ARCHITECT C-Peptide

REF 3L53-27

en

C-Peptide
3L53
G94303R01
B3L5W0

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 C-Peptide

INTENDED USE

The ARCHITECT C-Peptide assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of C-peptide in human serum, plasma and urine on the ARCHITECT iSystem. The ARCHITECT C-Peptide assay is used as an aid in the diagnosis and treatment of patients with abnormal insulin secretion including diabetes mellitus.

SUMMARY AND EXPLANATION OF THE TEST

Human C-peptide is a single chain polypeptide consisting of 31 amino acids. It connects the A and B chains of insulin in the precursor molecule proinsulin, which is stored in secretory granules of the pancreatic β-cells.[1-3] In insulin biosynthesis, it facilitates the formation of the correct secondary and tertiary structure of the hormone.[3, 4] C-peptide and insulin are secreted in equimolar amounts, however, C-peptide does not undergo significant hepatic extraction but is renally eliminated and therefore persists longer in the peripheral circulation. This results in a longer half-life (> 30 minutes) and less fluctuation of C-peptide compared to insulin (5 minutes).[4, 5] Hence, measurements of C-peptide more accurately reflect pancreatic insulin secretion rates than insulin. Moreover, C-peptide concentration is independent of exogenous insulin and is not subject to interference from insulin autoantibodies induced by insulin therapy.

Determination of the 24-hour urinary excretion of C-peptide is an additional option to monitor average β-cell insulin secretion. C-peptide is used as a test of β-cell function in human subjects in a variety of conditions including type 1 diabetes, and to aid in the differential diagnosis of hypoglycemia, and surreptitious insulin self-administration.[6-8] A low C-peptide level is expected if the insulin secretion is diminished as in insulin-dependent diabetes (type 1 diabetes, latent autoimmune diabetes of adults (LADA)). Elevated C-peptide levels may be found when β-cell activity is increased as in hyperinsulinism and insulinomas.[9] The C-peptide/insulin molar ratio can be considered as an estimation of hepatic clearance, since in liver insufficiency insulin metabolism is impaired, leading to an abnormally large proportion of insulin in the peripheral circulation.[10]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT C-Peptide assay is a two-step immunoassay for the quantitative determination of C-peptide in human serum, plasma and urine using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample, assay diluent, and anti-human C-peptide coated paramagnetic microparticles are combined. The C-peptide present in the sample binds to the anti-human C-peptide coated microparticles, forming an antigen-antibody complex.
2. After washing, anti-human C-peptide 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 C-peptide in the sample and the RLUs detected by the ARCHITECT iSystem optics.

Results are calculated automatically based on the previously established calibration curve.

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

REAGENTS

Kit Contents

ARCHITECT C-Peptide 3L53

REF	3L53-27
Σ	100
MICROPARTICLES	1 x 6.6 mL
CONJUGATE	1 x 5.9 mL
ASSAY DILUENT	1 x 10.0 mL

MICROPARTICLES Anti-human C-peptide (mouse, monoclonal) coated microparticles in TRIS buffer. Minimum Concentration: 0.05% solids. Preservatives: ProClin 300, ProClin 950.

CONJUGATE Anti-human C-peptide (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizers and detergent. Minimum Concentration: 0.1 µg/mL. Preservative: sodium azide.

ASSAY DILUENT Assay Diluent containing MES buffer with surfactant and protein (bovine, mouse) blockers. Preservatives: ProClin 300, ProClin 950.

Other Reagents

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

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

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

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

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[11-14]

The following warnings and precautions apply to: MICROPARTICLES / ASSAY DILUENT	
	
WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
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: 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.

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 C-Peptide assay file must be installed on the ARCHITECT iSystem from an ARCHITECT iSystem Assay CD-ROM prior to performing the assay.

