WHAT A DROP CAN DO: DRIED BLOOD SPOTS AS A MINIMALLY INVASIVE METHOD FOR INTEGRATING BIOMARKERS INTO POPULATION-BASED RESEARCH*

THOMAS W. MCDADE, SHARON WILLIAMS, AND J. JOSH SNODGRASS

Logistical constraints associated with the collection and analysis of biological samples in community-based settings have been a significant impediment to integrative, multilevel bio-demographic and biobehavioral research. However, recent methodological developments have overcome many of these constraints and have also expanded the options for incorporating biomarkers into population-based health research in international as well as domestic contexts. In particular, using dried blood spot (DBS) samples—drops of whole blood collected on filter paper from a simple finger prick—provides a minimally invasive method for collecting blood samples in nonclinical settings. After a brief discussion of biomarkers more generally, we review procedures for collecting, handling, and analyzing DBS samples. Advantages of using DBS samples—compared with venipuncture—include the relative ease and low cost of sample collection, transport, and storage. Disadvantages include requirements for assay development and validation as well as the relatively small volumes of sample. We present the results of a comprehensive literature review of published protocols for analysis of DBS samples, and 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.

Social, cultural, economic, and other environmental factors are widely recognized as critical determinants of human behavior, development, and health (Berkman and Kawachi 2000; Moen, Elder, and Luscher 1995; Mosley and Chen 1984; Whiting and Edwards 1988), but few studies have considered these factors in relation to objective measures of physiological function. Consequently, little is known about the processes or pathways through which these contextual factors "get under the skin" to shape our well-being.

To address this gap, a number of scholars, as well as recent initiatives from the National Institutes of Health, have advocated a more integrative, multimethod, interdisciplinary approach to human health research that draws on both biomedical and social/behavioral sciences (Anderson 1999; Cacioppo et al. 2000; Seeman and Crimmins 2001; Singer and Ryff 2001; Zerhouni 2003). The recent expansion of methodological options for collecting biological samples in nonclinical settings has greatly facilitated this effort (National Research Council 2001) and has encouraged a growing number of social scientists to consider integrating biomarkers into their research.

*Thomas W. McDade, Northwestern University, Department of Anthropology and Cells to Society (C2S): The Center on Social Disparities and Health at the Institute for Policy Research, 1810 Hinman Avenue, Evanston, IL 60208; E-mail: t-mcdade@northwestern.edu. Sharon Williams, Department of Anthropology, Purdue University. J. Josh Snodgrass, Department of Anthropology, University of Oregon. An earlier version of this paper was presented at the 2006 annual meeting of the Population Association of America in the session "Collection and Methods for Analysis of Biomarker Data for Demographic Studies." We thank session participants, Joanne Mei, and *Demography* editors and reviewers for insightful suggestions, as well as colleagues whose interest in biomarkers served as the motivation for this paper. We are also grateful to the National Science Foundation for financial support through a Presidential Early Career Award for Scientists and Engineers (TWM, BCS-0134225), and to the National Institute on Aging for postdoctoral research support (SW, 5 P30 AG 012857; JJS, P01 AG18911).

Table 1. Current Applications of Dried Blood Spot (DBS) Sampling in Large Population-Based Studies

Study	*N*[a]	Age Range	Biomarkers in DBS
Great Smoky Mountains Study http://devepi.mc.duke.edu/GSMS.html	1,000[c]	9–15 years	Androstenedione, CRP, DHEA-S, cortisol, EBV antibodies, estradiol, FSH, LH, testosterone
Health and Retirement Study http://hrsonline.isr.umich.edu/	7,000[b]	> 50 years	CRP, HbA1c, Total cholesterol, HDL
Los Angeles Family and Neighborhood Survey http://www.lasurvey.rand.org/	5,000[b]	> 3 years	CRP, EBV antibodies, HbA1c, Total cholesterol, HDL
National Longitudinal Study of Adolescent Health http://www.cpc.unc.edu/addhealth	17,000[b]	23–31 years	CRP, HbA1c, Total cholesterol, HDL, EBV antibodies
National Social Life, Health, and Aging Project http://www2.norc.org/nshap/	2,000	57–84 years	CRP, EBV antibodies, HbA1c, hemoglobin
Tsimane' Amazonian Panel Study (Bolivia) http://people.brandeis.edu/~rgodoy/	600[c]	2–15 years	CRP, transferrin receptor, leptin, EBV antibodies
Work and Iron Status Evaluation (Indonesia) http://chd.ucla.edu/WISE/index.html	16,000[b,c]	> 1 year	Transferrin receptor, CRP
Mexican Family Life Survey http://www.radix.uia.mx/ennvih/main.php?lang=en	17,700[b,c]	>15 years	CRP
Study of the Tsunami Aftermath and Recovery (Indonesia) http://chd.ucla.edu/STAR/STAR.html	35,000[b,c]	> 1 year	CRP, EBV antibodies

[a]Sample sizes are approximate and refer to the number of participants providing DBS samples.

[b]These studies are in the process of collecting or analyzing DBS samples, and specific plans for analyzing biomarkers in DBS samples are subject to change.

[c]DBS samples are collected from the same respondents multiple times over several months or years.

Dried blood spots (DBS)—drops of whole blood collected on filter paper from a finger prick—represent such an option and have been incorporated into a number of major data collection efforts in the United States as well as internationally (Table 1). The size, geographic distribution, and range of physiological measures of these applications is impressive and speaks to the feasibility of combining biological and contextual measures in survey research.

