Humidity | 12 weeks | |
| | 15°C to 45°C up to 85% Relative Humidity | 4 weeks | |
| | −25°C to −15°C | 12 weeks | |
| | −70°C or colder | Longer storage | |

### 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: Pre-analytical DBS Sample Elution

The pre-analytical sample elution is for DBS specimens only and not to be performed on calibrators or controls. Prior to loading DBS samples onto an Alinity m Universal Sample Rack (sample rack) for testing on the Alinity m System, the following procedures must be followed:

1) Hold perforated DBS card above the Alinity m Transport Tube or Alinity m Aliquot Tube.

2) Push the DBS circle out of the card using a clean pipette tip, one DBS circle per tube. Each DBS should be approximately one-half-inch (12 millimeters) in diameter. **Use a new pipette tip for each DBS sample to prevent cross contamination**.

Alternatively, from non-perforated DBS cards, cut out 1 entire DBS (approximately one-half-inch [12 millimeters] in diameter) for each specimen and transfer to the tube.

**NOTE: Avoid direct contact of the cutting surface with DBS specimens. Decontaminate the tool used to cut DBS between specimens if necessary, according to good laboratory practices.**

3) Fill the sample tube with 1.3 mL of Alinity m DBS Buffer from the Alinity m DBS Buffer Kit (09N66-001). **Note: Do not use any other lysis buffer or any other reagents for this step.**

4) Ensure that the DBS circle is fully submerged in the Alinity m DBS Buffer by tapping the tube or using a clean pipette tip to push the DBS into the buffer. **Note: If a pipette tip is used to push the DBS into the buffer, ensure that the pipette tip does not cause DBS buffer volume loss due to the liquid containment in the tip and/or absorption of the buffer by the tip filter.**

5) Manually shake or swirl the sample tubes and then place them in a heat block set at 55°C. Do not vortex the samples. Incubate for 30 minutes (± 2 minutes) at 55°C. Once incubation is complete, manually shake or swirl the sample tubes. Specimens may be stored for up to 24 hours at ambient temperature prior to loading them onto the sample rack for testing on the Alinity m System. If samples are stored beyond the 30 minute pre-analytical incubation, swirl the sample prior to loading on the sample rack.

All specimen 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 IFU or the Alinity m System Operations Manual, Section 4, for tube sizes.

## PROCEDURE

### Materials Required but not Provided

- 08N45-090 Alinity m HIV-1 AMP Kit
- 09N66-001 Alinity m DBS Buffer Kit
- 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
- Alinity m HIV-1 Application Specification File for DBS Supplement v2.0, 08N45-04A (v1.0) or higher
- 15.8 mm well diameter Heat Block capable of reaching 55˚C
- Calibrated pipettes capable of delivering 10 to 1000 µL
- Aerosol barrier pipette tips for 10 to 1000 µL pipettes
- 09N49-010 Alinity m Transport Tube Pierceable Capped
- 09N49-011 Alinity m Transport Tube
- 09N49-013 Alinity m Aliquot Tube

For information on additional materials required but not provided, refer to the Alinity m HIV-1 AMP Kit Package Insert.

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 IFU carefully before processing samples.
- Ensure the Alinity m HIV-1 AMP TRAY 1 is tapped prior to loading on the Alinity m System per instructions in the Alinity m HIV-1 AMP Kit Package Insert.
- Ensure the Alinity m HIV-1 ACT TRAY 2 is centrifuged prior to loading on the Alinity m System per instructions in the Alinity m HIV-1 AMP Kit Package Insert.
- Refer to the Alinity m HIV-1 AMP Kit Package Insert for additional procedural precautions before processing samples.

#### Assay Procedure

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 of the Alinity m HIV-1 AMP Kit Package Insert. 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) and select the assay (HIV-DBSV2). Select appropriate specimen type as shown in the table below.

| Select Specimen Type | When Testing |
|---|---|
| DBS | Dried Blood Spot (DBS) **Clinical Specimen** |
| PAI | Contrived DBS Sample (eg, DBS panel) |
| LDT | Laboratory Defined Test |

**Note: Selection of correct specimen type is important for generating accurate results. DBS is a default specimen selection.**

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.

