Alinity i Anti-HBc IgM (Anti-HBc M)-03

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SUPERSEDES: Procedure titled _____

INTENDED USE

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

The Alinity i Anti-HBc IgM assay is to be used as an aid in the diagnosis of acute or recent 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 IgM 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 IgM 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. The user is responsible for establishing their own assay performance characteristics in these populations.

SUMMARY AND EXPLANATION OF THE TEST

Virus specific IgM antibody has been detected in most acute viral infections and is a reliable marker for acute viral disease. High levels of IgM anti-HBc have been detected in patients with acute HBV infection[1], [2], [3], [4], [5], [6] and low levels have been detected in some patients with chronic HBV infection.[7], [8] Differentiation of acute and chronic HBV infection on the basis of viral markers such as HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc is difficult because most of these markers are seen during both acute and chronic disease.[1] In cases where these markers are present, acute illness with other agents such as hepatitis C, non-A, non-B, non-C hepatitis, and delta hepatitis may confuse the diagnosis.[9] Several studies have demonstrated that IgM anti-HBc is the only specific marker for the diagnosis of acute HBV infection.[4], [5], [10], [11], [12], [13]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

Sample and wash buffer are combined. An aliquot of the prediluted sample and anti-human IgM (mouse monoclonal) coated paramagnetic microparticles are combined and incubated. Human IgM present in the sample binds to the anti-human IgM (mouse monoclonal) coated microparticles. The mixture is washed. Recombinant hepatitis B virus core antigen (rHBcAg) acridinium-labeled conjugate is added to create a reaction mixture and incubated. Anti-HBc specific IgM binds to the conjugate. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

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

The presence or absence of IgM anti-HBc 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 IgM Reagent Kit 07P86

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

REF	07P8621
Tests per cartridge	100
Number of cartridges per kit	2
Tests per kit	200
MICROPARTICLES	5.6 mL
CONJUGATE	6.1 mL
MICROPARTICLES Anti-human IgM (mouse monoclonal) coated microparticles in TRIS buffer with protein (1.0% bovine serum albumin and 2.5% goat IgG) additives. Minimum concentration: 0.12% solids. Preservatives: sodium azide and antimicrobial agents.	
CONJUGATE Hepatitis B virus core antigen (*E. coli*, recombinant) acridinium-labeled conjugate in succinate buffer with protein (2.5% bovine serum albumin and 2.0% bovine calf serum) additives. Minimum concentration: 0.4 µg/mL. Preservatives: antimicrobial agents.	

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.[14, 15, 16, 17]

The following warnings and precautions apply to: CONJUGATE

WARNING	Contains Polyethylene glycol octylphenyl ether (Triton X-405).
H319	Causes serious eye irritation.
Prevention	
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice / attention.

Safety Data Sheets are available at www.abbottdiagnostics.com or/and SDS folder.

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 IgM 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)

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.

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.

To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at > 10,000 RCF (Relative Centrifugal Force) for 10 minutes before testing if

- they contain fibrin, red blood cells, or other particulate matter or
- they were frozen and thawed.

- 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 24 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.

If testing will be delayed more than 3 days for specimens stored at room temperature or more

than 7 days for specimens stored at 2-8°C, remove serum or plasma from the clot, red blood cells, or separator gel and store at -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 storage limitations listed above.

PROCEDURE

Materials Provided

07P86 Alinity i Anti-HBc IgM Reagent Kit

Materials Required but not Provided

- Alinity i Anti-HBc IgM assay file
- 07P8602 Alinity i Anti-HBc IgM Calibrators
- 07P8612 Alinity i Anti-HBc IgM Controls or other control material
- 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: 64 µL
- Sample volume for each additional test from same sample cup: 14 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: 14 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

- Refer to the Alinity i Anti-HBc IgM calibrator package insert and/or Alinity i Anti-HBc IgM 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 IgM assay.

Calibration

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

Calibrator 1 and Calibrator 2 are 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 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 IgM Controls are **in a serum** matrix made from recalcified plasma. The user should provide alternate control material for plasma when necessary.

The recommended control requirement for the Alinity i Anti-HBc IgM assay is that a single sample of each control level be tested once every day testing 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.*18*

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 IgM assay using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen and control.

Cutoff RLU = [(Calibrator 2 mean RLU- Calibrator 1 mean RLU) x 0.75] + Calibrator 1 mean RLU

The cutoff RLU is stored for each reagent lot calibration.

S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

S/CO	Instrument Interpretation	Interpretation
< 0.80	Nonreactive	IgM anti-HBc not detected. Does not exclude the possibility of exposure to or infection with HBV. No retest required.
0.80 to < 1.21	Grayzone	Antibodies to IgM anti-HBc may or may not be present. Patients with specimens exhibiting grayzone test results should be retested at approximately 1 week intervals.*
≥ 1.21	Reactive	Presumptive evidence of IgM anti-HBc. No retest required.

