ARCHITECT
CA 125 II Controls

REF 2K45-10

en

CA 125 II
2K45
602-016 8/14/R11
C2K450

Read Highlighted Changes: Revised November 2014.

INTENDED USE

The ARCHITECT CA 125 II Controls are for the verification of the accuracy and precision of the ARCHITECT iSystem when used for the quantitative determination of OC125 defined antigen in human serum and plasma.

Refer to the ARCHITECT CA 125 II reagent package insert for additional information.

CONTENTS

3 Bottles (8 mL each) of ARCHITECT CA 125 II Controls. The Low Control, Medium Control, and High Control contain OC125 defined antigen (human) prepared in TRIS buffer with protein (bovine) stabilizers. Preservatives: Sodium Azide and ProClin 300.

The following concentration ranges may be used for individual replicate control specifications on the ARCHITECT iSystem:

Control	Target CA 125 II Concentration (U/mL)	Range (U/mL)
CONTROL L	40	28.0 - 52.0
CONTROL M	300	210.0 - 390.0
CONTROL H	650	455.0 - 845.0

Each laboratory should establish its own concentration ranges for new control lots at each control level. This can be accomplished by assaying a minimum of 20 replicates over several (3-5) days. Sources of variation that can be expected should be included in this study in order to be representative of future system performance. These may include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules
- Data points collected at different times of the day

These results should be applied to your laboratory's quality control practices.

PRECAUTIONS

- IVD
- For *In Vitro* Diagnostic Use
- **CAUTION:** This product contains human-sourced and/or potentially infectious components. The controls contain antigen derived from a human cell line. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human-sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[1-4]

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

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

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

STORAGE

- Controls are stable until the expiration date when stored and handled as directed.
- Do not use past expiration date.
- 2°C – 8°C

PREPARATION FOR ANALYSIS

- Controls may be used immediately after removal from 2-8°C storage.
- Prior to use, mix by gentle inversion (5-10 times).
- After each use, tightly close the caps and return the controls to 2-8°C storage.

BIBLIOGRAPHY

1. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
2. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
3. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
4. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline–Third Edition*. CLSI Document M29-A3. Wayne, PA: CLSI; 2005.

Key to Symbols

	Caution
	Consult instructions for use
	Manufacturer
	Temperature limitation
	Use by/Expiration date
CONC	Concentration
CONTAINS AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL L	Control Low, Medium, High (L,M,H)
EC REP	Authorized Representative in the European Community
INFORMATION FOR USA ONLY	Information needed for United States of America only
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
PRODUCED FOR ABBOTT BY	Produced for Abbott by
PRODUCT OF USA	Product of USA
RANGE	Range
REF	List Number
WARNING: SENSITIZER	Warning: May cause an allergic reaction.


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Abbott

ARCHITECT CA 125 II

REF 2K45-23
REF 2K45-28
REF 2K45-38

en
CA 125 II
2K45
602-047 11/15/R09
B2K4U0

Revised November 2015.

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.

WARNING: CA 125 assay values obtained with different assay methods cannot be used interchangeably due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the CA 125 assay used. If, in the course of monitoring a patient, the assay method used for determining serial CA 125 levels is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory MUST confirm baseline values for patients being serially monitored.

NAME

ARCHITECT CA 125 II

INTENDED USE

The ARCHITECT CA 125 II assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of OC 125 defined antigen in human serum and plasma on the ARCHITECT iSystem.

The ARCHITECT CA 125 II assay is to be used as an aid in monitoring response to therapy for patients with epithelial ovarian cancer. Serial testing for patient CA 125 II assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer.

It is further intended to be used in conjunction with ARCHITECT HE4 as an aid in estimating the risk of epithelial ovarian cancer in premenopausal and postmenopausal women presenting with an adnexal mass who will undergo surgical intervention. The results must be interpreted in conjunction with other methods in accordance with standard clinical management guidelines.

SUMMARY AND EXPLANATION OF THE TEST

CA 125 II assay values are defined by using the OC 125 monoclonal antibody. OC 125 was generated through the hybridization of mouse myeloma cells to spleen cells from a mouse immunized with a human serous cystadenocarcinoma cell line called OVCA 433.[1] ARCHITECT CA 125 II is a second-generation assay for the detection of OC 125 defined antigen. The assay utilizes the OC 125 monoclonal antibody, as the capture antibody coated onto paramagnetic microparticles that bind molecules containing OC 125 defined antigen. These defined antigens are quantified using acridinium-labeled M11 antibody. The OC 125 monoclonal antibody is reactive with repeating OC 125 defined antigen expressed by a high percentage of nonmucinous ovarian carcinomas (serous, endometrioid, clear cell, and undifferentiated histologies) and epithelial ovarian carcinoma cell lines.[1, 2] OC 125 defined antigens were originally detected in normal peritoneal, pleural and pericardial tissues of both fetus and adult. In the fetus, OC 125 defined antigens have been localized in amniotic and umbilical epithelial and Müllerian epithelial tissues. In the adult, localization has been identified in endocervical and endometrial tissues and ovarian inclusion cysts and papillary excrescences. However, OC 125 defined antigens were not detected in fetal ovarian tissue or other normal adult ovarian tissues or benign mucinous ovarian tumors.[3] In serum, the OC 125 defined antigens are associated with high molecular weight glycoproteins heterogeneous in size and charge. The structure of the CA 125 molecule, including closely situated repeating epitopes for OC 125 and M11 antibodies has been proposed.[4]

Serum CA 125 II assay values are useful for monitoring the course of disease in patients with invasive epithelial ovarian cancer.[5] In a review of nine published studies, the overall correlation reported between CA 125 serum levels and the course of the disease was 87%.[6] Persistently rising CA 125 assay values may be associated with malignant disease and poor response to therapy, whereas decreasing CA 125 assay values may indicate a favorable response to therapy.[6-14]

A second-look, exploratory laparotomy may have been performed previously to assess response to therapy. The benefit has recently come into question because of high morbidity and low sensitivity in detecting residual or recurrent carcinoma.[15] In women with primary epithelial ovarian carcinoma who had undergone first-line therapy and were candidates for diagnostic second-look procedures, a CA 125 assay value greater than or equal to 35 U/mL was found to be indicative of the presence of residual tumor.[6, 9, 11, 13] However, a CA 125 assay value below 35 U/mL does not indicate the absence of residual ovarian cancer because patients with histopathologic evidence of ovarian carcinoma may have CA 125 assay values within the range of normal individuals.[7, 8]

Elevations of CA 125 assay values have been reported in approximately 1-2% of healthy individuals,[6, 7] and in individuals with nonmalignant conditions such as cirrhosis,[16, 17] hepatitis,[17, 18] endometriosis,[19-24] first trimester pregnancy,[25-27] ovarian cysts,[3, 28] and pelvic inflammatory disease.[10, 25] Elevations of CA 125 assay values during the menstrual cycle have also been reported.[23, 29] Non-ovarian malignancies in which CA 125 assay values have been reported include endocervical,[30] liver,[18] pancreatic,[18, 31] lung,[18] colon,[18, 31] stomach,[18, 31] biliary tract,[18, 31] uterine,[17] fallopian tube,[30] breast,[18] and endometrial carcinomas.[30, 32] The CA 125 assay is not recommended as a screening procedure to detect cancer in the general population; however, the use of CA 125 assay values as an aid in the management of ovarian cancer patients has been reported.[7-14]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT CA 125 II assay is a two-step immunoassay for the quantitative determination of OC 125 defined antigen in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample and OC 125 coated paramagnetic microparticles are combined. The OC 125 defined antigen present in the sample binds to the OC 125 coated microparticles.
2. After washing, M11 acridinium-labeled conjugate is added to create a reaction mixture.
3. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of OC 125 defined antigen in the sample and the RLUs detected by the ARCHITECT iSystem optics.

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

REAGENTS

Kit Contents

ARCHITECT CA 125 II 2K45

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

REF	2K45-28	2K45-33	2K45-38
Σ	100	400	500
MICROPARTICLES	1 x 6.6 mL	4 x 6.6 mL	1 x 27.0 mL
CONJUGATE	1 x 5.9 mL	4 x 5.9 mL	1 x 26.3 mL

MICROPARTICLES anti-CA 125 (mouse, monoclonal) coated microparticles in TRIS buffer with protein (bovine) stabilizers. Minimum concentration: 0.09% solids. Preservatives: Sodium Azide and ProClin 300.

CONJUGATE anti-CA 125 (mouse, monoclonal) acridinium-labeled conjugate in phosphate buffer with protein (bovine) stabilizers. Minimum concentration: 0.075 µg/mL. Preservatives: Sodium Azide and ProClin 300.

Other Reagents

MULTI-ASSAY MANUAL DILUENT 1 x 100 mL ARCHITECT Multi-Assay Manual Diluent, REF 7D82-50, containing phosphate buffered saline solution. Preservative: antimicrobial agent.

PRE-TRIGGER SOLUTION ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

WASH BUFFER ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[33-36]

The following warnings and precautions apply to: MICROPARTICLES and CONJUGATE

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

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

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

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
  - **To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.**
  - Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.

For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Reagent Storage

When stored and handled as directed, reagents are stable until the expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8°C	Until expiration date	May be used immediately after removal from 2-8°C storage. Store in upright position.
On board	System temperature	30 days	Discard after 30 days. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

* Reagents may be stored on or off the ARCHITECT iSystem. 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 any reagent bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The ARCHITECT CA 125 II assay file must be installed on the ARCHITECT iSystem prior to performing the assay.

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

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

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

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified sample types to be used with this assay:

Specimen Types	Collection Tubes
Serum	Serum Serum separator tubes
Plasma	Tripotassium EDTA Sodium Heparin Lithium Heparin

- Other specimen collection tube types have not been tested with this assay.
- The instrument 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 assay.
- When serial specimens are being evaluated, the same type of specimen should be used throughout the study.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - grossly hemolyzed
  - obvious microbial contamination
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter.
- 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.
- Performance has not been established using body fluids other than human serum and plasma.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for specimen collection tubes.
- 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, centrifuge specimens before testing if
  - they contain fibrin, red blood cells, or other particulate matter or
  - they were frozen and thawed.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- 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.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum/Plasma	2-8°C	≤ 7 days
	-20°C or colder	>7 days

- If testing will be delayed more than 24 hours, remove serum or plasma from the clot, serum separator or red blood cells.
- Specimens may be stored for up to 7 days at 2-8°C prior to being tested.
- If testing will be delayed more than 7 days, serum or plasma should be stored frozen at -20°C or colder.
- Avoid multiple freeze/thaw cycles.

