Collection, Processing, Storage, and Transport of Dry Blood Spots for the Assessment of Vitamin A Deficiency in the Uganda Demographic and Health Survey 2006

R Baingana [1], D Kasozi [1], D Garrett [2, 3], and A Fort [2, 3]

[1] Department of Biochemistry, Makerere University, Kampala, Uganda; [2] Macro International, Demographic and Health Surveys, Calverton, MD, USA; [3] PATH, Seattle, WA, USA

Background

Vitamin A deficiency (VAD) is a cause of significant morbidity and mortality among women and children in developing countries (Figure 1). Adequate epidemiologic data are critical for the development of appropriate VAD intervention programs; however, the collection of these data has been hampered by the lack of affordable, valid, and reliable assessment tools that are appropriate for low-income settings.

For example, the assessment of VAD is traditionally done on serum separated from venous whole blood, the collection and processing of which presents logistical challenges, especially in resource-poor countries. An alternative to serum is the dried blood spot (DBS). The collection of DBS from a finger prick is simple to perform, requires minimal training of field workers, and poses fewer risks of trauma and infection to respondents than venipuncture.

Methods

This study was conducted as part of the Uganda Demographic and Health Survey 2006. A representative probability sample of 9,864 households, including Internally Displaced People's camps, was selected for the survey. All women aged 15–49 who were either permanent residents of the households or visitors present in the household on the night before the survey and children aged 6–59 months were eligible for VAD assessment. Data were collected from early May 2006 to early October 2006. A total of 5,642 DBS samples were collected.

Training of Field Technicians

Fifteen female field workers were trained for 7 days to collect blood samples from a finger prick by spotting the blood drops onto special filter paper cards. Training was held in a one-room, classroom-style facility.

Collection of DBS

Blood samples were collected in the household from eligible women and children by finger or heel prick. The puncture site was cleaned with alcohol, air dried, and punctured with a sterile retractable lancet. Blood drops were allowed to fall freely inside preprinted circles on special filter paper cards. The filter paper cards were placed in drying boxes containing desiccants to protect the samples from moisture.

Storage and Transport of DBS

The DBS samples were kept in the field in drying boxes containing desiccants and a humidity indicator card to monitor the moisture buildup in the boxes. At the end of the day the blood samples were taken to the field house and dried further overnight (Figure 2).

The following morning the DBS were placed in plastic bags containing desiccants and a humidity indicator card and stored in a battery-operated refrigerator. Samples were sent to the laboratory in Kampala in portable refrigerators within 7–10 days of collection and stored at -20°C (Figure 3).

USAID FROM THE AMERICAN PEOPLE

Figure 1. Global prevelance of VAD

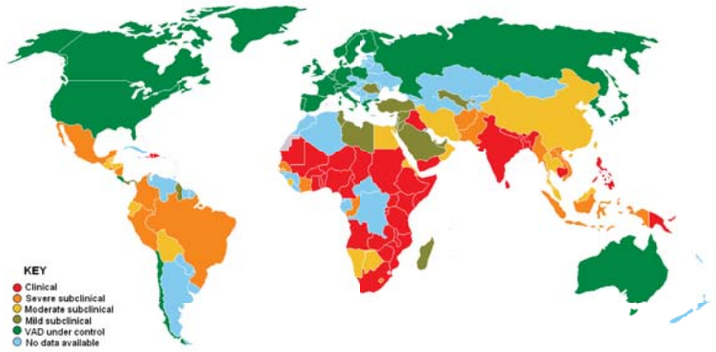


Figure 2. Blood spots drying in a storage box

Figure 3. Supplies for packaging the DBS cards

Analysis of Samples

On arrival at the Department of Biochemistry, Makerere University, samples were checked against transmittal sheets and stored at -20°C until analysis. On the day before analysis, one ¼" punch was taken from the center of each of two DBS circles and placed into a microcentrifuge tube. A 300 µL of sample diluent was added and the samples eluted for 18–20 hours at 4–8°C.

On the day of analysis, samples and reagents were removed from the refrigerator and left for 90–120 minutes to attain room temperature (22–25°C). Samples were analyzed using Scimedx RBP Assay (Scimedx Corporation, Denville, NJ, USA). Analysis followed the manufacturer's instructions with the following adjustments:

Conjugate: The conjugate concentration was increased to 1:1000 from 1:1500.

Washing: Wells were washed 6 times instead of 5 times.

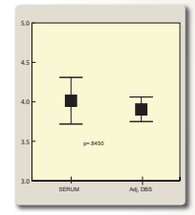
Calibration points: Calibration points were increased from 3 to 5 by including 15 µg/mL and 30 µg/mL calibration points.

Results

Validation Study

Matched DBS-serum samples from volunteers demonstrated that adjusted concentrations of RBP from DBS were comparable to RBP from serum (Figure 4). The means (±SEM) of serum RBP and DBS RBP were 39.74 ± 2.90 and 39.09 ± 1.69, respectively (p=0.35).

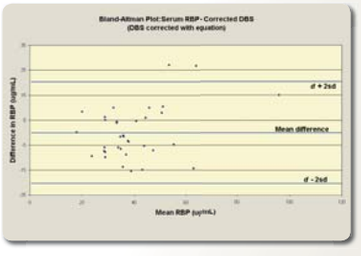
Figure 4. Box plot of serum and adjusted DBS RBP



Quality Control

1. A comparison of the experimental values of calibrators and controls with the expected values showed that there was good agreement (CV<20%) between experimental and expected calibrator values at all 5 calibrator points for 124 (88%) of 141 plates.
2. Bland-Altman analysis showed that results by different analysts on the same samples were within limits of agreement, and that differences within the limits of agreement were not important in this population-based survey (Figure 5).

Figure 5. Bland-Altman plot of paired serum and DBS



Conclusion

The adjusted concentrations of RBP from DBS were comparable to RBP from serum, suggesting that DBS is a viable alternative sample to serum for VAD assessment in population-based surveys. Analysis of RBP from DBS for VAD did not pose any analytical challenges to the laboratory staff. There were no logistical challenges associated with the collection, storage, or transfer of DBS under field conditions.