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

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

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

Alternate Result Units

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

Conversion formula:

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

Default result unit	Conversion factor	Alternate result unit
ng/mL	333.33	pmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay:

Serum and Plasma

Specimen Types	Collection Tubes
Human serum	Serum
	Serum separator tubes
Human plasma	Potassium-EDTA
	Lithium heparin
	Sodium heparin
	Ammonium heparin
	Sodium fluoride / potassium oxalate
	Plasma separator tubes (lithium heparin)

- Other specimen collection tube types have not been tested with this assay.
- Sodium citrate plasma tubes cannot be used with the ARCHITECT C-Peptide assay.
- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum, plasma and urine.
- 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.

Urine

Twenty-four-hour urine specimens may be used in the ARCHITECT C-Peptide assay. The urine specimens must be collected without preservatives over a 24-hour period in a clean, single container. Store at 2-8°C during collection process.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed (> 500 mg/dL hemoglobin)
  - obvious microbial contamination
- For accurate results, specimens should be free of fibrin, red blood cells, or 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 completely 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.
- 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

Serum and Plasma

Specimen Type	Storage Temperature	Maximum Storage Time
Serum and plasma	Room temperature	≤ 24 hours
	2-8°C	≤ 48 hours
	< -10°C	> 48 hours

If testing will be delayed more than 8 hours at 15-30°C or 48 hours at 2-8°C, remove serum or plasma from the clot, red blood cells, or separator gel.

Specimens stored frozen for 3 months showed no performance difference. Avoid more than 3 freeze/thaw cycles.

Urine

Twenty-four-hour urine specimens that cannot be tested within 24 hours after completion of collection have to be stored frozen at < -10°C.

Specimens stored frozen for 3 months showed no performance difference. Avoid more than 3 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

3L53 ARCHITECT C-Peptide Reagent Kit

Materials Required but not Provided

- ARCHITECT C-Peptide Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 3L53-02 ARCHITECT C-Peptide Calibrators
- 3L53-11 ARCHITECT C-Peptide Controls
- 7D82-50 ARCHITECT Multi-Assay Manual Diluent
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
- ARCHITECT Replacement Caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

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

Assay Procedure

- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
  - **Twenty-four-hour urine specimens must be diluted by selecting the automated dilution protocol "URINE 1:10".**
- 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

Serum and Plasma

- 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
- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

Urine

- Priority:

  Sample volume for first test: 100 µL

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

  Sample volume for first test: 150 µL

  Sample volume for each additional test from same sample cup: 50 µL
- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

- Prepare ARCHITECT C-Peptide Calibrators and Controls.
  - Mix calibrator(s) and controls by gentle inversion before use.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
  - Recommended volumes:

    for each calibrator: 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

Serum and Plasma

Serum or plasma specimens with a C-peptide concentration > 30.00 ng/mL will be flagged as ">30.00 ng/mL" and may be diluted with the Manual Dilution Procedure.

Manual Dilution Procedure

Suggested dilution: 1:2

NOTE: Samples diluted >1:2 (>50% Diluent) may result in an over-recovery >15%.

1. Add 75 µL of the patient specimen to 75 µL of ARCHITECT Multi-Assay Manual Diluent.
2. The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The result should be greater than 0.02 ng/mL (before the dilution factor is applied).

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

Urine

Twenty-four-hour urine specimens must be diluted by selecting the automated dilution protocol "URINE 1:10".

Twenty-four-hour urine specimens with a C-peptide concentration of >300.00 ng/mL will be flagged as ">300.00 ng/mL" and may be diluted with the Manual Dilution Procedure.

Manual Dilution Procedure

Suggested dilution: 1:20

1. Add 50 µL of the urine sample to 950 µL of ARCHITECT Multi-Assay Manual Diluent.
2. The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The result should be greater than 2.00 ng/mL (before the dilution factor is applied).

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

Calibration

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

  A single sample of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- Calibration Range: 0.00 - 30.00 ng/mL (0 - 10000 pmol/L)
- Once an ARCHITECT C-Peptide 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 C-Peptide 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 C-Peptide Control values must be within the acceptable ranges as 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 C-Peptide assay belongs to method group 1.

RESULTS

Calculation

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

The ARCHITECT iSystem calculates the Calibrator A through F mean chemiluminescent signal from two Calibrator A through F replicates, generates a calibration curve and stores the result. The default result unit for the ARCHITECT C-Peptide assay is ng/mL.

