Alinity i HBsAg Qualitative II (HBsAg Qual)-16

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity i HBsAg Qualitative II assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of hepatitis B surface antigen (HBsAg) in human adult and pediatric serum and plasma and neonate serum on the Alinity i analyzer.

The assay may also be used to screen for HBV infection in pregnant women to identify neonates who are at risk for acquiring hepatitis B during the perinatal period. Assay results, in conjunction with other laboratory results and clinical information, may be used to provide presumptive evidence of infection with the hepatitis B virus (HBV) (state of infection or associated disease not determined) in persons with signs and symptoms of hepatitis and in persons at risk for hepatitis B infection.

Not intended for use in screening blood, plasma, or tissue donors.

SUMMARY AND EXPLANATION OF THE TEST

The causative agent of serum hepatitis is HBV which is an enveloped DNA virus. During infection, HBV produces an excess of HBsAg, also known as Australia antigen, which can be detected in the blood of infected individuals. It is responsible for binding the virus to the liver cell and is the target structure of neutralizing antibodies.*1*, *2* HBsAg is the first serological marker after infection with HBV, appearing 1 to 10 weeks after exposure and 2 to 8 weeks before the onset of clinical symptoms.*3*, *4* HBsAg persists during this acute phase and clears late in the convalescence period. Failure to clear HBsAg within 6 months indicates a chronic HBsAg carrier state.

HBsAg assays are used to identify persons infected with HBV and to monitor the status of infected individuals in combination with other hepatitis B serological markers.*5* In most countries, testing for HBsAg is part of the antenatal screening program to identify HBV infected mothers and to prevent perinatal HBV infection by subsequent immunization.*6*

Specimens nonreactive by Alinity i HBsAg Qualitative II are considered negative for HBsAg. A reactive specimen must be retested in duplicate by Alinity i HBsAg Qualitative II to determine whether it is repeatedly reactive. Specimens found to be repeatedly reactive by the Alinity i HBsAg Qualitative II assay should be confirmed using the Alinity i HBsAg Qualitative II Confirmatory (08P11) assay, a neutralization procedure utilizing human anti-HBs. If the specimen is neutralized, the specimen is considered confirmed positive for HBsAg. It is recommended that confirmatory testing be performed before disclosing HBsAg status.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

(Note: Ancillary Wash Buffer is added in a second incubation step, so the assay file performs a two-step assay protocol).

Sample, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture and incubated. The HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled 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 direct relationship between the amount of HBsAg in the sample and the RLUs detected by the system optics.

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

If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, the sample is considered reactive for HBsAg.

For additional information on system and assay technology, refer to the Alinity ci-series

Operations Manual, Section 3.

REAGENTS

Kit Contents

Alinity i HBsAg Qualitative II Reagent Kit 08P10

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

REF	08P1021	08P1031
Tests per cartridge	100	600
Number of cartridges per kit	2	2
Tests per kit	200	1200
MICROPARTICLES	5.4 mL	24.8 mL
CONJUGATE	4.9 mL	24.3 mL
ANCILLARY WASH BUFFER	5.9 mL	24.5 mL

MICROPARTICLES Anti-HBs (mouse, monoclonal, IgM, IgG) coated microparticles in MES buffer with protein (bovine serum albumin) stabilizer. Minimum concentration: 0.08% solids. Preservatives: ProClin 300 and ProClin 950.

CONJUGATE Anti-HBs (mouse, monoclonal, IgG) and anti-HBs (goat, IgG) acridinium-labeled conjugate in phosphate buffer with human plasma and protein (bovine serum albumin, fetal bovine serum, goat IgG, mouse IgG) stabilizers. Minimum concentration: 0.35 µg/mL. Preservatives: ProClin 300 and ProClin 950.

ANCILLARY WASH BUFFER containing MES buffer. Preservatives: ProClin 300 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.[7, 8, 9, 10]

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

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

The following warnings and precautions apply to: ANCILLARY WASH BUFFER	
	
WARNING	Contains methylisothiazolones and dodecyltrimethylammonium bromide.
H317	May cause an allergic skin reaction.
H412	Harmful to aquatic life with long lasting effects.
Prevention	

P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
P273	Avoid release to the environment.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
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.

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

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 HBsAg Qualitative II 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.

Specimen Types	Collection Tubes
Serum	Serum
	Serum separator
Plasma	Lithium heparin
	Lithium heparin plasma separator
	Dipotassium EDTA
	Tripotassium EDTA
	Sodium heparin

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.
- Draw specimens from heparinized patients prior to heparin therapy. Specimens may be partially coagulated and erroneous results could occur due to the presence of fibrin.

- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- 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.
- Centrifuge mixed specimens as described below.

To ensure consistency in results, specimens must be centrifuged using an appropriate tube at a minimum 2500 RCF to obtain ≥ 100 000 g-minutes before testing if:

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

g-minutes = relative centrifugal force (RCF) (g) X centrifugation time (minutes). For Example:

Centrifugation Time (Minutes)	RCF (x g)	g-Minutes
10	10 000	100 000
20	5000	100 000
40	2500	100 000

- 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 (15 to 30°C)	24 hours	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	2 to 8°C	6 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.

If testing will be delayed more than 6 days, 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.

Lithium heparin tube type may demonstrate higher S/CO values for low positive specimens after freeze/thaw.

