LB400: Multiplexed Micronutrient Array for Population Surveillance

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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 C-reactive 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<.0001), improving on the values observed 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 WHO reference standards.

Conclusions: This 7-plex micronutrient 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[1] 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 (HRP-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 kit diluent containing competitors
  - Standards and true 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 with results from monoplex ELISAs for 170 plasma samples

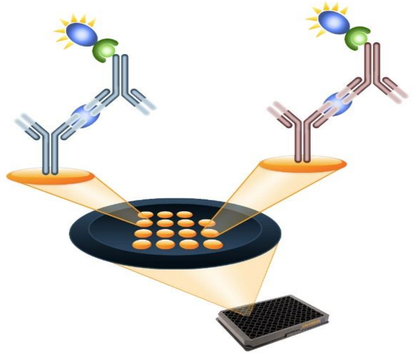

Figure 1. Illustration of the Quansys multiplex assay technology. Plates are coated with antibodies in discrete regions to create multiple assays per well. 350-500µM spots capture target proteins.

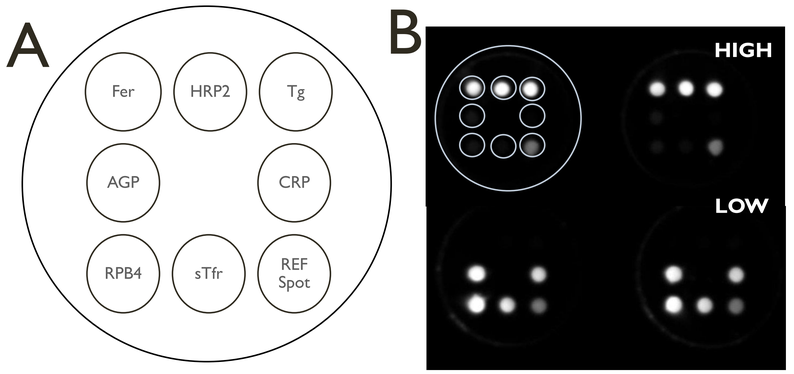

Figure 2. (A) Schematic of placement of assays in each test well; (B) images of developed signal in multiplex micronutrient assessment tool (MMAT) plate (analyte concentration descending).

RESULTS

Assay validations demonstrated adequate performance and range for all analytes (Table 1, Figure 3). Comparisons with established conventional ELISA methods gave excellent correlations with results from this new assay (Table 2, Figure 4).

Table 1. Multiplex micronutrient assessment tool (MMAT) assay precision (coefficient of variation [CV]), lower limits of quantification (LLOQ), and linearity.

Analyte	Average conc.	Units	Intra-assay precision (mean, CV)	Inter-assay precision (mean, CV)	LLOQ	Linearity (1:5, 1:10, 1:20)
Ferritin	1.60×10^{1}	µg/L	13%	4%	8.23×10^{-2} µg/L	107%,
	2.80×10^{0}		13%	10%		108%,
	6.44×10^{-1}		10%	10%		103%,
CRP	1.56×10^{-3}	mg/mL	13%	6%	8.23×10^{-6} mg/mL	76%,
	3.01×10^{-4}		12%	9%		76%,
	7.30×10^{-5}		14%	11%		83%
RBP4	1.45×10^{0}	mg/dL	11%	7%	4.15×10^{-3} mg/dL	83%,
	2.38×10^{-1}		13%	11%		91%,
	5.66×10^{-2}		15%	8%		100%
Tg	2.17×10^{0}	ng/mL	11%	7%	4.57×10^{-3} ng/mL	108%,
	4.37×10^{-1}		15%	9%		107%,
	1.07×10^{-1}		15%	9%		111%
sTfR	8.72×10^{0}	mg/L	10%	8%	4.94×10^{-2} mg/L	88%,
	2.28×10^{0}		14%	10%		86%,
	4.24×10^{-1}		11%	7%		91%
AGP	3.72×10^{-1}	mg/mL	12%	7%	8.23×10^{-4} mg/mL	122%,
	4.56×10^{-2}		12%	4%		114%,
	8.98×10^{-3}		12%	6%		107%
HRP2	7.62×10^{2}	pg/mL	7%	7%	1.83×10^{0} pg/mL	108%,
	1.52×10^{2}		14%	11%		86%,
	3.50×10^{1}		13%	10%		119%

