1. GENERAL INFORMATION

Device Generic Name:	In vitro reverse transcription polymerase chain reaction (PCR)-based assay for detection of HIV-1 RNA.
Device Trade Name:	Alinity m HIV-1 Alinity m System
Device Product Code:	MZF OOI
Applicant Name and Address:	Abbott Molecular Inc. 1300 E. Touhy Ave Abbott Molecular Inc.
Establishment Registration Number:	3005248192
Premarket Approval Application (PMA) Number:	BP200455
Date of Panel Recommendation:	Not Applicable

- □ **I concur with the summary review.**
- □ **I concur with the summary review and include a separate review to add further analysis.**
- □ **I do not concur with the summary review and include a separate review.**

Office's Signatory Authority:

Nicole Verdun, M.D.
Director, OBRR/CBER

Date of FDA Notice of Approval: July 02, 2020

Material Reviewed/Consulted: The PMA, amendments to the PMA, and other specific documentation used in developing the Summary of Safety and Effectiveness (SSE).

Review memos from the following reviewers were used in developing the SSE:

Discipline Reviewed	Reviewer Names
Product Design	Viswanath Ragupathy
Preclinical and Clinical Studies	Viswanath Ragupathy Jiangqin Zhao Nitin Verma Xue Wang Krishnakumar Devadas Zullo Susan
Chemistry/Manufacturing/Controls (CMC)	Viswanath Ragupathy Jiangqin Zhao Xue Wang
Instrumentation and Software	Nick Anderson
Statistician	Ho-Hsiang Wu
Bioresearch Monitoring Inspection (BIMO)	Bhanu Kannan
DMPQ/pre-approval inspection	Hsiaoling Wang
Product and Promotional Labeling (OCBQ/DCM/APLB)	Dana Jones Viswanath Ragupathy
Scientific and programmatic aspects	Pradip Akolkar David Leiby Julia Lathrop Indira Hewlett
Policy	Sayah Nedjar Hira Nakhasi J. Peyton Hobson

2. INDICATIONS FOR USE

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

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

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

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

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

3. DEVICE DESCRIPTION

The Alinity m HIV-1 assay utilizes real-time reverse transcription polymerase chain reaction (RT-PCR) to amplify and detect HIV-1 RNA genomic sequences that have been extracted from human plasma specimens. The Alinity m HIV-1 assay is designed to detect two highly conserved regions within the HIV-1 genome: the Integrase (INT) region of the pol gene and the Long Terminal Repeat (LTR) region. The steps of the Alinity m HIV-1 assay consist of sample preparation, amplification/detection, result calculation and reporting. All steps of the Alinity m HIV-1 assay procedure are executed automatically by the Alinity m System. Manual dilutions may be performed for low-volume specimens to meet the minimum volume requirement, and for high-titer specimens above the upper limit of quantitation (ULoQ).

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

HIV-1 RNA from human plasma is extracted using the Alinity m Sample Prep Kit 2, Alinity m Lysis Solution, and Alinity m Diluent Solution. The Alinity m System employs magnetic microparticle technology to facilitate nucleic acid capture, wash, and elution. The resulting purified RNA is then combined with Alinity m HIV-1 activation reagent and 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 Internal Control (IC) on the AMP Tray is rehydrated by the Alinity m System and delivered into each sample preparation reaction. IC is unrelated to HIV-1 target sequence and is derived from the (b) (4) The IC is then processed through the entire sample preparation and RT-PCR procedure along with the specimens, calibrators and controls to demonstrate proper sample processing and validity.

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

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

Assay controls are tested at or above an established minimum frequency to help ensure that instrument and reagent performance remains satisfactory. During each control event, a negative control, a low-positive control, and a high-positive control are processed through sample preparation and RT-PCR procedures that are identical to those used for specimens.

Alinity m HIV-1 is intended for use with the Alinity m System, a fully automated, self-contained system.

4. COMPONENTS OF THE ALINITY m HIV-1 ASSAY

4.1 Alinity m HIV AMP Kit

The Alinity m HIV AMP Kit consists of 2 types of multi-well trays:

- Alinity m HIV AMP TRAY 1 (4 trays x 48 tests): The Alinity m HIV AMP TRAY 1 contains separate wells of lyophilized, unit-dose RT-PCR amplification/detection reagents and lyophilized, unit-dose internal control.

- Alinity m HIV ACT TRAY 2 (4 trays x 48 tests): Alinity m HIV ACT TRAY 2 contains liquid activation reagent.

Each Alinity m HIV AMP TRAY 1 and Alinity m HIV ACT TRAY 2 is provided in a sealed foil pouch (4 pouches of each tray type per Alinity m HIV AMP Kit for up to 192 samples [patient specimens and/or assay controls or calibrators]). Both trays contain 48 unit-dose reagent wells (with reagents as listed above) of which one well of each reagent is used per test (48 tests per tray).

Additional materials required but purchased separately

4.2 Alinity m HIV CAL Kit

The Alinity m HIV-1 calibrators are for calibration of the Alinity m HIV-1 assay on the automated Alinity m System when used for the quantitative determination of HIV-1 RNA. The Alinity m HIV CAL Kit is composed of the following reagents:

- Alinity m HIV CAL A (4 tubes x 1.95mL)
- Alinity m HIV CAL B (4 tubes x 1.95mL)

The Alinity m HIV CAL A and Alinity m HIV CAL B tubes are intended for single-use only. The Alinity m System will process 3 replicates from each calibrator tube. The calibrators are assigned lot specific HIV RNA concentrations based on the results of testing against the Primary Calibrators.

4.3 Alinity m HIV CTRL Kit

The Alinity m HIV-1 controls are for validity determination of the Alinity m HIV-1 assay used on the automated Alinity m System. The Alinity m HIV-1 CTRL Kit is composed of the following reagents;

- Alinity m HIV-1 Negative CTRL (12 tubes x 1.15mL)
- Alinity m HIV-1 Low Positive CTRL (12 tubes x 1.15mL)
- Alinity m HIV-1 High Positive CTRL (12 tubes x 1.15mL)

The Alinity m HIV-1 control reagents are intended for single-use only. Controls are recommended to be tested at or above the minimum frequency of once every 24 hours.

4.4 Alinity m Sample Prep Kit 2

The Alinity m Sample Prep Kit 2 is provided in a liquid, multi-dose format and is shared with other Alinity m assays. It consists of 2 reagents:

- Alinity m Elution Buffer 2 (4 bottles x 22mL)
- Alinity m Microparticles 2 (4 bottles x 24mL)

The Alinity m Sample Prep Kit 2 is used in conjunction with Alinity m System Solutions as part of the sample preparation protocol to extract and concentrate

target nucleic acids from biological samples for subsequent Polymerase Chain Reaction (PCR) amplification, and to remove potential inhibitors from the resulting extract.

The sample preparation procedure consists of lysis/binding, washes, and elution. The sample preparation is performed within a disposable multi-well integrated reaction unit that is loaded onto an Assay Processing Unit on the Alinity m System.