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

08P10 Alinity i HBsAg Qualitative II Reagent Kit

Materials Required but not Provided

- Alinity i HBsAg Qualitative II assay file
- 08P1002 Alinity i HBsAg Qualitative II Calibrators
- 08P1012 Alinity i HBsAg Qualitative II Controls
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity i-series Concentrated Wash Buffer

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

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

Assay Procedure

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

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

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

Priority:

- Sample volume for first test: 106 µL
- Sample volume for each additional test from same sample cup: 56 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: 56 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

- Refer to the Alinity i HBsAg Qualitative II calibrator package insert and Alinity i HBsAg Qualitative II 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 HBsAg Qualitative II assay.

Calibration

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

Test calibrators 1 and 2 in replicates of 3.

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

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 recommended control requirement for the Alinity i HBsAg Qualitative II assay is that a single sample of each control level be tested once every day testing performed.

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) Document C24, 4th ed., for general quality control recommendations.[11]

If quality control results do not meet the acceptance criteria defined by laboratory procedure, sample results may be suspect. Follow the established quality control procedures to troubleshoot. Recalibration may be necessary. For troubleshooting information**, refer to the Alinity ci-series Operations Manual, Section 10**.

- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.
- To troubleshoot control values that fall outside the control range, **refer to the Alinity ci-series Operations Manual, Section 10.**

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

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

Cutoff RLU = (Calibrator 1 mean RLU x 0.0575) + (Calibrator 2 mean RLU x 0.8)

The cutoff RLU is stored for each reagent lot calibration.

S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

The cutoff is 1.00 S/CO.

Initial Results

S/CO	Instrument Interpretation	Retest Procedure
< 1.00	Nonreactive	No retest required.
≥ 1.00	Reactive	Retest in duplicate

A specimen with an S/CO of less than 1.00 is nonreactive; the specimen is considered negative for HBsAg.

Initially reactive specimens require retesting. Specimens that contain particulate matter should be recentrifuged according to directions in the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section in this package insert.

Retest Results

Instrument Interpretation	Specimen Classification
Both results nonreactive	Specimen considered negative for HBsAg.
One or both results reactive	**Specimen considered repeatedly reactive**

Instrument Interpretation	Specimen Classification
	for HBsAg; confirm using the Alinity i HBsAg Qualitative II Confirmatory assay.

Confirm repeatedly reactive specimens using the Alinity i HBsAg Qualitative II Confirmatory assay before disclosing HBsAg status to the patient.

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

- The effectiveness of the Alinity i HBsAg Qualitative II 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 HBsAg Qualitative II assay is used in conjunction with other manufacturers' assays for specific HBV markers. Users are responsible for establishing their own performance characteristics.
- Current methods for the detection of hepatitis B surface antigen may not detect all potentially infected individuals. A nonreactive test result does not exclude the possibility of exposure to or infection with hepatitis B virus. A nonreactive test result in individuals with prior exposure to hepatitis B may be due to antigen levels below the detection limit of this assay or lack of antigen reactivity to the antibodies in this assay.
- If the Alinity i HBsAg Qualitative II results are inconsistent with clinical evidence, additional testing is recommended.
- For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection.
- Results obtained with the Alinity i HBsAg Qualitative II assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[12] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous results may be observed. Additional information may be required for diagnosis.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[13, 14] Specimens containing HAMA may produce anomalous values when tested with assay kits such as Alinity i HBsAg Qualitative II that employ mouse monoclonal antibodies.[13]
- A reactive HBsAg result does not exclude co-infection by another hepatitis virus.
- 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 i2000SR and i2000 Systems.

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

Increased Risk Population

Of the 2800 specimens tested in the ARCHITECT HBsAg Qualitative clinical study, 1279 specimens were from individuals with increased risk of HBV infection. All 1279 individuals were at risk for HBV infection 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 3 clinical sites located in Hershey, PA; Fort Lauderdale, FL; and Aurora, CO.

The increased risk population (n=1279) consisted of the following race/ ethnic groups:

- 598 (46.76%) Caucasian
- 470 (36.75%) African American
- 159 (12.43%) Hispanic
- 25 (1.95%) Asian
- 2 (0.16%) American Indian/Alaska Native
- 25 (1.95%) Other

The percentage of specimens collected at each location and the percentage of reactive results from each location are presented in the following table.

Specimen Collection Site/ Vendor Location	**Percent of Specimens Collected at Each Location**	**Percent of Reactive Results from Each Location**
Site 1 Galveston, TX	26.66 (341/1279)	2.05 (7/341)
Site 2 Dallas, TX	9.46 (121/1279)	4.13 (5/121)
Site 3 Miami, FL	8.68 (111/1279)	18.92 (21/111)
Site 4 St. Petersburg, FL	33.39 (427/1279)	0.70 (3/427)
Site 5 Chicago, IL	5.47 (70/1279)	8.57 (6/70)
Site 6 Denver, CO	2.42 (31/1279)	3.23 (1/31)
Specimen Vendor Location		
High Point, NC	2.58 (33/1279)	3.03 (1/33)

Specimen Collection Site/ Vendor Location		Percent of Specimens Collected at Each Location	Percent of Reactive Results from Each Location
Colton, CA		2.66 (34/1279)	0.00 (0/34)
Plymouth, MA		8.68 (111/1279)	0.00 (0/111)
	Total	**100.00 (1279/1279)**	**3.44 (44/1279)**

Of the 1279 specimens, 607 (47.46%) were female and 672 (52.54%) were male. The age was not reported for 2 subjects. Of the remaining 1277 specimens, the mean age was 39 years (age range: 17 to 82 years).

