



HBsAg
Confirmatory V.1
REF 9C94
B9C940
77-3664/R9



HBsAg Confirmatory V.1

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

Note Changes Highlighted

IVD



Key to symbols used			
REF	List Number	LOT	Lot Number
IVD	<i>In Vitro</i> Diagnostic Medical Device		Expiration Date
	Store at 2-8°C	CONTROL NO.	Control Number
	CAUTION: Consult accompanying documents	REACTION VESSELS	Reaction Vessels
SN	Serial Number	SAMPLE CUPS	Sample Cups
	Manufacturer	SEPTUMS	Septums
		REPLACEMENT CAPS	Replacement Caps
		REAGENT LOT	Reagent Lot

NAME

ARCHITECT® HBsAg Confirmatory V.1

INTENDED USE

The ARCHITECT HBsAg Confirmatory V.1 assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the confirmation of the presence of Hepatitis B surface antigen (HBsAg) in human serum and plasma by means of specific antibody neutralization.

SUMMARY AND EXPLANATION OF TEST

Enzyme immunoassays for the detection of HBsAg were first described by Engvall and Perlmann¹⁻³ 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 HBsAg assays are routinely used to aid in the diagnosis of suspected hepatitis B viral (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. A sample which is found to be repeatedly reactive should be confirmed by the ARCHITECT HBsAg Confirmatory V.1 (9C94) assay, a neutralization procedure utilizing human anti-HBs. If the sample is neutralized in the ARCHITECT HBsAg Confirmatory V.1 assay, the sample is considered positive for HBsAg. It is recommended that confirmatory testing be performed prior to disclosure of HBV status.

ARCHITECT HBsAg Confirmatory V.1 uses the principle of specific antibody neutralization to confirm the presence of HBsAg in samples found to be repeatedly reactive. Antibody to hepatitis B surface antigen (anti-HBs) (human) is incubated with a sample. If HBsAg is present in the sample, it will be neutralized by the antibody. The neutralized HBsAg is subsequently blocked from binding to the anti-HBs coated microparticles. A reduction of signal occurs when compared to the signal of a paired sample that has not been treated with the antibody reagent. A sample is considered positive if the signal for the non-neutralized sample (incubated with Pretreatment 2 [Reagent 2]) result is greater than or equal to the cutoff ($S/CO \geq 1.00$) and the RLU of the neutralized sample is reduced by at least 50% compared to the non-neutralized sample.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT HBsAg Confirmatory V.1 assay is a two-step pretreatment immunoassay, using Chemiluminescent Microparticle Immunoassay (CMIA) technology, for the confirmation of the presence of HBsAg in human serum and plasma.

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 antibody in Pretreatment 1 (Reagent 1). An aliquot of the pretreated sample and anti-HBs coated paramagnetic microparticles are combined in another RV. 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 in the second step. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the ARCHITECT *i** optical system.

In a separate RV, sample and Pretreatment 2 (Reagent 2) are combined and incubated. An aliquot of the pretreated sample and anti-HBs coated paramagnetic microparticles are combined in another RV. 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. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as RLUs. A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the ARCHITECT *i* optical system.

If the signal for the non-neutralized sample (incubated with Pretreatment 2 [Reagent 2]) result is greater than or equal to the cutoff ($S/CO \geq 1.00$) 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.

* *i* = immunoassay

REAGENTS

Reagent Kit, 50 Tests

ARCHITECT HBsAg Confirmatory V.1 Reagent Kit (9C94-25)

- **MICROPARTICLES** 1 Bottle (6.6 mL) Anti-HBs (Mouse, Monoclonal, IgM, IgG) Coated Microparticles in MES buffer with protein stabilizers. Minimum concentration: 0.0675% solids. Preservative: ProClin® 300.
- **CONJUGATE** 1 Bottle (5.9 mL) Conjugate: Anti-HBs (Goat, IgG) Acridinium-Labeled Conjugate in MES buffer with protein stabilizers (Bovine and Human Plasma; nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV). Minimum concentration: 0.25 µg/mL. Preservative: ProClin 300.
- **PRE-TREATMENT 1** 1 Bottle (2.4 mL) Pretreatment 1 containing recalcified human plasma; reactive for anti-HBs and nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV. Preservative: ProClin 300.
- **PRE-TREATMENT 2** 1 Bottle (2.4 mL) Pretreatment 2 containing recalcified human plasma; nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, anti-HCV and anti-HBs. Preservative: Antimicrobial Agent and ProClin 300.