In this article, after a brief and general discussion of biomarkers, we review procedures for collecting, handling, and analyzing DBS samples. We highlight the advantages as well as disadvantages of using 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]

POTENTIAL CONTRIBUTIONS OF BIOMARKERS

Biological data drawn from representative samples as part of large national surveys (e.g., National Health and Nutrition Examination Survey [NHANES]) or more-focused epidemiological cohorts (e.g., Coronary Artery Risk Development in Young Adults [CARDIA]) reveal the population distribution of health and disease; illuminate the individual, household, and community-level variables that account for this distribution; and identify trends that can be used to predict patterns of morbidity and mortality. Recent integration of biomarkers into demographic research builds on longstanding attention to mortality as a primary health outcome and is a central component of efforts to understand the biological pathways through which social, economic, and community factors shape population health (Crimmins and Seeman 2001; Seeman and Crimmins 2001).

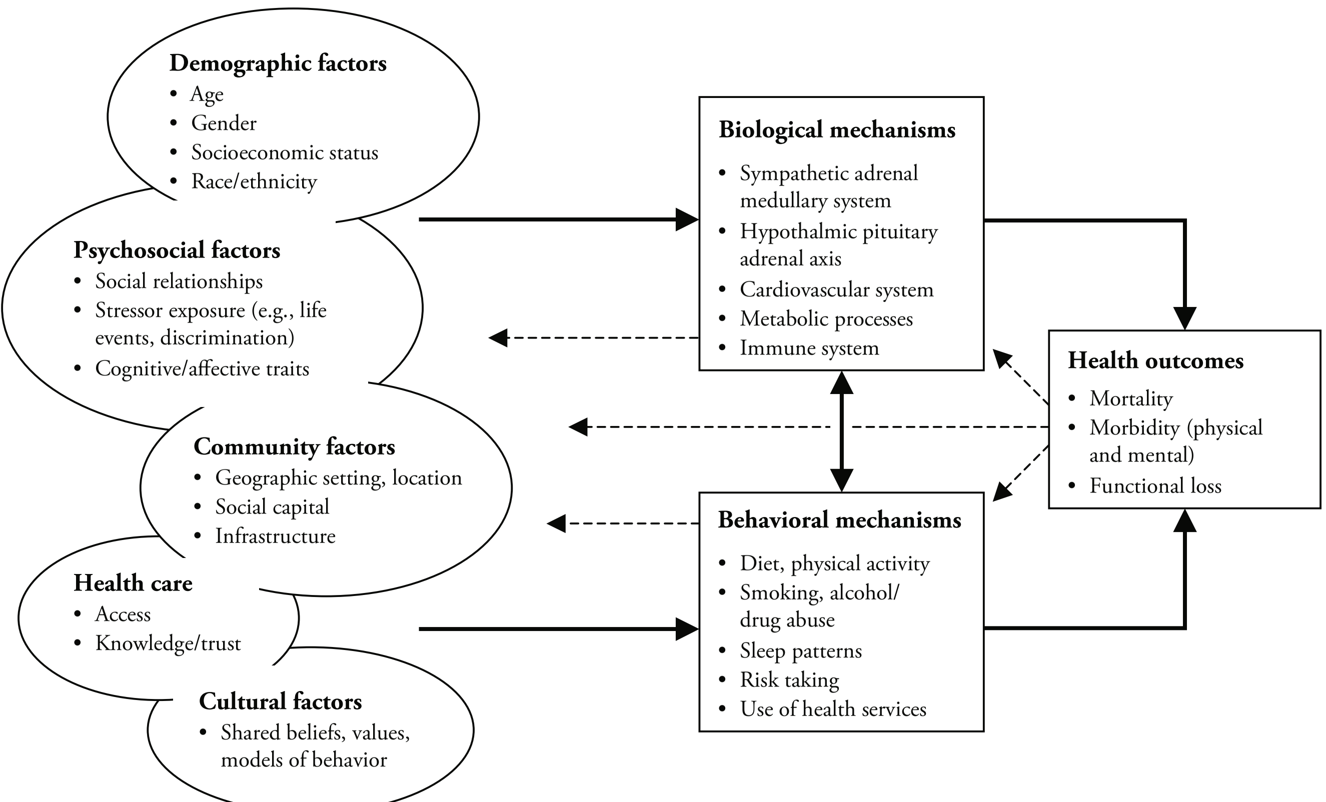
Figure 1 presents a conceptual model for incorporating biological data into population-based health research. It represents, in very general terms, the diverse theoretical and disciplinary perspectives that inform current efforts to investigate the dynamic associations among context, biology, behavior, and health. For the vast majority of this research, the causal impact of contextual factors on health is of primary interest, although the complex bidirectionality of these relationships is widely recognized. The conceptual models that guide the integration of biological and contextual measures require clear specification and will vary considerably, depending upon the pathways, outcomes, and populations of interest.

Historically, social and behavioral scientists have relied primarily on health data derived from self-reports, or from vital or clinical records. Although these data continue to be of central importance, there are many compelling reasons to consider direct biological measurement. First, biomarkers can shed light on the reciprocal links between environments and health by illuminating the specific physiological pathways through which socioeconomic, demographic, and psychosocial factors shape human health. They can help identify individuals who inhabit adverse psychosocial and physical environments, revealing 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 trajectories, with implications for selection into, for example, social or economic environments later in life. 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. 1995; Li et al. 2004). Measuring these biomarkers early in life may not uncover many clinical cases of CVD, but it will identify individuals who are most at risk for the development of disease. Further, biomarkers can provide insight into multiple physiological pathways—neuroendocrine,

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).