The distribution of ARCHITECT HBsAg Qualitative reactive and nonreactive results among the increased risk population by age and gender (n=1279) is summarized in the following table.

		ARCHITECT HBsAg Qualitative Result		
Age Range (Years)	**Gender**	**Number of Reactive (%)**	**Number of Nonreactive (%)**	**Total**
10 to 19	Female	1 (7.69)	12 (92.31)	13
	Male	2 (18.18)	9 (81.82)	11
20 to 29	Female	2 (1.12)	176 (98.88)	178
	Male	2 (1.36)	145 (98.64)	147
30 to 39	Female	3 (2.65)	110 (97.35)	113
	Male	8 (4.71)	162 (95.29)	170
40 to 49	Female	1 (0.63)	159 (99.38)	160
	Male	4 (1.90)	206 (98.10)	210
50 to 59	Female	5 (5.05)	94 (94.95)	99
	Male	12 (11.21)	95 (88.79)	107
60 to 69	Female	4 (11.11)	32 (88.89)	36
	Male	0 (0.00)	17 (100.00)	17
70 to 79	Female	0 (0.00)	5 (100.00)	5
	Male	0 (0.00)	9 (100.00)	9

Age Range (Years)	Gender	ARCHITECT HBsAg Qualitative Result		Total
		Number of Reactive (%)	Number of Nonreactive (%)	
80 to 89	Female	0 (0.00)	2 (100.00)	2
	Male	0 (0.00)	0 (0.00)	0
Unknown	Female	0 (0.00)	1 (100.00)	1
	Male	0 (0.00)	1 (100.00)	1
	Total	**44 (3.44)**	**1235 (96.56)**	**1279**

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 utilized the same reagents and sample/reagent ratios. Some performance characteristics for the Alinity i assay were established using the ARCHITECT i System.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

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.*15* Testing was conducted using 1 lot of Alinity i HBsAg Qualitative II Reagent Kit, 1 lot of Alinity i HBsAg Qualitative II Calibrators, 1 lot of HBsAg controls, and 1 instrument.* Two controls and 3 human plasma panels were assayed in a minimum of 2 replicates at 2 separate times per day on 12 different days.

Sample	n	Mean (S/CO)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Negative Control	72	0.35	0.035	N/A[b]	0.048	N/A[b]
Positive Control	72	3.06	0.078	2.6	0.085	2.8
High Negative	72	0.80	0.041	5.2	0.053	6.7

Sample	n	Mean (S/CO)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Panel						
Low Positive Panel	72	1.19	0.041	3.5	0.050	4.2
Moderate Positive Panel	72	3.56	0.117	3.3	0.135	3.8

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

[b] Not applicable

* The original study, performed on the ARCHITECT i System with the ARCHITECT HBsAg Qualitative assay, used 3 lots of ARCHITECT HBsAg Qualitative reagents, calibrators, and controls on 3 instruments

System Reproducibility

A 5-day precision study was performed for the Alinity i HBsAg Qualitative II assay based on guidance from CLSI EP05-A2*16* and CLSI EP15-A2*17*. Testing was conducted at 3 clinical sites using 1 lot each of the Alinity i HBsAg Qualitative II Reagent Kit, 1 lot of the Alinity i HBsAg Qualitative II Calibrators, and 1 lot of the Alinity i HBsAg Qualitative II Controls and 1 instrument.* Two controls and 3 human plasma panels were assayed in replicates of 4 at 2 separate times of day for 5 days.

Sample	N	Mean S/CO	Within-Run		Within-Day[a]		Within-Laboratory (Total)[b]		Reproducibility with Additional Component of Between-Site[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control	120	0.38	0.038	NA[d]	0.047	NA[d]	0.052	NA[d]	0.054	NA[d]
Positive Control	120	3.08	0.076	2.5	0.076	2.5	0.093	3.0	0.114	3.7
High Negative Panel	120	0.82	0.045	5.4	0.053	6.5	0.053	6.5	0.056	6.8
Low Positive	120	1.21	0.050	4.1	0.050	4.1	0.061	5.0	0.061	5.0

Sample	N	Mean S/CO	Within-Run		Within-Day[a]		Within-Laboratory (Total)[b]		Reproducibility with Additional Component of Between-Site[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Panel										
Moderate Positive Panel	120	3.17	0.079	2.5	0.090	2.8	0.094	3.0	0.120	3.8

[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

* The original study, performed on the ARCHITECT i System with the ARCHITECT HBsAg Qualitative assay, used 3 lots of ARCHITECT HBsAg Qualitative reagents, calibrators, and controls and one instrument per site.

Percent Agreement

Percent Agreement Between the Alinity i Analyzer and the ARCHITECT i2000/i2000SR System

A study was performed to compare the HBsAg Qualitative assay on the Alinity i analyzer and the ARCHITECT i2000SR system using 1 lot each of the Alinity i HBsAg Qualitative II Reagent Kit, Alinity i HBsAg Qualitative II Calibrators, and Alinity i HBsAg Qualitative II Controls. Of the 210 specimens tested, 110 were nonreactive and 100 were reactive based on the ARCHITECT HBsAg Qualitative 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.

