Automated High-throughput Clinical Proteomics Workflow using Hydrogel Nanotrap Particle Technology and Mass Spectrometry Analysis

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Hundreds of clinical samples are generally required for biomarker verification and validation studies. These many samples are necessary to characterize the concentration distribution of the candidate biomarker in a population and to establish the reference intervals required to define clinical utility. Consistent protein identifications are the cornerstone for these types of studies in a clinical proteomic setting and even slight variation in laboratory technique while carrying out the preparation protocol can often lead to unwarranted variation in protein identification. To help overcome these potential preparation vulnerabilities, we have developed a high-throughput automated system capable of preparing clinical samples for mass spectrometry analysis.

To further leverage the automation throughput for the analysis of protein biomarkers, hydrogel nanotrap particles, Figure 1, were incorporated into a workflow. These particles rapidly, in a single step, isolate low-molecular weight biomarkers from clinical samples. The utility of these particles becomes more evident since it is largely being found that the low-molecular weight low-abundance proteins present in biofluids is a rich fraction of the proteome to mine for new candidate biomarkers. When used to process biofluids the nanoparticle molecular sieving action along with their high affinity baits capture the relevant low-molecular weight low-abundance protein biomarkers while excluding the undesired higher molecular weight high-abundance proteins.

In this project, the functionality of this high-throughput automated preparation workflow is demonstrated using a novel nanoparticle-coupled analytical mass spectrometry scheme, Figure 2. Serum samples were spiked with a panel of cytokines, as well as other small (~10-30 kDa) cell signaling proteins, and processed using the automated system. Levels of the protein spikes, which were used to generate response curves, ranged from the low pg/mL to mid ng/mL levels and are within the physiological relevant levels of these proteins. SRM mass spectrometry analysis of the spiked proteins, along with the use of corresponding isotopically labeled peptides, show low total process CV's at the various response levels, and show the physiological relevant concentration of the proteins to be within the linear region of the response curves. This study demonstrates, for the first time, a simple high-throughput automated clinical proteomics workflow that does not require antibody-based target enrichment or complex sample processing, that is also capable of producing clinical grade process CVs.

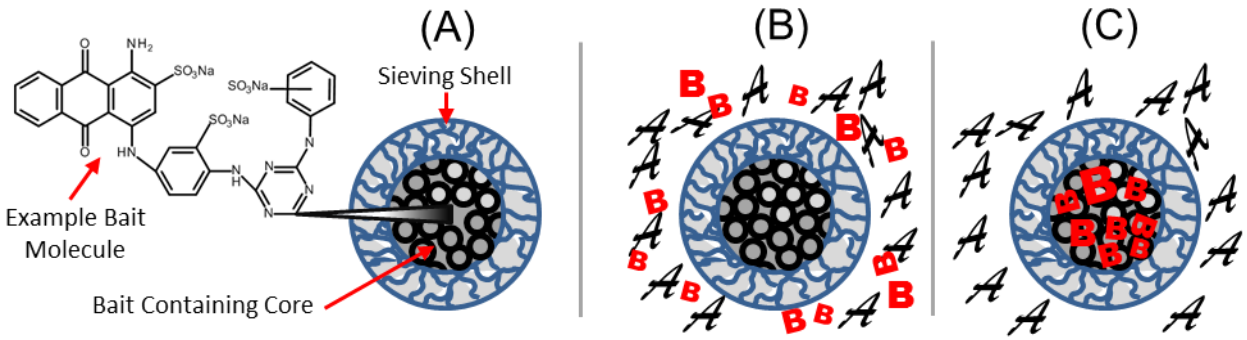

Figure 1. (A) Structure of the core shell particle. (B) Core shell particles in sample solution with 𝒜: albumin and **B**: biomarker molecules. (C) Biomarkers harvested by core shell particles.

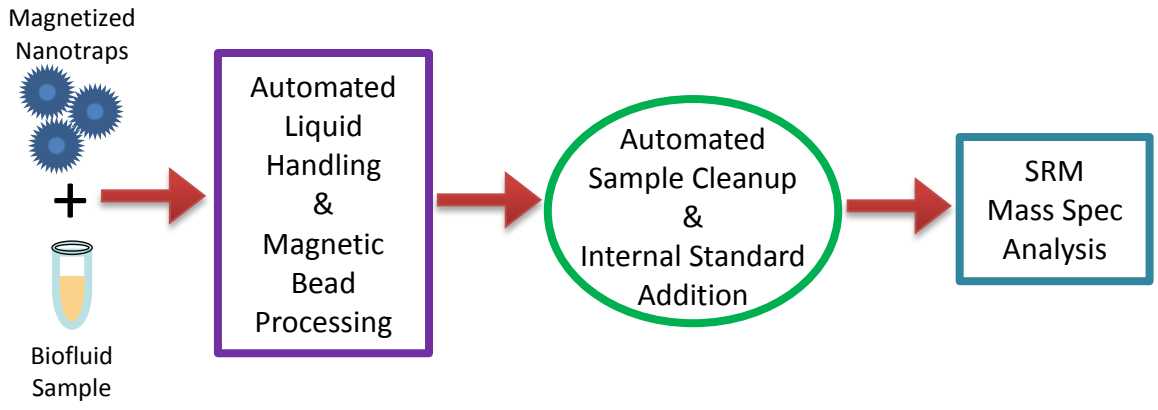

Figure 2. This scheme represents the high-throughput automated preparation workflow used to isolate biomarkers from clinical samples for downstream mass spectrometry analysis.