Multiplexed LC-MS/MS SRM Assay for Parathyroid Hormone (PTH) and Variants: Correlation with Current Clinical Immunoassay Methods

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Overview

**Purpose:** Apply an LC-MS/MS SRM assay in addition to two commercially available immunoassays to a cohort of clinical samples and monitor intact and truncated parathyroid (PTH) isoforms.

**Methods:** A single cohort of IRB approved clinical serum samples was distributed between three laboratories. Mass spectrometry and sample preparation were as previously described.

**Results:** Comparison of the MSIA–SRM assay with the commercial ELSA assays demonstrated good correlation.

Introduction

The heterogeneity of PTH has traditionally been an impediment to the development of assays that distinguish full length PTH (PTH1-84) from N-terminally truncated PTH (PTH 7-84 and others). Because intact and truncated forms of PTH vary in their biological activity, assays that can accurately quantify the ratio of intact hormone to its fragments are of increasing significance in the diagnosis of endocrine and osteological diseases. To date, most immunoassays used to monitor PTH levels are based on traditional sandwich ELISA methods and cannot accurately discriminate intact from truncated PTH. In addition, these methods typically employ primary antibodies to the N-terminus of the hormone, thereby preventing quantification of any fragments. Previously, we developed multiplexed SRM assays for PTH that allow quantification of four fully-tryptic monitoring peptides (that span the entire PTH sequence) and two semi-tryptic variant specific peptides. Using this approach, it is possible to monitor intact PTH and also the degree of N-terminal fragmentation. In this study, the objective was to apply the LC-MS/MS SRM assay in addition to two commercially available immunoassays to a cohort of clinical samples and monitor intact and truncated PTH isoforms. In addition the correlation between the three assay measurements was determined.

Methods

A single cohort of IRB approved clinical serum samples was distributed between three laboratories. Mass Spectrometry and sample preparation were as previously described. Immunoassays (Beckman, Cobas) were run according to manufacturer’s instructions. Samples were measured after refrigeration at all sites and a best fit algorithm was determined using log-transformed data. This optimized the fit at low values. Data were plotted as un-transformed data points.

Results

Peptides exhibited linear responses ($R^2 = 0.90–0.99$) relative to recombinant human PTH concentration. The limits of detection were 8 ng/L and limits of quantification were of 16 to 32 ng/L depending on the peptide. Comparison of the MSIA–SRM assay with the commercial ELSA assays demonstrated good correlation.
FIGURE 2. The large dynamic range of proteins in blood presents a technical hurdle for the development of low-abundance analytes. The reference range for parathyroid hormone (PTH) is 1 pmol/L or 10-60 pg/mL, making it one of the lowest abundance clinically important analytes.

FIGURE 2. MSIA (Mass Spectrometric Immunoassay)-SRM workflow for enrichment and quantification of low abundance proteins.

In order to develop a sensitive assay with sequence specificity for PTH, we coupled immuno-enrichment at the protein level with detection at the peptide level using SRM-MS. This approach allows rapid and automated enrichment with the selective detection and quantification of intact and variant forms of PTH. The active site of the PTH protein is from aa1-10. N-terminally truncated variants may confound immunoassays that do not distinguish intact from truncated forms of the protein.

- MSIA achieves higher sensitivity than any other methods tested
  - Easily accommodate large sample volume range (10 μL-10 mL)
  - Forced contact of analyte with AB in the tip increases binding efficiency
  - Repeated binding cycles (up and down in pipette tip) add capacity and sensitivity
- Scalable concentrations of antibody and multiplexing antibodies on tips
- Can use commercially available, FDA validated antibodies
- More economic and much more sensitive than magnetic or other beads
Previous top down analyses of PTH in clinical samples revealed a large degree of heterogeneity and truncated variants, principally at the N-terminus (1). In order to quantify intact and truncated forms, we chose 4 fully tryptic and 3 semi-tryptic (variant specific) peptides for the multiplexed SRM assay.

![PTH Variant Map](image)

**FIGURE 4.** Ten point calibration curve for peptide SVSEIQLMHNLGK. The values ranged from 0-2000pg/mL. CV’s of triplicate points ranged from 1-19%. The $R^2$ was 0.978. Calibration curves for peptides HLNSMER, ADVNLTK and LQDVHNFVALGAPLAPR demonstrated similar linearity and precision.

![Calibration Curve](image)
FIGURE 5. Correlation between Cobas immunoassay and MSIA-SRM assay.

![Correlation graph between Cobas immunoassay and MSIA-SRM assay.](image)

FIGURE 2. Correlation between Beckman and Cobas immunoassays.

![Correlation graph between Beckman and Cobas immunoassays.](image)

TABLE 1. Correlation Matrix

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FIGURE 5. Correlation between MSIA-SRM and Beckman immunoassay.

Conclusion

- The commercial immunoassays correlated well with each other.
- The MSIA-SRM assay correlated very well with both commercial immunoassays.

References


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