Site	Alinity i HBsAg Qualitative II	ARCHITECT HBsAg Qualitative		Negative % Agreement (95% Confidence Interval)[a]	Positive % Agreement (95% Confidence Interval)[a]
		Reactive	Nonreactive		
1	Reactive	100	0	100.00 (110/110)	100.00 (100/100)
	Nonreactive	0	110	(96.63,100.00)	(96.30, 100.00)
2	Reactive	100	1	99.09 (109/110)	100.00 (100/100)
	Nonreactive	0	109	(95.03, 99.84)	(96.30, 100.00)
3	Reactive	100	1	99.09 (109/110)	100.00 (100/100)

Site	Alinity i HBsAg Qualitative II	ARCHITECT HBsAg Qualitative		Negative % Agreement (95% Confidence Interval)[a]	Positive % Agreement (95% Confidence Interval)[a]
		Reactive	Nonreactive		
	Nonreactive	0	109	(95.03, 99.84)	(96.30, 100.00)
All	Reactive	300	2	99.39 (328/330)	100.00 (300/300)
	Nonreactive	0	328	(97.82, 99.83)	(98.74, 100.00)

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

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

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

Analytical Sensitivity

Analytical sensitivity was evaluated using serial dilutions of the **WHO Second International Standard (2003) for HBsAg, subtype *adw2*, genotype A NIBSC code: 00/588**. The dilutions ranged from 2 to 40 mIU/mL. Recalcified negative human plasma was used as the diluent. The dilutions were tested across 3 reagent lots on 1 Alinity i analyzer. The analytical sensitivity results ranged from 20 to 21 mIU/mL (0.020 to 0.021 IU/mL) for the 3 lots. The observed limit of detection (LoD), performed per CLSI EP17-A2*18* ranged **from 0.002 to 0.012 IU/mL.**

Seroconversion Sensitivity

To determine the seroconversion sensitivity, 32 seroconversion panels obtained from commercial vendors were tested on the Alinity i system using the Alinity i HBsAg Qualitative II and HBsAg Qualitative II Confirmatory assays. The panel results were evaluated against the ARCHITECT HBsAg Qualitative assay and data are summarized in the following table.

Panel ID	Days to HBsAg Reactive Result from Initial Draw Date		Difference in Days to HBsAg Reactive Result (Alinity – ARCHITECT)
	Alinity i HBsAg Qualitative II	ARCHITECT HBsAg Qualitative	
6271	7	7	0
6272	94	94	0
6273	14	14	0
6274	0	0	0
6275	7	7	0
6277	33	33	0
6278	12	12	0
6279	26	26	0
6283	29	26	**3**
6284	50	50	0
6285	40	40	0
6286	33	33	0
6288	10	10	0
6290	21	21	0
6292	29	29	0
6293	15	15	0
9072	128	128	0
9074	70	70	0
11000	21	21	0
11001	44	44	0
11002	7	7	0
11003	142	142	0
11004	48	48	0
11006	42	42	0
11007	36	34	**2**

Panel ID	Days to HBsAg Reactive Result from Initial Draw Date		Difference in Days to HBsAg Reactive Result (Alinity – ARCHITECT)
	Alinity i HBsAg Qualitative II	ARCHITECT HBsAg Qualitative	
11008	69	69	0
11009	79	79	0
11010	64	73	**-9**
11011	103	103	0
11012	18	18	0
11013	244	246	**-2**
11017	42	40	**2**

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 HBsAg Qualitative assay to detect HBsAg in a group of individuals that would normally be tested in a clinical situation. Of the 2800 specimens tested in the clinical study, 1279 specimens were obtained from individuals with increased risk of HBV infection due to lifestyle, behavior, occupation, or a known exposure event and 675 specimens were obtained from individuals exhibiting signs and symptoms of hepatitis infection.

Specimens (n=1954) from these populations consisted of the following race/ethnic groups:

- 970 (49.64%) Caucasian
- 598 (30.60%) African American
- 311 (15.92%) Hispanic
- 40 (2.05%) Asian
- 5 (0.26%) American Indian/Alaska Native
- 30 (1.54%) Other

Specimens (n=1954) from these specimen populations were obtained from the following collection locations:

- 359 (18.37%) Galveston, TX
- 200 (10.24%) Dallas, TX
- 179 (9.16%) Miami, FL

- 496 (25.38%) St. Petersburg, FL
- 284 (14.53%) Chicago, IL
- 137 (7.01%) Denver, CO
- 33 (1.69%) High Point, NC
- 35 (1.79%) Colton, CA
- 111 (5.68%) Plymouth, MA
- 100 (5.12%) Trinity, FL
- 20 (1.02%) Franklin, TN

Of the 1954 specimens from the increased risk and signs and symptoms populations, 893 (45.70%) were female and 1061(54.30%) were male. Age was not reported for 2 specimens. Of the remaining 1952 specimens, the mean age was 42 years (age range: 17 to 82 years).

The ARCHITECT HBsAg Qualitative assay was further evaluated by testing a total of 126 pre-selected specimens from acute and chronic HBV infections, which included 8 specimens from subjects with clinically diagnosed acute HBV infection, 29 specimens classified as acute based on four-marker HBV reference testing, 67 specimens from subjects with clinically diagnosed chronic HBV infection defined by the presence of HBsAg for ≥ 6 months, and 22 specimens classified as chronic based on four-marker HBV reference testing.

Each specimen was tested using a comparator HBsAg assay and 3 HBV reference assays, each detecting a unique serological marker (anti-HBc IgM, 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 testing was performed following manufacturer's instructions.

Each specimen was also tested at 1 of 3 clinical sites located in Hershey, PA; Fort Lauderdale, FL; and Aurora, CO, using the ARCHITECT HBsAg Qualitative assay.

