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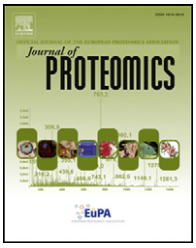

Profiling post-centrifugation delay of serum and plasma with antibody bead arrays☆,☆☆

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ABSTRACT

Several biobanking initiatives have emerged to create extensive collections of specimen for biomedical studies and various analytical platforms. An affinity proteomic analysis with antibody suspension bead arrays was conducted to investigate the influence of the pre-analytical time and temperature conditions on blood derived samples. Serum and EDTA plasma prepared from 16 individuals was centrifuged and aliquots were kept either at 4 °C or in ambient temperature for 1 h and up to 36 h prior to first storage. Multiplexed protein profiles of post-centrifugation delay were generated in 384 biotinylated samples using 373 antibodies that targeted 343 unique proteins. Very few profiles were observed as significantly altered by the studied temperature and time intervals. Single binder and sandwich assays revealed decreasing levels of caldesmon 1 (CALD1) related to EDTA standard tubes and prolonged post-centrifugation delay of 36 h. Indications from changes in CALD1 levels require further confirmation in independent material, but the current data suggests that samples should preferentially be frozen during the day of collection when to be profiled with antibody arrays selected for this study.

Biological significance

Affinity-based profiling of serum and plasma by microarray assays can provide unique opportunities for the discovery of biomarkers. It is though often not known how differences in sample handling after collection influence the downstream analysis. By profiling three types of blood preparations for alterations in protein profiles with respect to time and temperature post centrifugation, we addressed an important component in the analysis and of such specimen. We believe that this analysis adds valuable information to be considered when biobanking blood derived samples.

This article is part of a Special Issue entitled: Standardization and Quality Control in Proteomics.

Abbreviations: FDR, false discovery rate; MFI, median fluorescence intensity; HPA, Human Protein Atlas; nMFI, normalized median fluorescence intensity; LMM, linear mixed effects model; CV, coefficient of variance.

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1. Introduction

Over the recent years, biobanks have become more widely recognized as an essential and valuable resource in medical research. Prospective as well as retroprospective and high quality samples are being collected within specific research projects, routine health care or population cohorts. They provide powerful tools for exploring the etiology of common complex diseases, better understanding of the interplay between genetic and lifestyle determinants and the development of new diagnostic biomarkers and analytical methods. Several large-scale population cohorts, such as UK Biobank [1], Lifelines in the Netherlands and HUNT in Norway, are pushing these frontiers further. As for all new prospective biobanks, these studies have standard operations in place as well as IT systems enabling a complete tracking of time and temperature from needle to freezer. However, when utilizing old sample collections, such as legacy samples, stored for decades most of these samples have varying standards and lack documentation on how samples have been collected, processed and stored [2,3]. Success in identifying novel biomarkers may well depend on variability in sampling handling conditions between or within biobanks rather than disease states. Uncertainty still remains to what extent and how vulnerable different methodological approaches, such as the emerging affinity proteomic technologies, are in this aspect for their ability to discover and verify biomarkers.

For the widely used analysis based on mass spectrometric determination of a target of interest, proteolytic activities or other molecular modifications are important factors that alter the experimental outcome, hence standardized collection and handling procedures for proteomics [4] or metabolomics [5] have become important factors in study designs. In an emerging alternative to mass spectrometric analysis of proteins, namely affinity proteomics, the use of reagents such as antibodies is about to be established for a proteome-wide scale [6–8]. Alongside these efforts, technological advances have been taking place, and regarding the analysis of serum and plasma, the use of microarray technologies and the like are often at the forefront [9]. Among the different types of protein microarrays, the use of beads to create arrays in suspension in combination with direct sample labeling offers an attractive platform to now screen larger sample collections with up to 384 [10] or even 1728 antibodies per sample [11]. These high throughput methodologies are well suited for unbiased discovery proteomics, as they allow the analysis of many samples at a time.

The purpose of this study was to investigate the extent to which the pre-analytical phase between centrifuge and freezer affects antibody-based plasma profiling. We analyzed protein profiles generated by highly multiplexed single-binder assays, and focused on serum and two types of plasma collection tubes. A set of 16 healthy volunteers donated blood at a single occasion and upon collection the obtained specimens were handled differently from the time of centrifugation until being frozen for storage. A linear mixed model was used to assess each protein profile with regard to time and/or temperature and alterations in variance across time per temperature were evaluated.

2. Methods and material

2.1. Sample collection

Blood samples were collected from 16 donors, 8 males and 8 females in the age range 24–60 years. The study participants were a group of volunteers, matched for age and gender and were not diagnosed with a disease such as cancer or cardiovascular disorder. The ethical review board in Stockholm, Sweden, with giving consent by each participant, approved this study. From each sample donor, a total volume of about 50-ml blood was drawn into two 9-ml EDTA-Plasma Preparation Tube (K_2EDTA-PPT blood collection tubes: BD cat. no. 362799), two 9-ml EDTA standard (K_2EDTA blood collection tubes: BD cat. no. 367525) and two 9-ml serum venepuncture collection tubes (Serum blood collection tubes: SST-tubes BD cat. no. 367953). The two sample types plasma (PL1) and serum (SER) were thus prepared in three types of primary containers (PED ± gel and SST), here described according to SPREC version 2.0 [12]. All samples were gently mixed for one min and the plasma samples were centrifuged immediately at 2000 *g* for 10 min while serum was centrifuged within 40 min (SPREC; pre-centrifugation delay A and centrifugation A). The sample collection tubes were placed either in room temperature or at 4 °C. Plasma and serum were then aliquoted in 100-μl fractions into 2D bar coded tubes (REMP tubes in plate, SRBR-96-300, Brooks Life Science System) in the time intervals 1, 3, 8 and 36 h after sample collection (SPREC; post-centrifugation delay A-J) and stored at −80 °C until analysis (SPREC; long-term storage Z) (Fig. 1). Twelve individuals were included in the first phase of the study, while 16 individuals were included for verification.

2.2. Antibodies

To build a 384-plex bead array, 364 affinity-purified rabbit antibodies were selected from the Human Protein Atlas (HPA) [6] and provided by Atlas Antibodies AB. Additionally, rabbit IgG (Jackson ImmunoResearch Laboratories), albumin-binding protein (Affibody AB), anti-albumin antibody (rabbit IgG, Dako), anti-KLK3 antibodies 1H12, 5A6, 8A6 (all mouse IgG, HyTest Ltd.), anti-CNDP1 antibody AF2489 (goat IgG, R&D Systems), and anti-CNDP1 3A6, 5B2, 8A3 (all mouse IgG, Atlas Antibodies AB) were utilized. As summarized in Supplementary Table 1, 373 out of 380 antibodies were unique and a total of 343 unique proteins were targeted. The antibodies were selected based on their protein targets classified as serum/plasma proteins, such as a signal peptide predicting protein secretion, as well as in house indications from other plasma biomarker projects, such as Western blot analysis.

2.3. Bead coupling

Antibodies were coupled to carboxylated beads (Luminex Corp.) according to the manufacturer's protocol and as described previously [10]. The coupling efficiency for each antibody was determined via R-phycoerythrin-labeled anti-rabbit IgG antibody (Jackson ImmunoResearch Laboratories,) and a 383-plex bead mixture, denoted antibody suspension bead array was created as described previously [13]. A second coupling of 20 out