MEETING REPORT

Use of dried blood spot specimens to measure retinal-binding protein

March 1, 2018
Atlanta, GA
Acknowledgements

Thanks to USAID and CDC for convening the meeting, and to CDC and Emory University for hosting. A big thank you to Ms. Sarah Zimmerman for moderating. Thanks to all participants for their valuable input.

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## List of Key Terms

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<th>Term</th>
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<tr>
<td>Biomarker</td>
<td>an indicator of a biological state or condition</td>
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<td>Dried blood spot (DBS) sample</td>
<td>spot blood onto filter paper</td>
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<td>Enzyme immunoassay (EIA)</td>
<td>a method for the detection of antibodies or antigens in biological samples</td>
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<td>Enzyme-linked immunosorbent assay (ELISA)</td>
<td>a method of testing biomarkers</td>
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<td>High-performance liquid chromatography (HPLC)</td>
<td>a method of testing biomarkers</td>
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<tr>
<td>Modified Relative Dose Response (MRDR)</td>
<td>qualitative indicator of vitamin A liver reserves (low to adequate, does not measure toxicity)</td>
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<td>Serum Retinol</td>
<td>main circulating form of vitamin A, used to assess vitamin A status</td>
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<td>Retinol-binding protein (RBP)</td>
<td>a carrier protein for retinol that has been used as a proxy for retinol to assess vitamin A status</td>
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<tr>
<td>Vitamin A</td>
<td>a micronutrient that plays an important role in vision and immune health</td>
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<td>Vitamin A status</td>
<td>state of being vitamin A deficient or sufficient</td>
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<tr>
<td>VITAI-EQA – External Quality Assurance</td>
<td>a program to support external quality assurance for nutritional biomarkers</td>
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# Meeting Agenda

**Thursday, March 1, 2018:**

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<th>Time</th>
<th>Session</th>
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<td>08:30-09:00</td>
<td>Welcome, meeting objectives, and introductions</td>
<td>Dr. Omar Dary</td>
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<tr>
<td>09:00-09:30</td>
<td>Overview of retinol-binding protein assays and factors affecting the results of retinol-binding protein in dried blood spots</td>
<td>Dr. Neal Craft</td>
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<td>09:30-10:00</td>
<td>Principle, validation, and experiences of dried blood spots for retinol-binding protein</td>
<td>Dr. Dean Garrett</td>
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<td>10:00-10:30</td>
<td>Questions and Answers</td>
<td>Dr. Lisa Rogers (moderator)</td>
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<td><strong>10:30-11:00</strong></td>
<td><strong>Coffee/tea break</strong></td>
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<tr>
<td>11:00-11:30</td>
<td>Methodology used, and results from testing dried blood spots for retinol-binding protein in the different DHSs of Uganda</td>
<td>Ms. Rhona Baingana</td>
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<tr>
<td>11:30-12:30</td>
<td>Results of retinol-binding protein using dried blood spots and blood collected in microtainer tubes in Uganda</td>
<td>Ms. Rhona Baingana, Dr. Donnie Whitehead</td>
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<tr>
<td><strong>12:30-13:00</strong></td>
<td><strong>Lunch</strong></td>
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<td>13:00-14:00</td>
<td>Questions and Answers</td>
<td>Dr. Sarah Zimmerman (moderator)</td>
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<tr>
<td>14:30-15:00</td>
<td>Questions and Answers</td>
<td>Ms. Sarah Zimmerman (moderator)</td>
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<td><strong>15:00-15:30</strong></td>
<td><strong>Coffee/tea break</strong></td>
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<tr>
<td>15:30-17:30</td>
<td>Discussion and Next steps</td>
<td>Ms. Sarah Zimmerman (moderator)</td>
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Introduction

Vitamin A deficiency is a major public health problem that results in blindness, poor immune function, and in some cases death.\(^1\) The prevalence of vitamin A deficiency varies substantially by geographical setting, and is a problem in many countries in Africa and Southeast Asia.

