

A High-throughput Mass Spectrometry Multiplexed Assay to Measure Insulin and C-peptide

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The incidence of type 2 diabetes mellitus has increased markedly in the United States and worldwide in the past 2 decades. This increase is traceable largely to the dramatic increase in the prevalence of obesity and insulin resistance in developed nations.

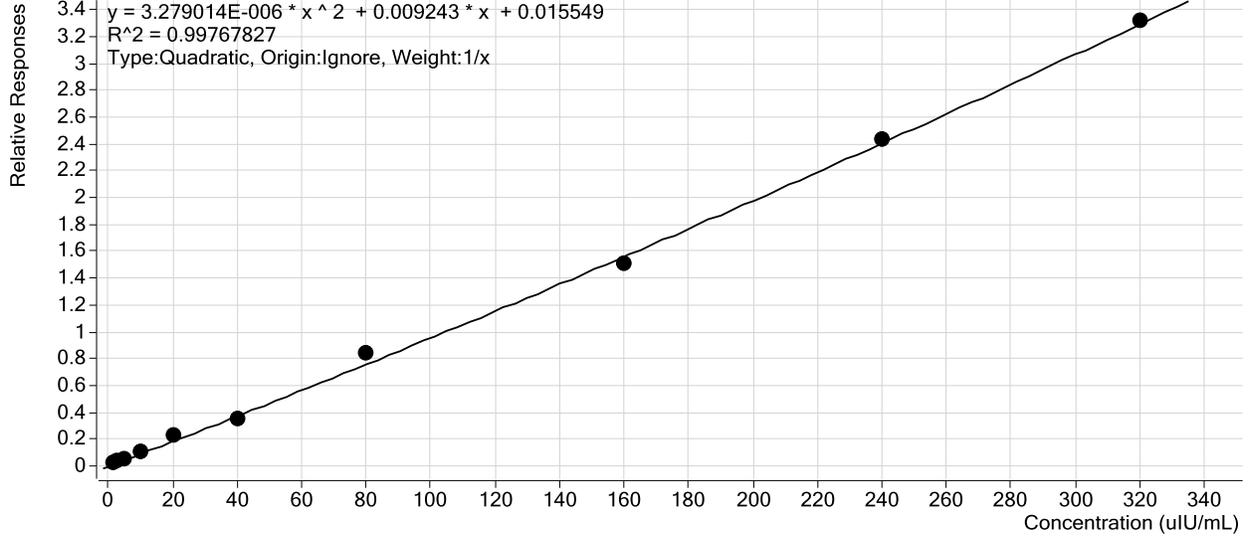
Elevated fasting insulin levels reflect the presence of insulin resistance and predict future development of diabetes mellitus. However, the clinical measurement of insulin levels has been largely relegated to specialized testing employed primarily in research and epidemiological studies examining insulin resistance, prediabetes, and early Type 2 diabetes. Although insulin measurements have a direct relation to more sophisticated assessments of insulin resistance, researchers have not yet defined specific insulin levels that are associated with specific degrees of insulin resistance. This is due at least in part to the marked variability among available assays. C-peptide measurements are less variable than insulin assays, but also suffer from a lack of standardization.

To address these aspects, we developed a multiplexed method to measure insulin and C-peptide using an LC tandem mass spectrometry assay. The assay involves enrichment of the peptides from patient sera using two different monoclonal antibodies immobilized on magnetic beads and processing on a robotic liquid handler. Eluted peptides are directly analyzed by LC-MS/MS on an Agilent 6490 triple quadrupole mass spectrometer. The assay has a clinical reportable range from 2.5 to 320 μ IU/mL for insulin and 0.11 to 27.2 ng/mL for C-peptide (Figure 1). Intra- and inter-day assay variation is less than 11% for both peptides. Of the five insulin analogues commonly prescribed to treat diabetes, only the recombinant human insulin drug Humalog® (insulin lispro) causes significant interference for the determination of endogenous insulin. There were no observed interferences for C-peptide.