Assay Diluent

ARCHITECT HBsAg Manual Diluent (6C36-40)

- **MANUAL DILUENT** 1 Bottle (100 mL) ARCHITECT HBsAg Manual Diluent containing recalcified human plasma; nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, anti-HCV and anti-HBs. Preservative: Antimicrobial Agent and 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 0.35 mol/L 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.

-  **CAUTION: This product contains human sourced infectious and/or potentially infectious components. Refer to the REAGENTS section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, it is recommended that all human sourced materials be considered potentially infectious and handled with appropriate biosafety practices.**

Safety Precautions

- Microparticles, Conjugate and Manual Diluent 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.

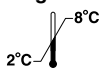
For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.

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

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not mix reagents from different reagent kits.**
- Prior to loading the ARCHITECT HBsAg Confirmatory V.1 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.**
- Prior to 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 an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

-  The ARCHITECT HBsAg Confirmatory V.1 Reagent Kit, Calibrators, and Controls must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT HBsAg Confirmatory V.1 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 on-board time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** After reagents are removed from the system, you must initiate a scan to update the on-board 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 will require retesting. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT HBsAg Confirmatory V.1 assay file must be installed on the ARCHITECT *i* System from the ARCHITECT *i* assay CD-ROM prior to performing the assay. For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human serum (including serum collected in serum separator tubes) or plasma collected in potassium EDTA, lithium heparin, sodium heparin, sodium citrate, ACD, CPDA-1, CP2D, CPD, and potassium oxalate may be used in the ARCHITECT HBsAg Confirmatory V.1 assay. Other anticoagulants have not been validated for use with the ARCHITECT HBsAg Confirmatory V.1 assay. Follow the tube manufacturer's processing instructions for serum or plasma collection tubes.

- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify the correct specimen types are used in the ARCHITECT HBsAg Confirmatory V.1 assay.
- Performance has not been established using cadaver specimens or body fluids other than human serum or plasma.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- This assay was designed and validated for use with human serum or plasma from individual patient and donor specimens. Pooled specimens must not be used since the accuracy of their test results has not been validated.
- Do not use heat-inactivated specimens.
- Do not use grossly hemolyzed specimens.
- For optimal results, inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- **For optimal results, serum and plasma specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give inconsistent results and must be transferred to a centrifuge tube and centrifuged at least 10,000 RCF (Relative Centrifugal Force) for 10 minutes.**
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- Specimens from heparinized patients may be partially coagulated and erroneous results could occur due to the presence of fibrin. To prevent this phenomenon, draw the specimen prior to heparin therapy.
- **Gravity separation is not sufficient for specimen preparation. Specimens must be separated from clots or red blood cells using centrifugation as recommended by the tube manufacturer.**
- Specimens may be stored on or off the clot or red blood cells for up to 14 days at 2-8°C.
- If testing will be delayed more than 14 days, remove serum or plasma from the clot, serum separator or red blood cells, and store frozen (-20°C or colder).
- **Frozen specimens must be mixed THOROUGHLY after thawing by LOW speed vortexing.**
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.
- No qualitative differences were observed between experimental controls and the 24 spiked reactive specimens subjected to 6 freeze-thaw cycles; however, multiple freeze-thaw cycles should be avoided.
- When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of specimens and infectious substances. Specimens may be shipped ambient, at 2-8°C (wet ice), or -20°C or colder (dry ice). Do not exceed the storage time limitations listed above. Prior to shipment, it is recommended that specimens be removed from the clot, serum separator or red blood cells.
- No qualitative performance differences were observed between experimental controls and the 21 spiked reactive specimens tested with elevated levels of triglycerides ($\leq 3,000$ mg/dL).
- No qualitative performance differences were observed between experimental controls and the 28 spiked reactive specimens tested with red blood cells at $\leq 0.4\%$ v/v.
- No qualitative performance differences were observed between experimental controls and the 21 spiked reactive specimens tested with elevated levels of protein (≤ 12 g/dL).
- No qualitative performance differences were observed between experimental controls and the 19 spiked reactive specimens tested with elevated levels of bilirubin (≤ 20 mg/dL).
- No qualitative performance differences were observed between experimental controls and the 22 spiked reactive specimens tested with elevated levels of hemoglobin (≤ 500 mg/dL).
- ARCHITECT HBsAg Confirmatory V.1 Calibrators and Controls must be mixed by gentle inversion prior to use.

