IMPROVED METHODS IN THE COLLECTION OF BIOLOGICAL SAMPLES FOR COMPLEX OCCUPATIONAL AND ENVIRONMENTAL SETTINGS

By

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ABSTRACT

Problem Statement: In the collection of traditional biological samples, such as liquid venous whole blood, plasma, and serum, the need for phlebotomy and cold chain often constrains their use in complex occupational and environmental settings. Less invasive methods that do not require phlebotomy or cold chain, such as dried blood spots (DBS), provide a potential alternative to traditional samples. Despite the advantages of DBS, scientific questions remain as to the range of potential applications of the method, as do technical challenges associated with field collection. Among these challenges, the requirement of open-air drying, which is not currently standardized and exposes DBS samples to potential contaminants while creating logistical hurdles in collection and storage, continues to hinder wider adoption of DBS.

Methods: In the first of three related manuscripts, we conducted a review of the current state of the science in DBS sampling using a scoping review of reviews methodology. In the second manuscript, we designed and demonstrated proof-of-concept for novel methods in field collection and storage of DBS samples. This study measured drying rates of DBS samples collected under novel methods through use of resistance sensors designed specifically for the study. In the third manuscript, we conducted a validation of assay protocol for comparing RNA measurements in DBS samples collected under our novel methods with those of the current methods recommended by the United States Centers for Disease Control and Prevention (CDC).

Results: In our first study, we identified approximately 2,000 (n=1,947) analytes that have been measured by one of more than 150 (n=169) different analytic methods. In our second study, we found that DBS samples collected under our novel methods in conditions of moderate and high humidity dried, on average, 30% and 50% faster respectively than DBS samples allowed to open-air dry under similar conditions as reported in the scientific literature. In our third study, our findings suggest that our novel methods demonstrated an overall improvement in performance on detection and quantification of RNA from DBS samples as compared with current methods.

Conclusions: DBS provide researchers and practitioners a wide-ranging tool with potential applications for biosampling in complex occupational and environmental settings. Our novel methods in DBS collection and storage provide several advantages over current methodologies, including removal of the requirement for open-air drying of samples, reduced risk of sample contamination, reduced variability in environmental conditions incurred by samples, and overall improvements in measurements derived from DBS. Our findings support adoption of our novel methods in the collection and storage of DBS samples.

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CHAPTER ONE

Introduction

RATIONALE FOR RESEARCH

Essential to the fields of public health and clinical medicine are the abilities to detect and measure potentially hazardous exposures and early indicators of adverse physiologic response. Measuring exposures and clinical effects both in the near-term and over the lifetime of individuals and populations are important contributors to advancing science and protecting health. In practice, exposures and their associated adverse health effects are most accurately measured using biological samples (biosamples), which may include blood, urine, and saliva among others [1]. Biosamples are also integral to the design of effective public health and clinical interventions, and enable practitioners to monitor, evaluate, and continuously improve their efforts [2]. Consequently, in the absence of biosampling, intervention efforts are not able to be implemented with full efficacy. Complex occupational and environments settings, defined here as any setting in which the ability to collect biosamples is limited, present an enormous challenge to the medical and public health communities, globally.

Complex occupational and environmentally challenging settings may include surroundings as complex as a war zone, or as simple as a person's home, both of which are outside of the traditional clinical and laboratory settings under which biological sampling is more easily and reliably conducted. Paradoxically, the more complex and potentially hazardous is the environment, the less likely are biosamples to be collected. For example, in war zones, where soldiers have historically been exposed to a variety of toxic chemicals, limitations in resources, time, and technical capacity have made the use of biosamples uncommon [3-4]. Beyond the military context, the need for biosampling in other

occupational settings is well established. For example, first responders during the Terrorist Attacks of September 11, 2001, many of whom developed a range of post-exposure adverse health conditions, were exposed to a variety of toxic chemicals, and yet few of the responders had any biosamples collected during the immediate response [5-6].

Though firefighters were among the 9/11 first responders, a rare event such as a terrorist attack is not required for them to encounter hazardous chemicals. The typical working environment for a firefighter presents an enormous risk of hazardous chemical exposure, and though the Occupational Safety and Health Administration (OSHA) mandates biological monitoring for specific chemicals in defined work settings, routine biosampling programs for firefighters are not currently in place nationally [7]. It is not surprising then, that biosampling among other potentially hazardous occupations, such as coal miners and oil rig workers, is also not routine [7]. The reasons for this may vary by occupation, but logistical constraints involved in the collection, transport, storage, and analysis of biological samples are shared across occupational settings. Many of these same constraints extend to non-occupational settings as well. For example, there is a demonstrated need in low resource countries for improvements in the collection and use of biosamples [8]. Even simple diagnostic tests for HIV and tuberculosis are limited in many low resource settings [9-11]. Whether in occupational or non-occupational settings, high or low resource settings, there is a well-established need for overcoming the challenges associated with the collection of biosamples in complex environmentally challenging settings.

One potential solution for addressing the logistical challenges of collecting biosamples in environmentally challenging settings is the use of dried blood spot (DBS) sampling, which involve the minimally invasive collection of a small drop of blood taken from either a finger or heel prick and placed on specially designed filter paper for drying until the time of analysis [12]. DBS sampling is a minimally invasive collection method that could potentially be deployed in a wide variety of settings [13]. Among the advantages of DBS sampling, the ability to collect a biosample without the need for phlebotomy or cold chain[1], are critical in environmentally challenging settings where such requirements often limit the ability to collect traditional biosamples, such as venous whole blood, plasma, and serum [12, 14]. DBS sampling may reduce interruptions in work flow and other challenges related to the collection of large volumes of blood. It also minimizes problems in sample transport and storage, which require more extensive inputs for maintaining integrity of traditional samples such as venous blood or urine.

DBS samples are reported to have a wide range of diagnostic capacity and have been shown to have advantages over venous blood, urine and other biological samples in terms of cost, ease of collection, and storage [15-17]. In recent years, as advancements in the quality and availability of highly sensitive laboratory instrumentation have been paired with high-powered statistical software programs, interest in the use of DBS by potential adopters has grown [18-20]. However, questions remain around the full range of DBS applications as no systematic assessment of this range is yet available in the scientific literature. What's more, as researchers, practitioners, and their respective institutions consider adoption, there

[1] Cold chain is a supply chain that is temperature controlled. Cold chain is a common requirement for transporting vaccines and other medical supplies that require constant refrigeration.

is a critical need for a more rigorous evaluation of the current state of the science in dried blood spots.

In addition to questions regarding the current state of the science, the technical challenges associated with DBS use must be addressed before wider adoption can occur. Though DBS samples have been shown in some cases to be comparable to other biosamples in terms of sample stability and reproducibility, the quickly emerging fields of transcriptomics, proteomics, and metabolomics present unique technical challenges to the use of DBS, especially in field settings [21-23]. Exposure to light, moisture, and other environmental conditions may all impact the precision and accuracy of certain biomarkers in DBS [24-26]. Additionally, the current protocol recommended by the United States Centers for Disease Control and Prevention (CDC) recommends open-air drying of DBS samples away from direct sunlight for a minimum of 3 hours after collection and before storage or transport of DBS samples [27]. In a well-resourced clinic or laboratory setting, these issues can be overcome; however, in complex occupational and environmental settings, the current protocol for DBS sampling is not sufficient. In addition to a higher risk of sample contamination from exposure to dust, insects and other environmental contaminants, space for open-air drying may not be available, and samples may need to be moved before they've been completely dried [28-29]. For DBS to be adopted more widely in complex occupational and environmental settings, improvements to drying methods must be developed and tested. In responding to the current gaps in the scientific literature, and the remaining technical challenges around DBS use in field settings, the objectives of this

dissertation was to characterize the current state of the science for DBS, and to resolve the issue of open-air drying in the collection of DBS samples in field settings.

STUDY AIMS

This dissertation has three specific aims and associated manuscripts:

1. Specific Aim 1: To apply a systematic approach to characterizing the current state of the science in dried blood spots.
2. Specific Aim 2: To develop and validate novel methods in DBS collection aimed at improving the reliability and stability of analyte measurements from DBS samples, especially those collected under challenging field conditions. ***Hypothesis****: We hypothesize that DBS samples collected under our novel methods will have average drying times of less than 90 minutes in conditions of low to moderate, or high humidity.*
3. Specific Aim 3: To investigate the performance of our novel methods in DBS collection on the detection and quantification of RNA in DBS samples compared with the current DBS methods recommended by CDC. ***Hypothesis****: We hypothesize that our novel methods in DBS collection will demonstrate an overall improvement in performance for the detection and quantification of RNA from DBS samples compared with current DBS methods recommended by CDC.*

DISSERTATION STRUCTURE

This body of this dissertation is comprised of three related manuscripts (chapters 2-4) each corresponding to one of the dissertation's three specific aims. Chapter one serves as

introduction and chapter five as conclusion. The order and specific content for each chapter is presented in Table 1.1.

Table 1.1. Dissertation structure and order of content.

No.	Title	Content
1.	Introduction	Rationale for Research; Specific Aims; Dissertation Structure
2.	Manuscript (Aim 1)	State of the Science in Dried Blood Spots
3.	Manuscript (Aim 2)	Improved Methods for Field Collection and Storage of Dried Blood Spots
4.	Manuscript (Aim 3)	Improved Methods in the Collection of Dried Blood Spots for RNA Detection and Quantification
5.	Conclusion	Summary Findings; Future Research and Concluding Remarks

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CHAPTER TWO

Manuscript 1

State of the Science in Dried Blood Spots

Freeman, J., Rosman, L., Ratcliff, J., Strickland, P., Graham, D., Silbergeld, E.

ABSTRACT

Background: Advancements in the quality and availability of highly sensitive analytical instrumentation and methodology has led to increased interest in the use of microsamples. Among microsample platforms, dried blood spots (DBS) are the most well-known and researched. While there have been a variety of review papers published on dried blood spots, there has been no attempt at describing and evaluating the full range of analytes measurable in DBS, nor any systematic approach published for characterizing the strengths, weaknesses, opportunities, and threats associated with adoption of the technology.

Objective: The objective of this review was to apply a systematic approach to characterizing the state of science in dried blood spots.

Methods: A scoping review of reviews (SRR) methodology was utilized for characterizing the state of the science in DBS. A snowball methodology was incorporated into the SRR methods in order to build a comprehensive database of analytes measured in DBS, and a SWOT analysis was included for describing strengths, weaknesses, opportunities, and threats commonly associated with dried blood spots.

Results: We identified 1,947 unique analytes measured by one or more of 169 different analytic methods. These analytes include a broad range of biomarkers from target genes to transcripts to proteins and metabolites among many others. The strengths of DBS enable its simple application in most clinical and laboratory settings, and the removal of the need

for phlebotomy and cold chain handling for stable analytes can potentially expand biosampling to hard-to-reach and otherwise vulnerable populations. Weaknesses may limit adoption in the near term as DBS is a nontraditional sample and often requires conversion of measurements to plasma or serum values. Opportunities presented by novel instruments, analytic and analyte stabilization approaches, however, may obviate many of the current limitations of DBS, but threats surrounding privacy, security, and ethical considerations in the use of DBS samples must be seriously considered by those adopting the technology. These threats are particularly problematic to DBS due to the improved stability of residual samples, which hold enormous potential value for research and application for DBS compared with traditional samples.

Conclusion: DBS provide a wide range of existing and potential applications that extend beyond the reach of traditional samples. Current limitations are serious, but not intractable. Likely technological advancements will continue to minimize constraints around DBS adoption.

BACKGROUND

Recent advancements in the quality and availability of highly sensitive analytical instrumentation has led to increased interest in the use of microsamples (i.e., biological samples of less than 50 microliters) [13, 18, 23]. Microsamples have been applied for basic research, public health, and clinical medicine [1, 5, 32-35]. Interest in microsampling has been driven, in part, by the development of sophisticated computer software programs and methodological platforms for improved qualitative and quantitative analysis [16, 38, 40,

73]. Among microsampling methods, dried blood spots (DBS), are the most well-known and researched. DBS are a minimally invasive method for the collection of small quantities of whole blood from finger or heel stick with application to specially prepared filter paper for drying [12, 22]. DBS samples do not require phlebotomy, and DBS can be stored and shipped under ambient conditions, although a comprehensive assessment of analyte stability has not been performed [43-44]. Existing stability studies for DBS, while limited, have also demonstrated a wide range of analyte stability even among similar storage conditions [84].

To date, DBS has a range of applications in clinical practice, basic research, and population-based research [1, 5, 22, 62, 70]. The most common and widely accepted clinical use of DBS is for newborn screening programs, which are primarily concerned with the detection of metabolic disorders [10]. Other clinical applications in the published literature have focused on HIV surveillance, therapeutic drug monitoring, and clinical chemistry [4, 10, 20, 28, 34]. Basic research applications for DBS, include biomarker development and validation, drug discovery and development, forensic science, systems biology, and toxicology [5, 9, 17, 44, 60]. Population-based research applications are variable, but may be broadly categorized into human epidemiological studies, including environmental population studies [5, 7, 44, 49].

As interest in DBS methodologies continues to increase, potential adopters will need to quickly, effectively, and systematically assess the utility of DBS for their respective purposes. Understanding strengths and weakness, as well as potential opportunities and

threats, is essential for adopters to avoid false starts and ensure effective and appropriate adoption of DBS sampling to a specific goal. Furthermore, a comprehensive list of current and potential analytes, as well as their respective analytic methods, could help adopters assess the potential of DBS. While there have been a variety of review papers published on DBS methods, there has been no systematic assessment of the strengths and weaknesses of DBS, and attempts at compiling a comprehensive list of analytes validated in DBS have been limited in scope.

The objective of this review was to apply a systematic approach to characterizing the state of the science in DBS for public health and medicine. We aimed to characterize the state of the science through identification of strengths, weaknesses, opportunities, and threats; and by compiling a comprehensive list of analytes and their respective analytic methods as identified in the published literature.

METHODS

A scoping review of reviews (SRR) is commonly used for identifying relevant evidence and mapping key concepts within a research area or domain when the available literature is vast and/or diverse [76]. Different from a systematic review, which typically focuses on a weight-of-the-evidence approach to a specific question, a scoping review does not attempt to "weigh" the evidence, and instead aims to identify the nature and extent of research around a broad question or field of science [77]. A seminal paper on scoping reviews in 2005 by Arksey and O'Malley defined unique stages for conducting scoping reviews [76]. These methods were further revised in a recent publication in 2015 by

Goertzen et al. which adapted the Arksey and O'Malley methods and put forward a protocol for conducting SRRs [78]. Goertzen et al. defined 5 stages, including:

1. Stage 1: Establishing the Research Questions
2. Stage 2: Identifying Relevant Studies
3. Stage 3: Study Selection
4. Stage 4: Charting the Data
5. Stage 5: Collating, Summarizing, and Reporting the Results

Consistent with the common uses of SRRs identified in the Arksey and O'Malley paper, our study aims to summarize the state of the science for dried blood spots (DBS), a topic with a wide range of applications. Our review characterizes the state of the science around DBS for policy makers, researchers, and practitioners that may otherwise lack the time, resources, or expertise to undertake such an endeavor [79].

To this end, we adapted the Goertzen et al. methods for use in our study. The Goertzen et al. methods have been modified to include: 1) snowball methods and SWOT (strengths, weaknesses, opportunities, and threats) methods for use in data extraction (*Stage 4: Charting the Data*); and 2) a form of quality assessment, (*Stage 5: Collating, Summarizing, and Reporting the Results*). We have included these modifications in response to a recent paper by Levac et al. aimed at advancing the methodology in SRRs [80]. The Levac et al. study identified the lack of quality assessment in study selection and the poorly defined analytical methods in charting the data as methodological challenges to SRRs [80]. A

rigorous and iterative approach to each stage, consistent with current SRR methods and with the stated modifications, is described below.

Stage 1: Establishing the Research Questions

We began our study with a general question, "What is the current state of the science for DBS in the published scientific literature?" After a preliminary search of the literature identified a broad range of domains and applications and consultation with subject matter experts in academia and government, we decided to limit our study to the following research questions:

1. What analytes have been measured in DBS and with which analytic methods?
2. What strengths, weaknesses, opportunities, and threats are commonly cited for DBS?

Stage 2: Identifying the Relevant Studies

The search process was conducted under the guidance of the medical librarian at Johns Hopkins University (JHU). A preliminary search of the literature was conducted on 3 September 2015, in 4 electronic databases, including PubMed, Embase, Toxline, and SciFinder. The preliminary search was designed to capture all DBS-related publications in the scientific literature within the databases selected. The preliminary search yielded 27,850 citations; 17,589 after duplicates were removed.

After review of the preliminary search (Appendix A, Search Strategy, Supplementary Materials), we determined our search strategy was too broad to characterize the state of the

science and map key concepts in DBS, and decided to limit our search strategy to include only review papers and/or validation/evaluation studies involving DBS. No search limiters for language or publication date were included in the final search strategy. Our final search took place on 16 November 2015, in 3 electronic databases, including PubMed, Embase, and SciFinder. In total, 2,776 citations were identified; 1,178 citations after duplicates were removed (Figure 2-1).

Stage 3: Study Selection

Search terms, limiters, databases, strategy by database, and inclusion/exclusion criteria are provided in Table 2-1. All citations identified in our final search were imported into the web-based bibliographic manager EndNote, and exported for uploading to Covidence, a web-based software program for managing literature reviews. We used Covidence for title/abstract and full text review. Title/abstract review included a dual review process with each reviewer blinded to the other reviewer's decision. Conflicts were resolved by a third reviewer. During title/abstract review, we selected only reviews, commentaries/short reports, or technical reports involving a broad category or domain of DBS. We included citations involving either human or animal subjects as long as animal subjects research had an explicitly stated relevance to human health. We excluded citations in which DBS were not a focus of the study.

Upon completion of title/abstract review we requested full text PDF files from the medical librarian. Publications not available directly through JHMI were requested through an interlibrary loan. Full text PDF files were uploaded to Covidence for completion of full

text review. Inclusion/exclusion criteria were confirmed during full text review and citations in which the full text was not available in English were excluded. Full text review included a dual review process with each reviewer blinded to the other reviewer's decision. Conflicts were resolved by a third reviewer. Due to the variability in the types of studies included in scoping reviews, and consistent with accepted SRR methodologies, no formal quality assessment component was included during study selection; however, a type of quality assessment, as described under *Stage* 5, was included during our review [77].

Stage 4: Charting the Data

Charting the data involves identification of key issues and themes found within information obtained from research reviewed in the SRR. Publications identified as relevant during full text review were included in this process. *Charting the Data* was completed in 2 phases, each conducted with two reviewers blinded to each other's work. After each phase, reviewers compared and reconciled findings. If differences could not be reconciled between the two reviewers, a third investigator made a final decision. Phase I of *Charting the Data* is designed to address the first research question: ***What analytes have been measured in DBS and with which analytic methods?***

Investigators utilized a 'snowball' technique for *Charting the Data* pertaining to analytes measured in DBS. Specifically, if an analyte was cited as has having been measured in DBS, investigators followed citations until the source material (i.e., original research) was identified. Once the original research had been identified, investigators extracted the

following information: analyte name, analytic method, SRR MLA stem citation, and source/original research MLA citation.

In order to improve the comprehensiveness of the analyte database, investigators cross-referenced the snowballed database with original research studies identified from the final search strategy and excluded at the title/abstract review stage. Analytes measured in DBS identified through these studies and not already found in the database were added. Phase II of *Charting the Data* is designed to address the second research question: ***What strengths, weaknesses, opportunities, and threats are commonly cited for DBS?***

Phase I utilizes SWOT methods for extraction. Though not yet commonly used in public health and medicine, SWOT methods were originally developed by the business community to enable strategic planning [81-82]. We selected these methods for their potential to aid policy makers, researchers, and practitioners in considering adoption of DBS for their respective purposes. SWOT provides a systematic approach to identification and review of both intrinsic issues (i.e., strengths and weaknesses) and extrinsic issues (i.e., opportunities and threats) as they pertain to a particular subject; in this case, DBS. Furthermore, the selection of a systematic method for conducting our review directly addresses one of the key limitations of SRRs: lack of a well-defined method for extraction of information. SWOT methods for extraction were applied to each included study individually during Phase I, and utilized an extraction table prepared by study investigators, which included the following items: first author and year, title, MLA citation, study type,

study purpose, study conclusions, strengths, weaknesses, opportunities, threats, and additional comments.

A component of quality assessment was included at this stage. Specifically, investigators reviewed the source reference (i.e., original research) cited for all information identified for SWOT extraction. Upon review, if the source material cited did not support the conclusion made in the publication reviewed in the SRR, this information was excluded from the SWOT extraction table.

Stage 5: Collating, Summarizing, and Reporting the Results

Upon review of the analyte database formed by the snowball extraction methods, analytes and analytic methods were given classifications. Analytes were classified as small molecule (molecular weight < 900 Daltons), large molecule (molecular weight =/> 900 Daltons), nucleic acid (i.e., DNA or RNA) or element. After review of all unique analytic methods identified in the literature, we devised broad categories of analytic methods as follows: mass spectrometry (MS), immunoassay, nucleic acid based, separation (chromatography), separation (electrophoresis), separation (other), spectroscopy, and other. Our classifiers were informed by a review of current relevant literature. A final analyte database with classified analytes and methods was then imported into Stata for calculation of the following: (1) total number of unique analytes, (2) number of unique analytic methods, (3) number of unique analyte-analytic method combinations, (4) percent of analytes by class, (5) percent of analytic methods by category, and (6) percent of unique analyte-analytic methods by method category.

Upon review of each included study SWOT extraction table, two investigators blinded to each other's work reviewed the SWOT tables to build a separate, summary SWOT table including common or reoccurring strengths, weaknesses, opportunities, and threats across all included studies. Investigators then compared their tables and rectified differences into a single, unified SWOT table.

RESULTS

Of the 1,178 citations identified for screening, 75 studies were selected for inclusion in the review (Figure 2-1). There were 62 review papers, 11 commentaries or short reports, and 2 technical reports included (Table 2-2).

Phase I - Analyte Database

We identified a total of 1,947 unique analytes in the literature to have been measured in DBS. A comprehensive list of analytes divided by class is provided in Table 2-3. Of the 1,947 unique analytes measured, 48% (n=942) were classified as 'Small Molecule', 34% (n=670) as 'Large Molecule', 16% (n=306) as 'Nucleic Acid', and 2% (n=29) as 'Element' (Figure 2-2). In terms of the range of analytes identified in the literature, Table 2-3 includes genomic, epigenomic, transcriptomic, proteomic, and metabolomic markers. In the area of infectious diseases, Table 2-3 includes analytes for viral, bacterial, parasitic, and protozoan detection [49, 57]. Additionally, Table 2-3 includes a wide range of analytes classified as markers of exposure as well as health and disease status [5, 7, 10, 33, 49].

We identified a total of 169 unique analytic methods in the literature to have been applied to DBS samples for bioanalysis. All major categories of common analytic methods were identified, including mass spectrometry, immunoassay, nucleic acid based methods (e.g., polymerase chain reaction), chromatography, electrophoresis, and spectroscopy among others. A comprehensive table of analytic methods divided by category of method is provided in Table 2-4. Of the 169 analytic methods applied, 33% (n=55) were measured by methods classified as 'Mass Spectrometry' (MS), 23% (n=38) as 'Immunoassay', 12% (n=21) as 'Nucleic Acid Based', 12% (n=20) as 'Separation (Chromatography)', 5% (n=8) as 'Separation (Electrophoresis)', 2% (n=4) as 'Separation (Other), 7% (n=12) as 'Spectroscopy', and 7% (n=11) as 'Other' (Figure 2-3). Methods identified as 'Other' included culture based assays (n=3), enzyme based assays (n=2), precipitation assays (n=2), and electric potential (n=1).

While an examination of the unique analytic methods applied to DBS may help characterize the range of potential DBS applications, it does not necessarily characterize common practices in the literature. It is important to note that many of the analytic methods in Table 2-4 were applied infrequently, while others were applied routinely. For example, Indirect Potentiometry was applied only once for the purpose of measuring a single analyte, ceruloplasmin; while Mass Spectrometry was applied to nearly 300 (n=292) large molecule analytes alone. In order to better characterize common DBS applications in the literature, we also examined the combination of unique analytes with their respective analytic methods. We found 3,073 unique analyte/analytic method combinations. Of the 3,073 combinations, 61% of analytes (n=1,867) were classified as having been measured by

'Mass Spectrometry' (MS), 18% (n=542) by 'Immunoassay', 12% (n=366) by 'Nucleic Acid Based', 6% (n=196) by 'Separation (Chromatography)', 1% (n=17) by 'Separation (Electrophoresis)', 0.5% (n=12) by 'Separation' (Other), 2% (n=50) by 'Spectroscopy', and 1% (n=23) by 'Other' (Appendix B - Analyte Database, Supplementary Materials).

A complete list of all analytes with their corresponding analytic methods, classifications, SRR stem references, and original research references can be found in the Analyte Database provided in Appendix B under Supplementary Materials.

Phase II - SWOT Analysis

For the purposes of this investigation, only those strengths, weaknesses, opportunities, and threats, that are specifically relevant to DBS, are provided in Table 2-5. The SWOT analysis applies to the most common type of DBS sampling (i.e., sampling by finger or heel stick followed by direct application to filter paper cards with ambient storage). Though it is possible to use blood collected by venipuncture for volumetric application of blood to filter paper cards, as well as cold storage to improve analyte stability, these modifications remove several of the key advantages of DBS methods, namely sampling without need for phlebotomist or cold chain.

Strengths

DBS sampling is minimally invasive, requires only a small volume of blood (i.e., < 50 uL), and utilizes simple collection methods (i.e., no centrifugation for plasma preparation prior to storage) [1, 12, 46]. DBS sampling typically involves prick of a finger or heel with a

small lancet followed by application of several drops of blood to filter paper cards for drying and storage. One of the key advantages to DBS sampling is the ability to derive a volumetric amount of blood from a non-volumetric application to filter paper [64]. This is achieved by punching a fixed diameter cylinder for analysis from a portion of the dried spot that is assumed to be fully saturated on the filter paper. This ability to derive a volumetric amount blood combined with minimally invasive methods, small sample volume, and simple collection, allow DBS to be collected in the absence of a trained phlebotomist or lab, and may enable self-sampling as well as sampling outside of the traditional clinic or lab setting [23-24, 34]. In terms of human sampling, these strengths make DBS a preferred method for collecting blood from difficult to sample populations, such as neonates, the elderly, persons with damaged veins, or persons in remote or under-resourced environments [7, 32, 34]. In terms of sampling from animals, DBS can allow for reduction and refinement in the use of small or juvenile animals [1-2, 6]. For example, by reducing the quantity of blood collected and the invasiveness of the method, DBS use in toxicological studies can allow for serial sampling from the same animal, which reduces the total number of animals required, and allows researchers to no longer rely on composite profiles[2], which improves overall data quality [29, 35, 55].

The dried matrix of DBS samples inactivates most pathogens and thereby reduces biohazard risks associated with samples in transport [9, 31, 32, 75]. Reductions in

[2] Composite profiles are commonly used in toxicological studies involving small or juvenile animals. Composite toxicological profiles are generated by combining toxicological data from multiple animals in order to simulate repeat sampling (i.e., serial sampling) from a single subject. They are often necessary when regulatory requirements limit blood sample volume or when the sample volume itself necessitates terminal sampling. In either of these cases, investigators are unable to conduct serial sampling, which diminishes data quality.

biohazard risks combined with simple methods in storage and transport (i.e., ambient conditions, no dry ice required) have allowed DBS to be considered exempt, non-regulated materials and are therefore not subject to hazardous material regulations in shipping [46]. Materials required for DBS sampling are relatively low cost, and have few material inputs and waste [22, 36, 66]. When taken together, the reduction in material inputs and waste, low cost, ambient storage and transport, simple collection, and minimally invasive methods, make DBS a suitable matrix for biosampling in large and/or complex population-based studies [5, 44].

DBS samples are compatible with most bioanalytical methodologies, which allows existing labs to easily adopt the technology with only minor modifications to their workflow [16]. Aside from a hole punch device to remove a portion of sample for processing, all other material requirements for analyzing DBS samples should be readily available in most labs [43]. DBS are also a versatile sample matrix. For example, anything that can be measured from liquid whole blood, plasma, or serum can, in principle, be measured in DBS [11]. Analytes representing a wide range of physicochemical properties have already been validated. To date, DBS samples have been used for a variety of viral, bacterial, protozoan, and helminthic agents [49]. DBS have also been used to measure DNA, RNA, antibodies, proteins, drugs, metabolites, and an assortment of environmental contaminants among other analytes (Table 2-3).

DBS as a method has been demonstrated to achieve similar levels of precision and reproducibility to that of traditional larger volume venous blood collection in vacutainer

tubes or capillary pipettes [11, 43]. Compared with liquid samples, several analyte classes in DBS have shown improved stability. For example, analytes susceptible to degradation due to hydrolysis, photolytic processes, and esterase as well as RNAase action [3, 7, 29, 57, 68]. Consequently, analyte stability in DBS compared with liquid samples is particularly pronounced for traditionally unstable analytes such as RNA, cytokines, and several classes of drug metabolites [41, 54, 62, 66].

The Center for Disease Control and Prevention (CDC) have established an independent quality control program, CDC's Newborn Screening Quality Assurance Program (NSQAP) [46]. NSQAP provides strict guidance to manufacturers and end-uses of DBS cards and helps to improve sensitivity and reproducibility of filter paper [43]. In addition to NSQAP, DBS have easy to understand federally established guidelines for collection and shipment [46]. Though no federal or international bioanalytical validation methods have yet been fully established, DBS validation methods were recommended in 2011 by the European Bioanalysis Forum (EBF) [45, 55, 59, 75].

Weaknesses

It is important for potential adopters to understand that DBS is not the same thing as traditional plasma or serum (Table 2-6). Differences between sample types may limit comparability of measurements from DBS and constrain their utility in public health and medicine. Differences of note between DBS and plasma or serum include the following: DBS comes from capillary blood versus venous blood; consists of whole blood versus centrifuged plasma or serum; is dried versus liquid or frozen; is typically less than 50 uL

versus several milliliters; requires open air drying prior to ambient storage versus immediate cold storage; is analyzed with modified protocols versus those which were originally designed for plasma or serum; and often has converted or adjusted measurement as compared to direct measurement for traditional samples [43-44, 49, 55, 57]. Each of these differences present the opportunity to introduce bias into converted measurements taken from DBS samples, and while DBS has often been successfully adjusted to corresponding plasma and serum values, the underlying assumptions for a valid adjustment must be validated before DBS can be reliably used [45, 54].

The small volume of blood in DBS requires highly sensitive analytical instrumentation for accurate quantification, and may limit DBS utility for repeat testing [45, 54]. The collection of DBS, while simple methodologically, may also be constrained by cold or dehydrated patients whereby the amount or viscosity of the blood can be problematic for application to filter paper cards through uneven saturation of the filter paper and ultimately inaccurate estimation of starting volume from a fixed diameter punch [5, 7]. As previously mentioned, DBS samples require open-air drying for a minimum of 2-3 hours before storage [47]. This is problematic for a several reasons. First, open-air drying may confound analyte measurements due to contamination, especially when the analyte of interest is DNA or an environmental contaminant [55]. Second, drying cards under open-air conditions requires extra space for drying racks and can be problematic in field-based collection [6]. Third, drying rates vary and are impacted by surrounding temperature and humidity conditions, which are particularly problematic in tropical, humid environments [22, 43]. The rate of drying not only impacts the ability to store samples in a reasonable time frame, but also

alters analyte measurements, especially for metabolites and other analytes susceptible to degradation by hydrolysis, as metabolism as well as hydrolytic processes will continue within the blood on the filter paper card and are not quenched until moisture has been removed from the spot [53-54]. It should also be noted that while most pathogens are inactivated by drying, some pathogens such as dengue, hepatitis B, and group A streptococci remain active for several days after drying [49, 57-58].