4.5 Alinity m Specimen Dilution Kit I

The Alinity m Specimen Dilution Kit I is intended to allow dilution of specimens for testing on the automated Alinity m System for measurement of nucleic acid. It consists of Alinity m specimen diluent tubes with a pierceable cap (24 tubes x 2.45mL). Each Specimen Dilution Kit I support dilution of up to 24 samples (patient specimens); each tube is for single use and must not be reused. The Alinity m specimen diluent tubes contain Abbott Molecular transport buffer which contains guanidine thiocyanate (GITC) in Tris Buffer.

4.6 Alinity m System Solutions

The Alinity m System Solutions are used as part of the sample preparation protocol to extract and concentrate target viral nucleic acid from biological samples for subsequent PCR amplification and to remove potential inhibitors from the resulting extract. The Alinity m system solutions are as follows;

- The Alinity m Lysis Solution: 1 bottle × 975mL.
- The Alinity m Diluent Solution: 4 bottles × 975mL.
- The Alinity m Vapor Barrier Solution: 1 bottle × 975 mL.

4.7 Alinity m Tubes and Caps

- The Alinity m LRV Tube consists of Low Residual Volume (LRV) Tubes closed with caps (12 capped tubes per kit).
- Alinity m Transport Tube Pierceable Capped consist of transport tubes closed with pierceable caps (1500 capped tubes per case, 10 boxes of 150 capped tubes).
- Alinity m Transport Tube consist of 1600 tubes per kit.
- Alinity m Pierceable Cap consist of 2000 caps per kit.
- Alinity m Aliquot Tube consist of 1600 tubes per kit.

5. INSTRUMENTATION AND SOFTWARE

The Alinity m System is a fully integrated and automated molecular diagnostics analyzer which utilizes real-time PCR technology in clinical laboratories. It provides sample-to-result uninterrupted processing workflow. The Alinity m

System enables continuous and random-access sample processing by using multiple sample processors and PCR thermal cycler/reader modules in parallel. Each individual sample occupies either one sample process lane or PCR Amplification and Detection (amp-detect) lane. Parallel lanes are provided to enable 300 tests in approximately 8 hours.

Each Alinity m System utilizes four (4) independent Assay Processing Units (APUs) to achieve the throughput and random-access requirements. Each APU consists of one extraction unit and one Amp-Detect unit, which automate the steps for nucleic acid purification/extraction and real-time PCR, respectively. This results in the ability to process up to twenty-four (24) different assay types simultaneously (i.e., up to 12 different assays types for purification/extraction and up to 12 different assay types for amplification and detection).

The Alinity m System software is the set of computer instructions that interprets system and assay information, calculates results, and provides the interface for controlling the system hardware.

The Alinity m System software interprets the assay information provided in the specific Application Specification File, along with system information, to control the system hardware and identify the appropriate algorithms for data reduction. Using application specifications, customers create orders for calibrators, controls, and specimens. Customers load racks of calibrators, controls, and specimens in the sample input to begin processing. Once the samples are processed, results are reviewed and released through the software user interface.

6. TEST PROCEDURE

Specimen Collection, Preparation and Storage

- The Alinity m HIV-1 assay can be performed on plasma collected in ethylenediamine tetra acetic acid (EDTA) or Acid Citrate Dextrose (ACD) anticoagulants and plasma preparation tubes (PPT).
- For blood collection and centrifugation, follow the specimen collection tube manufacturer’s instructions.
- For blood collection and centrifugation, follow the specimen collection tube manufacturer’s instructions.
- Whole blood may be stored in plasma tubes at 2°C to 8°C for up to 2 days or at 15°C to 30°C for up to 24 hours prior to centrifugation and testing with the Alinity m HIV-1 assay.
- Plasma can be tested on the Alinity m system in the primary tube or transferred to the secondary tube for storage.
- After centrifugation, the EDTA, PPT and ACD plasma samples may be stored in primary collection tubes at 15°C to 30°C for up to 24 hours or at 2°C to 8°C for up to 3 days. If transferred to the secondary tube, plasma may be stored at 2°C to 8°C for up to 3 days or stored frozen for up to 60

days at -20°C and longer storage at -70°C. Specimens in EDTA or ACD primary collection tubes should not be frozen.
- Plasma samples are stable for up to two freeze-thaw cycles.
- Specimens may be diluted manually for testing on the Alinity m System using the Alinity m Specimen Dilution Kit I.
- Low volume specimens with a minimum of 260µl volume available for Alinity m HIV-1 testing can be diluted 1:2.5. Specimens with 50 to (b) (4) µl volume available for Alinity m HIV-1 testing can be diluted 1:50. High-titer specimens above the upper limit of quantitation (> ULOQ) can also be diluted 1:50 before testing.
- Specimens must be tested within 2 hours after dilution and should not be frozen.

6.1 Running the Alinity m HIV assay

The minimum sample volume required for running the Alinity m HIV-1 assay is 750µL. Prior to testing specimens, the calibration and control status should be checked. 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. Procedural steps are summarized as follows;

- Prior to loading the specimen tubes on the Alinity m System ensure individual specimen tubes are labeled correctly with specimen ID barcodes.
- Specimens should be free of bubbles and foam. If found, bubbles and foam should be removed with a new sterile pipette tip for each tube to prevent cross-contamination.
- Load the ACT TRAY 2 onto the plate adapter (Eppendorf Catalog No. 022638955).
- Load the plate adapter (with the ACT TRAY 2) on a swing plate centrifuge capable of accommodating the plate adapter. Spin at 100 to 800g for 1 to 5 minutes to remove potential bubbles.
- 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.
- If disturbance occurs during transfer that could potentially introduce bubbles (dropping, bumping, inversion of the ACT TRAY 2), re-centrifuge the ACT TRAY 2.
- Proceed with the reagent and sample inventory management procedure as indicated in the Alinity m System Operations Manual.
- From the Specimen tab on the create order screen, enter the specimen ID (SID), select the assay (HIV-1).
- For a detailed description of how to run an assay, refer to the Alinity m System Operations Manual.

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

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

6.2 Procedural Notes

- Ensure the Alinity m HIV-1 AMP TRAY 1 is tapped prior to loading on the Alinity m System.
- Ensure the Alinity m HIV-1 ACT TRAY 2 is centrifuged prior to loading on the Alinity m System.
- 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.

7. RESULTS

7.1 Calculation

The Alinity m System automatically calculates the concentration of HIV-1 RNA for specimens and controls by comparing the results to a calibration curve. Quantitative viral load results reported are within the assay's quantitation range. The Alinity m System reports the results in Copies/mL, Log [Copies/mL], IU/mL or Log [IU/mL]. One copy of HIV-1 RNA is equal to 1.63 IU. The conversion factor for copies to International Unit (IU) traceable to the WHO 3rd International Standard for HIV-1 RNA (10/152) is stored in the instrument. For plasma specimens tested with the specimen dilution procedures, software will automatically report the neat result (prior to dilution) by using the dilution factor selected by the user.

7.2 QUALITY CONTROL PROCEDURES

The Alinity m HIV-1 assay contains three quality control procedures.

