A HealthTech Report

Validation of a Retinol-Binding Protein- Enzyme Immunoassay (RBP-EIA) Using Serum Specimens Collected From the Guinea-Bissau Health Project

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.

1. Introduction

This study was a collaboration between PATH, under the HealthTech program, and the The Royal Veterinary and Agricultural University (Denmark) which had recently completed operational research to assess the role of micronutrients in pregnancy on reproductive health in Guinea-Bissau. While the main objective of the original research was to examine the effects of antenatal supplements on adverse pregnancy outcome and peri- and neonatal mortality, the protocol collected a matched panel of serum and capillary dried blood spot (DBS) specimens from 750 pregnant women. These specimens were made available to PATH for further validation of the retinol-binding protein enzyme immunoassay test (RBP-EIA) (from serum) as well as to consider the feasibility of using DBS collected from capillary blood by finger puncture as a specimen for the analysis of RBP using the RBP-EIA.

This study used the existing serum specimens collected during a study of pregnant women in Bandim, Belem, and Mindara districts in Bissau, the capital of Guinea-Bissau. These areas had been under demographic surveillance by the Bandim Health Project for many years. The surveillance included a monthly visit to all houses in the area in order to identify new pregnancies. Hence, virtually all children born in the area had been identified prior to birth. A total of 2250 pregnant women were recruited at the first antenatal visit, between 16 and 24 weeks pregnant. Enrollment took place over a 1½ year period. For the sub-study to explore biological mechanisms responsible for the effects of the micronutrient supplementation, a cohort of 750 women was established. Nutritional status of these women was assessed at mid-pregnancy and two months after delivery. The serum specimens were collected in the period from May 2001 to February 2002.

Venous blood was collected in dry tubes, allowed to coagulate, and the serum was stored in cryotubes at –20°C in Bissau until they were transported (after a maximum of 6 months) to Copenhagen, Denmark, where they were stored at –80°C until analysis. Aliquots of the serum had already been subjected to analysis for ferritin, folic acid, CRP, transferrin-receptors, cholesterol, and retinol prior to specimens being sent to PATH. Capillary blood obtained by finger prick was collected in heparin-coated capillary tubes and placed on Schleicher & Schuell #903 filter papers, where it was allowed to dry before the samples were packed individually in plastic bags and stored at –20°C.

A total of 750 sera and 750 DBS specimens were shipped from Denmark to PATH where they were then prepared for analysis. Unfortunately, a large number of sera were not of sufficient quality to be analyzed due to hemolysis and loss of volume. In addition, there were problems with a substantial number of the DBS specimens where the filter paper matrix had been touched to the finger when blood was transferred, causing the paper to bunch up and render the DBS specimens unreliable. Consequently there was concern about having accurate volume estimates from punches taken from the cards. Of the total of 750 DBS specimens, less than 10% of the samples were able to be eluted and subjected to analysis for their RBP content.

2. Results

PATH completed analysis of a total of 254 serum samples for their RBP content. Of these, 251 had matched retinol samples and it was possible to establish the correspondence between RBP and retinol from the serum, as summarized in Table 1. There was no difference in the prevalence of vitamin A deficiency (VAD) estimated from the two indicators of VAD (11.6% vs. 10.8%), while the sensitivity of RBP-sera in screening for VAD as classified by retinol was 75.9% and the specificity was 97.7%. This is very much in accordance with other studies in which RBP analyzed by the RBP-EIA has been compared to retinol.

Table 1. Correspondence Between Retinol (Serum) and RBP (Serum), n = 251

	Retinol		
RBP-sera	< 0.70	> 0.70	
< 0.70	22	5	27
> 0.70	7	217	224
	29	222	251

Prevalence (retinol)	11.6%	(7.8,15.6)
Prevalence (RBP-sera)	10.8%	(6.8,14.2)
Sensitivity	75.9%	
Specificity	97.7%	

Correspondence between RBP from sera and DBS

As above, over 90% of the DBS specimens received had insufficient volume or had been compromised due to direct contact between the finger of the individual from whom the capillary blood was taken and the filter paper matrix. Consequently only 73 specimens were analyzed for their RBP content in order to assess the correspondence between RBP-DBS and RBP-sera and between RBP-DBS and retinol. Although these numbers are low, they provide an important initial impression of the feasibility of DBS specimens from capillary blood as a potential specimen type for use with the RBP-EIA. The data from these initial experiments are summarized below and must be treated with caution due to the small number of samples analyzed.

The characteristics of the subsample of individuals from whom DBS specimens could be analyzed were a little better than the general population, as evidenced by the fact that the VAD prevalence was only 6.8% (according to retinol) and 5.6% (according to RBP-sera), while for the full data set, the VAD prevalence was 11.6% (for retinol) and 10.6% (for RBP-sera) as above. Using both retinol as the reference standard (Table 2) and RBP-sera as the reference (Table 3), the specificity was still close to 95% indicating a good overall agreement. However, the sensitivity measures were not as robust as seen in the

correspondence between RBP and retinol from serum, which may have been due to the lower prevalence observed in the subsample or the poor quality of DBS specimens.

Table 2. Correspondence Between Retinol (Serum) and RBP (DBS)

	Retinol		
RBP-DBS	< 0.70	> 0.70	
< 0.70	3	4	7
> 0.70	2	64	66
	5	68	73

Prevalence (retinol)	6.8%	(7.8,15.6)
Prevalence (RBP-DBS)	9.6%	(6.8,14.2)
Sensitivity	60.0%	
Specificity	94.1%	

Table 3. Correspondence Between RBP (Serum) and RBP (DBS)

	RBP-Sera		
RBP-DBS	< 0.70	> 0.70	
< 0.70	3	4	7
> 0.70	1	65	66
	4	69	73

Prevalence (RBP-sera)	5.5%	(7.8,15.6)
Prevalence (RBP-DBS)	9.6%	(6.8,14.2)
Sensitivity	75.0%	
Specificity	94.2%	

3. Conclusions

The Guinea-Bissau data provide an additional validation of the RBP-EIA from serum samples, while also providing some initial data on the feasibility of using DBS as a specimen type for the assessment of VAD in the field. Unfortunately, the quality of the DBS specimens was not satisfactory sufficient for more than 90% of the samples those received, and this study highlighted the importance of ensuring good specimen collection procedures which are essential for the viability of DBS. This seems to be particularly important for DBS specimens where the total volume of whole blood stored on the filter paper matrix is relatively low so efforts to ensure full saturation of the matrix, complete drying prior to storage, and then proper storage, are all maintained. As further work is done to explore the utility of DBS, it will also be important to understand the optimal conditions required for storage, e.g.,

temperature, light, and humidity, to eliminate any degradation of the RBP content in the samples through the implementation of appropriate stability studies.

jgrp24476