

Quantitative Amino Acid Analysis in Insulin and C-peptide Assays

Letter Publication

What is already known?

- Measurement of insulin and C-peptide is not currently recommended for diagnosis and management of diabetes. This is due, in part, to a lack of standardization across laboratories.
- To enable comparisons between laboratories, assays must be standardized (ie, traceable to a common reference material).
- Ideally, the reference material is traceable to the International System of Units (SI). However, most peptide assays are standardized against World Health Organization (WHO) reference materials that are not SI-traceable (ie, there is uncertainty in the amount of pure material originally weighed).
- Furthermore, the reference materials previously used for the standardization of most insulin and C-peptide assays are no longer available.
- In a previous study aimed at improving standardization, the investigators developed and validated a multiplexed liquid chromatography tandem mass spectrometry (LC-MS/MS) assay that simultaneously measures intact insulin and C-peptide in patient sera.¹

What was done in this study?

- In this letter, the investigators provide more information about how they characterized peptides that were used to develop the LC-MS/MS assay to enable SI-traceability.
- The peptide content of insulin and C-peptide “calibrators” and “controls” was determined by quantitative amino acid analysis.
- Calibrators were used to measure the response of the assay to increasing concentrations of peptide. Controls were used to check the accuracy of the assay; different suppliers provided calibrators and controls.
- Insulin and C-peptide concentrations in patient samples were determined using the LC-MS/MS assay and immunoassays. Results were compared.

What were the new findings in this study?

- The peptide content based on quantitative amino acid analysis of calibrators and controls differed from the information provided by the commercial suppliers.
- The LC-MS/MS assay is accurate for insulin and C-peptide. When the controls were run through the assay, their concentrations matched values calculated based on peptide content.
- There was agreement between the LC-MS/MS assay and the immunoassay for insulin; the WHO reference material previously used to standardize the insulin immunoassay may have been SI-traceable.
- There was negative bias between the LC-MS/MS assay and the immunoassay for C-peptide; the WHO reference material previously used to standardize the C-peptide immunoassay may have been of uncertain peptide content and therefore not SI-traceable.

What were the conclusions from the study?

- Laboratories should independently characterize peptides used to develop their assays and not rely on information provided by commercial suppliers.
- The LC-MS/MS assay accurately measures C-peptide and insulin. It is based on SI-traceable peptide content and may help with diagnosis and management of diabetes.

Letter published in *Clinical Chemistry*

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Citation

Taylor SW, Clarke NJ, McPhaul MJ.
Clin Chem. 2016;62:1152-3.

Webpage

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1. Taylor SW, Clarke NJ, Chen Z, et al. *Clin Chim Acta.* 2016;455:202-208.