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J Nutr. Author manuscript; available in PMC 2023 January 11.

Published in final edited form as:

J Nutr. 2022 January 11; 152(1): 350–359. doi:10.1093/jn/nxab355.

The VitMin Lab Sandwich-ELISA Assays for Iron and Inflammation Markers Compared Well with Clinical Analyzer Reference-Type Assays in Subsamples of the Nepal National Micronutrient Status Survey

Christina M Fischer[1], **Ming Zhang**[1], **Maya R Sternberg**[1], **Maria E Jefferds**[2], **Ralph D Whitehead Jr**[2], **Zuguo Mei**[2], **Naveen Paudyal**[3], **Nira Joshi**[4], **Kedar R Parajuli**[5], **Debendra P Adhikari**[6], **Donna J LaVoie**[1], **Christine M Pfeiffer**[1]

[1]Division of Laboratory Sciences, National Center for Environmental Health, CDC, Atlanta, GA, USA;

[2]Division of Nutrition, Physical Activity, and Obesity, National Center for Chronic Disease Prevention and Health Promotion, CDC, Atlanta, GA, USA;

[3]Nutrition Section, UNICEF, Kathmandu, Nepal;

[4]New ERA, Kathmandu, Nepal;

[5]Ministry of Health and Population, Kathmandu, Nepal;

[6]United States Agency for International Development, Kathmandu, Nepal

Abstract

Background: The low cost and small specimen volume of the VitMin Lab ELISA assays for serum ferritin (Fer), soluble transferrin receptor (sTfR), C-reactive protein (CRP), and α−1-acid glycoprotein (AGP) have allowed their application to micronutrient surveys conducted in low-resource countries for ~2 decades.

Objectives: We conducted a comparison between the ELISA and reference-type assays used in the US NHANES.

Methods: Using the Roche clinical analyzer as a reference, we measured random subsets of the 2016 Nepal National Micronutrient Status Survey (200 serum samples from children aged 6–59 mo; 100 serum samples from nonpregnant women) for Fer, sTfR, CRP, and AGP. We compared the combined data sets with the ELISA survey results using descriptive analyses.

This work is written by (a) US Government employee(s) and is in the public domain in the US.

Address correspondence to MZ (mbz0@cdc.gov).

Author disclosures: The authors report no conflicts of interest.

The findings and conclusions in this article are those of the authors and do not necessarily represent the official views or positions of the CDC/Agency for Toxic Substances and Disease Registry, the Nepal Ministry of Health and Population, UNICEF, United States Agency for International Development, and New ERA. Use of trade names is for identification only and does not imply endorsement by the CDC.

Supplemental Figures 1–3, Supplemental Tables 1–2, and Supplemental Text 1 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.

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Results: The Lin's concordance coefficients between the 2 assays were 0.89 except for sTfR (Lin's $\rho = 0.58$). The median relative difference to the reference was as follows: Fer, −8.5%; sTfR, 71.2%; CRP, −19.5%; and AGP, −8.2%. The percentage of VitMin samples agreeing within ±30% of the reference was as follows: Fer, 88.5%; sTfR, 1.70%; CRP, 74.9%; and AGP, 92.9%. The prevalence of abnormal results was comparable between the 2 assays for Fer, CRP, and AGP, and for sTfR after adjusting to the Roche assay. Continued biannual performance (2007–2019) of the VitMin assays in CDC's external quality assessment program (6 samples/y) demonstrated generally acceptable performance.

Conclusions: Using samples from the Nepal survey, the VitMin ELISA assays produced mostly comparable results to the Roche reference-type assays for Fer, CRP, and AGP. The lack of sTfR assay standardization to a common reference material explains the large systematic difference observed for sTfR, which could be corrected by an adjustment equation pending further validation. This snapshot comparison together with the long-term external quality assessment links the survey data generated by the VitMin Lab to the Roche assays used in NHANES.

Keywords

micronutrients; iron deficiency; inflammation; ferritin; soluble transferrin receptor; C-reactive protein; α–1-acid glycoprotein

Introduction

According to the Global Burden of Disease Study 2016, iron deficiency anemia is 1 of the 5 leading causes of years lived with disability burden (1). Lower cognitive performance in children, altered physical capacity in adults, and a negative effect on immune status at all ages are the most common consequences of iron deficiency (2). According to a 2020 WHO guideline, measurement of serum ferritin (Fer) is an important and commonly used test to assess the iron status in otherwise apparently healthy individuals and in populations (3). In populations, Fer testing is usually performed along with measures of inflammation [e.g., C-reactive protein (CRP) and/or α–1-acid glycoprotein (AGP)] and additional iron indexes, such as soluble transferrin receptor (sTfR) (3).

In the early 2000s, the VitMin Lab, managed by Dr. Juergen Erhardt and based out of Willstaett, Germany, developed low-cost sandwich ELISA assays that only require small specimen volumes to measure the aforementioned biomarkers for iron status and inflammation (4). The availability of these assays has transformed biomarker assessments as part of micronutrient surveys and allowed for the generation of many comprehensive data sets in low- and middle-income countries in South and Southeast Asia, the Middle East, Africa, and Latin America (5–13). At their introduction, the VitMin ELISA assays were shown to have good agreement with the Fer RIA from Bio-Rad Laboratories ($n = 44$), the sTfR ELISA assay from Ramco Laboratories ($n = 119$), and the CRP ELISA assay from Immuno-Biological Laboratories Inc. ($n = 17$) (4). An AGP ELISA assay was added later. To allow for a systematic evaluation of the VitMin assays compared to the Roche Cobas clinical analyzer reference-type methods utilized to measure selected biomarkers in the US NHANES, we applied the Roche assays to analyze specimens from the 2016 Nepal National Micronutrient Status Survey (NNMSS; 200 serum samples from children aged 6–59 mo

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and 100 serum samples from nonpregnant women). We compared the results with those generated by the VitMin assays as part of these surveys (11). To provide context regarding the VitMin assay performance beyond this snapshot assessment, we also included data from >10 y of VitMin assay participation in CDC's external quality assessment program for serum micronutrients.

Methods

Biological specimens

The VitMin Lab analyzed Fer, sTfR, CRP, and AGP for the 2016 NNMSS postintervention during 2016 and 2017. Extra vials were stored frozen for samples with sufficient specimen volume to allow for potential future analyses. Simple random sample subsets of these samples were shipped frozen to the CDC laboratory in the fall of 2017 and kept at −70°C until the Roche assays were performed in the spring of 2018: 200 serum samples from children aged 6–59 mo and 100 serum samples from nonpregnant women aged 15–49 y from the NNMSS. Based on in-house tracking of serum quality control (QC) materials stored at −70°C, we know that these analytes are stable for at least a few years. The involvement of the CDC laboratory to analyze these human serum samples was determined not to constitute human subjects research, because CDC employees had no interaction with the study participants, specimens were not collected for this method comparison study, no extra specimens were collected, and CDC employees did not have access to the link between the data or specimens and the identity of the study participants.

