ARCHITECT

SYSTEM

en

Anti-HBs

REF 7C18-29, -39, -33

G80543R03

B7C1R0

Read Highlighted Changes
Revised May 2019

Anti-HBs

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

REF	List Number	REAGENT LOT	Reagent Lot
IVD	*In Vitro* Diagnostic Medical Device	CONTROL NO.	Control Number
LOT	Lot Number	REACTION VESSELS	Reaction Vessels
	Use by/Expiration date	SAMPLE CUPS	Sample Cups
SN	Serial Number	SEPTUM	Septum
	Temperature limitation	REPLACEMENT CAPS	Replacement Caps
	Caution	CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
	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 Anti-HBs

INTENDED USE

The ARCHITECT Anti-HBs assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of antibody to Hepatitis B surface antigen (anti-HBs) in human serum and plasma.

SUMMARY AND EXPLANATION OF TEST

The ARCHITECT Anti-HBs assay determines the concentration of antibody to Hepatitis B surface antigen (anti-HBs) present in human serum and plasma.

Anti-HBs assays are often used to monitor the success of Hepatitis B vaccination. The presence of anti-HBs has been shown to be important in protection against Hepatitis B virus (HBV) infection.[1] Numerous studies have demonstrated the effectiveness of the Hepatitis B vaccine to stimulate the immune system to produce anti-HBs and to prevent HBV infection.[2-4]

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

Based on the World Health Organisation recommendation, an Anti-HBs concentration ≥ 10 mIU/mL is regarded as being protective against Hepatitis B viral infection[5,6].

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

In the first step, sample and recombinant HBsAg (rHBsAg) coated paramagnetic microparticles are combined. Anti-HBs present in the sample binds to the rHBsAg coated microparticles. After washing, acridinium-labeled rHBsAg conjugate is added in the second step. 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 anti-HBs in the sample and the RLUs detected by the ARCHITECT *i** System optics.

The concentration of anti-HBs in the specimen is determined using a previously generated ARCHITECT Anti-HBs calibration curve.

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

* *i* = immunoassay

REAGENTS

Reagent Kit, 100/500 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 Anti-HBs Reagent Kit (7C18)

- **MICROPARTICLES** 1 or 4 Bottle(s) (4.56 mL per 100 test bottle/16.80 mL per 500 test bottle) Microparticles coated with Hepatitis B Surface Antigen (Subtypes *ad* and *ay*) (*E. coli* Recombinant DNA expressed in murine cells) in TRIS buffer with protein stabilizers. Minimum concentration: 0.08% solids. Preservatives: sodium azide and antimicrobial agents.
- **CONJUGATE** 1 or 4 Bottle(s) (5.9 mL per 100 test bottle/26.3 mL per 500 test bottle) Conjugate: Hepatitis B Surface Antigen (Subtypes *ad* and *ay*) (*E. coli* Recombinant DNA expressed in murine cells) labeled with Acridinium in MES buffer with protein stabilizers (Bovine and Human Plasma). Minimum concentration: 0.13 µg/mL. Preservatives: sodium azide and antimicrobial agents.

Assay Diluent

ARCHITECT Anti-HBs Specimen Diluent (7C18-40)

- **SPECIMEN DILUENT** 1 Bottle (100 mL) ARCHITECT Anti-HBs Specimen Diluent containing recalcified human plasma. Preservative: sodium azide 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**
- *In Vitro* Diagnostic Medical Device

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 human plasma used in the Conjugate is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, anti-HCV, anti-HBs and anti-HBc.

The following warnings and precautions apply to the following components:

- Microparticles
- Conjugate

Contains sodium azide.

EUH032 Contact with acids liberates very toxic gas.

P501 Dispose of contents/container in accordance with local regulations.