Serum retinol is the biomarker recommended by the World Health Organization (WHO) to assess vitamin A status at the population level. The use of high-performance liquid chromatography (HPLC) is the method of choice to measure serum retinol. Specifically, WHO recommends using at least two biological indicators, serum retinol and another biological indicator of vitamin A deficiency (e.g., night blindness, breast milk retinol, relative dose response, modified dose response or conjunctival impression cytology), to assess vitamin A status and to determine if vitamin A deficiency is a public health problem.\(^2\) Alternatively, assessing serum retinol and at least four demographic and ecological risk factors (i.e., two of which are nutrition or diet related) can be used to determine if vitamin A deficiency is a public health problem.\(^2\)

Retinol-binding protein (RBP) has been used as a proxy for serum retinol because of its biological relationship with retinol. RBP is a carrier protein for retinol. However, there is no method for measuring RBP that is agreed upon globally, nor WHO cutoffs to define vitamin A deficiency using RBP. Thus, if RBP is used to assess vitamin A status, the methods must be validated against serum retinol.

A meeting was convened by USAID and CDC to discuss the use of dried blood spot (DBS) specimens to measure RBP in the context of household surveys. The meeting reviewed the background of serum retinol and RBP measurement, use of assays and challenges, experiences using DBS, and comparison between assays and biomarkers to measure vitamin A.

This report highlights issues related to the use of RBP assays to measure vitamin A.

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Welcome and meeting objectives

Dr. Omar Dary from USAID opened the meeting by emphasizing that the accurate assessment of vitamin A status at the population level is critical to monitor programs, such as vitamin A-fortified sugar in Guatemala and vitamin A-fortified oil in Uganda. The approach used to measure vitamin A status is crucial to ensuring results accurately reflect vitamin A status. Limitations in methodological approaches can hinder the ability to evaluate the impact of vitamin A interventions, and result in providing too much—or too little—vitamin A to a population that may not be vitamin A deficient. With these programmatic implications in mind, USAID convened a meeting organized by The Demographic and Health Survey (DHS) program and the International Micronutrient Malnutrition Prevention and Control (IMMPaCt) program of the Centers for Disease Control and Prevention (CDC). The meeting objective was to review new data on the assessment of vitamin A status using a commercial retinol-binding protein assay. This stemmed from an interest in comparing vitamin A status data between Uganda’s newly established micronutrient surveillance system and the Uganda DHS survey.

A group of experts was convened to review the results of a study designed to assess the comparability between the results from the Uganda DHS survey and their nutrition surveillance system. The RBP commercial enzyme immunoassay (EIA) method (used in the 2006, 2011, and 2016 Uganda DHS) was unreliable based on the Makerere/CDC 2016-2017 assessment.

A brief history of vitamin A assessment was provided. The experience of Instituto de Nutrición de Centro América y Panamá (INCAP) in assessing vitamin A status in Guatemala was described. The use of fluorometry as an alternative to high-performance liquid chromatography (HPLC) to measure retinol was explored, but without success. In 2002, with the development of new technologies, a consultation was held by The USAID Micronutrient Program (MOST – Micronutrient Opportunities, Strategies and Technology). In this meeting, they discussed two simple and affordable laboratory methods for the assessment of vitamin A status. The first was the measurement of retinol in dried blood spots by HPLC performed by Craft Technologies, Wilson, North Carolina. While this method showed promise at the time, the challenge has been the lack of reproducibility of the method in other laboratories. DBS is appropriate for the qualitative detection of a disease (positive/negative response), but quantifying the respective biomarker concentrations is more difficult, which is also the case for RBP or retinol.

The second method discussed was the measurement of RBP, a proxy for retinol, using an EIA method developed by the Program for Appropriate Technologies in Health (PATH). RBP is easier to measure than retinol because it is based on an antigen-antibody reaction, whereas measuring retinol requires HPLC, which is very challenging to perform. There is still a lack of consensus in the micronutrient community about whether RBP reflects vitamin A status (e.g., RBP is an acute phase protein and the ratio between RBP and retinol may not always be consistent), but these are separate from our ability to accurately measure RBP concentrations. However, by 2002, the PATH-RBP method with serum/plasma samples showed the linear response was narrow; the monoclonal-RBP method was able to predict at the population level the proportion of samples below a certain cutoff, but as the variation was too large, the method was not valid for individual assessment. Nevertheless, despite this limitation, the same method was later applied to DBS, when acceptable performance would be at higher risk. This meeting was convened to discuss the results using the DBS RBP-EIA method [which PATH sold to another company once developed] as compared with others, to determine whether or not it is suitable to continue promoting it.
Overview of retinol-binding protein assays and factors affecting the results of retinol-binding protein in dried blood spots

