Alinity i Anti-HBs-05

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SUPERSEDES: Procedure titled _____

INTENDED USE

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

It is intended for quantitative measurement of antibody response following hepatitis B virus (HBV) vaccination, determination of HBV immune status, and for the laboratory diagnosis of HBV disease associated with HBV infection when used 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-HBs assay for use in screening blood, plasma, or tissue donors has not been established.

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

The Alinity i Anti-HBs assay determines the concentration of anti-HBs present in human serum and plasma.

HBV is a major cause of liver disease and is endemic worldwide. The virus can be transmitted through direct contact with blood and body fluids including sexual contact. The incubation period for HBV infection can range from 1 to 6 months averaging around 6 to 8 weeks. Typical acute clinical symptoms of HBV hepatitis include malaise, jaundice, gastroenteritis, and fever. However, HBV infection can also result in subclinical anicteric hepatitis, fulminant hepatitis, or chronic or persistent hepatitis. Although most adult patients with HBV infection completely recover from acute illness and clear the virus, 5 to 10% of patients with HBV may become chronic carriers. It is estimated that over 300 million people worldwide are chronic carriers of the virus. Chronic HBV infection is associated with the development of hepatocellular carcinoma. In HBV infected neonates, approximately 90% develop chronic hepatitis B infection.*1*, *2*, *3*

Anti-HBs assays are often used to determine the success of hepatitis B vaccination. The presence of anti-HBs has been shown to be important in protection against HBV infection.*4* Numerous studies have demonstrated the effectiveness of the hepatitis B vaccine to stimulate the immune system to produce anti-HBs and to prevent HBV infection.*5*, *6*, *7*

Assays for anti-HBs are also used to monitor the convalescence and recovery of hepatitis B infected individuals. The presence of anti-HBs after acute HBV infection and loss of hepatitis B virus surface antigen (HBsAg) can be a useful indicator of disease resolution. Detection of anti-HBs in an asymptomatic individual may indicate previous exposure to HBV or HBV vaccination.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the quantitative determination of **anti-HBs in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.**

Sample and recombinant HBsAg (rHBsAg) coated paramagnetic microparticles are combined and incubated. The anti-HBs present in the sample binds to the rHBsAg coated microparticles. The mixture is washed. Recombinant HBsAg 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 a direct relationship between the amount of anti-HBs in the sample and the RLUs detected by the system optics.

The concentration of anti-HBs in the specimen is determined using an active Alinity i Anti-HBs calibration curve.

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

REAGENTS

Kit Contents

Alinity i Anti-HBs Reagent Kit 07P88

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

REF	07P8851	07P8856
Tests per cartridge	100	600
Number of cartridges per kit	2	2
Tests per kit	200	1200
MICROPARTICLES	5.0 mL	19.9 mL
CONJUGATE	6.1 mL	31.6 mL
SPECIMEN DILUENT	5.9 mL	14.7 mL

MICROPARTICLES Hepatitis B surface (*E. coli*, recombinant) antigen (subtypes *ad* and *ay*) coated microparticles in TRIS buffer with protein (bovine) stabilizer (76 µM). Minimum concentration: 0.125% solids. Preservatives: antimicrobial agents and sodium azide.

CONJUGATE Hepatitis B surface (*E. coli*, recombinant) antigen (subtypes *ad* and *ay*) acridinium-labeled conjugate in MES buffer with protein (112.5 g/L bovine serum and 102.7 g/L human plasma) stabilizer. Minimum concentration: 0.10 µg/mL. Preservatives: antimicrobial agent and sodium azide.

SPECIMEN DILUENT Contains recalcified human plasma. Preservatives: sodium azide and ProClin 950.

Warnings and Precautions

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

Safety Precautions

CAUTION: This product contains human-sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human-sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be 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.[8, 9, 10, 11]

The human-sourced material used in the conjugate is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, anti-HCV, anti-HBs, and anti-HBc.

The human-sourced material used in the specimen diluent is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, anti- HCV, and anti-HBs.

The following warnings and precautions apply to: MICROPARTICLES and 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: SPECIMEN DILUENT	
	
WARNING	Contains methylisothiazolones 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.

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 2 hours 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

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
			cartridge 10 times and place in an upright position for 2 hours 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-HBs 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.**

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
mIU/mL	1	IU/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

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

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

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 other 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 21°C to 22°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 to 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 the storage limitations listed above.

PROCEDURE

Materials Provided

07P88 Alinity i Anti-HBs Reagent Kit

Materials Required but not Provided

- Alinity i Anti-HBs assay file
- 07P8801 Alinity i Anti-HBs Calibrators
- 07P8810 Alinity i Anti-HBs Controls
- 07P8841 Alinity i Anti-HBs Specimen Diluent
- 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: 125 µL
- Sample volume for each additional test from same sample cup: 75 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: 75 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

- Refer to the Alinity i Anti-HBs calibrator package insert and/or Alinity i Anti-HBs 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 with anti-HBs values exceeding 1000 mIU/mL (1000 IU/L) are flagged with the code "> 1000 mIU/mL" ("> 1000 IU/L") and **may be diluted** with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

For results up to 25,000 mIU/mL (25,000 IU/L).

The system performs a **1:25** dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor.