*Monitoring the level of IgM anti-HBc by retesting at approximately one week intervals will distinguish rapidly rising IgM anti-HBc levels associated with early acute hepatitis B infection from gradually decreasing or unchanging IgM anti-HBc levels often associated with late acute stage of HBV infection, 6 to 9 months from the appearance of HBsAg.

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 IgM anti-HBc may not detect all infected individuals. A nonreactive test result does not exclude the possibility of exposure to or infection with HBV.
- The Alinity i Anti-HBc IgM assay is limited to the detection of IgM anti-HBc in human

serum or plasma. It can be used to determine whether a patient has, or has recently had, acute or subclinical hepatitis B infection. Supportive clinical information, including other hepatitis B markers, should also be evaluated. The test cannot determine a patient's immune status to hepatitis B.

- Specimens from patients with high levels of IgM (e.g., specimens from patients with multiple myeloma) may show depressed values when tested with assay kits (such as Alinity i Anti-HBc IgM) that use reagents containing anti-human IgM.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[19, 20] Such specimens may show either falsely elevated or depressed values when tested with assay kits such as Alinity i Anti-HBc IgM that employ mouse monoclonal antibodies. [19]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[21] 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.
- Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section of this package insert for specimen limitations.

EXPECTED VALUES

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 2059 prospectively-collected specimens tested in the ARCHITECT CORE-M clinical study, 1207 were from individuals living in the United States with increased risk of HBV infection. All 1207 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=1207) consisted of the following race/ethnic groups:

- 582 (48.22%) Caucasian
- 396 (32.81%) African-American
- 176 (14.58%) Hispanic
- 26 (2.15%) Asian
- 4 (0.33%) American Indian/Alaska Native
- 21 (1.74%) Other
- 2 (0.17%) Unknown

The 1207 specimens from the increased risk population were obtained from the following collection locations:

- 417 (34.55%) from Galveston, TX
- 234 (19.39%) from St. Petersburg, FL
- 168 (13.92%) from Dallas, TX
- 121 (10.02%) from Plymouth, MA
- 107 (8.86%) from Miami, FL
- 47 (3.89%) from Chicago, IL
- 45 (3.73%) from Denver, CO
- 34 (2.82%) from Colton, CA
- 34 (2.82%) from High Point, NC

A total of 20 (1.66%) specimens in the increased risk population were reactive in the ARCHITECT CORE-M assay. The number of ARCHITECT CORE-M reactive results observed for the increased risk population at each collection location was:

- 5 (1.20%) from Galveston, TX
- 7 (2.99%) from St. Petersburg, FL
- 2 (1.19%) from Dallas, TX
- 2 (1.65%) from Plymouth, MA
- 1 (0.93%) from Miami, FL
- 3 (6.38%) from Chicago, IL
- 0 (0.00%) from Denver, CO
- 0 (0.00%) from Colton, CA
- 0 (0.00%) from High Point, NC

Of the 1207 specimens, 645 (53.44%) were female and 562 (46.56%) were male. The age was not reported for one specimen. Of the remaining 1206 specimens, the mean age was 39 years (age range: 17 to 82 years). The distribution of ARCHITECT CORE-M reactive, grayzone, and nonreactive results among the increased risk population by age and gender (n=1207) is summarized in the following table.

		ARCHITECT CORE-M Result			
Age Group (years)	**Gender**	**Reactive n (%)**	**Grayzone n (%)**	**Nonreactive n (%)**	**Total**

Age Group (years)	Gender	ARCHITECT CORE-M Result			Total
		Reactive n (%)	Grayzone n (%)	Nonreactive n (%)	
10-19	F	0 (0.00)	0 (0.00)	14 (100.00)	14
	M	0 (0.00)	0 (0.00)	7 (100.00)	7
20-29	F	4 (2.00)	1 (0.50)	195 (97.50)	200
	M	2 (1.72)	1 (0.86)	113 (97.41)	116
30-39	F	3 (2.00)	0 (0.00)	147 (98.00)	150
	M	1 (0.66)	1 (0.66)	149 (98.68)	151
40-49	F	3 (1.92)	2 (1.28)	151 (96.79)	156
	M	2 (1.15)	1 (0.57)	171 (98.28)	174
50-59	F	2 (2.06)	0 (0.00)	95 (97.94)	97
	M	1 (1.11)	1 (1.11)	88 (97.78)	90
60-69	F	2 (8.33)	0 (0.00)	22 (91.67)	24
	M	0 (0.00)	0 (0.00)	15 (100.00)	15
70-79	F	0 (0.00)	0 (0.00)	1 (100.00)	1
	M	0 (0.00)	0 (0.00)	8 (100.00)	8
80-89	F	0 (0.00)	0 (0.00)	3 (100.00)	3
	M	0 (0.00)	0 (0.00)	0 (0.00)	0
Unknown	F	0 (0.00)	0 (0.00)	0 (0.00)	0
	M	0 (0.00)	0 (0.00)	1 (100.00)	1
Total		**20 (1.66)**	**7 (0.58)**	**1180 (97.76)**	**1207**

