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What a drop can do:
Expanding options for the analysis of blood-based biomarkers in population health research

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Abstract

Methodological constraints associated with the collection and analysis of biological samples in community-based settings have been a significant impediment to integrative, multi-level biodemographic and biobehavioral research. However, recent methodological developments overcome many of these constraints, and have expanded the options for incorporating biomarkers into population-based health research. In particular, dried blood spots—drops of whole blood collected on filter paper from a simple prick of the finger—provide a minimally-invasive method for collecting blood samples in non-clinical settings. After a brief discussion of biomarkers more generally, we review procedures for collecting, handling, and analyzing dried blood spot samples. Advantages of dried blood spots—compared to venipuncture—include the relative ease and low cost of sample collection, transport, and storage. Disadvantages include requirements for assay development and validation, and the relatively small volumes of sample. We present the results of a comprehensive literature review that identified over 100 analytes with existing protocols for analysis in dried blood spot samples. We provide more detailed analysis of protocols for 45 analytes likely to be of particular relevance to population-level health research. Our objective is to provide investigators with the information they need to make informed decisions regarding the appropriateness of blood spot methods for their research interests

Introduction

In the social and behavioral sciences it has long been fashionable to claim that the nature-nurture controversy has been laid to rest, or to point to the fallacy of mind-body dualism. But rarely does current research embody these ideals. Social, cultural, and economic factors are widely recognized as critical determinants of human development and health (Berkman and Glass 2000; Moen et al. 1995; Mosley and Chen 1984; Seeman and Crimmins 2001), but few studies consider these factors in relation to objective measures of physiological function. Consequently, little is known about the processes or pathways through which social contexts "get under the skin" to influence our physical development, health, and well-being.

Biomedical research, on the other hand, features sophisticated assessment of physiology, but typically relies on clinic-based samples, and rarely incorporates detailed evaluation of social contexts beyond standard measures of socio-economic status or self-reported health behaviors. Epidemiology has a long-standing tradition of large-scale measurement of physiology and health, and social epidemiology in particular draws on conceptual tools from the social/behavioral sciences. An overarching emphasis on the more proximate determinants of disease distinguishes epidemiology from the broader mission of the social/behavioral sciences to conduct theory-based research into the biological, psychological, social, cultural, and political-economic contexts and processes that inform general principles of human behavior and social action.

However, these distinctions are becomingly increasingly difficult to make. A number of scholars, as well as recent initiatives from the National Institutes of Health, have advocated a more integrative, multi-method, interdisciplinary approach to research in human health that draws on the biomedical as well as social/behavioral sciences (Cacioppo et al. 2000; National Research Council 2001; Seeman and Crimmins 2001). The expansion of methodological options

for collecting biological samples in non-clinical settings has facilitated this effort (National Research Council 2000), and encouraged a growing number of social scientists to consider integrating biomarkers into their research agendas.

Dried blood spots—drops of whole blood collected on filter paper from a simple prick of the finger—represent such an option, and have been incorporated into a number of large population-based surveys (Table 1). After a brief discussion of biomarkers more generally, we review procedures for collecting, handling, and analyzing dried blood spot samples. We highlight the advantages as well as disadvantages of blood spots, and present the results of a comprehensive literature review of existing laboratory protocols for analyzing a wide range of biomarkers. Our objective is to provide investigators with the information they need to make an informed decision regarding the appropriateness of blood spot methods for their research interests.[1]

Why biomarkers?

There are many compelling reasons to consider integrating biomarkers into social and behavioral science research. First, biomarkers can shed light on the reciprocal links between environments and health by illuminating the physiological imprint of social experience. For example, biomarkers can document the physical consequences of poverty, unsupportive social relationships, or negative health behaviors. They can help identify individuals inhabiting adverse psychosocial environments, and reveal which aspects of these environments are most toxic. They can identify resiliency factors that buffer individuals from these exposures. Conversely, biomarkers may reveal the extent to which aspects of health shape individual life course

[1] Our emphasis is on blood-based biomarkers of physiological function. For excellent discussions of issues related to collecting genetic information in conjunction with survey research see Ewbank (2001) and Wallace (2001).

trajectories and inform selection into various social environments. The implementation of objective, "hard science" data may be particularly effective in mobilizing the attention of policy makers and informing interventions around important social issues.

Second, biomarkers provide direct information on predisease pathways that are causally proximate to a wide range of important health outcomes. Self-reports rely on subjective, conscious experience, whereas biomarkers tap into physiological processes that may be below the threshold of perception, but are nonetheless predictive of current or future disease. Longitudinal studies of cardiovascular disease (CVD) reveal that an individual's relative rank on biomarkers of CVD (e.g., blood pressure, lipids) tends to remain stable, or "track" from childhood into adulthood (Berenson et al.; Li et al. 2004). While the measurement of these biomarkers early in life may not uncover many clinical cases of CVD, it will identify individuals most at risk for the future development of disease. Biomarkers provide insight into critical physiological mechanisms through which social contexts exert their influence on health.

Third, biomarkers are not susceptible to many of the shortcomings associated with self-reported health measures. Since they represent objective indicators of health that are beyond the conscious control of research participants, they do not rely on the participant's ability to recall relevant health information, or their willingness to share this information. In some cases biomarkers may be useful in validating self-reports, but more often they will offer access to embodied information that is below the threshold perception, but that is nonetheless meaningful for physical health. This is a particular advantage for research with children, and for settings where linguistic and/or cultural factors help define idealized states of health, and may contribute to variation in the perception, experience, and/or reporting of health (Hahn 1995; Kleinman 1986). Along these lines, biomarkers provide a common metric for comparison across time and

space that is not confounded by issues related to self-report. This is not to say that biomarkers represent a higher order of evidence; rather, they are complementary to subjective measures, each of which has its own set of strengths and weaknesses.