Results by Specimen Classification

Following testing with the comparator HBsAg assay and 3 reference HBV assays, the 1954 specimens from the increased risk and signs and symptoms population plus 126 specimens from individuals with acute or chronic HBV infection were assigned an HBV classification according to the following table. There were 13 unique reference marker patterns observed in the ARCHITECT HBsAg Qualitative clinical study.

	HBV Reference Markers				
Number of Specimens	**HBsAg[a]**	**Anti-HBc IgM**	**Total Anti-HBc**	**Anti-HBs**	**HBV Classification**

Number of Specimens	HBV Reference Markers				HBV Classification
	HBsAg[a]	Anti-HBc IgM	Total Anti-HBc	Anti-HBs	
20	+	-	-	-	Acute
2	+	I	+	-	Acute
26	+	+	+	-	Acute
154	+	-	+	-	Chronic
6	+	-	+	+	Chronic
2	+	-	-	+	Chronic
5	+	+	+	+	Late Acute, Recovering
9	-	+	+	+	Recovering Acute
3	-	+	+	-	Recovering Acute, Undetectable HBsAg
118	-	-	+	-	Distantly Immune, Anti-HBs Not Detected
225	-	-	+	+	Immune Due to Natural Infection
414	-	-	-	+	Immune Due to HBV Vaccination
1096	-	-	-	-	Susceptible
2080					**Total**

+ = Positive/Reactive, – = Negative/Nonreactive, I = Indeterminate

[a] For HBsAg: + = Repeatedly reactive and confirmed by neutralization when required; – = Reference HBsAg test negative or not confirmed by neutralization.

Comparison of Results

The following table compares the ARCHITECT HBsAg Qualitative assay results with the comparator HBsAg assay final interpretation for each of the HBV classifications for the increased risk and signs and symptoms populations (n = 1954) and the acute or chronic HBV infection populations (n=126). The data are summarized in the following table.

HBV Classification	Comparator HBsAg Final Interpretation			
	Confirmed Positive[a]		Negative/Not Confirmed	
	ARCHITECT HBsAg Qualitative Result		ARCHITECT HBsAg Qualitative Result	
	Reactive	Nonreactive	Reactive	Nonreactive
	N	N	N	N
Acute	47	1	0	0
Chronic	160	2	0	0
Late Acute, Recovering	5	0	0	0
Recovering Acute	0	0	0	9
Recovering Acute, Undetectable HBsAg	0	0	0	3
Distantly Immune, Anti-HBs Not Detected	0	0	3	115
Immune Due to Natural Infection	0	0	5	220
Immune Due to HBV Vaccination	0	0	0	414
Susceptible	0	0	6	1090
Total	**212**	**3**[b]	**14**[c]	**1851**

[a] The comparator HBsAg final positive interpretation includes retesting and confirmatory testing according to the comparator package inserts.

[b] All 3 specimens were positive for an additional marker (anti-HBc or anti-HBs) or had DNA present (assay sensitivity of 169 copies/mL).

[c] Of these 14 specimens, 1 specimen was not confirmed on the ARCHITECT HBsAg Qualitative Confirmatory assay, 10 specimens were positive for an additional marker (anti-HBc, anti-HBs, or anti-HBe) or had DNA present, and 3 specimens had no additional markers or DNA present.

Percent Agreement

The percent agreement between the ARCHITECT HBsAg Qualitative assay results and the comparator HBsAg assay final interpretation for the increased risk and signs and symptoms

populations by HBV classification (n=1954) is summarized in the table below.

HBV Classification	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Acute	100.00 (7/7)	(59.04, 100.00)	NA	NA
Chronic	97.65 (83/85)	(91.76, 99.71)	NA	NA
Recovering Acute	NA	NA	100.00 (9/9)	(66.37, 100.00)
Recovering Acute, Undetectable HBsAg	NA	NA	100.00 (3/3)	(29.24, 100.00)
Distantly Immune, Anti-HBs Not Detected	NA	NA	97.44 (114/117)	(92.69, 99.47)
Immune Due to Natural Infection	NA	NA	98.21 (219/223)	(95.47, 99.51)
Immune Due to HBV Vaccination	NA	NA	100.00 (414/414)	(99.11, 100.00)
Susceptible	NA	NA	99.45 (1090/1096)	(98.81, 99.80)
Total	**97.83 (90/92)**	**(92.37, 99.74)**	**99.30 (1849/1862)**	**(98.81, 99.63)**

Percent Agreement for Individuals With Acute or Chronic HBV Infection

The percent agreement between the ARCHITECT HBsAg Qualitative assay results and the comparator HBsAg assay final interpretation for the pre-selected specimens from individuals with acute and chronic HBV infection (n=126) are presented in the table below.

Specimen Category	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Individuals with Acute HBV Infection	97.30 (36/37)	(85.84, 99.93)	NA	NA
Individuals with Chronic HBV	100.00 (86/86)	(95.80, 100.00)	66.67 (2/3)	(9.43, 99.16)

Specimen Category	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Infection				

Increased Risk Population Testing

In addition to the 1279 specimens from individuals at increased risk tested at 3 clinical sites, 498 specimens from hemodialysis patients were tested at Abbott Laboratories. The following table compares the ARCHITECT HBsAg Qualitative results and comparator HBsAg assay final interpretations for each risk factor for this overall increased risk population.