Measurement Range (Reportable Range)

The measurement range for the ARCHITECT C-Peptide assay is 0.01 ng/mL to 30.00 ng/mL for serum/plasma (defined by LoD and the maximum of the calibration range) and 0.10 ng/mL to 300.00 ng/mL for urine (defined by LoD and the maximum of the calibration range for urine prediluted 1:10).

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

- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the ARCHITECT C-Peptide results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT C-Peptide that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.[16, 17]
- 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.[15]

EXPECTED VALUES

A reference range study was conducted based on guidance from the Clinical and Laboratory Standards Institute (CLSI), Protocol C28-A2[18]. Serum specimens and twenty-four-hour urine from apparently healthy fasting individuals were evaluated in replicates of one using the ARCHITECT C-Peptide assay. The observed values are summarized in the following table.*

Specimen Type	n	Median	2.5th Percentile	97.5th Percentile	Unit
Serum	123	1.78	0.78	5.19	ng/mL
24-hour urine	123	35.26	8.20	116.28	ng/mL
	98[a]	75.60	23.74	206.96	µg/24 hours

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

[a] The volume of the 24-hour urinary excretion was measured for 98 out of the 123 urine specimens.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT C-Peptide assay is designed to have an assay precision of ≤ 10% total CV.

A study was performed with the ARCHITECT C-Peptide assay based on guidance from the CLSI Protocol EP5-A2[19]. Nine samples consisting of three ARCHITECT C-Peptide Controls, three serum based panels and three urine based panels were assayed, using two lots of reagents, on two instruments, in replicates of two at two separate times per day for 20 days. Data from this study are summarized in the following table.*

Sample	Instrument	Reagent Lot	n	Mean Conc. (ng/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
Low Control	1	1	80	0.96	0.019	1.9	0.031	3.2
	2	2	80	0.96	0.030	2.8	0.030	3.2
Medium Control	1	1	80	3.86	0.060	1.6	0.081	2.1
	2	2	80	3.96	0.090	2.3	0.100	2.5
High Control	1	1	80	17.22	0.308	1.8	0.359	2.1
	2	2	80	17.34	0.340	1.9	0.360	2.1
Serum Panel 1	1	1	80	0.69	0.014	2.0	0.027	3.9
	2	2	80	0.75	0.015	2.0	0.030	4.0
Serum Panel 2	1	1	80	3.46	0.060	1.7	0.118	3.4
	2	2	80	3.60	0.069	1.9	0.099	2.8
Serum Panel 3	1	1	80	14.63	0.225	1.5	0.404	2.8
	2	2	80	14.63	0.350	2.4	0.393	2.7
Urine Panel 1	1	1	80	8.45	0.239	2.8	0.316	3.7
	2	2	80	8.66	0.469	5.4	0.567	6.5
Urine Panel 2	1	1	80	44.93	0.838	1.9	1.170	2.6
	2	2	80	46.72	1.511	3.2	2.052	4.4
Urine Panel 3	1	1	80	140.51	2.856	2.0	4.763	3.4
	2	2	80	146.08	6.881	4.7	7.141	4.9

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

Recovery

The ARCHITECT C-Peptide assay is designed to have a mean recovery of 100 ± 15%.

Serum

A study was performed where known concentrations (0.05, 0.24, 1.20, and 6.00 ng/mL) of C-peptide were added to 10 pooled serum samples with C-peptide values ranging from 0.97 ng/mL to 16.33 ng/mL. The concentration of C-peptide was determined using the ARCHITECT C-Peptide assay and the resulting percent recovery was calculated. The percent recovery of the ARCHITECT C-Peptide assay ranged from 91.2% to 100.9% with a mean of 96.2%.*

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

Urine

A study was performed where known concentrations (25, 50, 100, and 200 ng/mL) of C-peptide were added to 5 urine samples with C-peptide values ranging from 6.12 ng/mL to 66.77 ng/mL. The concentration of C-peptide was determined using the ARCHITECT C-Peptide assay and the resulting percent recovery was calculated. The percent recovery of the ARCHITECT C-Peptide assay ranged from 98.9% to 101.2% with a mean of 99.8%.*

