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The Use of Dried Blood Spot Sampling in the National Social Life, Health, and Aging Project

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Objectives. This paper describes the methods used for and issues associated with collection and analysis of dried blood spot (DBS) samples for the National Social Life, Health, and Aging Project and provides the basic distributions of the resulting analytes.

Methods. DBSs from capillary finger sticks were collected by nonmedically trained interviewers from 2,044 individuals, aged 57–85 years. The quality and quantity of DBS samples were evaluated to allow for analysis of interviewer performance. Levels of C-reactive protein, antibodies to the Epstein–Barr virus, hemoglobin, and glycosylated hemoglobin were assayed using various analytic methods.

Results. Cooperation rate for DBS collection was 84.5%, with 99% of the cards yielding enough sample for at least one analysis. The distribution, mean, and standard deviation of the analytes obtained from DBSs are also presented in this paper.

Conclusions. The high cooperation rate and quality of the spots collected suggest that the collection of DBSs in population-based research is a feasible and viable alternative to venous blood draws. The relative ease of sample collection, transport, and storage are significant benefits. Care should be taken, however, when comparing results from analysis of DBS samples with those obtained from serum or plasma samples.

***Key Words*:** Biomeasures—Dried blood spots—Aging.

DRIED blood spots (DBSs)—drops of capillary whole blood collected on filter paper from a simple prick of the finger—provide a minimally invasive alternative to venous blood sampling that facilitates the collection of blood samples in home-based settings, by nonmedically trained personnel. Although the advantages of DBS in terms of sample collection, transport, and storage are substantial, care should be taken when using data obtained from DBS, as they are not identical to results obtained by more commonly applied methods using serum or plasma samples (McDade, Williams, & Snodgrass, 2007). DBSs are one of the 13 direct biological measures (biomeasures) collected in the National Social Life, Health, and Aging Project (NSHAP). These measures, which also include weight, waist circumference, height, blood pressure, smell, saliva collection, taste, a self-administered vaginal swab for female respondents, "Get Up and Go," distance vision, touch, and oral mucosal transudate (Orasure®) HIV test, were collected for analysis of measures associated with physiological functioning. The collection of biomeasures in conjunction with questionnaire items allows for the investigation of the interaction between the social, psychological, and environmental domains and their impact on health. The object of this paper is to provide pertinent information on the collection, transport, and laboratory analysis of DBS samples in NSHAP to promote the appropriate use of data derived from these samples.

Methods

In order to minimize participant burden and to maximize participation, the NSHAP interview and biomeasure collection process was designed to be completed in approximately 2 hr. To stay within these time constraints and still obtain the maximum amount of information, it was necessary to modularize the design of the survey. As a result, approximately 5/6 of NSHAP participants (2,494) were asked to provide blood spots. Of those asked, 84.5% (2,105) agreed. However, due to collection difficulties, blood spots were not collected from an additional 57 individuals who originally agreed to participate. As a result, DBSs were collected from a total of 2,048 individuals. Because of the variability in volume of blood available for analysis, the sample size for each analyte obtained from blood spots varies. A list of analytes obtained and the sample size for each analyte is listed in Table 1.

DBS Collection Protocol

Blood spots were collected from free-flowing capillary blood obtained by finger stick. The protocol is as follows:

1. Angle Respondent's hand below his/her lap.
2. Warm finger and stimulate circulation by gently kneading and squeezing the appropriate finger.

3. Ask Respondent to gently shake his/her hand a few times.
4. Wipe the index finger of the right hand with alcohol swab and wait a few seconds for the alcohol to dry (DO NOT blow on finger, wave hand, etc. to speed up drying).
5. Squeeze the finger just below the area to be pricked.
6. Firmly prick finger in the fleshy part of the pad, just off the center.
7. IMMEDIATELY dispose of the lancet into the sharps container.
8. Allow blood to well on tip of finger.
9. If necessary, apply gentle pressure below the site of the prick.
10. Place first drop in discard circle of filter paper—marked D.
11. Place three (if possible) additional drops on filter paper.
12. DO NOT reblot the circles on the filter paper (except discard circle).
13. If unable to fill three spots (+ discard spot), prick another finger. Place first drop in discard circle of filter paper (marked D) and place additional drops in remaining circles.
14. If necessary, ask Respondent to hold cotton ball on finger and apply pressure until bleeding stops.
15. Offer Respondent a bandage.

