Report from Guatemala

Mounting and Adaptation of a Fluorescent Rapid-Assay Device (iCHECK® FLUORO) for Vitamin A in Sugar and Biological Fluids

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Noel Solomons (right) with Klaus Kraemer outside *Sight and Life*'s office in Kaiseraugst, Switzerland

Sight and Life arranged to provide an iCHECK® FLUORO rapid assessment unit and a supply of iEX® extraction vials to the Center for Studies of Sensory Impairment, Aging and Nutrition, to strengthen their capacity-building program for exchange students and for analyzing the situation of vitamin A in contemporary Guatemala.

Through the efforts of the late Guatemalan biochemist, Guillermo Arroyave, a method for fortification of granulated table sugar with vitamin A was devised in the 1970s and subsequently adopted as a mandated public health program for Guatemala and the other countries of the Central America Isthmus. A rapid assay method would allow for convenient spot-checking on samples of sugar in the area to monitor the quality of compliance. Breast milk vitamin A has been advanced as a non-invasive and convenient indicator of population status. A user-friendly format for measuring milk vitamin A would contribute to the application of this biomarker.

A Canadian and Guatemalan student joined forces to adapt the application of iCHECK® FLUORO to the circumstances of Guatemala under the supervision of two senior professionals. It is with pleasure that we combine our institutional forces to share a report of these preliminary inquiries in measurement. It was originally presented in the periodical of CeSSIAM in Guatemala, and we reproduce it here for the wider, worldwide readership of *Sight and Life* magazine.

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Guatemala was once a country with a high prevalence of low circulating retinol levels, that is < 20 µg/dL. In the 1964–1965 Central American Survey of the International Committee for Nutrition in National Defense (ICNND), 26% of children under five years of age had retinol values below the cut-off criterion. This led to the mobilization of efforts at the Institute of Nutrition of Central America and Panama (INCAP) by Dr Guillermo Arroyave

for a method to add vitamin A to granulated table sugar in the national supply.[1] As part of the evaluation of the efficacy of fortified sugar, breast milk from lactating consumers was assayed for changes in vitamin A concentration.[2] Both of these studies in the 1970s depended on tedious and laborious extraction and colorimetric methods in the laboratory.

"Adaptation to the conditions of a field laboratory"

Advances in analytical technology

Analytical technology has advanced over the last three decades to allow for the emergence of rapid methods to assay food substances and biological fluids for their content of vitamin A. Prof. Florian Schweigert at the University of Potsdam in Germany conducted analytical chemistry, which led to a simplified system of solvents for extracting lipid-soluble compounds in a sealed vial. This has been manufactured and marketed by the BioAnalyt Company in Teltow, Germany, as the iEX® MILA vials. This extraction technology has been combined with a series of portable, compact battery-operated devices for the analysis of nutrients in foods or biological fluids in a rapid manner and at the site of the collection, if warranted. One of these is the iCHECK® FLUORO (BioAnalyt), which uses fluorescence as the analytic signal for quantifying retinol or retinyl esters. The developers in Potsdam collaborated with counterparts in Guatemala, to demonstrate the application of the iCHECK® FLUORO system with the iEX® MILA vials.[3]

The present mission was to take the systems from a sophisticated university laboratory and adapt them to the makeshift setting of a simulated field laboratory, while improvising the mixing and measuring equipment with items that can be purchased in pharmacies and supermarkets in low-income countries. In this exercise, we sought to prepare cow milk and heavy cream (as surrogates for human milk) and granulated sugar for rapid vitamin A analysis. A diverse array of plastic syringes was made for measurement instead of volumetric flasks or graduated cylinders. Clear plastic sandwich or storage bags were used for mixing, shaking, and dilution. Spoons were substituted for spatulas to transfer sugar, and medicine cups were tared on the balance to weigh out sugar samples. The formal equipment, aside from the iCHECK® device, was a digital balance (supplied by the manufacturer) and a Vortex mixer.

With respect to cow milk, 0.5 mL samples were delivered into iEX® vials and, after agitation and resedimentation, the fluorescence measurements of the clear phase containing the extracted vitamin A were made in the well of the device. After four readings, a digital value in µg/L was given. Although the commercial milk's label specified 900 µg/L, the average of 622 µg/L obtained by rapid assay was credible, given the notorious errors in dairy labeling of enriched nutrients. The coefficients of variation (CV) were 15% and 2.1%; interobserver agreement of average measures was excellent. The concentration of vitamin A in heavy cream was so high that the direct readings of 0.5 mL delivery exceeded the linear area of the device. When diluted to a suitable concentration, the average vitamin A concentration was 3,536 µg/L.

For the preparation of solutions of table sugar, we used 20 mL plastic syringes, previously plugged with silicon to make them into a water-tight vessel; 4 g specimens of an unknown sugar sample were weighed to a 0.01 g precision on a digital balance (DigiWeigh, Chino, CA, USA). When filled to the syringe volume, a 20% (w/v) solution was obtained. For this dilution, a correction factor of 200 applied to the digital reading converts it into the concentration of vitamin A in the original sugar, expressed as mg/kg. Samples of different sugars were obtained from different brands, and were mixed in plastic bags to assure homogeneity. Nevertheless, CVs within sample for the observers ranged from 0.1–4.2%.

Lessons and conclusions

This exercise in adapting the iCHECK® FLUORO system to a setting in a low-income country provided some lessons and conclusions. Interobserver agreement for overall averages is high. The CVs within and between observers were high, especially for sugar. Assuring consistent values requires multiple replicate preparations, and this raises the costs, as the iEX® MILA vials are a relatively expensive commodity.

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