Alinity i

HBsAg Qualitative II Reagent Kit

en
HBsAg Qual
08P10
G71231R03
B8P100

Revised March 2017.

REF 08P1022
REF 08P1032

Instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from these instructions.

NAME

Alinity i HBsAg Qualitative II Reagent Kit (also referred to as HBsAg Qual)

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 serum and plasma including specimens collected post-mortem (non-heart-beating) on the Alinity i analyzer.

The Alinity i HBsAg Qualitative II assay is to be used as an aid in the diagnosis of HBV infection and as a screening test to prevent transmission of HBV to recipients of blood, blood components, cells, tissue, and organs.

SUMMARY AND EXPLANATION OF THE TEST

The causative agent of serum hepatitis is hepatitis B virus (HBV) which is an enveloped DNA virus. During infection, HBV produces an excess of hepatitis B surface antigen (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 one to ten weeks after exposure and two to eight 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 six months indicates a chronic HBsAg carrier state.

HBsAg assays are used to identify persons infected with HBV and to prevent transmission of the virus by blood and blood products as well as 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]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a one-step immunoassay for the qualitative detection of HBsAg in human serum and plasma including specimens collected post-mortem (non-heart-beating) 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, ancillary wash buffer is added to the reaction mixture. Following another 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.

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

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

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

REF	08P1022	08P1032
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 Ancillary wash buffer containing MES buffer. Preservatives: ProClin 300 and ProClin 950.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

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.[7-10]

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.

Abbott

The following warnings and precautions apply to: MICROPARTICLES, CONJUGATE, and ANCILLARY WASH BUFFER	
	
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.

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

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

Reagent Handling

- Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.
  - Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
- After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

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

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.
Onboard			
100-Test Cartridge	System Temperature	29 days	Discard after 29 days.
600-Test Cartridge	System Temperature	30 days	Discard after 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 potential to compromise reagent performance.

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 or 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 on the ARCHITECT i System.

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

Specimen Types	Collection Tubes
Serum	Serum
	Serum separator
Plasma	Lithium heparin
	Lithium heparin (Plasma separator)
	Sodium heparin
	Dipotassium EDTA
	Tripotassium EDTA
	Sodium citrate
	Potassium oxalate / sodium fluoride plasma
	CPD
	CPDA-1
	ACD

- Performance has not been established for the use of bodily fluids other than human serum or plasma.

- Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual specimens.
- Performance has been established for the use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating) that have been collected up to 18.5 hours after death. Performance was established using 50 spiked and 50 non-spiked cadaveric blood specimens.[11]
- Testing of cadaveric blood specimens from patients with plasma dilution due to transfusions of > 2000 mL of blood or colloids within 48 hours, or > 2000 mL of crystalloids within 1 hour (or any combination thereof) prior to collection of the specimens have not been validated.
- The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

- Do not use:
  - heat-inactivated specimens
  - pooled specimens
  - grossly hemolyzed specimens
  - specimens with obvious microbial contamination
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- 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.

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

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

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 a minimum of 100 000 g-minutes.
- Examples of acceptable time and force ranges that meet this criterion are listed in the table below.

  Centrifugation time using alternate RCF values can be calculated using the following formula:

$$\text{Minimum Centrifugation time (minutes)} = \frac{100\,000 \text{ g-minutes}}{\text{RCF}}$$

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

$RCF = 1.12 \times r_{max}\ (rpm/1000)^2$

RCF - The relative centrifugal force generated during centrifugation.

rpm - The revolutions per minute of the rotor on which the specimens are being spun (usually the digital readout on the centrifuge will indicate the rpm).

Centrifugation Time - The time should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.

r_{max} - Radius of the rotor in millimeters. NOTE: If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used, then the radius (rmax) should be manually measured in millimeters and the RCF calculated.

g-minutes - The unit of measure for the product of RCF ($\times$ g) and centrifugation time (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 storage conditions were verified on the ARCHITECT i System.

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/ Plasma	Room temperature	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.
Cadaveric	Room temperature (15 to 30°C)	24 hours	If specimens are not processed directly after initial centrifugation, it is recommended to remove the supernatant from the clot, red blood cells or separator gel until further processing.
	2 to 8°C	6 days	If specimens are not processed directly after initial centrifugation, it is recommended to remove the supernatant from the clot, red blood cells or separator gel until further processing.