Sample tubes need to meet the requirements for sample volume when loaded on the Alinity m System. Alinity m Transport Tubes or Alinity m Aliquot Tubes with DBS sample may be placed on the sample rack onboard the system for up to 4 hours prior to processing.

| Tube Type[a] | List No. | DBS Buffer Volume Required | Cap Requirement on Instrument |
|---|---|---|---|
| Alinity m Aliquot Tube | 09N49-013 | 1.3 mL | Uncapped |
| Alinity m Transport Tube | 09N49-011 | 1.3 mL | Uncapped |
| Alinity m Transport Tube Pierceable Capped | 09N49-010 | 1.3 mL | Uncapped[b] |

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

[b] Cap must be removed prior to loading.

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

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

## QUALITY CONTROL PROCEDURES

#### Assay Calibration

A calibration curve is required to quantitate the HIV-1 RNA concentration in DBS samples and controls.

**Note: Calibration for the DBS assay cannot be shared with other approved specimen types (eg, plasma and serum).**

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 for DBS on the Alinity m System. **Do not process the Alinity m HIV-1 CAL A and Alinity m HIV-1 CAL B using the Pre-Analytical DBS Sample Elution Procedure in this IFU.**

For instructions on performing an assay calibration, refer to the Alinity m HIV-1 AMP Kit Package Insert, the Alinity m HIV-1 CAL Kit Package Insert, and 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 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.

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 for DBS Supplement v2.0 is installed.

#### Detection of Inhibition

For information on detection of inhibition, refer to the Alinity m HIV-1 AMP Kit Package Insert.

#### Negative and Positive Controls

For information on negative and positive controls, refer to the Alinity m HIV-1 AMP Kit Package Insert and the Alinity m HIV-1 CTRL Kit Package Insert.

**Note: Control status for the DBS assay cannot be shared with other specimen types (eg, plasma and serum).**

## RESULTS

#### Calculation

The concentration of HIV-1 RNA in a DBS sample is calculated by the Alinity m System software based on the calibration curve. The reported DBS concentration result represents the HIV-1 viral concentration in the plasma of the whole blood specimen from which the DBS specimen is obtained. 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.

### Interpretation of Results

For each DBS specimen the Alinity m System will report a result and a result interpretation as shown in the table below. If applicable, message codes or flags will also be displayed.

| HIV-1 Result for DBS | Interpretation | Flags |
|---|---|---|
| Not Detected | Target not detected | |
| < 2.60 Log Copies/mL | Detected < LLOQ | |
| 2.60 to 7.00 Log Copies/mL | | |
| > 7.00 Log Copies/mL | > ULOQ | |

### 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

**IVD**

- For In Vitro Diagnostic Use
- DBS specimens prepared using either capillary or venipuncture whole blood collected in an EDTA tube may be used with the Alinity m HIV-1 assay. For the use of other specimen types evaluated with the Alinity m HIV-1 assay, refer to the Alinity m HIV-1 AMP Kit Package Insert.
- Ahlstrom-Munktell TFN and Whatman 903 DBS cards may be used. The use of other cards has not been validated with Alinity m HIV-1.
- This test requires appropriate specimen collection and handling (refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this IFU).
- Only personnel proficient in DBS preparation and pre-processing, the procedures of a molecular diagnostic assay, and the Alinity m System should perform this procedure.
- Inappropriate collection, drying, storage, and handling of DBS cards may lead to inaccurate test results.
- If the HIV-1 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- The systems 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 IFU.

## SPECIFIC PERFORMANCE CHARACTERISTICS

Refer to the Alinity m HIV-1 AMP Kit (08N45-090) Package Insert for additional Performance Characteristics of the Alinity m HIV-1 assay.

### Limit of Detection

For DBS, the limit of detection (LoD) of Alinity m HIV-1 is 400 Copies/mL. The LoD was determined by testing dilutions of the 3rd World Health Organization (WHO) HIV-1 International Standard (NIBSC code: 10/152; group M subtype B) prepared in HIV-1 negative human whole blood and spotted onto DBS cards. Testing for each HIV-1 RNA concentration was performed with 4 AMP Kit lots 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) for DBS samples

| HIV-1 RNA (Copies/mL) | No. of Valid Replicates | No. Detected | Detection Rate (%) |
|---|---|---|---|
| 500 | 93 | 93 | 100.0 |
| 400 | 94 | 90 | 95.7 |
| 300 | 93 | 82 | 88.2 |
| 200 | 94 | 72 | 76.6 |
| 100 | 92 | 49 | 53.3 |
| 50 | 93 | 22 | 23.7 |

Probit analysis determined that the concentration of HIV-1 RNA detected with 95% probability was 393.15 Copies/mL (95% CI 331.92 to 487.93 Copies/mL).

