

Dried Blood Spot Testing for Atherosclerotic Cardiovascular Risk Assessment

Maxine Lang, Marianna Fernandes, Gregory Green, Margaret R. Diffenderfer, Lihong He, and Ernst J. Schaefer

Boston Heart Diagnostics, Framingham MA, USA

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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) or 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

- To validate the use of dried blood spot (DBS) cards after fingerstick sampling for multiple ASCVD risk markers and other parameters that are important including kidney and thyroid function testing.

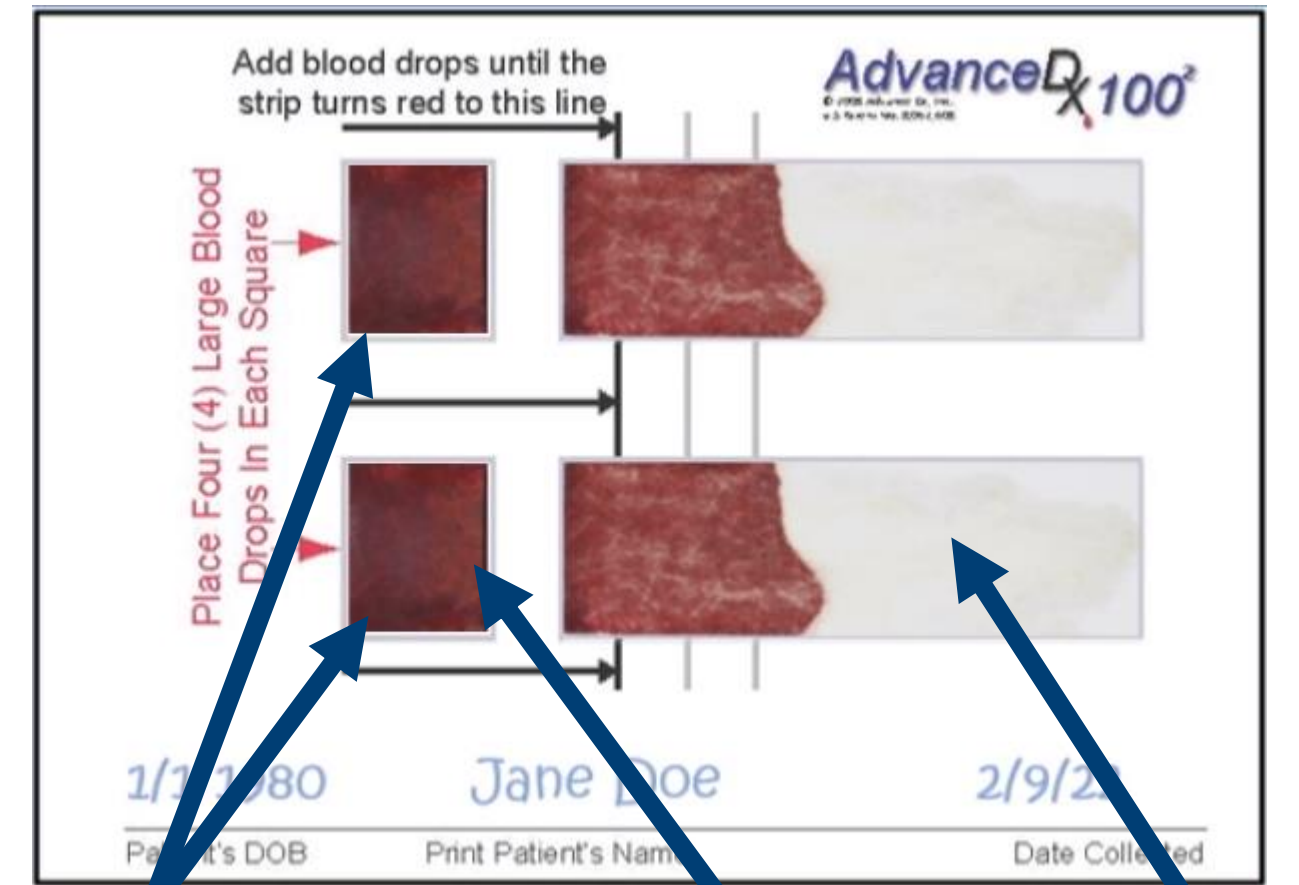
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METHODS-1

- 249 male and female fasting (>8 hours) volunteers did fingerstick testing (McKesson 17G, 2.0 mm blade lancets, cat. no. 16-PBSL17G) with 4-6 drops of capillary blood on ADX-100 (Advance Dx <https://adx100.com>) cards.
- Dried blood spot (DBS) cards were dried at room temperature for at least 30 min, sealed tightly in pouches containing desiccant and tested for up to 14 days after blood collection.
- Punches were obtained from the serum and red blood cell sections of the DBS cards (see below), solubilized, and analyzed as described below.
- Analyses were run on Beckman AU480 and ACCESS analyzers and compared with results obtained on serum collected after venipuncture using analyses on Roche COBAS analyzers.



