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Validation of Biomarkers of CVD Risk from Dried Blood Spots in Community-Based Research: Methodologies and Study-Specific Serum Equivalencies

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*Dried blood spot (DBS) methodology offers significant advantages over venipuncture in studies of vulnerable populations or large-scale studies, including reduced participant burden and higher response rates. Uncertainty about the validity of cardiovascular disease (CVD) risk biomarkers remains a barrier to wide-scale use. We determined the validity of DBS-derived biomarkers of CVD risk versus gold-standard assessments, and study-specific, serum-equivalency values for clinical relevance of DBS-derived values. Concurrent venipuncture serum and DBS samples (*n = *150 adults) were assayed in Clinical Laboratory Improvement Amendments–certified and DBS laboratories, respectively. Time controls of DBS standard samples were assayed single-blind along with*

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test samples. Linear regression analyses evaluated DBS-to-serum equivalency values; agreement and bias were assessed via Bland-Altman plots. Linear regressions of venipuncture values on DBS-to-serum equivalencies provided R^2 values for total cholesterol, high-density lipoprotein cholesterol (HDL-C), and C-reactive protein (CRP) of 0.484, 0.118, and 0.666, respectively. Bland-Altman plots revealed minimal systematic bias between DBS-to-serum and venipuncture values; precision worsened at higher mean values of CRP. Time controls revealed little degradation or change in analyte values for HDL-C and CRP over 30 weeks. We concluded that DBS-assessed biomarkers represent a valid alternative to venipuncture assessments. Large studies using DBS should include study-specific serum-equivalency determinations to optimize individual-level sensitivity, the viability of detecting intervention effects, and generalizability in community-level primary prevention interventions.

Introduction

Biomarkers are critical to cardiovascular and cardiometabolic disease prevention and stratification of risk. Important biomarker analytes for cardiovascular disease (CVD) include C-reactive protein (CRP), high-density lipoprotein cholesterol (HDL-C), and total cholesterol (TC). Numerous studies have demonstrated that cardiovascular risk is directly proportional to serum total cholesterol and inversely proportional to HDL cholesterol after accounting for other possible risk factors (Kannel et al. 1971; Gordon et al. 1977). CRP is a significant cardiovascular risk predictor, even among individuals with normal low-density lipoprotein (LDL) cholesterol levels (Ridker 2003), and is associated with other chronic diseases, such as type 2 diabetes (Ridker 2003; Pai et al. 2004; Boekholdt et al. 2006).

Traditionally, clinical measurement of these CVD biomarkers and interpretation of their prognostic value have been based on serum blood samples derived from venipuncture (Grundy et al. 2004), a distinct disadvantage in community-based research on health determinants. Venipunctures require specialized training and protocols that increase collection and processing costs and hamper collection opportunities in nonclinical settings. Moreover, venipuncture can be a significant barrier to participation for vulnerable populations such as children, the elderly, and individuals with existing comorbidities. Dried blood spot (DBS) collection, in which capillary blood samples are placed on filter paper following a simple finger prick, presents a viable alternative. Collection and storage protocols are simpler and less costly, and finger pricks are less of a barrier to participation (McDade, Williams, and Snodgrass 2007). These advantages increase the feasibility of conducting large-scale, community-based studies of biomarkers for CVD and other chronic diseases in vulnerable populations (Buxton et al. 2013).

Although the clinical level of accuracy of the DBS method compared to gold-standard venipuncture is clear for neonatal screening (De Jesus et al. 2009; Therrell et al. 1996; Chamoles et al. 2004), the use of DBS for measurement of cardiovascular risk biomarkers in adult population studies is still in its nascent stages. For DBS methods to attain clinical acceptance and regular implementation in studies of adult populations, it is important to repeatedly and reliably test whether DBS biomarker values significantly differ from standard clinical venipuncture values and, if so, the degree to which the discrepancy varies as a function of time and analyte.

Validation studies utilizing a stepped approach are necessary in order to verify critical assumptions that ensure accuracy and precision (McDade 2013). To date, the validation findings have been mixed. While strong correlations have been found between DBS and gold-standard venipuncture for some analytes, including CRP (Crimmins et al. 2014; Lacher et al. 2013), other studies have found poor correlations for analytes such as HDL-C

and total cholesterol (Lacher et al. 2013). DBS samples of certain analytes, such as CRP, appear to degrade with time and environmental exposure (Brindle et al. 2010). Specific laboratory procedures, such as time controls, may be used as quality measures of precision, substantiating the reproducibility of data and quantifying systematic error. Finally, establishing DBS-to-serum equivalence relative to whole blood methods is critical to ascertain both the quality and the veracity of DBS-assessed biomarker analytes and to improve interpretability of these values.

The present study was designed to address limitations to the use and interpretability of DBS-derived biomarkers of cardiovascular risk. We compared DBS- and serum venipuncture–assessed biomarker analytes including CRP, HDL-C, and TC in order to establish the validity of DBS-derived markers of CVD risk. A second goal was to quantify study-specific serum-equivalency values in order to establish the clinical relevance of DBS-derived values compared to venipuncture values from Certified Laboratory Improvement Amendment (CLIA) laboratories.