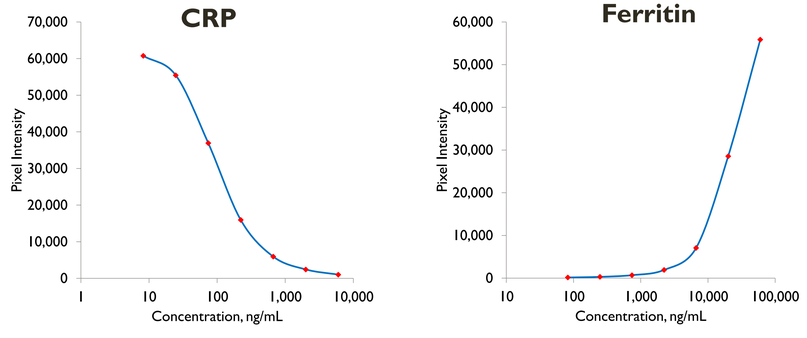

Figure 3. Examples of calibration curves for a sandwich assay (ferritin) and a competitive assay (CRP) using a five-parameter logistic model fit. Ferritin, HRP-2, and Tg use a sandwich assay format, while all other analytes are measured in a competitive format. Assay ranges were optimized to allow all analytes to be quantified at a single dilution of the sample (1:10).

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Table 2. MMAT versus conventional ELISA results for 170 plasma specimens.

Analyte	Pearson correlation	R^2	F statistic (p-value)	Monoplex assay
Ferritin	95%	0.91	< 2.2e-16	Bioreclamation
CRP	75%	0.91	< 2.2e-16	In house
RBP4	95%	0.91	< 2.2e-16	SciMedx
Tg	95%	0.91	< 2.2e-16	DRG International
sTfR	82%	0.68	2.6e-5	Ramco
AGP	72%	0.52	3.8e-7	GenWay

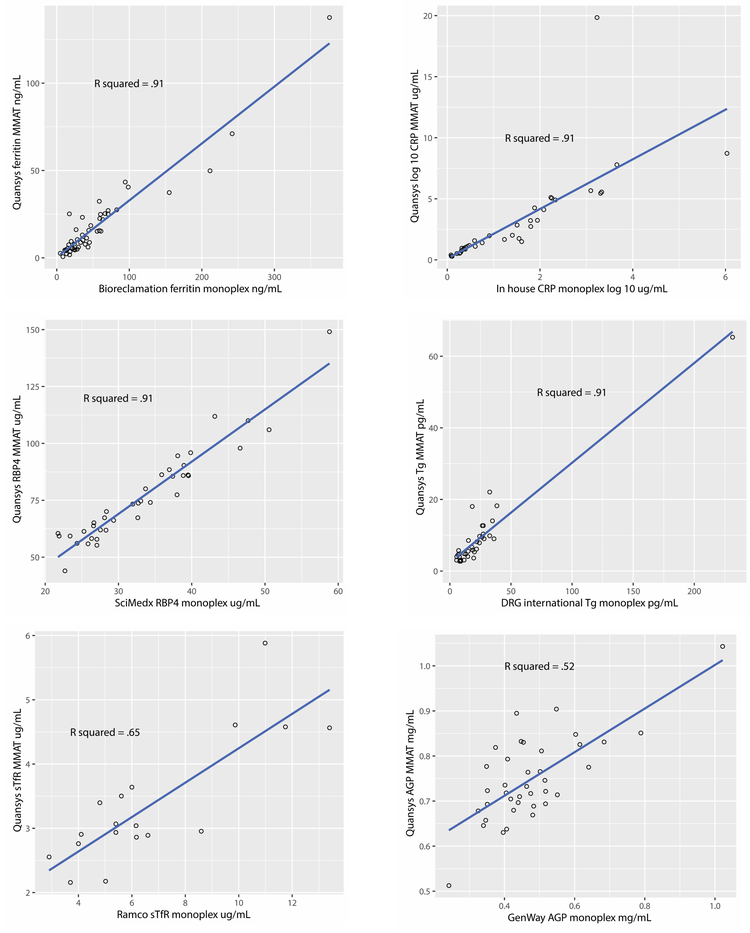

Figure 4. MMAT versus conventional ELISA. Concentrations of each analyte as measured in the Quansys MMAT (y-axes) plotted against concentrations measured using conventional assays (x-axes) for plasma specimens (log scales). Solid line indicates linear regression.

CONCLUSIONS

- Demonstrated ability to multiplex seven assays using only 5µL of sample
- Assays can detect across the range for deficiency, sufficiency and inflammation and can be used in any lab equipped for ELISAs
- Variable correlation to monoplex assays observed, but research on iterative assay designs and algorithms to correct variance between MMAT versus monoplex assay is ongoing
- Specimens used in assessment represented small region of dynamic range creating bias in analysis
  - Upon design lock field samples representing greater diversity of specimen types will be used to evaluate assay (Sonja Hess, UC Davis)

REFERENCES

1. Brindle E, Stevens D, Crudder C, Levin CE, Garrett D, Lyman C, Boyle DS. A Multiplex Immunoassay Method for Simultaneous Quantification of Iron, Vitamin A and Inflammation Status Markers. PLoS One. 2014 Dec 19;9(12)