	Comparator HBsAg Assay Final Interpretation				
	Confirmed Positive		Negative/Not Confirmed		
	ARCHITECT HBsAg Qualitative Result		ARCHITECT HBsAg Qualitative Result		
Specimen Category	Reactive (N)	Nonreactive (N)	Reactive (N)	Nonreactive (N)	Total (N)
Multiple Sex Partners	23	1	3	876	903
Injecting Drug User (IDU)	2	0	1	116	119
Men who have Sex with Men (MSM)	1	0	1	9	11
Sexual Contact with HBV	2	0	0	22	24
Household Contact with HBV	6	0	0	43	49
Occupational Exposure Incident	2	0	1	163	166
Hemodialysis Patient	2	0	0	499	501[a]
Perinatal Exposure to HBV	2	0	0	2	4
Total	**40**	**1**	**6**	**1730**	**1777**

[a] Of these 501 specimens, 3 specimens were tested at clinical sites and 498 specimens were tested at Abbott Laboratories.

Clinical Performance in Pregnant Females

The performance of ARCHITECT HBsAg Qualitative in detecting HBV infection in pregnant females was evaluated by testing serum specimens from pregnant females at low risk or increased risk of HBV infection due to lifestyle, behavior, or known exposure event. Of the 2800 specimens tested in the clinical study, 720 were from a pregnant female population. The specimens were obtained from commercial vendors. The 720 specimens, from pregnant females aged 16 to 45 years, were collected from collection sites in Colton, CA (n=161); Plymouth, MA (n=7); and Los Angeles, CA (n=552). Testing of these specimens was performed at the clinical sites located in Hershey, PA, Fort Lauderdale, FL, and Aurora, CO. The demographic profile of the pregnant female population is presented in the table below.

Category	Low Risk N (%)	Increased Risk N (%)	Total N (%)
Total	**544 (75.56)**	**176 (24.44)**	**720 (100.00)**
TRIMESTER			
First	24 (4.41)	6 (3.41)	30 (4.17)
Second	259 (47.61)	68 (38.64)	327 (45.42)
Third	261 (47.98)	102 (57.95)	363 (50.42)
RACE/ETHNIC GROUP			
Caucasian	10 (1.84)	38 (21.59)	48 (6.67)
African American	52 (9.56)	22 (12.50)	74 (10.28)
Hispanic	465 (85.48)	108 (61.36)	573 (79.58)
Asian	15 (2.76)	0 (0.00)	15 (2.08)
American Indian/Alaska Native	0 (0.00)	2 (1.14)	2 (0.28)
Other	2 (0.37)	6 (3.41)	8 (1.11)
AGE RANGE			
16 to 31	320 (58.82)	146 (82.95)	466 (64.72)
32 to 45	224 (41.18)	30 (17.05)	254 (35.28)

Agreement for Pregnant Females by Risk and Trimester

A comparison was performed between the ARCHITECT HBsAg Qualitative assay results

and the comparator HBsAg assay results using serum samples obtained from a total of 720 pregnant females at low risk or increased risk for HBV infection. Data were analyzed by risk and by trimester. The data are summarized in the tables below.

ARCHITECT and Comparator HBsAg Results by Trimester for Low Risk Pregnant Females

	First Trimester			Second Trimester			Third Trimester		
	Comparator HBsAg Final Interpretation			Comparator HBsAg Final Interpretation			Comparator HBsAg Final Interpretation		
ARCHITECT HBsAg Qualitative Result	**Confirmed Positive**	**Negative/Not Confirmed**	**Total**	**Confirmed Positive**	**Negative/Not Confirmed**	**Total**	**Confirmed Positive**	**Negative/Not Confirmed**	**Total**
Reactive	0	0	0	0	0	0	0	0	0
Nonreactive	0	24	24	0	259	259	0	261	261
Total	**0**	**24**	**24**	**0**	**259**	**259**	**0**	**261**	**261**

ARCHITECT and Comparator HBsAg Results by Trimester for Increased Risk Pregnant Females

	First Trimester			Second Trimester			Third Trimester		
	Comparator HBsAg Final Interpretation			Comparator HBsAg Final Interpretation			Comparator HBsAg Final Interpretation		
ARCHITECT HBsAg Qualitative Result	**Confirmed Positive**	**Negative/Not Confirmed**	**Total**	**Confirmed Positive**	**Negative/Not Confirmed**	**Total**	**Confirmed Positive**	**Negative/Not Confirmed**	**Total**
Reactive	0	0	0	0	0	0	1	0	1
Nonreactive	0	6	6	0	68	68	0	101	101
Total	**0**	**6**	**6**	**0**	**68**	**68**	**1**	**101**	**102**

Overall Summary and Percent Agreement for Pregnant Females

The percent agreement between the ARCHITECT HBsAg Qualitative assay results and the comparator HBsAg assay results for the pregnant female population are summarized in the table below.

Subjects	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Pregnant Females	100.00% (1/1)	(2.50%, 100.00%)	100.00% (719/719)	(99.49%, 100.00%)

A total of 3172 specimens from a diagnostic population (increased risk for HBV infection, signs and symptoms of hepatitis infection, and pregnant females) were tested using the ARCHITECT HBsAg Qualitative assay. The repeatedly reactive specimens were confirmed using the ARCHITECT HBsAg Qualitative Confirmatory assay. There were 122/3172 (3.85%) initially reactive results and 106/3172 (3.34%) repeatedly reactive results. Of the repeatedly reactive results, 104/106 (98.11%) results were confirmed.

