Alinity i Anti-HBc-04

Prepared by: Yusra Othman /Director/Supervisor-Chem **Date:** May/23/2024
signature/title

Reviewed by: Jordan Dillard /Instructor **Date:** June 27 2024
signature/title

Approved by: Sanford N. Bauch, M.D /Chairman **Date:** June 28 2024
signature/title

BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity i Anti-HBc assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgG and IgM antibodies to hepatitis B core antigen (anti-HBc) in human adult and pediatric serum and plasma (dipotassium EDTA, lithium heparin, sodium heparin) and neonatal serum on the Alinity i analyzer.

The Alinity i Anti-HBc assay is to be used as an aid in the diagnosis of acute, chronic, or resolved hepatitis B virus (HBV) infection in conjunction with other laboratory results and clinical information.

WARNING: Not intended for use in screening blood, plasma, or tissue donors. The effectiveness of the Alinity i Anti-HBc assay for use in screening blood, plasma, or tissue

donors has not been established.

Assay performance characteristics have not been established when the Alinity i Anti-HBc assay is used in conjunction with other manufacturers' assays for specific hepatitis markers. Users are responsible for establishing their own performance characteristics. Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients. Users are responsible for establishing their own assay performance characteristics in these populations.

SUMMARY AND EXPLANATION OF THE TEST

The Alinity i Anti-HBc assay utilizes microparticles coated with recombinant hepatitis B virus core antigen (rHBcAg) for the detection of anti-HBc antibodies. Anti-HBc antibody determinations can be used as an indicator of current or past HBV infection. Anti-HBc antibodies are found in serum shortly after the appearance of hepatitis B surface antigen (HBsAg) in acute HBV infections. They will persist after the disappearance of HBsAg and before the appearance of detectable antibody to HBsAg (anti-HBs).*1*, *2*, *3*, *4*, *5*, *6*, *7* In the absence of information about any other HBV markers, it must be considered that an individual with detectable levels of anti-HBc antibodies may be actively infected with HBV or that the infection may have resolved, leaving the person immune.*8* Anti-HBc antibodies may be the only serological marker of HBV infection and potentially infectious blood.*9*, *10*, *11*, *12*, *13*, *14*, *15*, *16*

The presence of anti-HBc antibodies does not differentiate between acute or chronic hepatitis B infection.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the qualitative detection of anti-HBc antibodies in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, rHBcAg coated paramagnetic microparticles, specimen diluent, and assay diluent are combined and incubated. The anti-HBc antibodies present in the sample binds to the rHBcAg coated microparticles. The mixture is washed. Anti-human IgG and IgM acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is relationship between presence of anti-HBc antibodies in the sample and the RLUs detected by the system optics.

The presence or absence of anti-HBc antibodies in the sample is determined by comparing the chemiluminescent RLU in the reaction to the cutoff RLU determined from an active calibration.

For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3.

REAGENTS

Kit Contents

Alinity i Anti-HBc Reagent Kit 07P84

NOTE: Some kit sizes are not available in all countries. Please contact your local distributor.

NOTE: This product is composed of 4 components, which are packaged as a 2 cartridge reagent set. Both cartridges are required to perform the assay.

Volumes (mL) listed in the table below indicate the volume per cartridge set.

REF	07P8421	07P8431
Tests per cartridge set	100	600
Number of cartridge sets per kit	2	2
Tests per kit	200	1200
MICROPARTICLES	6.6 mL	32.1 mL
CONJUGATE	11.0 mL	33.8 mL
ASSAY DILUENT	5.9 mL	28.7 mL
SPECIMEN DILUENT	5.6 mL	28.5 mL

MICROPARTICLES Hepatitis B core (*E. coli*, recombinant) antigen coated microparticles in TRIS buffer. Minimum concentration: 0.08% solids. Preservatives: ProClin 950 and sodium azide.

CONJUGATE Anti-human (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein stabilizers (bovine). Minimum concentration: 0.048 µg/mL. Preservatives: sodium alkyl paraben and sodium azide.

ASSAY DILUENT Assay diluent containing protein stabilizers (mouse) in MOPSO buffer. Preservatives: ProClin 950 and sodium azide.

SPECIMEN DILUENT Specimen diluent containing reductant in MOPSO buffer.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance

with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[17, 18, 19, 20]

The following warnings and precautions apply to: MICROPARTICLES	
	
WARNING	Contains methylisothiazolone and sodium azide.
H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: CONJUGATE	
Contains sodium azide.	
EUH032	Contact with acids liberates very toxic gas.
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: ASSAY DILUENT	
	
DANGER	Contains polyethylene glycol octylphenyl ether (Triton X-405) and methylisothiazolone and sodium azide.
H318	Causes serious eye damage.
H317	May cause an allergic skin reaction.

H412	Harmful to aquatic life with long lasting effects.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
P273	Avoid release to the environment.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor / physician.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Follow local chemical disposal regulations based on your location along with recommendations and content in the Safety Data Sheet to determine the safe disposal of this product.

For the most current hazard information, see the product Safety Data Sheet.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 8.

Reagent Handling

Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.

- Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
- After mixing, place reagent cartridges in an upright position for 1 hour before use to

allow bubbles that may have formed to dissipate.

- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 7.

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8 °C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use. May be used immediately after removal from 2-8°C storage.
Onboard	System Temperature	30 days	
Opened	2 to 8 °C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance. May be used immediately after removal from 2-8°C storage.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it

is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, refer to the Alinity ci-series Operations Manual, Section 5.

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The Alinity i Anti-HBc assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the Alinity ci-series Operations Manual, Section 2.

For information on printing assay parameters, refer to the Alinity ci-series Operations Manual, Section 5.