Assay Calibration

- Lot-specific concentration values can be automatically imported to the Alinity m System via Abbott Mail upon scanning the calibrators (HIV-1 CAL

A and HIV-1 CAL B) or controls (HIV-1 NEG CTRL, HIV-1 LOW POS CTRL, and HIV-1 HIGH POS CTRL) tube barcodes.
- Lot specific concentration values can also be obtained from the Abbott Molecular customer portal or provided by local Abbott Representative and imported via a USB drive.

A calibration curve is required to quantitate the HIV-1 RNA concentration. 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. If three replicates of each calibrators are not available, at the minimum one CAL A and one CAL B tube is required for performing an assay calibration.

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

- An Alinity m HIV-1 AMP Kit with a new lot number is used.
- An Alinity m Sample Prep Kit 2 or Alinity m Lysis Solution with a new lot number is used.
- The assay calibration has expired.
- A new version of the Alinity m HIV-1 Application Specification File is installed.
- This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Detection of Inhibition

An IC C_t assay validity parameter is established during a calibration run. A defined, consistent quantity of IC is introduced into each specimen, calibrator, and control at the beginning of sample preparation and measured on the Alinity m System to demonstrate proper specimen processing and assay validity. The median IC C_t value from calibrator samples establishes an IC C_t validity range for subsequently processed specimens and controls.

A message code is assigned to a specimen or control when its IC C_t value is outside of the IC C_t validity range. When the IC C_t value exceeds the upper limit of the IC C_t validity range, abnormal assay conditions, such as inhibition, are indicated. User is trained for corrective actions for message codes and additionally information is available in operator manual section 10.

Negative and Positive Controls

Alinity m HIV-1 Negative CTRL, Low Positive CTRL, and High Positive CTRL are recommended to be tested, at or above the minimum frequency of once every 24 hours, to monitor the performance of the assay and Alinity m System. Valid results for all control levels must be obtained before specimen results are reported. The assay controls are also tested following calibrators and valid results for controls are required to establish a new calibration curve. 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.

Additional controls may be tested in accordance with local, state, and/ or federal regulations or accreditation requirements and user laboratory's quality control policy.

The presence of HIV-1 must not be detected in the negative control. HIV-1 detection in the negative control is indicative of contamination by other samples or by amplified product. To avoid contamination, the Alinity m System should be cleaned for repeat sample processing of controls and specimens following the procedural precautions in this package insert. If negative controls are persistently reactive, an Abbott Representative may be contacted for resolution.

When a set of assay controls are being processed, the lot-specific concentration values of the Alinity m HIV-1 Low Positive CTRL and Alinity m High Positive CTRL can be automatically imported to the Alinity m System via Abbott Mail upon scanning the barcode labels on control tubes (HIV-1 LOW POS CTRL and HIV-1 HIGH POS CTRL) or obtained through customer portal or from Abbott local representative.

8. INTERPRETATION OF RESULTS

Undiluted Specimens

The Alinity m System will report a result and an interpretation for each specimen (Table 1). If applicable, message codes or flags will also be displayed.

Diluted Specimens

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

For diluted specimens 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. For diluted specimens with a result of < LLoQ (Lower Limit of Quantitation), it is recommended to collect and test another neat specimen.

Table 1. Result and Interpretation

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

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

Flags, Results Codes, and Message Codes

Some results may contain information in the flags and codes fields. For a description of the flags and result codes that may appear in these fields, user must refer to the Alinity m System operations manual.

9. WARNINGS AND PRECAUTIONS

- For In Vitro Diagnostic Use
- 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.
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.
- To reduce the risk of invalid results, carefully read the entire package insert and the Alinity m System Operator’s Manual prior to performing this assay.
- Use only supplied or specified required consumables to ensure optimal test performance.

10. PROCEDURAL LIMITATIONS

- Optimal performance of this test requires appropriate specimen collection and handling (refer to the Specimen Collection and Preparation for Analysis section of this package insert.)

- Human plasma (ACD, K2 EDTA, K3 EDTA, and PPT) specimens may be used with the Alinity m HIV-1 assay. The use of other anticoagulants has not been evaluated.
- Performance has not been established with serum specimens.
- Debris within plasma specimens (clots, fibrin strands) may interfere with sample processing.
- Diluted specimens must be tested within 2 hours after dilution and should not be frozen.
- If the HIV-1 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- HIV-1 RNA concentration (i.e., number of virus particles present in the samples) may be affected by patient factors (age, presence of symptoms), and/or stage of infection.
- Though rare, mutations within the highly conserved regions of a viral genome detected by Alinity m HIV may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus. To ensure assay robustness, the Alinity m HIV-1 assay is designed to target two highly conserved sequences within the HIV-1 genome.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to evaluate technology differences. Users should follow their own specific policies/procedures.
- The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the calibrators, positive controls, or specimens must be controlled by good laboratory practice and careful adherence to the procedures specified in this package insert.
- Assay linearity for HIV-1 Group M subtype BF, subtype H and Group N quantification was tested up to 10,000 Copies/mL for subtype BF, 300,000 Copies/mL for subtype H and 1,000,000 Copies/mL for Group N, respectively.

11. CONTRAINDICATIONS

There are no known contraindications for use for this test.

12. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently several FDA approved Class III in vitro diagnostic tests for the quantitation of HIV-1 RNA. Once the diagnosis of HIV has been established, $CD4^+$ cell count and HIV-1 viral load are the two most commonly utilized surrogate markers of HIV disease progression.

13. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

When used according to the instructions in the package insert, there are no known direct adverse effects of this device on the health of the user. No specific adverse effects occurred during conduct of the clinical studies.

An error with the quantification of viral load may occur with the Alinity m HIV-1 assay which may lead a clinician to believe that the current therapy is effective when it is not. Consequently, the clinician could fail to implement a more appropriate therapy. A high viral load result may lead a clinician to believe that the current therapy is not effective. Consequently, the clinician could implement an inappropriate change in therapy. There were no specific adverse events that occurred in the clinical studies.

14. MARKETING HISTORY

The Alinity m HIV-1 assay was CE-marked in 2019. The Alinity m HIV-1 AMP Kit, Alinity m HIV-1 CTRL Kit, Alinity m HIV-1 CAL Kit, Alinity m Sample Prep Kit 2, Alinity m Specimen Dilution Kit I, Alinity m Tubes and Caps and Alinity m System Solutions are identical in formulation to the US kits, except for kit labeling, and were introduced to multiple foreign markets outside of the United States.

The Alinity m System was self-certified for commercialization in the European Union, and the European Free Trade Association (EFTA) since December 2017.

The Alinity m HIV-1 kits and Alinity m System have not been withdrawn from the market for reasons related to safety or effectiveness.

15. SUMMARY OF NONCLINICAL STUDIES

15.1 Limit of Detection

The limit of detection (LOD) was determined by testing dilutions of the World Health Organization (WHO) 3rd International Standard for HIV-1 RNA (NIBSC code: 10/152) prepared in HIV-1 negative human plasma to create a 7-dilution panel. (b) (4) replicates of each panel members were run on (b) (4) Alinity m systems using four reagent lots over (b) (4) days for a total of 96 replicates for each dilution. The International Unit (IU) and copies/ml conversion used in this assay is traceable to WHO 3rd International Standard for HIV-1 RNA where one copy of HIV-1 RNA is equivalent to 1.63 IU. The results of the analytical sensitivity performance of Alinity m HIV-1, are summarized in Table 2.