Methods

Data were obtained from research participants in two studies at different research institutions: the Be Well Work Well (BWWW; $n = 102$) study at a Boston, Massachusetts–based hospital (Buxton et al. 2012; Sorensen et al. 2011), for which data were collected from August 2011 through October 2012; and the Sleep and Cardiovascular Disease Risk (Sleep CVD-R; $n = 48$) study at the University of Pittsburgh Medical Center, for which data were collected from November 2011 through August 2012. Institutional review boards at each study site provided approval for study procedures. Samples were processed within the flow of a larger workplace cohort study, the Work, Family and Health Study (WFHS)(Bray et al. 2013; King et al. 2013).

Venipuncture serum samples were collected from participants at both sites following identical procedures to ensure a uniform protocol for collection, processing, storage, and shipment. Trained study staff cleaned the DBS puncture site (middle or ring finger on the nondominant hand) using alcohol swabs prior to pricking the side of the participant's finger using a disposable, single-use micro-lancet (Unistik 2 Extra, Owen Mumford). Study staff wore sterile gloves and gently massaged the participant's hand to obtain up to five free-flowing blood spots of at least 3 mm in diameter. Spots were collected on a pre-printed filter paper designed for the collection of capillary whole blood (Whatman no. 903, GE Healthcare, Piscataway, NJ). Per standard procedures (Ostler, Porter, and Buxton 2014), collection cards were allowed to air-dry for at least 15 minutes, stored in a biohazard specimen bag with desiccant, and placed in a storage container at room temperature for weekly shipment to the Brigham and Women's Hospital (BWH) Division of Sleep Medicine in Boston. Samples were rated for quality, including proper packaging, presence of moisture, and sample adequacy, as determined by visual comparison of the size of each blood spot to a prescaled circle on a transparent rubric. Prior to assay, samples were stored at −80°C at the Harborview Medical Center at the University of Washington in Seattle. Samples were shipped as part of the flow of DBS samples from the WFHS (Bray et al. 2013; King et al. 2013), and all collection, processing, and storage procedures were identical to those used in the WFHS. The lab was blinded to the control samples (identical to study samples) that were shipped mixed among study samples.

Longitudinal Quality Assurance Measures

Blinded DBS Control Samples

While previous studies have tested the validity of DBS analytes compared to gold-standard venipuncture at a single time point, little is known about DBS assay degradation over time. In order to quantify variation in assay results over time, DBS control samples collected at the same time from three different individuals with known biomarker quantities were assayed periodically. These samples functioned as blinded time controls and were obtained from the venipuncture samples of three individuals, immediately applied to DBS paper, and stored at −80°C until assay. As described previously, the time controls were also processed, stored, and shipped in the flow of the main study's DBS samples.

Plasma Control Samples

As part of the DBS analysis laboratory's standard practice of error handling, selected assay runs included analyte controls from both undiluted pooled human plasma (high control) and pooled human plasma diluted with negligible analyte plasma (low control).

Serum-Equivalent DBS Values

Because translation of DBS-obtained biomarker quantities to serum quantities facilitates clinical interpretability of these values, generation of serum equivalents represented one major focus of the WFHS (Bray et al. 2013). Serum samples from non–clinically selected volunteers were assayed by local, independent, CLIA-certified laboratories (BWWW: LabCorp, Boston, MA; Sleep CVD-R: University of Pittsburgh Medical Center Clinical Laboratory, Pittsburgh, PA) (Buxton et al. 2012; Sorensen et al. 2011). These serum samples were used along with their paired finger-stick DBS results to generate study-specific serum-equivalent DBS values.

Assays

Dried blood spot samples were assayed at the University of Washington's Department of Laboratory Medicine. CRP assays employed a sandwich ELISA that was an adaptation of a previously published DBS method (McDade, Burhop, and Dohnal 2004). The UW laboratory performed HDL-C and TC fluorimetric assays with previously established reliability and validity (Crimmins et al. 2014). Concurrent serum venipuncture samples were sent to independent CLIA-certified laboratories (LabCorp, Boston, MA; University of Pittsburgh Medical Center Clinical Laboratory, Pittsburgh, PA) for analysis. Using standard laboratory techniques, CRP was assayed using a particle-enhanced turbidimetric assay (Roche Molecular Systems Inc.), HDL-C was assayed using a homogeneous enzymatic colorimetric test assay (Roche Molecular Systems Inc.), and TC was assayed with an enzymatic colorimetric test (Roche Molecular Systems Inc.).

Statistical Methods

To calculate DBS-to-serum equivalency values, venipuncture serum values were first regressed on DBS raw sample values to construct regression equations that predicted venipuncture serum values at each value of raw DBS. Serum-equivalent DBS values were

then calculated using these equations for each raw DBS value, thereby transforming the raw DBS values into clinically significant serum equivalencies. Simple linear regression models were then constructed for each analyte to estimate the variance in venipuncture serum concentrations explained by DBS serum-equivalent values, and *R*-squared values were obtained. In addition, bivariate correlations were run to assess the strength of the relationship between DBS serum-equivalent and venipuncture serum values.