SPECIFIC PERFORMANCE CHARACTERISTICS

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

The Alinity i Anti-HBc IgM assay/Alinity i analyzer and the ARCHITECT CORE-M assay/ARCHITECT i System utilize the same reagents and sample/reagent ratios. Some performance characteristics for this 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 CLSI EP05-A3.[22] Testing was conducted using 1 lot of the Alinity i Anti-HBc IgM Reagent Kit, 1 lot of Anti-HBc IgM Calibrators, and 1 lot of Anti-HBc IgM Controls and 1 instrument. Two 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 Precision (Total)[a]	
			SD	%CV	SD	%CV
Negative Control	72	0.05	0.005	NA[b]	0.005	NA[b]
Positive Control	72	3.19	0.105	3.3	0.112	3.5
Panel 1	72	0.76	0.029	3.9	0.031	4.1
Panel 2	72	1.14	0.042	3.7	0.042	3.7
Panel 3	72	1.91	0.068	3.5	0.071	3.7
Panel 4	72	1.29	0.046	3.5	0.053	4.1
Panel 5	72	0.59	0.022	3.7	0.026	4.4

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

[b] Not applicable

System Reproducibility

A study was performed based on guidance from CLSI EP05-A2 and CLSI EP15-A2.[23, 24] Testing was conducted at 3 clinical sites using 1 lot of the Alinity i Anti-HBc IgM Reagent Kit, 1 lot of the Alinity i Anti-HBc IgM Calibrators, and 1 lot of the Alinity i Anti-HBc IgM Controls and 1 instrument. Two 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 SD	Within-Run %CV	Within-Day[a] SD	Within-Day[a] %CV	Within-Laboratory Precision (Total)[b] SD	Within-Laboratory Precision (Total)[b] %CV	Precision with Additional Component of Between-Site[c] SD	Precision with Additional Component of Between-Site[c] %CV
Negative Control	120	0.06	0.003	NA[d]	0.003	NA[d]	0.004	NA[d]	0.005	NA[d]
Positive Control	120	3.39	0.105	3.1	0.113	3.3	0.118	3.5	0.153	4.5
High Negative Panel	120	0.70	0.024	3.5	0.026	3.8	0.026	3.8	0.028	4.0
Low Positive Panel	120	1.46	0.043	3.0	0.044	3.0	0.044	3.0	0.056	3.9
Moderate Positive Panel	120	2.13	0.072	3.4	0.072	3.4	0.072	3.4	0.089	4.2

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

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

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

[d] Not applicable

Percent Agreement

A study was performed to compare the anti-HBc IgM assay on the Alinity i analyzer and the ARCHITECT i2000SR using 1 lot each of the Anti-HBc IgM Reagent Kit, Anti-HBc IgM Calibrators, and Anti-HBc IgM Controls. Of the 204 specimens/samples tested, 98 were nonreactive, 12 were grayzone, and 94 were reactive based on the ARCHITECT CORE-M results on the ARCHITECT i2000SR instrument. An aliquot of each specimen/sample 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.

Site	Alinity i	ARCHITECT CORE-M	Negative %	Positive %

	Anti-HBc IgM	Reactive	Grayzone	Nonreactive	Agreement (95% Confidence Interval)[a]	Agreement (95% Confidence Interval)[a]
1	Reactive	93	0	0	100.00 (98/98) (96.23,100.00)	98.94 (93/94) (94.22,99.81)
	Grayzone	1	10	0		
	Nonreactive	0	2	98		
2	Reactive	93	0	0	100.00 (98/98) (96.23,100.00)	98.94 (93/94) (94.22,99.81)
	Grayzone	1	10	0		
	Nonreactive	0	2	98		
3	Reactive	94	0	0	100.00 (98/98) (96.23,100.00)	100.00 (94/94) (96.07,100.00)
	Grayzone	0	10	0		
	Nonreactive	0	2	98		
All	Reactive	280	0	0	100.00 (294/294) (98.71,100.00)	99.29 (280/282) (97.45,99.81)
	Grayzone	2	30	0		
	Nonreactive	0	6	294		

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

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

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

Seroconversion Sensitivity

To determine the seroconversion sensitivity, 9 seroconversion panels obtained from commercial vendors were tested on the Alinity i analyzer using the Alinity i Anti-HBc IgM assay. The panel results were evaluated against the ARCHITECT CORE-M assay and data are summarized in the following table.

Panel ID	Number of Days to Anti-HBc IgM First Reactive Result		Difference in Days to Anti-HBc IgM First Reactive Result (Alinity - ARCHITECT)
	Alinity i Anti-HBc IgM	ARCHITECT CORE-M Assay	
01	71	71	0
03	24	24	0
04	44	55	-11
05	42	42	0
06	41	41	0
07	43	43	0
10	43	43	0
11	140	140	0
12	59	59	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-M assay to detect IgM anti-HBc antibodies in a group of individuals that would normally be tested in a clinical situation. Of the 2159 specimens tested in the ARCHITECT CORE-M clinical study, 1207 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 545 specimens were obtained from individuals living in the United States exhibiting signs and symptoms of hepatitis infection (Population One).