VitMin ELISA assays

The sandwich ELISA assays for Fer, sTfR, and CRP were described previously and achieved assay variability between 5% and 14% (4). The ELISA assays were calibrated using a commercially available 3-level serum control material (Bio-Rad Liquichek Immunology Control) by using the mean concentrations listed in the manufacturer product insert and diluting the materials when needed further to generate calibration curves. Two calibration curves were used for each analyte to cover a wider range (Supplemental Figure 1). Owing to slight lot-to-lot concentration variations over time, the dilutions of the materials were modified to generate similar reportable ranges. Typical reportable ranges for each analyte were as follows: Fer, 0–350 μg/L; sTfR, 0–35 mg/L; CRP, 0–45 mg/L; and AGP, 0–2.5 g/L. Because the Liquichek material does not provide data for sTfR, the VitMin Lab determined the initial sTfR concentrations using a commercial ELISA kit from Ramco Laboratories. An in-house prepared serum QC sample with analyte concentrations in the mid-range was included with each plate (8 positions) and analyte to monitor assay stability. To minimize daily variations, the absorption of this known sample was used to calculate an adjuster factor for the daily calibration curves; different approximations (linear, quadratic, logarithmic) were used depending on the protein and for the lower and upper calibration curves. Retinol-binding protein, although part of the VitMin ELISA assays, was not included in this investigation because it is not measured on the clinical analyzer.

The VitMin Lab has continuously participated in CDC's Vitamin A Laboratory External Quality Assurance (VITAL-EQA) program since 2005. The program sends out 3 samples

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twice a year and provides assessments for Fer, sTfR, and CRP (not for AGP) using objective quality goals for method performance based on biological variation (Supplemental Table 1). The participant's assay results are considered acceptable if they fall at least within the minimum allowable relative difference as compared to the CDC target values, which are ±7.7% for Fer and ±32.7% for CRP. Although the minimum allowable relative difference based on biological variation is ±7.7% for sTfR, the VITAL-EQA program does not rate the participant's difference because there are large differences among sTfR assays due to these assays not yet being standardized to a common reference material (14). After the initial sTfR value assignment of the Liquichek materials based on analysis with the Ramco kit, the VitMin Lab used the results from the VITAL-EQA program for subsequent assay calibration and a factor of 1.6 to account for the Roche to VitMin (i.e., Ramco) assay difference determined at the onset of program participation. The factor of 1.6 is similar to the known Roche to Ramco assay difference [Roche = 0.631 × Ramco + 0.299; Roche ~30% lower than Ramco, leading to a factor of 1.43 (15)]. Thus, the recent discontinuation of the Ramco kit had no impact on the continued calibration of the VitMin sTfR assay. The VitMin Lab also utilized the VITAL-EQA results to occasionally correct assay shifts through recalibration. The minimum allowable relative difference based on biological variation is ±10.3% for AGP.

Reference-type Roche assays

We used the Cobas 6000 system (Roche Diagnostics) to measure Fer (Elecsys Ferritin electrochemiluminescence immunoassay) on the e601 immunology analyzer and sTfR (Tina-quant particle enhanced immunoturbidimetric assay), CRP (Gen.3 particle enhanced immunoturbidimetric assay), and AGP (Tina-quant Gen.2 immunoturbidimetric assay) on the c501 chemistry analyzer (16). The reportable range for each analyte was as follows: Fer, 0.5–2000 μg/L; sTfR, 0.5–40 mg/L; CRP, 0.3–350 mg/L; and AGP, 0.1–4.0 g/L. We monitored the long-term performance of these assays by including 3 levels of in-house serum QC samples measured in duplicate for each analytical run. The interday variability for the study period was 1.2%–1.9% for Fer (10.6–101 μg/L), 1.7%–2.4% for sTfR (2.17–14.8 mg/L), 1.3%–2.6% for CRP (1.24–23.0 mg/L), and 2.8%–6.0% for AGP (0.53–2.08 g/L). We successfully participated in the College of American Pathologists Ligand (Fer, 3 times/y), Immunology (CRP, 3 times/y), and Soluble Transferrin Receptor (sTfR, 2 times/y) proficiency testing challenges as well as the corresponding Linearity and Calibration Verification challenges. Lastly, we regularly (2 times/y) analyzed international reference materials from the WHO and the European Institute for Reference Material and Measurements (IRMM) to monitor potential shifts in the assays. Supplemental Text 1 describes the performance of the Roche assays with available reference materials and the standardization of the Roche assays.

Statistical analysis

We excluded incomplete pairs (5 for Fer, 5 for sTfR, 1 for CRP, and 5 for AGP). After initial data inspection we removed sTfR results for 1 highly influential outlying data pair (Roche: 72 mg/L; VitMin: 59.8 mg/L). All other outlying values were considered to be valid measurements. Our analysis included the following numbers of paired results: Fer, $n = 295$; sTfR, $n = 294$; CRP, $n = 299$; and AGP, $n = 295$. We present descriptive statistics such

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as median, IQR, and range (minimum to maximum concentration) for each sample set and overall.

We based our agreement analysis on paired sample results with numeric values and thus excluded 116 samples for CRP because the Roche results were less than the limit of detection (instrument output of <0.3); for 108 of these samples the VitMin results were <0.5 mg/L and the remaining VitMin results were <2 mg/L. A visual inspection of Bland–Altman plots using both the original and ln-transformed data suggested that combining the data from the 2 sample sets was reasonable because the relation between the 2 assays across the sample sets appeared similar (Supplemental Figure 2).

After reviewing the Bland–Altman plots, all analytes on the original scale appeared to violate 1 of the assumptions for the Bland–Altman limits of agreement (LoA) approach, such as nonconstant difference and/or nonconstant variance. The log transformation addressed these violations for some analytes, but the remaining analytes required a further modification of the standard Bland–Altman approach (17). Using the parameter estimates of a linear regression of the log difference between the assays on the average of the log-transformed pair, we derived prediction equations and 95% prediction intervals (18). These prediction equations (and 95% prediction intervals) were then back-transformed from the log scale. We used these prediction equations to calculate predicted values and 95% prediction intervals at selected measured values (minimum, 25th, 50th, 75th percentiles, and maximum as measured by the reference assay). Log-transformed data were also used to calculate the Pearson correlation and Lin's concordance coefficients. The Pearson correlation was used because the association between the methods was approximately linear after log transformation.

To supplement the information provided by the prediction equations, we used a nonparametric approach to describe the agreement between the 2 assays (17). We present the median, IQR, and the nonparametric LoA (2.5th and 97.5th percentiles) relative difference along with the proportion of samples where the relative differences fall within selected limits (e.g., within ±10% of the reference assay). We also calculated the latter for CRP with all 299 sample pairs included, using an imputed value of 0.3 for Roche. We assessed the acceptability of the median relative difference by comparing it with the minimum allowable difference based on biological variation: difference = 0.375 × (within-individual CV^2 + between-individual $CV^2)^{1/2}$ (19) (Supplemental Table 1).

Given that population-level nutrition status assessment often relies on dichotomization to derive estimates of deficiency/inflammation prevalence, we calculated the prevalence of abnormal analyte concentrations using all sample pairs (including CRP results less than the limit of detection for Roche) using the following common cutoff values: Fer <15 μg/L for women and <12 μg/L for children (4), CRP >5 mg/L (20), and AGP >1 g/L (20). For sTfR, we used a cutoff value of >8.3 mg/L for the original VitMin data because the VitMin sTfR assay was calibrated to the Ramco assay (4); however, when assessing the predicted results, we used the sTfR cutoff values applicable to the Roche assay (>5.33 mg/L for women and >6.00 mg/L for children) (21). We also generated folded empirical cumulative distribution function graphs to provide visual interpretation of the overlap between the Roche, original

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VitMin, and predicted VitMin concentration distributions. To provide additional individual-level assessment, we evaluated the diagnostic characteristics of the VitMin assays by calculating sensitivity, specificity, positive predictive value, and negative predictive value for the original and predicted results. P values 0.05 were considered statistically significant.