Figure 1. Insulin and C-peptide Calibration curves.

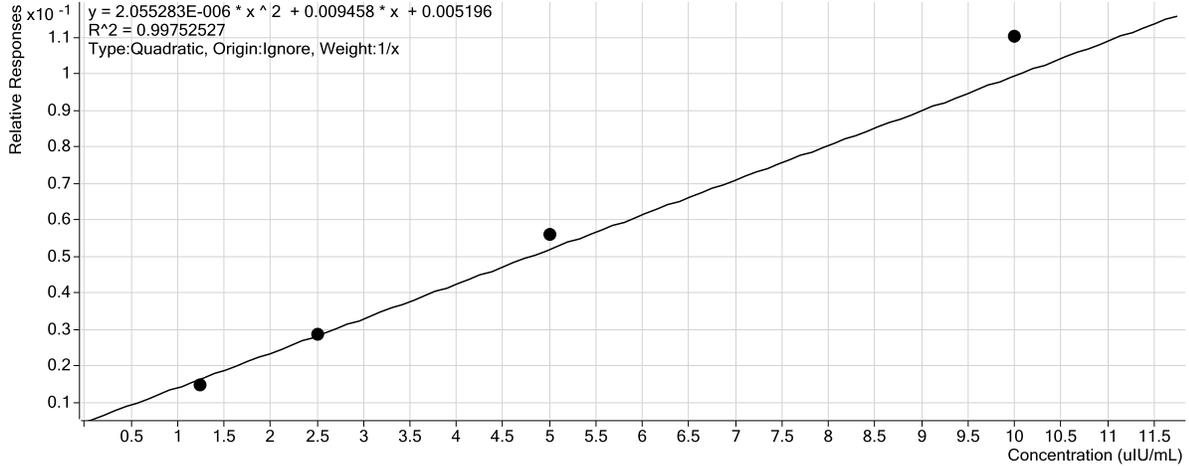
Insulin

Hu6 - 10 Levels, 10 Levels Used, 10 Points, 10 Points Used, 0 QCs



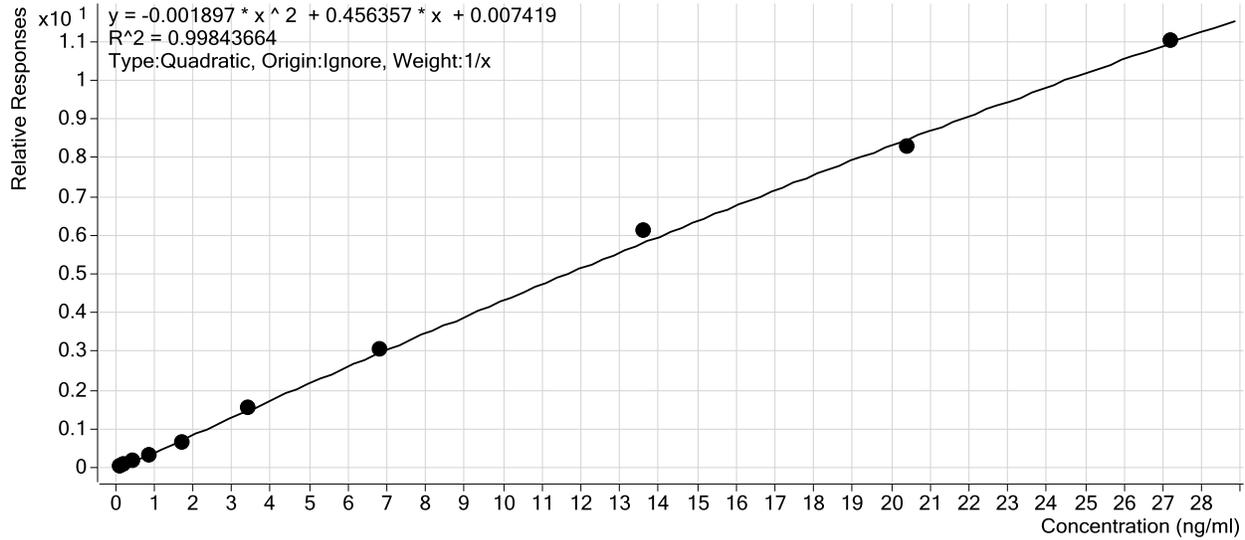
Low Concentration Region

Hu6 - 10 Levels, 10 Levels Used, 10 Points, 10 Points Used, 0 QCs



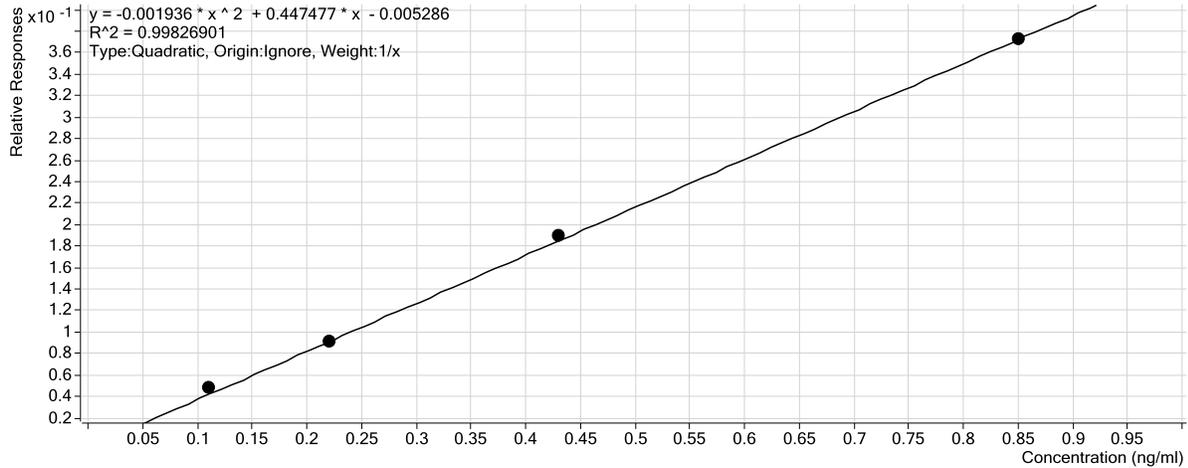
