

In pursuit of ‘normal baselines’: Longitudinal measurement of protein biomarkers in dried blood spots

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Dried blood spot (DBS) sampling is an attractive format for collecting clinical specimens since the procedure is minimally invasive, requires small amounts of blood and the handling is safe and inexpensive. Consequently, longitudinal sample collection for establishing personal baselines can be achieved using DBS. We have previously presented an automated workflow for reliable extraction of proteins from DBS samples, digestion of the proteins and measurement of proteotypic peptides using the SISCAPA technology. Using this workflow, proteins whose abundance spans 8 orders of magnitude were quantitated in a single ¼” DBS punch. A proprietary algorithm was used to normalize for spot-to-spot volume and hematocrit variations. In a set of longitudinal DBS samples from three individuals, we observed profound fluctuations in levels of the acute phase proteins (e.g. CRP, LPS binding protein, mannose binding lectin etc.) in response to such physiological conditions as a common cold or an infection. What remains to be shown is whether chronic health conditions that cause more subtle changes in the proteome can be monitored in longitudinal DBS samples. This is challenging on two fronts: a) the coefficient of variation of the assay used to measure a given protein has to be less than the ‘normal’ biological variation for that protein and b) ‘normal’ has to be defined on an individual basis.

To address these challenges, we have further optimized the SISCAPA workflow for DBS samples to measure a panel of 20 proteins with precisions below the ‘estimated’ biological variation for each analyte as reported in literature. The proteins that were included in this panel include ‘housekeeping’ proteins such as albumin as well as well known clinical biomarkers such as CRP. In most cases, we were able to decrease the total workflow CV to 5% or less. Our results also demonstrate that there is a high degree of parallelism between SISCAPA measurement of these proteins in capillary blood (DBS) vs. venous blood (Serum). To define ‘normal’ ranges for these proteins, we collected longitudinal DBS samples from 10 individuals for a period of 1 year (or more) with an average sampling frequency of about 40 samples/year.

Preliminary analysis of the data confirms that the average levels of the proteins under study vary significantly from one individual to another, and also that the scale of the 'normal' biological variation within an individual differs between subjects. The data strongly suggests that precise measurement of proteins in DBS format provides a baseline 'protein-fingerprint' unique to each individual, thus facilitating preventative, personalized medicine.