

ARCHITECT®

SYSTEM

In Vitro Test
REF 1.81-50
XX-XXX/R1

HBsAg Confirmatory

Manual Diluent

REF	Lot Number	LOT	Lot Number
LVD	In Vitro Diagnostic Medical Device		Expiration Date
	Store at 2-8°C	MANUAL DILUENT	Manual Diluent

 **CAUTION:** Consult accompanying documents



Abbott Laboratories
Diagnostics Division
Abbott Park, IL 60064 USA

INTENDED USE

The ARCHITECT HBSAg Confirmatory Manual Diluent is used for manually diluting specimens for testing on the ARCHITECT / System using the ARCHITECT HBSAg Confirmatory Reagent Kit. The performance of the ARCHITECT HBSAg Confirmatory Manual Diluent has not been established with any other HBSAg assays.

WARNINGS AND PRECAUTIONS
- For In Vitro Diagnostic Use.

CAUTION: This product contains human sourced infectious and/or potentially infectious components. 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^o or other appropriate biosafety practices* should be used for materials that contain or are suspected of containing infectious agents.

- The manual diluent is nonreactive for anti-HBs, HBSAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.
- The ARCHITECT HBSAg Confirmatory Manual Diluent contains methylisothiazolones (which are components of ProClin[®]) and is classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases:
 - R43 May cause sensitization by skin contact.
 - S24 Avoid contact with skin.
 - S35 This material and its container must be disposed of in a safe way.
 - S37 Wear suitable gloves.
 - S46 If swallowed, seek medical advice immediately and show this container or label.



MATERIALS PROVIDED
1. Bottle (100 mL) of ARCHITECT HBSAg Confirmatory Manual Diluent. The Manual Diluent contains recalled human plasma. Preservatives: antimicrobial agent, ProClin 300, and ProClin 950.

PREPARATION AND STORAGE

- The manual diluent is liquid ready-to-use. No preparation is required.
- The manual diluent may be used immediately after removal from 2-8°C storage.
- After each use, tightly close the cap and return the manual diluent to 2-8°C storage.
- When stored and handled as directed, the manual diluent is stable until the expiration date.



SPECIMEN DILUTION PROCEDURE

- To prepare a 1:500 dilution:
 - Add 25 µL of the patient specimen to 475 µL of ARCHITECT HBSAg Confirmatory Manual Diluent for a 1:20 dilution.
 - Add 20 µL of the 1:20 dilution to 480 µL of ARCHITECT HBSAg Confirmatory Manual Diluent for a 1:500 dilution.
- To prepare a 1:2000 dilution:
 - Add 25 µL of the 1:500 dilution to 975 µL of ARCHITECT HBSAg Confirmatory Manual Diluent.

Refer to the **Specimen Dilution Procedure and Interpretation of Results** sections of the ARCHITECT HBSAg Confirmatory reagent package insert for further information.

BIBLIOGRAPHY

1. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.
2. US Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed, Washington, DC: US Government Printing Office, May 1999.
3. World Health Organization, *Laboratory Biosafety Manual*, 3rd ed, Geneva: World Health Organization, 2004.
4. Clinical and Laboratory Standards Institute, *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition*, CLSI Document M29-A3, Wayne, PA: Clinical and Laboratory Standards Institute, 2005.

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Abbott Park, IL 60064 USA
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by
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ARCHITECT®

SYSTEM

In Vitro Test
REF 1L80-01
XX-XXXX/R1

HBSAg

Calibrators

ARCHITECT is a registered trademark of Abbott Laboratories.
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 Diagnostics Division
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 Manufactured for
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 by
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 August 2006
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Key to symbols used

REF	Lot Number	LOT	Lot Number
IVD	In Vitro Diagnostic Medical Device	EXP	Expiration Date
	Store at 2-8°C	CALL1	Calibrator 1
	CAUTION: Consult accompanying documents	CALL2	Calibrator 2

 Abbott Laboratories
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INTENDED USE

The ARCHITECT HBSAg Calibrators are used for calibration of the ARCHITECT / System when the system is used for the qualitative detection of hepatitis B surface antigen (HBSAg) using the ARCHITECT HBSAg and HBSAg Confirmatory Reagent Kits. The performance of the ARCHITECT HBSAg Calibrators has not been established with any other HBSAg assays.

PRINCIPLES OF PROCEDURE

The ARCHITECT HBSAg and HBSAg Confirmatory assays use Calibrator 1 and Calibrator 2 to assess calibration validity and Calibrator 2 to calculate the assay cutoff. The ARCHITECT / System calculates the cutoff Relative Light Unit (RLU) from the mean chemiluminescent signal of three replicates of Calibrator 2. The cutoff RLU is calculated using the following equation, as appropriate:

For the ARCHITECT HBSAg assay:
Cutoff RLU = 0.013 x (Calibrator 2 Mean RLU)

For the ARCHITECT HBSAg Confirmatory assay:
Cutoff RLU = 0.0175 x (Calibrator 2 Mean RLU)

The ARCHITECT HBSAg Confirmatory Calibrator 1 result is used to calculate the % neutralization results for the positive control and patient specimens.

The acceptable calibration is stored by the ARCHITECT / System for use with any reagent kit of that lot. The calibration should be used in conjunction with control ranges to determine the validity of the calibration.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.

- CAUTION: This product contains human sourced infectious and/or potentially infectious components. 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^o or other appropriate biohazard practices², should be used for materials that contain or are suspected of containing infectious agents.
- The human plasma in Calibrator 1 and Calibrator 2 is nonreactive for anti-HBs, HBSAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.
- Calibrator 2 is reactive for HBSAg.

The ARCHITECT HBSAg Calibrators contain methylisothiazolones (which are components of ProClin[®]) and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases:

P43 May cause sensitization by skin contact.

S24 Avoid contact with skin.

S35 This material and its container must be disposed of in a safe way.

S37 Wear suitable gloves.

S46 If swallowed, seek medical advice immediately and show this container or label.



MATERIALS PROVIDED

2 Bottles (4 mL each) of ARCHITECT HBSAg Calibrators (1 bottle each of Calibrators 1 and 2).

- Calibrator 1 is phosphate buffer with protein (bovine albumin and human plasma) additives; Preservatives; antimicrobial agent, ProClin 300, and ProClin 950.
- Calibrator 2 contains inactivated purified HBSAg (subtype ad) in phosphate buffer with protein (bovine albumin and human plasma) additives; Preservatives; antimicrobial agent, ProClin 300, and ProClin 950.

STANDARDIZATION

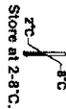
Calibrator 2 is manufactured by dilution and traceable to the World Health Organization (WHO) 1st International Standard (subtype ad; NIBSC Code 80/549)⁵.

The calibrators are at the following concentrations:

Calibrator	HBSAg Concentration (IU/mL)
CAL1	0
CAL2	5

PREPARATION AND STORAGE

- The calibrators are liquid ready-to-use. No preparation is required.
- When stored and handled as directed, the calibrators are stable until the expiration date.
- The calibrators must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- Refer to the ARCHITECT HBSAg or HBSAg Confirmatory reagent package insert for the maximum onboard stability requirements.



QUALITY CONTROL PROCEDURES

Refer to the ARCHITECT HBSAg and HBSAg Confirmatory reagent package inserts and ARCHITECT System Operations Manual for additional information.

- For the ARCHITECT HBSAg assay, a single sample of each control level must be tested to evaluate the assay calibration.
- For the ARCHITECT HBSAg Confirmatory assay, a single sample of the positive control level must be tested to evaluate the assay calibration.
- For information about ordering controls, refer to the appropriate reagent package insert.
- Ensure that assay control values are within the ranges specified in the control package insert.
- Once an ARCHITECT HBSAg 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.

PROCEDURE

Mix the calibrators by gentle inversion before use. To perform a calibration, test ARCHITECT HBSAg Calibrators 1 and 2 in triplicate. The calibrators should be priority loaded.

- To obtain the recommended volume requirements for the ARCHITECT HBSAg Calibrators, hold the bottles vertically and dispense
 - 14 drops into the respective sample cup for the ARCHITECT HBSAg assay or
 - 15 drops into the respective sample cup for the ARCHITECT HBSAg Confirmatory assay.

BIBLIOGRAPHY

- US Department of Labor: Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.
- US Department of Health and Human Services: *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. Washington, DC: US Government Printing Office; May 1999.
- World Health Organization: *Laboratory Biosafety Manual*, 3rd ed Geneva: World Health Organization; 2004.
- Clinical and Laboratory Standards Institute: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
- WHO Expert Committee on Biological Standardization. Geneva: World Health Organization; 1987:18. Technical Report Series 745.

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HBsAg Confirmatory

REF1L81

XX-XXXX/R1

HBsAg Confirmatory

CUSTOMER SERVICE

UNITED STATES: 1-877-4ABBOTT

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.

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

Key to symbols used		
	List Number	Reaction Vessels
	In Vitro Diagnostic Medical Device	Sample Cups
	Store at 2-8°C	Septums
	Lot Number	Replacement Caps
	Expiration Date	Serial Number
	CAUTION: Consult accompanying documents.	Control Number
		Reagent Lot

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

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NAME

ARCHITECT HBsAg Confirmatory

INTENDED USE

The ARCHITECT HBsAg Confirmatory assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative confirmation of the presence of hepatitis B surface antigen (HBsAg) in human adult and pediatric serum and plasma (dipotassium EDTA) that have been found to be repeatedly reactive by ARCHITECT HBsAg. Assay results, in conjunction with other laboratory results and clinical information, may be used to provide 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.

Warning: Not intended for use in screening blood, plasma, or tissue donors. The effectiveness of ARCHITECT HBsAg Confirmatory for use in screening blood, plasma, or tissue donors has not been established.

Assay performance characteristics have not been established when the ARCHITECT HBsAg Confirmatory assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

SUMMARY AND EXPLANATION OF THE TEST

ARCHITECT HBsAg Confirmatory uses the principle of specific antibody neutralization to confirm the presence of HBsAg in specimens found to be repeatedly reactive by the ARCHITECT HBsAg assay. Antibody to hepatitis B surface antigen (anti-HBs) (human) is incubated with a specimen. If HBsAg is present in the specimen, 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 specimen that has not been treated with the neutralizing antibody reagent. A specimen is considered confirmed positive if the signal for the non-neutralized specimen (incubated with Pretreatment 2 [Reagent 2]) result is greater than or equal to the cutoff and the relative light units (RLUs) of the neutralized specimen is reduced by at least 50% compared to the non-neutralized specimen. A sample is considered repeat reactive and nonconfirming for HBsAg if it is reactive and not neutralized in the ARCHITECT HBsAg Confirmatory assay.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT HBsAg Confirmatory assay is a two-step pretreatment immunoassay for the confirmation of the presence of HBsAg in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

Sample and Pretreatment 1 (Reagent 1) are combined in a reaction vessel (RV) and incubated. When HBsAg is present in the sample, it is neutralized by the HBs antibody in Pretreatment 1 (Reagent 1). An aliquot of the pretreated sample and anti-HBs coated paramagnetic microparticles are combined in another RV and incubated. Any non-neutralized HBsAg present in the sample binds to the anti-HBs coated microparticles. The neutralized HBsAg is blocked from binding to the anti-HBs coated microparticles. After washing, acridinium-labeled anti-HBs conjugate is added to the reaction mixture and incubated. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as RLU. A direct relationship exists between the amount of HBsAg in the sample and the RLU detected by the ARCHITECT i System optics.

This sequence is repeated for the sample and Pretreatment 2 (Reagent 2), except Pretreatment 2 (Reagent 2) does not neutralize HBsAg in the sample.