Manual Dilution Procedure

For results up to 100,000 mIU/mL (100,000 IU/L). Suggested dilution: 1:100

It is recommended that dilutions not exceed 1:100.

Add 10 µL of the sample to 990 µL of Alinity i Anti-HBs Specimen Diluent (07P8841).

The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. All assays selected for that order will be diluted. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result. The concentration reported by the Alinity i analyzer MUST be greater than or equal to 1000 mIU/mL (1000 IU/L). If the reported concentration is less than 1000 mIU/mL (1000 IU/L), make a smaller dilution.

Automated Dilution Protocol Combined with Manual Dilution Procedure

For results up to 2,500,000 mIU/mL (2,500,000 IU/L).

Suggested dilution: 1:100

It is recommended that dilutions not exceed 1:100.

Add 10 µL of the sample to 990 µL of Alinity i Anti-HBs Specimen Diluent (07P8841).

Order the Automated Dilution Protocol (1:25 dilution) using the manually diluted 1:100 sample.

The concentration reported by the Alinity i analyzer MUST be greater than 250 mIU/mL (250 IU/L). Multiply the result (from the Automated Dilution Protocol) by the manual dilution factor (e.g., 100) to obtain the final sample concentration. If the reported concentration is less than 250 mIU/mL (250 IU/L), make a smaller dilution.

For detailed information on ordering dilutions, **refer to the Alinity ci-series Operations Manual, Section 5.**

Calibration

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

Calibration Range: 0 - 1000 mIU/mL.

Calibrators A-F are tested in duplicate. Calibrator vials are placed directly on the instrument and automatically processed using the bar code on the calibrator vial. Alternatively, calibrators can be pipetted into sample cups. If the calibrators are pipetted into sample cups, the calibration must be manually ordered.

Each assay control must be tested to evaluate the assay calibration.

For instructions on ordering and loading controls on the instrument**, refer to the Alinity ci-series Operations Manual, Section 5.**

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

- Ensure that assay control values are within the concentration 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, as described in the Quality Control Procedures section of this package insert, used to monitor and control system performance.
- 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 also need to be recalibrated after specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the assay.

Quality Control Procedures

The Alinity i Anti-HBs 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-HBs assay is that a single sample of each control level be tested:

- Once every day testing performed
- After performing calibration
- After instrument service procedures or maintenance that may affect assay performance have been performed.

Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated sample results are invalid and the samples must be retested. Recalibration may be indicated.

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

These results should be applied to your laboratory's quality control practices. In addition, the laboratory must ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Unless specified, target values and ranges provided with the commercial control product insert are guidelines only and should not be used for quality control purposes.

To troubleshoot control values that fall outside the control range**, refer to the Alinity ci-series Operations Manual, Section 10, Observed Problems.**

For instructions on ordering patient samples, **refer to the Alinity ci-series Operations Manual, Section 5.**

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 for general quality control recommendations.[12]

RESULTS

Calculation

The Alinity i Anti-HBs and ARCHITECT AUSAB assays utilize a 4 Parameter Logistic Curve fit data reduction method (4PLC, X-weighted) to generate a calibration and results.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

Interpretation of Results

Initial Results		
Anti-HBs mIU/mL (IU/L)	**Instrument Interpretation**	**Retest Procedure**
< 8.00	Nonreactive	No retest required.
≥ 8.00 to < 12.00	Grayzone	Retest in duplicate.
≥ 12.00	Reactive	No retest required.

Anti-HBs Results

Initial Result mIU/mL (IU/L)	Retest Result mIU/mL (IU/L)	Result	Final Interpretation
< 8.00	No retest required.	Nonreactive	Individual is considered not immune to HBV infection.
≥ 8.00 to < 12.00	Both of the duplicate retest results are < 8.00.	Nonreactive	Individual is considered not immune to HBV infection.
	One or both of the duplicate retest results are ≥ 8.00 to < 12.00.	Grayzone	The immune status of the individual should be further assessed by considering other factors, such as clinical status, follow-up testing, associated risk factors, and the use of additional diagnostic information.
	Both of the duplicate retest results are ≥ 12.00.	Reactive	Individual is considered immune to HBV infection.
≥ 12.00	No retest required.	Reactive	Individual is considered immune to 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

- For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection.
- A non-reactive test result does not exclude the possibility of exposure to hepatitis B virus.
- Results obtained with the Alinity i Anti-HBs assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- Results from immunosuppressed patients should be interpreted with caution.
- Assay does not differentiate between vaccines and natural infections.
- Performance characteristics have not been established for therapeutic monitoring.

- A reactive anti-HBs result does not exclude co-infection by another hepatitis virus.

EXPECTED VALUES

Data in the Expected Values 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. When the ARCHITECT AUSAB assay was restandardized to the 2nd International WHO, the data/specimens from several clinical studies were reanalyzed/recalculated or retested.

Increased Risk Population

Of the 2387 specimens tested in the ARCHITECT AUSAB clinical study, 1312 were from individuals with increased risk of HBV infection. All 1312 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.