Manual DBS methods for processing and bioanalysis are time and labor intensive [56, 72]. DBS requires a series of preparation steps, including punching discs from cards, elution and extraction, filtration, and in some instances chemical derivatization [72-73]. These steps each add cost and complexity to DBS adoption. For example, use of punch devices for collecting fixed diameter discs from DBS cards for analysis may cause contamination if devices are not adequately cleaned between punches (i.e., carry-over effects) [27, 42]. Some steps, like the addition of an internal standard (IS) or sample dilution, may cause problems for traditional samples, but they also present challenges unique to DBS due to the use of a dried matrix [49]. For example, an IS cannot be added to blood and homogeneously mixed before bioanalysis when sampling directly by finger or heel stick, and while an IS may be added to the extraction solvent, this does not account for issues arising prior to extraction [3, 11]. In terms of storage, as DBS samples are typically stored under ambient conditions, they are more susceptible to extreme environmental conditions such as high temperature and humidity [75]. These conditions, if not properly managed, can facilitate bacterial growth and enhance the rate of analyte degradation rendering DBS sample results unreliable [53, 55].

The most commonly cited weakness of DBS is the hematocrit effect, which is the impact of varying percentages of red blood cells in whole blood spotted to filter paper [14-15]. High or low hematocrit has two primary issues. First, hematocrit can affect the blood-to-plasma ratio for target analytes, which can alter their measurements and bias any attempt at conversion from DBS to plasma [45, 74]. Second, hematocrit directly affects the viscosity of blood and can thus affect how a spot spreads and saturates filter paper, which in turn limits volumetric extraction of blood from a set diameter punch as well as extraction recovery [55-56]. In addition, or in combination with hematocrit effects, DBS measurements can be impacted by chromatographic or matrix effects within the filter paper card itself, which can lead to uneven spreading of blood or distribution of analytes within a spot depending on their particular physicochemical properties [54, 72]. Sample heterogeneity is also a particularly unique issue for DBS [55, 59]. Traditional liquid samples can be easily mixed to achieve a homogenous sample matrix, but DBS are a dried matrix, and when a portion of a spot is punched out from filter paper, lack of homogeneity within the spot can lead to different analyte measurements depending upon the location of the punch [30].

Differential analyte stability and degradation rates, as well as extraction efficiency, are not unique to DBS. However, open air drying and ambient storage is unique, and can exacerbate issues of differential analyte stability, degradation, and extraction [3, 6]. For example, measurement of analytes susceptible to oxidation can be impacted by atmospheric oxygen during drying, and extreme temperature or humidity conditions will

often have differential effects on analytes of different classes [74]. A major concern regarding analyte stability in DBS involves the inability to retain and detect volatile organic compounds (VOCs) in DBS samples [7, 55]. VOCs are often lost during drying, limiting the ability of investigators to measure VOCs from DBS, which in turns limits the utility of DBS for environmental studies [7]. Beyond stability of analytes, the matrix itself can be problematic in analysis. The added addition of filter paper to the matrix presents a challenge in bioanalysis as the filter paper can cause matrix effects at the point of analysis [45, 53, 63, 68]. For example, ion suppression is a commonly cited issue for DBS samples measured by mass spectrometry [48, 52].

Another relevant issue for potential adopters is the incomplete and emerging regulatory landscape for DBS. The current Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines for traditional samples are inadequate for DBS as bioanalytic validation of dried blood spots may require consideration of several additional parameters [29]. Added validation parameters may include type of card, volume applied to filter paper, homogeneity of spotting, effects of hematocrit, and comparison with gold standard traditional samples to name a few [45, 54, 59]. At present, FDA does not accept DBS as a stand-alone sample matrix and requires bridging studies for comparing DBS with traditional samples, which adds cost and work to adopters [59]. Lastly, of the assays validated in the literature, there is a wide range in quality of validation and often no comparison of DBS to an existing gold standard [54, 57].

Opportunities

A European Directive and concerns from US federal agencies have put pressure on researchers and drug developers to comply with the three 3Rs (i.e., reduction, refinement, replacement) in the use of animal subjects and may lead to wider adoption of DBS in preclinical and toxicology studies [6]. As stated previously, DBS can reduce the number of, and stress to, animal subjects in research and development [6, 60]. Other forces that may encourage DBS adoption are the trends toward centralization of labs and an increase in demand for outpatient or off-site clinical services [24]. As larger centralized lab facilities adopt DBS, the quality and availability of DBS analysis should improve. Furthermore, DBS are particularly suited to non-clinic or lab-based settings that may enable their use for off-site services such as home-based sampling [5, 32].

Traditionally, analytical instrumentation has lacked adequate sensitivity for accurate measurement of small quantity biosamples, but recent advancements in highly sensitive instrumentation such as LC-MS/MS and Digital Droplet q-PCR have helped resolve these issues [48-49]. Exponential reductions in the cost of sophisticated instruments have led to greater availability of the necessary methodologies for accurate use of DBS, and may also encourage adoption [41-42]. As interest has continued to grow, computer-based, robotic automation of DBS methods in bioanalysis have also emerged and can help resolve many of the issues of labor intensive methods involved in DBS [54, 72]. At present, there are a range of semi- and fully-automated systems commercially available for dried samples matrix processing and bioanalysis [54, 64]. Recent advancements in microfluidics and nanotechnology may also provide the next generation of DBS technology, and have already

been applied to DBS for achieving high-fidelity blood droplet manipulation without the need for manual intervention [14, 16, 54]. Such advancements can help resolve traditionally problematic issues in DBS, such as hematocrit and chromatographic or matrix effects. For example, membrane filtration technology has been designed into filter paper cards for filtering out a volumetric amount of plasma from a non-volumetric amount of whole blood taken from a finger stick [41, 45, 71]. Use of membrane filtration cards can simultaneously minimize the effects of hematocrit, while also providing plasma from whole blood without the need for centrifugation [41, 45].

The emergence of 'online' or direct analysis methods for DBS provide several advantages over traditional methods; namely, the removal of the need for punching or elution [42, 45]. Several technologies for online analysis are currently available, including desorption electrospray ionization (DESI), direct analysis in real time MS (DART), and paper spray-MS technologies [13, 26, 42, 45]. Though these methods have been shown to be less sensitive than off-line manual methods, their sensitivity has shown recent improvements [13]. Researchers have also improved microsample measurements from the data analytics side. For example, the use of endogenous indicators such as potassium have been used in DBS for estimating blood hematocrit and adjusting analyte measurements accordingly [14-15]. Furthermore, as multiplex platforms such as MS have become more routinely used, the use of multi-analyte molar ratios in clinical diagnostics have been demonstrated to be an effective data analytics approach for reducing variability and improving diagnostic performance. For example, diagnosis of phenylketonuria (PKU) from DBS samples in

newborn screening can be achieved by examining the relative amounts of phenylalanine with those of tyrosine or leucine [10].

Requirements of traditional blood sampling (i.e., phlebotomy and cold chain) have often precluded the use of biosampling in hard-to-reach or otherwise vulnerable populations. Use of DBS can help facilitate sampling within these populations without the need for phlebotomy or cold chain [33, 65]. What's more, DBS is particularly well-suited for use in large complex study designs where sampling may occur in multiple sites over an extended period of time [5, 36]. DBS provide a cost effective and logistically feasible method for such studies. DBS may also provide a viable sampling method for field-based forensics where proximity in time-to-events such as driving while under the influence or homicide may be important for obtaining accurate measurements from blood [9, 53-54, 60]. Finally, DBS techniques which yield a stable biosample in a dried matrix under ambient storage may also be applied to a variety of other kinds of biological samples, such as saliva, urine, or tissue [45].

Threats

Biobanking of DBS samples and their suitability for DNA analysis present a privacy and ethical dilemma around proper use of residual samples [47, 52]. This threat is enhanced by the predominant use of DBS for newborn screening, which are collected from newborns for metabolic screening purposes, but which can provide a wide range of applications beyond their intended use [25, 47]. Residual samples from newborn screening programs may afford researchers a powerful tool for retrospective study. However, serious questions

remain as to whether mothers could or should be adequately consented for use of their newborn child's DBS sample months to years after collection. Perceived improper use can and has led to public outrage and the mandated destruction of millions of residual DBS samples. For example, a settlement reached in Texas with a civil rights group led to the destruction of more than 5 million residual DBS samples [25]. DBS has also been put forward as a preferred sampling matrix for pediatric populations, but the potential enrollment of children in research studies could result in similar public outrage, particularly in the event of adverse health outcomes associated with pediatric clinical trials [50].

Another threat to DBS adoption is the dominance of traditional samples such as liquid plasma and serum in public health and medicine [28, 32, 40, 44]. Established labs are highly automated and are optimized for traditional samples [31]. Consequently, advantages of DBS may be overcome by resistance from labs due to the convenience and familiarity of using traditional samples. The lack of availability of a lab experienced with DBS samples may also constrain DBS use by researchers and clinicians [44]. If potential adopters cannot readily find an experienced lab for DBS bioanalysis, or if existing labs are unable to handle the added workload in a timely manner, then adopters may opt for traditional samples as a matter of convenience or even necessity. Finally, the most common threat identified in the literature around DBS use is regulatory uncertainty [14, 36]. At present, federal and international guidelines around DBS are lacking in comparison with traditional samples [27, 45]. The absence of clear regulatory guidance, and the potential for new or unexpected regulations will continue to constrain widespread DBS adoption [19, 23, 45, 60].

DISCUSSION

Analyte Database

With nearly 2,000 analytes measured in more than 150 different analytic methods, DBS presents potential adopters with a wide range of options for application. In the basic sciences, analytes measured in DBS have been published across the spectrum of 'omics-based analyses, including the genome, epigenome, transcriptome, proteome, and metabolome [13, 25, 34]. Beyond basic science, DBS has been applied in the field for use in public health and medicine for measuring markers of exposure (e.g. pathogens, environmental toxicants), physiological response, and health outcomes [5, 7, 33, 49]. From diagnosis to surveillance to retrospective study, the repertoire of DBS application continues to expand.

A majority of analytes measured in DBS and extracted for inclusion in the DBS database are classified as 'Small Molecule' and nearly two thirds of unique analyte/analytic method combinations in the database were measured by methods classified broadly as 'Mass Spectrometry' (Figure 2-4). These findings are not unexpected given the requirement of highly sensitive analytic instrumentation such as MS for measuring small quantity biosamples, and the wide range of analytes that can be measured in a single analytic run with MS methods. Though slightly less common, a range of large molecules have also been measured in DBS and can be found in the database, including therapeutic proteins, monoclonal antibodies, and a variety of carbohydrates among others (Table 2-3).

DBS for measuring nucleic acids (i.e., DNA and RNA), though much less common in the database than small or large molecule analytes, have been no less effective in their application. DNA has been demonstrated to be stable in DBS for more than 10 years, and RNA, while traditionally unstable in liquid samples, has shown remarkable stability in DBS [22, 66]. The stability of RNA in dried blood spots is a direct consequence of the absence of water in a dried matrix, as water is required for RNase action to degrade RNA [83]. Similar improvements in stability for analytes susceptible to hydrolysis suggests that DBS is not just an adequate replacement for plasma or serum, but in some instances, it may be a preferred matrix.

An examination of the range of analytic methods that have been applied to DBS confirm the theory that dried blood spots can, in principle, be applied to measuring anything you typically measure in liquid whole blood, plasma, or serum. Furthermore, our analyte database demonstrates an often-cited strength of DBS, that it can be applied to most common analytic instruments. In fact, analytes in the database have been measured with all the most common analytic methods. Still, the application of DBS in the literature and the rigor of validation methods applied are variable [20, 24, 27]. Potential adopters may consider the analyte database as a means for determining if DBS is a possible solution to their respective needs, but additional inspection of the specific analytes of interest and their validation of assay studies will be required prior to adoption. Original research for analytes measured in DBS can be found in the analyte database and may serve as a good first step for those considering adoption.

We have identified two potential limitations in our efforts to build a comprehensive analyte database for DBS. First, while our methods cast a wide net in terms of search strategies, we limited our study to three primary databases. Relevant publications not included within these databases would not have been captured by our review. However, we selected our databases in consultation with the medical librarian and after preliminary searches of other potential databases returned mostly duplicates and/or few relevant studies. Second, the size and comprehensiveness of the review required nearly 12 months to complete. Relevant studies published during the months between our final search and publication would not be captured in our review. We believe this is a common limitation of most review papers, and is an acceptable limitation of our study given the scope of our review.

SWOT Analysis

The combined strengths of DBS allow for removal of two often limiting components of traditional liquid samples: phlebotomy and cold chain handling [30]. This removal makes DBS a suitable alternative to traditional matrices for sampling outside of the clinic or lab, which allows DBS to be used in a range of settings [24]. DBS lends itself to sampling in situations as simple as the home or as complex as large longitudinal study designs in austere environments [5]. The use of simple to collect, minimally invasive, small volume samples like DBS also provide substantial benefit in the reduction and refinement in the use of animal subjects across the sciences [18, 35]. These same benefits allow DBS to improve sampling from hard-to-reach or otherwise vulnerable populations as well as problematic groups such as neonates and the elderly where large volumes of blood collected by venipuncture can be difficult [32, 34]. In addition to the advantages of DBS for sampling

in particularly problematic environments, improved stability for some analytes, such as RNA and other analytes susceptible to degradation due to hydrolysis, make DBS not just more suited than traditional samples to some environments, but more suited to entire classes of analytes as well [29, 53]. The strengths of DBS, therefore, make a compelling case for potential adopters.

The weaknesses involved with DBS sampling cannot be ignored, but may be better understood by potential adopters by considering them in the context of their opportunities. For example, limitations in the retention and detection of VOCs could be obviated by identification of relevant downstream metabolites [7]. Additionally, issues in limits of detection for small volume samples have largely been addressed through advancements in the quality and availability of highly sensitive analytical instrumentation [35]. Still, even when measured precisely, the variability inherent to dried microsamples stored under ambient conditions remains an impediment to wider adoption.

Current approaches to DBS rely on conversion of measurements for single analytes to corresponding plasma or serum values for the purposes of applying a clinically relevant diagnostic range to an individual analyte. However, as a nontraditional sample matrix, the differences between DBS and plasma or serum are substantial, and each present the possibility of introducing bias into converted measurements. What's more, even minimal bias when introduced to a microsample will have a large effect on the eventual converted value.

Alternatively, some of the variability in DBS measurements has been resolved by use of direct measurements rather than conversions to plasma or serum values, and through application of diagnostics that apply molar ratios, or relative values for multiple analytes, rather than single analyte ranges [10, 42]. This approach has been applied in newborn screening for PKU and has demonstrated improvements in diagnostic performance [10]. It may be the case that clinical application of DBS is better served by developing diagnostic criteria that are especially suited to DBS rather than attempting to apply DBS measurements to diagnostics originally developed for traditional plasma or serum. However, such an approach requires further investigation.

Another concern around DBS adoption is lack of consistency in methods for published validation studies; however, as recommendations for DBS validation have been recently put forth, and as regulatory agencies consider adoption of validation guidelines, the quality and availability of validation studies for DBS should improve [40, 45, 67-68]. In the meantime, potential adopters should become familiar with the parameters of a quality DBS bioanalytic validation [27, 55, 63]. Potential adopters must also remain aware of the regulatory landscape, which at present is poorly defined for DBS [45, 74]. However, as interest and use in the technology has expanded, scientific organizations as well as industry and regulatory agencies, have begun to take notice. For example, having recognized the need for greater collaboration and pooling of resources, the European Bioanalysis Forum created the Microsampling Topic Team, and the Global Bioanalysis Consortium recently began investigations specifically into DBS [59].

Though FDA in the US, and other regulatory agencies, do not yet accept DBS as a standalone sampling matrix for drug studies and most clinical applications, they have encouraged adopters to work closely with regulatory agencies while conducting bridging studies between traditional samples and DBS [74]. In the near term, these studies will undoubtedly add costs and complexity in adoption, but will be less necessary as more are completed and regulatory agencies grow more familiar with DBS application.

The advantages of DBS in terms of sampling from neonates or in austere environments, and their use in biobanking and retrospective investigation may also present one of the biggest threats to adoption [7, 44, 50, 52]. Consideration must be given to the feasibility and appropriateness of analyzing DBS samples collected from neonates or from persons in low resource for purposes other than they were originally intended. Though this could be resolved by a more expansive consenting process, questions around whether a person can truly consent for things neither they nor the person consenting them have yet considered need to be addressed. More restrictive use of residual samples could also be an effective measure for protecting against this threat, but would limit the utility of DBS samples for longitudinal and retrospective investigations.

Conclusion

DBS provide a wide range of existing and potential applications that extend beyond the reach of traditional samples. The utility of DBS for collection of blood outside of the clinic provide a range of possible applications from the research lab to the home to the most remote environments on earth. Current limitations though serious, are not intractable.

Issues of variability in measurements from DBS use in public health and medicine can be addressed by a variety of existing and emerging innovations. Technological advancements in material inputs for DBS and data analytic approaches for measurement have, and will likely continue, to minimize constraints around DBS adoption.

TABLES AND FIGURES

Table 2-1. Summary of review search terms, limiters, databases, inclusion and exclusion criteria.

Search Terms	A combination of controlled vocabulary and keyword terms were used to represent the two main concepts of DBS and validation studies. Terms used included: dried blood spot testing, blood spot, dried blood, guthrie, blood sampling paper, filter paper blotter, filter paper disk, dried filter paper, PKU card, blood collection card, validation studies, evaluation studies, validation
Search Limiters	Language: *None* Publication Date: *None*
Databases	PubMed, Embase, SciFinder
Inclusion Criteria	1. A review paper, commentary or short report 2. Discusses a category or domain of DBS (e.g. DBS in clinical trials, DBS methods development, etc.) 3. Involves human subjects or animals subjects with explicitly stated relevance to human health (e.g. animal toxicology or pharmacology studies)
Exclusion Criteria	1. DBS is not a focus of the study (i.e., DBS is not explicitly mentioned as relevant to the stated purpose, objective, or aims of the review) 2. Full text not available in English

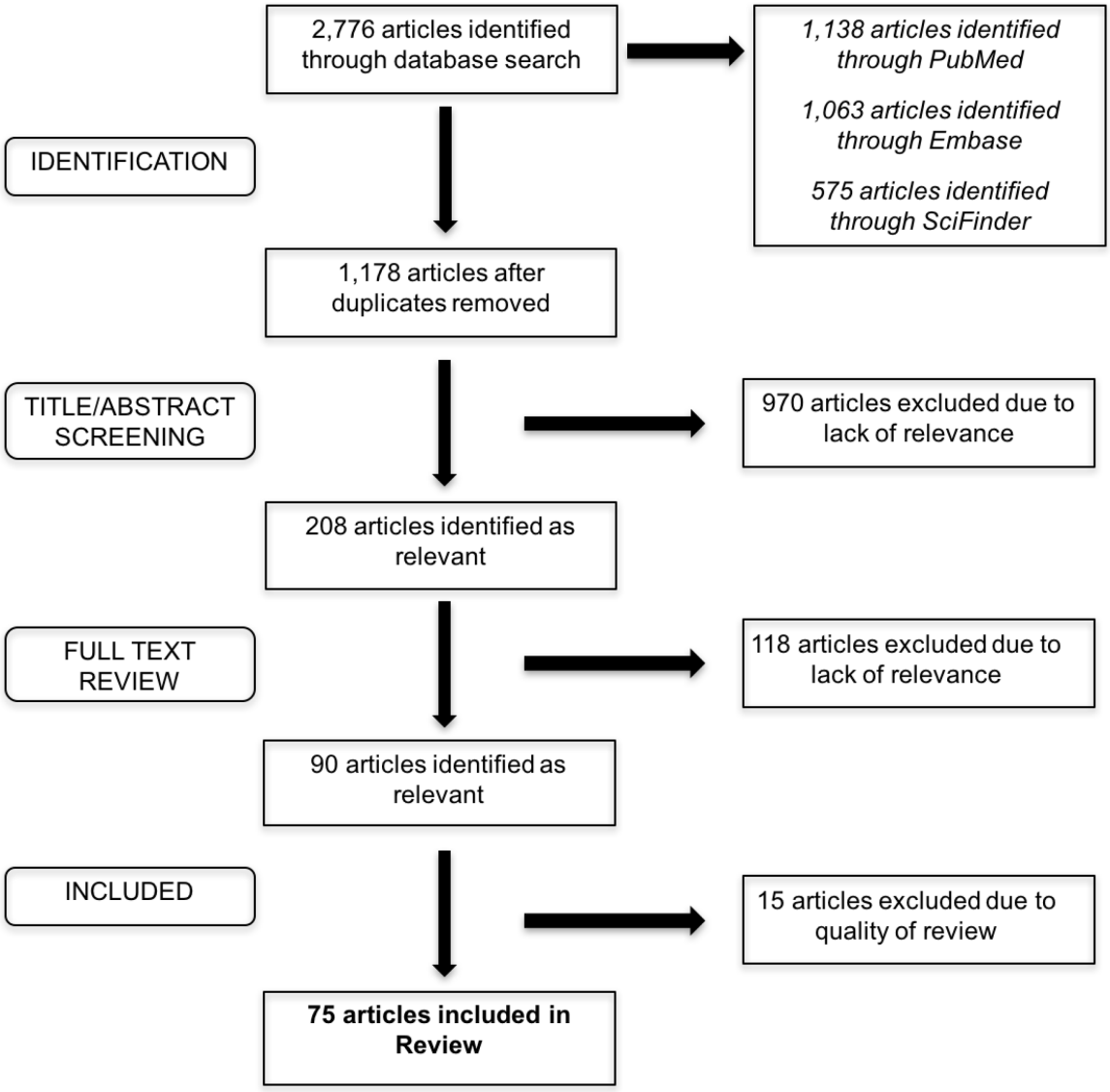

Figure 2-1. PRISMA flowchart of study selection process.

Table 2-2. Summary table of included studies.

Author & Year	Study Type	Title	Summary Conclusions
Amsterdam 2010 [1]	Commentary/ Short Report	The application of dried blood spot sampling in global clinical trials.	DBS adoption in clinical trials will likely see substantial savings in cost and improvements in overall data quality.
Barfield 2011 [2]	Review	GlaxoSmithKline's experience of incurred sample reanalysis for dried blood spot samples.	The future of DBS in drug studies is dependent upon solving issues of hematocrit effect and building confidence in DBS use in industry through good quality data.
Bowen 2014 [3]	Review	Challenges and Experiences with Dried Blood Spot Technology for Method Development and Validation.	New techniques in DBS are emerging, but more validation and vetting are required. Though challenges remain, in some instances, DBS offers immediate benefits to adopters.
Bowen 2011 [4]	Review	Investigations into the environmental conditions experienced during ambient sample transport: impact to dried blood spot sample shipments.	Data loggers are a feasible method for tracking environmental conditions in samples during transport and storage. DBS samples transported without controlled environments are likely to experience extreme conditions, especially in flight.
Brindle 2014 [5]	Review	Applications of Dried Blood Spots in General Human Health Studies.	DBS are a well suited biological matrix for human health studies, and their convenience enable adoption in a variety of field settings.
Burnett 2011 [6]	Review	Dried blood spot sampling: practical considerations and recommendation for use with preclinical studies.	DBS use in preclinical studies can provide substantial benefits towards principles of the 3Rs (reduction, refinement, replacement).
Calafat 2014 [7]	Review	Applications of dried blood spots in environmental population studies	DBS use allows biomonitoring in vulnerable and otherwise difficult to sample populations, however, limited data is available on DBS use for biomonitoring. Validated protocols for DBS use and further research to the suitability of DBS for epi studies are needed.
Chace 2003 [8]	Review	Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns.	MS/MS technologies are suitable for newborn screening and other mass screening programs. MS/MS improves detection of many diseases and may expand diagnostics to other important disorders in pediatric medicine.
Chace 2014a [9]	Review	The Use of Dried Blood Spots and Stains in Forensic Science.	DBS provide a several benefits for use in forensic science, especially in the ability to collect samples closer to the time of an event. However, small sample volumes remain a limitation for measuring some drugs.
Chace 2014b [10]	Review	Applications of Dried Blood Spots in Newborn and Metabolic Screening.	DBS analysis for newborn screening has helped to improve the lives of children and opened new opportunities in clinical chemistry and laboratory science.

Corso 2010 [11]	Commentary/ Short Report	A powerful couple in the future of clinical biochemistry: in situ analysis of dried blood spots by ambient mass spectrometry.	Current techniques in ambient ionization coupled with MS have enabled direct desorption/ionization of molecules from solid samples, such as DBS. It is likely that ambient MS methods will be increasingly adopted in clinical applications over the next 10 years.
Deep 2012 [12]	Review	Dry blood spot technique: a review	Though DBS is the dominant sample matrix for newborn screening and has proved convenient for therapeutic drug monitoring, due to limitations in sample volume and assay sensitivity, it is unlikely to fully replace traditional whole blood, plasma or serum collection in preclinical and clinical studies.
Déglon 2012 [13]	Review	Direct analysis of dried blood spots coupled with mass spectrometry: concepts and biomedical applications.	Recent advancements in direct MS/MS analysis of DBS samples offer competitive alternatives for high throughput and sensitivity compared with traditional plasma samples. Commercialization of automation methods in direct MS/MS for DBS indicate growing maturity of the technology.
De Kesel 2014 [14]	Commentary/ Short Report	Current strategies for coping with the hematocrit problem in dried blood spot analysis.	Several strategies for resolving issues of hematocrit have been developed, however, challenges remain. Differences between capillary and venous blood samples will continue to present a challenge to DBS even if issues of hematocrit are completely resolved.
De Kesel 2013 [15]	Review	Hemato-critical issues in quantitative analysis of dried blood spots: challenges and solutions.	DBS offer several advantages over traditional liquid samples, but despite these advantages, DBS still face substantial challenges. The issue of hematocrit effect remains a serious concern, however, multiple methods developed for dealing with hematocrit have been demonstrated to hold promise.
Demirev 2012 [16]	Review	Dried blood spots: analysis and applications.	Due to reductions in the cost and availability of technology, DBS use will continue to expand from health monitoring to rapid diagnostics to drug development and personalized point-of-care therapies.
Denniff 2014 [17]	Commentary/ Short Report	Bioanalysis Zone: DBS survey results.	At present, DBS use is more appropriate as a supplement than a replacement to plasma for pharmacokinetic studies. However, DBS may have a niche for studies with limited blood volume requirements (e.g. pediatrics) or in low resource settings where traditional sampling is not feasible..
Desai 2013 [18]	Review	Dried blood spot sampling analysis: recent advances and applications	DBS use in clinical trials will likely result in substantial savings in costs as well as overall improvements in data quality.

Dezateux 1998 [19]	Review	Evaluating newborn screening programmes based on dried blood spots: future challenges.	Due to advancements in technology such as MS/MS, newborn screening applications are expanding. However, such expansion should be based on unbiased benefits vs. harm estimates, which cannot be obtained solely by observational study.
Edelbroek 2009 [20]	Review	Dried blood spot methods in therapeutic drug monitoring: methods, assays, and pitfalls.	DBS has been applied for a range of medicines in therapeutic drug monitoring, but the benefits of DBS must be measured against potential errors due to sampling materials and methods. Standardization remains a critical gap in DBS applications.
Emmons 2010 [21]	Commentary/ Short Report	Pharmacokinetic considerations as to when to use dried blood spot sampling.	While DBS is a suitable matrix for pharmacokinetic studies, issues around blood: plasma ratio, hematocrit, and other physicochemical properties should be considered before DBS is adopted.
Hannon 2014 [22]	Review	Overview of the history and applications of dried blood samples.	DBS have a long history of use and are today in widespread use. Going forward, advancements in filter paper matrices and lab instrumentation are likely to improve the analytical precision and accuracy of DBS measurements.
Henion 2013 [23]	Review	Microsample analyses via DBS: challenges and opportunities.	DBS have several advantages over traditional samples. Advancements in polymer membranes and other substrate materials for filter paper cards will likely improve measurements derived from DBS samples; however, the current regulatory landscape will continue to hinder DBS adoption.
Hofman 2015 [24]	Review	Role of therapeutic drug monitoring in pulmonary infections: use and potential for expanded use of dried blood spot samples.	DBS is a promising method for improving therapeutic drug monitoring for pulmonary infections, especially for some drug classes; however, validation work remains to be done.
Ignjatovic 2014 [25]	Review	The utility of dried blood spots for proteomic studies: Looking forward to looking back.	Though DBS has already been effectively applied to epigenetic-based studies, it is important to develop improved technologies for DBS application in protein-based studies, which could allow for earlier detection of disease.
Ingels 2014 [26]	Review	Derivatization Techniques in Dried Blood Spot Analysis.	Due to the benefits associated with DBS sampling, the technique has already proven useful for a range of applications. The use of derivatization techniques, however, may be necessary to effectively apply DBS measurements. As automation increases, direct derivatization approaches are likely to gain importance in the future.
Jager 2014 [27]	Review	Procedures and practices for the validation of	Large differences exist in DBS validations conducted over the past decade. While DBS has several parameters above and beyond

		bioanalytical methods using dried blood spots: a review.	traditional samples, 40% of the published literature lack a single DBS-specific validation parameter.
Ji 2012 [28]	Review	What is next for dried blood spots?	Due to the scientific, social, ethical, and financial constraints of drug research, microsampling with ambient storage in dried matrices will be needed. Such adoption will require better understanding of plasma versus blood concentrations as well as innovations in technology.
Ji 2014 [29]	Review	Potential Role for Dried Blood Spot Sampling and Bioanalysis in Preclinical Studies.	As efforts to resolve current issues in DBS analysis continue to expand, DBS is likely to be well positioned to be the future matrix for nonclinical and clinical studies, though validations must first be successfully completed.
Kalou 2014 [30]	Review	Application of Enzyme Immunoassay Methods Using Dried Blood Spot Specimens.	DBS analysis through ELISA have been extensively evaluated and have been demonstrated to be effective for a range of biomarkers. Though constraints remain, limitations in DBS can be overcome by optimization and validation procedures prior to application in the field.
Keevil 2011 [31]	Review	The analysis of dried blood spot samples using liquid chromatography tandem mass spectrometry.	Combining LC-MS/MS with DBS can improve analyte stability and specificity, and could provide a powerful tool for future clinical research and application.
Kulmatycki 2014 [32]	Review	Application of Dried Blood Spot Sampling in Clinical Pharmacology Trials and Therapeutic Drug Monitoring.	DBS has become an established method for sampling aimed at therapeutic drug monitoring in developing countries. The method is particularly useful for special populations, such as pediatrics. DBS use in these settings can help improve personalized exposure-response strategies for patients.
Lakshmy 2014 [33]	Review	Role of dried blood spots in health and disease diagnosis in older adults.	DBS affords several advantages over traditional sampling methods, especially for older populations. Many of the current limitations in DBS can be overcome by advancements in technology for measurement and automation.
Lehmann 2013 [34]	Review	Current and future use of “dried blood spot” analyses in clinical chemistry.	DBS has advantages over traditional matrices in terms of sampling, transportation, storage, and biosafety. As a consequence, DBS is particularly advantageous for self-sampling at home. Innovations in microfluidics, multiplex systems, MS, and automation will continue to expand the potential of DBS application.
Liang 2010 [35]	Review	Dried blood spot (DBS) sampling technique and its applications.	Due to recent advancements in analytical instrumentation, such as LC-MS/MS, DBS is gaining wider attention and adoption in preclinical and population-based studies.