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

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a reagent kit or between reagent kits.**
- Prior to loading the ARCHITECT Anti-HBs 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 handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

- The ARCHITECT Anti-HBs Reagent Kit, Calibrators, and Controls 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, reagents are stable until the expiration date.
- The ARCHITECT Anti-HBs 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 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 will require retesting. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT Anti-HBs assay file 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.
- The default result unit for the ARCHITECT Anti-HBs assay is mIU/mL. An alternate result unit, IU/L, may be selected for reporting results by editing assay parameter "Result concentration units" to IU/L. The conversion factor used by the system is 1.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human serum (including serum collected in serum separator tubes) or plasma collected in dipotassium EDTA, sodium citrate, ACD, CPDA-1, lithium heparin, and sodium heparin may be used in the ARCHITECT Anti-HBs assay. Liquid anticoagulants may have a dilutional effect resulting in lower concentrations for individual patient samples. Other anticoagulants have not been validated for use with the ARCHITECT Anti-HBs assay. Follow the tube manufacturer's processing instructions for serum or plasma collection tubes.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify the correct specimen types are used in the ARCHITECT Anti-HBs assay.
- Performance has not been established using cadaver specimens or body fluids other than human serum or plasma.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- This assay was designed and validated for use with human serum or plasma from individual patient and donor specimens. Pooled specimens must not be used since the accuracy of their test results has not been validated.
- Do not use heat-inactivated specimens.
- Do not use grossly hemolyzed specimens.
- For optimal results, inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- **For optimal results, serum and plasma specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give inconsistent results and must be transferred to a centrifuge tube and centrifuged at a minimum of 10,000 RCF (Relative Centrifugal Force) for 10 minutes.**
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- Specimens from heparinized patients may be partially coagulated and erroneous results could occur due to the presence of fibrin. To prevent this phenomenon, draw the specimen prior to heparin therapy.
- **Gravity separation is not sufficient for specimen preparation. Specimens must be separated from clots or red blood cells using centrifugation as recommended by the tube manufacturer.**
- Specimens may be stored on or off the clot or red blood cells for up to 14 days at 2-8°C.
- If testing will be delayed more than 14 days, remove serum or plasma from the clot, serum separator, or red blood cells and store frozen (-20°C or colder).
- **Frozen specimens must be mixed THOROUGHLY after thawing by LOW speed vortexing.**
- 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.
- No qualitative performance differences were observed between experimental controls and the 24 nonreactive or 22 spiked reactive specimens subjected to 4 freeze-thaw cycles. The quantitative performance differences observed were within normal assay variability; however, multiple freeze-thaw cycles should be avoided.
- When shipped, specimens must be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of specimens and infectious substances. Specimens may be shipped ambient, at 2-8°C (wet ice), or -20°C or colder (dry ice). Do not exceed the storage time limitations listed above. Prior to shipment, it is recommended that specimens be removed from the clot, serum separator, or red blood cells.
- No qualitative performance differences were observed between experimental controls and the 23 nonreactive or 23 spiked reactive specimens tested with elevated levels of triglycerides (≤ 3000 mg/dL),* bilirubin (≤ 20 mg/dL),* and hemoglobin (≤ 500 mg/dL).*
- No qualitative performance differences were observed between experimental controls and the 30 nonreactive or 30 spiked reactive specimens tested with red blood cells at ≤ 0.4% v/v.*
- No qualitative performance differences were observed between experimental controls and the 21 nonreactive or 20 spiked reactive specimens tested with elevated levels of protein (≤ 12 g/dL).*
- ARCHITECT Anti-HBs Calibrators and Controls should be mixed by gentle inversion prior to use.

* The quantitative performance differences observed were within normal assay variability.

