Validation of collection and elution procedures for measurements of retinol binding protein in dried blood spot specimens

Running title: RBP in dried blood spots

Authors and institutions:

Eleanor Brindle[1]
Masako Fujita[2]
Dean Garrett[3]
Jonathan Gorstein[4]
Bettina Shell-Duncan[1,2]

[1] Center for Studies in Demography and Ecology, University of Washington, Seattle, WA 98195
[2] Department of Anthropology, University of Washington, Seattle, WA 98195
[3] PATH, Seattle, WA 98107
[4] Department of Health Services, University of Washington, Seattle, WA 98195

Corresponding author:
Eleanor Brindle
Center for Studies in Demography and Ecology
University of Washington
Seattle, WA 98195-3100
Phone: (206) 616-9369
Fax: (206) 543-3285

Abbreviations:
VA, VAD, RBP, DBS

EXTENDED ABSTRACT

Background

Biomarkers from blood specimens are vital and broadly useful tools for assessing many dimensions of health, but blood can be prohibitively difficult to collect, process, and transport in non-clinical research settings. Dried blood spot (DBS) specimens eliminate many of the obstacles to collecting blood specimens in non-clinical research settings, yet can provide comparable measures of many biomarkers, and thus offer a feasible alternative to serum or plasma collection.

Retinol, an indicator of vitamin A (VA) status, is one biomarker that presents logistical challenges for non-clinical research. Vitamin A deficiency (VAD) is a significant public health concern in many areas of the developing world where dietary sources of vitamin A are limited (1-4). VA is essential for vision, growth, cellular differentiation, immune function, and reproduction (4, 5). Symptoms in eye function such as night-blindness can identify clinical VAD while only biochemical markers can identify subclinical VAD (4). Both clinical and subclinical VAD are associated with elevated morbidity and mortality (4, 6, 7). Furthermore, in populations that face the dual challenges of VAD and HIV, maternal VAD is associated with increased mother-to-child transmission of HIV (8). For these reasons accurate and efficient assessment of subclinical VA status is crucial. Reliable methods that simplify assessment of VAD under field conditions are in great demand, as laboratory facilities are likely to be limited or non-existent in areas where VAD is most likely to occur.

Circulating retinol is the most direct indicator of VA status, but is relatively unstable in blood specimens, and can be difficult to measure. Retinol binding protein (RBP) is an excellent surrogate measure of retinol, as it is present at a 1 to 1 ratio to retinol, is more stable, and is relatively easy to measure (9, 10). Collection of DBS specimens on filter paper can further simplify assessment of VA status in resource-poor field settings. Unlike serum or plasma specimens, DBS do not require processing immediately following collection, pose relatively

small risks of infection to researchers and participants, and can be stored and transported without refrigeration, eliminating the need for immediate access to laboratory facilities . Capillary DBS can be collected from a small finger prick with a sterile disposable lancet, a procedure that is less invasive than venipuncture, and can be conducted by researchers themselves with minimal training, rather than by skilled phlebotomists.

To take full advantage of DBS collection methods, it is necessary to establish 1) that DBS specimens can provide biomarker measures comparable to serum or plasma measures, 2) how to process DBS prior to assay to ensure consistent results comparable to serum or plasma measures, and 3) that capillary blood collection provides results comparable to venous blood collection.

In this paper we validate the use of DBS for field studies of VAD by comparing RBP results from DBS with those of plasma and serum specimens. In addition, we compared RBP in capillary DBS to results from DBS prepared from venous blood collection, and test the effects of anti-coagulant on DBS made from venous blood spots. Further, to determine how to obtain results most similar to serum or plasma measures using DBS specimens, we tested the efficiency of several protocols for eluting blood from filter paper.

Methods

Plasma, serum, capillary DBS, and DBS from venous blood with and without anti-coagulant were collected on Whatman 903 filter paper cards from eight healthy US men and women, ages 25-45. Serum, plasma, eight elution protocols for both types of venous DBS, and four elution protocols for capillary DBS were assayed in quadruplicate using the Scimedx Scanlisa RBP-EIA kit.

Results

There was no significant difference ($p=0.342$) between RBP in serum (43.5 µg/mL ± 11.0) vs. plasma (40.9 µg/mL ±10.5). Recovery of RBP from DBS specimens was, on average,

90% of RBP in serum and 94% of RBP in plasma, and varied slightly by elution protocol used, but did not vary by blood collection method. Agreement with serum and plasma results was highest using two 1/8" punches from capillary DBS eluted to a 50-fold dilution (40.4 μg/mL ± 9.3). Elution procedures using high volumes of diluent produced results more similar to serum values, with mean (SD) RBP of 36.7 (1.9) μg/mL for 50- to 100-fold dilutions vs. 33.5 (1.2) μg/mL for 25-fold dilution.

Conclusions

Capillary or venous DBS RBP measurements adequately reflect serum and plasma values when DBS are properly eluted. This method is a crucial tool for assessing vitamin A status in field research settings where there are significant obstacles to serum or plasma collection.

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