Summary Basis for Regulatory Action

Date: July 18, 2019

From: Alain Debrabant, PhD, Chair of the Review Committee

BLA/ STN#: 125681.0

Applicant Name: ABBOTT GMBH & CO. KG

Date of Submission: May 31, 2018

Complete Response Letter: March 20, 2019

Resubmission: June 7, 2019

MDUFA Goal Date: August 7, 2019

Proprietary Name: Alinity s Anti-HBc

Established Name (common or usual name): Hepatitis B Virus Core Antigen (*E coli*, Recombinant)

Intended Use/Indications for Use: The Alinity s Anti-HBc assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of antibody to hepatitis B core antigen (anti-HBc) in human serum and plasma specimens on the Alinity s System. The Alinity s Anti-HBc 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-HBc. 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.**

Table 1 below indicates the material reviewed when developing the SBRA.

Table 1: Reviews Submitted

Document Title	Reviewer Name	Document Date
Product Review(s) (DETTD) • *Clinical* • *Non-Clinical*	 Babita Mahajan Robert Duncan Krishnakumar Devadas Susan Zullo Erica Silberstein Krishnakumar Devadas	 Jul 12, 2019 Jul 10, 2019 July 7, 2019 Jul 12, 2019 Jul 22, 2019 July 7, 2019
Statistical Review(s) • *Clinical* • *Non-Clinical*	 Tie-Hua Ng Zhen Jiang	 Mar 7, 2019 Jun 26, 2019
CMC Review • *CMC (DETTD)* • *Facilities Review (OCBQ/DMPQ)* • *Microbiology Review (OCBQ/DBSQC)* • *Establishment Inspection Report(s) (OCBQ/DMPQ)*	 Susan Zullo Robert Duncan Erica Silberstein Nicole Li Karla Garcia Nicole Li	 Jul 12, 2019 Jul 10, 2019 Jul 22, 2019 Feb 25, 2019 Nov 15, 2018 Jan 30, 2019
Labeling Review(s) • *Product Office* • *APLB (OCBQ/APLB)*	 Alain Debrabant Dana Jones	 Jul 23, 2019 May 14, 2018
Lot Release Protocols/Testing Plans	Varsha Garnepudi Kori Francis Karen Smith Swati Verma	Jul 12, 2019 Jul 23, 2019 Jul 23, 2019 Jul 16, 2019
Bioresearch Monitoring Review	Colonious King	July 3, 2019
Software and Instrumentation Review	Lisa Simone	Jul 17, 2019
Tissues and Advanced Therapies (OTAT)	Brychan Clark	Jul 16, 2019

1. Introduction

The Alinity s Anti HBc assay is manufactured at the Abbott GmbH & Co. KG facility, located in Wiesbaden, Germany. This biologics license application (BLA) for Alinity s Anti-HBc from Abbott Laboratories was received on June 1, 2018. The BLA was preceded by investigational new drug application (IND) 17643 received on August 3, 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 IND 17643 submission to discuss issues related to the IND. The BLA was submitted on June 1, 2018.

Table 2: Chronological Summary of Submission and FDA Correspondence

Date	Action	Amendment to BL 125681
Jun 1, 2018	BLA CBER Receipt	
Jun 11, 2018	Acknowledgment Letter	
Jul 23, 2018	Filing Notification Letter	
Aug 8, 2018	Information Request – DMPQ, DMPQ, Instrument and Software	
Aug 22, 2018	Sponsor response to August 8, 2018 information request	/0/1
Oct 30, 2018	Information Request - DMPQ	
Nov 8, 2018	Information Request – Preclinical, Clinical	
Nov 14, 2018	Sponsor response to October 30, 2018 information request	/0/2
Nov 16, 2018	Sponsor response to November 8, 2018 information request	/0/3
Dec 10, 2018	Advice Letter – Recommendation on precision, sensitivity and specificity additional studies	
Jan 15, 2019	Draft Meeting Minutes for Lot Release Protocol teleconference held on January 10, 2019	/0/4
Jan 18, 2019	Information Request – Software, preclinical, clinical, CMC, Cadaveric, Lot Release Protocol	
Jan 29, 2019	Advice Letter – Clarification on January 18, 2019 information request	
Feb 19, 2019	Sponsor response to Jan 18, 2019 information request	/0/5
Mar 12, 2019	Sponsor update to instrument and software activities	/0/6
Mar 20, 2019	Complete Response Letter	
Mar 22, 2019	Advice Letter – Clarification on complete response	
Jun 7, 2019	Sponsor response to March 20, 2019 Complete Response Letter	/0/7
Jun 18, 2019	Complete Response Classification Letter	
Jun 28, 2019	Information Request – Cadaveric claim, Labelling	
July 15, 2019	Sponsor response to June 28, 2019 information request including final labeling	/0/8