The 1752 specimens in Population One were obtained from the following collection location:

- 458 (26.14%) from Galveston, TX
- 287 (16.38%) from St. Petersburg, FL
- 267 (15.24%) from Dallas, TX
- 228 (13.01%) from Chicago, IL
- 165 (9.42%) from Denver, CO
- 153 (8.73%) from Miami, FL

- 124 (7.08%) from Plymouth, MA
- 36 (2.05%) from Colton, CA
- 34 (1.94%) from High Point, NC

Population One (n=1752) consisted of the following race/ethnic groups:

- 855 (48.80%) Caucasian
- 526 (30.02%) African-American
- 293 (16.72%) Hispanic
- 45 (2.57%) Asian
- 5 (0.29%) American Indian/Alaska Native
- 26 (1.48%) Other
- 2 (0.11%) Unknown

Of the 1752 specimens in Population One, 872 (49.77%) were female and 880 (50.23%) were male. The age was not reported for one specimen. Of the remaining 1751 specimens, the mean age was 42 years (age range: 17 to 83 years).

Specimens were also prospectively collected in Vietnam from 94 individuals at increased risk of HBV infection and 183 individuals with signs and symptoms of hepatitis infection (Population Two). The 277 specimens in Population Two were 100.00% Vietnamese, and 153 (55.23%) were female and 124 (44.77%) were male. The mean age was 36 years (age range: 18 to 68 years).

Each specimen was tested using a comparator IgM anti-HBc assay and 3 HBV reference assays, each detecting a unique serological marker (HBsAg, total anti-HBc, and anti-HBs). The HBV classification was determined for each specimen based on the reactivity patterns of the 4 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 3 clinical sites located in Galveston, TX; Hershey, PA; or Milwaukee, WI using the ARCHITECT CORE-M assay.

Results by Specimen Classification

Following testing with the comparator IgM anti-HBc assay and the 3 reference HBV assays, Population One specimens were assigned an HBV classification using the reactive (+) and nonreactive (-) patterns. There were 17 unique reference marker patterns observed in the ARCHITECT CORE-M clinical study for Population One.

	HBV Reference Markers				
n	**HBsAg**	**IgM Anti-HBc**	**Total Anti-HBc**	**Anti-HBs**	**HBV Classification**
8	+	-	-	-	Early Acute
17	+	+	+	-	Acute
1	+	+	+	I	Chronic
2	+	-	+	+	Chronic
51	+	-	+	-	Chronic
3	+	-	-	+	Chronic
7	-	+	+	+	Recovering Acute
2	-	+	+	-	Recovering Acute/Undetectable HBsAg
220	-	-	+	+	Immune Due to Natural Infection
34	-	-	+	I	Distantly Immune/Anti-HBs Unknown
107	-	-	+	-	Distantly Immune/Anti-HBs Not Detected
351	-	-	-	+	Immune Due to HBV Vaccination
897	-	-	-	-	Susceptible
1	+	+	+	+	Late Acute/Recovering
3	+	-	+	I	Chronic
3	-	+	+	I	Early Recovery
45	-	-	-	I	Unknown
1752					**Total**

I = Indeterminate

Following testing with the comparator IgM anti-HBc assay and the 3 reference HBV assays, Population Two specimens were assigned an HBV classification using the reactive (+) and nonreactive (-) patterns. There were 10 unique reference marker patterns observed in the ARCHITECT CORE-M clinical study for Population Two.

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

	HBsAg	IgM Anti-HBc	Total Anti-HBc	Anti-HBs	
1	+	-	-	-	Early Acute
3	+	-	+	+	Chronic
107	+	-	+	-	Chronic
1	+	-	-	+	Chronic
67	-	-	+	+	Immune Due to Natural Infection
5	-	-	+	I	Distantly Immune/Anti-HBs Unknown
12	-	-	+	-	Distantly Immune/Anti-HBs Not Detected
41	-	-	-	+	Immune Due to HBV Vaccination
37	-	-	-	-	Susceptible
3	+	-	+	I	Chronic
277					**Total**

I = Indeterminate

Comparison of Results

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

	IgM Anti-HBc Comparator						
	Reactive			Negative			
	ARCHITECT CORE-M Interpretation			ARCHITECT CORE-M Interpretation			
HBV Classification	**Reactive n (%)**	**Grayzone n (%)**	**Nonreactive n (%)**	**Reactive n (%)**	**Grayzone n (%)**	**Nonreactive n (%)**	**Total**
Early Acute	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	8 (0.46)	8 (0.46)
Acute	16 (0.91)	1[a] (0.06)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	17