PROCEDURE

Materials Provided

- 7C18 ARCHITECT Anti-HBs Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- ARCHITECT Anti-HBs Assay file, may be obtained from:
  - ARCHITECT *i* System e-Assay CD-ROM found on www.abbottdiagnostics.com
  - ARCHITECT *i* System Assay CD-ROM
- 7C18-03 ARCHITECT Anti-HBs Calibrators
- 7C18-13 ARCHITECT Anti-HBs Controls
- 7C18-40 ARCHITECT Anti-HBs **SPECIMEN DILUENT**
- 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**
- For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

Assay Procedure

- Before loading the ARCHITECT Anti-HBs Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment:
  - **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.
  - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Carefully snap the septum onto the top of the bottle.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
- Order calibration, if necessary.
- Order tests.
  - For information on ordering patient specimens, calibrators, and controls, and general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- Load the ARCHITECT Anti-HBs Reagent Kit on the ARCHITECT *i* System. Verify that all necessary reagents are present. Ensure that septums are present on all reagent bottles.
- 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 prior to running the test.
  - Priority: 125 µL for the first Anti-HBs test plus 75 µL for each additional Anti-HBs test from the same sample cup.
  - ≤ 3 hours on board: 150 µL for the first Anti-HBs test plus 75 µL for each additional Anti-HBs test from the same sample cup.
  - > 3 hours on board: additional sample volume is required. Refer to the ARCHITECT System Operations Manual, Section 5, for information on sample evaporation and volumes.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
  - ARCHITECT Anti-HBs Calibrators and Controls should be mixed by gentle inversion prior to use. To obtain the recommended volume requirements for the ARCHITECT Anti-HBs Calibrators and Controls, hold the bottles **vertically**, and dispense 7 drops of each Calibrator (for 2 replicates), or 5 drops of each Control (for 1 replicate) into each respective sample cup.
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN. The ARCHITECT *i* System performs the following functions:
  - Moves the sample to the aspiration point.
  - Loads a reaction vessel (RV) into the process path.
  - Aspirates and transfers sample into the RV.
  - Advances the RV one position and transfers microparticles into the RV.
  - Mixes, incubates, and washes the reaction mixture.
  - Adds conjugate to the RV.
  - Mixes, incubates, and washes the reaction mixture.
  - Adds Pre-Trigger and Trigger Solutions.
  - Measures chemiluminescent emission to determine the quantity of anti-HBs in the sample.
  - Aspirates contents of RV to liquid waste and unloads RV to solid waste.
  - Calculates the result.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ARCHITECT System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

- Specimens with an anti-HBs value exceeding 1,000 mIU/mL are flagged with the code ">1000.00 mIU/mL" and may be diluted with the Manual Dilution Procedure.

  Manual Dilution Procedure

  (for concentrations up to 100,000 mIU/mL)
  - The suggested manual dilution for Anti-HBs is 1:100. It is recommended dilutions not exceed 1:100.
  - For a 1:100 dilution, add 10 µL of the patient specimen to 990 µL of ARCHITECT Anti-HBs Specimen Diluent (7C18-40).
  - The operator must enter the dilution factor in the Patient or Control order screen. All assays selected for that order will be diluted. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The concentration reported by the ARCHITECT *i* System **MUST** be greater than 8.00 mIU/mL. If the reported concentration is less than 8.00 mIU/mL, make a smaller dilution.
- **For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.**

Calibration

- Test calibrators A - F in duplicate. The calibrators should be priority loaded. A single sample of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- Calibrator Range: 0 - 1000 mIU/mL.
- Once an ARCHITECT Anti-HBs calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used
  - Daily quality control results are outside of statistically-based quality control limits, as described in the Quality control Procedure section of this package insert, used to monitor and control system performance.
  - If statistically-based quality control limits are not available then the calibration should not exceed a 30-day limit for recalibration frequency.
  - The ARCHITECT Anti-HBs assay may also need to be recalibrated after specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the assay.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

NOTE: It is recommended that the ARCHITECT Anti-HBs Positive Control 1, Anti-HBs Positive Control 2, and Negative Control be run in order to verify the calibration.