Fourth, the development of minimally-invasive methods of sample collection facilitates the implementation of biomarkers into community-based research across a wider range of populations. The vast majority of current knowledge regarding human biology and health is based on research with clinical populations, or opportunistic samples of convenience. These samples fail to encompass the full range of human experience and, as cross-cultural research on human reproductive function has shown, a broader conceptualization of the ecology of human biology can lead to fundamental insight into the development and regulation of critical physiological systems (Campbell and Wood 1994; Ellison 2001; Konner and Worthman 1980). Drawing larger, more diverse, representative samples increases the generalizability of research findings, and may identify subgroups of individuals, or subsets of environments, that merit special attention. To the extent that this is a priority, the burden is on the researcher to bring our methods to people in the community, rather than relying on select individuals willing to come to the clinic or lab.

Lastly, biomarkers encourage productive collaboration among social, life, and biomedical scientists. Health is a quintessentially multidimensional, and collective efforts that bridge disciplinary boundaries promote innovation that may cast new light on intractable health problems, and provide new perspectives on important psychosocial, behavioral, and cultural processes.

Why not?

First, biomarkers add to respondent burden, and may impose additional risks to research participation. These burdens and risks will vary across methods of sample collection, but in many cases—particularly with the collection of saliva or finger prick blood samples—they are minimal. However, they are not non-existent, and it is important to consider the implications of biomarkers for sample recruitment and retention. Although we often think of biological measures as inherently more invasive than self-report measures, it is worth noting that for many respondents, slight physical discomfort may be preferable to the psychological discomfort associated with disclosure of embarrassing or otherwise sensitive information.

Second, biomarkers add to the logistical challenges associated with data collection. In many cases, survey interviewers can be trained to collect biomarkers from participants along with questionnaire data, but this will add to interview training requirements and increase the amount of time required for data collection. Important biosafety issues must be addressed to protect interviewers and other personnel handling biospecimens from potential risks of infection. Once collected, biological samples must be handled and transported following procedures that maintain sample integrity. These are tractable logistical issues, but they add to the complexity of data management.

Third, biomarkers are costly. In addition to laboratory costs for sample analysis, there are additional costs associated with sample collection, transport, and storage. Supplies for collecting DBS samples, for example, cost approximately $1.50-$2.00 per participant. Laboratory analysis, including labor and materials, ranges from approximately $5 to $20, depending on the biomarker and assay system. Laboratory-grade freezers for sample storage cost $3,000 to $5,000.

Fourth, as with any research involving potentially sensitive information, biomarkers raise important ethical issues that require careful consideration (Botkin 2001). For example, are appropriate safeguards in place to protect privacy? Will participants be informed of test results, particularly results with clinical implications? Will samples be stored for future analyses, and will investigators contact participants before conducting these analyses? Are risks associated with sample collection clearly outweighed by benefits to the participant and/or society?

In sum, the financial and logistical costs associated with biomarker collection and analysis require that they be implemented only in the service of a well-articulated research agenda. Many of these costs are attenuated considerably with the recent development of minimally-invasive methods of sample collection, and such methods may tip the balance of costs and benefits in favor of implementing biomarkers in some cases. However, if the scientific payoff of adding biomarkers to a particular project is not clear, then an investigator may be better off relying on well-established self-report methods.

Finger stick dried blood spots as an alternative to venipuncture

While blood spots are relatively new to population-level survey research, their application dates back to the early 1960's when Dr. Robert Guthrie first began collecting heel prick blood spot samples from newborns to detect phenylketonuria (Guthrie and Susi 1963). This effort has led to a nationwide screening program in which dried blood spot samples are collected from all newborns and then evaluated for a number of treatable metabolic disorders (Mei et al. 2001). Filter papers have been a central component of this major public health initiative for nearly forty years.

Collection papers are manufactured from high purity cotton linters, and are certified to meet performance standards for sample absorption and lot-to-lot consistency set by the National Committee on Clinical Laboratory Standards. The Centers for Disease Control and Prevention (CDC) maintains an independent quality control program, and notes that "The filter paper blood collection device has achieved the same level of precision and reproducibility that analytical scientists and clinicians have come to expect from standard methods of collecting blood, such as vacuum tubes and capillary pipettes" (Mei et al. 2001) p. 1631).

Sample collection, processing, and storage

Procedures for sample collection and processing are relatively uncomplicated. The participant's finger is cleaned with isopropyl alcohol, and then pricked with a sterile, disposable lancet of the type commonly used by diabetics to monitor blood glucose. These lancets are designed to deliver a controlled, uniform puncture that stimulates sufficient capillary blood flow with minimal injury. The first drop of blood is wiped away, and up to five drops (~50uL per drop) are applied to filter paper (formerly Schleicher and Schuell #903, now Whatman #903). The samples are allowed to dry (four hours to overnight), at which point they can be stacked and stored with desiccant in resealable plastic bags or plastic containers.

While sample collection is straightforward, potential sources of error should be recognized and steps taken to minimize their occurrence. First, proper placement of whole blood on the filter paper is essential. The uniform absorbing properties of the filter paper will be defeated if blood is blotted or smeared onto the paper, or if a drop of blood is placed on top of a previously collected drop. Instead, blood should be drawn onto the filter paper by capillary action, with no contact between the finger and paper.

