ARCHITECT

SYSTEM

en

HBsAg Qualitative II

REF 2G22

B2G220

G34444R06

Read Highlighted Changes
Revised April 2019

HBsAg Qualitative II

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

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	CONTROL NO.	Control Number
IVD	*In Vitro* Diagnostic Medical Device	REACTION VESSELS	Reaction Vessels
LOT	Lot Number	REAGENT LOT	Reagent Lot
	Expiration Date	REPLACEMENT CAPS	Replacement Caps
SN	Serial Number	SAMPLE CUPS	Sample Cups
2°C 8°C	Store at 2-8°C	SEPTUM	Septum
	Caution	WARNING: SENSITIZER	Warning: May cause an allergic reaction
	Consult instructions for use	GTIN	Global Trade Item Number
	Manufacturer	PRODUCT OF IRELAND	Product of Ireland

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

Abbott

NAME

ARCHITECT HBsAg Qualitative II

INTENDED USE

The ARCHITECT HBsAg Qualitative II assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum and plasma including specimens collected post-mortem (non-heart-beating).

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

SUMMARY AND EXPLANATION OF TEST

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

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

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT HBsAg Qualitative II assay is a one-step immunoassay for the qualitative detection of HBsAg in human serum and plasma using CMIA technology, with flexible assay protocols, referred to as Chemiflex. (Note: Ancillary Wash Buffer is added in a second incubation step, so the assay file performs a two-step assay protocol).

In the ARCHITECT HBsAg Qualitative II assay, sample, anti-HBs coated paramagnetic microparticles and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture. 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 to the reaction mixture. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the ARCHITECT *i* System optics.

The presence or absence of HBsAg in the sample 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 specimen is greater than or equal to the cutoff signal, the sample is considered reactive for HBsAg.

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

REAGENTS

Reagent Kit, 100 Tests/500 Tests

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT HBsAg Qualitative II Reagent Kit (2G22)

- **MICROPARTICLES** 1 or 4 bottles (6.6 mL per 100-test bottle/27.0 mL per 500-test bottle) anti-HBs (mouse, monoclonal, IgM, IgG) coated microparticles in MES buffer with protein (bovine serum albumin) stabilizer. Minimum concentration: 0.08% solids. Preservatives: ProClin 300 and ProClin 950.
- **CONJUGATE** 1 or 4 bottles (5.9 mL per 100-test bottle/26.3 mL per 500-test bottle) anti-HBs (mouse, monoclonal, IgG) and anti-HBs (goat, IgG) acridinium-labeled conjugate in phosphate buffer with human plasma and protein (bovine serum albumin, fetal bovine serum, goat IgG, mouse IgG) stabilizers. Minimum concentration: 0.35 µg/mL. Preservatives: ProClin 300 and ProClin 950.
- **ANCILLARY WASH BUFFER** 1 or 4 bottles (5.9 mL per 100-test bottle/ 26.3 mL per 500-test bottle) ancillary wash buffer containing MES buffer. Preservatives: ProClin 300 and ProClin 950.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-trigger solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

- **WASH BUFFER** Wash buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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[7]. Biosafety Level 2[8] or other appropriate biosafety practices[9,10] should be used for materials that contain or are suspected of containing infectious agents.
- The Conjugate contains human plasma that is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2 and anti-HCV.
- The following warnings and precautions apply to these components:
  - Microparticles
  - Conjugate
  - Ancillary Wash Buffer

WARNING:	Contains methylisothiazolones
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapours / 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.
P363	Wash contaminated clothing before reuse.

This material and its container must be disposed of in a safe way.

- Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.
- For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagents kits beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT HBsAg Qualitative II 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.
  - When handling conjugate vials, change gloves that have contacted human serum or plasma, since introduction of human IgG or IgM will result in a neutralized conjugate.
  - Once a septum has been placed on the 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 handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

- The ARCHITECT HBsAg Qualitative II Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, the reagents are stable until the expiration date.
- The ARCHITECT HBsAg Qualitative II Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°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. **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.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. 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 Qualitative II assay file (assay number 628) must be installed on the ARCHITECT *i* System before 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 collection tubes listed below were verified for use with the ARCHITECT HBsAg Qualitative II assay. Other specimen collection tubes have not been tested with this assay.
  - Human serum (including serum collected in serum separator tubes)
  - Human plasma collected in:
    - Lithium heparin
    - Dipotassium EDTA
    - Tripotassium EDTA
    - Sodium citrate
    - Plasma separator tubes (lithium heparin)
    - Sodium heparin
    - CPD
    - CPDA-1
    - ACD
    - Potassium oxalate / sodium fluoride plasma
- Performance has not been established for the use of body fluids other than human serum or plasma.
- Performance has been established for the use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating), for details refer to section **TESTING OF CADAVERIC BLOOD SPECIMENS.**
- Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient specimens.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the ARCHITECT HBsAg Qualitative II assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed
  - obvious microbial contamination
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- As specimens from heparinized patients may be partially coagulated and erroneous results could occur due to the presence of fibrin, draw the specimen prior to heparin therapy.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- No qualitative performance differences were observed between experimental controls and nonreactive or spiked reactive specimens tested with elevated levels of conjugated or unconjugated bilirubin (20 mg/dL), triglycerides (3000 mg/dL), protein (12 g/dL), or hemoglobin (500 mg/dL).

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Prepare frozen specimens as follows:
  - Frozen specimens must be completely thawed before mixing.
  - Mix thawed specimens thoroughly by inverting 10 times or by low speed vortexing. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous. If samples are not mixed thoroughly, inconsistent results may be obtained.
  - Centrifuge mixed specimens as described below.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at ≥10,000 RCF (Relative Centrifugal Force) for 10 minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter or
  - they were frozen and thawed.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.
- Transfer clarified specimen to a sample cup or secondary tube for testing.

Storage

- Specimens may be stored on or off the clot, red blood cells, or separator gel for
  - up to 24 hours at room temperature or
  - up to 6 days at 2-8°C.
- If testing will be delayed more than 6 days, remove serum or plasma from the clot, red blood cells, or separator gel and store at -20°C or colder.
- Avoid more than 3 freeze/thaw cycles.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator gel.
- When shipping specimens, package and label specimens in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped ambient, at 2-8°C (wet ice), or frozen (dry ice). Do not exceed the storage time limitations listed above.

TESTING OF CADAVERIC BLOOD SPECIMENS

- Performance has been established for the use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating) that have been collected up to 18.5 hours after death. Performance was established using 50 spiked and 50 non-spiked cadaveric blood specimens[11].
- Testing of cadaveric blood specimens from patients with plasma dilution due to transfusions of > 2000 mL of blood or colloids within 48 hours, or > 2000 mL of crystalloids within 1 hour (or any combination thereof) prior to collection of the specimens have not been validated.
- Follow general standards and/or regulations for collection, storage and handling.
- Follow the tube manufacturer's processing instructions for serum or plasma collection tubes. After initial centrifugation, transfer the supernatant to a centrifuge tube and centrifuge at ≥ 10,000 RCF (Relative Centrifugal Force) for 10 minutes. If specimens are not processed directly after initial centrifugation, it is recommended to remove the supernatant from the clot or red blood cells until further processing.
- Cadaveric blood specimens can be stored for up to 6 days at 2-8°C or up to 24 hours at 15-30°C following collection.
- No qualitative differences were observed for cadaveric blood specimens (nonreactive or spiked reactive) when subjected to up to 3 freeze/thaw cycles. However, multiple freeze/thaw cycles should be avoided.

