C-Peptide

Customer Service
United States: 1-877-4ABBOTT
International: Call your Abbott Representative

This package insert must be read carefully prior to use. Package insert instructions must be 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
- IVD: In Vitro Diagnostic Medical Device
- LOT: Lot Number
- SN: Serial Number
- REACTION VESSELS: Reaction Vessels
- SAMPLE CUPS: Sample Cups
- REPLACEMENT CAPS: Replacement Caps
- REAGENT LOT: Reagent Lot
- ASSAY CD-ROM: Assay CD-ROM
- CONTROL NO.: Control Number
- SEPTEUM: Septum
- Authorized Representative
- Manufacturer
- Store at 2-8°C
- Consult instructions for use

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

Read Highlighted Changes
Revised May, 2009
NAME
ARCHITECT C-Peptide

INTENDED USE
The ARCHITECT C-Peptide assay is a chemiluminescent microparticle immunoassay (CMA) for the quantitative determination of C-peptide in human serum, plasma and urine on the ARCHITECT i System. 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 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.1,2,3 In insulin biosynthesis, it facilitates the formation of the correct secondary and tertiary structure of the hormone.4,5 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.6,7,8 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

BIOLGICAL 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 CMA technology with flexible assay protocols, referred to as Chemiflex.

In the first step, sample, assay diluent, and anti-human C-peptide coated paramagnetic micro particles are combined. C-peptide present in the sample binds to anti-human C-peptide coated micro particles, forming an antigen-antibody complex. After washing, anti-human C-peptide acridinium-labeled conjugate is added to create a reaction mixture 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 C-peptide in the sample and the RLUs detected by the ARCHITECT i System 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
Reagent Kit, 100 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 C-Peptide Reagent Kit (3L53)

- **MICROPARTICLES**: 1 Bottle (6.6 mL) anti-human C-peptide (mouse, monoclonal) coated microparticles in TRIS buffer. Preservative: ProClin 300, ProClin 950.
- **CONJUGATE**: 1 Bottle (5.9 mL) anti-human C-peptide (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizers and detergent. Preservative: sodium azide.
- **ASSAY DILUENT**: 1 Bottle (10.0 mL) Assay Diluent containing MES buffer with surfactant and protein (bovine, mouse) blockers. Preservatives: ProClin 300, ProClin 950.

Assay Diluent

ARCHITECT i Multi-Assay Manual Diluent (7D82-50)

- **MULTI-ASSAY MANUAL DILUENT**: 1 Bottle (100 mL) ARCHITECT i Multi-Assay Manual Diluent containing phosphate buffered saline solution. Preservative: antimicrobial agent.

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. Preservative: antimicrobial agent.

WARNINGS AND PRECAUTIONS

- **IVD**

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.11 Biosafety Level 212 or other appropriate biosafety practices13,14 should be used for materials that contain or are suspected of containing infectious agents.

If the Microparticles and Assay Diluent contain methylisothiazolones, which are components of ProClin. These components are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases:

- R43 May cause sensitization by skin contact.
- S24 Avoid contact with skin.
- S35 This material and its container must be disposed of in a safe way.
- S37 Wear suitable gloves.
- S46 If swallowed, seek medical advice immediately and show this container or label.

- The Conjugate contains sodium azide. 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 sheet 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 C-Peptide Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend the 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

- **2°C-8°C**: The ARCHITECT C-Peptide 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 C-Peptide Reagent Kit may be stored on board the ARCHITECT / 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 / System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded. For information on tracking onboard time, 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 / System from the ARCHITECT / Assay CD-ROM Addition E before performing the assay. 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 the ARCHITECT System Operations Manual.

The default result unit for the ARCHITECT C-Peptide assay is ng/mL. An alternate result unit, pmol/L, may be selected for reporting results by editing assay parameter “Result concentration units” to pmol/mL. The conversion factor used by the system is 333.33.

Conversion formula:

\[
\text{Concentration in ng/mL} \times (333.33) = \text{pmol/L.}
\]

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types
The ARCHITECT / 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 C-Peptide assay.

Serum and Plasma
The specimen collection tubes listed below were verified to be used with the ARCHITECT C-Peptide assay. Other specimen collection tubes have not been tested with this assay.

- Human serum (including serum collected in serum separator tubes)
- Human plasma collected in:
  - Potassium-EDTA
  - Lithium heparin
  - Sodium heparin
- Sodium citrate plasma tubes cannot be used with the ARCHITECT C-Peptide assay.
- Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient specimens.