The recommended control requirement for the ARCHITECT Anti-HBs assay is a single sample of each control to be tested:

- Once every 24 hours each day of use.
- After performing calibration.
- After instrument service procedures or maintenance that may affect assay performance have been performed.
- If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures. Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.
- Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated sample results are invalid and the samples must be retested. Recalibration may be indicated.

- To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying a minimum of 20 replicates over several (3-5) days and using the reported results to establish the expected average (target) and variability about this average (ranges) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:
  - Multiple stored calibrations
  - Multiple reagent lots
  - Multiple calibrator lots
  - Multiple processing modules
  - Data points collected at different times of the day
- These results should be applied to your laboratory's quality control practices. In addition, the laboratory must ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.
- Unless specified, target values and ranges provided with the commercial control product insert are guidelines only and should not be used for quality control purposes.
- Refer to Clinical and Laboratory Standards Institute (CLSI) Document C24-A3[11].

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT Anti-HBs assay belongs to method group 4.

RESULTS

The ARCHITECT Anti-HBs assay utilizes a 4 Parameter Logistic Curve Fit data reduction method (4PLC, X-weighted) to generate a calibration curve.

Interpretation of Results

- Based on the World Health Organisation recommendation, an Anti-HBs concentration ≥ 10 mIU/mL is regarded as being protective against Hepatitis B viral infection[5,6].
- For additional information about the detection capability of the ARCHITECT Anti-HBs assay, refer to the **SPECIFIC PERFORMANCE CHARACTERISTICS** section, under **Limit of Blank, Limit of Detection, and Limit of Quantitation.**
- Follow your country-specific regulations and laboratory procedures for reporting of results.

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.

Measuring Interval

Measuring interval is defined as the range of values in mIU/mL which meets the limits of acceptable performance for both imprecision and bias for an undiluted sample. For the verification studies described in this package insert, the range was 2.50 mIU/mL* (Limit of Quantitation - LoQ) to 1000.00 mIU/mL.

* Representative data; results in individual laboratories may vary from these data

LIMITATIONS OF THE PROCEDURE

- If the Anti-HBs 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, chronic, or recovered infection.
- Samples containing particulate matter or red blood cells must be centrifuged prior to running the assay.
- Performance has not been established using cadaver specimens or body fluids other than human serum or plasma.
- Do not use heat-inactivated specimens.
- Specimens from heparinized patients may be partially coagulated and erroneous results could occur due to the presence of fibrin. To prevent this phenomenon, draw the specimen prior to heparin therapy.
- Quantitative values obtained using alternative assays (i.e. MEIA, EIA or RIA) may not be equivalent and cannot be used interchangeably. A new baseline, using the ARCHITECT Anti-HBs assay, should be established when monitoring vaccinees.

SPECIFIC PERFORMANCE CHARACTERISTICS

In order to calculate specificity and sensitivity specimens with result values of ≥ 10.00 mIU/mL were considered reactive and specimens with result values of < 10.00 mIU/mL were considered nonreactive.

Precision

The precision of ARCHITECT Anti-HBs was determined during clinical studies using three reagent lots. A panel composed of five unique members was tested in replicates of four with each reagent lot once daily for five days at three sites. Each daily run included the ARCHITECT Positive Controls each tested in duplicate at the beginning and end of the run. The intra-assay and inter-assay standard deviation (SD) and percent coefficient of variation (%CV) were determined with a variance component analysis[12] for a random effects model[13] (Table I*).

TABLE I
ARCHITECT Anti-HBs Precision

Panel Members	Total No. Replicates	Grand Mean mIU/mL	Intra-assay		Inter-assay[a]		Total[b]	
			SD	%CV	SD	%CV	SD	%CV
1	180	4.67	0.302	6.5	0.403	8.6	0.613	13.1
2	180	14.60	0.434	3.0	0.708	4.9	1.367	9.4
3	180	79.75	3.082	3.9	4.130	5.2	7.085	8.9
4	180	255.04	4.752	1.9	7.565	3.0	19.464	7.6
5	180	489.20	14.474	3.0	19.225	3.9	38.688	7.9
Positive Control 1	180	16.18	0.687	4.2	0.765	4.7	1.388	8.6
Positive Control 2	180	82.06	1.934	2.4	2.460	3.0	6.045	7.4

* Representative data; results in individual laboratories may vary from these data.

