ARCHITECT Anti-HCV

REF 6C37-27
REF 6C37-22
REF 6C37-37
REF 6C37-32

en
Anti-HCV
6C37
G5-3150/R11
B6C370

Read Highlighted Changes: Revised November 2014.

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-HCV

INTENDED USE

The ARCHITECT Anti-HCV assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of antibody to hepatitis C virus (anti-HCV) in human serum and plasma including specimens collected post-mortem (non-heart-beating). The ARCHITECT Anti-HCV assay is intended to be used as an aid in the diagnosis of Hepatitis C infection and as a screening test to prevent transmission of Hepatitis C virus (HCV) to recipients of blood, blood components, cells, tissue and organs.

SUMMARY AND EXPLANATION OF THE TEST

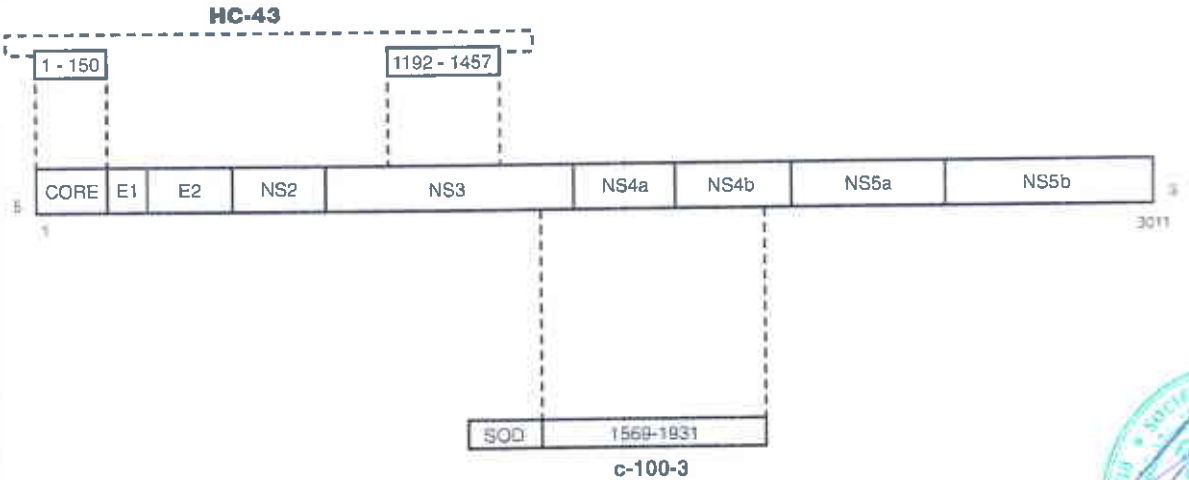
The ARCHITECT Anti-HCV assay is for the detection of antibodies to hepatitis C virus (HCV). Chemiluminescent immunoassays are a variation of the enzyme immunoassay (EIA) principle. Solid phase EIAs, first described in the early 1970s, use antigens and/or antibodies coated on a surface to bind complementary analytes.[1] The bound analyte is detected by a series of antigen-antibody reactions. EIAs are available to identify antigens and antibodies related to viral hepatitis infection. In the ARCHITECT Anti-HCV final reaction, bound acridinylated conjugates are used to generate a chemiluminescent signal.

HCV is a bloodborne virus.[2, 3] Serological studies employing EIAs for detection of antibodies to recombinant antigens of HCV have established HCV as the cause of most bloodborne[4-9] as well as community-acquired[10] non-A, non-B hepatitis. The presence of anti-HCV indicates that an individual may have been infected with HCV, may harbor infectious HCV, and/or may be capable of transmitting HCV infection.[11] Although the majority of infected individuals may be asymptomatic, HCV infection may develop into chronic hepatitis, cirrhosis, and/or increased risk of hepatocellular carcinoma.[12-15] The implementation of blood donation screening for anti-HCV by EIAs has led to a marked decline in the risk of transfusion-transmitted hepatitis.[16, 17]

ARCHITECT Anti-HCV has been designed to detect antibodies to putative structural and nonstructural proteins of the HCV genome. The relationship between the recombinant HCV proteins in ARCHITECT Anti-HCV and the putative structural and nonstructural proteins of the HCV genome is depicted below.[18]

- HCr43: The HCr43 protein is expressed in *Escherichia coli (E. coli)* and is composed of two noncontiguous coding regions of the HCV genome sequence. The first region represents amino acids 1192 to 1457 (33c) of the HCV sequence. The second of the two regions represents amino acids 1 to 150 (core) of the HCV sequence. Because of the similarity of the genomic organization of the flaviviruses, it is suggested that the first sequence is from the NS3 coding region and the second sequence is from the core coding region of HCV.
- c100-3: The c100-3 antigen is a recombinant HCV protein expressed in *Saccharomyces cerevisiae* (yeast). The genomic organization of flaviviruses suggests that the cloned sequence is contained within the putative nonstructural (NS3 and NS4) regions of HCV. The c100-3 protein is a chimeric fusion protein with 154 amino acids of human superoxide dismutase (hSOD), five linker amino acids, amino acids number 1569 to 1931 of the HCV polyprotein, and the additional five amino acid linker at the carboxyl terminus.

Hepatitis C antigens HCr43 and c100-3 are prepared under US license by Chiron Corporation under a shared manufacturing agreement. The ARCHITECT Anti-HCV assay is manufactured under contract agreement from Ortho Diagnostic Systems and Chiron Corporation.



BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

1. Sample, recombinant HCV antigen coated paramagnetic microparticles and Assay Diluent are combined. The anti-HCV present in the sample binds to the HCV coated microparticles.
2. After washing, anti-human 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 anti-HCV in the sample and the RLUs detected by the ARCHITECT iSystem optics.

The presence or absence of anti-HCV in the specimen 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 reaction is greater than or equal to the cutoff signal, the specimen is considered reactive for anti-HCV.

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

REAGENTS

Kit Contents

ARCHITECT Anti-HCV 6C37

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

REF	6C37-27	6C37-22	6C37-37	6C37-32
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
ASSAY DILUENT	1 x 10.0 mL	4 x 10.0 mL	1 x 50.9 mL	4 x 50.9 mL

MICROPARTICLES HCV (*E. coli*, yeast, recombinant) antigen coated microparticles in MES buffer. Minimum concentration: 0.14% solids. Preservatives: antimicrobial agents.

CONJUGATE Conjugate: murine anti-IgG/anti-IgM acridinium-labeled conjugate in MES buffer. Minimum concentration: (IgG) 8 ng/mL/ (IgM) 0.8 ng/mL. Preservatives: antimicrobial agents.

ASSAY DILUENT Anti-HCV Assay Diluent containing TRIS buffer with protein stabilizers. Preservatives: antimicrobial agents.

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 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.[19-22]

The following warnings and precautions apply to: CONJUGATE	
WARNING:	Contains polyethylene glycol octylphenyl ether (Triton X-405).
H319	Causes serious eye irritation.
Prevention	
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice / attention.

The following warnings and precautions apply to: ASSAY DILUENT	
DANGER:	Contains polyethylene glycol octylphenyl ether (Triton X-405).
H318	Causes serious eye damage.
H412	Harmful to aquatic life with long lasting effects.
Prevention	
P280	Wear protective gloves / protective clothing / eye protection.
P273	Avoid release to the environment.
Response	
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor / physician.
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.
- When handling conjugate vials, change gloves that have contacted human serum or plasma, since introduction of human IgG/IgM will result in a neutralized conjugate.