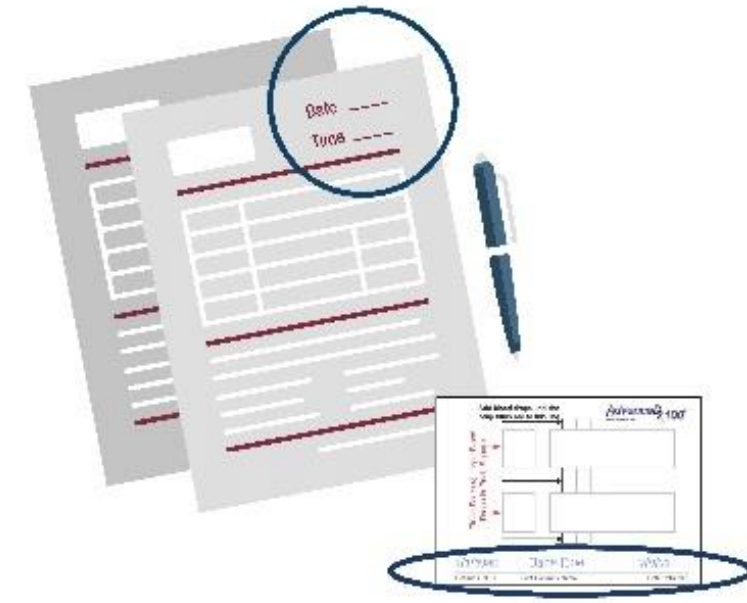
Blood Sample Placement Whole Blood for HbA1c and Genotyping Serum

METHODS – Dried Blood Spot Specimen Collection

1

FORMS AND LABELING

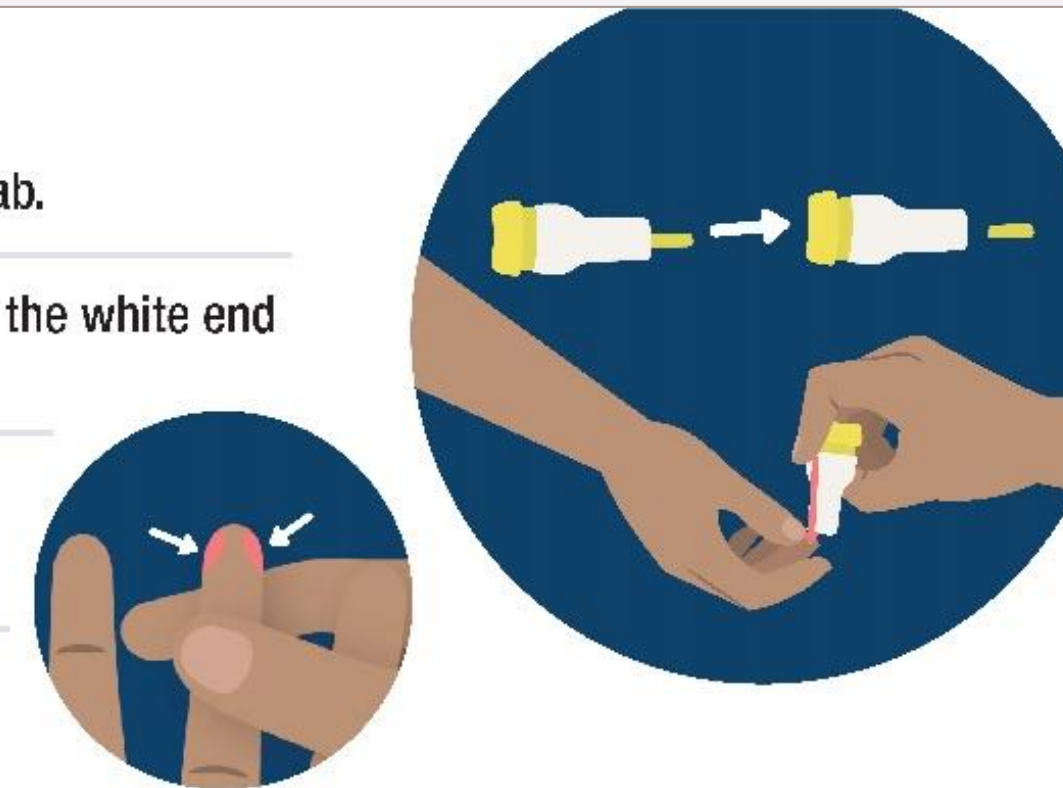
- Place all contents of the kit on a clean work surface.
- Fill in the Date and Time in the upper right-hand section of the Test Order Form supplied to you by your healthcare provider.
 - If you do not have a Test Order Form, reach out to your provider.
- Write your name, date, and date of birth on the order form AND sample collection cards.