For a detailed description of system procedures, refer to the Alinity ci-series Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Other specimen types and collection tube types have not been verified with this assay.

Specimen Types	Collection Tubes
Serum	Serum (glass and plastic)
	Serum separator (plastic)
Plasma	Lithium heparin plasma separator (plastic)
	Sodium heparin (plastic)
	Dipotassium EDTA (plastic)

- Performance has not been established for the use of cadaveric specimens or the use of bodily fluids other than human serum and plasma.
- The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

Do not use:

- heat-inactivated specimens
- pooled specimens
- grossly hemolyzed specimens
- specimens with obvious microbial contamination

- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge at >10,000 RCF (Relative Centrifugal Force) for 10 minutes.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/Plasma	Room temperature (study performed at 23 to 30°C)	3 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	2 to 8 °C	7 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.

Based on guidance from CLSI GP44-A4*21*, it is recommended that if testing will be delayed longer than the maximum storage time, remove serum or plasma from the clot, red blood cells, or separator gel and store frozen (-20°C or colder).

Avoid more than 3 freeze/thaw cycles.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

07P84 Alinity i Anti-HBc Reagent Kit

Materials Required but not Provided

- Alinity i Anti-HBc assay file
- 07P8401 Alinity i Anti-HBc Calibrator

- 07P8410 Alinity i Anti-HBc Controls
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, refer to the Alinity ci-series Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the Alinity ci-series Operations Manual, Section 9.

Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Maximum number of replicates sampled from the same sample cup: 10

Priority:

- Sample volume for first test: 75 µL
- Sample volume for each additional test from same sample cup: 25 µL

≤ 3 hours on the reagent and sample manager:

- Sample volume for first test: 150 µL
- Sample volume for each additional test from same sample cup: 25 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

- Refer to the Alinity i Anti-HBc calibrator package insert and/or Alinity i Anti-HBc control package insert for preparation and usage.
- For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

Samples **cannot be diluted for the Alinity i Anti**-HBc assay.

Calibration

For instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5.

Calibrator 1 is tested in triplicate.

A single sample of each control level must be tested to evaluate the assay calibration.

Ensure that assay control values are within the S/CO ranges specified in the control package insert.

Once a calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent kit with a new lot number is used.

Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.

- If statistically-based quality control limits are not available then the calibration should not exceed a 30-day limit for recalibration frequency.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

The Alinity i Anti-HBc Controls are in a serum matrix made from recalcified plasma. The user should provide additional control material for plasma when necessary.

The recommended control requirement for the Alinity i Anti-HBc assay is that a single sample of each control level be tested once every day teating performed.

Note: The insert ranges for the controls are not lot specific and represent the total range of values which may be generated throughout the life of the product. It is recommended that each laboratory establish its own means and acceptable ranges which should fall within the package insert ranges. Sources of variation that can be expected include:

- Calibration
- Control lot
- Instrument
- Calibrator lot

- Reagent lot

To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying a minimum of 20 replicates over several (3-5) days and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24, 4th ed. for general quality control recommendations.[22] Control values must be within the ranges specified in the control package insert. If a control result is out of its specified range, any test results generated since the last acceptable control results must be evaluated to determine if test results may have been adversely affected. Adversely affected test results are invalid, and these samples must be retested.

For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.

RESULTS

Calculation

The Alinity i analyzer calculates results for the Alinity i Anti-HBc assay using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen and control.

Cutoff RLU = Calibrator 1 Mean RLU x 1.0

The cutoff RLU is stored for each reagent lot calibration.

S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

Initial Results

S/CO	Instrument Interpretation	Retest Procedure
< 0.80	Nonreactive	No retest required.
0.80 to <1.21	Grayzone	Retest in duplicate.
≥ 1.21	Reactive	Retest in duplicate.

Final Interpretation

Initial Interpretation	Results with Retest	Final Interpretation
Nonreactive	No retest required.	Nonreactive
Grayzone	If two of the three results are < 1.00 S/CO	Nonreactive
	If two of the three results are ≥ 1.00 S/CO	Reactive
Reactive	If both retest results are < 1.00 S/CO	Nonreactive
	If two of the three results are ≥ 1.00 S/CO	Reactive

- A nonreactive final interpretation indicates that anti-HBc antibodies were not detected in the sample; it is possible that the individual is not infected with HBV.
- A reactive final interpretation indicates presumptive evidence of HBV; anti-HBc antibodies were detected in the sample which suggests either on-going or previous HBV infection.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- Current methods for the detection of anti-HBc antibodies may not detect all infected individuals. A nonreactive test result does not exclude the possibility of exposure to or infection with HBV.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as Alinity i Anti-HBc that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.*23*, *24*
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.*25*

Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section of this package insert for specimen limitations.

EXPECTED RESULTS

Data in the EXPECTED RESULTS section were generated using the ARCHITECT i2000 and i2000SR Systems.

Due to geographic locations or demographics, assay results obtained in individual laboratories may vary from data presented.

Of the 2159 prospectively-collected specimens tested and analyzed in the ARCHITECT CORE clinical study, 1254 were from individuals living in the United States with increased risk of HBV infection. All 1254 were at risk for HBV due to lifestyle, behavior, occupation, or a known exposure event but were asymptomatic and reported no current signs or symptoms of hepatitis. Testing of these specimens was performed at three clinical sites located in Galveston, TX; Hershey, PA; and Milwaukee, WI.