Figure 1. A General Conceptual Model for Incorporating Biological Data Into Population-Based Research Investigating the Dynamic Associations Among Contextual Factors and Health



Note: Dark arrows represent causal pathways of primary interest; dashed arrows acknowledge the bidirectionality of many of these pathways, particularly when considered over the life course.

cardiovascular, metabolic, and immune/inflammatory—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. Because biomarkers 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 on their willingness to share this information. In some cases, biomarkers may be useful in validating self-reports. More often, however, they will offer access to embodied information that is below the threshold of perception but that is nonetheless meaningful for physical health. This is a particular advantage for research with children, as well as for cross-cultural settings in which 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. Biomarkers do not 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 for sample collection facilitates the implementation of biomarkers into community-based research across a wider range of populations. Such methods may be particularly useful in many developing countries, where low rates of health service use, inadequate laboratory infrastructure, and logistical obstacles associated with sample collection and transport may hamper health-surveillance efforts (Boerma, Holt, and Black 2001). Furthermore, 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 and 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 these sampling issues are a priority, the burden is on the researcher to bring methods to people in the community instead of relying on select individuals willing to come to the clinic or lab. Although we often think of biological measures as inherently more invasive than self-report measures, for many respondents, slight physical discomfort may be preferable to the psychological discomfort associated with disclosure of embarrassing or otherwise sensitive information.

Lastly, biomarkers encourage productive collaboration among social, life, and biomedical scientists. Health is a quintessentially multidimensional phenomenon, 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.

POTENTIAL PITFALLS OF BIOMARKERS

First, collecting biomarkers adds to the burden placed on respondents and may impose additional risks to research participation. These burdens and risks for respondents 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, burdens and risks do exist, and considering the implications of biomarkers for sample recruitment and retention is important.

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 also be addressed to protect interviewers and other personnel handling biospecimens from

potential risks of infection. After they are 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, additional costs are 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 from $3,000 to $5,000.

Fourth, like with any research involving potentially sensitive information, the use of biomarkers raises important ethical issues that require careful consideration (Botkin 2001). Investigators pursue a wide range of strategies for addressing these issues in their research depending upon the biomarkers they are measuring, the populations they are working with, and the unique requirements of their institutional review boards. Specific issues to consider include the following: What is the risk to participants, and how will this risk be minimized? How will confidentiality be assured, particularly if samples are stored and analyzed across multiple sites? Will samples be stored for future analyses (as interesting new biomarkers emerge), or will they be destroyed immediately after analysis? Will participants be informed of their results, even if the clinical relevance is not clear? What action will be taken if a health problem or notifiable disease is detected? Few simple, widely accepted answers exist for these questions, and investigators are encouraged to work with their research communities and their institutional review boards to weigh the costs and benefits of different biomarker strategies.

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, an investigator may be better off relying on well-established self-report methods.

COLLECTION OF WHOLE BLOOD ON FILTER PAPER AS AN ALTERNATIVE TO VENIPUNCTURE

Although DBS sampling is relatively new to population-level survey research, their application dates back to the early 1960s 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 the implementation of a nationwide screening program in which DBS 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 40 years. More recently, DBS samples have played central roles in disease-surveillance efforts in several developing countries and have facilitated research on human biology and health in remote settings around the world (Boerma et al. 2001; Solomon et al. 2002; Worthman and Stallings 1997).

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 Clinical and Laboratory Standards Institute (Hannon et al. 1997). The Centers for Disease Control and Prevention (CDC), which maintains an independent quality-control program, noted 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. 2001:1631).

Sample Collection, Processing, and Storage

Procedures for sample collection and processing are relatively straightforward. 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 with gauze, and subsequent blood drops are applied to filter paper (formerly Schleicher and Schuell #903, now Whatman #903). The samples are allowed to dry (from four hours to overnight), at which point they can be stacked and stored with desiccant in resealable plastic bags or plastic containers. The desiccant should have a colored humidity indicator to show when it should be replaced.

Although sample collection is not complicated, researchers must receive adequate training to minimize potential sources of error. First, proper placement of whole blood on the filter paper is critical. 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, a drop of blood should be allowed to form on the finger and then absorbed into the filter paper without any direct contact between the finger and paper.

Second, an effort should be made to collect blood spots of comparable size because 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 from that spot (Adam et al. 2000). Variation in blood spot size can be minimized by collecting samples on filter papers with preprinted circles as guides to standardize the volume of whole blood collected from each individual. When filled to its border, each circle will contain approximately 50 μL of whole blood.

The filter paper matrix stabilizes most analytes in dried blood spots, but the rate of sample degradation will vary by analyte. Stability should be evaluated prior to sample collection because 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 one week of storage at 37°C. In contrast, concentrations of C-reactive protein decline significantly in DBS after three days at 37°C but are stable for at least two weeks at room temperature (20–23°C; McDade, Burhop, and Dohnal 2004). Although refrigerating or freezing samples promptly after drying is always advisable to minimize the chances of degradation, the stability of most analytes in DBS provides flexibility in the collection of samples in field settings. Keeping the blood spots dry with desiccant contributes to the stability of analytes.

