Q-Plex™ Human Micronutrient v2 (7-Plex)

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Introduction

Micronutrient deficiency (MND) can have systemic and enduring repercussions upon an individual as well as their offspring (1). MNDs affect populations across the globe, but disproportionately affect children and women of reproductive age in low- and middle-income countries (LMIC). MNDs can significantly reduce quality of life and have deadly consequences if left untreated. Public health surveillance programs are needed to identify populations at risk and determine the appropriate interventions to apply. However, it has been shown that the sustainability of such programs depends upon cost, capacity development, and location of the institutional base (2).
Several organizations have attempted to provide comprehensive platforms to address the needs of public health surveillance programs. Well characterized methods are often available only in testing centers outside the region or country. An important group to note here is the VitMin Lab (Willstätt, Germany), which is often considered the gold standard in measuring micronutrients via a traditional sandwich ELISA approach (3). However, this preference creates a high demand for services and creates an export related barrier to researchers in different situations (4). Thus, there exists a need for an affordable, accurate, and easy to implement and use assay that fits the needs of public health surveillance programs in LMICs.

Q-Plex™ Human Micronutrient (7-Plex) critical feedback

Originally introduced in 2014, the Multiplex Micronutrient Assessment Tool (MMAT), was a multiplex ELISA developed as a collaboration between PATH (Seattle, WA, USA) and Quansys Biosciences (Logan, UT, USA). The MMAT was renamed as the Q-Plex Human Micronutrient (5-Plex) upon official release. The assay simultaneously assessed the level of 5 analytes, including ferritin, C-reactive protein (CRP), α-1-acid glycoprotein (AGP), soluble transferrin receptor (sTfR), and retinol binding protein 4 (RBP4) (5). The multiplex ELISA allowed for more data to be generated and collected from small blood samples compared to traditional ELISAs. Additionally, unlike other multiplex ELISA platforms, such as the Luminex and MagPix platforms that rely on a fluidics system that may require specialized facilities, training, and service, the Q-Plex kit was designed upon and followed a conventional ELISA protocol, requiring relatively little training and specialized equipment. In 2017, the panel of analytes was expanded to include thyroglobulin (Tg) and histidine rich protein II (HRP2) and renamed as the Q-Plex Human Micronutrient (7-Plex). This broadened the assay's capabilities, allowing it to indicate iodine status as well as whether the patient was currently or recently infected with *Plasmodium falciparum* malaria (6,7). Additionally, the use with dry blood spots (DBS) as a specimen type was determined to work well for all analytes except for ferritin, which in DBS is known to reflect a mixture of ferritin from serum along with intracellular ferritin from lysed red blood cells, confounding test results (8).

The Q-Plex Human Micronutrient (7-Plex) assay was tested extensively by labs outside of Quansys Biosciences and PATH to verify performance before wide deployment in the field. External testing demonstrated good correlation for many of the analytes but identified concerns about data correlation with more established methods. One study demonstrated differences between data generated from the Q-Plex Human Micronutrient (7-Plex) and the VitMin Lab, and notably in differences between the sTfR, AGP, RBP4, along with the sensitivity of the ferritin assay (4). A second study completed by the CDC, compared Quansys ferritin, sTfR, CRP, and AGP with Roche clinical analyzer for ferritin, sTfR, CRP and AGP and a retinol HPLC reference standard supported some of these findings, highlighting the variability of sTfR and RBP4 (9).

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The CDCs ongoing external quality assurance efforts to improve standardization of MN assays provided a supportive platform to validate outside findings. Forty samples characterized in duplicate over 10 individual runs by a Roche clinical analyzer for CRP, sTfR, ferritin, AGP, and retinol HPLC were obtained from the CDC Method Performance Certification program. Data from these samples generated on the Q-Plex Human Micronutrient (7-Plex) show good agreement with the CDC's reference values for ferritin and CRP (Fig. 1-2), however, the analysis shows poor agreement for AGP, sTfR, and RBP4, correlating with the findings from previous studies (Fig. 3-5). Data is not shown here for thyroglobulin as the CDC reference samples have not been characterized for this analyte.