The increased risk population (n=1312) consisted of the following race/ethnic groups:

- 624 (47.56%) Caucasian
- 476 (36.28%) African-American
- 167 (12.71%) Hispanic
- 19 (1.45%) Asian
- 6 (0.46%) American Indian/Alaska Native
- 20 (1.52%) Other

The 1312 specimens from the increased risk population were obtained from the following collection locations:

- 741 (56.48%) from Galveston, TX
- 185 (14.08%) from High Point, NC
- 99 (7.53%) from Plymouth, MA
- 76 (5.78%) from Colton, CA
- 59 (4.49%) from Dallas, TX
- 56 (4.26%) from St. Petersburg, FL
- 52 (3.96%) from Miami, FL
- 36 (2.74%) from Denver, CO
- 8 (0.61%) from Chicago, IL

A total of 533 (40.63%) of the specimens in the increased risk population were reactive in the

ARCHITECT AUSAB assay. The number of ARCHITECT AUSAB reactive results observed for the increased risk population at each collection location was:

- 274 of 741 (36.98%) from Galveston, TX
- 103 of 185 (55.68%) from High Point, NC
- 29 of 99 (29.29%) from Plymouth, MA
- 35 of 76 (46.05%) from Colton, CA
- 16 of 59 (27.12%) from Dallas, TX
- 16 of 56 (28.57%) from St. Petersburg, FL
- 33 of 52 (63.46%) from Miami, FL
- 24 of 36 (66.67%) from Denver, CO
- 3 of 8 (37.50%) from Chicago, IL

Of the 1312 specimens, 815 (62.12%) were female and 497 (37.88%) were male. The age was not reported for 3 specimens. Of the remaining 1309 specimens, the mean age was 40 years (age range: 18 to 75 years). The distribution of ARCHITECT AUSAB reactive, grayzone, and nonreactive results among the increased risk population by age and gender (n=1309) is summarized in the following table.

		ARCHITECT AUSAB Result			
Age Group (years)	**Gender**	**Reactive n (%)**	**Grayzone n (%)**	**Nonreactive n (%)**	**Total**
10-19	F	9 (64.29)	1 (7.14)	4 (28.57)	14
	M	7 (63.64)	0 (0.00)	4 (36.36)	11
20-29	F	82 (44.57)	2 (1.09)	100 (54.35)	184
	M	35 (36.08)	1 (1.03)	61 (62.89)	97
30-39	F	77 (41.85)	2 (1.09)	105 (57.07)	184
	M	32 (29.91)	1 (0.93)	74 (69.16)	107
40-49	F	105 (42.00)	5 (2.00)	140 (56.00)	250
	M	55 (34.59)	1 (0.63)	103 (64.78)	159
50-59	F	66 (48.18)	5 (3.65)	66 (48.18)	137
	M	28 (25.93)	5 (4.63)	75 (69.44)	108
60-69	F	23 (65.71)	1 (2.86)	11 (31.43)	35
	M	3 (25.00)	0 (0.00)	9 (75.00)	12
70-79	F	3 (37.50)	1 (12.50)	4 (50.00)	8

Age Group (years)	Gender	ARCHITECT AUSAB Result			Total
		Reactive n (%)	Grayzone n (%)	Nonreactive n (%)	
	M	2 (66.67)	0 (0.00)	1 (33.33)	3
Total		**527 (40.26)**	**25 (1.91)**	**757 (57.83)**	**1309**[a]

[a] Age was not reported for three subjects.

Pediatric Population

Of the 2387 specimens tested in the ARCHITECT AUSAB clinical study, 114 were from a pediatric population. The specimens were obtained from a commercial vendor, which collected the specimens from a collection site located in Fall River, MA. The specimens were obtained from children ages greater than 1 month to 18 years.

The data are summarized by age and gender in the following table.

Age Group (years)[a]	Gender	Reactive n (%)	Grayzone n (%)	Nonreactive n (%)	Total
Under 2	F	8 (80.00)	1 (10.00)	1 (10.00)	10
	M	11 (84.62)	0 (0.00)	2 (15.38)	13
2 to 12	F	8 (44.44)*	2 (11.11)*	8 (44.44)	18
	M	7 (18.92)*	5 (13.51)	25 (67.57)	37
13 to 18	F	18 (75.00)	1 (4.17)	5 (20.83)	24
	M	8 (66.67)	0 (0.00)	4 (33.33)	12
Total		**60 (52.63)**	**9 (7.89)**	**45 (39.47)**	**114**

[a] Children with ages under 2 are at least 1 month old.

* A total of 6 specimens were excluded due to insufficient volume for retesting.

SPECIFIC PERFORMANCE CHARACTERISTICS

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

The Alinity i analyzer and the 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 CLSI EP05-A3.[13] Testing was conducted using 1 lot of the Alinity i Anti-HBs Reagent Kit, 1 lot of anti-HBs calibrators, and 1 lot of anti-HBs controls and 1 instrument. Two controls and 6 panels were assayed in replicates of 3 (to obtain a minimum of 2 replicates) at 2 separate times per day on 12 different days.

		Mean (mIU/mL)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
Sample	**n**	**(IU/L)**	**SD**	**%CV**	**SD**	**%CV**
Negative Control	71[b]	0.18	0.089	N/A[c]	0.096	N/A[c]
Positive Control	72	14.72	0.290	2.0	0.347	2.4
Panel 1	72	4.32	0.180	4.2	0.194	4.5
Panel 2	72	7.49	0.252	3.4	0.293	3.9
Panel 3	71[b]	11.04	0.255	2.3	0.349	3.2
Panel 4	71[b]	48.96	0.941	1.9	1.070	2.2
Panel 5	72	844.04	13.203	1.6	17.610	2.1
Panel 6	72	3.71	0.156	4.2	0.190	5.1

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

[b] Replicate lost due to instrument error.

