Created May 2022.

CAUTION: United States Federal Law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.

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

For laboratory professional use only.

NAME

ARCHITECT HBsAg Next Qualitative (also referred to as HBsAgNx)

INTENDED USE

The HBsAg Next Qualitative assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of Hepatitis B surface antigen (HBsAg) in human adult and pediatric (2 years to 21 years of age) serum, serum separator tube, and plasma (dipotassium EDTA, tripotassium EDTA, lithium heparin, lithium heparin separator, sodium heparin) on the ARCHITECT i System.

The assay may also be used to screen for hepatitis B virus (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 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.

WARNING: Not approved for use in screening blood, plasma, tissue donors, or cadaveric specimens.

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 10 weeks after exposure and 2 to 8 weeks before the onset of clinical symptoms.[2, 3] 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 or chronic HBV infection.

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.4 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.5

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.6 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.6, 7, 8, 9, 10, 11, 12 HBsAg mutations may result in a less favorable outcome in some patients6, 8, 13 and false negative results in some HBsAg assays.6, 7, 8

Specimens nonreactive by ARCHITECT HBsAg Next Qualitative are considered negative for HBsAg. A reactive specimen must be retested in duplicate by ARCHITECT HBsAg Next Qualitative to determine whether it is repeatedly reactive. Specimens found to be repeatedly reactive by the ARCHITECT HBsAg Next Qualitative assay should be confirmed using the ARCHITECT HBsAg Next Confirmatory (4P77) assay, a neutralization procedure utilizing sheep 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 adult and pediatric 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, assay specific diluent, 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 a relative light unit (RLU). There is a direct relationship between the amount of HBsAg in the sample and the RLU detected by the system optics.

The presence or absence of HBsAg in the specimen is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an active calibration. If the chemiluminescent signal in the reaction is greater than or equal to the cutoff signal, the specimen is considered reactive for HBsAg.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Kit Contents

ARCHITECT HBsAg Next Qualitative Reagent Kit 4P76

NOTE: Some kit sizes may not be available for use on all ARCHITECT i Systems. Please contact your local distributor.

Volumes (mL) listed in the following table indicate the volume per bottle.

REF	4P76-27	4P76-37	4P76-32
Tests per kit	100	500	500
Number of kits per box	1	1	4
Tests per box	100	500	2000
MICROPARTICLES	6.6 mL	27.0 mL	27.0 mL
CONJUGATE	3.3 mL	13.5 mL	13.5 mL
ASSAY SPECIFIC DILUENT	3.3 mL	13.5 mL	13.5 mL
ANCILLARY WASH BUFFER	5.9 mL	26.3 mL	26.3 mL

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

CONJUGATE anti-HBs (goat, IgG) acridinium-labeled conjugate in phosphate buffer with protein (bovine, goat, mouse) stabilizers. Minimum concentration: 0.75 µg/mL. Preservatives: ProClin 300 and ProClin 950.

ASSAY SPECIFIC DILUENT contains phosphate buffer with protein (bovine) stabilizer. Preservatives: ProClin 300 and ProClin 950.

ANCILLARY WASH BUFFER contains MES buffer. Preservatives: ProClin 300 and ProClin 950.

Warnings and Precautions

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

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[14, 15, 16, 17]

The following warnings and precautions apply to: MICROPARTICLES, CONJUGATE, ANCILLARY WASH BUFFER, and ASSAY SPECIFIC DILUENT

WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
H402	Harmful to aquatic life.
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.
P273	Avoid release to the environment.
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.

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

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

Safety Data Sheets are available at www.corelaboratory.abbott or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Reagent Handling

- Do not pool reagents within a kit or between kits.
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the PROCEDURE, Assay Procedure section of this package insert.

Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.

- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
- Once a septum has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.

For a detailed discussion of reagent handling precautions during system operation, refer to the ARCHITECT System 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.
Onboard	System	30 days	

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
	Temperature		
Opened	2 to 8°C	Until expiration date	Store in upright position. If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.

Reagents may be stored on or off the ARCHITECT i System. If reagents are removed from the system, store them at 2 to 8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright.

For information on unloading reagents, refer to the ARCHITECT System 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 ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The ARCHITECT HBsAg Next Qualitative assay file must be installed on the ARCHITECT i2000SR with Induction Heating prior to performing the assay.

The ARCHITECT System software version 9.25 or higher must be installed on the ARCHITECT i System prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.

For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.

For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

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

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

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

- Performance has not been established for the use of cadaveric specimens or the use of bodily fluids other than human serum/plasma.
- Liquid anticoagulants may have a dilution effect resulting in lower S/CO values for individual specimens.

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
- specimens with fungal growth

- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.

- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

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

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

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

  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

*To ensure consistency in results, specimens must be centrifuged using an appropriate tube at a minimum of 2500 RCF to obtain a minimum of 100 000 g-minutes.

- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/Plasma	Room temperature (15 to 30°C)	3 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	2 to 8°C	7 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.

If testing will be delayed more than 7 days, remove serum or plasma from the clot, red blood cells, or separator gel and store at -20°C or colder.

Do not subject the specimens to more than 3 freeze/thaw cycles

Specimen Shipping

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

Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

4P76 ARCHITECT HBsAg Next Qualitative Reagent Kit

Materials Required but not Provided

- ARCHITECT HBsAg Next Qualitative assay file found on www.corelaboratory.abbott.
- 4P76-01 ARCHITECT HBsAg Next Qualitative Calibrators
- 4P76-10 ARCHITECT HBsAg Next Qualitative Controls
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Septum

For information on materials required for operation of the instrument, refer to the ARCHITECT System Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

For a detailed description of how to run an assay, refer to the ARCHITECT System Operations Manual, Section 5.

- If using primary or aliquot tubes, refer to the ARCHITECT System Operations Manual, Section 5 to ensure sufficient specimen is present.
- Minimum sample cup volume is calculated by the system and printed on the Orderlist report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.

- **Invert the microparticle bottle 30 times.**
- Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
- **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**

- Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the Reagent Handling section of this package insert.

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

Priority:

- Sample volume for first test: 125 µL
- Sample volume for each additional test from same sample cup: 75 µL

≤ 3 hours on the reagent and sample manager:

- Sample volume for first test: 150 µL
- Sample volume for each additional test from same sample cup: 75 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

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

Sample Dilution Procedures

Samples cannot be diluted for the ARCHITECT HBsAg Next Qualitative assay.

Calibration

For instructions on performing a calibration, refer to the ARCHITECT System Operations Manual, Section 6.

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 quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.

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 ARCHITECT HBsAg Next Qualitative 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.

Refer to published guidelines for information on general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) or other published guidelines.

- 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 ARCHITECT System Operations Manual, Section 10.
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B.

The ARCHITECT HBsAg Next Qualitative assay belongs to method group 5.

RESULTS

Calculation

The ARCHITECT i System calculates results for the ARCHITECT HBsAg Next Qualitative 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 0.085) + (Calibrator 2 mean RLU x 0.25)

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.

Duplicate Retest Results

Instrument Interpretation	Specimen Classification
Both results nonreactive	Specimen considered negative for HBsAg.
One or both results reactive	Specimen considered repeatedly reactive; confirm using the ARCHITECT HBsAg Next Confirmatory assay. *

* Only the ARCHITECT HBsAg Next Confirmatory assay has been evaluated with the ARCHITECT HBsAg Next Qualitative assay.

The name(s) of the assays used to detect and confirm reactive results should be included in the results reported by the laboratory.

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 ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- The effectiveness of the ARCHITECT HBsAg Next Qualitative assay for use in screening blood, plasma, tissue donors, or cadaveric specimens has not been established.
- Assay performance characteristics have not been established when the ARCHITECT HBsAg Next Qualitative 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 ARCHITECT HBsAg Next Qualitative 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.
- Results obtained with the ARCHITECT HBsAg Next Qualitative assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical presentation.
- Potential interference has not been evaluated for substances other than those described in the SPECIFIC PERFORMANCE CHARACTERISTICS, Interference section of this package insert.
- **If specimens are not centrifuged according to the instructions in this package insert, depressed results may be obtained.**
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT HBsAg Next Qualitative that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.*18,19*
- 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.*20*
- Vaccination with a recombinant hepatitis B vaccine may cause transient positive results with a sensitive HBsAg assay such as ARCHITECT HBsAg Next Qualitative. These results are caused by a passive transfer of antigen by vaccination, not by viral replication. Positive results usually do not persist for more than 14 days after vaccination*21*, though positive signals up to 52 days have been reported*22*, and may not indicate clinical disease.
- 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

This study was performed on the ARCHITECT i2000SR System.

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

Increased Risk Population

Of the 2790 specimens tested in the ARCHITECT HBsAg Next Qualitative clinical study, 1205 specimens were from individuals with increased risk of HBV infection. All 1205 individuals were at risk for HBV infection due to lifestyle, behavior, occupation, or a known exposure event but reported no current signs or symptoms of hepatitis. Testing of these specimens was performed at 3 clinical sites located in San Antonio, TX; Indianapolis, IN; and Pompano Beach, FL.

The increased risk population (n = 1205) consisted of the following race/ ethnic groups:

- 229 (19.0%) White
- 741 (61.5%) Black or African American
- 152 (12.6%) Hispanic or Latino
- 8 (0.7%) Asian
- 1 (0.1%) American Indian/Alaska Native
- 1 (0.1%) Native Hawaiian/Other Pacific Islander
- 1 (0.1%) Lebanese
- 69 (5.7%) Mixed Race
- 2 (0.2%) Other
- 1 (0.1%) Unknown

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	Number of Specimens Collected (%)	Number of Reactive Results from Each Location (%)
Chicago, IL	18 (1.49)	0 (0.00)
Colton, CA	20 (1.66)	0 (0.00)
Dallas, TX	4 (0.33)	0 (0.00)
Denver, CO	1 (0.08)	0 (0.00)

Specimen Collection Site/ Vendor Location	Number of Specimens Collected (%)	Number of Reactive Results from Each Location (%)
Galveston, TX	88 (7.30)	2 (2.27)
Hyannis, MA	35 (2.90)	0 (0.00)
Los Angeles, CA	272 (22.57)	4 (1.47)
Metairie, LA	235 (19.50)	2 (0.85)
Miami, FL	4 (0.33)	0 (0.00)
New York, NY	242 (20.08)	1 (0.41)
Newark, NJ	281 (23.32)	3 (1.07)
St. Petersburg, FL	5 (0.41)	0 (0.00)
Total	**1205 (100.00)**	**12/1205 (1.00)**

Of the 1205 specimens, 431 (35.8%) were female and 774 (64.2%) were male. The median age was 39 years (age range: 17 to 72 years).

The distribution of ARCHITECT HBsAg Next Qualitative reactive and nonreactive results among the increased risk population by age and gender (n = 1205) is summarized in the following table.

		ARCHITECT HBsAg Next Qualitative Result		
Age Range (Years)	Gender	Number of Reactive (%)	Number of Nonreactive (%)	Total
18 and Younger	Female	0 (0.00)	6 (0.50)	6
	Male	0 (0.00)	6 (0.50)	6
19 to 30	Female	0 (0.00)	148 (12.28)	148
	Male	1 (0.08)	210 (17.43)	211
31 to 40	Female	2 (0.17)	95 (7.88)	97
	Male	3 (0.25)	176 (14.61)	179
41 to 50	Female	0 (0.00)	120 (9.96)	120
	Male	1 (0.08)	230 (19.09)	231
51 to 60	Female	0 (0.00)	51 (4.23)	51

Age Range (Years)	Gender	ARCHITECT HBsAg Next Qualitative Result		Total
		Number of Reactive (%)	Number of Nonreactive (%)	
	Male	5 (0.41)	120 (9.96)	125
61 to 70	Female	0 (0.00)	9 (0.75)	9
	Male	0 (0.00)	20 (1.66)	20
71 to 80	Male	0 (0.00)	2 (0.17)	2
Total		**12 (1.00)**	**1193 (99.00)**	**1205**

SPECIFIC PERFORMANCE CHARACTERISTICS

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

Unless otherwise specified, all studies were performed on the ARCHITECT i2000SR System.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A3.[23] Testing was conducted using 3 lots of the ARCHITECT HBsAg Next Qualitative reagent, 3 lots of the ARCHITECT HBsAg Next Qualitative Calibrators, and 3 lots of the ARCHITECT HBsAg Next Qualitative Controls and 2 ARCHITECT i2000SR instruments. Two controls and 3 human plasma panels were tested in replicates of 3 (to obtain a minimum of 2 replicates), twice per day on 20 days on 6 reagent lot/calibrator lot//instrument combinations, where a unique reagent lot and a unique calibrator lot is paired with 1 instrument. The results are summarized in the following table.