2. Background

Hepatitis B virus (HBV) is the causative agent of hepatitis B that is transmitted through exposure to infected blood, semen, and other body fluids through parenteral, sexual, and perinatal routes. Transmission may also occur through transfusion of HBV-contaminated blood and blood products. After infection with HBV, hepatitis B surface antigen (HBsAg) is the first antigenic marker that appears 1-12 weeks after exposure and 2-6 weeks before the onset of clinical symptoms. Antibody to the hepatitis B core antigen (anti-HBc) appears in the serum one to two weeks after the appearance of HBsAg. Because it generally remains detectable for the remainder of a patient's life, anti-HBc is an indicator of current (acute or chronic) or past infection. Anti-HBc assays are used to screen the donors of blood and blood products to prevent transmission of HBV infection to recipients of blood or blood products. Anti-HBc assays are also used to screen organ and tissue donors. The Alinity s Anti-HBc assay using CMIA technology is performed on a fully automated Alinity s System. This assay is for the qualitative detection of Anti-HBc in human serum and plasma. Once the samples are loaded on the Alinity s System, all the reaction steps are performed by the system. Any sample that is identified as initially reactive is tested in duplicate by the system.

3. Chemistry Manufacturing and Controls (CMC)

The manufacture of the Alinity s Anti-HBc assay is performed in accordance with Current Good Manufacturing Practices (cGMP) in an environmentally controlled facility.

a) Manufacturing Summary

The Alinity s Anti HBc assay is manufactured at the Abbott GmbH & Co. KG facility, located in Wiesbaden, Germany.

The Alinity s Anti-HBc Reagent Kit consists of the following components:

- Hepatitis B core (*E coli*, recombinant) antigen coated microparticles
- Murine anti-human IgG and IgM acridinium-labeled conjugates
- Assay Diluent
- Specimen Diluent

The Alinity s Anti-HBc Calibrator Kit consists of the following components:

- Calibrator 1 (recalcified, human plasma reactive for anti-HBc and anti-HBs)

The Alinity s Anti-HBc Assay Control Kit consists of the following components:

- Negative Control (negative recalcified human plasma)
- Positive Control (recalcified, human plasma reactive for anti-HBc and anti-HBs)

The Alinity s Anti-HBc Release Control Kit consists of the following component:

- Release Control (recalcified, human plasma reactive for anti-HBc and anti-HBs)

The Alinity s System Bulk Solutions listed below are not part of any Alinity s assay

kits, but are required to run the Alinity s assays 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 Alinity s Anti-HBc assay components 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 Anti-HBc assay is listed in the 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 Table for Alinity s Anti-HBc assays

Name/Address	FEI number	DUNS number	Inspection/ waiver	Justification /Results
Device Component Manufacturing, Finished Device Manufacturing, Instrument Solution Manufacture, Device Packaging/Labeling, QC and Release Testing Abbott GmbH & Co. KG, Max-Plank-Ring 2, Wiesbaden, Germany 65205	3002809144	315786293	Waived	DMPQ August 30 – 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 Anti-HBc assay was waived based on the favorable outcome of the aforementioned inspection.

e) Container Closure System
N/A

f) 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.

Review Issues and Resolution:

During the review of the CMC information, several issues regarding the characterization of the recombinant antigens, manufacture of the coated microparticles and conjugates, transport studies and kit expiration dating / stability claims were identified. Abbott has addressed these issues satisfactorily.

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.

a) Versioning
System Software v2.5.0. Assay Files Anti-HBc (List Number 06P06) version 140_002 .

b) 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.

c) Risk Management

The final risk profile of the Alinity s System includes 0 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.

d) 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.

e) 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 Anti-HBc 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.

f) 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:

i. 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 previously-collected preclinical and clinical data to support this submission.

ii. 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.

iii. 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.

iv. 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 Anti-HBc assay. The analytical studies were conducted in compliance with 21 CFR Part 58 (Good Laboratory Practices or GLPs), as applicable. All assay results were evaluated using the validity criteria for runs and specimens outlined in the package insert.

Sample Handling and Collection

a) Tube Type Equivalency and Matched Serum and Plasma

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. Depending on the type of tube a minimum of (b) (4) nonreactive and (b) (4) Anti-HBc spiked reactive samples were tested in (b) (4) using the Alinity s Anti-HBc assay. 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: In addition, anti-HBc positive specimens from (b) (4) individual donor sets were tested with the Alinity s Anti-HBc assay. The data provided and reviewed demonstrate acceptable performance of the assay supporting the use of serum specimens or plasma specimens.