The increased risk population (n=1254) consisted of the following race/ethnic groups:

- 635 (50.64%) Caucasian
- 385 (30.70%) African-American
- 177 (14.11%) Hispanic
- 28 (2.23%) Asian
- 2 (0.16%) American Indian/Alaska Native
- 25 (1.99%) Other
- 2 (0.16%) Unknown

The 1254 specimens from the increased risk population were obtained from the following collection locations:

- 399 (31.82%) from St. Petersburg, FL
- 250 (19.94%) from Galveston, TX
- 163 (13.00%) from Dallas, TX
- 121 (9.65%) from Miami, FL
- 111 (8.85%) from Plymouth, MA
- 94 (7.50%) from Chicago, IL
- 49 (3.91%) from Denver, CO
- 34 (2.71%) from High Point, NC
- 33 (2.63%) from Colton, CA

A total of 231 (18.42%) specimens in the increased risk population were reactive in the ARCHITECT CORE assay. The number of ARCHITECT CORE reactive results observed for the increased risk population at each collection location was:

- 67 (16.79%) from St. Petersburg, FL

- 28 (11.20%) from Galveston, TX
- 29 (17.79%) from Dallas, TX
- 34 (28.10%) from Miami, FL
- 19 (17.12%) from Plymouth, MA
- 38 (40.43%) from Chicago, IL
- 13 (26.53%) from Denver, CO
- 0 (0.00%) from High Point, NC
- 3 (9.09%) from Colton, CA

Of the 1254 specimens, 590 (47.05%) were female and 664 (52.95%) were male. The age was not reported for two specimens. Of the remaining 1252 specimens, the mean age was 39 years (age range: 17 to 82 years). The distribution of ARCHITECT CORE reactive and nonreactive results among the increased risk population by age and gender (n=1254) is summarized in the following table.

		ARCHITECT CORE Results		
Age Group (Years)	**Gender**	**Reactive n (%)**	**Nonreactive n (%)**	**Total**
10-19	F	1 (7.69)	12 (92.31)	13
	M	1 (12.50)	7 (87.50)	8
20-29	F	13 (7.22)	167 (92.78)	180
	M	6 (4.41)	130 (95.59)	136
30-39	F	8 (6.72)	111 (93.28)	119
	M	26 (14.53)	153 (85.47)	179
40-49	F	37 (25.00)	111 (75.00)	148
	M	51 (24.29)	159 (75.71)	210
50-59	F	18 (20.93)	68 (79.07)	86
	M	37 (36.63)	64 (63.37)	101
60-69	F	14 (40.00)	21 (60.00)	35
	M	9 (45.00)	11 (55.00)	20
70-79	F	3 (60.00)	2 (40.00)	5

Age Group (Years)	Gender	ARCHITECT CORE Results		Total
		Reactive n (%)	Nonreactive n (%)	
	M	6 (66.67)	3 (33.33)	9
80-89	F	1 (33.33)	2 (66.67)	3
Unknown	F	0 (0.00)	1 (100.00)	1
	M	0 (0.00)	1 (100.00)	1
Total		**231 (18.42)**	**1023 (81.58)**	**1254**

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i Anti-HBc assay/Alinity i analyzer and the ARCHITECT CORE assay/ARCHITECT i System utilize the same reagents and sample/reagent ratios. Some performance characteristics for the Alinity i assay were established using the ARCHITECT i System.

Alinity i Analyzer Specific Studies

The following results were generated using the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) EP05-A3.*26* Testing was conducted using 1 lot of the Alinity i Anti-HBc Reagent Kit, 1 lot of anti-HBc calibrator, and 1 lot of anti-HBc controls and 1 instrument. Two levels of controls and 5 panels were assayed in 3 replicates at 2 separate times per day on 12 different days.

Sample	n	Mean (S/CO)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Negative Control	72	0.15	0.007	N/A[b]	0.009	N/A[b]

Sample	n	Mean (S/CO)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Positive Control	72	2.74	0.040	1.4	0.049	1.8
Panel 1	71[c]	0.67	0.015	2.3	0.017	2.5
Panel 2	72	0.79	0.016	2.1	0.018	2.3
Panel 3	72	1.18	0.020	1.7	0.024	2.0
Panel 4	72	1.36	0.023	1.7	0.026	1.9
Panel 5	72	2.06	0.034	1.6	0.039	1.9

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

[b] Not applicable

[c] Replicate lost due to instrument or operator error.

System Reproducibility

A study was performed based on guidance from CLSI EP05-A2 and CLSI EP15-A2.[27, 28] Testing was conducted at 3 clinical sites using 1 lot of the Alinity i Anti-HBc Reagent Kit, 1 lot of the Alinity i Anti-HBc Calibrator, and 1 lot of the Alinity i Anti-HBc Controls and 1 instrument. Two levels of controls and 3 panels were assayed in replicates of 4 at 2 separate times per day for 5 days.

Sample	N	Mean S/CO	Within-Run		Within-Day[a]		Within-Laboratory Precision (Total)[b]		Precision with Additional Component of Between-Site[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control	120	0.11	0.007	N/A[d]	0.009	N/A[d]	0.010	N/A[d]	0.010	N/A[d]
Positive Control	120	2.48	0.041	1.6	0.043	1.7	0.061	2.5	0.063	2.5
High Negative Panel	120	0.62	0.016	2.6	0.017	2.7	0.019	3.1	0.019	3.1

Sample	N	Mean S/CO	Within-Run		Within-Day[a]		Within-Laboratory Precision (Total)[b]		Precision with Additional Component of Between-Site[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Positive Panel	120	1.27	0.026	2.1	0.029	2.2	0.032	2.5	0.032	2.6
Moderate Positive Panel	120	1.87	0.032	1.7	0.036	1.9	0.042	2.3	0.049	2.6

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

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

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

[d] Not applicable

Percent Agreement

A study was performed to compare the anti-HBc assay on the Alinity i analyzer and the ARCHITECT i2000SR system using 1 lot each of the Anti-HBc Reagent Kit, Anti-HBc Calibrator, and Anti-HBc Controls. Of the 300 specimens tested, 162 were negative, 15 were in the grayzone, and 123 were positive, based on the ARCHITECT CORE results on the ARCHITECT i2000SR instrument. An aliquot of each specimen was tested on 1 Alinity i analyzer at each of the 3 clinical testing sites and on 1 ARCHITECT i2000SR instrument at 1 clinical testing site.

