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Retinol-binding protein stability in dried blood spots

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Abstract

Background: Retinol-binding protein (RBP) is accepted as a substitute measure of retinol, a biochemical marker for vitamin A status. A recently developed enzyme immunoassay for RBP uses serum or whole blood stored as dried blood spots (DBS). However, the stability profile of RBP in DBS has not been examined.

Methods: A total of 63 DBS collected by finger prick, then stored sealed in a plastic bag with desiccant were exposed to one of 5 time and storage temperature treatments: 1) Baseline, 2) 30°C for 7days, 3) 30°C for 14 days, 4) 30°C for 28 days, and 5) 4°C for 38 days, and then assayed for RBP. Using linear mixed effects models, RBP concentrations at baseline were compared with storage time/temperature treatments.

Results: RBP stored at 30°C remained stable in DBS for 2-4 weeks at the group level although at 4 weeks there were some variations in RBP stability across subjects. By contrast, specimens stored at 4°C for 38 days produced values significantly lower than baseline ($p=.0023$).

Conclusion: RBP in DBS collected by finger-prick can withstand storage at a relatively high ambient temperature (30°C) up to 2-4 weeks and thus facilitates accurate vitamin A status assessments at the population level. In field settings where immediate cold storage or assay is unavailable, DBS is likely to be more stable stored at ambient temperature than in a refrigerator.

Vitamin A deficiency (VAD) is a serious public health problem in many developing countries where dietary intake of bioavailable sources of vitamin A is low (1-4). Vitamin A is essential for vision, growth, cellular differentiation, immune function, and reproduction (4, 5). Symptoms in eye function and structure such as night blindness and Bitot's spots 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, especially among preschoolers and women of reproductive age (4, 6, 7). Maternal VAD is particularly problematic for populations that face the dual challenges of VAD and HIV, because 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.

The relative instability of retinol, a biochemical marker of vitamin A status, precludes its use in field research on VAD. However, serum retinol-binding protein (RBP), the carrier protein of retinol, is accepted as a substitute measure given its approximately 1:1 molar relationship with retinol (9, 10). A recently developed enzyme immunoassay for RBP (11) uses serum or whole blood stored as dried blood spots (DBS). The DBS method eliminates the need for blood sample vials, and simplifies sample collection and transportation in field studies (12). While proteins like RBP are known to be more stable than retinol (13), the stability profile of RBP in DBS has not been examined. Therefore, we investigated the effects of storage temperature and duration on the recovery of RBP in DBS, simulating conditions in remote field settings with limited immediate access to cold storage.

Four female volunteers donated free-flowing capillary blood spots, collected by finger-prick with sterile disposable lancets. Research procedures were approved by the Institutional Review Board of the University of Washington. DBS collected on filter paper (Schleicher & Schuell 903) were dried completely (approximately 4hrs) at ambient laboratory temperature, and then placed in separate sealed plastic bags with desiccant in groups of 3 spots from each individual per bag. Each bag of DBS was

exposed to one of 5 time and storage temperature treatments: 1) Baseline (defined below), 2) 30°C for 7 days, 3) 30°C for 14 days, 4) 30°C for 28 days, and 5) 4°C for 38 days. These conditions allowed us to monitor a range of storage conditions and durations likely to be encountered in developing world field research settings, where VAD commonly occurs. The 30°C condition was maintained and monitored with an incubator. The baseline DBS were placed in a -20°C freezer immediately after drying and assayed after 42 days. After their respective treatments, specimens for 7, 14, and 28 days at 30°C, and those for 38 days at 4°C storage were frozen (-20°C) until assay.

The RBP enzyme immunoassay has been described in detail elsewhere (11). The inter- and intra-assay coefficients of variation for the assay are 8.9% and 6.7%, respectively (11, 14). Assay protocol followed Hix et al. 2004 (11) with appropriate modifications for DBS. From each DBS, a ¼” center disk was punched out for elution in assay buffer. All specimens were assayed in a single batch 42 days after specimen collection. Linear mixed effects models of RBP on storage treatment were used to determine if RBP concentrations differed by storage treatment.

Assay results showed that all specimens had RBP concentrations above the cut-off for subclinical VAD (0.96 µmol/L) as shown in **Figure 1**. Data from one subject were excluded from the analysis because baseline RBP values were above the assay upper limit of detection (1.92 µmol/L), and thus valid results for comparison were unavailable. A total of 63 observations were used for statistical analyses. **Table 1** summarizes estimated means based on linear mixed effects models, comparing the baseline to respective treatment condition. RBP remained stable in DBS stored in a sealed bag with desiccant for up to 4 weeks at 30°C. The estimated mean difference between day 7 or day 14 and baseline was less than 0.035 µmol/L ($p > .5$). RBP for day 28 specimens was 0.13 µmol/L lower than

baseline, but this difference was not statistically significant ($p=.083$). It should be noted, however, that there were significant variations in the relationship between storage duration and RBP across subjects ($p=.0001$). Specifically, one subject showed a sizable drop in RBP by day 28, but the other 2 subjects did not (see **Figure 1**). In comparison to 30°C storage, refrigeration had adverse effects on RBP stability. Specimens stored at 4°C for 38 days produced values significantly lower (0.2 umol/L) than baseline ($p=.0023$).

In summary, DBS collected by finger prick, then stored sealed in a plastic bag with desiccant at 30°C for up to 4 weeks, can be used to produce valid estimates of RBP concentrations at the group level. In contrast, RBP stored refrigerated for a similar duration may recover significantly lower concentrations than measured at baseline. In field research, where immediate assay or access to a freezer is not available, RBP in DBS specimens is likely to be more stable stored at ambient temperatures than in a refrigerator.

We also found that the RBP stability profile may vary significantly across subject beyond 2 weeks of storage at 30°C. In our study, DBS of the subject with the lowest baseline RBP concentration showed the greatest drop by 4 weeks. This may indicate that RBP stability is dependent on initial concentration, and that RBP of VAD people may decline at a greater rate than those of vitamin A sufficient people. If this were the case, our findings should be taken with caution since our study subjects were all vitamin A sufficient. Further studies should investigate contributing factors for the individual variation in the stability profile by including a larger number of subjects with varying VA status.

We tentatively conclude that RBP in DBS collected by finger-prick can withstand storage at a relatively high ambient temperature (30°C) up to 2-4 weeks and thus facilitates accurate vitamin A

status assessments at the population level in field settings where immediate cold storage or assay are unavailable. The minimally-invasive and field-friendly medium of DBS from finger prick provides impetus for public health researchers, as well as human biologists, to incorporate estimates of the vitamin A status in their field research.

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Table 1. Comparison of estimated means between baseline and storage time/temperature treatments based on linear mixed effects models.

	Baseline Mean	30°C for 7 days then frozen	30°C for 14 days then frozen	30°C for 28 days then frozen	4°C for 38 days, then frozen
Mean					
($\mu\text{mol/L}$)	1.742	1.706	1.722	1.611	1.539
P	n/a	0.551	0.624	0.082	<0.001
Df	56	56	56	56	56

Figure 1. RBP recovery by time-storage treatment conditions

Dried blood samples stored at 30°C up to 2-4 weeks recovered reliable RBP values. Presented in the figure are raw means, with error bars showing two standard errors for each subject per treatment.