C-peptide

cpp - 10 Levels, 10 Levels Used, 10 Points, 10 Points Used, 0 QCs



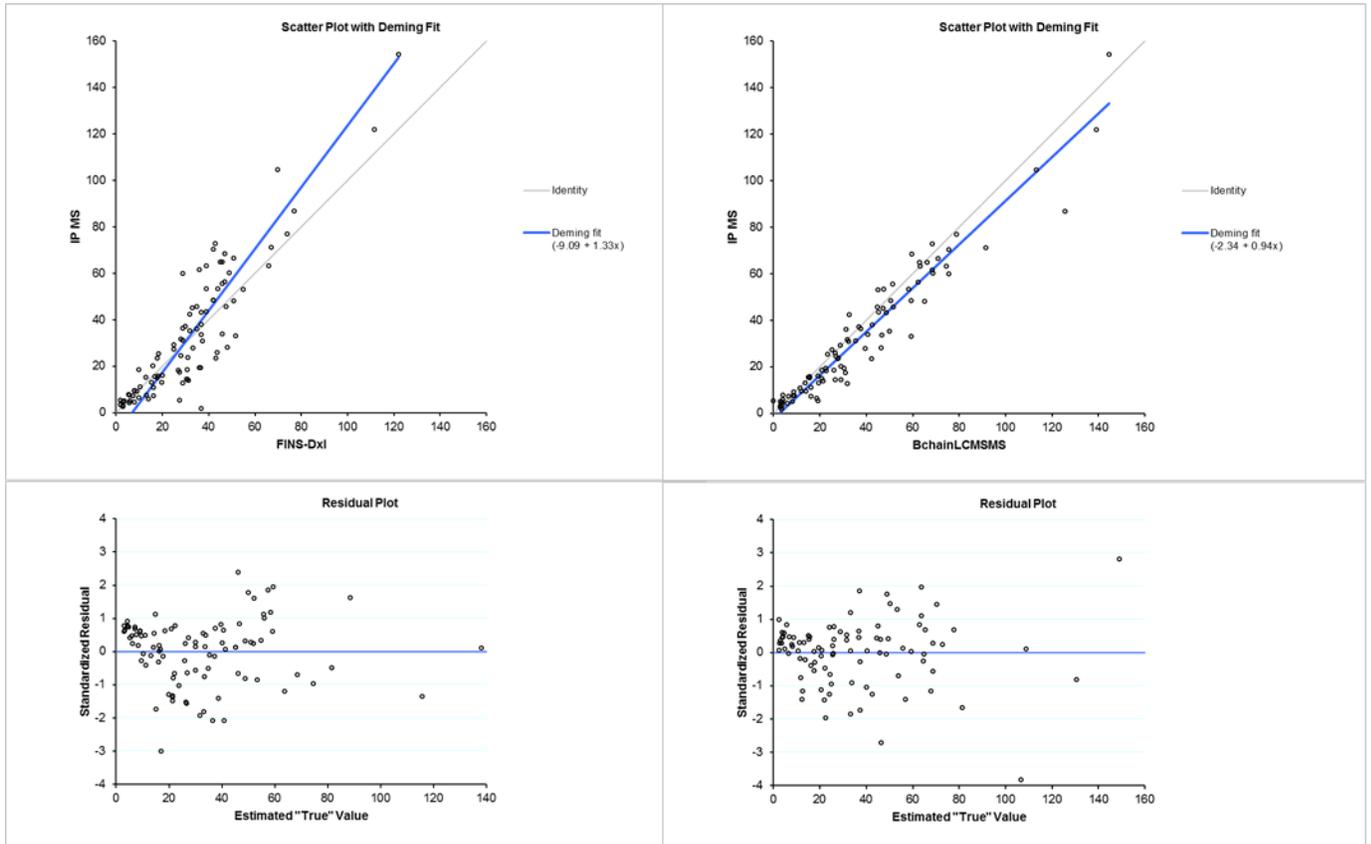
Low concentration region

cpp - 10 Levels, 10 Levels Used, 10 Points, 10 Points Used, 0 QCs



Using patient sample discards, we correlated our insulin assay against (a) a commercial immunoassay kit from Beckman and (b) an LC-MS/MS assay developed in our laboratory that involves using the insulin B chain, liberated on chemical reduction of insulin's inter-chain disulfide crosslinks, as a surrogate for intact insulin concentration. The latter assay yielded better correlation (Figure 2).

Figure 2. Insulin Correlations (n =94) Current Assay (IPMS) versus Beckman and B chain LC-MS/MS.