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.

No qualitative differences were observed for cadaveric blood specimens (nonreactive or spiked reactive) when subjected to up to 3 freeze/thaw cycles. However, multiple freeze/thaw cycles should be avoided.

Specimen Shipping

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

PROCEDURE

Materials Provided

08P10 Alinity i HBsAg Qualitative II Reagent Kit

Materials Required but not Provided

- Alinity i HBsAg Qualitative II assay file
- 08P1001 Alinity i HBsAg Qualitative II Calibrators
- 08P1010 Alinity i HBsAg Qualitative II Controls or other control material
- 06P1160 Alinity Trigger Solution
- 06P1265 Alinity Pre-Trigger Solution
- 06P1368 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.

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

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 24 hours each day of use.

Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

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-A3 or other published guidelines, for general quality control recommendations.[12]

- If more frequent control monitoring is required, follow the established quality control procedures for your laboratory.
- If quality control results do not meet the acceptance criteria defined by your laboratory, sample results may be suspect. Follow the established quality control procedures for your laboratory. 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.

Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[13]

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.

Initial Results

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

Duplicate Retest Results

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

*Confirm repeatedly reactive specimens using a neutralizing assay (Alinity i HBsAg Qualitative II Confirmatory assay is recommended) before disclosing HBsAg status to the patient.

For details on configuring the Alinity i analyzer to use grayzone interpretation, refer to the Alinity ci-series Operations Manual, Section 2. The grayzone interpretation is an editable parameter and should be utilized per end user requirements.

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

- If the Alinity i HBsAg Qualitative II results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection.
- Vaccination with a recombinant HBsAg Hepatitis B vaccine may cause transient positive results with a sensitive HBsAg assay such as Alinity i HBsAg Qualitative II. These results are caused by a passive transfer of antigen by vaccination, not by viral replication. Positive results usually don't persist for more than 14 days after vaccination[14], though positive signals up to 52 days have been reported[15], and may not indicate clinical disease.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[16, 17] Specimens containing HAMA may produce anomalous values when tested with assay kits such as Alinity i HBsAg Qualitative II that employ mouse monoclonal antibodies.[16]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.[18]

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.

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

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2. Testing was conducted using 3 lots of the Alinity i HBsAg Qualitative II Reagent Kit, 3 lots of the Alinity i HBsAg Qualitative II Calibrators, and 3 lots of the Alinity i HBsAg Qualitative II Controls (or commercially-available 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 20 different days.[19]

			Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
Sample	n	Mean (S/CO)	SD	%CV	SD (range[b])	%CV (range[b])
Negative Control	359	0.35	0.031	9.0	0.057 (0.038 - 0.077)	16.5 (11.7 - 21.1)
Positive Control	359	3.13	0.072	2.3	0.103 (0.078 - 0.116)	3.3 (2.5 - 3.7)
Panel 1	358	0.88	0.050	5.6	0.066 (0.051 - 0.088)	7.4 (5.7 - 10.0)
Panel 2	360	1.21	0.047	3.9	0.067 (0.050 - 0.083)	5.5 (4.1 - 7.0)
Panel 3	358	3.31	0.203	6.1	0.214 (0.201 - 0.230)	6.5 (6.0 - 6.8)

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

[b] Minimum and maximum SD or %CV for each reagent lot and instrument combination.

System Reproducibility

This study was performed on the ARCHITECT i System.

A 5-day precision study was performed for the ARCHITECT HBsAg Qualitative II assay based on guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP15-A2.[20]

Testing was conducted at 3 clinical sites using 3 lots each of ARCHITECT HBsAg Qualitative II reagents, calibrators and controls per site.

Two controls and 2 panels were assayed in replicates of 4 at 2 separate times of day for 5 days.

The data are summarized in the following table.

