ARCHITECT

SYSTEM

en

Anti-CCP

REF 1P65

ABBL174/R2

B1P650

Read Highlighted Changes
Revised September, 2009

Anti-CCP

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

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

Key to symbols used

REF	List Number	CONTROL NO.	Control Number
IVD	*In Vitro* Diagnostic Medical Device	ASSAY CD-ROM	Assay CD-ROM
LOT	Lot Number	REACTION VESSELS	Reaction Vessels
	Expiration Date	SAMPLE CUPS	Sample Cups
2°C 8°C	Store at 2-8°C	SEPTUM	Septum
	Consult instructions for use	REPLACEMENT CAPS	Replacement Caps
		SN	Serial Number
	Manufacturer	REAGENT LOT	Reagent Lot

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

Abbott

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 *i* System. 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 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).[6,7,8] 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.

In the first step, sample is prediluted with wash buffer. The prediluted sample, CCP coated paramagnetic microparticles, and sample diluent are combined. Anti-CCP antibodies present in the sample bind to the CCP coated microparticles. After washing, anti-human IgG acridinium-labeled conjugate is added in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of anti-CCP antibody in the sample and the RLUs detected by the ARCHITECT *i* System optics. For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Reagent Kit, 100/500 Tests

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

ARCHITECT Anti-CCP Reagent Kit (1P65)

- **MICROPARTICLES** 1 Bottle (6.5 mL/26.5 mL) CCP coated microparticles in phosphate buffer with surfactant and protein (bovine) stabilizer. Minimum concentration: 0.05% solids. Preservative: sodium azide.
- **CONJUGATE** 1 Bottle (5.8 mL/25.8 mL) 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** 1 Bottle (9.8 mL/50.0 mL) Phosphate buffer with surfactant and protein (bovine) stabilizer. Preservative: sodium azide.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

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

ARCHITECT *i* Trigger Solution

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

ARCHITECT *i* Wash Buffer

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

WARNINGS AND PRECAUTIONS

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

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens[17]. Biosafety Level 2[18] or other appropriate biosafety practices[19,20] should be used for materials that contain or are suspected of containing infectious agents.
- This product contains sodium azide; for a specific listing, refer to the **REAGENTS** section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.
- For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheets are available for professional user on request.
- For information on the safe disposal of sodium azide and a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT Anti-CCP Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that 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 the reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts, which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

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The ARCHITECT Anti-CCP Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT Anti-CCP Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

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

Indications of Reagent Deterioration

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

INSTRUMENT PROCEDURE

- The ARCHITECT Anti-CCP assay is designed for use on the ARCHITECT *i* System.
- The ARCHITECT Anti-CCP assay file must be installed on the ARCHITECT *i* System from an ARCHITECT assay CD-ROM before performing the assay (refer to the **PROCEDURE, Materials Required but not Provided** section). For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.
- The default result unit for the ARCHITECT Anti-CCP assay is U/mL.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen collection tubes listed below were verified to be used with the ARCHITECT Anti-CCP assay. Other specimen collection tubes have not been tested with this assay.

- Human serum and serum separator tubes
- Human plasma collected in:
  - lithium heparin plasma separator tubes
  - potassium EDTA
- Plasma specimens from different anticoagulant tube types should not be used interchangeably for monitoring anti-CCP.
- Liquid anticoagulant may have a dilution effect resulting in lower concentrations for individual patient specimens.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the ARCHITECT Anti-CCP assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed
  - obvious microbial contamination
  - cadaver specimens or body fluids other than human serum or plasma
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Preparation for Analysis

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

Storage

- Specimens may be stored on or off the clot, red blood cells, or separator gel for
  - up to 22 hours at room temperature (study performed at 30°C) or
  - up to 7 days at 2-8°C.
- 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.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator gel.
- When shipping, specimens must be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped on wet or dry ice. Do not exceed the storage time limitations listed above.

PROCEDURE

Materials Provided

- 1P65 ARCHITECT Anti-CCP Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- 1P38 ARCHITECT *i* System **ASSAY CD-ROM** - WW (excluding US) - Addition F, version 2.0 or higher (for use with ARCHITECT *i*2000 or *i*2000$_{SR}$ Systems)
- 1P39 ARCHITECT *i* System **ASSAY CD-ROM** - US - Addition F, version 2.0 or higher (for use with ARCHITECT *i*2000 or *i*2000$_{SR}$ Systems)
- 1P60 ARCHITECT *i*1000$_{SR}$ System **ASSAY CD-ROM** - US Special Edition, version 6.0 or higher
- 1P61 ARCHITECT *i*1000$_{SR}$ System **ASSAY CD-ROM** - WW (excluding US) Special Edition, version 5.01 or higher
- 1P65-01 ARCHITECT Anti-CCP Calibrators
- 1P65-10 ARCHITECT Anti-CCP Controls
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional)

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

Assay Procedure

- Before loading the ARCHITECT Anti-CCP Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that 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 remain adhered to the bottle, continue inverting 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 on placing septums on bottles refer to the **Handling Precautions** section of this package insert.

