CPAL
Central **P**ennsylvania **A**lliance
Laboratory

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Insulin
- New Test Introduction –

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Ordering Information:

Mnemonics:	**Insulin Level**
Test Name:	Insulin Level
Test Number:	1750124
Specimen:	1 ml serum from Fasting patient, stable frozen up to 7 days

Effective Date: Testing will begin on Monday, April 6, 2015.
Through May 31, 2015, samples will be held for 21 days from date of collection to accommodate patients who may be serially monitored. Please contact the laboratory if further testing is desired.

Performed: Monday through Friday

Reference Range: 2.0-20.0 μU/mL Fasting

Background:
Insulin is a polypeptide hormone (MW 6000) composed of two non-identical chains, A and B, which are joined by two disulfide bonds. Insulin is formed from a precursor, proinsulin (MW 9000), in the beta cells of the pancreas. In proinsulin, the A and B chains are joined by a connecting peptide, referred to as the C-peptide. Both insulin and C-peptide are stored in secretory granules of the islet cells of the pancreas and are then secreted.

Insulin secretion follows two basic mechanisms, tonic secretion and biphasic secretion. The basal or tonic secretion is independent of stimulation by exogenous glucose but is modulated by the fluctuations in physiological levels of glucose. The biphasic secretion is primarily a direct response from stimulation by exogenous glucose. Stimulation of insulin secretion can be caused by many factors including hyperglycemia, glucagon, amino acids, and by complex mechanisms involving growth hormone or catecholamines. Increased levels of Insulin are found with obesity, Cushing's Syndrome, oral contraceptives, acromegaly, insulinoma, and hyperthyroidism. Decreased levels of insulin are found in overt diabetes mellitus (although this may not be clearly expressed in early stages of the condition) and by part of a complex mechanism involving catecholamines.

"Immunoreactive insulin" (IRI) is a term often used to refer to the component of circulating insulin and insulin-like biological activity which can be measured using antibodies against insulin. Insulinomas may produce various forms of insulin and proinsulin-like material and show total immunoreactive insulin at normal or elevated levels. Since proinsulin and insulin both contain A and B polypeptide chains, there is a possible cross-reactivity with

antibodies generated against insulin. The ARCHITECT Insulin assay shows no cross-reactivity with proinsulin (≤ 0.1% at 10^6 pg/mL). Another possible interference is brought about by insulin antibodies which develop in patients treated with bovine or porcine insulin.

Immunoassays for insulin have been widely used to provide supplementary information, first, for the diagnosis of diabetes mellitus and, second, for differential diagnosis of fasting hypoglycemia to discriminate between insulinoma and factitious hypoglycemia. In these applications, the ratio of immunoreactive insulin to blood glucose (I/G) may be more valuable than the insulin level alone. Furthermore, a single random blood sample may provide insufficient information due to wide variations in the time responses of insulin levels and blood glucose which are found among individuals and various clinical conditions. Other uses of insulin assays have been suggested by the finding of an increase in risk factors for coronary artery disease among healthy persons with hyperinsulinemia and normal glucose tolerance.

Principle of Test:
The ARCHITECT Insulin assay is a one-step immunoassay to determine the presence of human insulin in human serum or plasma, using CMIA technology with flexible assay protocols, referred to as Chemiflex. Sample, anti-insulin coated paramagnetic microparticles, and anti-insulin acridinium-labeled conjugate are combined. Insulin present in the sample binds to the anti-insulin coated microparticles and anti-insulin acridinium-labeled conjugate. After washing, pre-trigger and trigger solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of insulin in the sample and the RLUs detected by the ARCHITECT *i* optical system.

Results Interpretation:
Reference range for fasting patient: 2.0-20.0 μU/mL

Limitations:
1. The presence of heterophilic (anti-animal) antibodies in human serum (whether due to repeated animal exposure or through therapies) can interfere with immunoassays such as this test. This result should be interpreted taking the entire clinical setting into account. Should unexpected results be obtained for this test, please contact the laboratory within seven days of specimen collection for further testing to rule out heterophilic antibody interference.
2. Specimens from patients treated with bovine or porcine insulin may contain insulin antibodies which could show interference in the assay.
3. The Insulin assay is performed on an Abbott Architect using an immunoassay procedure and is sensitive down to 1 μIU/mL.
4. Test results may vary by laboratory, therefore, for trending purposes, use sequential results from a single source.
5. Insulin levels may be measured lower in patients with insulin autoimmune syndrome of familial high pro-insulinemia.
6. Hemolyzed specimens should not be used, since enzymatic degradation of insulin may occur and result in lower assay values.

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.