Table 2. Alinity m HIV-1 Limit of Detection (LOD)

HIV-1 RNA (Copies/mL)	No. of Valid Replicates	No. of Detected Replicates	Detection Rate (%)
40.00	94	94	100.0

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

Summary: The LoD study as designed demonstrated that the Alinity m HIV-1 detected HIV-1 RNA at a concentration of 13.99 cp/mL (22.8 IU/mL), with a rate of detection of 95% as estimated by PROBIT analysis.
The claimed Lower Limit of Quantitation (LLoQ) and LOD for the Alinity m HIV-1 is 20 cp/mL (32.6 IU/mL) and has been demonstrated to generate positive results with a rate of detection of 96.7% (87/90).

15.2 Limit of Detection Across Groups and Subtypes

HIV-1 group M (subtypes A, BF, C, D, CRF01-AE, F, CRF02-AG, G and H), group O and group N panels were prepared by diluting HIV-1 viral stock or HIV-1 positive clinical specimens to 3 different concentrations (0.5x LoD, 1.0x LoD and 2.0x LoD) in HIV-1 negative human plasma. (b) (4) replicates of each diluted sample were tested using four Alinity m kit lots over (b) (4) days for a total of 96 replicates tested for each dilution. For HIV-1 groups/subtypes, an LOD of 20 cps/mL was demonstrated at a detection rate (hit rate) greater than or equal to 95%. The results are shown in Table 3.

Table 3. Alinity m HIV-1 Limit of Detection (LOD) Across Groups and Subtypes.

Group/Subtype	HIV-1 RNA (Copies/mL)	No. of Valid Replicates	No. of Detected Replicates	Detection Rate (%)
Group M, subtype A	40	93	93	100.0
	20	95	94	98.9
	10	94	88	93.6
Group M, subtype BF	40	94	94	100.0
	20	95	95	100.0
	10	96	82	85.4
Group M, subtype C	40	95	95	100.0
	20	95	95	100.0
	10	94	92	97.9
Group M, subtype D	40	95	95	100.0
	20	95	94	98.9
	10	96	86	89.6

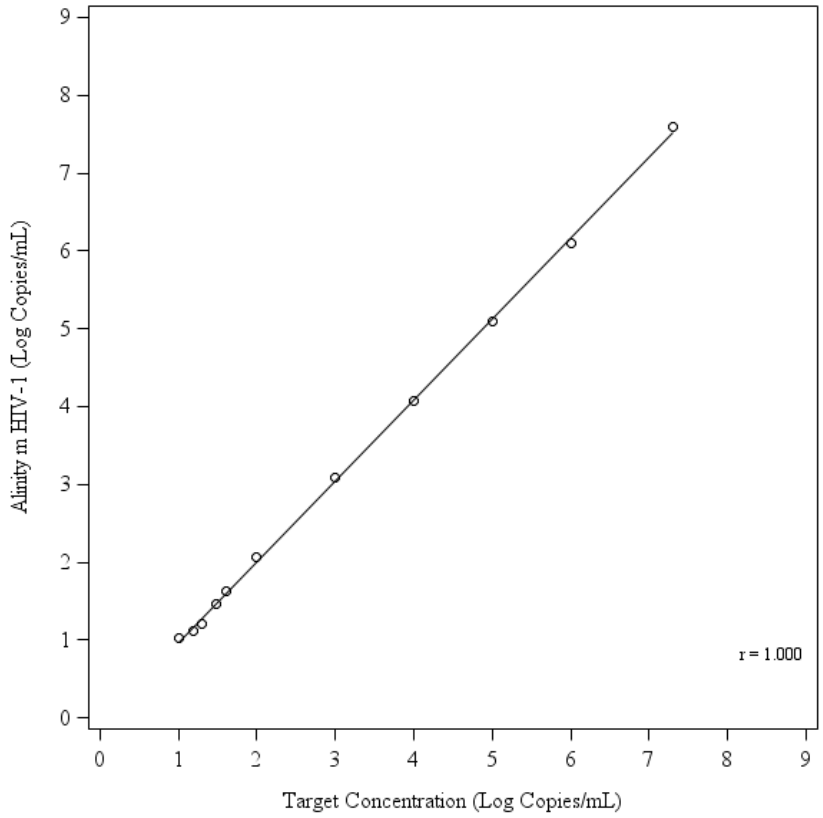
Group/Subtype	HIV-1 RNA (Copies/mL)	No. of Valid Replicates	No. of Detected Replicates	Detection Rate (%)
Group M, CRF01-AE	40	93	93	100.0
	20	96	96	100.0
	10	94	89	94.7
Group M, subtype F	40	94	94	100.0
	20	96	95	99.0
	10	93	88	94.6
Group M, CRF02-AG	40	93	93	100.0
	20	94	94	100.0
	10	94	90	95.7
Group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

Summary: The results from this study demonstrate that the detection rate at LOD (20 Copies/mL) ranged from 98.9% to 100.0% across HIV-1 groups /subtypes, and at 2x LOD (40 Copies/mL) was 100.0% for all HIV-1 groups / subtypes. The results support the LOD of 20 Copies/mL for the Alinity m HIV-1 assay in plasma for HIV-1 Group M Subtypes A, BF, C, D, CRF01-AE, F, CRF02-AG, G, H, Group O, and Group N.

15.3 Linear Range

Linearity was evaluated by testing 11 panel members formulated using HIV-1 Group M, subtype B high titer viral stock in HIV-1 negative plasma that spanned the intended dynamic range of the assay (20 copies/ml to 10,000,000 copies/ml), including a two members below the expected Lower Limit of Quantification (LLoQ, 20 cps/mL), and a member exceeding the expected Upper Limit of Quantification (ULOQ, 10,000,000 copies/ml). This range covers the medical decision points. All titers were calculated and converted into log titer. The data were analyzed for maximum deviation between the linear regression and the better fitting non-linear regression. In addition, the LLoQ was determined for the lowest concentration level (20 cp/mL) in the LOD panel with a detection rate ≥ 95 %. To determine LLoQ a statistical approach described in CLSI (b) (4) was used. Testing was conducted using (b) (4) .

