A HealthTech Report

Correspondence Between RBP and Retinol From DBS Specimens Prepared From Capillary Blood—Evidence From Zimbabwe

January 2006

1455 NW Leary Way
Seattle, WA 98107-5136, USA
Tel: 206.285.3500 Fax: 206.285.6619
www.path.org

USAID FROM THE AMERICAN PEOPLE

PATH A catalyst for global health

Acknowledgements

This document was made possible through support provided by the Office of Health, Infectious Diseases, and Nutrition of the United States Agency for International Development (USAID), under the terms of the HealthTech Award No. GPH-A-00-01-00005. The opinions expressed herein are those of the authors and do not necessarily reflect the views of USAID.

Introduction

The analysis of retinol binding protein (RBP) has been recognized as a valuable proxy indicator for retinol in assessing the prevalence of vitamin A deficiency (VAD) among preschool children at the population level. A new test for the analysis of RBP has been developed by PATH under the HealthTech program to meet the need for a low-cost, robust tool for VA assessment. The retinol binding protein enzyme immunoassay (RBP-EIA) can support health care workers in effectively assessing the prevalence of VAD in populations by alleviating major cost and time constraints inherent in evaluating specimens for retinol by high performance liquid chromatography (HPLC), which has hindered progress micronutrient programs in the past.

In order to validate the utility of the RBP-EIA, a series of field validation studies were undertaken to assess the correspondence between serum retinol and RBP in different populations. In light of this, PATH analyzed a subsample of dried blood spot (DBS) specimens collected as part of a small-scale, maize meal-fortification evaluation conducted in Midlands Province, Zimbabwe, in March 2002.

Background

In June 1998 CARE International began the implementation of its Nutrition Initiative in Communal Areas (NICA) project in Zvishavane district, Zimbabwe. NICA is a pilot project funded by the Canadian International Development Agency. The program aims to improve the VA and iron status among the population in Zvishavane district, Midlands Province. The main component of the NICA tested the feasibility of small-scale, maize meal-fortification at local hammer mills. In order to evaluate the impact and ascertain the sustainability of project activities, an evaluation was proposed to measure changes among the target population in key program parameters. CARE authorized PATH to obtain and use the DBS samples collected in the NICA evaluation in Zimbabwe for research purposes.

Finger-prick blood samples were collected using sterile, disposable lancets. DBS samples were prepared by dropping finger-prick blood on preprinted circles on a coated filter paper (Schleicher & Schuell # 903).

For the collection of the blood spots, hands were warmed, the finger was cleaned with alcohol and pierced with a lancet, and the first drop of blood was discarded until a free flow was observed. Two to five circles on each filter paper were filled depending upon the flow of blood. After marking the child’s identity and name, the filter papers were dried for two to three hours in plastic boxes closed air tight and covered with black paper to prevent any potential photo oxidation of the sample.

After the spots were completely dried, the filter papers were placed in zipper-locked polythene bags along with silica gel. Individual bags were placed in a black polythene bag and kept in a vaccine carrier containing ice packs. During each round of enumeration the samples were stored in deep freezers (< –20°C) available at different health facilities in the field, e.g., primary health centers and district hospitals. The stored samples were transported with dry ice to Craft Technologies, Inc. (Wilson, NC, USA) where they

were analyzed following established HPLC methodology.[1, 2] After analysis, Craft stored the samples under frozen conditions until they were shipped with ice packs to PATH. Samples were received by PATH with the original retinol results with identification data to facilitate linking of records.

The DBS samples were analyzed for their RBP content using the PATH RBP-EIA kit, with samples tested in duplicate and the mean value calculated. These results were compared to the retinol results, and statistical analysis was conducted to assess agreement between the two methods. Data were dichotomized using internationally accepted cut-off points to classify deficiency, and indices of agreement (sensitivity, specificity, and predictive value) were calculated.

Results

A total of 90 specimens were found suitable for reanalysis based on sample integrity. Many of the DBS specimens were not complete (including several which had punches indicating that they had been analyzed for their retinol content), some had mold, while several specimens had been collected on a different filter paper matrix (the majority had been spotted on S&S # 903 paper). Samples deemed inappropriate for analysis were excluded from the study. The prevalence of vitamin A deficiency (VAD) was calculated using both retinol (< 0.70 μmol/L) and RBP (using both < 0.70 μmol/L and < 0.775 μmol/L as cut-off points). Table 1 presents these data.

Table 1. Prevalence of VAD Using Retinol and RBP

Indicator	Cut-off point	Prevalence
Retinol	0.70 μmol/L	11.1 %
RBP	0.70 μmol/L	8.9 %
RBP	0.775 μmol/L	13.3 %

Two sets of 2x2 tables were prepared to evaluate the correspondence between the two indicators of VAD in classifying VAD. In Table 2, the prevalence of VAD is based on the same cut-off point, and a sensitivity of 60% and specificity of 97.5% was observed on the diagnosis of low retinol using the RBP test as a screening tool.

When the cut-off point was raised to 0.775 μmol/L for RBP as suggested from receiver operating curve analysis of previous studies evaluating the relationship between retinol and RBP, there was a significant improvement in sensitivity with only a minor compromise to specificity (Table 3).

Table 2. Correspondence Between Retinol and RBP (same cut-off point < 0.70 μmol/L)

	Retinol		
RBP	< 0.70 μmol/L	> 0.70 μmol/L	**Total**
< 0.70 μmol/L	6	2	8
> 0.70 μmol/L	4	78	82
	10	80	90

Sensitivity = 60.0%, Specificity = 97.5%, Predictive Value = 95.1%

Table 3. Correspondence Between Retinol and RBP (retinol < 0.70 μmol/L, RBP < 0.775 μmol/L)

	Retinol		
RBP	< 0.70 μmol/L	> 0.70 μmol/L	**Total**
< 0.775 μmol/L	8	4	12
> 0.775 μmol/L	2	76	78
	10	80	

Sensitivity = 80.0%, Specificity = 95.0%, Predictive Value = 97.4%

Conclusion

Based on this preliminary evaluation of 90 samples collected under field conditions, there was a significantly good correspondence between RBP and retinol in estimating the prevalence of VAD and in screening for deficiency. While the integrity of the specimens was not controlled and there is no way to verify the reliability of the retinol analysis, these results point to the fact that DBS samples prepared from capillary blood collected by a finger-prick method may prove to be invaluable specimens for analysis of RBP using a rapid ELISA test.

---

[1] Craft NE, Haitema T, Brindle LK, Yamini S, Humphrey JH, West KP. Retinol analysis in dried blood spots by high performance liquid chromatography: method development. *Journal of Nutrition*. 2000;130:882–885.

2 Craft NE, Bulux J, Valdez C, Li, Y, Solomons NW. Concentration of capillary blood spots from healthy volunteers: method of validation. *American Journal of Clinical Nutrition*. 2000;72:450–454.

jgrp24474