PROCEDURE

Materials Provided:

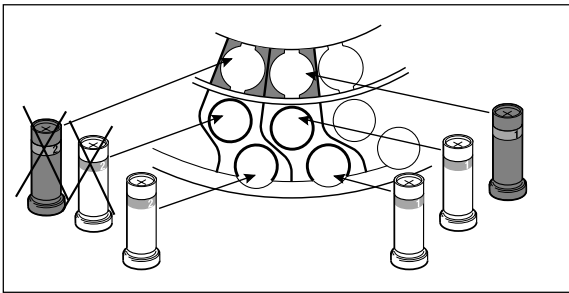
- 9C94-25 ARCHITECT HBsAg Confirmatory V.1 Reagent Kit

Materials Required but not Provided:

- ARCHITECT *i* System
- 9C94-01 ARCHITECT HBsAg Confirmatory V.1 Calibrators
- 9C94-10 ARCHITECT HBsAg Confirmatory V.1 Controls
- 6C36-40 ARCHITECT HBsAg **MANUAL DILUENT**
- 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**
- For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

Assay Procedure

- Before loading the ARCHITECT HBsAg Confirmatory V.1 Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment:
 - **Invert the microparticle bottle 30 times.**
 - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
 - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Squeeze the septum in half to confirm that the slits are open. Carefully snap the septum onto the top of the bottle.
 - **If the microparticles do not resuspend, DO NOT USE; contact your local Abbott representative.**
- Load the ARCHITECT HBsAg Confirmatory V.1 Reagent Kit on the ARCHITECT *i* System. Verify that all necessary reagents are present. Ensure that septums are present on all reagent bottles.
 - The reagent carousel has color coded rings which match the colored bands on the reagent bottle labels. The green bands on the Pretreatment 1 (Reagent 1) and Pretreatment 2 (Reagent 2) bottle labels have a #1 or #2 designation, respectively.
 - The diagram below can be used to facilitate reagent loading.



- Place the bottle with the yellow band (Conjugate) in the yellow ring of the carousel (the innermost ring - with the barcodes on the bottle facing away from you).
- Place the bottle with the pink band (Microparticles) in the pink ring of the carousel (the middle ring).
- Place the bottle with the green band #1 Pretreatment 1 (Reagent 1) in the green ring of the carousel (the outermost ring - with the barcodes on the bottle facing away from you).
- Place the bottle with the green band #2 Pretreatment 2 (Reagent 2) in the green ring of the carousel to the immediate **left** of the bottle with the #1 designation (the outermost ring - with the barcodes on the bottle facing away from you).