- Load the ARCHITECT Anti-CCP Reagent Kit on the ARCHITECT *i* System.
  - Verify that all necessary assay 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.
  - If utilizing ARCHITECT system software version 5.0 or higher, refer to the ARCHITECT System Operations Manual, Section 5 for information on ordering patient specimens and controls.
  - If utilizing an ARCHITECT system software version lower than 5.0, use the following instructions to order patient specimens and controls:
    - For information on ordering patient specimens and 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 *i* System if it is outside the acceptable control range.
- To troubleshoot control values that fall outside the control range, refer to the ARCHITECT System Operations Manual, Section 10.
- The minimum sample volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
  - Priority: 60 µL for the first ARCHITECT Anti-CCP test plus 10 µL for each additional ARCHITECT Anti-CCP test from the same sample cup.
  - ≤ 3 hours on board: 150 µL for the first ARCHITECT Anti-CCP test plus 10 µL for each additional ARCHITECT Anti-CCP test from the same sample cup.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare calibrators and controls.
  - ARCHITECT Anti-CCP Calibrators and Controls should be prepared according to their respective package inserts.
  - To obtain the recommended volume requirements for the ARCHITECT Anti-CCP Calibrators and Controls, hold the bottles **vertically** and dispense 4 drops of each calibrator or control into each respective sample cup.
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. When a laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

Patient specimens with anti-CCP values exceeding 200.0 U/mL are flagged with the code ">200.0 U/mL". To quantitate the concentration of these specimens, perform either the Automated Dilution or Manual Dilution Protocol.

Automated Dilution Procedure

If using the Automated Dilution Protocol, the system performs a 1:6 dilution of the specimen and automatically calculates the concentrations of the specimen before dilution and reports the result.

Manual Dilution Procedure

- The suggested dilution for the ARCHITECT Anti-CCP assay is 1:10.
- Add 50 µL of the patient specimen to 450 µL of the ARCHITECT Anti-CCP Negative Control.
- 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. This will be the reported result.
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT Anti-CCP calibration, test calibrators A, B, C, D, E, and F in replicates of two. A single sample of each ARCHITECT Anti-CCP control must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the control package insert. Calibrators should be priority loaded.
- 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.
  - 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 be tested once every 24 hours each day of use. If laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures. Additional controls may be tested in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

The ARCHITECT Anti-CCP 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.

ARCHITECT Anti-CCP Calibrators may be used in place of MasterCheck as described in the ARCHITECT System Operations Manual, Appendix B.

RESULTS

Calculation

The ARCHITECT Anti-CCP assay uses a 4 Parameter Logistic Curve Fit (4PLC, Y-weighted) data reduction method 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.*

		Reagent		Mean	Within Run		Total	
Sample	Instrument	Lot	n	(U/mL)	SD	%CV	SD	%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.

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.

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 (0.95 to 1.01)	-1.85 (-6.19 to 2.48)	0.9985

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

* 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*

Potential 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.*

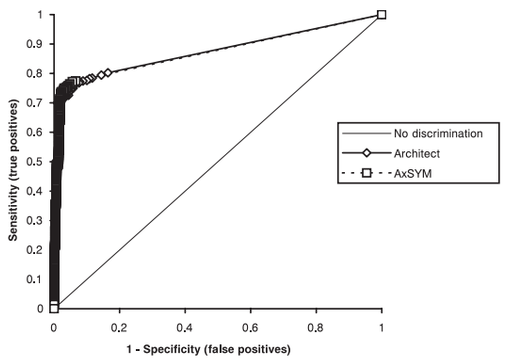
n = 995		AxSYM Anti-CCP	
		Positive	Negative
ARCHITECT Anti-CCP	Positive	356	3
	Negative	4	632

	Total n	% Positive Agreement (95% CI[a])	% Negative Agreement (95% CI[a])	% Total Agreement (95% CI[a])
Non-RA	499	100 (66.4-100)	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 (97.2-99.7)	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.

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