

# Derivatizing Serum Testosterone in Patient Samples Increases Throughput in a Multiplexed LC-MS/MS Assay

## Background

- Liquid chromatography-tandem mass spectrometry (LC-MS/MS) can be used to accurately measure levels of testosterone in patient samples.<sup>1</sup>
- Assay throughput may be increased by a process called “sample multiplexing,” which involves combining 2 or more patient samples before running them through a single LC-MS/MS column.
- A method of sample multiplexing is tagging samples with molecules of different masses called “derivatizing” agents. The tags allow differentiation of results from each patient sample.
- **Objective:** The investigators developed and validated an LC-MS/MS-based assay in which serum testosterone is derivatized to facilitate sample multiplexing.

## Methods

- A total of 269 discarded, de-identified patient samples were analyzed.
- Patient samples were derivatized by tagging with either hydroxylamine or methoxyamine.
- Two samples with different tags were combined, extracted, and injected into the multiplexed LC-MS/MS system.
- Testosterone was quantitated based on the chromatographic peaks of fragment ions corresponding to its derivatized form.
- Deming regression analysis was used to compare the results of multiplexed samples to results from underivatized samples run individually.

## Results

- Derivatization of the samples doubled LC-MS/MS throughput to 8 samples in 6.5 minutes.
- Assay characteristics were as follows:
  - Testosterone measurements were linear to 2,000 ng/dL, and calibration curves showed consistent and reproducible results.
  - For both the hydroxylamine and methoxyamine derivatives, the clinical reportable range was 1.0 to 10,000 ng/dL and the limit of quantitation was 1.0 ng/dL.
  - In the 8 to 1,200 ng/dL range, the imprecision of the testosterone measurements was 6% to 12% for the methoxyamine derivative and 5% to 9% for the hydroxylamine derivative.
- The regression analysis showed the performance of the LC-MS/MS assay using derivatized multiplexed samples to be equivalent to that using underivatized samples run individually.

## Conclusions

- The investigators showed that using 2 different derivatizing agents can increase the throughput of the testosterone LC-MS/MS assay without compromising accuracy.

## Poster presentation at the Endocrine Society 2018 Sessions

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### Endocrine Society 2018 Sessions

Chicago, IL  
March 17-20, 2018  
Date and time: Sunday, March 18;  
1:00 PM-3:00 PM

### Webpage

<http://www.abstractsonline.com/pp8/#!/4482/presentation/8136>

### References

1. Broccardo CJ, Schauer KL, Kohurt WM, et al. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2013;934:16–21.