[c] Not applicable

System Reproducibility

A study was performed based on guidance from CLSI EP05-A2 and EP15-A2.[14, 15] Testing was conducted at 3 clinical sites using 1 lot each of the Alinity i Anti-HBs Reagent Kit, the Alinity i Anti-HBs Calibrators, and the Alinity i Anti-HBs Controls and 1 instrument per site. Two controls and 5 panels were assayed in replicates of 4 at 2 separate times per day for 5 days.

Sample	N	Grand Mean (mIU/mL) (IU/L)	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	0.04	0.104	N/A[d]	0.104	N/A[d]	0.104	N/A[d]	0.104	N/A[d]
Positive Control	120	14.44	0.318	2.2	0.346	2.4	0.380	2.6	0.603	4.2
Panel 1	120	4.18	0.232	5.6	0.242	5.8	0.243	5.8	0.262	6.3
Panel 2	120	8.07	0.301	3.7	0.317	3.9	0.317	3.9	0.348	4.3
Panel 3	120	12.27	0.387	3.2	0.396	3.2	0.396	3.2	0.499	4.1
Panel 4	120	48.90	1.175	2.4	1.439	2.9	1.439	2.9	2.024	4.1
Panel 5	120	903.40	25.761	2.9	25.761	2.9	26.891	3.0	33.179	3.7

[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 variability and between site variance components.

[d] Not applicable

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2. Testing was conducted using 3 lots of the Alinity i Anti-HBs Reagent Kit on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.[16]

	mIU/mL (IU/L)
LoB[a]	0.33
LoD[b]	0.51
LoQ[c]	**3.31**

[a] The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b] The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ was determined from n ≥ 60 replicates of low-analyte level samples and is defined as the lowest concentration at which a total allowable error of **30%** was met.

Linearity

A study was performed based on guidance from CLSI EP06-A.[17]

This assay is linear from **3.31 to 1000.00** mIU/mL (3.31 to 1000.00 IU/L).

Percent Agreement

A study was performed to compare the anti-HBs assay on the Alinity i analyzer and the ARCHITECT i2000SR system using 1 lot each of the Alinity i Anti-HBs Reagent Kit, Alinity i Anti-HBs Calibrators, and Alinity i Anti-HBs Controls. Of the 255 specimens tested, 99 were negative, 146 were positive, and 10 were grayzone based on the ARCHITECT AUSAB results on the ARCHITECT i2000SR instrument. An aliquot of each 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.

		ARCHITECT AUSAB			Negative % Agreement (95% Confidence Interval)[a]	Positive % Agreement (95% Confidence Interval)[a]
Site	**Alinity i Anti-HBs**	**Reactive**	**Grayzone**	**Nonreactive**		
1	Reactive	145	0	0	98.99 (98/99) (94.50, 99.82)	99.32 (145/146) (96.22, 99.88)
	Grayzone	1	10	1		
	Nonreactive	0	0	98		
2	Reactive	145	0	0	100.00 (99/99) (96.26, 100.00)	99.32 (145/146) (96.22, 99.88)
	Grayzone	1	10	0		
	Nonreactive	0	0	99		
3	Reactive	146	0	0	100.00 (99/99) (96.26, 100.00)	100.00 (146/146) (97.44, 100.00)
	Grayzone	0	10	0		
	Nonreactive	0	0	99		
All	Reactive	436	0	0	99.66 (296/297)	99.54 (436/438)
	Grayzone	2	30	1		

Site	Alinity i Anti-HBs	ARCHITECT AUSAB			Negative % Agreement (95% Confidence Interval)[a]	Positive % Agreement (95% Confidence Interval)[a]
		Reactive	Grayzone	Nonreactive		
	Nonreactive	0	0	296	(98.12, 99.94)	(98.35, 99.87)

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

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

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

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 AUSAB assay to detect anti-HBs antibodies in a group of individuals that would normally be tested in a clinical situation. Of the 2387 specimens tested in the ARCHITECT AUSAB clinical study, 1312 specimens were obtained from individuals with increased risk of HBV infection due to lifestyle, behavior, occupation, disease state, or a known exposure event and 704 specimens were obtained from individuals exhibiting signs and symptoms of hepatitis infection.

The specimens (n=2016) consisted of the following race/ethnic groups:

- 1066 (52.88%) Caucasian
- 576 (28.57%) African-American
- 295 (14.62%) Hispanic

- 40 (1.98%) Asian
- 9 (0.45%) American Indian/Alaska Native
- 30 (1.49%) Other

The specimens (n=2016) were obtained from the following collection locations:

- 792 (39.29%) from Galveston, TX
- 341 (16.90%) from Plymouth, MA
- 185 (9.17%) from High Point, NC
- 166 (8.23%) from Chicago, IL
- 123 (6.10%) from Denver, CO
- 118 (5.85%) from Colton, CA
- 117 (5.80%) from Dallas, TX
- 89 (4.41%) from Miami, FL
- 85 (4.21%) from St. Petersburg, FL

Of the 2016 specimens, 1060 (52.58%) were female and 956 (47.42%) were male. The age was not reported for 3 specimens. Of the remaining 2013 specimens, the mean age was 41 years (age range: 18 to 83 years). Each specimen was tested using a comparator anti-HBs assay and 3 HBV reference assays, each detecting a unique serological marker (HBsAg, anti-HBc IgM, total anti-HBc). 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 manufacturers' instructions. Each specimen was also tested at 1 of 3 clinical sites located in Galveston, TX; Hershey, PA; or Milwaukee, WI using the ARCHITECT AUSAB assay.

