Dried Blood Spot Testing for Atherosclerotic Cardiovascular Risk Assessment

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INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD), mainly heart disease and stroke, is a leading cause of death in the United States.[1] Major ASCVD risk factors include hypertension, diabetes, smoking, and lipid abnormalities.[2,3] Laboratory assessment of ASCVD risk in routine standard of care entails mainly the measurement of fasting serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C), with low-density lipoprotein cholesterol (LDL-C) being calculated using a variety of formulas. We and others have documented that serum direct LDL-C, small dense LDL-C (sdLDL-C), lipoprotein(a) (Lp(a)), high sensitivity C-reactive protein (hs-CRP), homocysteine, and fatty acids add significant information about ASCVD risk above and beyond standard laboratory assessment.[4-8] Phlebotomy services have been increasingly costly, are sometimes unavailable for patients, and may not be an option for some healthcare providers, including those practicing telemedicine.

OBJECTIVE

Our objective was to validate the use of dried blood spot (DBS) cards after fingerstick sampling to measure multiple ASCVD risk markers and other parameters that are important, including those assessing kidney and thyroid function.

METHODS

Specimens. 249 male and female fasting (>8 hr) volunteers did fingerstick sampling (McKesson 17G, 2.0 min blade lancets, cat. no. 116-PBSL17G), collecting 4-6 drops of capillary blood on DBS cards (FIGURE 1) . Cards were dried at room temperature for at least 30 mins, sealed tightly in foil pouches containing desiccant, and tested for up to 14 days after blood collection.

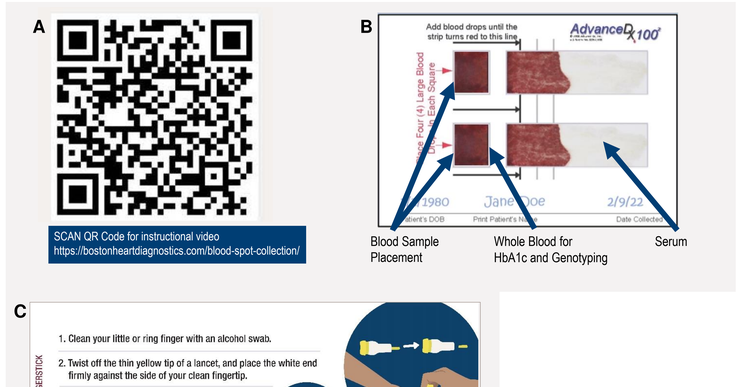

FIGURE 1. Dried blood spot specimen collection. Panel A, instructional video available at https://bostonheartdiagnostics.com/blood-spot-sample-collection/. Panel B, Dried blood spot collection card. Panel C, Instructions details. As blood is dropped into left hand rectangle and migrates to right hand rectangle, serum is separated from blood cells and clotting factors.

Analysis. Punches were obtained from the serum and red blood cell sections of the DBS cards (FIGURE 1B), solubilized, and analyzed. DBS-derived test results were compared with results derived from serum collected after venipuncture.

PARAMETERS MEASURED

7 lipids and apolipoproteins
TC, TG, HDL-C, direct LDL-C, sdLDL-C, Lp(a), apoB

1 inflammation marker
hs-CRP

2 diabetes markers
glucose, glycosylated hemoglobin (HbA1c)

1 kidney function marker
creatinine, calculated eGFR.

7 hormones and related markers
thyroid stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), dehydroepiandrosterone sulfate (DHEAS), testosterone, free testosterone, prostate-specific hormone (PSA)

5 vitamins and related markers
homocysteine, folate, vitamin B12, vitamin D, uric acid

6 fatty acid parameters
eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA), and omega-3, omega-6, and monounsaturated fatty acid indices.

11 genotyping tests
APOE (ASCVD risk and response), MTHFR (methylfolate), Factor V Leiden, Prothrombin (FACTOR II), SLCO1B1 (statin-induced myopathy risk), 4Q25 (arial fibrillation risk), LPA (aspirin benefit), 9P21 (ASCVD risk), KIF 6 (statin response), CYP2C19 (clopidogrel metabolism), haptoglobin.

DBS chemistry tests were run on Beckman AU4800 and ACCESS (Beckman Coulter, Brea, CA) analyzers and compared with venous serum testing run on COBAS (Roche, Indianapolis, IN) analyzers.[9] Fatty acids were measured by gas chromatography/mass spectrometry after lipid extraction; genotyping, by standard procedures.