			However, issues of sensitivity and sample homogeneity remain. Further instrument and method development may help resolve these issues and speed adoption.
Li 2012 [36]	Commentary/ Short Report	Will 'green' aspects of dried blood spot sampling accelerate its implementation and acceptance in the pharmaceutical industry?.	DBS technology affords several advantages over traditional samples in terms of reductions in material inputs and wastes. These advantages are likely to accelerate adoption by the pharmaceutical industry if data generated by DBS studies prove reliable.
Liu 2014 [37]	Review	Paper Spray Ionization for Direct Analysis of Dried Blood Spots.	Paper spray ionization provides a simple, rapid, and sensitive method for direct analysis of DBS.
Li 2014 [38]	Review	Considerations in Development and Validation of LC-MS/MS Method for Quantitative Analysis of Small Molecules in Dried Blood Spot Samples.	Due to the benefits of DBS, the technology is currently being explored as a sampling tool in bioanalytics. In order for DBS to achieve wider acceptance, issues of reliability in achieving accurate and reproducible results must be resolved.
Li 2010 [39]	Review	Dried blood spot sampling in combination with LC-MS/MS for quantitative analysis of small molecules.	DBS-LC-MS/MS for quantitative analysis of small molecules has emerged as an important tool, however issues of assay sensitivity due to small sample volume remain. Improvements in DBS cards and their bioanalysis are needed.
Majors 2011 [40]	Review	New directions in whole blood analysis: dried blood spot analysis and beyond.	DBS has several advantages over plasma or serum for drug discovery and development studies. The advantages of DBS for the pharmaceutical industry are likely to drive advancements in the technology going forward.
Martin 2014 [41]	Review	Challenges and opportunities in mass spectrometric analysis of proteins from dried blood spots.	Though traditional used for metabolite and small molecular analysis, DBS are a potential source of protein biomarkers. As methods for proteomic analyses of DBS continue to emerge, DBS may replace plasma as the sample of choice.
Mauch 2012 [42]	Review	Automation of DBS sampling for biopharmaceutical analysis.	DBS has a variety of benefits for pharmaceutical bioanalysis. Several systems for automating DBS analysis are currently available and have thus far focused on card handling, avoidance of carry-over, robustness of analysis, and traceability for workflow, all of which are required for DBS to be adopted as a robust system for bioanalysis.
McDade 2014 [43]	Review	Development and validation of assay protocols for use with dried blood spot samples.	DBS are a “field-friendly” method of biosample collection and can help bridge the gap between field-level survey data and biological mechanisms. However,

			convenience in the field must be balanced against challenges of quantification in the lab, which still requires more work in assay development and validation.
McDade 2007 [44]	Review	What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research.	DBS provide a field-ready tool for interdisciplinary research by allowing for social/behavioral data to be combined with biological data. However, the advantages of DBS use must be considered in the context of their added burden, however minimal, on researchers in the field.
Meesters 2013 [45]	Review	State-of-the-art dried blood spot analysis: an overview of recent advances and future trends.	DBS is still developing from a time and labor intensive technique to a sophisticated highly quantitative and reliable method for quantification of analytes in microsamples. Though advancements in DBS methods have spurred adoption in the life sciences, hurdles remain and must be overcome for wider adoption.
Mei 2014 [46]	Review	Dried blood spot sample collection, storage, and transportation.	As filter paper technology and analytical methods have improved, DBS use in biochemical and molecular testing has expanded. If collected, processed, and stored appropriately, DBS use in newborn screening can ensure timely and accurate results, less stress to infants and families, and decrease the overall burden on the health system.
Mei 2001 [47]	Review	Use of filter paper for the collection and analysis of human whole blood specimens.	CDC's NSQAP plays an important role in quality assurance for DBS in newborn screening, and also provide a vital resource for others considering application of new analytic methods to filter paper.
Nageswara 2014 [48]	Review	Emerging liquid chromatography–mass spectrometry technologies improving dried blood spot analysis.	LC-MS for DBS provides a rapid and high-through analysis tool, which could solve many of the issues around online extraction, high-throughput, sensitivity, and selectivity. Though LC-MS/MS is expected to play an important role in the future, further improvements will be required to achieve full automation and ultra-high performance.
Parker 1999 [49]	Review	The use of the dried blood spot sample in epidemiological studies.	DBS has advantages over other microsamples such as saliva and urine, and is particularly suited to surveillance uses in low resource environments around the world.
Patel 2010 [50]	Review	Facilitating pharmacokinetic studies in children: a new use of dried blood spots.	DBS has demonstrated a degree of accuracy and precision comparable to that of traditional samples. Use of high sensitive detection systems with DBS may enabled their use in PK studies with children, as well as several other important applications like toxicology and remote area sampling.

Politt 2010 [51]	Review	New technologies extend the scope of newborn blood-spot screening, but old problems remain unresolved.	New analytical technique and treatment methods have expanded newborn blood-spot screening, however, there are concerns that existing programs are being driven by analytical performance rather than clinical need. Furthermore, screening policies currently vary greatly between countries.
Politt 2009 [52]	Review	Newborn blood spot screening: New opportunities, old problems.	Newborn screening methods are evolving quickly and coverage is expanding. Screening programs vary greatly within and between countries. There is a need for evidence-based decisions around which diseases to include in screening as technology has enabled a wide-range of screening possibilities, but many may be inconsistent with clinical priorities.
Quraishi 2013 [53]	Review	The use of dried blood spot samples in screening drugs of abuse.	DBS application for detection of drugs of abuse has potential, but use must be measured against potential for error within the method. Quality of sampling paper, standardization, and sensitivity of analytic methods are critical factors in achieving reliable results from DBS.
Sadones 2014 [54]	Review	Spot them in the spot: analysis of abused substances using dried blood spots.	DBS methods are currently available for the detection of a wide range of drugs of abuse. A majority of these methods have demonstrated sufficient sensitivity for forensic applications, however, more experiments are required for implementation of DBS in routine analysis.
Sharma 2014 [55]	Review	Dried blood spots: concepts, present status, and future perspectives in bioanalysis.	Advanced in analytical tools combined with financial and ethical benefits make DBS a suitable sampling method for a range of applications. Though limitations remain, advantages in sample collection, storage, and shipment make DBS a preferred technique, however, regulatory issues and advancements in automation will be necessary for wider adoption for drug discovery.
Shi 2011 [56]	Commentary/ Short Report	Assay dynamic range for DBS: battles on two fronts.	Enhancements in LC-MS/MS methods have helped to improve the dynamic range of DBS assays, however, issues involving dilution remain a constraint.
Smit 2014 [57]	Review	An overview of the clinical use of filter paper in the diagnosis of tropical diseases.	DBS have demonstrated sensitivities and specificities comparable to gold standard methods; however, DBS has not consistently been used effectively due to a lack of standardized methodologies. DBS may prove to be an effective tool for empowering healthcare workers with improved lab-based diagnostics, but additional research and validation will be required.

Snijdewind 2012 [58]	Review	Current and future applications of dried blood spots in viral disease management.	DBS offers opportunities for diagnostics and treatment around viral disease. However, these opportunities require application of uniform and robust protocols along with defined treatment and interventions at the individual and population levels.
Spooner 2013 [59]	Commentary/ Short Report	A dried blood spot update: still an important bioanalytical technique?.	Despite challenge of hematocrit and spot homogeneity, DBS offers substantial benefits for sampling in some study types, particularly those involving pediatric patients, therapeutic drug monitoring, or sampling in remote locations.
Stove 2012 [60]	Review	Dried blood spots in toxicology: from the cradle to the grave?.	DBS sampling has been effectively used for toxicological purposes from birth through autopsy. Issues of contamination, hematocrit, spot volume, and site of punching remain, however, advancements in automation and direct analyses are helping to alleviate some concerns.
Suva 2014 [61]	Review	A brief review on dried blood spots applications in drug development	Simplicity of sampling in combination with financial and ethical benefits have led to DBS adoption in drug development. These advantages compared with traditional plasma will likely ensure DBS remains an increasingly important part of drug development.
Szapacs 2014 [62]	Review	Clinical Implications of Dried Blood Spot Assays for Biotherapeutics.	DBS has demonstrated to be an accurate and precise method for quantification of biotherapeutics. Additionally, some analytes such as peptides and proteins may even be more stable in DBS compared with plasma. Still, issues of hematocrit must still be resolved before DBS can be more widely adopted in PK and TK studies.
Taneja 2013 [63]	Review	Dried blood spots in bioanalysis of antimalarials: relevance and challenges in quantitative assessment of antimalarial drugs.	DBS is well suited to studies involving antimalarial drugs, but issues remain. Additional tests will be required in order to validate DBS for these purposes, and issues of card type, spot size, blood volume spotted, hematocrit, matrix effects, and chromatographic effects must be considered during development.
Tanna 2011 [64]	Review	Analytical methods used in conjunction with dried blood spots.	DBS have been applied with a wide range of different analytic methods, including newborn screening, drug discovery and development, and HIV studies in resource limited settings among others. Issues of quality assurance for filter paper, hematocrit effects, and proper protocol for drying, storage, and transport are essential to effective application of the technology. Going forward, advancements in automation paired with highly sensitive instruments will

			continue to expand the range DBS applications.
Tanna 2015 [65]	Commentary/ Short Report	Self-sampling and quantitative analysis of DBS: can it shift the balance in over-burdened healthcare systems?.	As populations continue to age, self sampling with DBS for quantitative analysis provides a valuable tool for shifting the balance and burdens in healthcare away from traditional hospitals and clinics, and thereby reducing pressure on acute care services, while allowing more convenient sampling for patients.
Tanna 2014 [66]	Review	Dried blood spot analysis to assess medication adherence and to inform personalization of treatment.	There is a paucity of research around DBS use for medication adherence, however, the opportunity to personalize health services through measuring adherence to treatment plans with DBS present a valuable opportunity.
Timmerman 2014 [67]	Technical Report	Update of the EBF recommendation for the use of DBS in regulated bioanalysis integrating the conclusions from the EBF DBS-microsampling consortium.	DBS is considered a developing technology and further innovation and improvements will be required to provide more balance between existing advantages and limitations. DBS is not yet viewed as a general alternative to traditional liquid samples, however, when appropriate applied, DBS may be a suitable matrix under some conditions.
Timmerman 2011 [68]	Technical Report	EBF recommendation on the validation of bioanalytical methods for dried blood spots.	DBS require several adaptations, enhancements and revisions to current validation methods in order to appropriately validate an assay for DBS. As interest in the technology increases it is important that successes and limitations continue to be shared.
Viswanathan 2012 [69]	Commentary/ Short Report	Perspectives on microsampling: DBS.	The ability of DBS to mimic existing traditional samples will be essential to its adoption. Regulatory approval of DBS is likely to remain a case-by-case situation whereby the quality and robustness of the data will be critical.
Wilcken 2012 [70]	Review	Screening for disease in the newborn: the evidence base for blood-spot screening.	MS/MS with DBS in newborn screening have been demonstrated to be effective for a range of disorders, however, concerns around anxiety due to screening, false positives, adverse effects of unwarranted treatments for mild variants and others remain a concern. Selection of diseases to include in screening would be more effective with full integration of screening programs, diagnostic labs, and clinical services.
Wilhelm 2014 [71]	Review	Therapeutic drug monitoring by dried blood spot: progress to date and future directions.	DBS has been used increasingly in therapeutic drug monitoring and methods have been applied effectively for dealing with the influence of hematocrit. However,

			additional work in clinical validation will be required.
Wong 2014 [72]	Review	Punching and Extraction Techniques for Dried Blood Spot Sample Analysis.	Punching and extraction methods for DBS are critical steps to effective application of the technology. Furthermore, investigation and understanding of the physicochemical properties of target analytes is essential to successful use of DBS.
Wong 2010 [73]	Commentary/ Short Report	Increasing efficiency for dried blood spot analysis: prospects for automation and simplified sample analysis.	Advantages of DBS for preclinical and clinical studies includes a marked reduction in blood volume requirements and simplified sampling logistics. At present, approaches are not adequate for large numbers of samples and improvements in efficiency are necessary for the benefits of DBS to be fully realized.
Xu 2013 [74]	Review	Merck's perspective on the implementation of dried blood spot technology in clinical drug development-why, when and how.	DBS use in PK studies requires understanding of several parameters, including blood-to-plasma ratio, hematocrit, plasma unbound fraction, and blood cell partition. When considering adoption, quick feasibility studies should be conducted. At present, bridging studies will be required before DBS can be applied as a stand alone sample matrix, and regulatory feedback is recommended on a case-by-case basis.
Zhang 2013 [75]	Review	Best Practices in LC-MS Method Development and Validation for Dried Blood Spots.	Due to the many benefits of the technology, DBS is increasingly being considered as a sampling tool in bioanalytics. However, issues of reliability in providing accurate and reproducible results must be resolved before wider adoption can occur. As the technology advances, efforts must be made to resolve challenges of hematocrit effects, spot homogeneity, extraction recovery, analyte stability, and automation.

Table 2-3. Comprehensive list of analytes identified in the literature to have been measured in dried blood spots.

SMALL MOLECULE
1 beta-Hydroxycholic acid
1-(3,4-Methylenedioxybenzyl)-piperazine
1-(9Z,12Z-Octadecadienoyl)-sn-glycero-3-phosphocholine
1-Arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine
1-Behenoyl-2-hydroxy-sn-glycero-3-phosphocholine
1-Dodecanoyl-2-tridecanoyl-sn-glycero-3-phosphate
1-Hexacosanoyl-2-hydroxy-sn-glycero-3-phosphocholine
1-Lignoceroyl-2-hydroxy-sn-glycero-3-phosphocholine
1-Methylhistidine
1-O-hexadecyl-2-hydroxy-sn-glycero-3-phosphocholine
1-O-octadecyl-2-hydroxy-sn-glycero-3-phosphocholine
1-Oleoyl-2-hydroxy-sn-glycero-3-phosphocholine
1-Oleoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine
1-Palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine
1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine
1,1-Dichloro-2,2-bis(4-chlorophenyl)ethene
1,1,1-Trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane
1,2-Diheptadecanoyl-sn-glycero-3-phosphate
1,2,3,7,8-Pentachlorodibenzo-p-dioxin
1,3-Benzodioxolyl-N-methylbutanamine
1'-Hydroxymidazolam
10-Hydroxydecenoic acid
11-Deoxycorticosterone
11-Deoxycortisol
11-Eicosenoic acid
11-Hydroxytetrahydrocannabinol
11-nor-9-Carboxy-tetrahydrocannabinol
11-nor-9-Carboxy-tetrahydrocannabinol glucuronide
13,16-Docosadienoic acid
17-alpha-Hydroxypregnenolone
17-alpha-Hydroxyprogesterone
2-(2,5-Dimethoxy-4-propylphenyl)ethanamine
2-(4-Iodo-2,5-dimethoxyphenyl)ethan-1-amine
2-[2,5-Dimethoxy-4-(propylsulfanyl)phenyl]ethan-1-amine
2-[4-(Ethylsulfanyl)-2,5-dimethoxyphenyl]ethan-1-amine
2-Aminoisobutyric acid
2-Deoxytetronic Acid
2-Ethyl-5-methyl-3,3-diphenylpyrroline
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
2-Furoic acid
2-Hydroxyadipic acid
2-Hydroxybutyric acid
2-Hydroxydocosanoic acid
2-Hydroxyisocaproate
2-Hydroxyisovalerate
2-Hydroxysebacic acid
2-Methylbutyrylcarnitine
2-Methylbutyrylglycine
2-Methylcitrate
2-Oxo-3-hydroxy-Lysergic acid diethyamide
2-Oxoadipic acid
2-Propylglutaric acid
2,2',3,3',4,4',5-Heptachlorobiphenyl
2,2',3,3',4,4',5,5'-Octachlorobiphenyl
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl
2,2',3,4,4',5,5'-Heptachlorobiphenyl
2,2',3,4,4',5'-Hexachlorobiphenyl
2,2',3,4',5,5',6-Heptachlorobiphenyl
2,2',4,4'-Tetrabromodiphenyl ether
2,2',4,4',5-Pentabromodiphenyl ether
2,2',4,4',5,5'-Hexabromodiphenyl
2,2',4,4',5,5'-Hexabromodiphenyl ether
2,2',4,4',5,5'-Hexachlorobiphenyl
2,2',4,4',5,6'-Hexabromodiphenyl ether
2,2',4,4',6-Pentabromodiphenyl ether
2,3,3',4,4'-Pentachlorobiphenyl
2,3,7,8-Tetrachlorodibenzo-p-dioxin
2,3',4,4',5-Pentachlorobiphenyl
2,4-Dihydroxybutanoic acid
2,4,4'-Tribromodiphenyl ether
2,4,4'-Trichlorobiphenyl
2,4,6-Trimethoxyamphetamine
2,5-Dimethoxy-4-bromophenethylamine
2,5-Dimethoxy-4-ethylphenethylamine
2,5-Dimethoxy-4-isopropylthiophenethylamine
2,5-Dimethoxy-4-methylamphetamine
2,5-Dimethoxy-4-methylphenethylamine
2,5-Dimethoxyphenethylamine
2'-Deoxyguanosine
2'-Deoxyinosine
2'R-ochratoxin A
21-Deoxycortisol
25-Hydroxyvitamin D2
26-Hydroxycholesterol-3-sulfate
3-epi-25-hydroxyvitamin D3
3-Fluoromethcathinone
3-Hexenedioic acid
3-Hydroxy quinine
3-Hydroxy-decanoylcarnitine
3-Hydroxy-dodecanoylcarnitine
3-Hydroxy-hexadecanoylcarnitine
3-Hydroxy-hexanoylcarnitine
3-Hydroxy-iso-/butyrylcarnitine
3-Hydroxy-isovalerylcarnitine
3-Hydroxy-octadecadienoylcarnitine
3-Hydroxy-octadecanoylcarnitine
3-Hydroxy-octadecenoylcarnitine
3-Hydroxy-octanoylcarnitine
3-Hydroxy-stearoylcarnitine
3-Hydroxy-tetradecanoylcarnitine
3-Hydroxydodecanedioic acid
3-Hydroxydodecanoyl
3-Hydroxyglutaric acid
3-Hydroxyisovaleric acid
3-Hydroxypalmitoylcarnitine
3-Hydroxyproline
3-Hydroxypropionate
3-Hydroxypropionic acid
3-Hydroxyquinine
3-Methyl-2-oxovaleric acid
3-Methyladipic acid
3-Methylbutanoic acid
3-Methylcrotonylglycine
3-Methylglutaconic acid
3-Methylglutaric acid

3-Methylglutarylcarnitine
3-Methylhistidine
3,12-Dihydroxy-cholenic acid
3,4-Dihydroxy-L-phenylalanine
3,4-Methylenedioxy-N-ethylamphetamine
3,4-Methylenedioxyamphetamine
3,4-Methylenedioxymethamphetamine
3,4-Methylenedioxypyrovalerone
3',4'-Methylenedioxy-alpha-pyrrolidinopropiophenone
3b-Hydroxy-5-cholenoic acid
4-Aminobenzoic acid
4-Aminophenyl-1-phenethylpiperidine
4-Androstene-3,6,17-trione
4-Androstenedione
4-Androsterone glucuronide
4-Chlorophenylbiguanide
4-Hydroxy propranolol Beta-D-glucuronide
4-Hydroxybenzoic acid
4-Hydroxyhippuric acid
4-Hydroxyphenyllactic acid
4-Hydroxyphenylpyruvic acid
4-Methylethcathinone
4-Methylthioamphetamine
4-Methylumbelliferyl beta-D-galactopyranoside
4-Nitrophthalic acid
4'-Hydroxyflurbiprofen
5-Hydroxyhexanoic acid
5-Hydroxyindoleacetic acid
5-Hydroxymethyl-2-furoic acid
5-Iodo-2-aminoindane
5-methoxy-N,N-dimethyltryptamine
5-Sulfosalicylic acid
5,6-Methylenedioxy-2-aminoindane
6-Monoacetylmorphine
6-Prenylnaringenin
7-Aminoclonazepam
7-Aminoflunitrazepam
7-Dehydrocholesterol
7-Demethylated centchroman
7-Ethoxycoumarin
7-Hydroxyoctanoic acid
7-Octenedoic acid
8-Dehydrocholesterol
8-epi-Prostaglandin F2 Alpha
8-Prenylnaringenin
Acebutolol
Acenocoumarol
Acetazolamide
Acetic acid
Acetoacetic acid
Acetonitrile
Aceturic acid
Acetylcarnitine
Aconitic acid
Acyl glucuronide mycophenolic acid
Adenosine
Adipic acid
Afimoxifene
Alanine
Aldrin
Alfentanil
Allantoin
Aloe emodine
Alpha cyano-4-hydroxycinnamic acid
Alpha-1 antitrypsin
Alpha-aminoadipate
alpha-Aminoadipic acid
alpha-Aminobutyric acid
alpha-Carotene
Alpha-galactosylceramide
alpha-Hexachlorocyclohexane
Alpha-hydroxyalprazolam
alpha-Hydroxyglutaric acid
Alpha-isoleucine
alpha-Ketoglutaric acid
alpha-Ketoisocaproic acid
alpha-Ketoisovaleric acid
Alpha-ketomethylvaleric acid
alpha-Ketooctanoic acid
alpha-Linolenic acid
alpha-Methyltryptamine
Alpha-N-acetylgalactosaminidase
Alprazolam
Alprenolol
Amiloride
Amiodarone
Amitriptyline
Amlodipine
Amodiaquine
Amphetamine
Amprenavir
Anabasine
Anastrazole
Andarine
Angiotensin converting enzyme
Apixaban
Arabinose
Arachidic acid
Arachidonic acid
Arachidoylcarnitine
Arginine
Argininosuccinic acid
Artemether
Ascomycin
Ascorbic acid
Asparagine
Aspartic acid
Atazanavir
Atenolol
Atracurium
Atropine
Azelaic acid
Behenic acid
Benazepril
Benazeprilate
Benzethonium chloride
Benzoic acid
Benzoylecgonine
Beta-alanine
beta-Carotene
beta-Hexachlorocyclohexane
Biopterin
Bisacodyl
Bisoprolol
Bisphenol A
Bosentan
Brallobarbital
Brevetoxin
Bromadiolone
Bromhexine
Budesonide
Bupivacaine
Buprenorphine
Buprenorphine glucuronide
Bupropion
Busulfan
Butylone
C20 lysophosphatidylcholine
C22 lysophosphatidylcholine
C24 lysophosphatidylcholine
Caffeine
Calcifediol
Cannabidiol
Canrenone
Caprylic acid
Captopril
Carbamazepine

Carbamazepine-10,11 epoxide
Carboxymefloquine
Carnitine
Carnosinase
Carnosine
Cathine
Cathinone
Cefotaxime
Ceftriaxone
Cerotic acid
Chenodeoxycholic acid
Chitotriosidase
Chlordiazepoxide
Chlorodehydromethyltestosterone
Chlorophacinone
Chloroquine
Chlorthalidone
Cholesterol
Cholesterol sulfate
Choline theophyllinate
Ciguatoxin
Cimetidine
Cinchocaine
Ciprofloxacin
Cis-2-decenoic acid
Cis-4-decenoic acid
Cis-5-tetradecenoic acid
Citalopram
Citric acid
Citrulline
Clarithromycin
Clenbuterol
Clobazam
Clomifene
Clomipramine
Clonazepam
Clonidine
Clopidorel
Clozapine
Cobalamin C
Cocaethylene
Cocaine
Codeine
Codeine-6-glucuronide
Colchicine
Corticosterone
Cortisol
Cortisone
Cotinine
Coumachlor
Coumatetralyl
Creatine
Creatinine
Crimidine
Cryptoxanthin
Cycloguanil
Cyclophosphamide
Cyclosporin A
Cystathionine
Cysteine
Cystine
D-allo-Isoleucine
D-Galactonic acid
D-galactose-1-phosphate
Daidzein
Dapsone
Darunavir
Dasatinib
DBD-F
Decadienoylcarnitine
Decanoate
Decanoylcarnitine
Decenoylcarnitine
Delta-8-tetrahydrocannabinol
Delta-9-tetrahydrocannabinol
Delta-alanine
delta-Aminolevulinic acid
delta-Hexachlorocyclohexane
Deoxyadenosine
Deoxycholic acid
Dermatan sulfate
Desalkylflurazepam
Desbutyl-lumefantrine
Desethyl-amodiaquine
Desethylchloroquine
Desipramine
Desmethyl bosentan
Desmethylclomipramine
Desmethylflunitrazepam
Desoxypipradol
Desvenlafaxine
Dexamethasone
Dextroamphetamine
Dextromethorphan
Dextrorphan
Dextrose
Diazepam
Dichlorodiphenyldichloroethylene
Dichlorodiphenyltrichloroethane
Diclofenac
Diclofenac acyl glucuronide
Digitoxin
Dihydroartemisinin
Dihydrocodeine
Dihydrotestosterone
Dihydroxy-cholestanoic acid
Dihydroxy-oxocholestenoic acid
Dihydroxyacetone phosphate
Diisopropyltryptamine
Dimethoxyamphetamine
Dimethoxybromoamphetamine
Dimethylone
Dimethylphenylpiperazinium
Dipropyltryptamine
Dithioerythritol
Dithiothreitol
DL-3-Phenyllactic acid
DL-Sulforaphane N-acetyl-L-cysteine
Docetaxel
Docosahexaenoic acid
Docosapentaenoic acid
Docosatetraenoic acid
Dodecanoylcarnitine
Dodecenoylcarnitine
Domoic acid
Donepezil
Dopamine
Doxazosin
Ecgonine methyl ester
Efavirenz
Eicosadienoic acid
Eicosapentaenoic acid
Eicosatrienoic acid
Emixustat hydrochloride
Emodine
Emtricitabine
Enalapril
Endoxifen
Enrofloxacin
Enterolactone
Ephedrine
Epinephrine
Equol
Ergocalciferol
Ertapenem
Erucic acid
Erythronic acid
Erythrose 4-phosphate
Estradiol
Ethambutol
Ethanolamine
Ethcathinone
Ethyl acetate
Ethyl glucuronide
Ethyl sulfate

Ethylenediaminetetraaceti c acid
Ethylmalonic acid
Ethylone
Etilamfetamine
Etiocholanolone glucuronide
Etoposide
Etravirine
Everolimus
Exadecanedioylcarnitine
Exemestane
Exenatide
Exendin-4
Fenfluramine
Fentanyl
Fexofenadine
Fibrinopeptide A
Flephedrone
Fluconazole
Flunitrazepam
Fluoxetine
Flupenthixol
Flurazepam
Flurbiprofen
Fluvoxamine
Folate
Formiminoglutamic acid
Formoterol
Frataxin
Free carnitine
Free erythrocyte porphyrins
Free triiodothyronine
Fructose 6-phosphate
Fumaric acid
Furosemide
Gabapentin
Galactitol
Galactose
gamma-Aminobutyric acid
gamma-Hydroxybutyric acid
Gemifloxacin
Genistein
Gentamicin
Gentisic acid
Gla domain
Glibenclamide
Glibornuride
Gliclazide
Glucocerebroside
Gluconic acid
Glucose
Glucose 6-phosphate
Glucose tetrasaccharide
Glutaconic acid
Glutamate
Glutamic acid
Glutamine
Glutaric acid
Glutarylcarnitine
Glutathione
Glyceraldehyde 3-phosphate
Glyceric acid
Glycerol
Glycine
Glycochenodeoxycholic acid
Glycocholic acid
Glycolic Acid
Guanfacine
Guanidineacetic acid
Guanidinoacetate
Guanosine
Haloperidol
Heparan sulfate
Heptadecanoic acid
Heptanoylcarnitine
Hexachlorobenzene
Hexachlorocyclohexane
Hexacosanoyl lysophosphatidylcholine
Hexadecanoylcarnitine
Hexadecenoylcarnitine
Hexanoic acid
Hexanoylcarnitine
Hexanoylglycine
Hexose
Hippuric acid
Histidine
Homocysteine
Homoserine
Homovanillic acid
Hydrazine monohydrate
Hydrochlorothiazide
Hydrocodone
Hydromorphone
Hydroxy bosentan
Hydroxy desmethyl bosentan
Hydroxy-cholestanoic acid
Hydroxy-palmitoleylcarnitine
Hydroxybupropion
Hydroxychloroquine
Hydroxyflurbiprofen
Hydroxyisovalerylcarnitine
Hydroxyomeprazole
Hydroxypropranolol glucuronide
Hydroxysuberic acid
Hydroxyzine dihydrochloride
Hymecromone
Ibuprofen
Imatinib
Imipramine
Indinavir
Indole-3-acetic acid
Inosine
Irbesartan
Irinotecan
Iso-/butyrylcarnitine
Iso-/butyrylcarnitine hydrochloride
Isocitric acid
Isoleucine
Isoniazid
Isovaleryl-/2-Methylbutyrylcarnitine
Isovaleryl-/2-Methylbutyrylcarnitine hydrochloride
Isovalerylglycine
Isoxanthohumol
Isoxanthopterin
Ketamine
L-allo-Isoleucine
L-allo-Isoleucine
L-Arginine monohydrochloride
L-Hydroxyproline
Labetalol
Lactic Acid
Lamivudine
Lamotrigine
Lansoprazole
Lapatinib
Leucine
Levamisole
Levetiracetam
Levomepromazine
Lidocaine
Lignoceric acid
Lindane
Linezolid
Linoleic acid
Lomefloxacin hydrochloride
Loperamide
Loperamide hydrochloride
Lopinavir
Loratadine
Lorazepam
Lormetazepam
Losartan
Losartan carboxylic acid
Lumefantrine
Lutein

Lycopene
Lysergic acid diethylamide
Lysine
Lysophosphatidylethanolamine
Maleic acid
Malic acid
Malonyl
Malonylcarnitine
Mavoglurant
Mefenamic acid
Mefloquine
Mephedrone
Mepivacaine
Mesocarb
meta-Chlorophenylpiperazine
Metandienone
Metformin
Methadone
Methamphetamine
Methanol
Methcathinone
Methedrone
Methionine
Methotrexate
Methotrexate polyglutamates
Methylcitrate
Methylcitric acid
Methylecgonine
Methylene blue
Methylene violet
Methylenedioxypyrovalerone
Methylephedrine
Methylhexaneamine
Methylisopropyltryptamine
Methylmalonic acid
Methylmalonyl-/succinylcarnitine
Methylone
Methylphenidate
Methylsuccinic acid
Metoprolol
Metronidazole
Mevalonic acid
Midazolam
Mirtazapine
Mono-2-ethylhexyl phthalate
Mono-3-methyl-5-dimethylhexyl phthalate
Mono-3-methyl-7-methyloctyl phthalate
Monoacetyldapsone
Monobenzyl phthalate
Monobutyl phthalate
Monocyclohexyl phthalate
Monodesethylchloroquine
Monoethyl phthalate
Monomethyl phthalate
Monooctyl phthalate
Morphine
Morphine-3-glucuronide
Morphine-6-glucuronide
Moxifloxacin
Mycophenolic acid
Mycophenolic acid glucuronide
Mycotoxin ochratoxin A
Myristic acid
N-Acetylaspartic acid
N-Acetylgalactosamine
N-Acetylhexosamine
N-Desalkylflurazepam
N-Desmethylflunitrazepam
N-Desmethyltamoxifen
N-Glycan
N-Propionylglycine
N,N-diallyl-5-methoxy tryptamin
N,N-Dimethylphenylalanine
N,N-Dimethyltryptamine
N'-(4-Acetylaminophenyl)-N,N-dimethylacetamidine
N'-(4-Aminophenyl)-N,N-dimethylacetamidine
Nadolol
Nalbuphine
Naphyrone
Naproxen
Nelfinavir
Nelfinavir mesylate hydrate
Neopterin
Netilmicin
Nevirapine
Nicotine
Nifedipine
Nikethamide
Nilotinib
NIM811
Nitisinone
Nitrazepam
Non-ayl-l-carnitine
Norbuprenorphine
Norbuprenorphine glucuronide
Norcodeine
Nordiazepam
Norfentanyl
Norfluoxetine
Norketamine
Nortriptyline
O-desmethyl metoprolol
Octadecadienoylcarnitine
Octadecanoylcarnitine
Octadecenoylcarnitine
Octanoate
Octanoylcarnitine
Octenoylcarnitine
Olanzapine
Oleic acid
Omeprazole
Ormeloxifene
Ornithine
Orotic acid
Oseltamivir
Oseltamivir acid
Oseltamivir carboxylate
Oxalic Acid
Oxazepam
Oxazepam glucuronide
Oxcarbazepine
Oxepin
Oxprenolol
Oxycodone
Oxyphencyclimine
Paclitaxel
Paliperidone
Palmitic acid
Pantothenic acid
para-Fluorophenylpiperazine
para-Methoxy-N-methylamphetamine
para-Methoxyamphetamine
para-Methoxyphenylpiperazine
Paracetamol
Paracetamol glucuronide
Paracetamol sulfate
Paraxanthine
Paroxetine
Pazopanib
Peginesatide
Pentylone
Perchlorate
Perchloric acid
Perfluorohexane sulfonate
Perfluorononanoic acid
Perfluorooctane sulfonamide
Perfluorooctane sulfonate
Perfluorooctanoic acid
Phencyclidine
Phenobarbital
Phenol
Phenol glucuronide mycophenolic acid