Taken together, these data and the feedback provided by third party assessments clearly highlighted the areas in need of refinement in order for the MMAT assays to be a viable alternative to existing methods. Briefly, the main points are as follows:

- Sensitivity of ferritin assay
- Precision of RBP4 assay
- Correlation of sTfR and AGP data with established methods

Figure 1 - Ferritin Correlation

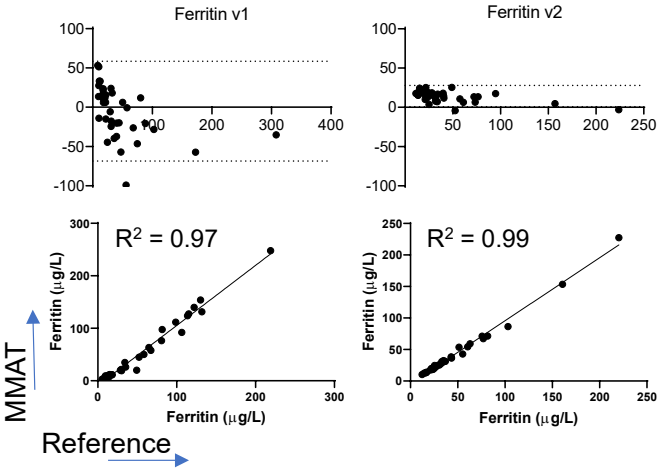


Figure 2 - CRP Correlation

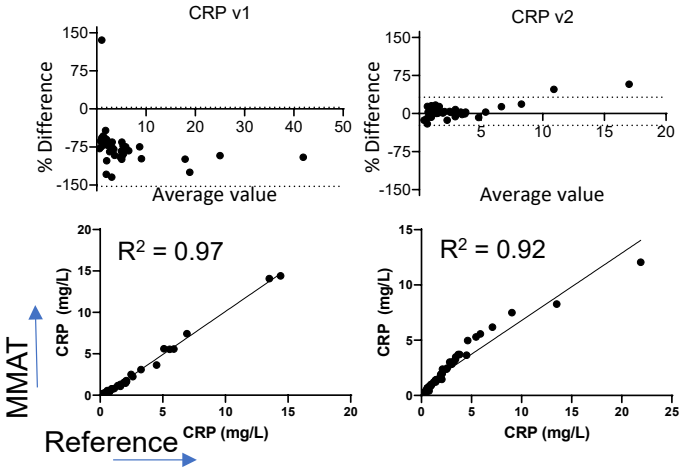


Figure 3 - AGP Correlation

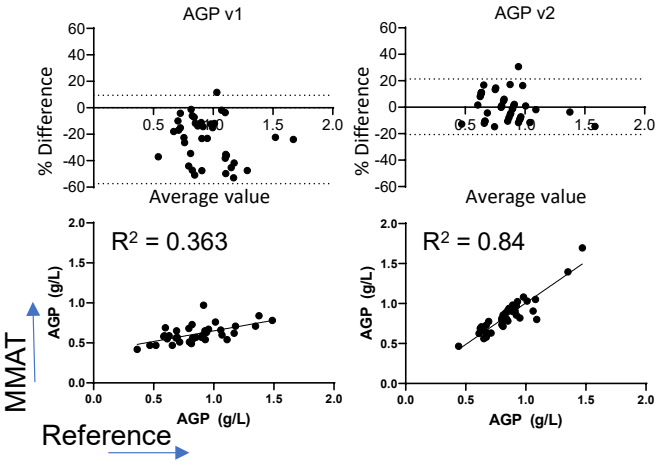
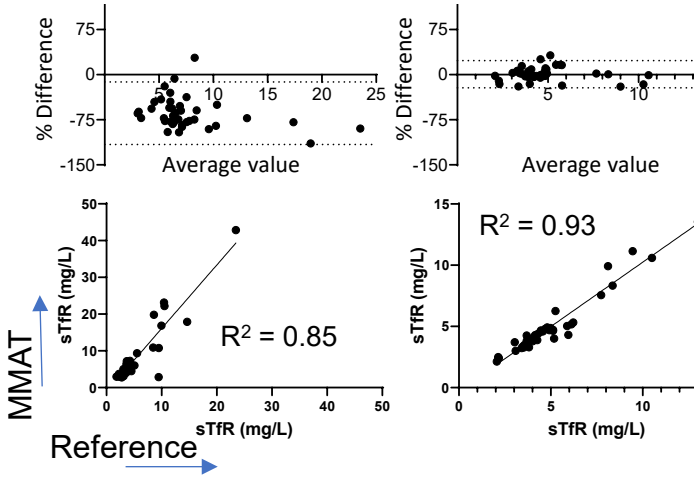


Figure 4 - sTfR Correlation



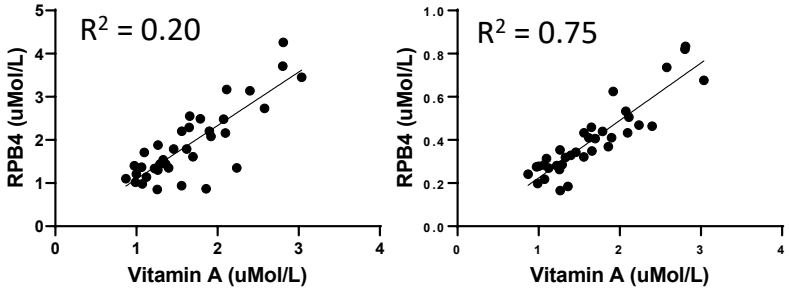
Q-Plex™ Human Micronutrient (7-Plex) data

To address the areas in need of improvement, Quansys refined the tool to generate Q-Plex Human Micronutrient v2 (7-Plex). Specifically, new capture antibody improved sensitivity of the ferritin assay, while still maintaining the correlation with the reference samples (Fig. 1). This has shifted the assay range to ensure that the sample distribution falls more consistently into the middle of the quantifiable range, which leads to more accurate and precise measurement of samples with low levels of ferritin. (Fig. 6A).