Specimen Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

2K45 ARCHITECT CA 125 II Reagent Kit

Materials Required but not Provided

- ARCHITECT CA 125 II Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com
- 2K45-01 ARCHITECT CA 125 II Calibrators
- 2K45-10 ARCHITECT CA 125 II Controls
- 7D82-50 ARCHITECT Multi-Assay Manual Diluent
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
- ARCHITECT Replacement Caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

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

Assay Procedure

- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.

- Minimum sample cup volume is calculated by the system and printed on the Orderlist report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

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

  - Priority:

    Sample volume for first test: 75 µL

    Sample volume for each additional test from same sample cup: 25 µL

  - ≤ 3 hours on board:

    Sample volume for first test: 150 µL

    Sample volume for each additional test from same sample cup: 25 µL

  - > 3 hours on board: additional sample volume is required. Refer to the ARCHITECT System Operations Manual, Section 5 for information on sample evaporation and volumes.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare ARCHITECT CA 125 II Calibrators and Controls.
  - Mix calibrator(s) and controls by gentle inversion before use.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
  - Recommended volumes:

    To obtain the recommended 150 µL volume requirement for the ARCHITECT CA 125 II Calibrators dispense 4 drops.

    To obtain the recommended 150 µL volume requirement for the ARCHITECT CA 125 II Controls dispense 4 drops.
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures

Specimens with CA 125 II value exceeding 1000 U/mL are flagged with the code "> 1000.0" and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a 1:10 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.

Manual Dilution Procedure

Suggested dilution: 1:10

An additional 1:10 dilution may be made if needed.

1. Add 50 µL of the patient specimen to 450 µL of ARCHITECT Multi-Assay Manual Diluent.
2. The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The dilution should be performed so that the diluted result reads greater than 20 U/mL.

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

Calibration

- Test Calibrators A-F in duplicate. The calibrators should be priority loaded.

  A single sample of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- Calibration Range: 0 - 1000 U/mL.
- Once an ARCHITECT CA 125 II calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used or
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

Quality Control Procedures

The recommended control requirement for the ARCHITECT CA 125 II assay is that a single sample of each control level be 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 concentration ranges specified in the package insert.

Verification of Assay Claims

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

The ARCHITECT CA 125 II assay belongs to method group 1.

RESULTS

Calculation

The ARCHITECT CA 125 II assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

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.

Measurement Range (Reportable Range)

The measurement range for the ARCHITECT CA 125 II assay is 1.0 U/mL to 1000 U/mL.

LIMITATIONS OF THE PROCEDURE

- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the ARCHITECT CA 125 II results are inconsistent with clinical evidence, additional testing is recommended.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.[37]
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT CA 125 II that employ mouse monoclonal antibodies. Additional clinical or diagnostic information may be required to determine patient status.[38-40]
- Patients with confirmed ovarian carcinoma may have pretreatment CA 125 assay values in the same range as healthy individuals. Elevations in circulating OC 125-defined antigen may be observed in patients with nonmalignant disease. For these reasons, a CA 125 assay value, regardless of level, should not be interpreted as absolute evidence for the presence or absence of malignant disease. The CA 125 assay value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures. **The ARCHITECT CA 125 II assay should not be used as a cancer screening test.**
- Representative performance data are given in the **EXPECTED VALUES** and **SPECIFIC PERFORMANCE CHARACTERISTICS** sections. Results obtained in individual laboratories may vary.

EXPECTED VALUES

The distribution of CA 125 II assay values determined in 811 specimens is shown in the table below:

Distribution of ARCHITECT CA 125 II Assay Values

	Number of Subjects	Percent (%) 0-35 U/mL	35.1-65 U/mL	65.1-100 U/mL	>100 U/mL
APPARENTLY HEALTHY					
Females (Premenopausal)	99	89.9	6.1	4.0	0.0
Females (Postmenopausal)	97	99.0	1.0	0.0	0.0
MALIGNANT CONDITIONS					
Ovarian Cancer	166	49.9	14.3	4.8	32.8
Breast Cancer	50	80.0	20.0	0.0	0.0
Colorectal Cancer	50	84.0	4.0	10.0	2.0
Endometrial Cancer	25	96.0	4.0	0.0	0.0
Lung Cancer	50	60.0	16.0	10.0	12.0
NONMALIGNANT CONDITIONS					
Ovarian Disease	100	90.0	8.0	1.0	0.0
Urogenital Disease	49	83.7	14.3	2.0	0.0
Hypertension/CHD	100	88.0	11.0	0.0	1.0
Benign Endometrial	25	84.0	8.0	4.0	4.0

In this study, 94.4% of the healthy female subjects had CA 125 II assay values at or below 35.0 U/mL (mean = 16.4, SD = 13.0). It is recommended that each laboratory establish its own reference value for the population of interest.

Monitoring of Disease Status in Patients Diagnosed with Ovarian Cancer

Changes observed in serial CA 125 assay values when monitoring ovarian cancer patients should be evaluated in conjunction with other clinical methods used for monitoring ovarian cancer patients.

The effectiveness of the ARCHITECT CA 125 II assay as an aid in the monitoring of disease status in ovarian cancer patients was determined by assessing changes in CA 125 levels in serial serum samples from 63 patients compared to changes in disease status. A study involving a total of 306 observations was performed with an average number of 4.9 observations per patient. A significant change in CA 125 level was defined as at least a 10.75% increase in assay value [i.e., 2.5 times greater than the assay's total %CV (4.3%)]. Seventy-seven percent (77% or 85/111) of the positive patient samples correlated with disease progression while sixty-one percent (61% or 81/132) of serial samples showing no significant change in CA 125 assay value correlated with no progression. The total concordance in this study was sixty-eight percent (68% or 166/243). The following table presents the data in a 2 x 2 classification scheme.

Change in Disease State per Sequential Pair

Change in CA 125 Concentration	Progression	No Progression	Total
≥ 10.75%	85	51	136
< 10.75%	26	81	107
Total	111	132	243

The following table provides the per patient distribution. Ninety-eight percent (98% or 46/47) of the significantly increased serial samples per patient correlated with disease progression while thirty-eight percent (38% or 6/16) of serum sets showing no significant change in CA 125 level correlated with no progression. The total concordance in this study was eighty-three percent (83% or 52/63).

Change in Disease State per Patient

Change in CA 125 Concentration	Progression	No Progression	Total
≥ 10.75%	46	10	56
< 10.75%	1	6	7
Total	47	16	63

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT CA 125 II assay precision is ≤ 10% total CV. A study was performed as described per the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP5-A.[41] Three defibrinated plasma-based panels were assayed, using two lots of reagents, in replicates of two at two separate times per day for 20 days on two separate instruments. Each reagent lot used a single calibration curve throughout the study. Data from this study are summarized below.*

Sample	Reagent Lot	Instrument	n	Mean Conc. (U/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
Panel 1	1	1	80	43.5	1.1	2.4	1.7	3.9
	2	2	80	49.7	0.8	1.5	0.8	1.7
Panel 2	1	1	80	303.3	9.8	3.2	11.9	3.9
	2	2	80	340.7	5.5	1.7	6.7	2.0
Panel 3	1	1	80	598.0	18.6	3.1	25.8	4.3
	2	2	80	678.3	12.4	1.8	13.5	2.0

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

Recovery

The ARCHITECT CA 125 II assay mean recovery is 100 ± 15%. A study was performed based on guidance from Tietz Textbook of Clinical Chemistry[42] for the ARCHITECT CA 125 II assay. Known concentrations of OC 125 defined antigen were added to normal human serum samples. The concentration of CA 125 was determined using the ARCHITECT CA 125 II assay, and the resulting percent recovery was calculated. Representative data from this study are summarized in the table below.*

Sample	Endogenous Assay Value (U/mL)	OC 125 Defined Antigen Added (U/mL)	Observed CA 125 Assay Value (U/mL)	% Recovery*
1	36.8	165	193.7	94
		715	704.7	94
2	31.3	165	160.2	82
		715	618.6	83
3	40.2	165	186.5	91
		715	685.8	92

Average Recovery across two separate spiked concentrations shown above = 90%

$$\% \text{ Recovery} = \frac{\text{Observed CA 125 Conc. (U/mL)}}{\text{Endogenous CA 125 Conc. (U/mL)} + \text{CA 125 Added (U/mL)}} \times 100$$

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

Dilution Linearity

The ARCHITECT CA 125 II assay mean dilution linearity is 100 ± 15%. A study was performed for the ARCHITECT CA 125 II assay modeled after the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP6-P2.[43] Samples with known elevated CA 125 concentrations were diluted with Multi-Assay Manual Diluent. The CA 125 concentration was determined for each dilution and the percent (%) recovery was calculated. Representative data from this study are summarized below.*

Sample	Final Dilution Factor	Expected Value (U/mL)	Value Obtained (U/mL)	% Recovery*
1	Undiluted	846.4	846.4	—
	1:1.4	604.6	631.4	104.4
	1:2	423.2	466.2	110.6
	1:3.3	256.5	282.6	110.3
	1:5	169.3	182.8	108.0
	1:10	84.6	92.7	109.5
	1:20	42.3	46.0	108.7
2	Undiluted	903.8	903.8	—
	1:1.4	645.6	631.6	97.8
	1:2	451.9	446.4	98.8
	1:3.3	273.9	274.0	100.1
	1:5	180.8	186.7	103.3
	1:10	90.4	95.4	105.5
	1:20	45.2	47.5	105.0
3	Undiluted	935.3	935.3	—
	1:1.4	668.1	645.9	96.7
	1:2	467.7	450.4	96.3
	1:3.3	283.4	284.7	100.5
	1:5	187.1	185.6	99.2
	1:10	93.5	95.8	102.4
	1:20	46.8	50.0	106.9

Average recovery across the three diluted samples above = 103.6%

$$^{a}\%\ \text{Recovery} = \frac{\text{Values Obtained} \times \text{Dilution Factor}}{\text{Undiluted Concentration}} \times 100$$

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

Analytical Sensitivity

The sensitivity of the ARCHITECT CA 125 II assay is ≤ 1.0 U/mL (n=24 runs, in replicates of 10). Analytical sensitivity corresponds to the upper limit of the 95% confidence interval and represents the lowest concentration of OC 125 defined antigen that can be distinguished from zero.