Second, an effort should be made to collect blood spots of comparable size, since the volume of whole blood applied to filter paper as a blood spot has a small effect on the volume of serum contained within a single disc punched out of that spot (Adam et al. 2000). Variation in blood spot size can be minimized by collecting samples on filter papers with pre-printed circles as guides to standardize the volume of whole blood collected from each individual. When filled to its border, each circle will contain approximately 50uL of whole blood.

The filter paper matrix stabilizes most analytes in dried blood spots, but the rate of sample degradation will vary by analyte. It is essential that stability be evaluated prior to sample collection, as this has direct implications for sample handling and storage. For example, antibodies against the Epstein-Barr virus (an indirect measure of cell-mediated immunity) are stable in blood spots stored at room temperature for at least eight weeks (McDade et al. 2000). However, samples begin to deteriorate after 1 week of storage at 37°C. In contrast, concentrations of C-reactive protein decline significantly in dried blood spots after 3 days at 37°C, but are stable for at least two weeks at room temperature (McDade et al. 2004). While it is always advisable to refrigerate or freeze samples when possible to minimize the chances of degradation, the stability of most analytes in dried blood spots provides flexibility in the collection of samples in field settings.

For long term storage, it is important to freeze samples in a reliable laboratory grade freezer to ensure sample integrity. Food freezers are not acceptable since these units typically do not maintain a consistent temperature, and may have automatic defrost cycles that lead to sample thaw. As with any biological sample, repeated cycles of freezing and thawing are to be avoided, although the filter paper matrix appears to provide a degree of protection against sample degradation that is not present with liquid blood samples. For example, prior validation studies

have found that concentrations of C-reactive protein, antibodies against the Epstein-Barr virus, and transferrin receptor in blood spots show no evidence of deterioration through at least six cycles of freeze/thaw (McDade et al. 2000; McDade et al. 2004; McDade and Shell-Duncan 2002). A standard 27 cubic foot lab freezer can hold 8,000 to 10,000 samples.

Requirements for shipping blood spot samples are relatively minimal unless the samples are known to contain an infectious or etiologic agent. Samples from normal, healthy individuals are considered "diagnostic specimens," and must be labeled as such for shipment. Filter papers stored in plastic bags and sealed in a secondary container (e.g., bond envelope, cardboard box) can be shipped domestically without special packaging or permitting. With respect to importing samples from overseas, the Centers for Disease Control and Prevention (CDC) will issue importation permits, although such permits may only be required under certain circumstances. Up-to-date shipping and importation guidelines are available from the CDC (http://www.cdc.gov/od/ohs/biosfty/biosfty.htm).

Analysis of blood spot samples and requirements for assay development

Laboratory protocols for the analysis of blood spot samples are comparable to plasma/serum protocols, with some important exceptions. First, since the sample has been dried on filter paper, analytes must first be brought into solution. A standard hole punch is typically used to cut out discs of whole blood of uniform size (occasionally an entire blood spot is cut into smaller pieces), and one or more discs are placed into an elution buffer for a fixed amount of time. In effect, the dried blood spot is reconstituted as hemolyzed liquid whole blood, which can then be used in various protocols across multiple assay systems, much as plasma or serum would be.

In most cases, an analyte that can be measured in serum or plasma can also be assayed in dried blood spot samples. However, there are potential obstacles that in some cases may prove to be insurmountable exceptions. First, will the presence of red and/or white blood cells interfere with the assay? Whole blood is comprised of liquid and cellular fractions, and centrifugation of samples collected through venipuncture removes cellular components to yield serum or plasma (serum and plasma are comparable in this regard, the difference being that clotting factors have also been removed in serum). When whole blood samples are dried on filter paper, cellular elements rupture and their components are subsequently released into solution when blood spot samples are reconstituted. Different assay systems and specific analytes will vary in their sensitivity to potential interference, and some assays may require additional processing prior to analysis. This is not a common problem, although the presence of lysed red blood cells has proven to be an insurmountable obstacle in the measurement of ferritin in dried whole blood (Ahluwalia 1998).

Second, will the analyte come off the filter paper and enter solution in a form suitable for analysis? Drying may alter the biochemical structure of a molecule, and the efficiency with which analytes enter solution will vary. Elution protocols need to be evaluated to establish the optimal combination of elution duration (e.g., 2 hours, 4 hours, overnight), mixing (e.g., end-over-end, orbital, no mixing), temperature (e.g., room temperature, 4ºC), and buffer constituents (e.g., phosphate-buffered saline, Tris, Tween-20) that maximizes the efficiency of elution.

Lastly, will the analyte come of the filter paper in sufficient quantities for analysis? The relatively recent development of highly sensitive and specific immunoassays has facilitated analysis of biomarkers in small, microliter quantities of blood, but there are limits. One 3.2 mm disc punched out of a dried blood spot will contain approximately 1.4 uL of serum (Mei et al.

2001). Multiple discs, or a larger hole punch, can be used to increase sample volume, but an assay that normally requires 50 or 100 uL of undiluted plasma is not likely to be easily adapted to use with blood spots.

Above and beyond these obstacles are standard aspects of assay performance that should be used to evaluate the performance of any analytic protocol (Nexo et al. 2000; Vikelsoe et al. 1974). While investigators can expect performance that is comparable to that obtained with serum/plasma samples, this may not always be possible. In such cases, the benefits of blood spot methods with regard to sample collection and handling will have to be weighed against the degree of potential error introduced during sample analysis.