After collection of the blood spots, field interviewers (FIs) were instructed to record in the computer the number of blood spots collected and finger pricks attempted and to set the filter paper aside and allow the blood spots to dry for the remainder of the interview. At the end of the interview, the FI was instructed to flip the cover of the filter paper over the blood spots and place the filter paper in a plastic bag with a desiccant pack. Upon returning home, the blood spots were removed from the bag and allowed to air dry overnight. They were then returned to the bag and stored at 4 °C until shipped.

Field Interviewer Training

The majority of FIs used for data collection in NSHAP did not have any previous medical training. However, most of them did have previous experience in field interviewing. FIs were brought to a central location, in three groups, for 8 days of training. FIs were trained in preventing disease transmission and the use of universal precautions as well as the specific techniques necessary for blood spot collection. Although one of the key benefits of this method of blood collection is the minimal risk to both the participant and the collector, every effort was made to train the interviewers to minimize their exposure to potential risks. Further, only single-use safety lancets were used to prevent exposure to used lancet blades, FIs were well supplied with alcohol pads, and all materials were collected in biohazard and sharps containers and properly disposed.

Table 1. Analytes Obtained From Dried Blood Spots

Analyte	Sample Size
C-reactive protein	1,940
Epstein–Barr virus antibody titers	1,981
Hemoglobin	1,859
HbA1c	1,739

Basic techniques in the use of lancets, the proper positioning of the finger stick, and proper application of the blood to the card were demonstrated to FIs in small groups during the larger NSHAP training. Individual FIs were then given time to practice on themselves and each other under the supervision of experienced training personnel. Additional time with training personnel was available for those who needed extra training. On the final day of training, each FI was required to demonstrate adequacy in blood spot collection before training was considered complete.

Continual feedback on the quality of spots was also provided to the FIs throughout the study period. As the blood spots were received in the laboratory for analysis, the number, quality, and condition of the spots was recorded by trained personnel. If consistent problems with collection were observed for a single FI, he or she was contacted to discuss potential problems and techniques that could be used to improve the quality of the blood spot collection.

Key issues in the collection of DBSs.—Sample volume is a key issue in the usability of blood spots for biomeasure analysis. In order to obtain the maximum amount of blood, FIs were instructed to allow large drops of blood to pool on the finger before dropping them onto the filter paper with the goal of filling each of the five premarked circles entirely. Analysis of DBSs typically requires that either a 3.2-mm or a 6.0-mm disk of dried blood be punched out of the filter paper. A full drop of blood provides sufficient sample for either a 1- to 6.0-mm disk of dried blood or up to 7- to 3.2-mm disks. Smaller volumes collected means less sample is available for analysis. Further, proper techniques used for promoting blood flow were stressed during training. The idea that "some people just don't bleed well" was discouraged, and FIs were taught that with proper technique—including having the individual rigorously shake his/her hand before the stick and firmly pressing the lancet to the finger for maximum contact with the blade—five drops of blood is possible from almost anyone.

In addition to volume, it is necessary that the blood be collected appropriately to ensure that the blood is absorbed evenly across the filter paper. This ensures that the analyte of interest is evenly distributed across the blood spot. It is necessary, therefore, for the blood to be freely dropped onto the filter paper. If the finger is touched to the filter paper or blood is blotted onto the filter paper, the blood cannot flow evenly across the paper matrix and the spot is not usable for

Table 2. DBS Card Quality

Card Quality	% of Total Cards	% Average by Field Interviewer
Usable (≥1 punch)	98.6	100.0
Acceptable (≥4 punches)	88.8	100.0
Good (≥10 punches)	56.4	66.0
Excellent (≥20 punches)	13.6	3.3

most analyses. Further, if more than one drop is applied to the same area of the filter paper and the spots are doubled or overlapping, these spots are also unusable for most analyses. Upon careful examination of the spots, those that have been blotted or double spotted will appear asymmetric. Only spots that appear symmetric and circular on both sides of the filter paper are appropriate for most analyses. The exceptions to these general guidelines are spots that will be used for DNA extraction or for other analyses where quantification of the analyte in question is not necessary. A more detailed discussion of important issues in the collection and use of DBSs can be found in McDade et al. (2007).

