CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

Technical Bulletin
No. 139

March 20, 2015

C-Peptide
- New Test Introduction –

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Ordering Information:

Mnemonics:	**C Peptide**
Test Name:	C-Peptide
Test Number:	1750126
Specimen:	1 ml serum, refrigerated up to 48 hours, frozen up to 3 months

Effective Date: Testing will begin on Monday, April 6, 2015.

Performed: Monday through Friday

Reference Range: Fasting patient 0.78-5.19 ng/mL

Background:
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. In insulin biosynthesis, it facilitates the formation of the correct secondary and tertiary structure of the hormone. 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). 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. 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. 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.

Principle of Test:
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.

In the first step, sample, assay diluent, and anti-human C-peptide coated paramagnetic microparticles are combined. C-peptide present in the sample binds to anti-human C-peptide coated mircoparticles, 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* optical system. Results are calculated automatically based on the previously established calibration curve.

Results Interpretation:
Fasting Reference Range: 0.78-5.19 ng/mL

Limitations:
1. For diagnostic purposes, results should be used in conjunction with other data: *e.g.*, symptoms, results of other tests, clinical impression, etc.
2. 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.
3. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Specimens containing HAMA may produce anomalous values when tested with assay kits that employ mouse monoclonal antibodies.
4. If the C-peptide results are inconsistent with clinical evidence, additional testing is suggested to confirm the result. Please contact the laboratory within seven days of specimen collection for further testing to rule out heterophilic antibody interference.
5. C-peptide concentration is independent of exogenous insulin and is not subject to interference from insulin autoantibodies induced by insulin therapy.

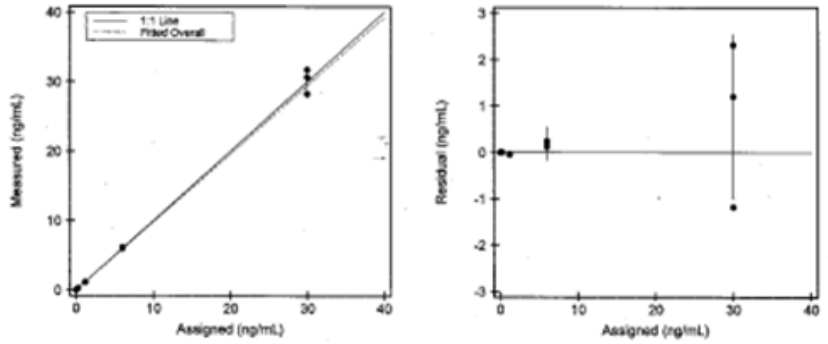
Validation Data:
Precision:
To assess within-run precision, two levels of controls were run in the same run ten times each. To assess between-run precision, two levels of controls were run in duplicate on five different days. (Table 1) The tests performance was within manufacturer's precision claims of <10% CV.

Table 1		**C-Peptide Precision**					
Within Run Precision				**Between Run Precision**			
ng/mL	**%CV**	**ng/mL**	**%CV**	**ng/mL**	**%CV**	**ng/mL**	**%CV**
0.92	2.44%	17.5	3.25%	0.96	2.34%	17.48	4.92%

Linearity and Analytical Measurement Range:
To verify the analytical measurement range of the assay, six levels of C-Peptide calibrator (of a different lot than the lot used to calibrate) were run in triplicate. (Figure 1)

Figure 1:

Linearity



Linearity Summary

	N	Slope	Intercept	Error
Overall	6	0.982	0.002	0.00 ng/mL or 3.2%

LINEAR within Allowable Systematic Error of 0.005 ng/mL or 5.8%

Statistical Analysis and Experimental Results

	Assigned	Est	Mean	Residual	Linear?	Measured Concentrations		
CAL-01	0.00	0.002	0.000	-0.002	Pass	0	0	0
CAL-02	0.05	0.051	0.053	0.003	Pass	.06	.05	.05
CAL-03	0.24	0.237	0.240	0.003	Pass	.24	.24	.24
CAL-04	1.20	1.179	1.143	-0.036	Pass	1.13	1.15	1.15
CAL-05	6.00	5.891	6.083	0.192	Pass	6.1	6.01	6.14
CAL-06	30.00	29.449	30.227	0.777	Pass	28.27	31.76	30.65

See User's Specifications for Pass/Fail criteria

X: Excluded from calculations

User's Specifications

Allowable Total Error	0.01 ng/mL or 11.7%
Systematic Error Budget	50%
Allowable Systematic Error	0.005 ng/mL or 5.8%

Supporting Data

Analyst	SJ
Date	25 Aug 2014
Value Mode	Pre-Assigned
Units	ng/mL
Controls	--
Reagent	--
Calibrators	--
Comment	

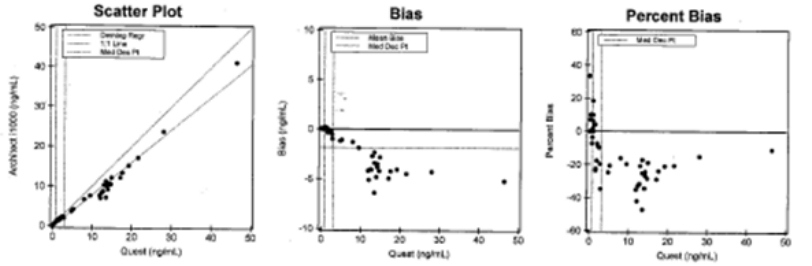
Method Comparison

A total of 50 specimens were split and processed utilizing Abbott Diagnostics' Architect *i*1000 assay and compared to Quest Diagnostics' C-Peptide assay. Quantitative analysis yielded a correlation coefficient (R) of 0.9892 with a slope of 0.819 and an intercept of -0.397. Overall bias for CPAL versus Quest was -1.850 ng/mL. (Figure 2)

Figure 2:

Alternate (Quantitative) Method Comparison

X Method: Quest Y Method: Architect i1000



Regression Analysis

	Deming	Regular
Slope:	0.819 (0.784 to 0.855)	0.812 (0.777 to 0.847)
Intercept:	-0.397 (-0.825 to 0.031)	-0.340 (-0.767 to 0.087)
Std Err Est:	1.111	1.109

95% Confidence Intervals are shown in parentheses

Reference Range Validation:
Twenty-two specimens were collected from apparently healthy individuals that had fasted for 8 hours. Twenty-one out of twenty-two results fell within Abbott's published reference range. The reference range was verified based upon CLSI EP29-A3c, section 11.2 guidelines, which describes transference "using small number of reference individuals".

References:

1. Architect System C-Peptide package insert; 6/12.
2. Architect System C-Peptide Calibrators package insert; 6/12.
3. Architect System C-Peptide Controls package insert; 6/12.
4. Ricos C, et al. *Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation.* From: "Current databases on biologic variation: pros, cons and progress." Scand J Clin Lab Invest 1999;59:491-500. Databases updated 2014.
5. CLSI. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory*; *Approved Guideline—Third Edition.* CLSI document EP28-A3c. Wayne, PA: Clinical and Laboratory Standards Instititute; Nov 2008. Corrected Version Oct 2010.