- Do not place HBsAg Confirmatory V.1 Pretreatment 1 (Reagent 1) bottle with a #1 designation in green ring position 25 of the reagent carousel and Pretreatment 2 (Reagent 2) bottle with a #2 designation in green ring position 1 of the reagent carousel.
- **NOTE: HBsAg Confirmatory Reagent Kits have only one each of the yellow and pink colored bottles. The carousel positions to the immediate left of the yellow and pink bottles are to be left empty.**
- For information on ordering patient specimens, calibrators and controls, and general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- Order calibration, if necessary (refer to the ARCHITECT System Operations Manual, Section 5, Subsection Loading - Loading Samples and Sample Carriers).
- To perform an ARCHITECT HBsAg Confirmatory V.1 calibration, test Calibrators 1 and 2 in triplicate. The minimum sample cup volume required for ordering 3 replicates of each of the Calibrators is 338 μ L for each calibrator. This volume is also reflected on the **Calibrator Order** screen and on the Orderlist report. A single sample of both levels of HBsAg Confirmatory V.1 controls must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the Control package insert. Calibrators should be priority loaded.
 - ARCHITECT HBsAg Confirmatory V.1 Calibrators must be mixed by gentle inversion prior to use. To obtain the recommended volume requirements for the ARCHITECT HBsAg Confirmatory V.1 Calibrators, hold the bottles **vertically**, and dispense 15 drops of each Calibrator (for three replicates) into each respective sample cup.
- Once an ARCHITECT HBsAg Confirmatory V.1 calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
 - A reagent kit with a new lot number is used
 - Controls are out of range
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

Controls and Specimens

- Order tests (refer to the ARCHITECT System Operations Manual, Section 5, Subsection Ordering).
- **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 a patient specimen, after selecting the HBsAg Confirmatory V.1 assay, select **F5 - Assay Options**, select Module - Auto (default mode if working with *i2000*), Select Manual (if working with *i4000* or *i8000*) and select the Module of choice, then type the number of replicates for Reagent 1 and Reagent 2 (same as Pretreatment 1 and Pretreatment 2). **Please note that in the default method no number of replicates is specified for Reagent 2. In order to obtain a valid test result, this number needs to be defined manually.**
- The minimum sample cup volume required to perform the HBsAg Confirmatory V.1 assay on the ARCHITECT *i* System is 242 μ L (sample volume for Pretreatment 1 [Reagent 1] and Pretreatment 2 [Reagent 2]) for the first HBsAg confirmation plus 200 μ L for each additional HBsAg confirmation from the same sample cup. No more than 5 replicates may be sampled from the same sample cup. Verify the minimum sample volume is present in the sample cup prior to running the test. The minimum sample cup volume is calculated by the system and is printed on the Order list report.
 - ARCHITECT HBsAg Confirmatory V.1 Controls must be mixed by gentle inversion prior to use. To obtain the recommended volume requirements for ARCHITECT HBsAg Confirmatory V.1 Controls, hold the bottles vertically, and dispense 6 drops of each Control (for one replicate) into each respective sample cup.
 - To minimize the effects of evaporation, all samples (patient specimens, calibrators, and controls) must be tested within 3 hours of being placed on-board the ARCHITECT *i* System. If the sample is on-board the system for longer than 3 hours, additional sample volume is required. For additional information on sample evaporation and volumes, refer to the ARCHITECT System Operations Manual, Section 5.

- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Load samples.
 - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN. The ARCHITECT *i* System performs the following functions:
 - Moves the sample to the aspiration point.
 - Loads a RV into the process path.
 - Aspirates and transfers sample into the RV.
 - Advances the RV one position and adds Pretreatment 1 (Reagent 1) to the sample.
 - Mixes and incubates.
 - Transfers an aliquot of the pretreated sample to a second RV.
 - Advances the RV one position and transfers microparticles into the RV.
 - Mixes, incubates, and washes the reaction mixture.
 - Adds conjugate to the RV.
 - Mixes, incubates, and washes the reaction mixture.
 - Adds Pre-Trigger and Trigger Solutions.
 - Measures chemiluminescent emission to confirm the presence of HBsAg in the sample.
 - Aspirates contents of RV to liquid waste and unloads RV to solid waste.
 - Calculates the S/CO result.
 - Repeats sequence for sample and Pretreatment 2 (Reagent 2).
- Operator needs to calculate % Neutralization using results from both Pretreatment 1 and 2 (Reagent 1 and 2) tests.
 - Obtain Calibrator 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 results or select **Print F4**.
 - If **Print F4** was selected, select **Calibration Details Report** to Print Calibrator mean RLU values.
 - Obtain sample Pretreatment (1 and/or 2) same as Reagent (1 and/or 2) RLU and S/CO from the ARCHITECT *i* System as follows:
 - Select **Results** icon, then select **Results review** from the drop down menu. If your system is configured for auto release of results, you can find the results in the **Store Results** screen.
 - Select the required specimen(s).
 - Select **Details F5** to view results or select **Print F4**.
 - If **Print F4** was selected, select **Result Details Report** to Print Sample RLU and S/CO values.
- See **RESULTS** section for % Neutralization calculation.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ARCHITECT System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

