

Development of Proteomics-Based Methods for Protein Quantification

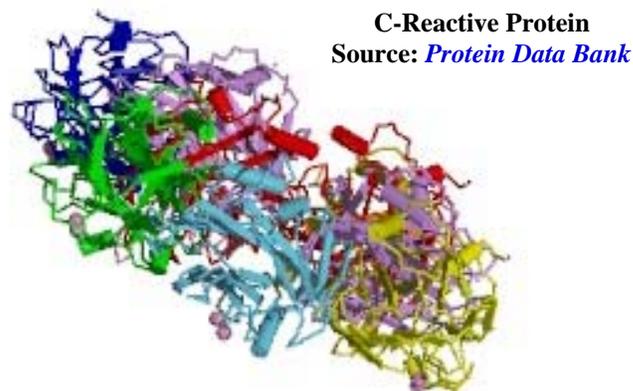
NIST is developing reference methods for the sensitive and direct measurement of proteins in blood plasma and serum. This is a first step in the development of new reference methods employing modern analytical methods such as capillary electrophoresis, liquid chromatography and mass spectrometry, to help meet the standardization needs of clinical medicine and drug discovery research. Only through this and similar types of activities, will the full potential of proteomics be realized.

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Reference measurement procedures are an important part of clinical measurement standardization. They can be used to directly assess the accuracy of routine methods or to assign or verify the concentrations of controls and calibrators used in routine methods. Reference measurement procedures also provide a means to demonstrate traceability of routine methods and materials to higher-order reference materials. Such traceability is required by the European Directive on in vitro diagnostics (IVD) for all measurements in laboratory medicine. Lists of such higher-order methods are published by the Joint Committee for Traceability in Laboratory Medicine (JCTLM).

While there are many reference measurement procedures for clinical inorganic and small organic species, few exist for clinically relevant proteins. A recent survey of clinical reference methods by the JCTLM indicated approximately 25 documented reference methods for serum proteins, most using immunological methodologies or an enzyme activity measurement. Only two reference methods, both for HbA1c, use modern analytical measurement procedures – such as capillary electrophoresis, liquid chromatography, and mass spectrometry – for the sensitive and direct measurement of the clinical analyte. In order to meet the standardization needs for clinically relevant proteins, new reference measurement procedures must be developed.

In this work, an approach typically used in “bottom-up” proteomics is used for the quantification of clinically relevant proteins in serum. Specifically, quantification is achieved through the measurement of peptides generated from the enzymatic digestion of the target protein in serum. Because the analyte (protein) and measurand (pep-



tides) are different, care must be taken to identify potential sources of bias in the enzymatic digestion process that converts analyte to measurand. Therefore, much of the focus in the development of this approach has been in the exploration of the enzymatic digestion process.

Because proteolytic digests, particularly trypsin digests, are at the core of our proteomics-based approach, we have devoted a large effort to understanding the practical nature of trypsin through fundamental studies of how experimental factors affect trypsin digests of analyte proteins. In collaboration with scientists from the national metrology institutes of the United Kingdom (LGC) and Germany (PTB), we have explored the quantitative nature of tryptic digestion. Successful quantitative measurements have been made on serum C-reactive protein using this approach.

To develop a methodology capable of quantifying proteins present at low concentrations in complex clinical matrices such as plasma or serum, work has been started to incorporate affinity-based extraction techniques, in the form of antibody-coupled magnetic beads, into the measurement approach. Additionally, using *in vitro* methods for protein production, isotopically labeled proteins are being prepared for use as internal standards in this quantitative methodology.

As the field of proteomics matures, it is very likely that more protein biomarkers will be discovered and used for clinical diagnoses. New immunoassays for protein biomarkers will require validation through more metrologically sound approaches.



Accurate and precise protein quantitation may help answer fundamental biological questions regarding protein expression and its relation to the genome and environment. Additionally, the techniques developed for protein quantitation can also be used in the areas of drug discovery and biotechnology.

Future Plans:

Because protein quantification is a critical measurement capability, NIST will continue research in the development of proteomic-based approaches to protein quantification. Further development of the use of affinity reagents and isotopically labeled proteins as internal standards is needed to validate this approach and to apply it to clinically relevant protein measurements.