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

Dilution Linearity

Serum

The ARCHITECT C-Peptide assay is designed to have a mean recovery of 100 ± 15% of the expected result for samples diluted < 1:2 (< 50% Diluent). Samples diluted > 1:2 (> 50% Diluent) may result in an over-recovery > 15%. A dilution linearity study was performed using pooled serum samples with C-peptide values that ranged between 0.63 ng/mL and 15.66 ng/mL. These samples were diluted manually using ARCHITECT Multi-Assay Manual Diluent at various dilutions (1:2.0 to 1:1.1) to result in 50% to 90% of the original C-peptide value. Data from this study are summarized in the following table.*

Sample	Dilution	Mean Observed Value (ng/mL)	% Mean Recovery[a]
1	Undiluted	0.63	---
	1:2.0 – 1:1.1	0.34 - 0.56	104.0
2	Undiluted	3.53	---
	1:2.0 – 1:1.1	1.85 - 3.10	100.9
3	Undiluted	10.19	---
	1:2.0 – 1:1.1	5.39 - 9.77	106.5
4	Undiluted	15.66	---
	1:2.0 – 1:1.1	8.18 - 14.06	101.5

In addition, a dilution linearity study was performed using pooled low and high level serum samples with C-peptide values ranging from 0.82 ng/mL to 21.20 ng/mL. The low level sample was used to dilute the high level sample to different concentrations. Data from this study are summarized in the following table.*

Sample Pair	Undiluted Value (ng/mL)	Diluted Value (ng/mL)	% Mean Recovery[a]
1	Low 0.82 High 6.95	2.36 - 5.62	102.2
2	Low 1.56 High 13.20	4.08 - 10.10	95.4
3	Low 2.62 High 21.20	7.39 - 16.79	102.7

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

[a] % Recovery = $\frac{\text{Mean Observed Value (ng/mL)}}{\text{Mean Expected Value (ng/mL)}} \times 100$

% Mean Recovery = Mean of % Recovery of all dilutions of a sample

Urine

The ARCHITECT C-Peptide assay is designed to have a mean recovery of 100 ± 15% of the expected result.

A dilution linearity study was performed using urine samples with C-peptide values that ranged between 6.80 ng/mL and 284.48 ng/mL. These samples were diluted manually using ARCHITECT Multi-Assay Manual Diluent at various dilutions (1:10.0 to 1:1.1) to result in 10% to 90% of the original C-peptide value. Data from this study are summarized in the following table.*

Sample	Dilution	Mean Observed Value (ng/mL)	% Mean Recovery[a]
1	Undiluted	6.80	---
	1:10.0 – 1:1.1	0.74 - 6.40	107.0
2	Undiluted	75.64	---
	1:10.0 – 1:1.1	7.47 - 70.30	100.6
3	Undiluted	126.60	---
	1:10.0 – 1:1.1	12.64 - 117.36	100.8
4	Undiluted	284.48	---
	1:10.0 – 1:1.1	25.47 - 252.22	98.7

In addition, a dilution study was performed using low and high level urine samples with C-peptide values ranging from 12.04 ng/mL to 208.16 ng/mL. The low level sample was used to dilute the high level sample to different concentrations. Data from this study are summarized in the following table.*

Sample Pair	Undiluted Value (ng/mL)	Diluted Value (ng/mL)	% Mean Recovery[a]
1	Low 17.16 High 200.84	62.03 - 155.55	100.1
2	Low 40.98 High 158.30	68.38 - 122.87	97.5
3	Low 12.04 High 208.16	69.66 - 176.80	113.6

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

[a] % Recovery = $\frac{\text{Mean Observed Value (ng/mL)}}{\text{Mean Expected Value (ng/mL)}} \times 100$

% Mean Recovery = Mean of % Recovery of all dilutions of a sample

Sensitivity

Analytical Sensitivity

The ARCHITECT C-Peptide assay is designed to have a sensitivity of ≤ 0.01 ng/mL. Analytical sensitivity is estimated as the mean of the blank sample (Calibrator A) plus two times the SD obtained on the blank sample.