HBV Classification	IgM Anti-HBc Comparator						
	Reactive			Negative			
	ARCHITECT CORE-M Interpretation			ARCHITECT CORE-M Interpretation			
	Reactive n (%)	Grayzone n (%)	Nonreactive n (%)	Reactive n (%)	Grayzone n (%)	Nonreactive n (%)	Total
Chronic	1 (0.06)	0 (0.00)	0 (0.00)	0 (0.00)	4[d] (0.23)	55 (3.14)	60 (3.42)
Recovering Acute	7 (0.40)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	7 (0.40)
Recovering Acute/Undetectable HBsAg	2 (0.11)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.11)
Immune Due to Natural Infection	0 (0.00)	0 (0.00)	0 (0.00)	14[b] (0.80)	5[e] (0.29)	201 (11.47)	220 (12.56)
Distantly Immune/Anti-HBs Unknown	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1[f] (0.06)	33 (1.88)	34 (1.94)
Distantly Immune/Anti-HBs Not Detected	0 (0.00)	0 (0.00)	0 (0.00)	2[c] (0.11)	1[f] (0.06)	104 (5.94)	107 (6.11)
Immune Due to HBV Vaccination	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	351 (20.03)	351 (20.03)
Susceptible	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	897 (51.20)	897 (51.20)
Late Acute/Recovering	1 (0.06)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.06)
Early Recovery	3 (0.17)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.17)
Unknown	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	45 (2.57)	45 (2.57)
Total	**30 (1.71)**	**1 (0.06)**	**0 (0.00)**	**16 (0.91)**	**11 (0.63)**	**1694 (96.69)**	**1752 (100.00)**

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

[b] Two specimens were tested and determined to be negative for HBeAg; positive for anti-

HBe and HBV DNA; and nonreactive by a second FDA-approved IgM anti-HBc assay. Two specimens were tested and determined to be negative for HBeAg; positive for anti-HBe and HBV DNA; and grayzone by a second FDA-approved IgM anti-HBc assay. Four specimens were tested and determined to be negative for HBeAg and HBV DNA; positive for anti-HBe; and nonreactive by a second FDA-approved IgM anti-HBc assay. Five specimens were tested and determined to be negative for HBeAg and HBV DNA; positive for anti-HBe; and grayzone by a second FDA-approved IgM anti-HBc assay. One specimen was tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and grayzone by a second FDA-approved IgM anti-HBc assay.

[c] 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 IgM anti-HBc assay. One specimen was tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA; and grayzone by a second FDA-approved IgM anti-HBc assay.

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

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

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

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

	IgM Anti-HBc Comparator						
	Reactive			**Negative**			
	ARCHITECT CORE-M Interpretation			**ARCHITECT CORE-M Interpretation**			
HBV Classification	**Reactive n (%)**	**Grayzone n (%)**	**Nonreactive n (%)**	**Reactive n (%)**	**Grayzone n (%)**	**Nonreactive n (%)**	**Total**
Early Acute	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.36)	1 (0.36)
Chronic	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1[a] (0.36)	113 (40.79)	114 (41.16)

	IgM Anti-HBc Comparator						
	Reactive			Negative			
	ARCHITECT CORE-M Interpretation			ARCHITECT CORE-M Interpretation			
HBV Classification	**Reactive n (%)**	**Grayzone n (%)**	**Nonreactive n (%)**	**Reactive n (%)**	**Grayzone n (%)**	**Nonreactive n (%)**	**Total**
Immune Due to Natural Infection	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	67 (24.19)	67 (24.19)
Distantly Immune/Anti-HBs Unknown	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	5 (1.81)	5 (1.81)
Distantly Immune/Anti-HBs Not Detected	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	12 (4.33)	12 (4.33)
Immune Due to HBV Vaccination	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	41 (14.80)	41 (14.80)
Susceptible	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	37 (13.36)	37 (13.36)
Total	**0 (0.00)**	**0 (0.00)**	**0 (0.00)**	**0 (0.00)**	**1 (0.36)**	**276 (99.64)**	**277 (100.00)**

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

Percent Agreement

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

HBV Classification	**Positive Percent Agreement %**	**95% Confidence Interval**	**Negative Percent Agreement %**	**95% Confidence Interval**

HBV Classification	Positive Percent Agreement %	95% Confidence Interval	Negative Percent Agreement %	95% Confidence Interval
Early Acute	NA	NA	100.00 (8/8)	63.06 - 100.00
Acute	94.12 (16/17)	71.31 - 99.85	NA	NA
Chronic	100.00 (1/1)	2.50 - 100.00	93.22 (55/59)	83.54 - 98.12
Recovering Acute	100.00 (7/7)	59.04 - 100.00	NA	NA
Recovering Acute/Undetectable HBsAg	100.00 (2/2)	15.81 - 100.00	NA	NA
Immune Due to Natural Infection	NA	NA	91.36 (201/220)	86.84 - 94.72
Distantly Immune/Anti-HBs Unknown	NA	NA	97.06 (33/34)	84.67 - 99.93
Distantly Immune/Anti-HBs Not Detected	NA	NA	97.20 (104/107)	92.02 - 99.42
Immune Due to HBV Vaccination	NA	NA	100.00 (351/351)	98.95 - 100.00
Susceptible	NA	NA	100.00 (897/897)	99.59 - 100.00
Late Acute/Recovering	100.00 (1/1)	2.50 - 100.00	NA	NA
Early Recovery	100.00 (3/3)	29.24 - 100.00	NA	NA
Unknown	NA	NA	100.00 (45/45)	92.13 - 100.00
Total	**96.77 (30/31)**	**83.30 - 99.92**	**98.43 (1694/1721)**	**97.73 - 98.96**

