Summary Basis for Regulatory Action

Date: July 22, 2019

From: Babita Mahajan, Chair of the Review Committee

BLA/ STN#: 125679

Applicant Name: Abbott GmbH & Co. KG

Date of Submission: May 24, 2018

Complete Response Letter: March 01, 2019

Resubmission: May 24, 2019

MDUFA Goal Date: July 24, 2019

Proprietary Name: Alinity s HIV Ag/Ab Combo

Established Name (common or usual name): Human Immunodeficiency Virus Types 1 and 2 (*E coli, B megaterium*, Recombinant) Antigen, Antibody (p24) and Synthetic Peptides

Intended Use/Indications for Use: The Alinity s HIV Ag/Ab Combo assay is a chemiluminescent microparticle immunoassay (CMIA) used for the simultaneous qualitative detection of human immunodeficiency virus (HIV) p24 antigen and antibodies to HIV type 1 (HIV-1 group M and group O) and/or type 2 (HIV-2) in human serum and plasma specimens on the Alinity s System. The Alinity s HIV Ag/Ab Combo assay is intended to screen individual human donors, including volunteer donors of whole blood and blood components, and other living donors for the presence of anti-HIV-1/HIV-2 and HIV-1 p24 antigen. The assay is also intended for use in testing serum and plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing serum specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens.

Recommended Action: The Review Committee recommends licensure of this product.

Review Office Signatory Authority: Nicole Verdun, M.D., Director, OBRR/CBER

☐ **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.**

The table below indicates the material reviewed when developing the SBRA.

Table 1: Reviews Submitted

Document Title	Reviewer Name	Document Date
Product Review(s) (product office) • *Clinical* • *Non-Clinical*	Alain Debrabant Robert Duncan Krishna Devadas Erica Silberstein Krishna Devadas Susan Zullo	Jun 18, 2019 Jun 10, 2019 Jun 11, 2019 Jun 10, 2019 Jun 11, 2019 Jun 27, 2019
Statistical Review(s) • *Clinical* • *Non-Clinical*	Linye Song	Jun 11, 2019
CMC Review • *CMC (Product Office)* • *Facilities Review (OCBQ/DMPQ)* • *Microbiology Review (OCBQ/DBSQC)* • *Establishment Inspection Report(s) (OCBQ/DMPQ)*	 Alain Debrabant Robert Duncan Susan Zullo Nicole Li Claire H. Wernly Nicole Li	 Jun 18, 2019 Jun 10, 2019 Jun 27, 2019 Feb 13, 2019 Oct 23, 2019 Jan 30, 2019
Labeling Review(s) • *Product Office* • *APLB (OCBQ/APLB)*	 Babita Mahajan Dana Jones	 Jul 12, 2019 Oct 26, 2019
Lot Release Protocols/Testing Plans	Sean Younker Kori Francis Varsha Garnepudi	Jul 17, 2019 Jul 17, 2019 Jul 10, 2019
Bioresearch Monitoring Review	Colonious King	Jul 2, 2019
Software and Instrumentation	Lisa Simone	Jul 11, 2019
Tissues and Advanced Therapies (OTAT)	Bruce Crise	Jun 6, 2019

1. Introduction

The Alinity s HIV Ag/Ab Combo assay is manufactured at the Abbott facility in Wiesbaden, Germany. This biologics license application (BLA) for HIV Ag/Ab Combo assay from Abbott Laboratories, Abbott Park, IL, on behalf of Abbott GmbH & Co. KG was received on May 24, 2018. The BLA was preceded by investigational new drug application (IND) 17494 received on May 11, 2017. An overview of the Alinity s System instrumentation and software is included in this original BLA submission.

Multiple pre-submission discussions on the regulatory pathway were conducted with FDA (May 18, 2012 - Type C Pre-IND meeting request; July 25, 2012 - Face-

to-Face Meeting with Abbott (CRMTS 8519); February 21, 2013 - Type B meeting (CRMTS 8793); July 30, 2015 - Pre-submission meeting telecon BQ150276; May 8, 2017 - Pre-submission meeting BQ170022). Multiple Pre-submission meetings (BQ170158; BQ180168) were conducted following the submission of IND 17494 (May 11, 2017) to discuss issues related to the IND.

Table 2: Chronological Summary of Submission and FDA

Date	Action	Amendment to BL125679
May 24, 2018	BLA CBER receipt	
Jun 1, 2018	Acknowledgement letter	
Jul 13, 2018	Filing notification letter	
Jul 26, 2018	FDA IR on software, DBSQC, and CMC	
Aug 14, 2018	Sponsor response to IR	/0/1
Oct 24, 2018	FDA IR - DMPQ	
Nov 02, 2018	Sponsor response to IR	/0/2
Nov 08, 2018	FDA response to Abbott telecon of Sep 25, 2018	
Nov 16, 2018	Sponsor information on studies with software update	/0/3
Dec 10, 2018	Advice letter - studies with software upgrade	
Jan 04, 2019	FDA IR	
Jan 15, 2019	Meeting minutes - lot release protocol	/0/4
Feb 05, 2019	Sponsor response to IR	/0/5
Mar 01, 2019	Complete Response (CR) letter	
Mar 12, 2019	Sponsor email about change to software version 2.5	/0/6
May 24, 2019	Sponsor response to CR	/0/7
Jun 3, 2019	Resubmission classification letter (Class 1)	
Jul 8, 2019	Sponsor updated lot release template	/0/8
Jul 10, 2019	FDA IR – revision to package insert	
Jul 12, 2019	Sponsor updated the package insert	/0/9

2. Background

Acquired immunodeficiency syndrome (AIDS) is caused by two types of human immunodeficiency viruses, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). HIV is transmitted by sexual contact, exposure to blood or blood products, and prenatal or perinatal infection of a fetus or newborn. During early infection, the first marker to be detected in HIV infected individuals is HIV RNA followed several days later by HIV core protein p24 antigen. Several days after the appearance of HIV p24 antigen, antibodies against HIV are detectable. HIV RNA levels peak prior to antibody seroconversion, and then decline to steady state levels. HIV p24 antigen levels also peak prior to seroconversion and then become undetectable

consistent with the immune complexing of the antigen with the emerging antibodies. After seroconversion, antibodies against HIV are nearly always detected in HIV infected asymptomatic individuals and AIDS patients. HIV antigen and antibody combination assays are used to identify individuals infected with HIV and to prevent transmission of the virus to recipients of blood, blood components, cells, tissues, and organs. In addition to antigens for detecting antibodies to HIV-1 groups M and O and HIV-2, Alinity s HIV Ag/Ab Combo uses anti-HIV-1 p24 antibodies as reagents to detect HIV-1 p24 antigen prior to seroconversion, thereby decreasing the seroconversion window by early detection of HIV infection. This will be the first FDA approved antigen-antibody combo donor screening assay that detects both p24 antigen and antibodies to HIV-1 groups M and O, and HIV-2.

The Alinity s HIV Ag/Ab Combo assay is a chemiluminescent microparticle immunoassay for the simultaneous qualitative detection of human immunodeficiency virus (HIV) p24 antigen and antibodies to HIV type 1 (HIV-1 Group M and Group 0) and/or type 2 (HIV-2) in human serum and plasma specimens. This assay is designed to be performed on the Alinity s System, a high throughput, fully automated immunoassay analyzer that provides routine and priority processing while allowing continuous access and automated retesting.

3. Chemistry Manufacturing and Controls (CMC)

The manufacture of the Alinity s HIV Ag/Ab assay is performed in accordance with Current Good Manufacturing Practices (cGMP) in an environmentally controlled facility.

a) Manufacturing Summary

The Alinity s HIV Ag/Ab Combo assay is manufactured at the Abbott GmbH & CO. KG facility located at Max-Planck-Ring 2, 65205 Wiesbaden, Germany.

The Alinity s HIV Ag/Ab Combo Reagent Kit (List Number 06P0160) consists of the following components:

- HIV-1/HIV-2 antigen and HIV-1 p24 antibody coated microparticles
- HIV-1 antigens, HIV-1/HIV-2 synthetic peptides, and HIV-1 p24 antibody acridinium-labeled conjugate
- Assay Diluent

The Alinity s HIV Ag/Ab Combo Calibrator Kit (List Number 06P0102) consists of the following component:

- Calibrator 1 (purified HIV-1 viral lysate prepared in TRIS buffered saline with protein stabilizer)

The Alinity s HIV Ag/Ab Combo Assay Control Kit (List Number 06P0110) consists of the following components:

- Negative Control (negative recalcified human plasma)

- Positive Control 1 (recalcified, inactivated, human plasma reactive for anti-HIV-1)
- Positive Control 2 (recalcified, inactivated, human plasma reactive for anti-HIV-2)
- Positive Control 3 (purified HIV-1 viral lysate prepared in TRIS buffered saline with protein stabilizer
- Positive Control 4 (purified HIV-1 group O mouse monoclonal antibody prepared in negative recalcified human plasma)

The Alinity s HIV Ag/Ab Combo Release Control Kit (List Number 06P0112) consists of the following component:

- Release Control (purified HIV-1 viral lysate prepared in TRIS buffered saline with protein stabilizer)

The Alinity s System Bulk Solutions listed below are not part of the Alinity s HIV Ag/Ab Combo Reagent Kit, Calibrator Kit, Assay Control Kit, or Release Control Kit but are required to run the Alinity s HIV Ag/Ab Combo assay on the Alinity s System.

- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity s Concentrated Wash Buffer

Product Quality

b) Testing Specifications

The analytical methods and their validations and/or qualifications reviewed for the Alinity s HIV Ag/Ab Combo kit were found to be adequate for their intended use.

c) CBER Lot Release

The lot release protocol template was submitted to CBER for review and found to be acceptable after revisions. A lot release testing plan was developed by CBER and will be used for routine lot release.

d) Facilities Review/Inspection

Facility information and data provided in the BLA were reviewed by CBER and found to be sufficient and acceptable. The facility involved in the manufacture of the Alinity s HIV Ag/Ab Combo assay is listed in table below. The activities performed, and inspectional histories are noted in the table and are further described in the paragraphs that follow.

Table 3: Manufacturing Facilities for Alinity s HIV Ag/Ab Combo Assay

Name/Address	FEI Number	DUNS number	Inspection/ Waiver	Justification /Results
Device Component	3002809144	315786293	Waived	DMPQ August 30 –

Manufacturing, Finished Device Manufacturing, Instrument Solution Manufacture, Device Packaging / Labeling, QC and Release Testing Abbott GmbH & Co. KG Max-Planck-Ring 2 Wiesbaden, Germany 65205				September 7, 2018 VAI

CBER/DMPQ conducted a pre-license inspection (PLI) of Abbott GmbH & Co. KG from August 30 – September 7, 2018 for a similar BLA for Human T-Lymphotropic Virus Types I and II (E coli, Recombinant) Antigen and Synthetic Peptides. At the end of this inspection, a Form FDA 483 was issued. The firm responded to the observations and the corrective actions were reviewed and found to be adequate. All inspectional issues were resolved, and the inspection was classified as voluntary action indicated (VAI). The PLI for Alinity s HIV Ag/Ab Combo assay was waived based on the favorable outcome of the aforementioned inspection.

e) Environmental Assessment

The BLA included a request for categorical exclusion from an Environmental Assessment under 21 CFR 25.31(c). The FDA concluded that this request is justified as the manufacturing of this product will not alter significantly the concentration and distribution of naturally occurring substances, and no extraordinary circumstances exist that would require an environmental assessment.

f) Container Closure

N/A

4. Software and Instrumentation

The following is a summary overview of software, instrumentation and risk management information provided to support a reasonable assurance that the device is safe and effective for its intended uses and conditions of use.

Versioning: System Software v2.5.0. Assay Files HIV Ag/Ab (List Number 06P160) version 180_002.

Device Description: This fully-automated immunoassay analyzer is intended to perform high throughput routine and priority testing while allowing continuous access and automated retesting. The processing for each assay type is controlled by an assay-specific protocol, where parameter information is version-controlled. Positive sample ID is maintained with a barcode reader and all consumables are tracked for availability, stability and expiration. All consumables may be accessed for loading during normal assay operation, and liquid waste requires a laboratory drain outlet. The analyzer may interface with a Laboratory Information System to exchange test order information and results, and with a Laboratory Automation System to allow automated delivery of test samples, where sample ID is reconfirmed by barcode. The system is connected to the customer network with a required ethernet firewall for all external access. The Alinity PRO web-based application allows remote management of multiple instruments in one site. The AbbottLink application allows transfer of instrument data and system updates.

Risk Management: The final risk profile of the Alinity s System includes o red (unacceptable) risks, (b) (4) yellow risks (that required assessment of acceptability) and (b) (4) green (acceptable) risks. Of the (b) (4) yellow risks, (b) (4) are related to false negative results (due to compromised consumables, incorrect instrument processing, and non-conforming lab facilities), and (b) (4) are related to a delay in donor results (due to user delay/interruption). The applicant stated that all risk control measures are implemented and verified and that the labeling notifies the user of residual risks. The applicant concluded the overall residual risk of the Alinity s System is acceptable. This assessment appears to be supported by the evidence provided.

Short-term and long-term risks were evaluated related to donor test results, and to biological, chemical (including toxicological), physical and environmental hazards. Major hazards include: false positive and false negative screening results, delayed screening results, and various physical hazards to the operator (e.g., exposure to infectious materials; chemical, caustic or toxic exposure; slips, trips and falls; sharp/piercing object; clothing or jewelry entrapment; heat/hot parts/magnetic radiation; sprays and air borne matter; generation of metal azides that become explosive upon percussion; electricity; repetitive motion; manual handling of heavy items; and exposure to noise). Moderate hazards include inappropriate disposal of waste.

Significant risk controls for incorrect results include use of barcodes for sample and reagent tracking, sample and reagent handling quality checks, checks to detect errors in assay protocol execution, checks to minimize sampling errors (e.g., clot, fibrin and gel aspiration or short sampling). Labeling control measures to address use issues are also provided (e.g., instructions related to sample quality, sample preparation, material handling and storage). Control measures for delayed results focus on ensuring data are protected through power outages, minimizing use errors, and automated maintenance procedures. Cybersecurity risk control measures span those for confidentiality, integrity and availability; primarily user authentication, hardware firewall, operating system lockout (kiosk mode),

encryption over the AbbottLink connection, platform hardening and monitoring to isolate allowed functionality, and configuration management to ensure release of malware-free software.

Unresolved Anomalies: Software version v2.5.0 contains 210 non-safety-related open anomalies, and two safety-related open anomalies. The safety-related anomalies were both evaluated to represent low risk to the operator and no risk to the donor or recipient. In the first, the operator may be exposed to a chemical hazard, caused when a jam occurs in the loading of reaction vessels. The instrument provides an operator warning. There is no potential exposure to biohazard material, because no sample is present in the reaction vessel at that time. In the second case, the operator may be exposed to a chemical and/or biological hazard if a robotic collision inside the instrument occurs during a maintenance operation. When this situation was observed, the system detected the failure and issued a warning message. The manual contains operator information for chemical and biological hazards. Both defects will be corrected in the next software version.

Testing: Design verification was performed to confirm the design elements meet the specified requirements and includes verification of the effectiveness of risk control measures for potential causes of failure modes. This included software verification, software validation, and system integration. Over 600 protocols were performed. Representative test runs were provided, which corresponded to the highest risks identified in the system. System integration testing confirmed the Alinity s System met requirements using the Alinity s HIV Ag/Ab Combo assay reagents and assay files, and instrument accessories. A human-factors validation assessment identified two safety-related changes that required updates to the System Operations Manual (for proper handling of dry ice) and to the user interface (for search functionality of the On-line Help Browser). These changes were successfully validated. The assay files also met the acceptance criteria for unit (parameter) testing, integration testing, and system testing.

Development Management: The software development activities included establishing detailed software requirements, linking requirements with associate verification tests, verification and validation testing, defect tracking, configuration management and maintenance activities to ensure the software conforms to user needs and intended uses.

Review Issues and Device Changes for Safety and Effectiveness:
During this review, the following issues were raised and resolved to improve safety and effectiveness of the device:

1. System software was upgraded three times over the review cycle (for a total of six software versions) to address 12 CAPAs and 422 software changes. Eleven of these defects had the potential to impact assay results. Of the hardware changes made: six had the potential to impact EMC and/or safety certifications, and six had the potential to impact assay results. Adequate justification was provided to

support the use of most previously-collected preclinical and clinical data to support this submission.

2. The applicant did not originally disclose the high risks associated with the system, which prevented a risk-based review.
   a. Risks processes were updated to comply with ISO 14971, and the improved risk documentation allowed the review to focus on the highest risks to use.
   b. As a result of the new risk process, the applicant stated several improvements are in progress; for example, to ensure risk control measures always have explicit requirements. This will ensure risk control measures are always implemented and verified.
3. Existing anomalies prior to v2.5.0 were reassessed based on the new risk management processes for their connection to risk controls and to system stability. A total of 167 software changes were made in the final version alone, where six had the potential to impact assay results.
4. The original submission was missing information related to the final assay file version, instrument and robot controls, discussion of how the device interoperates with other devices and software in the use environment, verification and validation for the highest risks in the system, impact of outstanding anomalies on system and assay performance, description of configuration management and maintenance to ensure malware free development and shipping, and documentation linking cybersecurity related risks to implemented controls. These were all provided, and all issues were resolved.

5. Analytical Studies

Non-clinical studies were performed at Abbott Diagnostics, Abbott Park, Illinois to evaluate the performance of the Alinity s HIV Ag/Ab Combo assay. The analytical studies were conducted in compliance with 21 CFR Part 58 (Good Laboratory Practices or GLPs), as applicable.

Sample Handling and Collection

a) Tube Type Equivalency and Matched Serum and Plasma

Tube Type Equivalency: Assay performance when used to test blood specimens collected from individual donors in tubes containing: ACD-A, ACD-B, CP2D, CPDA-1, CPD, dipotassium EDTA, lithium heparin, sodium citrate, sodium heparin, dipotassium EDTA (plasma preparation tube), lithium heparin (plasma separator tube), serum (separator tube), and tripotassium EDTA was compared to performance when used to test specimens collected in serum tubes. A minimum of (b) (4) nonreactive and (b) (4) spiked reactive samples (anti-HIV-1 group M, anti-HIV-1 group O, anti-HIV-2, and HIV-1 p24 antigen) for each test condition and the control condition were tested. The samples were spiked to a target concentration of (b) (4) S/CO. The data provided and reviewed demonstrate acceptable performance of the assay supporting the use of specimens collected in all tube types listed above.