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-HCV 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.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Validated specimen types to be used with this assay:

Specimen Types	Collection Tubes
Human serum	Serum Serum separator tubes
Human plasma	potassium EDTA lithium heparin sodium heparin sodium citrate ACD CPDA-1 CPD CP2D potassium oxalate

- Other anticoagulants have not been validated for use with the ARCHITECT Anti-HCV 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.
- 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.**
- 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:

- heat-inactivated
- pooled
- 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.

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.

To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

No qualitative performance differences were observed between experimental controls and the 23 nonreactive or 23 spiked reactive specimens tested with elevated levels of bilirubin (≤ 20 mg/dL), hemoglobin (≤ 500 mg/dL), triglycerides (≤ 3,000 mg/dL), or protein (≤ 12 g/dL).

No qualitative performance differences were observed between experimental controls and the 25 nonreactive or 25 spiked reactive specimens tested with red blood cells at ≤ 0.4% v/v.

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.**
- **For optimal results, serum and plasma specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give inconsistent results and must be transferred to a centrifuge tube and centrifuged at least 10,000 RCF (Relative Centrifugal Force) for 10 minutes.**
- 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	≤ 7 days
	- 20°C or colder	--

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

Remove serum or plasma from the clot, serum separator, or red blood cells if stored longer than the maximum 2-8°C storage time and store at - 20°C or colder.

No qualitative differences were observed between experimental controls and the 25 nonreactive or 25 spiked reactive specimens subjected to 6 freeze/thaw cycles; 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.
- It is recommended that specimens be removed from the clot, serum separator or red blood cells.
- Specimens may be shipped ambient, at 2-8°C (wet ice), or -20°C or colder (dry ice).
- Do not exceed the storage time limitations listed above.

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 15 hours after death. Performance was established using 50 spiked and 50 non-spiked cadaveric blood specimens. [23]
- 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 7 days at 2-8°C or up to 3 days 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

6C37 ARCHITECT Anti-HCV Reagent Kit

Materials Required but not Provided

- ARCHITECT Anti-HCV Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 6C37-01 ARCHITECT Anti-HCV Calibrator
- 6C37-10 ARCHITECT Anti-HCV Controls
- 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:
  - **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, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Carefully snap the septum onto the top of the bottle.
- 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.
- The minimum sample cup volume required to perform a single anti-HCV test on the ARCHITECT iSystem is 150 µL for the first anti-HCV test plus 20 µL for each additional anti-HCV test from the same sample cup. No more than 10 replicates may be sampled from the same sample cup. Verify the minimum sample volume is present in the sample cup prior to running the test. The minimum sample cup volume is calculated by the system and is displayed on the Patient, Calibrator, and Control order screens and on the Orderlist report.

- For a sample that is priority loaded, with 3 or fewer replicates ordered, a smaller sample cup volume than is displayed on the order screen may be used. In this case, the minimum sample cup volume is 70 µL for the first anti-HCV test plus 20 µL for each additional replicate. For additional information on priority loading, refer to the ARCHITECT System Operations Manual, Section 5.
- To minimize the effects of evaporation, all samples (patient specimens, calibrator, and controls) must be tested within 3 hours of being placed on board the ARCHITECT iSystem. If the sample is on board the system for longer than 3 hours, replace with fresh sample. For additional information on sample evaporation and volumes, refer to the ARCHITECT System Operations Manual, Section 5.
- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

- Prepare ARCHITECT Anti-HCV Calibrator 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: 5 drops
    for each control: 6 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 cannot be diluted for the ARCHITECT Anti-HCV assay.

Calibration

- Test Calibrator 1 in replicates of three. The calibrator 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.
- Once an ARCHITECT Anti-HCV 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

NOTE: It is recommended that the ARCHITECT Anti-HCV Positive Control and the Negative Control be run to verify the calibration. The recommended control requirement for the ARCHITECT Anti-HCV assay is a single sample of both ARCHITECT Anti-HCV Controls tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory specific procedures. Ensure that assay Control values are within the ranges specified in the Controls package insert.

Verification of Assay Claims

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

The ARCHITECT Anti-HCV assay belongs to method group 5.

RESULTS

The ARCHITECT iSystem calculates the Anti-HCV Calibrator 1 mean chemiluminescent signal from three Calibrator 1 replicates and stores the result.

Calculation

The ARCHITECT Anti-HCV assay calculates a result based on S/CO.

- Cutoff calculation:
  Calibrator 1 Mean RLU Value x 0.074 = Cutoff RLU
- S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

Initial Results

S/CO	Instrument Interpretation	Retest Procedure
< 1.00	Nonreactive	No retest required.
≥ 1.00	Reactive	Retest in duplicate.

Duplicate Retest Results

Instrument Interpretation	Specimen Classification
Both results nonreactive	Specimen considered nonreactive for anti-HCV.
One or both results reactive	Specimen considered repeatedly reactive for anti-HCV by the criteria of ARCHITECT Anti-HCV.

Repeatedly reactive anti-HCV specimens should be investigated further in supplemental tests such as other HCV specific immunoassays and immunoblot assays or a combination thereof and/or NAT tests.

NOTE: For details on configuring the ARCHITECT iSystem to use grayzone and high reactive interpretations, refer to the ARCHITECT System Operations Manual, Section 2. The grayzone and high reactive 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

- False positive results can be expected with any test kit. The proportion of these falsely reactive specimens is dependent upon the specificity of the test kit, specimen integrity, and on the prevalence of HCV antibodies in the population being screened.
- If the ARCHITECT Anti-HCV results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute or chronic infection.
- 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.

SPECIFIC PERFORMANCE CHARACTERISTICS

NOTE: Representative performance data are shown. Results obtained in individual laboratories and with different populations may vary.

Precision

The precision of ARCHITECT Anti-HCV was determined using three reagent lots. A panel composed of four unique members was tested in replicates of four with each reagent lot once daily for five days across three instruments. Each daily run also included the ARCHITECT Positive Control run in duplicate at the beginning and end of the run. The intra-assay, inter-assay, total standard deviation (SD) and percent coefficient of variation (%CV) were determined with a variance component analysis[24] for a random effects model[25] (Table I).

Table I: ARCHITECT Anti-HCV Precision

Panel member	Total No. Replicates	Grand Mean (S/CO)	Intra-assay		Inter-assay[a]		Total[b]	
			SD	%CV	SD	%CV	SD	%CV
1	180	7.39	0.351	4.7	0.385	5.3	0.447	6.0
2	180	3.92	0.138	3.5	0.169	4.3	0.209	5.3
3	180	1.50	0.056	3.7	0.067	4.4	0.095	6.4
4	180	0.08	0.005	5.6	0.007	8.4	0.011	13.4
Positive Control	180	3.27	0.127	3.9	0.147	4.5	0.166	5.1

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

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

Specificity

A total of 8,942 serum and plasma specimens from volunteer whole blood and plasmapheresis donors were evaluated. The specimens from volunteer whole blood donors were collected from European blood centers and the plasmapheresis specimens from US blood centers (Table II). There was a total of 59 repeatedly reactive specimens. Following supplemental testing with an anti-HCV immunoblot assay, 28 specimens were anti-HCV positive (reactive to two or more gene products), 15 were indeterminate (reactive to one gene product), and 16 were negative (no gene products detected). Ninety-nine of the 1,500 specimens obtained from hospital patients were repeatedly reactive, of which 88 were anti-HCV positive, five indeterminate, and six negative by supplemental testing. In 65 specimens from individuals with medical conditions unrelated to HCV infection and specimens containing potentially interfering substances, three specimens were repeatedly reactive, and all were anti-HCV positive by supplemental testing.