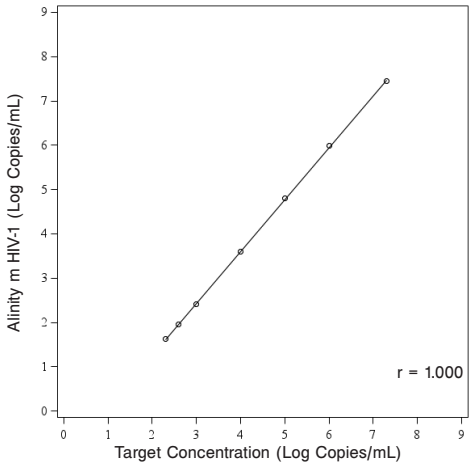
### Linear Range

For DBS, Alinity m HIV-1 linearity was assessed by testing a dilution series of an HIV-1 viral stock representing group M subtype B in negative human whole blood spotted on DBS cards. The dilution series consisted of 7 panel members spanning from 200 Copies/mL to 20,000,000 Copies/mL.

Representative results for Alinity m HIV-1 linearity performance for DBS samples are shown in **Figure 1**.

The Alinity m HIV-1 assay demonstrated linearity across the entire range of DBS sample HIV-1 RNA concentrations tested (from 200 Copies/mL to 20,000,000 Copies/mL). The upper limit of quantitation (ULoQ) for the Alinity m HIV-1 assay is 10 million Copies/mL and the lower limit of quantitation (LLoQ) is equivalent to the LoD (400 Copies/mL) for the DBS claim.

**Figure 1.** Linearity of DBS Samples[a]




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

### Specificity

For DBS, the Alinity m HIV-1 specificity was determined by testing HIV-1 negative whole blood specimens from individual donors spotted onto DBS cards. A total of 111 DBS specimens were analyzed. The specificity was 100.0% (95% CI: 96.7 to 100.0%).

### Carryover

For DBS, the carryover rate for the Alinity m HIV-1 assay was determined in two studies. Study 1 evaluated the carryover rate in the Sample Input Rack and Sample Processing Unit by analyzing 376 valid replicates of HIV-1 negative DBS samples processed from alternating positions in the Sample Input Rack with 376 valid replicates of high concentration HIV-1 positive DBS samples at 1,000,000 Copies/mL, across multiple runs. HIV-1 RNA was not detected in any of the HIV-1 negative DBS 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 360 valid replicates of HIV-1 negative DBS samples processed from alternating positions at the AMP Tray with 360 valid replicates of high concentration HIV-1 positive DBS samples at 1,000,000 Copies/mL across multiple runs. HIV-1 RNA was not detected in any of the HIV-1 negative DBS samples, resulting in a carryover rate of 0.0% (95% CI: 0.0 to 1.1%).

HIV-1 RNA was not detected in any of the total 736 HIV-1 negative DBS samples, resulting in an overall Alinity m HIV-1 carryover rate of 0.0% (95% CI: 0.0 to 0.5%).

### Precision

For DBS, 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 3.60 to 7.00 Log Copies/mL (4000 to 10,000,000 Copies/mL), and less than or equal to 0.46 Log Copies/mL at three times the LLoQ.

Precision of Alinity m HIV-1 assay testing DBS samples was determined by analyzing an 8-member DBS panel, which was prepared by diluting an HIV-1 viral stock into HIV-1 negative human whole blood. 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.

The results, representative of the precision of Alinity m HIV-1, are summarized in **Table 2**.