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-HBc spiked reactive samples for each sample type were evaluated using the Alinity s Anti-HBc assay. For both reactive and nonreactive samples, the data provided and reviewed demonstrate acceptable performance of the assays supporting the use of serum and

plasma specimens that have been stored at 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 samples for each sample type and each storage condition were evaluated. The data provided and reviewed demonstrate acceptable performance of the Alinity s Anti-HBc assay supporting the use of 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) Anti-HBc spiked reactive samples for each interferent were evaluated with a minimum of (b) (4) replicates using the Alinity s Anti-HBc assay. 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 and positive control were spiked with biotin to a concentration of 4,250 ng/mL. No interference was observed using the Alinity s Anti-HBc 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. (b) (4) specimens for each interferent were used. Nonreactive and Anti-HBc spiked reactive samples with naturally occurring elevated levels of each interferent were compared to specimens with normal levels of each. The samples were tested using the Alinity s Anti-HBc assay. The data provided and reviewed demonstrate acceptable performance of the assays 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 = 207) unrelated to hepatitis B infection was evaluated (Table 4).

Table 4: Alinity s Anti-HBc Other Disease States (Analytical Specificity) Summary

Other Disease States or Specimen Conditions	Alinity s Anti-HBc				(b) (4)		
	Total	IR	RR	Confirmed	Total	IR	RR
Anti-HIV-1/HIV-2 Positive	10	0	0	0	10	0	0
Anti-HTLV I/II Positive	10	0	0	0	10	0	0
Anti-HCV Positive	10	4	4	3	10	4	4
Anti-HAV Positive	10	3	3	3	10	3	3
Co-infected CMV/EBV/HSV	10	1	1	1	10	1	1
Anti-HDV Positive	9	8	8	8	9	9	9
Anti-*T. pallidum* Positive	10	2	2	2	10	2	2
Non-viral Hepatitis	10	0	0	0	10	0	0
Rheumatoid Factor Positive	10	0	0	0	10	0	0
Anti-ds DNA Positive	10	0	0	0	10	0	0
Pregnant Females	14	0	0	0	14	0	0
Multiparous Females	10	0	0	0	10	0	0
Hyper IgG/IgM	9	3	3	3	6	3	3
Influenza Vaccine Recipient	10	0	0	0	10	0	0
Hemodialysis Patients	10	0	0	0	10	0	0
HAMA positive	10	1	1	1	10	1	1
Escherichia coli Infection	10	2	2	2	10	2	2
Heterophilic Antibody Positive	25	7	7	6	23	6	6
Fungal (Yeast) Infection	10	0	0	0	10	0	0
Total	**207**	**31**	**31**	**29**	**202[a]**	**31**	**31**

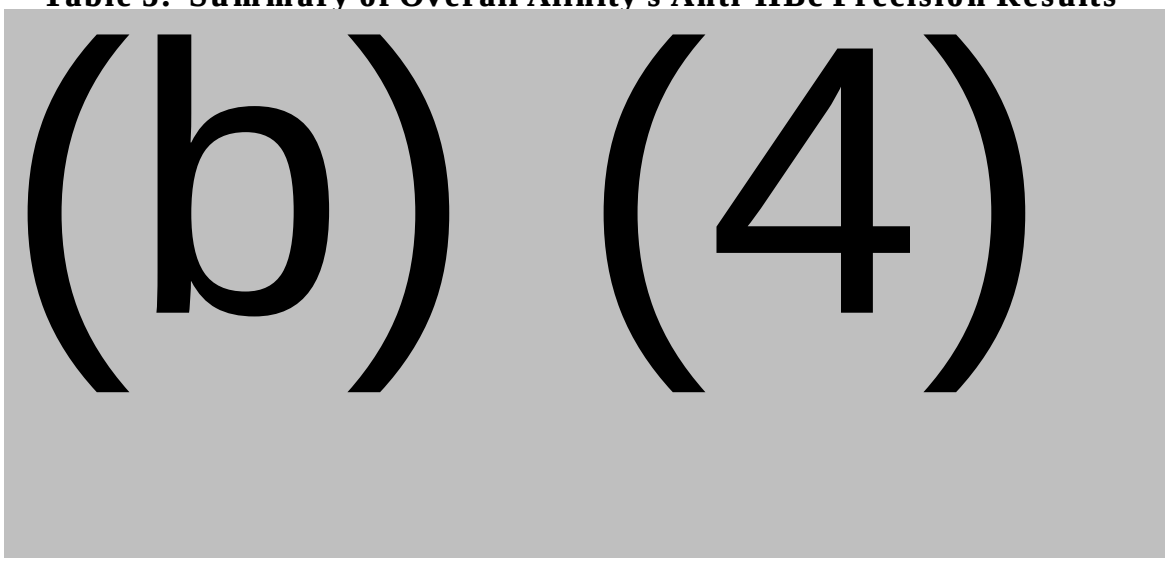
[a] Results were not obtained for 5 specimens due to instrument errors

Each specimen was tested (b) (4) using the Alinity s Anti-HBc assay and with the (b) (4) assay. The initial reactive (IR) rate and repeat reactive (RR) rate of the Alinity s Anti-HBc were 14.98% (31/207). The 31 repeatedly reactive specimens were repeatedly reactive using the (b) (4) assay, and 29 specimens were confirmed positive by additional testing. Additional testing included the following tests, (b) (4) HBsAg/HBsAg Confirmatory assay, (b) (4) assay, (b) (4) assay, (b) (4) assay, and the (b) (4) Anti-HBc assay. One anti-HCV positive specimen and 1 heterophilic antibody positive specimen were repeatedly reactive with the Alinity s Anti-HBc assay and negative by additional testing. The anti-HDV results were expected since HDV requires HBV for replication.

b) Precision

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 table 5. The Alinity s Anti-HBc assay using Alinity s System software version 2.5.0 demonstrated acceptable precision.

