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Educational Discussion: Insulin and C-peptide Wild Cards Results

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Insulin and C-peptide are cleaved from proinsulin and secreted in a 1:1 molar ratio, and are both important biomarkers in the pathophysiology of diabetes mellitus. Measurement of circulating insulin concentrations may improve the classification and management of diabetes and aid in defining and treating individuals with varying levels of insulin resistance. Assessment of C-peptide has clinical utility in monitoring endogenous insulin production in diabetics receiving exogenous insulin therapy, and the Diabetes Control and Complications Trial (DCCT) established the importance of C-peptide as a key biomarker of beta cell function and depletion, primarily in Type 1 diabetics. Insulin is cleared by the liver but C-peptide is not, making C-peptide a superior marker of insulin secretion.

Presently neither insulin nor C-peptide are standardized or harmonized, although there are active efforts working simultaneously to achieve standardization of both analytes. Ultimately standardization of insulin and C-peptide assays will allow data and information from numerous epidemiological studies and clinical trials to be aggregated and ultimately incorporated into clinical practice guidelines.

The INGW-B Survey included two serum specimens (a low pool and a high pool) to evaluate the current state of insulin and C-peptide immunoassay results, which are not expected to be comparable between manufacturers or peer groups because neither assay is presently fully standardized. Serum specimens eliminate any matrix effects observed from use of non-commutable materials.

The insulin low and high serum pool mean concentrations across all peer groups was 49.9 pmol/L (8.31 uIU/mL) and 340.5 pmol/L (56.8 uIU/mL), respectively. The range of mean values for the low pool was 37.4 – 64.6 pmol/L and the high pool was 279.8 – 410.1 pmol/L. For a majority of peer groups the inter-laboratory comparability of results was better using the serum pools compared to the CAP ING specimens which were spiked with insulin (Y-04, Y-05, Y-06).

The C-peptide low and high serum pool mean concentrations across all peer groups was 0.49 nmol/L and 1.99 nmol/L, respectively. One peer group's C-peptide results (Tosoh AIA) were markedly different compared to the other peer groups in the spiked CAP specimens (ING-04, ING-05, ING-06) but not in the pooled sera. There are two generations of Tosoh C-peptide assays likely being utilized, with the first generation assay being phased out over time. The material spiked into the ING Survey samples is synthetic human C-peptide but is the 35-mer peptide (proinsulin 55-89) which separates the insulin A and B chains, while C-peptide actually circulates as a 31 amino acid molecule. The 2nd generation Tosoh assay is specific for the 31 amino acid circulating form therefore has low cross-reactivity the CAP spiked materials but detects C-peptide in the serum pools to a similar extent compared to other peer groups. The other C-peptide assays have variable cross-reactivity with proinsulin, therefore those assays have higher C-peptide concentrations in the spiked samples.

Finally, we have discovered that some of the pooled serum results from the various C-peptide methods may have been affected by the storage conditions of the specimens prior to shipment. Ongoing stability studies will help resolve any outstanding questions related to C-peptide stability.

Further evaluation of the performance, harmonization, and standardization of both insulin and C-peptide assays will continue to be an ongoing process.

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