		Grand Mean	Within-Run		Within-Day		Within-Laboratory Precision (Total)	
Sample	n	S/CO	SD	%CV	SD	%CV	SD	%CV
Negative Control	360	0.17	0.028	NA	0.031	NA	0.031	NA
Positive Control	360	3.45	0.066	1.9	0.070	2.0	0.073	2.1
High Negative Panel	360	0.77	0.037	4.8	0.061	7.9	0.061	7.9
Low Positive Panel	360	1.28	0.066	5.1	0.066	5.1	0.066	5.1

Specificity

Blood Donor Specimens

A total of 5110 serum and plasma specimens collected from 2 blood banks were tested using the Alinity i HBsAg Qualitative II assay and the ARCHITECT HBsAg Qualitative II assay. Two specimens were repeatedly reactive on both the Alinity i HBsAg Qualitative II assay and the ARCHITECT HBsAg Qualitative II assay. Specimens repeatedly reactive on the Alinity i HBsAg Qualitative II assay were further tested using the Alinity i HBsAg Qualitative II Confirmatory assay. Specimens repeatedly reactive on the ARCHITECT HBsAg Qualitative II assay were further tested using the ARCHITECT HBsAg Qualitative II Confirmatory assay. Based on supplemental test results, the 2 repeatedly reactive specimens were negative for HBsAg.

		Alinity i HBsAg Qualitative II				ARCHITECT HBsAg Qualitative II
Category	n	IR (% of Total)	RR (% of Total)	Number Positive by Supplemental Testing (% of RR)	Specificity (95% CI)	Specificity (95% CI)
Blood Donors - Serum	2561	1 (0.04)	1 (0.04)	0 (0.00)	99.96% (2560/2561) (99.78 - 100.00)	99.96% (2561/2562) (99.78 - 100.00)
Blood Donors - Plasma	2549	2 (0.08)	1 (0.04)	0 (0.00)	99.96% (2548/2549) (99.78 - 100.00)	99.96% (2549/2550) (99.78 - 100.00)
Total Donors	5110	3 (0.06)	2 (0.04)	0 (0.00)	99.96% (5108/5110) (99.86 - 100.00)	99.96% (5110/5112)[a] (99.86 - 100.00)

IR = Initially Reactive, RR = Repeatedly Reactive, CI = Confidence Interval

[a] None of the 2 specimens additionally tested on the ARCHITECT HBsAg Qualitative II assay were initially reactive.

Diagnostic Specimens

A total of 218 randomly selected diagnostic (hospitalized) patients were tested using the Alinity i HBsAg Qualitative II assay and the ARCHITECT HBsAg Qualitative II assay. Two specimens were repeatedly reactive on both the Alinity i HBsAg Qualitative II assay and the ARCHITECT HBsAg Qualitative II assay. Specimens repeatedly reactive on the Alinity i HBsAg Qualitative II assay were further tested using the Alinity i HBsAg Qualitative II Confirmatory assay. Specimens repeatedly reactive on the ARCHITECT HBsAg Qualitative II assay were further tested using the ARCHITECT HBsAg Qualitative II Confirmatory assay. Based on supplemental test results, the presence of HBsAg was confirmed by specific neutralization with anti-HBs on the 2 repeatedly reactive specimens.

		Alinity i HBsAg Qualitative II				ARCHITECT HBsAg Qualitative II
Category	n	IR (% of Total)	RR (% of Total)	Number Positive by Supplemental Testing (% of RR)	Specificity[a] (95% CI)	Specificity[a] (95% CI)
Hospitalized Patients	218	2 (0.92)	2 (0.92)	2 (100.00)	100.000% (216/216) (98.31 - 100.00)	100.000% (216/216) (98.31 - 100.00)

IR = Initially Reactive, RR = Repeatedly Reactive, CI = Confidence Interval

[a] Two repeatedly reactive specimens determined to be positive by supplemental testing were excluded from these calculations.

Unrelated Medical Conditions

This study was performed on the ARCHITECT i System.

The ARCHITECT HBsAg Qualitative II assay was evaluated for potential cross-reactivity for specimens from individuals with medical conditions unrelated to HBV infection. A total of 294 specimens from 28 different categories were tested. Two hundred ninety specimens were nonreactive and 4 specimens were reactive by the ARCHITECT HBsAg Qualitative II and commercially-available HBsAg assays. All 4 reactive specimens were confirmed positive for HBsAg by the ARCHITECT HBsAg Qualitative II Confirmatory and commercially-available HBsAg confirmatory assays. The data are summarized by final interpretation in the following table.