The concordance analysis for the Alinity i analyzer and the ARCHITECT system are summarized in the table below, per site and across sites:

Site	Alinity i Anti-HBc	ARCHITECT CORE			Negative % Agreement (95% Confidence Interval)[a]	Positive % Agreement (95% Confidence Interval)[a]
		Reactive	Grayzone	Nonreactive		
1	Reactive	121	3	0	97.53 (158/162) (93.82,99.04)	98.37 (121/123) (94.26,99.55)
	Grayzone	2	12	4		
	Nonreactive	0	0	158		

Site	Alinity i Anti-HBc	ARCHITECT CORE			Negative % Agreement (95% Confidence Interval)[a]	Positive % Agreement (95% Confidence Interval)[a]
		Reactive	Grayzone	Nonreactive		
2	Reactive	121	4	3	93.83 (152/162) (89.01,96.61)	98.37 (121/123) (94.26,99.55)
	Grayzone	2	11	7		
	Nonreactive	0	0	152		
3	Reactive	121	3	0	100.00 (162/162) (97.68,100.00)	98.37 (121/123) (94.26,99.55)
	Grayzone	2	12	0		
	Nonreactive	0	0	162		
All	Reactive	363	10	3	97.12 (472/486) (95.22,98.28)	98.37 (363/369) (96.50,99.25)
	Grayzone	6	35	11		
	Nonreactive	0	0	472		

[a] The 95% confidence intervals for negative percent agreement and positive percent agreement were estimated using Wilson Score method.

$$\text{Positive Percent Agreement} = \frac{\text{[Number of initially reactive Alinity i results in agreement with ARCHITECT i2000SR results]}}{\text{[Total number of initially reactive ARCHITECT i2000SR results]}} \times 100\%$$

$$\text{Negative Percent Agreement} = \frac{\text{[Number of initially nonreactive Alinity i results in agreement with ARCHITECT i2000SR results]}}{\text{[Total number of initially nonreactive ARCHITECT i2000SR results]}} \times 100\%$$

Carryover

The Alinity i Anti-HBc assay was evaluated for susceptibility to within-assay sample carryover comparing the results of a protected low sample to an unprotected low sample. The protected low sample was tested before a high sample, and the unprotected low sample was tested after the high sample. Fourteen iterations across 5 runs were tested. The low sample, which was used as both the protected and unprotected sample, was a mixture of anti-HBc positive plasma and anti-HBc negative plasma and was targeted to 0.80 S/CO (range of 0.60

S/CO to 0.99 S/CO). The high sample was a mixture of anti-HBc positive plasma and anti-HBc negative plasma and had an antibody level near a concentration of 7500 PEI U/mL (target: 10 S/CO). The difference between the protected sample and the unprotected sample was 0.02 S/CO. Carryover was not observed in the Alinity i Anti-HBc assay.

Analytical Sensitivity

Analytical sensitivity was evaluated using dilutions of the WHO 1st International Standard for anti-Hepatitis B core antigen (anti-HBc), plasma, human NIBSC code: 95/522. The dilutions ranged from 1.00 to 0.05 IU/mL. The dilutions were tested with 1 reagent lot on the Alinity i instrument and 1 reagent lot on the ARCHITECT i2000SR instrument. The analytical sensitivity was 0.61 IU/mL with the Alinity i instrument as compared to 0.58 IU/mL with the ARCHITECT i2000SR instrument.

Seroconversion Sensitivity

To determine the seroconversion sensitivity, 8 seroconversion panels obtained from commercial vendors were tested on the Alinity i system using the Alinity i Anti-HBc assay. The panel results were evaluated against the comparator assay (ARCHITECT CORE) and data are summarized in the following table.

Panel ID	Days to Anti-HBc First Reactive Result from Initial Draw Date		Difference in Days to Anti-HBc First Reactive Result (Alinity-ARCHITECT)
	Alinity i Anti-HBc	ARCHITECT CORE	
13867/3482	41	41	0
26982/14399	24	24	0
43527/3453	34	34	0
26022/14518	37	37	0
6278	37	37	0
6281	41	41	0
SCP-HBV-001	29	29	0
SCP-HBV-004	71	71	0

ARCHITECT i2000/i2000SR System Specific Studies

The following results were generated using the ARCHITECT i2000/i2000SR System.

Clinical Performance

A prospective multi-center study was conducted to evaluate the ability of the ARCHITECT CORE assay to detect IgG and IgM antibodies to anti-HBc in a group of individuals that would normally be tested in a clinical situation. Of the 2259 specimens tested and analyzed in the ARCHITECT CORE clinical study, 1254 specimens were obtained from individuals living in the United States with increased risk of HBV infection due to lifestyle, behavior, occupation, disease state, or a known exposure event, and 625 specimens were obtained from individuals living in the United States exhibiting signs and symptoms of hepatitis infection (Population 1).

The 1879 specimens in Population 1 were obtained from the following collection locations:

- 470 (25.01%) from St. Petersburg, FL
- 329 (17.51%) from Chicago, IL

- 278 (14.80%) from Galveston, TX
- 264 (14.05%) from Dallas, TX
- 182 (9.69%) from Miami, FL
- 176 (9.37%) from Denver, CO
- 111 (5.91%) from Plymouth, MA
- 35 (1.86%) from Colton, CA
- 34 (1.81%) from High Point, NC

Population 1 (n=1879) consisted of the following race/ethnic groups:

- 937 (49.87%) Caucasian
- 531 (28.26%) African-American
- 323 (17.19%) Hispanic
- 48 (2.55%) Asian
- 4 (0.21%) American Indian/Alaska Native
- 34 (1.81%) Other
- 2 (0.11%) Unknown

Of the 1879 specimens in Population 1850 (45.24%) were female and 1029 (54.76%) were male. The age was not reported for two specimens. Of the remaining 1877 specimens, the mean age was 42 years (age range: 17 to 83 years).