- A Manual Dilution Procedure may be performed if the ARCHITECT HBsAg Confirmatory V.1 assay result is reactive but is not neutralized (specimen pretreated with Pretreatment 2 [Reagent 2] S/CO \geq 1.00 and % Neutralization < 50%).
- Refer to **RESULTS** section for further information.
 - The suggested dilution for ARCHITECT HBsAg Confirmatory V.1 assay is 1:500.
 - Add 25 μ L of the patient specimen to 475 μ L of ARCHITECT HBsAg Manual Diluent for a 1:20 dilution.
 - Add 20 μ L of the 1:20 dilution to 480 μ L of ARCHITECT HBsAg Manual Diluent for a 1:500 dilution.
 - Additional specimen dilutions may be performed if the 1:500 dilution result is still reactive but not neutralized.
 - For a 1:20000 dilution, add 25 μ L of the 1:500 dilution to 975 μ L of ARCHITECT HBsAg Manual Diluent.
- **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.**
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

QUALITY CONTROL PROCEDURES

NOTE: It is recommended that the ARCHITECT HBsAg Confirmatory V.1 Positive Control and Negative Control be run in order to verify the calibration.

The recommended control requirement for the ARCHITECT HBsAg Confirmatory V.1 assay is a single sample of each control tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures. Ensure that assay Control values are within the ranges specified in the Control package insert.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT HBsAg Confirmatory V.1 assay belongs to method group 4.

RESULTS

The ARCHITECT *i* System calculates the Calibrator 1 and Calibrator 2 mean RLU from three replicates of each calibrator and stores the result.

Calculation

The ARCHITECT HBsAg Confirmatory V.1 assay calculates results based on a cutoff and sample to cutoff (S/CO) determination by the following calculations:

- Cutoff = Calibrator 1 Mean RLU + (0.0075 x [Calibrator 2 Mean RLU – Calibrator 1 Mean RLU])
- S/CO = Sample RLU/Cutoff RLU

The % Neutralization is calculated by the operator as described below.

- Match up the Pretreatment 1 and 2 (Reagent 1 and 2) values for the same sample to calculate % Neutralization.

$$\% \text{ Neutralization}^* = \frac{(\text{Sample's Pretreatment 2 [Reagent 2] RLU} - \text{Sample's Pretreatment 1 [Reagent 1] RLU})}{(\text{Sample's Pretreatment 2 [Reagent 2] RLU} - \text{Calibrator 1 Mean RLU})} \times 100$$

* Calculate out to a single decimal place.

Interpretation of Results

Positive - If the non-neutralized sample (incubated with Pretreatment 2 [Reagent 2]) S/CO result \geq 1.00 and the percent neutralization is \geq 50%, the sample is considered confirmed positive for HBsAg.

Negative - If the non-neutralized sample (incubated with Pretreatment 2 [Reagent 2]) S/CO result < 1.00, the sample is considered repeatedly reactive, not confirmed for HBsAg.