For long-term storage, samples should be packed with desiccant and frozen in a reliable laboratory-grade freezer to ensure sample integrity. Food freezers are not acceptable because 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 between 8,000 and 10,000 DBS samples.

Requirements for shipping DBS 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 with desiccant 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 shipping samples internationally, the CDC will issue importation permits although such permits may be required only 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. Because the sample has been dried on filter paper, analytes must first be brought into solution. A standard hole punch is typically used to cut discs of whole blood of uniform size, although occasionally an entire blood spot is cut into smaller pieces. 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 DBS samples. However, there are potential obstacles that may prove to be insurmountable exceptions in some cases. First, will the presence of red and/or white blood cells interfere with the assay? Whole blood comprises 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 is 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 DBS 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 the 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., two hours, four 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 off 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 from a dried blood spot will contain approximately 1.4 μL of serum (Mei et al. 2001). Multiple discs, or a larger hole punch, can be used to increase sample quantity, but an assay that normally requires 50 or 100 μL 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, Bechgaard, and Magid 1974). Although 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 Using Dried Blood Spots for Field-Based Research

The primary advantage of using DBS samples is that they provide access to physiological information that would not otherwise be attainable in nonclinical settings. Saliva and urine are frequently used for the subset of biomarkers that enter these fluids in a measurable form (e.g., cortisol, catecholamines), but this is not an option for the majority of analytes that are accessible only through blood. The collection of several milliliters of plasma or serum through venipuncture is the current clinical standard, but finger-prick blood sampling is a viable alternative that offers the following advantages.

First, sample collection is relatively painless and noninvasive, and can be conducted in the participant's home by interviewers who are not medically trained 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) as well as for research in remote or underserved communities where the logistics of venipuncture may limit access to willing participants.

Second, unlike plasma or serum, DBS 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 one week or more, providing considerable flexibility in procedures for sample collection and transport.

Third, DBS 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 μL of whole blood and will result in a DBS sample 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 10–20 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, Lindau, and McDade 2006).

Fourth, a single finger prick can provide capillary whole blood for spots on filter paper and for the onsite assessment of biomarkers by using portable point-of-care instruments. Affordable, portable instruments for the analysis of hemoglobin, HbA1c, and lipid profiles are available that provide an opportunity to collect physiological information away from the lab. Using the same finger-prick sampling procedure detailed earlier, 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, researchers can collect biomarker results onsite and share them with participants, and can assay blood spots 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 Using Dried Blood Spots

Advantages of using DBS samples compared with venipuncture need to be weighed against potential disadvantages, including the following:

First, the vast majority of standard laboratory protocols require serum or plasma, and assay protocols must therefore be developed specifically for DBS and validated for accuracy, precision, reliability, and limits of detection. As outlined earlier, this is a relatively methodical process that can take several weeks of dedicated effort. In some cases, quantifying an analyte of interest in DBS samples may not be possible. Furthermore, many standard clinical assays (e.g., total cholesterol, glycosylated hemoglobin) are performed

on automated, high-throughput analyzers designed for use with serum or plasma samples. These instruments offer increased speed and reduced costs of analysis but currently are not likely to accommodate DBS samples.

Second, from a clinical perspective, DBS samples are a nonstandard diagnostic substance, and DBS results may not be directly comparable with those derived from serum or plasma. Assays of DBS samples provide results that represent the concentration of an analyte in whole blood. For serum and plasma samples, the cellular fraction of whole blood is removed following centrifugation. The concentration of analytes in these samples is, therefore, higher relative to whole blood. Because the correlations between results derived from matched serum/plasma and DBS samples are linear and high for most analytes, correction factors can be applied to DBS values to derive plasma equivalents if desired. Such corrections will not be necessary for internal comparisons but will be important for any attempts to compare data to prior research based on results from serum or plasma samples. Furthermore, established clinical cut-points used to identify individuals at risk for disease (e.g., C-reactive protein > 3 mg/L) are typically based on serum/plasma samples collected via venipuncture. When using these cut-points or making direct comparisons to results from prior research is essential, extra efforts will have to be taken to develop correction factors specific to the DBS methods used and the populations to which they are applied.

Third, because of requirements for assay development, DBS 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 DBS 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 become available.

Fourth, although the technical demands associated with the laboratory analysis of DBS samples are similar to those required to analyze serum or plasma samples, few labs (commercial or academic) have direct experience with DBS analytic methods. Therefore, finding a collaborating lab for sample analysis can be a challenge, although this situation is likely to improve as interest in DBS sampling grows.

Evaluation Criteria for Available Protocols

In this section, we provide an overview of current options for the analysis of biomarkers in DBS samples and discuss issues related to methods evaluation that can help investigators decide on the appropriateness of a method for their research. As is apparent in Table 1, recent incorporation of DBS measures in large surveys has focused primarily on markers that provide insight into the cardiovascular/metabolic (total cholesterol, high density lipoprotein, HbA1c) and immune/inflammatory (C-reactive protein, Epstein-Barr virus antibodies) pathways that link environments, behavior, and health. In most cases, these measures are used in conjunction with non-DBS biomarkers (e.g., salivary cortisol, blood pressure, anthropometric measures) to provide summary assessments of health across multiple physiological systems, analogous to current applications of the concept of allostatic load (McEwen 1998; Seeman et al. 2001). Several studies—in the United States and internationally—have included more diverse panels of DBS measures specifically tailored to their unique research questions related to, for example, iron status and productivity, socioeconomic predictors of infectious disease risk, and psychosocial dynamics related to reproductive development and function (Almeida et al. 2001; Rowe et al. 2004; Thomas and Frankenberg 2002).

