

Using Dry Blood Spots to Evaluate Serum Cytokines and Chemokines in Humans via Multiplex Technology

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Introduction: Dried Blood Spot (DBS) analysis has been used routinely since 1963 for the assessment of metabolic diseases in neonates; however, recent efforts have focused on the refinement and validation of DBS for other patient populations. The purpose of this study was to adapt existing DBS methods to analyze 38 serum cytokines/chemokines in human subjects. Validation will be completed by comparing DBS to serum for a given analyte.

Methods: After providing informed consent, subjects (N=21) provided a finger-stick DBS or venous serum sample using standard technique. Subjects were apparently healthy, non-obese, and had no known disease. Finger-stick capillary blood samples were collected on Whatman 903 Protein Saver Card (Maidstone, U.K.) and frozen with desiccant in sealed bags prior to elution. DBS samples (2, 6 mm punches) were eluted and transferred through 96-well Multiscreen and Ultracel plates (EMD Millipore) using elution buffer (PBS with 0.5 M NaCl and 0.1% Tween-20) for 16-18 h in the refrigerator. The resultant DBS elute was resuspended in 90 μ L of nuclease-free water. Serum samples were thawed overnight in the refrigerator to prevent protein loss. Prior to Milliplex analysis, protein and lipid content were analyzed using a IR-based spectrometer (EMD Millipore Direct Detect). DBS solutions were also analyzed for hemoglobin concentration. Samples were analyzed in pairs to determine the concentration 38 cytokines/chemokines using a Magnetic Multiplex Kit (Milliplex Map Kit High Sensitivity Human Cytokine; Billerica, MA). A minimum of 50 beads of each targeted analyte were collected on a Luminex MagPix (Austin, TX) and analyzed using Milliplex Analyst Software. Bi-variate correlations were completed in SPSS to compare DBS with serum.

Results: Of the 38 markers, 13 were measurable in both DBS and serum, and 3 of these were significantly correlated with each other: Eotaxin (.755), MDC (.446), IP-10 (.831). Based on a post-hoc sample size analysis we need to expand our sample size by approximately 40 subjects in order to establish significant correlations the other 10 DBS measurable cytokines/chemokines.

Conclusion: Utilization of DBS may make it possible to obtain previously unobtainable blood samples in a field setting. Despite the potential for DBS all serum markers have not been validated in this sample source. An increase in validated biomarkers detectable in DBS could make the decision to utilize DBS over

venipuncture easier. Now that we have identified changes in resting cytokines/chemokines, the next logical step is to evaluate the effect of exercise.