To assess the agreement between the serum-equivalent DBS and serum venipuncture measures, we calculated the mean difference of the DBS serum equivalents and venipuncture serum analyte measurements along with corresponding standard deviations. Bland-Altman plots were created to assess agreement for the TC, HDL-C, and CRP biomarkers by plotting the means of the DBS serum-equivalent and gold-standard venipuncture serum values against difference scores between the two measures (Bland and Altman 1999). A positive or negative mean difference represents a serum-equivalent DBS overestimation or underestimation of venipuncture-assessed biomarker concentrations, respectively, and the degree of scatter in difference values on the Y-axis indicates the amount of variability between methods.

Study Schematics

Figure 1 represents a visual depiction of the conceptual framework, providing an overview of the operationalization of precision and accuracy within the substudy.

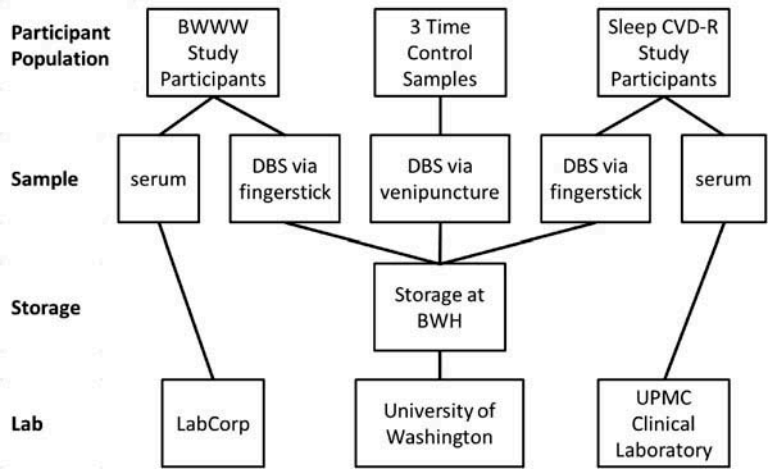

Figure 1. Schematic of the dried blood spot biomarker serum equivalency and quality control substudy depicting blood sample types for the Be Well Work Well (BWWW) study, the Sleep and Cardiovascular Disease Risk (Sleep CVD-R) study, and a blinded time control sample. All three of these control samples were processed and shipped to the DBS lab using procedures identical to those used for the main study samples, including the use of main study blood spot cards, and were mixed among the main study sample shipments. The University of Washington lab was thus kept blind to the use of any control samples, as all samples appeared identical. For the paired control samples, the serum venipuncture collection occurred within minutes of the DBS finger-stick collection. Serum samples were assayed at independent CLIA-certified labs.

Results

The sample included 150 participants of varying ethnicities, BMI values, and ages (Table 1). The means, standard deviations, and minimum and maximum values for raw DBS, DBS-to-serum equivalents, and serum values for TC, HDL-C, and CRP are shown in Table 2. Figure 2 displays the laboratory-assessed serum-equivalent values for the blinded high, medium, and low controls over a period of 30 weeks. Since each set of points represents the same control, evidence of a slope or nonzero R^2 value signifies a variation in the values recorded from the same source at different times. CRP and HDL-C had slopes and regression coefficients close to zero, while TC had rather large slopes, such as −2.32 and −1.09, for two of its controls.

Table 1
Sample characteristics ($n = 150$)

	Mean ± SD (range)
Age (years)	46.60 ± 13.83 (21−74)
BMI (kg/m^2)	27.19 ± 6.44 (18−68)
	Number (percent)
Female	134 (89.3%)
Race	
Hispanic or Latino	3 (2.0%)
African American	8 (5.3%)
Asian	2 (1.3%)
White	135 (90.0%)
Unknown	2 (1.3%)

Table 2
Distributions of raw dried blood spots (DBS) compared to DBS-to-serum equivalents and serum blood

Analyte (unit)	Specimen	Mean	SD	Minimum value	Maximum value
Total cholesterol (mg/dL)	Raw DBS	308.85	61.98	162.97	555.59
	DBS-to-serum	193.03	28.38	126.22	306.04
	Serum	192.81	40.68	126.00	444.00
HDL cholesterol (mg/dL)	Raw DBS	86.70	21.67	49.83	150.42
	DBS-to-serum	61.66	5.89	51.63	78.99
	Serum	62.25	17.52	26.00	119.00
C-reactive protein (mg/L)	Raw DBS	3.31	4.73	0.14	31.19
	DBS-to-serum	1.40	2.12	−0.02	13.89
	Serum	1.41	2.59	0.02	17.55