Lastly, we summarized the performance of the VitMin Fer, sTfR, and CRP assays in the VITAL-EQA program starting with round 8 (2007) and continuing to round 33 (2019). We plotted the relative difference to the CDC target value for the 3 samples in each round (level 1, low concentration; level 2, medium concentration; level 3, high concentration). Different rounds measured different samples with low, medium, and high concentrations. For sTfR, the VitMin Lab reported adjusted results (measured result/1.6) to account for the VitMin to Roche assay difference. We readjusted the VitMin results (reported result × 1.6) to their originally measured concentration to appropriately reflect the relative difference to the CDC target values.

Results

Descriptive data for the VitMin and Roche assays

Whereas the 2 Nepal sample subsets covered somewhat different concentration ranges, combining the 2 sets covered a wide range of normal and abnormal values (Table 1). In addition, the relation between the Roche and VitMin assays was similar for each analyte regardless of the sample set (Supplemental Figure 2).

Agreement between the VitMin and Roche assays

Using the original data, the Bland–Altman plot between each VitMin and Roche assay showed the existence of nonconstant variance for all 4 analytes and nonconstant difference for Fer, sTfR, and AGP (Supplemental Figure 1A–D), also supported by the significant P values for nonconstant difference and nonconstant variance (Table 2). After we log-transformed the data (Supplemental Figure 2E–H, Table 2), these violations appeared to resolve for Fer (constant difference and constant variance), partially resolve for AGP (constant variance but still nonconstant difference), and noticeably improve for CRP and sTfR (nonconstant difference and nonconstant variance).

Based on the log models, we observed high Pearson correlation coefficients (r 0.92) between the VitMin and Roche assay results for each analyte (Table 2). We also observed high concordance for Fer, CRP, and AGP (Lin's ρ 0.89), but not for sTfR (Lin's $\rho = 0.58$), indicating that the 2 assays have a strong linear relation but poor agreement, pointing to the existence of a systematic bias. The mean relative difference was −11.5% for Fer, but it varied over the concentration range for sTfR, CRP, and AGP (Table 2, Figure 1A–D).

Based on a nonparametric approach, the median relative difference between the 2 assays was relatively small for AGP (−8.2%) and Fer (−8.5%). It was somewhat larger for CRP (−19.5%) and large for sTfR (71.2%) (Table 2, Figure 1E–H). We estimated that for Fer and AGP nearly 50% of the VitMin Nepal results agreed within ±10% of the Roche results and ~ 90% agreed within ±30% (Table 3). For CRP, nearly 25% of the VitMin results agreed with ±10% of the Roche results and nearly 75% agreed within ±30%. When assessing all

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299 CRP sample pairs, 8.4%, 16.7%, 23.7%, 33.1%, 44.1%, and 52.5% agreed within ±5%, ±10%,±15%,±20%,±25%, and ±30%, respectively. Among the CRP sample pairs where the Roche results were less than the limit of detection ($n = 116$), 17.2% and 8.6% of the VitMin results agreed within ± 30% and ±10% of the Roche results, respectively. For sTfR, only 1.70% of the VitMin results agreed within ±30% of the Roche results.

Predicting reference-equivalent VitMin assay results

We established prediction equations for each analyte using the log-transformed Nepal data to convert VitMin assay results to reference-equivalent data (Table 4). Fer had a slope of 1 and a nonzero intercept, reflecting the constant difference. The linear prediction line for Fer was reasonably close to the line of identity (Figure 2A). sTfR, CRP, and AGP all had a slope slightly different from 1 and a nonzero intercept, reflecting the nonconstant difference. The nonlinear prediction line for CRP was reasonably close to the line of identity, whereas for sTfR and AGP it moved further away as the concentration increased (Figure 2B–D). We applied the prediction equations to 5 selected VitMin assay results for each analyte to calculate reference-equivalent predicted values with 95% prediction limits (Table 5). For example, we have 95% confidence that a future reference measurement of Fer will fall between 13.8 μg/L and 43.3 μg/L when using a value of 21.7 μg/L measured by the VitMin assay. For Fer, the analyte with constant difference across the log-transformed concentration range, we observed a constant relative difference of ~13% for the 5 selected values (Table 5). However, for sTfR, CRP, and AGP, the 3 analytes with nonconstant difference, we noted a slight concentration-dependent relative difference for sTfR (from −36.7% to −44.4%) and strong concentration-dependent relative difference for CRP (from 35.8% to −4%) and AGP (from 12.1% to −5.0%) across the concentration range (Table 5).

Population-level assessment by comparing prevalence of abnormal VitMin and Roche concentrations and distribution curves

The prevalence of abnormal results was comparable (overlapping 95% CI) between the 2 assays for Fer, CRP, and AGP, for both the original and the predicted VitMin results (Table 6). The prevalence of abnormal sTfR results was also comparable between the VitMin (original and predicted) and Roche assays for nonpregnant women; however, for children the prevalence was only comparable after adjusting the VitMin assay to the Roche assay. The folded empirical cumulative distribution function graphs showed good overlap between the Roche, original VitMin, and predicted VitMin results for Fer, CRP, and AGP, but for sTfR only between the Roche and predicted VitMin results (Supplemental Figure 3).

Individual-level assessment through diagnostic performance of VitMin assays

Between 15% and 22% of the Nepal samples had abnormal analyte results based on the Roche assays (Supplemental Table 2). We observed sensitivity and specificity of >80% for each VitMin assay using the original results as well as the predicted results. However, we observed an improvement in the positive predictive value for sTfR (from 66.3% to 88.1%) when using predicted results.

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Performance of VitMin assays in CDC's VITAL-EQA program

Over a period of 12 y [2007 (round 8) to 2019 (round 33)], the VitMin Fer assay achieved 52 out of 78 (66.7%) acceptable results (within ±7.7% of the CDC target) and showed a mean difference of –0.1% (Figure 3A). Unacceptable Fer results were either high or low with no apparent time trend. The majority of VitMin sTfR results were within a range of 40%–90% higher than the CDC target value, with a mean difference of 66.2% and no apparent time trend (Figure 3B). The VitMin CRP assay achieved 70 out of 78 (89.7%) acceptable results (within ±33% of the CDC target) and showed a mean difference of –7.9% (Figure 3C). There appeared to be a trend toward lower CRP results over time, with a few unacceptable low results in more recent rounds. The Nepal survey samples were analyzed around rounds 26–28.

Discussion

To our knowledge, this is the first systematic study to compare the VitMin ELISA assays with the well-established reference-type Roche clinical analyzer assays used for NHANES. Our results showed high correlations for all 4 VitMin assays with the Roche assays, good concordance for 3 of the 4 assays (Fer, CRP, and AGP), and a potentially correctable systematic bias for the sTfR assay. Furthermore, the VitMin assays displayed mainly overlapping distribution curves with the Roche assays, comparable prevalence estimates for abnormal concentrations, and generally acceptable long-term performance for Fer, sTfR, and CRP in CDC's VITAL-EQA program.