CONCLUSIONS

Dried blood spot testing is an effective and accurate method for advanced ASCVD risk assessment and management.

Our data demonstrate that DBS-derived measurements had excellent correlations with results obtained with venous blood for 29 biomarkers and 11 genetic variants.

DBS technology has many advantages:

- **No centrifugation of specimens is required.**
- **Specimens are stable for up to 14 days at room temperature and can be shipped without ice.**
- **Specimens can be collected at home, thereby, providing an attractive alternative for telemedicine patients.**

RESULTS

- DBS-derived concentrations for lipids, inflammation, diabetes, vitamins, kidney function, and hormones were all highly correlated (Pearson $R \geq 0.95$; $P < 0.00001$) with values obtained by standard venipuncture (FIGURE 2). All assays had intra- and inter-assay coefficients of variation (CV) <5%.
- DBS measurements for EPA, DHA, AA, omega-3 index, omega-6 index, and monounsaturated fat index were highly correlated (Pearson $R > 0.90$; $P < 0.0001$) with standard venipuncture results.
- Genotyping results were 100% correlated between DBS and phlebotomy assessments.

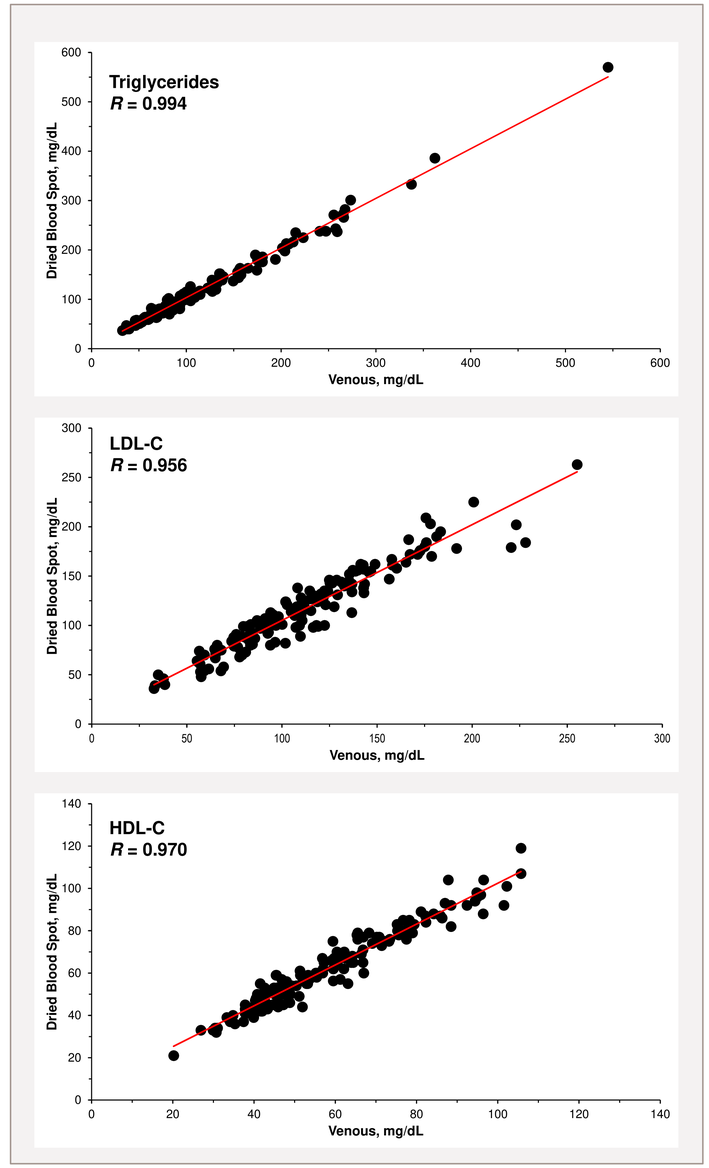

FIGURE 2. Representative correlations between DBS-derived and venipuncture-derived concentrations. Concentrations of triglycerides, LDL-C, and HDL-C measured in serum collected by DBS were highly correlated (Pearson $R > 0.95$; $P < 0.0001$) with those measured in serum collected by standard venipuncture.

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All authors have been or are employees or consultants of Boston Heart Diagnostics.