Phenolphthalein
Phenprocoumon
Phenylacetate
Phenylacetic acid
Phenylalanine
Phenyllactate
Phenylpropanolamine
Phenylpropionylglycine
Phenylpyruvic acid
Phenytoin
Phosphatidylcholine
Phosphatidylethanol
Phosphoethanolamine
Phosphoric acid
Phosphoserine
Phytanic acid
Pimelic acid
Pindolol
Pioglitazone
Pipecolic acid
Piperacillin/tazobactam
Piperaquine
Pivalic Acid
Pivaloylcarnitine
Posaconazole
Pramipexole
Prasugrel
Prazepam
Praziquantel
Prazosin
Pregabalin
Pristanic acid
Procaine
Progesterone
Proguanil
Proline
Propionic Acid
Propionylcarnitine
Propionylcarnitine hydrochloride
Propionylglycine
Propranolol
Propranolol hydrochloride
Proprionylcarnitine
Propylglutarylcarnitine
Propyphenazone
Prostaglandin A1
Prostaglandin E2
Prostaglandin F2 alpha
Pseudoephedrine
Pterin
Pyrimethamine
Pyroglutamic acid
Pyrovalerone
Pyruvic acid
Quetiapine
Quinidine
Quinine
R-trans-4-hydroxy-praziquantel
Raltegravir
Ramipril
Ramoplanin
Ranitidine
Reboxetine
Retinol
Rhein
Ribavirin
Ribavirin-5'-diphosphate
Ribavirin-5'-monophosphate
Ribavirin-5'-triphosphate
Ribose 5-phosphate
Ribulose 5-phosphate
Rifampicin
Rifapentine
Rifaximin
Risperidone
Ritalinic acid
Ritonavir
Ropiramate
Rosiglitazone
Rufinamide
Salbutamol
Salicylic acid
Salmeterol
Saquinavir
Sarcosine
Sebacic acid
Sebacylcarnitine
Sedoheptulose
Sedoheptulose 7-phosphate
Serine
Serotonin
Sertraline
Simvastatin
Sirolimus
Sisomicin
Sitagliptin
Sitamaquine
Sodium sulfite
Sodium valproate
Somatomedin C
Sorafenib
Sotalol
Sotalol hydrochloride
Sphingomyelin
Stanozolol
Stearic acid
Stearidonic acid
Stearoylcarnitine
Strychnine
Suberic acid
Suberylcarnitine
Suberylglycine
Succinic acid
Succinylacetone
Sufentanil
Sulconazole
Sulfadoxine
Sulfamethoxazole
Sulfate
Sulforaphane
Sulforaphane glutathione
Sulphadoxine
Sunitinib
Tacrolimus
Tafenoquine
Tamoxifen
Tasquinimod
Taurine
Taurochenodeoxycholic acid
Taurocholic acid
Telaprevir
Telmisartan
Temazepam
Temsirolimus
Tenofovir
Tenofovir diphosphate
Tenofovir disoproxil
Terfenadine
Testosterone
Testosterone glucuronide
Testosterone undecanoate
Tetrabromobisphenol A
Tetracosahexaenoic acid
Tetracosapentaenoic acid
Tetradecadienoylcarnitine
Tetradecanoylcarnitine
Tetradecenoylcarnitine
Tetrahydrocannabinol
Tetrahydroxy-cholestenoic acid
Tetrasaccharide
Theobromine
Theophylline
Thioridazine
Threonic acid
Threonine
Tiglylcarnitine
Tiglylglycine
Timolol

Tolbutamide
Topiramate
Topotecan
Torasemide
Tramadol
trans-3'-Hydroxycotinine
Triazolam
Tribendimidine
Trichloroacetic acid
Trifluoromethylphenylpiperazine
Triglyceride
Trihydroxy-cholestenoic acid
Triiodothyronine
Trimethoxyamphetamine
Trimipramine
Triprolidine
Tris(2-carboxyethyl)phosphine
Tryptophan
Tubocurarine
Tyrosine
Unconjugated 4'-hydroxyflurbiprofen
Unconjugated testosterone
Uracil
Urea
Uric acid
Uridine
Urocanic acid
Ursodeoxycholic acid
Valeric Acid
Valine
Valproate
Valproic acid
Valsartan
Vancomycin
Vanillic acid
Vanillylmandelic acid
Vasoactive intestinal peptide
Vecuronium
Vemurafenib
Venlafaxine
Verapamil
Verapamil hydrochloride
Very long fatty acid chain
Vincristine
Vitamin A
Vitamin B12
Vitamin C
Vitamin D
Voriconazole
Warfarin
Xanthine
Xanthohumol
Xylulose 5-phosphate
Zaleplon
Zatebradine
Zeaxanthin
Zidovudine
Ziprasidone
Zolpidem
Zopiclone
Zuclopenthixol

LARGE MOLECULE

1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol
14-3-3 Protein beta/alpha
14-3-3 Protein theta
14-3-3 Protein zeta/delta
26S Protease regulatory subunit 8
3-Hydroxy-3-methylglutaryl-CoA lyase
3-Hydroxyhexadecanoic acid
3-Mercaptopyruvate sulfurtransferase
6-Phospho-D-gluconate dehydrogenase, decarboxylating
a-Thrombin
Acetlyated hemoglobin
Acid alpha-glucosidase
Acid sphingomyelinase
Actin, cytoplasmic-1
Acylamino-acid-releasing enzyme
Adenine phosphoribosyltransferase
Adenosine deaminase
Adenosylhomocysteinase
Adenylate kinase isoenzyme 1
Adenylosuccinate lyase
Adiponectin
Adrenomedullin
Afamin
Alanine transaminase
Aldolase C
Alpha 1-antichymotrypsin
Alpha 2-antiplasmin
Alpha actin
Alpha-1-antichymotrypsin
Alpha-1-B glycoprotein
Alpha-1-microglobulin/bikunin precursor
Alpha-2-HS-glycoprotein
Alpha-2-macroglobulin
Alpha-enolase
Alpha-fetoprotein
Alpha-galactosidase
Alpha-glycosidase
Alpha-hemoglobin-stabilizing protein
Alpha-soluble NSF attachment protein
Alpha-synuclein
AMG 162 (therapeutic monoclonal antibody)
AMG 517 (therapeutic monoclonal antibody)
AMG A (therapeutic monoclonal antibody)
AMG B (therapeutic monoclonal antibody)
Amphiregulin
Angiopoietin-1 receptor
Angiotensinogen
Ankyrin
Annexin A7
anti-B-lymphocyte antigen CD20 monocodal antibody drug
anti-MSP-119 antibody
anti-MSP2 antibody
Anti-Mullerian hormone
Antinuclear antibodies
Antithrombin-III
Apolipoprotein A1
Apolipoprotein A2
Apolipoprotein A4
Apolipoprotein B
Apolipoprotein B100
Apolipoprotein C1
Apolipoprotein C2
Apolipoprotein C3
Apolipoprotein D
Apolipoprotein E
Apolipoprotein L1
Aquaporin 1
Arginase 1
Arylsulfatase A
Arylsulfatase B
Aspartate aminotransferase
B-cell activitating factor
Babesia microti antibody
Band 3 anion transport protein
Bartonella quintana antibody
Beta galactosidase
Beta globin
Beta-2-glycoprotein 1
Beta-actin-like protein 2
Beta-glucocerebrosidase
Beta-glucosidase
Beta-lipoprotein
Betacellulin
Bifunctional purine biosynthesis protein PURH
Biotinidase

Bisphosphoglycerate mutase
Blood group Rh(CE) polypeptide
Brain-derived neurotrophic factor
Brucella antibody
C-C motif chemokine 19
C-C motif chemokine 21
C-C motif chemokine 24
C-peptide
C-reactive protein
C-X-C motif chemokine 10
C-X-C motif chemokine 11
C-X-C motif chemokine 13
C-X-C motif chemokine 5
C-X-C motif chemokine 9
C1 Inactin
C3B Inhibitor
C4b-binding protein alpha chain
CA 242
Calcitonin gene-related peptide
Calpain small subunit 1
Calpastatin
Campylobacter antibody
Cancer antigen 125
Carbonic anhydrase 1
Carbonic anhydrase 2
Carbonic anhydrase 3
Carbonic anhydrase 9
Carcinoembryonic antigen
Carnitine-acylcarnitine translocase
Caspase-3
Catalase
Cathepsin D
CD154
Ceruloplasmin
Chemokine (C-C motif) ligand 2
Chemokine (C-C motif) ligand 3
Chemokine (C-C motif) ligand 4
Chemokine (C-C motif) ligand 5
Chemokine (C-C motif) ligand 8
Chemokine (C-X-C motif) ligand 2
Chikungunya virus antibody
Chlamydia trachomatis antibody
Chloride intracellular channel protein 1
Cholinesterase
Chylomicron
Cluster of differentiation 3
Cluster of differentiation 3 zeta antibody
Cluster of differentiation 4
Clusterin
Coagulation factor XIIa heavy chain
Coagulation factor XIII A chain
Cofilin-1
Colony stimulating factor 1
Complement component C1 inactivator
Complement component C1q subcomponent subunit C
Complement component C1s
Complement component C1s subcomponent
Complement component C2
Complement component C3
Complement component C3B inhibitor
Complement component C4
Complement component C4 beta chain
Complement component C4 gamma chain
Complement component C5
Complement component C8 beta chain
Complement component C9
Complement factor 1
Complement factor B
COP9 signalosome complex subunit 3
Coxiella burnetii antibody
Creatine kinase
Creatine kinase B-type
Creatine kinase MM isoenzyme
Cryptosporidium antibody
Cyclic AMP-responsive element-binding protein 3-like protein 4
Cystatin B
Cystatin C
Cytomegalovirus antibody
D-dopachrome decarboxylase
Dactinomycin
Dehydroepiandrosterone sulfate
Delta-aminolevulinic acid dehydratase
Dematin
Dengue virus antibody
Dermcidin
Dihydropteridine reductase
Diphtheria antitoxin
Diptheria antibody
Disialotransferrin
Drebrin-like protein
E-selectin
Early activation antigen cluster of differentiation 69
Echinococcus antibody
Echinococcus granulosus antibody
Enolase 1
Entamoeba histolytica antibody
Enterotoxigenic Escherichia coli antibody
Epidermal growth factor
Epidermal growth factor receptor
Epididymal secretory protein E4
Epiregulin
Epithelial cell adhesion molecule
Epstein-Barr virus antibody
Erythrocyte acetylcholinesterase
Erythrocyte band 7 integral membrane protein
Erythrocyte membrane protein band 4.2
Erythropoietin
Estrogen receptor
Etanercept
Eukaryotic translation initiation factor 5A-1
Extracellular matrix metalloproteinase inducer
F-actin capping protein subunit beta
F-box only protein 7
Factor H
Factor V Leiden
Fas antigen ligand
Fasciola hepatica antibody
Fatty acid binding protein 4
Fc-fusion protein
Ferritin
Fetal hemoglobin
Fibrinogen
Fibrinogen alpha chain
Fibrinogen beta chain
Fibrinogen gamma chain
Fibroblast growth factor 2
Fibronectin
Filamin A, alpha
Filarioidea antibody
Flavin reductase
Fms-related tyrosine kinase 3 ligand
Folate receptor 1
Follicle-stimulating hormone
Follistatin
Free-beta human chorionic gonadotropin
Fructose-bisphosphate aldolase A
FT03
FT04

FT05

Fumarylacetoacetase

Galactocerebroside beta-galactosidase

Galactose-1-phosphate uridyltransferase

Galactosylceramidase

Galectin-3

Gelsolin

Giardia antibody

Giardia duodenalis antibody

Giardia lamblia antibody

Glial fibrillary acidic protein

Glucose-6-phosphate dehydrogenase

Glutamate-cysteine ligase regulatory subunit

Glutaredoxin-1

Glutaryl-CoA dehydrogenase

Glutathione peroxidase 1

Glutathione S-transferase A1

Glutathione S-transferase omega-1

Glutathione S-transferase P

Glycated hemoglobin

Glyceraldehyde 3-phosphate dehydrogenase

Glycophorin A

Glycophorin C

Granulocyte macrophage colony-stimulating factor

Granulocyte-colony stimulating factor

Granulocyte-macrophage colony-stimulating factor

Green fluorescent protein

Growth Hormone

Growth/differentiation factor 15

Haptoglobin

Heat shock 70 kDa protein 1

Heat shock 70 kDa protein 2

Heat shock 70 kDa protein 8

Heat shock protein 27

Heat shock protein 90

Helicobacter pylori antibody

Heme-binding protein 1

Hemoglobin

Hemoglobin A

Hemoglobin A1

Hemoglobin A2

Hemoglobin C

Hemoglobin D

Hemoglobin D-Punjab

Hemoglobin E

Hemoglobin Lepore

Hemoglobin O Arab

Hemoglobin S

Hemoglobin subunit alpha

Hemoglobin subunit delta

Hemoglobin subunit gamma-1

Hemoglobin subunit zeta

Hemopexin

Hemozoin

Heparin cofactor 2

Heparin-binding EGF-like growth factor

Hepatitis A virus antibody

Hepatitis B virus core antibody

Hepatitis B virus core antigen maternal antibody

Hepatitis B virus envelope antibody

Hepatitis B virus envelope antigen

Hepatitis B virus surface antibody

Hepatitis B virus surface antigen

Hepatitis C virus antibody

Hepatitis C virus antigen

Hepatitis C virus core antigen

Hepatocyte growth factor

Hepatocyte growth factor receptor

Herpes simplex virus 2 antibody

Herpes simplex virus antibody

High-density lipoprotein

Histidine-rich glycoprotein

Histone H2A type 1-H

HIV antibody

HIV p24 antigen

HIV-1 antibody

HIV-1 envelope peptide

HIV-1 full length core recombinant protein

HIV-1 polymerase

HIV-2 antibody

Human chorionic gonadotropin

Human papillomavirus antibody

Human T-Cell lymphotropic virus type 1 antibody

Human T-Cell lymphotropic virus type 2 antibody

Hydroxyacylglutathione hydrolase

Hypoxanthine-guanine phosphoribosyltransferase

Iduronate 2-sulfatase

Iduronidase

Immunoglobulin 64

Immunoglobulin A

Immunoglobulin alpha-1 chain C region

Immunoglobulin E

Immunoglobulin G

Immunoglobulin gamma-1 chain C region

Immunoglobulin gamma-2 chain C region

Immunoglobulin gamma-3 chain C region

Immunoglobulin gamma-4 chain C region

Immunoglobulin heavy chain V-1 region EU

Immunoglobulin heavy chain V-III region CAM

Immunoglobulin heavy chain V-III region GA

Immunoglobulin heavy chain V-III region GAL

Immunoglobulin heavy chain V-III region TEI

Immunoglobulin heavy chain V-III region TIL

Immunoglobulin heavy chain V-III region TRO

Immunoglobulin heavy chain V-III region WEA

Immunoglobulin J chain

Immunoglobulin kappa chain C region

Immunoglobulin kappa chain V-I region CAR

Immunoglobulin kappa chain V-I region DEE

Immunoglobulin kappa chain V-I region Lay

Immunoglobulin kappa chain V-I region Mev-like

Immunoglobulin kappa chain V-I region Ni

Immunoglobulin kappa chain V-II region MIL

Immunoglobulin kappa chain V-II region RPMI 6410

Immunoglobulin kappa chain V-III region B6

Immunoglobulin kappa chain V-III region HAH

Immunoglobulin kappa chain V-III region LOI

Immunoglobulin kappa chain V-III region SIE

Immunoglobulin kappa chain V-III region VG (fragment)

Immunoglobulin kappa chain V-III region VH (fragment)

Immunoglobulin kappa chain V-IV region (fragment)

Immunoglobulin kappa chain V-IV region Len

Immunoglobulin lambda chain V-I region HA

Immunoglobulin lambda chain V-I region WAH

Immunoglobulin lambda chain V-III region LOI

Immunoglobulin lambda chain V-III region SH

Immunoglobulin lambda chain V-IV region Hil

Immunoglobulin lambda chain V-IV region MOL

Immunoglobulin lambda-1 chain C region

Immunoglobulin lambda-2 chain C region
Immunoglobulin lambda-7 chain C region
Immunoglobulin lamda chain V region 4A
Immunoglobulin M
Immunoglobulin mu chain C region
Immunoglobulin mu heavy chain disease protein
Immunoglonin gamma-3 chain C region
Immunoreactive trypsin
Immunoreactive trypsinogen
Importin subunit beta-1
Influenza A pdm09 virus antibody
Insulin
Insulin-like growth factor 1
Insulin-like growth factor binding protein 1
Insulin-like growth factor binding protein 2
Insulin-like growth factor binding protein 3
Inter-alpha-trypsin inhibitor heavy chain H1
Inter-alpha-trypsin inhibitor heavy chain H2
Inter-alpha-trypsin inhibitor heavy chain H3
Inter-alpha-trypsin inhibitor heavy chain H4
Interferon gamma
Interleukin 1
Interleukin 1 alpha
Interleukin 1 beta
Interleukin 1 receptor antagonist protein
Interleukin 10
Interleukin 11
Interleukin 12
Interleukin 13
Interleukin 17 receptor B
Interleukin 17A
Interleukin 18
Interleukin 2
Interleukin 2 receptor subunit alpha
Interleukin 3
Interleukin 4
Interleukin 5
Interleukin 6
Interleukin 6 receptor
Interleukin 6 receptor subunit alpha
Interleukin 7
Interleukin 8
Interleukin 9
Isovaleryl-CoA dehydrogenase
Japanese encephalitis virus antibody
John Cunningham polyomavirus antibody
KAI-9803
Kallikrein-11
Kallikrein-6
Keratin, type I cytokskeletal 10
Keratin, type I cytoskeletal 13
Keratin, type I cytoskeletal 14
Keratin, type I cytoskeletal 9
Keratin, type II cytoskeletal 1
Keratin, type II cytoskeletal 2 epidermal
Keratin, type II cytoskeletal 2 oral
Keratin, type II cytoskeletal 5
Keratin, type II cytoskeletal 6A
Kininogen-1
L-lactate dehydrogenase A chain
L-lactate dehydrogenase B chain
L-selectin
Lactoferrin
Latency-associated peptide transforming growth factor beta-1
Leishmania antibody
Leishmania donovani promastigote antigen
Leptin
Leptospira antibody
Lipoprotein(a)
Liver carboxylesterase 1
Long chain polyunsaturated fatty acids
Low molecular weight phosphotyrosine protein phosphatase
Lumican
Luteinizing hormone
Lymphatic filariasis antibody
Lymphocyte
Lysosomal Acid Lipase
Lysosomal b-d-galactosidase
Lysosomal-associated membrane protein 1
Lysozyme C
M protein
Macrophage colony-stimulating factor 1
Macrophage inflammatory protein-1 alpha
Macrophage inflammatory protein-1 beta
Macrophage inflammatory protein-1 delta
Macrophage migration inhibitory factor
Macrophage-derived chemokine
Matrix metallopeptidase-9
Matrix metalloproteinase-3
Measles antibody
Medium-chain acyl-CoA dehydrogenase
Melanoma-derived growth regulatory protein
Methylcrotonyl-CoA carboxylase
MHC class I polypeptide-related sequence A
Midkine
MK-1775
Mucin-like protein 1
Mumps virus antibody
Mycobacterium leprae antibody
Mycobacterium leprae phenolic glycolipid I antibody
Myeloid differentiation primary response protein MyD88
Myeloperoxidase
Myosin-9
Myotrophin
N-acetylgalactosamine-4-sulfatase
N-acetylgalactosamine-6-sulfatase
N-acetylmuramoyl-L-alanine amidase
Neurotrophin-3
Neurotrophin-4
Neutrophil activating peptide 2
Nicotinate phosphoribosyltransferase
Norovirus antibody
NSFL1 cofactor p47
Nucleoside diphosphate kinase A
Nucleoside-diphosphate kinase B
Obg-like ATPase 1
Orientia tsutsugamushi antibody
Orosomucoid
Osteoprotegerin
Pancreatitis associated protein
Pappalysin-1
PEGylated-adnectin
Pentasialotransferrin
Peptidyl-prolyl cis-trans isomerase FKBP1A
Peptidylprolyl isomerase A
Peroxiredoxin-1
Peroxiredoxin-2
Peroxiredoxin-6
Phosphatidylethanolamine-binding protein
Phosphoglycerate kinase 1
Placental growth factor
Plasma cholinesterase
Plasma kallikrein

Plasma protease C1 inhibitor
Plasma retinol-binding protein
Plasminogen
Plasmodium falciparum antibody
Plasmodium vivax antibody
Platelet basic protein
Platelet endothelial cell adhesion molecule
Platelet-derived growth factor
Platelet-derived growth factor B homodimer
Platelet-derived growth factor subunit B
Pregnancy-associated plasma protein A
Procalcitonin
Programmed cell death protein 5
Prolactin
Prolidase
Properdin
Propionyl-CoA carboxylase
Prostasin
Prostate-specific antigen
Proteasome activator complex subunit 2
Proteasome inhibitor PI31 subunit
Proteasome subunit alpha type-2
Proteasome subunit alpha type-3
Proteasome subunit beta type-1
Proteasome subunit beta type-4
Proteasome subunit beta type-6
Protein 4.1
Protein C
Protein deglycase DJ-1
Protein disulfide-isomerase A2
Protein S
Protein S100-A9
Protein tyrosine phosphatase, receptor type
Protein-glutamine gamma-glutamyltransferase
Proteins Induced by Vitamin K Absence
Prothrombin
Pseudomonas aeruginosa antibody
Purine nucleoside phosphorylase
Putative protein FAM10A4
Pyruvate kinase isozymes R/L
Rab GDP dissociation inhibitor beta
RAs-related nuclear protein
Ras-related protein Rab-14
Ras-related protein Rab-1A
Receptor tyrosine-protein kinase erbB-2
Receptor tyrosine-protein kinase erbB-3
Receptor tyrosine-protein kinsne erbB-4
Regenerating islet-derived protein 4
Respiratory syncytial virus antibody
Retinal dehydrogenase 1
Retinol-binding protein
Ribonuclease inhibitor
Rickettsia conorii antibody
Rickettsia typhi antibody
Rift Valley Fever Virus antibody
Rubella virus antibody
S-adenosyl-L-methionine:protein-L-isoaspartate O-methyltransferase
S-formylglutathione hydrolase
S100 calcium-binding protein A4
S100 calcium-binding protein A6
S100 calcium-binding protein A8
S100 calcium-binding protein A9
Salmonella LPS Group B antibody
Salmonella LPS Group D antibody
Saponin C
Saposin C
Schistosoma antibody
Selenium-binding protein 1
Semenogelin-1
Semenogelin-2
Serine/threonine-protein kinase OSR1
Serine/threonine-protein phosphatase 2A 65 kDa regulatroy subunit A alpha isoform
Serotransferrin
Serpin B3
Serum albumin
Serum amyloid A
Serum amyloid A-4 protein
Serum amyloid P
Serum amyloid P component
Serum paraoxonase/arylesterase 1
Sex hormone binding globulin
SH3 domain-binding glutamic acid-rich-like protein 3
Soluble transferrin receptor
Solute carrier family 2, facilitated glucose transporter member 1
Somatotropin
Sorcin
Spectrin alpha chain, erythrocyte
Spectrin beta, erythrocytic
Stathmin
Stem cell factor
Steroid 21-hydroxylase
Stress induced phosphoprotein 1
Strongyloides stercoralis antibody
Substance P
Succinyladenosine
Succinylaminoimidazole carboxamide riboside
Sulfamidase
Superoxide dismutase
Systemic lupus erythematosus antibody
T-complex protein 1 subunit beta
T-complex protein subunit epsilon
T-complex protein subunit zeta
Taenia solium antibody
Talin-1
Tartrate-resistant acid phosphatase
Tetanus antibody
Tetanus antitoxin
Tetrasialotransferrin
Thioredoxin
Threonine antibody
Thrombin
Thrombin antibody
Thrombopoietin
Thymosin beta-4-like protein 3
Thyroglobulin
Thyroid antibody
Thyroid peroxidase antibody
Thyroid-stimulating hormone
Thyrotropin
Thyroxine
Thyroxine-binding globulin
Tissue factor
Tissue plasminogen activator
Toxoplasma gondii antibody
Transaldolase
Transcortin
Transcription elongation factor SPT6
Transferrin
Transferrin receptor
Transforming growth factor alpha
Transforming growth factor beta

Transforming growth factor beta 1
Transgelin-2
Transitional endoplasmic reticulum ATPase
Transthyretin
Treponema pallidum antibody
Treponema pallidum antigen
Treponema pertenue antigen
Trichomonas vaginalis antibody
Triggering receptor expressed on myeloid cells 1
Triose-phosphate isomerase
Trisialotransferrin
Tropomyosin alpha-1 chain
Tropomyosin alpha-3 chain
Trypanosoma brucei antibody
Trypanosoma brucei antigen
Trypanosoma cruzii antibody
Trypsin
Trypsin 1
Trypsinogen
Tubulin-specific chaperone A
Tumor necrosis factor alpha
Tumor necrosis factor beta
Tumor necrosis factor ligand superfamily member 14
Tumor necrosis factor ligand superfamily member 8
Tumor necrosis factor receptor 1
Tumor necrosis factor receptor 2
Tumor necrosis factor receptor superfamily member 4
Tumor necrosis factor receptor superfamily member 6
Ubiquitin carboxyl-terminal hydrolase 14
Ubiquitin carboxyl-terminal hydrolase 5
Ubiquitin carboxyl-terminal hydrolase isozyme L3
Ubiquitin thioesterase OTU1
Ubiquitin-C
Ubiquitin-conjugating enzyme E2 variant 1
UDP-glucose 4-epimerase
UMP-CMP kinase
Uncharacterized protein C6orf163
Uncharacterized protein C9orf40
Urokinase plasminogen activator surface receptor
Uroporphyrinogen I Synthetase
Uroporphyrinogen III decarboxylase
UV excision repair protein RAD23 homolog A
Vascular endothelial growth factor
Vascular endothelial growth factor A
Vascular endothelial growth factor receptor 2
Vascular endothelial growth factor-D
Vibrio cholerae antibody
Vitamin D-binding protein
Vitronectin
von Willebrand factor
Zinc alpha 2-glycoprotein
Zinc finger protein 410
Zinc finger protein 611
Zymogen granule protein 16 homolog B

NUCLEIC ACID

-173 G/C SNP
-794 CATT(5-8)
1078delT gene mutation
1717 1G->A gene mutation
1726G>A gene mutation
1898+1G->A gene mutation
2183-AA->G gene mutation
22q11.2 gene deletion
2789+5G->A gene mutation
3659delC gene mutation
3849+10kbC->T gene mutation
3849+4A->G gene mutation
3905insT gene mutation
621+1G->T gene mutation
711+1G->T gene mutation
A455E gene mutation
A985G gene mutation
ABCB1 C1236T polymorphism
Alpha-1 antitrypsin gene S mutation
Alpha-1 antitrypsin gene Z mutation
Alpha-globin gene
Amelogenin gene locus
Apolipoprotein E polymorphism
ASL:p.Q354STOP gene mutation
BCHE gene
Beta-actin gene
Beta-globin gene
BMP7 gene mutation
Brugia malayi DNA
BTD gene
C-X-C motif chemokine 10 mRNA
c.[1726A; 2065A]
c.1000C>T (R334W)
c.1003G>T
c.1022_1023insTC (1154insTC)
c.1040G>A (R347H)
c.1040G>C (R347P)
c.1116+1G>A (1248+1G->A)
c.1156G>T
c.115C>T (Q39X)
c.1364C>A (A455E)
c.1400T>C (L467P)
c.1475C>T (S492F)
c.1519_1521delATC (I507del)
c.1521_1523delCTT (F508del)
c.1585-1G>A (1717-1G->A)
c.1624G>T (G542X)
c.1646G>A (S549N)
c.1652G>A (G551D)
c.1657C>T (R553X)
c.1675G>A (A559T)
c.1679G>C (R560T)
c.1679T>G
c.178G>T (E60X)
c.1898delC
c.1905 + 1G>A
c.2051_2052delAAinsG (2183AA->G)
c.2052delA (2184delA)
c.220C>T (R74W)
c.223C>T (R75X)
c.2440C>T
c.254G>A (G85E) c
c.262_263delTT (394delTT)
c.2630A>T
c.2657+5G>A (2789+5G->A)
c.2767G>C
c.295_298delTCAT
c.2988+1G>A (3120+1G->A)
c.2989-1G>A (3121-1G->A)
c.3140-26A>G (3272-26A->G)
c.3196C>T (R1066C)
c.3197G>A (R1066H)
c.3276C>A (Y1092X(C>A))
c.3454G>C (D1152H)
c.3472C>T (R1158X)
c.3484C>T (R1162X)
c.350G>A (R117H)
c.3528delC (3659delC)
c.3612G>A (W1204X)
c.3659delC (3791delC)

c.3705T>G (S1235R)
c.3717+12191C>T (3849+10kbC->T)
c.3773_3774insT (3905insT)
c.3846G>A (W1282X)
c.3909C>G (N1303K)
c.489+1G>T (621+ 1G->T)
c.496A>G
c.54-5940_273+10250del21kb (CFTRdele2,3)
c.579+1G>T (711+ 1G->T)
c.617T>G (L206W)
c.703C>T
C3435T polymorphism
C59R mutation
CFTR gene
Chikungunya RNA
Chlamydia trachomatis DNA
Cluster E6 gene mutation
CSF1PO locus
CYP2D*15 gene mutation
CYP2D6*11 gene mutation
CYP2D6*12 gene mutation
CYP2D6*14 gene mutation
CYP2D6*19 gene mutation
CYP2D6*20 gene mutation
CYP2D6*3 gene mutation
CYP2D6*4 gene mutation
CYP2D6*6 gene mutation
CYP2D6*8 gene mutation
CYP3A4 A-392G polymorphism
CYP3A5 A6986G polymorphism
Cytomegalovirus DNA
D10S1248 locus
D12S391 locus
D13S317 locus
D16S539 locus
D18S51 locus
D19S433 locus
D1S1656 locus
D1S80 gene locus
D21S11 locus
D22S1045 locus
D2S1338 locus
D2S441 locus
D3S1358 locus
D5S818 locus
D6S1043 locus
D7S820 locus
D8S1179 locus
Del 8 bp E3 gene mutation
Del 8bp E3 gene mutation
delta-F508 gene mutation
Dengue virus RNA
DNA
DNA methylation
Enteroviral RNA
FAT1 gene mutation
FGA locus
FGF12 gene mutation
Fragile X mental retardation 1 gene
G2677T/A polymorphism
G542X gene mutation
G551D gene mutation
G6PD gene
G85E gene mutation
GADPH gene
GAMT gene
GAPDH gene
GJB2 gene mutation
GJB6 gene mutation
GLA gene
GSTM1 gene
GSTT1 gene
GTPCH gene mutation
Hepatitis B virus
Hepatitis B virus DNA
Hepatitis C virus RNA
Hepatitis E virus RNA
Herpes simplex virus DNA
HIV RNA
HIV-1 DNA
HIV-1 genotyping
HIV-1 resistance genotyping
HIV-1 RNA
HIV-1 total nucleic acid
HLA-B*27 gene locus
HLA-DPA1 gene
HLA-DPB1 gene
HLA-DQA1 gene
HLA-DQA1*05 gene
HLA-DQB1*02 gene
HLA-DQB1*0302 gene
HLA-DRB1 gene
HLA-DRB3 gene
HLA-DRB4 gene
HLA-DRB5 gene
HMGCL:p.R41Q gene mutation
HPRT1 gene mutation
HTR2A gene locus
Human cytomegalovirus DNA
Human herpesvirus type 6 DNA
Human leukocyte antigen genotyping
Human T-lymphotropic virus DNA
I164L mutation
I172N gene mutation
I2 splice (655A/C>G) gene mutation
I2 splice gene mutation
I236N/V237E/M239K (Cluster E6) gene mutation
I507del gene mutation
IDUA gene
IL-1B-31 gene
IL-1B-511 gene
IL28 gene mutation
Interferon gamma mRNA
K285N gene mutation
KCNA5 gene
L307Frameshift gene mutation
Leishmania donovani promastigote DNA
Leishmania RNA
Loa loa DNA
Mansonella ozzardi DNA
Mansonella perstans DNA
MBL2 gene
Measles virus DNA
N1303K gene mutation
N314D gene mutation
N51I gene mutation
Onchocerca volvulus DNA
p.I172N gene mutation
p.L307fs gene mutation
p.P30L gene mutation
p.P453S gene mutation
p.Q318X gene mutation
p.R356W gene mutation
p.R483P gene mutation
p.V281L gene mutation
P30L gene mutation
P453 gene mutation
PAX8 gene
Penta D STR locus
Penta E STR locus
Pfcrt K76T polymorphism
Pfdhfr gene
Pfdhps gene
PIK3R3 gene mutation
Plasmodium falciparum 18S rRNA
Plasmodium falciparum DNA
Plasmodium falciparum gametocyte mRNA
Plasmodium falciparum lactate dehydrogenase gene
Plasmodium falciparum multidrug resistance protein
Plasmodium knowlesi DNA

Plasmodium malariae DNA
Plasmodium ovale DNA
Plasmodium vivax DNA
Polymorphic N-acetyltransferase gene
Puumala RNA
Q188R gene mutation
Q318Stop gene mutation
Q493X gene mutation
R1162X gene mutation
R117H gene mutation
R334W gene mutation
R347H gene mutation
R347P gene mutation
R356W gene mutation
R483P gene mutation
R553X gene mutation
R560T gene mutation
Rift Valley fever virus RNA
rs1020636 gene locus
rs1111366 gene locus
rs11249784 gene locus
rs11706962 gene locus
rs1361861 gene locus
rs1403294 gene locus
rs1479530 gene locus
rs1500098 gene locus
rs1620329 gene locus
rs1674139 gene locus
rs17379 gene locus
rs17407 gene locus
rs1860665 gene locus
rs1894697 gene locus
rs1924609 gene locus
rs222 gene locus
rs228043 gene locus
rs2282739 gene locus
rs2289105 gene locus
rs230 gene locus
rs2303025 gene locus
rs234 gene locus
rs240 gene locus
rs276922 gene locus
rs326414 gene locus
rs3784740 gene locus
rs4240868 gene locus
rs4306954 gene locus
rs4358717 gene locus
rs4763188 gene locus
rs544021 gene locus
Rubella virus RNA
RYR2 gene locus
S108N gene mutation
S135L gene mutation
S549N gene mutation
S549R gene mutation
SCN5A gene
SERPINA1 gene
SMN1 gene
SMN2 gene
Streptococcus pneumoniae DNA
T-cell receptor excision circle
TBX2 gene mutation
TBX4 gene mutation
TH01 locus
Toxoplasma gondii DNA
TPOX locus
TSPAN1 gene mutation
V281L gene mutation
V520F gene mutation
Visceral leishmaniasis DNA
vWA locus
W1282X gene mutation
West nile virus RNA
Wuchereria bancrofti DNA
Y122X gene mutation
Yellow fever virus RNA

ELEMENT

Antimony
Arsenic
Barium
Beryllium
Bismuth
Cadmium
Calcium
Cesium
Chromium
Cobalt
Copper
Iron
Lead
Lithium
Magnesium
Manganese
Mercury
Molybdenum
Nickel
Phosphorus
Potassium
Rubidium
Selenium
Sodium
Sulfur
Thallium
Titanium
Vanadium
Zinc

Table 2-4. Comprehensive list of analytic methods identified in the literature to have been applied to dried blood spots.