Instrument	Reagent Lot	Sample	n	Mean (S/CO)	Within- Run (Repeatability)		Within-Laboratory[a]	
					SD	%CV	SD	%CV
i2000SR (1)	1	Negative Control	120	0.36	0.027	N/A	0.039	N/A
		Positive Control	120	3.15	0.065	2.1	0.076	2.4
		High Negative	120	0.82	0.027	3.2	0.052	6.3

Instrument	Reagent Lot	Sample	n	Mean (S/CO)	Within- Run (Repeatability)		Within-Laboratory[a]	
					SD	%CV	SD	%CV
		Panel						
		Low Positive Panel	120	1.31	0.039	3.0	0.064	4.9
		Moderate Positive Panel	120	2.92	0.076	2.6	0.080	2.8
	2	Negative Control	119	0.19	0.023	N/A	0.035	N/A
		Positive Control	119	3.25	0.077	2.4	0.099	3.0
		High Negative Panel	119	0.72	0.027	3.7	0.049	6.8
		Low Positive Panel	119	1.28	0.037	2.9	0.058	4.5
		Moderate Positive Panel	119	3.11	0.072	2.3	0.087	2.8
	3	Negative Control	120	0.31	0.021	N/A	0.031	N/A
		Positive Control	119	3.21	0.090	2.8	0.101	3.1
		High Negative Panel	120	0.80	0.027	3.4	0.041	5.1
		Low Positive Panel	120	1.31	0.040	3.0	0.056	4.2
		Moderate Positive Panel	119	3.03	0.066	2.2	0.079	2.6

Instrument	Reagent Lot	Sample	n	Mean (S/CO)	Within- Run (Repeatability)		Within-Laboratory[a]	
					SD	%CV	SD	%CV
i2000SR (2)	1	Negative Control	120	0.35	0.032	N/A	0.042	N/A
		Positive Control	119	3.18	0.083	2.6	0.093	2.9
		High Negative Panel	119	0.80	0.044	5.5	0.051	6.4
		Low Positive Panel	116	1.26	0.042	3.4	0.051	4.1
		Moderate Positive Panel	118	2.92	0.075	2.6	0.085	2.9
	2	Negative Control	120	0.19	0.023	N/A	0.040	N/A
		Positive Control	120	3.23	0.138	4.3	0.145	4.5
		High Negative Panel	120	0.68	0.026	3.8	0.034	5.0
		Low Positive Panel	119	1.20	0.038	3.2	0.041	3.4
		Moderate Positive Panel	118	3.04	0.087	2.8	0.103	3.4
	3	Negative Control	119	0.30	0.014	N/A	0.028	N/A
		Positive Control	120	3.13	0.083	2.7	0.095	3.0
		High Negative Panel	119	0.76	0.074	9.7	0.077	10.2

Instrument	Reagent Lot	Sample	n	Mean (S/CO)	Within- Run (Repeatability)		Within-Laboratory[a]	
					SD	%CV	SD	%CV
		Low Positive Panel	119	1.24	0.041	3.3	0.047	3.8
		Moderate Positive Panel[b]	118	2.89	0.121	4.2	0.129	4.4

N/A = Not applicable

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

[b] An outlying run was observed. A replacement run was performed and the results are shown in the preceding table. Without the replacement run, the within-run (repeatability) %CV was 130.3% and the within-laboratory precision %CV was 130.4%.

System Reproducibility

A 5-day precision study was performed for the ARCHITECT HBsAg Next Qualitative assay based on guidance from CLSI EP05-A3. 23Testing was conducted at 3 clinical sites using 3 lots each of ARCHITECT HBsAg Next Qualitative reagents, calibrators, and controls and one ARCHITECT i2000SR per site. Two controls and 3 panels were assayed in replicates of 4 at 2 separate times of day for 5 days. The results are summarized in the following table.

			Within-Run		Between-Run		Between-Day		Within-Laboratory [a]			Between-Site		Between-Lot		Reproducibility[b]	
Sample	n	Mean S/CO	SD	%CV	SD	%CV	SD	%CV	SD	%CV	%CV Upper CL[c]	SD	%CV	SD	%CV	SD	%CV
Negative Control	359	0.32	0.030	N/A	0.005	N/A	0.000	N/A	0.030	N/A	N/A	0.011	N/A	0.098	N/A	0.103	N/A
Positive Control	360	3.18	0.081	2.5	0.021	0.6	0.008	0.3	0.084	2.6	2.8	0.030	0.9	0.033	1.0	0.096	3.0
High Negative Panel	360	0.80	0.026	3.2	0.016	2.0	0.000	0.0	0.030	3.8	4.1	0.022	2.7	0.062	7.8	0.074	9.2
Low Positive Panel	360	1.31	0.044	3.4	0.024	1.8	0.000	0.0	0.050	3.8	4.1	0.031	2.4	0.036	2.8	0.070	5.3
Moderate Positive Panel	359	3.07	0.083	2.7	0.056	1.8	0.022	0.7	0.102	3.3	3.6	0.055	1.8	0.101	3.3	0.156	5.1

N/A = Not applicable

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

[b] Includes within-run, between-run, between-day, between-site, between-lot, and the site-lot interaction variability.

[c] One-sided upper 95% confidence limit for %CV with degrees of freedom calculated by Satterthwaite method.

Clinical Performance

A multi-center study was conducted to evaluate the ability of the ARCHITECT HBsAg Next Qualitative assay to detect HBsAg in a group of individuals that would normally be tested in a clinical situation. Of the 2790 specimens tested and analyzed in the clinical study, 1827 specimens were obtained from individuals with increased risk of HBV infection due to lifestyle, behavior, occupation, or a known exposure event (n = 1205) and individuals exhibiting signs and symptoms of hepatitis infection (n = 622).

Specimens (n = 1827) from the increased risk and signs and symptoms populations consisted of the following race/ethnic groups:

- 551 (30.2%) White
- 962 (52.7%) Black or African American
- 200 (10.9%) Hispanic or Latino
- 13 (0.7%) Asian
- 4 (0.2%) American Indian/Alaska Native
- 4 (0.2%) Native Hawaiian/Other Pacific Islander
- 1 (0.1%) Lebanese
- 86 (4.7%) Mixed Race
- 5 (0.3%) Other
- 1 (0.1%) Unknown

Specimens (n = 1827) from the increased risk and signs and symptoms populations were obtained from the following collection locations:

- 21 (1.15%) Chicago, IL
- 20 (1.09%) Colton, CA
- 15 (0.82%) Dallas, TX
- 16 (0.88%) Denver, CO
- 249 (13.63%) Fall River, MA
- 88 (4.82%) Galveston, TX
- 35 (1.92%) Hyannis, MA
- 416 (22.77%) Los Angeles, CA
- 371 (20.31%) Metairie, LA

- 6 (0.33%) Miami, FL
- 267 (14.61%) New York, NY
- 318 (17.41%) Newark, NJ
- 5 (0.27%) St. Petersburg, FL

Each specimen was tested at 1 of 3 clinical sites located in San Antonio, TX; Indianapolis, IN; and Pompano Beach, FL, using the ARCHITECT HBsAg Next Qualitative assay. Each specimen was also tested using a comparator HBsAg assay at 1 of 2 clinical sites located in San Antonio, TX or Indianapolis, IN.

Of the 1827 specimens from the increased risk and signs and symptoms populations, 727 (39.8%) were female and 1100 (60.2%) were male. The median age was 40 years (age range: 17 to 84 years).

The ARCHITECT HBsAg Next Qualitative assay was further evaluated by testing a total of 129 pre-selected specimens from acute and chronic HBV infections.

The HBV classification was determined for each specimen based on the reactivity patterns of the 4 HBV serological marker results (HBsAg, anti-HBc IgM, total anti-HBc, and anti-HBs). This testing was performed using FDA approved assays from 3 manufacturers, and testing was performed following the manufacturer's instructions.

Results by Specimen Classification

For the increased risk and signs and symptoms populations (n = 1827) and the pre-selected diagnosed acute and chronic population (n = 129), the HBV classification was determined for each specimen according to the following table based on the reactivity patterns of the 4 HBV serological marker results (HBsAg, anti-HBc IgM, total anti-HBc, and anti-HBs) (total n = 1956). There were 13 unique reference marker patterns observed in the ARCHITECT HBsAg Next Qualitative clinical study.

	HBV Reference Markers				
Number of Specimens	**HBsAg[a]**	**Anti-HBc IgM**	**Total Anti-HBc**	**Anti-HBs**	**HBV Classification**
27	+	-	-	-	Early acute
35	+	+	+	-	Acute
5	+	I	+	-	Acute
1	-	+	+	-	Recovering acute/undetectable HBsAg
8	-	+	+	+	Recovering acute

Number of Specimens	HBV Reference Markers				HBV Classification
	HBsAg[a]	Anti-HBc IgM	Total Anti-HBc	Anti-HBs	
1	-	+	-	-	Possible recovering acute/undetectable HBsAg
1	+	-	+	+	Chronic
71	+	-	+	-	Chronic
165	-	-	+	+	Immune due to natural infection
554	-	-	-	+	Immune due to HBV vaccination
983	-	-	-	-	Susceptible
51	-	-	+	-	Uninterpretable
11	-	-	+	I	Uninterpretable
39	-	-	-	I	Uninterpretable
4	-	I	+	+	Uninterpretable
1956					**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 Next Qualitative assay results with the ARCHITECT HBsAg Qualitative and ARCHITECT HBsAg Qualitative Confirmatory assays final interpretation for each of the HBV classifications for the increased risk and signs and symptoms populations (n = 1827) and the acute or chronic HBV infection populations (n = 129). The combined results are summarized in the following table.

HBV Classification	Comparator ARCHITECT HBsAg Qualitative Final Interpretation			
	Confirmed Positive		Negative/Not Confirmed	
	ARCHITECT HBsAg Next Qualitative Result		ARCHITECT HBsAg Next Qualitative Result	
	Repeatedly Reactive	Nonreactive	Repeatedly Reactive	Nonreactive
	N	N	N	N
Early acute	26	0	0	0
Acute	38	0	0	0
Recovering acute / undetectable HBsAg	0	0	0	1
Recovering acute	0	0	0	8
Possible recovering acute / undetectable HBsAg	0	0	0	1
Chronic	68	0	0	0
Immune due to natural infection	0	0	0	165
Immune due to HBV vaccination	0	0	1[a]	553
Susceptible	0	0	1[b]	982
Uninterpretable	0	0	2	103
Total[c]	**132**	**0**	**4[d]**	**1813**

[a] Specimen was PCR positive, consistent with vaccine breakthrough or recent vaccination.*24*

[b] Specimen was PCR positive, consistent with an early acute infection or an occult infection. *25,26*

[c] Seven specimens were excluded due to no ARCHITECT HBsAg Qualitative Confirmatory results.

[d] The 4 specimens were confirmed reactive by the ARCHITECT HBsAg Next Confirmatory assay.

Percent Agreement

The percent agreement between the ARCHITECT HBsAg Next Qualitative assay results and the ARCHITECT HBsAg Qualitative assay final interpretation for the increased risk and signs and symptoms populations by HBV classification (n = 1827) is summarized in the following table.

HBV Classification	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Acute	100.00 (3/3)	(43.85, 100.00)	N/A[a]	N/A
Recovering acute/ undetectable HBsAg	N/A	N/A	100.00 (1/1)	(20.65, 100.00)
Recovering acute	N/A	N/A	100.00 (8/8)	(67.56, 100.00)
Possible recovering acute / undetectable HBsAg	N/A	N/A	100.00 (1/1)	(20.65, 100.00)
Chronic	100.00 (7/7)	(64.57, 100.00)	N/A	N/A
Immune due to natural infection	N/A	N/A	100.00 (165/165)	(97.72, 100.00)
Immune due to HBV vaccination	N/A	N/A	99.82 (553/554)	(98.98, 99.97)
Susceptible	N/A	N/A	99.90 (982/983)	(99.43, 99.98)
Uninterpretable	N/A	N/A	98.10 (103/105)	(93.32, 99.48)
Total	**100.00 (10/10)**	**(72.25, 100.00)**	**99.78 (1813/1817)**	**(99.44, 99.91)**

[a] Not applicable

The percent agreement between the ARCHITECT HBsAg Next Qualitative assay results and the ARCHITECT HBsAg Qualitative assay final interpretation for the increased risk population by HBV classification (n = 1205) is summarized in the following table.

HBV Classification	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Acute	100.00 (3/3)	(43.85, 100.00)	N/A[a]	N/A
Recovering acute	N/A	N/A	100.00 (5/5)	(56.55, 100.00)
Chronic	100.00 (6/6)	(60.97, 100.00)	N/A	N/A
Immune due to natural infection	N/A	N/A	100.00 (122/122)	(96.95, 100.00)
Immune due to HBV vaccination	N/A	N/A	99.73 (365/366)	(98.47, 99.95)
Susceptible	N/A	N/A	99.84 (635/636)	(99.11, 99.97)
Uninterpretable	N/A	N/A	98.51 (66/67)	(92.02, 99.74)
Total	**100.00 (9/9)**	**(70.09, 100.00)**	**99.75 (1193/1196)**	**(99.27, 99.91)**

[a] Not applicable

The percent agreement between the ARCHITECT HBsAg Next Qualitative assay results and the ARCHITECT HBsAg Qualitative assay final interpretation for the signs and symptoms population by HBV classification (n = 622) is summarized in the following table.