After drying, the blood spots were refrigerated until shipment to the laboratory for analysis. DBSs were shipped in batches to the laboratory every 2 weeks. Shipment within the United States of blood (including blood spots, plasma, and serum) requires samples to be labeled and shipped as "diagnostic specimens." These samples must be packed with enough absorbable material to collect any sample leakage and packed in a sealed, leak-proof bag or container. This container must be packed inside an additional sealed container clearly marked as diagnostic specimens on the outside of the package. Diagnostic specimens can be shipped through commercial carriers or through standard U.S. mail when correctly packaged (contact the United States Department of Transportation [USDOT] or commercial shipper for details). Upon arriving in the laboratory, blood spot cards were catalogued, analyzed for quantity and quality, and frozen at −25 °C until analysis.

Collection Results

There were no significant differences between individuals who agreed to provide blood spots and those who refused with respect to gender, race, ethnicity, age, education, income, or marital status. There was also no difference between the two groups with respect to self-reported mental or physical health or the number of doctor visits reported by the participants. However, the experience of the FI did have an impact on the percentage of their participants who refused to provide blood spots. Of the 131 FIs, the average success rate (measured by percentage of total number of subjects asked who agreed to provide a DBS) was 84. However, interviewers had significantly higher response rates if they had more than 2 years of survey experience (82% vs. 86%). Neither the race of the FIs nor the age of the FIs, relative to the age group of the NSHAP participants, was associated with their success rate.

Individuals who refused to participate in blood spot collection reported various reasons for doing so. However, these responses followed a few general themes. Of the 158 refusal responses noted, 25% refused because of a reported medical condition, most commonly due to blood thinners, recent blood work, or daily glucose checks due to diabetes. Of the remaining refusals, 20% reported unease with potential pain, fear of needles, or dislike of blood, and 17% reported that they (or in three cases a family member) did not think providing blood was appropriate or believed the measure was too invasive or dangerous.

DBS sample quality.—Each card contains five preprinted circles of standard size (1/2-inch diameter) for suggested collection. Interviewers in this study were instructed to blot the first drop on the filter paper in the rightmost circle. This spot will not be used for analysis and is not described in the current paper. Remaining spots are counted and measured (in relation to the size of punches possible), and any obviously blotted or double dropped spots are noted. Both individual spots and entire cards will be described. Spots are considered to be too small if they are not large enough to allow for a single small (3.2 mm) hole punch. Spots are considered good if they will allow for a single large (6 mm) hole punch. Spots are considered excellent if they fill the entire circle. For the purposes of the NSHAP study, one large and three small punches are required for analysis of proposed biomarkers. Cards are considered to be *inadequate* if they cannot support the necessary punches, *adequate* if they will support only the required punches, and *excellent* if they will support more than the required amount of punches. Statistical analysis in the following section was completed using STATA 9.2 (StataCorp, College Station, TX). A summary of DBS card quantity and average quantity of blood collected by each FI is presented in Table 2.

Blood Spot Analysis

Biomarkers for analysis from DBSs in NSHAP were selected based on several criteria, including availability of a valid assay, cost, and relative value of the measure with respect to the objectives of the survey. Analytes quantified in NSHAP DBS samples include C-reactive protein (CRP), antibodies to the Epstein–Barr virus (EBV), hemoglobin, and HbA1c. In most cases, there was more sample collected than used, allowing for the potential future use of these DBSs for more analyses. The consent process was designed with this in mind, and participants were asked for permission to store their DBS samples for future use. The majority of participants, 86%, agreed to allow storage of any remaining sample for future use. Many additional analytes have been obtained from DBS, and improvements in technology may result in new analyses or methods for analyses. However, length of storage and stability of a new analyte must be

Table 3. Summary Statistics for C-reactive Protein Levels (mg L^{-1})

C-Reactive Protein (mg L^{-1})	Range	Mean (Weighted)	*SD*
Men (years)			
Ages 57–65	0–38.2	2.80	3.93
Ages 66–75	0.06–100	2.87	6.53
Ages 76+	0.03–39.8	2.51	4.69
Women (years)			
Ages 57–65	0–100	4.17	8.93
Ages 66–75	0–41.8	3.28	5.14
Ages 76+	0–100	3.20	6.05

considered before any future analyses. A comprehensive list of potential DBS analytes, available protocols, validity, and stability are available in McDade et al. (2007). Unless otherwise noted, all assays were conducted in the Laboratory for Human Biology Research at Northwestern University (Evanston, IL).