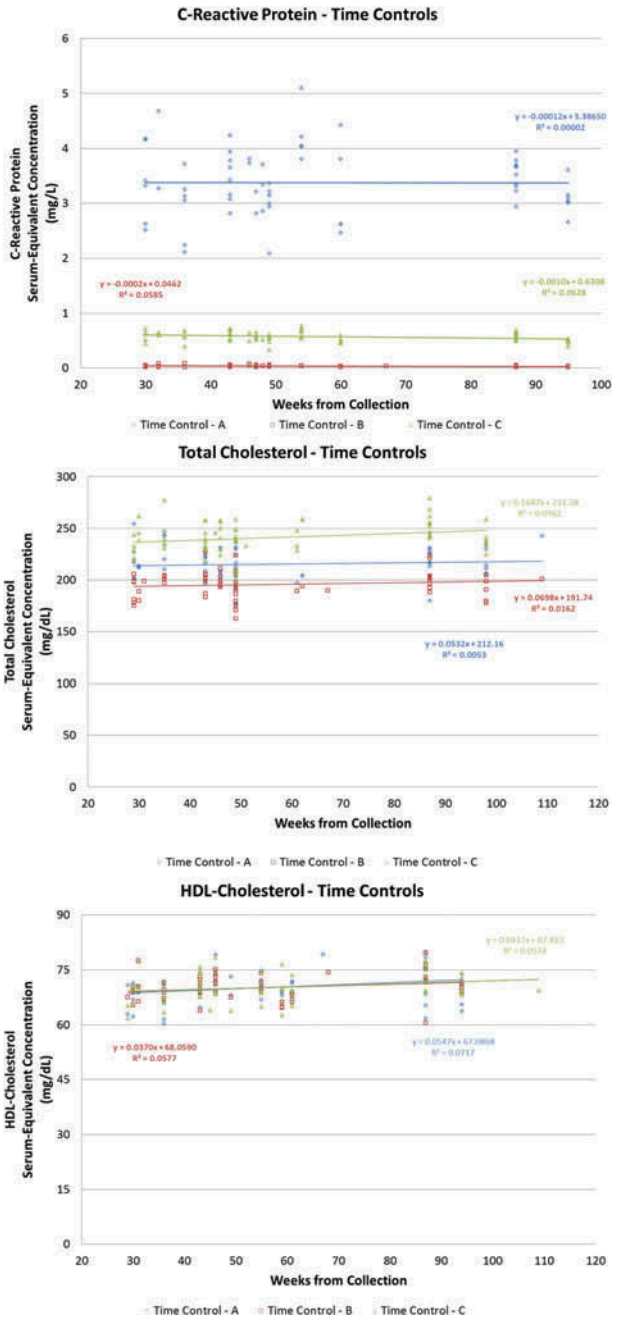

Figure 2. Serum equivalent concentrations for C-reactive protein, total cholesterol, and high-density lipoprotein cholesterol levels in three different standards compared to time of collection. Each set of colored points represents the serum-equivalent concentration from the same source, collected at one time, tested over a period of 65 weeks. DBS samples were created at one time using venipuncture blood and were stored frozen at −80°C until assay between 29 and 109 weeks following collection. A distribution of the same colored points along the Y-axis signifies different values recorded for serum-equivalent concentrations from the same source.