Dr. Neal Craft from Eurofins Craft Technologies presented background on the history of RBP, use of assays to measure RBP, and associated challenges, and provided an overview of vitamin A absorption and transport in the body. Dietary vitamin A is mainly stored in the liver as retinol. RBP4, a transport protein, is primarily produced in the liver in the apo-form (RBP without retinol) and is released from the liver in the holo-form (retinol bound to RBP). Serum retinol has been a longstanding biomarker for vitamin A, and consistent relationship has been found between lower levels of serum retinol and clinical manifestation of vitamin A deficiency.

The Carr-Price assay was the first assay used to measure serum retinol, and was based on colorimetry. Later the Futterman assay was used, which measures fluorescence to detect holo-RBP. However, assessing vitamin A in a field setting with these assays was not practical; neither was the HPLC method for measuring serum retinol. In the 1990s it was established that holo-RBP (i.e., retinol bound to RBP) could be measured in dry blood spots (DBS) using capillary electrophoresis with laser-excited fluorescence detection. This established that retinol was protected in DBS while associated with RBP. With adequately sensitive equipment, it was possible to measure retinol from intact DBS using HPLC, but it was difficult to extract an adequate amount.

The use of RBP has increasingly been used as a proxy for retinol because RBP is transported in a 1:1 molar ratio with retinol (unless vitamin A stores in the liver are very low), and RBP is more stable than retinol, although retinol bound to RBP in DBS holds up well. RBP is used as a surrogate for retinol because it is less expensive to measure, and requires lower-tech equipment. Assays used to detect RBP4 react not only to holo-RBP but also to the apo-RBP.

Dr. Craft then explained the common features of assays. He described how assays require an anti-RBP to bind RBP, which may be monoclonal or polyclonal, and that binding sites have different affinities and bind to different parts of the RBP molecule. Thus, it is important for users to research the composition of the kits. The assays measure RBP indirectly and require a form of detection to measure the amount of bound antibody. Sandwich enzyme-linked immunosorbent assays (ELISAs) use an enzyme-substrate complex to form a colored end product, and the intensity of the colored product is proportional to the amount of analyte in the sample. Some RBP assays are competitive ELISAs, which means that the RBP in the blood sample competes with conjugated RBP in the assay reagent. Thus, the amount of RBP in the sample is inversely proportional to the color (optical density reading) of the end product. Additionally, some assays are calibrated to different forms of RBP (truncated, complete, or RBP:TTR). The most desirable calibration would be with the form that is measured in the blood, RBP:TTR complex. All of the assays require calibration and must also have a measure of the indicator (e.g., colorimetric, fluorescence, etc.).

There are different types of antibodies to RBP. Polyclonal antibodies bind to multiple sites on RBP, whereas monoclonal antibodies bind a single site on RBP. Currently, the VitMin Laboratory is using assays with polyclonal antibodies that are no longer commercially manufactured. Common types of ELISA for RBP are the competitive, or sandwich ELISA. Briefly, with some forms of competitive assays, RBP is adsorbed to the bottom of the wells of a microtiter plate, and competes with RBP (in the sample) for binding sites on RBP antibodies. To perform the assay, samples, calibrators and controls are added to respective individual wells. Immediately after, a monoclonal, polyclonal anti-RBP or IgG antibody conjugated to an indicator is added to the wells. The RBP in the sample competes with the RBP adsorbed on the bottom of the wells of the microtiter plate. With sandwich
ELISAs, the wells of the microtiter plate are coated with an antibody against RBP. Samples, calibrators, and controls are added to respective wells and the RBP in the samples, calibrators, and controls bind to the antibody in the well and reflect the amount of RBP in the sample. Next, a second antibody against RBP that is conjugated to an indicator is added to the wells. It is important to know that there are several types of commercially available RBP assays; however, there is no standardization in how they are manufactured.