[a] Inter-assay variability contains intra-assay variability.

[b] Total assay variability contains intra-assay, inter-assay, inter-lot, inter-site variability.

Sensitivity

A total of 389 specimens from 248 HBV vaccine recipients, 41 individuals recovered from HBV infection, and 100 individuals at risk for HBV infection were tested. Of the 389 specimens, 340 (87.40%) were repeatedly reactive and positive by supplemental testing (Table II*).

TABLE II
Reactivity of the ARCHITECT Anti-HBs Assay in Specimens from HBV Vaccine Recipients, Individuals who have Recovered from HBV Infection, and Individuals at Increased Risk for HBV Infection

Category	Number of Specimens Tested	Number of Repeatedly Reactive (% of total)	Number of Positive by Supplemental Testing (% of Repeatedly Reactive)
HBV Vaccine Recipients	248	245 (98.79%)	245 (100.00%)
Recovered HBV Infection	41	39[a] (95.12%)	39 (100.00%)
Increased Risk for HBV Infection[b]	100	56 (56.00%)	56 (100.00%)
TOTAL	389	340 (87.40%)	340 (100.00%)

* Representative data; results in individual laboratories may vary from these data.

[a] Two specimens were reactive for anti-HBc and anti-HBe but also nonreactive for anti-HBs by RIA.

[b] Category included the following: intravenous drug users (34), hemodialysis patients (33), and hemophilia patients (33).

HBV Vaccine Recipient Serial Bleed Panels

A total of 90 specimens comprising 15 serial bleed panels from HBV vaccine recipients were tested. The vaccine was administered in three injections over a six-month period. All specimens drawn one month following the third and final injection were reactive by the ARCHITECT Anti-HBs assay.

Specificity

Three clinical sites tested a total of 1,716 serum and plasma specimens from the following categories: volunteer whole blood donors, matched serum and plasma pairs, random hospital patients, medical conditions unrelated to HBV infection and potentially interfering substances. A total of 259 (15.09%) of the 1,716 specimens were repeatedly reactive, and 254 (98.07%) of the 259 specimens were positive by supplemental testing (Table III*).

TABLE III
Reactivity of the ARCHITECT Anti-HBs Assay in Specimens from Whole Blood Donors, Plasma Specimens from Matched Serum/Plasma Pairs, Hospital Patients, Individuals with Medical Conditions Unrelated to HBV Infection and in Specimens Containing Potentially Interfering Substances

Category	Number of Specimens Tested	Number of Repeatedly Reactive (% of total)	Number of Positive by Supplemental Testing[a] (% of Repeatedly Reactive)
Volunteer Whole Blood Donors	1006	154 (15.31%)	151 (98.05%)
Plasma Specimens from Matched Serum/Plasma Pairs	50	8 (16.00%)	8 (100.00%)
Hospital Patients	500	65 (13.00%)	63 (96.92%)
Medical Conditions Unrelated to HBV Infection and Potentially Interfering Substances[b]	160	32 (20.00%)	32 (100.00%)
TOTAL	1716	259 (15.09%)	254 (98.07%)

* Representative data; results in individual laboratories may vary from these data.

[a] Supplemental testing for anti-HBc, HBsAg and anti-HBe was performed to support the presence of anti-HBs in an ARCHITECT Anti-HBs reactive specimen. Detection of anti-HBs by RIA was also performed. A specimen was defined as anti-HBs positive if one or more of the following HBV markers were detected: anti-HBs (detected by the comparator method or RIA), anti-HBc, HBsAg, or anti-HBe.