HBV Classification	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Recovering acute/ Undetectable HBsAg	N/A[a]	N/A	100.00 (1/1)	(20.65, 100.00)
Recovering acute	N/A	N/A	100.00 (3/3)	(43.85, 100.00)
Possible recovering acute/ Undetectable HBsAg	N/A	N/A	100.00 (1/1)	(20.65, 100.00)
Chronic	100.00 (1/1)	(20.65, 100.00)	N/A	N/A
Immune due to natural infection	N/A	N/A	100.00 (43/43)	(91.80, 100.00)

HBV Classification	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Immune due to HBV vaccination	N/A	N/A	100.00 (188/188)	(98.00, 100.00)
Susceptible	N/A	N/A	100.00 (347/347)	(98.91, 100.00)
Uninterpretable	N/A	N/A	97.37 (37/38)	(86.51, 99.53)
Total	**100.00 (1/1)**	**(20.65, 100.00)**	**99.84 (620/621)**	**(99.09, 99.97)**

[a] Not applicable

Percent Agreement for Individuals With Acute or Chronic HBV Infection

The percent agreement between the ARCHITECT HBsAg Next Qualitative assay results and the ARCHITECT HBsAg Qualitative assay final interpretation for the pre-selected specimens from individuals with acute and chronic HBV infection (n = 129) 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	100.00 (55/55)	(93.47, 100.00)	N/A[a]	N/A
Individuals with chronic HBV infection	100.00 (74/74)	(95.07, 100.00)	N/A	N/A

[a] Not applicable

Increased Risk Population Testing

There were 1205 specimens from individuals at increased risk tested at 3 clinical sites. The following table compares the ARCHITECT HBsAg Next Qualitative results and the ARCHITECT HBsAg Qualitative and ARCHITECT HBsAg Qualitative Confirmatory assays final interpretations for each risk factor for this overall increased risk population.

	Comparator ARCHITECT HBsAg Qualitative Assay Final Interpretation				
	Confirmed Positive		Negative/Not Confirmed		
	ARCHITECT HBsAg Next Qualitative Result		ARCHITECT HBsAg Next Qualitative Result		
Specimen Category	**Repeatedly Reactive (N)**	**Nonreactive (N)**	**Repeatedly Reactive (N)**	**Nonreactive (N)**	**Total (N)**
Multiple sex partners	8	0	3	916	927
Intravenous drug user	0	0	0	50	50
Men who have sex with men (MSM)	0	0	0	33	33
Sexual contact with HBV infected individual	1	0	0	14	15
Household contact with HBV infected individual	0	0	0	37	37
Diagnosed or treated for a sexually transmitted disease	0	0	0	58	58
Current or past residence in a Hepatitis B endemic region	0	0	0	6	6
History of incarceration	0	0	0	79	79
Total	**9**	**0**	**3**[a]	**1193**	**1205**

[a] Specimens were confirmed positive using the ARCHITECT HBsAg Next Confirmatory assay. Two of the 3 repeatedly reactive specimens were PCR positive. The third specimen was anti-HBc positive.

Clinical Performance in Pregnant Females

The performance of ARCHITECT HBsAg Next 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 2790 specimens tested in the clinical study, 706 were from a pregnant female population. The specimens were obtained from commercial vendors. The 706 specimens, from pregnant females aged 18 to 43 years, were collected from collection sites in City of Industry, CA (n = 88); Los Angeles, CA (n = 76); Santa Ana, CA (n = 10); Fall River, MA (n = 358); and New York City, NY (n = 174). Testing of these specimens was performed at the clinical sites located in San Antonio, TX; Indianapolis, IN; and Pompano Beach, FL. The demographic profile of the pregnant female population is presented in the table below.

	Low Risk	**Increased Risk**	**Total**
Category	**N (%)**	**N (%)**	**N (%)**
Total	**524 (74.2)**	**182 (25.8)**	**706 (100.00)**
TRIMESTER			
First	194 (37.0)	66 (36.3)	260 (36.8)
Second	178 (34.0)	33 (18.1)	211 (29.9)
Third	152 (29.0)	83 (45.6)	235 (33.3)
RACE/ETHNIC GROUP			
White	142 (27.1)	25 (13.7)	167 (23.7)
Black or African American	94 (17.9)	45 (24.7)	139 (19.7)
Hispanic or Latino	251 (47.9)	97 (53.3)	348 (49.3)
Asian	14 (2.7)	2 (1.1)	16 (2.3)
American Indian/Alaska Native	1 (0.2)	N/A[a]	1 (0.1)
Native Hawaiian/Pacific Islander	1 (0.2)	N/A	1 (0.1)
Mixed Race	2 (0.4)	2 (1.1)	4 (0.6)
Other	8 (1.5)	6 (3.3)	14 (2.0)
Unknown	11 (2.1)	5 (2.7)	16 (2.3)
AGE RANGE			
18 to 31	363 (69.3)	151 (83.0)	514 (72.8)

Category	Low Risk N (%)	Increased Risk N (%)	Total N (%)
32 to 45	161 (30.7)	31 (17.0)	192 (27.2)

[a] Not applicable

Agreement for Pregnant Females by Risk and Trimester

A comparison was performed between the ARCHITECT HBsAg Next Qualitative assay results and the ARCHITECT HBsAg Qualitative assay results using samples obtained from a total of 706 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 following tables.

ARCHITECT HBsAg Next Qualitative and ARCHITECT HBsAg Qualitative and ARCHITECT HBsAg Qualitative Confirmatory Results by Trimester for Low Risk Pregnant Females

	First Trimester			Second Trimester			Third Trimester		
	Comparator ARCHITECT HBsAg Qualitative Final Interpretation			Comparator ARCHITECT HBsAg Qualitative Final Interpretation			Comparator ARCHITECT HBsAg Qualitative Final Interpretation		
ARCHITECT HBsAg Next 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	194	194	0	178	178	0	152	152
Total	**0**	**194**	**194**	**0**	**178**	**178**	**0**	**152**	**152**

ARCHITECT HBsAg Next Qualitative and ARCHITECT HBsAg Qualitative and ARCHITECT HBsAg Qualitative Confirmatory Results by Trimester for Increased Risk Pregnant Females

	First Trimester			Second Trimester			Third Trimester		
	Comparator ARCHITECT HBsAg Qualitative Final Interpretation			Comparator ARCHITECT HBsAg Qualitative Final Interpretation			Comparator ARCHITECT HBsAg Qualitative Final Interpretation		
ARCHITECT HBsAg Next 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	66	66	0	33	33	0	83	83
Total	**0**	**66**	**66**	**0**	**33**	**33**	**0**	**83**	**83**

Overall Summary and Percent Agreement for Pregnant Females

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

Subjects	Negative Percent Agreement	95% Confidence Interval	Overall Percent Agreement	95% Confidence Interval
Pregnant Females – Increased Risk	100.00 (182/182)	(97.93 - 100.00)	100.00 (182/182)	(97.93 - 100.00)
Pregnant Females – Low Risk	100.00 (524/524)	(99.27 - 100.00)	100.00 (524/524)	(99.27 - 100.00)
Pregnant Females - Total	100.00 (706/706)	(99.46, 100.00)	100.00 (706/706)	(99.46, 100.00)

A total of 2533 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 Next Qualitative assay. The repeatedly reactive specimens were confirmed using the ARCHITECT HBsAg Next Confirmatory assay. There were 21/2533 (0.83%) initially reactive results and 14/2533 (0.55%) repeatedly reactive results. Of the repeatedly reactive results, 14/14 (100.00%) results were confirmed.

Specimen Equivalence in Pregnant and Non-pregnant Individuals

A study was conducted to evaluate the results observed when samples from pregnant women were tested with the ARCHITECT HBsAg Next Qualitative. A total of 32 serum specimens from pregnant females and 32 serum specimens from non-pregnant females of child-bearing age were spiked with an HBsAg positive specimen to a target of 3.00 S/CO. The distribution by trimester is: 14 specimens from women in the 1st trimester, 11 in the 2nd trimester, and 7 in the 3rd trimester of pregnancy. The ages ranged from 17 to 41 years. The average recovery for the specimens from pregnant individuals was 93% and ranged from 81.0% to 101.4%. The results of the study suggest that specimens from pregnant individuals react in the same manner as specimens from non-pregnant individuals for HBsAg detection.

Clinical Performance in a Pediatric Population

Of the 2790 specimens in the clinical study, 128 specimens were from a pediatric population aged 0 to 21. There were 117 specimens (out of the 128) in the 2 to 21 year age range. For the 117 specimens, the negative percent agreement was 99.14% (115/116) with a 95% confidence interval of 95.28% to 99.85% and the positive percent agreement was 100.00% (1/1) for the ARCHITECT HBsAg Next Qualitative result versus the ARCHITECT HBsAg Qualitative final interpretation. The ARCHITECT HBsAg Next Qualitative results are summarized by age and gender in the following table.

		ARCHITECT HBsAg Next Qualitative Result		
Age Range (Years)	**Gender**	**Reactive N (%)**	**Nonreactive N (%)**	**Total**
2 to 12	Female	1 (0.85)[a]	20 (17.09)	21
	Male	0 (0.00)	42 (35.90)	42
>12 to 21	Female	1 (0.85)[b]	35 (29.91)	36
	Male	0 (0.00)	18 (15.38)	18
Total		**2 (1.71)**	**115 (98.29)**	**117**

[a] The specimen was confirmed by ARCHITECT HBsAg Next Confirmatory. The corresponding comparator ARCHITECT HBsAg Qualitative result is nonreactive.

[b] The specimen was not confirmed by ARCHITECT HBsAg Next Confirmatory. The corresponding comparator ARCHITECT HBsAg Qualitative result is repeatedly reactive/not confirmed.

Pediatric versus Adult Specimen Comparison

A study was conducted to evaluate the results observed when pediatric samples were tested with the ARTCHITECT HBsAg Next Qualitative. A total of 46 negative pediatric specimens (15 serum and 31 plasma) were used in the study and one adult reference plasma specimen. There were 14 (30%) specimens from individuals in the 2 to 12 years age range and 32 (70%) in the 13 to 21 years age range. The pediatric specimens and a single adult specimen were spiked with an HBsAg positive specimen to a target of 3.00 S/CO. Averaged results for each pediatric specimen were compared to results obtained from adult specimens. The average recovery for all pediatric specimens was 94% and ranged from 47.9% to 112.8% (A single specimen obtained a recovery of 48%. The specimen was anti-HBs nonreactive at 0.27 mIU/mL. There was insufficient sample volume to perform follow up testing). The results of the study suggest that pediatric specimens react in the same manner as adult specimens for HBsAg detection.

Analytical Sensitivity

The analytical sensitivity of the ARCHITECT HBsAg Next Qualitative and ARCHITECT HBsAg Next Confirmatory assays was determined using serial dilutions of the WHO Second International Standard for HBsAg, subtype *adw2*, genotype A, NIBSC code: 00/588. The dilutions ranged from 3 to 40 mIU/mL. Recalcified negative human plasma was used as the diluent. Each dilution was tested in a minimum of 4 replicates across 3 reagent lots, 3 calibrator lots, and 1 control lot on 1 ARCHITECT i2000SR instrument. The analytical sensitivity ranged from 4.62 to 6.14 mIU/mL with the ARCHITECT i2000SR instrument.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.*27* Testing was conducted using 3 lots of the ARCHITECT HBsAg Next Qualitative reagent kit on 1 ARCHITECT i2000SR instrument over a minimum of 3 days. The maximum observed limit of blank (LoB) and limit of detection (LoD) values for each instrument are summarized below.

	mIU/mL
LoB[a]	1.07
LoD[b]	2.34

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

[b] The LoD presented in the table is in alignment with the ARCHITECT HBsAg Next Qualitative assay on the ARCHITECT i2000SR System. The observed LoD on the ARCHITECT i System was 2.34 mIU/mL and represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

Analytical Specificity

The ARCHITECT HBsAg Next Qualitative assay and confirmation by the ARCHITECT HBsAg Next Confirmatory assay were evaluated for potential interference using specimens from individuals with medical conditions unrelated to hepatitis B.

The ARCHITECT HBsAg Next Qualitative and ARCHITECT HBsAg Qualitative assays and their respective confirmatory assays (for repeatedly reactive specimens) evaluated 288 specimens from 27 other disease states categories. Of the 288 specimens tested, 277 specimens were concordant nonreactive on both the ARCHITECT HBsAg Next Qualitative and ARCHITECT HBsAg Qualitative assays, while 10 of the 288 specimens were concordant repeatedly reactive and confirmed on both assays. One HIV-1 sample was repeatedly reactive on the ARCHITECT HBsAg Next Qualitative assay and confirmed positive by the ARCHITECT HBsAg Next Confirmatory assay and elevated nonreactive on the ARCHITECT HBsAg Qualitative assay.