To document the range of current DBS methods, we compiled a comprehensive bibliography of published methods for over 100 analytes by drawing on previous reviews, searching Medline and Web of Science, and pursuing methods referenced in papers col-

lected through this process. From this list, we selected for closer inspection protocols for those analytes most likely to be of interest to researchers conducting population-level, community-based health research (Appendix Table A1).[2] We base inclusion in Appendix Table A1 on four criteria. First, the method must use capillary whole blood collected on filter paper. This criterion eliminates analytes (e.g., ferritin) that require the separation of red blood cells prior to application to filter paper because this step adds to the burden of sample collection. Second, we focus on markers of physiological function and health that are broadly relevant across a wide range of ages. Therefore, we 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). Even though DNA and RNA are readily extracted from DBS (Caggana, Conroy, and Pass 1998), we do not review these methods because they are relatively straightforward and comparable, differing primarily with respect to the application of specific primers for molecular markers of interest. Third, some attempt at assay validation must be evident, including a report of accuracy, precision, reliability, and/or analysis of matched DBS and serum/plasma samples; all of these need not be present, but we are looking for attention to the importance of evaluating assay performance. And finally, we require that methods that appear in Appendix Table A1 were subjected to peer review.

Appendix Table A1 includes information on multiple aspects of assay performance and implementation. Our goal is to document the current range of validated methods and provide key information for each biomarker that would be useful when making decisions regarding its utility and feasibility. 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 50 μL of whole blood. Most assays use a hole punch to produce a disc of dried blood of a given size for analysis, but others use the entire spot. In this column, we report the amount of DBS sample required for analysis. Linear dimensions (i.e., mm or inches) pertain to methods that use a hole punch, and volume measures (i.e., μL) are presented for methods that use an entire blood spot containing a premeasured quantity of whole blood.

Stability. Here, we report the stability of analytes in DBS stored at room temperature and when refrigerated (~4°C). No standardized criteria exist 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. Therefore, actual stability may be significantly longer; we use > to indicate these cases. In addition, for some analytes, stability information is presented in supplemental publications not included here because they do not meet our criteria for inclusion in Appendix Table A1.

Analytic method. Multiple platforms are available 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 because this factor may be limiting for some labs.

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 a lower

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

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 (Inczedy, Lengyel, and Ure 1998).

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 requirement may prove to be an impediment to the development of a DBS method because of the relatively low volume of collected sample.

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

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 measured on different days. As with precision, we present the average inter-assay CV as an approximation of assay reliability.

DBS versus venous blood. Comparing DBS assay results with those from matched, simultaneously collected serum or plasma samples via venipuncture 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, 1999). Analysis of matched DBS and plasma/serum samples can also be used to generate a conversion formula to derive plasma-equivalent values from results with DBS samples (Worthman and Stallings 1997). However, caution should be used in the application of plasma equivalents because the relationship will vary across analytic methods and may vary across populations (e.g, Shirtcliff et al. 2001). In some cases, liquid whole blood is used for comparison with DBS results instead of serum/plasma.

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

Reagent availability. We also ask whether all the materials required for the assay are commercially available or if instead key reagents (e.g., antibodies, calibrators) were developed in-house. Our answer is "yes" if all reagents could be purchased from established suppliers at the time of publication. This is subject to change because 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.

We provide information on assay performance and implementation for 45 analytes that are most likely to be of relevance to biosocial and demographic research (Appendix Table A1). 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 associations between psychosocial/behavioral contexts and multiple physiological systems.

These protocols use standard clinical chemistry methods. 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 reemphasizing, however, that investigators should independently evaluate any protocol before assuming it can be used in their research.

Stability of DBS samples 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 two weeks. Refrigeration and storage with desiccant tends to extend this period.

A few of the methods listed in Appendix Table A1 take advantage of recent innovations in immunoassay technology that make it possible to quantify multiple analytes simultaneously in one sample rather than analyzing one analyte at a time (Bellisario, Colinas, and Pass 2000). This advance is made possible by particle-based flow cytometry, 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 using DBS and promise to expand the range of factors that can be measured.

CONCLUSION

Methodological tools that advance interdisciplinary, multilevel research on health in population-based settings are 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 innovative, new research directions that will ultimately lead to a richer, multidimensional understanding of human biology and health.

Dried blood spot sampling represents such a method, and a growing number of population-based studies, internationally and in the United States, are adding DBS to data-collection protocols. Field-based research that collects biological specimens in participants' homes places a premium on the ease of sample collection, storage, and transport. For many biomarkers, DBS sampling provides a viable alternative to using venipuncture, particularly as the long list of analytes that can be quantified in blood spot samples grows.

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 using DBS so that investigators can make informed decisions regarding the appropriateness of using DBS 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. We 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.