Matched Serum and Plasma: Anti-HIV-1 positive specimens from a minimum of (b) (4) individual donor sets were tested with a minimum of (b) (4) replicates using the

Alinity s HIV Ag/Ab Combo assay. The data provided and reviewed demonstrate acceptable performance of the assay supporting the use of serum specimens or plasma specimens collected in tubes containing tripotassium EDTA.

b) Specimen Storage

Assay performance when used to test serum and plasma specimens stored at various temperatures was evaluated. A minimum of (b) (4) nonreactive and (b) (4) anti-HIV-1 group M reactive, anti-HIV-1 group O reactive, anti-HIV-2 reactive, and HIV-1 p24 antigen reactive samples were evaluated using the Alinity s HIV Ag/Ab Combo assay. For both reactive and nonreactive samples, the data provided and reviewed demonstrate acceptable performance of the assay supporting the use of serum and plasma specimens that have been stored at approximately 30°C for up to 7 days, 2 to 8°C for up to 14 days, -20°C or colder for up to 3 months, and up to 6 freeze/thaw cycles.

c) Specimen Processing

Assay performance when used to test centrifuged non-frozen and previously frozen serum and plasma specimens was evaluated. A minimum of (b) (4) nonreactive and (b) (4) reactive (anti-HIV-1 group M, anti-HIV-1 group O, anti-HIV-2, and HIV-1 p24 antigen) samples for each sample type and each storage condition were evaluated. The data provided and reviewed demonstrate acceptable performance of the Alinity s HIV Ag/Ab Combo assay supporting the use with non-frozen and previously frozen serum and plasma specimens that have been tested up to (b) (4) hours after centrifugation at either 30,000 or 75,000 g-minutes.

Potentially Interfering Substances

a) Endogenous Interferences (Spiked)

Assay performance when used to test specimens containing high levels (spiked) of conjugated and unconjugated bilirubin, hemoglobin, triglycerides, or total protein was evaluated. A minimum of (b) (4) nonreactive and (b) (4) reactive (anti-HIV-1 group M reactive, anti-HIV-1 group O reactive, anti-HIV-2 reactive, and HIV-1 p24 antigen reactive) samples for each interferent were evaluated. The data provided and reviewed demonstrate acceptable performance of the assay for both nonreactive and reactive samples supporting the use of specimens containing up to 20 mg/dL of conjugated or unconjugated bilirubin, up to 500 mg/dL of hemoglobin, up to 3,000 mg/dL of triglycerides, and up to 12 g/dL of total protein. In addition, a negative control, an anti-HIV-1 positive control, and an HIV-1 antigen positive control were spiked with biotin to a concentration of 4,250 ng/mL. No interference was observed using the Alinity s HIV Ag/Ab Combo assay.

b) Endogenous Interferences (Native)

Assay performance when used to test specimens containing naturally occurring elevated levels of total bilirubin, hemoglobin, triglycerides or total protein were evaluated. A minimum of (b) (4) specimens for each interferent were used. Nonreactive and reactive samples with naturally occurring elevated levels of each interferent were compared to specimens with normal levels of each. The data provided and reviewed demonstrate acceptable performance of the assay for both

nonreactive and reactive samples supporting the use of specimens that contain greater than (b) (4) of total bilirubin (range tested (b) (4) greater than (b) (4) of hemoglobin (range tested (b) (4) , greater than (b) (4) of triglycerides (range tested (b) (4) , and greater than (b) (4) of total protein (range tested (b) (4)

Specific Performance Characteristics

a) Analytical Specificity (Other Disease States)

Assay performance when used to test specimens from individuals with other conditions or disease states (n = 242) unrelated to HIV infection was evaluated.

Table 4: Alinity s HIV Ag/Ab Combo with Other Disease States (Analytical Specificity) Summary

Other Disease States or Specimen Conditions	Alinity s HIV Ag/Ab Combo				ABBOTT PRISM HIV O Plus			
	Total	IR	RR	Confirmed RR	Total	IR	RR	Confirmed RR
Anti-HTLV I/II Positive	10	0	0	0	10	0	0	0
Anti-HCV Positive	10	0	0	0	10	0	0	0
Anti-HAV Positive	10	0	0	0	10	0	0	0
HBV Positive	10	0	0	0	10	0	0	0
Co-infected CMV/EBV/HSV	10	0	0	0	10	0	0	0
Anti-*T. pallidum* Positive	10	0	0	0	10	0	0	0
Rheumatoid Factor Positive	10	0	0	0	10	0	0	0
Anti-ds DNA Positive	10	0	0	0	10	0	0	0
Pregnant Females	14	0	0	0	14	0	0	0
Multiparous Females	10	0	0	0	10	0	0	0
Hyper IgG/IgM	10	0	0	0	7[a]	0	0	0
Influenza Vaccine Recipients	10	0	0	0	10	0	0	0
Hemodialysis Patients	10	0	0	0	10	0	0	0
HAMA positive	10	0	0	0	10	0	0	0
Escherichia coli Infection	10	0	0	0	10	0	0	0
Heterophilic Antibody Positive	8	0	0	0	8	0	0	0
Anti-gonococcus Positive	10	0	0	0	10	0	0	0
Anti- *C. trachomatis* Positive	10	0	0	0	10	0	0	0
Anti-*T. gondii* Positive	10	0	0	0	10	0	0	0
Fungal (Yeast) Infection	10	0	0	0	10	0	0	0
Anti-nuclear Antibody Positive	10	0	0	0	10	0	0	0
Crohn's Disease	10	0	0	0	10	0	0	0
Anti-VZV Positive	10	0	0	0	10	0	0	0
Anti-rubella Positive	10	0	0	0	10	0	0	0
Total	242	0	0	0	239	0	0	0

[a] Only seven Hyper IgG/IgM samples were tested with Abbott PRISM HIV O Plus assay

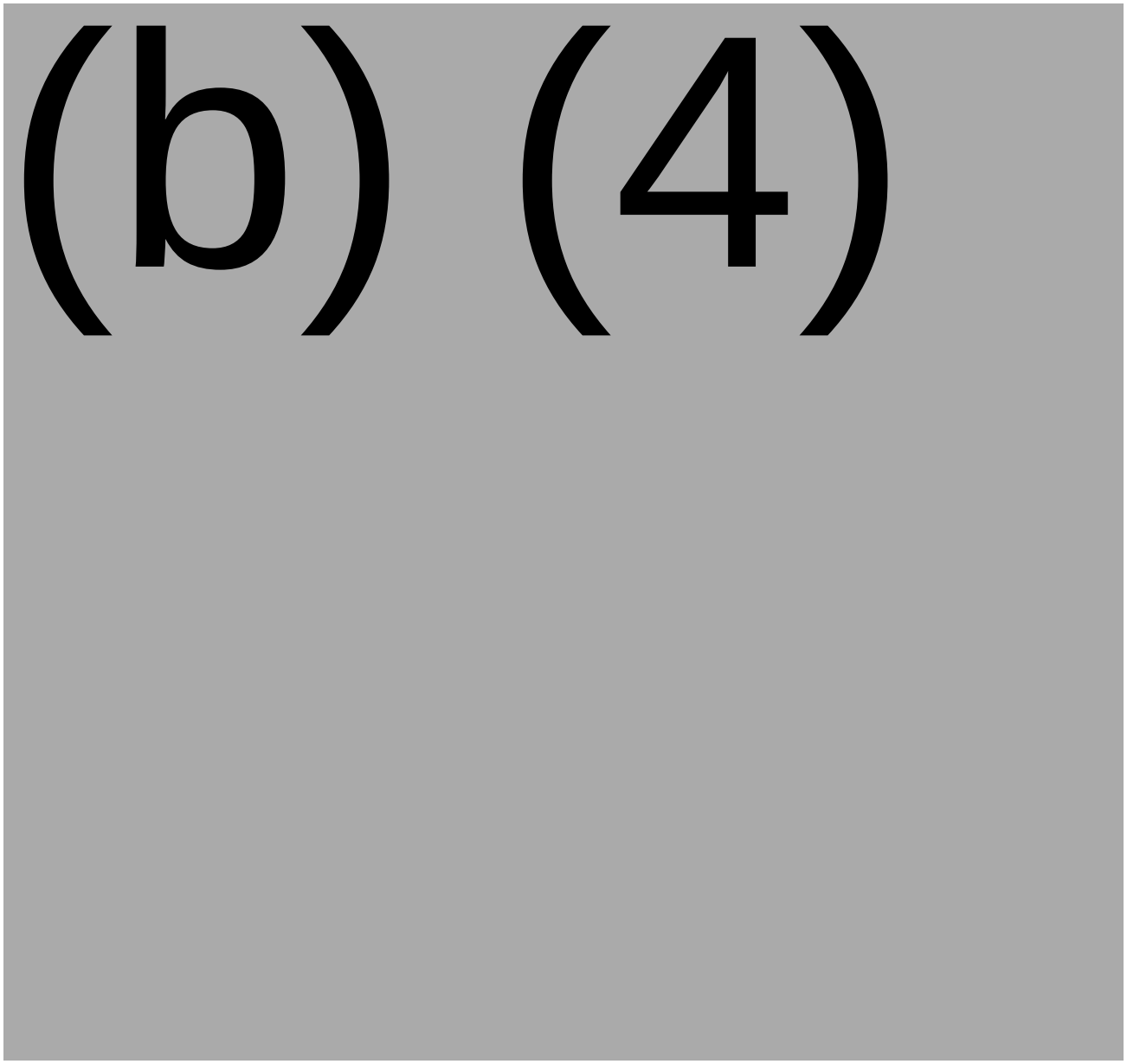
Each specimen was tested (b) (4) using the Alinity s HIV Ag/Ab Combo and Abbott PRISM HIV O Plus assays. The initial and repeat reactive rates were 0.00% (0/242) for the Alinity s HIV Ag/Ab Combo assay.