Advantages of dried blood spots for field-based research

Key biomarkers of physiological function and health are accessible only through the analysis of blood, and the collection of several milliliters of plasma or serum through venipuncture is the current clinical standard. The collection of dried blood spot samples via finger prick is a viable alternative, and offers the following advantages:

1) Sample collection is relatively painless and non-invasive, and can be conducted by non-medically trained interviewers in the participant's home, or in some cases, by participants themselves. This is a particular advantage for research with infants, children, and the elderly, for whom venipuncture may be particularly problematic, and for research in remote or underserved communities where the logistics of venipuncture may limit access to willing participants.

2) Unlike plasma or serum, blood spot samples do not need to be centrifuged, separated, or immediately frozen following collection. Similarly, requirements for shipping are minimal, and a cold chain from the point of sample collection to receipt in the laboratory is not required.

Drops of whole blood are simply applied to filter paper, allowed to dry, and then stacked and stored. Most analytes remain stable at room temperature for a week or more, providing considerable flexibility in procedures for sample collection and transport.

3) Blood spot samples remain stable in laboratory freezers for long periods of time, and can be analyzed down the road as new biomarkers of interest emerge. A typical drop of blood will contain approximately 50 uL of whole blood, and will result in a dried blood spot approximately 12 mm in diameter. Such a spot will yield seven 3.2 mm discs of blood. A full card of five blood spots will therefore contain enough sample to analyze 35 analytes requiring one 3.2 mm disc, or 17 analytes requiring two such discs. However, in practice, five perfect blood spots are rarely obtained, and sufficient sample for 10 to 20 3.2 mm discs is a more reasonable expectation for a single finger prick. In a recent analysis of nearly 2,000 samples, participants provided blood spots that yielded on average 14 usable 3.2 mm discs (Williams et al. 2006).

4) A single finger prick can provide capillary whole blood for spots on filter paper, and for the onsite assessment of biomarkers using portable "point-of-service" instruments. Affordable, portable instruments for the analysis of hemoglobin, HbA1c, and lipid profiles are currently available that provide an opportunity to collect physiological information away from the lab. Using the same finger prick sampling procedure detailed above, a fraction of a drop of blood can be placed into one of these instruments, with subsequent drops applied to filter paper. By combining these procedures, biomarker results can be collected onsite and shared with participants, while blood spots can be assayed in the lab for a broader range of biomarkers. In some cases this may provide a valuable health screening service, and act as an incentive for research participation.

Disadvantages of dried blood spots

Advantages of blood spots vis-à-vis venipuncture need to be weighed against potential disadvantages, including the following:

1) The vast majority of standard laboratory protocols require serum or plasma, and assay protocols must therefore be developed specifically for blood spots, and validated for accuracy, precision, reliability, and limits of detection. This is a relatively methodical process that can take several weeks of dedicated effort. However, as noted above, most analytes that can be measured in serum or plasma can also be quantified in dried blood spots, subject to some important limitations.

2) From a clinical perspective, dried blood spots are a non-standard diagnostic substance, and blood spot results may not be directly comparable to those derived from plasma. However, correlations between plasma and blood spot concentrations of most analytes in matched samples derived from the same individuals are linear and high (e.g., Pearson R=0.93 to 0.99 for nine analytes related to reproductive function; Worthman and Stallings 1997)), and correction factors can be applied to blood spot values to derive plasma equivalents if desired.

3) Due to requirements for assay development, blood spot samples may constrain flexibility for future biomarker measurement. Assays for cutting edge biomarkers will almost certainly first be available for serum/plasma, and there will be a lag before comparable methods are developed for blood spots. The relatively small quantity of sample collected with blood spots may also be an insurmountable limitation for some analytes that require large volumes of blood, particularly in the early stages of research before more sensitive protocols come online.

Available protocols for the analysis of dried blood spots

In this section we provide an overview of current options for the analysis of biomarkers in dried blood spot samples. Table 2 includes a list of all analytes for which a blood spot method has been published. We compiled this list by drawing on previous reviews, searching Medline, and pursuing methods referenced in papers collected through this process.

From this list we selected for closer inspection the methods of those analytes most likely to be of interest to researchers conducting population-level, community-based health research (Table 3). We based this selection on four criteria. First, the method has to use capillary whole blood collected on filter paper. This criterion eliminated analytes (e.g., ferritin) that require the separation of red blood cells prior to application to filter paper since this step adds to the burden of sample collection.

Second, we focused on markers of physiological function and health that are broadly relevant across a wide range of ages. We therefore do not include markers of inborn errors of metabolism commonly used for neonatal screening, nor do we evaluate markers of toxicology. We also do not include clinical markers of specific diseases, unless they are likely to be relevant at the population level (e.g., HIV, hepatitis). DNA and RNA are readily extracted from dried blood spots, although we did not review these methods since they are relatively straightforward and comparable, and differ primarily with respect to the application of specific primers for molecular markers of interest.