CRP.—CRP is an acute-phase protein and a nonspecific marker of inflammation. CRP has been used as a marker of immune activation (McDade, Burhop, & Dohnal, 2004) as well as a risk factor for cardiovascular disease (Smith et al., 2004). Although CRP is correlated with other biological indicators of health, it is independently related to cardiovascular risk, even when accounting for age, smoking, blood pressure, and diabetes and lipids (Koenigh, Sund, Frohlich, & Fischer, 1999; Pai, Pischon, Ma, & Manson, 2004; Ridker, 2003; Ridker Cushman, Stampfer, Tracy, & Hennekens, 1997; Ridker, Rifai, Rose, Buring, & Cook, 2002). CRP values were assayed using an enzyme-linked immunosorbent assay (ELISA) protocol previously developed for use with DBS (McDade et al., 2004). Validation studies indicate that the blood spot CRP method has a lower detection limit of 0.028 mg L^{-1} and a high correlation between matched plasma and blood spot samples. These matched samples can be used to convert DBS results to plasma/serum equivalents using the linear regression equation reported in McDade et al. (2004).

However, care should be taken when comparing DBS values with plasma or serum values, as the relationship will vary across analytic methods and may vary by population (e.g., Shirtcliff, Reavis, Overman, & Granger, 2001). As reported in Pearson et al. (2003), the Centers for Disease Control and Prevention (CDC) has recently recommended classifications of cardiovascular risk as derived from CRP values. Plasma or serum CRP values >10 mg L^{-1} are presumed to represent the presence of acute, active infection and should be excluded from analyses that are using CRP as an indicator of chronic, low-grade inflammation. Based on the relationship between DBS and plasma/serum CRP values evaluated as part of the DBS CRP assay validation, a DBS value of 8.6 mg L^{-1} corresponds to a plasma/serum value of 10 mg L^{-1}. Distribution of CRP levels in the NSHAP sample are provided in Table 3.

Table 4. Summary Statistics for EBV Antibody Titers

EBV Antibody Titers (ELISA Units)	Range	Mean (Weighted)	*SD*
Men (years)			
Ages 57–65	13.2–318.7	155	75.7
Ages 66–75	14.2–371	158	72.8
Ages 76+	13.1–317	157	75.3
Women (years)			
Ages 57–65	13.7–318	151	73.4
Ages 66–75	14.4–331	159	77.9
Ages 76+	12.7–314	156	83.0

Note: EBV, Epstein–Barr virus; ELISA, enzyme-linked immunosorbent assay.

EBV antibody.—EBV is a ubiquitous human herpes virus that may cause mononucleosis, although most primary infections are asymptomatic. EBV is present in at least 90% of humans, although rates of seropositivity are lower among younger individuals and in more affluent populations. Once infected, an individual carries the virus for his or her entire life (Callan, 2004). Although most individuals remain asymptomatic, EBV has also been linked to chronic fatigue syndrome and some lymphomas (Thompson & Kurzrock, 2004). Increased levels of psychosocial stress have been associated with viral activation due to reductions in cell-mediated immune function, and measuring antibodies to EBV therefore provides an indirect measure of an aspect of cell-mediated immune function (Glaser et al., 1991, 1993). Levels of immunoglobulin G antibody to the viral capsid antigen complex of the EBV were determined using an ELISA protocol previously validated for use with DBS (McDade, Stallings, & Worthman, 2000). Distribution of EBV levels in the NSHAP sample are provided in Table 4.

HbA1c.—Glucose is a sugar molecule that circulates in blood until it is used by cells for energy. Effective control over glucose concentrations is important for health, and high blood sugar after fasting is a sign of diabetes. Hemoglobin (Hb) is the protein that carries oxygen in the blood, contained in red blood cells. Normally, a small percentage of the Hb molecules in red blood cells become glycosylated (that is, chemically linked to glucose). The percent of glycosylated hemoglobin (HbA1c) increases over time in response to higher concentrations of circulating glucose. HbA1c, thus, can provide an integrated measure of blood sugar levels over the past 2–3 months. Recent work has linked elevated HbA1c levels to cardiovascular disease, independent of diabetes status (see Grant et al., 2004). Here, HbA1c is used to measure long-term blood glucose control—useful as a marker of blood sugar regulation, and therefore of how well diabetes is being managed, and as a marker of "prediabetes" (Yoshinaga & Kosaka, 1996). An HbA1c level of >7% (in total Hb) is associated with diabetes (Ford & Mokdad, 2003). According to the 3rd wave of the National Health and Nutrition Examination Survey (NHANES III), 4.2% of the total population have reached this level of HbA1c, whereas the percentage