Table II: Reactivity of the ARCHITECT Anti-HCV Assay in Specimens from Whole Blood Donors, Plasmapheresis Donors, Hospital Patients, Individuals with Medical Conditions Unrelated to HCV Infection, and in Specimens Containing Potentially Interfering Substances

Category	Number Tested	IR (% of Total)	RR (% of Total)	Number of Positive by Supplemental Testing[a] (% of Repeatedly Reactive)
Volunteer Whole Blood Donors				
Serum[b]	3,000	14 (0.47)	12 (0.40)	0
Plasma	2,508	11 (0.44)	11 (0.44)	0
Plasmapheresis Donors	3,434	37 (1.08)	36 (1.05)	28 (77.78)
Total Donors	8,942	62 (0.69)	59 (0.66)	28 (47.46)
Hospital Patients	1,500	100 (6.67)	99 (6.60)	88 (88.89)
Medical Conditions Unrelated to HCV Infection and Potentially Interfering Substances[c]	65	3 (4.62)	3 (4.62)	3 (100.00)

IR = Initially Reactive; RR = Repeatedly Reactive

[a] A positive result was defined as reactive to two or more gene products by an immunoblot assay.

[b] Includes a subset of 500 matched serum/plasma pairs; only the serum results were included in the specificity calculation.

[c] Category included the following: anti-CMV positive (5), anti-EBV positive (5), anti-HAV positive (5), HBsAg positive (5), anti-HIV-1 positive (5), syphilis (5), rheumatoid factor (5), alcoholic liver disease (5), anti-HBc positive (5), anti-HTLV-I positive (5), human anti-mouse antibody positive (10), and influenza vaccine recipients (5).

Sensitivity

A total of 117 specimens from 50 individuals with chronic HCV infection, 42 individuals that were anti-HCV and HCV RNA positive, and 25 individuals at increased risk for HCV infection were tested. Of the 117 specimens, 100 were repeatedly reactive, and were anti-HCV positive by supplemental testing (Table III).

Table III: Reactivity of the ARCHITECT Anti-HCV Assay in Selected Populations with Chronic HCV Infection, Anti-HCV/HCV RNA Positive, and at Increased Risk for HCV Infection

Category	Number Tested	Number Repeatedly Reactive (% of Total)	Number of Positive by Supplemental Testing[a] (% of Repeatedly Reactive)
Chronic HCV Infection	50	50 (100.00)	50 (100.00)
Anti-HCV/HCV RNA Positive	42	42 (100.00)	42 (100.00)
Increased Risk for HCV Infection[b]	25	8 (32.00)	8 (100.00)
TOTAL	117	100 (85.47)	100 (100.00)

[a] A positive result was defined as reactive to two or more gene products by an immunoblot assay.

[b] Category included the following: intravenous drug users (5), hemophilia patients (10), men sex men (5), and female prostitutes (5).

Overall Specificity and Sensitivity

Overall specificity and sensitivity were estimated from the results of 10,624 serum and plasma specimens summarized in Tables II and III. The overall specificity was 99.60% (10,361/10,403) with a 95% confidence interval of 99.45% to 99.71%. Specificity observed from different sites ranged between 99.20% (496/500) to 99.70% (1994/2000). The sensitivity was 99.10% with a 95% confidence interval of 96.77% to 99.89%.

Seroconversion

The ability of the ARCHITECT Anti-HCV assay to detect anti-HCV was evaluated by testing 20 HCV seroconversion panels from blood and plasmapheresis donors who seroconverted over the course of their donation history. The panels were also tested by an approved assay. The ARCHITECT Anti-HCV assay detected anti-HCV three days (one bleed) earlier than the comparator assay in 1 of the 20 panels. The comparator assay detected anti-HCV five to six days (one bleed) earlier than ARCHITECT Anti-HCV in 3 of the 20 panels. Both assays exhibited equivalent detection of anti-HCV in 16 of the 20 panels.

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Key to Symbols

	Consult Instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
ASSAY DILUENT	Assay Diluent
CONJUGATE	Conjugate
CONTROL NO.	Control Number
ECO HAZARD	Ecological hazard
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCT OF GERMANY	Product of Germany
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: EYE IRRITANT	Warning: Causes serious eye irritation.
WASH BUFFER	Wash Buffer

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Revised November 2014.

Abbott

ARCHITECT Anti-CCP

REF 1P65-25
REF 1P65-35

en
Anti-CCP
1P65
ABBL174/R04
B1P650

Read Highlighted Changes: 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.

NAME

ARCHITECT Anti-CCP

INTENDED USE

The ARCHITECT Anti-CCP assay is a chemiluminescent microparticle immunoassay (CMIA) for the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma on the ARCHITECT iSystem. Detection of anti-CCP antibodies is used as an aid in the diagnosis of Rheumatoid Arthritis (RA) and should be used in conjunction with other clinical information. Autoantibody levels represent one parameter in a multicriterion diagnostic process, encompassing both clinical and laboratory-based assessments.

SUMMARY AND EXPLANATION OF THE TEST

Rheumatoid Arthritis (RA) is a common, systemic autoimmune disease affecting 0.5-1% of the population. It is characterized by chronic inflammation of the synovium, which commonly leads to progressive joint destruction and in most cases, to disability and reduction of quality of life.[1] Evidence gained over the last few years suggests that aggressive therapy given early in the disease has the greatest therapeutic potential.[2, 3]

The serum of RA patients contains a variety of antibodies directed against self-antigens. The most widely known of these autoantibodies is the rheumatoid factor (RF) antibody directed against the constant domain of IgG molecules. The presence of RF is one of the American College of Rheumatology's (ACR) criteria for the classification of RA.[4] Although the RF test has good sensitivity for RA, it is not very specific for the disease as it can also be detected in the serum of patients with other rheumatic or inflammatory diseases and even in a substantial percentage of the healthy (elderly) population.[5] For several years it has been recognized that antibodies to anti-perinuclear factor (APF) and anti-keratin (AKA) are highly specific for RA. It was subsequently reported that both of these antibodies reacted with native filaggrin and are now referred to as anti-filaggrin antibodies (AFA).[6-8] More recently it has been shown that all of these antibodies are directed to citrulline-containing epitopes.[9] Citrulline is a non-standard amino acid, as it is not incorporated into proteins during protein synthesis. It can, however, be generated via post-translational modification of arginine residues by the enzyme peptidyl arginine deiminase (PAD).[10] In 1998, Schellekens and colleagues reported that linear peptides containing citrulline (CP) were very specific for RA antibodies (96%) in an ELISA based assay.[11] Subsequent work demonstrated that cyclic variants of these peptides, termed cyclic citrullinated peptides (CCP), were equally specific for RA, but with a higher sensitivity than linear peptides.[12] To improve the sensitivity of the CCP test further, several dedicated libraries of citrulline-containing peptides were screened with RA sera and a new set of peptides (CCP2) were discovered which gave superior performance compared to the CCP1 test.[13] Over the last few years, many independent studies have confirmed the diagnostic performance of the CCP2 test.[14, 15] In 2007, the European League against Rheumatism (EULAR) published guidelines for the diagnosis of early RA, and the measurement of antibodies to anti-CCP was included as a serology marker.[16]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT Anti-CCP assay is a two-step immunoassay with an automated sample pretreatment for the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample is prediluted with wash buffer. The prediluted sample, CCP coated paramagnetic microparticles and sample diluent are combined. The Anti-CCP antibodies present in the sample binds to the CCP coated microparticles.
2. After washing, anti-human IgG 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 anti-CCP antibody 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 Anti-CCP 1P65

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

REF	1P65-25	1P65-35
Σ	100	500
MICROPARTICLES	1 x 6.5 mL	1 x 26.5 mL
CONJUGATE	1 x 5.8 mL	1 x 25.8 mL
SAMPLE DILUENT	1 x 9.8 mL	1 x 50.0 mL

MICROPARTICLES CCP coated microparticles in phosphate buffer with surfactant and protein (bovine) stabilizer. Minimum concentration: 0.05% solids. Preservative: sodium azide.