If the signal for the non-neutralized sample (incubated with Pretreatment 2 [Reagent 2]) result is greater than or equal to the cutoff ($S/CO \geq 0.80$) and the RLU of the neutralized sample (incubated with Pretreatment 1 [Reagent 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

Reagent Kit, 50 Tests

ARCHITECT HBsAg Confirmatory Reagent Kit (1L81-25)

- **MICROPARTICLES** 1 Bottle (6.6 mL) anti-HBs (mouse, monoclonal, IgM, IgG) coated microparticles in MES buffer with protein additives (152 µM bovine serum albumin, 10.0% bovine calf serum) and surfactant. Minimum concentration: 0.0675% solids. Preservatives: ProClin® 300 and ProClin 950.
- **CONJUGATE** 1 Bottle (5.9 mL) anti-HBs (goat, IgG) acridinium-labeled conjugate in MES buffer with protein additives (30.3 µM bovine serum albumin, 12.5% bovine calf serum, 2.0% human plasma) and surfactant. Minimum concentration: 0.25 µg/mL. Preservative: ProClin 300.
- **PRE-TREATMENT 1** 1 Bottle (2.4 mL) Pretreatment 1 contains recalcified human plasma and surfactant. Preservatives: sodium azide and ProClin 950.
- **PRE-TREATMENT 2** 1 Bottle (2.4 mL) Pretreatment 2 contains recalcified human plasma and surfactant. Preservatives: antimicrobial agent, 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 0.35N sodium hydroxide.

ARCHITECT i Wash Buffer

- **WASH BUFFER** Wash buffer containing phosphate buffered saline solution. Preservative: antimicrobial agent.

WARNINGS AND PRECAUTIONS

- **For In Vitro Diagnostic Use.**

Safety Precautions

-  **CAUTION: This product contains human sourced infectious and/or potentially infectious components. 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,¹ Biosafety Level 2² or other appropriate biosafety practices^{3,4} should be used for materials that contain or are suspected of containing infectious agents.**
- The human plasma used in the conjugate and Pretreatment 2 is nonreactive for anti-HBs, HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.
- The human plasma used in Pretreatment 1 is reactive for anti-HBs and nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.
- The ARCHITECT HBsAg Confirmatory reagents contain methylisothiazolones (which are components of ProClin) and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.



- R43 May cause sensitization by skin contact.
- S24 Avoid contact with skin.
- S35 This material and its container must be disposed of in a safe way.
- S37 Wear suitable gloves.
- S46 If swallowed, seek medical advice immediately and show this container or label.

- Pretreatment 1 contains sodium azide. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.
- 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.

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Handling Precautions

- Do not use reagents beyond the expiration date.
- **Do not pool reagents from different reagent kits.**
- Before loading the ARCHITECT HBsAg Confirmatory 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.**
- Before placing the septum on an uncapped reagent bottle, squeeze the septum in half to confirm that the slits are open. If the slits appear sealed, continue to gently squeeze the septum to open the slits.
- 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

- 
- **2°C - 8°C** The ARCHITECT HBsAg Confirmatory 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 Confirmatory 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.** After reagents are removed from the system, initiate a scan to update the onboard stability timer.

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 Confirmatory assay file must be installed on the ARCHITECT *i* System from the ARCHITECT *i* System Assay CD-ROM 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 following specimen tube types may be used with the ARCHITECT HBsAg Confirmatory assay:

Glass	Plastic
<ul style="list-style-type: none"> • Serum • Serum separator 	<ul style="list-style-type: none"> • Serum • Serum separator • Dipotassium EDTA

- 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 Confirmatory assay.

Specimen Conditions

- Do not use specimens with the following conditions:
 - heat-inactivated
 - pooled
 - grossly hemolyzed
 - obvious microbial contamination
- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum and plasma.
- **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.
- 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.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- 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

- The Clinical and Laboratory Standards Institute (CLSI) provides the following recommendations for storing blood specimens.³
 - Store samples at 22°C (72°F) for no longer than 8 hours.
 - If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C (36-46°F).
 - If the assay will not be completed within 48 hours, freeze the sample at -20°C (-4°F).
 - Specimens that are not tested within the specified time period listed above must be removed from the clot or red blood cells and stored frozen (-20°C or colder).
- Avoid more than three 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.

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PROCEDURE

Materials Provided:

- 1L81 ARCHITECT HBsAg Confirmatory Reagent Kit

Materials Required but not Provided:

- ARCHITECT *i* System
- ARCHITECT *i* System Assay CD-ROM
- 1L81-50 ARCHITECT HBsAg Confirmatory Manual Diluent
- 1L80-01 ARCHITECT HBsAg Calibrators
- 1L80-10 ARCHITECT HBsAg 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*: **SEPTUMS**
- 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 Confirmatory 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 still adhere 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 **Handling Precautions** section of this package insert.
- Load the ARCHITECT HBsAg Confirmatory Reagent Kit on the ARCHITECT *i* System.
 - Verify that all necessary reagents are present.
 - Ensure that septums are present on all reagent bottles.
 - For the reagent bottle loading instructions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- Order calibration, if necessary.
 - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Mix ARCHITECT HBsAg Calibrators by gentle inversion before use.
- To perform an ARCHITECT HBsAg Confirmatory calibration, test ARCHITECT HBsAg Calibrators 1 and 2 in triplicate. To obtain the recommended volume requirement for the ARCHITECT HBsAg Calibrators, hold the bottles **vertically** and dispense 15 drops of each calibrator into each respective sample cup. Calibrators should be priority loaded.
- A single sample of positive control must be tested to evaluate the assay calibration.
 - Order the control as described in the **Control and Specimens** section of this package insert.
 - Ensure that assay control S/CO and % neutralization values are within the ranges specified in the control package insert.
- Once an ARCHITECT HBsAg Confirmatory 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.
 - Positive control is out of range.

Control and Specimens

- Order tests.
 - **Use the following instructions to order a control:**
 - **Order the control as a patient specimen, not as a Control.**
 - **Manually verify the validity of the control every time it is run. Because the control is run as a patient specimen, a result will not be flagged by the ARCHITECT *i* System if it is outside the acceptable control range.**

- To troubleshoot a control value that falls outside the control range, refer to the ARCHITECT System Operations Manual, Section 10.
- For information on ordering patient specimens and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- **NOTE:** For each confirmatory result, two tests are performed, one with Pretreatment 1 (Reagent 1) and one with Pretreatment 2 (Reagent 2). Therefore, when ordering a patient specimen (after selecting the HBsAg Confirmatory assay),
 - **Select F5 - Assay Options.**
 - Select Module-Auto (default mode) or, if working with a multi-module *i* system, select Manual and then select the Module of choice.
 - Enter the number of replicates for Pretreatment 1 (Reagent 1) and Pretreatment 2 (Reagent 2).
- **Note: in the default method, the number of replicates is not specified for Reagent 2. In order to obtain a valid test result, this number needs to be entered manually.**
- 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: 242 μ L for the first HBsAg confirmation test plus 192 μ L for each additional HBsAg confirmation test from the same sample cup.
 - \leq 3 hours onboard: 242 μ L for the first HBsAg confirmation test plus 192 μ L for each additional HBsAg confirmation test from the same sample cup.
 - $>$ 3 hours onboard: 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 control.
 - The ARCHITECT HBsAg Positive Control must be mixed by gentle inversion before use.
 - To obtain the recommended volume requirement for the ARCHITECT HBsAg Positive Control, hold the bottle **vertically** and dispense 6 drops into the sample cup.
- 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.
- Calculate % neutralization using results from both Pretreatment 1 and 2 (Reagent 1 and 2) tests.
 - Obtain the Calibrator 1 (Cal 1) mean RLU from the ARCHITECT *i* System as follows:
 - Select the **Qc-Cal** icon, then select **Calibration status** from the drop down menu.
 - Select the correct reagent lot number to be used for the corresponding sample neutralization calculation.
 - Select **Details F5** to view the calibrator RLU values or select **Print F4**.
 - If **Details F5** was selected, record the value for the Calibrator 1 mean RLU.
 - If **Print F4** was selected, select **Calibration Details Report** to print the calibrator mean RLU values.
 - Obtain sample Pretreatment 1 (Reagent 1) RLU, Pretreatment 2 (Reagent 2) RLU, and Pretreatment 2 (Reagent 2) S/CO from the ARCHITECT *i* System as follows:
 - Select **Results** icon, then select **Results review** from the drop down menu. If the system is configured for the auto release of results, the results can be viewed in the **Stored Results** screen.
 - Select the required specimen(s).
 - Select **Details F5** to view results or select **Print F4**.
 - If **Details F5** was selected, record the values for the sample's Reagent 1 RLU, Reagent 2 RLU, and Reagent 2 S/CO.
 - If **Print F4** was selected, select **Result Details Report** to print the sample RLU and S/CO values.
 - See the **RESULTS** section for the % neutralization calculation, and refer to the **% Neutralization Calculation Worksheet** at the end of this package insert.

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- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. When a laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedure

- Perform a 1:500 manual dilution if the ARCHITECT HBsAg Confirmatory assay result is reactive but is not neutralized (specimen treated with Pretreatment 2 [Reagent 2] S/CO \geq 0.80 and % neutralization < 50%).
 - Add 25 μ L of the patient specimen to 475 μ L of ARCHITECT HBsAg Confirmatory Manual Diluent for a 1:20 dilution.
 - Add 20 μ L of the 1:20 dilution to 480 μ L of ARCHITECT HBsAg Confirmatory Manual Diluent for a 1:500 dilution.
- Perform an additional specimen dilution if the 1:500 dilution result is reactive but is not neutralized.
 - For a 1:20000 dilution, add 25 μ L of the 1:500 dilution to 975 μ L of ARCHITECT HBsAg Confirmatory Manual Diluent.
- Refer to **Interpretation of Results** section of this package insert for further information.
- 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.**

QUALITY CONTROL PROCEDURES

The ARCHITECT HBsAg positive quality control material is in a matrix that consists of 5% BSA and 7.5% recalcified human plasma in phosphate buffer. A study was performed that demonstrated the ARCHITECT HBsAg positive quality control material detects reagent failure (induced by heat-stress of reagents) comparably to a positive control in a serum or plasma matrix. The positive quality control material may not adequately monitor the assay under all potential conditions of reagent failure when testing serum and plasma specimens. The user should provide alternate positive quality control material for testing of serum and plasma matrices.

The recommended control requirement for the ARCHITECT HBsAg Confirmatory assay is that a single sample of positive control 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.

The positive control values must be within the concentration and % neutralization 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 result 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.

RESULTS

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

Note: If the sample's Pretreatment 2 (Reagent 2) S/CO is < 0.80, % neutralization is not applicable and need not be calculated. Obtain the final interpretation of results directly from the table in the **Interpretation of Results** section of this package insert.

Calculations

Calculations Performed by the ARCHITECT i System

- The ARCHITECT i System calculates the cutoff RLU from the mean RLU of three replicates of Calibrator 2. The cutoff RLU is stored for each reagent lot calibration.

$$\text{Cutoff RLU} = 0.0175 \times A$$

where A = Calibrator 2 Mean RLU

- The ARCHITECT i System calculates the S/CO result for each sample as follows:

$$\text{S/CO} = \text{Sample RLU} / \text{Cutoff RLU}$$

Calculation Performed by the Operator

- The % neutralization is calculated by the operator as follows:
 - Match up the Pretreatment 1 and 2 (Reagent 1 and 2) values for each sample.

$$\% \text{ Neutralization} = \frac{D - C}{D - B} \times 100\%$$

where B = Calibrator 1 Mean RLU

C = Sample's Pretreatment 1 [Reagent 1] RLU

D = Sample's Pretreatment 2 [Reagent 2] RLU

- Note: Round to the nearest whole number.