b) Precision Alinity s HIV Ag/Ab Combo assay

Panels and controls were tested with a minimum of (b) (4) replicates (b) (4) times per day (separated by a minimum of (b) (4) on (b) (4) instruments, on at least (b) (4) different

days, for a minimum of (b) (4) required measurements. The within-laboratory imprecision results (which include within-run, between-run, and between-day variance components), between-instrument imprecision results, and the reproducibility imprecision results (which include within-run, between-run, between-day, and between-instrument variance components) are presented in the Table 5. The Alinity s HIV Ag/Ab Combo assay using Alinity s System software version 2.5.0 demonstrated acceptable precision.

Table 5: Summary of Overall Alinity s HIV Ag/Ab Combo assay Precision Results



Review Issue: Abbott's original precision study using software version 1.2.0. was evaluated using (b) (4) noting that each Alinity s System contains two process paths with two lanes per process path. All four lanes on (b) (4)

were used in this study for Alinity s HIV Ag/Ab Combo assay. As each of the four lanes on one system has its own independent set of wash zones and optics, the data for each lane were analyzed as a separate instrument. The review committee did not agree as many of the earlier steps in the assay such as reagent dispense 1 and sample mixing are not separate for each process path. Further, the review committee conveyed to Abbott that Alinity s is a new instrument and the study fails to capture the precision among different instruments. A request to repeat the study was conveyed to the sponsor in an Information Request followed by a Complete Response letter dated March 1, 2019 because the study data had not yet been received. The precision study was repeated using (b) (4) separate Alinity s Systems with software version 2.5.0. The data from the new study were received in the response to the Complete Response letter on May 24, 2019 (Amendment 7) with acceptable variances among the instruments, and the issue was resolved.

c) In-House Specificity (Donors)

The specificity of the Alinity s HIV Ag/Ab Combo assay was determined by testing a minimum of (b) (4) plasma specimens from blood donors using (b) (4) reagent kit lots. There were no initially reactive specimens. The specificity of the Alinity s HIV Ag/Ab Combo assay was (b) (4) (95% CI: (b) (4)

d) Group, Subtype and Unique Recombinant Form Detection

A total of (b) (4) preselected anti-HIV-1 and HIV-1 p24 antigen positive specimens which included (b) (4) anti-HIV-1 subtype positive samples, (b) (4) anti-HIV-1 groups (N, O, and P) samples, (b) (4) anti-HIV-1 unique recombinant form (URF) subtype samples, (b) (4) HIV-1 antigen subtype positive samples (human), and (b) (4) HIV-1 antigen group and subtype positive samples (viral isolates) were tested using HIV Ag/Ab Combo assay and (b) (4) using the Abbott PRISM HIV O plus assay. All (b) (4) specimens were detected by the Alinity s HIV Ag/Ab Combo assay. (b) (4) group P subtype positive, (b) (4) HIV-1 antigen subtype positive (human), and (b) (4) HIV-1 antigen group and subtype positive samples (viral isolates) were nonreactive on the ABBOTT PRISM HIV O Plus assay. The Alinity s HIV Ag/Ab Combo assay is designed to detect HIV-1 p24 antigen whereas the ABBOTT PRISM HIV O Plus assay is an antibody detection method only. Of the (b) (4) discordant samples, (b) (4) are HIV-1 p24 antigen containing samples that the ABBOTT PRISM HIV O Plus assay does not detect.

e) Analytical Sensitivity

The analytical sensitivity of the Alinity s HIV Ag/Ab Combo assay was evaluated using the WHO 1st International Standard for HIV-1 p24 antigen (NIBSC Code 90/636), diluted to target concentrations between (b) (4) The dilutions were tested across (b) (4) lots of the Alinity s HIV Ag/Ab Combo Reagent Kit on one Alinity s System. The analytical sensitivity ranged from (b) (4)

f) Dilution Sensitivity

The dilution sensitivity of the Alinity s HIV Ag/Ab Combo assay and the Abbott PRISM HIV O Plus assay were compared. (b) (4) anti-HIV-1, (b) (4) anti-HIV-1 group O, (b) (4) anti-HIV-2, and (b) (4) HIV-1 p24 specimens were serial diluted with nonreactive human plasma to create samples with final dilution factors ranging from (b) (4) A total of (b) (4) samples (neat and diluted) were tested in a minimum of (b) (4) replicates using both the Alinity s HIV Ag/Ab Combo and ABBOTT PRISM HIV O Plus assays. For (b) (4) of the positive (b) (4) anti-HIV-1, (b) (4) anti-HIV-1 group O, and (b) (4) anti-HIV-2) specimens, the ABBOTT PRISM HIV O Plus assay detected additional dilutions not detected by the Alinity s HIV Ag/Ab Combo assay. For (b) (4) of the positive (b) (4) anti-HIV-1 and (b) (4) HIV-1 p24 antigen) specimens, the Alinity s HIV Ag/Ab Combo assay detected additional dilutions not detected by the ABBOTT PRISM HIV O Plus assay. The difference is associated with assay design and HIV Ag/Ab Combo assay is designed and optimized for detection of HIV-1 antigen and HIV antibodies in neat specimens.

g) Limit of Detection

The limit of blank (LoB) and limit of detection (LoD) of the Alinity s HIV Ag/Ab Combo assay were evaluated. (b) (4)-analyte level samples were prepared by diluting the WHO 1st International Standard for HIV-1 p24 antigen (NIBSC Code 90/636), to target concentrations between (b) (4) -analyte sample and (b) (4)-analyte level samples were tested in multiple replicates using (b) (4) Alinity s HIV Ag/Ab Combo assay lots on a minimum of (b) (4) days for a minimum of (b) (4) replicates of each sample. The maximum LoB value was (b) (4) S/CO and ranged from (b) (4) S/CO. The maximum LoD value was (b) (4) S/CO and ranged from (b) (4) S/CO.

h) Seroconversion

The seroconversion detection of the Alinity s HIV Ag/Ab Combo assay were compared to the Abbott PRISM HIV O Plus assay. Twenty seroconversion panels were tested. There were 30 panel members with discordant results between the Alinity s HIV Ag/Ab Combo assay and the ABBOTT PRISM HIV O Plus assay. All 30 discordant panel members were repeatedly reactive for the Alinity s HIV Ag/Ab Combo assay and nonreactive for the ABBOTT PRISM HIV O Plus assay. The difference was associated with difference in assay design. ABBOTT PRISM HIV O Plus assay is not designed to detect HIV-1 p24 antigen. For 3 of the 20 panels, the first reactive time point for the Alinity s HIV Ag/Ab Combo assay occurred at the same time as the first reactive time point for the ABBOTT PRISM HIV O Plus assay. For the remaining 17 panels, the Alinity s HIV Ag/Ab Combo assay demonstrated earlier detection than the ABBOTT PRISM HIV O Plus assay (Table 6).

Table 6: Alinity s HIV Ag/Ab Combo Seroconversion Summary of Reactivity Results

	Vendor ID	Number of Panel Members	Alinity s HIV Ag/Ab Combo			ABBOTT PRISM HIV O Plus			Difference in Days[a]
			Number of RR Panel Members	Number of Days to First RR	Percent of RR Panel Members	Number of RR Panel Members	Number of Days to First RR	Percent of RR Members	

				Result			Result		
01	HIV9013	7	1	25	14.3	0		0.0	
02	HIV9015	7	2	26	28.6	1	31	14.3	-5
03	HIV9018	11	3	28	27.3	2	32	18.2	-4
04	HIV9020	22	3	90	13.6	1	97	4.5	-7
05	HIV9021	17	4	47	23.5	1	57	5.9	-10
06	HIV9022	9	2	25	22.2	1	32	11.1	-7
07	HIV9023	22	3	78	13.6	0		0.0	
08	HIV9024	12	1	53	8.3	0		0.0	
09	HIV9030	16	3	47	11.8	1	54	6.3	-7
10	HIV9032	8	4	38	50.0	4	38	50.0	0
11	HIV9077	24	13	45	54.2	10	57	41.7	-12
12	PRB945	6	3	13	50.0	3	13	50.0	0
13	PRB946	4	2	7	50.0	0		0.0	
14	PRB949(M)	4	1	18	25.0	0		0.0	
15	PRB953	4	2	7	50.0	1	10	25.0	-3
16	PRB955	5	4	3	80.0	2	12	40.0	-9
17	PRB956	5	2	47	40.0	0		0.0	
18	PRB958	6	4	7	66.7	2	15	33.3	-8
19	PRB963	7	2	17	28.6	0		0.0	
20	PRB969	10	3	70	30.0	3	70	30.0	0