Analytical Specificity

The ARCHITECT CA 125 II mean assay specificity is ≤ 12%. Recovery studies were performed to compare sera containing the following compounds at the indicated concentrations with control sera.*

INTERFERING SUBSTANCE

Test Compound	Test Concentration
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Total Protein	12 g/dL
Triglycerides	3 g/dL

CHEMOTHERAPEUTIC AGENTS

Test Compound	Test Concentration
Carboplatin	500 µg/mL
Cisplatin	165 µg/mL
Clotrimazole	0.3 µg/mL
Cyclophosphamide	500 µg/mL
Dexamethasone	10 µg/mL
Doxorubicin	1.16 µg/mL
Leucovorin	2.68 µg/mL
Melphalan	2.8 µg/mL
Methotrexate	45 µg/mL
Paclitaxel	3.5 mg/mL

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

POTENTIALLY INTERFERING CLINICAL CONDITIONS

The ARCHITECT CA 125 II assay was evaluated using specimens with HAMA and Rheumatoid Factor (RF) to further assess the assay specificity. Five specimens positive for HAMA and five specimens positive for RF were evaluated for % recovery with OC 125 defined antigen spiked into each specimen at 35 and 250 U/mL; mean % recovery results are summarized in the following table.*

Clinical Condition	Number of Specimens	Mean % Recovery
HAMA	10	96
RF	10	97

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

High Dose Hook

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For the ARCHITECT CA 125 II assay, no high dose hook effect was observed when samples containing up to approximately 180,000 U/mL of OC 125 defined antigen were assayed.

Method Comparison

The ARCHITECT CA 125 II assay method comparison correlation coefficient is ≥ 0.90 and the slope is 1.0 ± 0.15 for the full range of the assay. The ARCHITECT CA 125 II assay was compared to the Abbott AxSYM CA 125 assay. The results of the specimen testing are shown in the following table.*

ARCHITECT CA 125 II vs. Abbott AxSYM CA 125

Regression Method	n	Slope (99% CI)	Intercept (99% CI)	Correlation Coefficient
Passing-Bablok †	279**	1.06 (1.03 to 1.11)	4.0 (2.0 to 4.9)	0.985
	167***	1.23 (1.16 to 1.30)	0.4 (-0.9 to 1.8)	0.967

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

** Sample Range: 4.5 - 4085.9 U/mL (ARCHITECT); 2.7 - 3436.1 U/mL (AxSYM)

*** Sample Range: 4.5 - 110.5 U/mL (ARCHITECT); 2.7 - 95.4 U/mL (AxSYM)

† A linear regression method with no special assumptions regarding the distribution of the samples and the measurement errors.[44]

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Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number
EC REP	Authorized Representative in the European Community
VD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
MULTI-ASSAY MANUAL DILUENT	Multi-Assay Manual Diluent
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCED FOR ABBOTT BY	Produced for Abbott by
PRODUCT OF USA	Product of USA
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
TRIGGER SOLUTION	Trigger Solution
WARNING: SENSITIZER	Warning: May cause an allergic reaction.
WASH BUFFER	Wash Buffer

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Revised November 2015

ARCHITECT SYSTEM

Total PSA Controls

en

Total PSA

REF 7K70-10

C7K700

G5-3210/R03

Read Highlighted Changes
Revised January 2015

INTENDED USE

The ARCHITECT Total PSA Controls are for the verification of the accuracy and precision of the ARCHITECT *i* System when used for the quantitative determination of total prostate specific antigen (both free PSA and PSA complexed to alpha-1-antichymotrypsin) in human serum. Refer to the ARCHITECT Total PSA reagent package insert for additional information.

CONTENTS

3 Bottles (8.0 mL each) of ARCHITECT Total PSA Controls (CONTROL L, CONTROL M, CONTROL H) containing PSA (human) prepared in TRIS buffer with protein (bovine) stabilizer. Preservatives: Sodium Azide and Antimicrobial Agents.

The following concentration ranges may be used for individual replicate control specifications on the ARCHITECT *i* System:

Control	Target Concentration CONC (ng/mL)	Range RANGE (ng/mL)
CONTROL L	0.5	0.325 - 0.675
CONTROL M	4.0	2.600 - 5.400
CONTROL H	23.0	14.950 - 31.050

Each laboratory should establish its own concentration ranges for new control lots at each control level. This can be accomplished by assaying a minimum of 20 replicates over several (3-5) days. Sources of variation that can be expected should be included in this study in order to be representative of future system performance. These may include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules
- Data points collected at different times of the day

These results should be applied to your laboratory's quality control practices.

STANDARDIZATION

The Controls are manufactured by dilution of Prostate Specific Antigen (PSA) of known concentration to obtain a target concentration. The target concentration is referenced against the World Health Organization (W.H.O.) 1st International Standard for Prostate Specific Antigen (90:10) 96/670 at each concentration level.

PRECAUTIONS

- IVD
- For *In Vitro* Diagnostic Use.
- **CAUTION:** This product contains human sourced and/or potentially infectious components. For a specific listing, refer to the **CONTENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens[1]. Biosafety Level 2[2] or other appropriate biosafety practices[3,4] should be used for materials that contain or are suspected of containing infectious agents.
- The Controls contain human PSA; donor material of which has been tested and found negative for HIV-1, HIV-2, Hepatitis B and Hepatitis C.
- The following warnings and precautions apply to the Controls: Contains sodium azide.

EUH032	Contact with acids liberates very toxic gas.
P501	Dispose of contents / container in accordance with local regulations.

- Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.
- For information on safe disposal of sodium azide containing materials, refer to the ARCHITECT System Operations Manual, Section 8.

STORAGE

- ARCHITECT Total PSA Controls are stable until the expiration date when stored and handled as directed.
- Do not use past expiration date.
- 2°C – 8°C

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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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CE 0843

January 2015

Abbott

Key to symbols used

GTIN	Global Trade Item Number
PRODUCT OF IRELAND	Product of Ireland
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.

ARCHITECT SYSTEM

Total PSA Calibrators

en

Total PSA

REF 7K70-01

S7K700

G5-3211/R03

Read Highlighted Changes
Revised January 2015

INTENDED USE

The ARCHITECT Total PSA Calibrators are for calibration of the ARCHITECT *i* System when used for the quantitative determination of total prostate specific antigen (both free PSA and PSA complexed to alpha-1-antichymotrypsin) in human serum. Refer to the ARCHITECT Total PSA reagent package insert for additional information.

CONTENTS

2 Bottles (4.0 mL each) of ARCHITECT Total PSA Calibrators. Calibrator 1 (CAL 1) contains TRIS buffer with protein (bovine) stabilizer. Calibrator 2 (CAL 2) contains PSA (human) prepared in TRIS buffer with protein (bovine) stabilizer.

Preservatives: Sodium Azide and Antimicrobial Agents.

The calibrators yield the following concentrations:

Calibrator	Total PSA Concentration (ng/mL)
CAL 1	0
CAL 2	15

STANDARDIZATION

The Calibrators are manufactured by dilution of Prostate Specific Antigen (PSA) of known concentration to obtain a target concentration. The target concentration is referenced against the World Health Organization (W.H.O.) 1st International Standard for Prostate Specific Antigen (90:10) 96/670 at each concentration level.

PRECAUTIONS

- IVD
- For *In Vitro* Diagnostic Use.
- **CAUTION:** This product contains human sourced and/or potentially infectious components. For a specific listing, refer to the **CONTENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens[1]. Biosafety Level 2[2] or other appropriate biosafety practices[3,4] should be used for materials that contain or are suspected of containing infectious agents.
- Calibrator 2 contains human PSA; donor material of which has been tested and found negative for HIV-1, HIV-2, Hepatitis B and Hepatitis C.
- The following warnings and precautions apply to the Calibrators:
  Contains sodium azide.

EUH032	Contact with acids liberates very toxic gas.
P501	Dispose of contents / container in accordance with local regulations.
- Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.
- For information on safe disposal of sodium azide containing materials, refer to the ARCHITECT System Operations Manual, Section 8.

STORAGE

- ARCHITECT Total PSA Calibrators are stable until the expiration date when stored and handled as directed.
- Do not use past expiration date.
- 2°C–8°C

BIBLIOGRAPHY

1. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
2. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
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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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CE 0843

January 2015

Abbott

Key to symbols used

GTIN	Global Trade Item Number
PRODUCT OF IRELAND	Product of Ireland
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.

ARCHITECT Total PSA

REF 7K70-25
REF 7K70-20
REF 7K70-35
REF 7K70-30

en
Total PSA
7K70
G47685R06
B7K700

Revised September 2017

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.

WARNING: The concentration of Total PSA in a given specimen, determined with assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the Total PSA assay used. Values obtained with different assay methods, including Abbott PSA assays, cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining Total PSA levels serially is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory MUST confirm baseline values for patients being serially monitored.

NAME

ARCHITECT Total PSA (Prostate Specific Antigen)

INTENDED USE

The ARCHITECT Total PSA assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of Total PSA (both Free PSA and PSA complexed to alpha-1-antichymotrypsin) in human serum:

1. As an aid in the detection of prostate cancer when used in conjunction with digital rectal exam (DRE) in men 50 years or older. Prostatic biopsy is required for diagnosis of cancer.
2. As an adjunctive test to aid in the management of prostate cancer patients.