MASS SPECTROMETRY

- Atmospheric pressure thermal desorption chemical ionization mass spectrometry
- Capillary electrophoresis mass spectrometry
- Capillary electrophoresis-electrospray ionization-mass spectrometry
- Desorption electrospray Ionization mass spectrometry
- Digital microfluidics mass spectrometry
- Electrospray ionization mass spectrometry
- Electrospray ionization tandem mass spectrometry
- Electrospray ionization triple-quadrupole mass spectrometry
- Fast atom bombardment tandem mass spectrometry
- Flow injection analysis electrospray ionization tandem mass spectrometry
- Flow injection analysis tandem mass spectrometry
- Gas chromatography electron capture mass spectrometry
- Gas chromatography mass spectrometry
- Gas chromatography mass spectroscopy selective ion monitoring
- Gas chromatography tandem mass spectrometry
- Hydrophilic interaction liquid chromatography mass spectrometry
- Hydrophilic interaction liquid chromatography tandem mass spectrometry
- Inductively coupled plasma mass spectrometry
- Ion chromatography tandem mass spectrometry
- Ion trap mass spectrometry
- Isotope-dilution mass spectrometry
- Isotope-dilution tandem mass spectrometry
- Laser ablation inductively coupled plasma mass spectrometry
- Laser ablation inductively coupled plasma time-of-flight mass spectrometry
- Laser desorption mass spectrometry
- Laser diode thermal desorption atmospheric pressure chemical ionization tandem mass spectrometry
- Laser diode thermal desorption tandem MS
- Liquid chromatography atmospheric pressure chemical ionization mass spectrometry
- Liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry
- Liquid chromatography electrospray ionization mass spectrometry
- Liquid chromatography electrospray ionization tandem mass spectrometry
- Liquid chromatography multiple reaction monitoring mass spectrometry
- Liquid chromatography porous graphitized carbon time-of-flight mass spectrometry
- Liquid chromatography time-of-flight mass spectrometry
- Liquid chromotagraphy mass spectrometry
- Liquid chromotagraphy tandem mass spectrometry
- Liquid microjunction surface sampling probe
- Liquid secondary ion tandem mass spectrometry
- Mass spectrometry
- Matrix-assisted laser desorption/ionization high-resolution accurate mass mass spectrometry
- Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
- Matrix-assisted laser desorption/ionization triple quadrupole mass spectrometry
- Microwave-assisted silylation gas chromatography mass spectrometry
- Nanoelectrospray ionization mass spectrometry
- Nanoelectrospray ionization mass spectrometry digital microfluidics
- Negative-ion chemical ionization mass spectrometry
- Negative-ion chemical ionization tandem mass spectrometry
- Paper spray ionization multiple reaction monitoring mass spectrometry
- Paper spray mass spectrometry
- Reverse phase liquid chromatography electrospray ionization mass spectrometry
- Sector field inductively coupled plasma mass spectrometry
- Speciated isotope dilution mass spectrometry
- Tandem mass spectrometry
- Top down proteomics mass spectrometry

IMMUNOASSAY

- Antibody microarray
- Chemiluminescent immunoassay
- Competitive enzyme-linked immunosorbent assay
- Direct agglutination test

Dissociation-enhanced lanthanide fluorescence immunoassay
Electroimmunodiffusion
Enzyme immunoassay
Enzyme multiplied immunoassay technique
Enzyme-linked immunosorbent assay
Fluorescence polarization immunoassay
Fluorometric enzyme immunocapture assay
Gelatin particle agglutination
gG-Capture enzyme immunoassay
High-throughput multiplex enzyme assay
Immunoaffinity capillary electrophoresis
Immunoassay
Immunoassay with xMAP technology
Immunochemiluminometric assay
Immunochromatographic assays
Immunofluorescence assay
Immunofluorescence assay with digital microfluidics
Immunonephelometric assay
Immunonephelometry
Immunoturbidimetric assay
Indirect immunoenzyme
Indirect immunofluorescence
Latex aggluniation test
Micro-card agglutination test for Trypanosomiasis
Microscopic agglutination
Microtiter plate-based immunofluorescence assay
Multiplexed fluorescent microsphere immunoassay
Protein microarray
Proximity extension immunoassay
Radial immunodiffusion
Radioimmunoassay
Recycling immunoaffinity chromatography
Silver-enhanced gold-labelled immunosorbent assay
Treponema pallidum particle agglutination assay

NUCLEIC ACID BASED

Helicase dependent amplification assay
High-throughput sequencing
Hybridization protection assay
Loop mediated isothermal amplification
Methylated DNA immunoprecipitation next generation sequencer
Multiplex polymerase chain reaction
Nested polymerase chain reaction
Next-generation sequencing
Nucleic acid sequence-based-amplification
Polymerase chain reaction
Polymerase chain reaction oligonucleotide ligation assay
Polymerase chain reaction-restriction fragment length polymorphism
Proximity ligation assay
Proximity-dependent DNA ligation
Pyrosequencing
Quantitative (or real time) polymerase chain reaction
Real time reverse transcriptase polymerase chain reaction
Real-time quantitative nucleic acid sequence-based amplification
Reverse transcription polymerase chain reaction
Selective acid lipase inhibitor enzyme assay
TaqMan 5'-nuclease assay
Transcription mediated aplification hybridization protection assay
Transferrin receptor assay

SEPARATION (CHROMATOGRAPHY)

Affinity chromatography
Column chromatography
Gas chromatography
Gas chromatography flame ionization detector
Gas-liquid chromatography
Gel chromatography
High performance capillary electrophoresis with fluoresence detection
High-performance anion-exchange chromatography-pulsed amperometric detection
High-performance liquid chromatography
High-performance liquid chromatography ultraviolet radiation detection
High-performance liquid chromatography with diode-array detection

High-performance liquid chromatography with fluorescence detection

Hydrophilic interaction liquid chromatography

Ion exchange chromatography

Liquid chromatography

Liquid chromatography colorimetry

Liquid chromatography with fluorescence detection

Liquid chromatography with ultraviolet radiation detection

Reverse phase high-performance liquid chromatography ultraviolet radiation

Reverse phase ultra-performance liquid chromatography

SEPARATION (ELECTROPHORESIS)

Capillary zone electrophoresis

Discontinuous electrophoresis

Gel electrophoresis

Isoelectric focusing

Lateral flow immuno-chromatographic antigen-detection test

Southern blot

Visual automated fluorescence electrophoresis

Western blot

SEPARATION (OTHER)

Digital microfluidics

Ligand binding assay

Microfluidics

Microtiter transfer plates

SPECTROSCOPY

Atomic absorption assay

Colorimetric ultramicroassay

Colorimetry

Electrogenerated chemiluminescence

Fluorometric assay

Fluorometry

Graphite furnace atomic absorption assay

Microtiter plate fluorometry

Reflectometery

Solid sampling graphite furnace atomic absorption assay

Spectrofluorometry

OTHER

Bacterial inhibition assay

Cholesterol oxidase/p-aminophenazone method

Enzymatic colorimetric analysis

Glycerophosphate oxidase-peroxidase/aminophenazone method

Hemagglutination assay

Hemagglutination inhibition assay

Indirect potentiometry

Microbiological assay

Neuroblastoma cell-based assay

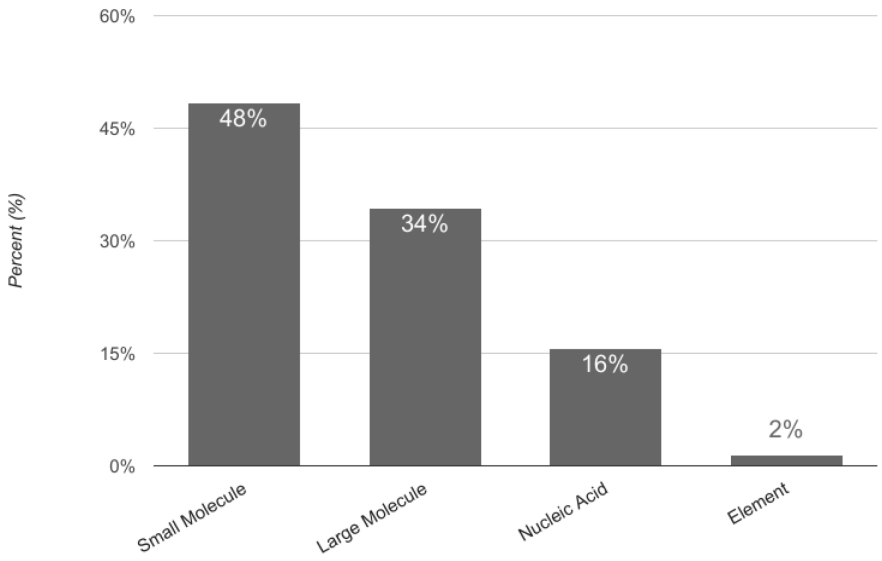

Figure 2-2. Percentage of analyte classes assigned to unique analytes identified in the literature.

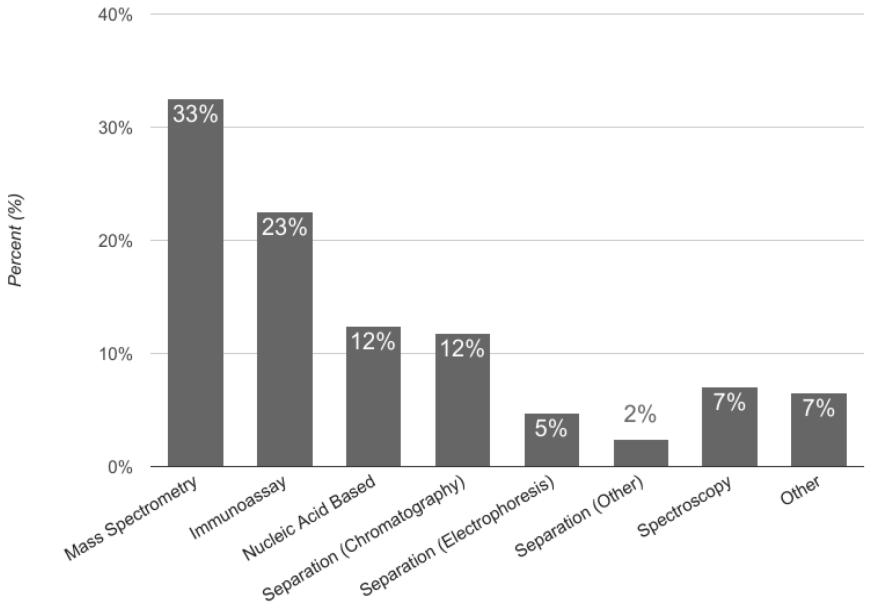

Figure 2-3. Percentage of analytic method categories assigned to unique analytic methods identified in the literature.

Table 2-5. SWOT analysis of common strengths, weaknesses, opportunities, and threats identified in the literature for dried blood spots.

Strengths	**Weaknesses**
Minimally invasive Small sample volume Volumetric measurement Simple collection, transport, and storage Reduced biohazard risk Low cost Reduced material input and waste Compatible with most bioanalytical methods Versatile matrix Wide range of analytes validated Good precision and reproducibility Improved analyte stability Federal quality assurance program Federally established guidelines Published recommendations for validation methods	Nontraditional sample matrix Small sample volume Sampling from cold or dehydrated persons Required drying Pathogenicity of agents Time, space, and labor intensive processing Susceptibility to environmental conditions Hematocrit effects Chromatographic effects Sample heterogeneity Differential analyte stability Differential analyte extraction efficiency Poorly defined regulatory landscape Additional validation steps Variability in validation methods applied
Opportunities	**Threats**
Compliance with the 3Rs Centralization of labs Increased outpatient and offsite services Advancements in bioanalytical instruments Cost and availability of sophisticated instrumentation Microfluidics and nanotechnology Online/direct analyses Endogenous indicators of blood hematocrit Use of molar ratios Sampling in hard-to-reach and vulnerable populations Large, complex study design needs In-field forensics Other dried matrices	Use of residual samples Pediatric involvement in studies Existing assays and workflows Availability of experienced labs Regulatory uncertainty

Table 2-6. Comparison of dried blood spots to traditional liquid plasma and serum.

Characteristics	**Dried Blood Spots**	**Traditional Plasma/Serum**
Matrix source	Capillary blood (contains interstitial and intracellular fluids)	Venous blood
Matrix type	Whole blood	Plasma or serum
Matrix state	Dried	Liquid or frozen
Volume	< 50 microliters (i.e., microsample)	Several milliliters
Collection	Ambient *delayed* storage (i.e., open air drying followed by ambient storage)	Cold *immediate* storage (i.e., cold storage immediately after preparation)
Bioanalytical workflow	Converted/modified	Original/optimized
Measurement	Converted/adjusted	Direct

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CHAPTER THREE

Manuscript 2

Improved Methods for Field Collection and Storage of Dried Blood Spots

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ABSTRACT

Background: Dried blood spots (DBS) are a minimally invasive method for the collection of small quantities of whole blood, including cells and plasma, from finger or heel stick with application to specially designed filter paper cards for drying and storage. Existing guidelines for DBS collection require open-air drying for a minimum of 3 hours prior to storage. This requirement limits the use of DBS in field settings as logistical constraints and environmental conditions in the field may not be conducive to open-air drying. Samples left out for extended periods of time can be exposed to dust, insects, and other environmental contaminants, which may impact measurements from DBS samples. Additionally, highly humid environments can more than double drying times, which add substantial bias to sample measurements. Therefore, the ability to store DBS samples quickly after collection while achieving reductions in the variability of drying conditions could substantially improve analyte measurements from DBS samples.

Objective: The objective of this study was to develop and validate new methods in DBS collection aimed at improving the reliability and stability of analyte measurements from DBS samples, especially those collected under challenging field conditions. We hypothesized that DBS samples stored quickly after collection in novel DBS kits would have average drying times of less than 90 minutes in conditions of low to moderate, or high humidity.

Methods: We measured drying times of blood spots collected in novel DBS collection kits. Kits were tested under ambient lab conditions of moderate humidity and ambient field simulation conditions of high humidity in the rainforest exhibit of the National Aquarium in Baltimore, Maryland. Drying times of blood spots were measured by use of resistance sensors that measure the resistance of currents in an electrical circuit that we specially designed for use with filter paper cards for this experiment.

Results: Novel DBS collection kits were developed and tested under both moderate and high humidity conditions. Our kits demonstrated blood spot drying times of less than 90 minutes. Moreover, DBS samples collected with these kits under moderate and high humidity ambient conditions had blood spot drying times approximately 30% and 50% faster respectively than open-air drying under similar conditions reported in the literature. Our kits remove the requirement of open-air drying, and may improve data quality by removal of potential bias introduced to DBS samples drying under variable environmental conditions.

Conclusion: Our novel DBS collection and storage kits can enable improved field use of DBS by allowing for storage quickly after collection rather than open-air drying for 3 hours. Moreover, our kits showed overall improvements in blood spot drying times that compare favorably with existing literature that indicate blood spots require a minimum of 90 minutes to dry under conditions of low to moderate humidity, and greater than 150

minutes in conditions of high humidity. Findings suggest our kits allow for storage of DBS samples immediately after collection, thereby preventing exposure to environmental contaminants, enabling movement of samples immediately following spotting to filter paper cards, and may provide overall improvements in data quality due to the removal of potential bias introduced to DBS samples from variable drying conditions.

BACKGROUND

Dried blood spots (DBS) are a minimally invasive method for the collection of small volumes of blood (< 50 microliters) from finger or heel stick and transfer to filter paper cards for drying and storage [1-2]. In principle, anything that can be measured from liquid whole blood, plasma or serum, can be measured in dried blood spots [3]. All major categories of analytic methods have been applied for measuring analytes in DBS, including electrophoresis, immunoassay, chromatography, mass spectrometry, spectroscopy, and polymerase chain reaction (PCR) [4]. To date, DBS samples have been used for measuring over 1,900 different analytes, and have been applied to a variety of uses in basic research, public health, and clinical medicine [4].

The simplicity of collection methods and small volume of blood sample enable DBS to be collected without the need for a trained phlebotomist [5-6]. Additionally, the use of a dried matrix allows for ambient shipping and storage, and removes the requirement for cold chain, which is necessary for traditional liquid samples such as whole blood, plasma, and

serum [5-6]. These benefits make DBS a preferred method for the collection of biosamples in the field (i.e. outside of the traditional clinic or lab setting). However, some limitations in the methods for DBS collection continue to impede wider adoption in a range of settings. Variability inherent in rates of drying blood samples, and a minimum requirement of several hours open-air drying prior to sample storage or shipment are often cited as constraints on wider adoption of DBS [7-9]. As the quality and availability of highly sensitive analytical instrumentation, such as mass spectrometry, continue to improve, constraints around detection limits and variability in measuring analytes from DBS have become less of an impediment to adoption [10-11]. Yet the issue of open-air drying remains unresolved.

The current collection protocol recommended by the United States Centers for Disease Control and Prevention (CDC) requires a minimum open-air drying time of 3 hours prior to sample storage [12]. This requirement is problematic in many settings of sample collection, including field collection studies. Open-air drying is logistically difficult during field collection as space for drying racks may not be available, conditions are often not conducive to drying (e.g. high humidity in tropical climates), and in the case of household surveys or other technically challenging environments such as occupational or austere settings, open-air drying for several hours may not be acceptable or feasible [6, 13-14]. Open-air drying in field settings also increase the risk of sample exposure to dust, chemical contaminants ubiquitous in the environment, airborne pathogens, and small insects to name

a few [15-16]. These risks are particularly problematic when target analytes include DNA or environmental contaminants, as sample measurements of either may actually reflect sample exposures during drying rather than host exposures prior to sample collection [16]. Variable ambient conditions of humidity also affect the drying times of DBS samples, which is problematics as drying times have a direct impact on measurements for a range of target analytes, particularly metabolites, RNA, and all classes of analytes susceptible to hydrolysis, or other processes utilizing water for analyte degradation [7, 17-19]. In conditions of low or moderate humidity (i.e. relative humidity of less than 60%) drying times of 90 minutes are reported, whereas in conditions of high humidity (i.e. relative humidity equal to or greater than 60%) drying times as high as 150 minutes or more have been reported [20-21]. The ability to dry and store DBS samples shortly after blood collection, without compromising drying times, while also reducing variability of drying conditions that affect analyte stability, could enable wider adoption of DBS sampling in a range of settings, especially field settings, and may provide better quality measurements.

The objective of this study was to demonstrate proof-of-concept for a novel DBS collection kit aimed at enabling field collection and storage. We hypothesized that our novel collection kits will enable storage of DBS samples quickly after collection without compromising drying times. Specifically, we hypothesized that DBS samples stored in our kits immediately after collection would have average drying times of less than 90 minutes in conditions of low to moderate, or high humidity. Storage quickly after blood collection

in kits protected from environmental exposures, and which improve drying times, could remove some of the major impediments to wider adoption of DBS sampling, especially in field settings.

METHODS

Kit Selection and Justification

The goal of kit selection and optimization was to design and optimize a kit that maintained the simplicity of DBS methods such that they could be used effectively in field settings. Inclusion criteria for kit design and fabrication were as follows:

1. Kit materials must be commercially available.
2. Kit contents must not require any additional manufacturing or engineering beyond the point of procurement/purchasing.
3. Kit contents must be easily put together by end users in the field.

Novel DBS collection kits were designed with a closed-system (i.e. airtight containers protected from the external environment) by inclusion of an opaque, airtight, cylindrical container with an optimized amount of molecular sieve desiccant, and use of a DBS filter paper card. The container selected included a 644 mL aluminum, opaque, cylindrical bottle (75 mm diameter and 152 mm height) with screw-on cap from Elemental Container (Figure 3-1a; product # 0075152). The use of an opaque container was selected in order to meet current recommendations for drying DBS samples away from direct sunlight, which could

be an issue during field collection as personnel may need to move blood samples shortly after collection. The use of an airtight container was selected in order to allow control and modulation of the moisture conditions within the kit. The cylindrical shape and size of the container was selected to allow enough space for inclusion of desiccant, filter paper card, and wireless sensors with data loggers for measuring drying times of blood spots, and tracking relative humidity and temperature during experimentation. Molecular sieve MiniPax absorbent packets from Multisorb Technologies (Figure 3-1b; product # 02-00041AG19) were used as the desiccant of choice, as opposed to the more common silica gel desiccant, due to their ability to absorb moisture faster and maintain moisture within the desiccant under dynamic or extreme temperature conditions, which were directly tested in our study as described herein under *Stress Testing Methods*. Whatman 903 filter paper cards from GE Healthcare Life Sciences (Figure 3-1c; product # 10531018) were selected for inclusion in the kits due to being the most commonly used type of filter paper card in DBS studies, as well as their history of rigorous quality assurance testing from CDC [22-23]. The optimal amount of molecular sieve (i.e. 40 grams) was determined experimentally and was based on the volume of air within the selected kit container, type of filter paper card, and expected amount of moisture introduced into the closed-system by a freshly spotted filter paper card. More specifically, in preparation for our study, we conducted a series of experiments with increasing amounts of molecular sieve desiccant within our kits in order to determine the optimal amount of sieve for removing moisture from the closed-

system. We have detailed these methods and reported our findings in Appendix A under Supplementary Materials.

Drying Rate Methods

Goal: The goal of the drying rate experiments was to determine the time required for blood on filter paper cards to dry within our novel DBS collection kits. We defined drying in our experiment as the time at which specially designed resistance sensors, as described below, achieved a stable measurement, which indicated all detectable moisture had evaporated from the blood spot.

Outcome Measures: The outcome measures of interest included (1) time required for freshly spotted human whole blood to dry on filter paper cards within DBS kits and (2) time required for the relative humidity (RH) inside kits to reach near zero moisture levels, defined here as an RH level of less than 0.01%. As we were interested in the time required for spots to dry within a closed system, common approaches such as periodic weight measurements of filter paper cards could not be used. In consultation with the Biomedical Engineering Department at Johns Hopkins University, we developed a novel method for measuring drying time. Specifically, we utilized resistance, which is a measure of opposition to passage of electric current through a media, in this case, blood on filter paper

[24]. As spots dry on filter paper cards and conductivity of the current reduces, resistance measurements will eventually begin to drop and stabilize once the spot is dry[3].

Materials: Kit containers were procured directly from Elemental Container; 10 g molecular sieve desiccant packets and Whatman 903 cards were procured from Sigma-Aldrich; wireless bluetooth enabled RH/temperature HOBO data loggers (Figure 3-1d; product # MX1101) were procured from ONSET; and 200 microliter adjustable pipettes (product # 3121000082) were procured from Eppendorf. For measuring drying rate of blood spots, a resistance measuring and storage system was designed and built from scratch by assembling components procured from the online digital electronics retailers Adafruit and Sparkfun. Specifically, we assessed drying through resistance across blood spots as measured by applying a constant DC voltage of 3.7V with the help of a lithium polymer battery (product #2011). The data obtained from this system was stored in a data logger (product #1895). The data collection, storage, and retrieval was managed using an Arduino pro mini microcontroller (product #2377). Additionally, a circuit scribe conductive ink pen (product # COM-13254) was procured from Sparkfun, and mini alligator clips (product # CZACA) were procured from Amazon. After procurement, all components were soldered together and the sensor designed as show in the circuit diagram (Figure 3-2). We then designed and wrote an Arduino program for logging resistance data from sensors during

[3] We cross-validated the use of resistance for measuring drying weights (data not shown) outside of the closed-system kits by real time monitoring of resistance measurements at 1-minute intervals followed by weight measurements of filter paper cards with a microscale before and after resistance levels stabilized.

experimentation. 30 mL of fresh donor human whole blood (i.e. collected less than 48 hours from time of experimental use) with sodium citrate anticoagulant was procured from Innovative Research (product # IPLA-WB1).

Process: Drying rate experiments were conducted under ambient lab conditions, which included a temperature range of 22-24℃ and moderate humidity (30-50% RH), and under simulated field conditions of 24-25℃ and high humidity conditions (>50% RH) in the rainforest exhibit of the National Aquarium in Baltimore, Maryland. All experiments utilized the same study design, which included 6 replicate kits with optimized amounts of molecular sieve desiccant and filter paper cards freshly spotted with human whole blood. Specifically, we used circuit pens (product # COM-13254) to draw an electric circuit onto filter paper cards (Figure 3-1e) and attached mini alligator clips, which were connected to the microcontroller circuit. We would then start HOBO and resistance sensor data logging at 1-minute measurement intervals, spot a total of four 30 uL spots of human whole blood via micropipette onto Whatman 903 filter paper cards, and immediately placed the spotted cards, HOBO sensors, resistance sensors, and optimized amount of molecular sieve desiccant into the containers and sealed them (Figure 3-1f). Experiments were carried out for 24 hours, after which time containers were unsealed, HOBO and resistance sensors were stopped, and data was downloaded in Excel and CSV formats to a desktop computer. Data was then imported into Stata version 13.1 for analysis.

Analysis: We inspected resistance measurements and recorded drying time by measure of the Time to Stability (TTS), defined here as the minutes required for resistance measurements to decrease and stabilize. The mean and standard deviation for drying times of all 6 replicates in each experiment was calculated. The mean and standard deviation for time required to reach near zero moisture levels was also calculated. Two-sample t-tests were conducted to determine if the differences in drying times between the three experiments was significant.

Extended Storage Methods

Goal: The goal of the extended storage experiment was to determine if near zero moisture levels (<0.001% RH) inside DBS kits was maintained for at least 14 days of storage.

Outcome Measure: The outcome measure for the extended storage experiment was RH.

Materials: Containers, desiccant, DBS cards, RH/temperature data loggers, pipettes, and human whole blood were procured as previously specified.

Process: 6 replicate kits with optimized amounts of molecular sieve desiccant and filter paper cards were freshly spotted with human whole blood and included in the extended storage experiment. Specifically, we began by starting HOBO sensor data logging at 1-minute measurement intervals, then spotted four 30 uL spots of human whole blood via

micropipette onto Whatman 903 filter paper cards, and immediately placed the spotted cards, HOBO sensors, and optimized amount of molecular sieve desiccant into the containers and sealed them. The experiment was carried out for 14 days, after which time containers were unsealed, HOBO sensors were stopped, and data was downloaded in Excel and CSV formats to a desktop computer. Data was then imported into Stata version 13.1 for analysis.

Analysis: RH values were inspected to determine time required for near zero moisture levels to be achieved, and determined if RH levels rose above near zero moisture levels at any time thereafter.

Stress Test Methods

Goal: The goal of the stress test experiment was to determine if near zero moisture levels were maintained by molecular sieve desiccants under temperature extremes (i.e. does moisture escape from the sieve under extreme heat or cold).

Outcome Measure: The outcome measure for the extended storage experiment was RH.

Materials: Containers, desiccant, DBS cards, RH/temperature data loggers, pipettes, and human whole blood were procured as previously specified. An environmental chamber was

procured on loan from the Johns Hopkins University Applied Physics Laboratory (Figure 3-1H), and a minus twenty freezer was already present in the lab for experimentation.

Process: 6 replicate kits with optimized amounts of molecular sieve desiccant and filter paper cards were freshly spotted with human whole blood and included in the stress testing experiment. Specifically, we began by starting HOBO sensor data logging at 1-minute measurement intervals, then spotted 4-30 uL spots of human whole blood via micropipette onto Whatman 903 filter paper cards, and immediately placed the spotted cards, HOBO sensors, and optimized amount of molecular sieve desiccant into the containers and sealed them. We allowed moisture levels inside the kits to reach near zero levels before beginning stress testing. After near zero moisture levels were achieved, kits were placed inside of an environmental chamber and heated to > 38℃. Kits were then removed from the environmental chamber and allowed to return to ambient conditions. After returning to ambient conditions, kits were placed inside a freezer and cooled to below 0℃, after which time containers were removed from the freezer and allowed to return to ambient temperatures. After returning to ambient temperatures, kits were unsealed, HOBO sensors stopped, and data downloaded in Excel and CSV formats to a desktop computer. Data was then imported into Stata version 13.1 for analysis.

Analysis: RH values were inspected to determine if RH levels rose above near zero moisture levels at any time during the stress test.

RESULTS

Drying Rate

Lab-based drying rate experiments were conducted under ambient lab conditions. Experimental data is reported in Table 3-1. Temperatures in the lab during drying for lab-based experiment 1 ranged between 22 and 24℃ with an ambient RH between 33 and 35%. Mean resistance-based blood spot drying time for lab-based experiment 1 was calculated at 47.6 minutes (n = 5, SD = 4.51)[4]. The mean time required to achieve near zero moisture conditions inside kit containers for lab-based experiment 1 was calculated at 603.8 minutes (n = 6, SD = 100.9), or approximately 10 hours. Ambient conditions for lab-based experiment 2 were similar to experiment 1, as were the recorded blood spot drying times. Specifically, temperatures in the lab during drying for lab-based experiment 2 ranged between 22 and 23℃ with an ambient RH between 33 and 35%. Mean resistance-based blood spot drying time for lab-based experiment 2 was calculated at 53.3 minutes (n = 6, SD = 6.95). The mean time required to achieve near zero moisture conditions inside kit containers for lab-based experiment 2 was calculated at 423.2 minutes (n = 6, SD = 61.1), or approximately 7 hours. A two sample t test was calculated for comparing experiments 1 and 2. No significant difference in blood spot drying times was detected between

[4] Lab-based experiment 1 and field simulation have an n of 5 for mean drying time due to resistance sensor failures during experimentation. RH sensors operating during these same experiments functioned for all 6 replicates, and therefore time required to achieve near zero moisture has an n of 6 for all three experiments.

experiments 1 and 2 (df = 9, t = 1.58, p = 0.1482). Visual inspection of RH curves for experiments 1 and 2 had similar findings (Figure 3-3 and Figure 3-4). Specifically, an initial increase above starting RH levels of 5-10% was detected for all 6 replicates, followed by rapid RH depletion until RH fell below 20%, after which time RH depletion slows until near zero moisture is achieved.