**Table 2.** DBS Precision

| Panel | n[e] | Mean Concentration (Log Copies/mL) | Within-Run[a] Component SD | Between-Run Component SD | Between-Day Component SD | Within-Laboratory[b] SD | Between- Instrument Component[c] SD | Total[d] SD |
|---|---|---|---|---|---|---|---|---|
| 01 | 127 | 1.79 | 0.36 | 0.07 | 0.10 | 0.38 | 0.08 | 0.39 |
| 02 | 140 | 2.42 | 0.22 | 0.00 | 0.07 | 0.23 | 0.00 | 0.23 |
| 03 | 144 | 2.81 | 0.16 | 0.06 | 0.03 | 0.17 | 0.06 | 0.18 |
| 04 | 144 | 3.06 | 0.16 | 0.02 | 0.01 | 0.16 | 0.04 | 0.17 |
| 05 | 137 | 3.36 | 0.11 | 0.05 | 0.05 | 0.14 | 0.02 | 0.14 |
| 06 | 143 | 4.74 | 0.08 | 0.04 | 0.04 | 0.10 | 0.02 | 0.10 |
| 07 | 138 | 6.06 | 0.07 | 0.00 | 0.02 | 0.08 | 0.00 | 0.08 |
| 08 | 143 | 7.53 | 0.06 | 0.00 | 0.03 | 0.07 | 0.06 | 0.09 |

[a] Within-Run SD is also known as repeatability.
[b] Within-Laboratory includes Within-Run, Between-Run, and Between-Day Components.
[c] Between-Instrument component consists of Alinity m System, AMP Kit lot, and operator.
[d] Total includes Within-Run, Between-Run, Between-Day, and Between-Instrument Components. Total SD is also known as reproducibility.
[e] Valid and detected replicates.

Repeatability and reproducibility for Panel Member 02 (near LoD) were 0.22 Log Copies/mL and 0.23 Log Copies/mL, respectively. Repeatability and reproducibility for Panel Member 07 (near ULoQ) were 0.07 Log Copies/mL and 0.08 Log Copies/mL, respectively, and for Panel Member 08 (near ULoQ) were 0.06 Log Copies/mL and 0.09 Log Copies/mL, respectively.

### Clinical Performance

HIV-1 RNA quantitation was compared between DBS results and matched plasma results obtained from testing with the Alinity m HIV-1 assay. Plasma and DBS specimens from a total of 269 subjects were included in the analysis. For each HIV-1 infected subject, dried blood spots were prepared from both capillary blood and venous blood. The quantitative results from specimens that fell within the common assay dynamic range were analyzed by the least squares linear regression method (capillary DBS versus plasma N=155, venous DBS versus plasma N=154, and capillary DBS versus venous DBS N=156). For the correlation between capillary DBS and plasma, the correlation coefficient was 0.921, the slope was 1.02 (95% CI: 0.95, 1.09), and the intercept was -0.35 Log Copies/mL (95% CI: -0.67, -0.03) (**Figure 2**). For the correlation between venous DBS and plasma, the correlation coefficient was 0.914, the slope was 1.02 (95% CI: 0.95, 1.09), and the intercept was -0.40 Log Copies/mL (95% CI: -0.73, -0.06) (**Figure 3**). For the correlation between capillary DBS and venous DBS, the correlation coefficient was 0.973, the slope was 0.97 (95% CI: 0.93, 1.01), and the intercept was 0.16 Log Copies/mL (95% CI: -0.00, 0.32) (**Figure 4**). Additionally, Bland-Altman plots for these same comparisons are shown in **Figures 5, 6,** and **7**. The mean bias was -0.26 Log Copies/mL between capillary DBS versus plasma (**Figure 5**), -0.29 Log Copies/mL between venous DBS versus plasma (**Figure 6**), and 0.03 Log Copies/mL between capillary DBS versus venous DBS (**Figure 7**). Predicted bias between the capillary DBS and plasma was evaluated at LLoQ and ULoQ target levels. In addition, predicted bias between venous DBS and plasma was evaluated at LLoQ and ULoQ target levels. Refer to **Table 3** for the predicted bias summary.

**Table 3.** Predicted Bias between DBS (Capillary or Venous) and Plasma at LLoQ and ULoQ

| Comparison | Level | Viral Load Target Level (Log Copies/mL) | Predicted Bias |
|---|---|---|---|
| Capillary DBS vs Plasma | LLoQ | 2.60 | -0.30 |
| | ULoQ | 7.00 | -0.21 |
| Venous DBS vs Plasma | LLoQ | 2.60 | -0.34 |
| | ULoQ | 7.00 | -0.24 |