Dr. Craft summarized challenges of commercial RBP assays including inconsistent methods of calibration; variation in the protein used for calibration; variation in the type of EIA; variation in the type of antibody used; and variation in the type of detection antibody (conjugated anti-RBP, labeled anti-IgG, Avidin-biotin). Despite these challenges, there are steps that can be taken to improve the reliability and standardization of RBP assays, including providing proof of assay validation; using consistent methods of calibration; documenting historical performance (e.g., manufacturer, type of EIA, type of detection antibodies); testing of batch-to-batch variability; and the availability of common set of serum samples with a wide range of known concentrations.

Discussion:

I. There seemed to be a good relationship between RBP and retinol historically, but more recent studies show the 1:1 molar ratio between retinol and RBP does not always hold.

II. The presence of obesity and liver disease may potentially alter the 1:1 molar ratio. Although study results are mixed, the majority of studies show RBP4 released from adipocytes to be elevated among obese individuals, and much of this RBP4 is in the apo-RBP4 form.

III. Participants stated the integrity of sample could be compromised, and this would result in there not being a 1:1 molar ratio.

IV. The majority of experts agreed little is known about assay calibration, and different assays seem to give different results. The ELISA kits are designed to measure RBP, but it is not always clear from the manufacturer if the assay measures holo-RBP, apo-RBP, or RBP:TTR.

V. It is possible to get a molar ratio that is greater or less than 1, and the correlations can still be strong, but the RBP value can be off by as much as 50% of the true value. Addressing this in the field can be a challenge, and it was suggested not to trust RBP, but the retinol. Dr. Craft noted if there is a 1:1 molar ratio of RBP and retinol, using correlation plots at 1 µmol/L RBP you expect to see close to 1µmol/L retinol (because retinol should be ~90% saturation).

VI. The group recognized that the use of DBS samples raises concerns.

   a. Most acknowledged that although using DBS-RBP to measure vitamin A can be done, there are several limitations to using this methodology.
   
   b. The VitMin Laboratory no longer accepts DBS because of concerns with the quality of the DBS samples.
   
   c. Most literature indicates that there is ~20-30% loss of retinol recovery from DBS during the first 7-14 days. This decline seems to occur for RBP, but it may alter the retinol:RBP 1:1 molar ratio.
   
   d. If the DBS sample is not of good quality, then there is no way to generate good-quality data. For example, if the preprinted circle on the filter paper card is not saturated through with blood, you cannot get a consistent, known volume of serum or plasma.
Principle, validation, and experiences of dried blood spots for retinol-binding protein

Dr. Dean Garett from PATH provided a summary of The DHS Program’s experience using DBS to measure RBP. He described the basis for using RBP as a surrogate marker for retinol to measure vitamin A status. RBP transports retinol in the plasma, and the ratio of RBP to retinol has been found to be closely related. While retinol is the biomarker of choice, RBP is more feasible to measure in field settings. The DHS Program uses an EIA for the detection of RBP, which had been validated against retinol in the early 2000s. The correlation between retinol by HPLC and RBP by EIA was found to be approximately 0.80. The RBP assay that the DHS uses is a commercially available competitive assay for use with serum and DBS.

The DHS Program measures RBP on DBS in a few countries and supports the countries’ laboratories to conduct these tests; countries include Tanzania, Uganda, and Zimbabwe. They have not found major challenges for sample collection and storage. The in-country laboratories have highly skilled staff with experience in the EIA method, and biochemical analyses.

The main difficulty encountered has been identifying a second laboratory in country with high enough capacity to conduct the external quality control. In an attempt to mitigate this, The DHS Program has created standard procedures that laboratories are trained to carefully follow. A second challenge has been the sourcing of assay kits – at times there have been delays from the manufacturer. Further, not all commercial assay kits perform well, and in Uganda 2011 the supplier had trouble sourcing the antibody used in the assay. There is a need for more rigorous standardization of the manufacture of assay kits, such as the quality of antibodies, consistency in the antibody used to manufacture the kits, and better labeling of kits to clearly identify the type and source of antibodies used to make the kits.