Table 5: Summary of Overall Alinity s Anti-HBc Precision Results



Review Issues and Resolution:

i. 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 (b) (4) were used in this study for the Alinity s Anti-HBc. As each of the four lanes on one system has its own independent sets 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 20, 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 June 7, 2019 (Abbott Amendment 5) with acceptable variances among the instruments, and the issue was resolved.

c) In-House Specificity (Donors)

The specificity of the Alinity s Anti-HBc 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 Anti-HBc assay was (b) (4) (lower one-sided 95% confidence limit of (b) (4) .

d) Analytical Sensitivity

The analytical sensitivity of the Alinity s Anti-HBc assay was evaluated using the WHO 1st International Standard for hepatitis B core antigen (anti-HBc) NIBSC

Code: 95/522. The standard was diluted to target concentrations between 0.25 and 2.50 IU/mL and tested in a minimum of (b) (4) replicates using 3 Alinity s Anti-HBc Reagent Kit lots. The analytical sensitivity of the Alinity s Anti-HBc assay ranged from 0.53 IU/mL to 0.56 IU/mL.

e) Dilution Sensitivity

The dilution sensitivity of the Alinity s Anti-HBc assay compared to the (b) (4) was evaluated. A total of (b) (4) anti-HBc reactive specimens were serial diluted with recalcified nonreactive human plasma to create samples with final dilution factors ranging from (b) (4). A total of (b) (4) dilutions were tested in a minimum of (b) (4) replicates using both the Alinity s Anti-HBc and (b) (4) assays. For 5 of the (b) (4) anti HBc positive specimens, the Alinity s Anti-HBc assay detected the same dilutions as the (b) (4) assay. For the remaining (b) (4) anti-HBc positive specimens, the (b) (4) assay detected one additional dilution not detected by the Alinity s Anti-HBc assay. Of the (b) (4) total dilutions, (b) (4) were reactive by the Alinity s Anti-HBc assay, and (b) (4) were reactive by the (b) (4) assay.

f) Seroconversion

The seroconversion detection of the Alinity s Anti-HBc was compared to the (b) (4) assay. Ten seroconversion panels obtained from commercial vendors were tested using the 2 assays. The total number of specimens reactive by the Alinity s Anti-HBc assay was greater than the number of specimens reactive by the (b) (4) assay. For 8 of 10 panels, the first reactive time point for the Alinity s Anti-HBc assay occurred at the same time as the first reactive time point for the (b) (4) assay. The remaining 2 panels showed discordant interpretations between the two assays: for 1 panel, the Alinity s Anti-HBc assay detected anti-HBc antibodies 7 days earlier than the (b) (4) assay; for 1 panel the (b) (4) assay detected anti-HBV antibodies 7 days earlier than the Alinity s Anti-HBc assay.

g) Reagent Onboard Stability and Calibration Storage

Assay performance when reagents are stored on board the Alinity s System, and the acceptability of a calibration generated using the Alinity s Anti-HBc 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 Anti HBc Reagent kit was used to generate a calibration on Day 0 and stored on board the Alinity s System. The Anti-HBc panel prepared by diluting an anti-HBc positive specimen to an S/CO value of (b) (4), Negative Control, Positive Control, and Release Control tested at each time point (test conditions) were compared to the same samples tested at Day 0 (control condition) with a minimum of (b) (4) replicates for (b) (4) time points over a period of (b) (4) days. The data provided and reviewed support the use of Alinity s Anti-HBc Reagent Kits that have been stored on board the Alinity s System for 15 days, and the use of calibration generated using the Alinity s Anti-HBc assay and stored on the Alinity s System for up to 14 days.

h) Specimen Onboard Stability (Primary Tube)

The performance of the Alinity s Anti-HBc 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) anti-HBc spiked reactive samples for each sample type (serum and plasma (b) (4) were tested with a minimum of (b) (4) replicates using the Alinity s Anti-HBc assay. 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.

i) Specimen Onboard Stability (Sample Cup)

The performance of the Alinity s Anti-HBc assay when used to test serum and plasma specimens stored onboard the Alinity s System in sample cups was evaluated. The Alinity s Anti-HBc Negative Control and Positive Control 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 a minimum of 10 sample cups for each timepoint and tested (b) (4) using the Alinity s Anti-HBc 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.

j) 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-HBc positive spiked sample were used for the study. The following assays were used as potentially contaminating assays to the Alinity s Anti-HBc: (b) (4) . The results demonstrated that the reagent (b) (4) are effective in controlling reagent cross contamination from a potentially contaminating Alinity s assay to the Alinity s Anti-HBc assay.