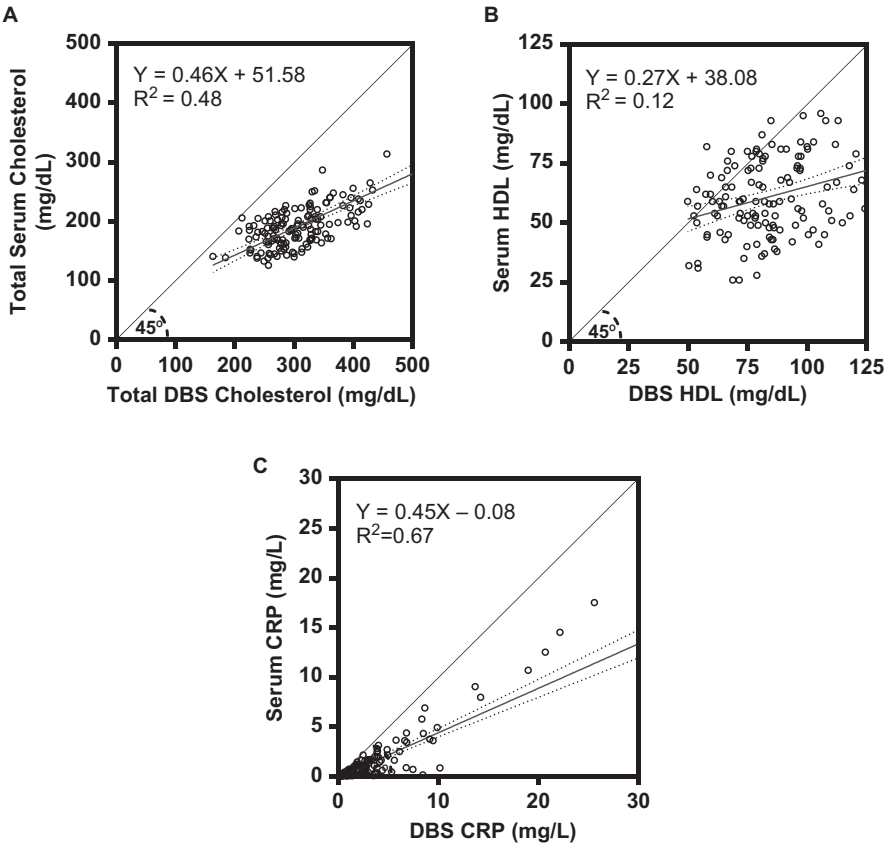

Figure 3. Analyte levels measured by dried blood spots compared to gold-standard venipuncture serum measurements. (**A**) Levels of total cholesterol, (**B**) high-density lipoprotein cholesterol, and (**C**) C-reactive protein. Individual data points represent the DBS levels (X-axis) compared to corresponding venipuncture serum measurements from the same individual and assayed by a CLIA-certified laboratory (Y-axis) for each analyte.

For each analyte, linear regression analyses obtained the following DBS-to-serum equivalency equations [Figure 3]:

$$Y_{\text{TC serum}} = 0.458 * \text{TC DBS raw} + 51.581 \; (p < .001)$$

$$Y_{\text{HDL-C serum}} = 0.272 * \text{HDL-C DBS raw} + 38.075 \; (p < .001)$$

$$Y_{\text{CRP serum}} = 0.448 * \text{CRP DBS raw} - 0.084 \; (p < .001)$$

Using these relationships, each raw DBS value was then transformed into a serum-equivalent DBS value to provide clinically relevant serum equivalencies. The scatter plots for each analyte in Figure 4 compare the serum-equivalent DBS values from the designated laboratory to the paired venipuncture serum samples for each participant. The R^2 values and standard errors of the estimate (SEE) for the analytes TC, HDL-C, and CRP were 0.484 ($SEE_{TC} = 29.389$), 0.118 ($SEE_{HDL} = 16.075$), and 0.666 ($SEE_{CRP} = 1.518$), respectively. Figure 5 displays the Bland-Altman plots of mean serum-equivalent DBS

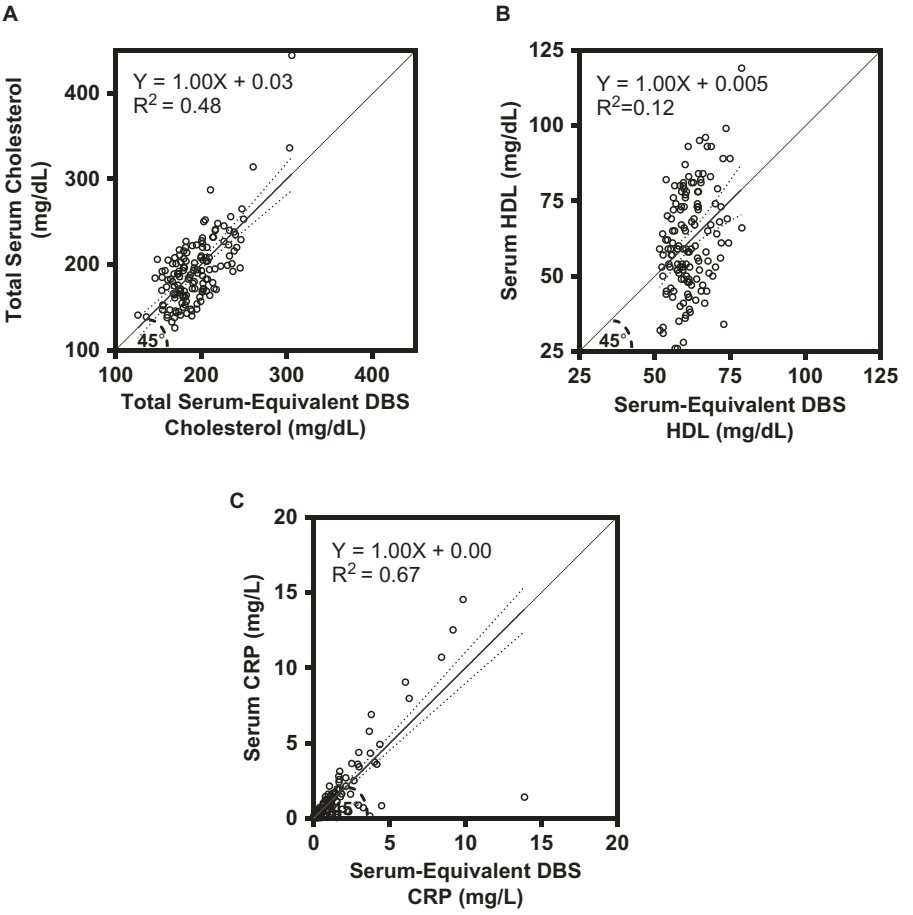

Figure 4. DBS-to-serum equivalent analyte levels compared to gold-standard venipuncture serum measurements. (**A**) Total cholesterol, (**B**) high-density lipoprotein cholesterol, and (**C**) C-reactive protein. Raw DBS values were transformed into serum-equivalent DBS values using empirically derived calibrations between these measures (see Results section) to provide clinically relevant serum equivalencies for each analyte.

