MODULAR AUTOMATED PROCESSING SYSTEM (MAPS) FOR HIGH-THROUGHPUT AUTOMATED SERUM PROCESSING

Sandia National Laboratories

Dan Throckmorton, Jim Brennan, Steve Branda, Joe Schoeniger, Gabriela Chir

Sandia National Laboratories, Livermore, CA, 7011 East Avenue, Livermore, CA, 94550

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Introduction

Microfluidic separations and mass spectrometry enable rapid and sensitive detection of potential biomarkers. Success in this approach depends upon meeting strict sample requirements: a limited number of components per sample; minimal amounts of salts, surfactants, and other contaminants; and significant enrichment of low abundance species. Current sample preparation practices involve complex, typically manual, multistep operations; these methods are too slow and too costly to adequately support comprehensive, high throughput analysis of biological samples in clinical settings.

Sandia introduced a **modular automated sample processing system (MAPS)** that enables reliable, high-throughput analysis. MAPS consists of a set of microfluidic modules connected with custom-designed fittings, manifolds, and miniature flow-switching valves assembled on a breadboarded platform. The modules are optimized to carry out individual sample processing functions: protein concentration, interferent removal, buffer exchange, enzymatic digestion, viral, bacterial concentration and cell lysis. The modular architecture enable application- and sample-customized processing, while microfluidic processing renders improved sensitivity and small footprint for portable diagnostics and/or mass-spectrometric detection.

New MAPS modules are currently developed to enable standardized, high-throughput processing of microliter-volumes of mouse serum.

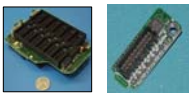
The analysis of blood is problematic, particularly when processed in microliters, due to its viscosity, high salt (~150mM) and protein (~75mg/ml plasma) content. We are presenting here results obtained using 3 new modules : a) desalting module, b) immunodepletion module and c) size fractionation module. The ability to precisely control fluid flow and balance the strengths and weaknesses of each processing methods can deliver the desired compromise between sensitive, cost-effective and comprehensive analysis.

Hardware infrastructure for processing ml → µl → nl volume samples

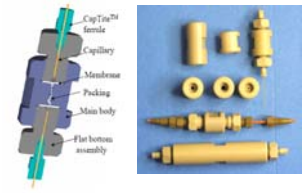
Custom-built hardware including CaptiteTM fittings, manifolds, and flow-switching valves provide nanoliter dead volume connections for precise handling of microliter volumes.

Electronically actuated valves

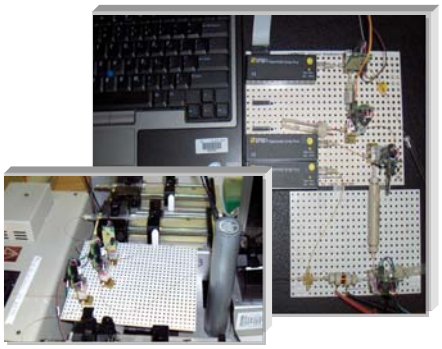
Breadboard and CapTite™ microfluidic fittings



Miniature HV power supply, drivers and controllers

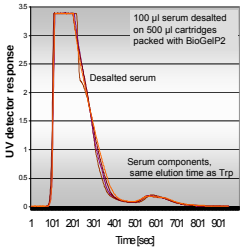


Sandia designed µl-ml cartridges packed with any sorbent use highly permeable biocompatible membranes to afford processing of real samples and minimize clogging.



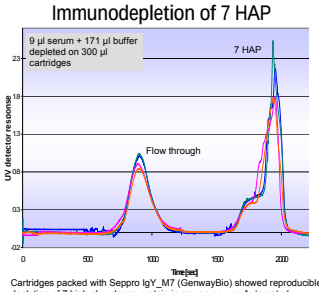
MAPS µl-volume serum processing modules

Desalting/buffer exchange



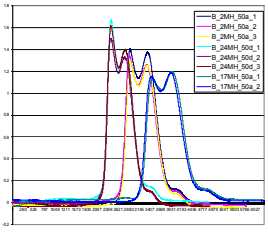
Rapid on-line desalting enables electrophoretic, microfluidic and mass spectrometric analysis, while buffer exchange resolves compatibility issues between various enrichment/separation dimensions.

Immunodepletion of 7 HAP

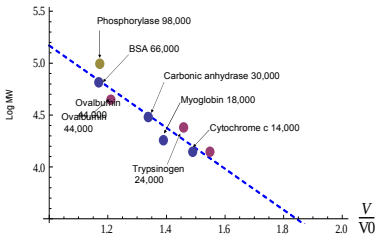


Cartridges packed with Seppro IgY_M7 (GenwayBio) showed reproducible depletion of 7 high abundance protein in mouse serum. Automated operation and the use of highly permeable membranes significantly improved the lifetime of the packing and reduce the run-to-run sample losses recorded with the compared to commercial spin-columns.

Size fractionation of native serum proteins



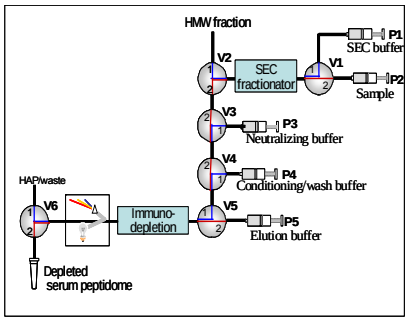
50 ul serum samples spiked with myoglobin were injected on cartridges packed with ToyoPearl HW-50. The reproducible separations enable automated fractionation of high-molecular weight proteins (HMW) and the peptidome (<2% HMW).



Calibration curve for the SEC module was obtained on two columns:blue points on column A, red points on column B and yellow point average on column B and A.

Combining size exclusion and immunodepletion

improves throughput, sample capacity and significantly reduces the cost of peptidome analysis

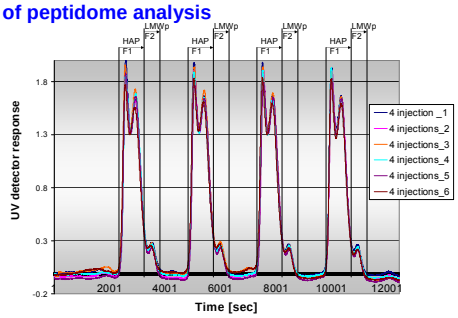


Current immunodepletion methods have two major disadvantages: they require 15-20 x dilution of the serum sample and the cost of the depletion material is very high. Although size exclusion is a dispersive method, packing materials are less expensive and HAPs are also high molecular weight proteins.

By coupling the size fractionation (SEC) and immunodepletion (ID) modules we significantly improve the throughput and sample capacity, while reducing the cost (~20x) and the final dilution factor of the depleted sample. Our size fractionation method renders 80% recovery of ~ 20kDa proteins with 98% depletion of the higher molecular weight proteins.

In a typical immunodepletion protocol 1 ml of packing depletes 15 µl serum. SEC-ID can process 100 µl serum each run while using only 130 µl of immunodepletion packing.

SEC-ID enables analysis of small proteins and peptides unassociated in serum as well as those carried by higher abundance proteins.



Overlay of 6 runs each including 4 multi-threaded injections of 50 ul serum spiked with myoglobin. Switching valve 6 at indicated collection times allows very reproducible collection of both the peptidome and higher abundance proteins fractions for further processing. Multithreaded injections reduce the SEC run time with 30%.

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