Figure 1. Linearity



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

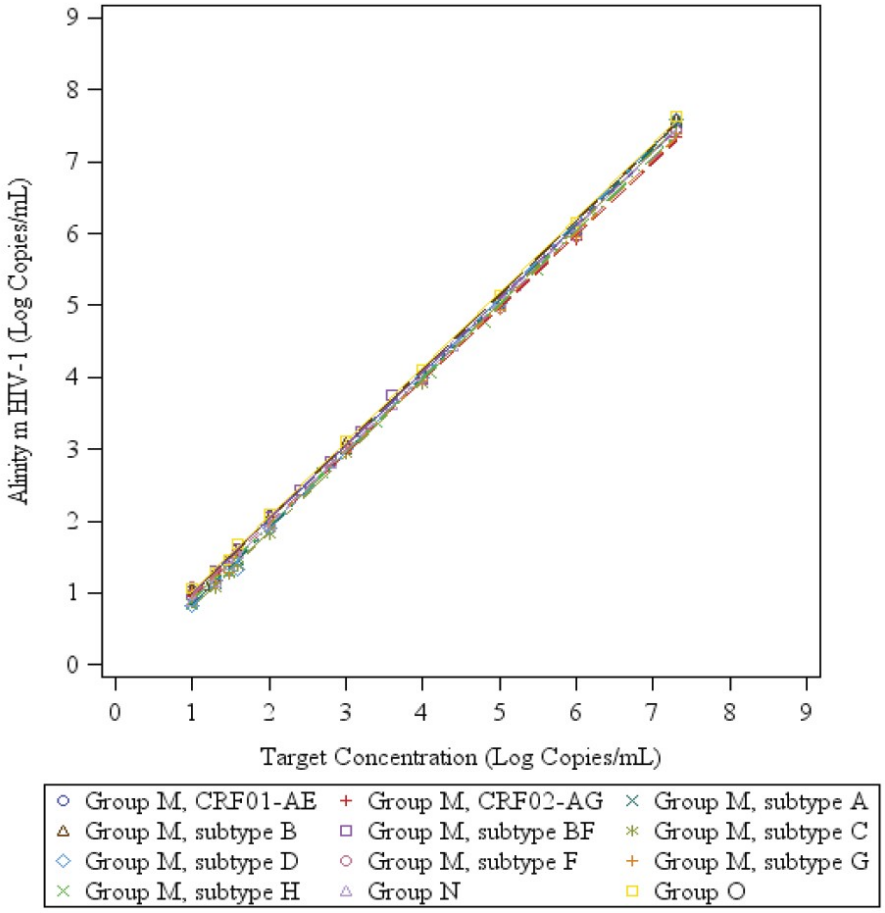
Summary: As shown in Figure 1, the Alinity m HIV-1 assay was linear across the range of HIV-1 RNA concentrations tested ranging from 10 Copies/mL to 20,000,000 Copies/mL (1.0 log copies/mL to 7.30 log copies/mL) and the LLoQ was determined as 20cp/mL using the input sample volume 0.6mL. The difference in the predicted concentration between the fitted nonlinear model and the linear model was less than (b) (4) Log Copies/mL for each panel member tested. The performance of Alinity m HIV-1 assay is acceptable.

15.4 Linearity Across Groups and Subtypes

Linearity of Alinity m HIV-1 assay for HIV-1 group M (subtypes A, BF, C, D, CRF01-AE, F, CRF02-AG, G and H), group O and group N was confirmed by testing a dilution series consisting of 10 panel members for each group/subtype, prepared using HIV-1 cultured virus or HIV-1 positive clinical specimens diluted in HIV-1 negative human plasma. Linearity panel members spanned the intended dynamic range of the assay (20 Copies/mL to 10,000,000 Copies/mL). However, for Group M/subtype BF, H and Group N the targeted concentration for testing

ranged from 10 - 10,000 copies/ml, 10 – 300,000 copies/ml and 10 – 1,000,000 copies/ml, respectively. All titers were calculated and converted into log titer.

Figure 2. Linearity Across Groups and Subtypes



Summary: As shown in Figure 2, Alinity m HIV-1 assay was linear between 10 copies/ml to 20,000,000 copies/ml for HIV-1 Group M subtypes A, C, D, CRF01-AE, CRF02-AG, F, G and Group O. For HIV-1 Group M subtypes BF, H and Group N, the assay was linear from 10 copies/ml to the highest concentration tested. For each subtype analyzed the maximum deviation between the linear regression and the better fitting non-linear regression was less than (b) (4) Log cps/mL.

15.5 Precision

The precision of the Alinity m HIV-1 assay was determined by analyzing an 8-member plasma panel, prepared by diluting an HIV-1 viral stock into HIV-1 negative human plasma that spanned the dynamic range of the assay (b) (4) copies/ml to 10,000,000 copies/ml). Each panel member was tested in 5 replicates, twice each day for 12 days, on 3 Alinity m Systems with 3 Alinity m HIV-1 AMP Kit lots by 3 operators for a total of 360 replicates per panel member(5 replicates x 2 runs/day x 3 kit lots x 12 days). A statistical approach of

total assay variability was determined. The overall precision results shown in Table 4.

Table 4. Precision

			Within-Run Component		Between-Run Component		Between-Day Component		Within-Laboratory[b]		Between-Instrument Component		Total[c]	
Panel	N[a]	Mean Conc (Log Copies/mL)	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
08	349	7.34	0.04	0.5	0.01	0.2	0.00	0.1	0.04	0.6	0.08	1.1	0.09	1.2
07	347	6.02	0.04	0.7	0.00	0.0	0.01	0.2	0.04	0.7	0.05	0.8	0.07	1.1
06	348	5.04	0.04	0.8	0.02	0.3	0.01	0.2	0.04	0.9	0.02	0.5	0.05	1.0
05	353	4.04	0.05	1.3	0.00	0.0	0.01	0.3	0.05	1.3	0.03	0.8	0.06	1.5
04	353	3.11	0.05	1.5	0.01	0.5	0.01	0.2	0.05	1.6	0.04	1.2	0.06	2.0
03	353	2.40	0.09	3.6	0.02	1.0	0.00	0.0	0.09	3.7	0.04	1.5	0.10	4.0
02	352	1.87	0.15	8.2	0.00	0.0	0.03	1.5	0.16	8.4	0.04	1.9	0.16	8.6
01	353	1.34	0.27	19.8	0.02	1.7	0.00	0.0	0.27	19.9	0.05	3.4	0.27	20.2

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

Summary: The Alinity m HIV-1 assay results showed acceptable precision across the dynamic range of assay (1.34 cp/mL to 7.34 cps/mL) and total variance is less than 0.5 log cps/mL when tested with three lots of reagents.

15.6 Performance with HIV-1 negative specimens (Specificity)

The specificity of the Alinity m HIV-1 assay was determined by testing 250 HIV-1 negative plasma obtained from HIV-1 negative whole blood donors (as determined using an FDA approved Ag/Ab assay) and demonstrated to be HIV-1 RNA negative using the Abbott RealTime HIV-1 assay. The study was conducted using (b) (4) of Alinity m HIV-1 AMP kit reagents and (b) (4) Alinity m System.

Summary: All 250 HIV-1 negative specimens reported a Not Detected interpretation when tested on Alinity m HIV-1 assay. Diagnostic specificity was 100.0% (95% CI: 98.5%, 100.0%) for HIV-1 negative plasma specimens. The specificity of Alinity m HIV-1 assay was determined to be acceptable.

15.7 Carryover

The carryover rate for the Alinity m HIV-1 assay was determined by testing 720 samples (360 HIV-1 negative and 360 HIV-1 positive samples) across 15 runs. Each run consisted of (b) (4) replicates of the HIV-1 high-positive sample (b) (4) cp/mL) and (b) (4) replicates of the HIV-1 negative sample. HIV-1 high-positive and HIV-1 negative sample replicates were placed in alternating positions within the sample input rack, so that each negative sample replicate was processed

adjacent to a positive sample replicate during sample preparation and RT-PCR processes.