**Figure 2.** Correlation: Capillary DBS Versus Plasma

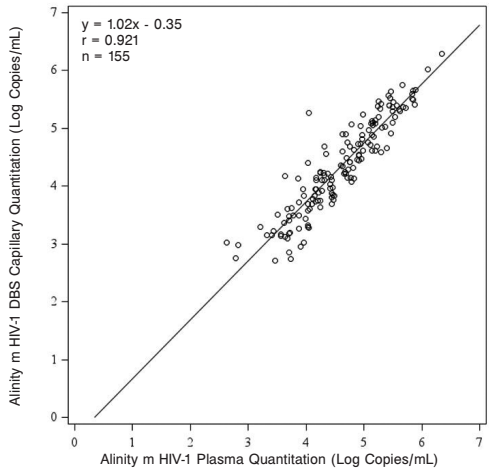



**Figure 3.** Correlation: Venous DBS Versus Plasma

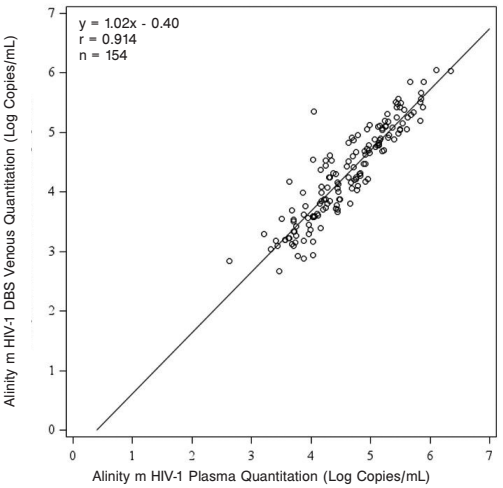


**Figure 4.** Correlation: Capillary DBS Versus Venous DBS

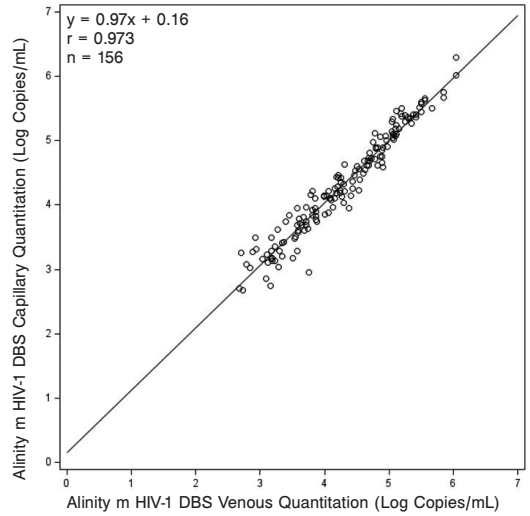



**Figure 5.** Bias: Capillary DBS Versus Plasma

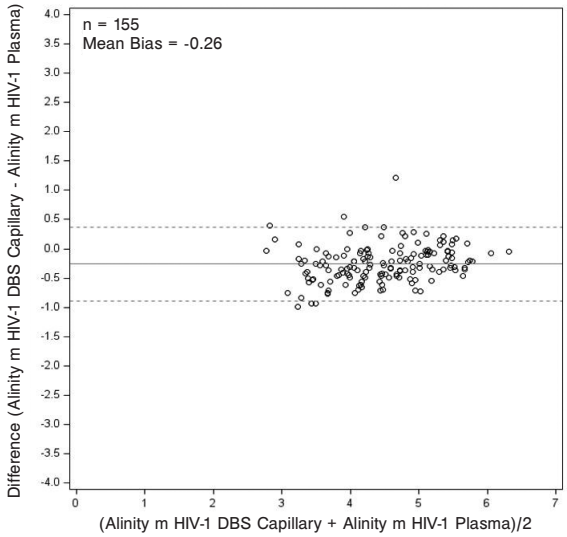



**Figure 6.** Bias: Venous DBS Versus Plasma

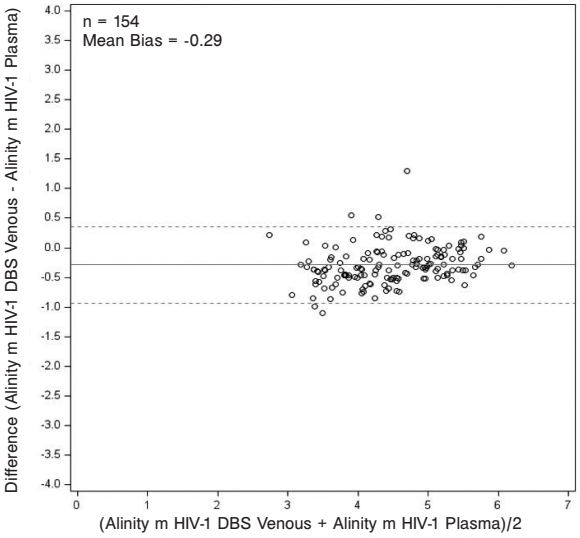
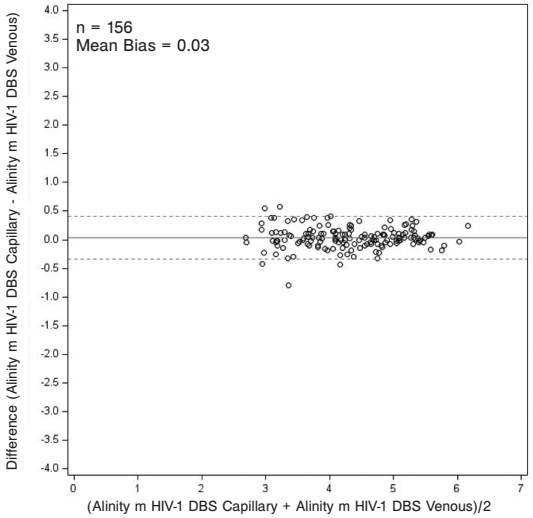



**Figure 7.** Bias: Capillary DBS Versus Venous DBS




Agreement between DBS results and matched plasma results were assessed at the 1,000 Copies/mL (3.0 Log Copies/mL) clinical threshold for detecting virological failure (**Tables 4** and **5**).[10] The positive percent agreement and negative percent agreement between capillary DBS and matched plasma results was 94.3% (95% CI: 89.5 to 97.0%) and 97.3% (95% CI: 92.4 to 99.1%), respectively. The