NA = not applicable

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

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

The table below summarizes the percent agreement between ARCHITECT CORE-M and the comparator IgM anti-HBc assay for Population Two 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	NA	NA	99.12 (113/114)	95.21 - 99.98
Immune Due to Natural Infection	NA	NA	100.00 (67/67)	94.64 - 100.00
Distantly Immune/Anti-HBs Unknown	NA	NA	100.00 (5/5)	47.82 - 100.00
Distantly Immune/Anti-HBs Not Detected	NA	NA	100.00 (12/12)	73.54 - 100.00
Immune Due to HBV Vaccination	NA	NA	100.00 (41/41)	91.40 - 100.00
Susceptible	NA	NA	100.00 (37/37)	90.51 - 100.00
Total	**NA**	**NA**	**99.64**	**98.01 - 99.99**

HBV Classification	Positive Percent Agreement %	95% Confidence Interval	Negative Percent Agreement %	95% Confidence Interval
			(276/277)	

NA = not applicable

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

Percent of Positive Specimens and Percent Agreement for Individuals Diagnosed with Acute HBV Infection and Pre-Selected IgM Anti-HBc Positive Specimens

The performance of the ARCHITECT CORE-M assay was evaluated by testing prospectively-collected specimens from 14 individuals diagnosed with acute HBV infection and 16 pre-selected IgM anti-HBc positive specimens. Acute status was defined for the 30 specimens by the 4 HBV serological marker results. The percent of positive ARCHITECT CORE-M specimens for individuals with documented acute HBV infection was 100.00% (14/14, with a 95% confidence interval of 76.84% to 100.00%). The percent of positive ARCHITECT CORE-M specimens for the pre-selected IgM anti-HBc positive specimens was 100.00% (16/16, with a 95% confidence interval of 79.41% to 100.00%).

For individuals diagnosed with acute HBV infection and for the pre-selected IgM anti-HBc positive specimens combined, the positive percent agreement between the ARCHITECT CORE-M assay results and the comparator IgM anti-HBc assay results was 100.00% (30/30, with a 95% confidence interval of 88.43% to 100.00%).

Clinical Performance in a Pediatric Population

The performance of the ARCHITECT CORE-M 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 125 prospectively-collected pediatric specimens from Population One (n = 81), Population Two (n = 36), and pre-selected IgM anti-HBc positive specimens (n = 8).

For the surplus pediatric specimens, the negative percent agreement between the ARCHITECT CORE-M assay results and the comparator IgM anti-HBc assay results was 100.00% (100/100, with a 95% confidence interval of 96.38% to 100.00%). The distribution

of the ARCHITECT CORE-M reactive, grayzone, and nonreactive results for the surplus pediatric population is summarized by age and gender in the following table.

Age Group (years)	Gender	ARCHITECT CORE-M Result			Total
		Reactive n (%)	Grayzone n (%)	Nonreactive n (%)	
2-12	F	0 (0.00)	0 (0.00)	25 (100.00)	25
	M	0 (0.00)	0 (0.00)	25 (100.00)	25
13-18	F	0 (0.00)	0 (0.00)	32 (100.00)	32
	M	0 (0.00)	0 (0.00)	18 (100.00)	18
Total		**0 (0.00)**	**0 (0.00)**	**100 (100.00)**	**100**

For the prospectively-collected pediatric specimens (Population One [n = 81], Population Two [n = 36], and pre-selected IgM anti-HBc positive specimens [n = 8]), the positive percent agreement between the ARCHITECT CORE-M assay results and the comparator IgM anti-HBc assay results was 100.00% (8/8, with a 95% confidence interval of 63.06% to 100.00%) and the negative percent agreement between the ARCHITECT CORE-M assay results and the comparator IgM anti-HBc assay results was 99.15% (116/117, with a 95% confidence interval of 95.33% to 99.98%). The distribution of the ARCHITECT CORE-M reactive, grayzone, and nonreactive results for the prospectively-collected pediatric population is summarized by age and gender in the following table.