Appendix Table A1. Detailed Information on Blood Spot Protocols Developed for Select Analytes

Analyte and Volume/Size	Stability: Room Temperature	Stability: Refrigerated (at ~4ºC)	Method	Lower Detection Limit	Intra-Assay	Inter-Assay	DBS vs. Venous	Protocol	Reagents	Source
Androstenedione										
4 × 2.5 mm	4 wks.	> 8 wks.	RIA	0.012 ng/mL	9.5	10.7	yes	yes	yes	Worthman and Stallings 1997
1 × 5.0 mm			RIA	4 nmol/L	< 10	< 10	no	yes	no	Thomson et al. 1989
2 × 6.0 mm	> 3 mos.	> 3 mos.	RIA	0.6 nmol/L			yes	yes	no	Egan et al. 1989
Apolipoprotein A-I										
1 × 3.0 mm	1 day	1 day	ELISA		5.2	14	yes	yes	yes	Wang et al. 1989
2 × 6.0 mm		1 mo.	immunonephelometry				yes	yes	no	Van Biervliet et al. 1982
1 × 3.2 mm			immunoelectrophoresis		3.9	13.9	yes	yes	no	Micic et al. 1988
Apolipoprotein B										
1 × 3.0 mm		20 days	ELISA		3.4	5.6	yes	yes	yes	Wang et al. 1989
2 × 6.0 mm		1 mo.	immunonephelometry				yes	yes	no	Van Biervliet et al. 1982
1 × 3.0 mm			immunoelectrophoresis				yes	yes	yes	Dudman et al. 1985
1 × 3.0 mm		2 wks.	immunoturbidimetry		7.8	12.5	yes	yes	yes	Bangert et al. 1992
1 × 3.0 mm			ELISA		5.2	7.8	yes	yes	no	Ohta et al. 1988
1× 3.2 mm			immunoelectrophoresis		4.9	16.5	yes	yes	no	Micic et al. 1988
Cortisol										
1 × 2.5 mm	4 wks.	> 8 wks.	RIA	0.46 ug/dL	9	9.2	yes	yes	yes	Worthman and Stallings 1997
2 × 3.2 mm			RIA	83 nmol/L	< 11	< 15	yes	yes	yes	Mitchell and Hermonths 1998
CD4+ lymphocytes										
?			ELISA				yes	no	yes	Mwaba et al. 2003
C-reactive protein										
1 × 5.0 mm	> 12 wks.	> 12 wks.	ELISA	0.019 mg/L			yes	no	yes	Beesley et al. 2000
1 × 3.2 mm	> 14 days	> 14 day	ELISA	0.028 mg/L	5.8	8.2	yes	yes	yes	McDade et al. 2004

2 × 3.2 mm			Luminex	1.1 ug/L	7.5	8.9	no	yes	yes	Skogstrand et al. 2005
1 × 6.0 mm		> 21 days	ELISA	7.3 ng/mL	5.1	10.2	yes	yes	yes	Cordon et al. 1991
DHEA-S										
1 × 2.5 mm	4 wks.	> 8 wks.	RIA	8.0 ng/mL	7.5	9.4	yes	yes	yes	Worthman and Stallings 1997
Folate										
2 × 6.35 mm	1 wk.	1 wk.	microbiological		6.5	7.7	yes	yes	yes	O'Broin and Gunter 1999
FSH										
1 × 2.5 mm	> 8 wks.	> 8 wks.	FIA	0.13 IU/L	7.7	7.9	yes	yes	yes	Worthman and Stallings 1994
Epstein-Barr virus antibodies										
1 × 3.2 mm	> 8 wks.	> 8 wks.	ELISA		5.6	7.7	yes	yes	yes	McDade et al. 2000
Estradiol										
8 × 1/8 in.			RIA	2 pg/mL	7.57	8.22	yes	yes	yes	Shirtcliff et al. 2000
4 × 2.5 mm	3 wks.	> 8 wks.	RIA	9 pg/mL	8.6	7.5	yes	yes	yes	Worthman and Stallings 1997
Glucose										
1 × 6 mm	3 wks.		colorimetric		3.5	6.4	yes	yes	yes	von Schenck et al. 1985
1 × 6.5 mm	7 days		colorimetric		3.6	4.2	yes	yes	yes	Abyholm 1981
1 × 6.0 mm			enzymic	0.26 mmol/L	2.9	3.1	yes	yes	yes	Burrin and Price 1984
1 × 10 mm		> 10 days	enzymatic fluorometric		3.0		yes	yes	yes	Burrin et al. 1981
Glycosylated Hemoglobin/HbA1c										
1 × 3.0 mm	5 days	> 10 days	HPLC				yes	yes	yes	Jeppsson et al. 1996
1 × 1/2 in.	3 mos.		colorimetric		< 6.0	< 6.0	no	no	yes	Eross et al. 1984
1 × 6.0 mm			affinity chromatography		5.28		yes	yes	yes	Little et al. 1986
Hemoglobin										
2 × 6.35 mm			spectrophotometric		2.1	3.4	no	yes	yes	O'Broin and Gunter 1999

(continued)

(Appendix Table A1, continued)