To overcome the challenge of not having an external QC lab, a viable alternative is to validate the RBP results against serum retinol by measuring serum retinol by HPLC in 5-10% of the survey population. Although sub-sampling 5-10% of samples for testing for retinol has the potential to improve the quality of the VA data, The DHS Program does not do HPLC in their surveys since many of the countries in which the DHS works do not have the equipment or technical laboratory knowledge. If this validation approach were used in every survey where RBP was measured, there would need to be a laboratory assessment and capacity building for this measurement. The competitive assay used by The DHS Program requires substantially less expertise than the HPLC method. CDC has worked with The DHS Program to increase data quality for HIV testing (e.g., by providing well-characterized DBS controls and a QC panel, always using controls and paired samples, and obtaining a panel from the CDC for the laboratories to samples before they are allowed to test HIV on DBS) – something like this could be done for micronutrient assessment.

Discussion:

I. The use of the DBS and blood from a Microtainer® was compared using the same assay in a small sample in Uganda with support from The DHS Program.

II. The manufacturers of the RBP kits have not compared RBP using DBS against retinol, and the RBP: retinol molar ratio using DBS is unknown.
Methodology and results of dried blood spots for retinol-binding protein in the different DHS surveys of Uganda

Ms. Rhona Baingana from Makerere University described the assessment of vitamin A in three DHS surveys in Uganda, including her collaboration with Dr. Craft for the 2001 Uganda DHS. She highlighted the importance of being able to monitor vitamin A and other micronutrients using inexpensive techniques that are able to be conducted in country.

She summarized the DHS survey protocol used in Uganda for sample collection, storage, and transportation. Briefly, blood drops are placed directly onto a filter paper card from a finger-prick; samples are dried overnight in boxes with desiccant and, when dried completely, packed individually in Ziploc bags with a desiccant and humidity indicator card. Samples for a cluster are gathered in a larger Ziploc bag with desiccant, stored in a portable freezer in the field, and delivered every 8-10 days to Makerere University where samples are stored at -20°C until analysis. Poor spots are identified based on how filled the preprinted circle is with blood, and if the sample is not packed with desiccant and a humidity indicator card. These samples are not tested.

As mentioned earlier, the laboratory uses a commercially available RBP EIA kit. The kits are optimized with paired serum and DBS samples from a minimum of 20 volunteers. Slight modifications from the manufacturer’s instructions have been made (e.g., six washes instead of five, and increasing the calibration points from three to five). Briefly, to perform the assay, two 6mm discs are punched from a single blood spot (preprinted circle on the filter paper card) and the discs are eluted overnight in phosphate buffer. The next day, the eluates, pre-prepared calibrators, and conjugate are added to the plate, and the plate is incubated for 15 minutes. After 15 minutes, unwanted materials are removed from the plate with a manual aspirator before the addition of a substrate. The plates are then incubated for 10 minutes before stopping the reaction with acid. The color produced in the wells is read using a microplate reader to determine the amount of RBP in the samples.

A validation of RBP in paired DBS and serum (or matching DBS and serum samples) was conducted for all surveys. Samples differed by survey. In 2006 and 2011 they used 50 adults, and in 2016 they used 75 children. The coefficient of determination was 0.63 in 2006, 0.56 in 2011, and 0.66 in 2016; the slopes were 0.64, 0.49, and 0.82, respectively. Vitamin A deficiency adjusted for inflammation was 13.9% in 2006, 32.6% in 2011, and 8.9% in 2016 among children (unadjusted was 20.4%, 38%, and 15.1%, respectively). Vitamin A deficiency was 19.4% in 2006 and 35% in 2011 among women (not adjusted for inflammation because the difference in mean RBP between the normal CRP and raised CRP groups was not statistically significant), and was not measured in 2016 among women. Challenges with dried-out calibrator stock and conjugate occurred in 2006 that affected entire lots. In 2011, there were problems with the assay kits, delays, and in some cases no antigen bound the plates, although only a small percentage of assay kits were not able to be used.