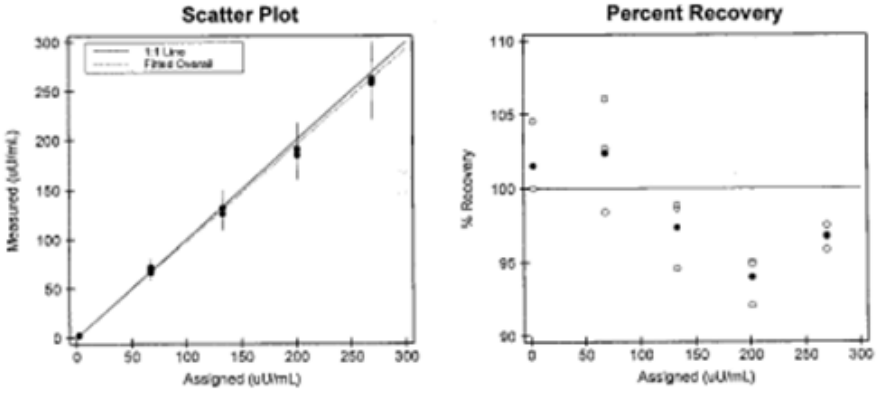
Table 1		**Insulin Precision**					
Within Run Precision				**Between Run Precision**			
μU/mL	**%CV**	**μU/mL**	**%CV**	**μU/mL**	**%CV**	**μU/mL**	**%CV**
8.2	2.07%	124.0	1.47%	8.63	3.13%	122.24	1.77%

Linearity and Analytical Measurement Range:

To verify the linearity and analytical measurement range of the assay, a high patient sample was diluted with a low patient sample to cover the reportable range of 1.0 µU/mL to 300.0 µU/mL. The dilutions were run in triplicate. (Figure 1)

Figure 1:

Accuracy and Linearity



Linearity Summary

	N	Slope	Intercept	Error
Overall	5	0.976	0.38	0.30 uU/mL or 4.1%

LINEAR within Allowable Systematic Error of 1.1 uU/mL or 15.0%

Statistical Analysis and Experimental Results

	Assigned	Mean	% Rec.	Est	Resid	Linear?	Measured Concentrations		
CPAL Lin-01	2.2	2.23	101.5	2.53	-0.30	Pass	2.2	2.3	2.2
CPAL Lin-02	67.6	69.20	102.4	66.39	2.81	Pass	71.7	69.4	66.5
CPAL Lin-03	133.1	129.60	97.4	130.34	-0.74	Pass	131.3	131.6	125.9
CPAL Lin-04	201.2	189.13	94.0	196.83	-7.69	Pass	185.3	191.2	190.9
CPAL Lin-05	269.3	260.53	96.7	263.32	-2.78	Pass	258.2	260.8	262.6

See User's Specifications for Pass/Fail criteria

X: Excluded from calculations

User's Specifications

Allowable Total Error:	2.2 uU/mL or 30.0%
Systematic Error Budget:	50%
Allowable Systematic Error:	1.1 uU/mL or 15.0%

Supporting Data

Analyst:	SAF
Date:	09 Feb 2015
Value Mode:	Preassigned
Units:	uU/mL
Lot Number:	
Comment:	

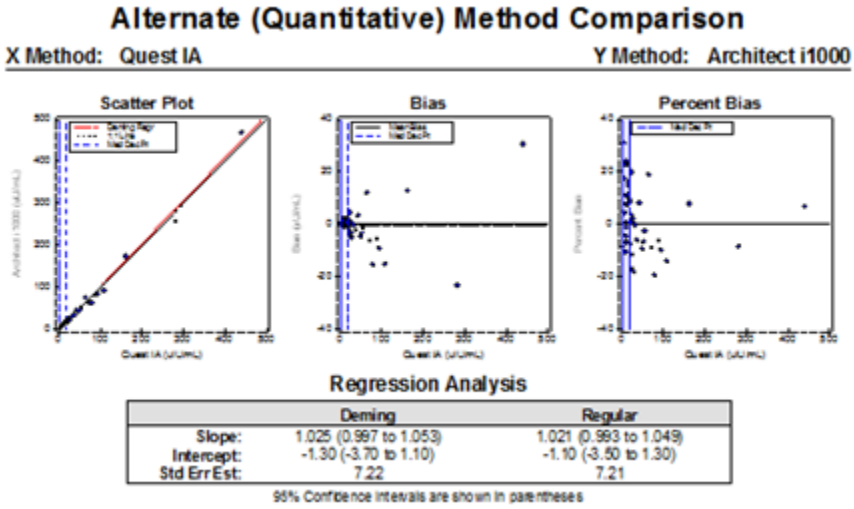
Analytical Claim

The Accuracy and Linearity of Insulin were analyzed on Architect i1000 over a measured range of 2.23 to 260.53 uU/mL. This analysis assumes accurate defined values. Allowable systematic error (SEa) was 1.1 uU/mL or 15.0%. The accuracy test PASSED. The maximum deviation for a mean recovery from 100% was 6.0%. 5 of 5 mean recoveries were accurate within the SEa. 15 of 15 results were accurate within the allowable total error (TEa) of 2.2 uU/mL or 30.0%. The results are LINEAR.

Method Comparison

A total of 50 specimens were split and processed utilizing the Architect *i*1000 Insulin assay and compared to the results of Quest Diagnostics' Insulin assay. Quantitative analysis yielded a correlation coefficient (R) of 0.9957 with a slope of 1.025 and an intercept of -1.30. Overall bias for CPAL versus Quest was -0.21. U/mL. (Figure 2)

Figure 2:



Reference Range Validation:

Abbott Diagnostics does not provide a published reference range for the Insulin assay. Therefore, medical decision point analysis was used to verify a normal range of 2-20 µU/mL, which correlated very well with the published literature.

References:

1. Architect System Insulin package insert; 9/12.
2. Architect System Insulin Calibrators package insert; 9/12.
3. Architect System Insulin Controls package insert; 9/12.