		Comparator ARCHITECT HBsAg Qualitative Final Interpretation			
		Nonreactive		**Repeatedly Reactive and Confirmed**	
		ARCHITECT HBsAg Next Qualitative Final Interpretation		**ARCHITECT HBsAg Next Qualitative Final Interpretation**	
Category	**n**	**Nonreactive**	**Repeatedly Reactive and Confirmed**	**Nonreactive**	**Repeatedly Reactive and Confirmed**
HTLV-1/2	10	10	0	0	0
CMV	10	10	0	0	0
HCV	10	10	0	0	0
EBV	10	10	0	0	0
HIV-1	10	8	1	0	1
HIV-2	10	10	0	0	0
HAV	10	10	0	0	0
T.pallidum (Syphilis)	10	10	0	0	0
Rheumatoid Factor (RF)	10	10	0	0	0
Antinuclear Autoantibodies	10	10	0	0	0

		Comparator ARCHITECT HBsAg Qualitative Final Interpretation			
		Nonreactive		Repeatedly Reactive and Confirmed	
		ARCHITECT HBsAg Next Qualitative Final Interpretation		ARCHITECT HBsAg Next Qualitative Final Interpretation	
Category	n	Nonreactive	Repeatedly Reactive and Confirmed	Nonreactive	Repeatedly Reactive and Confirmed
(ANA)					
Anti-dsDNA autoantibodies	10	10	0	0	0
Pregnant females 1st trimester	10	10	0	0	0
Pregnant females 2nd trimester	10	10	0	0	0
Pregnant females 3rd trimester	20	20	0	0	0
Multiparous females	10	9	0	0	1
Immunoglobulin from monoclonal gammopathy for IgG	7	7	0	0	0
Immunoglobulin from multiple myeloma	10	8	0	0	2
Influenza vaccine recipients	20	20	0	0	0
Hemodialysis patients	10	9	0	0	1
Human anti-mouse antibody (HAMA)	20	20	0	0	0
Non-viral liver disease / alcoholic liver disease	10	10	0	0	0

		Comparator ARCHITECT HBsAg Qualitative Final Interpretation			
		Nonreactive		Repeatedly Reactive and Confirmed	
		ARCHITECT HBsAg Next Qualitative Final Interpretation		ARCHITECT HBsAg Next Qualitative Final Interpretation	
Category	**n**	**Nonreactive**	**Repeatedly Reactive and Confirmed**	**Nonreactive**	**Repeatedly Reactive and Confirmed**
Autoimmune hepatitis	10	10	0	0	0
Fatty liver disease	10	10	0	0	0
Hepatocellular carcinoma (HCC)*	10	5	0	0	5
Obstructive jaundice and smooth muscle antibody positive (SMA)	6	6	0	0	0
ANCA (neutrophil cytoplasmatic antibodies)	8	8	0	0	0
AMA (anti-mitochondrial antibodies) or histology	7	7	0	0	0
Total	**288**	**277**	**1**	**0**	**10**

* Of the 10 HCC samples, 5 were concordant nonreactive and 5 were concordant reactive. Chronic and/or persistent infection with hepatitis B and/or hepatitis C are known risk factors for hepatocellular cancer.*2829*

Interference

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07-A2.*30* Each substance was tested at 2 levels of the analyte (approximately 0.80 S/CO and 1.20 S/CO). No significant interference (interference within ± 20%) was observed at the following concentrations:

Potentially Interfering Endogenous Substance	Interferent Level
Unconjugated Bilirubin	40 mg/dL
Conjugated Bilirubin	40 mg/dL
Hemoglobin	1000 mg/dL
Triglycerides	3000 mg/dL
Total Protein	15 g/dL

Interferences from medication or endogenous substances may affect results.*31*

Potentially Interfering Substances

A study was performed based on guidance from CLSI EP07-A2.*30* Each substance was tested at 2 levels of the analyte (approximately 0.80 S/CO and 1.20 S/CO). No significant interference (interference within ± 20%) was observed at the following concentrations:

Potentially Interfering Substance	Interferent Level
Acetaminophen	250 mg/L
Acetylcysteine	150 mg/L
Acetylsalicylic Acid	1000 mg/L
Adefovir	10 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic Acid	300 mg/L
Biotin	4250 ng/mL
Ca-dobesilate	200 mg/L
Cefoxitin	2500 mg/L
Cyclosporine	5 mg/L
Doxycycline	50 mg/L
Entecavir	0.5 mg/L

Potentially Interfering Substance	Interferent Level
Ibuprofen	500 mg/L
Lamivudin	300 mg/L
Levodopa	20 mg/L
Methyldopa	20 mg/L
Metronidazole	200 mg/L
PEG interferon-alpha	180 ug/L
Phenylbutazone	400 mg/L
Rifampicin	60 mg/L
Sodium Heparin	10 U/mL
Telbivudine	600 mg/L
Tenofovir	245 mg/L*
Theophylline (1,3-dimethylxanthine)	100 mg/L

* Tenofovir concentration tested exceeds the Test Concentration Level listed in EP37 1st Edition.[32]

Tube Type Matrix Comparison

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

- Serum, including serum separator
- Dipotassium EDTA
- Tripotassium EDTA
- Lithium heparin
- Lithium heparin separator
- Sodium heparin

On average, the tube types listed in the table below showed less than a 20% difference when compared to the control tube type (plastic serum) for low positive samples (target: 1.20 S/CO) and less than a 0.20 S/CO difference for high negative samples (target: 0.80 S/CO). The ARCHITECT HBsAg Next Qualitative assay showed the following distribution of percent differences when compared to the plastic serum tube type.

Evaluation Tube Type	Distribution of Differences for High Negative Samples			Distribution of %Differences for Low Positive Samples		
	< 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	100.00% (29/29)	0.0% (0/29)	0.0% (0/29)	0.0% (0/30)	0.0% (0/30)	100.0% (30/30)
Dipotassium EDTA	100.00% (29/29)	0.0% (0/29)	0.0% (0/29)	0.0% (0/30)	3.3% (1/30)	96.7% (29/30)
Tripotassium EDTA	100.00% (29/29)	0.0% (0/29)	0.0% (0/29)	0.0% (0/30)	10.0% (3/30)	90.0% (27/30)
Lithium heparin	100.00% (29/29)	0.0% (0/29)	0.0% (0/29)	0.0% (0/30)	13.3% (4/30)	86.7% (26/30)
Sodium heparin	100.00% (29/29)	0.0% (0/29)	0.0% (0/29)	0.0% (0/30)	6.7% (2/30)	93.3% (28/30)
Lithium heparin plasma separator	100.00% (29/29)	0.0% (0/29)	0.0% (0/29)	0.0% (0/30)	3.3% (1/30)	96.7% (29/30)

Seroconversion Sensitivity

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

HBsAg was first detected by the ARCHITECT HBsAg Next Qualitative assay and confirmed by the ARCHITECT HBsAg Next Confirmatory assay 2 to 43 days earlier than it was first detected by the ARCHITECT HBsAg Qualitative assay in 24 seroconversion panel sets and coincident with the first day detected by the ARCHITECT HBsAg Qualitative assay in 8 seroconversion panel sets. Data are summarized in the following table.

In 25 of 32 panels (78%) the number of days to the first repeatedly reactive and confirmed result was less for the ARCHITECT HBsAg Next Qualitative assay compared to the ARCHITECT HBsAg Qualitative assay. Of 483 panel members tested, the ARCHITECT HBsAg Next Qualitative and ARCHITECT HBsAg Next Confirmatory assays detected 271 specimens as repeatedly reactive and confirmed positive. The ARCHITECT HBsAg Qualitative assay detected a total of 223 of the 483 specimens.

Panel ID	Days to HBsAg Repeatedly Reactive Result from Initial Draw Date		Difference in Days (ARCHITECT HBsAg Next Qualitative - Comparator ARCHITECT HBsAg Qualitative)[a]
	ARCHITECT HBsAg Next Qualitative	Comparator ARCHITECT HBsAg Qualitative Assay	
6271	0	7	**-7**
6272	51	94	**-43**
6273	14	14	0
6275	2	7	**-5**
6277	26	33	**-7**
6278	4	8	**-4**
6279	21	26	**-5**
6281	13	13	0
6282	12	14	**-2**
6284	46	50	**-4**
6285	38	40	**-2**
6286	29	29	0[b]
6290	7	14	**-7**
9074	66	70	**-4**
11000	0	26	**-26**
11001	44	44	0
11002	0	7	**-7**
11003	133	142	**-9**
11006	35	42	**-7**
11007	29	34	**-5**
11008	62	69	**-7**
11012	18	18	0
11013	239	244	**-5**

Panel ID	Days to HBsAg Repeatedly Reactive Result from Initial Draw Date		Difference in Days (ARCHITECT HBsAg Next Qualitative - Comparator ARCHITECT HBsAg Qualitative)[a]
	ARCHITECT HBsAg Next Qualitative	Comparator ARCHITECT HBsAg Qualitative Assay	
11014	37	51	**-14**
11017	34	40	**-6**
11026	36	39	**-3**
11029	32	35	**-3**
13867/3482	0	0	0
1807/3463	4	4	0
43527/3453	0	0	0
26022/14518	0	7	**-7**
0994/3457	0	4	**-4**

[a] Negative values indicate an earlier detection by the Investigational Method.

[b] For the ARCHITECT HBsAg Qualitative assay, the first repeat reactive bleed (bleed 4) did not confirm so was repeatedly reactive only. Therefore, the investigational assay detected this panel earlier as repeat reactive and confirmed.

HBsAg Mutant Detection

A panel consisting of 71 internally prepared recombinant mutant samples and 2 wild type controls, and a panel of 95 native mutant samples collected through Abbott's Global Viral Surveillance Program[33, 34] were obtained. Samples had been diluted with recalcified negative human plasma to an S/CO of approximately 2.00 in the ARCHITECT HBsAg Qualitative assay. All recombinant mutant samples were antigens with amino acid sequences representing native mutants of hepatitis B surface antigen. Across the recombinant and native panels, 4 of the 166 mutant samples shared the same mutation pattern.

One hundred fifty-one of the samples contained at least one substitution or insertion in the region spanning amino acids (aa) 120 – 145 within the 'a' determinant of the surface antigen. Forty-two of the samples had single substitutions, 32 had double substitutions, 86 had 3 to a maximum of 18 substitutions or insertions, and 6 had insertions following aa 122 or 123 of the surface antigen. Thirty-nine samples contained mutants Gln-129-His, Met-133-Leu, Asp-144-Ala, Gly-145-Arg, and Thr-123-Ala or insertion mutants 122NT, 122RA, P142L+G145R, P142S+G145R.

All mutant specimens were evaluated with the ARCHITECT HBsAg Next Qualitative and ARCHITECT HBsAg Qualitative assays and their respective confirmatory assays. All specimens

were initial and repeatedly reactive in both assays and were confirmed positive in the respective confirmatory assays. The mean S/CO across all mutant specimens was about 7-fold higher for the ARCHITECT HBsAg Next Qualitative assay compared to the ARCHITECT HBsAg Qualitative assay (about 10 fold for the recombinant mutant specimens and about 5 fold higher for the native mutant specimens). There was one native mutant specimen that could not be confirmed (48% neutralization) in the ARCHITECT HBsAg Qualitative Confirmatory assay but was confirmed in the ARCHITECT HBsAg Next Confirmatory assay.

Mutation Type	Amino Acid Position Substitution
Single	C137Y, C138W, C147S, C124R, Q129H, M133L, D144A, G145R, T123A, T123N, K141E, P120Q, G145K, T143L, T123S, F161L, C76Y, C121Y, C137A, G145E, C149Y, S154W, T126S, M133T, S136T, C139W, G145A, C147Y, T126I, S143L, Q129H, L127V, A128V, G145AG, L88P, T126S, T126NT, T118A, M133L, G145A, S143L, G130R
Double	T123N+C124R, P142L+G145R, P142S+G145R, T123A+G145R, C76Y+F83S, M133L+G145R, P108H+S113T, T123N+T143S, T126A+M133I, P127T+G145R, F134V+D144G, D144E+G145R, C147Y+C149Y, T127P+T131I, L77LQ+G145GR, Y100C+M133I, F134FL+E164EG, S140L+T189IT, Y100C+Q129QR, T123AT+E164EG, L127P+D144E, S143L+G145GR, G145A+S174N, N59S+Q129H, Q101H+I110L, T127P+S143L, S143L+P211H, G145A+T189I, Q101H+Q129H, S143L+V177A, P127L+Q129H, S143L+T189I
Three to eighteen	P120S+T125M+P127Y+S143L, C121Y+K122L+T123N+G130E+M133I+D144G+G145R, P120S+D144E+G145R+T189I, F134H+P142L+D144E+G145R, P120Q+N131K+G145R, M133I+Y134H+T143M, T143L+Y206G+S207R, T126I+F134H+P142L+G145R, T143L+V190A+Y200C+Y206R, L109I+G112K+S113A+P120T+F134S, I110R+K122Y+F134S+P142L+D144A, T114S+K122R+N131T+F134Y+T143S, I110M+T116N+S117T+T118S+T140S+T143L, T115N+P120L+M133I+F134H+D144V+S154P, T118V+M133I+F1`34N+P142S+T143L+G145R, P120T+S132F+F134N+P135A+D144G+I150T, T114S+S117T+T123S+N131T+M133I+F134R+I150T+A194V+Y200S+S207N+V209L+I213M, JPA, T125M+T126N+P127T, Q101R+G112K+.T118V+R122K+T126N+S136Y+S143L G112K+S114L+T115N+T118R+Q129P, R122K+P127S+G130E, Q101H+P111S+T118P+P120T, L110I+T118M+S154LS+R160KR, L77Q+L88P+P120PT+K122T+T126S, P120AP+G145AG+E164EG, Q101R+S154PS+K160N, E164G L91HL+I92IT+Q101H, F134FV+G145A+S167LS, Y100C+Q101PQ+P120PT Y100C+M103I+N131S+E164G+R169HR, Y100C+S113FS+T116IT+C121CGS+T123P+M133IM+F134FL+P135H, M103I+T126I+Q129H+E164G, T27KT+A45AT+I110IL+S113KT+S114PS+P120PT+T126I+L127P+S140LS+D144DE+G145GR+I150T+F158FL+A168AV+L175LS+V177AEPQ+V180AV Y72F+P120T+L127P+K160R N59S+Q129R+S136SY T57I+L127P+D144E+L216StopL T118M+D144A+I150T V47G+L49P+R122K+F134L+Y200F+I213L+L216stop S55F+Y100C+T123A+T126I+L127P+S143L+S174N+L176P+V177A+P178L+S193LS+P214L+V224AV L49P+Y72F+Q101R+I110L+D144AD+G145A+K160N+E164V+S174N+V177A+P178L+Q181R+I213T S34L+F41S+S117T+Q129P+N131K+S154P+S155Y+E164D+A166AG+V168A+S174N+V177A+Y200F+I213S+P217L+I226T s3n+F8L+G44E+A128V+L175S G18V+V96G+L127P+S143L+K160N+E164G+S167L+R169H+S174N+P178L+S210NS+P214H+L216FL