Third, some attempt at assay validation had to be evident, including a report of accuracy, precision, reliability, and/or analysis of matched blood spot and serum/plasma samples. All of these need not be present, but we were looking for attention to the importance of evaluating assay performance. And finally, we required that methods in Table 3 were subjected to peer review. We recognize a certain subjectivity in our decisions regarding what to include in Table

3, and we therefore list references to all blood spot methods in Table 2 so investigators can evaluate other protocols that may be of interest.[2]

Table 3 includes information on multiple aspects of assay performance and implementation. Our goal was to document the current range of validated methods, and to provide key information for each biomarker that would be useful in making decisions regarding its utility and feasibility. We made an effort to stay as close to the original publication as possible, and investigators should refer to the original publications for additional details. Specifically, we extracted information on the following:

Volume of sample: A typical drop of capillary blood collected from a finger stick includes approximately 50uL of whole blood. Most assays use a hole punch to produce a disc of dried blood of a given size for analysis, while others use the entire spot. In this column we report the amount of dried blood sample required for analysis. Linear dimensions (i.e., mm or inches) pertain to methods using a hole punch, while volume measures (i.e., uL) are presented for methods that use an entire blood spot containing a pre-measured quantity of whole blood.

Stability: Here we report the stability of analytes in dried blood spots stored at room temperature, and when refrigerated (~4°C). There are no standardized criteria for acceptable levels of sample degradation, so we rely on the stability determination as published. In many cases, the reported stability reflects the maximum period of time evaluated, and therefore actually stability may be significantly longer. In addition, for some analytes, stability

[2] Regularly updated versions of these tables are available at http://www.northwestern.edu/ipr/c2s/biomarkers. The corresponding author welcomes suggestions for additional blood spot methods to include.

information is presented in other publications not included here because they did not meet our criteria for inclusion in Table 3.

Analytic method: There are multiple platforms for biomarker analyses, and labs vary in capabilities according to their investment in specific analytic systems and technologies. We note, in general terms, the analytic methods applied to each analyte since this may be a limiting factor for some labs.

Intra-assay CV: The precision of an assay can be estimated by calculating the coefficient of variation (CV; standard deviation/mean) of multiple determinations of a single sample, all run in a single assay (NCCLS). This is typically done with multiple samples across the full range of measurable values, but for ease of presentation, and since investigators differ in the number of samples they use to determine precision, we present the simple average intra-assay CV for each method. It is important to note, however, that the precision of an assay may vary across the assay range, and precision is often poorer at lower concentrations.

Inter-assay CV: The day-to-day variation, or reliability, of a method can be estimated by calculating the CV of multiple determinations of a single sample run on different days. As with precision, we present the average inter-assay CV as an approximation of assay reliability.

Lower detection limit: Sometimes referred to as analytical sensitivity[3], the lower detection limit of an assay is the smallest concentration of analyte that can be differentiated from zero with confidence. This is typically defined as the quantity of analyte that corresponds to a signal that is two or three standard deviations above the mean signal derived from multiple determinations of a sample free of analyte. The evaluation of lower detection limit is particularly important for analytes that circulate at low concentrations. In such cases higher volumes of sample may be necessary for acceptable assay performance, and this may prove to be an impediment to the development of a blood spot method due to the relatively low volume of collected sample.

Blood spot/plasma comparison: The comparison of blood spot assay results with those from matched, simultaneously collected serum or plasma samples using a previously established, "gold standard" method is an excellent validation tool. Statistical evaluation of this relationship is typically performed with linear regression, or by inspecting residual plots for evidence of bias or inconsistent variability across the range of measurement {Bland and Altman 1986). Analysis of matched blood spot and plasma/serum samples can also be used to generate a conversion formula to derive plasma-equivalent values from results with blood spot samples (Worthman and Stallings 1997). However, caution should be used in the application of plasma equivalents, since the relationship will vary across analytic methods, and may vary across populations. In some cases, liquid whole blood is used for comparison with blood spot results instead of serum/plasma. These cases are indicated in the table.

[3] Analytical sensitivity is technically defined as the degree to which a method produces a change in signal for a defined change in analyte quantity (e.g., the slope of the calibration curve), whereas lower detection limit is the smallest quantity of analyte that can be reasonably distinguished from zero (ref: International Union of Pure and Applied Chemistry)

Protocol: Is the blood spot method presented in sufficient detail that a lab with appropriate analytic capabilities could reasonably expect to implement the method with success? We answer "no" if key information is missing that would require investigators to contact the method's developers, or implement additional assay development steps prior to application.

Reagent availability: Are all the materials required for the assay commercially available, or were key reagents (e.g., antibodies, calibrators) developed in-house? We answer "yes" if all reagents could be purchased from established suppliers at the time of publication. This is subject to change, as in-house reagents (or acceptable substitutes) may become available over time, and investigators are often generous in sharing their reagents. Conversely, previously available reagents for older methods may be difficult to obtain.

Our literature search yielded published blood spot methods for over 100 analytes, not including methods for DNA or RNA (Table 2). We provide additional information on assay performance and implementation for 45 analytes most likely to be of relevance to population-based health research (Table 3). A wide range of biomarkers are represented, including important indicators of endocrine, immune, reproductive, and metabolic function, as well as measures of nutritional status and infectious disease. Many of these biomarkers are applied clinically, and may be used in population research to determine risk for the development of disease, or to gain insight into the impact of psychosocial/behavioral contexts across multiple physiological systems.

These protocols use standard clinical chemistry methods, and for many analytes multiple protocols have been developed that allow analysis with different assay systems. Reagents are

readily available and protocols are published with sufficient detail for most analytes such that their implementation is feasible, assuming access to the appropriate equipment and technical expertise. It is worth re-emphasizing, however, that investigators should independently evaluate any protocol before assuming it can be used in their research.