SUMMARY AND EXPLANATION OF THE TEST

Prostate specific antigen (PSA), a member of the human kallikrein gene family, is a serine protease with chymotrypsin-like activity. The mature form of PSA is a single chain glycoprotein of 237 amino acids containing 7-8% carbohydrate as a single N-linked oligosaccharide side chain. PSA has a molecular weight of approximately 30,000 daltons.[1, 8, 37, 38]

The major site of PSA production is the glandular epithelium of the prostate. PSA has also been found in breast cancers, salivary gland neoplasms, periurethral and anal glands, cells of the male urethra, breast milk, blood and urine.[1, 2] PSA produced in the prostate is secreted into the seminal fluid in high concentrations. A major function of PSA is the proteolytic cleavage of gel-forming proteins in the seminal fluid, resulting in the liquefaction of the seminal gel and increased sperm mobility.[1] Low levels of PSA are found in the blood as a result of leakage of PSA from the prostate gland. Increasing levels of serum PSA are associated with prostatic pathology, including prostatitis, benign prostatic hyperplasia (BPH), and cancer of the prostate.[1, 3-7]

PSA occurs in three major forms in blood. The major immunodetectable form is PSA complexed with the serine protease inhibitor, alpha-1-antichymotrypsin (PSA-ACT). Uncomplexed, or Free PSA, is the other immunodetectable form of PSA in serum. The majority of Free PSA in serum appears to be an inactive form that cannot complex with protease inhibitors and may be either a PSA zymogen or an enzymatically-inactive, cleaved form of PSA. Equimolar-response PSA assays have an equivalent response to both Free PSA and PSA-ACT.[1] The ARCHITECT Total PSA assay is an equimolar assay. A third form of PSA, a complex with alpha-2-macroglobulin, is not detectable with current immunoassays for PSA due to the engulfment and subsequent masking of PSA epitopes by the alpha-2-macroglobulin molecule.[1, 8, 9]

Prostate cancer is the most frequently diagnosed cancer and the second leading cause of cancer deaths in men in the United States.[10] Early diagnosis of carcinoma of the prostate is hindered by the lack of symptoms in men with localized tumors. Therefore, early detection requires a simple, safe, and inexpensive test for the disease in asymptomatic men. The traditional method for detection of prostate cancer is the digital rectal examination (DRE). However, only 30 to 40% of cancers detected by DRE screening are expected to be confined to the prostate. The frequent finding of locally advanced prostate cancer in screened patients may be due to the inability of DRE to detect tumors of small volume that are most likely to be confined to the prostate.[11] Since patients with small tumors are believed to have the best prognosis, it can be concluded that DRE has limited sensitivity in detecting those tumors with the greatest potential for cure.[12]

In a 1990 publication by Cooner et al., data was presented regarding the clinical use of other diagnostic modalities such as prostate ultrasonography and serum prostate specific antigen for early detection of prostate cancer. This study found that there was a significant increase in predictability for cancer when the DRE and PSA tests were abnormal.[13] Several other studies have shown that the measurement of serum PSA concentrations offers several advantages in the early detection of prostate cancer. The procedure is more acceptable to patients, the result is objective and quantitative, and is independent of the examiners skill. In several recent studies of healthy men 50 years or older, serum PSA levels had the greatest ability to predict prostate cancer. These studies concluded that not only is serum PSA measurement a useful addition to rectal examination and ultrasonography in the detection of prostate cancer, but that it is also the most accurate of the three tests for this purpose.[14, 15] In January 1992, the American Urological Association endorsed annual examination with DRE and PSA, for early detection of prostate cancer, beginning at age 50.[16] This was reaffirmed by the American Cancer Society in November 1992.[17] The combined use of DRE and PSA has been shown to result in an increased detection of early stage prostate cancer; however, the benefit of early detection on patient outcome has not been proven and is the subject of ongoing clinical trials.[4-7, 10-15, 18, 19]

PSA testing can have significant value in detecting metastatic or persistent disease in patients following surgical or medical treatment of prostate cancer. Persistent elevation of PSA following treatment, or an increase in a post-treatment PSA level is indicative of recurrent or residual disease. PSA testing is widely accepted as an adjunctive test in the management of prostate cancer patients.[3-7]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT Total PSA assay is a two-step immunoassay to determine the presence of Total PSA (both Free PSA and PSA complexed to alpha-1-antichymotrypsin) in human serum using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample and anti-PSA coated paramagnetic microparticles are combined to create a reaction mixture. The PSA present in the sample binds to the anti-PSA coated microparticles.
2. After washing, anti-PSA acridinium-labeled conjugate is added. Pre-Trigger and Trigger Solutions are then added to the reaction mixture.
3. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of Total PSA in the sample and the RLUs detected by the ARCHITECT iSystem optics.

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

REAGENTS

Kit Contents

ARCHITECT Total PSA 7K70

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

REF	7K70-25	7K70-20	7K70-35	7K70-30
Σ	100	400	500	2000
MICROPARTICLES	1 x 6.6 mL	4 x 6.6 mL	1 x 27.0 mL	4 x 27.0 mL
CONJUGATE	1 x 5.9 mL	4 x 5.9 mL	1 x 26.3 mL	4 x 26.3 mL

MICROPARTICLES Anti-PSA (mouse, monoclonal) coated Microparticles in TRIS buffer with protein (bovine) stabilizer. Preservative: antimicrobial agents.

CONJUGATE Anti-PSA (mouse, monoclonal) acridinium-labeled Conjugate in MES buffer with protein (bovine) stabilizer. Minimum concentration: 10 ng/mL. Preservative: antimicrobial agents.

Other Reagents

MULTI-ASSAY MANUAL DILUENT 1 x 100 mL ARCHITECT Multi-Assay Manual Diluent, REF 7D82-50, containing phosphate buffered saline solution. Preservative: antimicrobial agent.

PRE-TRIGGER SOLUTION ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

WASH BUFFER ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

NOTE: Bottle and volume varies based on order.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[20-23]

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

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

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
  - To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.

For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Reagent Storage

When stored and handled as directed, reagents are stable until the expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8°C	Until expiration date	May be used immediately after removal from 2-8°C storage. Store in upright position.
On board	System temperature	30 days	Discard after 30 days. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

* Reagents may be stored on or off the ARCHITECT iSystem. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The ARCHITECT Total PSA assay file must be installed on the ARCHITECT iSystem from an ARCHITECT iSystem 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.

Alternate Result Units

Edit assay parameter "Result concentration units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
ng/mL	1.0	µg/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

- Only human serum may be used in the ARCHITECT Total PSA assay.
- The instrument 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 assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - grossly hemolyzed
  - obvious microbial contamination
- For accurate results, serum specimens should be free of fibrin, red blood cells, or other particulate matter. Centrifuge specimens containing fibrin, red blood cells, or particulate matter prior to use to ensure consistency in the results.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum collection tubes.
- It is recommended to obtain specimens for PSA testing prior to procedures involving manipulation of the prostate.
- Follow these package insert instructions as well as the specimen collection tube manufacturer's instructions for specimen collection and preparation for analysis. Refer to the specimen collection tube manufacturer's instructions for centrifugation time and speed.
- Insufficient processing of sample, or disruption of the sample during transportation may cause depressed results.
- 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 specimens are centrifuged before a complete clot forms, the presence of fibrin or particulate matter may cause erroneous results. Centrifuge specimens containing fibrin, red blood cells, or particulate matter. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
- If proper specimen collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter. Aliquots poured versus pipetted from specimen tube types that do not include serum separators are at higher risk of including particulates and generating depressed results.
- Failure to follow these instructions may result in depressed specimen results.
- Specimens must be mixed THOROUGHLY after thawing, by vortexing. Thawed samples containing red blood cells or particulate matter, or **which are hazy or cloudy in appearance** must be centrifuged prior to use to ensure consistency in the 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.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum	2-8°C	≤ 24 hours

If testing will be delayed more than 24 hours, specimens should be removed from the clot or serum separator and stored frozen at -20°C or colder.[24, 25]

NOTE: Samples which may be tested for Free PSA should be removed from the clot within 3 hours.

Avoid multiple freeze/thaw cycles.

Specimen Shipping

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

  Specimens that will not be assayed within 24 hours should be stored/shipped frozen. Prior to shipment, it is recommended that specimens be removed from the clot or serum separator.

PROCEDURE

Materials Provided

7K70 ARCHITECT Total PSA Reagent Kit

Materials Required but not Provided

- ARCHITECT Total PSA Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 7K70-01 ARCHITECT Total PSA Calibrators
- 7D82-50 ARCHITECT Multi-Assay Manual Diluent
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
- ARCHITECT Replacement Caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

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

Materials Available but not Provided

- 7K70-10 ARCHITECT Total PSA Controls

Assay Procedure

- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.

- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- Minimum sample cup volume is calculated by the system and printed on the Orderlist report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

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

    Sample volume for first test: 100 µL

    Sample volume for each additional test from same sample cup: 50 µL
  - ≤ 3 hours on board:

    Sample volume for first test: 150 µL

    Sample volume for each additional test from same sample cup: 50 µL
  - > 3 hours on board: Additional sample volume is required. Refer to the ARCHITECT System Operations Manual, Section 5 for information on sample evaporation and volumes.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare ARCHITECT Total PSA Calibrators and Controls.
  - Mix calibrator(s) and controls by gentle inversion before use.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
  - Recommended volumes:

    for each calibrator: 7 drops

    for each control: 4 drops
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures

Specimens with a Total PSA value exceeding 100 ng/mL are flagged with the code "> 100.000" and may be diluted using either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a 1:10 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.

Dilutions other than 1:10 should be done manually.

Manual Dilution Procedure

Suggested dilution: 1:20

1. Add 50 µL of the patient specimen to 950 µL of ARCHITECT Multi-Assay Manual Diluent.
2. The operator must enter the dilution factor in the Patient or Control order screen. All assays selected for that order will be diluted. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The dilution should be performed so that the diluted result reads greater than 0.4 ng/mL.

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

Calibration

- Test Calibrators 1 and 2 in duplicate. The calibrators should be priority loaded.

  A single replicate of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- Calibration Range: 0 - 50 ng/mL.
- The assay protocol allows for the range to be extended to 100 ng/mL.
- Once an ARCHITECT Total PSA 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 or
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

Quality Control Procedures

The recommended control requirement for the ARCHITECT Total PSA assay is that a single replicate of each control level be 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.

Verification of Assay Claims

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

The ARCHITECT Total PSA assay belongs to method group 1.