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)
  - obvious microbial contamination
  - cadaver specimens or body fluids other than human serum, plasma and urine
- 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.
- 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 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. Care must be taken to transfer only the clarified specimen without the lipemic material.

Storage
Serum and Plasma
- 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 may be stored for up to 24 hours at 15-30°C or 48 hours refrigerated at 2-8°C prior to being tested. If testing will be delayed more than 48 hours, store frozen at ≤ -10°C.
- 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.

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

PROCEDURE

Materials Provided
- 3L53 ARCHITECT C-Peptide Reagent Kit

Materials Required but not Provided
- ARCHITECT / System
- 1L65 ARCHITECT / ASSAY CD-ROM - US - Addition E
- 1L66 ARCHITECT / ASSAY CD-ROM - WW (excluding US) - Addition E
- 3L53-01 ARCHITECT C-Peptide Calibrators
- 3L53-10 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

...
To the ARCHITECT System Operations Manual, Section 9.

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

**Assay Procedure**

- Before loading the ARCHITECT C-Peptide Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend the 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 C-Peptide 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.
- 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”.

**Serum and Plasma**

- Priority: 75 μL for the first Architect C-Peptide test plus 25 μL for each additional ARCHITECT C-Peptide test from the same sample cup.
- ≤ 3 hours on board: 150 μL for the first ARCHITECT C-Peptide test plus 25 μL for each additional ARCHITECT C-Peptide test from the same cup.
- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

**Urine**

- Priority: 100 μL for the first ARCHITECT C-Peptide test plus 50 μL for each additional ARCHITECT C-Peptide test from the same sample cup.
- ≤ 3 hours on board: 150 μL for the first ARCHITECT C-Peptide test plus 50 μL for each additional ARCHITECT C-Peptide 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.
- Mix ARCHITECT C-Peptide Calibrators and Controls by gentle inversion before use.
- To obtain the recommended volume requirements for the ARCHITECT C-Peptide Calibrators and Controls, hold the bottles vertically and dispense 4 drops of each calibrator and 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 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. When a laboratory requires more frequent maintenance, follow those 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 dilutions should be performed as follows:
  - The suggested dilution for the ARCHITECT C-Peptide assay is 1:2.
  - NOTE: Samples diluted > 1:2 (> 50% Diluent) may result in an over-recovery > 15%.
  - Add 75 μL of the patient specimen to 75 μL of ARCHITECT i/Multi-Assay Manual Diluent.
  - 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 (before the dilution factor is applied) should be greater than 0.02 ng/mL.
  - 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 dilutions should be performed as follows:
  - The suggested dilution for the ARCHITECT C-Peptide assay is 1:20.
  - Add 50 μL of the urine sample to 950 μL of ARCHITECT i/Multi-Assay Manual Diluent.
  - 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 (before the dilution factor is applied) should be greater than 2.00 ng/mL.
  - For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

**Calibration**

- To perform an ARCHITECT C-Peptide calibration, test calibrators A to F in replicates of two. A single sample of each ARCHITECT C-Peptide control level 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: 0.00 - 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 laboratory quality control procedures require more frequent use of controls to verify test results, follow those 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 belongs to method group 1.

Use ARCHITECT C-Peptide Calibrators in place of MasterCheck as described in the ARCHITECT System Operations Manual, Appendix B.

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

Calculation
The ARCHITECT i System 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
• If the ARCHITECT C-Peptide 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.
• 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 that employ mouse monoclonal antibodies.

EXPECTED VALUES
A reference range study was conducted based on guidance from the Clinical and Laboratory Standards Institute (CLSI), Protocol C28-A2. 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.*

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>n</th>
<th>Median</th>
<th>2.5th Percentile</th>
<th>97.5th Percentile</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>123</td>
<td>1.78</td>
<td>0.78</td>
<td>5.19</td>
<td>ng/mL</td>
</tr>
<tr>
<td>24-hour urine</td>
<td>123</td>
<td>35.28</td>
<td>8.20</td>
<td>116.28</td>
<td>ng/mL</td>
</tr>
</tbody>
</table>

* 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.

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. 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.*

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

* 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%.