Discussion:

I. The DBS-RBP and serum RBP were poorly correlated, particularly in 2006 and 2011.

II. Removing RBP > 40ug/ml, which is beyond the limit of detection of the assay, would result in improvement for both the slope and correlations.

III. A 0.8 slope (as in 2016) makes sense because a 20% difference may occur due to the incomplete recovery of RBP from the DBS sample (which the DHS accounts for). However, for the 2001 and 2006 surveys, where slope is lower, there could be issues with degradation of the sample or the quality of the RBP kits.
IV. Using a microplate washer instead of manual washing results in more reproducible results and reduces substantial work, but they have been manually washed because the washer was not working properly.

V. The group discussed the rationale for using RBP-DBS with the kits. The kit was validated against serum retinol when developed by PATH and showed a strong correlation between retinol (HPLC) and RBP when measured in serum. The limitation is not knowing whether there is a consistent correlation between RBP-DBS and serum retinol.
Results of retinol-binding protein using dried blood spots and microtainer tubes in Uganda

Ms. Baingana and Dr. Donnie Whitehead co-presented results of a study in Uganda comparing vitamin A methods. The objective was to compare the use of the assay used to assess vitamin A status by Uganda DHS and the Uganda surveillance system. They compared DBS-RBP (using the same kits used in the 2016 Uganda DHS, Makerere University), serum RBP (VitMin Laboratory), and serum retinol/modified relative dose response (MRDR) assays (HPLC) (University of Wisconsin).

The study was conducted in 2016, in which they collected paired data on children and women in Amuria District in Eastern Uganda. Vitamin A samples were collected from children (12-23 months) and nonpregnant women (age 15-49 years). The study compared DBS RBP (commercial-EIA), plasma RBP (commercial-EIA), and serum RBP (VitMin Laboratory-ELISA) each to serum retinol. The sample sizes for the DBS RBP (commercial-EIA) and serum RBP (VitMin Laboratory-ELISA) were total n=321, child n=162, and women n=159, and for comparing the RBP assays against serum retinol (HPLC) total n=79, child n=44, and women n=35. These sample sizes were considered sufficient by the group for the purposes of comparing assays. A capillary sample was placed into a Microtainer® tube, and blood from the same tube was used to create DBS samples to measure RBP using the commercial RBP-EIA. The remaining sample in the Microtainers® was kept cold at ~2-4°C and processed at end of day for RBP (ELISA-VitMin Laboratory). After the first blood draw, vitamin A2 dosing was done for MRDR and then a second blood draw was done for MRDR analyzed by HPLC at U of Wisconsin, providing a value for serum retinol.

Table 1 provides a summary of the main findings. In addition, the study examined the molar ratio between serum RBP and serum retinol and found it was not a 1:1 molar ratio (ELISA-VitMin Laboratory) and retinol (HPLC). The ratio was 0.75 among children and 0.45 for women. The sensitivity and specificity were also examined using MRDR as the gold standard. The RBP (commercial) was not sensitive for detecting vitamin A deficiency, nor was the RBP (ELISA-VitMin Laboratory). The sensitivity for serum retinol-HPLC was higher, but remained low (50%). The specificity was 94.6 for the DBS-RBP (commercial), 78.4 for the Serum-RBP (ELISA-VitMin Laboratory), and 75.7 for the retinol (HPLC).

Table 1: Key findings and implications raised during this meeting

<table>
<thead>
<tr>
<th>Blood specimen and method used to assess RBP</th>
<th>Relationship (R²) to serum retinol using HPLC</th>
<th>Implications for use of RBP as a proxy for retinol¹²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum RBP (ELISA)</td>
<td>0.73</td>
<td>Acceptable for assessing vitamin A status</td>
</tr>
<tr>
<td>DBS RBP (commercial)</td>
<td>0.33</td>
<td>Serious concerns for assessing vitamin A status</td>
</tr>
<tr>
<td>Plasma RBP (commercial)</td>
<td>0.39</td>
<td>Serious concerns for assessing vitamin A status</td>
</tr>
</tbody>
</table>

¹ It is important to always include a serum retinol subsample at a minimum for comparison, and if warranted, determine cutoff.
² As per WHO recommendations, at least two biological biomarkers of vitamin A status are required to determine if vitamin A deficiency is a public health problem requiring intervention.