3

FINGERSTICK

- Clean your little or ring finger with an alcohol swab.
- Twist off the thin yellow tip of a lancet, and place the white end firmly against the side of your clean fingertip.
- Press the yellow button until the lancet clicks. This will push a tiny needle into your fingertip.
- Remove the first drop of blood using a piece of gauze.



5

SHIP TODAY

- Tear open the white Blood Sample Return Bag at the notches.
- Place the dried collection card into the Sample Return Bag(s) and seal.
 - If you have more than one card, each goes into a separate sample return bag.
- Place the Blood Sample Return Bag inside of the provided clear plastic bag.
- Place the Test Order Form into the outer pouch of the clear plastic bag.
- Place the clear plastic bag inside the pre-labeled mailer (either FedEx or U.S. Postal Service) and seal closed.
- Ship the same day as sample collection.
- If you have a U.S. Postal Service mailer, place in a standard mailbox or bring to your local post office.
 - If you have a FedEx mailer, find the nearest FedEx drop box by:
 - Going to: bit.ly/fedexdropbox (Mon-Fri only) or
 - Calling: 1.800.GoFedEx to schedule a FedEx Ground pickup.
- Take note of the return label tracking number on the FedEx label to know when your kit reaches the lab.



2

PREP

- Scan QR code or go to bostonheartdiagnostics.com/blood-spot-sample-collection for a video demonstration.



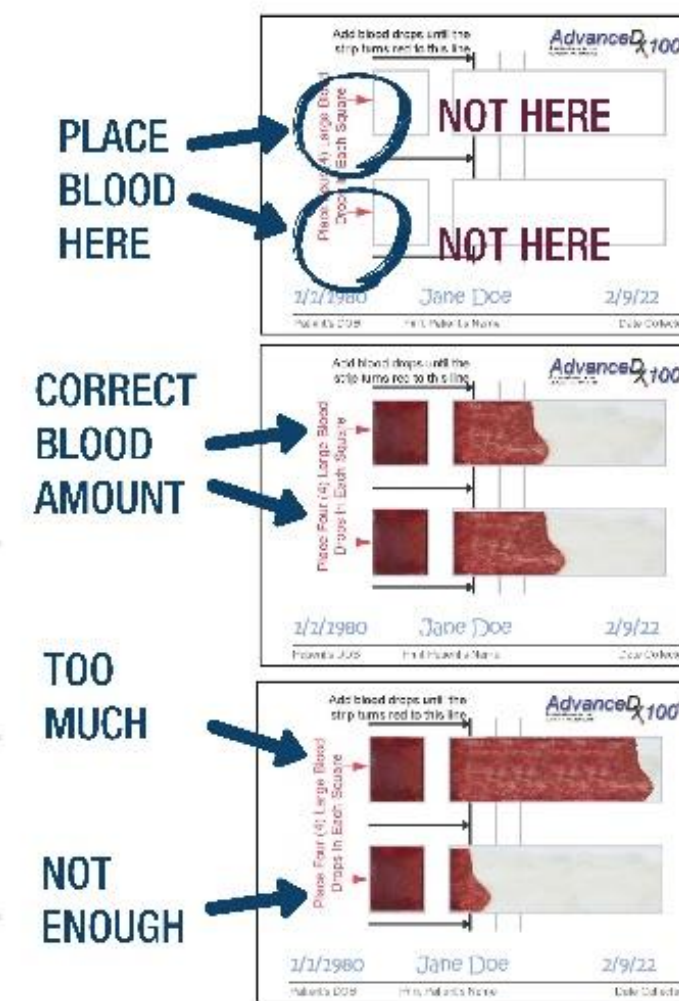
- Wash your hands with very warm water for at least 2 minutes to get your blood flowing.
 - This is very important for adequate sample collection.



4

COLLECT

- Allow 4-7 large free-falling blood drops to land on the top left square of the card. Do NOT touch your finger directly on the collection square.
 - The illustration shows how a proper collection looks.
 - Blood will migrate from the square to the rectangle.
 - Do not put blood directly on the rectangle.
 - Please be careful to add the correct amount of blood. Do not add too much or not enough blood to the card.
- Place 4-7 additional drops of blood on the lower left square by repeating the steps above using a new lancet if needed.
- If you have a second collection card then repeat the process above for the upper and lower collection squares.
- Allow the collection card(s) to dry for 1 hour.



