Methods to Minimize Confounding Effects of Hematocrit and Hemoglobin when using Dried Blood Spots

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ABSTRACT

Dried blood spots (DBS) are an alternative method of collecting venous blood samples that can be used to measure blood biomarkers. Two confounding factors, hemoglobin and hematocrit, limit the validity of DBS in comparison to the gold standard serum sample. The saturation of biomarkers on DBS filter paper is affected by the sample's hematocrit and hemoglobin. Also hemoglobin contamination is known to confounder for antibody binding in assay systems. The purpose of this study was two-fold: 1) to evaluate a DBS punching technique designed to limit the effects of hematocrit whilst minimizing sample volume and 2) to evaluate a novel device designed to remove hemoglobin from plasma during DBS collection (Seraform™). A bead-based multiplex assay of nine cardiovascular disease risk (CVD) biomarkers (C-reactive protein, fibrinogen, L-selectin, Haptoglobin, serum amyloid protein, von Willebrand factor, adipsin, α2-macroglobulin, and α1-acid glycoprotein) was measured and compared using the various DBS treatments. Outcomes were compared using linear regression analysis examining the R2 change with hematocrit and hemoglobin as covariates. Significance was set at P<0.05. The "edge to edge" punching technique employed in this report resulted in a majority of the biomarkers having the same concentration in comparison to whole spot punching. The Seraform™ device can effectively remove hemoglobin from analysis an accurately predict the concentration of C-reactive protein, fibrinogen, and von Willebrand factor. The other 6 biomarkers had lower predicative potential between the Seraform and the whole DBS sample. The key findings of the present study demonstrate alternate methodologies for collecting/processing DBS samples for routine outcome measures in clinical population studies.