	Q101R+L127P+S140L+G145A+S154L+K160R+S174N+F219S+F220Y+V224A
	F8FL+T45NT+L49LR+P120S+T189IT+S193LS+M198MT+P203PQ+S207NS+P211PR+P217LP
	F8FL+S64FS+C69stopC+I110IL+T127P+S143L+T189IT
	F8L+P120S+T127P+P135LP+S204N+Y206C+F220C+V224A
	Q30K+S31N+T127P+G130R+T131N+M133T+I208T
	P142FLPS+G145KR+S174NS+A194V
	Q30K+L49P+N59S+L91H+Y100S+Q101R+I110L+T123V+T126I+L127P+M133L+K160N+L175S+P178L+P211L+I213M+I218L
	L94S+V96G+R122K+G145A+G159V
	I68T+S114P+L127P+A128V+S174N+S204G+V224A
	L127P+S143L+D144DE+K160N+E164V+L175S+V177A+P211L
	Q101QR+S143L+F219L
	M133IM+P142LP+S143L+D144E
	T27K+Y100C+Q129R+L175S+W199L
	V96AG+Y100C+T116I+G119R+P120L+F134L+P135H+S174N+S210DG
	S31N+Q101R+G145A+S154QR+K160KN+F220L
	L49R+Q101H+T126I+E164G
	R79H+L91HL+F93S+L98LR+Y100C+G102S+Q129L+F134S+P135H+W182stop
	T23I+L127I+M133I
	L49LR+G145A+S204RS+I226IT
	C76W+P120S+S132F
	E2EG+F8FL+Q101R+P127IT+G145A+F219S+F220L
	L49R+R79HR+P120T+M133IM
	D144E+V184I+T189I
	L21LS+R24KR+Q101QR+P120S+T189I+S204K+P217LP
	D144E+S204N+S207N
	F8P+T45NT+F83CF+Y100C+Q129R+Y134ADFSVY
	S143L+S204R+L209LV+L213IL+P214LP
	P70T+S143L+S193L
	T126IT+Y134NY+G145R
	G145A+T189I+F212Y
	G145A+S207N+I208T+P214L
	M103IM+S114AS+T131AT+S143L+L175LS+V184AV
	T57I+M103I+D144A+W172L+V177A+Y206C+S207R+P214Q+Y225F
	I92T+L109M+M133I+F134I+D144A+G145A+I208T+P217L
	F8L+T126S+M197T+S204N+N207R
	F83S+V96G+M103I+F134V+I150M+S174N+S204R+N207T
	S64FS+V96GV+K141KR+P142PS+G145R
	S31RS+Q101R+T131P+M133I+K141KT+G145A+K160N+E164G+Y206HY+F220Y+I226T
	G11AG+S53L+P62L+M103I+P105L+T113AT+P120K+T123ATP127PS+A128AV+M133I+W165R+S174N+F179Y+Q181R+V184G+G185E+S210N
	T118K+T140I+C149Y

	P135L+C139Y+D144A+G145R+S171Y+V180A F93C+M103I+G145R+S174N
Insertion after 122 or 123	122DT 122NT 122RA insertion 123NSTGPCTT 123RGA G145R/122DT Insertion

HBV Genotype Detection

A total of 109 HBsAg genotype panels (genotypes A through H) were tested including 15 samples that were prepared as dilutions from the 1st WHO International Reference Panel for Hepatitis B Virus (HBV) Genotypes for HBsAg Assays PEI code 6100/09. Ninety-four were HBsAg native based genotype panels. All samples were tested with the ARCHITECT HBsAg Next Qualitative and ARCHITECT HBsAg Qualitative assays and their respective confirmatory assays. All samples were repeatedly reactive and confirmed positive in both HBsAg assays. There were no discordant samples.

Genotype	N
Genotype A	26
Genotype B	15
Genotype C	22
Genotype D	23
Genotype E	7
Genotype F	13
Genotype G	1
Genotype H	2
Total	109

BIBLIOGRAPHY

1. Neurath AR, Kent SB, Strick N, et al. Identification and chemical synthesis of a host cell receptor binding site on hepatitis B virus. *Cell* 1986;46:429-436.
2. Szmuness W, Stevens CE, Harley EJ, et al. Hepatitis B vaccine: Demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N Engl J Med* 1980;303:833-841.
3. Krugman S, Giles JP. Viral hepatitis, type B (MS-2-Strain): further observations on natural history and prevention. *N Engl J Med* 1973;288:755-760.
4. Perrillo RP, Aach RD. The clinical course and chronic sequelae of hepatitis B virus infection. *Seminars in Liver Disease* 1981;1:15-25.
5. CDC. A comprehensive immunization strategy to eliminate transmission of Hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) Part 1: Immunization of Infants, Children, and Adolescents. *MMWR* 2005;54 (RR-16):1-23.

6. Hunt CM, McGill JM, Allen MI, et al. Clinical relevance of hepatitis B viral mutations. *Hepatology* 2000;31(5):1037-1044.

7. Zuckerman AJ. Effect of hepatitis B virus mutants on efficacy of vaccination. *Lancet* 2000;355:1382-1384.

8. Carman WF, Trautwein C, Van Deursen FJ, et al. Hepatitis B virus envelope variation after transplantation with and without hepatitis B immune globulin prophylaxis. *Hepatology* 1996;24(3):489-493

9. Grethe S, Monazahian M, Böhme I, et al. Characterization of unusual escape variants of hepatitis B virus isolated from a hepatitis B surface antigen-negative subject. *J Virology* 1998;72(9):7692-7696.

10. Nainan OV, Stevens CE, Taylor PE, et al. Hepatitis B virus (HBV) antibody resistant mutants among mothers and infants with chronic HBV infection. In: Rizzetto M, Purcell RH, Gerin JL, et al., eds. *Viral Hepatitis and Liver Disease*. Minerva Medica: Torino;1997:132-134

11. Jongerius JM, Wester M, Cuypers HTM, et al. New hepatitis B virus mutant form in a blood donor that is undetectable in several hepatitis B surface antigen screening assays. *Transfusion* 1998;38:56-59.

12. Bock CT, Tillmann HL, Torresi J, et al. Selection of hepatitis B virus polymerase mutants with enhanced replication by lamivudine treatment after liver transplantation. *Gastroenterology* 2002;122:264-273.

13. Locarnini SA. Hepatitis B virus surface antigen and polymerase gene variants: potential virological and clinical significance. *Hepatology* 1998;27(1):294-297.

14. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.

15. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 6th ed. Washington, DC: US Government Printing Office; June 2020.

16. World Health Organization. *Laboratory Biosafety Manual*. 4th ed. Geneva: World Health Organization; 2020.

17. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition.* CLSI Document M29-A4. Wayne, PA: CLSI; 2014.

18. Primus FJ, Kelley EA, Hansen HJ, et al. “Sandwich”-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* 1988;34(2):261-264.

19. Schroff RW, Foon KA, Beatty SM, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985;45(2):879-885.

20. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin*

Chem 1988;34(1):27-33.

21. Rysgaard CD, Morris CS, Drees D, et al. Positive hepatitis B surface antigen tests due to recent vaccination: a persistent problem. *BMC Clin Pathol* 2012;12(1):15-20.

22. Calisti G, Herman O, Powley M, et al. Persistence of hepatitis B surface antigen in blood in a chronic haemodialysis patient following vaccination booster. *BMJ Case Rep* Published online: 2014 June 10. doi:10.1136/bcr-2013-202191.

23. Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline—Third Edition.* CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.

24. Kuhns MC, Holzmayer V, McNamara AL, et al. Molecular and serological characterization of hepatitis B vaccine breakthrough infections in serial samples from two plasma donors. *Virol J* 2019;16(1):1-9.

25. Kuhns MC, Holzmayer V, McNamara AL, et al. Improved detection of early acute, late acute, and occult Hepatitis B infections by an increased sensitivity HBsAg assay. *J Clin Virol* 2019;118:41-45.

26. Raimondo G, Locarnini S, Pollicino T, et al. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. *J Hepatol* 2019;71(2):397-408.

27. Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition.* CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.

28. Adult primary liver cancer treatment (PDQ®)–health professional version. NIH National Cancer Institute. Updated January 19, 2022. Accessed May 17, 2022. https://www.cancer.gov/types/liver/hp/adult-liver-treatment-pdq

29. Liver cancer causes, risk factors, and prevention. American Cancer Society. Revised March 31, 2022. Accessed May 17, 2022. https://www.cancer.org/content/dam/CRC/PDF/Public/8699.00.pdf

30. Clinical and Laboratory Standards Institute (CLSI). *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition.* CLSI Document EP07-A2. Wayne, PA: CLSI; 2005.

31. Young DS. *Effects of Drugs on Clinical Laboratory Tests*, 4th ed. Washington, DC: AACC Press; 1995:3-16–3-22.

32. Clinical and Laboratory Standards Institute (CLSI). *Supplemental Tables for Interference Testing in Clinical Chemistry.* 1st ed. CLSI supplement EP37. Wayne, PA: CLSI; 2018.

33. Harris BJ, Holzmayer V, Qureshi H, et.al. Hepatitis B Genotypes and Surface Antigen Mutants Present in Pakistani Blood Donors. PLOS ONE. Epub 2017 Jun 5. Doi: 10.1371/journal.pone.0178988.

34. Rodgers MA, Vallari AS, Harris B, et al. Identification of rare HIV-1 Group N, HBV AE,

and HTLV-3 strains in rural South Cameroon. *Virology* 2017;504:141-151.

Key to Symbols

ISO 15223 Symbols	
	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
REF	List Number
SN	Serial number

Other Symbols	
ANCILLARY WASH BUFFER	Ancillary Wash Buffer
ASSAY SPECIFIC DILUENT	Assay Specific Diluent
CONJUGATE	Conjugate
CONTROL NO.	Control Number
DISTRIBUTED IN THE USA BY	Distributed in the USA by
INFORMATION FOR USA ONLY	Information needed for United States of America only
MICROPARTICLES	Microparticles
PRODUCT OF IRELAND	Product of Ireland
REAGENT LOT	Reagent Lot
Rx ONLY	For use by or on the order of a physician only (applicable to USA classification only).

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%).

Abbott Ireland

Diagnostics Division

Finisklin Business Park

Sligo

Ireland

+353-71-9171712

DISTRIBUTED IN THE USA BY

Abbott Laboratories

Abbott Park, IL 60064 USA

Customer Service: Contact your local representative or find country-specific contact information on www.corelaboratory.abbott

Created May 2022.

Created March 2022.

CAUTION: United States Federal Law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.

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

For laboratory professional use only.

NAME

ARCHITECT HBsAg Next Confirmatory (also referred to as HBsAgNx C)

INTENDED USE

The HBsAg Next Confirmatory assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative confirmation of the presence of hepatitis B surface antigen (HBsAg) in human adult and pediatric (2 years to 21 years of age) serum, serum separator, and plasma (dipotassium EDTA, tripotassium EDTA, lithium heparin, lithium heparin separator, sodium heparin) by means of specific antibody neutralization on the ARCHITECT i System

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.

It is intended to be used for the confirmation of samples found to be repeatedly reactive by HBsAg Next Qualitative.

WARNING: Not approved for use in screening blood, plasma, tissue donors, or cadaveric specimens. The effectiveness of the ARCHITECT HBsAg Next Confirmatory assay for use in screening blood, plasma, or tissue donors has not been established.