Analyte and Volume/Size	Stability: Room Temperature	Stability: Refrigerated (at ~4°C)	Method	Lower Detection Limit	Intra-Assay	Inter-Assay	DBS vs. Venous	Protocol	Reagents	Source
Hepatitis A antibodies										
?			ELISA				yes	no	yes	Gil et al. 1997
1 × 1 cm			RIA				yes	no	yes	Zoulek et al. 1985
2 × 25 mm			ELISA				yes	yes	yes	de Almeida et al. 1999
Hepatitis B antigen										
25 μL	> 30 days	> 30 days	RIA				yes	no	no	Farzadegan et al. 1978
1 × 3.0 mm			Luminex		21.0		yes	yes	yes	Lukacs et al. 2005
1 × 1 cm			RIA				yes	no	yes	Zoulek et al. 1985
~ 8 mm	14 days	14 days	RIA				yes	no	yes	Villa et al. 1980
Hepatitis B antibodies										
1 × 1 cm			RIA				yes	no	yes	Zoulek et al. 1985
2 × 3.0 mm			haemagglutination				yes	yes	yes	Tappin et al. 1998
~ 8 mm	14 days	14 days	RIA				yes	no	yes	Villa et al. 1980
Hepatitis C antibodies										
1 × 3.0 mm			Luminex		21.0		yes	yes	yes	Lukacs et al. 2005
1 × 5.5 mm			ELISA				yes	yes	yes	Parker et al. 1997
1 × 3.0 mm			ELISA				yes	yes	yes	McCarron et al. 1999
HIV antibodies										
1 × 3.2 mm			Luminex			6.0	no	yes	yes	Bellisario et al. 2001
1 × 3.0 mm			Luminex		21.0		yes	yes	yes	Lukacs et al. 2005
1 × 6.0 mm			Luminex			6.5	yes	yes	yes	Faucher et al. 2004
5 × 1.0 cm			ELISA				yes	yes	yes	Varnier et al. 1988
1 × 5.0 mm			ELISA				yes	yes	yes	Thongcharoen et al. 1992

1 × 14 mm		> 3 mos.	ELISA/immunoblot				yes	no	no	Lindhardt et al. 1987
1 × 1/8 in.			EIA				yes	yes	yes	Fortes et al. 1989
1 × 20 μL			SEGLISA				yes	yes	yes	Rocks et al. 1991
Homocysteine										
1 × 3 mm			LC-MS/MS		6.0	10.2	yes	yes	yes	McCann et al. 2003
IFNg										
2 × 3.2 mm			Luminex	50 ng/L	6.7	24.5	no	yes	yes	Skogstrand et al. 2005
IgE										
50 μL			RAST				yes	yes	no	Stapel et al. 2004
1 × 3.2 mm	< 4 days	> 14 days	EIA	55 ng/mL	4.6	8.4	no	yes	yes	Tanner and McDade 2007
IGF-I										
1 × 8 mm	> 4 wks.	> 4 wks.	ELISA		7.5	11.4	yes	yes	yes	Diamandi et al. 1998
1 × 8 mm	> 40 days	> 40 days	ELISA				yes	yes	yes	Jones 2001
2 × 3.18 mm	> 5 days		RIA		8.1	8.7	yes	yes	yes	Schutt et al. 2003
1 × 6.0 mm			ELISA				yes	yes	yes	Nindl et al. 2003
IGFBP-2										
2 × 3.18 mm	> 5 days		RIA		4.2	6.0	yes	yes	yes	Schutt et al. 2003
IGFBP-3										
1 × 8 mm	> 4 wks.	> 4 wks.	ELISA		8.2	7.5	yes	yes	yes	Diamandi et al. 1998
1 × 3.18 mm	> 5 days		RIA		7.6	4.6	yes	yes	yes	Schutt et al. 2003
IL-1b										
2 × 3.2 mm			Luminex	26 ng/L	6.4	16.5	no	yes	yes	Skogstrand et al. 2005
IL-6										
2 × 3.2 mm			Luminex	24 ng/L	7.6	16.5	no	yes	yes	Skogstrand et al. 2005
Insulin										
3 × 0.5 in.			RIA		14.0	25.0	yes	yes	yes	Dowlati et al. 1998
2 × 3.0 mm			chemiluminescent	5.9 pmol/L	18.1	12.9	yes	yes	yes	Butter et al. 2001

(continued)

(Appendix Table A1, continued)

Analyte and Volume/Size	Stability: Room Temperature	Stability: Refrigerated (at ~4°C)	Method	Lower Detection Limit	Intra-Assay	Inter-Assay	DBS vs. Venous	Protocol	Reagents	Source
Lipoprotein (a)										
1 × 3.0 mm			ELISA	22 mg/L	4.5	4.4	yes	yes	yes	Wang et al. 1992
LH										
1 × 2.5 mm	> 8 wks.	> 8 wks.	FIA	0.26 IU/L	7.3	8.9	yes	yes	yes	Worthman and Stallings 1994
PSA										
5 × 3.0 mm	> 24 days	> 24 days	chemiluminescent	0.35 ug/L		11.7	yes	yes	yes	Hoffman et al. 1996
Progesterone										
8 × 1/8 in.			RIA	0.015 ng/mL	8.8	12.7	yes	yes	yes	Shirtcliff et al. 2001
1 × 5 mm	> 9 wks.	> 9 wks.	RIA	2.5 nmol/L	7.0	9.2	yes	yes	no	Petsos et al. 1985
Prolactin										
2 × 12 mm	> 7 days		immunoenzymetric				yes	yes	yes	Fisher et al. 1991
1 × 7.5 mm			IRMA				yes	yes	yes	Ehsan et al. 1996
2 × 3 mm	7 days	> 12 wks.	RIA	2 ug/L	7.3	12.2	yes	yes	yes	Bassett et al. 1986
1 × 2.5 mm	3 wks.	8 wks.	FIA	0.11 ng/mL	6.1	7.2	yes	yes	yes	Worthman and Stallings 1997
Retinol										
1 × 6.35 mm	< 1 wk.		HPLC	0.1 umol/L	< 6	< 6	yes	yes	yes	Erhardt et al. 2002
SHBG										
1 × 2.5 mm	2 wks.	> 8 wks.	FIA	0.2 nmol/L	13.2	14.5	yes	yes	yes	Worthman and Stallings 1997
Somatomedin-C										
1 × 3.2 mm			RIA	0.05 unit/mL	6.2	6.5	yes	yes	no	Mitchell et al 1987
Testosterone										
8 × 1/8 in.			RIA	0.015 ng/mL	6.2	8.1	yes	yes	yes	Shirtcliff et al. 2001
4 × 2.5 mm	3 wks.	> 8 wks.	RIA	6.3 ng/dL	7.6	12.7	yes	yes	yes	Worthman and Stallings 1997
2 × 20 µL	> 1 wk.		GCMS				yes	yes	yes	Peng et al. 2000