k) Within-Assay Carryover

The performance of the Alinity s Anti-HBc assay when exposed to potential within-assay sample carryover from a sample with high levels of Anti-HBc (b) (4) was evaluated by comparing the results of a protected negative sample to an unprotected negative sample. The protected negative sample was tested before a high positive sample, and the unprotected negative sample was tested after the high positive sample. The high positive sample was pipetted for each assay before the sample probe was cleaned to simulate a worst-case scenario for sample carryover. A total of (b) (4) iterations of alternating contaminating assay and susceptible assay were performed. The results demonstrated that no within-assay sample carryover was observed with the Alinity s Anti-HBc 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 Anti-HBc Reagent Kit, Calibrator Kit, Assay Control Kit, Release Control Kit. The data support expiration dating (shelf life) of 12 months for all kits. In addition, studies for the following stability conditions were also provided: (b) (4) lot of each assay component stored (b) (4) to cause (b) (4) between the product and the container closure), (b) (4) lot of calibrators, assay controls, and release control 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 Anti-HBc Reagent Kit, Calibrator Kit, Assay Control Kit, and Release Control Kit. All criteria were met. The data support ambient shipping of the Alinity s Anti-HBc Reagent Kit, Calibrator Kit, Assay Control Kit, and Release Control Kit.

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 Anti-HBc 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 with the exception of the specimen diluent. Results for the specimen diluent were bactericidal, and neither fungicidal nor fungistatic. A (b) (4) log increase in microbial counts were measured between Day (b) (4) and Day (b) (4) for (b) (4) when the bioburden test was done at the expiration date of the component.

b) Microbial Interference

The performance of the Alinity s Anti-HBc 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) 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.

Microbial Challenge Conclusion: The combined results of the antimicrobial effectiveness and microbial interference studies show that all Alinity s Anti-HBc 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. Assessments for plasma dilution and hemolysis were made prior to initiating the studies.

a) Cadaveric Reproducibility

Assay reproducibility when used to test cadaveric serum specimens was evaluated. A total of 23 cadaveric and 22 living donor serum specimens were tested (Table 6). The duration between the time of death and time of draw ranged from (b) (4) hour, (b) (4) minutes to 14 hours, 34 minutes. Both random living donor serum samples and cadaveric serum samples were spiked with (b) (4) different unique sources of anti-HBc (b) (4) to create reactive samples. Samples were tested once daily for 6 days using 3 Alinity s Anti-HBc Reagent Kit lots for a total of 6 runs (n=18 total replicates per sample). The total %CV (coefficient of variation expressed as a percentage) of 9.2 for the test cadaveric serum samples was less than the %CV of 9.0 for the living donor serum samples demonstrating acceptable reproducibility of the Alinity s Anti-HBc. Since the cadaveric total %CV result was greater than the living donor total %CV result, the lower limit of the 95% CI around the SD (standard deviation) ratio was evaluated. Because the lower limit of the 95% CI around the SD ratio was (b) (4), the cadaveric total %CV result was not considered statistically greater than the living donor total %CV result. Two acceptance criteria were met demonstrating that the cadaveric reproducibility results were acceptable: 1) the cadaveric total %CV result was only slightly greater than the living donor total %CV result, but the lower limit of the 95% CI around the SD ratio was (b) (4); and 2) the cadaveric total %CV and the living donor total %CV were both less than or equal to (b) (4).

Table 6: Alinity s Anti-HBc Cadaveric Reproducibility

Specimen Category	Number of Replicates	Mean S/CO	Total [a] SD	Total %CV
Cadaveric [b]	414	3.63	0.334	9.2
Living Donor	396	3.62	0.324	9.0

CV = coefficient of variation
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 14.6 hours after death.

b) Cadaveric Specificity

The specificity of the assay when used to test cadaveric serum specimens compared to living donor specimens was evaluated. Specificity was determined by testing 55 cadaveric serum specimens and 55 living donor serum specimens (Table 7). Each specimen was tested once using each of 3 lots of Alinity s Anti-HBc Reagent Kit. Two cadaveric donor specimens and one living donor specimen were repeatedly positive per supplemental testing and were excluded from the specificity calculation. Two additional living donor specimens were repeatedly reactive by the investigational method but had insufficient sample volume to complete all supplemental testing. This specimen was treated as a negative or false reactive and included in the specificity analysis. A total of 53 cadaveric and 54 living donor serum specimens were used for the specificity analysis (Table 7). 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 Anti-HBc Reagent Kit lots. Specificity was 100.0% (53/53) for all reagent lots for the cadaveric serum specimens with 95% confidence intervals (CI) of 93.28 to 100.00. Specificity was 96.30% (52/54) for all 3 reagent lots for the living donor serum specimens, with 95% confidence intervals for clinical specificity of 87.25 to 99.55