Example: Sample's Pretreatment 2 [Reagent 2] RLU = 2118

Sample's Pretreatment 1 [Reagent 1] RLU = 431

Calibrator 1 Mean RLU = 454

$$\% \text{ Neutralization} = \frac{(2118 - 431)}{(2118 - 454)} \times 100\%$$

$$\% \text{ Neutralization} = 101\%$$

NOTE: To assist in calculations, a % Neutralization Calculation Worksheet is provided at the end of this package insert.

Interpretation of Results

Confirmed Positive - If the Pretreatment 2 (Reagent 2) S/CO result is \geq 0.80 and the % neutralization is \geq 50%, the specimen is considered confirmed positive for HBsAg.

Repeatedly Reactive, Not Confirmed - If the Pretreatment 2 (Reagent 2) S/CO result is < 0.80 or if the Pretreatment 2 (Reagent 2) S/CO result is \geq 0.80 and the % neutralization is < 50%, the specimen is considered repeatedly reactive, not confirmed for HBsAg.

The table below summarizes the various final interpretations from the neat and dilution results:

Dilution	Pretreatment 2 (Reagent 2) S/CO	% Neutralization	Final Interpretation
Neat (Undiluted)	< 0.80 \geq 0.80 \geq 0.80	Not applicable \geq 50% < 50%	Repeatedly reactive, not confirmed Confirmed positive Repeat test using a 1:500 dilution
1:500	< 0.80 \geq 0.80 \geq 0.80	Not applicable \geq 50% < 50%	Repeatedly reactive, not confirmed Confirmed positive Repeat test using a 1:20000 dilution
1:20000	< 0.80 \geq 0.80 \geq 0.80	Not applicable \geq 50% < 50%	Repeatedly reactive, not confirmed Confirmed positive Repeatedly reactive, not confirmed

NOTE: Follow the dilution and final interpretation routine as outlined in the table above, even if % neutralization results of < 0% or > 100% are obtained. For specimen dilution instructions, refer to the **Specimen Dilution Procedures** section of this package insert.

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

- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).^{6,7} Such specimens may show either falsely elevated or depressed values when tested with assay kits (such as ARCHITECT HBsAg Confirmatory) that employ mouse monoclonal antibodies.⁸
- 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 Confirmatory assay may not be used interchangeably with the values obtained with different manufacturers' assay methods.
- Heparinized plasma cannot be used with the ARCHITECT HBsAg Confirmatory assay.
- 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.

EXPECTED RESULTS

Not applicable.

SPECIFIC PERFORMANCE CHARACTERISTICS

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

ARCHITECT HBsAg Confirmatory Performance

In a multi-center study, 167 repeatedly reactive specimens that were tested using the ARCHITECT HBsAg assay were also tested with the ARCHITECT HBsAg Confirmatory assay. Of the 167 specimens, 157 were confirmed positive using the ARCHITECT HBsAg Confirmatory assay, and 149 were reported positive by the comparator assay. The data are summarized in the following table.

Specimen Category	Comparator HBsAg/HBsAg Confirmatory Final Interpretation								Total	
	Positive				Negative					
	ARCHITECT HBsAg/ARCHITECT HBsAg Confirmatory Final Interpretation				ARCHITECT HBsAg/ARCHITECT HBsAg Confirmatory Final Interpretation					
	Confirmed Positive		Repeatedly Reactive Not Confirmed		Confirmed Positive		Repeatedly Reactive Not Confirmed			
	n	%	n	%	n	%	n	%		
Individuals Diagnosed With Acute HBV Infection	6	3.59	0	0.00	0	0.00	0	0.00	6	3.59
Individuals Diagnosed With Chronic HBV Infection	41	24.55	0	0.00	1 ^c	0.60	0	0.00	42	25.15
Individuals At Increased Risk For HBV Infection	18	10.78	1 ^a	0.60	3 ^d	1.80	2	1.20	24	14.37
Individuals From Vietnam	53	31.74	0	0.00	0	0.00	0	0.00	53	31.74
Individuals With Signs And Symptoms Of Hepatitis Infection	27	16.17	1 ^b	0.60	4 ^e	2.40	3	1.80	35	20.96
Pregnant Females	2	1.20	0	0.00	2 ^f	1.20	3	1.80	7	4.19
TOTAL	147	88.02	2	1.20	10	5.99	8	4.79	167	100.0

- ^a This specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, HBeAg, anti-HBe, and HBV DNA, but positive for anti-HBs.
- ^b This specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, anti-HBs, HBeAg, and anti-HBe, but positive for HBV DNA.
- ^c This specimen was tested and determined to be negative for anti-HBc IgM, HBeAg, and HBV DNA, but positive for anti-HBc, anti-HBs, and anti-HBe.
- ^d Two specimens were tested and determined to be negative for anti-HBc IgM, anti-HBc, HBeAg, and anti-HBe, but positive for anti-HBs and HBV DNA; one specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, HBeAg, and anti-HBe, but positive for anti-HBs and HBV DNA.
- ^e Two specimens were tested and determined to be negative for anti-HBc IgM, anti-HBc, anti-HBs, HBeAg, and anti-HBe, but positive for HBV DNA; one specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, anti-HBs, HBeAg, and HBV DNA, but positive for anti-HBc and anti-HBe; one specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, anti-HBs, HBeAg, anti-HBe, and HBV DNA.
- ^f One specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, HBeAg, anti-HBe, and HBV DNA, but indeterminate for anti-HBs; one specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, HBeAg, anti-HBe, and HBV DNA, but positive for anti-HBs.

Of the 167 specimens tested above, 59 specimens from the increased risk and signs and symptoms populations were classified by HBV infection. A comparison of the ARCHITECT HBsAg/HBsAg Confirmatory specimens versus the comparator HBsAg/HBsAg Confirmatory final interpretation by HBV classification are summarized in the following table.

HBV Classification	Comparator HBsAg/HBsAg Confirmatory Final Interpretation								Total	
	Positive				Negative					
	ARCHITECT HBsAg/ARCHITECT HBsAg Confirmatory Final Interpretation				ARCHITECT HBsAg/ARCHITECT HBsAg Confirmatory Final Interpretation					
	Confirmed Positive		Repeatedly Reactive Not Confirmed		Confirmed Positive		Repeatedly Reactive Not Confirmed			
	n	%	n	%	n	%	n	%		
Early Acute	1	1.69	1 ^a	1.69	0	0.00	0	0.00	2	3.39
Acute	4	6.78	0	0.00	0	0.00	0	0.00	4	6.78
Chronic	39	66.10	1 ^b	1.69	0	0.00	0	0.00	40	67.80
Late Acute/Recovering	1	1.69	0	0.00	0	0.00	0	0.00	1	1.69
Distantly Immune/Anti-HBs Not Detected	0	0.00	0	0.00	1 ^c	1.69	0	0.00	1	1.69
Immune Due to HBV Vaccination	0	0.00	0	0.00	1 ^d	1.69	0	0.00	1	1.69
Susceptible	0	0.00	0	0.00	5 ^e	8.47	5	8.47	10	16.95
TOTAL	45	76.27	2	3.39	7	11.86	5	8.47	59	100.00

- ^a This specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, anti-HBs, HBeAg, and anti-HBe, but positive for HBV DNA.
- ^b This specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, HBeAg, anti-HBe, and HBV DNA, but positive for anti-HBs.
- ^c This specimen was tested and determined to be negative for anti-HBc IgM, anti-HBs, HBeAg, and HBV DNA, but positive for anti-HBc and anti-HBe.
- ^d This specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, HBeAg, and anti-HBe, but positive for anti-HBs and HBV DNA.
- ^e Four specimens were tested and determined to be negative for anti-HBc IgM, anti-HBc, anti-HBs, HBeAg, and anti-HBe, but positive for HBV DNA; one specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, anti-HBs, HBeAg, anti-HBe, and HBV DNA.

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6. Primus FJ, Kelley EA, Hansen HJ, Goldenberg DM. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* 1988;34:261-264.
7. Schrott RW, Foon KA, Beatty SM, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985;45:879-885.

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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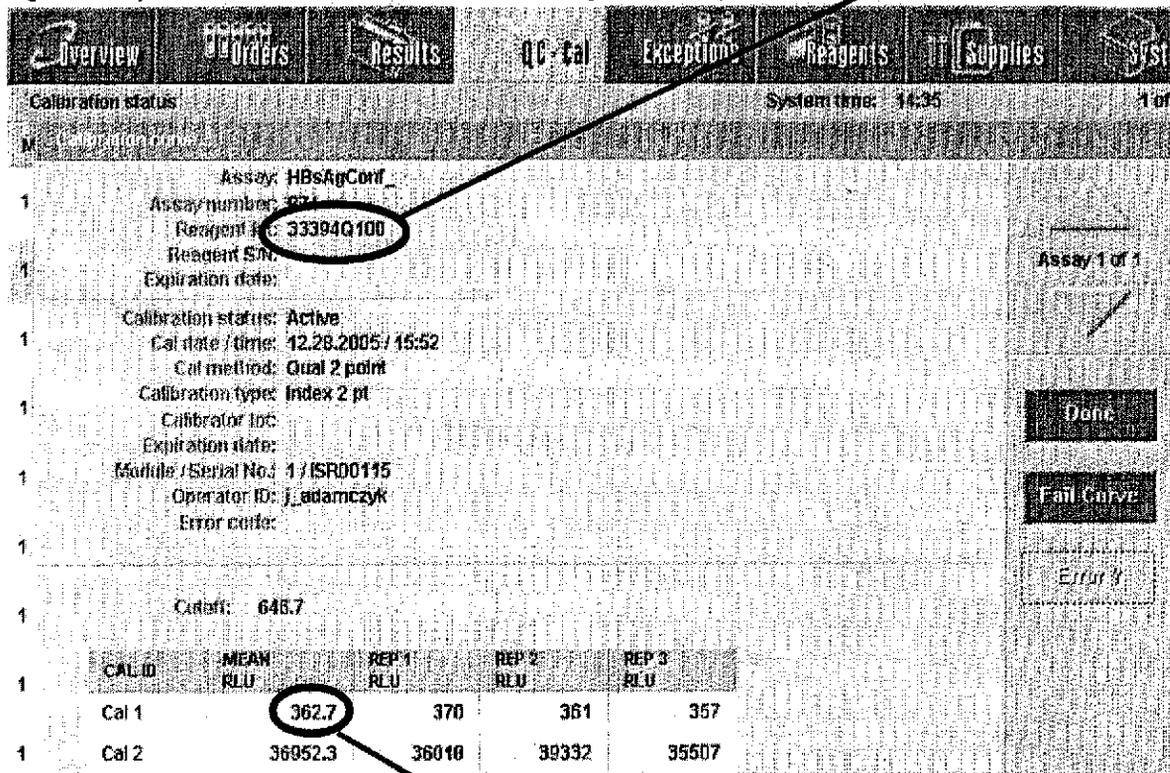
% Neutralization Calculation Worksheet

In order to complete the % neutralization worksheet, you will need the following:

- **Sample Dilution Factor** – Neat (undiluted), 1:500, or 1:20000.
- **Calibration curve data** for the HBsAgConf_ assay. See Fig. 1.
- **Two Result Details** reports for the patient specimen (report for **Reagent 1** dilution and report for **Reagent 2** dilution). See Fig. 2.