Summary: HIV-1 RNA was not detected in any of the HIV-1 negative samples, resulting in an overall carryover rate of 0.0% (95% CI: 0.0 to 1.1%). The Alinity m HIV-1 assay was shown to be not impacted by carryover contamination from previous runs or from the high positive samples analyzed in the same run.

15.8 Potentially Interfering Microbial Contaminants

The impact of potential cross-reactivity and/or interference of pathogens in the Alinity m HIV assay was evaluated. The study was designed according to CLSI (b) (4). HIV-1 negative or positive test specimens were spiked with microorganisms (b) (4) from microorganisms to achieve a final titer of (b) (4) units/mL for viruses and yeast, and (b) (4) CFU/mL for bacteria (Table 5). Cross-reactivity was analyzed using an HIV negative sample and microbial interference was analyzed using an HIV positive sample at 60 cps/mL (3x LLoQ) and from the 200 cps/mL sample. (b) (4) replicates for each cross reactant were tested.

Table 5. Potential Cross-Reactants

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

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

Summary: The results demonstrates no cross-reactivity of the Alinity m HIV-1 assay with the potential cross-reactants tested. In addition, there was no impact on the detection or quantitation by the Alinity m HIV-1 assay by the organisms listed.

15.9 Potentially Interfering Substances (Endogenous)

The impact of potentially interfering endogenous substances, presence of autoimmune disorders, and markers of other diseases on the analytical specificity and detection/quantitation of the Alinity m HIV-1 assay was evaluated by testing spiked samples as well as patient samples with naturally elevated levels of endogenous substances. (b) (4) donor samples were tested for each interfering substance with (b) (4) replicate each. As a control, (b) (4) replicate of each donor sample was also tested without the addition of any potentially interfering endogenous substance. HIV-1 positive samples were prepared by adding HIV-1 viral stock to HIV-1 negative plasma at a final concentration of 60 cps/mL (3x LLoQ) of assay (detection) and at 200 cps/mL (quantitation).

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

15.10 Potentially Interfering Substances (Exogenous)

The impact of potentially interfering drugs commonly prescribed for the treatment of HIV-1 and other disease states on the performance of Alinity m HIV-1 assay was evaluated. (b) (4) donor samples were tested for each interfering drug pool or single drug with (b) (4) replicate each. As a control, (b) (4) replicate of each donor sample was also tested without the addition of any potentially interfering drug compounds. The HIV-1 positive samples were prepared at two HIV-1 levels by adding HIV viral stock to HIV-1 negative plasma at a final concentration of 60

cps/mL (3x LLoQ) of assay (detection) and at 200 cps/mL (quantitation). The drug compounds listed in Table 6 were tested at three times the reported maximum concentration (Cmax) evaluated with and without HIV-1 viral targets.

Table 6. Drugs Tested for Interference with the Quantitation of HIV-1 RNA by the Alinity m HIV-1 assay

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

Summary: No interference was observed in the presence of drug compounds tested in pools that are listed in Table 6, at a concentration of 3 times the reported Cmax or higher.

15.11 Alinity m HIV-1 Testing Using Dilution Procedure

The Alinity m HIV-1 assay design provides optional manual dilution procedures for low volume or for high viral load specimens (upper limit of quantification). To verify that the Alinity m HIV-1 assay provides accurate quantitation, dilution procedures were evaluated by comparing quantitation of neat specimens and specimens tested using the Alinity m HIV-1 dilution procedure (1:2.5 and 1:50). Specimens were diluted using Alinity m Specimen Dilution Kit I. A Ten-member

panel was prepared for assay's linear range (2.18 to 7.3 log cps/mL) and tested both neat as well as diluted (Table 7).

Table 7. Alinity m HIV-1 Results for Plasma Samples Tested Using Dilution Procedure

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

Summary: This study demonstrated that the mean quantitation differences between the diluted (test) and undiluted specimens (control condition) are within the medical decision threshold level <0.5log cps/mL. Thus, Alinity m HIV-1 assay provides accurate quantitation of specimens when tested using the dilution procedure described in the package insert.

15.12 Precision of Alinity m HIV-1 Using Dilution Procedures

The purpose of this study was to demonstrate the within-laboratory precision of the Alinity m HIV-1 assay for diluted specimens. Precision was evaluated in plasma by testing 3 panel members with HIV-1 target concentrations of 2.8 Log Copies/mL, 4.7 Log Copies/mL, and 6.7 Log Copies/mL, that were diluted using Alinity m Specimen Dilution Kit I. Panel member 2.8 log cps/mL was diluted for 1:25 and other two panel members are diluted for 1:50. Testing was performed using 3 replicates per panel member using 3 kit lots on 3 Alinity m systems for 12 days and 2 runs per day. A total of 360 replicates tested per panel member(5 replicates x 2 runs/day x 3 kit lots x 12 days).The results are summarized in Table 8.

Table 8. Precision of Alinity m HIV-1 Using Dilution Procedures for Plasma

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

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

Summary: The analyses demonstrated that the Alinity m HIV-1 assay has a within-laboratory SD of 0.25 Log Copies/mL or less for samples tested using dilution procedures. The performance of Alinity m HIV-1 assay is acceptable because the differences are within the threshold of medical decision.

15.13 Specimen and collection tube type equivalency

The Alinity m HIV-1 test uses plasma samples collected in either Di-Potassium Ethylenediaminetetraacetic Acid (K2 EDTA) tubes, Tri-Potassium EDTA (K3 EDTA) tubes, Acid Citrate Dextrose (ACD) tubes and Plasma Preparation Tubes (PPT). For each tube type, matched specimens from (b) (4) unique HIV-1 negative blood donors were collected in each of the 4 tube types. HIV-1 was spiked into each specimen to a target HIV-1 level of approximately 60 cps/mL(3x LLoQ) or 200 Copies/mL. The levels used included HIV-1 RNA detection and quantification. In addition, (b) (4) HIV-1 matched negative, un-infected specimens were also analyzed.

Summary: The Alinity m HIV-1 test demonstrated comparable performance (b) (4) for matched HIV-1 positive RNA plasma collected in EDTA, ACD and PPT. All (b) (4) matched HIV-1 negative specimens collected from uninfected individuals collected in EDTA, ACD and PPT tested negative. Viral loads were comparable for HIV-1 positive paired samples collected in EDTA or ACD and PPT. The tube equivalence study demonstrated acceptable performance between the different tubes.

15.15 Real-Time Reagent Stability

Realtime stability studies were performed to establish the shelf-life for the Alinity m HIV-1 assay. Three (3) lots of reagent kits were stored at the intended storage temperature indicated in Table 9 and then tested at various time points throughout the study. Performance was assessed against clinically relevant acceptance criteria (within (b) (4) log cps/mL) using controls, calibrators and an internal stability panel consisting of three panel members PM1 (b) (4) log cps/mL),

PM2 (b) (4) log cps/mL), PM3 (b) (4) log cps/mL).The Shelf life study included the assessment of an (b) (4) condition, as well as a condition that simulated

fluctuating (hot/cold) temperature extremes during shipping.