positive percent agreement and negative percent agreement between venous DBS and matched plasma results was 94.9% (95% CI: 90.3 to 97.4%) and 100.0% (95% CI: 96.7 to 100.0%), respectively.

**Table 4**. Capillary DBS and Plasma Agreement

| | | Plasma (Log Copies/mL) | |
|---|---|---|---|
| | | ≥ **3.00** | < **3.00** |
| **Capillary DBS (Log Copies/mL)** | ≥ **3.00** | 148 | 3 |
| | < **3.00** | 9 | 109 |
| **Positive Percent Agreement: 94.3% (148/157), 95% CI (89.5%, 97.0%)** | | | |
| **Negative Percent Agreement: 97.3% (109/112), 95% CI (92.4%, 99.1%)** | | | |

**Table 5**. Venous DBS and Plasma Agreement

| | | Plasma (Log Copies/mL) | |
|---|---|---|---|
| | | ≥ **3.00** | < **3.00** |
| **Venous DBS (Log Copies/mL)** | ≥ **3.00** | 149 | 0 |
| | < **3.00** | 8 | 112 |
| **Positive Percent Agreement: 94.9% (149/157), 95% CI (90.3%, 97.4%)** | | | |
| **Negative Percent Agreement: 100.0% (112/112), 95% CI (96.7%, 100.0%)** | | | |