PROCEDURE

Materials Provided

- 2G22 ARCHITECT HBsAg Qualitative II Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- ARCHITECT HBsAg Qualitative II Assay file, may be obtained from:
  - ARCHITECT *i* System e-Assay CD-ROM found on www.abbottdiagnostics.com
  - ARCHITECT *i* System Assay CD-ROM
- 2G22-01 ARCHITECT HBsAg Qualitative II Calibrators
- 2G22-10 ARCHITECT HBsAg Qualitative II Controls or other control material
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional) to deliver the specified volumes.

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

Assay Procedure

- Before loading the ARCHITECT HBsAg Qualitative II 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 Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Handling Precautions** section of this package insert.
- Load the ARCHITECT HBsAg Qualitative II Reagent Kit on the ARCHITECT *i* System.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample cup volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present before running the test.
  - Priority: 125 µL for the first HBsAg Qualitative II test plus 75 µL for each additional HBsAg Qualitative II test from the same sample cup.
  - ≤ 3 hours on-board: 150 µL for the first HBsAg Qualitative II test plus 75 µL for each additional HBsAg Qualitative II test from the same sample cup.
  - > 3 hours on-board: replace with a fresh sample (patient specimens, controls and calibrators).
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare calibrators and controls.
  - Mix the ARCHITECT HBsAg Qualitative II Calibrators and Controls by gentle inversion before use.
  - To obtain the recommended volume requirements for the ARCHITECT HBsAg Qualitative II Calibrators and Controls, hold the bottles **vertically** and dispense 11 drops of each calibrator and 6 drops of each control into each respective sample cup.
  - If commercially available control material is used, follow the manufacturer's instructions for preparation.
- Load samples
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- 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.

Specimen Dilution Procedure

Specimens cannot be diluted for the ARCHITECT HBsAg Qualitative II assay.

Calibration

- To perform an ARCHITECT HBsAg Qualitative II calibration, test calibrators 1 and 2 in replicates of 3. The calibrators should be priority loaded.
- A single sample of each control level must be tested to evaluate the assay calibration.
  - Order controls as described in the **Assay Procedure** section.
  - Ensure that assay control values are within the ranges specified in the control package insert.
- Once an ARCHITECT HBsAg Qualitative II 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.
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT HBsAg Qualitative II assay is that a single sample of each control be tested once every 24 hours each day of use. If your laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures. Additional controls may be tested in conformance with local, state and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

Control values must be within the ranges specified in the control package insert. If a control result is out of its specified range, any test results generated since the last acceptable control results must be evaluated to determine if test results may have been adversely affected. Adversely affected test results are invalid and these samples must be retested. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT HBsAg Qualitative II assay belongs to method group 5, except functional sensitivity.

RESULTS

Calculations

- The ARCHITECT *i* System calculates the result for the ARCHITECT HBsAg Qualitative II assay using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen and control.
  - Cutoff RLU = (0.0575 x Calibrator 1 Mean RLU) + (0.8 x Calibrator 2 Mean RLU)
  - S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

ARCHITECT HBsAg Qualitative II Initial Result

Initial Result (S/CO)	Instrument Interpretation	Retest Procedure
< 1.00	NONREACTIVE	No retest required.
≥ 1.00	REACTIVE	Retest in duplicate.

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

ARCHITECT HBsAg Qualitative II 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 a neutralizing assay.*

* The ARCHITECT HBsAg Qualitative II Confirmatory assay is recommended.

- Confirm repeatedly reactive specimens using a neutralizing assay (*e.g.*, ARCHITECT HBsAg Qualitative II Confirmatory) before disclosing HBsAg status to the patient.

For details on configuring the ARCHITECT *i* System to use gray zone interpretations, refer to the ARCHITECT System Operations Manual, Section 2. The grayzone interpretations are editable parameters and should be utilized per end user requirements.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- If the ARCHITECT HBsAg Qualitative II results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[12,13] Specimens containing HAMA may produce anomalous values when tested with assay kits such as ARCHITECT HBsAg Qualitative II that employ mouse monoclonal antibodies.[12]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[14] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous results may be observed. Additional information may be required for diagnosis.
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert for specimen limitations.