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

A study was performed based on guidance from the CLSI Protocol EP17-A[20] and resulted in an LoB of 0.002 ng/mL, an LoD of 0.01 ng/mL and an LoQ of 0.08 ng/mL.*

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

Specificity

The specificity of the ARCHITECT C-Peptide assay is designed to have ≤ 0.01% cross-reactivity when tested with compounds listed in the table below. For proinsulin the assay is designed to have ≤ 40% cross-reactivity. A study was performed with the ARCHITECT C-Peptide assay based on guidance from the CLSI Protocol EP7-A2[21]. Aliquots of ARCHITECT C-Peptide Calibrator A and samples were supplemented with potential cross-reactants at the concentrations listed and tested for C-peptide. Data from this study are summarized in the following table.*

Cross-Reactant	Concentration (ng/mL)	% Cross-Reactivity[a]
Human insulin	8660	0.00
Glucagon	10000	0.00
Human proinsulin	100	12.80
Secretin	15000	0.00
Somatomedin-C (IGF-1)	1000	0.00

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

[a] % Cross-Reactivity = $\frac{\text{Mean Value spiked (ng/mL) - Mean Value non spiked (ng/mL)}}{\text{Concentration of Cross-Reactant (ng/mL)}} \times 100$

Interference

Serum and Plasma

Potential interference in the ARCHITECT C-Peptide assay from hemoglobin, bilirubin, triglycerides, protein, rheumatoid factor, HAMA, and red blood cells is designed to be ≤ 10%.

Interference was demonstrated by a study based on guidance from the CLSI Protocol EP7-A2. Data from this study are summarized in the following table.*

Potentially Interfering Substance	Concentration	% Mean Recovery[a]
Hemoglobin	500 mg/dL	101.8
Bilirubin	20 mg/dL	99.6
Triglycerides	5000 mg/dL	102.3
Protein (Human Albumin)	12 g/dL	91.9
Rheumatoid Factor[b]	100 IU/mL	93.1
HAMA	1000 ng/mL	99.7
Red blood cells	0.4% (v/v)	100.5

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

[a] % Recovery = $\frac{\text{Observed Value (ng/mL)}}{\text{Expected Value (ng/mL)}} \times 100$

% Mean Recovery = Mean of % Recovery of all tested samples

[b] For samples with rheumatoid factors with concentrations between 200 IU/mL and 800 IU/mL, the % Mean Recovery ranged from 89.3% to 80.9%.

Urine

Potential interference in the ARCHITECT C-Peptide assay from creatinine, urea, glucose, NaCl, acetone, and leukocytes is designed to be ≤ 10%.

Interference was demonstrated by a study based on guidance from the CLSI Protocol EP7-A2. Data from this study are summarized in the following table.*

Potentially Interfering Substance	Concentration	% Mean Recovery[a]
Creatinine	600 mg/dL	100.7
Urea	6 g/dL	94.6
Glucose	300 mg/dL	100.1
NaCl	6 g/dL	95.1
Acetone	6 mg/dL	100.1
Leukocytes	20 cells/µL	100.1

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

[a] % Recovery = $\frac{\text{Observed Value (ng/mL)}}{\text{Expected Value (ng/mL)}} \times 100$

% Mean Recovery = Mean of % Recovery of all tested samples

Accuracy

The ARCHITECT C-Peptide Assay is designed to maintain accuracy through standardization to an internationally recognized reference standard.

A study was performed where WHO C-Peptide was added at various concentrations to serum and urine samples with known C-peptide values. The percent recovery of the WHO C-Peptide material, using the ARCHITECT C-Peptide assay, in serum ranged from 93% to 106% with a mean of 99% and in urine ranged from 98% to 107% with a mean of 102%.*

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

Method Comparison

Serum

The ARCHITECT C-Peptide assay is designed to have a correlation coefficient of ≥ 0.95 for serum samples when evaluated against a commercially available diagnostic assay.

