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Comparison of serum fractionation methods by data independent label-free proteomics

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ABSTRACT

Off-line sample prefractionations applied prior to biomarker discovery proteomics are options to enable more protein identifications and detect low-abundance proteins. This work compared five commercial methods efficiency to raw serum analysis using label-free proteomics. The variability of the protein quantities determined for each process was similar to the unprefractionated serum. A 49% increase in protein identifications and 12.2% of reliable quantification were obtained. A 61 times lower limit of protein quantitation was reached compared to protein concentrations observed in raw serum. The concentrations of detected proteins were confronted to estimated reference values.

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1. Introduction

The research of proteomic clinical biomarker was often undertaken on serum or plasma to highlight biomarkers available at the systemic level. Indeed clinical trials often collect serum samples which are sometimes available for ancilary biomarkers discovery studies. The low-concentration protein range of serum contains proteins as secretion or tissue leakage products. These are expected to be more specific and sensitive potential disease biomarkers than more abundant proteins [1–3]. The main issue inherent to the complexity of serum analysis is the limitations of the technologies used for the discovery analysis: limits of detection (LOD) and quantification (LOQ) and the linear range of response. Indeed, serum and plasma are very complex biological matrices in which the protein concentration covers a large dynamic range higher than 10 orders of magnitude [4]. Currently no high end mass spectrometer can cover such a large range [5]. Prefractionation strategies offer an alternative by modifying the original sample protein distribution inducing shifting/shrinking effects and allowing proteins initially present at low concentrations to be accessible for analysis. Several commercial solutions for sample prefractionation are proposed to remove abundant proteins which saturate signal during proteomic analysis and alsofor reaching the low-abundance proteins [2,6–8]. Of course, off-line or on-line fractionation steps enhance the number of proteins identified by increasing the separation power. But this is at the cost of longer acquisition times, a modified limit of quantification and a higher global variability; all due to the additional preprocessing steps applied [9]. Such multistep strategy is certainly better adapted for cell lines or animal models complete proteome characterization without downstream differential analysis [10]. However for differential analysis and therefore for related clinical proteomics, a higher number of consistent protein identifications and more accurate quantification increase the probability to highlight a significant potential biomarker while keeping reasonable data aquisition time and run length. Therefore, sample preprocessing steps appear mandatory, but must involve at least a good repeatability, reasonable cost and manageable processing time for allowing a medium to high number of clinical sample preparations.

Among the strategies available are the immuno-affinity depletion of the abundant proteins as simple IgG and/or albumin or the depletion of up to the twenty most abundant plasma proteins using IgY chicken antibodies. These IgY decrease the risk for aspecific protein co-depletions potentially occurring with mammalian

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antibodies based depletion [11–15]. Another option is based on the principle of the equalization technique, utilizing a random synthetic hexapeptides library cross-linked to micro-beads [16–21] which actually appears to work according to a general hydrophobic binding mechanism [4]. The large volume of sample which is required and the fact that the "equalization mechanism" shrinks the sample protein concentration dynamic range, can be seen as a problem for data interpretation after differential proteomic discovery analysis and further validation of the results. The enrichment of glycoproteins on lectines may also be used as an alternative approach. Many studies were performed on various systems dedicated to differential analysis [12,22–24]. However, beside works done for protocols testing [14,20,23,25,26], only one study comparing plasma preprocessing reproducibility using two commercial kits under spin column format and applying a downstream label-free proteomic analysis was published [27]. Therefore, in this context, the aim of our work was to compare five different commercial methods proposed under disposable spin column format, involving reasonable processing time and costs and tested using the same serum pool originated from healthy and diseased individuals. The qualitative and quantitative results obtained for the five conditions were compared together as well as with those obtained with the raw serum analysis. The results are discussed in the context of the application of the prefractionation methods on serum before a clinical biomarker discovery study by label-free proteomics. It can be viewed as a tool to select the most convenient method to apply on clinical sera before proteomic analysis.

2. Materials and methods

2.1. Composition of the sample serum pool

All the serum samples of patients were collected with signed informed consent and with the ethic approval of our university hospital. Three healthy subjects, five patients with colorectal cancer, five Crohn's disease and three ulcerative colitis patient samples were selected to prepare a pool of 5 mL. The serum pool aliquots of each patient were stored for several years at −80 °C (maximum 5 years) and thawed on ice prior to mixing. This raw serum pool (RS* pool) was tested for total protein quantitation using the RC DC kit (BioRad, Inc., Hercule, CA, USA). Aliquots of suitable volumes were prepared for each protein depletion kit applications and stored at −80 °C until further use.