RR = Repeat Reactive; Blank spaces indicate NA

[a] Difference in Days = Alinity s Days to First Repeatedly Reactive Result – ABBOTT PRISM Days to First Repeatedly Reactive Result. Negative value indicates an earlier detection by the Alinity s HIV Ag/Ab Combo

i) Reagent Onboard Stability and Calibration Storage

The performance of the Alinity s HIV Ag/Ab Combo assay when the reagents are stored onboard the Alinity s System and the acceptability of a calibration generated using the Alinity s HIV Ag/Ab Combo assay and stored on the Alinity s System were evaluated. The reagents were subjected to transport/motion stress during shipping from the manufacturing site to the testing site. The Alinity s HIV Ag/Ab Combo Reagent Kit was used to generate Day 0 calibration and stored onboard the Alinity s System. The Anti-HIV-1, Anti-HIV-2, Anti-HIV Group O, and HIV-1 p24 Antigen Panels (prepared by diluting respective positive specimens to an S/CO target value of (b) (4), Negative Control, Positive Control (1,2, 3 and 4), and Release Control tested at each timepoint were compared to the same samples at Day 0 with a minimum of (b) (4) replicates for (b) (4) timepoints over a period of (b) (4) days. The data provided and reviewed demonstrate acceptable performance of the assay for all samples supporting the use of Alinity s HIV Ag/Ab Combo Reagent Kit that have been stored onboard the Alinity s System for (b) (4) days, and the use of a calibration generated using the Alinity s HIV Ag/Ab Combo assay and stored on the Alinity s System for up to 14 days.

j) Specimen Onboard Stability (Primary Tube)

The performance of the Alinity s HIV Ag/Ab Combo assay when used to test serum and plasma specimens stored onboard the Alinity s System in primary tubes was evaluated. A minimum of (b) (4) nonreactive and (b) (4) reactive (anti-HIV-1 group M, anti-HIV-1 group O, anti-HIV-2, and HIV-1 p24 antigen) samples for each sample type (serum and plasma (sodium citrate)) were tested. The nonreactive and reactive specimens stored for (b) (4) hours in primary tubes onboard the Alinity s System were compared to the same specimens tested at baseline. The data provided and reviewed demonstrate acceptable performance of the assay for both the nonreactive and reactive samples supporting the use of serum and plasma specimens that have been stored onboard the Alinity s System in primary tubes for up to 10 hours.

k) Specimen Onboard Stability (Sample Cup)

The performance of the Alinity s HIV Ag/Ab Combo assay when used to test serum and plasma specimens stored onboard the Alinity s System in sample cups was evaluated. The Alinity s HIV Ag/Ab Combo Negative Control, Positive Control 1, Positive Control 2, Positive Control 3, and Positive Control 4 were used for this study. Controls stored for (b) (4) hours in sample cups onboard the Alinity s System were compared to the same specimens tested at baseline. Each control was pipetted into (b) (4) sample cups for each timepoint and tested (b) (4) using the Alinity s HIV Ag/Ab assay. The data provided and reviewed demonstrate acceptable performance of the assay for both the Negative and Positive Controls supporting the use of serum and plasma specimens that have been stored onboard the Alinity s System in sample cups for up to 3 hours.

l) Reagent Cross Contamination

Potential cross contamination between assay reagents was evaluated by verifying the effectiveness of the Alinity s System reagent (b) (4) . A negative sample and anti-HIV-1 positive spiked sample, anti-HIV-1 group O positive spiked sample, anti-HIV-2 positive spiked sample, and HIV-1 p24 viral lysate positive spiked sample were used for the study. The following assays were used to evaluate potential cross contamination of any reagents with the Alinity s HIV Ag/Ab Combo assay: (b) (4)

The results demonstrated that the reagent wash stations are effective in controlling reagent cross contamination from a potentially contaminating Alinity s assay to the Alinity s HIV Ag/Ab Combo assay.

m) Within-Assay Carryover

The performance of the Alinity s HIV Ag/Ab Combo assay when exposed to potential within-assay sample carryover interference from a sample with high levels of positive antibody (b) (4) and high positive HIV-1 p24 antigen sample was evaluated. The results of an unprotected negative sample that was tested after the high positive sample (test condition) were compared to the results of a protected negative sample tested before the high positive sample (control condition). A total of (b) (4) iterations of alternating contaminating assay and susceptible assay were

performed for the high antibody sample and (b) (4) iterations were performed for the high antigen sample. The results demonstrated that no within-assay sample carryover was observed with the Alinity s HIV Ag/Ab assay.

Stability

The stability studies were performed using a real-time stability study design. The studies were conducted through Month (b) (4) using 3 lots each of Alinity s HIV Ag/Ab Combo Reagent Kit, Calibrator Kit, Assay Control Kit, and Release Control Kit. The stability limits of the test were met for all lots for (b) (4) months allowing them to claim 12-month expiration dating. In addition, studies for the following stability conditions were also provided: (b) (4) of each assay component stored (b) (4) to cause (b) (4) between the product and the container closure), (b) (4) of calibrators, assay controls, and release controls subjected to simulated customer-use conditions, with repeated cycles of opening, use, closure, and storage, including time the container is open when onboard the instrument), and (b) (4) lots of reagents and release control subjected to (b) (4) the instrument). Testing for these stability conditions has been completed through Month 12 and all criteria were met. The transport stability study was conducted through Month 12 using (b) (4) each of the Alinity s HIV Ag/Ab Combo Reagent Kit, Calibrator Kit, Assay Control Kit, and Release Control Kit. All criteria were met.

Microbial Challenge

The following organisms were used in both the antimicrobial effectiveness and microbial interference studies. (b) (4)

a) Antimicrobial Effectiveness

The level of antimicrobial protection provided by the preservative system used in the components of the Alinity s HIV Ag/Ab Combo assay was evaluated. The assay kit components were (b) (4) listed above to a (b) (4) at each timepoint, evaluated and compared to a control sample (b) (4). Bioburden levels were determined at (b) (4) days and (b) (4) days after inoculation. The preservative was considered cidal if there was at least a (b) (4) log reduction in microbial counts between Day 0 and Day (b) (4) and no increase greater than (b) (4) log between Day (b) (4) and Day (b) (4). The preservative was considered static if there was no increase greater than (b) (4) log in microbial counts between Day 0 and Day (b) (4) or between Day (b) (4) and Day (b) (4). The results for all components were either cidal or static for all organisms.

b) Microbial Interference

The performance of the Alinity s HIV Ag/Ab Combo assay was evaluated using kit components that had been exposed to (b) (4). All kit components were (b) (4) listed above to a (b) (4)

(b) (4) and compared to control samples (b) (4) the components with (b) (4). All (b) (4) and control samples were stored for (b) (4) days at the recommended storage condition of (b) (4) and then tested within (b) (4) days after Day (b) (4) None of the components were sensitive to microbial contamination.

The combined results of the antimicrobial effectiveness and microbial interference studies show that all Alinity s HIV Ag/Ab Combo Reagent Kit, Calibrator Kit, Assay Control Kit, and Release Control Kit components were adequately protected from microbial contamination through expiration for all organisms tested.

Cadaveric Studies

All cadaveric serum specimens used in the studies were previously frozen and stored frozen until their use. The living donor serum specimens used as control samples were either previously frozen or collected in-house and stored frozen after collection.

a) Cadaveric Reproducibility

The reproducibility of the Alinity s HIV Ag/Ab Combo assay when used to test cadaveric serum specimens was evaluated. Twenty-three cadaveric and 23 living donor serum specimens were tested (Table 7). The duration between the time of death and time of draw ranged from (b) (4) hour, (b) (4) minutes to 21 hours, 35 minutes. Both random living donor and cadaveric serum samples were spiked with only one analyte (anti-HIV-1 group M, anti-HIV-1 group O, anti-HIV-2, or HIV-1 p24 antigen) using (b) (4) of (b) (4) to create reactive samples. Samples were tested once daily for six days using three Alinity s HIV Ag/Ab Combo Reagent Kit lots for a total of six runs (n=18 total replicates per sample). For anti-HIV-1 group M and anti-HIV-1 group O samples, the cadaveric total %CV result was less than or equal to the living donor total %CV result. For anti-HIV-2 and HIV-1 p24 antigen samples, the cadaveric total %CV result was greater than the living donor %CV result, but the lower limit of the 95% CI around the SD ratio was (b) (4) The Alinity s HIV Ag/Ab Combo assay demonstrated acceptable reproducibility when comparing cadaveric serum specimens to living donor serum specimens.