Table 7: Specificity in Cadaveric and Living Donors

Specimen Category	Lot	Nonreactive	Repeatedly Reactive	Specificity (%) (95% CI)
Cadaveric [a] (N=55)	Lot 1	53	2[b]	100.00 (93.28 – 100.00)
Cadaveric [a] (N=55)	Lot 2	53	2[b]	100.00 (93.28 – 100.00)
Cadaveric [a] (N=55)	Lot 3	53	2[b]	100.00 (93.28 – 100.00)
Living Donor (N=55)	Lot 1	52	3[c]	100.00 (87.25 – 99.55)
Living Donor (N=55)	Lot 2	52	3[c]	100.00 (87.25 – 99.55)
Living Donor (N=55)	Lot 3	52	3[c]	100.00 (87.25– 99.55)

CI = confidence interval
[a] Cadaveric serum specimens were collected up to 23.7 hours after death.
[b] Two cadaveric donor specimens were positive per supplemental testing; therefore, they were excluded from the specificity calculation.
[c] One living donor specimen was positive per supplemental testing; therefore, it was excluded from the specificity calculation. Two additional living donor specimens were repeatedly reactive by the investigational method but had insufficient sample volume to complete all supplemental test methods; therefore, were treated as negative for the purposes of the analysis and included in the specificity calculation.

c) Cadaveric Sensitivity

The analytical sensitivity of the Alinity s Anti-HBc 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) hour, (b) (4) minutes to 23 hours, (b) (4) minutes. Both random living donor serum samples and cadaveric serum samples were spiked with (b) (4) different anti-HBc (b) (4) to create reactive samples. Samples were tested once within 24 hours of spiking using 3 Alinity s Anti-HBc Reagent Kits. All samples were reactive. Sensitivity was 100.0% (52/52) for all reagent lots and both sample types with 95% confidence intervals of 93.15 to 100.00 (Table 8).

Table 8: Analytical Sensitivity in Cadaveric and Living Donors by Lot

Specimen Category	Lot	Nonreactive	Mean S/CO	Sensitivity (%) (95% CI)
Cadaveric [a] (N=52)	Lot 1	52	3.76	100.00 (93.15 – 100.00)
Cadaveric [a] (N=52)	Lot 2	52	3.97	100.00 (93.15 – 100.00)
Cadaveric [a] (N=52)	Lot 3	52	4.48	100.00 (93.15 – 100.00)
Living Donor (N=52)	Lot 1	52	3.74	100.00 (93.15 – 100.00)
Living Donor (N=52)	Lot 2	52	4.00	100.00 (93.15 – 100.00)
Living Donor (N=52)	Lot 3	52	4.44	100.00 (93.15 – 100.00)

CI = confidence interval

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

d) Cadaveric Specimen Storage

The performance of the Alinity s Anti-HBc 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 14 hours, 30 minutes for the cadaveric serum samples used for the -20°C or colder storage condition and (b) (4) hour, (b) (4) minutes to 10 hours, 0 minutes for the cadaveric serum samples used for other storage conditions. Random cadaveric serum specimens were spiked with (b) (4) different anti-HBc (b) (4) (b) (4) to create reactive samples. A minimum of twelve nonreactive and 12 spiked reactive samples were used. 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 Anti-HBc 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 and Resolution:

i. **Data exclusions and invalidation rates**: The pre-clinical evaluation report lacked information on data exclusions and invalidation rate observed during the pre-clinical studies. The line data of some studies did include line listing of excluded data. An information request was forwarded to Abbott on January 18, 2019, requesting information relevant to data exclusion, invalid rates and instrument flagged errors for each study listed under non-clinical study section. Abbott provided the line data, information on data exclusions and invalidation rate observed during the pre-clinical studies in Abbott Amendment 4, received on February 18, 2019. The issue was resolved

ii. **Interference by other disease states or conditions**: In this study, (b) (4) heterophilic antibody positive specimens were included and tested with the Alinity s Anti-HBc. Of these, 6 were repeatedly reactive with the Alinity s Anti-HBc and positive by additional testing. Of the (b) (4) remaining heterophilic antibody positive specimens (b) (4) were non-reactive with the Alinity s Anti-HBc and (b) (4) was reactive with the Alinity s Anti-HBc and negative by additional testing. To further evaluate the potential cross-reactivity of heterophilic antibodies with the assay, Abbott was requested on January 18, 2019 to test 10-20 additional heterophilic antibody positive specimens. In their response received on February 19, 2019, Abbott tested (b) (4) additional heterophilic antibody positive samples. None of these specimens were reactive with the Alinity s Anti HBc. Together, these results indicate that no interference to the Alinity s Anti-HBc was detected.