The lack of precision observed for RBP4 was determined to be associated with RBP4 sample values falling at or above the upper limit of quantification of the assay. To address this challenge, the assay was re-calibrated to ensure that all endogenous samples, not just samples from individuals deficient in Vitamin A, fall into the linear portion of the standard curve where quantitation is more precise (Fig. 6B). This was made possible by the heightened sensitivity of the ferritin assay, which allowed an increase in the working dilution of the sample from 1:10 to 1:40. Additionally, data from tests using the CDC's samples from the Serum Micronutrient Performance Verification program show strong correlation between levels of RBP4 and retinol (R2 = 0.75) (Fig. 5).

AGP, CRP, Tg, HRP2 and sTfR were all re-optimized under the 1:40 sample dilution method established for RBP4 and ferritin. Performance characteristics for CRP and Tg did not significantly change with the new sample dilution factor (Fig. 2), however, AGP results showed that optimization improves correlation to the reference values while also shifting data points away from the limits of quantification (Fig. 6C-D). To improve the sTfR assay, a new capture and detection antibody set was identified and used to improve correlation to the CDC reference values (Fig. 4).

Figure 5 - RBP4 correlates with Vitamin A

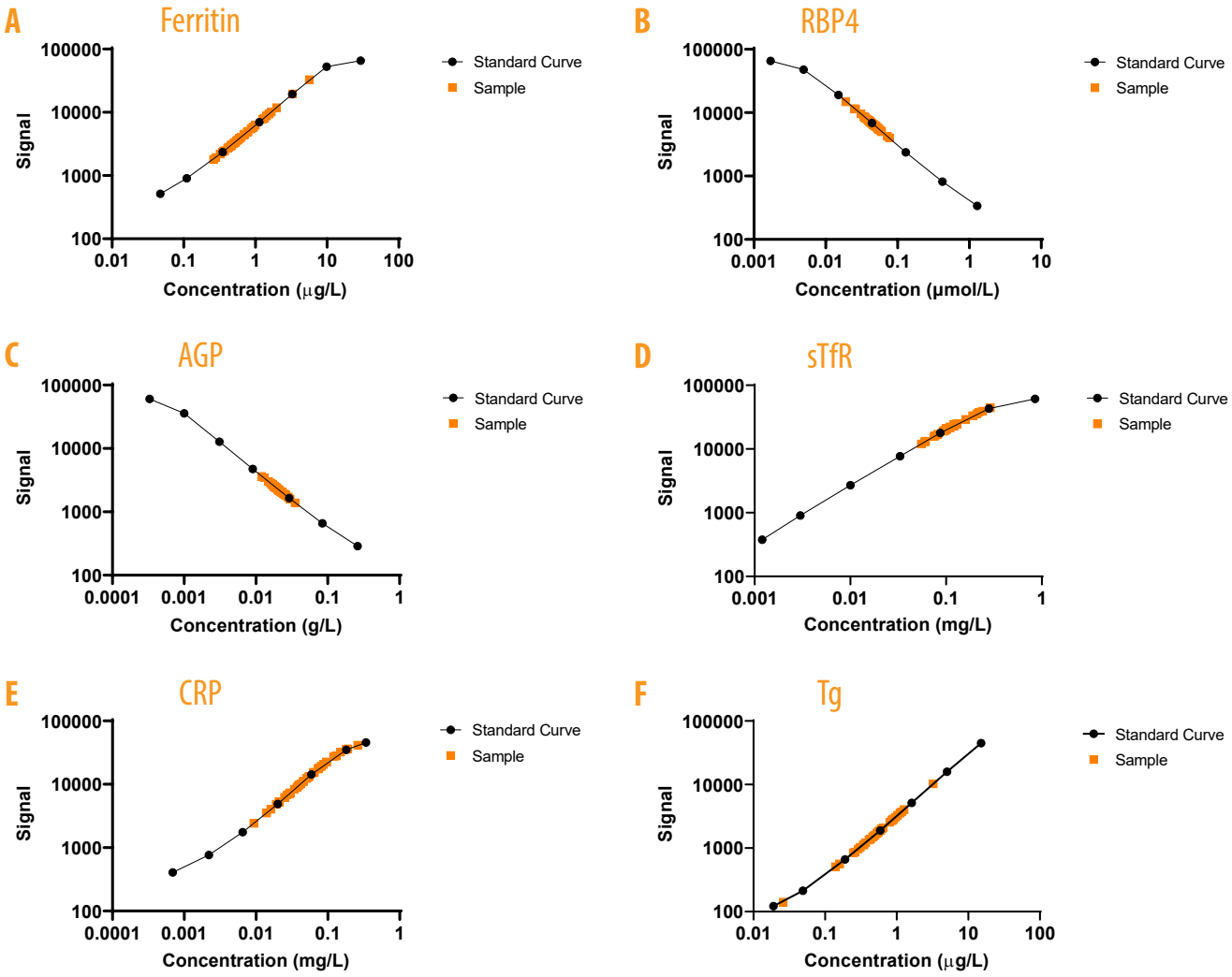


The final step in improving the Micronutrient assay was to remove a technological barrier that may cause lab to lab variation when processing samples with the same kits and materials. The assay is optimized without the need for a plate shaker throughout the incubation steps of the protocol. This not only eases the burden of equipment requirements but also eliminates any variability generated from differences between plate shakers operating at variable speeds. Additionally, Quansys has made available control samples to insure assay performance.

Taken together, the newly optimized Q-Plex Human Micronutrient v2 (7-Plex) assay addresses feedback in the following ways:

- *Improved sensitivity of ferritin assay*
- *Improved precision of RBP4 assay*
- *Improved correlation of sTfR and AGP data with established methods*
- *Removed need for plate shaker*

Figure 6 - Performance Verification data falls along readable portion of standard curve



Discussion and Future Directions

The data presented here demonstrate the significant improvements to the Q-Plex Human Micronutrient assay arising from the combined efforts of Quansys working closely with PATH, the CDC, and several individual labs focused on addressing global MND concerns. The assay is an accurate and affordable tool for measuring micronutrients in serum and plasma sample types collected as part of related public health surveillance programs.