RESULTS

Calculation

The ARCHITECT Total PSA assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

For information on alternate result units, refer to the **INSTRUMENT PROCEDURE, Alternate Result Units** 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). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT Total PSA that employ mouse monoclonal antibodies.[26, 27] ARCHITECT Total PSA reagents contain a component that reduces the effect of HAMA reactive specimens. Additional clinical or diagnostic information may be required to determine patient status.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.[28]
- The concentration of PSA in a given specimen, determined with assays from different manufacturers, can vary due to differences in assay methods, calibration, and reagent specificity.[1, 29, 30]

- Quality control samples may be produced by introducing seminal fluid PSA into a human serum matrix. PSA in serum and seminal fluid may exist in different forms. The concentration of PSA in these controls, determined with assays from different manufacturers, can vary due to differences in assay methods, calibration, reagent specificity, and the form of PSA that is present; therefore, it is important to use assay-specific values to evaluate control results.
- Hormonal therapy may affect PSA expression; therefore, a low PSA level after any treatment that includes hormonal therapy may not adequately reflect the presence of residual or recurrent disease.[31]
- In most instances, specimens obtained from patients immediately following digital rectal examination show no clinically significant increases in PSA levels.[32] However, prostatic massage, ultrasonography, and needle biopsy may cause clinically significant elevations.[33] PSA levels may also be increased following ejaculation.[34]
- Active Free PSA in the serum at the time of blood sampling can continue to complex with serum protease inhibitors, especially alpha-2-macroglobulin, resulting in a rapid decrease in PSA levels of the active form of Free PSA.[35]
- Serum PSA concentrations should not be interpreted as absolute evidence for the presence or absence of prostate cancer. Elevated concentrations of PSA may be observed in the serum of patients with benign prostatic hyperplasia or other nonmalignant disorders as well as in prostate cancer. Furthermore, low PSA concentrations are not always indicative of the absence of cancer. The PSA value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures such as DRE. Some early cases of prostate cancer will not be detected by PSA testing; the same is true for DRE. Prostatic biopsy is required for the diagnosis of cancer.

EXPECTED VALUES FOR DETECTION OF PROSTATE CANCER

[Values developed for the ARCHITECT i2000 analyzer.]

A prospective study was conducted at seven clinical sites to demonstrate the usefulness of PSA in the detection of prostate cancer when used in conjunction with DRE. All clinical data presented supporting the detection claim were generated using the ARCHITECT iSystem and ARCHITECT Total PSA assay reagents. A total of 531 men 50 years of age or older participated in the study. All subjects were biopsied based on an initial elevated PSA value and/or suspicious DRE result. A distribution of the ARCHITECT Total PSA results is presented in the following table:

Distribution of Results from ARCHITECT Total PSA

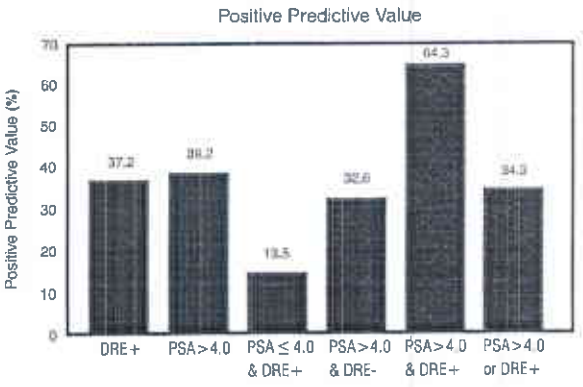
	PSA ≤ 4.0	PSA > 4.0	Total
DRE-[b]	32	319	351
	6.0%	60.1%	66.1%
DRE+[a]	96	84	180
	18.1%	15.8%	33.9%
Total	128	403	531
	24.1%	75.9%	100.0%

NOTE: 499 patients tested positive by DRE and/or PSA.

[a] DRE+: Digital Rectal Examination (Suspicious for cancer)

[b] DRE-: Digital Rectal Examination (Not suspicious for cancer)

The positive predictive values for various combinations of DRE and PSA are presented graphically in the figure below and table below.



Positive Predictive Values

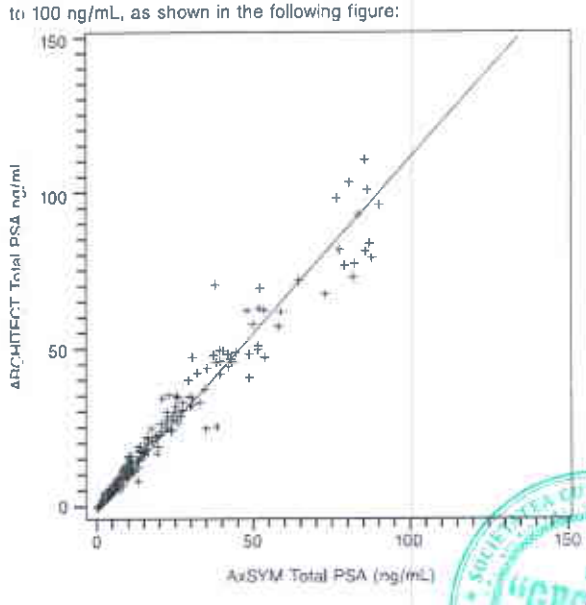
Detection Method	Positive Predictive Value (%)[a]	Number of Subjects with Cancer/Number of Subjects Suspicious for Cancer
DRE+	37.2 (30.1-44.7)	67/180
PSA > 4.0	39.2 (34.4-44.2)	158/403
PSA ≤ 4.0 and DRE+	13.5 (7.4-22.0)	13/96
PSA > 4.0 and DRE-	32.6 (27.5-38.0)	104/319
PSA > 4.0 and DRE+	64.3 (53.1-74.4)	54/84
PSA > 4.0 or DRE+	34.3 (30.1-38.6)	171/499

[a] 95% Confidence Interval (Lower Limit - Upper Limit)

Cancers were detected in 177 of the 531 subjects. The overall cancer detection rate was 96.6% (171/177) when at least one test was suspicious, 30.5% (54/177) when both tests were suspicious, 53.8% (104/177) for PSA alone, and 7.3% (13/177) for DRE alone.

CORRELATION

To demonstrate that the ARCHITECT Total PSA assay results are comparable to the results from the AxSYM Total PSA assay, a least squares linear regression analysis was performed comparing the PSA values from both assays for 1,798 clinical specimens. The analysis yielded a correlation coefficient of 0.987, a slope of 1.06, and a Y-intercept of 0.344 for the specimens covering the range up to 100 ng/mL, as shown in the following figure:



These results demonstrate that the ARCHITECT Total PSA assay yields equivalent results compared to those obtained using the AxSYM Total PSA assay.

Serum PSA concentrations, regardless of the value, should not be interpreted as definitive evidence for the presence or absence of prostate cancer. In addition, PSA testing should be done in conjunction with DRE because PSA and DRE together detected the greatest number of cancers. Prostatic biopsy is required for the diagnosis of cancer.

EXPECTED VALUES

[Values developed for the ARCHITECT i2000 analyzer.]

The distribution of ARCHITECT Total PSA values determined in 2,287 specimens is shown in the following table.

Distribution of ARCHITECT Total PSA Values

			Percent (%)				
		Number of Subjects	0 - 4.0 (ng/mL)	> 4.0 - 10 (ng/mL)	>10 - 30 (ng/mL)	>30-60 (ng/mL)	> 60 (ng/mL)
Apparently Healthy Subjects	Females	296	100.0	0.0	0.0	0.0	0.0
	Males Ages 40 to 49	99	100.0	0.0	0.0	0.0	0.0
	Males Ages 50 to 59	120	97.5	2.5	0.0	0.0	0.0
	Males Ages 60 to 69	123	93.5	6.5	0.0	0.0	0.0
	Males Ages 70 to 79	124	91.9	7.3	0.8	0.0	0.0
Nonmalignant Disease	BPH	352	42.6	42.3	12.8	1.1	1.1
	Cirrhosis	89	94.4	3.4	1.1	0.0	1.1
	Genitourinary	151	90.7	7.3	1.3	0.7	0.0
	Prostatitis	142	46.5	40.1	11.3	1.4	0.7
	Renal	140	90.0	5.7	2.9	1.4	0.0
Malignant Disease	Prostate Stage A	94	46.8	30.9	17.0	1.1	4.3
	Prostate Stage B	166	30.1	44.0	23.5	0.6	1.8
	Prostate Stage C	141	26.2	22.7	29.1	12.8	9.2
	Prostate Stage D	95	15.8	12.6	32.6	10.5	28.4
	Genitourinary	155	82.9	3.9	1.9	0.6	0.6

In this study, 95.5% of the specimens from apparently healthy male subjects (n=466) had values of 4.0 ng/mL or less.

It is recommended that each laboratory establish its own expected reference range for the population of interest.

The malignant disease portion of the distribution table is derived primarily from carcinoma patients representing both active (clinical evidence of disease progression) and inactive (no clinical evidence of disease progression) disease states. When changing PSA assay methods in the course of monitoring a patient, additional sequential testing should be carried out to confirm baseline values.

SPECIFIC PERFORMANCE CHARACTERISTICS

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

Precision

[Values developed for the ARCHITECT i2000 analyzer.]

ARCHITECT Total PSA assay precision is ≤ 8%. Precision was determined as described in the National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP5-A.[36] Six samples, consisting of three serum based panels and three Total PSA controls, were assayed using three instruments in replicates of two at two separate times per day for twenty days (n=80 for each sample), using a single lot of reagents and a single calibration. Data from this study are summarized in the following table.*

Reproducibility of ARCHITECT Total PSA

			Within Run		Total	
Sample	Instrument	Mean Total PSA (ng/mL)	SD	%CV	SD	%CV
Low Control	1	0.498	0.0087	1.8	0.0109	2.2
	2	0.511	0.0203	4.0	0.0237	4.6
	3	0.504	0.0131	2.6	0.0194	3.9
Medium Control	1	4.030	0.1036	2.6	0.1107	2.7
	2	4.104	0.1517	3.7	0.1836	4.5
	3	4.101	0.1218	3.0	0.1714	4.2
High Control	1	24.565	0.7187	2.9	0.8121	3.3
	2	24.558	1.0663	4.3	1.1691	4.8
	3	24.210	0.7742	3.2	1.5808	6.5
Panel 1	1	4.130	0.1129	2.7	0.3230	7.8
	2	4.109	0.1479	3.6	0.1665	4.1
	3	4.139	0.1042	2.5	0.2099	5.1
Panel 2	1	49.191	1.6925	3.4	1.8405	3.7
	2	46.943	2.0034	4.3	2.6271	5.6
	3	47.770	1.5792	3.3	3.4934	7.3
Panel 3	1	66.952	2.0804	3.1	4.1157	6.1
	2	62.631	3.1461	5.0	3.2269	5.2
	3	61.632	1.5534	2.5	5.5307	9.0

* Representative performance data are shown. Results obtained at individual laboratories may vary.