SCAN QR Code for instructional video available at <https://bostonheartdiagnostics.com/blood-spot-sample-collection/>



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METHODS-2

The following 42 parameters were assessed:

- 7 Lipids and Apolipoproteins: TC, TG, HDL-C, direct LDL-C, sdLDL-C, Lp(a), and apoB.
- 2 Inflammation Markers: hs-CRP, lipoprotein associated phospholipase A2 (LpPLA2).
- 3 Diabetes Markers: glucose, insulin, glycosylated hemoglobin (HbA1c), calculated HOMA-IR and HOMA- β .
- 1 Kidney Function: creatinine, calculated eGFR.
- 5 Other Markers: homocysteine, folate, vitamin B12, uric acid, vitamin D
- 7 Hormones & Related Markers: TSH, FSH, LH, DHEAS, testosterone, free testosterone & PSA. \
- 6 Fatty acid parameters by gas chromatography/mass spectrometry after lipid extraction: EPA, DHA, omega-3, omega-6, and monounsaturated fatty acid indices, and arachidonic acid.
- 11 Genotyping Tests: APOE (risk and response), MTHFR (methylfolate), Factor V Leiden, Prothrombin (Factor II), SLC01B1 (statin-induced myopathy risk), 4Q25 (atrial fibrillation risk), LPA (aspirin benefit), 9P21 (ASCVD risk), KIF6 (statin response), and CYP2C19 (clopidogrel metabolism), and haptoglobin.

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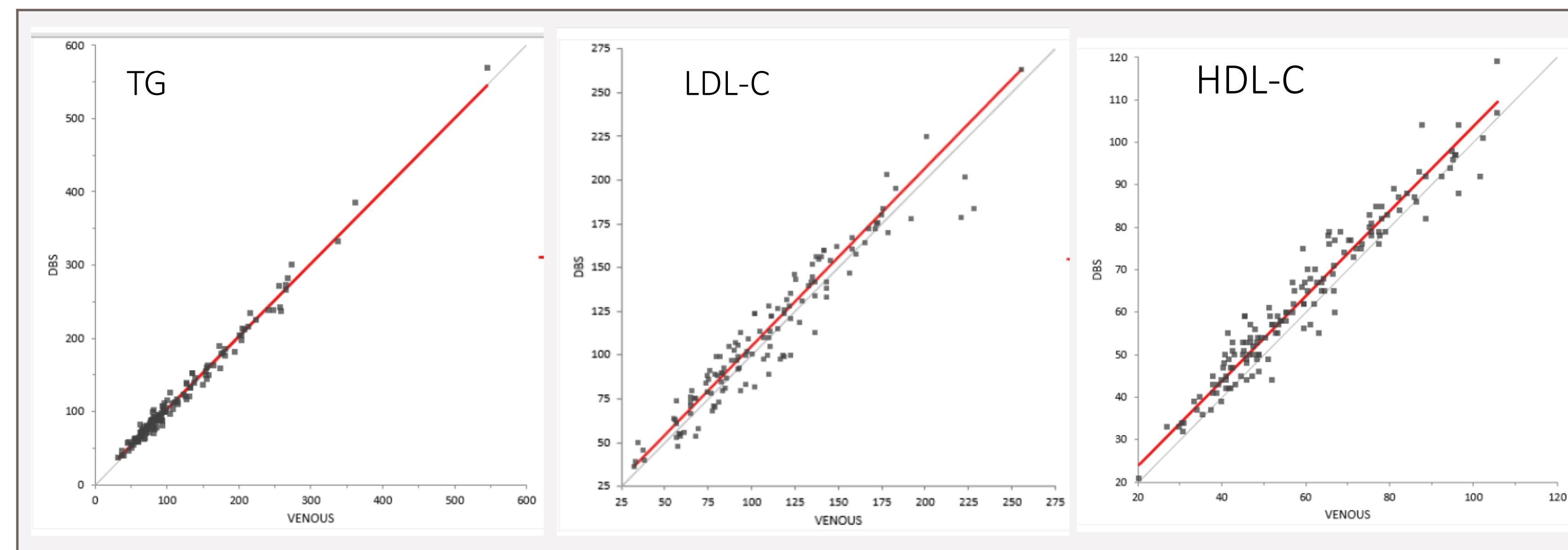
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RESULTS

- 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 (**See examples below for TG, LDL-C and HDL-C**). All assays had intra- and inter-assay coefficient of variation (CV) $< 5\%$.



- DBS measurements for eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (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.

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CONCLUSIONS

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- 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 31 biomarkers and 11 genetic variants.
- DBS technology has many advantages:
 - No centrifugation of samples is required.
 - Samples are stable for up to 14 days at room temperature and can be shipped without ice.
 - Sample can be collected at home, thereby, providing an attractive alternative for telemedicine patients.

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¹National Center for Health Statistics

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