Age Group (years)	Gender	ARCHITECT CORE-M Result			Total
		Reactive n (%)	Grayzone n (%)	Nonreactive n (%)	
2-12	F	0 (0.00)	0 (0.00)	0 (0.00)	0
	M	1 (100.00)	0 (0.00)	0 (0.00)	1
13-18	F	1 (14.29)	0 (0.00)	6 (85.71)	7
	M	1 (16.67)	0 (0.00)	5 (83.33)	6
19-21	F	1 (1.54)	0 (0.00)	64 (98.46)	65
	M	5 (10.87)	0 (0.00)	41 (89.13)	46
Total		**9 (7.20)**	**0 (0.00)**	**116 (92.80)**	**125**

Analytical Specificity

The ARCHITECT CORE-M 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-M assay and the comparator IgM 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.

		Comparator IgM Anti-HBc Assay					
		Nonreactive			**Reactive**		
		ARCHITECT CORE-M			**ARCHITECT CORE-M**		
Category	**n**	**NR[a]**	**GZ[a]**	**R[a]**	**NR[a]**	**GZ[a]**	**R[a]**
Anti-nuclear antibody (ANA)	10	10	0	0	0	0	0
Cytomegalovirus (anti-CMV positive)	10	10	0	0	0	0	0
Elevated IgG	10	10	0	0	0	0	0
Elevated IgM	5	5	0	0	0	0	0
Epstein-Barr Virus (anti-EBV positive)	10	10	0	0	0	0	0
HBV vaccine recipient	8	8	0	0	0	0	0
Hepatitis A Virus (anti-HAV IgM positive)	10	10	0	0	0	0	0
Hepatitis C Virus (anti-HCV positive)	10	10	0	0	0	0	0
Herpes Simplex Virus (anti-HSV positive) IgG	4	4	0	0	0	0	0
Human Anti-Mouse Antibodies (HAMA) positive	7	7	0	0	0	0	0
Human	10	10	0	0	0	0	0

		Comparator IgM Anti-HBc Assay					
		Nonreactive			Reactive		
		ARCHITECT CORE-M			ARCHITECT CORE-M		
Category	**n**	**NR[a]**	**GZ[a]**	**R[a]**	**NR[a]**	**GZ[a]**	**R[a]**
Immunodeficiency Virus (anti-HIV-1 positive)							
Human Immunodeficiency Virus (anti-HIV-2 positive)	10	10	0	0	0	0	0
Influenza vaccine recipient	10	10	0	0	0	0	0
Multiparous female	10	10	0	0	0	0	0
Multiple myeloma	2	2	0	0	0	0	0
Mumps virus	10	10	0	0	0	0	0
Non-Hodgkin's lymphoma	6	6	0	0	0	0	0
Non-viral liver disease	12	12	0	0	0	0	0
Rheumatoid factor positive	10	9	0	0	1[b]	0	0
Rubella	10	10	0	0	0	0	0
Rubeola virus	9	9	0	0	0	0	0
Syphilis	10	10	0	0	0	0	0
Systemic Lupus Erythematosus (SLE)	9	9	0	0	0	0	0
Toxoplasmosis IgG positive	9	9	0	0	0	0	0
Varicella Zoster Virus (anti-VZV positive)	4	4	0	0	0	0	0
Yeast infection	7	7	0	0	0	0	0
Total	**222**	**221**	**0**	**0**	**1**	**0**	**0**

[a] NR = Nonreactive, GZ= Grayzone, R = Reactive

[b] This specimen was tested and determined to be reactive for HBsAg, but did not confirm; negative for total anti-HBc; and positive for anti-HBs. A second FDA-approved IgM anti-HBc assay was performed and the specimen was determined to be negative.

Interference

At the concentrations listed below, bilirubin (conjugated and unconjugated), hemoglobin, total protein, and triglycerides showed less than 10% interference in the ARCHITECT CORE-M 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-M 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 Percent Differences		
Tube Type	**< 10%**	**> 10% to < 20%**	**> 20%**
Glass Serum	87.8% (36/41)	12.2% (5/41)	0.0% (0/41)
Plastic Serum Separator	82.9% (34/41)	14.6% (6/41)	2.4% (1/41)

Tube Type	Distribution of Absolute Percent Differences		
	< 10%	> 10% to < 20%	> 20%
Plastic Dipotassium EDTA	80.5% (33/41)	17.1% (7/41)	2.4% (1/41)
Plastic Sodium Heparin	82.9% (34/41)	14.6% (6/41)	2.4% (1/41)
Plastic Lithium Heparin Plasma Separator	80.5% (33/41)	17.1% (7/41)	2.4% (1/41)

Seroconversion Panels

The ability of the ARCHITECT CORE-M assay to detect IgM anti-HBc was evaluated by testing 8 seroconversion panels obtained from 2 commercial vendors.

The results were compared to the results of an FDA-approved IgM anti-HBc assay (reference). IgM anti-HBc was detected by ARCHITECT CORE-M coincident with the reference IgM anti-HBc assay in 8 panels.

The profiles of the 8 seroconversion panels were characteristic of an acute HBV infection progressing to eventual recovery and immunity to HBV. ARCHITECT CORE-M detected IgM anti-HBc following detection of HBsAg in all panels during the acute stage of the disease.