A study was performed based on guidance from the CLSI Protocol EP5-A2. 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.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Specimen</th>
<th>Reagent</th>
<th>Lot</th>
<th>n</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Serum</td>
<td>Low</td>
<td>1</td>
<td>1</td>
<td>99.8%</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2</td>
<td>2</td>
<td>98.9%</td>
<td>101.2%</td>
</tr>
<tr>
<td>Medium</td>
<td>Serum</td>
<td>Medium</td>
<td>1</td>
<td>1</td>
<td>90.0%</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2</td>
<td>2</td>
<td>88.9%</td>
<td>101.2%</td>
</tr>
<tr>
<td>High</td>
<td>Serum</td>
<td>High</td>
<td>1</td>
<td>1</td>
<td>91.8%</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2</td>
<td>2</td>
<td>91.2%</td>
<td>101.2%</td>
</tr>
<tr>
<td>Serum</td>
<td>Panel 1</td>
<td>1</td>
<td>1</td>
<td>88.0%</td>
<td>101.2%</td>
</tr>
<tr>
<td></td>
<td>Panel 2</td>
<td>2</td>
<td>2</td>
<td>90.0%</td>
<td>101.2%</td>
</tr>
<tr>
<td>Serum</td>
<td>Panel 1</td>
<td>1</td>
<td>1</td>
<td>88.0%</td>
<td>101.2%</td>
</tr>
<tr>
<td></td>
<td>Panel 2</td>
<td>2</td>
<td>2</td>
<td>90.0%</td>
<td>101.2%</td>
</tr>
</tbody>
</table>

* 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%.

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.

Dilution Linearity

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.
In addition, a dilution linearity study was performed using pooled low and high level serum samples with C-peptide values ranging from 8.02 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.*

<table>
<thead>
<tr>
<th>Sample Pair</th>
<th>Undiluted Value (ng/mL)</th>
<th>Diluted Value (ng/mL)</th>
<th>% Mean Recoverya</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low 0.82 High 6.95</td>
<td>2.36 - 5.62</td>
<td>102.2</td>
</tr>
<tr>
<td>2</td>
<td>Low 1.56 High 13.20</td>
<td>4.08 - 10.10</td>
<td>95.4</td>
</tr>
<tr>
<td>3</td>
<td>Low 2.62 High 21.20</td>
<td>7.39 - 16.79</td>
<td>102.7</td>
</tr>
</tbody>
</table>

* 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

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 (1:1.0 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.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Mean Observed Value (ng/mL)</th>
<th>% Mean Recoverya</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Undiluted</td>
<td>6.80</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>1:1.0 – 1:1.1</td>
<td>0.74 - 6.40</td>
<td>107.0</td>
</tr>
<tr>
<td>2</td>
<td>Undiluted</td>
<td>75.64</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>1:1.0 – 1:1.1</td>
<td>7.47 - 70.30</td>
<td>100.6</td>
</tr>
<tr>
<td>3</td>
<td>Undiluted</td>
<td>126.60</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>1:1.0 – 1:1.1</td>
<td>12.64 - 117.36</td>
<td>100.8</td>
</tr>
<tr>
<td>4</td>
<td>Undiluted</td>
<td>284.48</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>1:1.0 – 1:1.1</td>
<td>25.47 - 252.22</td>
<td>98.7</td>
</tr>
</tbody>
</table>

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.*

<table>
<thead>
<tr>
<th>Sample Pair</th>
<th>Undiluted Value (ng/mL)</th>
<th>Diluted Value (ng/mL)</th>
<th>% Mean Recoverya</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low 17.18 High 200.84</td>
<td>62.03 - 155.55</td>
<td>100.1</td>
</tr>
<tr>
<td>2</td>
<td>Low 40.98 High 158.30</td>
<td>68.38 - 122.87</td>
<td>97.5</td>
</tr>
<tr>
<td>3</td>
<td>Low 12.04 High 208.16</td>
<td>69.66 - 176.80</td>
<td>113.6</td>
</tr>
</tbody>
</table>

* 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 EP7-A2 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. 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.*

<table>
<thead>
<tr>
<th>Cross-Reactant</th>
<th>Concentration (ng/mL)</th>
<th>% Cross-Reactivitya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human insulin</td>
<td>8660</td>
<td>0.00</td>
</tr>
<tr>
<td>Glucagon</td>
<td>10000</td>
<td>0.00</td>
</tr>
<tr>
<td>Human proinsulin</td>
<td>100</td>
<td>12.80</td>
</tr>
<tr>
<td>Secretin</td>
<td>15000</td>
<td>0.00</td>
</tr>
<tr>
<td>Somatomedin-C (IGF-1)</td>
<td>1000</td>
<td>0.00</td>
</tr>
</tbody>
</table>