and venipuncture serum values against difference values between these two measures for each biomarker. Visual inspection of the HDL-C plot indicates no overall systematic bias, although DBS serum-equivalent values overestimate the gold standard at lower mean HDL-C levels and underestimate gold-standard values at higher mean HDL-C levels (overall bias = 0.004 mg/dL, 95 percent limits of agreement = −31.38–31.40). Visual inspection of the TC plot suggests that serum-equivalent DBS values are evenly clustered around venipuncture serum values across all mean TC levels (overall bias = 0.67 mg/dL, 95 percent limits of agreement = −57.34–57.47), indicating minimal bias. While the CRP plot is also suggestive of an overall minimal bias (overall bias = −.001 mg/L, 95 percent limits of agreement = −3.00–2.96), visual inspection reveals greater variability between the methods across the distribution of mean CRP levels, such that there is increasing overestimation and underestimation of venipuncture serum values at higher mean levels. For all three of the studied biomarkers, the bivariate correlations between serum-equivalent DBS

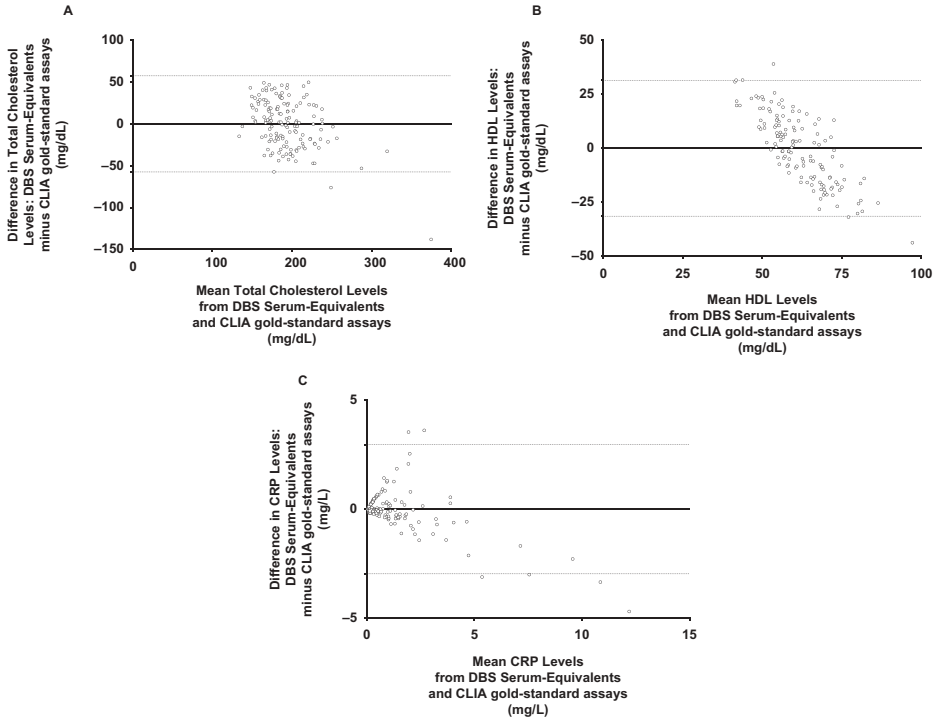

Figure 5. Bland-Altman plots of analyte levels measured by dried blood spots compared to gold-standard venipuncture serum measurement. (**A**) Total cholesterol, (**B**) high-density lipoprotein cholesterol, and (**C**) C-reactive protein. These plots depict the difference between serum-equivalent DBS and venipuncture serum measurements compared to the corresponding means of the values derived from both measures. The horizontal line at zero represents zero bias, and the additional reference lines represent the upper and lower 95 percent confidence limits for the difference scores. Equal point distribution on either side of the zero bias and within the standard deviation limits of difference indicates minimal difference between values derived from the two methods.

and venipuncture serum values were significant at the $p < .01$ level ($r_{TC} = 0.696$, $r_{HDL} = 0.344$, $r_{CRP} = 0.816$).

Discussion

One of the primary aims of this study was to translate DBS-assessed biomarker values into a clinically relevant scale (per CLIA laboratory gold-standard determinations) in order to improve interpretability with other studies. DBS sample values are based on whole blood, and the concentration of analyte is reduced in comparison with serum because of the presence of a large volume of red blood cells, which may dilute the sample (McDade, Williams, and Snodgrass 2007). The DBS-to-serum equivalency is therefore necessary to ensure clinically interpretable results from DBS values when serum values represent the clinical standard, although this translation may not result in reduced variability within the samples all of the time.

