Development of a Reference Method for the Quantification of Serum C-Reactive Protein

Coronary heart disease is the major cause of death in the United States. Traditionally, cholesterol testing has been used as a tool to identify individuals at risk for developing coronary heart disease. However, nearly half of the time, this approach does not identify those individuals who develop myocardial infarction when cholesterol levels are only moderately increased. Recently, the diagnosis of coronary heart disease has been improved through the measurement of the serum concentration of C-reactive protein (CRP), however the lack of suitable standards hampers the effectiveness of its clinical application. To address this need, NIST has developed a reference method for the quantitation of CRP in serum. This reference method will be used to certify reference materials for this important clinical diagnostic marker.

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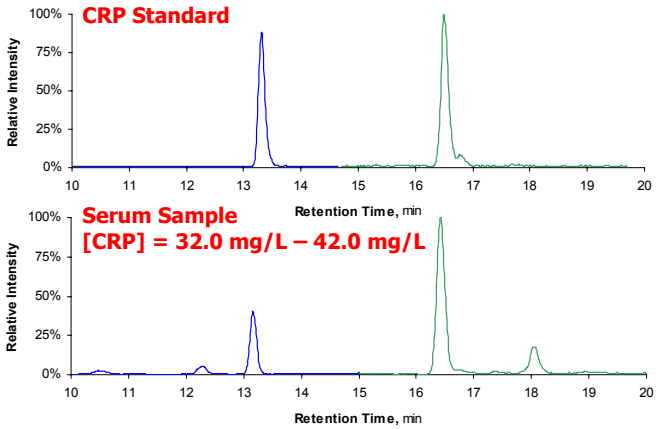
Clinical studies have shown that slightly elevated levels of C-reactive protein (CRP), measured with high sensitivity C-reactive protein (hsCRP) assays, are associated with coronary heart disease. When hsCRP is considered along with traditional total cholesterol/HDL-cholesterol ratios, the ability to assess relative risk of future coronary events is greatly improved.

In this work, quantification of human CRP in serum is achieved through the measurement of CRP tryptic peptides generated from the digestion of unfractionated serum. This approach has been called "bottom-up" proteomics. In order to minimize the bias and variability that can be introduced by enzymatic digestion, the method of standard additions is employed. Liquid chromatography with tandem mass spectrometry (LC/MS/MS) provides the sensitivity to measure CRP down to levels found in normal patient serum and the selectivity to measure the CRP tryptic peptides among the tryptic peptides from other serum proteins. The method bias was evaluated by comparing quantitative results obtained from different CRP tryptic peptides and accuracy assessed by the measurement of a variety of CRP quality control materials.



The figure shows an LC/MS/MS analysis of a tryptic digest of a CRP standard, and a serum sample.

The reference method was developed and evaluated using pooled serum CRP quality control materials. Through this method, quantitation of serum CRP was possible even at the low concentrations found in normal, healthy patient samples. Measurement accuracy and specificity was determined to be excellent. However, measurement precision would still need improvement before this reference method could be used to certify a serum reference material for clinical use.

NIST researchers have demonstrated the feasibility of a "bottom-up" proteomics approach for accurately quantifying CRP in serum - a key step toward producing a reference material for clinical use.

The success of this method to measure a clinically relevant protein in a complex matrix such as serum demonstrated that this measurement approach, specifically protein quantification at the peptide level, can be potentially applied to the measurement of other clinically-relevant proteins. Through the development of this measurement approach, NIST will be able to, with high specificity and measurement sensitivity, directly quantify proteins in complex matrices that have only been amenable to affinity methods such as immunoassay.

Future Plans: **Additional work is needed to improve the precision of the method and validate the accuracy. There are several technical difficulties that need to be solved, including the precipitation of serum proteins during sample preparation and the steady and substantial loss of the labeled internal standard peptides (and presumably the tryptic peptides from CRP) during the course of the tryptic digestion. Elimination of serum protein precipitation would improve the repeatability of the method. Elimination of the peptide loss, possibly through the use of protease inhibitors, would substantially improve the limits of detection of the method and improve overall method precision.**