Results by Specimen Classification

Following testing with the comparator anti-HBs assay and the 3 reference HBV assays, the 2016 specimens from the increased risk and signs and symptoms populations were assigned an HBV classification according to the following table. There were 19 unique reference marker patterns observed in the ARCHITECT AUSAB clinical study.

HBV Reference Markers				
HBsAg[a]	**Anti-HBc IgM[a]**	**Total Anti-HBc[a]**	**Anti-HBs[a]**	**HBV Classification**
+	-	-	-	Early Acute
+	+	+	-	Acute
+	+	+	I	Chronic
+	-	+	+	Chronic
+	-	+	-	Chronic
+	-	-	+	Chronic
+	-	+	I	Chronic
-	+	+	+	Recovering Acute
-	+	-	+	Recovering Acute
-	+	+	-	Recovering Acute/Undetectable HBsAg
-	+	+	I	Early Recovery
+	+	+	+	Late Acute/Recovering
-	+	-	-	Possible Recovering Acute/Undetectable HBsAg
-	-	+	+	Immune Due to Natural Infection
-	-	+	I	Distantly Immune/Anti-HBs Unknown
-	-	+	-	Distantly Immune/Anti-HBs Not Detected
-	-	-	+	Immune Due to HBV Vaccination
-	-	-	I	Unknown
-	-	-	-	Susceptible

[a] + = reactive, - = nonreactive, I = Indeterminate

Comparison of Results

The following table compares the ARCHITECT AUSAB assay results with comparator anti-

HBs assay results for each of the HBV classifications for the increased risk and signs and symptoms populations. The data are summarized in the following table.

	Comparator Anti-HBs Interpretation																			
	Positive						Indeterminate						Negative							
	ARCHITECT AUSAB Interpretation						ARCHITECT AUSAB Interpretation						ARCHITECT AUSAB Interpretation							
	R[a]		GZ[a]		NR[a]		R[a]		GZ[a]		NR[a]		R[a]		GZ[a]		NR[a]		Total	
HBV Classification	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Early Acute	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	2	0.10	2	0.10
Acute	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	5	0.25	5	0.25
Late Acute/Recovering	0	0.00	0	0.00	1	0.05	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.05
Early Recovering	0	0.00	0	0.00	0	0.00	2	0.10	0	0.00	1	0.05	0	0.00	0	0.00	0	0.00	3	0.15
Recovering Acute	4	0.20	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	4	0.20
Chronic	3	0.15	0	0.00	0	0.00	1	0.05	1	0.05	2	0.10	0	0.00	0	0.00	35	1.74	42	2.08
Immune Due to Natural Infection	190	9.42	3	0.15	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	193	9.57
Distantly Immune/ Anti-HBs Unknown	0	0.00	0	0.00	0	0.00	20	0.99	7	0.35	4	0.20	0	0.00	0	0.00	0	0.00	31	1.54
Distantly Immune/ Anti-HBs Not Detected	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	11	0.55	13	0.64	83	4.12	107	5.31
Immune Due to HBV Vaccination	494	24.50	13	0.64	1	0.05	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	508	25.20
Unknown	0	0.00	0	0.00	0	0.00	31	1.54	20	0.99	15	0.74	0	0.00	0	0.00	0	0.00	66	3.27
Susceptible	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	9	0.45	5	0.25	1040	51.59	1054	52.28
Total	691	34.28	16	0.79	2	0.10	54	2.68	28	1.39	22	1.09	20	0.99	18	0.89	1165	57.79	2016	100.00

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

Percent Agreement

The table below summarizes the positive percent agreement and negative percent agreement data for the increased risk and signs and symptoms populations by HBV classification. For the purposes of calculating percent agreement, indeterminate results for the comparator anti-HBs assay were not included in the calculation.

HBV Classification	Positive Percent Agreement % (x/n)	95% Confidence Interval	Negative Percent Agreement % (x/n)	95% Confidence Interval
Early Acute	NA (0/0)	NA	100.00 (2/2)	15.81 - 100.00
Acute	NA (0/0)	NA	100.00 (5/5)	47.82 - 100.00
Late Acute/Recovering	0.00 (0/1)	0.00 - 97.50	NA (0/0)	NA
Early Recovering	NA (0/0)	NA	NA (0/0)	NA
Recovering Acute	100.00 (4/4)	39.76 - 100.00	NA (0/0)	NA
Chronic	100.00 (3/3)	29.24 - 100.00	100.00 (35/35)	90.00 - 100.00
Immune Due to Natural Infection	98.45 (190/193)	95.52 - 99.68	NA (0/0)	NA
Distantly Immune/Anti-HBs Unknown	NA (0/0)	NA	NA (0/0)	NA
Distantly Immune/Anti-HBs Not Detected	NA (0/0)	NA	77.57 (83/107)	68.49 - 85.07
Immune Due to HBV Vaccination	97.24 (494/508)	95.42 - 98.49	NA (0/0)	NA
Unknown	NA (0/0)	NA	NA (0/0)	NA
Susceptible	NA (0/0)	NA	98.67 (1040/1054)	97.78 - 99.27
All	97.46 (691/709)	96.02 - 98.49	96.84 (1165/1203)	95.69 - 97.76