Reagent Lot Number

Fig. 1. Example of Calibration Curve Data for the HBsAgConf_ assay



B (Cal 1 Mean RLU)

% Neutralization Calculation Worksheet

Fig 2. Example of Result Details reports for the Reagent 1 dilution and Reagent 2 dilution

Example report for Reagent 1 dilution

Reagent lot number

Sample ID

Sample ID: PA1109A1		Assay: HBsAgConf_					
Patient ID:		Assay number: 874					
Draw date / time:		Module / Serial no.: 1 /ISR00115					
Sample comment:		<u>Calibration information</u> Cal. lot: Cal. date/time: 12.28.2005 15:52 Reagent master lot: 33394Q100 Serial no.:					
Date completed: 01.12.2006		Location:					
Time completed: 15:24		Doctor:					
Operator ID: R_DUBLER							
C / P	Result	Units	Range	Dilution	Flags	Code	RLU
R201/4	32.50	S/CO		REAGENT 1	IUO		21018
Positive							

C (Reagent 1 RLU)

Example report for Reagent 2 dilution

Reagent lot number

Sample ID

Sample ID: PA1109A1		Assay: HBsAgConf_					
Patient ID:		Assay number: 874					
Draw date / time:		Module / Serial no.: 1 /ISR00115					
Sample comment:		<u>Calibration information</u> Cal. lot: Cal. date/time: 12.28.2005 15:52 Reagent master lot: 33394Q100 Serial no.:					
Date completed: 01.12.2006		Location:					
Time completed: 15:24		Doctor:					
Operator ID: R_DUBLER							
C / P	Result	Units	Range	Dilution	Flags	Code	RLU
R201/4	791.93	S/CO		REAGENT 2	IUO		512114
Positive							

D (Reagent 2 RLU)

Reagent 2 S/CO

For this example, % Neutralization equals:

$$\frac{D - C}{D - B} \times 100\%$$

$$= \frac{512,114 - 21,018}{512,114 - 362.7} \times 100\%$$

$$= \frac{491,096}{511,751.3} \times 100\%$$

$$= 0.960 \times 100\%$$

$$= 96\%$$

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% Neutralization Calculation Worksheet

Reagent Lot Number: _____

Sample ID: _____

Sample Dilution Factor (circle one): Neat (undiluted) 1:500 1:20000

Step 1. Record Calibration Data

B = Cal 1 Mean RLU _____

Step 2. Record Sample Data

Reagent 2 S/CO result _____

NOTE: If Reagent 2 S/CO result is < 0.80, then % neutralization is not applicable, go directly to Step 4.

C = Sample's Reagent 1 RLU _____

D = Sample's Reagent 2 RLU _____

Step 3. Calculate % Neutralization

$$\% \text{ Neutralization} = \frac{D - C}{D - B} \times 100\%$$

A. Calculate **D - C** and record the difference in the upper box below.

D - C = _____

B. Calculate **D - B** and record the difference in the lower box below.

D - B = _____

C. Complete the calculation by dividing and then multiplying the results by 100.

$$\frac{D - C}{D - B} = \frac{\boxed{}}{\boxed{}} = \boxed{} \times 100 = \boxed{} \%$$

Step 4. Interpret Results

For interpretation of the final test result, select the appropriate line in the table below based on the Sample Dilution Factor and values for Reagent 2 S/CO and % neutralization.

Sample Dilution Factor	Reagent 2 S/CO	% Neutralization	Interpretation of Results
Neat (undiluted)	< 0.80	Not applicable	Repeatedly reactive, not confirmed
	≥ 0.80	≥ 50%	Confirmed positive
	≥ 0.80	< 50%	Repeat test using a 1:500 dilution
1:500	< 0.80	Not applicable	Repeatedly reactive, not confirmed
	≥ 0.80	≥ 50%	Confirmed positive
	≥ 0.80	< 50%	Repeat test using a 1:20000 dilution
1:20000	< 0.80	Not applicable	Repeatedly reactive, not confirmed
	≥ 0.80	≥ 50%	Confirmed positive
	≥ 0.80	< 50%	Repeatedly reactive, not confirmed

Final Test Result _____

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HBSAG

Controls

BIBLIOGRAPHY

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3. World Health Organization, *Laboratory Biosafety Manual*, 3rd ed. Geneva: World Health Organization, 2004.
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by
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- The % neutralization is calculated by the operator as follows:
 - Match up the Pretreatment 1 and 2 (Reagent 1 and 2) values for each sample.
 - % Neutralization = $\frac{D - C}{D - B} \times 100\%$
- where B = Calibrator 1 Mean RLU
 C = Sample's Pretreatment 1 [Reagent 1] RLU
 D = Sample's Pretreatment 2 [Reagent 2] RLU
- Note: Round to the nearest whole number.

NOTE: To assist in calculations, a % Neutralization Calculation Worksheet is provided at the end of the ARCHITECT HBSAg Confirmatory reagent package insert.

To troubleshoot control values that fall outside the control range, refer to the ARCHITECT System Operations Manual, Section 10.

LIMITATIONS

- The ARCHITECT HBSAg positive quality control material is in a matrix that consists of 5% BSA and 7.5% recalcified human plasma in phosphate buffer. A study was performed that demonstrated the ARCHITECT HBSAg positive quality control material detects reagent failure (induced by heat-stress of reagents) comparably to a positive control in a serum or plasma matrix. The positive quality control material may not adequately monitor the assay under all potential conditions of reagent failure when testing serum and plasma specimens. The user should provide alternate positive quality control material for testing of serum and plasma matrices.
- The ARCHITECT HBSAg negative quality control material is in a serum matrix made from recalcified plasma. The user should provide alternate negative quality control material for plasma when necessary.
- Control values have not been established for assays other than ARCHITECT HBSAg and HBSAg Confirmatory. If the user wishes to use this control material with other assays, it is their responsibility to establish the appropriate ranges.
- The controls are not calibrators and should not be used for assay calibration.

EXPECTED RESULTS

The controls must fall within the following ranges:

For ARCHITECT HBSAg (tLR0) values, refer to the following table:

Control	Color	Target (S/CO)	Target Range (S/CO)
CONTROL -	Natural	0.57	< 1.00
CONTROL +	Blue	4.25	2.13 - 6.37

For ARCHITECT HBSAg Confirmatory (tLR1) values, refer to the following table:

Control	Color	Target (S/CO)	Target Range (S/CO)	Neutralization %
CONTROL -	Blue	3.28	1.84 - 4.92	≥ 50%

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
- Calibrator lot
- Control lot
- Reagent lot
- Instrument

Key to symbols used

	Lot Number		Lot Number
	In Vitro Diagnostic Medical Device		Expiration Date
	Store at 2-8°C		Negative Control
	CAUTION: Consult accompanying documents		Positive Control

Color: PMS 329 C
Size: 8 1/2 x 14
Editor: DTP

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INTENDED USE

The ARCHITECT HBSAg Controls are used for monitoring the performance of the ARCHITECT System (reagents, calibrators, and instrument) when used for the qualitative detection of hepatitis B surface antigen (HBsAg) using the ARCHITECT HBSAg and HBSAg Confirmatory assays. The performance of the ARCHITECT HBSAg Controls has not been established with any other HBSAg assays.

WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use.

CAUTION: This product contains human sourced infectious and/or potentially infectious components. No known test method can offer complete assurance that products derived from human sources or inoculated 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.

- The negative control is nonreactive for anti-HBs, HBSAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.

- The positive control is reactive for HBSAg. The human plasma used in the positive control is nonreactive for anti-HBs, HBSAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.

- The ARCHITECT HBSAg Controls contain methylisothiazolones (which are components of ProClin®) and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases:

R43 May cause sensitization by skin contact.

S24 Avoid contact with skin.

S35 This material and its container must be disposed of in a safe way.

S37 Wear suitable gloves.

S46 If swallowed, seek medical advice immediately and show this container or label.



MATERIALS PROVIDED

- 2 Bottles (8 mL each) of ARCHITECT HBSAg Controls (1 bottle of negative control and 1 bottle of positive control).

- The negative control is recalibrated HBSAg-negative human plasma. Preservative: antimicrobial agent, ProClin 300, and ProClin 950.

- The positive control contains inactivated purified HBSAg (subtypes ad/ay) in phosphate buffer with protein additives (5% bovine albumin and 7.5% human plasma). The positive control is blue and contains Acid Blue No. 9 dye. Preservatives: antimicrobial agent, ProClin 300, and ProClin 950.

PREPARATION AND STORAGE

- The controls are liquid ready-to-use. No preparation is required.
- When stored and handled as directed, the controls are stable until the expiration date.
- The controls must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- Refer to the ARCHITECT HBSAg or HBSAg Confirmatory reagent package insert for the maximum onboard stability requirements.

2-8°C
Store at 2-8°C

QUALITY CONTROL PROCEDURES

The ARCHITECT HBSAg positive quality control material is in a matrix that consists of 5% BSA and 7.5% recalibrated human plasma in phosphate buffer. A study was performed that demonstrated the ARCHITECT HBSAg positive quality control material detects reagent failure (induced by heat-stress of reagents) comparably to a positive control in a serum or plasma matrix. The positive quality control material may not adequately monitor the assay under all potential conditions of reagent failure when testing serum and plasma specimens. The user should provide alternate positive quality control material for testing of serum and plasma matrices.

Refer to the ARCHITECT HBSAg and HBSAg Confirmatory reagent package inserts and ARCHITECT System Operations Manual for additional information.

- For the ARCHITECT HBSAg assay, the recommended control requirement is that a single sample of each control be tested once every 24 hours each day of use.

- For the ARCHITECT HBSAg Confirmatory assay, the recommended control requirement is that a single sample of the positive control be tested once every 24 hours each day of use.

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

PROCEDURE

The following applies to the ARCHITECT HBSAg assay:

- Mix the controls by gentle inversion before use.

- To obtain the recommended volume requirements for the ARCHITECT HBSAg Controls, hold the bottles vertically and dispense 6 drops of each control into each respective sample cup.

- For information on ordering patient specimens and the positive control and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.

- Use the following instructions to order an ARCHITECT HBSAg Negative Control (nonreactive for HBSAg):

- Order the negative control as a patient sample. The negative control cannot be ordered as a Control.

- Manual verification of the validity of the negative control is required any time the negative control is run.

- Because the negative control is run as a patient sample, patient values will not be flagged by the ARCHITECT System if a negative control is outside of its control range. Only release patient results if a valid negative control value is obtained.

- To troubleshoot control values that fall outside the control ranges in this package insert, refer to the ARCHITECT System Operations Manual, Section 10.

The following applies to the ARCHITECT HBSAg Confirmatory assay:

- Mix the controls by gentle inversion before use.
- To obtain the recommended volume requirements for the ARCHITECT HBSAg Positive Control, hold the bottle vertically and dispense 6 drops into a sample cup.
- Use the following instructions to order an ARCHITECT HBSAg Positive Control:

- Order the positive control as a patient sample. The positive control cannot be ordered as a Control.

- For information on ordering patient specimens and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.

- Manual verification of the validity of the positive control is required any time the positive control is run.

- Because the positive control is run as a patient sample, patient values will not be flagged by the ARCHITECT System if a positive control is outside of its control range. Only release patient results if a valid positive control value is obtained.

- Note: For each confirmatory result, two tests are performed, one for Pretreatment 1 (Reagent 1) and one for Pretreatment 2 (Reagent 2). Therefore, when ordering the positive control (after selecting the HBSAg Confirmatory assay),

- Select F5 - Assay Options.

- Select Module-Auto (default mode) or, if working with a multi-module / system, select Manual and then select the Module of choice.

- Enter the number of replicates for Pretreatment 1 (Reagent 1) and Pretreatment 2 (Reagent 2).