## BIBLIOGRAPHY


1. Barré-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science*. 1983;220(4599):868-871. doi:10.1126/science.6189183
2. Popovic M, Sarngadharan MG, Read E, et al. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science*. 1984(4648);224:497-500. doi:10.1126/science.6200935
3. Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science*. 1984;224(4648):500-503. doi:10.1126/science.6200936
4. Curran JW, Jaffe HW, Hardy AM, et al. Epidemiology of HIV infection and AIDS in the United States. *Science*. 1988;239(4840):610-616. doi:10.1126/science.3340847
5. Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N Engl J Med*. *1991;324(14):961-964.* doi:10.1056/NEJM199104043241405
6. Clark SJ, Saag MS, Decker WD. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *N Engl J Med*. 1991;324(14):954-960. doi:10.1056/NEJM199104043241404
7. Albert J, Abrahamsson B, Nagy K, et al. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. *AIDS*. 1990;4(2):107-112. doi:10.1097/00002030-199002000-00002
8. Horsburgh CR Jr, Ou CY, Jason J, et al. Duration of human immunodeficiency virus infection before detection of antibody. *Lancet*. 1989;2(8664):637-640. doi:10.1016/s0140-6736(89)90892-1
9. Pantaleo G, Graziosi C, Fauci AS. The immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med*. 1993;328(5):327-335. doi:10.1056/NEJM199302043280508
10. World Health Organization. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach, Second Edition 2016. June 1, 2016. Accessed June 27, 2024. http://www.who.int/hiv/pub/arv/arv-2016/en/
11. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature*. 1995;373(6510):123-126. doi:10.1038/373123a0
12. Wei X, Ghosh SK, Taylor ME, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature*. 1995;373(6510):117-122. doi:10.1038/373117a0
13. Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science*. 1996;272(5265):1167-1170. doi:10.1126/science.272.5265.1167
14. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med*. 1997;126(12):946-954. doi:10.7326/0003-4819-126-12-199706150-00003
15. Chêne G, Sterne JA, May M, et al. Prognostic importance of initial response in HIV-1 infected patients starting potent antiretroviral therapy: analysis of prospective studies. *Lancet*. 2003;362(9385):679-686. doi:10.1016/s0140-6736(03)14229-8
16. Egger M, May M, Chêne G, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet*. 2002;360(9327):119-129. doi:10.1016/s0140-6736(02)09411-4
17. Wood E, Hogg RS, Yip B, et al. Higher baseline levels of plasma human immunodeficiency virus type 1 RNA are associated with increased mortality after initiation of triple-drug antiretroviral therapy. *J Infect Dis*. 2003;188(10):1421-1425. doi:10.1086/379201
18. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV. Department of Health and Human Services. Updated February 27, 2024. Accessed June 27, 2024. https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-arv
19. World Health Organization. Updated Recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV: interim guidelines. Supplement to the 2016 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. December 2018. Accessed July 25, 2024. https://www.who.int/publications/i/item/WHO-CDS-HIV-18.51
20. Perelson AS, Essunger P, Cao Y, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature*. 1997;387(6629):188-191. doi:10.1038/387188a0
21. European AIDS Clinical Society (EACS). EACS Guidelines Version 12.0, October 2023. Accessed June 27, 2024. https://www.eacsociety.org/guidelines/eacs-guidelines/
22. Mulder J, McKinney N, Christopherson C, Sninsky J, Greenfield L, Kwok S. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. *J Clin Microbiol*. 1994;32(2):292-300. doi:10.1128/jcm.32.2.292-300.1994
23. Dewar RL, Highbarger HC, Sarmiento MD, et al. Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. *J Infect Dis*. 1994;170(5):1172-1179. doi:10.1093/infdis/170.5.1172
24. Van Gemen B, Kievits T, Schukkink R, et al. Quantification of HIV-1 RNA in plasma using NASBA™ during HIV-1 primary infection. *J Virol Methods*. 1993;43(2):177-187. doi:10.1016/0166-0934(93)90075-3
25. Taylor BS, Sobieszczyk ME, McCutchan FE, Hammer SM. The challenge of HIV-1 subtype diversity. *N Engl J Med*. 2008;358(15):1590-1602. doi:10.1056/NEJMra0706737
26. World Health Organization. WHO Manual for HIV Drug Resistance Testing Using Dried Blood Spot Specimens. 3rd ed. World Health Organization; 2020. Accessed June 27, 2024. https://www.who.int/publications/i/item/9789240009424


## Key to Symbols

| | |
|---|---|
| REF | Reference Number |
| IVD | In Vitro Diagnostic Medical Device |
| LOT | Lot Number |
| In Vitro Test | In Vitro Test |
| AMP TRAY | AMP TRAY |
| ACT TRAY | ACT TRAY |
| UNIT | Unit |
|  | Health Hazard |
|  | Exclamation Mark |
|  | Caution |
|  | Consult Instructions for Use or Consult Electronic Instructions for Use |
|  | Temperature Limit |
|  | Contains sufficient for <*n*> tests |
|  | Use By |
| EC REP | Authorized Representative in the European Community/European Union |
|  | 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 Dried Blood Spot (DBS) Supplement v2.0 to the Alinity m HIV-1 Assay.

## SUMMARY OF SAFETY AND PERFORMANCE STATEMENT

A summary of safety and performance (SSP) for this device is available at https://ec.europa.eu/tools/eudamed. This is the SSP location after the launch of European Database on Medical Devices. Search for device using UDI-DI provided on the outer package of the device.

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

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

CE
2797

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



53-608536/R1
December 2024

(01)00884999051584
(240)08N45-040



Abbott