One commonly used approach to assess the acceptability of assay differences is to use objective quality goals for method performance based on biological variation (19). For Fer, the acceptability limits are fairly tight at ±7.7% (minimum allowable difference). The VitMin Fer assay slightly exceeded this limit (–8.5% median relative difference from nonparametric model; –11.5% mean relative difference from log model). VitMin Fer results in the VITAL-EQA program showed a minimal difference (–0.1%) to the Roche assay and no apparent time trend from 2007 to 2019.

For CRP, on the other hand, the minimum allowable difference is much wider (±32.7%) owing to the large biological variation. Thus, even though the median relative difference for CRP was more than double that of Fer (–19.5% compared with –8.5%) for the Nepal samples, the difference was well within the minimum criterion and even met the desirable criterion (±21.8%). VitMin CRP results in the VITAL-EQA program confirmed the modest difference to the Roche assay (–7.9%). The noticeable downward trend of CRP results in more recent years, although mostly within acceptability limits, could alter the interpretation of epidemiologic data where determining the prevalence of abnormal results is often an objective and CRP data are used in models to adjust Fer or other nutritional biomarkers for inflammation (22).

The VitMin AGP assay showed the smallest median relative difference of the 4 assays (–8.21%) and met the minimum criterion (±10.3%). Because of the lack of published validation data for the VitMin AGP assay, these findings are important to linking the AGP assay performance to a reference-type assay. However, owing to the lack of AGP data in

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the VITAL-EQA program, we do not know whether the snapshot assessment with the Nepal samples is representative of other time periods.

A practical approach to assess the closeness of 2 assays is to determine the percentage of results by the test assay that agree with the reference assay within certain limits. The majority of Fer, CRP, and AGP VitMin results (~75%–90%) agreed within ±30% of Roche results. Considering that FDA Bioanalytical Method Validation acceptability criteria for Incurred Sample Reanalysis conducted with the same bioanalytical method procedures specify that for ligand binding assays 67% of results should be within ±30% of the mean (23); and considering that the Roche and VitMin assays use different assay principles, antibodies, and calibrators; and that the VitMin assays are carried out manually, whereas the Roche assays are automated, the agreement found in our study seems acceptable.

Using the Nepal samples, the VitMin sTfR assay showed a large difference of ~70% to the Roche assay. Because the VitMin assay was originally calibrated to the Ramco assay and the 2 assays showed good agreement [$n = 119$; VitMin = 0.727 × Ramco + 2.891; $r = 0.92$; mean Bland–Altman difference close to 0, but evidence of nonconstant difference (4)], we expected the VitMin sTfR results to be ~45% higher than the Roche results based on earlier findings showing that the Roche assay performed ~30% lower than the Ramco assay (21). We regularly analyze the WHO sTfR reference material with the Roche assay and know that the assay remained largely stable over the last decade, still producing the same value as during the material characterization in 2008 (Supplemental Text 1). Based on the VITAL-EQA data, the VitMin sTfR assay also appears to have been stable over the period 2007–2019 and the mean relative difference (66.2%) was nearly identical to the difference found with the Nepal samples. This speaks to the value of an independent external quality assessment program that uses unaltered samples to monitor assay performance over time. Even when the standardization of assays is still outstanding, as is the case for sTfR, and we are unable to assess the acceptability of results with regards to their “trueness,” it is helpful to determine whether the performance over time is stable.

The reference-type assays used in this study, those of the Roche clinical analyzer, have been in use at the CDC laboratory for many years to analyze biomarkers in NHANES and other epidemiologic studies and their performance is well-documented. We applied a thorough process to characterize the relation between the VitMin and Roche assays and to ensure that statistical assumptions were not violated when generating prediction equations. Furthermore, we utilized available data on the long-term performance of the VitMin assays from the VITAL-EQA program to gain insight on whether the prediction equations are valid beyond the time point when the VitMin Lab analyzed the Nepal survey samples. Because of the acceptable long-term performance of the VitMin assays, we have confidence that the presented prediction equations could be used to generate reference-equivalent VitMin results. This facilitates the comparison of population iron status data from various micronutrient surveys analyzed by the VitMin Lab with data from NHANES, although caution should be used if the range of biomarker concentrations is different in other studies. Furthermore, before these equations can be universally applied, they should be validated in 1 independent sample set that includes samples from other demographic groups than children and nonpregnant women. The relatively wide 95% prediction intervals calculated

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for selected example results demonstrate, however, that data adjustments come with a cost and should be avoided when possible. Of the 4 analytes studied, sTfR would benefit most from a data alignment to the reference assay to address the systematic assay difference. CRP may also benefit from a data adjustment because of the strong concentration-dependent bias which alters results at the low end by ~30%, but hardly at all at the high end of measurement. It is worth noting, however, that the CRP prediction equation does not apply to concentrations <0.3 mg/L, the detection limit for the Roche assay. The other 2 analytes, Fer and AGP, appear to have a small enough difference to the Roche assays that data adjustments may not be necessary.

Our study has several strengths and limitations. We used a large, combined sample set that covered a wide range of concentrations for all analytes to allow for a thorough assessment of the relation between the assays and the generation of prediction equations. The combined sample set contained a large enough portion of samples with abnormal biomarker concentrations to evaluate the prevalence of the VitMin assays in comparison to the Roche assays. The combined sample set included samples from different age groups (children and women), further demonstrating the robustness of the assay relations. Lastly, we carefully conducted the statistical analysis to assess the agreement between the test and the reference assays, ensuring that we appropriately addressed assumption violations such as nonconstant difference and nonconstant variance. Nonetheless, even after a log transformation, both CRP and sTfR continued to show a violation of nonconstant variance. Although some ad hoc methods have been proposed such as a regression approach modeling the variability in the SD across the range of measurement (16), this approach has limitations, such as needing a fair amount of data at the higher concentration end to be confident that any trend detected is not a consequence of a few outliers or data points that are poorly captured by a linear model. We elected to accept this model violation in reporting our final results. Therefore, the LoA for CRP and sTfR should be interpreted with caution because they may be over- or underestimated. A further limitation of our study is that we were not able to confirm whether the VitMin AGP assay performance holds up across time. A broader limitation that applies to any method comparison study is related to how one assesses the acceptability of the test assay results. We chose to use the approach based on biological variation. However, as we have shown, it comes with limitations when the acceptability limits are either wide or tight because of high or low biological variation.

The VitMin assays have been used widely throughout the years to analyze samples from micronutrient surveys in low- and middle-income countries. Our study provides data from a snapshot comparison using Nepal samples and a long-term comparison using VITAL-EQA samples, and thus links the data generated by the VitMin Lab to assays used for NHANES. The prediction equations could be used to convert survey results to the reference-type assay results, thus allowing a better comparison and interpretation of iron status data across populations.