Specimens were also prospectively collected in Vietnam from 97 individuals at increased risk of HBV infection and 127 individuals with signs and symptoms of hepatitis infection (Population 2). The 224 specimens in Population 2 were 100.00% Vietnamese, and 124 (55.36%) were female and 100 (44.64%) were male. The mean age was 37 years (age range: 18 to 68 years).

Each specimen was tested using a comparator anti-HBc assay and three HBV reference assays, each detecting a unique serological marker (HBsAg, IgM anti-HBc, and anti-HBs). The HBV classification was determined for each specimen based on the reactivity patterns of the four HBV serological marker results. The comparator and reference assays were from a single manufacturer and during the clinical study, all comparator and reference testing was performed following manufacturer's instructions. Each specimen was also tested at one of three clinical sites located in Galveston, TX; Hershey, PA; or Milwaukee, WI using the ARCHITECT CORE assay.

Results by Specimen Classification

Following testing with the comparator anti-HBc assay and the three reference HBV assays, Population 1 specimens were assigned an HBV classification using the reactive (+) and nonreactive (-) patterns. There were 15 unique reference marker patterns observed in the ARCHITECT CORE clinical study for Population 1.

	HBV Reference Markers				
n	**HBsAg**	**IgM Anti-HBc**	**Total Anti-HBc**	**Anti-HBs**	**HBV Classification**
14	+	-	-	-	Early Acute
11	+	+	+	-	Acute
4	+	-	+	+	Chronic
73	+	-	+	-	Chronic
2	+	-	-	+	Chronic
6	-	+	+	+	Recovering Acute
4	-	+	+	-	Recovering Acute/ Undetectable HBsAg
219	-	-	+	+	Immune Due to Natural Infection
37	-	-	+	I	Distantly Immune/Anti-HBs Unknown
107	-	-	+	-	Distantly Immune/Anti-HBs Not Detected
341	-	-	-	+	Immune Due to HBV Vaccination
1004	-	-	-	-	Susceptible
4	+	-	+	I	Chronic
1	-	+	+	I	Early Recovery
52	-	-	-	I	Unknown
1879					**Total**

I = Indeterminate

Following testing with the comparator anti-HBc assay and the three reference HBV assays, Population 2 specimens were assigned an HBV classification using the reactive (+) and nonreactive (-) patterns. There were 10 unique reference marker patterns observed in the ARCHITECT CORE clinical study for Population 2.

n	**HBV Reference Markers**	**HBV Classification**

	HBsAg	IgM Anti-HBc	Total Anti-HBc	Anti-HBs	
1	+	-	-	-	Early Acute
2	+	-	+	+	Chronic
65	+	-	+	-	Chronic
1	+	-	-	+	Chronic
61	-	-	+	+	Immune Due to Natural Infection
5	-	-	+	I	Distantly Immune/Anti-HBs Unknown
16	-	-	+	-	Distantly Immune/Anti-HBs Not Detected
40	-	-	-	+	Immune Due to HBV Vaccination
31	-	-	-	-	Susceptible
2	+	-	+	I	Chronic
224					**Total**

I = Indeterminate

Comparison of Results

The following table compares the ARCHITECT CORE assay results with comparator anti-HBc assay results for each of the HBV classifications for Population 1. The data are summarized in the following table.

	Anti-HBc Comparator				
	Reactive		**Negative**		
	ARCHITECT CORE Interpretation		**ARCHITECT CORE Interpretation**		
HBV Classification	**Reactive n (%)**	**Nonreactive n (%)**	**Reactive n (%)**	**Nonreactive n (%)**	**Total**
Early Acute	0 (0.00)	0 (0.00)	4 (0.21)[a]	10 (0.53)	14 (0.75)
Acute	11 (0.59)	0 (0.00)	0 (0.00)	0 (0.00)	11 (0.59)
Chronic	81 (4.31)	0 (0.00)	0 (0.00)	2 (0.11)	83 (4.42)
Recovering Acute	6 (0.32)	0 (0.00)	0 (0.00)	0 (0.00)	6 (0.32)
Recovering Acute/ Undetectable HBsAg	4 (0.21)	0 (0.00)	0 (0.00)	0 (0.00)	4 (0.21)
Immune Due to Natural Infection	213 (11.34)	6 (0.32)[b]	0 (0.00)	0 (0.00)	219 (11.66)

	Anti-HBc Comparator				
	Reactive		Negative		
	ARCHITECT CORE Interpretation		ARCHITECT CORE Interpretation		
HBV Classification	**Reactive n (%)**	**Nonreactive n (%)**	**Reactive n (%)**	**Nonreactive n (%)**	**Total**
Distantly Immune/Anti-HBs Unknown	37 (1.97)	0 (0.00)	0 (0.00)	0 (0.00)	37 (1.97)
Distantly Immune/Anti-HBs Not Detected	102 (5.43)	5 (0.27)[c]	0 (0.00)	0 (0.00)	107 (5.69)
Immune Due to HBV Vaccination	0 (0.00)	0 (0.00)	17 (0.90)[d]	324 (17.24)	341 (18.15)[e]
Susceptible	0 (0.00)	0 (0.00)	7 (0.37)[f]	997 (53.06)	1004 (53.43)
Early Recovery	1 (0.05)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.05)
Unknown	0 (0.00)	0 (0.00)	2 (0.11)[g]	50 (2.66)	52 (2.77)
Total	**455 (24.22)**	**11 (0.59)**	**30 (1.60)**	**1383 (73.60)**	**1879 (100.00)**

[a] Two specimens were tested and determined to be negative for HBeAg and anti-HBe; positive for HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay. One specimen was tested and determined to be negative for HBeAg and anti-HBe; positive for HBV DNA; and reactive by a second FDA-approved total anti-HBc assay. One specimen was tested and determined to be positive for HBeAg; negative for anti-HBe; positive for HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay.