The effect of the On-Board Storage (OBS) on reagent performance was also assessed by testing one lot of reagents at the maximum on-board (b) (4) days) temperature/humidity conditions allowed by the Alinity m Instrument System (i.e., 30ºC (b) (4) ºC], (b) (4) relative humidity [RH]) for the intended OBS of the reagents. Results of the OBS conditions were compared to the results when the reagents were stored at their intended storage condition. Real time shelf life and on-board storage conditions are summarized in Table 9.

Table 9. Reagent Shelf Life and On-Board Stability for the Alinity m HIV-1 and Alinity m Accessory Kits

Kit/Reagents	Shelf Life and Intended Storage Condition	On-board Stability
Alinity m HIV-1 AMP Kit	15 months 2°C to 8°C	30 days
Alinity m HIV-1 CTRL Kit	15 months -15°C to -25°C	4 hours
Alinity m HIV-1 CAL Kit	15 months -15°C to -25°C	4 hours
Alinity m Sample Prep Kit 2 (Elution Buffer 2 and Microparticles 2)	15 months 2°C to 8°C	10 days
Alinity m System Solutions (Lysis Solution, Diluent Solution and Vapor Barrier Solution)	15 months 15°C to 30°C	Lysis Solution: 30 days Diluent Solution: 30 days Vapor Barrier Solution: until expiration
Alinity m Specimen Dilution Kit I	15 months 15°C to 30°C	N/A

Summary: Study results demonstrate that reagents are stable at their intended storage condition and continue to meet acceptance criteria fifteen (15) months after the date of manufacture including when shipped upon exposure to fluctuating temperature extremes. In addition, on-board study results demonstrate that reagents are stable on-board the Alinity m Instrument and continue to meet acceptance criteria for the intended on-board storage time.

16. SUMMARY OF PRIMARY CLINICAL STUDIES

To assess clinical performance of Alinity m HIV-1 assay when 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, the applicant performed following three clinical studies to establish a reasonable assurance of safety and effectiveness:

- Clinical Specificity
- Method Comparison
- Reproducibility.

All clinical studies were performed at three US clinical sites.

16.1 Clinical Specificity

Retrospectively collected plasma specimens from a total of 596 HIV-1 negative volunteer whole blood donors were included in the evaluation for Alinity m HIV-1 assay specificity. The HIV-1 negative whole blood donors were confirmed to be ”Not Detected” with an FDA approved viral load assay. The HIV-1 negative specimens were tested at three clinical testing sites with two Alinity m HIV-1 reagent kit lots. Clinical specificity was calculated as the percentage of HIV-1 negative specimens with the results of “Not Detected”.

Summary: HIV-1 RNA was not detected in all 596 specimens tested. Clinical Specificity of Alinity m HIV was determined to be 100.0% (596/596, 95% CI: 99.4% to 100.0%).

16.2 Validation of Viral Load Quantitation (Method Comparison Study)

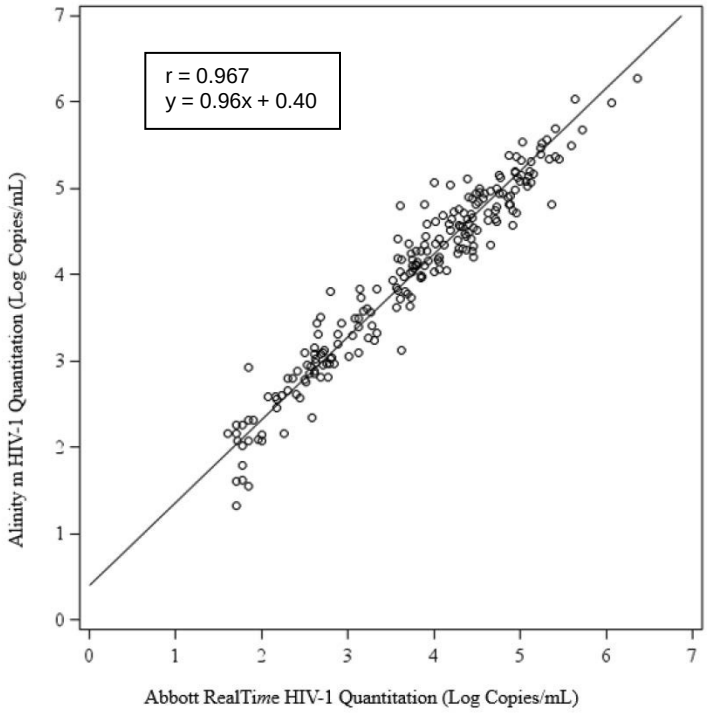
The performance of Alinity m HIV-1 assay was compared to an FDA-approved viral load assay. The evaluation was performed at 3 clinical sites using a total of 326 specimens. Of these, 236 (72.4%) specimens were tested using frozen aliquots and 90 (27.6%) were tested fresh. The test specimens were obtained from 231 (70.9%) males, 94 (28.8%) females, and 1 of unknown gender (0.3%) with an age range of 5 to 75 years. Sample characteristics were shown in Table 10.

Table 10. Sample Characteristics

Antiviral Medication	**Statistics, n(%)**
	(N=326)
Yes	217 (66.6%)
No	109 (33.4%)
CD4+ Cell Count (cells/µL)	
< 200	78 (23.9%)
200-500	113 (34.7%)
> 500	110 (33.7%)
Unknown	25 (7.7%)

Summary: Of 326 specimens tested, 216 had viral load measurements within the linear range (20 cp/mL to 1.00E+07 cp/mL) for both assays and were used for method comparison analysis. The remaining 110 specimens had viral load levels outside of the quantifiable ranges for both assays and could not be used for the validation study. The results demonstrate that the Alinity m HIV-1 assay and the FDA approved assay are comparable based on the Deming regression analysis (Fig 3) with a correlation coefficient of 0.967 and mean paired viral load difference between the two assays is 0.25 Log Copies/mL with a 95% CI of (0.21, 0.28). These differences are lower than the clinically meaningful difference of 0.5 log10 cp/ml.

Figure 3. Deming regression analysis



Viral Load Discrimination at Clinically Meaningful Thresholds

In the method comparison study, concordance between the Alinity m HIV-1 assay and the FDA approved comparator assay was evaluated at the medical decision points of 50 Copies/mL, 200 Copies/mL, and 1000 Copies/mL. Using the results from the method comparison study, the bias at medical decision points was calculated from the Deming regression line and results are presented in Table 11.

Table 11. Bias Analysis for Alinity m HIV-1 Assay at Medical Decision Points

Medical Decision Point		Bias	SE	95% CI of Bias
Copies/mL	Log Copies/mL			
50	1.70	0.33	0.050	(0.23, 0.43)
200	2.30	0.31	0.044	(0.22, 0.39)
1000	3.00	0.28	0.040	(0.20, 0.36)

Summary: The bias and 95% confidence intervals (CI) are within ± 0.5 Log Copies/mL for the 50 Copies/mL, 200 Copies/mL, and 1000 Copies/mL medical decision points. The concordance analysis demonstrates that the Alinity m HIV-1 assay can be used to accurately quantitate HIV-1 RNA in HIV-1 infected patients and define virological outcomes of antiviral therapy.