Supplementary Material

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Acknowledgments

We acknowledge Dr. Juergen Erhardt for conducting the VitMin ELISA analyses for the Nepal survey samples. We also thank Sweta Patel (ORISE) for conducting the Roche laboratory analyses. The authors' responsibilities were as follows—MZ and CMP: designed the overall research project; DJL: planned and oversaw the Roche analyses and verifications with reference materials; MZ, CMF, MRS, and CMP: performed the data analysis; CMF and MZ: wrote the initial draft, which was modified based on critical review from all coauthors; MEJ, ZM, and RDW: provided technical assistance for the planning and implementation of the Nepal surveys; NP, NJ, KRP, and DPA: were the in-country partners carrying out the planning and implementation of the Nepal surveys and provided critical review of the paper from the country perspective; CMP: has primary responsibility for the content; and all authors: read and approved the final manuscript.

This work was performed under employment of the US Federal government and the authors did not receive any outside funding for it. Funding and technical support to the Nepal National Micronutrient Status Survey were provided by the United States Agency for International Development, CDC, UNICEF, the European Union, and Nepal Ministry of Health and Population.

Abbreviations used:

AGP	α–1-acid glycoprotein
CRP	C-reactive protein
Fer	ferritin
LoA	limits of agreement
NNMSS	Nepal National Micronutrient Status Survey
QC	quality control
sTfR	soluble transferrin receptor
VITAL-EQA	Vitamin A Laboratory External Quality Assurance

References

1. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017;390(10100):1211–59. [PubMed: 28919117]
2. Lynch S, Pfeiffer CM, Georgieff MK, Brittenham G, Fairweather-Tait S, Hurrell RF, Hurrell RF, McArdle HJ, Raiten DJ. Biomarkers of Nutrition for Development (BOND)—iron review. J Nutr 2018;148(suppl_1):1001S–67S. [PubMed: 29878148]
3. World Health Organization. WHO guideline on the use of ferritin concentrations to assess iron status in individuals and populations. Geneva (Switzerland): WHO; 2020.
4. 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;134(11):3127–32. [PubMed: 15514286]
5. Shinoda N, Sullivan KM, Tripp K, Erhardt JG, Haynes BMH, Temple VJ, Woodruff B. Relationship between markers of inflammation and anaemia in children of Papua New Guinea. Public Health Nutr 2013;16(2):289–95. [PubMed: 22607654]
6. Rohner F, Northrop-Clewes C, Tschannen AB, Bosso PE, Kouassi-Gohou V, Erhardt JG, Bui M, Zimmermann MB, Mascie-Taylor CG. Prevalence and public health relevance of micronutrient deficiencies and undernutrition in pre-school children and women of reproductive age in Côte d'Ivoire, West Africa. Public Health Nutr 2014;17(9):2016–28. [PubMed: 24171836]

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7. Engle-Stone R, Haskell MJ, Ndjebayi AO, Nankap M, Erhardt JG, Gimou MM, Brown KH. Plasma retinol-binding protein predicts plasma retinol concentration in both infected and uninfected Cameroonian women and children. J Nutr 2011;141(12):2233–41. [PubMed: 22049292]
8. National Statistical Office. Malawi Micronutrient Survey Key Indicators Report 2015–16. [Internet]. Zomba (Malawi); Lilonge (Malawi); and Atlanta (GA): National Statistical Office; Ministry of Health; and CDC; 2017; [cited October 2020]. Available from: https://dhsprogram.com/pubs/pdf/FR319/FR319m.pdf.
9. Petry N, Al-Maamary S, Woodruff B, Alghannami S, Al-Shammakhi S, Al-Ghammari I, Tyler V, Rohner F, Wirth J. National prevalence of micronutrient deficiencies, anaemia, genetic blood disorders and over- and undernutrition in Omani women of reproductive age and preschool children. Sultan Qaboos Univ Med J 2020;20(2):e151–64. [PubMed: 32655907]
10. National Nutrition Agency (NaNA)-Gambia, UNICEF, Gambia Bureau of Statistics, GroundWork. Gambia Micronutrient Survey 2018. [Internet]. Banjul (Gambia): NaNA-Gambia; 2019; [cited October 2020]. Available from: https://groundworkhealth.org/wp-content/uploads/2019/03/GNMS2018-Final-Report_190325.pdf.
11. Ministry of Health and Population, Nepal, New ERA, UNICEF, European Union, US Agency for International Development, CDC. Nepal National Micronutrient Status Survey, 2016. [Internet]. Kathmandu (Nepal): Ministry of Health and Population, Nepal; 2018; [cited October 2020]. Available from: https://www.unicef.org/nepal/media/1206/file.
12. Instituto de Nutrición de Centro América y Panamá (INCAP). Informe del Sistema de Vigilancia Epidemiológica de Salud y Nutrición (SIVESNU): augusto 2018–abril 2019: módulo 2: salud y nutrición infantil, informe final. Guatemala City (Guatemala): INCAP; 2020.
13. Instituto de Nutrición de Centro América y Panamá (INCAP). Informe del Sistema de Vigilancia Epidemiológica de Salud y Nutrición (SIVESNU): augusto 2018–abril 2019: módulo 3: nutrición y salud de la mujer, informe final. Guatemala City (Guatemala): INCAP; 2020.
14. Pfeiffer CM, Looker AC. Laboratory methodologies for indicators of iron status: strengths, limitations, and analytical challenges. Am J Clin Nutr 2017;106(Suppl 6):1606S–14S. [PubMed: 29070545]
15. Pfeiffer CM, Cook JD, Mei Z, Cogswell ME, Looker AC, Lacher DA. Evaluation of an automated soluble transferrin receptor (sTfR) assay on the Roche Hitachi analyzer and its comparison to two ELISA assays. Clin Chim Acta 2007;382(1–2):112–6. [PubMed: 17511979]
16. Cobas® 6000 Operator’s Manual. Roche Diagnostics.
17. Bland JM, Altman DG. Measuring agreement in method comparison studies. Stat Methods Med Res 1999;8(2):135–60. [PubMed: 10501650]
18. Carstensen B. Comparing clinical measurement methods: a practical guide. Chichester (United Kingdom): John Wiley & Sons; 2011.
19. Fraser CG, Petersen PH, Libeer JC, Ricos C. Proposals for setting generally applicable quality goals solely based on biology. Ann Clin Biochem 1997;34(1):8–12. [PubMed: 9022883]
20. Raiten DJ, Sakr Ashour FA, Ross AC, Meydani SN, Dawson HD, Stephensen CB, Brabin BJ, Suchdev PS, van Ommen B; INSPIRE Consultative Group. Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence (INSPIRE). J Nutr 2015;145(5):1039S–108S. [PubMed: 25833893]
21. Mei Z, Pfeiffer CM, Looker AC, Flores-Ayala RC, Lacher DA, Mirel LB, Grummer-Strawn LM. Serum soluble transferrin receptor concentrations in US preschool children and non-pregnant women of childbearing age from the National Health and Nutrition Examination Survey 2003–2010. Clin Chim Acta 2012;413(19–20):1479–84. [PubMed: 22705806]
22. Namaste SML, Rohner F, Huang J, Bhushan NL, Flores-Ayala R, Kupka R, Mei Z, Rawat R, Williams AM, Raiten DJ, et al. Adjusting ferritin concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2017;106(Suppl 1):359S–71S. [PubMed: 28615259]
23. US Department of Health and Human Services. Bioanalytical method validation: guidance for industry. [Internet]. Beltsville (MD): Food and Drug Administration; 2018; [cited September 2021]. Available from: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf.