[b] Three specimens were tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay. Two specimens were tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and reactive by a second FDA-approved total anti-HBc assay.

One specimen was tested and determined to be negative for HBeAg and anti-HBe; positive for HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay.

[c] These specimens were tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay.

[d] Eight specimens were tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay. Three specimens were tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and reactive by a second FDA-approved total anti-HBc assay. Two specimens were tested and determined to be negative for HBeAg and HBV DNA; positive for anti-HBe; and nonreactive by a second FDA-approved total anti-HBc assay. One specimen was tested and determined to be negative for HBeAg and anti-HBe; positive for HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay. One specimen was tested and determined to be negative for HBeAg and anti-HBe; not tested for HBV DNA due to insufficient sample volume; and nonreactive by a second FDA-approved total anti-HBc assay. One specimen was tested and determined to be negative for HBeAg; positive for anti-HBe and HBV DNA; and reactive by a second FDA-approved total anti-HBc assay. One specimen was tested and determined to be negative for HBeAg and HBV DNA; equivocal for anti-HBe; and nonreactive by a second FDA-approved total anti-HBc assay.

[e] Although serological HBV classification indicates immune due to HBV vaccination, 142 were recorded as vaccinated, 113 were recorded as unknown, and 86 were recorded as not vaccinated.

[f] Three specimens were tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay. Two specimens were tested and determined to be negative for HBeAg and anti-HBe; positive for HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay. One specimen was tested and determined to be negative for HBeAg and HBV DNA; positive for anti-HBe; and nonreactive by a second FDA-approved total anti-HBc assay. One specimen was tested and determined to be negative for HBeAg; positive for anti-HBe; not tested for HBV DNA due to insufficient sample volume; and nonreactive by a second FDA-approved total anti-HBc assay.

[g] These specimens were tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay.

The following table compares the ARCHITECT CORE assay results with comparator anti-HBc assay results for each of the HBV classifications for Population 2. The data are summarized in the following table.

	Anti-HBc Comparator				
	Reactive		Negative		
	ARCHITECT CORE Interpretation		ARCHITECT CORE Interpretation		
HBV Classification	**Reactive n (%)**	**Nonreactive n (%)**	**Reactive n (%)**	**Nonreactive n (%)**	**Total**
Early Acute	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.45)	1 (0.45)
Chronic	69 (30.80)	0 (0.00)	1 (0.45)[a]	0 (0.00)	70 (31.25)
Immune Due to Natural Infection	61 (27.23)	0 (0.00)	0 (0.00)	0 (0.00)	61 (27.23)
Distantly Immune/Anti-HBs Unknown	5 (2.23)	0 (0.00)	0 (0.00)	0 (0.00)	5 (2.23)
Distantly Immune/Anti-HBs Not Detected	16 (7.14)	0 (0.00)	0 (0.00)	0 (0.00)	16 (7.14)
Immune Due to HBV Vaccination	0 (0.00)	0 (0.00)	19 (8.48)[b]	21 (9.38)	40 (17.86)[c]
Susceptible	0 (0.00)	0 (0.00)	3 (1.34)[d]	28 (12.50)	31 (13.84)
Total	**151 (67.41)**	**0 (0.00)**	**23 (10.27)**	**50 (22.32)**	**224 (100.00)**

[a] One specimen was tested and determined to be positive for HBeAg; negative for anti-HBe; positive for HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay.

[b] Nine specimens were tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay. Four specimens were tested and determined to be negative for HBeAg and anti-HBe; not tested for HBV DNA due to insufficient sample volume; and nonreactive by a second FDA-approved total anti-HBc assay. Three specimens were tested and determined to be negative for HBeAg and HBV DNA; positive for anti-HBe; and nonreactive by a second FDA-approved total anti-

HBc assay. One specimen was tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and reactive by a second FDA-approved total anti-HBc assay. One specimen was tested and determined to be negative for HBeAg and anti-HBe; positive for HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay. One specimen was tested and determined to be negative for HBeAg and HBV DNA; equivocal for anti-HBe; and nonreactive by a second FDA-approved total anti-HBc assay.

[c] Although serological HBV classification indicates immune due to HBV vaccination, all 40 were recorded as not vaccinated.

[d] Two specimens were tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and nonreactive by a second FDA-approved total anti-HBc assay. One specimen was tested and determined to be negative for HBeAg; positive for anti-HBe; not tested for HBV DNA due to insufficient sample volume; and reactive by a second FDA-approved total anti-HBc assay.

Percent Agreement

The table below summarizes the percent agreement between ARCHITECT CORE and the comparator anti-HBc assay for Population 1 by HBV classification.