In order to simulate high humidity field conditions, a field simulation experiment was conducted in the rain forest exhibit of the National Aquarium in Baltimore, Maryland. Data for the field simulation is reported in Table 3-1. Temperatures in the rain forest exhibit during drying ranged between 24 and 25℃ with an ambient RH between 49 and 61%. Mean resistance-based drying time for the field simulation was calculated at 72.4 minutes (n = 5, SD = 13.39). The mean time required to achieve near zero moisture conditions inside kit containers for the field simulation was calculated at 558.5 minutes (n = 6, SD = 139.8), or approximately 9 hours. Two sample t-tests were calculated for comparing blood spot drying times in the field simulation with the lab-based experiments. A significant difference in drying times was detected for the field simulation versus lab-based experiment 1 (df = 8, t = 3.93, p = 0.0044). A significant difference in drying times was also detected for the field simulation versus lab-based experiment 2 (df = 9, t = 3.05, p = 0.0138). Visual inspection of the RH curves for the field simulation found a different shape than what was found for the lab-based experiments (Figure 3-5). Specifically, there is an immediate drop of 5-10% in detected RH levels inside kit containers for all 6 replicates

followed by a leveling off of RH, and then a rapid decline until RH falls below 20%, after which time RH depletion slows until near zero moisture is achieved.

Extended Storage and Stress Test

Extended storage and stress test experimental findings were unremarkable. After achieving near zero moisture conditions within containers, all 6 replicates maintained near zero moisture throughout the 14-day storage period (Figure 3-6). Under stress testing, near zero moisture conditions were maintained for all 6 replicates under conditions of extreme heat and cold. Specifically, neither heating kits to > 38℃ nor cooling kits to below freezing (i.e. < 0℃) resulted in any detectable moisture being released by the molecular sieve desiccant during experimentation (Figure 3-7).

DISCUSSION

Consistent with the study's hypothesis, findings suggest that our novel DBS collection and storage kits can remove the requirement of open-air drying, while reliably drying DBS samples in less than 90 minutes in low to moderate or high humidity conditions. Though low humidity conditions were not directly tested in this experiment, both moderate and high humidity conditions demonstrated mean drying times of less than 90 minutes, and would suggest that low humidity conditions would perform similarly well, if not better.

Both experiments conducted under ambient conditions of moderate humidity demonstrated mean drying times of less than 60 minutes, which compares favorably with previous studies citing 90-minute drying times for similar ambient conditions [20-21]. These findings represent approximately a 30% improvement in blood spot drying compared with open-air drying. Kit performance under ambient conditions of high humidity compared with open-air drying under similar conditions was even more pronounced. Mean drying times for DBS samples in kits during field simulation in the high humidity environment of the rainforest exhibit at the National Aquarium in Baltimore were less than 75 minutes, which represents approximately a 50% improvement in blood spot drying compared with previous studies citing as high as 150 minutes or more under high humidity [20-21].

In contrast to ambient conditions of moderate humidity, which demonstrate an immediate increase in the internal RH conditions of kits, ambient conditions of high humidity demonstrated an immediate decrease in the internal RH conditions of kits. In the case of an immediate increase in internal RH conditions inside kits compared with the external environment, it could be inferred that drying conditions for DBS samples inside our kits used in moderate or low humidity conditions are initially worse than open-air drying in similar conditions. However, drying times suggest this is not the case. The initial increase in RH detected within kits may simply be a result of moisture being transferred quickly through the air as it is removed from blood spots and absorbed by the molecular sieve. In the case of an immediate decrease in internal RH conditions inside kits compared with the

external environment, as was detected in the RH curves for the field simulation, it is reasonable to assume that drying conditions inside the container are better than open-air drying under similar conditions of high humidity within a minute of sealing the container. These findings suggest our kits would be a preferred method of DBS collection over the current protocol in conditions low to moderate or high humidity.

Stress tests indicate that molecular sieve desiccants do, in fact, retain moisture even under extreme temperature conditions, and may therefore be the preferred desiccant of choice for maintaining a near moisture free environment around DBS samples when in transport and storage, particularly in field settings where temperature conditions cannot be controlled. Extended storage tests suggest that kits can be effectively used as storage containers for a minimum of 14 days. Taken together with improvements in drying times, study findings suggest that our novel DBS collection and storage kits may be a preferred method for DBS sampling in field settings by removing the requirement of open-air drying, allowing for immediate storage, and potentially improving data quality by stabilizing analytes and preventing contamination. Potential improvements in analyte stability due to faster or more consistent drying times, particularly for metabolites, RNA, and other classes of analytes, which are susceptible to degradation by hydrolysis, should be demonstrated experimentally. We did not directly measure analyte stability for DBS samples collected in our novel kits compared with open-air drying. Future studies in this area will need to be conducted.

Study limitations around ambient conditions for experimentation, storage times and stressing should be noted. As molecular sieve desiccant action is temperature dependent, additional experimentation with use of our novel kits should include a wider range of ambient temperatures [25]. Longer storage times would also provide a benefit to potential end-users. Stress testing of kits under extremes greater than 38℃ may also be warranted as field conditions could easily exceed the upper limit of the heat conditions of our stress test. Use of fresh blood, rather than sodium citrate treated blood should also be tested as anticoagulant use may affect drying rate of blood spots.

Conclusion

Our novel DBS collection and storage kits developed can enable improved field use of DBS by removing the requirement for open-air drying and allowing quick storage after collection with overall improvements in blood spot drying times. Immediate storage and faster drying times could reduce the logistical constraints around DBS collection in the field, prevent possible sample contamination, and provide for overall improvements in data quality.

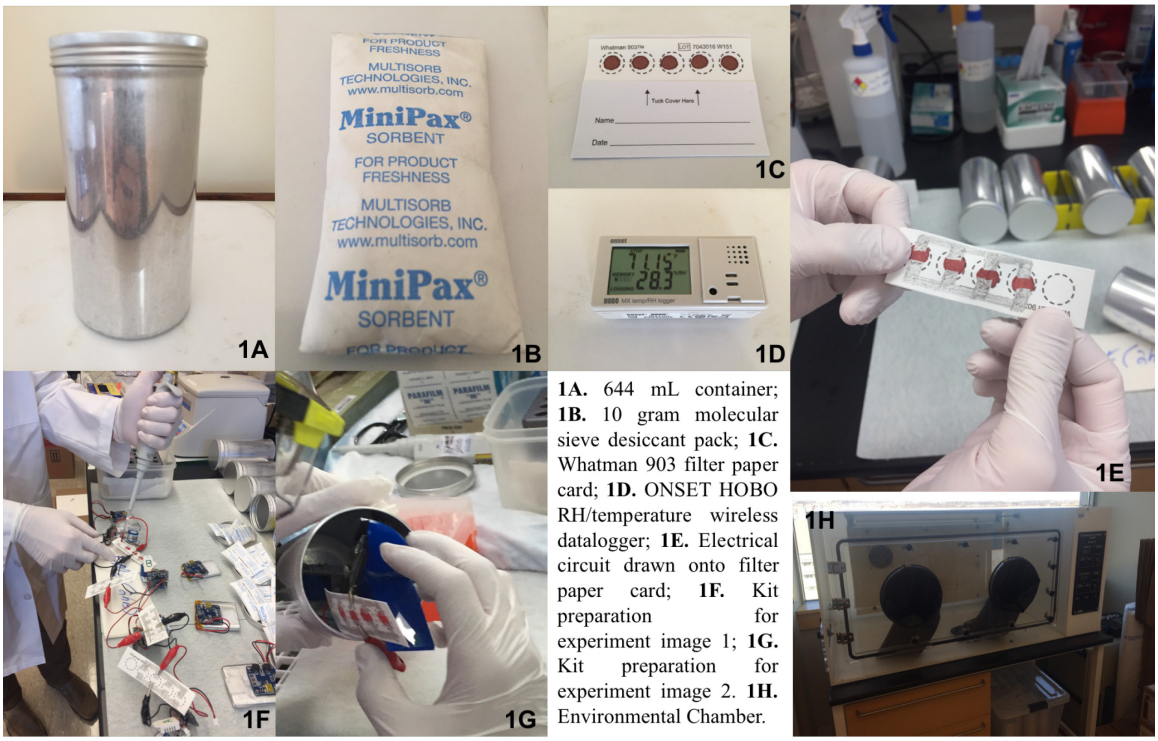
SPECIAL THANKS

We would like to express our sincere thanks and gratitude to the National Aquarium in Baltimore, Maryland for allowing our research team to conduct a field simulation in their rainforest exhibit. Specifically, we'd like to acknowledge the director the rainforest exhibit, Ken Howell, as well as the other members of the aquarium's research committee: Jill Arnold, Leigh Clayton, Diana Fridberg, Alan Henningsen, Jennie Janssen, Jessica Nelson, Mark Turner, and Brent Whitaker. We would also like to thank the Johns Hopkins Department of Biomedical Engineering for their support in conducting this study. Specifically, we'd like to thank Dr. Soumyadipta Acharya for recommending the use of resistance as a potential method for measuring blood spot drying times, as well as Neha Goal for connecting our team with Dr. Acharya. We'd also like to thank Emily Eggert for building the prototype sensors for our study. Lastly, we'd like to express our gratitude to Dr.'s Christopher Bradburne and Zahra Chaudhry from the Johns Hopkins Applied Physics Laboratory for loaning our team an environmental chamber for conducting our study.

TABLES AND FIGURES

Table 3-1. Drying time and time required to achieve near zero moisture for drying rate experiments.

Experimental Setting	External Ambient Conditions During Drying	Resistance-Based Drying Time (Mean + SD)	Time Required to Achieve Near Zero Moisture in Kits (Mean + SD)
Lab-Based 1	RH = 33-35% T=22-24℃	47.6 + 4.5 min (n=5)	604 + 101 min or ~10 hours (n=6)
Lab-Based 2	RH = 33-35% T = 22-23℃	53.3 + 7.0 min (n=6)	423 + 61 min or ~7 hours (n=6)
Field Simulation (Rainforest)	RH = 49-65% T = 24-25℃	72.4 +13.4 min (n=5)	559 + 140 min or ~9 hours (n=6)



1A. 644 mL container; **1B.** 10 gram molecular sieve desiccant pack; **1C.** Whatman 903 filter paper card; **1D.** ONSET HOBO RH/temperature wireless datalogger; **1E.** Electrical circuit drawn onto filter paper card; **1F.** Kit preparation for experiment image 1; **1G.** Kit preparation for experiment image 2. **1H.** Environmental Chamber.

Figure 3-1. Images for kit contents and experimental methods.

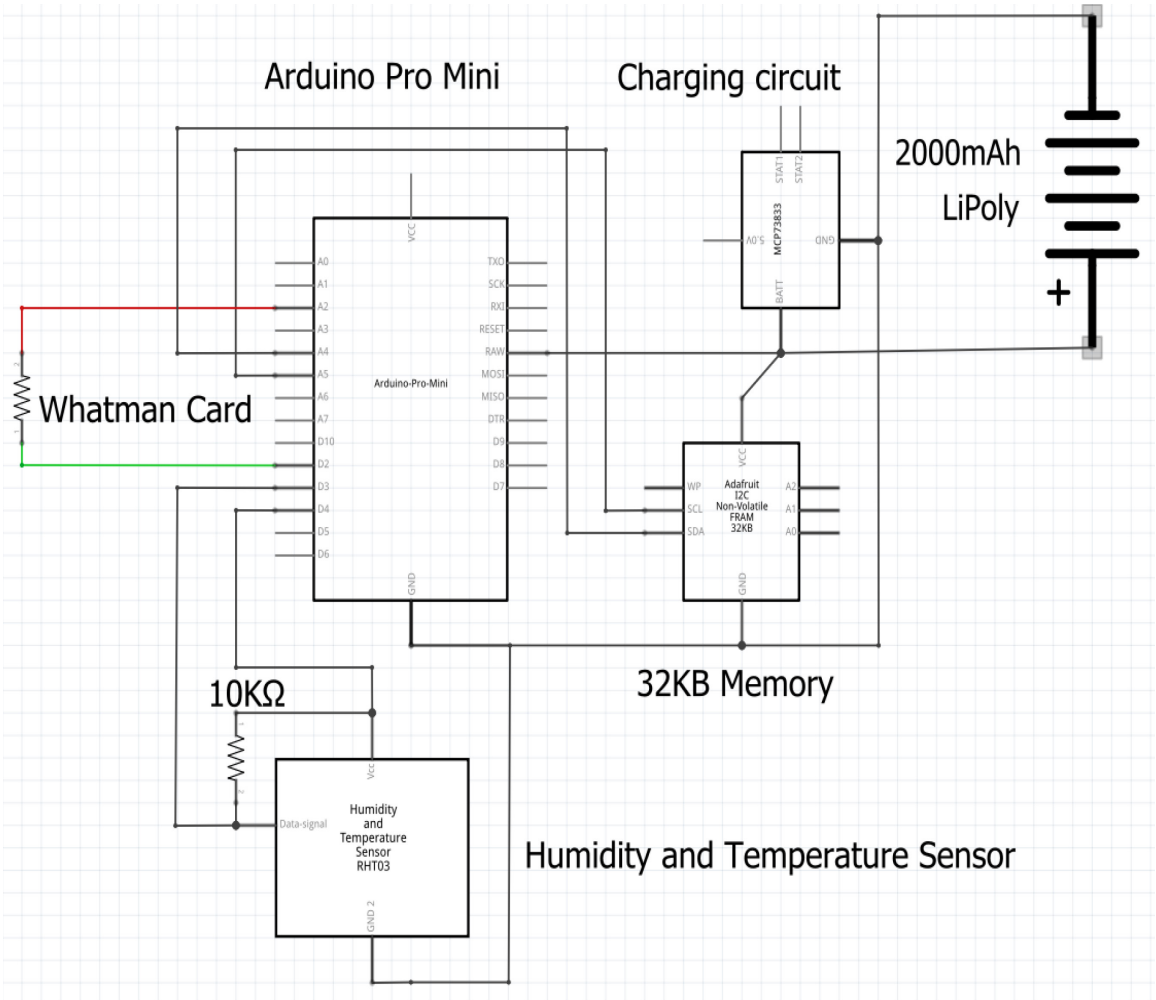


Figure 3-2. Circuit diagram of resistance sensor for measuring drying rate of blood spots.

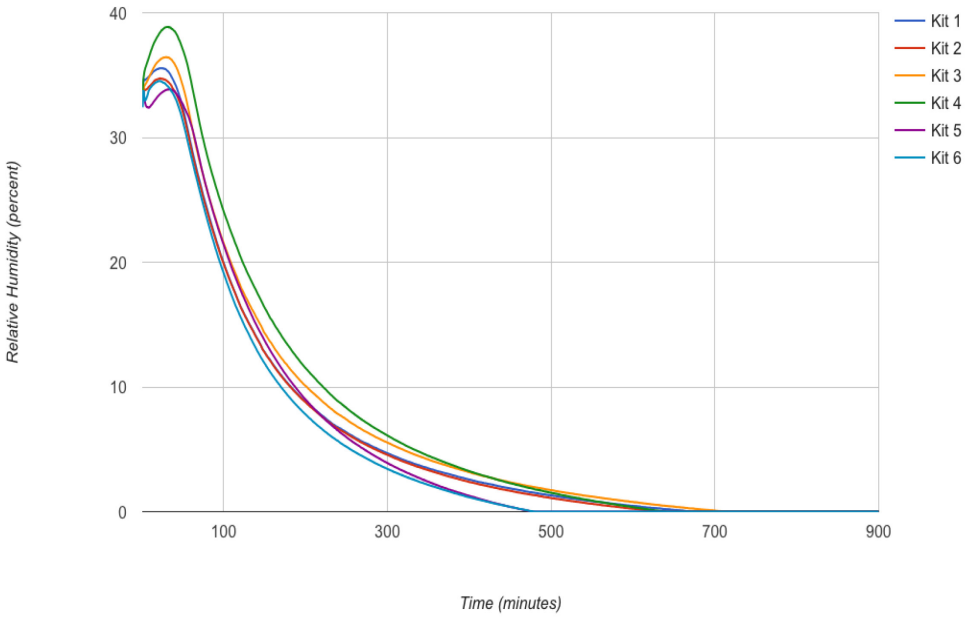


Figure 3-3. Internal moisture conditions for DBS kits during lab-based drying rate experiment 1.

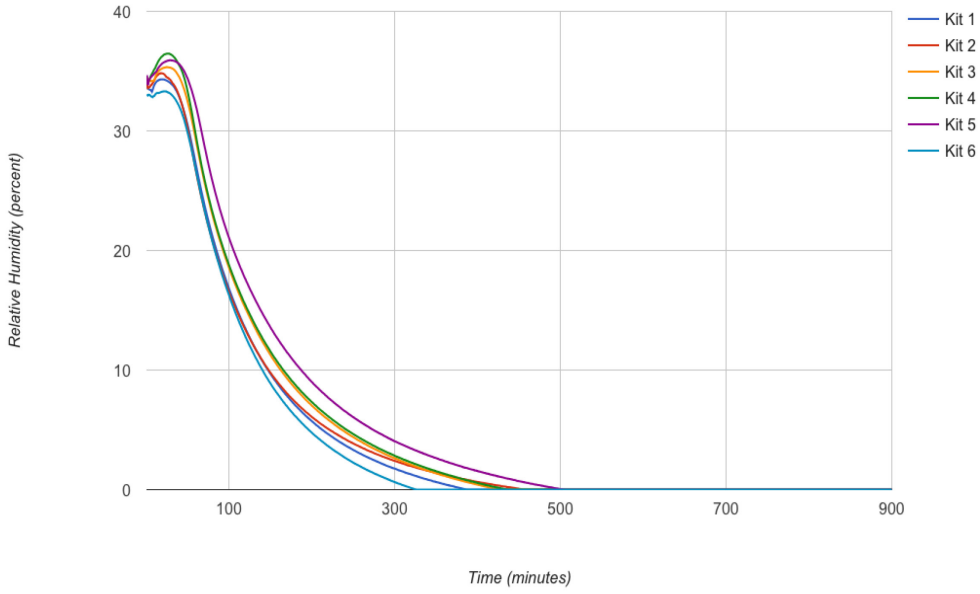


Figure 3-4. Internal moisture conditions for DBS kits during lab-based drying rate experiment 2.

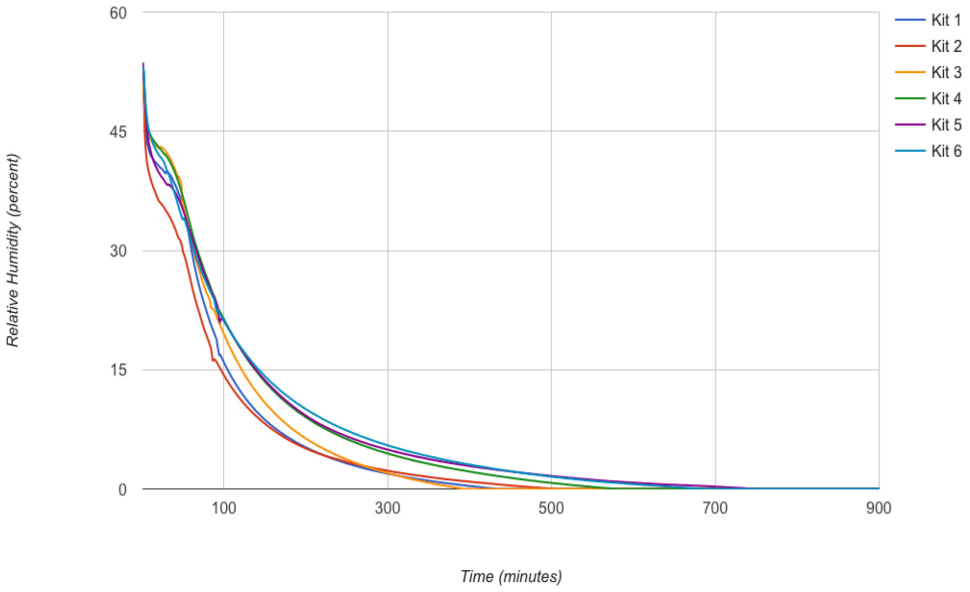


Figure 3-5. Internal moisture conditions for DBS kits during field simulation drying rate experiment in the Rainforest Exhibit of the National Aquarium (Baltimore, Maryland, USA).

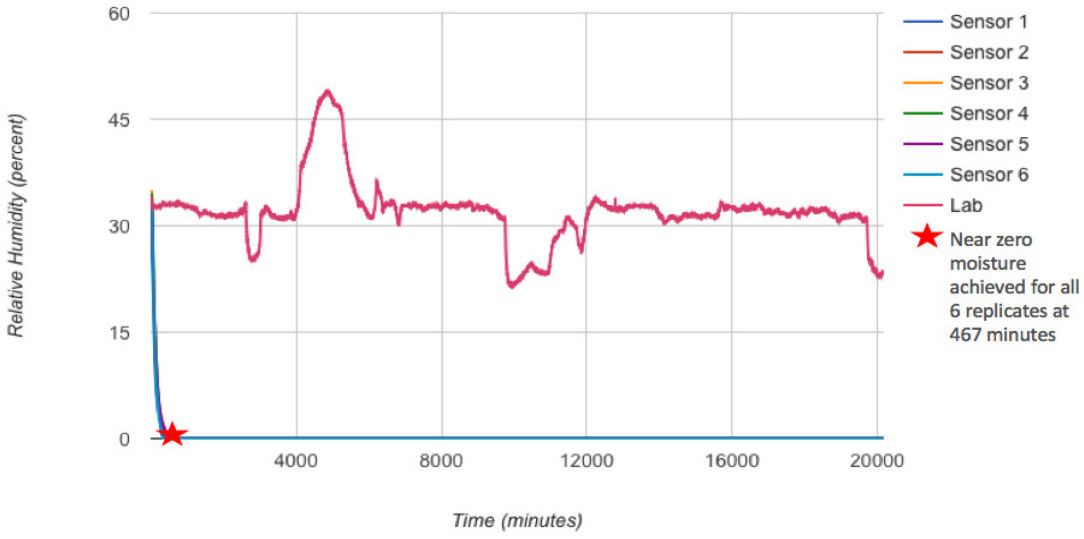


Figure 3-6. Internal moisture conditions for DBS kits during 14 day extended storage experiment.

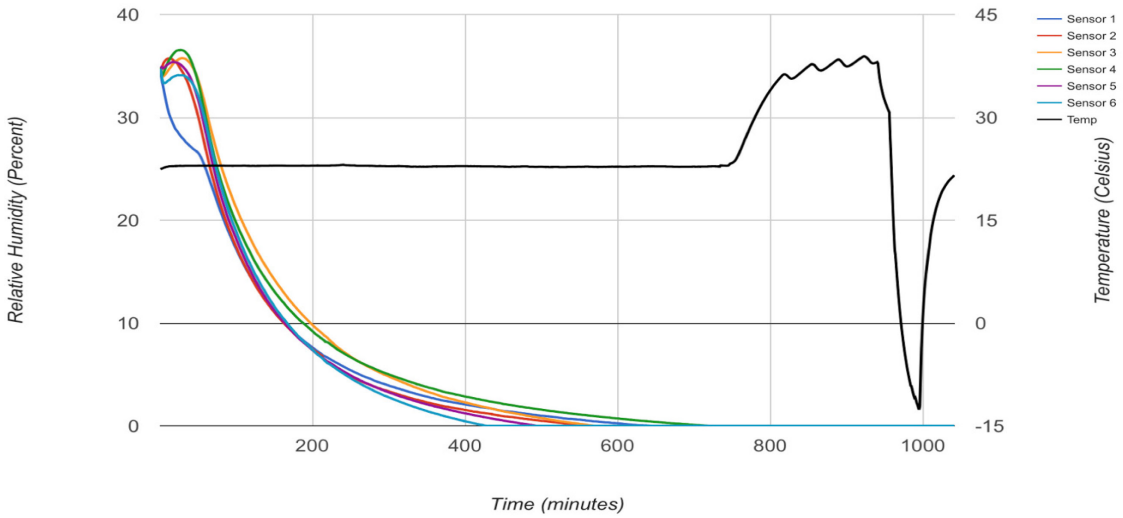


Figure 3-7. Internal moisture conditions for DBS kits during stress test experiment.

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CHAPTER FOUR

Manuscript 3

Improved Methods for Collection and Storage of Dried Blood Spots for RNA Detection and Quantification

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ABSTRACT

Background: The ability to collect and analyze biosamples in settings outside of the traditional clinic or lab environment is essential to basic research, public health, and clinical medicine. Unfortunately, collection of traditional samples such as liquid whole blood, plasma, and serum require phlebotomy and cold chain, neither of which may be available outside of the lab or clinic. Dried blood spots provide a minimally invasive alternative method for the collection of biosamples without the need for phlebotomy or cold chain. However, the current methods recommended by CDC for DBS collection require open-air drying of samples for a minimum of 3 hours, which may not be feasible in the field, and can lead to sample contamination. We have recently reported on novel methods in DBS collection that allow storage of DBS samples immediately after sampling, removing the requirement of open-air drying. This paper reports on the application of these methods to the measurement of gene transcripts in blood samples.

Objective: The objective of our study was to investigate the performance of our novel DBS methods with the current methods recommended by CDC, as well as the current gold standards for venous blood collection, on the detection and quantification of messenger RNA (mRNA) in DBS samples.

Methods: We used a validation of assay protocol for investigating the performance of our novel DBS collection methods on the detection and quantification of mRNA compared

with current DBS methods. We used quantitative RT-PCR (qRT-PCR) to assess abundance of 3 target gene transcripts (*GBP5, DUSP3, KLF2*) and 1 housekeeping gene transcript (*GAPDH*). Our study included samples from 16 individual donors. Samples included paired aliquots from each individual donor biosample prepared under novel and current methods, as well as a gold standard sampling method for mRNA analysis (PAXgene Blood RNA Tubes). Our analysis included percentage detection of mRNA above threshold levels for each sampling modality as well as analysis of transcript abundance measures by descriptive statistics (mean delta C_t values and coefficient of variation), correlation statistics and linear regression, and Bland-Altman analysis.

Results: Our data suggests our novel methods provide as good or an improvement in performance for mRNA detection and quantification compared with current methods. Specifically, we found similar performance in mRNA detection for our novel methods compared with current methods, and improved performance in mRNA quantification over current methods. We found no significant differences in mean delta C_t values for our novel methods compared with gold standard measurements for 2 of 3 transcripts, whereas mean delta C_t values for current methods were significantly different from gold standard for all 3 transcripts. We also found less variation in our novel methods compared with current methods for all 3 transcripts, suggesting our novel methods help reduce some of the technical noise associated with DBS sampling.

Conclusion: Our novel methods in DBS collection and storage demonstrated an overall improvement in the detection and quantification of mRNA from DBS. We recommend our novel methods for additional bioanalytical validation work and field testing.

BACKGROUND

Methods for the collection and use of biological specimens (biosamples) for biological markers (biomarkers) measurement are essential tools in public health and medicine. Biosamples are used routinely in basic research, as well as in public health practice for surveillance and population-based studies among other applications [1-6]. Biosamples are also critical tools in clinical medicine. For example, biomarkers are commonly used for characterizing health status, diagnosing disease, and therapeutic drug treatment monitoring [2, 7]. Traditional biosamples, such as venous whole blood, plasma, and serum, however, may pose signficant challenges in collection and storage outside of the clinic, hospital, or laboratory settings [8]. Venous blood sample collection requires a trained phlebotomist as well as refrigeration or freezing of blood or blood constituents from time of collection until time of analysis, i.e., sustaining a reliable cold chain. In many environments, especially in remote or austere settings such as the Sahel where humanitarian efforts are common, phlebotomy and/or cold chain may not be available nor financially or logistically feasible [8-9].

An alternative method to the collection of venous whole blood, plasma, and serum in nontraditional, remote, or austere settings is dried blood spot (DBS) sampling. DBS is a minimally invasive method for the collection of small quantities of whole blood from finger or heel stick with transfer to specially designed filter paper cards for storage and transport [10]. Historically, DBS has been used in newborn screening programs, and is the most commonly used type of dried microsample in research, public health, and medicine [11-12]. Among the advantages of DBS sampling methods, the ability to obtain a blood sample without a trained phlebotomist or cold chain has positioned DBS as a potential biosample matrix for use in non-traditional environments, and especially in remote and austere settings [8-9]. In recent years, as advancements in highly sensitive lab instrumentation and analytic software have emerged, interest in the use of DBS has increased, however, challenges in field collection remain an impediment to wider adoption [13-15]. Specifically, current recommendations in DBS methods require open-air drying for a minimum of 3 hours prior to storage and transport [16]. This requirement can be especially problematic in the field where space for drying may not be available, and prolonged open-air drying could allow for sample contamination from dust, insects, and other environmental exposures [17-20]. Furthermore, variable drying conditions can significantly alter biomarker measurements. This is particularly problematic for biomarkers that are susceptible to hydrolysis or other processes that utilize water for degradation as greater variability in drying times result in greater variability in biomarker

measurements [21-24]. Evidence-based improvements in field collection of DBS samples would greatly facilitate wider adoption of the methodology.

We recently developed novel methods for the field collection of DBS samples that control contamination and air drying [25]. These methods utilize small, opaque, air-tight kits with optimized amounts of molecular sieve desiccant for quickly drying DBS samples within a protected and closed environment. We reported faster drying times compared with open-air drying in similar environments, and an ability to reduce variability in drying conditions - i.e., the ability to consistently dry DBS samples in less than 90 minutes in low, moderate, or high humidity conditions. Additionally, the ability to store DBS samples quickly after collection removes the requirement of open-air drying, which is likely to reduce the chance for sample contamination.

In theory, faster drying rates in combination with less variation in drying conditions between sample collections should improve the precision of biomarker measurements from DBS samples, especially for biomarkers that are susceptible to degradation by hydrolysis or other processes utilizing water. Less variation in drying conditions alone is an incredibly important improvement as it can improve overall data quality. However, this hypothesis had not been tested for our novel DBS methods. Therefore, the objective of our study was to examine the performance of novel methods in DBS collection compared with current methods with respect to a particular analyte that is both broadly relevant to biomedical

research and clinical health monitoring, and subject to hydrolytic degradation in storage – messenger RNA (mRNA). We defined current methods as those presently recommended by the United States Centers for Disease Control and Prevention (CDC), which requires open-air drying for a minimum of 3 hours followed by storage in sealable, airtight, plastic bags with silica gel desiccant [16].

Our selection of mRNA as the biomarker for this study was based on two factors. First, mRNA is a particularly problematic biomarker in traditional biosamples due to the effect of RNAse, which is ubiquitous in the biosamples themselves, as well as in the environment, and quickly degrade mRNA [23, 26]. As RNAse requires water for its degradation processes, faster or less variable drying rates could result in improved performance for DBS compared with traditional samples [26-28]. Second, current gold standard methods in mRNA analysis often require use of vacutainers and RNA stabilizing agents (e.g. PAXgene RNA Blood Tubes, RNAlater, etc.) following by freezing the biosample, which increases the overall cost and technical requirements for field collection and storage of biosamples, which may not be feasible in some settings [29]. The ability to use novel DBS collection methods in the field for detection and quantification of mRNA in biosamples could remove many of the existing hurdles to DBS adoption. We hypothesize that our novel DBS collection methods will demonstrate an overall improvement in performance for the detection and quantification of mRNA from DBS samples compared with current methods.

METHODS

Study Context and Design

Study subjects were recruited and samples collected under informed consent at Johns Hopkins Medical Institutions in Baltimore, Maryland. Research protocols were approved by the Institutional Review Board (IRB) at the Johns Hopkins Bloomberg School of Public Health (IRB No: 00007621). A total of 18 subjects were enrolled prior to sample collection, and all biosamples were collected on a single day (24 March 2017). Samples from two subjects were removed from the study due to protocol deviations during biosampling. Sample size and the use of triplicate assay determinations (see Nucleic Acid Amplification and Determination) were based on best practices in the scientific literature and FDA guidance for bioanalytical method development [31-32]. The only inclusion criteria for subjects was that they be between the ages of 18 and 49 years. Our study design included a validation of assay protocol for comparing mRNA measurements between DBS methodologies (aka, sampling modalities) and liquid venous blood samples collected under gold standard laboratory methods using PAXgene Blood RNA Tubes (see Sample Collection and Preparation). We selected 3 gene transcripts associated with immune function as our target mRNA biomarkers (*GBP5, DUSP3, KLF2*), and 1 well-established housekeeping gene transcript for normalization of mRNA measurements (*GAPDH*) [33-36]. We selected our target transcripts based on commercial availability of probes for qRT-PCR and the requirement that they be constitutively expressed - i.e., we selected transcripts

that should be present at detectable levels in all study subjects irrespective of their individual health status or other factors.