CONJUGATE Mouse anti-human IgG: acridinium-labeled conjugate in MES buffer with surfactant and protein (bovine) stabilizer. Minimum concentration: 10 ng/mL. Preservatives: Nipasept and Sarafloxacin.

SAMPLE DILUENT Phosphate buffer with surfactant and protein (bovine) stabilizer. Preservative: sodium azide.

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 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.[17-20]

The following warnings and precautions apply to: MICROPARTICLES and SAMPLE DILUENT	
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 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-CCP 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

The default result unit for the ARCHITECT Anti-CCP assay is U/mL.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay:

Specimen Types	Collection Tubes
Serum	Serum Serum separator tubes
Plasma	Lithium heparin plasma separator tubes Potassium EDTA

- Other specimen collection tube types have not been tested with this assay.
- Plasma specimens from different anticoagulant tube types should not be used interchangeably for monitoring anti-CCP.
- 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.

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.
- 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.

- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at ≥ 10,000 RCF (Relative Centrifugal Force) for 10 minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter,
  - they require repeat testing, 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	Room temperature (study performed at 30°C)	≤ 22 hours
	2-8°C	≤ 7 days
	-20°C or colder	

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

If testing will be delayed more than 22 hours for specimens stored at room temperature or more than 7 days for specimens stored at 2-8°C, remove serum or plasma from the clot, red blood cells, or separator gel and store at -20°C or colder.

Avoid more than three 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

1P65 ARCHITECT Anti-CCP Reagent Kit

Materials Required but not Provided

- ARCHITECT Anti-CCP Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 1P65-01 ARCHITECT Anti-CCP Calibrators
- 1P65-10 ARCHITECT Anti-CCP Controls
- 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: 60 µL

    Sample volume for each additional test from same sample cup: 10 µL
  - ≤ 3 hours on board:

    Sample volume for first test: 150 µL

    Sample volume for each additional test from same sample cup: 10 µL
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare ARCHITECT Anti-CCP Calibrators and Controls.
  - ARCHITECT Anti-CCP Calibrators and Controls should be prepared according to their respective package inserts.
  - Recommended volumes:

    for each calibrator: 4 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 anti-CCP value exceeding 200.0 U/mL are flagged with the code "> 200.0 U/mL" and may be diluted using either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a 1:6 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.

1. Add 50 µL of the patient specimen to 450 µL of ARCHITECT Anti-CCP Negative Control.

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.

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

Calibration

- Test Calibrators A-F in replicates of two. 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: 0.0 - 200.0 U/mL.
- Once an ARCHITECT Anti-CCP 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 Anti-CCP 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.

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

Control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

Verification of Assay Claims

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

The ARCHITECT Anti-CCP assay belongs to method group 1.

RESULTS

Calculation

The ARCHITECT Anti-CCP 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 of the ARCHITECT Anti-CCP assay is 0.5 U/mL to 200.0 U/mL.

LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes, the ARCHITECT Anti-CCP results should be used in conjunction with other clinical data; e.g., symptoms, medical history, etc.
- If the ARCHITECT Anti-CCP results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- The value of anti-CCP in juvenile arthritis has not been determined.
- Some specimens may not dilute linearly because of the heterogeneity of the autoantibodies with respect to physiochemical properties.
- **ARCHITECT Anti-CCP results should not be used interchangeably with other manufacturers' methods for anti-CCP determinations.**
- Plasma specimens from different anticoagulant tube types should not be used interchangeably for monitoring anti-CCP.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[21, 22] Specimens containing HAMA may produce anomalous values when tested with assay kits such as ARCHITECT Anti-CCP that employ mouse monoclonal antibodies.[21]
- Heterophilic antibodies in human specimens can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[23] 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.
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this package insert for specimen limitations.

EXPECTED VALUES

In a representative study, serum specimens from 199 asymptomatic, apparently healthy males (n=126) and females (n=73), with an age range of 19 to 67 years, were tested with the ARCHITECT Anti-CCP assay. No differences attributable to gender or age were observed. Specimen values ranged from < 0.5 U/mL to 2.5 U/mL. A cut-off of 5.0 U/mL was chosen, whereby a result of ≥ 5.0 U/mL is considered positive and a result of <5.0 U/mL is considered negative.*

* Representative data; results in individual laboratories may vary from these data. It is recommended that each laboratory establish its own expected range, which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT Anti-CCP assay is designed to have an imprecision of < 10% total CV.

A study was performed based on guidance from the National Committee for Clinical Laboratory Standards (NCCLS) document EP5-A2.[24] Seven samples consisting of the ARCHITECT Anti-CCP Positive Control, four human plasma panels, and two human plasma samples were assayed on two instruments, in replicates of two at two separate times per day for 20 days (n = 80 for each sample), using two lots of reagents and a single calibration for each instrument/reagent lot combination. Data from this study are summarized in the following table.*

Sample	Instrument	Reagent Lot	n	Mean (U/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
Positive Control	1	1	80	24.5	0.73	3.0	0.81	3.3
	2	2	80	26.7	0.68	2.6	0.74	2.8
Panel 1	1	1	80	10.9	0.30	2.7	0.61	5.6
	2	2	80	11.3	0.26	2.3	0.58	5.2
Panel 2	1	1	80	28.6	0.63	2.2	1.95	6.8
	2	2	80	30.3	0.60	2.0	1.68	5.5
Panel 3	1	1	80	66.7	1.40	2.1	4.03	6.0
	2	2	80	72.7	1.92	2.6	4.85	6.7
Panel 4	1	1	80	135.3	6.36	4.7	8.11	6.0
	2	2	80	154.1	5.02	3.3	11.82	7.7
Sample 1	1	1	80	2.8	0.07	2.6	0.11	4.0
	2	2	80	2.7	0.07	2.4	0.11	4.0
Sample 2	1	1	80	181.4	6.67	3.7	9.69	5.3
	2	2	80	195.3	7.20	3.7	12.14	6.2

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

Sensitivity

Sensitivity is defined as the limit of detection (LoD). The ARCHITECT Anti-CCP assay is designed to have a LoD of ≤ 0.5 U/mL. The LoD and the limit of blank (LoB) of the ARCHITECT Anti-CCP assay were determined based on guidance from the NCCLS document EP17-A[25]

using proportions of false positives (α) less than 5% and false negatives (β) less than 5%. These determinations were performed using one blank (60 replicates) and five low level anti-CCP samples (20 replicates each); LoB = 0.02 U/mL and LoD = 0.11 U/mL.*

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

Linearity

The ARCHITECT Anti-CCP assay is designed to be linear across the measurement range of 0.5 to 200.0 U/mL.