SPECIFIC PERFORMANCE CHARACTERISTICS

All performance studies were conducted using the ARCHITECT *i*2000/*i*2000$_{SR}$ Systems. Additionally, the Within-Laboratory Precision, Analytical Sensitivity and Seroconversion studies were conducted using the ARCHITECT *i*1000$_{SR}$.

Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT HBsAg Qualitative II assay is designed to have an imprecision of ≤ 10% within-laboratory (Total) CV for the positive control and low positive panel and a standard deviation (SD) of ≤ 0.10 S/CO for the high negative panel.

Within-Laboratory Precision

A study was performed based on guidance from the National Committee for Clinical Laboratory Standards (NCCLS) document EP5-A2.[15] Testing was conducted using 3 lots of ARCHITECT HBsAg Qualitative II reagents, calibrators and controls and 4 instruments. Two controls and two panels were assayed in a minimum of 2 replicates at 2 separate times per day for 20 different days. Each reagent lot used a single calibration curve throughout the study. The ranges for all instruments and reagent lots are summarized across instruments and reagent lots in the following table.

Sample	n	Mean Range S/CO	Within-Run Range		Within-Laboratory Precision (Total) Range	
			SD	%CV	SD	%CV
Negative Control	956	0.15 - 0.18	0.012 - 0.016	NA	0.014 - 0.030	NA
Positive Control	958	3.26 - 3.45	0.056 - 0.082	1.7 - 2.5	0.072 - 0.103	2.1 - 3.2
High Negative Panel	955	0.71 - 0.77	0.021 - 0.024	NA	0.025 - 0.033	NA
Low Positive Panel	956	1.17 - 1.27	0.026 - 0.040	2.1 - 3.4	0.029 - 0.048	2.3 - 4.1

NA = Not applicable

System Reproducibility

A 5-day precision study was performed for the ARCHITECT HBsAg Qualitative II assay based on guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP15-A2.[16] Testing was conducted at 3 clinical sites using 3 lots each of ARCHITECT HBsAg Qualitative II reagents, calibrators and controls per site. Two controls and 2 panels were assayed in replicates of 4 at 2 separate times of day for 5 days. The data are summarized in the following table.

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

NA = not applicable

Specificity

Blood Donor Specimens

The ARCHITECT HBsAg Qualitative II assay is designed to have a specificity of > 99.5% on blood donor specimens.

A study was performed at three external sites on a total of 5401 serum and plasma specimens collected from two blood-donation centers. For 1 specimen which was tested as initial and repeat reactive on ARCHITECT HBsAg Qualitative II, the presence of HBsAg was confirmed by specific neutralization with anti-HBs. The specificity on the remaining 5400 blood donors was assessed to be 99.91% (5395/5400) with an assumed zero prevalence of HBV infection. The data are summarized in the following table.

Category	n	IR[a] (%)	RR[a] (%)	Specificity	95% Confidence Interval
Overall Blood Donors	5401[b]	7 (0.13%)	6 (0.11%)	99.91% (5395/5400)	99.78% - 99.97%
Blood Donors Plasma	2700	4 (0.15%)	3 (0.11%)	99.89% (2697/2700)	99.68% - 99.98%
Blood Donors Serum	2701[b]	3 (0.11%)	3 (0.11%)	99.93% (2698/2700)	99.73% - 99.99%

[a] IR = Initially Reactive, RR = Repeatedly Reactive

[b] One specimen confirmed positive.

Diagnostic Specimens

A study was performed using a total of 1499 randomly selected diagnostic patients, including specimens from hospitalized and hemodialysis patients. For 16 specimens which were tested as initial and repeat reactives on ARCHITECT HBsAg Qualitative II, the presence of HBsAg was confirmed by specific neutralization with anti-HBs. The specificity on the remaining 1483 diagnostic specimens was assessed to be 99.93% (1482/1483) with an assumed zero prevalence of HBV infection. The data are summarized in the following table.