[b] Category included the following: anti-CMV positive (10), anti-EBV positive (10), anti-HSV (10), anti-HAV (10), anti-HCV (10), anti-HIV-1 (10), rubella antibody positive (10), toxoplasma antibody positive (10), *E. coli* infections (10), yeast infections (10), syphilis positive (10), anti-nuclear antibody positive (10), rheumatoid factor (10), multiple myeloma (10), HBsAg positive (10) and alcoholic liver disease (10).

Overall Specificity and Sensitivity

Overall specificity and sensitivity were estimated from the results of 2,105 specimens tested with ARCHITECT Anti-HBs at five clinical sites. In order to represent unique specimens, results from the HBV vaccine recipient serial bleed panels and the serum specimens from the matched serum/plasma pairs were excluded from these calculations. The overall specificity was estimated to be 99.67% (1,491/1,496) with a 95% confidence interval of 99.22% to 99.89%. The overall sensitivity was estimated to be 97.54% (594/609) with a 95% confidence interval of 95.97% to 98.62%.

Limit of Blank, Limit of Detection, and Limit of Quantitation

The Limit of Blank (LoB) and Limit of Detection (LoD) of the ARCHITECT Anti-HBs assay were determined, based on guidance from CLSI Protocol EP17-A2[14], using proportions of false positives (α) less than 5% and false negatives (β) less than 5%. These determinations were performed using 4 blanks (15 replicates each) and 8 low level anti-HBs samples (30 replicates each); LoB = 0.50 mIU/mL* and LoD = 0.98 mIU/mL*.

The Limit of Quantitation (LoQ) of the ARCHITECT Anti-HBs assay was determined based on guidance from CLSI Protocol EP17-A2[14]. The LoQ is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a Percent Total Allowable Error of 30%; LoQ = 2.50 mIU/mL.

* Representative data; results in individual laboratories may vary from these data

BIBLIOGRAPHY

1. Wainwright RB, McMahon BJ, Bulkow LR, *et al.* Duration of Immunogenicity and Efficacy of Hepatitis B Vaccine in a Yupik Eskimo Population-Preliminary Results of an 8-year Study. In: Hollinger FB, Lemon SM, Margolis HS, editors. *Viral Hepatitis and Liver Disease.* Baltimore: Williams & Wilkins, 1991:762-6.
2. Ambrosch F, Frisch-Niggemeyer W, Kremsner P, *et al.* Persistence of Vaccine-induced Antibodies to Hepatitis B Surface Antigen and the Need for Booster Vaccination in Adult Subjects. *Postgrad Med J* 1987;63(S2):129-35.
3. Krugman S, Giles JP, Hammond J. Viral Hepatitis Type B (MS-2 Strain): Studies on Active Immunization. *JAMA* 1971;217:41-5.
4. Jilg W, Schmidt M, Deinhardt F. Immune Response to Hepatitis B Revaccination. *J Med Virol* 1988;24:377-84.
5. World Health Organization. Hepatitis B vaccines. Weekly epidemiological record No. 40, 2009, 84, 405–420.
6. Jack AD, Hall AJ, Maine N, Mendy M and Whittle HC. What Level of Hepatitis B Antibody Is Protective? Journal of Infectious Diseases 1999;179:489–92.
7. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
8. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
9. World Health Organization. *Laboratory Biosafety Manual.* 3rd ed. Geneva: World Health Organization; 2004.
10. 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.
11. Clinical and Laboratory Standards Institute. Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline – Third Edition. CLSI Document C24-A3. Wayne, PA: CLSI, 2006.
12. Box GEP, Hunter WG, Hunter JS. *Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building.* New York: John Wiley & Sons, Inc, 1978:510-39, 571-83.
13. SAS Institute Inc. The MIXED Procedure. In: SAS Technical Report P-229, *SAS/STAT Software: Changes and Enhancements, Release 6.07.* Cary, NC: SAS Institute Inc, 1992:289-366.
14. Clinical and Laboratory Standards Institute. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI, 2012.

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May 2019