Measurement Range

The measurement (reportable) range of the ARCHITECT Total PSA assay is 0.008 ng/mL to 100 ng/mL, as defined by the analytical sensitivity lower limit and the upper limit of the extended calibration range. For patient specimens with a Total PSA assay value exceeding 100 ng/mL refer to the **Specimen Dilution Procedures** section of this package insert.

Recovery

[Values developed for the ARCHITECT i2000 analyzer.]

Known concentrations of serum PSA were added to ten normal human serum samples. Each sample was spiked at a low and a high level. The concentration of Total PSA was determined using the ARCHITECT Total PSA assay and the resulting percent recovery was calculated. The mean recovery was 95.9% with values ranging from 89.8% to 99.6%.

Sensitivity

[Values developed for the ARCHITECT i2000 analyzer.]

Functional

Functional sensitivity is defined as the lowest concentration that can be measured with an inter-assay coefficient of variation (CV) less than or equal to 20%. The calculated %CV for one reagent lot from all sites was plotted against the mean concentration of each panel. A parametric curve was fitted through the data, and the functional sensitivity was determined to be less than 0.05 ng/mL, which corresponded to less than 20% CV on the fitted curve.

Analytical

The analytical sensitivity of the ARCHITECT Total PSA assay was calculated to be less than 0.008 ng/mL. This sensitivity is defined as the concentration at two standard deviations above the mean RLU for the ARCHITECT Total PSA MasterCheck Level 0 and represents the lowest measurable concentration of Total PSA that can be distinguished from zero.

Analytical Specificity

[Values developed for the ARCHITECT i2000 analyzer.]

The analytical specificity of the ARCHITECT Total PSA assay was determined by testing sera containing the following compounds. These compounds showed less than or equal to 10% interference in the ARCHITECT Total PSA assay at the levels indicated.

INTERFERING SUBSTANCES

Test Compound	Concentration
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Total Protein	2.0 g/dL & 12.0 g/dL
Prostatic Acid Phosphatase	1000 ng/mL
Triglycerides	3000 mg/dL
Hytrin	10 µg/mL
Proscar	25 µg/mL
Flomax	1 µg/mL

CHEMOTHERAPEUTIC AGENTS

Test Compound	Concentration
Cyclophosphamide	700 µg/mL
Diethylstilbestrol	2 µg/mL
Doxorubicin-HCl	16 µg/mL
Estramustine Phosphate	200 µg/mL
Flutamide	10 µg/mL
Goserelin Acetate	100 ng/mL
Lupron	100 µg/mL
Megestrol Acetate	90 µg/mL
Methotrexate	30 µg/mL

Carryover

[Values developed for the ARCHITECT i2000 analyzer.]

No detectable carryover (less than 0.5 PPM) was observed when a sample containing 16,791 ng/mL of PSA was assayed.

High Dose Hook

[Values developed for the ARCHITECT i2000 analyzer.]

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For the ARCHITECT Total PSA assay, no high dose hook effect was observed when samples containing up to approximately 48,000 ng/mL of PSA were assayed.

Accuracy by Correlation

The ARCHITECT Total PSA assay reagents were compared on the ARCHITECT i2000/i2000SR and the ARCHITECT i1000SR platforms. The results of specimen testing are shown below.

Statistical Method	Number of Observations	Intercept	Slope	Correlation Coefficient
Least Squares	151	-0.06	1.05	0.996
Passing-Bablok*	151	-0.03	1.04	0.996

* A linear regression method with no special assumptions regarding the distribution of the samples and the measurement errors.[39]

In this evaluation, serum specimens tested ranged from 0.046 ng/mL to 81.710 ng/mL, by the i1000SR platform.

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Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
CONJUGATE	Conjugate
CONTROL NO.	Control Number
GTIN	Global Trade Item Number
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
MULTI-ASSAY MANUAL DILUENT	Multi-Assay Manual Diluent
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCT OF IRELAND	Product of Ireland
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
TRIGGER SOLUTION	Trigger Solution
WASH BUFFER	Wash Buffer

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ARCHITECT SYSTEM

HBsAg Qualitative II Controls

en

HBsAg Qualitative II

REF 2G22-10

C2G220

G3-4430/R02

Read Highlighted Changes
Revised March 2013

INTENDED USE

The ARCHITECT HBsAg Qualitative II Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT *i* System when used for the qualitative detection and for the confirmation of the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma using the ARCHITECT HBsAg Qualitative II and HBsAg Qualitative II Confirmatory Reagent Kits. Refer to the ARCHITECT HBsAg Qualitative II and ARCHITECT HBsAg Qualitative II Confirmatory Reagent package inserts and the ARCHITECT *System* Operations Manual for additional information.

CONTENTS

2 Bottles (8.0 mL each) of ARCHITECT HBsAg Qualitative II Controls. The Negative Control (CONTROL −) contains recalcified human plasma. Preservatives: ProClin 950 and sodium azide. The Positive Control (CONTROL +) contains inactivated purified human HBsAg (subtype *ad/ay*) in phosphate buffer with human plasma and protein (bovine serum albumin) stabilizers. Preservatives: ProClin 300 and ProClin 950.
The controls are at the following ranges:

For the ARCHITECT HBsAg Qualitative II assay:

		S/CO	
Control	Color	Target (TARGET)	Range (RANGE)
CONTROL −	Natural	N/A	≤ 0.85
CONTROL +	Blue*	3.50	1.75 - 5.25

* Dye: Acid Blue No. 9

For the ARCHITECT HBsAg Qualitative II Confirmatory assay:

		HBsAgQ2 C2 S/CO**		% Neutralization (NEUTRALIZATION)
Control	Color	Target	Range	
CONTROL +	Blue*	3.31	1.65 - 4.96	≥ 50%

* Dye: Acid Blue No. 9
** A target and a range are not defined for HBsAgQ2 C1 S/CO.

PRECAUTIONS

- IVD
- *For In Vitro* Diagnostic Use
- **CAUTION:** This product contains human sourced and/or potentially infectious components. Refer to the **CONTENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens[1], Biosafety Level 2[2] or other appropriate biosafety practices[3,4] should be used for materials that contain or are suspected of containing infectious agents.
- Negative Control contains human plasma that is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.
- Positive Control contains purified HBsAg (inactivated). The human plasma used is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.
- WARNING: SENSITIZER Warning: May cause an allergic reaction.
- CONTAINS: AZIDE Contains Sodium Azide. Contact with acids liberates very toxic gas.
- This material and its container must be disposed of in a safe way.
- The following warnings and precautions apply to the Controls.

WARNING: Contains methylisothiazolones.
H317 May cause an allergic skin reaction.

Prevention
P261 Avoid breathing mist / vapours / spray.
P272 Contaminated work clothing should not be allowed out of the workplace.
P280 Wear protective gloves / protective clothing / eye protection.

Response
P302+P352 IF ON SKIN: Wash with plenty of water.
P333+P313 If skin irritation or rash occurs: Get medical advice / attention.
P363 Wash contaminated clothing before reuse.

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

- Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

STORAGE

- ARCHITECT HBsAg Qualitative II Controls are stable until the expiration date when stored and handled as directed.
- Do not use past expiration date.
- ARCHITECT HBsAg Qualitative II Controls must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- 2°C – 8°C

QUALITY CONTROL PROCEDURE

Refer to the ARCHITECT HBsAg Qualitative II and ARCHITECT HBsAg Qualitative II Confirmatory Reagent package inserts for information on ordering controls and for the recommended volume requirements for the controls for each assay.

PREPARATION FOR USE

- The ARCHITECT HBsAg Qualitative II Controls are liquid ready-to-use.
- ARCHITECT HBsAg Qualitative II Controls must be mixed by gentle inversion before use.
- After each use, tightly close the caps and return the controls to 2-8°C storage.

BIBLIOGRAPHY

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

GTIN	Global Trade Item Number
PRODUCT OF IRELAND	Product of Ireland

ARCHITECT SYSTEM

HBsAg Qualitative II Calibrators

en

HBsAg Qualitative II

REF 2G22-01

S2G220

G3-4431/R02

Read Highlighted Changes
Revised March 2013

INTENDED USE

The ARCHITECT HBsAg Qualitative II Calibrators are for the calibration of the ARCHITECT *i* System when used for qualitative detection and confirmation of the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma using the ARCHITECT HBsAg Qualitative II and HBsAg Qualitative II Confirmatory Reagent Kits. Refer to the ARCHITECT HBsAg Qualitative II and ARCHITECT HBsAg Qualitative II Confirmatory Reagent package inserts and the ARCHITECT System Operations Manual for additional information.

CONTENTS

2 Bottles (4.0 mL each) of ARCHITECT HBsAg Qualitative II Calibrators. Calibrator 1 (CAL 1) contains inactivated purified human HBsAg (subtype *ad*) in phosphate buffer with human plasma and protein (bovine serum albumin) stabilizers. Preservatives: ProClin 300 and ProClin 950. Calibrator 2 (CAL 2) contains recalcified human plasma. Preservatives: ProClin 950 and sodium azide.

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

STANDARDIZATION

The ARCHITECT HBsAg Qualitative II Calibrator 1 is referenced to the World Health Organization (WHO) Second International Standard for HBsAg (subtype *adw2*, genotype A, NIBSC Code 00/568).

PRECAUTIONS

- IVD
- For *In Vitro* Diagnostic Use
- ⚠ **CAUTION:** This product contains human sourced and/or potentially infectious components. Refer to the **CONTENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens[1]. Biosafety Level 2[2] or other appropriate biosafety practices[3,4] should be used for materials that contain or are suspected of containing infectious agents.
- Calibrator 1 contains purified HBsAg (inactivated). The human plasma used is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.
- Calibrator 2 contains human plasma that is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.
- WARNING: SENSITIZER Warning: May cause an allergic reaction.
- CONTAINS: AZIDE Contains Sodium Azide. Contact with acids liberates very toxic gas.
- This material and its container must be disposed of in a safe way.
- The following warnings and precautions apply to the Calibrators:

WARNING:	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapours / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P363	Wash contaminated clothing before reuse.

