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Technical note

Protein expression profiling by antibody array analysis with use of dried blood spot samples on filter paper

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ABSTRACT

Dried blood spot samples (DBSS) on filter paper offer several advantages compared to conventional serum/plasma samples: they do not require any phlebotomy or separation of blood by centrifugation; they are less invasive; they allow sample stability and shipment at room temperature; and they pose a negligible risk of infection with blood-borne viruses, such as HIV, HBV and HCV, to those who handle them. Therefore dried blood spot samples (DBSS) on filter paper can be a quick, convenient and inexpensive means of obtaining blood samples for biomarker discovery, disease screening, diagnosis and treatment monitoring in non-hospitalized, public health settings. In this study, we investigated for the first time the potential application of dried blood spot samples (DBSS) in protein expression profiling using antibody array technology. First, optimal conditions for array assay performance using dried blood spot samples (DBSS) was established, including sample elution buffer, elution time, elution temperature and assay blocking buffer. Second, we analyzed dried blood spot samples (DBSS) using three distinct antibody array platforms, including sandwich-based antibody arrays, quantitative antibody arrays and biotin-label-based antibody arrays. In comparison with paired serum samples, detection of circulating proteins in dried blood spot samples (DBSS) correlated well for both low- and high-abundance proteins on all three antibody array platforms. In conclusion, our study strongly indicates the novel application of multiplex antibody array platforms to analyze dried blood spot samples (DBSS) on filter paper represents a viable, cost-effective method for protein profiling, biomarker discovery and disease screening in a large, population-based survey.

1. Introduction

Dried blood spot samples (DBSS) were first introduced by Drs. Guthrie and Susi in the 1960s for screening of phenylketonuria (PKU) in newborn infants (Guthrie and Susi, 1963). DBSS on filter paper offer several advantages compared to conventional methods of serum/plasma sample collection. First, since they do not require any phlebotomy or separation of blood by centrifugation, no special training or cumbersome laboratory equipment is needed to obtain or process the samples. Second,

Abbreviations: DBSS, dried blood spot samples; BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor; MCP-1, monocyte chemoattractant protein-1; MDC, macrophage-derived chemokine; PDGF-BB, platelet-derived growth factor B homodimer; RANTES, regulated upon activation, normal T cell expressed and secreted; IGFBP-1, insulin-like growth factor binding protein 1; IL-1 alpha, interleukin-1 alpha; MIP-1 delta, macrophage inflammatory protein-1 delta; Nap-2, neutrophil activating peptide 2; PKU, phenylketonuria; MFI, mean fluorescent intensity.

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they require only a few drops of blood and, therefore, are much less invasive. Third, DBSS can be stored and shipped at room temperature with minimal risk of sample degradation, so no refrigeration or freezer packs are necessary. Fourth, DBSS pose an extremely low risk of infection with blood-borne viruses, such as HIV, HBV and HCV. Therefore dried blood spots on filter paper is a quick and convenient way for collecting large numbers of blood samples for disease screening, diagnosis and monitoring of treatment efficacy in a public health setting (Therrell et al. 1996; McDade et al. 2007). For this reason, DBSS have become an important screening resource for clinical and epidemiological testing. It has been reported that over 100 analytes has been tested using DBSS, including DNA and RNA molecules (Caggana et al. 1998; Van der Auwera et al. 2010; Hollegaard et al. 2013), biochemical biomarkers (Mei et al. 1998; Cizdziel, 2007), bacterial and viral pathogens (Gwinn et al. 1991; Parker et al. 1999a), and auto-antibodies (Parker and Cubitt, 1999b). However, the majority of these studies employing DBSS focused on single analytes and biomarkers (Mei et al. 2001; McDade et al. 2007).

Protein microarray technology has drawn great attention for biomarker discovery due to its high-throughput, flexibility and cost effectiveness. Protein arrays have been widely used for detecting protein expression profiles, screening for protein–protein interactions, analyzing post-translational protein modifications, and characterization auto-antibody expression profiles. This technology has demonstrated great promise in advancing biomedical research and protein biomarker discovery and validation, as well as drug discovery and development programs (Huang 2003; Borrebaeck and Wingren, 2007, 2009, Jiang and Huang, 2012).