16.3 Reproducibility

Reproducibility of the Alinity m HIV-1 assay was evaluated by testing a 10-member reproducibility panel. All panel members were prepared using HIV-1 virus diluted in negative human plasma with concentration levels targeted to span the linear quantitation range of the assay. A total of 3 different Alinity m HIV-1 AMP Kit lots were used. Each of the 3 clinical sites tested 2 Alinity m HIV-1 AMP Kit lots, on 5 non-consecutive days for each lot. Five replicates of each panel member were tested on each of 5 days. The design (3 sites x 5 replicates in one panel x 1 run/day x 5 days x 2 lots/site) accounts for a total of 150 replicates per panel member. Results are shown in Table 12.

Table 12. Reproducibility

		Mean Concentration	Within-Run Component		Between-Run Component		Within-Laboratory[b]		Between-Lot Component		Between-Site Component		Total[c]	
Panel	N[a]	(Log Copies /mL)	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
10	149	7.35	0.06	0.8	0.04	0.5	0.07	1.0	0.05	0.7	0.00	0.0	0.09	1.2
9	150	6.47	0.06	1.0	0.05	0.7	0.08	1.2	0.03	0.5	0.01	0.2	0.09	1.4
8	149	5.81	0.09	1.5	0.05	0.9	0.10	1.8	0.00	0.0	0.02	0.4	0.11	1.8
7	150	5.14	0.07	1.3	0.06	1.2	0.09	1.7	0.00	0.0	0.03	0.6	0.09	1.8
6	150	4.45	0.08	1.8	0.05	1.1	0.09	2.1	0.01	0.2	0.01	0.2	0.09	2.1
5	149	3.78	0.08	2.0	0.04	1.1	0.09	2.3	0.02	0.5	0.00	0.0	0.09	2.4
4	150	3.09	0.08	2.7	0.05	1.7	0.10	3.1	0.03	1.0	0.02	0.6	0.10	3.4
3	149	2.43	0.10	4.1	0.06	2.3	0.11	4.7	0.06	2.4	0.00	0.0	0.13	5.3

2	150	1.85	0.16	8.6	0.07	3.6	0.17	9.3	0.05	2.5	0.04	2.0	0.18	9.8
1	149	1.38	0.31	22.3	0.10	7.1	0.32	23.4	0.05	3.7	0.00	0.0	0.33	23.7

[a] Number of valid replicates.
[b] Within-Laboratory includes Within-Run and Between-Run Components.
[c] Total includes Within-Run, Between-Run, Between-Lot, and Between-Site Components.

Summary: Table 12 shows the reproducibility and precision of Alinity m HIV-1 assay results for each positive panel members within-run, between-run, within-laboratory, between-lot, and between-site variations. The overall analysis of data from all three sites shows a total standard deviation (SD) rate of 0.09 to 0.33 with a total percent coefficient of variation (%CV) range of 1.2% to 23.7% across panel members. When clinically relevant viral load of 2.43 to 7.35 log cps/mL (panel 3-10) included, total variance is <6% and SD is 0.09-0.13. For viral load 1.38 and 1.85 log cps/mL (~1 to 3x LLoQ) total variance was >20% and SD, 0.18-0.33. The within-laboratory component contributed the most variability for low viral panel members 1 and 2 which was acceptable. The overall estimated detectable viral load differences were well below the ,medical decision threshold (0.5 log cps/mL), therefore performance of Alinity m HIV-1 assay is acceptable.

INSPECTIONS

17.1 Manufacturing Facilities Review/Inspection

Facility information and data provided in the PMA were reviewed by CBER and found to be sufficient and acceptable. The facilities involved, activities performed in the manufacture of Alinity m HIV-1 Assay and inspectional histories are listed in the table below.

Table 13: Manufacturing Facilities Alinity m HIV-1 Quant assay and Alinity m System

Facility	Activity	Inspection/ Waiver	Most Recent Inspection
Abbott Molecular Inc. 1300 E Touhy Avenue, Des Plaines, IL 60018 (FEI # 3005248192)	Device Component Manufacturing; Finished Device Manufacturing; Device Packaging / Labeling; QC and Release Testing	Waived	July 2019 ORA Post market Inspection NAI

NAI – No Action Indicated
Based on the Team Biologics recent inspection, DMPQ recommends an inspection waiver for this PMA.

17.2 Bioresearch Monitoring (BIMO) Inspections

CBER Bioresearch Monitoring (BIMO) waived inspection assignments for all three sites. CBER BIMO review of the FDA inspections noted that two of the three clinical testing sites participating for the applicant's current CBER PMA submission were recently inspected in January 2020 for CDRH's (b) (4) PMA submission and the inspections did not note any violative study conduct at the two testing sites. The third clinical testing site that participated for the applicant's current CBER PMA submission was inspected in 2017 and did not note significant inspectional observations at the testing site. These inspections did not reveal significant problems that impact the data submitted in this PMA. The inspections were classified as No Action Indicated (NAI).

18. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

18.1 Effectiveness Conclusions

The clinical study results, in combination with the non-clinical performance evaluations strongly support the effectiveness of the Alinity m HIV-1 assay for the medical intended use in the clinical management of HIV-1 infected individuals by measuring the baseline HIV-1 level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.

18.2 Safety Conclusions

The risk of the device is based on data collected in the clinical study conducted to support PMA approval as described above. Based on the results of the clinical studies, the Alinity m HIV-1 assay, when used according to the labeling and in conjunction with other serological and clinical information, is safe to use and poses minimal risk to the patient due to false test results.

19. Benefit-Risk Determination

The benefits outweigh the risks at the level of performance observed in the pivotal clinical study. Complimentary analytical studies strengthen this conclusion. Accurate quantitation of HIV-1 RNA is an essential component of the treatment of HIV-1 infection. In an era of highly active antiretroviral therapy for HIV infection, accurate quantitation of viral load to monitor treatment and assess sustained virological suppression has substantial individual benefit (i.e., reduction of the risk of disease progression). Risk related to inaccurate quantitation was substantially mitigated by device design (i.e., use of controls). Appropriate warnings to address routine risks encountered in the laboratory practice are contained in the labeling and package inserts for the device. Standard good laboratory practices are considered sufficient to mitigate the risks to the end user.

In conclusion, given the available information above, the data support that for the management of HIV-1 patients who are undergoing antiretroviral therapy, the probable benefits outweigh the probable risks.

20. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The data from the nonclinical studies demonstrated acceptable analytical sensitivity, linearity, precision, and analytical specificity of the Alinity m HIV-1 assay when used according to the instructions for use as stated in the labeling, the warnings and precautions, and limitations sections of the labeling. The clinical studies and the statistical analysis of clinical data in this application has shown that the Alinity m HIV-1 assay is informative in assessing viral response to antiretroviral treatment by measuring changes in plasma HIV-1 RNA levels, and that the assay is safe and effective when used according to the directions for use in the labeling.

21. APPROVAL SPECIFICATIONS

- Directions for use: See device labeling.
- Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.
- Post-approval Requirements and Restrictions: See approval order.

22. PANEL RECOMMENDATIONS

Not Applicable – This product was not submitted for review by the Blood Products Advisory Committee.

23. FDA/CBER DECISION

The PMA BP200455 is recommended for approval.