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Dried blood spots: An evaluation of utility in the field

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ABSTRACT

Background: Use of dried blood spots (DBS) offers advantages over serum samples in studies conducted in resource-poor settings. The aim of this study was to compare the number of adequate spots collected across different demographic groups.
Methods: Five DBS were collected from 3316 individuals aged 0–49 years in Tianjin, China for a measles antibody study; DBS were rated "adequate" or "inadequate." Linear regression, with the number of adequate DBS on a card as the outcome variable, was used to test for predictors of DBS adequacy.
Results: There were 0 adequate DBS for 23% of participants and 5 adequate DBS for 24%. Mean number of adequate DBS was 1.68 in infants (<12 months), 2.57 (1–9 years), 3.49 (10–29 years), 3.08 (30–49 years). The number of adequate DBS increased over the study; the mean number of adequate DBS for the five years 2011–2015 were 1.21, 2.52, 3.40, 2.22, and 3.62, respectively. DBS quality was not related to measles IgG antibodies.
Conclusions: DBS are an alternative for adults and children but pose challenges in infants, and improve with experience. In a resource-limited environment or in a scenario where more invasive techniques like venipuncture may be less accepted by the study population, DBS can be the preferred technique to efficiently obtain serum specimens for analyte testing.

Introduction

Serological testing of antibody levels can be used for disease surveillance and diagnostics and generally involves obtaining a sample through a standard blood draw. Performing traditional serological testing requires trained phlebotomists as well as a centrifuge and other supplies to extract the serum from blood specimens soon after collection. The additional step of re-packaging the serum risks labeling errors and specimens transported to a central laboratory for testing must be kept at cool temperatures (4–6 °C). If serum specimens are stored frozen before shipping, temperatures below 0 °C must be maintained to avoid antibody-destroying freeze–thaw cycles [1]. All of these represent potential challenges in countries and settings without available skilled workers or adequate laboratory resources.

An alternative to blood draws for serological testing of antibody levels are dried blood spots (DBS), in which blood from a finger prick is dripped onto marked circles on a filter paper card. DBS-based tests offer several advantages over serum testing when resource and environmental conditions are challenging. DBS collection is less expensive, relies on more portable equipment, and can be done effectively by a minimally trained individual [2]. DBS collection is also safer and less invasive than blood draws, and may be more acceptable to participants, particularly when they are not directly benefitting from the procedure [2]. The DBS card is labeled at the time of the DBS collection with patient- and study-identifying information, and this information remains with the specimen until testing is initiated, decreasing the opportunity for mislabeling. Importantly, many analytes, including antibodies, are stabilized once dried on filter paper, even given fluctuating shipping temperatures. The DBS can also be stored at room temperature

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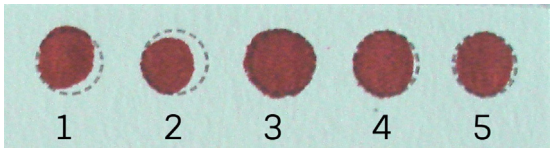

Fig. 1. Examples of dried blood spots. Spots 1 and 2 are inadequate; spots 3–5 are adequate.

for a period of time prior to testing. DBS have been successfully used in diagnostic testing for measles, HIV, hepatitis C, hepatitis E, and polio [3–7].

Because DBS collection requires a large drop of blood (~100 μl) it can be a challenge to collect in the field, particularly in infants and in the presence of colder ambient temperatures. Here, we describe our experience using DBS to measure measles antibodies (IgG and IgM) and vitamin A levels as part of a measles antibody seroprevalence study in Tianjin, China. Based on the 5 DBS obtained per person, we counted the number of adequate DBS for each study participant and compared these numbers by demographic groups and across the study period to inform the feasibility of using DBS sampling for future studies.

Materials and methods

From November 2011 to May 2015 we interviewed and collected DBS samples from 3318 people, age 0–49 in Tianjin, China. We used a single-use lancet on the finger and collected five DBS on a single filter paper card (for a total of 16,590 DBS) from each participant. DBS cards were transported to the Tianjin CDC laboratory for evaluation of quality and testing for measles IgG antibodies [8]. Each of the five DBS was evaluated and rated as "adequate" or "inadequate." An adequate DBS was at least 11 mm in diameter, i.e., completely filled the pre-printed circle on the filter paper. Each card was scored based on the number of adequate DBS. An example of a DBS card is shown in Fig. 1. Measles IgG testing was conducted after the quality assessment with a SERION ELISA classic measles IgG (quantitative) test from Institut Virion/Serion GmbH, Würzberg, Germany. The laboratory results were considered positive based on guidelines from the Standardization Administration of the People's Republic of China [9]. The thresholds were >200 IU/ml for positive, 150–200 IU/ml for borderline, and <150 IU/ml for negative.