Table 7: Alinity s HIV Ag/Ab Combo Cadaveric Reproducibility

Analyte	Specimen Category	Number of Replicates	Mean S/CO	Total [a]	
				SD	CV
Anti-HIV-1 Group M	Cadaveric [b]	414	5.28	0.439	8.3
	Living Donor	414	4.86	0.442	9.1
Anti-HIV-1 Group O	Cadaveric [b]	414	4.36	0.551	12.6
	Living Donor	414	4.31	0.583	13.5
Anti-HIV-2	Cadaveric [b]	414	3.49	0.228	6.5
	Living Donor	414	3.52	0.213	6.0
HIV-1 p24	Cadaveric [b]	414	3.94	0.286	7.3

Antigen	Living Donor	414	4.00	0.175	4.4

CV = coefficient of variation expressed as a percentage
SD = standard deviation
[a] Total variability contains within-specimen, between-lot and lot specimen interaction variance components.
[b] Cadaveric serum specimens were collected up to 21.6 hours after death.

b) Cadaveric Specificity

The specificity of the Alinity s HIV Ag/Ab Combo assay when used to test cadaveric serum specimens by comparing them to living donor specimens was evaluated. A total of 55 cadaveric and 55 living donor serum specimens were tested (Table 8). The duration between the time of death and time of draw ranged from (b) (4) hour, (b) (4) minutes to 23 hours, (b) (4) minutes. Both random living donor serum samples and cadaveric serum samples were tested once using three Alinity s HIV Ag/Ab Combo Reagent Kit lots. All samples were nonreactive. Specificity was 100.0% (55/55) for all reagent lots for both sample types with 95% confidence intervals of 93.51 to 100.00.

Table 8: Analytical Specificity in Cadaveric and Living Donors

Specimen Category	Lot	Nonreactive	Repeatedly Reactive	Specificity (%) (95% CI)
Cadaveric [a] (N=55)	Lot 1	55	0	100.00 (93.51 – 100.00)
	Lot 2	55	0	100.00 (93.51 – 100.00)
	Lot 3	55	0	100.00 (93.51 – 100.00)
Living Donor (N=55)	Lot 1	55	0	100.00 (93.51 – 100.00)
	Lot 2	55	0	100.00 (93.51 – 100.00)
	Lot 3	55	0	100.00 (93.51 – 100.00)

[a] Cadaveric serum specimens were collected up to 23.7 hours after death

c) Cadaveric Sensitivity

The analytical sensitivity of the Alinity s HIV Ag/Ab Combo assay when used to test cadaveric serum specimens was evaluated. The duration between the time of death and time of draw ranged from (b) (4) hours, (b) (4) minutes to 26 hours, 30 minutes. Each cadaveric and living donor serum specimen were divided into four aliquots. An individual aliquot was supplemented with only one analyte (anti-HIV-1 group M, anti-HIV-1 group O, anti-HIV-2, or HIV-1 p24 antigen) using (b) (4) Samples were tested once within 24 hours of spiking using three Alinity s HIV Ag/Ab Combo Reagent Kits. All samples were reactive. Sensitivity was 100.0% for all reagent lots (Table 9) with 95% confidence intervals of 93.51 to 100.00 for both cadaveric and living donor samples.

Table 9: Analytical Sensitivity in Cadaveric and Living Donors by Lot

Analyte	Specimen Category	Lot	Number of Specimens	Mean S/CO	Sensitivity (%) (95% CI)
Anti-HIV-1 Group M	Cadaveric [a] (N=55)	Lot 1	55	4.84	100.00 (93.51 – 100.00)
		Lot 2	55	5.31	100.00 (93.51 – 100.00)
		Lot 3	55	5.24	100.00 (93.51 – 100.00)
	Living Donor (N=55)	Lot 1	55	4.44	100.00 (93.51 – 100.00)
		Lot 2	55	5.03	100.00 (93.51 – 100.00)
		Lot 3	55	4.95	100.00 (93.51 – 100.00)
Anti-HIV-1 Group O	Cadaveric [a] (N=55)	Lot 1	55	3.84	100.00 (93.51 – 100.00)
		Lot 2	55	4.28	100.00 (93.51 – 100.00)
		Lot 3	55	4.30	100.00 (93.51 – 100.00)
	Living Donor (N=55)	Lot 1	55	3.74	100.00 (93.51 – 100.00)
		Lot 2	55	4.25	100.00 (93.51 – 100.00)
		Lot 3	55	4.23	100.00 (93.51 – 100.00)
Anti-HIV-2	Cadaveric [a] (N=52)	Lot 1	52	3.27	100.00 (93.15 – 100.00)
		Lot 2	52	3.25	100.00 (93.15 – 100.00)
		Lot 3	52	3.35	100.00 (93.15 – 100.00)
	Living Donor (N=55)	Lot 1	55	3.01	100.00 (93.51 – 100.00)
		Lot 2	55	3.09	100.00 (93.51 – 100.00)
		Lot 3	55	3.17	100.00 (93.51 – 100.00)
HIV-1 p24 Antigen	Cadaveric [a] (N=55)	Lot 1	55	3.96	100.00 (93.51 – 100.00)
		Lot 2	55	3.90	100.00 (93.51 – 100.00)
		Lot 3	55	4.19	100.00 (93.51 – 100.00)

	Living Donor (N=55)	Lot 1	55	3.77	100.00 (93.51 – 100.00)
		Lot 2	55	3.73	100.00 (93.51 – 100.00)
		Lot 3	55	3.98	100.00 (93.51 – 100.00)

[a] Cadaveric serum specimens were collected up to 26.5 hours after death

d) Cadaveric Specimen Storage

The performance of the Alinity s HIV Ag/Ab Combo assay when used to test cadaveric serum specimens that have been stored at various storage conditions was evaluated. The duration between the time of death and time of draw ranged from (b) (4) hours, (b) (4) minutes to 21 hours, 21 minutes for the cadaveric serum samples used for the -20°C or colder storage condition and (b) (4) minutes to 32 hours, (b) (4) minutes for the cadaveric serum samples used for other storage conditions. Random cadaveric serum specimens were spiked with (b) (4) unique sources (b) (4) anti-HIV-1 group M, (b) (4) anti-HIV-1 group O, (b) (4) anti-HIV-2, and (b) (4) HIV-1 p24 antigen viral lysate) of (b) (4) to create reactive samples. Twelve nonreactive serum samples and 12 each low-level reactive serum samples (anti-HIV-1 group M, anti-HIV-1 group O, anti-HIV-2, and HIV-1 p24 antigen) were evaluated. Both sample types stored for a period of time at various storage temperatures were compared to samples tested at baseline. The samples were tested at least (b) (4) at each timepoint using the Alinity s HIV Ag/Ab Combo assay. For both nonreactive and reactive samples, the data provided and reviewed demonstrate acceptable performance of the assay supporting the use of cadaveric serum specimens that have been stored at approximately 30°C for up to 3 days, 2 to 8°C for up to 14 days, -20°C or colder for up to 3 months, and up to 6 freeze/thaw cycles.

Review Issues: During this review, the following issues were raised and resolved.
1. Cadaveric Reproducibility: The data submitted for cadaveric reproducibility study showed that the number of samples tested for each anti-HIV-1 group M, anti-HIV-1 group O, anti-HIV-2, and HIV-1 p24 antigen viral lysate spiked specimens were not adequate to ensure assay reproducibility. This was communicated in FDA IR (Jan 4, 2019) and FDA CR letter (March 1, 2019). Abbott provided additional testing data in amendment 7 (received on May 24, 2019), in which 23 cadaveric and 23 living donor samples were spiked separately with anti-HIV-1 group M, anti-HIV-1 group O, anti-HIV-2, and HIV-1 p24 antigen and tested. This additional testing resolved the issue.

2. Cadaveric Sensitivity: The data submitted for cadaveric sensitivity study showed that the number of samples tested for each anti-HIV-1 group M, anti-HIV-1 group O, anti-HIV-2, and HIV-1 p24 antigen viral lysate spiked specimens were not adequate to ensure assay sensitivity for each analyte. This was communicated in FDA IR (Jan 4, 2019) and FDA CR letter (March 1, 2019). Abbott provided additional testing data in amendment 7 (received on May 24, 2019), in which 55 cadaveric and 55 living donor samples were spiked separately with anti-HIV-1 group M, anti-HIV-1 group O, and HIV-1 p24 antigen and tested. 52 cadaveric and

55 living donor samples spiked with anti-HIV-2, and this additional testing resolved the issue.

6. Clinical Studies

Clinical studies were conducted to evaluate assay specificity, sensitivity and reproducibility to demonstrate performance and intended use of the Alinity s HIV Ag/Ab Combo assay. Testing was performed at four blood donor testing laboratories using specimens collected at three whole blood collection sites and one plasmapheresis collection site. A minimum of three lots each of the Alinity s HIV Ag/Ab Combo Reagent Kit, Alinity s HIV Ag/Ab Combo Calibrator Kit, Alinity s HIV Ag/Ab Combo Assay Control Kit, and Alinity s HIV Ag/Ab Combo Release Control Kit were used for the studies at testing sites. The FDA-licensed PRISM HIV O Plus assay was used as the comparator test.

Clinical Specificity

A prospective multicenter study was conducted to evaluate the clinical specificity of the Alinity s HIV Ag/Ab Combo assay on the Alinity s System using a total of 13,858 whole blood donor specimens from three sites. Of these, 7,347 were fresh serum and 6,511 were fresh plasma. An additional 3,138 plasmapheresis specimens were also collected from a separate site. The testing was performed using the Alinity s Alinity s HIV Ag/Ab Combo assay and the ABBOTT PRISM HIV O Plus assay. There were 15 donors that were eligible for follow-up; four of the 15 donors provided a follow-up specimen. For Alinity s HIV Ag/Ab Combo, the initial reactive rate was 0.09% (15/16,996), and the repeat reactive rate was 0.08% (14/16,996). Repeatedly reactive specimens were further tested using the following supplemental assays: HIV-1 qualitative RNA assay, HIV-1 Western blot/HIV-1 IFA, HIV-2 EIA, and HIV 1/2 immunochromatographic assay. Based on supplemental test results, 13 specimens were negative, and one specimen was indeterminate. For ABBOTT PRISM HIV O Plus, the initial reactive rate was 0.08% (14/16,996) and the repeat reactive rate was 0.07% (12/16,996). The final agreement between Alinity s HIV Ag/Ab Combo and ABBOTT PRISM HIV O Plus was 99.87% (16,974/16,996). Specificity in blood and plasmapheresis donors was calculated to be 99.92% (16,981/16,994) with a 95% confidence interval of 99.87% to 99.96% (Table 10).