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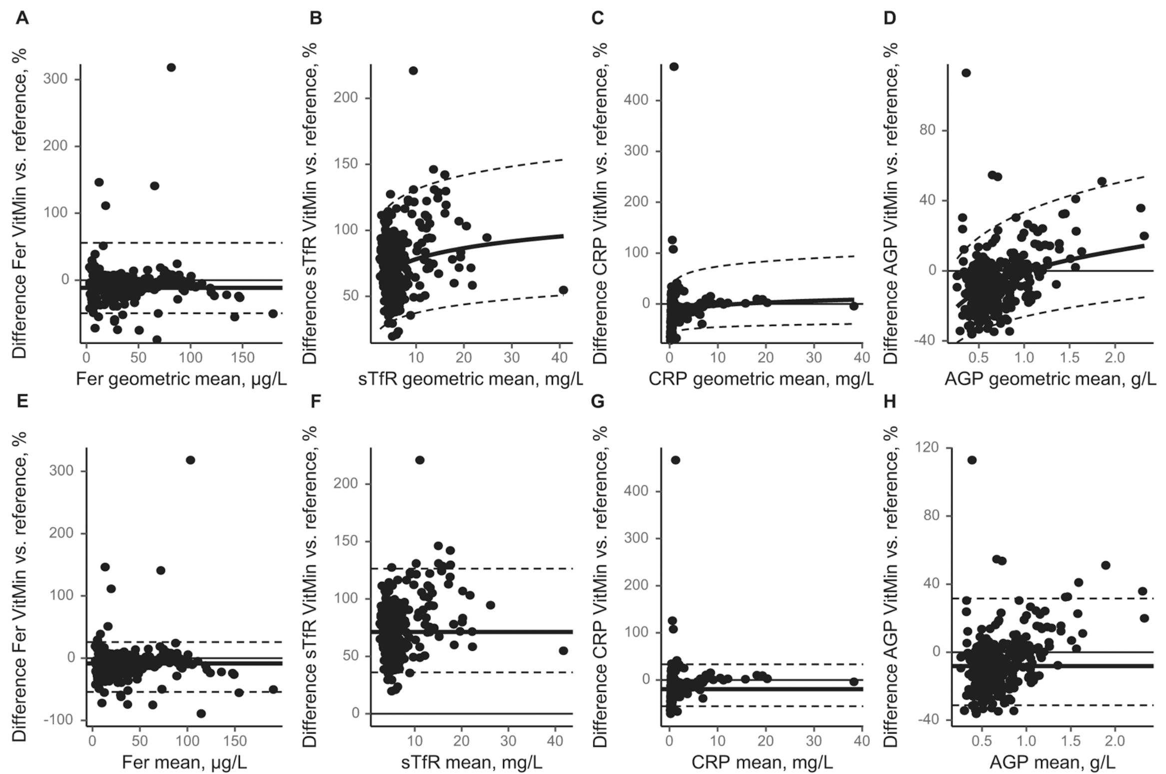

FIGURE 1.

Method comparison plots for VitMin ELISA against Roche reference assays. (A–D) Percentage difference from the reference on the *y* axis against the geometric mean on the *x* axis. The heavy solid line is derived through a back-transformation from the selected model of the log-transformed differences on the average of the log-transformed values of each pair. The dashed lines are the corresponding LoA from the same linear regression model. (E–H) Percentage difference from the reference on the *y* axis against the mean on the *x* axis and showing the median with the 2.5th and 97.5th percentiles as the nonparametric LoA. The solid horizontal line represents the 0 line on all plots. AGP, α−1-acid glycoprotein; CRP, C-reactive protein; Fer, ferritin; LoA, limits of agreement; sTfR, soluble transferrin receptor.

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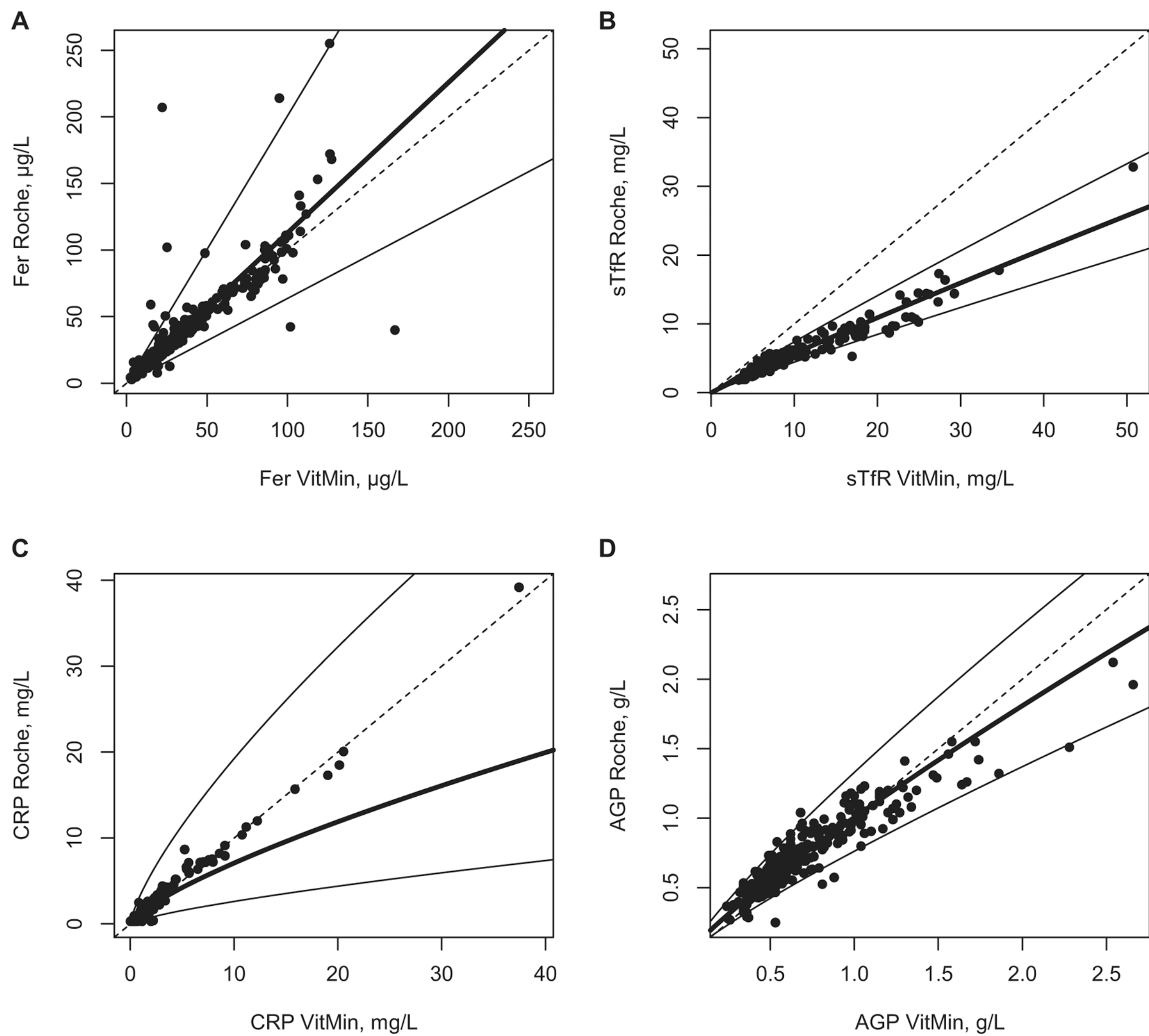

FIGURE 2.