1 × 7.9 mm	> 7 days		RIA	0.4 nmol/L	41.2	18.3	yes	yes	yes	Howe and Handelsman 1997
Transferrin receptor										
2 × 3.2 mm	> 4 wks.	> 4 wks.	ELISA	0.55 mg/L	6.6	8.2	yes	yes	yes	McDade and Shell-Duncan 2002
25 μL	> 4 wks.	> 4 wks.	ELISA		4.5	5.6	yes	yes	no	Cook et al. 1998
Thyrotropin (TSH)										
2 × 3.2 mm			Luminex	0.7 mIU/L	4.9		no	yes	yes	Bellisario et al. 2000
1 × 3.0 mm			RIA	10 mIU/L	7.1	12.7	yes	yes	no	Nagataki et al. 1980
1 × 5.0 mm			chemiluminescence	2.9 mIU/L	10.4	7.4	no	yes	yes	Neto and Schulte 1998
1 × 3.0 mm	< 7 wks.	> 11 wks.	FIA				yes	yes	yes	Elnagar et al. 1997
1 × 5.0 mm			EIA/FIA	1.25 mIU/L	7.8	6.9	yes	yes	yes	Tuuminen et al. 1991
1 × 6.5 mm			EIA	3.5 mU/L	7.6	11.7	no	yes	yes	Torresani, Qui, and Illig 1986
? × 3.0 mm			FIA		7.6	11.0	no	yes	yes	Torresani and Scherz 1986
1 × 4.0 mm			IRMA	1.0 mU/L	7.8	7.7	no	yes	no	Jirkalova et al. 1996
1 × 4.8 mm			immunoenzymetric	2.4 mU/L	7.5	8.7	no	yes	no	Jirkalova et al. 1996
1 × 1.0 cm			RIA	5 uU/mL			yes	yes	no	Irie et al. 1975
2 × 5.0 mm			RIA	15 mU/L			yes	yes	yes	Beckers et al. 1979
3 × 3.0 mm			EIA		11.8	15.0	no	yes	yes	Miyai et al. 1984
Thyroxine (T4)										
2 × 3.2 mm			Luminex	10.3 nmol/L	8.2		no	yes	yes	Bellisario et al. 2000
1 × 3.0 mm			RIA	10 ug/L	11.7	9.8	yes	yes	no	Nagataki et al. 1980
1 × 4.25 mm			RIA	0.8 pmol/L	9.6	13.2	yes	yes	yes	Lemonnier et al. 1991
1 × 9 mm	4 wks.	4 wks.	RIA	0.8 ng/L	5.3	6.2	yes	yes	yes	Mizuta et al. 1982
2 × 3 mm	4 wks.	1 wk.	EIA	1.9 ng/L	7.6	6.4	yes	yes	no	Hata et al. 1985
1 × 7.0 mm			RIA	1.5 pg/mL	6.6	9.0	yes	yes	yes	Pacchiarotti et al. 1988
1 × 5.0 mm			RIA	8.37nmol/L			yes	yes	yes	Beckers et al. 1979
1 × 3.0 mm			ELISA	1.25 ug/L	5.8	6.8	no	yes	no	Maeda et al. 1985
1 × 2.0 mm			RIA				yes	yes	no	Obregon 1982

(continued)

(Appendix Table A1, continued)

Analyte and Volume/Size	Stability: Room Temperature	Stability: Refrigerated (at ~4ºC)	Method	Lower Detection Limit	Intra-Assay	Inter-Assay	DBS vs. Venous	Protocol	Reagents	Source
Thyroglobulin										
1 × 4.75 mm			FIA	1.42 ug/L	< 10	< 20	yes	yes	yes	Zimmermann et al. 2003
Thyroxine binding globulin										
1 × 6.5 mm			RIA	1.83 mg/L	6.7	5.7	yes	yes	yes	Torresani et al. 1983
Triglycerides										
10 μL	30 days	> 90 days	enzymatic		6.1	7.4	yes	yes	yes	Quraishi et al. 2006
Triiodothyronine (T3)										
1 × 4.25 mm		8 wks.	RIA	0.48 pmol/L	13.6	16.0	yes	yes	yes	Laroche et al. 1988
5 × 7.0 mm			RIA	1.5 pg/mL	9.4	9.8	yes	yes	yes	Pacchiarotti et al. 1988
TNFa										
2 × 3.2 mm			Luminex	34 ng/L	5.6	18.0	no	yes	yes	Skogstrand et al. 2005

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