HBV Classification	Positive Percent Agreement %	95% Confidence Interval	Negative Percent Agreement %	95% Confidence Interval
Early Acute	NA	NA	71.43 (10/14)	41.90 - 91.61
Acute	100.00 (11/11)	71.51 - 100.00	NA	NA
Chronic	100.00 (81/81)	95.55 - 100.00	100.00 (2/2)	15.81 - 100.00
Recovering Acute	100.00 (6/6)	54.07 - 100.00	NA	NA
Recovering Acute/Undetectable HBsAg	100.00 (4/4)	39.76 - 100.00	NA	NA
Immune Due to Natural Infection	97.26 (213/219)	94.13 - 98.99	NA	NA
Distantly Immune/Anti-HBs Unknown	100.00 (37/37)	90.51 - 100.00	NA	NA
Distantly Immune/Anti-HBs Not Detected	95.33 (102/107)	89.43 - 98.47	NA	NA
Immune Due to HBV Vaccination	NA	NA	95.01 (324/341)	92.14 - 97.07

HBV Classification	Positive Percent Agreement %	95% Confidence Interval	Negative Percent Agreement %	95% Confidence Interval
Susceptible	NA	NA	99.30 (997/1004)	98.57 - 99.72
Early Recovery	100.00 (1/1)	2.50 - 100.00	NA	NA
Unknown	NA	NA	96.15 (50/52)	86.79 - 99.53
Total	**97.64 (455/466)**	**95.82 - 98.82**	**97.88 (1383/1413)**	**96.98 - 98.56**

NA = not applicable

$$\text{Positive percent agreement} = \frac{\text{[No. of ARCHITECT CORE reactive results in agreement with the comparator anti-HBc reactive results]}}{\text{[Total number of comparator anti-HBc reactive results]}} \times 100\%$$

$$\text{Negative percent agreement} = \frac{\text{[No. of ARCHITECT CORE nonreactive results in agreement with the comparator anti-HBc negative results]}}{\text{[Total number of comparator anti-HBc negative results]}} \times 100\%$$

The table below summarizes the percent agreement between ARCHITECT CORE and the comparator anti-HBc assay for Population 2 by HBV classification.

HBV Classification	Positive Percent Agreement %	95% Confidence Interval	Negative Percent Agreement %	95% Confidence Interval
Early Acute	NA	NA	100.00 (1/1)	2.50 - 100.00
Chronic	100.00 (69/69)	94.79 - 100.00	0.00 (0/1)	0.00 - 97.50
Immune Due to Natural Infection	100.00 (61/61)	94.13 - 100.00	NA	NA

HBV Classification	Positive Percent Agreement %	95% Confidence Interval	Negative Percent Agreement %	95% Confidence Interval
Distantly Immune/Anti-HBs Unknown	100.00 (5/5)	47.82 - 100.00	NA	NA
Distantly Immune/Anti-HBs Not Detected	100.00 (16/16)	79.41 - 100.00	NA	NA
Immune Due to HBV Vaccination	NA	NA	52.50 (21/40)	36.13 - 68.49
Susceptible	NA	NA	90.32 (28/31)	74.25 - 97.96
Total	**100.00 (151/151)**	**97.59 - 100.00**	**68.49 (50/73)**	**56.56 - 78.87**

NA = not applicable

$$\text{Positive percent agreement} = \frac{\text{[No. of ARCHITECT CORE reactive results in agreement with the comparator anti-HBc reactive results]}}{\text{[Total number of comparator anti-HBc reactive results]}} \times 100\%$$

$$\text{Negative percent agreement} = \frac{\text{[No. of ARCHITECT CORE nonreactive results in agreement with the comparator anti-HBc negative results]}}{\text{[Total number of comparator anti-HBc negative results]}} \times 100\%$$

Percent of Positive Specimens and Percent Agreement for Individuals Diagnosed with Acute and Chronic HBV Infection

The performance of the ARCHITECT CORE assay was evaluated by testing prospectively-collected specimens from six individuals diagnosed with acute HBV infection and 50 individuals diagnosed with chronic HBV infection. Acute status was defined for the six specimens by the four HBV serological marker results. The percent of positive ARCHITECT CORE specimens for individuals with documented acute HBV infection was 100.00% (6/6, with a 95% confidence interval of 54.07% to 100.00%). The percent of positive ARCHITECT CORE specimens for individuals with documented chronic HBV infection was 100.00% (50/50, with a 95% confidence interval of 92.89% to 100.00%).

Clinical Performance in a Pediatric Population

The performance of the ARCHITECT CORE assay in a pediatric population was evaluated by testing 100 surplus specimens from a pediatric population collected in Fall River, MA by a specimen vendor, and from the 112 prospectively-collected pediatric specimens from Population 1, Population 2, and the chronic population.

For the surplus pediatric specimens, the negative percent agreement between the ARCHITECT CORE assay results and the comparator anti-HBc assay results was 98.99% (98/99, with a 95% confidence interval of 94.50% to 99.97%). The positive percent agreement between the ARCHITECT CORE assay results and the comparator anti-HBc assay results was 100.00% (1/1, with a 95% confidence interval of 2.50% to 100.00%). The distribution of the ARCHITECT CORE reactive and nonreactive results for the surplus pediatric population is summarized by age and gender in the following table.

		ARCHITECT CORE Result		
Age Group (Years)	**Gender**	**Reactive n (%)**	**Nonreactive n (%)**	**Total**
2 to 12	Female	0 (0.00)	17 (100.00)	17
	Male	0 (0.00)	33 (100.00)	33
13 to 18	Female	0 (0.00)	22 (100.00)	22
	Male	2 (18.18)	9 (81.82)	11
19 to 21	Female	0 (0.00)	12 (100.00)	12
	Male	0 (0.00)	5 (100.00)	5
Total		**2 (2.00)**	**98 (98.00)**	**100**

For the prospectively-collected pediatric specimens (Population 1, Population 2, and chronic specimens), the negative percent agreement between the ARCHITECT CORE assay results and the comparator anti-HBc assay results was 96.63% (86/89, with a 95% confidence interval of 90.46% to 99.30%). The positive percent agreement between the ARCHITECT CORE assay results and the comparator anti-HBc assay results was 100.00% (23/23, with a 95% confidence interval of 85.18% to 100.00%). The distribution of the ARCHITECT CORE reactive and nonreactive results for the prospectively-collected pediatric population is summarized by age and gender in the following table.