6. Clinical Studies

Clinical studies were conducted to evaluate assay specificity, sensitivity, and reproducibility to demonstrate performance and intended use of the Alinity s Anti-HBc assay. Testing was performed at four blood donor testing laboratories using specimens collected at three whole blood collection sites. A minimum of three lots each of the Alinity s Anti-HBc Reagent Kit, Alinity s Anti-HBc Calibrator Kit, Alinity s Anti-HBc Assay Control Kit, and Alinity s Anti-HBc Release Control Kit were used for the studies at testing sites. The FDA-licensed (b) (4) assay was used as the comparator test.

Clinical Specificity

A prospective multicenter study was conducted to evaluate the clinical specificity of the Alinity s Anti-HBc assay on the Alinity s System using a total of 15,877 whole blood specimens from three sites. Of these, 9,365 were fresh serum and 6,512 were fresh plasma. The testing was performed using the Alinity s Anti-HBc and the (b) (4) assays. Repeatedly reactive specimens were further tested for one or more additional markers: HBV qualitative DNA, HBsAg, anti-HBc IgM, anti-HBs, and anti-HBe. Based on additional test results for the 44 repeatedly reactive (RR) specimens with one or both assays, 28 were positive (9 blood donor serum and 19 blood donor plasma) and 16 were negative (11 blood donor serum and 5 blood donor plasma).

All 28 RR specimens found to be positive by additional testing were excluded from the specificity calculations. Specificity in blood donors was calculated to be 99.90% (15,833/15849) with a 95% confidence interval of 99.84% to 99.94% (Table 9).

Table 9: Alinity s Anti-HBc Clinical Study Assay Reactivity

Category	Number Tested	IR (% of Total) (95% CI)	RR (% of Total) (95% CI)	Number Confirmed Positive (% of RR)	Specificity (%)[a] (95% CI)
Volunteer Blood Donors - Serum	9,365	20 (0.21) (0.13 - 0.33)	20 (0.21) (0.13 - 0.33)	9 (45.00)	99.88 (9,345 / 9,356) (99.79 - 99.94)
Volunteer Blood Donors - Plasma	6,512	24 (0.37) (0.24 - 0.55)	24 (0.37) (0.24 - 0.55)	19 (79.17)	99.92 (6,488 / 6,493) (99.82 – 99.97)
Total Donors	**15,877**	**44 (0.28) (0.20 - 0.37)**	**44 (0.28) (0.20 - 0.37)**	**28 (63.64)**	**99.90 (15,833 / 15,849) (99.84- 99.94)**

IR = initially reactive; RR = repeatedly reactive; CI = confidence interval

[a] Based on additional test results for the 44 RR specimens, 28 specimens were positive (9 blood donor serum and 19 blood donor plasma) and 16 specimens were negative (11 blood donor serum and 5 blood donor plasma). All 28 RR specimens found to be positive by additional testing were excluded from the specificity calculations.

Clinical Sensitivity

Assay sensitivity was calculated by analyzing test results from frozen specimens provided by Abbott Laboratories to the testing sites. A total of 807 specimens were tested with the Alinity s Anti-HBc assay at three sites and with the (b) (4) assay at one site. The specimens were of the following categories:

- Preselected Anti-HBc Positive – Acute HBV Infection = 28
- Preselected Anti-HBc Positive – Chronic HBV Infection = 97
- Preselected Anti-HBc Positive – Recovered HBV Infection = 279
- Individuals at Increased Risk of HBV Infection = 403

Assay sensitivity was estimated to be 100% for preselected Anti-HBc positive specimens (404/404) with a 95% confidence interval of 99.09% to 100.00% (Table 10). Repeatedly reactive specimens from individuals at increased risk of HBV infection were further tested for one or more additional markers: HBV DNA, HBsAg, anti-HBc IgM, anti HBs, and anti-HBe. Of the 403 specimens at increased risk of HBV infection, 84 were repeatedly reactive with the Alinity s Anti-HBc. Of those 84, 70 were positive by additional testing (Table 10).

Table 10: Alinity s Anti-HBc Clinical Study Overall Sensitivity Summary

Category	Number Tested	Number Positive	Number RR (% of Total)	Number RR Positive by Supplemental Testing (% of RR)	Sensitivity (%) (95% CI)
Preselected Anti-HBc Positive – Acute HBV Infection[a]	28	28	28 (100.00)	28 (100.00)	100.00 (28/28) (87.66 – 100.00)
Preselected Anti-HBc Positive – Chronic HBV Infection[a]	97	97	97 (100.00)	97 (100.00)	100.00 (97/97) (96.27 – 100.00)
Preselected Anti-HBc Positive – Recovered HBV Infection[a]	279	279	279 (100.00)	279 (100.00)	100.00 (279/279) (98.69 – 100.00)
Subtotal	**404**	**404**	**404 (100.00)**	**404 (100.00)**	**100.00 (404/404) (99.09 – 100)**
Increased Risk of HBV Infection[b]	403	70	84[c] (20.84)	70 (83.33)	100.00 (70/70) (94.87 – 100.00)
Total	**807**	**474**	**488 (60.47)**	**474 (97.13)**	**100.00 (474/474) (99.22 – 100.00)**

RR = Repeatedly Reactive

[a] Preselected Anti-HBc positive specimens were previously identified as reactive by two FDA approved anti-HBc assays. Acute, chronic and recovered HBV classifications were determined using four HBV reference markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs).