Table 10: Alinity s HIV Ag/Ab Combo Clinical Study Reactivity of the Alinity s HIV Ag/Ab Combo Assay in Donors

Specimen Category	N	IR (% of Total) (95% CI)	RR (% of Total) (95% CI)	Number Positive by Supplemental Testing (% of RR)	Specificity (%) [a] (95% CI)
Volunteer Blood Donors - Serum	7,347	6 (0.08) (0.03 - 0.18)	5 (0.07) (0.02 - 0.16)	0 (0.00)	99.93 (7,342 / 7,347) (99.84 - 99.98)
Volunteer Blood Donors - Plasma	6,511	6 (0.09) (0.03 - 0.20)	6 (0.09) (0.03 - 0.20)	0 (0.00)	99.91 (6,504 / 6,510) (99.80 - 99.97)

Specimen Category	N	IR (% of Total) (95% CI)	RR (% of Total) (95% CI)	Number Positive by Supplemental Testing (% of RR)	Specificity (%) [a] (95% CI)
Total Volunteer Blood Donors	**13,858**	**12 (0.09) (0.04 - 0.15)**	**11 (0.08) (0.04 - 0.14)**	**0 (0.00)**	**99.92 (13,846 / 13,857) (99.86 - 99.96)**
Plasmapheresis Donors	3,138	3 (0.10) (0.02 - 0.28)	3 (0.10) (0.02 - 0.28)	0 (0.00)	99.94 (3,135 / 3,137) (99.77 - 99.99)
Total Donors	**16,996**	**15 (0.09) (0.05 - 0.15)**	**14 (0.08) (0.05 - 0.14)**	**0 (0.00)**	**99.92 (16,981 / 16,994) (99.87 - 99.96)**

N = Number tested; IR = initially reactive; RR = repeatedly reactive; CI = confidence interval

[a] Based on supplemental test results for the 14 repeatedly reactive specimens, 1 specimen was indeterminate (plasmapheresis donor) and 13 specimens were negative (5 blood donor serum, 6 blood donor plasma, and 2 plasmapheresis donors). The 1 repeatedly reactive specimen found to be indeterminate by supplemental testing was excluded from the specificity calculations. One additional Alinity s HIV Ag/Ab Combo nonreactive specimen was indeterminate (blood donor plasma) by supplemental testing and was excluded from the specificity calculations.

Clinical Sensitivity

Assay sensitivity was calculated by analyzing test results from frozen specimens provided by Abbott Laboratories. A total of 2,476 specimens were tested with the Alinity s HIV Ag/Ab Combo assay at three sites. These specimens were also tested at one site with the Abbott PRISM HIV O Plus assay. Repeatedly reactive specimens from individuals at increased risk of HIV-1/2 infection and individuals at increased risk of HIV infection from HIV-2 endemic areas were tested using the following supplemental assays: HIV-1 qualitative RNA assay, HIV-1 IFA, and HIV 1/2 immunochromatographic assay. Sensitivity was estimated to be 100.00% (1336/1336) with a 95% confidence interval of 99.72% to 100.00% for preselected positive specimens and HIV-1 viral isolates (Table 11).

The agreement between the Alinity s HIV Ag/Ab Combo assay and the ABBOTT PRISM HIV O Plus assay for specimens from individuals at increased risk of HIV-1/2 infection was 98.02% (593/605). There were 23 specimens that were Alinity s HIV Ag/Ab Combo repeatedly reactive and ABBOTT PRISM HIV O Plus repeatedly reactive. Twenty-one of the 23 specimens were positive and the remaining 2 specimens were negative by supplemental testing. There were 12 specimens that were ABBOTT PRISM HIV O Plus repeatedly reactive, Alinity s HIV Ag/Ab Combo nonreactive, and negative by supplemental testing.

Table 11: Alinity s HIV Ag/Ab Combo Clinical Study Overall Sensitivity Summary

Specimen Category	N	Number Positive	Alinity s HIV Ag/Ab Combo		
			Number RR (% of Total)	Number RR that were Positive (% of RR)	Sensitivity (%) (95% CI)
Preselected Anti-HIV-1 Positive [a]	1,016	1,016	1,016 (100.00)	1,016 (100.00)	100.00 (1016/1016) (99.64 - 100.00)
Preselected Anti-HIV-2 Positive [b]	232	232	232 (100.00)	232 (100.00)	100.00 (232/232) (98.42 - 100.00)
Preselected HIV-1 Antigen Positive [c]	35	35	35 (100.00)	35 (100.00)	100.00 (35/35) (90.00 - 100.00)
HIV-1 Viral Isolates [d]	53	53	53 (100.00)	53 (100.00)	100.00 (53/53) (93.28 - 100.00)
Subtotal	**1,336**	**1,336**	**1,336 (100.00)**	**1,336 (100.00)**	**100.00 (1336/1336) (99.72 - 100.00)**
Individuals at Increased Risk of HIV-1/2 Infection [e]	605	21	23 (3.80)	21 (91.30)	NA [i]
Individuals at Increased Risk of HIV Infection from HIV-2 Endemic Areas [f]	535	49 [g]	61 [h] (11.40)	49 (80.33)	100.00 (49/49) (92.75 - 100.00)
Total	**2,476**	**1,406**	**1,420 (57.35)**	**1,406 (99.01)**	**100.00 (1406/1406) (99.74 - 100.00)**

N = number tested; RR = Repeatedly Reactive

[a] Specimens were confirmed positive for HIV-1 antibody by HIV-1 Western blot. The preselected anti-HIV-1 positive category included 488 specimens from individuals with stage 1 HIV infection, 427 specimens from individuals with stage 2 HIV infection and 101 specimens from individuals with stage 3 HIV infection.

[b] The preselected anti-HIV-2 positive specimens were confirmed positive for HIV-2 antibody by HIV-2 Western blot and differentiated by a rapid enzyme immunoassay that differentiates HIV-1 and HIV-2.

[c] All 35 were HIV-1 p24 antigen positive; 32 specimens were Western blot negative, and 3 were Western blot indeterminate.

[d] 53 unique viral isolates that were propagated in cell culture and classified as HIV-1 group M (subtypes A, B, C, D, F, G, H, J, CRF01, CRF02, CRF06 and URFs), HIV-1 group N, HIV-1 group O, and HIV-1 group P.

[e] The following risk factors were included: diagnosed or treated for a sexually transmitted disease, heterosexual contact with a high-risk individual, heterosexual

contact with an infected individual, history of incarceration, intravenous drug user, men who have sex with men, multiple sex partners, and sexual contact with HIV infected individual.

[f] The following risk factors were included: intravenous drug user, multiple sex partners, and unprotected sex with an HIV infected individual. Individuals from HIV-2 endemic areas included specimens from the following areas: Ivory Coast (285) and Sierra Leone (250).

[g] The 49 specimens that were positive by supplemental testing included 32 anti-HIV-1 positive specimens, 2 anti-HIV-2 positive specimens, 6 anti-HIV-2 positive with anti-HIV-1 cross-reactivity specimens, and 9 undifferentiated anti-HIV positive specimens.

[h] Of the 61 repeatedly reactive specimens, 49 were positive, 10 were indeterminate and 2 were negative by supplemental testing.

[i] The sensitivity calculation and/or confidence interval are not meaningful due to the small number of specimens.

Reproducibility Studies

Reproducibility of the Alinity s HIV Ag/Ab Combo assay was evaluated at three sites with one instrument per site using three lots each of Alinity s HIV Ag/Ab Combo Reagent Kit, Calibrator Kit, Assay Control Kit, and Release Control Kit per CLSI EP15-A2. The panels were tested twice a day for 5 days in replicates of 4 at 3 sites using 3 lots each to obtain 360 replicates for each sample (i.e., 360 = 2 runs/day × 5 days × 4 replicates × 3 sites × 3 lots). The testing was conducted for 5 nonconsecutive days with a minimum of one break of at least 1 day. The unique panel members included:

- Low antibody panels (HIV-1 Group M, HIV-2, HIV-1 Group O) with target S/CO 1.50 to 2.00
- Low HIV-1 p24 Antigen with target S/CO 1.50 to 2.00
- High antibody panels (HIV-1 Group M, HIV-2) with target S/CO 8.00 to 10.00
- High HIV-1 p24 Antigen with target S/CO 8.00 to 10.00
- Negative Control with target S/CO $\leq$ 0.61
- Positive Control 1 with target S/CO 1.00 to 7.5
- Positive Control 2 and 3 with target S/CO 1.29 to 6.60
- Positive Control 4 with target S/CO 1.16 to 5.9

Low positive and high positive panel members were prepared by spiking negative recalcified human plasma with human-sourced material reactive for anti-HIV-1 group M, anti-HIV-2, or anti-HIV-1 group O or with HIV-1 p24 antigen viral lysate. There was 100% agreement observed in all seven panel members (Table 12).