		ARCHITECT CORE Result		
Age Group (Years)	**Gender**	**Reactive n (%)**	**Nonreactive n (%)**	**Total**

Age Group (Years)	Gender	ARCHITECT CORE Result		Total
		Reactive n (%)	Nonreactive n (%)	
2 to 18	Female	1 (25.00)	3 (75.00)	4
	Male	2 (50.00)	2 (50.00)	4
19 to 21	Female	17 (28.81)	42 (71.19)	59
	Male	6 (13.33)	39 (86.67)	45
Total		**26 (23.21)**	**86 (76.79)**	**112**

Analytical Specificity

The ARCHITECT CORE assay was evaluated for potential cross-reactivity for specimens from individuals with medical conditions unrelated to HBV infection. The specimens were tested using the ARCHITECT CORE assay and the comparator anti-HBc assay. The final results for each of the specimens were compared between the two assays. The data are summarized in the following table.

Reactivity of the ARCHITECT CORE Assay in Individuals with Medical Conditions Unrelated to HBV Infection

Category	n	Comparator Anti-HBc Assay			
		Negative		Reactive	
		ARCHITECT CORE		ARCHITECT CORE	
		NR[a]	R[a]	NR[a]	R[a]
Anti-Cytomegalovirus (Anti-CMV positive)	10	8	0	1[b]	1
Anti-*Escherichia coli* (Anti-*E. coli*)	2	0	0	0	2
Anti-nuclear antibody (ANA)	7	7	0	0	0
Epstein-Barr Virus (anti-EBV positive)	6	3	0	0	3
Hepatitis A Virus (anti-HAV IgM positive)	8	6	0	0	2
Hepatitis C Virus (anti-HCV positive)	10	9	0	0	1

Reactivity of the ARCHITECT CORE Assay in Individuals with Medical Conditions Unrelated to HBV Infection

		Comparator Anti-HBc Assay			
		Negative		Reactive	
		ARCHITECT CORE		ARCHITECT CORE	
Category	**n**	**NR[a]**	**R[a]**	**NR[a]**	**R[a]**
Herpes Simplex Virus (HSV) positive	10	9	0	1[b]	0
Human Anti-Mouse Antibodies (HAMA) positive	5	5	0	0	0
Human Immunodeficiency Virus (anti-HIV-1 positive)	8	2	0	0	6
Influenza vaccine recipient	9	9	0	0	0
Mumps Virus positive	10	10	0	0	0
Non-viral liver disease	5	3	0	0	2
Rheumatoid factor positive	10	7	0	0	3
Rubella Virus positive	10	7	0	0	3
Rubeola Virus (Measles) positive	9	9	0	0	0
Syphilis	9	9	0	0	0
Systemic Lupus Erythematosus (SLE)	4	4	0	0	0
Toxoplasmosis IgG positive	2	2	0	0	0
Varicella Zoster Virus (anti-VZV positive)	10	8	0	0	2
Yeast infection	9	8	0	0	1
Total	**153**	**125**	**0**	**2**	**26**

[a] **NR = Nonreactive, R = Reactive**

[b] These specimens were tested and determined to be nonreactive for HBsAg; nonreactive for anti-HBs; and nonreactive for IgM anti-HBc. A second FDA-approved total anti-HBc assay was performed and the specimens were determined to be nonreactive.

Interference

At the concentrations listed below, bilirubin (conjugated and unconjugated), hemoglobin, total protein, and triglycerides showed less than 10% interference in the ARCHITECT CORE assay for high negative samples (S/CO range: 0.60 to 0.99) and low positive samples (S/CO range: 1.00 to 1.40):

Interferent	Interferent Concentration
Bilirubin	≤ 20 mg/dL
Hemoglobin	≤ 500 mg/dL
Total Protein	≤ 12 g/dL
Triglycerides	≤ 3000 mg/dL

Tube Type Matrix Comparison

The following tube types are acceptable for use with the ARCHITECT CORE assay:

- Glass: serum
- Plastic: serum, serum separator, lithium heparin plasma separator, sodium heparin, and dipotassium EDTA

On average, the tube types evaluated showed less than a 10% difference when compared to the control tube type (plastic serum). The distribution of the percent differences per tube type is listed in the following table.

	Distribution of Absolute % Differences	
Tube Type	**≤ 10%**	**> 10% to ≤ 20%**
Glass Serum	98.0% (50/51)	2.0% (1/51)
Plastic Serum Separator	98.0% (50/51)	2.0% (1/51)
Plastic Dipotassium EDTA	98.0% (50/51)	2.0% (1/51)
Plastic Sodium Heparin	96.1% (49/51)	3.9% (2/51)
Plastic Lithium Heparin Plasma Separator	100.0% (51/51)	0.0% (0/51)

Neonate Serum

A study was conducted to evaluate whether neonate samples may be tested with the ARCHITECT CORE assay. Cord blood serum was used as a surrogate for neonate serum. Twenty-two matched cord blood and maternal serum samples were spiked with anti-HBc positive stock to yield a high negative sample (target S/CO 0.80) and a low positive sample (target S/CO 1.20). The distribution of the percent differences per analyte level is listed in the following table.

	Distribution of Absolute % Differences		
Analyte Level S/CO	**< 10%**	**≥ 10% to < 20%**	**≥ 20% to < 30%**
0.80	59.1% (13/22)	31.8% (7/22)	9.1% (2/22)
1.20	86.4% (19/22)	13.6% (3/22)	0.0% (0/22)

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