- Note: In the default method, the number of replicates is not specified for Reagent 2. In order to obtain a valid test result, this number needs to be defined manually.

- Calculate the % neutralization for the positive control using the results from both the Pretreatment 1 and 2 (Reagent 1 and 2) tests.

- Obtain the Calibrator 1 (Cal 1) mean RLU from the ARCHITECT System as follows:

- Select the Qc-Cal icon, then select Calibration status from the drop down menu.
- Select the correct reagent lot number to be used for the corresponding sample neutralization calculation.

- Select Details F5 to view the calibrator RLU values or select Print F4.

- If Details F5 was selected, record the value for the Calibrator 1 mean RLU.

- If Print F4 was selected, select Calibration Details Report to print the calibrator mean RLU values.

- Obtain sample Pretreatment 1 (Reagent 1) RLU, Pretreatment 2 (Reagent 2) RLU, and Pretreatment 2 (Reagent 2) S/CO from the ARCHITECT System as follows:

- Select Results Icon, then select Results review from the drop down menu. If the system is configured for the auto release of results, the results can be viewed in the Stored Results screen.

- Select the required specimen(s).

- Select Details F5 to view results or select Print F4.

- If Details F5 was selected, record the values for the sample's Reagent 1 RLU, Reagent 2 RLU, and Reagent 2 S/CO.

- If Print F4 was selected, select Result Details Report to print the sample RLU and S/CO values.

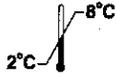
HBsAg

CUSTOMER SERVICE

UNITED STATES: 1-877-4ABBOTT

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.

This package insert must be read carefully before product use. Package insert instructions must be followed accordingly. 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
IVD	<i>In Vitro</i> Diagnostic Medical Device
	Store at 2-8°C
LOT	Lot Number
	Expiration Date
	CAUTION: Consult accompanying documents.
REACTION VESSELS	Reaction Vessels
SAMPLE CUPS	Sample Cups
SEPTUMS	Septums
REPLACEMENT CAPS	Replacement Caps
SN	Serial Number
CONTROL NO.	Control Number
REAGENT LOT	Reagent Lot

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

NAME

ARCHITECT HBsAg

INTENDED USE

The ARCHITECT HBsAg assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of hepatitis B surface antigen (HBsAg) in human adult and pediatric serum and plasma (dipotassium EDTA). The assay may also be used to screen for HBV infection in pregnant females to identify neonates who are at risk of acquiring hepatitis B during the perinatal period. Assay results, in conjunction with other laboratory results and clinical information, may be used to provide presumptive evidence of infection with the hepatitis B virus (HBV) (state of infection or associated disease not determined) in persons with signs and symptoms of hepatitis and in persons at risk for hepatitis B infection.

Warning: Not intended for use in screening blood, plasma, or tissue donors. The effectiveness of ARCHITECT HBsAg for use in screening blood, plasma, or tissue donors has not been established.

Assay performance characteristics have not been established when the ARCHITECT HBsAg assay is used in conjunction with other manufacturers' assays for specific HBV markers. Users are responsible for establishing their own performance characteristics.

SUMMARY AND EXPLANATION OF THE TEST

Enzyme immunoassays for the detection of antigens (for example, HBsAg) were first described by Engvall and Perlmann^{1,3} and Van Weemen and Schuurs⁴ in 1971. In 1976 and 1977, solid phase "sandwich" enzyme immunoassays were developed in which HBsAg was captured on a solid phase coated with polyclonal antibodies against HBsAg (anti-HBs) and then detected with anti-HBs conjugated to an enzyme.^{5,7} In the early 1980s, monoclonal anti-HBs based assays were developed for the detection of HBsAg.⁸⁻¹³ ARCHITECT HBsAg is a CMIA that uses microparticles coated with monoclonal anti-HBs for the detection of HBsAg.

HBsAg assays are routinely used to aid in the diagnosis of suspected HBV infection and to monitor the status of infected individuals, *i.e.*, whether the patient's infection has resolved or the patient has become a chronic carrier of the virus.¹⁴ For the diagnosis of acute or chronic hepatitis, HBsAg reactivity should be correlated with patient history and the presence of other hepatitis B serological markers. Prenatal testing has been recommended by the United States Centers for Disease Control and Prevention (CDC) to identify newborns from HBV carrier mothers that may benefit from prophylactic treatment.^{15,16}

Specimens nonreactive by ARCHITECT HBsAg are considered negative for HBsAg. A reactive specimen must be retested in duplicate by ARCHITECT HBsAg to determine whether it is repeatedly reactive. A specimen which is found to be repeatedly reactive should be confirmed by the ARCHITECT HBsAg Confirmatory (1L81) assay, a neutralization procedure utilizing human anti-HBs. If the specimen is neutralized, the specimen is considered confirmed positive for HBsAg. It is recommended that confirmatory testing be performed before disclosing HBsAg status.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT HBsAg assay is a two-step immunoassay for the qualitative detection of HBsAg in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

In the first step, sample and anti-HBs coated paramagnetic microparticles are combined. HBsAg present in the sample binds to the anti-HBs coated microparticles. After washing, acridinium-labeled anti-HBs conjugate is added in the second step and combines with any HBsAg bound to the microparticles. 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 ARCHITECT HBsAg calibration curve. If the chemiluminescent signal of the sample is greater than or equal to the cutoff signal, the sample is considered reactive for HBsAg. If the chemiluminescent signal is less than the cutoff signal, the sample is considered negative for HBsAg.

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

REAGENTS

Reagent Kit, 100/500 Tests

Note: Reagent kit configuration varies based on order.

ARCHITECT HBsAg Reagent Kit (1L80)

- **MICROPARTICLES** 1 or 4 Bottle(s) (6.6 mL/27.0 mL) anti-HBs (mouse, monoclonal, IgM, IgG) coated microparticles in MES buffer with protein additives (152 µM bovine serum albumin, 10.0% bovine calf serum) and surfactant. Minimum concentration: 0.0675% solids. Preservatives: ProClin[®] 300 and ProClin 950.
- **CONJUGATE** 1 or 4 Bottle(s) (5.9 mL/26.3 mL) anti-HBs (goat, IgG) acridinium-labeled conjugate in MES buffer with protein additives (30.3 µM bovine serum albumin, 12.5% bovine calf serum, 2.0% human plasma) and surfactant. Minimum concentration: 0.25 µg/mL. Preservative: ProClin 300.

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 0.35N sodium hydroxide.

ARCHITECT *i* Wash Buffer

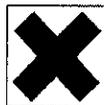
- **WASH BUFFER** Wash buffer containing phosphate buffered saline solution. Preservative: antimicrobial agent.

WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use.

Safety Precautions

-  **CAUTION:** This product contains human sourced infectious and/or potentially infectious components. 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,¹⁷ Biosafety Level 2¹⁸ or other appropriate biosafety practices^{19,20} should be used for materials that contain or are suspected of containing infectious agents.
- The human plasma used in the conjugate is nonreactive for anti-HBs, HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.
- The ARCHITECT HBsAg reagents contain methylisothiazolones (which are components of ProClin) and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.



R43 May cause sensitization by skin contact.

S24 Avoid contact with skin.

S35 This material and its container must be disposed of in a safe way.

S37 Wear suitable gloves.

S46 If swallowed, seek medical advice immediately and show this container or label.

- 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 beyond the expiration date.
- Do not pool reagents within a reagent kit or between reagent kits.
- Before loading the ARCHITECT HBsAg 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.

BS

- 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 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

- 
- **2°C** The ARCHITECT HBsAg 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 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.** After reagents are removed from the system, initiate a scan to update the onboard stability timer.

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 assay file must be installed on the ARCHITECT *i* System from the ARCHITECT *i* System Assay CD-ROM 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 following specimen tube types may be used with the ARCHITECT HBsAg assay:

Glass	Plastic
<ul style="list-style-type: none"> • Serum • Serum separator 	<ul style="list-style-type: none"> • Serum • Serum separator • Dipotassium EDTA

- 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 assay.

Specimen Conditions

- Do not use specimens with the following conditions:
 - heat-inactivated
 - pooled
 - grossly hemolyzed
 - obvious microbial contamination

- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum and plasma.
- 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.
- 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.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- 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

- The Clinical and Laboratory Standards Institute (CLSI) provides the following recommendations for storing blood specimens.²¹
 - Store samples at 22°C (72°F) for no longer than 8 hours.
 - If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C (36-46°F).
 - If the assay will not be completed within 48 hours, freeze the sample at -20°C (-4°F).
 - Specimens that are not tested within the specified time period listed above must be removed from the clot or red blood cells and stored frozen (-20°C or colder).
- Avoid more than three 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.

PROCEDURE

Materials Provided:

- 1L80 ARCHITECT HBsAg Reagent Kit

Materials Required but not Provided:

- ARCHITECT *i* System
- ARCHITECT *i* System Assay CD-ROM
- 1L80-01 ARCHITECT HBsAg Calibrators
- 1L80-10 ARCHITECT HBsAg 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* **SEPTUMS**
- 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.

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Assay Procedure

- Before loading the ARCHITECT HBsAg 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 still adhere 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 **Handling Precautions** section of this package insert.
- Load the ARCHITECT HBsAg 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 positive controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
 - Use the following instructions to order a negative control (nonreactive for HBsAg):
 - Order the negative control as a patient specimen, not as a Control.
 - Manually verify the validity of the negative control every time it is run. Because the control is run as a patient specimen, a result will not be flagged by the ARCHITECT *i* System if it is outside the acceptable control range.
 - To troubleshoot control values that fall outside the control range, refer to the ARCHITECT System Operations Manual, Section 10.
- 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 first HBsAg test plus 75 µL for each additional HBsAg test from the same sample cup.
 - ≤ 3 hours onboard: 150 µL for the first HBsAg test plus 75 µL for each additional HBsAg test from the same sample cup.
 - > 3 hours onboard: 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.
 - ARCHITECT HBsAg Calibrators and Controls must be mixed by gentle inversion before use.
 - To obtain the recommended volume requirements for the ARCHITECT HBsAg Calibrators and Controls, hold the bottles **vertically** and dispense 14 drops of each calibrator or 6 drops of each control into each respective sample cup.
- 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. When a laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedure

- Specimens cannot be diluted for the ARCHITECT HBsAg assay.

Calibration

- To perform the calibration, test ARCHITECT HBsAg Calibrators 1 and 2 in triplicate. 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 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.

QUALITY CONTROL PROCEDURES

The ARCHITECT HBsAg positive quality control material is in a matrix that consists of 5% BSA and 7.5% recalcified human plasma in phosphate buffer. A study was performed that demonstrated the ARCHITECT HBsAg positive quality control material detects reagent failure (induced by heat-stress of reagents) comparably to a positive control in a serum or plasma matrix. The positive quality control material may not adequately monitor the assay under all potential conditions of reagent failure when testing serum and plasma specimens. The user should provide alternate positive quality control material for testing of serum and plasma matrices.

The ARCHITECT HBsAg negative quality control material is in a serum matrix made from recalcified plasma. The user should provide alternate negative quality control material for plasma when necessary.

The recommended control requirement for the ARCHITECT HBsAg assay is that a single sample of each control 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.

Control values must be within the concentration 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.

RESULTS

Calculations

- The ARCHITECT *i* System calculates the cutoff RLU from the mean RLU of three replicates of Calibrator 2. The cutoff RLU is stored for each reagent lot calibration.

$$\text{Cutoff RLU} = 0.013 \times (\text{Calibrator 2 mean RLU})$$

- The ARCHITECT *i* System calculates the S/CO result for each sample as follows:

$$\text{S/CO} = \text{Sample RLU/Cutoff RLU}$$

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.