The Q-Plex Human Micronutrient assay is one of several tools designed in collaboration with PATH to aid those in low- and middle-income countries. Research and studies directly benefitting these groups qualify for special pricing. With ease of use of a traditional ELISA protocol, the platform will help facilitate testing within the survey country.

Bibliography

1. Bailey RL, West KP, Black RE. The epidemiology of global micronutrient deficiencies. Ann Nutr Metab. 2015 Jun 2;66 Suppl 2:22–33.

2. Tuffrey V. A perspective on the development and sustainability of nutrition surveillance in low-income countries. BMC Nutr. 2016 Dec;2(1):15.

3. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. J Nutr. 2004 Nov;134(11):3127–3132.

4. Karakochuk CD, Henderson AM, Samson KLI, Aljaadi AM, Devlin AM, Becquey E, et al. Comparison of a New Multiplex Immunoassay for Measurement of Ferritin, Soluble Transferrin Receptor, Retinol-Binding Protein, C-Reactive Protein and α1-Acid-glycoprotein Concentrations against a Widely-Used s-ELISA Method. Diagnostics (Basel). 2018 Feb 2;8(1).

5. Brindle E, Stevens D, Crudder C, Levin CE, Garrett D, Lyman C, et al. A multiplex immunoassay method for simultaneous quantification of iron, vitamin A and inflammation status markers. PLoS One. 2014 Dec 19;9(12):e115164.

6. Zimmermann MB, Aeberli I, Andersson M, Assey V, Yorg JAJ, Jooste P, et al. Thyroglobulin is a sensitive measure of both deficient and excess iodine intakes in children and indicates no adverse effects on thyroid function in the UIC range of 100-299 μg/L: a UNICEF/ICCIDD study group report. J Clin Endocrinol Metab. 2013 Mar;98(3):1271–1280.

7. Beadle C, Long GW, Weiss WR, McElroy PD, Maret SM, Oloo AJ, et al. Diagnosis of malaria by detection of Plasmodium falciparum HRP-2 antigen with a rapid dipstick antigen-capture assay. Lancet. 1994 Mar 5;343(8897):564–568.

8. Brindle E, Lillis L, Barney R, Bansil P, Lyman C, Boyle DS. Measurement of micronutrient deficiency associated biomarkers in dried blood spots using a multiplexed immunoarray. PLoS One. 2019 Jan 8;14(1):e0210212.

9. Esmaeili R, Zhang M, Sternberg MR, Mapango C, Pfeiffer CM. The Quansys multiplex immunoassay for serum ferritin, C-reactive protein, and α-1-acid glycoprotein showed good comparability with reference-type assays but not for soluble transferrin receptor and retinol-binding protein. PLoS One. 2019 Apr 29;14(4):e0215782.