Table 5. Summary Statistics for HbA1c Levels (% of Total Hemoglobin)

HbA1c (% of Total Hb)	Range	Mean (Weighted)	*SD*
Men (years)			
Ages 57–65	4.70–13.4	6.21	1.39
Ages 66–75	4.60–11.4	6.07	0.89
Ages 76+	4.20–14.2	6.09	1.02
Women (years)			
Ages 57–65	4.50–12.5	5.99	0.99
Ages 66–75	4.50–11.4	5.91	0.82
Ages 76+	4.60–13.5	6.04	0.82

of those with diagnosed diabetes with >7% HbA1c is 56.4%. In a sample of inner-city residents, Grant et al. found that 3.4% of people without diabetes had elevated HbA1c levels (Grant et al., 2004). However, these associations have not been demonstrated for values HbA1c levels obtained from DBS samples. To prevent further glycosylation of hemoglobin after collection on filter paper, one spot on the filter paper was pretreated with a proprietary solution provided by Flexsite Diagnostics (Palm City, FL). Analyses of HbA1c values were determined at Flexsite Diagnostics using an in-house protocol developed for the Cobas Integra blood chemistry analyzer. Based on previous research, Flexsite reports high correlation of results when compared with clinical standard methods (see http://www.flexsite.com/Professional_A1c.html for details). Distribution of HbA1c levels in the NSHAP sample are provided in Table 5.

Hemoglobin.—Hemoglobin is the protein that carries oxygen to the blood, contained in red blood cells. Both high and low levels of hemoglobin signal defects in the balance of red blood cells in the blood. Anemia, indicated by hemoglobin deficiency, is an increasing health concern in the United States. In older populations, anemia is an independent risk factor for death (Lipschitz, 2003) and decline in physical performance (Penninx et al., 2003). It is associated with decrease in myocardial function (Penninx et al., 2003), poor outcomes in many chronic diseases (Goodnough & Nissenson, 2004), and muscle density (Cesari et al., 2004). Prevalence of anemia increases with age (Penninx et al., 2003), but estimated prevalence rates for older populations in the United States vary widely from 3.9% to 59.9% depending on the population studied and criteria used for defining anemia (Beghe, Wilson, & Ershler, 2004). Hemoglobin was quantified in DBS samples using a colorimetric method based on the conversion of hemoglobin to cyanmethemoglobin in the presence of Drabkin's solution (O'Broin & Gunter, 1999). Distribution of hemoglobin levels in the NSHAP sample are provided in Table 6.

Table 6. Summary Statistics for Hemoglobin Levels (mg dL^{-1})

Hemoglobin (mg dL^{-1})	Range	Mean (Weighted)	*SD*
Men (years)			
Ages 57–65	8.89–18.5	13.8	1.76
Ages 66–75	7.65–18.7	13.2	1.92
Ages 76+	7.56–17.0	12.6	1.72
Women (years)			
Ages 57–65	7.89–18.3	12.6	1.63
Ages 66–75	4.50–18.4	12.3	1.81
Ages 76+	6.58–16.4	12.0	1.66

Analytical Issues

Because of the modularized design, blood spots were not requested of all NSHAP participants, and other sections of the survey were not completed on all individuals from which blood spots were requested. For example, on 500 of the individuals from which blood spots were requested, no sensory function or "Get Up and Go" tests were administered. As a result, sample sizes might be significantly different for analyses using these variables.

Care should be taken when attempting to compare values derived from DBS samples and other sources. Although these methods have been previously validated against clinical standards, such as plasma or serum, they are not equivalent to these methods, and the relationship may vary across populations (Shirtcliff et al., 2001). The source and method of comparison, sample size used for validation, and strength of statistical relationship should be considered before comparisons are made.

Conclusions

DBS sampling was used as an alternative to venipuncture in the NSHAP to obtain several biomeasures useful in the assessment of health and risk for development of chronic diseases. In most cases, this method proved to be a viable alternative to venipuncture for both FIs and study participants, although proper and sufficient training for interviewers is vital to the success of this method. The feasibility of both collection and analysis of DBS provides results that are valid and useful in community-based studies. However, care should be taken when using these data because these methods are not directly comparable to clinically standard methods. By providing these measures, we hope that when combined with the breadth of available social measures and self-reported measures collected in NSHAP, we are providing useful and important data to explore in an attempt to understand health outcomes in aging adults.

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