Based on a study performed by guidance from the NCCLS document EP6-A,[26] the ARCHITECT Anti-CCP assay demonstrated linearity from 0.5 to 200.0 U/mL.*

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

Concentration Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	r^2
0.1 - 257.4	0.98	-1.65	0.9985
	(0.95 to 1.01)	(-6.19 to 2.48)	

Autodilution Verification

The ARCHITECT Anti-CCP automated dilution method is designed to have a mean difference of ±10% versus the manual dilution method when performed on samples with values > 50.0 U/mL.

The ARCHITECT Anti-CCP assay was evaluated with the 1:6 autodilution method versus the 1:10 manual dilution method using 12 human serum samples with anti-CCP levels ranging from 58.7 to 785.0 U/mL. Five replicates each of the autodiluted and manually diluted samples were assayed on one instrument using the ARCHITECT Anti-CCP assay. The mean percent difference across all samples was 2.6%. The percent difference results are summarized in the following table.*

Sample	Mean Automated Diluted Value x Dilution Factor of 6 (U/mL)	Mean Manually Diluted Value x Dilution Factor of 10 (U/mL)	% Difference[a]
1	456.4	453.0	0.7
2	504.1	482.7	4.4
3	796.8	743.8	7.1
4	734.6	785.0	-6.4
5	220.2	209.9	4.9
6	192.0	187.9	2.2
7	213.9	207.0	3.3
8	196.8	194.6	1.1
9	65.3	61.4	6.3
10	70.1	69.2	1.3
11	72.0	69.0	4.4
12	167.2	165.2	1.2

[a] % Difference = $\frac{[\text{Mean Automated Diluted Value x 6 (U/mL)} - \text{Mean Manually Diluted Value x 10 (U/mL)}]}{\text{Mean Manually Diluted Value x 10 (U/mL)}} \times 100$

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

Interference

The ARCHITECT Anti-CCP assay is designed to have a maximum deviation in anti-CCP concentration from the following potentially interfering compounds within:

- ±15% for anti-CCP concentrations > 10.0 U/mL
- ±10% for anti-CCP concentrations ≥ 5.0 U/mL to ≤ 10.0 U/mL
- ±0.5 U/mL for anti-CCP concentrations < 5.0 U/mL

A study was performed based on guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP7-A2[27] for the ARCHITECT Anti-CCP assay. Serum samples with anti-CCP levels across the assay range of 0.5 U/mL to 200.0 U/mL were supplemented with the potentially interfering compounds listed in the table below. The maximum deviation of anti-CCP concentration observed in serum samples during these studies ranged from:

- -7.6% to 0.8% for anti-CCP concentrations > 10.0 U/mL
- -1.0% to 7.5% for anti-CCP concentrations ≥ 5.0 U/mL to ≤ 10.0 U/mL
- -0.3 U/mL to 0.2 U/mL for anti-CCP concentrations < 5.0 U/mL*

Potentially Interfering Substance	No Interference Found up to the Following Concentration
Bilirubin	20 mg/dL
Hemoglobin	800 mg/dL
Total Protein	12 g/dL
Triglycerides	3000 mg/dL
Rheumatoid Factor	200 IU/mL
Red Blood Cells	0.4%

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

Cross-Reactivity

To assess the potential cross-reactivity of the CCP antigen used in the ARCHITECT Anti-CCP assay with other autoantibodies, the assay was evaluated with 20 samples positive for various other autoantibodies and negative for CCP antibodies. The following autoantibodies (1-4 samples of each) were tested in the assay: SSA, SSB, Sm, RNP, ds-DNA, Jo-1, Scl-70, Ribo-P, TPO, ANA, and AMA. The study showed no significant cross-reactivity of the CCP antigen with any of these other autoantibodies.

Tube Type Matrix Comparison

The specimen collection tubes listed below were verified for use with the ARCHITECT Anti-CCP assay.

- serum, serum separator, lithium heparin plasma separator, and potassium EDTA.

When compared to the control tube type (serum), the tube types evaluated for samples with anti-CCP values < 5.0 U/mL showed less than a 0.5 U/mL difference on average, and the tube types evaluated for samples with anti-CCP values ranging from 5.3 to 178.8 U/mL showed less than a 10% difference on average. The distribution of the differences or percent differences per tube type is listed in the following table.*

Tube Type	Distribution of Absolute Differences < 0.5 U/mL for Samples with Anti-CCP Values < 5.0 U/mL	Distribution of Absolute Percent Differences for Samples with Anti-CCP Values 5.3 to 178.8 U/mL		
		< 10%	≥ 10% to ≤ 20%	> 20%
Serum Separator	100% (19/19)	76% (19/25)	16% (4/25)	8% (2/25)
Potassium EDTA	100% (19/19)	72% (18/25)	24% (6/25)	4% (1/25)
Lithium Heparin Plasma Separator	100% (19/19)	80% (20/25)	16% (4/25)	4% (1/25)

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

Clinical Sensitivity and Specificity

The clinical sensitivity was determined for 496 confirmed RA individuals, and clinical specificity was determined for 499 non-RA specimens (299 from patients with other rheumatic and non-rheumatic disorders and 200 from asymptomatic apparently healthy individuals). Using a cut-off of 5.0 U/mL, the sensitivity was calculated to be 70.6% with a specificity of 98.2%. The results are summarized in the following tables.*

Specimen Category	Total n	ARCHITECT Anti-CCP Positive n	% Sensitivity
Confirmed RA[a]	496	350	70.6

[a] RA patients were classified according to the ACR Criteria.[4]

Specimen Category	Total n	ARCHITECT Anti-CCP Positive n	% Specificity
Non-RA Specimens in Total	499	9	98.2
Non-RA Healthy Asymptomatic	200	1	99.5
Non-RA Disease Specimens[a]	299	8	97.3

[a] The non-RA diseases were Ankylosing Spondylitis, Autoimmune Thyroiditis/Hashimoto's Disease, Crohn's Disease, Dermatomyositis, Epstein-Barr Virus, Lyme Disease, Osteoarthritis, Polymyalgia Rheumatica, Polymyositis, Psoriatic Arthritis, Reactive Arthritis/ Reiter's Syndrome, Scleroderma, Sjögren's Syndrome, Systemic Lupus Erythematosus, and Ulcerative Colitis.