[b] The following risk factors were included: current or past residence in a Hepatitis B endemic region, diagnosed or treated for a sexually transmitted disease, hemodialysis patient, heterosexual contact with a high-risk individual or an infected individual, history of incarceration, household contact with HBV infected individual, intravenous drug user, men who have sex with men, and multiple sex partners.

[c] Of the 14 specimens that were Alinity s Anti-HBc repeatedly reactive and negative by supplemental testing, 13 were also repeatedly reactive with a commercially available anti-HBc assay.

Reproducibility Studies

Reproducibility of the Alinity s Anti-HBc assay was evaluated at three sites with one instrument per site using three lots each of Alinity s Anti-HBc Reagent Kits, Calibrator Kits, Control Kits, and Release Control Kits per CLSI EP15-A2. The Low Anti-HBc panel (Target S/CO (b) (4)), High Anti-HBc panel (Target S/CO (b) (4)), Positive Control (Target S/CO 1.41 to 7.20), and Negative Control (Target S/CO $\leq$ 0.65), 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. Low and High Anti-HBc panel members were made by spiking recalcified human plasma with human-sourced material positive for Anti-HBc. There was 100% agreement observed in all four panel members. The within-run, between-run, between-day, within-laboratory, between-site, and between-lot variance components were determined based on CLSI EP15-A2. For Low Anti-HBc, High Anti-HBc, and Positive Control panel members the overall %CV were 7.4, 13.8, and 5.2%

respectively (Table 11). These data demonstrate Alinity s Anti-HBc assay reproducibility across three sites with three lots of reagents across a range of reactivity.

Table 11: Alinity s Anti-HBc 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 HBc Antibody	360	1.96	0.043	2.2	0.016	0.8	0.017	0.9	0.049	2.5	0.009	0.5	0.129	6.6	0.144	7.4
High HBc Antibody	360	9.93	0.222	2.2	0.000	0.0	0.079	0.8	0.235	2.4	0.051	0.5	1.339	13.5	1.374	13.8
Positive Control	360	2.86	0.063	2.2	0.015	0.5	0.032	1.1	0.072	2.5	0.000	0.0	0.118	4.1	0.148	5.2
Negative Control	360	0.10	0.006	NA	0.002	NA	0.000	NA	0.006	NA	0.004	NA	0.010	NA	0.013	NA

N = number of replicates; NA = not applicable; %CV = coefficient of variation expressed as a percentage; SD = standard deviation; %CVs are not meaningful when S/CO approaches zero
[a] Includes within-run, between-run, and between-day variability
[b] Includes within run, between-run, between-day, between-site, between-lot, and site-lot interaction variability

Review Issues and Resolution:

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.

i. **In-House Specificity Study Comparing Software Versions.** An in-house specificity study using (b) (4) blood donor specimens obtained from vendors ((b) (4) serum specimens and (b) (4) plasma specimens nonreactive for anti-HBc and nonreactive by HBV (b) (4)) was performed on (b) (4) Alinity s Systems with (b) (4) each of Alinity s Anti-HBc 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) The (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 Anti-HBc assay to test blood donor specimens. Abbott's response was acceptable, and the issue was satisfactorily resolved.

ii. **In-House Sensitivity Study Comparing Software Versions.** An in-house sensitivity study was performed in which (b) (4) sensitivity samples were tested on (b) (4) Alinity s Systems with (b) (4) each of Alinity s Anti-HBc reagent kits, calibrators, and controls. The samples were tested on both the new (version 2.5.0) and the previous (version 1.2.0) software versions to allow side-by-side comparison of results for each specimen. The (b) (4) sensitivity samples included (b) (4) positive samples from the clinical study, (b) (4) Anti-HBc panel members from

seroconversion panel HBV(b) (4), and (b) (4)-member CBER HBc Reference Panel (b) (4). For all samples, there was no qualitative difference in the final interpretation between software versions. The Alinity s System software versions 1.2.0 and 2.5.0 demonstrated equivalent performance when used with the Alinity s Anti-HBc assay to test sensitivity samples. Abbott's response was acceptable, and the issue was satisfactorily resolved.

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.

Pediatrics
N/A

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 Anti-HBc assay.

b) Risk/Benefit Assessment
The benefit/risk analysis demonstrates that the benefit of the Alinity s Anti-HBc 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 99.09% -

100.00%) with preselected anti-HBc positive specimens, indicating a low probability of a false negative result. Among 15,877 blood donors tested with the Alinity s Anti-HBc assay, the assay specificity of 99.90% (95% CI of 99.84-99.94%) in clinical trials suggests a low probability of a false positive result.

c) Recommendation for Postmarketing Activities

No postmarketing activities have been proposed for this application.