B111 Data Interpretation Guidelines for Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) - Based Forensic Proteomics

Kristin Jarman, PhD, Pacific Northwest National Laboratory, Richland, WA 99352; Eric D. Merkley, PhD, Pacific Northwest National Laboratory, Richland, WA 99354; Karen L. Wahl, PhD, Pacific Northwest National Laboratory, Richland, WA 99354*

Learning Overview: After attending this presentation, attendees will: (1) gain an appreciation of the unique data interpretation challenges of forensic proteomics; and (2) take home specific guidelines for constructing objective and defensible proteomics assays.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by establishing common practices among the relevant communities and continue to increase visibility and acceptance of this emerging discipline.

Proteomics has become a standard approach to the study of cellular function. Based on LC/MS/MS measurements of fragmented proteins (called peptides) coupled with bioinformatics tools to identify those peptides, proteomics can be used to detect and quantify proteins in a sample. LC/MS/MS instrumentation provides high molecular specificity across a broad range of peptides, allowing for the identification of a virtually unlimited variety of proteins in a single sample. This makes proteomics a desirable alternative to the more traditional single-agent protein assays, such as Enzyme-Linked Immuno-Sorbent Assay (ELISA) and immunoelectrophoresis. As a result, the field of forensic proteomics is a steadily growing area of research. To date, a number of forensic proteomics applications have been explored, including microbial identification, characterization of microbiological growth media, human hair analysis, bodily fluid identification, species identification from bones and other tissues, identification of protein toxins,such as ricin and the botulinum neurotoxin, and the characterization of various historical and archaeological artifacts.

There are two common types of proteomics methods that employ LC/MS/MS: targeted and untargeted. With targeted methods, the instrumentation is tuned to detect a small number of pre-specified peptides. The data produced by targeted LC/MS/MS assays amounts to a list of peak intensities corresponding to the peptides searched for by the instrument. Research and development of targeted assays involves selecting the most protein-specific peptide markers to measure, constructing standard operating protocols, characterizing the limit of detection, and measuring the sensitivity and specificity of the overall method. In this way, targeted proteomics assays are similar to traditional MS-based chemical assays. Untargeted LC/MS/MS methods, while less sensitive that their targeted counterparts, offer the ability to detect a virtually unlimited variety of peptides in a single measurement. They achieve this by operating in data-dependent mode, where the instrumentation is set to detect as many peptides as possible, producing thousands of mass spectra in a single experiment. Because of the vast number and diversity of fragmentation spectra involved, untargeted proteomics methods rely heavily on search databases and bioinformatics tools to identify peptides in an unknown sample and associate those peptides with proteins and/or protein sources. This creates unique data analysis and interpretation challenges that must be addressed when establishing the statistical defensibility of untargeted forensic proteomics methods.

In this presentation, statistical issues associated with the emerging field of targeted and untargeted forensic proteomics will be discussed. Key considerations for meeting the *Daubert* criteria will be presented, including establishing reliability of a method and developing a framework for interpreting the strength of proteomics evidence. The importance of standardized data analysis protocols, objective data interpretation criteria, and appropriate search databases will be demonstrated. An untargeted proteomics method for the detection of protein toxins, such as ricin and abrin, will be presented and used to illustrate the potential of this emerging discipline.

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*Presenting Author