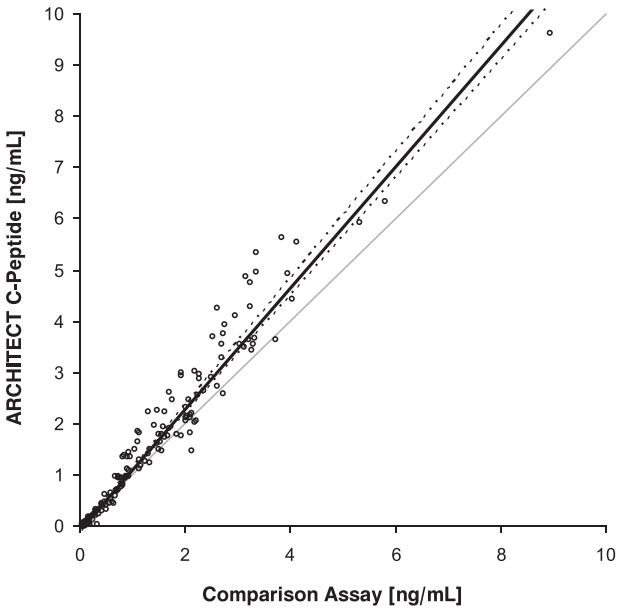
A study was performed with the ARCHITECT C-Peptide assay where regression analysis was performed using the Passing-Bablok[22] method. The following representative data are provided to aid in understanding differences between ARCHITECT C-Peptide versus a commercially available diagnostic assay and is not intended to demonstrate accuracy of the ARCHITECT C-Peptide assay. Data from this study are summarized in the following table and graph.*

Regression Method	n	Slope	Intercept	Correlation Coefficient
Passing-Bablok [a]	176	1.19	-0.10	0.98

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

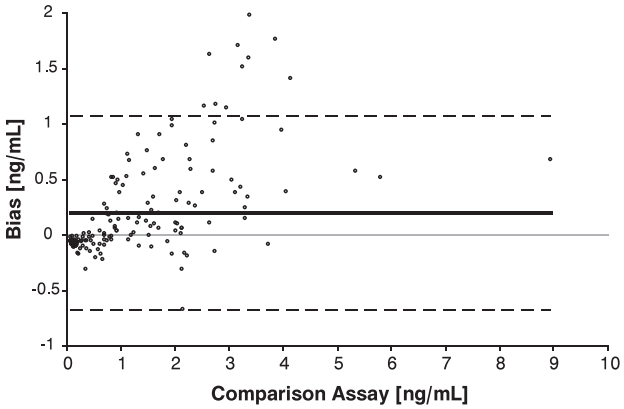
In this evaluation, serum specimen concentrations ranged from 0.01 ng/mL to 9.63 ng/mL with the ARCHITECT C-Peptide assay and from 0.06 ng/mL to 8.95 ng/mL with the commercially available diagnostic assay. The specimens included in the study were from patients with an abnormal insulin secretion.

ARCHITECT C-Peptide vs. Comparison Assay (Passing-Bablok)



A bias analysis of ARCHITECT C-Peptide versus a commercially available diagnostic assay was performed on the same 176 specimens. The following representative data are provided to aid in the understanding the difference between the two assays. The average concentration bias exhibited by ARCHITECT C-Peptide versus the commercially available diagnostic assay in this study was 0.20 ng/mL. The 95% confidence interval of that average concentration bias was -0.68 ng/mL to 1.07 ng/mL. Data from this study are summarized in the following graph.*

ARCHITECT C-Peptide concentration Bias to Comparison Assay



* Representative data; variables such as differences in sampling size, sample population and test method may impact the correlation of the assay, therefore, 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
ASSAY DILUENT	Assay Diluent
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
MULTI-ASSAY MANUAL DILUENT	Multi-Assay Manual Diluent
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCED FOR ABBOTT BY	Produced for Abbott by
PRODUCT OF SPAIN	Product of Spain
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
TRIGGER SOLUTION	Trigger Solution
WASH BUFFER	Wash Buffer

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