Stability on filter paper has been evaluated for most analytes, and varies widely. For the vast majority, sample degradation is minimal at room temperature for at least 2 weeks. Refrigerator storage tends to extend this period.

A few of the methods in Table 3 take advantage of recent innovations in immunoassay technology that make it possible to quantify simultaneously multiple analytes in one sample, rather than analyzing one analyte at a time (Bellisario et al. 2000). This advance is made possible by the Luminex flow analyzer, which incubates samples with multiple sets of polystyrene microspheres, each of which has a unique fluorescent signature. Data are acquired by running the samples through the flow analyzer which identifies each microsphere set, and quantifies the amount of bound analyte. The increased sensitivity, reduced cost, and low sample volume requirements afforded by this technology ameliorate some of the limitations of blood spots, and promise to expand the range of factors that can be measured.

Conclusion

Methodological tools that advance interdisciplinary, multi-level research in population health are currently in high demand. Survey research has historically relied on self-reports of health, but minimally-invasive methods that facilitate the direct, objective measurement of physiological processes in naturalistic settings are expanding the range of possibilities. These methods bridge the biomedical and social/behavioral sciences—drawing on the strengths of

both—to open up innovative new research directions that will ultimately lead to a richer, multidimensional understanding of human biology and health.

Dried blood spots represent such a method, and a growing number of population-based studies are adding them to their data collection protocols. Community-based research that collects biological specimens in participants' homes places a premium on the ease of sample collection, storage, and transport. For many biomarkers, blood spot sampling provides a viable alternative to venipuncture, particularly as the long list of physiological and genetic markers that can be assayed in blood spot samples continues to grow.

Any approach to collecting information on health represents a reasonable compromise between the desire to maximize accuracy and validity, while minimizing costs in terms of time, money, and participant burden. We review the advantages and disadvantages of blood spots so investigators can make informed decisions regarding the appropriateness of blood spots for their own research goals and settings. Dried blood spots provide a "field-friendly" option that may alter the terms of this compromise for some investigators. It is our hope that these methods help pave the way for a new generation of research that investigates the complex intersections of human behavior, society, and health.

Table 1. Recent applications of dried blood spot (DBS) sampling in large population-based studies in the U.S..

Study	N*	Age range	Biomarkers in DBS
Great Smoky Mountains Study	1071	9-15 years	Androstenedione, DHEA-S, EBV antibodies, estradiol, FSH, LH, testosterone
Health and Retirement Study	7,000+	>50 years	CRP, HbA1c, Total cholesterol, HDL
Los Angeles Family and Neighborhood Survey	5,000+	3 years and up	CRP, EBV antibodies, HbA1c, Total cholesterol, HDL
National Longitudinal Study of Adolescent Health	17,000+	32-31 years	CRP, EBV antibodies, HbA1c
National Social Life, Health, and Aging Project	2,000	57-84 years	CRP, EBV antibodies, HbA1c, hemoglobin

*For DBS sampling
+Projected

Table 2. List of analytes for which a dried blood spot method has been published (not including analytes listed in Table 3) (*references pending*).

Acyl Glycines
Adenine Phosphoribosyltransferase
Adenosine Deaminase
Amino Acids
Amodiaquine
Benzoylecgonine (Cocaine)
Biotinidase
Brucella Antibodies
Carnatine/Acylcarnatine
Ceruloplasmin
Chloroquine
Chlorpheniramine
Creatinine Kinase
Cytokines
Cysticercus Antibodies
Cytomegalovirus Antibodies
Dichlorodiphenyldichloroethylene
Dihydropteridine Reductase
Diptheria Antibodies
Erythrocyte Protoporphyrin
Fatty Acids
α-Fetoprotein
Filariasis Antibodies
Galactose-1-phosphate Uridyltransferase
α-D-galactosidase A
Gentamicin
Hemoglobin Variants
Homocystine
Hexosaminidase A
β-Human Chorionic Gonadotripin
3-hydroxybutyrate
Hypoxanthine-guanine Phosporibosyltransferase
Lactate
17-Hydroxyprogesterone
Immunoreactive Trypsin
α-L-Iduronidase
Lead
Leishmania Antibodies
Measles Antibodies
Mefloquine
Netilmicin
Oligosaccharides
Onchocerca volvulus Antibodies
Phytanic Acid
Pristanic Acid
Proguanil
Protoporphyrin IX

Pseudomonas aeruginosa Antibodies
Purine Nucleoside Phosphorylase
Pyrimethamine
Quinine
Ring-infected Erythrocyte Surface Antigen Antibodies
Respiratory Syncytial Virus Antibodies
Rickettsial Antibodies
Rubella Antibodies
Sisomicin
Sulfadoxine
Syphilis Antibodies
Tetanus Antibodies
Theophylline
Thyroxine-binding Globulin
Toxoplasma gondii Antibodies
Treponemal Antibodies
Trichomonas vaginalis Antibodies
Trypanosoma cruzi Antibodies
Urea
Wuchereria bancrofti Antigen
Zinc Protoporphyrin

Table 3. Analytes most likely to be of relevance for population-based health research, and aspects of assay performance reported for their measurement in dried blood spots (*references pending*).