Discussion:
I. There is a lack of guidance from the WHO for RBP cutoffs. There were mixed views on whether the previously assumed 1:1 molar ratio between RBP and retinol holds. The group expressed this could be the result of biological or contextual differences that may influence the ratio, and others raised potential analytical issues.

II. The majority expressed concern that the RBP in DBS correlated poorly with serum retinol (HPLC), while the serum RBP ELISA correlation with serum retinol (HPLC) was 0.73 for children and 0.55 for women. Another participant raised the possibility that the poor performance of DBS-RBP could be because the iteration of kits was not working and suggested going back to manufacturer to get evidence on quality control methods.

III. Overall, the group agreed the results from this study indicate the commercial assay performance was very poor; some raised the possibility that when PATH did the validation, maybe the assay performed better, but now no longer does.

IV. The group also emphasized the results should be viewed in light of this being one study, and while the commercial assay should not be used at this time, future research is needed.

V. They further agreed it is not valid to present RBP data without measuring serum retinol in a subsample, and developing a cutoff based on the relationship between RBP and serum retinol.

VI. The group raised concern about the low sensitivity of RBP or retinol to define vitamin A deficiency compared to MRDR as the standard, which has also been seen in other studies.

VII. The group was undecided on whether it is necessary to do MRDR in a subsample, but generally the group agreed that MRDR was needed in most contexts to at least understand the situation if little is known about the vitamin A status population, especially if the data was needed to make decisions regarding phasing out vitamin A programming.

VIII. Participants also noted the aims of this study were not to assess the prevalence of vitamin A deficiency in Uganda, and larger studies designed to be representative of the population would be needed to estimate the prevalence of vitamin A deficiency in Uganda.
Conclusions from Meeting

Uganda has been a pioneer in the introduction of vitamin A assessment in the DHS. The 2000-01 Uganda DHS measured serum retinol using HPLC. However, the 2006, 2011, and 2016 Uganda DHS measured RBP using a commercial assay. In the mid-2000s the RBP EIA method was validated against serum retinol using HPLC. Uganda has now established a micronutrient surveillance system. In the surveillance system, vitamin A status is being assessed using RBP with the noncommercial sandwich ELISA, which requires shipping samples out of the country to VitMin Laboratory, retinol in the first blood draw, and also MRDR in the second blood draw (University of Wisconsin). The VitMin Laboratory ELISA was validated against serum retinol, and VitMin Laboratory routinely participates in the CDC VITAL-EQA interlaboratory comparison rounds. The surveillance system uses a different method (VitMin Laboratory ELISA) to assess RBP than the 2006, 2011, and 2016 Uganda DHS surveys (commercial assay).

A group of experts was convened to review the results of a study designed to assess the comparability between the results from the Uganda DHS survey and their nutrition surveillance system. The RBP commercial EIA method (used in the 2006, 2011, and 2016 Uganda DHS) was unreliable based on the Makerere/CDC 2016-2017 assessment. Moreover, the commercial EIA method was unreliable irrespective of the type of blood specimen (plasma or DBS) used. The Makerere University and CDC study data presented during this meeting strongly indicated that RBP concentrations, using the commercial RBP assay, should be interpreted with extreme caution. Further, based on the performance of the assays, the meeting participants did not think the commercial RBP assay should be used until there is more information on the calibration of the assay.

It was agreed by the majority of experts that based on the meeting and study results, a statement advising caution when interpreting the vitamin A results in the 2016 Uganda DHS was warranted. The DHS agreed to assist the Uganda Bureau of Statistics with drafting a one-page statement summarizing the concerns with the vitamin A data in the Uganda 2016 DHS survey, and that the one-pager would be available for distribution at the nutrition dissemination event for the 2016 Uganda DHS and included online.

Not all commercial assays may be equally valid when assessing vitamin A status using RBP. The inconsistency in quality of commercially available kits is a current challenge. Suggestions for overcoming this challenge included developing a minimum set of criteria for manufacturers to meet.
List of Participants

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