- This material and its container must be disposed of in a safe way.
- Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

STORAGE

- ARCHITECT HBsAg Qualitative II Calibrators are stable until the expiration date when stored and handled as directed.
- Do not use past expiration date.
- ARCHITECT HBsAg Qualitative II Calibrators must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.

PREPARATION FOR USE

- The ARCHITECT HBsAg Qualitative II Calibrators are liquid ready-to-use.
- ARCHITECT HBsAg Qualitative II Calibrators must be mixed by gentle inversion before use.
- To perform a calibration, test the calibrators in replicates of 3. The calibrators should be priority loaded.
- For information on ordering calibrations and for the recommended volume requirements for the calibrators for each assay, refer to the ARCHITECT HBsAg Qualitative II and ARCHITECT HBsAg Qualitative II Confirmatory package inserts.
- After each use, tightly close the caps and return the calibrators to 2-8°C storage.

QUALITY CONTROL PROCEDURE

A single sample of each control level must be tested to evaluate the assay calibration. For information on ordering controls, refer to the ARCHITECT System Operations Manual, Section 5.

- Ensure that assay control values are within the ranges specified in the control package insert.

BIBLIOGRAPHY

1. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
2. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
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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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Key to symbols used

GTIN	Global Trade Item Number
PRODUCT OF IRELAND	Product of Ireland

ARCHITECT SYSTEM

en

HBsAg Qualitative II

REF 2G22

B2G220

G3-4444/R05

Read Highlighted Changes
Revised September 2013

HBsAg Qualitative II

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

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

Key to symbols used

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

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

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NAME

ARCHITECT HBsAg Qualitative II

INTENDED USE

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

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

SUMMARY AND EXPLANATION OF TEST

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

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

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

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

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

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

REAGENTS

Reagent Kit, 100 Tests/500 Tests

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

ARCHITECT HBsAg Qualitative II Reagent Kit (2G22)

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

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

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

ARCHITECT *i* Trigger Solution

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

ARCHITECT *i* Wash Buffer

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

WARNINGS AND PRECAUTIONS

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

Safety Precautions

- **CAUTION:** This product contains human sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens[7], Biosafety Level 2[8] or other appropriate biosafety practices[9,10] should be used for materials that contain or are suspected of containing infectious agents.
- The Conjugate contains human plasma that is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2 and anti-HCV.
- The following warnings and precautions apply to these components:
  - Microparticles
  - Conjugate
  - Ancillary Wash Buffer

WARNING:	Contains methylisothiazolones
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapours / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P363	Wash contaminated clothing before reuse.

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

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

Handling Precautions

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

- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - When handling conjugate vials, change gloves that have contacted human serum or plasma, since introduction of human IgG or IgM will result in a neutralized conjugate.
  - Once a septum has been placed on the reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

- The ARCHITECT HBsAg Qualitative II Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, the reagents are stable until the expiration date.
- The ARCHITECT HBsAg Qualitative II Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT HBsAg Qualitative II assay file (assay number 628) must be installed on the ARCHITECT *i* System before performing the assay.
- For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

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

Specimen Conditions

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

Preparation for Analysis

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

Storage

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

Shipping

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

TESTING OF CADAVERIC BLOOD SPECIMENS

- Performance has been established for the use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating) that have been collected up to 18.5 hours after death. Performance was established using 50 spiked and 50 non-spiked cadaveric blood specimens[1].
- Testing of cadaveric blood specimens from patients with plasma dilution due to transfusions of > 2000 mL of blood or colloids within 48 hours, or > 2000 mL of crystalloids within 1 hour (or any combination thereof) prior to collection of the specimens have not been validated.
- Follow general standards and/or regulations for collection, storage and handling.
- Follow the tube manufacturer's processing instructions for serum or plasma collection tubes. After initial centrifugation, transfer the supernatant to a centrifuge tube and centrifuge at ≥ 10,000 RCF (Relative Centrifugal Force) for 10 minutes. If specimens are not processed directly after initial centrifugation, it is recommended to remove the supernatant from the clot or red blood cells until further processing.
- Cadaveric blood specimens can be stored for up to 5 days at 2-8°C or up to 24 hours at 15-30°C following collection.
- No qualitative differences were observed for cadaveric blood specimens (nonreactive or spiked reactive) when subjected to up to 3 freeze/thaw cycles. However, multiple freeze/thaw cycles should be avoided.

PROCEDURE

Materials Provided

- 2G22 ARCHITECT HBsAg Qualitative II Reagent Kit

Materials Required but not Provided

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

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

Assay Procedure

- Before loading the ARCHITECT HBsAg Qualitative II Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Handling Precautions** section of this package insert.
- Load the ARCHITECT HBsAg Qualitative II Reagent Kit on the ARCHITECT *i* System.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample cup volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present before running the test.
  - Priority: 125 µL for the first HBsAg Qualitative II test plus 75 µL for each additional HBsAg Qualitative II test from the same sample cup.
  - ≤ 3 hours on-board: 150 µL for the first HBsAg Qualitative II test plus 75 µL for each additional HBsAg Qualitative II test from the same sample cup.
  - > 3 hours on-board: replace with a fresh sample (patient specimens, controls and calibrators).
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare calibrators and controls.
  - Mix the ARCHITECT HBsAg Qualitative II Calibrators and Controls by gentle inversion before use.
  - To obtain the recommended volume requirements for the ARCHITECT HBsAg Qualitative II Calibrators and Controls, hold the bottles **vertically** and dispense 11 drops of each calibrator and 6 drops of each control into each respective sample cup.
  - If commercially available control material is used, follow the manufacturer's instructions for preparation.
- Load samples
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedure

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

Calibration

- To perform an ARCHITECT HBsAg Qualitative II calibration, test calibrators 1 and 2 in replicates of 3. The calibrators should be priority loaded.
- A single sample of each control level must be tested to evaluate the assay calibration.
  - Order controls as described in the **Assay Procedure** section.
  - Ensure that assay control values are within the ranges specified in the control package insert.
- Once an ARCHITECT HBsAg Qualitative II calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

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

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

Verification of Assay Claims

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

RESULTS

Calculations

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

Interpretation of Results

ARCHITECT HBsAg Qualitative II Initial Result

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

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

ARCHITECT HBsAg Qualitative II Retest Results

Instrument Interpretation	Specimen Classification
Both results nonreactive	Specimen considered negative for HBsAg.
One or both results reactive	Specimen considered repeatedly reactive; confirm using a neutralizing assay.*

* The ARCHITECT HBsAg Qualitative II Confirmatory assay is recommended.

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

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

Flags

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

LIMITATIONS OF THE PROCEDURE

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

SPECIFIC PERFORMANCE CHARACTERISTICS

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

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

Precision

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

Within-Laboratory Precision

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

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

NA = Not applicable

System Reproducibility

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

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

NA = not applicable

Specificity

Blood Donor Specimens

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

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

Category	n	IR[a] (%)	RR[a] (%)	Specificity	95% Confidence Interval
Overall Blood Donors	5401[b]	7 (0.13%)	6 (0.11%)	99.91% (5395/5400)	99.76% - 99.97%
Blood Donors Plasma	2700	4 (0.15%)	3 (0.11%)	99.89% (2697/2700)	99.68% - 99.98%
Blood Donors Serum	2701[b]	3 (0.11%)	3 (0.11%)	99.93% (2698/2700)	99.73% - 99.99%

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

[b] One specimen confirmed positive.

Diagnostic Specimens

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

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

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

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

[c] Sixteen specimens confirmed positive.

[d] Ten specimens confirmed positive.

[e] Six specimens confirmed positive.

Sensitivity

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

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

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

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

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

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

Analytical Sensitivity

The ARCHITECT HBsAg Qualitative II assay is designed to have a mean analytical sensitivity value that is less than or equal to the lower limit of the 95% confidence interval around the mean analytical sensitivity of a commercially available HBsAg assay. Analytical sensitivity was evaluated using serial dilutions of the WHO 2nd International HBsAg Standard (subtype *adw2*, genotype A, NIBSC Code 00/586). The dilutions ranged from 0.010 to 0.5 IU/mL. Recalcified negative human plasma/serum was used as the diluent and represented the 0 IU/mL sample. The dilutions were tested across 3 reagent lots on 3 instrument types (1 *i*2000$_{SR}$, 1 *i*2000 and 1 *i*1000$_{SR}$). In this study, the lower limit of the 95% confidence interval for the commercially available HBsAg assay was 0.021 IU/mL. The analytical sensitivity results for ARCHITECT HBsAg Qualitative II, calculated by linear regression, ranged from 0.017 to 0.022 IU/mL. The mean analytical sensitivity ranged from 0.019 to 0.020 IU/mL across instrument types.

Analytical Specificity

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

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

[a] NR = Nonreactive, R = Reactive

Seroconversion Sensitivity

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

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

HBsAg Mutant Detection

The hepatitis B virus, unlike other DNA viruses, replicates through reverse transcription. The reverse transcription process lacks proofreading capability; therefore, HBV is subject to a mutation rate 10 times higher than the mutation rate of other DNA viruses.[17] Some of these mutations may cause changes in the antigenic structure of HBsAg, resulting in epitopes that are no longer recognized by anti-HBs. HBsAg mutants have been reported in a wide range of patient populations, including blood donors, vaccine recipients, renal dialysis patients, orthotopic liver transplant recipients, infants born to HBsAg-positive mothers and patients undergoing nucleoside analog treatment for HBV.[17-24] HBsAg mutations may result in a less favorable outcome in some patients[17,18,20] and false negative results in some HBsAg assays.[17-19]

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

Mutant	Final Interpretation: ARCHITECT HBsAg Qualitative II	Final Interpretation: Commercially Available HBsAg
Gln-129-His	Repeatedly Reactive	Repeatedly Reactive
Met-133-Leu	Repeatedly Reactive	Repeatedly Reactive
Asp-144-Ala	Repeatedly Reactive	Nonreactive
Gly-145-Arg	Repeatedly Reactive	Repeatedly Reactive
Thr-123-Ala	Repeatedly Reactive	Nonreactive
P142L+G145R	Repeatedly Reactive	Repeatedly Reactive
P142S+G145R	Repeatedly Reactive	Repeatedly Reactive
I22NT	Repeatedly Reactive	Repeatedly Reactive
I22RA	Repeatedly Reactive	Repeatedly Reactive

HBV Genotype Detection

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

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

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

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ARCHITECT Anti-HBs

REF 7C18-41
REF 7C18-42

en
Anti-HBs
7C18
G8-0542/R02
B7C110

Read Highlighted Changes: Revised November 2016.

