ABSTRACT
Objective: Deficiencies of vitamin A, iron and iodine affect a significant portion of the world’s population and are often associated with adverse health outcomes, particularly among pregnant women and children. Efforts to characterize deficiency patterns have been hampered by a lack of measurement tools appropriate for large-scale use. Since many of these micronutrients are not easily measured directly, reliable proxy biomarkers indicative of deficiency status have been identified and widely adopted. Inflammation or infection biomarker levels must also be measured, as inflammation affects the levels of vitamin A and iron status biomarkers. Furthermore, malaria infection is known to deplete iron levels, thus screening for malaria is also recommended. We previously developed a prototype multiplex immunoassay for the simultaneous measurement of five biomarkers relevant to assessing vitamin A, iron status and inflammation; retinol binding protein, soluble transferrin receptor, ferritin, alpha-1-acid glycoprotein and Creatine protein. Here we present an improved version of the immunoassay which has also been expanded to include measurement of the biomarkers for iodine deficiency (thyroglobulin) and malarial parasitemia (Histidine Rich Protein II).

Method: Using affordable technology from Quansys Biosciences, antibodies are coated in seven discrete regions of the well of a microtiter plate and the seven analytes are assayed in a single volume of sample. A control standard was developed for the assay that reflected the clinical range of each biomarker being assayed. Assay performance was evaluated by comparing multiplex and conventional assay results for plasma from 170 US volunteers.

Results: The new multiplex immunoassay and established conventional assay methods showed high correlation for all analytes tested (Average 0.77, p<0.001), improving on the values reported with the original 5-plex plate. Use of a control standard specially designed for the multiplex assay in place of a commercially available standard allowed for more accurate quantitation of each analyte. The assay was also validated against the available ELISA. The results demonstrated adequate performance and range for all analytes (Table 1, Figure 3). Comparisons with established conventional ELISA methods give excellent correlations with results from this new assay (Table 2, Figure 4).

Conclusions: This 7-plex multiplex assay has excellent potential for use as a cost effective tool for population surveillance of vitamin A, iron and iodine deficiencies as well as malaria infectivity rates.

INTRODUCTION

- Micronutrient (MN) malnutrition is a significant health problem
- There is a need for better tools to identify subpopulations at greatest risk:
  - Pinpoint where interventions are most needed
  - Monitor the impact of programs after implementation
- We improved upon a previously described multiplex micronutrient assessment tool (MMAT) to measure a panel of markers widely used to determine iron, iodine, vitamin A and malaria status.
  - Alpha-1-acid glycoprotein (AGP), C-reactive protein (CRP), ferritin, soluble transferrin receptor (sTfR) and retinol binding protein (RBP4), thyroglobulin (Tg) and Histidine Rich Protein II (HRP2-2).
- This tool uses simple, affordable equipment along with the advantages of multiplex technology:
  - Eliminates the need to individually assess inflammatory and MN biomarkers
  - Reduces sample volume needed to measure all analytes
  - Increases efficiency and decreases laboratory costs

METHODS

- Quansys Biosciences developed a prototype kit whereby the antibodies for 7 assays were printed in discrete areas of a microtiter plate well (Figures 1 and 2).
  - Allows for independent measurements of 7 analytes simultaneously
  - Each well requires 5µL of sample pre-diluted 1:10 in diluent containing competitors
  - Standards and zero included to create a calibration curve for each analyte
  - Assay takes ~3 hours to run
- Evaluated the performance of this new assay by comparing results from the MMAT web results from monoplex ELISA's for 170 plasma samples.