A HealthTech Report

RBP-EIA: Validation and Establishment of a Rapid, Field-Based Tool for the Assessment of Vitamin A Deficiency: *A Summary of Available Evidence*

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Introduction

The retinol-binding protein enzyme immunoassay (RBP-EIA) test has undergone intensive validation following standard methods[1], both in laboratory settings and in the field. The primary focus of the validation studies has been to establish the correspondence between retinol-binding protein (RBP) and retinol as measured by high performance liquid chromatography (HPLC), which has long been the gold reference standard for the assessment of vitamin A deficiency (VAD) in populations.

The RBP-EIA test was developed with the distinct aim of being able to help researchers and program managers estimate the extent or prevalence of VAD in order to plan and evaluate interventions, such as vitamin A (VA) supplementation and VA fortification. While other more sophisticated (and expensive) tools are capable of measuring VA status with greater precision, the main objective of the RBP-EIA is to discriminate between those in a population with deficiency from those whose VA status is adequate. Prior to the development of the RBP-EIA, several field studies had demonstrated the utility of RBP as an alternative marker of VA status in different population groups.[2,3,4]

1. Validation studies using venous blood samples

Retinol has been recommended by the World Health Organization, UNICEF, and the International Vitamin A Consultative Group for tracking progress towards the elimination of VAD in populations. Thus, it is important to establish the ability of the RBP-EIA to provide similar estimates of VAD prevalence in populations. The test was originally developed to measure RBP in serum that had been separated from whole venous blood collected by venipuncture, so initial validation work was done with this specimen type.

Six studies have assessed the correspondence between retinol and RBP from venous blood samples (Appendix 1). These evaluations of the prognostic capacity of RBP to estimate VAD relative to retinol using venous specimens were conducted in diverse geographical settings in Nicaragua,[5] Cambodia,[6] Tanzania,[7] Senegal,[8] Guinea-Bissau,[9] and Thailand.[10] The prevalence of VAD in the six population groups included in these studies varied from 2.9% to 67.4%, providing a good representation of the degrees of severity of the VAD problem. Across all studies the proficiency of RBP to predict the prevalence of VAD (based on retinol as the reference by HPLC analysis) was very good with sensitivity between 70% and 80% and specificity over 90%.

2. Validation studies using capillary blood samples

Subsequent work has been done to explore the feasibility of using whole capillary blood collected by finger prick as a viable specimen type for VA assessment, stored either as dried blood spots (DBS) on filter paper or in microcapillary tubes after separation of serum in the field. There has been a great deal of interest in the possibility of undertaking finger-prick sampling, since collection of finger-prick blood in the field is relatively painless and noninvasive, and capillary blood sampling eliminates the need for a trained phlebotomist to collect samples. Two validation studies have been undertaken using capillary blood. The first, in Zimbabwe,[11] established the correspondence between retinol and RBP from the same

sample of DBS specimens prepared from capillary blood. In the analysis of a matched panel of 90 DBS specimens, the sensitivity of RBP was 60.0% and the specificity was 97.5% in estimating the prevalence of VAD by retinol, which was 11.1% in the population.

A second, more extensive study undertaken in northeastern Thailand[10] aimed to evaluate the biological equivalence of capillary and venous blood for the assessment of VAD using both retinol and RBP. Researchers collected matched panels of venous and capillary specimens (using microcapillary tubes) from the same cohort of preschool children. The study found a very close correspondence between the three test parameters of VA status, e.g., retinol-capillary, RBP-venous, and RBP-capillary, with retinol from venous blood (taken as the gold reference standard) in classifying VAD prevalence.

The study also collected data on inflammation to determine whether infection would alter the ability of RBP to estimate population VAD. Appendix 2 provides summary results from this study. The prevalence of VAD in the study was 7.2% based on retinol from venous blood. Given the low prevalence, the specificity of all parameters was very high, all above 96.5%. For retinol from capillary blood, the sensitivity was 71.4%, while the sensitivity was 92.9% for RBP from venous blood, and the sensitivity was 85.7% for RBP from capillary blood. For purposes of population assessment, the proficiency of all three test parameters seemed to perform as well as one another in screening for VAD relative to retinol from venous blood.

The significance of the Thailand study was that, for the assessment of VAD prevalence among populations, capillary samples provided good reliable results and could be used as an alternative to venous blood. This is the first time such a result has been demonstrated. Taken together, the Thailand and Zimbabwe studies provide evidence of the feasibility of using capillary blood, although further work is still needed to establish the proficiency of using capillary blood stored as DBS specimens for VAD assessment, including the identification of minimum collection and storage requirements to ensure that there is no degradation of sample quality. The study also found a pronounced association between VAD and both chronic and acute inflammation, an observation that is not surprising given the fact that many acute-phase proteins, such as RBP, are depressed in the presence of infection. This study confirmed earlier findings where RBP was found to be responsive to changes in VA status due to the inflammatory process associated with HIV infection.[12,13]

3. Stability studies

In addition to the validation studies establishing the correspondence of the RBP-EIA with retinol, there have been two studies undertaken to determine the stability of RBP in serum and DBS samples exposed to different environmental conditions, including light and temperature. In the initial test development, PATH conducted stability studies both on serum samples collected from healthy VA-replete volunteers and on DBS specimens prepared from whole blood collected from healthy volunteers. In both sets of specimens, exposure to direct sunlight and high temperature (> 25°C) led to rapid and significant degradation, while samples maintained in the dark at 4ºC and 8°C retained over 90% of the RBP content for as long as 32 hours of exposure. In a subsequent study carried out with serum samples collected from a population with high VAD in Tanzania,[7] there were similar results with rapid loss of RBP as soon as three days following storage when exposed to 37°C, continuing to < 60%

retention by one week. Of interest was the fact that there was little difference in the stability of RBP in samples maintained at 4°C relative to those that were frozen at –20°C through two weeks of exposure. Thus far, stability studies have only been conducted on serum and DBS samples from venous blood and have not been replicated or extended to capillary samples.

Appendix 1. Studies of Correspondence Between Retinol and RBP-Venous Specimens

Country/ Population group	Prevalence of VAD[a]	Sensitivity[b]	Specificity[b]
Nicaragua/ Preschool children (n = 70)	2.9%	68.2	98.1
Cambodia/ Preschool children (n = 359)	22.3%	70.0	93.2
Tanzania/ Preschool children (n = 472)	67.4%	75.5	93.5
Senegal/ School-aged children (n = 70)	38.6%	81.8	84.0
Guinea-Bissau/ Pregnant women (n = 251)	11.6%	75.9	97.7
Thailand/ Preschool children (n = 195)	7.2%	71.4	99.3

[a] Retinol < 0.70 µmol/L.

[b] Sensitivity and specificity of RBP relative to retinol < 0.70 µmol/L.

Appendix 2. Proficiency in Estimating VAD by Different VA Parameters—Controlling for Inflammation Status

(All comparisons are relative to retinol-venous < 0.70 µmol/L.)

	All children (n = 196)		Early convalescence (n = 17)		Late convalescence (n = 47)	
Parameter	**Se[a]**	**Sp[b]**	**Se**	**Sp**	**Se**	**Sp**
Retinol-capillary	71.4	97.2	100.0	76.9	66.7	100.0
RBP-venous	71.4	99.5	75.0	92.3	66.7	100.0
RBP-capillary	64.3	98.9	50.0	100.0	100.0	97.7

[a] Sensitivity

[b] Specificity

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