$$\text{Positive percent agreement} = \frac{\text{[Number of ARCHITECT AUSAB reactive results in agreement with the comparator anti-HBs positive results]}}{\text{[Total number of comparator anti-HBs positive results]}} \times 100$$

$$\text{Negative percent agreement} = \frac{\text{[Number of ARCHITECT AUSAB nonreactive results in agreement with the comparator anti-HBs negative results]}}{\text{[Total number of comparator anti-HBs negative results]}} \times 100$$

The table below summarizes the positive percent agreement and negative percent agreement data for the pediatric population. For the purposes of calculating percent agreement, indeterminate results for the comparator anti-HBs assay were not included in the calculation.

ARCHITECT AUSAB Results versus Comparator Anti-HBs Results Percent Agreement for Pediatric Subgroup n=114a

ARCHITECT AUSAB Interpretation	**Comparator Anti-HBs Interpretation**		
	Positive	**Indeterminate**	**Negative**
Reactive	57 (A)	3 (B)	0 (C)
Grayzone	3 (D)	4 (E)	2 (F)
Nonreactive	1 (G)	6 (H)	38 (I)

[a] NOTE: Six specimens in ARCHITECT AUSAB grayzone were excluded due to insufficient volume for retesting.

Negative Percent Agreement = (I / (C+F+I)) x 100 = 95.00%

Positive Percent Agreement = (A / (A+D+G)) x 100 = 93.44%

95% Confidence Interval for Negative Percent Agreement = (83.08%, 99.39%)

95% Confidence Interval for Positive Percent Agreement = (84.05%, 98.18%)

HBV Post-Vaccine Recipient Population

Of the 2387 specimens tested in the ARCHITECT AUSAB clinical study, 211 specimens were obtained from individuals who had received a full course of injections (three) of one of the following vaccines:

- GlaxoSmithKline Engerix-B (n = 106, 50.24%)
- Merck & Co., Inc. RECOMBIVAX HB (n = 49, 23.22%)
- Sanofi Pasteur MSD (n = 12, 5.69%)
- Merck & Co., Inc. HEPTAVAX-B (n = 9, 4.27%)
- Merck & Co., Inc. trade name unknown (n = 8, 3.79%)
- Other (includes different combinations of manufacturer and trade name types for the three doses) (n = 5, 2.37%)
- GlaxoSmithKline Twinrix (n = 1, 0.47%)

- Unknown (n = 21, 9.95%)

Each specimen was tested using the comparator anti-HBs assay following manufacturer's instructions. These specimens were tested using a reference anti-HBc assay and found to be negative. Each specimen was also tested at one of the clinical sites located in Galveston, TX and Milwaukee, WI using the ARCHITECT AUSAB assay. Of the 211 specimens in the post vaccine recipient population, 154 (72.99%) specimens were reactive by the ARCHITECT AUSAB assay and 146 (69.19%) specimens were reactive by the comparator anti-HBs assay. The positive percent agreement between the ARCHITECT AUSAB assay results and the comparator anti-HBs assay results for the post vaccine recipient population was 100.00% (146/146, with a 95% confidence interval of 97.51% to 100.00%). The negative percent agreement between the ARCHITECT AUSAB assay results and the comparator anti-HBs assay results for the post vaccine recipient population was 84.78% (39/46, with a 95% confidence interval of 71.13% to 93.66%). For the purposes of calculating percent agreement, indeterminate results for the comparator anti-HBs assay were not included in the calculation. The data are summarized in the following table.

ARCHITECT AUSAB Results versus Comparator Anti-HBs Results Percent Agreement for Vaccine Recipients Subgroup n=211

ARCHITECT AUSAB Interpretation	**Comparator Anti-HBs Interpretation**		
	Positive	**Indeterminate**	**Negative**
Reactive	146 (A)	6 (B)	4 (C)
Grayzone	0 (D)	8 (E)	3 (F)
Nonreactive	0 (G)	5 (H)	39 (I)

- Negative Percent Agreement = (I / (C+F+I)) x 100 = 84.78%
- Positive Percent Agreement = (A / (A+D+G)) x 100 = 100.00%
- 95% Confidence Interval for Negative Percent Agreement = (71.13%, 93.66%)
- 95% Confidence Interval for Positive Percent Agreement = (97.51%, 100.00%)

HBV Pre-Vaccine and Post-Vaccine Recipient Population

Of the 2387 specimens tested in the ARCHITECT AUSAB clinical study, matched pre-vaccination and post-vaccination specimens were obtained from 20 hepatitis B vaccine recipients. The pre-vaccination specimens were tested using a reference anti-HBs and anti-HBc assay and found to be negative by both. Each specimen was tested using the comparator

anti-HBs assay following manufacturer's instructions. Each specimen was also tested at the clinical site located in Milwaukee, WI using the ARCHITECT AUSAB assay. The table below summarizes the positive percent agreement and negative percent agreement data for the pre and post hepatitis B vaccine recipient population. For the purposes of calculating percent agreement, indeterminate results for the comparator anti-HBs assay were not included in the calculation.