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

DILUTION	S/CO (Pretreatment 2 [Reagent 2])	% NEUTRALIZATION	FINAL INTERPRETATION
NEAT	< 1.00	N/A*	Repeatedly Reactive, Not Confirmed
	\geq 1.00	\geq 50%	Confirmed Positive
	\geq 1.00	< 50%	Go to 1:500 Dilution
1:500	< 1.00	N/A	Repeatedly Reactive, Not Confirmed
	\geq 1.00	\geq 50%	Confirmed Positive
	\geq 1.00	< 50%	Go to 1:20000 Dilution
1:20000	< 1.00	N/A	Repeatedly Reactive, Not Confirmed
	\geq 1.00	\geq 50%	Confirmed Positive
	\geq 1.00	< 50%	Repeatedly Reactive, Not Confirmed

* N/A = Not Applicable

NOTE: Follow the dilution and final interpretation routine as outlined in the table above, even if negative neutralization or > 100% neutralization results are obtained.

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). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.^{15,16} Additional clinical or diagnostic information may be required to determine patient status.
- If the HBsAg Confirmatory V.1 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute or chronic infection.
- Samples containing particulate matter or red blood cells must be centrifuged prior to running the assay.
- Performance has not been established using cadaver specimens or body fluids other than human serum or plasma.
- Do not use heat-inactivated specimens.
- Specimens from heparinized patients may be partially coagulated and erroneous results could occur due to the presence of fibrin. To prevent this phenomenon, draw the specimen prior to heparin therapy.
- If you run ARCHITECT HBsAg Confirmatory on the same module as the ARCHITECT B12 assay, refer to labeling of the B12 assay for additional information and instructions regarding accumulation of protein in the sample probe.

SPECIFIC PERFORMANCE CHARACTERISTICS

Confirmation of HBsAg Reactive Specimens

A total of 6379 specimens from the following categories were evaluated with the ARCHITECT HBsAg assay: volunteer whole blood donors, hospital patients, medical conditions unrelated to HBV infection and potentially interfering substances, preselected HBsAg positive, acute HBV infection, chronic HBV infection, and increased risk for HBV infection (Table I). There were a total of 426 repeatedly reactive specimens tested with the ARCHITECT HBsAg Confirmatory V.1 assay to confirm the presence of HBsAg. In 418 of the 426 specimens (98.12%), the presence of HBsAg was confirmed.

TABLE I
Confirmation of ARCHITECT HBsAg Reactive Specimens by ARCHITECT HBsAg Confirmatory V.1

Category	Number Tested	ARCHITECT HBsAg Assay Number of Repeatedly Reactive (% of Total)	ARCHITECT HBsAg Confirmatory V.1 Positive ^a (% of Repeatedly Reactive)
Volunteer Whole Blood Donors	5043	8 (0.16%)	1 (12.50%)
Hospital Patients	500	3 (0.60%)	3 (100.00%)
Medical Conditions Unrelated to HBV Infection and Potentially Interfering Substances ^b	333	7 (2.10%)	6 (85.71%)
Preselected HBsAg Positive	343	343 ^c (100.00%)	343 (100.00%)
Acute HBV Infection	10	10 (100.00%)	10 (100.00%)
Chronic HBV Infection	50	50 (100.00%)	50 (100.00%)
Increased Risk for HBV Infection ^d	100	5 (5.00%)	5 (100.00%)
TOTAL	6379	426 (6.68%)	418 (98.12%)

^a A specimen is considered positive if the signal for the non-neutralized specimen (incubated with Pretreatment 2 [Reagent 2]) result is greater than or equal to the cutoff ($S/CO \geq 1.00$) and the RLU of the neutralized specimen is reduced by at least 50% compared to the non-neutralized specimen.

^b Category included the following: anti-CMV positive (10), anti-EBV positive (10), anti-HSV (10), anti-HAV (10), anti-HCV (10), anti-HIV-1 (10), HBV vaccine recipients (10), rubella antibody positive (10), toxoplasma antibody positive (10), *E. coli* infections (10), yeast infections (10), syphilis positive (10), anti-nuclear antibody positive (10), rheumatoid factor (10), multiple myeloma (10), multiparous females (10), alcoholic Cirrhosis (10), and pregnant females (163).

^c Specimens were tested once.

^d Category included the following: intravenous drug users (25), hemodialysis patients (25), hemophilia patients (25), men sex men (25).

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

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