Interpretation of Results

ARCHITECT HBsAg Initial Results	
Instrument Interpretation	Retest Procedure
Nonreactive (S/CO value < 1.00)	No retest required.
Reactive (S/CO value ≥ 1.00)	Retest in duplicate.

- A specimen with an S/CO of less than 1.00 is nonreactive; the specimen is considered negative for HBsAg.
- Initially reactive specimens require retesting. Specimens that contain particulate matter should be recentrifuged according to directions in the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert and then retested in duplicate.

ARCHITECT HBsAg Retest Results	
Instrument Interpretation	Interpretation of Retest Results
Both results nonreactive (both S/CO values < 1.00)	Specimen considered negative for HBsAg.
One or both results reactive (one or both S/CO values ≥ 1.00)	Specimen considered repeatedly reactive; confirm using the ARCHITECT HBsAg Confirmatory assay.

- If both retest results are nonreactive; the specimen is considered negative for HBsAg.
- If one or both retest results are reactive, the specimen is considered repeatedly reactive for the presence of HBsAg.
- Confirm repeatedly reactive specimens using the ARCHITECT HBsAg Confirmatory assay before disclosing HBsAg status to the patient.

LIMITATIONS OF THE PROCEDURE

- 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.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).^{22,23} Such specimens may show either falsely elevated or depressed values when tested with assay kits (such as ARCHITECT HBsAg) that employ mouse monoclonal antibodies.²²
- 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 assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- The reaction vessel to reaction vessel carryover for the ARCHITECT HBsAg assay is ≤ 0.35 parts per million.
- The magnitude of an ARCHITECT HBsAg assay result cannot be correlated to an end point titer.
- Heparinized plasma cannot be used with the ARCHITECT HBsAg assay.
- A reactive HBsAg result does not exclude co-infection by another hepatitis virus.

EXPECTED RESULTS

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

Increased Risk Population

Of the 2946 specimens tested in the ARCHITECT HBsAg clinical study, 1313 specimens were from individuals with increased risk of HBV infection. All 1313 individuals were at risk for HBV due to lifestyle, behavior, occupation, or a known exposure event but were asymptomatic and reported no current signs or symptoms of hepatitis. Testing of these specimens was performed at three clinical sites located in Galveston, TX; Hershey, PA; and Milwaukee, WI.

The increased risk population (n=1313) consisted of the following race/ethnic groups:

- 625 (47.60%) Caucasian
- 476 (36.25%) African-American
- 167 (12.72%) Hispanic
- 19 (1.45%) Asian
- 6 (0.46%) American Indian/Alaska Native
- 20 (1.52%) Other

The 1313 specimens from the increased risk population were obtained from the following collection locations:

- 742 (56.51%) from Galveston, TX
- 185 (14.09%) from High Point, NC
- 99 (7.54%) from Plymouth, MA
- 76 (5.79%) from Colton, CA
- 59 (4.49%) from Dallas, TX
- 56 (4.27%) from St. Petersburg, FL
- 52 (3.96%) from Miami, FL
- 36 (2.74%) from Denver, CO
- 8 (0.61%) from Chicago, IL

A total of 23 (1.75%) of the specimens in the increased risk population were reactive in the ARCHITECT HBsAg assay. The number of ARCHITECT HBsAg reactive results observed for the increased risk population at each collection location was:

- 9 of 742 (1.21%) from Galveston, TX
- 1 of 185 (0.54%) from High Point, NC
- 0 of 99 (0.00%) from Plymouth, MA
- 0 of 76 (0.00%) from Colton, CA
- 6 of 59 (10.17%) from Dallas, TX
- 3 of 56 (5.36%) from St. Petersburg, FL
- 4 of 52 (7.69%) from Miami, FL
- 0 of 36 (0.00%) from Denver, CO
- 0 of 8 (0.00%) from Chicago, IL

Of the 1313 specimens, 815 (62.07%) were female and 498 (37.93%) were male. The age was not reported for three specimens. Of the remaining 1310 specimens, the mean age was 40 years (age range: 18 to 75 years). The distribution of ARCHITECT HBsAg reactive and nonreactive results among the increased risk population by age and gender (n=1310) is summarized in the following table.

Age Group (Years)	Gender	ARCHITECT HBsAg Result		Total
		Reactive n (%)	Nonreactive n (%)	
10 to 19	F	0 (0.00)	14 (100.00)	14
	M	0 (0.00)	11 (100.00)	11
20 to 29	F	2 (1.09)	182 (98.91)	184
	M	0 (0.00)	97 (100.00)	97
30 to 39	F	0 (0.00)	184 (100.00)	184
	M	5 (4.67)	102 (95.33)	107
40 to 49	F	4 (1.60)	246 (98.40)	250
	M	4 (2.52)	155 (97.48)	159
50 to 59	F	2 (1.46)	135 (98.54)	137
	M	5 (4.59)	104 (95.41)	109
60 to 69	F	0 (0.00)	35 (100.00)	35
	M	1 (8.33)	11 (91.67)	12
70 to 79	F	0 (0.00)	8 (100.00)	8
	M	0 (0.00)	3 (100.00)	3
Total		23 (1.76)	1287 (98.24)	1310[†]

[†] Age was not reported for three subjects.

SPECIFIC PERFORMANCE CHARACTERISTICS

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

Precision

The ARCHITECT HBsAg assay is designed to have a Within Laboratory Precision (Total) CV of $\leq 10\%$ for the ARCHITECT HBsAg positive control and $\leq 15\%$ for samples targeted to 1.20 S/CO (low positive panel) and to have a Total SD ≤ 0.15 S/CO for samples targeted to 0.80 S/CO (high negative panel).

Within Laboratory Precision

A 20-day precision study was performed for the ARCHITECT HBsAg assay based on guidance from the CLSI document EP5-A2.²⁴ Testing was conducted at Abbott Laboratories using three ARCHITECT HBsAg assay reagent lots, three calibrator lots, one control lot, and two instruments. Two controls and two panels were assayed in replicates of two at two separate times of day for 20 different days. The data are summarized in the following table.

Instrument	Sample	n	Grand Mean S/CO	Within-Run		Within-Day		Within Laboratory Precision (Total)	
				SD	%CV	SD	%CV	SD	%CV
1	Negative Control	240	0.52	0.029	NA	0.031	NA	0.035	NA
	Positive Control	240	4.00	0.078	2.0	0.099	2.5	0.147	3.7
	High Negative Panel	240	0.74	0.036	5.0	0.036	5.0	0.046	6.3
	Low Positive Panel	240	1.15	0.042	3.7	0.043	3.8	0.059	5.1
2	Negative Control	240	0.56	0.038	NA	0.038	NA	0.039	NA
	Positive Control	240	4.02	0.159	4.0	0.176	4.4	0.206	5.1
	High Negative Panel	240	0.83	0.073	8.9	0.076	9.2	0.078	9.5
	Low Positive Panel	240	1.24	0.074	6.0	0.076	6.2	0.082	6.6

NA = not applicable

System Reproducibility

A five-day precision study was performed for the ARCHITECT HBsAg assay based on guidance from the CLSI document EP15-A2.²⁵ Testing was conducted at three clinical testing sites using three ARCHITECT HBsAg assay reagent, calibrator, and control lots per site. Two controls and two panels were assayed in replicates of four at two separate times of day for five days. The data are summarized in the following table.

Sample	n	Grand Mean S/CO	Within-Run		Within-Day		Within Laboratory Precision (Total)		Precision with Additional Component of Between-Site		Precision with Additional Component of Between-Lot		Precision with Additional Component of Site and Lot (Overall)	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control	360	0.59	0.051	NA	0.051	NA	0.054	NA	0.072	NA	0.101	NA	0.111	NA
Positive Control	360	4.25	0.183	4.3	0.186	4.4	0.195	4.6	0.216	5.1	0.254	6.0	0.254	6.0
High Negative Panel	360	0.83	0.066	7.9	0.066	8.0	0.068	8.1	0.100	12.0	0.107	12.9	0.128	15.4
Low Positive Panel	360	1.25	0.079	6.3	0.084	6.8	0.096	7.7	0.148	11.8	0.124	10.0	0.162	13.0

NA = not applicable

Clinical Performance

A prospective multi-center study was conducted to evaluate the ability of the ARCHITECT HBsAg assay to detect HBsAg in a group of individuals that would normally be tested in a clinical situation. Of the 2946 specimens tested in the ARCHITECT HBsAg clinical study, 1313 specimens were obtained from individuals with increased risk of HBV infection due to lifestyle, behavior, occupation, or a known exposure event and 690 specimens were obtained from individuals exhibiting signs and symptoms of hepatitis infection.

Specimens (n=2003) from these populations consisted of the following race/ethnic groups:

- 1060 (52.92%) Caucasian
- 40 (2.00%) Asian
- 576 (28.76%) African-American
- 9 (0.45%) American Indian/Alaska Native
- 288 (14.38%) Hispanic
- 30 (1.50%) Other

Specimens (n=2003) from these specimen populations were obtained from the following collection locations:

- 791 (39.49%) from Galveston, TX
- 116 (5.79%) from Colton, CA
- 341 (17.02%) from Plymouth, MA
- 116 (5.79%) from Dallas, TX
- 185 (9.24%) from High Point, NC
- 84 (4.19%) from Miami, FL
- 166 (8.29%) from Chicago, IL
- 82 (4.09%) from St. Petersburg, FL
- 122 (6.09%) from Denver, CO

Of the 2003 specimens from the increased risk and signs and symptoms populations, 1055 (52.67%) were female and 948 (47.33%) were male. Age was not reported for three specimens. Of the remaining 2000 specimens, the mean age was 41 years (age range: 18 to 83 years). Each specimen was tested using a comparator HBsAg assay and three HBV reference assays, each detecting a unique serological marker (anti-HBc IgM, total anti-HBc, and anti-HBs). The HBV classification was determined for each specimen based on the reactivity patterns of the four HBV serological marker results. The comparator and reference assays were from a single manufacturer, and testing was performed following manufacturer's instructions. Each specimen was also tested at one of three clinical sites located in Galveston, TX; Hershey, PA; or Milwaukee, WI, using the ARCHITECT HBsAg assay.

Results by Specimen Classification

Following testing with the comparator HBsAg assay and three reference HBV assays, the 2003 specimens from the increased risk and signs and symptoms population plus 117 individuals with acute or chronic HBV infection were assigned an HBV classification according to the following table. There were 15 unique reference marker patterns observed in the ARCHITECT HBsAg clinical study.

Number of Specimens	HBV Reference Markers				HBV Classification
	HBsAg	Anti-HBc IgM	Total Anti-HBc	Anti-HBs	
17	+	-	-	-	Early Acute
10	+	+	+	-	Acute
3	+	-	+	+	Chronic
85	+	-	+	-	Chronic
2	+	-	-	+	Chronic
3	+	-	+	I	Chronic
43	Presence of HBsAg ≥ 6 Months				Chronic
1	+	+	+	+	Late Acute/Recovering
4	-	+	+	+	Recovering Acute
2	-	+	+	I	Early Recovery
192	-	-	+	+	Immune Due to Natural Infection
31	-	-	+	I	Distantly Immune/Anti-HBs Unknown
107	-	-	+	-	Distantly Immune/Anti-HBs Not Detected
504	-	-	-	+	Immune Due to HBV Vaccination
63	-	-	-	I	Unknown
1053	-	-	-	-	Susceptible
2120					Total

+ = Positive/Reactive, - = Negative, I = indeterminate

Comparison of Results

The following table compares the ARCHITECT HBsAg assay results with the comparator HBsAg/HBsAg Confirmatory assay final interpretation for each of the HBV classifications for the increased risk and signs and symptoms populations (n = 2003) plus individuals with acute or chronic HBV infection (n=117). The data are summarized in the following table.