Category	n	IR[a] (%)	RR[a] (%)	Specificity[b]	95% Confidence Interval
Overall Diagnostics	1499[c]	18 (1.20%)	17 (1.13%)	99.93% (1482/1483)	99.62% - 100.00%
Hospitalized/ Diagnostics	999[d]	12 (1.20%)	11 (1.10%)	99.90% (988/989)	99.44% - 100.00%
Hemodialysis	500[e]	6 (1.20%)	6 (1.20%)	100.00% (494/494)	99.26% - 100.00%

[a] IR = Initially Reactive, RR = Repeatedly Reactive

[b] One aberrant result was observed and the specificity was 99.93% (1481/1482) with this specimen excluded.

[c] Sixteen specimens confirmed positive.

[d] Ten specimens confirmed positive.

[e] Six specimens confirmed positive.

Sensitivity

The ARCHITECT HBsAg Qualitative II assay is designed to show sensitivity performance that is greater than or equal to the lower limit of the 95% confidence interval for a commercially available HBsAg assay on the same population of HBsAg positive specimens.

For the 402 HBsAg positive specimens from patients with unknown disease status evaluated in this study, the lower limit of the 95% confidence interval for the commercially available HBsAg assay was 99.09%. In this study, the sensitivity of the ARCHITECT HBsAg Qualitative II assay was 100.00% (402/402).

The assay was further evaluated by testing a total of 126 pre-selected specimens from acute and chronic HBV infections.

Specimen Category	Number of Specimens	Number of Positive Results	Clinical Sensitivity (%)	95% Confidence Interval (%)
Individuals with Acute* HBV Infection	8	8	100.00	(63.06, 100.00)
Individuals with Chronic** HBV Infection	118	118	100.00	(96.92, 100.00)
Total	126	126	100.00	(97.11, 100.00)

* Samples were positive for HBsAg, total anti-HBc, anti-HBc IgM and negative for anti-HBs by commercially available assays.

** Samples were positive for HBsAg, total anti-HBc and negative for anti-HBc IgM and anti-HBs by commercially available assays.

Analytical Sensitivity

The ARCHITECT HBsAg Qualitative II assay is designed to have a mean analytical sensitivity value that is less than or equal to the lower limit of the 95% confidence interval around the mean analytical sensitivity of a commercially available HBsAg assay. Analytical sensitivity was evaluated using serial dilutions of the WHO 2nd International HBsAg Standard (subtype *adw2*, genotype A, NIBSC Code 00/588). The dilutions ranged from 0.010 to 0.5 IU/mL. Recalcified negative human plasma/serum was used as the diluent and represented the 0 IU/mL sample. The dilutions were tested across 3 reagent lots on 3 instrument types (1 $i2000_{SR}$, 1 *i*2000 and 1 $i1000_{SR}$). In this study, the lower limit of the 95% confidence interval for the commercially available HBsAg assay was 0.021 IU/mL. The analytical sensitivity results for ARCHITECT HBsAg Qualitative II, calculated by linear regression, ranged from 0.017 to 0.022 IU/mL. The mean analytical sensitivity ranged from 0.019 to 0.020 IU/mL across instrument types.

Analytical Specificity

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

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

[a] NR = Nonreactive, R = Reactive

Seroconversion Sensitivity

The ARCHITECT HBsAg Qualitative II assay is designed to have a seroconversion sensitivity that is better than or equivalent to the seroconversion sensitivity of a commercially available HBsAg assay. To determine the seroconversion sensitivity, 30 HBV seroconversion panels obtained from commercial vendors were tested using the ARCHITECT HBsAg Qualitative II and ARCHITECT HBsAg Qualitative II Confirmatory assays. The results were compared to a commercially available HBsAg assay and representative data from 6 panels are summarized in the following table.