Sample Collection and Preparation

A total volume of 30 mL venous blood was collected from each study subject by a trained phlebotomist with a standard venipuncture collection protocol at JHMI. The first 10 mL of blood were collected directly into a PAXgene RNA blood tube from BD Biosciences (Product No. 762165) containing anticoagulant and an RNA stabilizing agent. The remaining 20 mL were collected into a syringe containing citrate dextrose anticoagulant solution. PAXgene RNA blood tube samples, hereafter referred to as gold standard, served as our gold standard comparison for mRNA measurements. Gold standard samples for all study subjects were paired with matched DBS samples prepared under two different protocols. Both the PAXgene tubes and syringes were transported from the phlebotomy room to the lab (on the same floor as the phlebotomy room) for sample preparation and storage immediately following collection. PAXgene tubes were placed into a -20℃ freezer while the blood in the syringe, which contained citrate dextrose anticoagulant solution, was divided into four 5 mL conicals for DBS sample preparation. Four 30 uL drops of blood were spotted by micropipette onto Whatman 903 filter paper cards under ambient lab conditions (47-53% RH, 22-23℃).

DBS samples were prepared and stored under two different DBS protocols. DBS protocol 1, hereafter referred to as novel methods, included use of the novel methodologies we developed for enabling field collection of DBS samples. These methods include use of Whatman 903 filter paper cards from GE Healthcare Life Sciences (Product No. 10531018) with immediate storage after blood spotting into 644 mL aluminum, opaque, cylindrical containers with screw-on caps from Elemental Container (Product No. 0075152). Each kit contains an experimentally optimized 40 g of molecular sieve desiccant, as described in Chapter 3, from Multisorb Technologies (Product No. 02-00041AG19) for the purpose of quickly drying freshly spotted DBS samples within a closed system.

DBS protocol 2, hereafter referred to as current methods, is the current protocol for DBS collection and storage as recommended by CDC [16]. The current methods include use of Whatman 903 filter paper cards, which are open-air dried on a rack for a minimum of 3 hours prior to storage. Once dry, DBS cards are placed inside glassine envelopes and plastic bags along with humidity indicator cards, and silica gel desiccants for maintaining a low-moisture environment during storage and transport. Our study used Whatman glassine envelopes (Product No. 28417400), Whatman plastic bags (Product No. 28417398), Humonitor humidity indicator cards (Product No. 2291DG03), and silica gel desiccant packs (Product No. 02-00040AG45). We procured all DBS materials for current methods from Sigma-Aldrich. After 24 hours following spotting, we prepared samples for shipment to the UCLA Social Genomics Core in Los Angeles, California for mRNA extraction and

analysis. PAXgene tubes were shipped on dry ice, while DBS samples collected under both novel and current methods were shipped overnight under ambient conditions (not recorded). After receipt at UCLA, PAXgene tubes were stored in a -20℃ freezer and DBS samples stored under ambient lab conditions (not recorded) for two weeks until mRNA extraction and assay.

Nucleic Acid Extraction

Total mRNA was extracted from PAXgene Blood RNA Tube samples using an automated nucleic acid processing system (Qiagen QIAcube) following a standard protocol derived from the PAXgene Blood RNA Kit Handbook from Qiagen (UCLA Social Genomics Core Laboratory SOP-45, Appendix A, Supplementary Materials) [50]. Total mRNA was extracted from DBS samples by suspending sheared DBS filter papers for 30 min in Qiagen RLT buffer (at 37°C with agitation) followed by extraction using an automated nucleic acid processing system (Qiagen QIAcube) following a standard protocol derived from the RNeasy® Micro Handbook from Qiagen and modified per manufacturer's instructions for DBS processing (UCLA Social Genomics Core Laboratory SOP-44, Appendix B, Supplementary Materials) [51].

Nucleic Acid Amplification and Determination

mRNA samples were assayed using standard qRT-PCR protocols implemented on a Bio-Rad iQ5 real-time PCR system using reverse transcriptase and polymerase chain reaction

enzymes and buffers appropriate for fluorescent probe-based detection (Qiagen QuantiTect® Probe PCR Kit) and standard commercially available primer/probe systems (Applied Biosystems TaqMan® Gene Expression Assays Hs00369472_m1, Hs01115776_m1, Hs00360439_g1, and Hs02786624_g1). Reverse transcription and PCR thermal cycling protocols followed the assay manufacturer's specified time/temperature profiles. Procedures for mRNA amplification and quantitation followed a UCLA protocol adapted from the QuantiTect® Probe PCR Handbook from Qiagen (UCLA SOP-24, Appendix C, Supplementary Materials) [52]. All measurements were conducted in triplicate with median C_t values reported as final mRNA measurements for each of the gene transcripts. C_t values were then normalized to delta C_t values for statistical analysis. Delta C_t values were calculated for each of the 3 target gene transcripts by subtracting the corresponding C_t values of the housekeeping transcript (*GAPDH*), for the same sample and analytical run, from the target transcript. Due to our study design, Delta Delta C_t values were not considered for analysis. Delta Delta C_t calculations require an assumption of equal amplification efficiency, which could not be assumed due to our use of different collection modalities, which was required for testing our study hypothesis.

Statistical Analysis

We evaluated performance of both DBS methods against each other as well as against the gold standard. Detection performance was evaluated by percentage of samples detected above threshold levels, defined here as the percentage of samples within a sampling

modality that yielded detectable mRNA measurements above an auto-calculated threshold level (i.e., we used the Bio-Rad iQ5 PCR instrument's default algorithm for identifying a valid detection threshold above background). These measurements enabled us to examine the extent to which our novel methods impacted the ability to detect mRNA from DBS samples. Quantitative performance was evaluated by descriptive statistics for each sampling modality (mean Delta C_t values and corresponding coefficient of variation), correlation and linear regression, Bland-Altman analysis, and Wilcoxon matched-pairs signed rank tests [37-39]. We used mean delta C_t and corresponding CV values in order to examine how our methods impact variability of mRNA measurements from DBS. We used correlation and linear regression to examine the degree to which our methods effect comparability of DBS measurements with gold standard. We used Bland-Altman analysis to examine the respective bias of our novel methods compared with gold standard in contrast to the current methods compared with gold standard. Finally, we used Wilcoxon matched-pairs signed rank tests to determine if mRNA measurements derived from our methods were significantly different current methods as well as from the gold standard. Significance levels for comparative analyses were set at α= 0.05. All analyses were conducted with statistical software program, GraphPad Prism 7.0b.

RESULTS

Results are presented for mRNA measurements for 3 target gene transcripts (*GBP5, DUSP3, KLF2*), and 1 housekeeping transcript (*GAPDH*). mRNA measurements were

taken from blood samples from 16 subjects for two sets of matched DBS samples collected under our novel and current methods paired with PAXgene Blood RNA Tubes as gold standard. No personal identifying information of any kind was collected nor reported on study subjects.

Percentage Detection Above Threshold

To assess sampling modality performance on mRNA detection, the percent of samples achieving detectable mRNA above threshold levels were reported for all 16 samples collected under each of the 3 sampling modalities (Table 4-1). 100% of samples collected under gold standard methods yielded detectable mRNA levels for all 3 target gene transcripts as well as for the housekeeping transcript. As expected, gold standard methods outperformed both DBS sampling modalities. Findings suggest our novel methods had comparable performance in detection with current methods. Specifically, 100% of samples collected under novel methods versus 93.8% for current methods yielded detectable levels of *GBP5*. 81.3% yielded detectable levels of *DUSP3* for both novel and current methods. 81.3% for novel methods versus 93.8% for current methods yielded detectable levels of *KLF2*. Lastly, 93.8% of samples collected by both novel and current methods yielded detectable levels of mRNA for *GAPDH* (housekeeper).

Mean Delta C_t Values and Coefficient of Variation

Mean delta C_t values and corresponding coefficients of variation (CV) were reported for comparing quantitative performance. Mean delta C_t values for novel methods were closer to gold standard measurements for 2 of 3 transcripts, and CVs for all 3 transcripts were less dispersed than were values from samples collected under current methods (Table 4-2). Specifically, *GBP5* (n=14) mean delta C_t values were reported as 1.64 (CV=62.9%), 1.14 (CV=137.2%), and 0.62 (CV=241.2%) for gold standard, novel methods, and current methods respectively. Mean delta C_t values for *DUSP3* (n=8) were reported as 3.12 (15.6%), 2.82 (45.2%), and 2.11 (66.0%) for gold standard, novel, and current methods respectively. Mean delta C_t values for *KLF2* (n=12) were reported as 1.20 (CV=38.3%), 2.14 (41.9%), and 1.41 (48.0%) for gold standard, novel, and current methods respectively.

Wilcoxon Matched-Pairs Signed Rank Tests

Wilcoxon matched-pairs signed rank tests were also used to compare matched delta C_t values for each of the 3 target gene transcripts for novel vs. gold standard, current vs. gold standard, and novel vs. current methods (Table 4-3). Delta C_t values for novel methods were not significantly different from gold standard for *GBP5* (p=0.1205, n=15) or *DUSP3* (p=0.2810, n=13), whereas delta C_t values for current methods were significantly different for both *GBP5* (**p=0.0045**, n=15), and *DUSP3* (**p=0.0339**, n=13). Delta C_t values for novel and current methods were both significantly different than gold standard for *KLF2*

(**p=0.0005**, n=13; **p=0.0026**, n=15); however, they were not significantly different than each other (p=0.3013, n=12).

Correlation Statistics and Linear Regression

Correlation statistics are reported for novel and current methods compared with gold standard (Table 4) as well as compared with each other (Table 5). Linear regression plots are reported for novel and current methods compared with gold standard (Figure 1). Findings suggest novel methods had a neutral effect on quantification of mRNA compared with current methods as it pertains to correlation with gold standard. Specifically, both novel and current methods yielded significant positive correlations with gold standard for *GBP5* (r=0.66, **p=0.0070**; r=0.60, **p=0.0178**), whereas neither method yielded significant correlation with gold standard for *DUSP3* or *KLF2*. Novel and current methods were also significant positively correlated with each other for *GBP5* (r=0.7442, **p=0.0023**), but not for *DUSP3* or *KLF2* (r=0.499, p=0.1182; r=-0.5076, p=0.920).

Inspection of linear regression plots (Figure 1) and CV values (Table 2) suggests that the relatively poor correlation of DBS-derived results with gold standard results for *DUSP3* and *KLF2* may stem in part from the relatively restricted range of underlying biological variation for these two transcripts relative to *GBP5*. Regression analysis shows clustering of values within a limited range of biological variation for both *DUSP3* and *KLF2* (both

~4-fold range of variation) whereas *GBP5* showed substantially greater variation across participants (~16-fold range). Note that these differences do not represent any decrement in assay precision for *DUSP3* and *KLF2* as replicate determinations actually showed lower CV values for these two analytes than did *GBP5*.

Bland-Altman Analysis

Findings from Bland-Altman analysis suggest novel methods had an overall neutral to positive effect on mRNA quantification as estimated biases for novel methods were smaller than for current methods in absolute terms (and comparable in confidence interval length) for 2 of 3 transcripts (*GBP5* and *DUSP3*) (Table 6). Specifically, novel methods yielded biases of -0.4827 (-2.717 to 1.752) for *GBP5*, -0.3923 (-2.565 to 1.78) for *DUSP3*, and 1.982 (-1.142 to 5.107) for *KLF2*. These values compare favorably with current methods' bias statistics of -0.9927 (-3.267 to 1.282), -0.7115 (-3.026 to 1.603), and 1.121 (-1.151 to 3.392), respectively. Bland-Altman plots show similar distribution of biases for both DBS methods. Specifically, bias for novel and current methods cluster around zero for both *GBP5* and *DUSP3*, with greater bias detected at lower delta C_t values. In contrast, bias for novel and current methods cluster above zero for *KLF2*, with greater detected bias at higher delta C_t values.

DISCUSSION

These findings suggest that our novel methods in DBS collection and storage had a neutral effect on performance for detection and a positive effect on the quantification of mRNA by RT-PCR when compared with current DBS methods recommended by CDC. As previously noted, even a neutral effect on assay results would be valuable as our novel methods eliminate one of the biggest impediments to wider adoption of DBS sampling in the field, specifically, the requirement for extended open-air drying. By enabling immediate storage after sample collection, DBS can be used in a range of complex environmental settings, including tropical climates, remote or austere environments, and occupational settings, to name a few. In each of these environments, the technical requirement for extended (3h) open-air drying may often be infeasible and could thus prohibit use. Moreover, the elimination of extended open-air drying also substantially reduces the chance of sample contamination. The present findings provide an opportunity to significantly broaden the array of fields in which microsampling is employed, and may enable wider adoption of DBS sampling in non-traditional settings such as remote, austere, and occupational environments.

Though improvements in quantification over current DBS methods were modest, and differences between novel DBS and gold standard methods clearly remain, it should be noted that our study design worked against detecting any material advantages for the novel DBS approach because the laboratory setting employed here lacked many of the ecological

challenges that complicate analysis of field-collected biosamples. For example, this experiment was conducted in a lab-based environment under conditions of moderate humidity (limiting the hydrolytic advantage of immediate storage relative to extended drying times). This experiment also compared novel DBS methods not just against current DBS methods but also with gold standard venipuncture sampling. The gold standard methodology benefited from a substantially greater sample volume collected directly into a vacutainer with a stabilizing agent specific for RNA. The gold standard method also benefited from its requirement to freeze samples shortly after collection, whereas both novel and current-method DBS samples were stored under ambient conditions for 2 weeks prior to RNA extraction and analysis. Despite the technical advantages for the gold standard sampling method (which would not be feasible in many field settings), novel and standard DBS assays showed reasonable quantitative correspondence with gold standard results, particularly for the transcript that showed the widest range of intrinsic biological variation (i.e., *GBP5*, which showed ~4 times greater magnitude inter-individual variation than did other assayed transcripts).

The primary advantages of our novel DBS methods over current DBS methods are their ability to dry samples more quickly, and thereby remove some of the variation in drying conditions, while also minimizing potential sample contamination. Each of these advantages was reduced in the present laboratory setting. However, under the more challenging and variable conditions of field collection, these advantages should in principle

reduce the technical variation or noise associated with current DBS collection and storage methods, and thereby increase the assay signal-to-noise ratio by providing a more stable environment. It is reasonable to expect that the observed advantage of novel DBS methods would be more evident in high-humidity field environments where drying conditions would be more variable and humidity more deleterious to RNA integrity [8, 40-42]. Follow-on studies with field application of novel methods may help clarify the analytic impact of these advantages over current methods.

Performance in mRNA detection suggests that novel DBS methods provide a suitable sampling modality when qualitative detection alone is the priority. There are a wide range of nucleic acid-based amplification tests available for infectious disease diagnostics, many of which are most prevalent in tropical or austere environments where traditional sampling modalities are more problematic [43]. Use of novel methods could also allow for substantial improvements in the quality and availability of infectious disease diagnostics in remote or other vulnerable populations where access to basic diagnostic services remains constrained [8-9, 17, 43].

Performance improvements in the quantification of mRNA abundance were less convincing for the novel DBS methods, particularly for 2 of the 3 target gene transcripts (i.e., *DUSP3* and *KLF2*). These 2 transcripts are notable in showing substantially less "true" biological variation across study participants than did *GBP5*, which showed more

impressive correlation in quantitative estimates across sampling modalities. The relatively poor performance of DBS methods in quantification of *DUSP3* and *KLF2* mRNA did not stem from poorer assay performance, as replicate determination CV values actually showed superior performance for these two assays. Instead, visual inspection of linear regression scatter plots showed substantial less dispersion in the magnitude of true individual differences in average mRNA abundance. Correlations are essentially a ratio of "true" variation across individuals relative to "noise" variation stemming from sampling variability and/or measurement (assay) error. Holding constant the technical accuracy of an assay, as the range of true biologic variation in the sampled observations goes down ("range restriction"), the correlation between sampling modalities will be reduced, as was seen here for *DUSP3* and *KLF2*. The cause of the differences in biological variation between gene transcripts is less understood. However, *GBP5* is known to track innate antiviral responses, so it is possible that the relatively large variation in average expression of this transcript may stem from substantial variation in the number and activity of subclinical viral infections [33, 44-45]. *KLF2* and *DUSP3* transcripts may be less sensitive to the same kinds of common latent viral infections and thus show relatively less true variation in the generally healthy sample examined here.

We believe these findings bode well for the potential field application of novel DBS methods for two related reasons. First, as noted above, the quantitative performance of novel DBS methods will likely benefit from (or more accurately, suffer less from) the

greater technical challenges of complex environments. The primary benefit of novel DBS methods over current DBS methods is removal of the extended open-air drying requirement. This should help reduce technical variation in field settings as novel DBS methods should be less impacted by contamination and/or variable desiccation rates than are current DBS methods. Second, it is important to note that DBS methods will inherently be noisier than gold standard venipuncture methods due to the reduced biosample volume available. However, comparison of DBS accuracy with gold standard venipuncture sample accuracy is not the appropriate conceptual frame from which to consider wider adoption of DBS. In complex occupational and environmental settings, and especially in austere environments, biosampling is often not conducted at all due in large part to the technical and logistical infeasibility of venipuncture as well as costs associated with the immediate processing and storage of those samples [8, 46-47]. The appropriate reference point for assessing the relative value of DBS sampling is, therefore, not the more accurate measurements theoretically available from gold standard, but rather measurements from novel DBS methods compared with no measurements at all (i.e., when no gold standard measurement is feasible).

Our study design had several limitations. First, as a proof-of-concept study, our sample size was relatively small compared with full bioanalytical validation studies, which would likely involve a minimum of 40 subjects per best practices in the scientific literature [31, 38]. Second, as previously discussed, we conducted our study in a lab-based environment

under conditions of moderate humidity, which limited our ability to detect larger differences between novel and current methods that might have been detected under more variable conditions in the field. Lastly, we prepared DBS samples by precise application of venous blood to filter paper cards by micropipette, whereas the more common application of DBS, especially in field settings, would come from capillary blood by finger stick with direct application to filter paper cards. Though measurements from capillary blood are often found to be highly correlated with venous blood, the additional variability associated with the sampling method could introduce bias [48-49]. For our purposes, however, we chose to prevent introduction of bias from finger stick application by use of micropipette application in order to more accurately assess the differences between collection and storage protocols for novel and current methods (i.e., holding constant the blood source). This approach was justified by the fact that the present study aimed to measure the variation associated with collection, storage, and assay protocols per se, rather than the additional biological variation associated with capillary versus venous blood, which would apply to both modalities in the field.

Given our study findings, we suggest the following three areas as immediate priorities for future research. First, we recommend a full bioanalytical validation of novel methods compared with current methods, as well as gold standard methods, not just for mRNA measurements, but also for other categories of biomarkers, such as genes (DNA polymorphisms), metabolites, lipids, and other markers of basic research, public health,

and clinical relevance. Second, we recommend follow-on studies with field applications to determine if novel methods will demonstrate improved performance over current methods in the field (particularly in the presence of clinically meaningful variations in health status, which were not present here). Third, as our novel methods were originally optimized for maximizing drying rate, irrespective of the target biomarker, we would suggest additional exploration into further optimization of novel methods that are based on analytic categories rather than maximal drying rates alone. It is possible, for instance, that some analytes may benefit from drying rates optimized for analyte stability rather than speed of cell lysis or desiccation.

Conclusion

Our findings suggest our novel DBS methods had an overall neutral to positive effect on performance for detection and quantification of mRNA from DBS samples. We recommend full bioanalytic validation, and field testing of novel methods for mRNA and other biomarkers of basic research, public health, and clinical relevance.

TABLES AND FIGURES

Table 4-1. Percentage of samples achieving detectable RNA above threshold levels.

	RNA Detection		
Gene Transcript	*Gold Standard (PAXgene Tube)*	*Novel Methods*	*Current Methods*
GBP5 (n=16)	100%	100%	93.8%
DUSP3 (n=16)	100%	81.3%	81.3%
KLF2 (n=16)	100%	81.3%	93.8%
GAPDH, housekeeper (n=16)	100%	93.8%	93.8%

Table 4-2. Descriptive statistics for mRNA measurements.

	Mean Delta C_t Values + Coefficient of Variation		
Gene Transcript	*Gold Standard (PAXgene)*	*Novel Methods*	*Current Methods*
GBP5 (n=14)	1.64 + 62.9%	1.14 + 137.2%	0.62 + 241.2%
DUSP3 (n=8)	3.12 + 15.6%	2.82 + 45.2%	2.11 + 66.0%
KLF2 (n=12)	1.20 + 38.3%	2.14 + 41.9%	1.41 + 48.0%

Table 4-3. Wilcoxon matched-pairs signed rank tests for comparing mRNA measurements between sampling modalities.

	P Values (significance level set at P<0.05), Number of Pairs		
Gene Transcript	*Novel vs. Gold Standard (PAXgene)*	*Current vs. Gold Standard (PAXgene)*	*Novel vs. Current*
GBP5	0.1205, n=15	**0.0045**, n=15	0.1575, n=14
DUSP3	0.2810, n=13	**0.0339**, n=13	0.1475, n=11
KLF2	**0.0005**, n=13	**0.0026**, n=15	0.3013, n=12

Table 4-4. Correlation statistics for mRNA measurements of novel and current DBS methods compared with Gold Standard (PAXgene).

Protocol/Transcript	Pearson r	95% Confidence Limits	R squared	P value
Novel/GBP5 (n=15)	0.66	0.23 to 0.88	0.44	**0.0070**
Current/GBP5 (n=15)	0.60	0.13 to 0.85	0.36	**0.0178**
Novel/DUSP3 (n=13)	0.16	-0.43 to 0.65	0.02	0.6132
Current/DUSP3 (n=13)	0.33	-0.28 to 0.74	0.11	0.2804
Novel/KLF2 (n=13)	-0.14	-0.64 to 0.45	0.02	0.6583
Current/KLF2 (n=15)	0.10	-0.44 to 0.58	0.01	0.7343

Table 4-5. Correlation statistics for mRNA measurements from novel DBS methods compared with current methods.

Gene Transcript	Pearson r	95% Confidence Limits	R squared	P value
GBP5 (n=14)	0.7442	0.353 to 0.9139	0.5539	**0.0023**
Novel/DUSP3 (n=11)	0.499	-0.144 to 0.8457	0.249	0.1182
Novel/KLF2 (n=12)	-0.5076	-0.8375 to 0.0935	0.2577	0.0920

Table 4-6. Detected bias (Bland-Altman) for mRNA measurements from novel and current DBS methods compared with Gold Standard (PAXgene).

Protocol/Transcript	Bias	95% Confidence Limits
Novel/GBP5 (n=15)	-0.4827	-2.717 to 1.752
Current/GBP5 (n=15)	-0.9927	-3.267 to 1.282
Novel/DUSP3 (n=13)	-0.3923	-2.565 to 1.78
Current/DUSP3 (n=13)	-0.7115	-3.026 to 1.603
Novel/KLF2 (n=13)	1.982	-1.142 to 5.107
Current/KLF2 (n=15)	1.121	-1.151 to 3.392

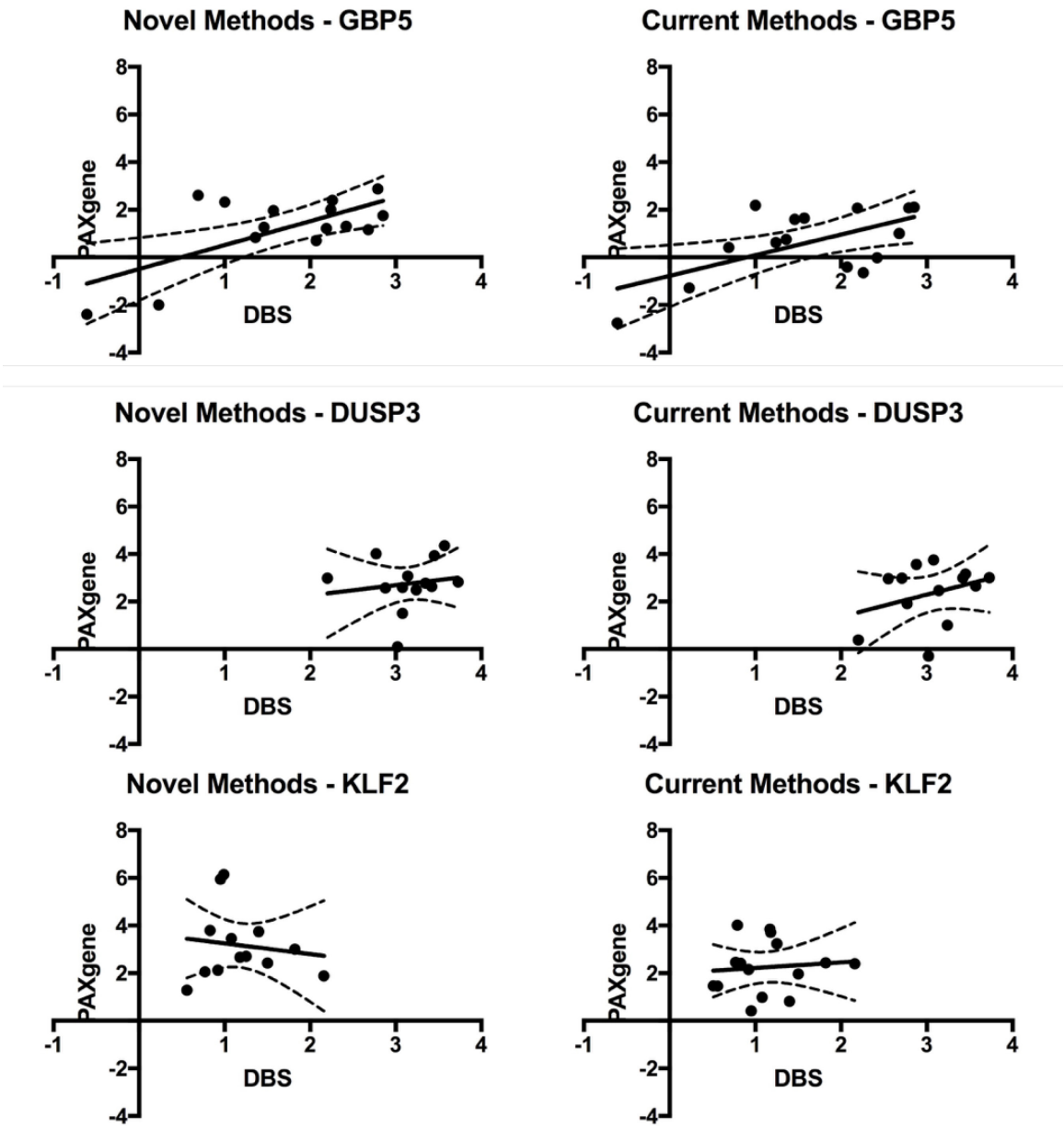

Figure 4-1. Regression analyses for mRNA measurements in novel and current DBS methods compared with Gold Standard (PAXgene).

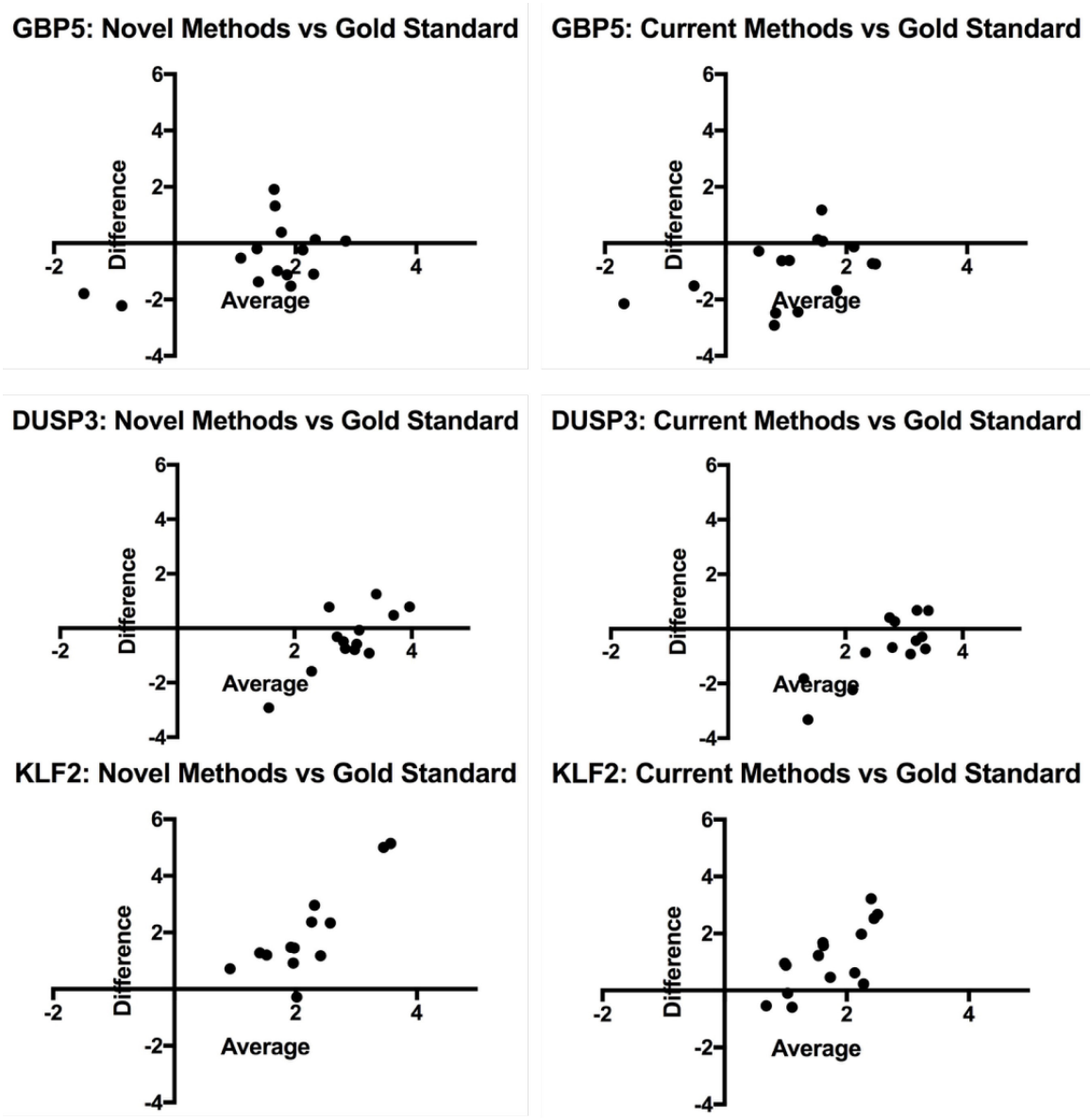

Figure 4-2. Bland-Altman Analyses for mRNA measurements comparing DBS samples to Gold Standard (PAXgene).

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CHAPTER FIVE

Conclusion

SUMMARY FINDINGS

Aim 1 – State of the Science in Dried Blood Spots

In our attempts to systematically characterize the current state of the science for dried blood spots, we identified in the scientific literature nearly 2,000 unique analytes (n=1,947) measured by one of more than 150 different analytic methods (n=169). In our examination of the strengths, weaknesses, opportunities, and threats associated with potential adoption of DBS, we noted that the strengths of the sampling method, and especially the removal of the need for phlebotomy and cold chain, make DBS a valuable tool for potential application in the field. Problems with analyte quantification due to small sample volume, issues of blood hematocrit, and the requirement for highly sensitive analytic instrumentation have largely been obviated by recent advancements in the quality and availability of such technologies. While issues of differential degradation of some classes of analytes, and the logistical challenges, such as open-air drying, of the current DBS collection protocol remain, we believe these constraints will largely be resolved by continued methodological improvements and the fast pace of technological advancement.