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

Method Comparison

The ARCHITECT Anti-CCP assay is designed to have a concordance of ≥ 95% for RA and non-RA specimens when compared to the AxSYM Anti-CCP assay. The RA and non-RA specimens described in the Clinical Sensitivity and Specificity section were used to compare the ARCHITECT Anti-CCP assay to the AxSYM Anti-CCP assay. The cut-off employed for the AxSYM Anti-CCP assay was 5.0 U/mL, as stated in the manufacturer's package insert. Using a cut-off of 5.0 U/mL for the ARCHITECT Anti-CCP assay, the concordance was calculated to be 99.3%. The results are summarized in the following tables.*

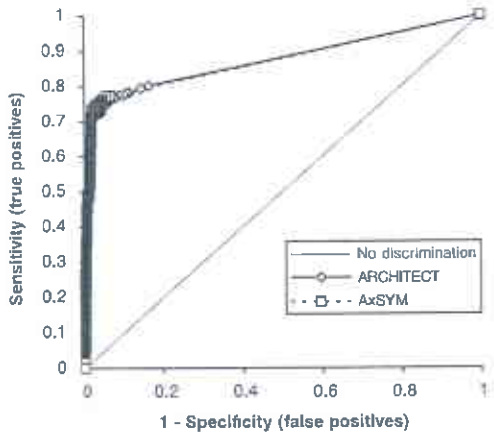
n = 995		AxSYM Anti-CCP Positive	AxSYM Anti-CCP Negative
ARCHITECT Anti-CCP	Positive	356	3
	Negative	4	632

	Total n	% Positive Agreement (95% CI[a])	% Negative Agreement (95% CI[a])	% Total Agreement (95% CI[a])
Non-RA	499	100 (56.4-100)	100 (99.2-100)	100 (99.3-100)
RA	496	98.9 (97.1-99.7)	97.9 (94.1-99.6)	98.6 (97.1-99.4)
All Samples	995	98.9 (97.3-99.7)	99.5 (98.6-99.9)	99.3 (98.6-99.7)

[a] CI = Confidence Interval.

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

A Receiver Operator Characteristic (ROC) analysis was carried out using the above data obtained for the two assays. The area under the curve (AUC) for the ARCHITECT Anti-CCP assay was 0.873 (95% confidence interval: 0.849-0.897) and 0.872 (95% confidence interval: 0.848-0.896) for the AxSYM Anti-CCP assay, thus indicating that both assays are comparable with respect to their clinical differentiation. The ROC analysis curve is shown below.*



* 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 Anti-CCP assay, no high dose hook effect was observed when a sample containing approximately 2000 U/mL of anti-CCP antibody was assayed.*

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

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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
DISTRIBUTED IN THE USA BY	Distributed in the USA by
INFORMATION FOR USA ONLY	Information needed for United States of America only
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCED FOR ABBOTT BY	Produced for Abbott by
PRODUCT OF UK	Product of United Kingdom
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SAMPLE DILUENT	Sample Diluent
SEPTUM	Septum
SN	Serial number
TRIGGER SOLUTION	Trigger Solution
WASH BUFFER	Wash Buffer

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Revised November 2015.

Abbott

ARCHITECT Anti-CCP Controls

REF 1P65-10

en

Anti-CCP
1P65
ABBL179 / R03
C1P650

Read Highlighted Changes: Revised September 2015.

INTENDED USE

The ARCHITECT Anti-CCP Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT iSystem (reagents, calibrators and instrument) when used for the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma.

Refer to the ARCHITECT Anti-CCP reagent package insert and the ARCHITECT System Operations Manual for additional information.

CONTENTS

2 Bottles (7.0 mL each) of ARCHITECT Anti-CCP Controls contain either anti-CCP positive or anti-CCP negative human plasma in phosphate buffer. Preservative: sodium azide.

The controls yield the following concentrations:

Control	Anti-CCP Concentration (U/mL)	Range (U/mL)
CONTROL +	24.0	13.2 - 34.8
CONTROL -	0.1	≤ 1.6

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

The following warnings and precautions apply to: CONTROL + and CONTROL -	
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 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.
- Gently invert the bottles 5-10 times prior to dispensing to ensure mixing.
- After each use, tightly close the caps and return the controls to 2-8°C storage.

PROCEDURE

- If utilizing ARCHITECT system software version 5.0 or higher, refer to the ARCHITECT System Operations Manual, Section 5 for information on ordering positive and negative controls.
- If utilizing an ARCHITECT system software version lower than 5.0, use the following instructions to order controls:
  - For information on ordering the positive control, refer to the ARCHITECT System Operations Manual, Section 5.
  - Order the negative control as a patient specimen, not as a Control.
  - Manually verify the validity of the negative control every time it is run. Because the control is run as a patient specimen, a result will not be flagged by the ARCHITECT iSystem if it is outside the acceptable control range.
- To troubleshoot control values that fall outside the control range, refer to the ARCHITECT System Operations Manual, Section 10.

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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--Fourth Edition.* CLSI Document M29-A4. Wayne, PA: CLSI; 2014.

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 -	Negative Control
CONTROL +	Positive Control
DISTRIBUTED IN THE USA BY	Distributed in the USA by
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 UK	Product of United Kingdom
RANGE	Range
REF	List Number

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Revised September 2015.

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ARCHITECT Anti-CCP Calibrators

REF 1P65-01

en

Anti-CCP
1P65
ABBL181 / R03
S1P650

Read Highlighted Changes: Revised September 2015.

INTENDED USE

The ARCHITECT Anti-CCP Calibrators are for the calibration of the ARCHITECT iSystem when used for the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma.

Refer to the ARCHITECT Anti-CCP reagent package insert and the ARCHITECT System Operations Manual for additional information.

CONTENTS

6 Bottles (4.3 mL each) of ARCHITECT Anti-CCP Calibrators. Calibrator A is phosphate buffer with protein (bovine) stabilizers. Calibrators B-F contain anti-CCP positive human plasma in phosphate buffer with protein (bovine) stabilizers. Preservative: sodium azide.

The calibrators yield the following concentrations:

Calibrator	Anti-CCP Concentration (U/mL)
CAL A	0.0
CAL B	5.0
CAL C	25.0
CAL D	50.0
CAL E	100.0
CAL F	200.0

STANDARDIZATION

Due to the absence of an internationally recognized standard for anti-CCP antibody, the units of anti-CCP antibody per mL (U/mL) within a human plasma sample demonstrating high levels of anti-CCP IgG activity were defined by Axis-Shield Diagnostics using a commercially available second-generation anti-CCP assay. The calibrators for the ARCHITECT Anti-CCP assay are manufactured by dilution and are referenced to this high-level sample.

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. 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 human plasma used in calibrators B through F is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2 and anti-HCV or HCV RNA.

The following warnings and precautions apply to: CAL A, CAL F	
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 a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

STORAGE

- Calibrators 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

- Calibrators may be used immediately after removal from 2-8°C storage.
- Gently invert the bottles (5-10 times) prior to dispensing to ensure mixing.
- After each use, tightly close the caps and return the calibrators 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--Fourth Edition*. CLSI Document M29-A4. Wayne, PA: CLSI; 2014.

Key to Symbols

	Caution
	Consult instructions for use
	Manufacturer
	Temperature limitation
	Use by/Expiration date
CAL A	Calibrator (A,B,C,D,E or F)
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
DISTRIBUTED IN THE USA BY	Distributed in the USA by
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 UK	Product of United Kingdom
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ARCHITECT
Anti-HBc II Calibrator

REF 8L44-01

en
Anti-HBc II
8L44
G4-7702 / R04
S8L440

Read Highlighted Changes: Revised November 2014.

INTENDED USE

The ARCHITECT Anti-HBc II Calibrator 1 is for the calibration of the ARCHITECT iSystem when used for the qualitative detection of antibody to hepatitis B core antigen (anti-HBc) in human serum and plasma.

Refer to the ARCHITECT Anti-HBc II reagent insert and the ARCHITECT System Operations Manual for additional information.

CONTENTS

1 Bottle (4.0 mL) of ARCHITECT Anti-HBc II Calibrator 1 containing recalcified human plasma and dyes. The calibrator is reactive for anti-HBc. Preservatives: ProClin 950 and sodium azide.