Prediction plots for VitMin ELISA compared with Roche reference assays showing the prediction line and 95% prediction intervals for Fer (A), sTfR (B), CRP (C), and AGP (D). The dashed line represents the line of identity. AGP, α–1-acid glycoprotein; CRP, C-reactive protein; Fer, ferritin; sTfR, soluble transferrin receptor.

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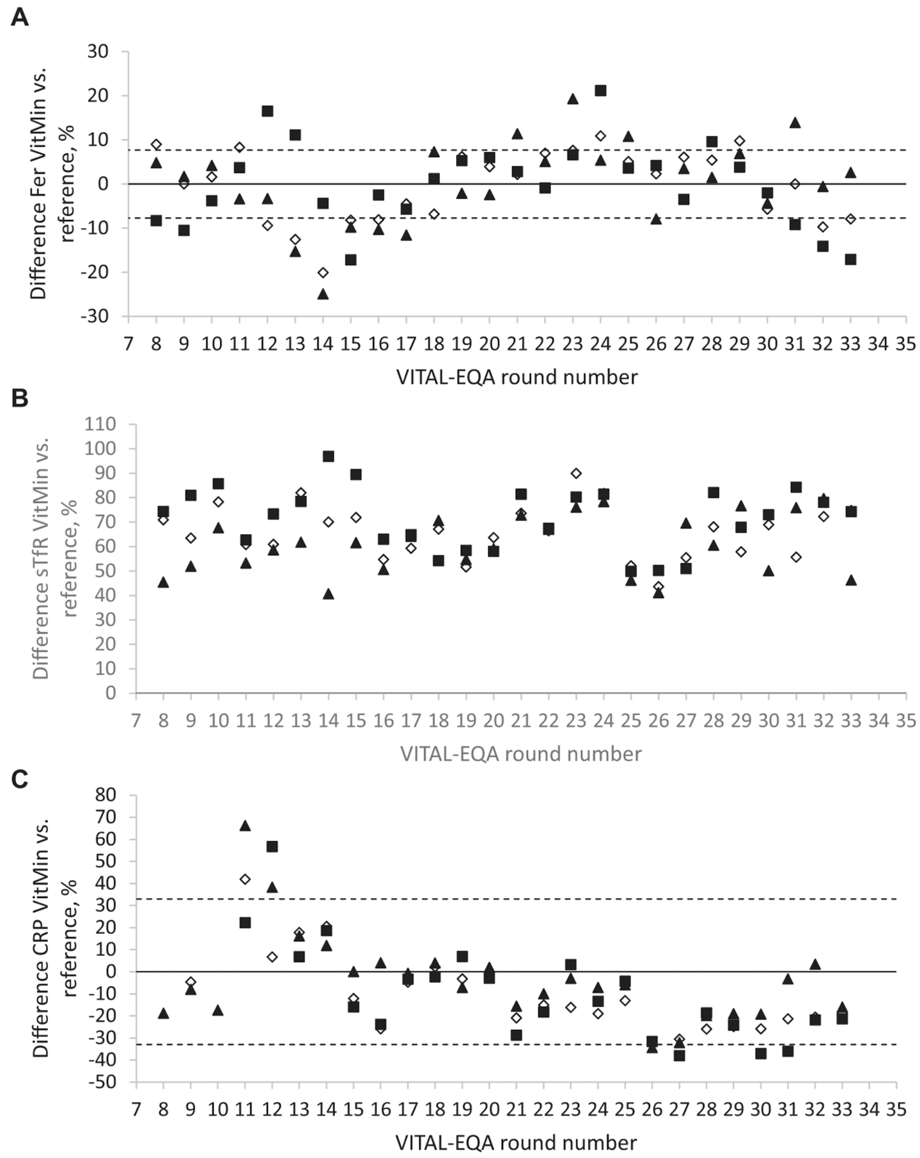

FIGURE 3.

Performance of VitMin ELISA assays over time in the CDC VITAL-EQA program with the relative difference compared to the CDC reference assay shown on the *y* axis, for Fer (A), sTfR (B), and CRP (C). The solid horizontal line represents the 0 line, whereas the dashed lines represent the acceptability limits (±7.7% for Fer and ±33% for CRP). No acceptability limits are shown for sTfR because of large assay differences due to the lack of standardization to a common reference material. Data are shown for 3 samples/round: level 1 (low concentration), solid square; level 2 (medium concentration), open diamond; and level 3 (high concentration), solid triangle. Different samples are used in each round. The Nepal survey samples were analyzed around rounds 26–28. CRP, C-reactive protein; Fer, ferritin; sTfR, soluble transferrin receptor; VITAL-EQA, Vitamin A Laboratory External Quality Assurance.

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TABLE 1

Descriptive parameters for serum Fer, sTfR, CRP, and AGP measured by Roche reference and VitMin ELISA assays[1]

Analyte	Parameter	All	Children 6–59 mo	Nonpregnant women
Fer	n[2]	295	195	100
	Roche median [IQR], μg/L	36.6 [17.8–55.3]	31.8 [17.1–50.9]	40.3 [18.9–61.8]
	Roche range, μg/L	2.72–255	3.31–214	2.72–255
	VitMin median [IQR], μg/L	30.4 [16.0–48.2]	27.4 [14.5–44.3]	36.5 [18.7–65.6]
	VitMin range, μg/L	2.44–167	2.44–119	3.24–167
sTfR	n[2]	294	195	99
	Roche median [IQR], mg/L	4.13 [3.25–5.38]	4.52 [3.69–5.71]	3.27 [2.71–4.50]
	Roche range, mg/L	1.86–32.8	2.20–32.8	1.86–14.4
	VitMin median [IQR], mg/L	6.70 [5.54–8.85]	7.13 [6.16–10.3]	5.37 [4.68–7.36]
	VitMin range, mg/L	3.30–50.8	4.21–50.8	3.30–29.3
CRP	n[2]	299	199	100
	Roche median [IQR], mg/L	0.45 [0.30–1.58]	0.48 [0.30–1.86]	0.39 [0.30–1.29]
	Roche range, mg/L	0.30–39.2	0.30–39.2	0.30–17.3
	VitMin median [IQR], mg/L	0.40 [0.18–1.35]	0.42 [0.18–1.57]	0.37 [0.17–1.11]
	VitMin range, mg/L	0.00–37.4	0.00–37.4	0.00–19.0
AGP	n[2]	295	195	100
	Roche median [IQR], mg/L	0.68 [0.56–0.89]	0.72 [0.57–0.96]	0.62 [0.51–0.76]
	Roche range, mg/L	0.25–2.12	0.32–2.12	0.25–1.04
	VitMin median [IQR], mg/L	0.61 [0.48–0.82]	0.64 [0.50–0.95]	0.56 [0.40–0.70]
	VitMin range, mg/L	0.24–2.66	0.31–2.66	0.24–1.10

[1] AGP, α–1-acid glycoprotein; CRP, C-reactive protein; Fer, ferritin; sTfR, soluble transferrin receptor.