All study personnel who collected the DBS attended multiple training sessions conducted by both US- and Chinese-trained physicians and scientists, and were provided with opportunities to practice and repeatedly demonstrate proficiency before performing DBS collection in the field.

We used linear regression, with the number of adequate DBS on a card as the outcome variable, to test for predictors of DBS adequacy. Covariates included age, urbanicity (rural/urban/suburban), sex, district of residency, measles immunity status, and year and season of collection. We report the predicted population marginal means with 95% confidence intervals (CI), for each level of these covariates. This estimate is the average number of DBS for a given group, after controlling for other demographic groups in the regression model. A global Wald Type 3 chi-square test was used to evaluate whether there were significantly differences across levels within a variable. Additionally, in a subsample of adults 20 years of age and older, we ran a model including occupation (categorized into manual workers, service workers, professionals, and others). In order to examine the relationship between DBS adequacy and IgG seropositivity, we also estimated a logistic regression model, with DBS adequacy as one of the predictors, alongside other confounders. A significance level of 0.05 was used. Model fit was confirmed with residual plots and investigation of influential observations.

The OpenClinica software version 3.3 (OpenClinica LLC, Waltham, MA, USA) was used for data collection from participants and Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) was used for recording DBS results. Data analyses were conducted using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Ethical approval

The study was approved by both the University of Michigan Institutional Review Board (IRB) and the Tianjin Centers for Disease Control and Prevention Ethics Committee.

Results

A total of 3318 participants were enrolled in the study; DBS quality data were available for 3316 (2 DBS cards were unavailable for quality review). For all DBS cards, 23% were rated as having 0 adequate DBS (of 5 total DBS), 6% had 1 adequate DBS, 9% had 2 adequate DBS, 18% had 3 adequate DBS, 21% had 4 adequate DBS and 24% had 5 adequate DBS. By age group, 43% of infants had no adequate DBS compared with 25% of those 1–9 years, 11% of those 10–29 years and 18% of those 30–49 years.

Table 1 shows the mean number of adequate DBS by demographic groups. Overall, the mean number of adequate DBS for all study participants was 2.81 (standard deviation = 1.87). According to the linear regression model, the mean number of DBS varied by age of the participant, urbanicity, sex, and year and season of study enrollment.

Children younger than 9 years of age, and especially young infants, had a lower number of adequate DBS than older people; for example, infants under 12 months of age had on average less than 2 adequate DBS, whereas for all age groups 10 years and above, the average number of adequate DBS was approximately 3 or greater.

People living in suburban (3.20 DBS) and rural districts (2.85 DBS) had higher mean numbers of adequate DBS than those living in urban areas (2.64 DBS), and females had more adequate DBS (3.11) than males (2.30).

Participants who enrolled in the study during the latter years had significantly higher numbers of adequate DBS than those enrolled earlier. The mean number of adequate DBS for the five years 2011–2015 were 1.21, 2.52, 3.40, 2.22, and 3.62, respectively.

There was no significant difference in the mean number of adequate DBS by residency status ($p = 0.2769$) nor between those who were measles IgG positive and those measles IgG borderline or negative ($p = 0.4252$).

In a subanalysis of adults 20 years of age and older with an additional factor added into the model, occupation, results were similar. Moreover, occupation was significantly related to the number of adequate DBS ($p = 0.0003$). Manual workers (n = 561, mean = 2.96 DBS, 95% CI = 2.83, 3.10) and service workers (n = 193, mean = 3.15 DBS, 95% CI = 2.93, 3.37) had the lowest number of adequate DBS, followed by individuals in a professional (n = 572, mean = 3.40 DBS, 95% CI = 3.27, 3.52) or some other occupation (n = 653, mean = 3.49 DBS, 95% CI: 3.37, 3.61).

In order to assess the potential impact of spot quality we looked at the difference in IgG results by the number of adequate spots on each card (Table 2). This analysis showed that there was no difference in IgG results by number of adequate DBS specimens ($P = 0.2127$).

Discussion

We found that using DBS to measure antibodies as part of a large population-based susceptibility study was feasible, while

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