Analyte	Volume/size	Stability @ RT	Stability @ 4C	Method	Lower Det	Intra assay	Inter assay	DBS/ plasma	Protocol	Reagent avail
androstenedione	4 x 2.5mm	4 wks	8 wks	RIA	0.012 ng/mL	9.5	10.7	yes	yes	yes
	1 x 5.0mm			RIA	4 nmol/L	<10	<10	no	yes	no
	2 x 6.0 mm	> 3 mos	> 3 mos	RIA	0.6 nmol/L			yes	yes	no
apolipoprotein A-I	1 x 3.0mm	1 day	1 day	ELISA		5.2	14	yes	yes	yes
	2 x 6.0 mm		1 mo	immunonephelometry				yes	yes	no
	1 x 3.2 mm			immunoelectrophoresis		3.9	13.9	yes	yes	no
Lipoprotein (a)	1 x 3.0mm			ELISA	22 mg/L	4.5	4.4	yes	yes	yes
apolipoprotein B	1 x 3.0mm		20 days	ELISA		3.4	5.6	yes	yes	yes
	2 x 6.0 mm		1 mo	immunonephelometry				yes	yes	no
	1 x 3.0 mm			immunoelectrophoresis				yes	yes	yes
	1 x 3.0 mm		2 wks	immunoturbidimetry		7.8	12.5	yes	yes	yes
	1 x 3.0 mm			ELISA		5.2	7.8	yes	yes	no
	1x 3.2 mm			immunoelectrophoresis		4.9	16.5	yes	yes	no
cortisol	1 x 2.5mm	4 wks	8 wks	RIA	0.46 ug/dL	9	9.2	yes	yes	yes
	2 x 3.2			RIA	83 nmol/L	<11	<15	yes	yes	yes
CD4+ lymphocytes	?			ELISA				yes	no	yes
C-reactive protein	1 x 5.0 mm	>12 wks	>12 wks	ELISA	0.019 mg/L			yes	no	yes
	1 x 3.2 mm	>14 d	>14 d	ELISA	0.028 mg/L	5.8	8.2	yes	yes	yes
	2 x 3.2 mm			Luminex	1.1 ug/L	7.5	8.9	no	yes	yes
DHEA-S	1 x 2.5mm	4 wks	8 wks	RIA	8.0 ng/mL	7.5	9.4	yes	yes	yes
Folate	2 x 6.35mm	1 wk	1 wk	Microbiological assay		6.5	7.7	yes	yes	yes
FSH	1 x 2.5mm	8 wks	8 wks	FIA	0.13 IU/L	7.7	7.9	yes	yes	yes
Epstein-Barr virus antibodies	1 x 3.2 mm	8 wks		ELISA		5.6	7.6	yes	yes	yes
estradiol	8 x 1/8in			RIA	2 pg/mL	7.57	8.22	yes	yes	yes
	4 x 2.5mm	3 wks	8 wks	RIA	9 pg/mL	8.60	7.50	yes	yes	yes
glucose	1 x 6mm	3 wks		colorimetric		3.50	6.40	yes	yes	yes
	1 x 6.5 mm	7 d		colorimetric		3.60	4.20	yes	yes	yes
	1 x 6.0 mm			enzymic	0.26 mmol/L	2.90	3.10	yes	yes	yes
	1 x 10 mm		> 10 d	enzymatic fluorometric		3.00		yes	yes	yes
glycosylated hemoglobin/HbA1c	1 x 3.0 mm	5 d	10 d	HPLC				yes	yes	yes
	1 x 1/2 in	3 mos		colorimetric		< 6.0	< 6.0	no	no	yes
	1 x 6.0 mm			affinity chromatography		5.28		yes	yes	yes
hepatitis A antibodies				ELISA				yes	no	yes
				RIA				yes	no	yes
	2 x 25mm			ELISA				yes	yes	yes
hepatitis B antigen	25uL	>30d	>30d	RIA				yes	no	
	1 x 3.0mm			Luminex		21.00		yes	yes	yes
				RIA				yes	no	yes
	~ 8 mm	14 d	14 d	RIA				yes	no	yes
hepatitis B antibodies				RIA				yes	no	yes
	2 x 3.0mm			haemagglutination				yes	yes	yes
	~ 8 mm	14 d	14 d	RIA				yes	no	yes
hepatitis C antibodies	1 x 3.0mm			Luminex		21.00		yes	yes	yes

