Quantitative evaluation of serum high-abundance protein depletion

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Introduction

Serum and plasma are rich sources of biomarkers. Detection of biomarker proteins can aid early on diagnosis and therapy monitoring by a minimally invasive procedure for the patient. The serum proteome is spread over a wide dynamic range of 10 orders of magnitude from proteins of high abundance like albumin and immunoglobulins to those present at minor concentrations like cytokines (Fig. 1). High abundance proteins can mask diagnostically relevant proteins and hamper their identification and measurement. One possibility to overcome this masking effect is by the selective removal or the depletion of high abundance proteins by bio-affinity based techniques. We have evaluated the performance of commercially available kits in their efficacy and specificity.

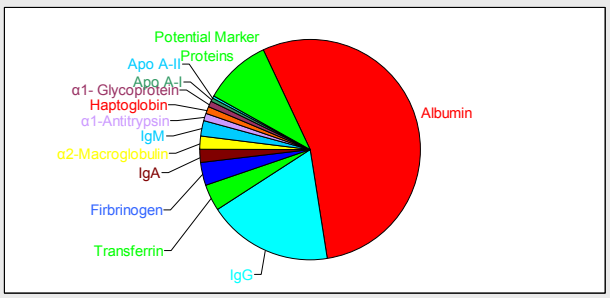


Figure 1: Abundances of serum proteins. The albumin and IgG fraction comprises approximately 75% of the serum proteome. Potential disease markers are of low abundance and their discovery by classical proteomic techniques requires relative enrichment.

Figure 2: Multiple Affinity Removal Spin Column for the removal of albumin, IgG, transferrin, haptoglobin, IgA and antitrypsin (courtesy image of Agilent Technologies).

Materials and Methods

Systems combining high specificity with high affinity were chose from Vivapure (Anti HSA/IgG Kit), Calbiochem (ProteoExtract Albumin/IgG Removal Kit), Amersham (Albumin and IgG Removal Kit), Qiagen (Qproteome Albumin/IgG Depletion Kit), GenWay (IgY Microbeads for HSA and IgG) and Agilent (Multiple Affinity Removal Spin Column, MARSC). Binding of albumin and IgG to the matrix of the spin and flow through columns was mediated by antibodies and bacterial protein A and G. The MARSC additionally removed transferrin, haptoglobin, IgA and antitrypsin (Fig. 2). For the removal of larger particles serum was passed through 0.45 µm cellulose acetate filters. Fractions yielded from the depletion process were stored at -20°C and resolved on 2-D gels. Whole protein concentrations were determined with the DC protein kit from Bio-Rad. Concentrations of albumin and IgG were measured with ELISAssays. To estimate unspecific binding of potential marker proteins to the matrices a panel of recombinant inflammatory cytokines was spiked into serum. Recovery rates of the cytokines were determined with a cytometric bead assay on a FACS device (Beckman Coulter).

Results

The most effective system in the depletion of albumin and IgG is the avian antibody based kit from GenWay with 91.3-95.6% (albumin) and 93.3-96.9% (IgG) removal and the Qiagen kit with a removal of 88.0-95.3% (albumin) and 92.0-96,4% (IgG). 2-D electrophoresis demonstrates the successful removal of 6 high abundance proteins from serum with the MARSC from Agilent Technolgies (Fig. 3). Previously unresolved spots appear on the 2-D gel (Fig. 4). The recovery of spiked-in cytokines after the depletion was quantified with a FACS-based CBA assay indicating high specificity of the kit from GenWay Biotech (Fig. 5). Only low levels of cytokines were detected in the albumin and IgG fractions signalling low reversible binding to the matrices. Antibody based systems have higher recovery rates than the less specific systems of Vivapure and ProteoExtract relying on bacterial protein A and G.

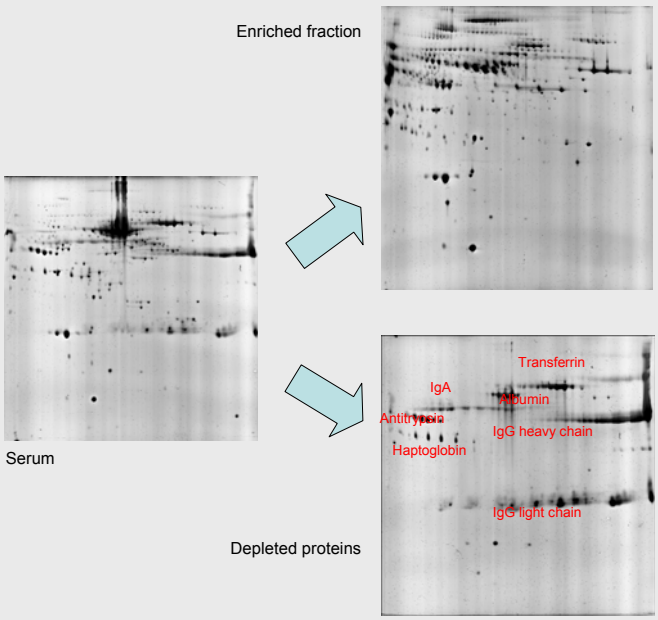
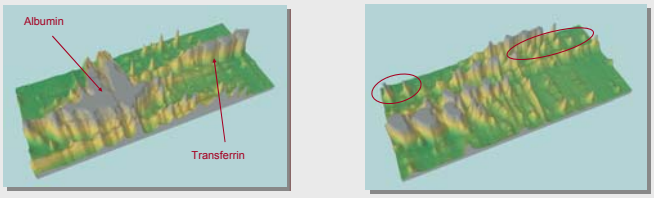


Figure 3: 2-D images of 300 µg of protein from serum and the two fractions yielded by the depletion of 6 high-abundance proteins trough the Multiple Affinity Removal Spin Column of Agilent Technologies. The enriched fraction offers a radically different perspective towards serum proteins compared to the initial serum sample.



Pre-Depletion **Post-Depletion**

Figure 4: Enlarged albumin peak of a 2-D gel. Before the depletion step the albumin spot covers a large area and thereby masks other proteins of lower abundance. After the depletion of albumin and transferrin new spots appear (encircled).

	IL-12	TNF-α	IL-10	IL-6	IL-1β	IL-8
Vivapure	**Recovery**	**Recovery**	**Recovery**	**Recovery**	**Recovery**	**Recovery**
Markerfraction	**48,7**	**23,0**	**32,4**	**62,5**	**100,0**	**48,9**
Amersham						
Markerfraction	**63,7**	**46,9**	**16,6**	**35,7**	**100,0**	**77,1**
ProteoExtract						
Markerfraction	**18,3**	**57,1**	**24,4**	**71,2**	**100,0**	**0,0**
Agilent						
Markerfraction	**47,7**	**37,5**	**37,7**	**63,2**	**66,9**	**65,9**
Qiagen						
Markerfraction	**59,1**	**70,2**	**62,6**	**61,7**	**100,0**	**48,6**
GenWay						
Markerfraction	**56,9**	**100,0**	**55,0**	**72,3**	**100,0**	**65,0**

Figure 5: Recovery rates [%] of spiked-in Cytokines measured with a cytometic bead array (BD Biosciences). IL-1β had the highest recovery in the marker fractions. The IgY based product of GenWay showed the least unspecific binding of cytokines to the matrix during depletion.

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