Table 12: Alinity s HIV Ag/Ab Combo Assay 5-Day Reproducibility Agreement Results

Sample		N	≥ 1.00 S/CO	< 1.00 S/CO
			Agreement (%) (95% CI)	Agreement (%) (95% CI)
Low HIV-1 Group M Antibody		360	100.0 (360 / 360) (99.0 - 100.0)	NA
High HIV-1 Group M Antibody		360	100.0 (360 / 360) (99.0 - 100.0)	NA
Low HIV-2 Antibody		360	100.0 (360 / 360) (99.0 - 100.0)	NA
High HIV-2 Antibody		360	100.0 (360 / 360) (99.0 - 100.0)	NA
Low HIV-1 Group O Antibody		360	100.0 (360 / 360) (99.0 - 100.0)	NA
Low HIV-1 p24 Antigen		360	100.0 (360 / 360) (99.0 - 100.0)	NA
High HIV-1 p24 Antigen		360	100.0 (360 / 360) (99.0 - 100.0)	NA
Positive Control 1		359 [a]	100.0 (359 / 359) (99.0 - 100.0)	NA
Positive Control 2		360	100.0 (360 / 360) (99.0 - 100.0)	NA
Positive Control 3		360	100.0 (360 / 360) (99.0 - 100.0)	NA
Positive Control 4		360	100.0 (360 / 360) (99.0 - 100.0)	NA
Negative Control		360	NA	100.0 (360 / 360) (99.0 - 100.0)

CI = Confidence Interval

[a] One replicate was missing due to a wash zone aspiration failure

The within-run, between-run, between-day, within-laboratory, between-site, and between-lot variance components were determined based on CLSI EP15-A2. The results of the reproducibility panel and control testing demonstrated that the

Alinity s HIV Ag/Ab Combo assay was reproducible across three sites and three lots of reagents across a range of reactivity (Table 13).

Table 13: Alinity s HIV Ag/Ab Combo Assay Variance Components Analysis Results

Sample	N	Mean S/CO	Within-Run		Between-Run		Between-Day		Within-Laboratory [a]		Between-Site		Between-Lot		Reproducibility [b]	
			SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
Low HIV-1 Group M Antibody	360	1.69	0.060	3.5	0.029	1.7	0.024	1.4	0.071	4.2	0.089	5.2	0.057	3.3	0.127	7.5
High HIV-1 Group M Antibody	360	8.92	0.305	3.4	0.107	1.2	0.000	0.0	0.323	3.6	0.498	5.6	0.320	3.6	0.676	7.6
Low HIV-2 Antibody	360	1.71	0.058	3.4	0.000	0.0	0.024	1.4	0.063	3.7	0.086	5.1	0.162	9.5	0.194	11.4
High HIV-2 Antibody	360	9.07	0.300	3.3	0.000	0.0	0.118	1.3	0.322	3.5	0.442	4.9	0.808	8.9	0.976	10.8
Low HIV-1 Group O Antibody	360	1.64	0.055	3.4	0.002	0.2	0.030	1.8	0.063	3.8	0.063	3.8	0.178	10.9	0.199	12.2
Low HIV-1 p24 Antigen	360	1.76	0.047	2.7	0.000	0.0	0.012	0.7	0.049	2.8	0.035	2.0	0.011	0.6	0.061	3.5
High HIV-1 p24 Antigen	360	8.89	0.222	2.5	0.084	0.9	0.000	0.0	0.238	2.7	0.195	2.2	0.042	0.5	0.311	3.5
Positive Control 1	359[c]	2.99	0.103	3.4	0.025	0.8	0.028	0.9	0.109	3.6	0.118	3.9	0.127	4.3	0.205	6.8
Positive Control 2	360	2.30	0.071	3.1	0.000	0.0	0.026	1.2	0.076	3.3	0.076	3.3	0.219	9.5	0.244	10.6
Positive Control 3	360	2.72	0.077	2.8	0.020	0.7	0.016	0.6	0.081	3.0	0.025	0.9	0.030	1.1	0.090	3.3
Positive Control 4	360	1.92	0.076	3.9	0.000	0.0	0.014	0.7	0.077	4.0	0.045	2.3	0.183	9.6	0.204	10.6
Negative Control	360	0.08	0.013	NA	0.003	NA	0.000	NA	0.014	NA	0.007	NA	0.008	NA	0.018	NA

N = number of replicates; NA = not applicable; CV = coefficient of variation expressed as a percentage (CVs are not meaningful when S/CO approaches zero); SD = standard deviation

[a] Includes within-run, between-run, and between-day variability.

[b] Includes within-run, between-run, between-day, between-site, between-lot and the site-lot interaction variability.

[c] One replicate was missing due to a wash zone aspiration failure.

Review Issues: The clinical studies submitted in the original BLA were completed using software version 1.2.0. Due to several changes in software versions that are described in the software and instrumentation section, smaller in-house studies to confirm the clinical sensitivity and specificity were requested to help determine if the upgrade to software version 2.5.0 had an effect on the previously evaluated performance of the assays.

In-House Specificity Study Comparing Software Versions

An in-house specificity study using (b) (4) blood donor specimens obtained from specimen vendors (b) (4) serum specimens and (b) (4) plasma specimens, nonreactive for anti-HIV-1, anti-HIV-2, and anti-HIV-1 Group O, and nonreactive by HIV-1 Group M, HIV-2, HIV-1 Group O nucleic acid testing) was performed on (b) (4) Alinity s Systems with (b) (4) lot each of reagent kits, calibrators, and controls. The samples were tested on both the new (2.5.0) and the previous (1.2.0) software versions. The % agreement between the two software versions was (b) (4) One serum specimen and one

plasma specimen were repeatedly reactive with both software versions, and the remaining (b) (4) specimens were nonreactive with both software versions. The Alinity s System software versions 1.2.0 and 2.5.0 demonstrated equivalent performance when used with the Alinity s HIV Ag/Ab Combo to test blood donor specimens.

In-House Sensitivity Study Comparing Software Versions
An in-house sensitivity study was performed in which (b) (4) sensitivity samples (b) (4) positive samples from the clinical study including: (b) (4) HIV-1, (b) (4) HIV-2, and (b) (4) HIV-1 Group O dilution samples, (b) (4) HIV-1 panel members from seroconversion panel PRB (b) (4) the (b) (4) -member CBER HIV-1 Panel (b) (4), and (b) (4) -member CBER HIV-2 Panel (b) (4) were tested on both on software versions 1.2.0 and 2.5.0 to allow side-by-side comparison of results for each specimen. The study was performed on (b) (4) Alinity s Systems with (b) (4) each of reagent kits, calibrators, and controls. For all samples except one, there was no qualitative difference in the final interpretation between software versions. (b) (4) dilution panel member with S/CO values near the cutoff was discordant but the results fell into the zone of equivalence. The Alinity s System software versions 1.2.0 and 2.5.0 demonstrated equivalent performance when used with the Alinity s HIV Ag/Ab Combo assays to test sensitivity samples.

BIMO – Clinical/Statistical/Pharmacovigilance
Bioresearch Monitoring (BIMO) clinical investigator inspections were conducted at two domestic clinical study sites participating in the conduct of Study Protocol 9DY-02-14U01-03. The inspections did not reveal substantive problems that impact the data submitted in the application.

a) Pediatrics
N/A

b) Other Special Populations
N/A

7. Advisory Committee Meeting

N/A

8. Other Relevant Regulatory Issues

N/A

9. Labeling

The Advertising and Promotional Labeling Branch (APLB) found the proposed Instructions for Use (IFU), and the package and container labeling, acceptable from a promotional and comprehension perspective.

10. Recommendations and Risk/ Benefit Assessment

a) Recommended Regulatory Action

The Review Committee reviewed the original submission and related Amendments. All review issues have been resolved; therefore, the Review Committee recommends licensure of the Alinity s HIV Ag/Ab Combo assay.

b) Risk/Benefit Assessment

The risk/benefit analysis demonstrates that the benefit of the Alinity s HIV Ag/Ab Combo assay outweighs any risk to the blood donor and the safety of the nation's blood supply. The clinical studies demonstrate a sensitivity of 100% (95% CI of 99.72% - 100.00%), indicating a low probability of a false negative result. Among 16,994 blood and plasmapheresis donors tested with the Alinity s HIV Ag/Ab Combo assay, the assay specificity of 99.92% (95% CI of 99.87-99.96%) in clinical trials suggests a low probability of a false positive result. The Alinity s HIV Ag/Ab Combo assays demonstrate better performance to the currently licensed Abbott PRISM HIV O Plus assay. This is due to design difference among the two assays, Alinity s HIV Ag/Ab Combo assay is designed to detect HIV-1 p24 antigen whereas the ABBOTT PRISM HIV O Plus assay detects antibody only. When licensed, this will be the first FDA approved antigen-antibody combo donor screening assay that detects both p24 antigen and antibodies to HIV-1 groups M and O, and HIV-2.

c) Recommendation for Postmarketing Activities

No postmarketing activities have been proposed for this application