	1 x 5.5mm			ELISA				yes	yes	yes
	1 x 3.0mm			ELISA				yes	yes	yes
HIV antibodies	1 x 3.2mm			Luminex			6.00	no	yes	yes
	1 x 3.0mm			Luminex		21.00		yes	yes	yes
				Luminex			6.50	yes	yes	yes
	5 x 1.0cm			ELISA				yes	yes	yes
	1 x 5.0 mm			ELISA				yes	yes	yes
	1 x 14 mm		> 3mos	ELISA/immunoblot				yes	no	no
	1 x 1/8 in			EIA				yes	yes	yes
	1 x 20 uL			SEGLISA				yes	yes	yes
HIV antigen	1 x 6.0mm			Luminex				yes	yes	yes
homocysteine	1 x 3mm			HPLC		6.00	10.20	yes	yes	yes
	1 x 5.0 mm		4 wks	HPLC	51 nM	4.00	5.50	yes	yes	yes
IFNg	2 x 3.2 mm			Luminex	50 ng/L	6.70	24.50	no	yes	yes
IgE	50 uL			RAST				yes	yes	no
IGF-I	1 x 8mm	>4 wks	>4 wks	ELISA		7.50	11.40	yes	yes	yes
	1 x 8mm	>40 d	>40 d	ELISA				yes	yes	yes
	2 x 3.18mm	>5 d		RIA		8.10	8.70	yes	yes	yes
IGFBP-2	2 x 3.18mm	>5 d		RIA		4.20	6.00	yes	yes	yes
IGFBP-3	1 x 8mm	>4 wks	>4 wks	ELISA		8.20	7.50	yes	yes	yes
	1 x 3.18mm	>5d		RIA		7.60	4.60	yes	yes	yes
IL-1b	2 x 3.2mm			Luminex	26 ng/L	6.40	16.50	no	yes	yes
IL-6	2 x 3.2mm			Luminex	24 ng/L	7.60	16.50	no	yes	yes
insulin	3 x 0.5in			RIA		14.00	25.00	yes	yes	yes
	2 x 3.0mm			chemiluminescence	5.9 pmol/L	18.10	12.90	yes	yes	yes
LH	1 x 2.5mm	8 wks	8 wks	FIA	0.26 IU/L	7.30	8.90	yes	yes	yes
PSA	5 x 3.0mm	>24d	>24d	chemiluminescence	0.35 ug/L		11.70	yes	yes	yes
progesterone	8 x 1/8in			RIA	0.015 ng/mL	8.79	12.70	yes	yes	yes
	1 x 5mm	>9 wks	>9 wks	RIA	2.5 nmol/L	7.00	9.20	yes	yes	no
prolactin	2 x 12mm	>7d		immunoenzymetric				yes	yes	yes
	1 x 7.5mm			IRMA				yes	yes	yes
	2 x 3mm	7d	>12 wks	RIA	2 ug/L	7.33	12.23	yes	yes	yes
	1 x 2.5mm	3 wks	8 wks	FIA	0.11ng/mL	6.10	7.20	yes	yes	yes
retinol	1 x 6.35mm	< 1 wk		HPLC	0.1 umol/L	<6	<6	yes	yes	yes
SHBG	1 x 2.5mm	2 wks	8 wks	FIA	0.2 nmol/L	13.20	14.50	yes	yes	yes
somatomedin-C	2 x 3.2mm			RIA	0.05 unit/mL	6.20	6.50	yes	yes	no
testosterone	8 x 1/8in			RIA	0.015 ng/mL	6.18	8.06	yes	yes	yes
	4 x 2.5mm	3 wks	8 wks	RIA	6.3 ng/dL	7.60	12.70	yes	yes	yes
	2 x 20 uL	>1 wk		GCMS				yes	yes	yes
	1 x 7.9mm	>7 d		RIA	0.4 nmol/L	41.20	18.30	yes	yes	yes
transferrin receptor	2 x 3.2	>4 wks	>4 wks	ELISA	0.55 mg/L	6.60	8.20	yes	yes	yes
	25 uL	>4 wks	>4 wks	ELISA		4.48	5.55	yes	yes	no
thyrotropin (TSH)	2 x 3.2			Luminex	0.7 mIU/L	4.90		no	yes	yes
	1 x 3.0			RIA	10 mIU/L	7.10	12.70	yes	yes	no
	1 x 5.0 mm			chemiluminescence	2.9 mIU/L	10.40	7.40	no	yes	yes
	1 x 3.0 mm	< 7wks	> 11 wks	FIA				yes	yes	yes
	1 x 5.0 mm			EIA/FIA	1.25 mIU/L	7.80	6.90	yes	yes	yes
	1 x 6.5 mm			EIA	3.5 mU/L	7.60	11.70	no	yes	yes
	? x 3.0 mm			FIA		7.60	11.00	no	yes	yes
	1 x 4.0 mm			IRMA	1.0 mU/L	7.80	7.70	no	yes	no
	1 x 4.8 mm			immunoenzymetric	2.4 mU/L	7.50	8.70	no	yes	no

	1 x 1.0 cm			RIA	5 uU/mL			yes	yes	no
	2 x 5.0 mm			RIA	15 mU/L			yes	yes	yes
	3 x 3.0 mm			EIA		11.80	15.00	no	yes	yes
thyroxine (T4)	2 x 3.2			Luminex	10.3 nmol/L	8.20		no	yes	yes
	1 x 3.0			RIA	10 ug/L	11.70	9.75	yes	yes	no
	1 x 4.25mm			RIA	0.8 pmol/L	9.60	13.20	yes	yes	yes
	1 x 9mm	4 wks	4 wks	RIA	0.8 ng/L	5.30	6.20	yes	yes	yes
	2 x 3mm	4 wks	1 wk	EIA	1.9 ng/L	7.60	6.40	yes	yes	no
	1 x 7.0 mm			RIA	1.5 pg/mL	6.60	9.00	yes	yes	yes
	1 x 5.0 mm			RIA	8.37nmol/L			yes	yes	yes
	1 x 3.0 mm			ELISA	1.25 ug/L	5.80	6.80	no	yes	no
	1 x 2.0 mm			RIA				yes	yes	no
thyroglobulin	1 x 4.75mm			FIA	1.42 ug/L	<10	<20	yes	yes	yes
thyroxin binding globulin	1 x 6.5 mm			RIA	1.83 mg/L	6.70	5.70	yes	yes	yes
triiodothyronine (T3)	1 x 4.25mm		8 wks	RIA	0.48 pmol/L	13.60	16.00	yes	yes	yes
	5 x 7.0 mm			RIA	1.5 pg/mL	9.40	9.80	yes	yes	yes
TNFa	2 x 3.2mm			Luminex	34 ng/L	5.60	18.00	no	yes	yes

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