Clinical Performance in a Pediatric Population

Of the 2800 specimens in the clinical study, 142 specimens were from a pediatric population aged 17 to 21. In addition, 68 specimens from pediatric individuals aged 4 to 18 who were at

increased risk of HBV infection were tested at Abbott Laboratories. For all 210 specimens, the negative percent agreement was 99.51% (203/204) with a 95% confidence interval of 97.30% to 99.99% and the positive percent agreement was 83.33% (5/6) with a 95% confidence interval of 35.88% to 99.58% for the ARCHITECT HBsAg Qualitative result versus the comparator HBsAg final interpretation. The ARCHITECT HBsAg Qualitative results are summarized by age and gender in the following table.

		ARCHITECT HBsAg Qualitative Result		
Age Range (Years)	**Gender**	**Reactive N (%)**	**Nonreactive N (%)**	**Total**
>4 to 12	Female	1 (5.26)	18 (94.74)	19
	Male	0 (0.00)	22 (100.00)	22
>12 to 18	Female	0 (0.00)	23 (100.00)	23
	Male	0 (0.00)	7 (100.00)	7
>18 to 21	Female	2 (1.75)	112 (98.25)	114
	Male	3 (12.00)	22 (88.00)	25
Total		**6 (2.86)**	**204 (97.14)**	**210**

Neonate Serum

A study was conducted to evaluate whether neonate samples may be tested with the ARCHITECT HBsAg Qualitative assay. Cord blood serum was used as a surrogate for neonate serum. Twenty-three matched cord blood and maternal serum samples were spiked with HBsAg positive stock to yield a high negative sample (target S/CO 0.80) and a low positive sample (target S/CO 1.20).

The distribution of the percent differences per analyte level is listed in the following table.

		Distribution of Percent Differences			
Analyte Level	**N**	**< 10%**	**≥ 10% to < 20%**	**≥ 20% to < 30%**	**≥ 30%**
0.80 S/CO	23	91.3% (21/23)	8.7% (2/23)	0.0% (0/23)	0.0% (0/23)
1.20 S/CO	23	91.3% (21/23)	8.7% (2/23)	0.0% (0/23)	0.0% (0/23)

Analytical Specificity

The ARCHITECT HBsAg Qualitative assay was evaluated for potential cross-reactivity for specimens from individuals with medical conditions unrelated to HBV infection. A total of 301 specimens from 28 different categories were tested. Two hundred ninety-eight (298) specimens were nonreactive and 3 specimens were reactive by the ARCHITECT HBsAg Qualitative and comparator HBsAg assays. All 3 reactive specimens were confirmed positive for HBsAg by the ARCHITECT HBsAg Qualitative Confirmatory and comparator HBsAg confirmatory assays. The data are summarized by final interpretation in the following table.

		Comparator HBsAg Assay			
		Negative/Not Confirmed		**Positive[a]**	
		ARCHITECT HBsAg Qualitative		**ARCHITECT HBsAg Qualitative**	
Category	**N**	**Nonreactive**	**Reactive**	**Nonreactive**	**Reactive**
Anti-nuclear antibodies (ANA)	10	10	0	0	0
Auto-immune hepatitis	10	10	0	0	0
C. trachomatis	7	7	0	0	0
Cytomegalovirus (CMV)	10	10	0	0	0
Epstein-Barr virus (EBV)	10	10	0	0	0
Fatty liver disease	10	10	0	0	0
Hemodialysis patient	10	10	0	0	0
Hepatitis A virus (HAV)	10	10	0	0	0
Hepatitis C virus (HCV)	10	10	0	0	0
Hepatocellular carcinoma	10	10	0	0	0
Herpes simplex virus (HSV)	10	10	0	0	0
HIV-1	10	10	0	0	0

		Comparator HBsAg Assay			
		Negative/Not Confirmed		Positive[a]	
		ARCHITECT HBsAg Qualitative		ARCHITECT HBsAg Qualitative	
Category	**N**	**Nonreactive**	**Reactive**	**Nonreactive**	**Reactive**
HIV-2	17	14	0	0	3
Human anti-mouse antibodies (HAMA) positive	15	15	0	0	0
Human T-lymphotropic virus (HTLV-1/2)	9	9	0	0	0
IgG monoclonal gammopathy	10	10	0	0	0
IgM monoclonal gammopathy	10	10	0	0	0
Influenza vaccine recipients	10	10	0	0	0
Multiparous pregnancies	10	10	0	0	0
Multiple myeloma	10	10	0	0	0
Multiple transfusion recipients	10	10	0	0	0
N. gonorrhea	9	9	0	0	0
Pregnancy 1st trimester	15	15	0	0	0
Pregnancy 2nd trimester	14	14	0	0	0
Pregnancy 3rd trimester	15	15	0	0	0
Rheumatoid arthritis (RF)	10	10	0	0	0
T. cruzi	10	10	0	0	0

		Comparator HBsAg Assay			
		Negative/Not Confirmed		Positive[a]	
		ARCHITECT HBsAg Qualitative		ARCHITECT HBsAg Qualitative	
Category	**N**	**Nonreactive**	**Reactive**	**Nonreactive**	**Reactive**
T. pallidum	10	10	0	0	0
Total	**301**	**298**	**0**	**0**	**3**

[a] The comparator HBsAg final positive interpretation includes retesting and confirmatory testing according to the comparator package inserts.