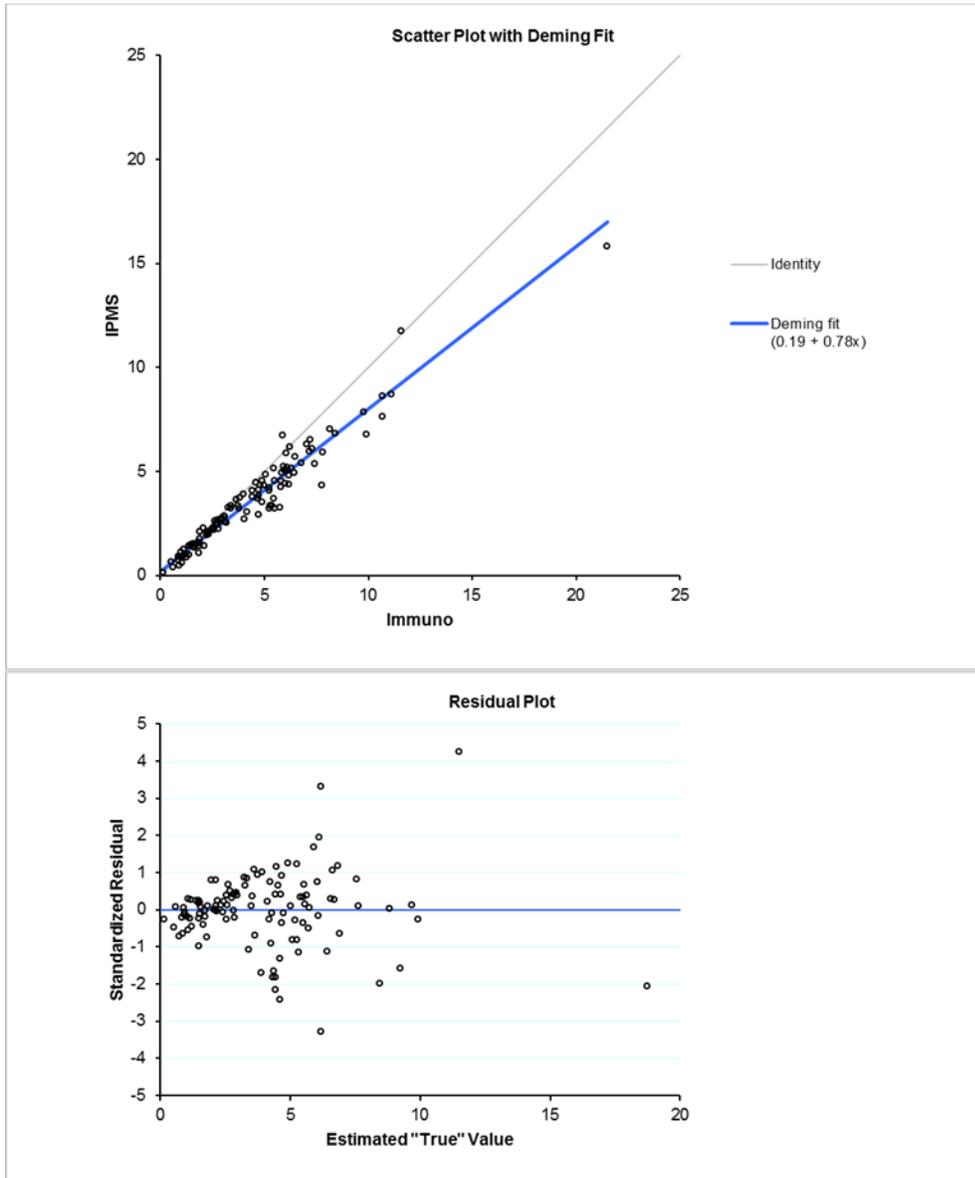
IPMS vs Beckman

IPMS vs B chain LC-MS/MS

IPMS=Immuno-capture(-precipitation) LC-MS/MS (Current Assay)

For C-peptide we correlated our assay against the C-peptide Immulite® assay. The correlation was tight but strongly biased toward the immunoassay by at least 20%, reflecting the specificity of LC-MS/MS only detecting intact C-peptide and not partially processed, truncated or modified forms (Figure 3). In contrast, there was much better agreement of the values obtained for LC-MS/MS calibrators in the immunoassay (not shown, Deming regression $(0.93x+0.08)$).

Figure 3. C-peptide Correlations (n =115) Current Assay (IPMS) versus C-peptide Immulite



In summary, we developed a multiplexed method to measure insulin and C-peptide using an LC tandem mass spectrometry assay. This approach offers a highly accurate and reproducible tool to measure insulin and C-peptide. Applying this tool to clinical samples will permit the standardization of both measurements using physicochemical properties of the analytes. This in turn will facilitate comparison of results obtained in different studies and to highly characterized standard reference materials.