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

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

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.*

<table>
<thead>
<tr>
<th>Potentially Interfering Substance</th>
<th>Concentration</th>
<th>% Mean Recoverya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>500 mg/dL</td>
<td>101.8</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>20 mg/dL</td>
<td>99.6</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>5000 mg/dL</td>
<td>102.3</td>
</tr>
<tr>
<td>Protein (Human Albumin)</td>
<td>12 g/dL</td>
<td>91.9</td>
</tr>
<tr>
<td>Rheumatoid Factorb</td>
<td>100 IU/mL</td>
<td>93.1</td>
</tr>
<tr>
<td>HAMA</td>
<td>1000 ng/mL</td>
<td>99.7</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>0.4% (v/v)</td>
<td>100.5</td>
</tr>
</tbody>
</table>

* 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.*

<table>
<thead>
<tr>
<th>Potentially Interfering Substance</th>
<th>Concentration</th>
<th>% Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>600 mg/dL</td>
<td>100.7</td>
</tr>
<tr>
<td>Urea</td>
<td>6 g/dL</td>
<td>94.6</td>
</tr>
<tr>
<td>Glucose</td>
<td>300 mg/dL</td>
<td>100.1</td>
</tr>
<tr>
<td>NaCl</td>
<td>6 g/dL</td>
<td>95.1</td>
</tr>
<tr>
<td>Acetone</td>
<td>6 mg/dL</td>
<td>100.1</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>20 cells/μL</td>
<td>100.1</td>
</tr>
</tbody>
</table>

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

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

Method Comparison

Serum
The ARCHITECT C-Peptide assay is designed to have a slope of 1.0 ± 0.15 and 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-Bablok22 and Least Squares methods. Data from this study are summarized in the following table and graph.*

<table>
<thead>
<tr>
<th>Regression Method</th>
<th>n</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passing-Bablok</td>
<td>310</td>
<td>1.02</td>
<td>0.03</td>
<td>0.99</td>
</tr>
<tr>
<td>Least Squares</td>
<td>310</td>
<td>0.99</td>
<td>0.14</td>
<td>0.99</td>
</tr>
</tbody>
</table>

* 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 23.31 ng/mL with the ARCHITECT C-Peptide assay and from 0.62 ng/mL to 23.03 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 vs. a commercially available diagnostic assay was performed on the same 310 specimens. The following representative data are provided to aid in understanding the difference between the two assays. The average bias exhibited by ARCHITECT C-Peptide vs. a commercially available diagnostic assay in this study was 6.44%. The 95% confidence interval of that average bias was -11.28 to 39.81%. Data from this study are summarized in the following graph.*

ARCHITECT C-Peptide % Bias to Comparison Assay

Urine
The ARCHITECT C-Peptide assay is designed to have a slope of 1.0 ± 0.20 and a correlation coefficient of ≥ 0.95 for urine 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-Bablok22 and Least Squares methods. Data from this study are summarized in the following table and graph.*

<table>
<thead>
<tr>
<th>Regression Method</th>
<th>n</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passing-Bablok</td>
<td>113</td>
<td>1.15</td>
<td>1.62</td>
<td>0.99</td>
</tr>
<tr>
<td>Least Squares</td>
<td>113</td>
<td>1.07</td>
<td>3.16</td>
<td>0.99</td>
</tr>
</tbody>
</table>

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

In this evaluation, urine specimen concentrations ranged from 1.34 ng/mL to 286.64 ng/mL with the ARCHITECT C-Peptide assay and from 0.92 ng/mL to 261.65 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 vs. a commercially available diagnostic assay was performed on the same 113 specimens. The following representative data are provided to aid in understanding the difference between the two assays. The average bias exhibited by ARCHITECT C-Peptide vs. a commercially available diagnostic assay in this study was 24.30%. The 95% confidence interval of that average bias was -18.40 to 71.41%. Data from this study are summarized in the following graph.*

* Representative data; variables such as differences in sampling size and sample population may impact the correlation of the assay, therefore, results in individual laboratories may vary from these data.

BIBLIOGRAPHY