The calibrator is at the following concentration:

Calibrator	Color	Concentration (PEI U/mL)
CAL 1	Green*	0.5

* Dyes: Acid Yellow No.23 and Acid Blue No.9

STANDARDIZATION

The ARCHITECT Anti-HBc II Calibrator 1 concentration is standardized against the Anti-HBc IgG reference standard of the Paul Ehrlich Institute, Langen, Germany.

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

The following warnings and precautions apply to: CAL 1	
WARNING	Contains methylisothiazolone 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 B.

STORAGE

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

PREPARATION FOR ANALYSIS

Calibrator may be used immediately after removal from 2-8°C storage.

Prior to each use, mix by gentle inversion.

After each use, tightly close the cap and return the calibrator 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.
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Key to Symbols

	Caution
	Consult instructions for use
	Manufacturer
	Temperature limitation
	Use by/Expiration date
CAL 1	Calibrator 1
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
PRODUCT OF GERMANY	Product of Germany
REF	List Number
WARNING: SENSITIZER	Warning: May cause an allergic reaction.


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Revised November 2014.

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ARCHITECT
Anti-HBc II Controls

REF 8L44-10

en

Anti-HBc II
8L44
G4-7709 / R03
C8L440

Read Highlighted Changes: Revised November 2014.

INTENDED USE

The ARCHITECT Anti-HBc II Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT iSystem when used for the qualitative detection of antibody to hepatitis B core antigen (anti-HBc) in human serum and plasma. Refer to the ARCHITECT Anti-HBc II reagent insert and the ARCHITECT System Operations Manual for additional information.

CONTENTS

2 Bottles (8.0 mL each) of ARCHITECT Anti-HBc II Controls: Negative Control and Positive Control. The Negative Control contains recalcified human plasma. The Positive Control contains recalcified human plasma and dye, and is reactive for anti-HBc. Preservatives: ProClin 950 and sodium azide.

The controls are at the following ranges:

Control	Color	Target S/CO	Control Range S/CO
CONTROL −	Natural	N/A	0.00 - 0.80
CONTROL +	Blue*	2.73	1.50 - 3.96

* Dye: Acid Blue No. 9

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. 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 human plasma used in the Negative Control is nonreactive for anti-HBc, HBsAg, anti-HIV-1/HIV-2, anti-HCV and HIV-1 RNA or HIV-1 Ag.
- The human plasma used in the Positive Control is reactive for anti-HBc, and nonreactive for HBsAg, anti-HIV-1/HIV-2, anti-HCV and HIV-1 RNA or HIV-1 Ag.

The following warnings and precautions apply to: CONTROL − / CONTROL +	
WARNING	Contain methylisothiazolone 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 each use, mix by gentle inversion.

After each use, tightly close the caps and return the controls to 2-8°C storage.

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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
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL −	Negative Control
CONTROL +	Positive Control
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
PRODUCT OF GERMANY	Product of Germany
RANGE	Range
REF	List Number
WARNING: SENSITIZER	Warning: May cause an allergic reaction.

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Abbott

ARCHITECT Anti-HBc II

REF 8L44-25
REF 8L44-35
REF 8L44-30

en
Anti-HBc II
8L44
G4-7715/R08
B8L440

Read Highlighted Changes: Revised January 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.

NAME

ARCHITECT Anti-HBc II

INTENDED USE

The ARCHITECT Anti-HBc II assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of antibody to hepatitis B core antigen (anti-HBc) in human serum and plasma, including specimens collected post-mortem (non-heart-beating). The ARCHITECT Anti-HBc II assay is intended to be used as an aid in the diagnosis of hepatitis B infection and as a screening test to prevent transmission of hepatitis B virus (HBV) to recipients of blood, blood components, cells, tissue and organs.

SUMMARY AND EXPLANATION OF THE TEST

The ARCHITECT Anti-HBc II assay utilizes microparticles coated with recombinant hepatitis B virus core antigen (rHBcAg) for the detection of anti-HBc. Anti-HBc determinations can be used as an indicator of current or past HBV infection. Anti-HBc is found in serum shortly after the appearance of hepatitis B surface antigen (HBsAg) in acute HBV infections. It will persist after the disappearance of HBsAg and before the appearance of detectable antibody to HBsAg (anti-HBs).[1-7] In the absence of information about any other HBV markers, it must be considered that an individual with detectable levels of anti-HBc may be actively infected with HBV or that the infection may have resolved, leaving the person immune.[8] Anti-HBc may be the only serological marker of HBV infection and potentially infectious blood.[9-15]

The presence of anti-HBc does not differentiate between acute or chronic hepatitis B infection.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

1. Sample, assay diluent, specimen diluent, and rHBcAg coated paramagnetic microparticles are combined. Anti-HBc present in the sample binds to the rHBcAg coated microparticles.
2. The reaction mixture is washed and anti-human acridinium-labeled conjugate is added.
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-HBc in the sample and the RLUs detected by the ARCHITECT iSystem optics.

The presence or absence of anti-HBc in the specimen 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 reaction is greater than or equal to the cutoff signal, the specimen is considered reactive for anti-HBc.

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

REAGENTS

Kit Contents

ARCHITECT Anti-HBc II 8L44

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

REF	8L44-25	8L44-35	8L44-30
Σ	100	500	2000
MICROPARTICLES	1 x 6.6 mL	1 x 27.0 mL	4 x 27.0 mL
CONJUGATE	1 x 11.0 mL	1 x 28.8 mL	4 x 28.8 mL
ASSAY DILUENT	1 x 5.36 mL	1 x 23.72 mL	4 x 23.72 mL
SPECIMEN DILUENT	1 x 5.36 mL	1 x 23.72 mL	4 x 23.72 mL

MICROPARTICLES Hepatitis B core (*E. coli*, recombinant) antigen coated microparticles in TRIS buffer. Minimum concentration: 0.08% solids. Preservatives: ProClin 950 and sodium azide.

CONJUGATE Murine acridinium-labeled anti-human conjugate in MES buffer with protein stabilizers. Minimum concentration: 0.04 µg/mL. Preservatives: sodium alkyl paraben and sodium azide.

ASSAY DILUENT Assay diluent containing murine protein stabilizers in MOPSO buffer. Preservatives: ProClin 950 and sodium azide.

SPECIMEN DILUENT Specimen diluent containing reductant in MOPSO buffer.

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 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.[16-19]

The following warnings and precautions apply to: MICROPARTICLES	
	
WARNING	Contains methylisothiazolone 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.

The following warnings and precautions apply to: ASSAY DILUENT	
	
DANGER	Contains polyethylene glycol octylphenyl ether (Triton X-405), methylisothiazolone and sodium azide.
H318	Causes serious eye damage.
H317	May cause an allergic skin reaction.
H412	Harmful to aquatic life with long lasting effects.
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.
P273	Avoid release to the environment.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor / physician.
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.

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

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

For 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.
- 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.

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-HBc II 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.

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	Sodium heparin
	Lithium heparin (PST)
	Potassium-EDTA
	Sodium citrate
	Potassium oxalate
	CPD
	CPDA-1
	ACD

- ACD tubes may show a positive bias up to 20 % relative to serum.
- Other specimen collection tube types have not been tested with this assay.
- 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.
- 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**.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed (> 500 mg/dL hemoglobin)
  - obvious microbial contamination
  - body fluids other than human serum and plasma
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.
- Patient specimens should be tested within 3 hours of being placed on board the ARCHITECT iSystem.
- No interference was observed between experimental controls and nonreactive or reactive specimens tested with elevated levels of bilirubin (20 mg/dL), triglycerides (3000 mg/dL), protein (4.5 - 12 g/dL), red blood cells (0.4% v/v), or hemoglobin (500 mg/dL).