Aim 2 – Improved Methods for Field Collection and Storage of Dried Blood Spots

In our efforts to facilitate the adoption of DBS sampling for complex occupational and environmental settings, we sought to modify the current collection protocol by designing novel methods for DBS collection that remove the need for open-air drying of samples. In doing so, we hypothesized that our novel methods would dry DBS spots in our closed-

system kits in less than 90 minutes in conditions of low to moderate and high humidity. Our findings suggest that our hypothesis was correct as our novel methods demonstrated average drying times in conditions of moderate and high humidity of approximately 30% and 50% faster respectively than open-air drying times under similar conditions reported in the literature. These findings suggest our novel methods could be used in a range of complex occupational and environmental settings, and should provide a benefit in field collection by removal of the need for open-air drying, prevention of possible sample contamination, and potential overall improvements in data quality. For example, in humanitarian settings in the Sahel where surrounding environments can pose contamination risks and high humidity conditions are common, the technical noise associated with collection of DBS can be problematic; however, our methods should minimize this noise and allow for more accurate measurements in the field.

Aim 3 – Improved Methods in the Collection and Storage of Dried Blood Spots for RNA Detection and Quantification

In our efforts to demonstrate improvements in data quality associated with our novel DBS collection methods, we sought to conduct a validation of assay protocol for comparing our novel DBS collection methods with the current methods recommended by CDC. We hypothesized that our novel methods would demonstrate an overall improvement in performance for RNA detection and quantification from DBS samples compared with current methods. Our findings suggest that our hypothesis was correct. Our novel methods

demonstrated a comparable performance for detection, and an improved performance on the quantification of RNA from DBS samples. Furthermore, our findings suggest that our novel methods will have an even more pronounced improvement when applied in the field, as they will likely yield greater benefit from increased biological variation in sample populations, due to minimizing increases in technical and environmental noise often associated with field collection of biosamples.

FUTURE RESEARCH, PUBLIC HEALTH IMPLICATIONS, AND CONCLUDING REMARKS

Future Research

We have identified three priority areas for future research, each associated with one of the specific aims from this dissertation. First, associated with aim 3 (State of the Science in Dried Blood Spots), we believe there is a need for a follow-on review study that attempts to characterizes the quality of validation studies identified from our comprehensive analyte database. Such a review will need to incorporate assessment criteria not just for bioanalytical validation studies of traditional biosamples, but also for criteria specific to DBS, which go above and beyond the parameters required for traditional samples. For example, blood hematocrit and spot-to-spot variance should be considered with DBS [1-2].

Second, associated with aim 2 (Improved Methods for Field Collection and Storage of Dried Blood Spots), we believe there is a need for a more comprehensive approach to

optimization of our novel methods whereby inclusion of additional evaluation parameters specific to problematic classes of analytes might be considered. For example, some analytes in DBS samples are degraded due to oxidative processes resulting from exposure to atmospheric oxygen while drying [3-7]. While our novel methods may reduce overall oxygen exposure compared with open-air drying, they do not completely remove oxygen exposure. Modification to our methods might be considered for resolving the issue of oxygen exposure. Additionally, our novel methods were developed to maximize the rate of drying, however, it may be the case that some classes of analytes demonstrate greater stability when dried at slower rates. This would need to be investigated.

Third, associated with aim 3 (Improved Methods in the Collection and Storage of Dried Blood Spots for RNA Detection and Quantification), we believe our findings provide sufficient evidence to justify a full bioanalytic validation of our novel methods as well as immediate adoption by researchers in field settings where the alternative to data collected from samples under our novel methods is no data at all, which is often the case in complex occupational and environmentally challenging settings [8-9].

Public Health Implications

Review findings for our State of the Science in DBS manuscript documented the extent to which DBS could inform epidemiological and biomedical research, and demonstrated a wide range of current and potential applications in public health and clinical medicine.

Experimental findings for our novel methods in papers 2 and 3 could further expand the potential range of applications, particularly for those purposes for which DBS are already in use, such as newborn screening and population-based sampling in austere environments, as well as in settings where biosampling has been historically constrained due to either cost or feasibility, such as complex occupational and environmentally challenging settings. Where DBS is already in place, our novel methods should help reduce technical variation associated with the current sampling methodologies, which could significantly improve data quality. Where biosampling has historically been constrained due to cost or feasibility, we believe our novel methods provide a low cost and technically feasible option for adoption of biosampling in settings where no sampling, and consequently, no data are available. Should our methods be adopted in these areas, DBS can provide a valuable tool for assessing health and disease status, occupational and environmental exposures, and conducting both retrospective and longitudinal data collection for research and health surveillance purposes. Furthermore, our findings justify a full bioanalytical validation of our novel methods, which could pave the way for their integration into existing health systems as a clinical resource for enabling home-sampling, generally, and sampling in remote or otherwise underserved populations, more specifically. Adoption in these areas could substantially expand access to and utilization of essential health services.

Concluding Remarks

It is clear from our findings that DBS provide researchers and practitioners with a wide-ranging tool for a variety of potential applications in complex occupational and environmental settings. Though remaining challenges are substantial, they are not intractable, and the recent history of DBS use seems to suggest that the real question surrounding the enduring issues is not "if" they will be resolved, but "when". As this dissertation has demonstrated, even some of the longest held technical challenges, such the hematocrit effect, or in the case of this dissertation, the requirement for open-air drying, can be resolved simply and with existing or emerging technologies. Recent trends may also play a role in the eventual routine adoption of DBS. As current economic and social pressures continue to shift the provision of health services away from the traditional clinical or laboratory settings, and as researchers and practitioners continue to extend the reach of biosampling to remote, underserved, or otherwise vulnerable populations, DBS will likely play an increasingly important role.

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APPENDICES

APPENDIX 2-A - SEARCH STRATEGIES

Preliminary Search - PubMed (~9,041)

Date run: 9/3/15 (9,238 hits – only 9,237 imported. Tested twice – must be a duplicate)

"Dried Blood Spot Testing"[Mesh] OR blood spot*[tw] OR bloodspot*[tw] OR "dried blood"[tw] OR Guthrie[tw] OR "blood sampling paper"[tw] OR "filter paper blotter"[tw] OR "filter paper disk"[tw] OR "dried filter paper"[tw] OR PKU card*[tw] OR Blood Collection Card*[tw] OR ((Filter paper*[tw] OR filter card*[tw] OR filter plate*[tw]) AND (blood*[tw] OR "Blood"[Mesh] OR "blood" [Subheading])) OR (("Paper"[Mesh]) AND ("Blood Specimen Collection"[Mesh] OR "Blood Chemical Analysis"[Mesh] OR "Tandem Mass Spectrometry"[Mesh] OR "Biological Markers/blood"[Mesh] OR "Blood Preservation"[Mesh])) OR ("Blood Specimen Collection"[Mesh] AND dried[tw]) OR "Blood Stains"[Mesh] OR bloodstain*[tw] OR blood stain*[tw] OR (DBS[tw] AND ("blood"[Subheading] OR "blood"[All Fields] OR "blood"[MeSH Terms] OR "mass screening"[MeSH Terms] OR "screening"[tw]))

Final Search Strategy - Review of Reviews/Validation Studies/Evaluation Studies

Date: 11/16/15, 1,138 hits

Reviews – filter

Systematic Reviews - filter

"Validation Studies as Topic"[Mesh] OR "Validation Studies" [Publication Type] OR "Evaluation Studies" [Publication Type] OR validation[tw]

****

****

Embase (~12,194)

Date run: 12,520 hits

'dried blood spot testing'/exp OR 'blood stain'/exp

((blood NEXT/1 spot*) OR bloodspot* OR "dried blood" OR Guthrie OR "blood sampling paper" OR "filter paper blotter" OR "filter paper disk" OR "dried filter paper" OR (PKU NEXT/1 card*) OR ("Blood Collection" NEXT/1 Card*) OR bloodstain* OR (blood NEXT/1 stain*)):ti,ab

((Filter NEXT/1 paper*) OR (filter NEXT/1 card*) OR (filter NEXT/1 plate*)):ti,ab

AND (blood*:ti,ab OR 'blood'/exp)

('paper'/exp) AND ('blood sampling'/exp OR 'blood specimen collection kit'/exp OR 'blood analysis'/exp OR 'tandem mass spectrometry'/exp OR 'blood storage'/exp)

(('blood sampling'/exp OR 'blood specimen collection kit'/exp) AND dried:ti,ab)

DBS:ti,ab AND ('blood'/exp OR blood:ti,ab OR 'screening'/exp OR screening:ti,ab)

****

Review of Reviews/Validation Studies/Evaluation Studies

Date: 11/16/15, 1,063 hits

Filters – Review, Systematic Reviews, Meta-Analysis

'validation study'/exp OR 'evaluation study'/exp OR validation:ti,ab

****

****

Toxline

Date run: 9/3/15. 1,048 without PubMed. (2,974 with all results)

All combined:

"blood spot" OR "blood spots" OR "blood spotted" OR bloodspot* OR "dried blood" OR Guthrie OR "blood sampling paper" OR "filter paper blotter" OR "filter paper disk" OR "dried filter paper" OR "PKU card" OR "PKU cards" OR "Blood Collection Card" OR "Blood Collection Cards" OR bloodstain* OR "blood stain" OR "blood stains" OR "blood stained" OR (("Filter paper" OR "filter papers" OR "filter card" OR "filter cards" OR "filter plate" OR "filter plates") AND blood*) OR (Paper AND ("Blood Specimen Collection" OR "Blood Chemical Analysis" OR "Tandem Mass Spectrometry" OR **"Biological Markers/blood"** OR "Blood Preservation")) OR ("Blood Specimen Collection" AND dried) OR (DBS AND (Blood OR Screening))

11/16/15: Validation Studies – 29 hits, none relevant

("blood spot" OR "blood spots" OR "blood spotted" OR bloodspot* OR "dried blood" OR Guthrie OR "blood sampling paper" OR "filter paper blotter" OR "filter paper disk" OR "dried filter paper" OR "PKU card" OR "PKU cards" OR "Blood Collection Card"

OR "Blood Collection Cards" OR bloodstain* OR "blood stain" OR "blood stains" OR "blood stained" OR (("Filter paper" OR "filter papers" OR "filter card" OR "filter cards" OR "filter plate" OR "filter plates") AND blood*) OR (Paper AND ("Blood Specimen Collection" OR "Blood Chemical Analysis" OR "Tandem Mass Spectrometry" OR **"Biological Markers/blood"** OR "Blood Preservation")) OR ("Blood Specimen Collection" AND dried) OR (DBS AND (Blood OR Screening))) AND (validation)

****

SciFinder (lrosman1; Welch123)

https://scifinder.cas.org/help/scifinder/R36/index.htm

Refine: "CAPLUS"

All combined: 5,090 – remove duplicates: 5,045

"dried blood spot" (2,096)

"dried bloodspot" (21)

Guthrie (554)

"dried bloodstain" (78)

"dried blood stain" (171)

"dried blood sample" (2,179)

("Blood Specimen Collection" and dried) (136)

(DBS and Blood) (925)

(DBS and screening) (323)

"PKU card" (5)

"Blood Collection Card" (704)

DBS biomarker (36)

DBS marker (186)

****

11/16/15: Validation studies/Reviews: 575 total

Validation

"dried blood spot" 376

"dried bloodspot" 1

"dried blood sample" 256

Review

"dried blood spot" 142

"dried bloodspot" 1

"dried blood sample" 83

APPENDIX 2-B - ANALYTE DATABASE (refer to accompanying spreadsheet**)**

APPENDIX 3-A - OPTIMIZATION EXPERIMENT

OPTIMIZATION EXPERIMENTAL METHODS & RESULTS

Goal: The goal of the optimization experiments was to determine an optimal amount of molecular sieve for drying the internal conditions of our novel DBS kits as based on the volume of space within the container, choice of filter paper card, and likely moisture content introduced into the closed system by inclusion of a freshly spotted filter paper card.

Outcome Measure: The outcome measure of interest for optimization experiments was Time to Decline (TTD), defined here as the minutes required from the start of the experiment for the relative humidity (RH) inside kits to begin to reduce suggesting desiccant is effectively controlling internal moisture and drying the environment around the spotted filter paper card.

Materials: Kit containers were procured directly from Elemental Container (product # 0075152); 10 g molecular sieve desiccant packets (product # 02-00041AG19) and Whatman 903 cards (product # 10531018) were procured from Sigma-Aldrich; wireless bluetooth enabled RH/temperature HOBO data loggers (product # MX1101) were procured

from ONSET; and 200 microliter adjustable pipettes (product # 3121000082) were procured from Eppendorf.

Process: Increasing amounts of molecular sieve were included inside kit containers along with filter paper cards freshly spotted with 4 drops of 30 uL amounts of water by micropipette, and a HOBO sensor for measuring relative humidity (RH) and temperature inside the closed-system containers. External temperature and humidity were controlled at 25.6℃ and 35% RH respectively. The moisture inside the kit containers was measured throughout experimentation with relative humidity by HOBO sensors at 1-minute increments. To carry out the experiment, investigators started the data logger, then spotted filter paper cards, and immediately stored the card inside the kit along with a pre-defined amount of molecular sieve, and an ONSET data logger. Experiments were carried out under ambient lab conditions (25.6℃ and 35% RH), and included 6 replicates. Temperature and RH conditions in the lab were monitored throughout experiments. Experiments were carried out for 24 hours, after which time containers were unsealed, HOBO sensors stopped, and data downloaded in Excel and CSV formats. Data was then imported into Stata version 13.1 for analysis. Each experiment contained a pre-defined amount of molecular sieve for each 10 gram increment between 0 grams and 100 grams.

Analysis: The mean and standard deviation for TTD of each experimental run (i.e. 6 replicates of pre-defined 10 gram molecular sieve amount) was calculated. A two-sample t-test for comparing mean TTDs between experimental groups was used for determining the optimal amount of sieve, which is defined here as the lowest 10 gram increment of sieve with a significantly faster TTD above the previous 10 gram increment, plus 10 grams excess sieve for long-term storage. The 10 grams of excess sieve was included in the kit's optimal sieve amount in order to maintain a near moisture free environment over extended periods of storage. Near moisture free environment is defined here as a detected RH level of less than 0.01%.

Results

The lowest incremental amount of molecular sieve with a significantly faster TTD compared with the previous increment was 30 grams ($df = 10$, $t = 3.77$, $p = 0.0037$, 95% CI; Table A-1). Based on predefined criteria for optimal desiccant quantity, investigators selected 40 grams molecular sieve as optimal amount of desiccant for inclusion in kits. 40 grams molecular sieve represents 10 grams in excess of the lowest sieve amount with a significantly faster TTD above the previous 10 gram increment of sieve.

Table A-1. Optimization experimental findings for mean TTD with two sample t-test comparisons between experimental group means.

Amount desiccant	Mean TTD, standard deviation (n=6)	Two sample t test *df = 10, 95% CI*
10 grams	33.50 minutes, 3.51	Not applicable
20 grams	31.17 minutes, 1.33	t = 1.52, p = 0.1585 (compared w/ 10 g)
30 grams	26.83 minutes, 2.48	t = 3.77, **p = 0.0037** (compared w/ 20 g)
40 grams	28.67 minutes, 0.82	t = 1.72, p = 0.1166 (compared w/ 30 g)
50 grams	27.17 minutes, 1.72	t = 0.27, p = 0.7925 (compared w/ 30 g)
60 grams	23.67 minutes, 4.37	t = 1.54, p = 0.1536 (compared w/ 30 g)
70 grams	25.33 minutes, 3.27	t = 0.90, p = 0.3915 (compared w/ 30 g)
80 grams	29.50 minutes, 1.87	t = 2.10, p = 0.0620 (compared w/ 30 g)
90 grams	32.50 minutes, 2.07	t = 4.29, **p = 0.0016**[5] (compared w/ 30 g)
100 grams	26.00 minutes, 4.69	t = 0.38, p = 0.7086 (compared w/ 30 g)

[5] The difference in TTD for 90 grams compared with 30 grams was significant, however, the mean TTD for 90 grams was significantly *slower* than 30 grams and is therefore *not* considered optimal.

APPENDIX 4-A. SOP-45

SOP – 45- PAXgene Blood RNA Isolation

PAXgene Blood RNA Tube: Catalog # 762165 (Qiagen/BD Company)

PAXgene Blood RNA Kit: Catalog# 762164 762165 (Qiagen/BD Company)

NOTE: Store the PAXgene Blood RNA tube upright @ RT for a minimum of 2 hrs and a maximum of 72 hrs before processing or transferring to refrigerator (2°C - 8°C) or freezer (-20°C). Stand the tubes upright in a wire rack. Do not freeze in a Styrofoam tray as this may cause the tubes to crack.

Performance Characteristics: RNA profile remains stable for 3 days @ RT (18°C - 25°C), 5 days @ 2°C - 8°C, or for a minimum of 50 months @ -20°C or -70°C/-80°C.

Things to do before starting

- If the PAXgene Blood RNA Tube was stored at 2-8°C or -20°C or **-70°C** after blood collection, first equilibrate it to room temperature, and then store it at room temperature for 2 hours before starting the procedure.
- Buffer BR4 is supplied as a concentrate. Before using for first time, add 4 volumes of ethanol (96%-100%) as indicated in the bottle to obtain a working solution.

1. Close the QIAcube door, and switch on the power switch.
2. Open the QIAcube door, and load the necessary reagents and plasticware into the QIAcube, pages 29-39 in the PAXgene Blood RNA Kit Handbook. To save time, loading can be performed during one or both of the following 10 min centrifugation steps (steps 3 and 5).
3. Centrifuge the PAXgene Blood RNA tube for 10 min @ 3000-5000 x g using a swing-out rotor. It will be **Program#3** in the Sorval ST 40R centrifuge.

   NOTE: Ensure that the blood sample has been incubated for a minimum of 2 hrs @ RT, in order to achieve complete lysis of blood cells. Excessive centrifugation speed (over 10,000 RCF) may cause PAXgene Blood RNA Tube breakage.
4. Remove the supernatant by decanting or pipetting. Add 4 ml RNAse-free water to the pellet, and close the tube using a fresh secondary BD Hemogard closure (supplied with the kit).
5. Vortex until the pellet is visibly dissolved, and centrifuge for 10 min @ 3000-5000 x g (**Program#3**). Remove and discard the entire supernatant.

   NOTE: Incomplete removal of the supernatant will inhibit lysis and dilute the lysate, and therefore affect the conditions for binding RNA to the PAXgene membrane.
6. Add 350ul resuspension buffer (Buffer BR1), and vortex until the pellet is

visibly dissolved.

7. Pipet the sample into a 2 ml safe-lock tubes processing tube.
8. Load the open 2ml processing tubes containing sample into the QIAcube shaker (see Fig. 13, pg30 and Fig.17, pg33). The sample positions are numbered for ease of loading. This enables detection of samples during the load check.

   NOTE: Make sure that the correct shaker adapter (Shaker Adapter marked with a "2") is installed.
9. Close the QIAcube instrument door.
10. Select the "PAXgene Blood RNA Part A" protocol, and start the protocol. Follow the instructions given on the QIAcube touchscreen. NOTE: Make sure that both program parts (part A and part B) are installed on the QIAcube instrument.

    NOTE: The QIAcube will perform load checks for samples, tips, rotor adapters, and reagent bottles.
11. After the "PAXgene Blood RNA PartA protocol is finished, as indicated by a display message, open the QIAcube instrument door. Remove and discard the PAXgene RNA spin columns from the rotor adapters and the empty processing tubes from the shaker. NOTE: During the run, spin columns are transferred from the rotor adapter position 1 (L1) to rotor adapter position 3 (L2) by the instrument.

12. Close the lids of all 1.5 ml microcentrifuge tubes containing the purified RNA in the rotor adapters. Transfer the 1.5ml microcentrifuge tubes into the corresponding positions in the QIAcube shaker adapter.
13. Close the QIAcube instrument door.
14. Select the "PAXgene Blood RNA Part B" protocol, and start the protocol. Follow the instructions given on the QIAcube screen.

    NOTE: This program incubates the samples @ 65°C and denatures the RNA for downstream applications. Even if the downstream application includes a heat denaturation step, do not omit this step. Sufficient RNA denaturation is essential for maximum efficiency in downstream applications.
15. After the "PAXgene Blood RNA Part B" program is finished, as indicated by a display message, open the QIAcube instrument door. Immediately place the microcentrifuge tubes containing the purified RNA on ice.

    NOTE: Do not let the purified RNA remain in the QIAcube. Since the samples are not cooled, the purified RNA can be degraded. Unattended overnight sample preparation runs are therefore not recommended.
16. Measure the RNA concentration (e.g., using PicoGreen dye fluorescence or spectrophotometry on a Nanodrop instrument).
17. If the RNA samples will not be used immediately, store at -20°C or -70°C.
18. Remove the reagent bottle rack from QIAcube worktable, and close all bottles with the appropriately labeled lids. Buffer in bottles can be stored @ RT for

up to 3 months. Remove and discard remaining reagents in the processing tubes in the QIAcube microcentrifuge tube slots. Remove and discard rotor adapters from the centrifuge. Empty the QIAcube waste drawer. Close the QIAcube instrument door, and switch off the instrument with the power switch.

APPENDIX 4-B. SOP-44

SOP – 44- Dried Blood Spot RNA Isolation

1. Take the dried blood spot sample (Whatman filter paper), place on the small plastic weighing boat. Cut out all available blood spots using a sterile sharp surgery scissors. Further cut into little strips of 0.3 cm^2. Place surgery scissors in the 50mL Falcon tube with 100% alcohol to decontaminate.
2. Place the cut out blood spot using a forceps in a 2ml safe-lock microcentrifuge tube.
3. Add 360-370 ul RLT buffer and completely submerge the filter paper.
4. Turn On QIAcube machine.
5. Select Tools. Select Shaker. Press Edit.
6. Time = 1800 sec

   Temp = 37°C

   Frequency = 1000 rpm
7. Incubate the sample for 30 min @ 37°C with agitation using the QiaCube Shaker @ 1000 rpm.
8. Transfer RLT sample (360- 370ul everything including the cut up filter paper) into the QIAshreddrer. Spin it for in the microcentrifuge for 1 min, maximum speed.
9. Transfer all the liquid which should be around 360 ul into the 2ml safe-lock

microcentrifuge tube.

10. Proceed to RNA extraction using the QIAcube **RNeasy Micro Kit** (cat. # 74004). Choose the **QiaCube Protocol under RNA and choose RNeasy Micro kit (with the DNase)**.
11. **Do not Nano drop RNA** sample (the very low RNA concentration will generally fall below the limit of detection by current assays).

APPENDIX 4-C. SOP-24

SOP – 24- QuantiTect Probe RT-PCR Procedure

1. Preparation of reagents:

1.1 **Always wear gloves** when handling reagents. Prepare ice bucket before getting reagents from -20 freezer. Place reagents on ice. Don't touch the enzyme's tube or inside the tube with bare hands!

1.2 **Wear appropriate PPE** (lab gown, goggles, mask, gloves)

1.3 Vortex reagent-mixture before use.

1.4 QuantiTect Probe RTPCR Kit - cat# 204445 (Qiagen)

1.5 Optical PCR Plate (96 well) – cat# 2239441 (Bio-Rad)

1.6 Microseal "B" Film – cat# MSB1001 (Bio-Rad)

2. Preparation of Master Mix: multiply MM by the number of rxns

QuantiTect Probe MM (2X)	= 12.5 uL
RNAse Free H2O	= 6.0 uL
Taqman Gene Expression Assay (20X)	= 1.25 uL
RT mix	= 0.25 uL
RNA template (1pg-500ng/rxn)	= 5 uL
	= 25 uL

3. Label the optical PCR 96 well plate. Make sure to put the probe, study name, date, and initials.

4. Add 5ul of RNA sample to the bottom surface of the optical reaction plate.

5. Add 20ul of the Master Mix to each well containing 5ul of RNA template.

6. Place the optical tape on top of the wells. Don't touch the top with gloves. Use the flat edge tool to seal.

7. Place the PCR samples in the Bio-Rad iCycler machine.

7.1 Before running the machine, make sure that on the bottom of the screen it says, "HOST CONTROL MODE" to make sure the machine is ready to take pictures of the reactions.

8. Reaction Protocol

8.1 Review SOP-23b- BioRad iCycler RT-PCR Machine before using the machine.

8.2 Use *QuantiTectPCR.tmo* or *AB 1-Step RTPCR.tmo* for running the Reverse Transcriptase PCR.

9. When finished with the PCR reaction, don't take samples back in the PCR/laminar hood.

9.1 Open the iCycler lid and check the labels on the plate. Verify the primer/probe used for the PCR run, samples (RNA/DNA), study name, and the date.

9.2 Don't throw PCR products away, wrap them up in foil and place in the fridge with your initial and date.

CURRICULUM VITAE

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Professional Summary

Currently completing doctoral studies in Environmental Health and Engineering at Johns Hopkins Bloomberg School of Public Health. Doctoral research is focused on the development of field-ready methods for the collection and preservation of biological samples in complex occupational and environmentally challenging settings. Advanced training and certification in Public Health Informatics, Risk Sciences and Public Policy, and Global Complex Humanitarian Emergencies. In addition to doctoral studies, currently serving as a Research Associate in the Johns Hopkins Center for Humanitarian Health; an Instructor in the Public Health Studies Program teaching courses focused on disasters and humanitarian response; and a member of the Johns Hopkins Go Team, which provides rapid response capacity for regional and national disasters. Most recently began a project for which I am a co-Principal Investigator, and which is aimed at the development of a regional biosample referral program for Madagascar. The project is specially designed to be scalable, sustainable, and transferable to other resource limited environments.

Professional Experience

01/27/2017 – Present	*Emerging Leader in Biosecurity Initiative Fellow*, Center for Health Security, Johns Hopkins University, Baltimore, MD.
09/01/2016 – Present	*Gordis Teaching Fellow*, Public Health Studies, Johns Hopkins University, Baltimore, MD. Developed and directs a new undergraduate course titled, Environmental Health and Disasters.
08/01/2016 – Present	*Team Member*, Go Team, Johns Hopkins Office of Critical Event Preparedness and Response, Baltimore, MD. Providing technical support in response to regional and national disasters.
05/01/2015 – Present	*Research Associate*, Center for Refugee and Disaster Response, Johns Hopkins Bloomberg School of Public

	Health, Baltimore, MD. Conducting research focused on humanitarian emergencies and environmental disasters.
01/15/2015 – Present	*Instructor*, Public Health Studies Program, Johns Hopkins University, Baltimore, MD. Course director for three classes including, Health in Complex Humanitarian Emergencies, Responding to Disasters – from Earthquakes to Ebola, and Environmental Health and Disasters.
09/03/2013 – 09/02/2014	*ORISE Research Fellow*, Emergency Response and Recovery Branch, United States Centers for Disease Control and Prevention, Atlanta, GA. Primary appointment with the Health Systems Recovery Team (HSRT) focused on rebuilding Haiti's health system. Worked in maternal death surveillance, tuberculosis, and water, sanitation and hygiene (WASH). Also worked on development of a WASH training program for UNHCR among other duties related to complex humanitarian emergencies.
08/28/2013 – 08/28/2016	*Adjunct Faculty Member*, Global Health Culture and Society Program, Emory University, Atlanta, GA. Course director for Health in Complex Humanitarian Emergencies.
12/01/2012 – 08/31/2013	*Graduate Researcher*, International Emergency and Refugee Health Branch, United States Centers for Disease Control and Prevention, Atlanta, GA. Served as a graduate researcher working on a WASH epidemiology-training course provided to UNHCR in Kenya. Provided logistical support to the joint Emory/CDC graduate certificate program in global complex humanitarian emergencies. Assisted the branch in collating and communicating information pertaining to the violent conflict in northern Syria.
09/01/2012 – 05/16/2013	*Researcher*, Human Sciences Research Council, Cape Town, South Africa. Worked with the Human Sciences Research Council in Cape Town on a CDC funded project to assess and redesign the maternal and child health surveillance systems for South Africa. Was commissioned by the South African Statistician General to conduct a comprehensive assessment of the civil registration and vital statistics systems for RSA based on WHO technical

guidelines. Other projects included building a gender inequity index specific for RSA, formative research for development of a national health insurance program, design of a cross-sectional cluster sample survey for use in collecting data necessary for the 2014 MDG country assessment reports, a paper on sustainable development and the green economy presented at Rio+20, and a publication on the social determinants of HIV clustering of infections in South Africa.

09/01/2011 – 08/31/2013 *Graduate Coordinator*, Global Complex Humanitarian Emergencies Program, Hubert Department of Global Health, Emory University, Atlanta, GA. Worked with the Associate Director of Academic Programs for the Hubert Department of Global Health to coordinate the joint Rollins School of Public Health and CDC International Emergency and Refugee Health Branch Graduate Certificate in Global Complex Humanitarian Emergencies. Also provided logistical support to the GH Department upon request.

Education

08/2017 (expected) *Doctor of Philosophy (PhD)*, Environmental Health and Engineering, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

08/09/2013 *Master of Public Health (MPH)*, Global Health, Rollins School of Public Health, Emory University, Atlanta, GA

05/16/2009 *Bachelor of Science (BS)*, Psychology, School of Arts and Sciences, West Virginia University, Morgantown, WV

Specialized Training & Skills

12/22/2016 *Public Health Informatics*, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

05/18/2016 *Risk Sciences and Public Policy*, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

08/09/2013 *Global Complex Humanitarian Emergencies*, Rollins School of Public Health, Emory University, Atlanta, GA

Languages	English, French
Coding	Arduino, MATLAB, Prism, Python, R, SAS, Stata

Contributions to Science

1. My investigations into the range of dried blood spot (DBS) analyses and their potential application in environmentally challenging settings have produced findings that indicate DBS is a potentially field-ready, scalable method for biosampling in a variety of settings. These methods may be applied for clinical diagnosis, disease surveillance, and epidemiologic study.
2. I am currently in the process of studying differential degradation of analytes in dried blood spots compared with venous blood samples. Differential degradation rates have led me to the development of a biomedical informatics platform aimed at the removal of degradation as a variable of concern for clinical diagnostics. These efforts are achieved through exploration of clinically relevant signatures of disease through measurement of relative quantities of analytes with similar degradation rates.
3. While at CDC and under the guidance and support of Dr. Tom Handzel and Dr. Farah Hussein, I assisted in the development of a technical training curriculum for water, sanitation and hygiene assessment among refugee populations.
4. While working for the Human Sciences Research Council, I directed a national assessment of civil registration and vital statistics (CRVS) for South Africa. My findings were presented in 2012 at an intercontinental conference on CRVS in Durbin, South Africa.

Publications

2016	Tappis H, **Freeman J**, Glass N, Doocy S. Effectiveness of Interventions, Programs and Strategies for Gender-based Violence Prevention in Refugee Populations: An Integrative Review. *PLOS Currents Disasters*. 2016 Apr 19. Edition 1. doi: 10.1371/currents.dis.3a465b66f9327676d61eb8120eaa5499.
2016	Errett, N., Thompson, C., Rutkow, L., Garrity, S., Stauss-Riggs, K., Altman, B., Walsh, L., **Freeman, J.**, Balicer, R., Schor, K., Barnett, D. "Examining public health workers' perceptions toward participating in disaster recovery after Hurricane Sandy: a

quantitative assessment." *Disaster medicine and public health preparedness* (2016).

2013 Wabiri, N., Shisana, O., Khangelani, Z., **Freeman, J.** Exploring the Social Determinants of Spatial Clustering of HIV Infections in South Africa. *Human Sciences Research Council Library*. 2013.

Research Support

Development of low-cost, low-tech, systems-level collection devices for biological sampling in environmentally challenging environments
National Institutes of Health (R21 CA183623)
January 2016 – May 2017
PI: Dr. David Graham (director, Center for Resources in Integrative Biology, Johns Hopkins Medicine)
Role: co-Investigator (thesis research)

Development of a regional biological sample referral program with validation of a novel, gene-based diagnostic tool for tuberculosis in Madagascar.
PIVOT, Boston, MA (pivotworks.org)
February 2017 – August 2017
PI: Paul Strickland
Role: co-Principal Investigator

Volunteer Experience

06/2005 – 08/2010 EMERGENCY, Milan, Italy, Volunteer. In addition to advocacy and fund raising, I created a clinical referral program between US-based NGO CIVIC and international NGO Emergency for victims of violent conflict.

Honors

04/2013 CDC Millennial Health Leader, The King Center and the United States Centers for Disease Control and Prevention, Atlanta, GA.

09/2005 Selected Representative, National Student Leadership Forum, United States Congress, Washington, D.C.