Vaccination Status	Positive Percent Agreement % (x/n)	95% Confidence Interval	Negative Percent Agreement % (x/n)	95% Confidence Interval
Pre-Vaccination	NA (0/0)	NA	100.00 (20/20)	83.16 - 100.00
Post-Vaccination	100.00 (18/18)	81.47 - 100.00	100.00 (2/2)	15.81 - 100.00
Combined	100.00 (18/18)	81.47 - 100.00	100.00 (22/22)	84.56 - 100.00

WHO Standard Linearity

A study was conducted to evaluate dilutions of the **World Health Organization (WHO) Second International Reference Preparation, 2008 (code 07/164) for Anti-HBs** with the ARCHITECT AUSAB assay calibrated with Abbott internal reference calibrators. A linear regression analysis was performed using mean concentration results from 18 replicates each of 7 WHO Reference Preparation dilutions versus the expected concentrations. Predicted concentrations were determined using the slope equation from various concentrations of the WHO Reference Preparation dilutions. The data are summarized in the following tables.

ARCHITECT AUSAB WHO Standard Linearity

				Slope		Intercept	
Lot	Regression Type	Group	N	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
1	Least Square Linear Regression	Samples up to 250 mIU/mL	5[a]	0.94	(0.89, 0.98)	2.33	(-3.06, 7.73)
	Least Square Linear Regression	Samples up to 500 mIU/mL	7[a]	0.96	(0.94, 0.98)	0.83	(-4.32, 5.98)
	Least Square Linear Regression	Samples up to 800 mIU/mL	8[a]	0.98	(0.96, 0.99)	-1.62	(-8.60, 5.37)
2	Least Square Linear Regression	Samples up to 250 mIU/mL	5[a]	1.01	(0.97, 1.04)	1.30	(-2.95, 5.55)
	Least Square Linear Regression	Samples up to 500 mIU/mL	7[a]	1.01	(1.00, 1.02)	1.26	(-1.15, 3.68)
	Least Square Linear Regression	Samples up to 800 mIU/mL	8[a]	0.99	(0.97, 1.01)	4.01	(-2.63, 10.66)

[a] Each sample value was created from 18 replicates.

ARCHITECT AUSAB WHO Standard Linearity Least Squares Regression Predicted Values

Group	Expected Concentration (mIU/mL)	Predicted Concentration (mIU/mL)	
		Lot 1	Lot 2
Samples up to 250 mIU/mL	0	2.33	1.30
	10	11.69	11.39
	50	49.13	51.76
	100	95.93	102.22
	250	236.32	253.60
Samples up to 500 mIU/mL	0	0.83	1.26
	10	10.39	11.35
	50	48.65	51.72
	100	96.47	102.17
	250	239.93	253.54
	350	335.57	354.45
	500	479.04	505.82
Samples up to 800 mIU/mL	0	-1.62	4.01
	10	8.14	13.89
	50	47.15	53.40
	100	95.92	102.79
	250	242.22	250.97
	350	339.75	349.75
	500	486.06	497.92
	800	778.66	794.26

Analytical Specificity

The ARCHITECT AUSAB assay was evaluated for potential crossreactivity for specimens from individuals with medical conditions unrelated to HBV infection and specimens containing potentially interfering substances. The data are summarized in the following tables.

		Comparator Anti-HBs Assay					
		Negative			Positive		
		ARCHITECT AUSAB			ARCHITECT AUSAB		
Category	n	NR[a]	GZ[a]	R[a]	NR[a]	GZ[a]	R[a]
Cytomegalovirus (anti-	9	4	0	0	0	0	5

		Comparator Anti-HBs Assay					
		Negative			Positive		
		ARCHITECT AUSAB			ARCHITECT AUSAB		
Category	**n**	**NR[a]**	**GZ[a]**	**R[a]**	**NR[a]**	**GZ[a]**	**R[a]**
CMV positive)							
Epstein-Barr Virus (anti-EBV positive)	9	2	0	0	0	0	7
Hepatitis A Virus (anti-HAV virus)	10	9	0	0	0	0	1
Hepatitis C Virus (anti-HCV virus)	10	6	0	0	0	0	4
Human Immunodeficiency Virus (anti-HIV-1 positive)	10	6	0	0	0	0	4
Herpes Simplex Virus (anti-HSV positive)	6	4	0	0	0	0	2
Elevated bilirubin	9	9	0	0	0	0	0
Elevated protein	8	5	0	0	0	0	3
Human Anti-Mouse Antibodies (HAMA) positive	10	10	0	0	0	0	0
Influenza vaccine recipients	10	4	0	0	0	0	6
Multiparous female	10	10	0	0	0	0	0
Non-viral liver disease	8	6	0	0	0	0	2
Rheumatoid factor positive	6	5	0	0	0	0	1
Rubella antibody positive	10	7	0	0	0	0	3
Syphilis	10	5	0	0	0	0	5
Toxoplasmosis IgG positive	9	5	0	0	0	0	4
Varicella Zoster Virus	7	4	0	0	1[b]	0	2

		Comparator Anti-HBs Assay					
		Negative			Positive		
		ARCHITECT AUSAB			ARCHITECT AUSAB		
Category	**n**	**NR[a]**	**GZ[a]**	**R[a]**	**NR[a]**	**GZ[a]**	**R[a]**
(VZV) positive							
Yeast infection	9	6	0	0	0	0	3
TOTAL	160	107	0	0	1	0	52

[a] NR = nonreactive, GZ = grayzone, R = reactive

[b] The final interpretation of the VZV positive specimen was anti-HBs negative when tested using the supplemental AUSAB enzyme immunoassay.