In addition, a minimum of 10 serum samples were supplemented with antigens from hepatitis A virus, cytomegalovirus, Epstein-Barr virus, herpes simplex virus-1, rubella, *Toxoplasma gondii*, and varicella-zoster virus. The viral or parasitic antigens were spiked to 1.0 µg/mL except for the hepatitis A virus, which was spiked to 0.1 µg/mL. The prepared samples were tested in replicates of one. All replicates of the serum samples spiked with viral or parasitic antigens were nonreactive.

Interference

At the concentrations listed below, the ARCHITECT HBsAg Qualitative assay showed interference from unconjugated bilirubin, conjugated bilirubin, protein, hemoglobin, and triglycerides for high negative samples (targeted to an S/CO of 0.80) of ≤ +0.15 S/CO and low positive samples (targeted to an S/CO of 1.20) of ≥ -15%.

Interferent	**Interferent Concentration**
Unconjugated Bilirubin	≤ 20 mg/dL
Conjugated Bilirubin	≤ 20 mg/dL
Triglycerides	≤ 3000 mg/dL
Protein	≤ 12 g/dL
Hemoglobin	≤ 500 mg/dL

Tube Type Matrix Comparison

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

· Serum, including serum separator

- Plasma: dipotassium EDTA, tripotassium EDTA, lithium heparin, lithium heparin separator, and sodium heparin

On average, the tube types listed in the table below showed less than a 15% difference when compared to the control tube type (plastic serum) for low positive samples (S/CO range: 1.00 to 1.40) and less than a 0.15 S/CO difference for high negative samples (S/CO range: 0.60 to 0.99). The ARCHITECT HBsAg Qualitative assay showed the following distribution of percent differences when compared to the plastic serum tube type.

	Distribution of Differences for High Negative Samples			**Distribution of Percent Differences for Low Positive Samples**		
Evaluation Tube Type	**< 0.10 S/CO**	**≥ 0.10 S/CO to ≤ 0.20 S/CO**	**> 0.20 S/CO**	**< -20%**	**≥ -20% to ≤ -10%**	**> -10%**
Serum Separator, Plastic	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	0.0% (0/26)	0.0% (0/26)	100.0% (26/26)
Dipotassium EDTA	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	0.0% (0/27)	3.7% (1/27)	96.3% (26/27)
Tripotassium EDTA	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
Lithium Heparin	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
Sodium Heparin	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	0.0% (0/27)	3.7% (1/27)	96.3% (26/27)
Lithium Heparin Plasma Separator	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)

HBsAg Mutant Detection

The ARCHITECT HBsAg Qualitative assay is designed to have the ability to better detect (as reactive) the HBsAg mutant Thr-123-Ala and to have the equivalent or better ability to detect (as reactive) other HBsAg mutants (including Gly-145-Arg) when compared to the comparator HBsAg assay. The hepatitis B virus, unlike other DNA viruses, replicates through reverse transcription. The reverse transcription process lacks proofreading capability; therefore, HBV is subject to a mutation rate 10 times higher than the mutation rate of other DNA viruses.[20] Some of these mutations may cause changes in the antigenic structure of HBsAg, resulting in epitopes that are no longer recognized by anti-HBs. HBsAg mutants have been reported in a wide range of patient populations, including blood donors, vaccine recipients, renal dialysis patients, orthotopic liver transplant recipients, infants born to HBsAg-positive mothers, and patients undergoing nucleoside analog treatment for HBV.[20], [21], [22], [23], [24], [25], [26], [27] HBsAg mutations may result in a less favorable outcome in some

patients[20], [21], [23] and false negative results in some HBsAg assays.[20], [21], [22]

A panel of 9 HBsAg recombinant proteins containing defined mutations between amino acid positions 122 and 145 were prepared in a fetal calf serum containing tissue culture media as described by Coleman, Chen, and Mushahwar[28] except for the Thr-123-Ala mutation, which was expressed in serum free tissue culture media. Each mutant was diluted with recalcified negative human plasma to an S/CO of 2.0 ± 0.5 and tested with the ARCHITECT HBsAg Qualitative assay and with a comparator HBsAg assay. The data are summarized in the following table.

	Final Results	
Mutant	**ARCHITECT HBsAg Qualitative**	**Comparator HBsAg**
Gln-129-His	Repeatedly Reactive	Repeatedly Reactive
Met-133-Leu	Repeatedly Reactive	Repeatedly Reactive
Asp-144-Ala	Repeatedly Reactive	Nonreactive
Gly-145-Arg	Repeatedly Reactive	Repeatedly Reactive
Thr-123-Ala	Repeatedly Reactive	Nonreactive
P142L+G145R	Repeatedly Reactive	Repeatedly Reactive
P142S+G145R	Repeatedly Reactive	Repeatedly Reactive
122NT	Repeatedly Reactive	Repeatedly Reactive
122RA	Repeatedly Reactive	Repeatedly Reactive

HBV Genotype Detection

A study was performed to evaluate the ability of the ARCHITECT HBsAg Qualitative assay to detect HBV genotypes A through H. A total of 19 panel members (3 panel members each of A, B, C, D, and E; 2 panel members of F; and 1 panel member each of G and H) were tested using the ARCHITECT HBsAg Qualitative and ARCHITECT HBsAg Qualitative Confirmatory assays. All genotypes were reactive by the ARCHITECT HBsAg Qualitative assay and confirmed positive by the ARCHITECT HBsAg Qualitative Confirmatory assay.

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