IgM anti-HBc remained detectable over a range of 2 to 10 months in the 8 panels. The overall ARCHITECT CORE-M results were consistent with the known serological profile of each panel.

Neonate Serum

A study was conducted to evaluate whether neonate samples may be tested with the ARCHITECT CORE-M assay. Cord blood serum was used as a surrogate for neonate serum. Twenty-one matched cord blood and maternal serum samples were spiked with IgM 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). None of the samples were initially reactive. The data obtained upon spiking are summarized in the following table, showing the amount of bias for the cord blood serum samples from the matched maternal serum samples. For cord blood serum samples with ≥ 10% bias, one sample exhibited negative bias and the remaining samples exhibited positive bias when compared to the matched maternal serum samples.

Analyte Level S/CO	Distribution of % Bias			
	< 10%	≥ 10% to < 20%	≥ 20% to < 30%	≥ 30%

Analyte Level S/CO	Distribution of % Bias			
	< 10%	≥ 10% to < 20%	≥ 20% to < 30%	≥ 30%
0.80	66.7% (14/21)	28.6% (6/21)	4.8% (1/21)	0.0% (0/21)
1.20	52.4% (11/21)	38.1% (8/21)	9.5% (2/21)	0.0% (0/21)

BIBLIOGRAPHY

1. Overby LR, Ling C-M, Decker RH, et al. Serodiagnostic profiles of viral hepatitis. In: Szmuness W, Alter HJ, Maynard JE, editors. *Viral Hepatitis*. Philadelphia, PA: Franklin Institute Press, 1981:169-182.
2. Kryger P, Aldershvile J, Mathiesen LR, et al. Acute type B hepatitis among HBsAg negative patients detected by anti-HBc IgM. *Hepatology* 1982;2(1):50-53.
3. Mortimer PP, Vandervelde EM, Parry JV, et al. The anti-HBc IgM response in the acute and convalescent phases of acute hepatitis. *J Infect* 1981;3:339-347.
4. Lemon SM, Gates NL, Simms TE, et al. IgM antibody to hepatitis B core antigen as a diagnostic parameter of acute infection with hepatitis B virus. *J Infect Dis* 1981;143(6):803-809.
5. Chau KH, Hargie MP, Decker RH, et al. Serodiagnosis of recent hepatitis B infection by IgM class anti-HBc. *Hepatology* 1983;3(2):142-149.
6. Hoofnagle JH, Ponzetto A, Mathiesen LR, et al. Serological diagnosis of acute viral hepatitis. *Dig Dis Sci* 1985;30(11):1022-1027.
7. Sjogren M, Hoofnagle JH. Immunoglobulin M antibody to hepatitis B core 7. antigen in patients with chronic type B hepatitis. *Gastroenterology* 1985;89: 252-258.
8. Surrenti C, Ambu S, Patussi V, et al. Diagnostic significance of anti-HBc IgM (RIA) in healthy HBsAg carriers and in chronic hepatitis B. *J Med Virol* 1986;18:229-234.
9. Perrillo RP, Chau KH, Overby LR, et al. Anti-hepatitis B core immunoglobulin M in the serologic evaluation of hepatitis B virus infection and simultaneous infection with type B, delta agent, and non-A, non-B viruses. *Gastroenterology* 1983;85:163-167.
10. Hawkes RA, Boughton CR, Ferguson V, et al. Use of immunoglobulin M antibody to hepatitis B core antigen in diagnosis of viral hepatitis. *J Clin Microbiol* 1980;11(6):581-583.
11. Roggendorf M, Deinhardt F, Frösner GG, et al. Immunoglobulin M antibodies to hepatitis B core antigen: Evaluation of enzyme immunoassay for diagnosis of hepatitis B virus infection. *J Clin Microbiol* 1981;13(4):618-626.
12. Kryger P. Significance of anti-HBc IgM in the differential diagnosis of viral hepatitis. *J Virol Methods* 1985;10:283-289.

13. Govindarajan S, Ashcavai M, Chau KH, et al. Evaluation of enzyme immunoassay for anti-HBc IgM in the diagnosis of acute hepatitis B virus infection. *Am J Clin Pathol* 1984;82(3):323-325.
14. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
15. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
16. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
17. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition.* CLSI Document M29-A4. Wayne, PA: CLSI; 2014.
18. Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions.* 4th ed. CLSI Guideline C24. Wayne, PA: CLSI; 2016.
19. Primus FJ, Kelley EA, Hansen HJ, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* 1988;34(2):261-264.
20. Schroff RW, Foon KA, Beatty SM, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985;45(2):879-885.
21. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem* 1988;34(1):27-33.
22. Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline—Third Edition.* CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.
23. Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition.* CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.
24. Clinical and Laboratory Standards Institute (CLSI). *User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition.* CLSI Document EP15-A2. Wayne, PA: CLSI; 2005.
25. Wayne, PA: Clinical and Laboratory Standards Institute, 2010.