		ARCHITECT HBsAg Qualitative II			
		Nonreactive		Reactive	
Category	n	NR[a]	R[a]	NR[a]	R[a]
Cytomegalovirus (CMV)	10	10	0	0	0
Epstein-Barr Virus (EBV)	10	10	0	0	0
Multiple Transfusion Recipients	10	10	0	0	0
Hepatitis A Virus (HAV)	10	10	0	0	0
Human Anti-Mouse Antibodies (HAMA) Positive	15	15	0	0	0
Hepatitis C Virus (HCV)	10	10	0	0	0
Human Immunodeficiency Virus (HIV-1)	10	10	0	0	0
Autoimmune Hepatitis	10	10	0	0	0
Human Immunodeficiency Virus (HIV-2)	17	14	0	0	3
Fatty Liver Disease	10	10	0	0	0
Herpes Simplex Virus (HSV)	10	10	0	0	0
Hepatocellular Carcinoma	10	10	0	0	0
Human T-Lymphotropic Virus (HTLV-1/2)	9	9	0	0	0
T. pallidum	2	2	0	0	0
N. gonorrhea	9	9	0	0	0
C. trachomatis	7	7	0	0	0
T. cruzi	10	10	0	0	0
Rheumatoid Factor (RF)	10	10	0	0	0
Anti-Nuclear Antibodies (ANA)	10	10	0	0	0
Pregnancy 1st Trimester	15	15	0	0	0
Pregnancy 2nd Trimester	15	14	0	0	1
Pregnancy 3rd Trimester	15	15	0	0	0
Multiparous Females	10	10	0	0	0
IgM Monoclonal Gammopathy	10	10	0	0	0
IgG Monoclonal Gammopathy	10	10	0	0	0
Multiple Myeloma	10	10	0	0	0
Influenza Vaccine Recipients	10	10	0	0	0
Hemodialysis Patient	10	10	0	0	0
Total	294	290	0	0	4

[a] NR = Nonreactive, R = Reactive

Sensitivity

A study was performed based on guidance from CLSI EP12-A2.[21] A total of 496 specimens from the following categories were evaluated with the Alinity i HBsAg Qualitative II assay and the ARCHITECT HBsAg Qualitative II assay: HBV genotypes (A-F, H), acute HBV infection, chronic HBV infection, known high positive specimens, known low positive specimens, other HBsAg positive specimens, and HBsAg mutant panel. The overall sensitivity of the Alinity i HBsAg Qualitative II assay was found to be 100% (496/496) with a two sided 95% confidence interval of 99.26 to 100.00%. The overall sensitivity of the ARCHITECT HBsAg Qualitative II assay was found to be 99.80% (495/496) with a two sided 95% confidence interval of 98.88 to 99.99%.

		Alinity i HBsAg Qualitative II			ARCHITECT HBsAg Qualitative II	
Specimen Category	n	RR (% of Total)	Sensitivity	95% CI	Sensitivity	95% CI
Genotypes A, B, C, D, E, F, H	47	47 (100%)	100% (47/47)	92.45 - 100.00	100% (47/47)	92.45 - 100.00
Acute HBV Infection	25	25 (100%)	100% (25/25)	86.28 - 100.00	100% (25/25)	86.28 - 100.00
Chronic HBV Infection	72	72 (100%)	100% (72/72)	95.01 - 100.00	100% (72/72)	95.01 - 100.00
High Positive	210	210 (100%)	100% (210/210)	98.26 - 100.00	100% (210/210)	98.26 - 100.00
Low Positive	22	22 (100%)	100% (22/22)	84.56 - 100.00	100% (22/22)	84.56 - 100.00
Other HBsAg Positive	52	52 (100%)	100% (52/52)	93.15 - 100.00	98.08% (51/52)	89.74 - 99.95
HBsAg Mutant Panel[a]	68	68 (100%)	100% (68/68)	94.72 - 100.00	100% (68/68)	94.72 - 100.00

RR = Repeatedly Reactive, CI = Confidence Interval

[a] Includes 13 mutants containing Thr-123-Ala and/or Gly-145-Arg mutations.

