Background

- Liquid chromatography-tandem mass spectrometry (LC-MS/MS) can be used to accurately measure levels of testosterone in patient samples.  
- 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:  
  o Testosterone measurements were linear to 2,000 ng/dL, and calibration curves showed consistent and reproducible results.  
  o 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.  
  o 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.