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 10 weeks after exposure and 2 to 8 weeks before the onset of clinical symptoms.[2, 3] 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 or chronic HBV infection.

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.[4] 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.[5]

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.[6] 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.[6, 7, 8, 9, 10, 11, 12] HBsAg mutations may result in a less favorable outcome in some patients[6, 8, 13] and false negative results in some HBsAg assays.[6, 7, 8]

It is recommended that confirmatory testing be performed prior to disclosure of HBV status. HBsAg Next Confirmatory uses the principle of specific antibody neutralization to confirm the presence of HBsAg in samples found to be repeatedly reactive. Antibody to hepatitis B surface antigen (anti-HBs) is incubated with a sample. If HBsAg is present in the sample, it will be neutralized by the antibody. The neutralized HBsAg is subsequently blocked from binding to the anti-HBs coated microparticles. A reduction of signal occurs when compared to the signal of a paired sample that has not been treated with the antibody reagent. A sample is considered confirmed if the signal for the non-neutralized sample (incubated with Pre-Treatment 2) result is greater than or equal to the cutoff of 0.70 S/CO and the relative light unit (RLU) of the neutralized sample is reduced by at least 50% compared to the non-neutralized sample.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay consists of two single tests (HBsAgNx_C1, also referred to as C1 and HBsAgNx_C2, also referred to as C2) that are both one-step pre-treatment immunoassays for the confirmation of the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma using chemiluminescent microparticle immunoassays (CMIA) technology with flexible assay protocols, referred to as Chemiflex.

(Note: As ancillary wash buffer is added in a second incubation step, the assay files perform a two-step assay.)

C1:

Sample and Pre-Treatment 1 are combined and incubated. The HBsAg present in the sample is neutralized by the anti-HBs in Pre-Treatment 1.

An aliquot of the pretreated sample, anti-HBs coated paramagnetic microparticles, assay specific diluent, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture and incubated. Any remaining non-neutralized HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled conjugate. The neutralized HBsAg is blocked from forming a sandwich with acridinium-labeled anti-HBs conjugate and

anti-HBs coated microparticles. After washing, ancillary wash buffer is added and the mixture is incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as a relative light unit (RLU). There is a direct relationship between the amount of non-neutralized HBsAg in the sample and the RLU detected by the system optics.

C2:

Sample and Pre-Treatment 2 are combined and incubated. Pre-Treatment 2 does not contain anti-HBs and will not neutralize HBsAg present in the sample.

An aliquot of the pretreated sample, anti-HBs coated paramagnetic microparticles, assay specific diluent, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture and incubated. HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled conjugate. After washing, ancillary wash buffer is added and the mixture is incubated. Following another wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as an RLU. There is a direct relationship between the amount of HBsAg in the sample and the RLU detected by the system optics.

If the signal for the non-neutralized sample (incubated with Pre-Treatment 2) result is greater than or equal to the cutoff of 0.70 S/CO and the RLU of the neutralized sample (incubated with Pre-Treatment 1) is reduced by at least 50% compared to the non-neutralized sample, the sample is considered confirmed positive for HBsAg.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Kit Contents

ARCHITECT HBsAg Next Confirmatory Reagent Kit 4P77

Volumes (mL) listed in the following table indicate the volume per bottle.

REF	4P77-27
Tests per kit	100 tests (50 determinations)
Number of kits per box	1
Tests per box	100 tests (50 determinations)
MICROPARTICLES	6.6 mL
CONJUGATE	3.3 mL
ASSAY SPECIFIC DILUENT	3.3 mL

REF	4P77-27
PRE-TREATMENT 1	2.4 mL
PRE-TREATMENT 2	2.4 mL
ANCILLARY WASH BUFFER	5.9 mL

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

CONJUGATE anti-HBs (goat, IgG) acridinium-labeled conjugate in phosphate buffer with protein (bovine, goat, mouse) stabilizers. Minimum concentration: 0.75 µg/mL. Preservatives: ProClin 300 and ProClin 950.

ASSAY SPECIFIC DILUENT contains phosphate buffer with protein (bovine) stabilizer. Preservatives: ProClin 300 and ProClin 950.

PRE-TREATMENT 1 contains recalcified sheep plasma reactive for anti-HBs and recalcified human plasma. Preservatives: ProClin 950 and sodium azide.

PRE-TREATMENT 2 contains recalcified human plasma and recalcified sheep plasma. Preservatives: ProClin 950 and sodium azide.

ANCILLARY WASH BUFFER contains 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.[14, 15, 16, 17]

The human-sourced material used in Pre-Treatment 1 and Pre-Treatment 2 are 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, ANCILLARY WASH BUFFER, and ASSAY SPECIFIC DILUENT	
	
WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
H402	Harmful to aquatic life.
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.
P273	Avoid release to the environment.
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: PRE-TREATMENT 1 and PRE-TREATMENT 2


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

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

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

Safety Data Sheets are available at www.corelaboratory.abbott or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Reagent Handling

- Do not pool reagents within a kit or between kits.
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the PROCEDURE, Assay Procedure section of this package insert.

Septums MUST be used to prevent reagent evaporation and contamination and to ensure

reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.

- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
- Once a septum has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.

For a detailed discussion of reagent handling precautions during system operation, refer to the ARCHITECT System 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.
Onboard	System Temperature	30 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.

Reagents may be stored on or off the ARCHITECT i System. If reagents are removed from the system, store them at 2 to 8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright.

For information on unloading reagents, refer to the ARCHITECT System 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 ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The ARCHITECT HBsAg Next Confirmatory assay files must be installed on the ARCHITECT i System with i2000SR Induction Heating installed on the system prior to performing the assay.

Install the software and assay files in the following order:

- ARCHITECT System Software Version 9.25 or higher
- HBsAgNx_C2
- HBsAgNx_C1

When the assay files referenced above are installed, the HBsAgNx_%N assay file is installed automatically and the HBsAgNx_Pa panel is made available.

- HBsAgNx_%N allows the instrument to automatically calculate a percent neutralization result from the HBsAgNx_C2 and HBsAgNx_C1 results.
- HBsAgNx_Pa provides a convenient method to order HBsAg confirmatory tests so the ARCHITECT i System will report the S/CO and % neutralization results required for the interpretation.

Recommended system configuration steps:

Configure result units and decimal places.

- It is recommended to configure the result unit to % and the decimal places to 0 decimal places for HBsAgNx_%N.
- For information on configuring a result unit and decimal places, refer to the ARCHITECT System Operations Manual, Section 2.

Configure the positive control as a multiconstituent control (MCC).

- It is recommended to configure the ARCHITECT HBsAg Next Qualitative Positive Control as an MCC with the HBsAgNx_%N and HBsAgNx_C2 assays.
- For information on configuring a multiconstituent control, refer to the ARCHITECT System Operations Manual, Section 2.

- Refer to the PROCEDURE, Assay Procedure section of this package insert for information on ordering tests.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.

For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.

For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

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

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

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

- Performance has not been established for the use of cadaveric specimens or the use of bodily fluids other than human serum/plasma.
- Liquid anticoagulants may have a dilution effect resulting in lower S/CO values for individual specimens.

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
- specimens with fungal growth

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

Preparation for Analysis

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

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

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

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

Prepare frozen specimens as follows:

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

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge at a minimum of 100 000 g-minutes.
- Examples of acceptable time and force ranges that meet this criterion are listed in the following table.

  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

- 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)	3 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	2 to 8°C	7 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.

If testing will be delayed more than 7 days, remove serum or plasma from the clot, red blood cells, or separator gel and store at -20°C or colder.

Do not subject the specimens to more than 3 freeze/thaw cycles.

Specimen Shipping

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

Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

4P77 ARCHITECT HBsAg Next Confirmatory Reagent Kit

Materials Required but not Provided

- ARCHITECT HBsAg Next Confirmatory assay file found on www.corelaboratory.abbott.
- 4P76-01 ARCHITECT HBsAg Next Qualitative Calibrators
- 4P76-10 ARCHITECT HBsAg Next Qualitative Controls

- 4P77-40 ARCHITECT HBsAg Next Confirmatory Manual Diluent
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Septum

For information on materials required for operation of the instrument, refer to the ARCHITECT System Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

For a detailed description of how to run an assay, refer to the ARCHITECT System Operations Manual, Section 5.

Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.

- **Invert the microparticle bottle 30 times.**
- Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
- **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
- Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the Reagent Handling section of this package insert.

Order tests.

- For each HBsAg Next Confirmatory result, two tests must be performed by the ARCHITECT i System: HBsAgNx_C1 and HBsAgNx_C2. The percent neutralization result is calculated from these two test results.
- It is recommended that patient specimens be ordered using the patient panel HBsAgNx_Pa. HBsAgNx_Pa automatically selects the necessary assay files to test and reports the results required for assay interpretation (HBsAgNx_C2 S/CO and percent neutralization).

If a rerun is required, ensure that the calculated % neutralization result is based on constituent assay results which were generated on the same day. To do this

- perform the rerun on the same day the exception is generated or

  - perform the rerun on a different day using the calculated assay (HBsAgNx_%N) and both constituent assays (HBsAgNx_C1 and HBsAgNx_C2).

- If using primary or aliquot tubes, refer to the ARCHITECT System Operations Manual, Section 5 to ensure sufficient specimen is present.
- Minimum sample cup volume is calculated by the system and printed on the Order List report. 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: 242 µL (sample volume for HBsAgNx_C1 and HBsAgNx_C2)
  - ≤ 3 hours on the reagent and sample manager: 242 µL (sample volume for HBsAgNx_C1 and HBsAgNx_C2)
  - > 3 hours on the reagent and sample manager: Replace with a fresh aliquot of sample, controls, and calibrators.

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

Sample Dilution Procedures

A Manual Dilution Procedure may be performed on a neat sample if the ARCHITECT HBsAg Next Confirmatory assay has a HBsAgNx_C2 S/CO of ≥ 10.00 and a % Neutralization of < 50%. Refer to the RESULTS section of this package insert for further information.

Manual Dilution Procedure

Suggested dilution: 1:500

Add 25 µL of the sample to 475 µL of ARCHITECT HBsAg Next Confirmatory Manual Diluent for a 1:20 dilution.

Add 20 µL of the 1:20 dilution to 480 µL of ARCHITECT HBsAg Next Confirmatory Manual Diluent for a 1:500 dilution.

Additional specimen dilutions may be performed if the 1:500 dilution result is still reactive but not neutralized.

For a 1:20 000 dilution, add 25 µL of the 1:500 dilution to 975 µL of ARCHITECT HBsAg Next

Confirmatory Manual Diluent.

Avoid contamination of ARCHITECT HBsAg Next Confirmatory Manual Diluent when transferring volume for manual dilution.

NOTE: Manual dilution factors cannot be entered into the Patient or Control order screen. However, for maintenance of detailed information (records) - Select Patient Order then select the appropriate Assay. Select Sample Details F2. Enter the Dilution Factor in the Comments Box.

Refer to Interpretation of Results section of this package insert for further information.

For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

For instructions on performing a calibration, refer to the ARCHITECT System Operations Manual, Section 6.

- Test Calibrators 1 and 2 in replicates of 3 with the HBsAgNx_C2 assay. The HBsAgNx_C1 assay uses the calibration generated from HBsAgNx_C2. The calibrators should be priority loaded.

A single replicate of the positive control **only** must be tested to evaluate the assay calibration.

- Order the positive control as described in the Assay Procedure section of this reagent insert.
- Ensure that the positive control S/CO and % Neutralization results are within the ranges specified in the control insert.

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

- A reagent kit with a new lot number is used.
- Daily quality control results are outside of quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.

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 ARCHITECT HBsAg Next Confirmatory assay is that a single sample of the positive control level **only** 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.

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) or other published guidelines.

- 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 ARCHITECT System Operations Manual, Section 10.
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B.

The ARCHITECT HBsAg Next Confirmatory assay belongs to method group 5.

RESULTS

The ARCHITECT HBsAg Next Confirmatory result is based on the sample to cutoff ratio (S/CO) and % neutralization of the sample.

Note: If the sample HBsAgNx_C2 S/CO is < 0.70, % neutralization is not applicable. Obtain the final interpretation of results directly from the table in the Interpretation of Results section in this package insert.

Calculation

The ARCHITECT i System calculates results for the ARCHITECT HBsAg Next Confirmatory 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 0.085) + (Calibrator 2 mean RLU x 0.25)

The cutoff RLU is stored for each reagent lot calibration.