2.2. Prefractionation of the RS* pool

The methods of depletion tested were: ProteoPrep® 20 Plasma kit (Sigma St. Louis, USA), under the spin column format, the ProteoMiner™ (BioRad, Inc., Hercule, CA, USA) designed for 200 μL of sample volume, IgG & Albumin Spin Trap column (GE Healthcare, USA) and WGA Glycoprotein isolation kit (Thermo-Fisher Scientific Inc., USA) with or without prior IgG & Albumin Depletion Spin Trap (GE Healthcare). Fig. 1 summarizes the workflow with the main sample processing steps applied to the RS* pool before performing the proteomic analysis.

2.2.1. ProteoMiner™ kit (BioRad Inc., Hercule, CA, USA)

The small capacity kit (designed for 200 μL of sample) was used for the three technical replicates which were run in parallel. In brief, a 700 μL volume aliquot of the RS* pool was thawed on ice. After centrifugation, 3 × 200 μL of RS* pool were treated in parallel, as recommended by the manufacturer, and resulted in three successive elution steps per 200 μL of RS* treated. All three eluted fractions were pooled together per replicate and stored at −80 °C.

2.2.2. ProteoPrep® 20 Plasma kit (Sigma, St. Louis, USA)

This kit was applied as recommended by the manufacturer. We performed three replicates of process consecutively on the same column (and on the same day). Briefly, for each process replicate

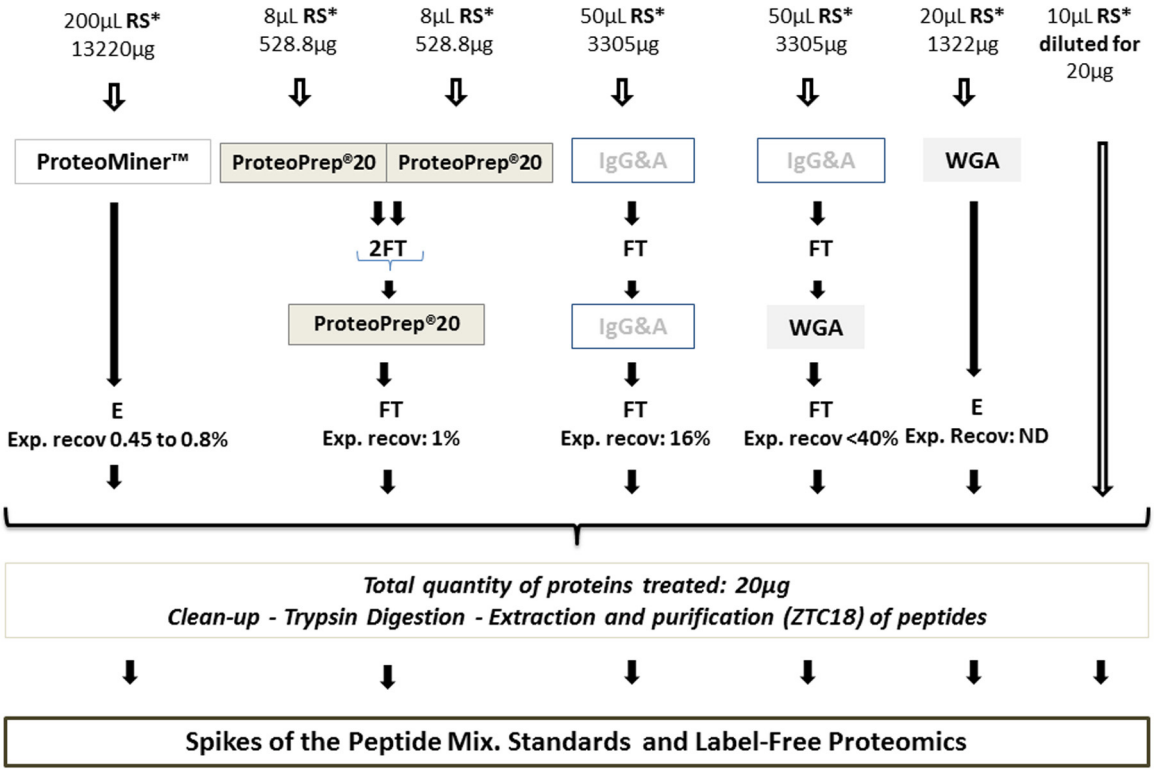

Fig. 1. Experimental workflow detailing the commercial prefractionation and other processing steps applied to the RS* pool.

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