HBV Classification	Comparator HBsAg/HBsAg Confirmatory Final Interpretation								Total	
	Positive†				Negative					
	ARCHITECT HBsAg Results†									
	Reactive		Nonreactive		Reactive		Nonreactive			
	n	%	n	%	n	%	n	%	n	%
Early Acute	16	0.75	1 ^a	0.05	0	0.00	0	0.00	17	0.80
Acute	10	0.47	0	0.00	0	0.00	0	0.00	10	0.47
Chronic	133	6.27	1 ^a	0.05	1 ^e	0.05	1	0.05	136	6.42
Late Acute/Recovering	1	0.05	0	0.00	0	0.00	0	0.00	1	0.05
Recovering Acute	0	0.00	0	0.00	0	0.00	4	0.19	4	0.19
Early Recovery	0	0.00	0	0.00	0	0.00	2	0.09	2	0.09
Immune Due to Natural Infection	0	0.00	0	0.00	0	0.00	192	9.06	192	9.06
Distantly Immune/Anti-HBs Unknown	0	0.00	0	0.00	0	0.00	31	1.46	31	1.46
Distantly Immune/Anti-HBs Not Detected	0	0.00	0	0.00	1 ^e	0.05	106	5.00	107	5.05
Immune Due to HBV Vaccination	0	0.00	0	0.00	1 ^a	0.05	503	23.73	504	23.77
Unknown	0	0.00	0	0.00	0	0.00	63	2.97	63	2.97
Susceptible	0	0.00	0	0.00	10 ^f	0.47	1043	49.20	1053	49.67
Total	160	7.55	2	0.09	13	0.61	1945	91.75	2120	100.00

† The comparator HBsAg final positive interpretation is based on the comparator HBsAg assay repeatedly reactive results or the comparator HBsAg confirmatory assay results, when required.

‡ Includes retesting of initial reactives.

a This specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, anti-HBs, HBeAg, and anti-HBe, but positive for HBV DNA.

b This specimen was tested and determined to be negative for anti-HBc IgM, anti-HBc, HBeAg, anti-HBe, and HBV DNA, but positive for anti-HBs.

c This specimen was tested and determined to be negative for anti-HBc IgM, but positive for anti-HBc and anti-HBs.

d This specimen was tested and determined to be confirmed positive by the ARCHITECT HBsAg Confirmatory assay and determined to be negative for anti-HBc IgM, anti-HBs, HBeAg, and HBV DNA, but positive for anti-HBc and anti-HBe.

e This specimen was tested and determined to be confirmed positive by the ARCHITECT HBsAg Confirmatory assay and determined to be negative for anti-HBc IgM, anti-HBc, HBeAg, and anti-HBe, but positive for anti-HBs and HBV DNA.

f Five specimens were tested and determined to be repeatedly reactive, not confirmed by the ARCHITECT HBsAg Confirmatory assay and determined to be negative for anti-HBc IgM, anti-HBc, and anti-HBs; four specimens were tested and determined to be confirmed positive by the ARCHITECT HBsAg Confirmatory assay and determined to be negative for anti-HBc IgM, anti-HBc, anti-HBs, HBeAg, and anti-HBe, but positive for HBV DNA; one specimen was tested and confirmed positive by the ARCHITECT HBsAg Confirmatory assay but determined to be negative for anti-HBc IgM, anti-HBc, anti-HBs, HBeAg, anti-HBe, and HBV DNA.

Percent Agreement

The table below summarizes the percent agreement between the ARCHITECT HBsAg assay results and the comparator HBsAg assay final interpretation for the increased risk and signs and symptoms populations by HBV classification (n=2003).

HBV Classification	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Early Acute	50.00 (1/2)	1.26 - 98.74	---	---
Acute	100.00 (4/4)	39.76 - 100.00	---	---
Chronic	97.50 (39/40)	86.84 - 99.94	---	---
Late Acute/Recovering	100.00 (1/1)	2.50 - 100.00	---	---
Recovering Acute	---	---	100.00 (4/4)	39.76 - 100.00
Early Recovery	---	---	100.00 (2/2)	15.81 - 100.00
Immune Due to Natural Infection	---	---	100.00 (192/192)	98.10 - 100.00
Distantly Immune/Anti-HBs Unknown	---	---	100.00 (31/31)	88.78 - 100.00
Distantly Immune/Anti-HBs Not Detected	---	---	99.07 (106/107)	94.90 - 99.98
Immune Due to HBV Vaccination	---	---	99.80 (503/504)	98.90 - 99.99
Unknown	---	---	100.00 (63/63)	94.31 - 100.00
Susceptible	---	---	99.05 (1043/1053)	98.26 - 99.54
Total	95.74 (45/47)	85.46 - 99.48	99.39 (1944/1956)	98.93 - 99.68

Percent Agreement for Individuals With Acute or Chronic HBV Infection

ARCHITECT HBsAg performance was further evaluated using serum specimens that were: prospectively collected in the U.S. from six individuals clinically diagnosed with acute HBV infection and 43 individuals clinically diagnosed with chronic HBV infection defined by the presence of HBsAg for ≥ 6 months; prospectively collected in Vietnam from 53 individuals classified as chronic by four-marker HBV reference testing; and 15 seroconversion panel members classified as acute by four marker HBV reference testing. The percent agreement between the ARCHITECT HBsAg assay results and the comparator HBsAg assay final interpretation for the individuals with acute and chronic HBV infection (n=117) 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	21/21 (100.00)	83.89-100.00	NA	NA
Individuals With Chronic HBV Infection	94/94 (100.00)	96.15-100.00	1/2 (50.00)	1.26-98.74
Total	115/115 (100.00)	96.84-100.00	1/2 (50.00)	1.26-98.74

Clinical Performance in Pregnant Females

The performance of ARCHITECT HBsAg in detecting HBV infection in pregnant females was evaluated by testing prospectively-collected serum specimens from pregnant females at low risk or increased risk of HBV infection due to lifestyle, behavior, or known exposure event. Of the 2946 specimens tested in the ARCHITECT HBsAg clinical study, 741 were from a pregnant female population. The specimens were obtained from commercial vendors. The 741 specimens, from pregnant females ages 16 to 45 years, were collected from collection sites in Colton, CA (178); Plymouth, MA (7); and Los Angeles, CA (556). Testing of these specimens was performed at the clinical sites located in Galveston, TX and Milwaukee, WI. Of the pregnant female population, 4.18% were obtained during the first trimester, 45.21% during the second trimester, and 50.61% during the third trimester. The demographic profile of the pregnant female population is presented in the table below.

	Low Risk n (%)	Increased Risk n (%)	Total n (%)
TOTAL	548 (73.95)	193 (26.05)	741 (100.00)
TRIMESTER			
First	24 (4.38)	7 (3.63)	31 (4.18)
Second	261 (47.63)	74 (38.34)	335 (45.21)
Third	263 (47.99)	112 (58.03)	375 (50.61)
AGE			
16-31	323 (58.94)	159 (82.38)	482 (65.05)
32-45	225 (41.06)	34 (17.62)	259 (34.95)
RACE/ETHNIC GROUP			
Caucasian	10 (1.82)	41 (21.24)	51 (6.88)
African-American	52 (9.49)	24 (12.44)	76 (10.26)
Hispanic	468 (85.40)	120 (62.18)	588 (79.35)
Asian	16 (2.92)	0 (0.00)	16 (2.16)
American Indian/ Alaska Native	0 (0.00)	2 (1.04)	2 (0.27)
Other	2 (0.36)	6 (3.11)	8 (1.08)

Agreement for Pregnant Females by Risk and Trimester

A comparison was performed between the ARCHITECT HBsAg assay results and the comparator HBsAg assay results using serum samples obtained from a total of 741 females at low risk or increased risk for HBV infection. Data were analyzed by risk and by trimester. The data are summarized in the tables below.

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

ARCHITECT HBsAg Results ¹	First Trimester			Second Trimester			Third Trimester		
	Comparator HBsAg Results ²			Comparator HBsAg Results ²			Comparator HBsAg Results ²		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Reactive	0	0	0	1	2 ³	3	0	2 ³	2
Nonreactive	0	24	24	0	258	258	0	261	261
Total	0	24	24	1	260	261	0	263	263

² Includes retesting and confirmatory testing performed according to the comparator package insert.
¹ Includes retesting of initial reactives.
³ One specimen was tested and determined to be repeatedly reactive, not confirmed by the ARCHITECT HBsAg Confirmatory assay but was not further tested. One specimen was tested and determined to be confirmed positive by the ARCHITECT HBsAg Confirmatory assay and was determined to be negative for anti-HBc IgM, anti-HBc, HBeAg, anti-HBe, and HBV DNA, but positive for anti-HBs.
⁴ One specimen was tested and determined to be repeatedly reactive, not confirmed by the ARCHITECT HBsAg Confirmatory assay but was not further tested. One specimen was tested and determined to be confirmed positive by the ARCHITECT HBsAg Confirmatory assay and was determined to be negative for anti-HBc IgM, anti-HBc, HBeAg, anti-HBe, and HBV DNA, but indeterminate for anti-HBs.

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

ARCHITECT HBsAg Results ¹	First Trimester			Second Trimester			Third Trimester		
	Comparator HBsAg Results ²			Comparator HBsAg Results ²			Comparator HBsAg Results ²		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Reactive	0	0	0	0	0	0	1	1 ³	2
Nonreactive	0	7	7	0	74	74	0	110	110
Total	0	7	7	0	74	74	1	111	112

² Includes retesting and confirmatory testing performed according to the comparator package insert.
¹ Includes retesting of initial reactives.
³ This specimen was tested and determined to be repeatedly reactive, not confirmed by the ARCHITECT HBsAg Confirmatory assay but was not further tested.

Overall Summary and Percent Agreement for Pregnant Females

The table below summarizes the frequency of reactivity of the ARCHITECT HBsAg assay and the comparator HBsAg assay from a total of 741 females at low risk and increased risk for HBV infection.

ARCHITECT HBsAg Results ¹	Comparator HBsAg Results ²		Total n (%)
	Positive n (%)	Negative n (%)	
Reactive	2 (100.0)	5 (0.68)	7 (0.94)
Nonreactive	0 (0.00)	734 (99.32)	734 (99.06)
Total	2 (0.27)	739 (99.73)	741 (100.00)

² Includes retesting and confirmatory testing performed according to the comparator package insert.
¹ Includes retesting of initial reactives.

The table below summarizes the percent agreement between the ARCHITECT HBsAg assay results and the comparator HBsAg assay results for the pregnant female population and shows the 95% confidence intervals.

Subjects	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Pregnant Females	100.00% (2/2)	15.81 - 100.00	99.32% (734/739)	98.43 - 99.78

Clinical Performance in a Pediatric Population

Of the 2946 specimens tested in the ARCHITECT HBsAg clinical study, 100 specimens were from a pediatric population. The specimens were obtained from a commercial vendor, which collected the specimens from a collection site located in Fall River, MA. The specimens were obtained from children ages 2 to 18 years. Testing of these specimens was performed at the clinical site located in Galveston, TX.

The data are summarized by age and gender in the following table.

Age Group (Years)	Gender	Reactive n (%)	Nonreactive n (%)	Total
2 to 12	F	0 (0.00)	28 (100.00)	28
	M	0 (0.00)	22 (100.00)	22
13 to 18	F	0 (0.00)	41 (100.00)	41
	M	0 (0.00)	9 (100.00)	9
Total		0 (0.00)	100 (100.00)	100

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Detectable Concentration of HBsAg at the Cutoff

The sensitivity of the ARCHITECT HBsAg assay is designed to be ≤ 0.2 ng/mL.