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.

NAME

ARCHITECT Anti-HBs

INTENDED USE

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

SUMMARY AND EXPLANATION OF THE TEST

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

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

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

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

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

1. Sample and recombinant HBsAg (rHBsAg) coated paramagnetic microparticles are combined. The Anti-HBs present in the sample binds to the rHBsAg coated microparticles.
2. After washing, acridinium-labeled rHBsAg conjugate is added to create a reaction mixture.
3. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of anti-HBs in the sample and the RLUs detected by the ARCHITECT iSystem optics.

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

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

REAGENTS

Kit Contents

ARCHITECT Anti-HBs 7C18

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

REF	7C18-41	7C18-42
Σ	100	500
MICROPARTICLES	1 x 4.56 mL	1 x 16.80 mL
CONJUGATE	1 x 5.9 mL	1 x 26.3 mL
SPECIMEN DILUENT	1 x 3.01 mL	1 x 11.99 mL

MICROPARTICLES Microparticles coated with Hepatitis B Surface Antigen (Subtypes *ad* and *ay*) (*E. coli* Recombinant DNA expressed in murine cells) in TRIS buffer with protein stabilizers. Minimum concentration: 0.08% solids. Preservatives: sodium azide and antimicrobial agents.

CONJUGATE Hepatitis B Surface Antigen (Subtypes *ad* and *ay*) (*E. coli* Recombinant DNA expressed in murine cells) labeled with Acridinium in MES buffer with protein stabilizers (Bovine and Human Plasma). Minimum concentration: 0.13 µg/mL. Preservatives: sodium azide and antimicrobial agents.

SPECIMEN DILUENT ARCHITECT Anti-HBs Specimen Diluent containing recalcified human plasma. Preservatives: sodium azide and ProClin 950.

Other Assay-Specific Reagents

SPECIMEN DILUENT 1 x 100 mL ARCHITECT Anti-HBs Specimen Diluent, REF 7C18-40, containing recalcified human plasma. Preservatives: sodium azide and ProClin 950.

Other Reagents

PRE-TRIGGER SOLUTION ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

WASH BUFFER ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

Safety Precautions

CAUTION: This product contains human-sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human-sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[7-10]

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

The following warnings and precautions apply to: MICROPARTICLES / CONJUGATE	
Contains sodium azide.	
EUH032	Contact with acids liberates very toxic gas.
P501	Dispose of contents / container in accordance with local regulations.

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

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

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

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
  - To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.

For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Reagent Storage

When stored and handled as directed, reagents are stable until the expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8°C	Until expiration date	May be used immediately after removal from 2-8°C storage. Store in upright position.
On board	System temperature	30 days	Discard after 30 days. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

* Reagents may be stored on or off the ARCHITECT iSystem. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The ARCHITECT Anti-HBs assay file must be installed on the ARCHITECT iSystem 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.

Alternate Result Units

Edit assay parameter "Result concentration units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default result unit	Conversion factor	Alternate result unit
mIU/mL	1	IU/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay.

Specimen Types	Collection Tubes
Human serum	Serum Serum separator tubes
Human plasma	Dipotassium EDTA Sodium citrate ACD CPDA-1 Lithium heparin Sodium heparin

- Other specimen collection tube types have not been tested with this assay.
- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum or plasma.
- Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient specimens.
- The instrument 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 assay.
- 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.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - grossly hemolyzed
- 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.
- **For optimal results, serum and plasma specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give inconsistent results and must be transferred to a centrifuge tube and centrifuged at a minimum of 10,000 RCF (Relative Centrifugal Force) for 10 minutes.**
- Samples containing particulate matter or red blood cells must be centrifuged prior to running the assay.
- 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.
- No qualitative performance differences were observed between experimental controls and the 23 nonreactive or 23 spiked reactive specimens tested with elevated levels of triglycerides (≤ 3000 mg/dL),* bilirubin (≤ 20 mg/dL),* and hemoglobin (≤ 500 mg/dL).*
- No qualitative performance differences were observed between experimental controls and the 30 nonreactive or 30 spiked reactive specimens tested with red blood cells at ≤ 0.4% v/v.*
- No qualitative performance differences were observed between experimental controls and the 21 nonreactive or 20 spiked reactive specimens tested with elevated levels of protein (≤ 12 g/dL).*

  * The quantitative performance differences observed were within normal assay variability.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.

  Specimens must be separated from clots or red blood cells using centrifugation as recommended by the tube manufacturer.
- **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.
- 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.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum/Plasma	2-8°C	≤ 14 days

Specimens may be stored on or off the clot or red blood cells.

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).

No qualitative performance differences were observed between experimental controls and the 24 nonreactive or 22 spiked reactive specimens subjected to 4 freeze-thaw cycles. The quantitative performance differences observed were within normal assay variability; however, multiple freeze-thaw cycles should be avoided.

Specimen Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

7C18 ARCHITECT Anti-HBs Reagent Kit

Materials Required but not Provided

- ARCHITECT Anti-HBs Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com
- 7C18-03 ARCHITECT Anti-HBs Calibrators
- 7C18-13 ARCHITECT Anti-HBs Controls
- 7C18-40 ARCHITECT Anti-HBs Specimen Diluent
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
- ARCHITECT Replacement Caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

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

Assay Procedure

- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.

- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- Minimum sample cup volume is calculated by the system and printed on the Orderlist report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

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

    Sample volume for first test: 125 µL

    Sample volume for each additional test from same sample cup: 75 µL
  - ≤ 3 hours on board:

    Sample volume for first test: 150 µL

    Sample volume for each additional test from same sample cup: 75 µL
  - > 3 hours on board: Additional sample volume required.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare ARCHITECT Anti-HBs Calibrators and Controls.
  - Mix calibrator(s) and controls by gentle inversion before use.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
  - Recommended volumes:

    for each calibrator: 7 drops

    for each control: 5 drops
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press **RUN**.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures

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

Automated Dilution Protocol

The system performs a 1:25 dilution (for concentrations up to 25,000 mIU/mL) of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.

Manual Dilution Procedure

(for concentrations up to 100,000 mIU/mL)

Suggested dilution: 1:100

It is recommended that dilutions not exceed 1:100.

1. Add 10 µL of the patient specimen to 990 µL of ARCHITECT Anti-HBs Specimen Diluent (7C18-40).
2. The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The concentration reported by the ARCHITECT iSystem **MUST** be greater than 8.00 mIU/mL. If the reported concentration is less than 8.00 mIU/mL, make a smaller dilution.

   Specimens with an anti-HBs value reported as greater than 25,000 mIU/mL may be diluted using either the Automated Dilution Protocol (1:25 dilution) or the Manual Dilution Procedure. The Manual Dilution Procedure detailed above can be used for concentrations up to 100,000 mIU/mL.

Automated Dilution Protocol and the Manual Dilution Procedure

(for concentrations up to 2,500,000 mIU/mL)

Suggested dilution: 1:100

It is recommended that dilutions not exceed 1:100.

1. Add 10 µL of the patient specimen to 990 µL of ARCHITECT Anti-HBs Specimen Diluent (7C18-40)
2. Order the Automated Dilution Protocol (1:25 dilution) using the manually diluted 1:100 sample.

   The concentration reported by the ARCHITECT iSystem **MUST** be greater than 8.00 mIU/mL. **Multiply the result (from the Automated Dilution Protocol) by the manual dilution factor (e.g. 100) to obtain the final sample concentration.** If the concentration reported by the ARCHITECT iSystem is less than 8.0 mIU/mL, make a smaller dilution.

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

Calibration

- Test Calibrators A-F in duplicate. The calibrators should be priority loaded.

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

Quality Control Procedures

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

- The recommended control requirement for the ARCHITECT Anti-HBs assay is that a single sample of each control level be tested:
  - Once every 24 hours each day of use.
  - After performing calibration.
  - After instrument service procedures or maintenance that may affect assay performance have been performed.
- If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures.

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

  Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated sample results are invalid and the samples must be retested. Recalibration may be indicated.

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

Verification of Assay Claims

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

The ARCHITECT Anti-HBs assay belongs to method group 4.

RESULTS

Calculation

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

Interpretation of Results

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

For additional information about the detection capability of the ARCHITECT Anti-HBs assay, refer to the **SPECIFIC PERFORMANCE CHARACTERISTICS** section, under **Limit of Blank, Limit of Detection, and Limit of Quantitation.**

Follow your country-specific regulations and laboratory procedures for reporting of results.

Flags

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

Measuring Interval

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

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

LIMITATIONS OF THE PROCEDURE

- If the Anti-HBs results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with patient history and other Hepatitis markers for diagnosis of acute, chronic, or recovered infection.
- Quantitative values obtained using alternative assays (i.e. MEIA, EIA or RIA) may not be equivalent and cannot be used interchangeably. A new baseline, using the ARCHITECT Anti-HBs assay, should be established when monitoring vaccinees.

SPECIFIC PERFORMANCE CHARACTERISTICS

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

Precision

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

TABLE I: ARCHITECT Anti-HBs Precision

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

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

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

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

Sensitivity

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

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

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

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

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

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

HBV Vaccine Recipient Serial Bleed Panels

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

Specificity

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

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

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

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

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

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

Overall Specificity and Sensitivity

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

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

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

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

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

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Key to Symbols

	Caution
	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number
GTIN	Global Trade Item Number
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCT OF IRELAND	Product of Ireland
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
SPECIMEN DILUENT	Specimen Diluent
TRIGGER SOLUTION	Trigger Solution
WARNING: SENSITIZER	Warning: May cause an allergic reaction.
WASH BUFFER	Wash Buffer

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.

5,458,646	5,543,324	5,545,739
5,565,570	5,669,819	5,783,699

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