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at ≥ 10,000 RCF (Relative Centrifugal Force) for 10 minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter or
  - they were frozen and thawed.

  Transfer clarified specimen to a sample cup or secondary tube for testing.
- 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	15-30°C	≤ 3 days
	2-8°C	≤ 14 days
	-20°C or colder	—

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

Remove serum or plasma from the clot, red blood cells, or separator gel if stored longer than the maximum 15-30°C or 2-8°C storage time and store frozen at -20°C or colder.

No qualitative performance differences were observed between experimental controls and nonreactive or spiked reactive specimens subjected to 6 freeze/thaw cycles; 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.
- It is recommended that specimens be removed from the clot, red blood cells, or separator gel.
- Ship on wet ice or 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 17.5 hours after death. Performance was established using 50 spiked and 50 non-spiked cadaveric blood specimens. [23]
- 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, red blood cells or separator gel until further processing.
- Cadaveric blood specimens can be stored for up to 7 days at 2-8°C or up to 3 days 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

8L44 ARCHITECT Anti-HBc II Reagent Kit

Materials Required but not Provided

- ARCHITECT Anti-HBc II Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 8L44-01 ARCHITECT Anti-HBc II Calibrator
- 8L44-10 ARCHITECT Anti-HBc II Controls
- 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, discard the cap and place a septum on the bottle. For instructions on 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: 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 ARCHITECT Anti-HBc II Calibrator 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: 5 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 cannot be diluted for the ARCHITECT Anti-HBc II assay.

Calibration

- Test calibrator in replicates of three. The calibrator 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.
- Once an ARCHITECT Anti-HBc 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 Anti-HBc 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.

The ARCHITECT Anti-HBc II control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and samples must be retested. Recalibration may be indicated.

Verification of Assay Claims

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

The ARCHITECT Anti-HBc II assay belongs to method group 5, except functional sensitivity.

RESULTS

Calculation

- The ARCHITECT iSystem calculates the cutoff RLU from the mean RLU of three replicates of Calibrator 1 and stores the result. The cutoff RLU is determined by multiplying the Anti-HBc II Calibrator 1 mean RLU by 1.0.
  Cutoff RLU = Calibrator 1 Mean RLU x 1.0
- The ARCHITECT iSystem calculates the S/CO result for each specimen and control as follows.
  S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

Initial ARCHITECT Anti-HBc II Results

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

Final ARCHITECT Anti-HBc II Interpretation

Initial Interpretation	Results with Retest	Final Interpretation
Nonreactive	No retest required.	**Nonreactive**
Reactive	If two of the three results are < 1.00 S/CO	**Nonreactive**
Reactive	If two of the three results are ≥ 1.00 S/CO	**Reactive**

For details on configuring the ARCHITECT iSystem to use grayzone interpretations, refer to the ARCHITECT System Operations Manual, Section 2.

The grayzone interpretation from the ARCHITECT interpretations screen is not used by the ARCHITECT iSystem unless a grayzone is configured.

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 anti-HBc results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- 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.[20]
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Specimens containing HAMA may produce anomalous values when tested with assay kits that employ mouse monoclonal antibodies.[21, 22]

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT Anti-HBc II assay is designed to have an imprecision of ≤10% total** CV for specimens at 1.20 S/CO and for the Positive Control. The study was performed at one internal and two external evaluation sites each using one instrument. A panel consisting of three different control lots and two human plasma specimens was tested in replicates of four across three reagent lots and three calibrator lots per site. Each combination of instruments, panel members, and reagent lots was tested in four runs. Data from this study are summarized in Table 1*.

Table 1: ARCHITECT Anti-HBc II Precision

Panel member	n	Mean (S/CO)	Within Run SD	Within Run %CV	Total** SD	Total** %CV
Negative Control	432	0.22	0.01	6.52	0.02	7.57
Positive Control	431	2.97	0.08	2.63	0.09	2.87
Human Plasma Panel 1	144	0.81	0.02	2.73	0.03	3.24
Human Plasma Panel 2	144	1.18	0.03	2.52	0.03	2.87

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

** Total is an accumulation of within run, between run and between day.

Specificity

The ARCHITECT Anti-HBc II assay is designed to have an overall specificity of ≥ 99.5% on a blood donor population and ≥ 98.0% on a hospitalized/diagnostic population. A study was performed at one internal and two external evaluation sites. A total of 5141 serum and plasma specimens collected from five blood-donation centers and 260 hospitalized/diagnostic specimens were evaluated to assess specificity.

From the blood donor population a total of 26 specimens were classified as reactive. Two additional specimens were excluded from specificity calculation as final specimen disposition could not be determined. From the hospitalized/diagnostic specimens a total of 28 specimens were classified as reactive. One additional specimen was excluded from specificity calculation as final specimen disposition could not be determined. Data from this study are summarized in Table 2*.

Table 2: ARCHITECT Anti-HBc II Specificity

Category	N	IR [%]	RR [%]	Clinical Specificity	95% Confidence Interval
Overall Blood Donors	5141	44 [0.86]	41 [0.80]	99.71% (5098/5113)	99.52 - 99.84%
Blood Donor Serum	3584	25 [0.70]	22 [0.61]	99.75% (3561/3570)	99.52 - 99.88%
Blood Donor Plasma	1557	19 [1.22]	19 [1.22]	99.61% (1537/1543)	99.16 - 99.86%
Hospitalized/ Diagnostic Specimens	260	28 [10.77]	28 [10.77]	100% (231/231)	98.42 - 100%

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

Sensitivity

A total of 406 anti-HBc positive specimens from patients with acute, chronic and recovered HBV infection and signs and symptoms of HBV infection were tested, resulting in a sensitivity of 100% (406/406), 95% confidence interval: 99.10% - 100%. (Representative data; results in individual laboratories may vary from these data).

Analytical Sensitivity

The ARCHITECT Anti-HBc II assay is designed to show an analytical sensitivity of less than 1.0 PEI U/mL. The sensitivity of the ARCHITECT Anti-HBc II assay was evaluated with a four-member panel that was standardized against reference serum from the Paul-Ehrlich-Institute (PEI). The panel was tested with three reagent lots. The ARCHITECT Anti-HBc II assay sensitivity ranged from 0.4 to 0.5 PEI U/mL. (Representative data; results in individual laboratories may vary from these data).

Interference

Additional studies were performed to evaluate other potential interfering disease states on the ARCHITECT Anti-HBc II assay. A total of 104 specimens were tested from the following categories: antinuclear antibodies (ANA), Epstein-Barr virus (anti-EBV positive), hepatitis A virus (anti-HAV IgM positive), hepatitis C virus (anti-HCV positive), human immunodeficiency virus (anti-HIV-1 positive), human anti-mouse antibodies (HAMA) positive, influenza vaccine recipients, non-viral liver disease, rheumatoid factor positive, syphilis, systemic lupus erythematosus (SLE), toxoplasmosis IgG positive, varicella zoster (anti-VZV positive), anti-*E. coli* positive and yeast infection. With these specimens, ARCHITECT Anti-HBc II showed the same qualitative results as the comparator method.

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Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
ASSAY DILUENT	Assay Diluent
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number
ECO HAZARD	Ecological hazard
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCT OF GERMANY	Product of Germany
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

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