Interference

At the concentrations listed below, bilirubin (conjugated and unconjugated), hemoglobin, total protein, and triglycerides showed less than 10% interference in the ARCHITECT AUSAB assay using samples ranging in anti-HBs concentration from 6.0 to 14.0 mIU/mL.

	Interferent Level
Potentially Interfering Substance	**Default Units**
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 AUSAB assay:

- Glass: serum and serum separator
- 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 (glass serum). The distribution of the percent differences per tube type is

listed in the following table.

Evaluation Tube Type	Distribution of %Differences		
	< 10%	**≥ 10% to ≤ 20%**	**> 20%**
Glass Serum Separator	81.8% (36/44)	15.9% (7/44)	2.3% (1/44)
Plastic Serum	79.5% (35/44)	18.2% (8/44)	2.3% (1/44)
Plastic Serum Separator	79.5% (35/44)	20.5% (9/44)	0.0% (0/44)
Plastic Lithium Heparin Plasma Separator	86.0% (37/43)	14.0% (6/43)	0.0% (0/43)
Plastic Sodium Heparin	85.7% (36/42)	14.3% (6/42)	0.0% (0/42)
Plastic Dipotassium EDTA	78.0% (32/41)	19.5% (8/41)	2.4% (1/41)

Neonate Serum

A study was conducted to evaluate neonate samples when tested with the ARCHITECT AUSAB assay. Twenty-one neonate serum (cord blood) samples, whose final interpretation was determined by consensus testing with 3 anti-HBs assays, were obtained and tested using the ARCHITECT AUSAB assay and the results were compared. The data are summarized in the following table.

	Consensus Anti-HBs Assay Result					
	Negative			Positive		
	ARCHITECT AUSAB			ARCHITECT AUSAB		
n	**NR[a]**	**GZ[a]**	**R[a]**	**NR[a]**	**GZ[a]**	**R[a]**
21	15	0	0	0	1[b]	5

[a] NR = nonreactive, GZ = grayzone, R = reactive

[b] The final interpretation of the grayzone specimen was anti-HBs positive after supplemental testing.

Seroconversion Sensitivity

To determine the seroconversion sensitivity, HBV seroconversion panels obtained from commercial vendors were tested using the ARCHITECT AUSAB assay. The study was performed on the ARCHITECT i2000SR. AUSAB was first detected by the ARCHITECT AUSAB assay 12 to 29 days earlier than it was first detected by the comparator AUSAB

assay in 3 seroconversion panel sets and coincident with the first day detected by the comparator AUSAB assay in 5 seroconversion panel sets.

The data are summarized in the following table.

Panel	Days to AUSAB Reactive Result from Initial Draw Date		Difference in Days to Anti-HBs Reactive Result Comparator AUSAB Assay ARCHITECT AUSAB (Comparator – ARCHITECT)
	Comparator AUSAB Assay	ARCHITECT AUSAB	
HBV 11000	0	0	0
HBV-001	156	127	29
43527/3453	365	365	0
13867/3782	63	63	0
26982/14399	24	24	0
HBV-002	81	69	12
26022/14518	54	54	0
1807/3463	138	122	16

BIBLIOGRAPHY

1. Gitlin N. Hepatitis B: diagnosis, prevention, and treatment. *Clinical Chemistry* 1997;43:8(B):1500-1506.
2. Mahoney FJ. Update on Diagnosis, Management, and Prevention of Hepatitis B Virus Infection. *Clinical Microbiology Review*s 1999;12(2):351-366.
3. Juszczyk J. Clinical course and consequences of hepatitis B infection. *Vaccine* 2000;18:S23-S25.
4. Wainwright RB, McMahon BJ, Bulkow LR, et al. Duration of immunogenicity and efficacy of hepatitis B vaccine in a Yupik Eskimo population-preliminary results of an 8-year study. In: Hollinger FB, Lemon SM, Margolis HS, editors. *Viral Hepatitis and Liver Disease*. Baltimore: Williams & Wilkins, 1991:762-766.
5. Ambrosch F, Frisch-Niggemeyer W, Kremsner P, et al. Persistence of vaccine-induced antibodies to hepatitis B surface antigen and the need for booster vaccination in adult subjects. *Postgrad Med J* 1987;63(S2):129-135.

6. Krugman S, Giles JP, Hammond J. Viral hepatitis type B (MS-2 strain): studies on active immunization. *JAMA* 1971;217:41-45.
7. Jilg W, Schmidt M, Deinhardt F. Immune response to hepatitis B revaccination. *J Med Virol* 1988;24:377-384.
8. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
9. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
10. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
11. 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.
12. Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition.* CLSI Document C24-A3. Wayne, PA: CLSI; 2006.
13. 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.
14. 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.
15. 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.
16. Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition.* CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.
17. Clinical and Laboratory Standards Institute (CLSI). *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.