S/CO = Sample RLU/Cutoff RLU

The ARCHITECT i System calculates the % Neutralization result for the ARCHITECT HBsAg Next Confirmatory assay using the HBsAgNx_C1 and HBsAgNx_C2 results for each specimen and the positive control using the following equation:

$$\% \text{ Neutralization} = \frac{(\text{Sample HBsAgNx\_C2 RLU}) - (\text{Sample HBsAgNx\_C1 RLU})}{(\text{Sample HBsAgNx\_C2 RLU}) - (\text{Cal 2 HBsAgNx\_C2 Mean RLU})} \times 100$$

Interpretation of Results

Dilution	HBsAgNx_C2 S/CO	% Neutralization*	Final Interpretation
Neat (Undiluted)	< 0.70	Not applicable	Not confirmed
	< 10.00	< 50%	Not confirmed
	≥ 0.70	≥ 50%	Confirmed positive
	≥ 10.00	< 50%	Repeat test using a 1:500 dilution
1:500	< 0.70	Not applicable	Not confirmed
	≥ 0.70	≥ 50%	Confirmed positive
	≥ 0.70	< 50%	Repeat test using a 1:20,000 dilution
1:20 000	< 0.70	Not applicable	Not confirmed
	≥ 0.70	≥ 50%	Confirmed positive
	≥ 0.70	< 50%	Not confirmed

* If the % neutralization is < -15% and the C2 value is less than 350.00 S/CO, then the results should be considered invalid and the specimen should be retested. Perform the retest using the calculated assay (HBsAgNx_%N) and both constituent assays (C1 and C2). If the % neutralization is < -15% and the C2 value is equal to or greater than 350.00 S/CO the specimen should be retested per dilution instructions.

NOTES:

- Follow the dilution and final interpretation routine as outlined in the table above, even if % neutralization results > 100% are obtained.
- For specimen dilution instructions, refer to the Sample Dilution Procedures section of this

package insert.

- The interpretation of not confirmed for HBsAg indicates the presence of HBsAg cannot be confirmed via neutralization. The repeatedly reactive result obtained with the ARCHITECT HBsAg Next Qualitative assay may be the result of a nonspecific reaction (false reactive). As the presence of nonspecific binding may obscure low levels of HBsAg in the specimen due to early infection or early recovery, it is recommended that the patient be evaluated for other serologic markers of HBV infection (i.e., total anti-HBc or IgM anti-HBc)18 and that the patient be retested for HBsAg in 4 to 6 weeks.19
- Although there is an association between the presence of HBsAg, infectivity and a reactive result, it is recognized that presently available methods for HBsAg confirmation may not confirm all possible cases of HBV infection.
- The name(s) of the assays used to detect and confirm reactive results should be included in the results reported by the laboratory.

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 ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- The effectiveness of the ARCHITECT HBsAg Next Confirmatory assay for use in screening blood, plasma, tissue donors, or cadaveric specimens has not been established.
- Assay performance characteristics have not been established when the ARCHITECT HBsAg Next Confirmatory assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.
- If the ARCHITECT HBsAg Next Confirmatory 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.
- Results obtained with the ARCHITECT HBsAg Next Confirmatory assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical presentation.
- Potential interference has not been evaluated for substances other than those described in the SPECIFIC PERFORMANCE CHARACTERISTICS, Interference section of this package insert.
- **If specimens are not centrifuged according to the instructions in this package insert, depressed results may be obtained.**
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT HBsAg Next Confirmatory that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.*20, 21*
- 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.*22*
- Vaccination with a recombinant hepatitis B vaccine may cause transient positive results with a sensitive HBsAg assay such as ARCHITECT HBsAg Next Qualitative. 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*23*, though positive signals up to 52 days have been reported*24*, and may not indicate clinical disease.
- Although there is an association between the presence of HBsAg infectivity and a reactive result, it is recognized that presently available methods for HBsAg confirmation may not detect all possible cases of HBV infection.

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

SPECIFIC PERFORMANCE CHARACTERISTICS

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

Unless otherwise specified, all studies were performed on the ARCHITECT i2000SR System.

ARCHITECT HBsAg Next Confirmatory Performance

In a multi-center study, 145 specimens were tested using the ARCHITECT HBsAg Next Confirmatory assay and/or the ARCHITECT HBsAg Qualitative Confirmatory assay. Of the 145 specimens, 135 were confirmed positive using the ARCHITECT HBsAg Next Confirmatory assay, and 132 were reported positive by the ARCHITECT HBsAg Qualitative Confirmatory assay. The data are summarized in the following table.

	Comparator ARCHITECT HBsAg Qualitative Confirmatory Final Interpretation			
	Confirmed Positive [a]		**Negative/Not Confirmed**	
	ARCHITECT HBsAg Next Confirmatory		**ARCHITECT HBsAg Next Confirmatory**	
Specimen Category	**Confirmed Positive**	**Negative/Not Confirmed**	**Confirmed Positive**	**Negative/Not Confirmed**
	N	**N**	**N**	**N**
Increased Risk of HBV Infection	9	0	3	0
Signs and Symptoms of Hepatitis Infection	1	0	1	0
Individuals with Acute HBV Infection	50	0	0	0
Individuals with Chronic HBV Infection	69	0	0	0
Pregnant Females	0	0	0	2

Specimen Category	Comparator ARCHITECT HBsAg Qualitative Confirmatory Final Interpretation			
	Confirmed Positive [a]		Negative/Not Confirmed	
	ARCHITECT HBsAg Next Confirmatory		ARCHITECT HBsAg Next Confirmatory	
	Confirmed Positive	Negative/Not Confirmed	Confirmed Positive	Negative/Not Confirmed
	N	N	N	N
Total	**129**	**0**	**4**	**2**[b]

[a] A specimen is considered as confirmed if the signal for the non-neutralized specimen (incubated with Pre-Treatment 2) result is greater than or equal to the cutoff (S/CO ≥ 0.70) and the RLU of the neutralized specimen is reduced by at least 50% compared to the non-neutralized specimen.

[b] Two specimens were repeatedly reactive in the ARCHITECT HBsAg Qualitative assay but not confirmed in the ARCHITECT HBsAg Qualitative Confirmatory assay. The specimens were nonreactive in the ARCHITECT HBsAg Next Qualitative assay.

Eight specimens from the acute and chronic populations were excluded due to an invalid ARCHITECT HBsAg Next Confirmatory result, 7 specimens were excluded due to an invalid ARCHITECT HBsAg Next Qualitative Confirmatory results (5 of those were concordantly invalid).

Of the 145 specimens tested above, 133 specimens from the increased risk, signs and symptoms, and acute and chronic HBV infection populations were classified by HBV infection. A comparison of the ARCHITECT HBsAg Next Confirmatory interpretation versus the ARCHITECT HBsAg Qualitative Confirmatory interpretation by HBV classification based on HBV reference markers (anti-HBc IgM, total anti-HBc, anti-HBs, and HBsAg results) are summarized in the following table.

HBV Classification	Comparator ARCHITECT HBsAg Qualitative Confirmatory Interpretation			
	Confirmed Positive [a]		Negative/Not Confirmed	
	ARCHITECT HBsAg Next Confirmatory Result		ARCHITECT HBsAg Next Confirmatory Result	
	Confirmed Positive	Not Confirmed	Confirmed Positive	Not Confirmed
	N	N	N	N
Early acute	26	0	0	0
Acute	35	0	0	0
Chronic	68	0	0	0
Immune due to HBV vaccination	0	0	1	0
Susceptible	0	0	1	0
Uninterpretable	0	0	2	0
Total	**129**	**0**	**4**	**0**

[a] A specimen is considered as confirmed if the signal for the non-neutralized specimen (incubated with Pre-Treatment 2) result is greater than or equal to the cutoff (S/CO ≥ 0.70) and the RLU of the neutralized specimen is reduced by at least 50% compared to the non-neutralized specimen.

Other Disease States

Of the 288 specimens tested, 277 specimens were nonreactive on the ARCHITECT HBsAg Next Qualitative assay, while 11 of the 288 specimens were repeatedly reactive. All 11 repeatedly reactive specimens were tested on the ARCHITECT HBsAg Next Confirmatory assay and were confirmed positive.

	ARCHITECT HBsAg Next Qualitative		ARCHITECT HBsAg Next Confirmatory Confirmed Positive
Specimen Category	**N (Total Number Tested)**	**Number of Repeatedly Reactive (RR) (% of Total)**	**(% of RR)**
Other Disease Specimens	288	11* (3.82)	11 (100.00)

* The confirmed positive specimens belonged to the following categories: HIV-1 (2), multiparous females (1), immunoglobulin from multiple myeloma (2), hemodialysis patients (1), hepatocellular carcinoma (5).

Interference

Potentially Interfering Endogenous Substances

For the following potentially interfering endogenous substances, it was demonstrated that the ARCHITECT HBsAg Next Confirmatory assay is not susceptible to interference at the following interferent levels:

Potentially Interfering Endogenous Substance	Interferent Level
Unconjugated Bilirubin	40 mg/dL
Conjugated Bilirubin	40 mg/dL
Hemoglobin	1000 mg/dL
Triglycerides	3000 mg/dL
Total Protein	15 g/dL

Analytical Sensitivity

The analytical sensitivity of the ARCHITECT HBsAg Next Qualitative and ARCHITECT HBsAg Next Confirmatory assays was determined using serial dilutions of the WHO Second International Standard for HBsAg, subtype *adw2*, genotype A, NIBSC code: 00/588. The dilutions ranged from 3 to 40 mIU/mL. The dilutions that were reactive by the ARCHITECT HBsAg Next Qualitative assay were tested using 3 ARCHITECT HBsAg Next Confirmatory reagent lots. In this study, the ARCHITECT HBsAg Next Confirmatory assay confirmed as positive all dilutions detected as reactive by the ARCHITECT HBsAg Next Qualitative assay.

BIBLIOGRAPHY

1. Neurath AR, Kent SB, Strick N, et al. Identification and chemical synthesis of a host cell receptor binding site on hepatitis B virus. *Cell* 1986;46:429-436.
2. Szmuness W, Stevens CE, Harley EJ, et al. Hepatitis B vaccine: Demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N Engl J Med* 1980;303:833-841.
3. Krugman S, Giles JP. Viral hepatitis, type B (MS-2-Strain): further observations on natural history and prevention. *N Engl J Med* 1973;288:755-760.
4. Perrillo RP, Aach RD. The clinical course and chronic sequelae of hepatitis B virus infection. *Seminars in Liver Disease* 1981;1:15-25.
5. CDC. A comprehensive immunization strategy to eliminate transmission of Hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) Part 1: Immunization of Infants, Children, and Adolescents. *MMWR* 2005;54 (RR-16):1-23.
6. Hunt CM, McGill JM, Allen MI, et al. Clinical relevance of hepatitis B viral mutations. *Hepatology* 2000;31(5):1037-1044.
7. Zuckerman AJ. Effect of hepatitis B virus mutants on efficacy of vaccination. *Lancet* 2000;355:1382-1384.
8. Carman WF, Trautwein C, Van Deursen FJ, et al. Hepatitis B virus envelope variation after transplantation with and without hepatitis B immune globulin prophylaxis. *Hepatology* 1996;24(3):489-493
9. Grethe S, Monazahian M, Böhme I, et al. Characterization of unusual escape variants of hepatitis B virus isolated from a hepatitis B surface antigen-negative subject. *J Virology* 1998;72(9):7692-7696.
10. Nainan OV, Stevens CE, Taylor PE, et al. Hepatitis B virus (HBV) antibody resistant mutants among mothers and infants with chronic HBV infection. In: Rizzetto M, Purcell RH, Gerin JL, et al., eds. *Viral Hepatitis and Liver Disease*. Minerva Medica: Torino;1997:132-134
11. Jongerius JM, Wester M, Cuypers HTM, et al. New hepatitis B virus mutant form in a blood donor that is undetectable in several hepatitis B surface antigen screening assays. *Transfusion* 1998;38:56-59.
12. Bock CT, Tillmann HL, Torresi J, et al. Selection of hepatitis B virus polymerase mutants with enhanced replication by lamivudine treatment after liver transplantation. *Gastroenterology* 2002;122:264-273.
13. Locarnini SA. Hepatitis B virus surface antigen and polymerase gene variants: potential virological and clinical significance. *Hepatology* 1998;27(1):294-297.
14. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part

1910.1030, Bloodborne pathogens.

15. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 6th ed. Washington, DC: US Government Printing Office; June 2020.

16. World Health Organization. *Laboratory Biosafety Manual*. 4th ed. Geneva: World Health Organization; 2020.

17. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition.* CLSI Document M29-A4. Wayne, PA: CLSI; 2014.

18. Centers for Disease Control and Prevention. *Guidelines for Viral Hepatitis Surveillance and Case Management*. Atlanta, GA: 2005.

19. Koziel MJ, Siddiqui A. Hepatitis B virus and hepatitis delta virus. In: *Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases*. 6th ed. New York, NY: Elsevier/Churchill Livingstone; 2005:1864-1890.

20. Primus FJ, Kelley EA, Hansen HJ, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* 1988;34(2):261-264.

21. Schroff RW, Foon KA, Beatty SM, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985;45(2):879-885.

22. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem* 1988;34(1):27-33.

23. Rysgaard CD, Morris CS, Drees D, et al. Positive hepatitis B surface antigen tests due to recent vaccination: a persistent problem. *BMC Clin Pathol* 2012;12(1):15-20.

24. Calisti G, Herman O, Powley M, et al. Persistence of hepatitis B surface antigen in blood in a chronic haemodialysis patient following vaccination booster. *BMJ Case Rep* Published online: 2014 June 10. doi:10.1136/bcr-2013-202191.