Panel ID	Days since 1st bleed	ARCHITECT HBsAg Qualitative II S/CO Reactive ≥ 1.00 S/CO	Commercially Available HBsAg Assay S/CO Reactive ≥ 1.00 S/CO
6271	0	0.31	0.39
	3	0.74	0.70
	7	**1.88**	**1.81**
	12	**14.41**	**9.49**
	18	**113.86**	**56.70**
PHM 925	0	0.59	0.64
	4	**1.32**	0.91
	8	**2.48**	**1.87**
	14	**5.69**	**4.10**
	17	**6.72**	**3.46**
PHM 930	0	0.50	0.41
	3	**4.95**	**2.28**
	8	**43.38**	**19.73**
	12	**124.59**	**47.42**
	15	**321.30**	**112.32**
PHM 933	2	0.79	0.69
	7	**4.01**	**2.26**
	9	**9.07**	**4.85**
	16	**45.03**	**22.30**
	144	**2715.52**	**823.14**
6273	0	0.17	0.72
	3	0.16	0.39
	7	0.25	0.55
	14	**1.05**	**1.02**
	25	**20.99**	**13.84**
	30	**158.83**	**73.40**
11002	0	0.36	0.42
	2	0.49	0.60
	7	**1.59**	**1.55**
	9	**2.40**	**2.08**
	35	**1612.66**	**379.66**
	39	**403.92**	**232.20**

HBsAg Mutant Detection

The hepatitis B virus, unlike other DNA viruses, replicates through reverse transcription. The reverse transcription process lacks proofreading capability; therefore, HBV is subject to a mutation rate 10 times higher than the mutation rate of other DNA viruses.[17] 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.[17-24] HBsAg mutations may result in a less favorable outcome in some patients[17,18,20] and false negative results in some HBsAg assays.[17-19]

The ARCHITECT HBsAg Qualitative II assay is designed to have the ability to better detect (as reactive) the HBsAg mutant Thr-123-Ala and to have the equivalent or better ability to detect (as reactive) other HBsAg mutants when compared to the comparator assay. A panel of 9 recombinant HBsAg mutant samples was obtained. Each panel member was diluted with recalcified negative human plasma to an S/CO of 2.0 ± 0.5 and tested with the ARCHITECT HBsAg Qualitative II assay and with a comparator assay. The data are summarized in the following table.

Mutant	Final Interpretation: ARCHITECT HBsAg Qualitative II	Final Interpretation: Commercially Available HBsAg
Gln-129-His	Repeatedly Reactive	Repeatedly Reactive
Met-133-Leu	Repeatedly Reactive	Repeatedly Reactive
Asp-144-Ala	Repeatedly Reactive	Nonreactive
Gly-145-Arg	Repeatedly Reactive	Repeatedly Reactive
Thr-123-Ala	Repeatedly Reactive	Nonreactive
P142L+G145R	Repeatedly Reactive	Repeatedly Reactive
P142S+G145R	Repeatedly Reactive	Repeatedly Reactive
122NT	Repeatedly Reactive	Repeatedly Reactive
122RA	Repeatedly Reactive	Repeatedly Reactive

HBV Genotype Detection

The ARCHITECT HBsAg Qualitative II assay is designed to detect HBV genotypes A through F and H. A study was performed to evaluate the ability of the ARCHITECT HBsAg Qualitative II assay to detect different HBV genotypes by testing a commercially available genotype panel containing genotypes A through F and H. A total of 18 panel members (3 panel members each of A, B, C, D and E; 2 panel members of F and 1 panel member of H) were tested using the ARCHITECT HBsAg Qualitative II and ARCHITECT HBsAg Qualitative II Confirmatory assays. All genotypes were reactive by the ARCHITECT HBsAg Qualitative II assay and confirmed positive by the ARCHITECT HBsAg Qualitative II Confirmatory assay.

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The following U.S. Patents are relevant to the ARCHITECT System or its components. There are other such patents and patent applications in the United States and worldwide.

5 468 646	5 543 524	5 545 739
5 565 570	5 669 819	5 783 699

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