Recent studies have reported analyses of protein expression in DBSS using methods of multiplexed detection of proteins, including bead-based protein arrays (Skogstrand et al. 2005, 2008; Kofoed et al. 2006; Chase et al. 2012) and reverse-phase peptide arrays (Faucher et al. 2004). These studies showed the promising application of multiplexed protein analysis to archived DBSS collections. However, there have been no reports of the application of planar-based protein array technology to these DBSS samples. In the present study, we investigated the potential application of multiplexed detection of proteins eluted from dried blood spots on filter papers using three distinct planar antibody array formats. First we determined the optimal assay conditions to elute the proteins from the dried sample for detection with these three different antibody array formats. Second, by comparison of paired blood samples, collected as serum and DBSS from the same individuals, we determined if any of these antibody array technologies were suitable for analysis of serum proteins in eluted DBSS samples.

2. Material and methods

2.1. Antibodies and reagents

All antibodies and recombinant proteins were either purchased from commercial sources or were available from in-house production. The commercial vendors included PeproTech (Rocky Hill, NJ, USA), BD Pharmingen (San Diego, CA, USA) and R&D Systems (Minneapolis, MN, USA).

2.2. DBSS and serum sample preparation

Serum samples from the healthy subjects were venipunctured, collected, centrifuged, and stored at −80 °C until use. Paired DBSS were prepared by pricking the middle fingers of the same subjects and blotting a small amount of blood onto Whatman 903 filter paper. After the first drops of blood from the pricked finger were discarded, three drops of fresh blood were collected by spotting on a clean filter paper (each filter representing one healthy subject). After blood collection, the filter papers were dried at room temperature on a benchtop for 24 h and then stored at −80 °C until used. Proteins bound to each DBSS filter were sampled as 10-mm paper punch and eluted in 1.5-ml Eppendorf tubes containing 150 μl PBS with agitation on a Vertex Genie 2 for 4 h at room temperature. Elution volumes were selected to represent an effective 1:10 dilution of serum (i.e., DBSS filter paper punches were determined to absorb approximately 15 μl of fresh serum each). Following elution, the filter papers were removed, and the eluted samples were stored at 4 °C until use. Pooled DBSS samples from two healthy subjects were used for the array assays in the study.

2.3. Sandwich-based antibody array technology

Commercially available, off-the-shelf semi-quantitative (RayBio® G-Series) and quantitative (Quantibody®) sandwich-based antibody arrays manufactured by RayBiotech (Norcross, GA) were used to detect a panel of 60 (catalog # AAH-CYT-G6) and 20 serum markers (catalog # QAH-CYT-1) respectively (see also, Huang, 2003). A pair of antibodies is required to detect each analyte. Capture antibodies were first printed onto the glass-slide matrices using a pre-determined grid map in which each antibody was printed in duplicate for semi-quantitative arrays and quadruplicate for quantitative arrays. To allow for normalization of fluorescent signal intensities between arrays, positive controls, consisting of biotin-labeled bovine IgG, were printed in each array. To assess non-specific binding to antibody spots, negative controls, consisting of BSA in PBS were also printed in each array. To allow for incubation of multiple samples, 8 or 16 identical antibody array maps were printed on each glass slide, which was subsequently mounted in a 16-well chamber assembly. Serum and eluted DBBS samples were then incubated with pre-printed antibody chips. To allow for quantitative analysis, a calibration standard mix, consisting of known amounts of recombinant proteins representing all 20 markers of the quantitative panel, was diluted serially with blocking buffer. A total of seven standard 3× dilutions and a zero-concentration "blank" were incubated with the pre-printed antibody chips, alongside the samples (see Supplementary Table S2.1 for the list of 20 cytokines and their diluted standard concentrations). After a blocking step, a total of seven standard dilutions and a zero-concentration "blank" were incubated with the pre-printed antibody chips, alongside the samples. Following by extensive washes with blocking buffer to remove non-specific binding of sample, cocktails of mixed biotinylated detection antibodies were added to the arrays. After washing away unbound biotinylated antibodies, the array slides were incubated with HiLyte Fluor™ 555 (Anaspec, Fremont, CA) conjugated with streptavidin. The signals were then visualized using fluorescence scanner system and analyzed