Key to Symbols

ISO 15223 Symbols	
	**Caution**
	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
REF	List Number
SN	Serial number

Other Symbols	
ANCILLARY WASH BUFFER	Ancillary Wash Buffer
ASSAY SPECIFIC DILUENT	Assay Specific Diluent
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number
DISTRIBUTED IN THE USA BY	Distributed in the USA by
INFORMATION FOR USA ONLY	Information needed for United States of America only
MICROPARTICLES	Microparticles

Other Symbols

PRE-TREATMENT 1	Pre-Treatment 1
PRE-TREATMENT 2	Pre-Treatment 2
PRODUCT OF IRELAND	Product of Ireland
REAGENT LOT	Reagent Lot
Rx ONLY	For use by or on the order of a physician only (applicable to USA classification only).

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%).

Abbott Ireland

Diagnostics Division

Finisklin Business Park

Sligo

Ireland

+353-71-9171712

DISTRIBUTED IN THE USA BY

Abbott Laboratories

Abbott Park, IL 60064 USA

Customer Service: Contact your local representative or find country-specific contact information on www.corelaboratory.abbott

Created March 2022.

General Instructions

Created December 2019.

INTENDED USE

The HBsAg Next Qualitative Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT iSystem when used for the qualitative detection and for the confirmation of the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma.

For additional information, refer to the HBsAg Next Qualitative and HBsAg Next Confirmatory reagent package inserts and the ARCHITECT System Operations Manual.

CONTENTS

The CONTROL - contains recalcified human plasma. Preservatives: ProClin 950 and sodium azide.

The CONTROL + contains inactivated purified human HBsAg (subtype *ad/ay*) in phosphate buffer with a protein (bovine) stabilizer. Preservatives: ProClin 300 and ProClin 950.

The controls are at the following targets and ranges:

ARCHITECT HBsAg Next Qualitative assay (4P76):

	Quantity	TARGET S/CO	RANGE S/CO
CONTROL -	1 x 8.0 mL	-	≤ 0.85
CONTROL +	1 x 8.0 mL	3.20	1.60 - 4.80

ARCHITECT HBsAg Next Confirmatory assay (4P77):

		C2 S/CO*		
	Quantity	TARGET	RANGE	% NEUTRALIZATION
CONTROL +	1 x 8.0 mL	2.90	1.45 - 4.35	≥ 50%

* A target and a range are not defined for C1 S/CO.

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

- Calibration
- Control lot
- Reagent lot
- Calibrator lot
- Instrument

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 **CONTENTS** 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.[1, 2, 3, 4]
- The Negative Control contains human plasma that is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.
- The purified HBsAg (inactivated) used in the Positive Control was derived from human donor units tested and found to be nonreactive for HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2 and anti- HCV.

The following warnings and precautions apply to: CONTROL –	
WARNING	Contains methylisothiazolones and sodium azide.
H317	May cause an allergic skin reaction.

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

The following warnings and precautions apply to: CONTROL +

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.

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

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

Safety Data Sheets are available at www.corelaboratory.abbott or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

STORAGE

· Do not use past expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	
Opened	2 to 8°C	Until expiration date	Store tightly capped. Return to refrigerated storage after use.

INDICATIONS OF INSTABILITY OR DETERIORATION

Instability or deterioration should be suspected if there are precipitates, visible signs of leakage, or turbidity, or if controls do not meet the appropriate package insert and/or ARCHITECT System Operations Manual criteria.

PREPARATION FOR USE

· This product is liquid ready-to-use.

· This product may be used immediately after removal from 2 to 8°C storage.

· Prior to each use, mix by gentle inversion.

INSTRUMENT PROCEDURE

- For information on configuring the positive control for the ARCHITECT HBsAg Next Confirmatory assay refer to the ARCHITECT HBsAg Next Confirmatory Reagent package insert.
- To obtain the required volume of controls for the ARCHITECT HBsAg Next Qualitative assay, hold the control bottles **vertically** and dispense 6 drops of each control into each respective sample cup.
- To obtain the required volume of controls for the ARCHITECT HBsAg Next Confirmatory assay, hold the positive control bottle **vertically** and dispense 10 drops of Positive Control **only** (for two replicates, one for C1 and one for C2) into a sample cup.
- For information on ordering controls, refer to the ARCHITECT System Operations Manual, Section 5.

BIBLIOGRAPHY

1. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
2. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 6th ed. Washington, DC: US Government Printing Office; June 2020.
3. World Health Organization. *Laboratory Biosafety Manual*. 4th ed. Geneva: World Health Organization; 2020.
4. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition.* CLSI Document M29-A4. Wayne, PA: CLSI; 2014.

Key to Symbols

ISO 15223 Symbols	
	Caution
	Consult instructions for use
	Manufacturer
	Temperature limitation

ISO 15223 Symbols	
	Use by/Expiration date
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
REF	List Number

Other Symbols	
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTAINS: METHYLISOTHIAZOLONES	Contains Methylisothiazolones
CONTROL –	Negative Control
CONTROL +	Positive Control
DISTRIBUTED IN THE USA BY	Distributed in the USA by
INFORMATION FOR USA ONLY	Information needed for United States of America only
NEUTRALIZATION	Neutralization
PRODUCT OF IRELAND	Product of Ireland
RANGE	Range
Rx ONLY	For use by or on the order of a physician only (applicable to USA classification only).
TARGET	Target
WARNING: SENSITIZER	Warning: May cause an allergic reaction.

Abbott Ireland

Diagnostics Division

Finisklin Business Park

Sligo

Ireland

+353-71-9171712

CE

0123

DISTRIBUTED IN THE USA BY

Abbott Laboratories

Abbott Park, IL 60064 USA

Customer Service: Contact your local representative or find country-specific contact information on www.abbottdiagnostics.com

Created December 2019.

Abbott

General Instructions

Created December 2019.

INTENDED USE

The HBsAg Next Qualitative Calibrators are for the calibration of the ARCHITECT iSystem when used for qualitative determination and confirmation of the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma.

For additional information, refer to the HBsAg Next Qualitative and HBsAg Next Confirmatory reagent package inserts and the ARCHITECT System Operations Manual.

CONTENTS

The CAL 1 contains recombinant HBsAg (subtype *ad*) in phosphate buffer with protein (bovine) stabilizer.

The CAL 2 contains phosphate buffer with a protein (bovine) stabilizer.

Preservatives: ProClin 300 and ProClin 950.

The ARCHITECT HBsAg Next Qualitative and HBsAg Next Confirmatory assays use Calibrator 1 and Calibrator 2 to assess calibration validity and to calculate the assay cutoff. The ARCHITECT HBsAg Next Confirmatory assay uses Calibrator 2 to calculate the % Neutralization.

The calibrators are at the following target concentrations:

Calibrator	Quantity	HBsAg CONC (IU/mL)
CAL 1	1 x 4.0 mL	0.1
CAL 2	1 x 4.0 mL	0.0

STANDARDIZATION

The ARCHITECT HBsAg Next Calibrator 1 is referenced to the World Health Organization (WHO) Second International Standard for HBsAg (subtype *adw2*, genotype A, NIBSC Code 00/588).

PRECAUTIONS

· IVD

·For *In Vitro* Diagnostic Use

· Rx ONLY

Safety Precautions

The following warnings and precautions apply to: CAL 1 / CAL 2	
	
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.

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

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

Safety Data Sheets are available at www.corelaboratory.abbott or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

STORAGE

· Do not use past expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	
Opened	2 to 8°C	Until expiration date	Store tightly capped. Return to refrigerated storage after use.

INDICATIONS OF INSTABILITY OR DETERIORATION

Instability or deterioration should be suspected if there are precipitates, visible signs of leakage, or turbidity, if calibration does not meet the appropriate package insert criteria and/or the ARCHITECT System Operations Manual criteria, or if controls do not meet the appropriate criteria.

PREPARATION FOR USE

· This product is liquid ready-to-use.

· This product may be used immediately after removal from 2 to 8°C storage.

· Prior to each use, mix by gentle inversion.

QUALITY CONTROL PROCEDURES

Refer to the ARCHITECT HBsAg Next Qualitative and HBsAg Next Confirmatory reagent package inserts and ARCHITECT System Operations Manual, Section 5, for additional information.

· For the ARCHITECT HBsAg Next Qualitative assay, a single replicate of each control level must be tested to evaluate the assay calibration.

- For the ARCHITECT HBsAg Next Confirmatory assay, a single replicate of the positive control **only** must be tested to evaluate the assay calibration.
- Ensure that assay control values are within the ranges specified in the control package insert.

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

- A reagent kit with a new lot number is used.
- Daily quality control results are outside of quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of the ARCHITECT HBsAg Next Qualitative reagent package insert.

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

INSTRUMENT PROCEDURE

Perform an ARCHITECT HBsAg Next Qualitative calibration as follows:

- Test calibrators 1 and 2 in triplicate. To obtain the recommended volume requirements for the HBsAg Next Qualitative Calibrators, hold the bottles **vertically** and dispense 11 drops into each respective sample cup.
- The calibrators should be priority loaded.

Perform an ARCHITECT HBsAg Next Confirmatory calibration as follows:

- Test calibrators 1 and 2 in triplicate with the C2 assay. The C1 assay uses the calibration generated from the C2 assay. To obtain the recommended volume requirements for the HBsAg Next Qualitative Calibrators, hold the bottles **vertically** and dispense 14 drops into each respective sample cup.
- The calibrators should be priority loaded.

- For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.

Key to Symbols

ISO 15223 Symbols	
	Consult instructions for use
	Manufacturer
	Temperature limitation
	Use by/Expiration date
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
REF	List Number

Other Symbols	
CAL 1	Calibrator 1
CAL 2	Calibrator 2
CONC	Concentration
CONTAINS: METHYLISOTHIAZOLONES	Contains Methylisothiazolones
DISTRIBUTED IN THE USA BY	Distributed in the USA by
INFORMATION FOR USA ONLY	Information needed for United States of America only
PRODUCT OF IRELAND	Product of Ireland
Rx ONLY	For use by or on the order of a physician only (applicable to USA classification only).
WARNING: SENSITIZER	Warning: May cause an allergic reaction.

Abbott Ireland

Diagnostics Division

Finisklin Business Park

Sligo

Ireland

+353-71-9171712

0123

DISTRIBUTED IN THE USA BY

Abbott Laboratories

Abbott Park, IL 60064 USA

Customer Service: Contact your local representative or find country-specific contact information on www.abbottdiagnostics.com

Created December 2019.

Abbott

General Instructions

Created December 2019.

INTENDED USE

The HBsAg Next Confirmatory Manual Diluent is used for manually diluting specimens for testing on the ARCHITECT iSystem.

For additional information, refer to the HBsAg Next Confirmatory reagent package insert and the ARCHITECT System Operations Manual.

CONTENTS

REF	**4P77-40**
MANUAL DILUENT	1 x 100 mL
MANUAL DILUENT	contains recalcified human plasma. Preservatives: ProClin 950 and sodium azide.

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 **CONTENTS** 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.[1], [2], [3], [4]

- The manual diluent contains human plasma that is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.

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

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

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

Safety Data Sheets are available at www.corelaboratory.abbott or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

STORAGE

· Do not use past expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	
Opened	2 to 8°C	Until expiration date	Store tightly capped. Return to refrigerated storage after use.

INDICATIONS OF INSTABILITY OR DETERIORATION

Instability or deterioration should be suspected if there are precipitates, visible signs of leakage, or turbidity.

PREPARATION FOR USE

· This product is liquid ready-to-use.

· This product may be used immediately after removal from 2 to 8°C storage.

SAMPLE DILUTION PROCEDURE

· Refer to the ARCHITECT HBsAg Next Confirmatory Reagent package insert for the sample dilution procedure.

BIBLIOGRAPHY

1. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
2. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
3. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
4. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From*

Occupationally Acquired Infections; Approved Guideline—Fourth Edition. CLSI Document M29-A4. Wayne, PA: CLSI; 2014.

Key to Symbols

ISO 15223 Symbols	
	Caution
	Consult instructions for use
	Manufacturer
	Temperature limitation
	Use by/Expiration date
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
REF	List Number

Other Symbols	
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTAINS: METHYLISOTHIAZOLONES	Contains Methylisothiazolone
DISTRIBUTED IN THE USA BY	Distributed in the USA by
INFORMATION FOR USA ONLY	Information needed for United States of America only
MANUAL DILUENT	Manual Diluent
PRODUCT OF IRELAND	Product of Ireland
Rx ONLY	For use by or on the order of a physician only (applicable to USA classification only).
WARNING: SENSITIZER	Warning: May cause an allergic reaction.

Abbott Ireland

Diagnostics Division

Finisklin Business Park

Sligo

Ireland

+353-71-9171712

DISTRIBUTED IN THE USA BY

Abbott Laboratories

Abbott Park, IL 60064 USA

Customer Service: Contact your local representative or find country-specific contact information on www.abbottdiagnostics.com

Created December 2019.

Abbott