The sensitivity of the ARCHITECT HBsAg assay was evaluated using a 17-member sensitivity panel consisting of eight HBsAg subtype *ad* members, eight HBsAg subtype *ay* members, and a nonreactive blank. The panel members were tested in replicates of five using three reagent lots across two instruments. The HBsAg level at the assay's cutoff was estimated from a linear regression analysis. The data are summarized in the following table.

Sample	Mean Sensitivity (ng/mL)	Mean Sensitivity (Approximate IU/mL)	Upper One-Sided 95% Confidence Limit (ng/mL)
HBsAg Sensitivity Panel (<i>ad</i> subtype)	0.15	0.03	0.18
HBsAg Sensitivity Panel (<i>ay</i> subtype)	0.13	0.02	0.15

In addition, the sensitivity of the ARCHITECT HBsAg assay was evaluated using serial dilutions of the WHO 1st International Standard. The dilutions ranged from 0.0195 to 2.5 IU/mL. Recalcified nonreactive human plasma was used as the diluent and represented the 0 IU/mL sample. The dilutions were tested in replicates of five using three reagent lots across two instruments. The HBsAg level at the assay's cutoff was estimated from a linear regression analysis. The data are summarized in the following table.

Sample	Mean Sensitivity (Approximate IU/mL)	Upper One-Sided 95% Confidence Limit (IU/mL)
Dilutions of WHO HBsAg Standard	0.02	0.03

Analytical Specificity

The ARCHITECT HBsAg assay was evaluated for potential cross-reactivity for specimens from individuals with medical conditions unrelated to HBV infection. A total of 161 specimens from 17 different categories were tested. The first 13 of 17 categories were antibody, antigen, or PCR positive. One hundred fifty-nine specimens were nonreactive and two specimens were reactive by ARCHITECT HBsAg. One of the two reactive specimens was confirmed positive for HBsAg by ARCHITECT HBsAg Confirmatory. The data are summarized by final interpretation in the following table.

Category	n	Comparator HBsAg Assay					
		Negative			Positive		
		ARCHITECT			ARCHITECT		
		NR*	RR*	POS*	NR*	RR*	POS*
Cytomegalovirus (Anti-CMV Positive)	9	9	0	0	0	0	0
Epstein-Barr Virus (Anti-EBV Positive)	10	10	0	0	0	0	0
Hepatitis A Virus (Anti-HAV Positive)	10	10	0	0	0	0	0
Hepatitis C Virus (Anti-HCV Positive)	10	9	0	1	0	0	0
Herpes Simplex Virus (HSV) IgG	10	10	0	0	0	0	0
Human Anti-Mouse Antibodies (HAMA)	10	10	0	0	0	0	0
Human Immunodeficiency Virus (Anti-HIV-1 Positive)	10	9	1	0	0	0	0
Parvovirus B19 Infection	9	9	0	0	0	0	0
Rheumatoid Factor Positive	10	10	0	0	0	0	0
Rubella	10	10	0	0	0	0	0
Syphilis	10	10	0	0	0	0	0
Systemic Lupus Erythematosus (SLE)	10	10	0	0	0	0	0
Toxoplasmosis IgG Positive	8	8	0	0	0	0	0
Influenza Vaccine Recipient	10	10	0	0	0	0	0
Non-Viral Liver Disease: Alcoholic Liver Disease	10	10	0	0	0	0	0
Non-Viral Liver Disease: Hepatocellular Carcinoma	5	5	0	0	0	0	0
Non-Viral Liver Disease: Obstructive Jaundice	10	10	0	0	0	0	0
Total	161	159	1	1	0	0	0

*NR = Nonreactive, RR = Repeatedly Reactive Not Confirmed, POS = Positive

Interference

At the concentrations listed below, the ARCHITECT HBsAg assay showed interference from bilirubin, total protein, hemoglobin, and triglycerides for high negative samples (targeted to an S/CO of 0.80) of ≤ 0.11 S/CO and low positive samples (targeted to an S/CO of 1.20) of $\leq 10\%$.

Interferent Interferent Concentration

- Bilirubin ≤ 20 mg/dL
- Total Protein ≤ 12 g/dL
- Hemoglobin ≤ 400 mg/dL
- Triglycerides ≤ 1100 mg/dL

In addition, high negative (0.80 S/CO target) and low positive (1.20 S/CO target) serum samples were supplemented with viral or parasitic antigens (cytomegalovirus, Epstein-Barr virus, herpes simplex virus, rubella, varicella-zoster virus, and *Toxoplasma gondii*) and with bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). The viral or parasitic antigens were spiked to 1 ng/mL and 1 μ g/mL. The bacteria were supplemented to $10^{5.5}$, $10^{3.4}$, and $10^{2.3}$ colony-forming units per mL. All samples were tested in replicates of 22. All replicates of the high negative samples (0.80 S/CO target) remained nonreactive and all replicates of the low positive samples (1.20 S/CO target) remained reactive.

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Tube Type Matrix Comparison

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

- Glass: serum and serum separator
- Plastic: serum, serum separator, dipotassium EDTA

On average, the tube types listed in the table below showed less than a 10% difference when compared to the control tube type (glass serum) for high negative samples (S/CO range: 0.60 to 0.99) and low positive samples (S/CO range: 1.00 to 1.40).

The ARCHITECT HBsAg assay showed the following distribution of percent differences when compared to the glass serum tube type.

Tube Type	Distribution of the Differences		
	< 10%	≥ 10% to ≤ 20%	> 20%
Glass Serum Separator	82.2% (37/45)	13.3% (6/45)	4.4% (2/45)
Plastic Serum	87.0% (40/46)	10.9% (5/46)	2.2% (1/46)
Plastic Serum Separator	82.6% (38/46)	10.9% (5/46)	6.5% (3/46)
Plastic Dipotassium EDTA	89.1% (41/46)	8.7% (4/46)	2.2% (1/46)

Seroconversion Panels

To determine the seroconversion sensitivity, 15 HBV seroconversion panels obtained from commercial vendors were tested using the ARCHITECT HBsAg and ARCHITECT HBsAg Confirmatory assays. HBsAg was first detected by the ARCHITECT HBsAg assay and confirmed by the ARCHITECT HBsAg Confirmatory assay 5 to 35 days earlier than it was first detected by the comparator assays in nine seroconversion panel sets and coincident with the first day detected by the comparator assays in six seroconversion panel sets. The data are summarized in the following table.

Panel ID	Days to HBsAg Reactive Result from Initial Draw Date		Difference in Days to HBsAg Reactive Result (Comparator - ARCHITECT)
	Comparator HBsAg Assay	ARCHITECT HBsAg Assay	
PHM903	17	10	7
PHM909	14	9	5
PHM915	54	19	35
PHM916	69	62	7
PHM917	43	43	0
PHM920	26	26	0
PHM923	21	15	6
6271	12	7	5
6272	115	97	18
6273	25	25	0
6274	167	156	9
6275	22	22	0
0994/3457	14	4	10
26982/14399	0	0	0
43527/3453	0	0	0

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.²⁶ 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.²⁶⁻³¹ HBsAg mutations may result in a less favorable outcome in some patients^{26,27,29} and false negative results in some HBsAg assays.^{26,28}

The immunodominant "a" determinant portion of the HBsAg protein spans the region bound by amino acids 100-158.³⁴ This region includes at least two antigenic loops; the second loop (amino acids 139-147) binds a large proportion of anti-HBs in immune serum.^{26,29} Immunological pressure by anti-HBs, whether induced by natural infection, vaccination, or therapeutic administration, may be a method by which HBsAg mutants are selected.^{26,29} The most frequent and stable mutation reported is the glycine to arginine mutation at amino acid position 145 (Gly 145 to Arg) in the second loop of the "a" determinant.²⁶

A panel of 26 recombinant HBsAg mutant samples were prepared as described by Coleman *et al.*³⁵ except for the Thr 123 to Ala panel member, which was expressed in serum-free tissue culture media instead of a fetal calf serum-containing tissue culture media. Twenty-four of the 26 samples spanned the "a" determinant region; the other two samples have mutations outside of the "a" determinant region. Each sample was prepared to a concentration of approximately 2 ng/mL and tested by ARCHITECT HBsAg. One of the 24 "a" determinant samples was nonreactive by ARCHITECT HBsAg. All of the remaining 25 samples were repeatedly reactive by the ARCHITECT HBsAg assay and confirmed positive by the ARCHITECT HBsAg Confirmatory assay. These results are consistent with the detection of the most common HBsAg mutants as reported in the literature.³⁵⁻³⁷ The data are summarized in the following table.

Mutant	ARCHITECT HBsAg Results	ARCHITECT HBsAg Confirmatory Final Interpretation	Mutant (Continued)	ARCHITECT HBsAg Results	ARCHITECT HBsAg Confirmatory Final Interpretation
Asn 40 to Ser	Repeatedly Reactive	Positive	Pro 142 to Ser	Repeatedly Reactive	Positive
Pro 111 to Thr	Repeatedly Reactive	Positive	Asp 144 to Ala	Repeatedly Reactive	Positive
Thr Thr 115, 116 to Ile Ile	Repeatedly Reactive	Positive	Gly 145 to Ala	Repeatedly Reactive	Positive
Thr 118 to Ser	Repeatedly Reactive	Positive	Gly 145 to Arg	Repeatedly Reactive	Positive
Thr 123 to Ala	Nonreactive	NA	Gly 145 to Lys	Repeatedly Reactive	Positive
Thr 123 to Ile	Repeatedly Reactive	Positive	Thr 126 to Ser + Gly 145 to Arg	Repeatedly Reactive	Positive
Asn 40 to Ser + Thr 123 to Ile	Repeatedly Reactive	Positive	Pro 142 to Leu + Gly 145 to Arg	Repeatedly Reactive	Positive
Gln 129 to His	Repeatedly Reactive	Positive	Pro 142 to Ser + Gly 145 to Arg	Repeatedly Reactive	Positive
Thr 131 to Ile	Repeatedly Reactive	Positive	Asp 144 to Ala + Gly 145 to Arg	Repeatedly Reactive	Positive
Met 133 to Leu	Repeatedly Reactive	Positive	Thr 148 to His	Repeatedly Reactive	Positive
Phe 134 to Ala	Repeatedly Reactive	Positive	Ser 154 to Trp	Repeatedly Reactive	Positive
Pro 135 to Ser	Repeatedly Reactive	Positive	Ser 155 to Tyr	Repeatedly Reactive	Positive
Pro 142 to Leu	Repeatedly Reactive	Positive	Met Met Met 197, 198, 199 to Ser Ser Ser	Repeatedly Reactive	Positive

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HBV Genotype Detection

The ARCHITECT HBsAg assay is designed to detect HBV genotypes A through G.

A binding epitope for ARCHITECT HBsAg is conserved across all known genotypes of HBV.^{34,36,38} A study was performed to evaluate the ability of the ARCHITECT HBsAg assay to detect different HBV genotypes by testing a commercially-available genotype panel containing genotypes A through G. All genotypes were detected by ARCHITECT HBsAg and ARCHITECT HBsAg Confirmatory. The data are summarized in the following table.

Genotype	Number of Genotypes Tested	Number of ARCHITECT HBsAg Reactive/ ARCHITECT HBsAg Confirmatory Positive
A	5	5
B	1	1
C	7	7
D	3	3
E	6	5*
F	11	11
G	1	1
Total	34	33

* One genotype E sample was nonreactive by ARCHITECT HBsAg and an FDA-licensed HBsAg 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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