Furthermore, our use of blinded controls to create a study-specific DBS-to-serum equivalent relationship allowed for a study-specific representation of serum-equivalent values perhaps more useful than laboratory controls, which may use less directly

comparable samples. This study-specific approach can increase the power of DBS data to detect individual-level differences in longitudinal studies, rather than just large group– or population-level differences, which is particularly important in the presence of an individual-level intervention. To ensure DBS quality control and increase reliability in future studies, we recommend that approximately 10 percent of additional, blinded study samples be collected independently and be subjected to the same pre- and post-collection procedures as the main study samples in order to calculate serum equivalents. Blinded samples should be reinserted into the main study assay flow. While collection of samples for quality control might be considered burdensome, we argue that the benefits of having study-specific serum-equivalent values from DBS assay results outweigh this cost for some study designs. Our confidence in the overall process and procedures for the larger study was strengthened by the minimal variability in control (blinded) samples assessed over time.

Dried Blood Spot Method Considerations

Although the DBS methodology offers advantages over venipuncture, certain aspects of DBS should be considered before its implementation within a study. Because of their higher variability as compared to venipuncture, some DBS assays are more appropriate in population-level, public health research, while venipuncture sampling remains the gold standard for blood-based, diagnostic testing of individuals. As new biomarkers become available for clinical standard measurement, they will first need to be adapted to DBS assays before they can be reliably measured by DBS. However, this opportunity to expand biomarker DBS serum equivalents illustrates the flexibility of this new methodology. In addition, it should be noted that some labs currently lack a DBS sample protocol or the capacity to analyze DBS samples while maintaining clinical applicability through validation and verification (McDade, Williams, and Snodgrass 2007). There are costs associated with the DBS methodology, including the purchase of laboratory-grade freezers for storage and DBS protocol training for non–medically trained staff (McDade, Williams, and Snodgrass 2007). Nonetheless, the total costs of DBS are generally lower than those of serum venipuncture methods.

Limitations to the Study

It is possible that increasing the number of samples set aside for quality control may have resulted in a more accurate study-specific DBS-to-serum equivalence relationship. In addition, although our current sample size ($n = 150$) was of sufficiently good quality, additional samples used as blinded controls may have produced more strongly correlated equivalence relationships. Because of the relatively small sample size, it is possible that we did not have a full distribution of each analyte, resulting in relatively few data points at the tails of each distribution, particularly for CRP. While some DBS collection protocols recommend that the cards be allowed to dry for two hours prior to packaging, all cards in the present study followed a previously established and validated protocol, which found that 15 minutes was sufficient to fully air-dry the cards (Ostler, Porter, and Buxton 2014).

Importance to Biomarker Research

Our study provides further validation for the use of DBS methodology for TC, HDL-C, and CRP biomarker collection, contributing to the growing literature on DBS biomarker validation (e.g., Brindle et al. 2010; Crimmins et al. 2014; Lacher et al. 2013). The present study translated raw DBS values to serum equivalents, which we argue will allow for the

interpretation of DBS values on a clinical scale, providing valuable diagnostic and predictive capability of cardiometabolic disease risk biomarkers. This equivalence method allows for the identification and consideration of pre-analytic sources of error in the final values, as control samples and study samples undergo identical processing. Nonetheless, DBS does exhibit greater variation and assay error for the tested analytes, suggesting that DBS may have greater utility in group or population studies and may be less useful in individual clinical decisions than standard venipuncture samples. A particular strength of the present study is its demonstration through the use of time controls that DBS samples remain fairly constant over time, particularly for the analytes CRP and HDL-C. The DBS method allows for biomarker assays to be implemented in studies using larger, community-based samples and those that target more vulnerable populations, broadening the applicability and generalizability of biomarker use in CVD research.

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References

Bland, J. M., and D. G. Altman. 1999. Measuring agreement in method comparison studies. *Statistical Methods in Medical Research* 8 (2):135–60. doi:10.1191/096228099673819272.

Boekholdt, S. M., C. E. Hack, M. S. Sandhu, R. Luben, S. A. Bingham, N. J. Wareham, R. J. Peters, et al. 2006. C-reactive protein levels and coronary artery disease incidence and mortality in apparently healthy men and women: The EPIC-Norfolk prospective population study 1993–2003. *Atherosclerosis* 187 (2):415–22. doi:10.1016/j.atherosclerosis.2005.09.023.

Bray, J. W., E. Kelly, L. B. Hammer, D. M. Almeida, J. W. Dearing, R. Berkowitz King, and O. M. Buxton. 2013. *An integrative, multilevel, and transdisciplinary research approach to challenges of work, family, and health*. Research Triangle Park, NC: RTI Press.

Brindle, E., M. Fujita, J. Shofer, and K. A. O'Connor. 2010. Serum, plasma, and dried blood spot high-sensitivity C-reactive protein enzyme immunoassay for population research. *Journal of Immunological Methods* 362:112–20. doi:10.1016/j.jim.2010.09.014.

Buxton, O. M., K. Hopcia, G. Sembajwe, J. H. Porter, J. T. Dennerlein, C. Kenwood, A. M. Stoddard, D. Hashimoto, and G. Sorensen. 2012. Relationship of sleep deficiency to perceived pain and functional limitations in hospital patient care workers. *Journal of Occupational and Environmental Medicine* 54 (7):851–58. doi:10.1097/JOM.0b013e31824e6913.