HBsAg Mutant Detection

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. 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.[22-29] HBsAg mutations may result in a less favorable outcome in some patients[22, 23, 25] and false negative results in some HBsAg assays.[22-24]

The panel of 68 samples was comprised of two recombinant wild-type controls and 65 different recombinant HBsAg mutant samples. One mutation pattern was common in two panel members; mutant 113 (JPA) and mutant 104 share an identical mutant pattern. All mutant samples were recombinant antigen with amino acid sequences representing native mutants of hepatitis B s antigen. 57/66 recombinant mutant samples included substitutions or insertions in the region spanning aa 120 – 145 within the 'a' determinant. The panel contained 28 samples with single substitutions, 12 samples with two substitutions, 21 samples with 3 to 12 substitutions, and 5 samples with insertions following aa 122 or 123 of the s antigen. All samples had been diluted in recalcified negative human plasma to low reactive S/CO levels in ARCHITECT HBsAg Qual II. This mutant panel included 13 HBsAg mutants containing Thr-123-Ala and/or Gly-145-Arg mutations.

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 5 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 instrument. The analytical sensitivity results ranged from 19.93 to 20.87 mIU/mL for the 3 lots.

Seroconversion Sensitivity

To determine the seroconversion sensitivity, 32 seroconversion panels obtained from commercial vendors were tested on the Alinity i system using the HBsAg Qualitative II and HBsAg Qualitative II Confirmatory assays. The results were compared to the ARCHITECT HBsAg Qualitative II assay and representative data from 5 panels are summarized in the following table.

Panel ID	Days Since 1st Bleed	Alinity i HBsAg Qualitative II S/CO (Reactive ≥ 1.00 S/CO)	ARCHITECT HBsAg Qualitative II S/CO (Reactive ≥ 1.00 S/CO)
6271	0	0.41	0.44
	3	0.91	0.83
	7	**2.44**	**2.52**
	12	**14.52**	**16.28**
	18	**116.22**	**126.96**
6273	0	0.37	0.23
	3	0.32	0.22
	7	0.38	0.34
	14	**1.14**	**1.18**
	25	**22.52**	**25.19**
	30	**158.66**	**168.52**
6275	0	0.59	0.49
	2	0.66	0.59
	7	**1.31**	**1.90**
	9	**1.61**	**1.60**
	22	**15.81**	**1.62**
	27	**38.20**	**41.64**
	29	**55.26**	**63.53**
11000	0	0.40	0.28
	3	0.34	0.32
	12	0.51	0.46
	14	0.68	0.70
	19	0.88	0.87
	21	**1.35**	**1.47**
	26	**3.27**	**3.47**
	29	**5.05**	**5.23**
	33	**20.66**	**23.44**
11002	0	0.51	0.48
	2	0.64	0.57
	7	**1.96**	**1.96**
	9	**2.59**	**2.77**
	35	**1753.02**	**1821.14**
	39	**490.52**	**515.05**

Interference

Potentially Interfering Endogenous Substances

This study was performed on the ARCHITECT i System.

No qualitative performance differences were observed between experimental controls and nonreactive or spiked reactive specimens tested with elevated levels of conjugated or unconjugated bilirubin, triglycerides, protein, or hemoglobin.

Potentially Interfering Substance	Interferent Level
Conjugated Bilirubin	≤ 20 mg/dL
Unconjugated Bilirubin	≤ 20 mg/dL
Triglycerides	≤ 3000 mg/dL
Protein	≤ 12 g/dL
Hemoglobin	≤ 500 mg/dL

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Note for number formatting:

- A space is used as thousands separator (example: 10 000 specimens).
- A period is used to separate the integer part from the fractional part of a number written in decimal form (example: 3.12%).

Key to Symbols

	Caution
	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
ANCILLARY WASH BUFFER	Ancillary Wash Buffer
CONJUGATE	Conjugate
IVD	*In Vitro* Diagnostic Medical Device
INVERSIONS PERFORMED	Inversions Performed
LOT	Lot Number
MICROPARTICLES	Microparticles
PRODUCT OF IRELAND	Product of Ireland
REF	List Number
SN	Serial number

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