Buxton, O. M., L. C. Klein, J. Whinnery, S. Williams, and T. McDade. 2013. Biomarkers in work and family research. In *New frontiers in work and family research*, ed. J. G. Grzywacz and E. Demerouti, 170–90. East Sussex, UK: Psychology Press.

Chamoles, N. A., G. Niizawa, M. Blanco, D. Gaggioli, and C. Casentini. 2004. Glycogen storage disease type II: Enzymatic screening in dried blood spots on filter paper. *Clinica Chimica Acta* 347 (1–2):97–102. doi:10.1016/j.cccn.2004.04.009.

Crimmins, E., J. K. Kim, H. McCreath, J. Faul, D. Weir, and T. Seeman. 2014. Validation of blood-based assays using dried blood spots for use in large population studies. *Biodemography and Social Biology* 60 (1):38–48. doi:10.1080/19485565.2014.901885.

De Jesus, V. R., X. K. Zhang, J. Keutzer, O. A. Bodamer, A. Muhl, J. J. Orsini, M. Caggana, R. F. Vogt, and W. H. Hannon. 2009. Development and evaluation of quality control dried blood spot materials in newborn screening for lysosomal storage disorders. *Clinical Chemistry* 55 (1):158–64. doi:10.1373/clinchem.2008.111864.

Gordon, T., W. P. Castelli, M. C. Hjortland, W. B. Kannel, and T. R. Dawber. 1977. High density lipoprotein as a protective factor against coronary heart disease. The Framingham study. *American Journal of Medicine* 62 (5):707–14. doi:10.1016/0002-9343(77)90874-9.

Grundy, S. M., J. I. Cleeman, C. N. Merz, H. B. Brewer Jr., L. T. Clark, D. B. Hunninghake, R. C. Pasternak, S. C. Smith Jr., and N. J. Stone. 2004. Implications of recent clinical trials for the national cholesterol education program adult treatment panel III guidelines. *Arteriosclerosis, Thrombosis, and Vascular Biology* 24 (8):e149–61. doi:10.1161/01.ATV.0000133317.49796.0E.

Kannel, W. B., W. P. Castelli, T. Gordon, and P. M. McNamara. 1971. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. *Annals of Internal Medicine* 74 (1):1–12. doi:10.7326/0003-4819-74-1-1.

King, R. B., G. T. Karuntzos, L. M. Casper, P. Moen, K. D. Davis, L. Berkman, M. Durham, and E. E. Kossek. 2013. Work-family balance issues and work-leave policies. In *Handbook of occpational health and wellness*, ed. R. J. Gatchel and I. Z. Schultz, 323–40. New York: Springer.

Lacher, D. A., L. E. Berman, T. C. Chen, and K. S. Porter. 2013. Comparison of dried blood spot to venous methods for hemoglobin A1c, glucose, total cholesterol, high-density lipoprotein cholesterol, and C-reactive protein. *Clinica Chimica Acta* 422:54–58. doi:10.1016/j.cca.2013.03.032.

McDade, T. W. 2013. Development and validation of assay protocols for use with dried blood spot samples. *American Journal of Human Biology* 26 (1):1–9. doi:10.1002/ajhb.22463.

McDade, T. W., J. Burhop, and J. Dohnal. 2004. High-sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. *Clinical Chemistry* 50 (3):652–54. doi:10.1373/clinchem.2003.029488.

McDade, T. W., S. Williams, and J. J. Snodgrass. 2007. What a drop can do: Dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. *Demography* 44 (4):899–925. doi:10.1353/dem.2007.0038.

Ostler, M. W., J. H. Porter, and O. M. Buxton. 2014. Dried blood spot collection of health biomarkers to maximize participation in population studies. *Journal of Visualized Experiments* 28 (83):e50973.

Pai, J. K., T. Pischon, J. Ma, J. E. Manson, S. E. Hankinson, K. Joshipura, G. C. Curhan, et al. 2004. Inflammatory markers and the risk of coronary heart disease in men and women. *New England Journal of Medicine* 351 (25):2599–610. doi:10.1056/NEJMoa040967.

Ridker, P. M. 2003. Rosuvastatin in the primary prevention of cardiovascular disease among patients with low levels of low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: Rationale and design of the JUPITER trial. *Circulation* 108 (19):2292–97. doi:10.1161/01.CIR.0000100688.17280.E6.

Sorensen, G., A. M. Stoddard, S. Stoffel, O. M. Buxton, G. Sembajwe, D. Hashimoto, J. T. Dennerlein, and K. Hopcia. 2011. The role of the work context in multiple wellness outcomes for hospital patient care workers. *Journal of Occupational and Environmental Medicine* 53 (8):899–910. doi:10.1097/JOM.0b013e318226a74a.

Therrell, B. L., W. H. Hannon, K. A. Pass, F. Lorey, C. Brokopp, J. Eckman, M. Glass, et al. 1996. Guidelines for the retention, storage, and use of residual dried blood spot samples after newborn screening analysis: Statement of the council of regional networks for genetic services. *Biochemical and Molecular Medicine* 57 (2):116–24. doi:10.1006/bmme.1996.0017.