[2] Reflects the number of paired results available for both assays.

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TABLE 2

Agreement between VitMin ELISA and Roche reference assays[1]

Parameter	Fer	sTfR	CRP	AGP
Nonconstant difference[2]				
Original data *P* value	<0.001	<0.001	0.78	<0.001
Log-data *P* value	0.56	<0.001	<0.001	<0.001
Nonconstant variance[3]				
Original data *P* value	<0.001	<0.001	<0.001	<0.001
Log-data *P* value	0.27	0.0054	<0.001	0.056
Selected model[4]	Log assuming constant difference	Log assuming nonconstant difference	Log assuming nonconstant difference	Log assuming nonconstant difference
Ln model				
Pearson *r*[5]	0.95	0.96	0.97	0.92
Lin's concordance ρ (95% CI)[5]	0.94 (0.93, 0.95)	0.58 (0.54, 0.62)	0.95 (0.93, 0.96)	0.89 (0.87, 0.91)
Mean relative difference (LoA) to reference,[6] %	−11.5 (−49.7, 55.9)	Varies	Varies	Varies
Nonparametric model				
Median [IQR] relative difference to reference,[7] %	−8.51 [−16.9 to −0.93]	71.2 [57.5–87.1]	−19.5 [−29.1 to −4.25]	−8.21 [−15.9 to 1.78]
Nonparametric LoA, %	−54.2, 25.8	36.0–126.3	−56.1 to 33.3	−31.3 to 31.6

[1]Roche assays used as reference. AGP, α−1-acid glycoprotein; CRP, C-reactive protein; Fer, ferritin; LoA, limits of agreement; sTfR, soluble transferrin receptor.

[2]The *P* value tests the null hypothesis that the slope coefficient is 0 from a regression of the differences on the averages.

[3]The *P* value tests the null hypothesis that the slope coefficient is 0 from a regression of the absolute residuals on the averages, where the residuals are computed from a regression of the difference on the averages.

[4]Model selected to present the final method comparison results between VitMin and Roche.

[5]Data analysis performed on a ln scale (Pearson correlation, Lin's concordance).

[6]Mean relative difference and LoA have been back-transformed from the log scale.

[7]Relative difference calculated as (VitMin − reference)/reference and expressed as percentages.

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TABLE 3

Percentages of the VitMin ELISA sample results that agree with the Roche reference assay results within selected limits[1]

Selected limits	Fer	sTfR	CRP[2]	AGP
±5%	21.7	0.000	12.0	25.8
±10%	49.8	0.000	23.5	44.4
±15%	64.4	0.000	33.9	63.7
±20%	76.6	0.340	45.9	76.3
±25%	82.7	1.02	62.3	88.1
±30%	88.5	1.70	74.9	92.9

[1] Roche assays were used as the reference. AGP, α−1-acid glycoprotein; CRP, C-reactive protein; Fer, ferritin; sTfR, soluble transferrin receptor.

[2] Assessment excludes all values less than the limit of detection for Roche. Among the pairs of measurements where Roche was less than the limit of detection ($n = 116$), 17.2% of VitMin samples were within ±30% and 8.6% of VitMin samples were within ±10% of an imputed Roche value of 0.3. When assessing all 299 CRP sample pairs, 8.4%, 16.7%, 23.7%, 33.1%, 44.1%, and 52.5% agreed within ±5%, ±10%, ±15%, ±20%, ±25%, and ±30% of an imputed Roche result of 0.3, respectively.

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TABLE 4

Prediction equations between VitMin ELISA and Roche reference assays[1]

Parameter	Fer, μg/L	sTfR, mg/L	CRP, mg/L	AGP, g/L
VitMin is x, reference is y[2]	$\ln y = 0.12 + \ln x$ (0.29)	$\ln y = -0.41 + 0.94 \times \ln x$ (0.13)	$\ln y = 0.21 + 0.92 \times \ln x$ (0.28)	$\ln y = 0.01 + 0.85 \times \ln x$ (0.14)

[1]Roche assays used as reference. AGP, α–1-acid glycoprotein; CRP, C-reactive protein; Fer, ferritin; PE, prediction error; sTfR, soluble transferrin receptor.

[2]Log-transformed equations to convert VitMin results to the reference assay results. PE (provided in parentheses) can be used to construct 95% prediction intervals for a selected value x using $\pm t_{n-1,0.025}$PE, where $t_{n-1,0.025}$ is the 97.5th percentile from the Student t distribution with $n - 1$ degrees of freedom.

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TABLE 5

Predictions for VitMin ELISA assays and 95% prediction intervals from conversion equations[1]

Biomarker (unit)	Future measured VitMin value	Predicted VitMin value (95% prediction interval) after aligning to reference	Relative difference predicted vs. measured,[2] %
Fer, $\mu g/L$	6.80	7.70 (4.3, 13.6)	12.7
	13.6	15.3 (8.7, 27.1)	12.7
	21.7	24.5 (13.8, 43.3)	12.7
	44.6	50.3 (28.4, 89.0)	12.7
	122	137.6 (77.7, 243.4)	12.7
sTfR, mg/L	2.10	1.3 (1.0, 1.8)	−36.7
	3.80	2.3 (1.8, 3.0)	−39.1
	4.70	2.8 (2.1, 3.7)	−39.9
	5.80	3.4 (2.6, 4.5)	−40.7
	15.9	8.8 (6.7, 11.6)	−44.4
CRP, mg/L	0.30	0.4 (<LOD, 0.7)	35.8
	1.00	1.2 (0.7, 2.1)	23.4
	2.70	3.1 (1.8, 5.3)	13.9
	4.40	4.8 (2.8, 8.4)	9.6
	23.0	22.1 (12.7, 38.4)	−4.0
AGP, g/L	0.50	0.6 (0.4, 0.7)	12.1
	0.70	0.7 (0.6, 1.0)	6.6
	0.80	0.8 (0.6, 1.1)	4.4
	1.00	1.0 (0.8, 1.3)	1.0
	1.50	1.4 (1.1, 1.9)	−5.0

[1] Results in this table can be interpreted as follows: for a selected value measured by the VitMin assay there is a 95% confidence that the future reference equivalent value is within the presented prediction interval. Roche clinical analyzer assays were used as the reference. AGP, α−1-acid glycoprotein; CRP, C-reactive protein; Fer, ferritin; LOD, limit of detection; sTfR, soluble transferrin receptor.

[2] Calculated as $100 \times \left(\frac{e^{(a + b \times \ln(\text{measured}))} - \text{measured}}{\text{measured}} \right)$, where a and b are the estimated intercept and slope from the selected prediction equation, respectively.

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TABLE 6

Prevalence of abnormal results for original and predicted VitMin data and for Roche data[1]

			Prevalence (95% CI), %		
Analyte	***n***	**Cutoff**	**Roche**	**Original VitMin**	**Predicted VitMin**
Fer (NPW)	100	<15 μg/L	21.0 (13.0, 29.0)	19.0 (11.3, 26.7)	17.0 (9.6, 24.4)
Fer (6–59 mo)	195	<12 μg/L	20.0 (14.4, 25.6)	22.1 (16.2, 27.9)	21.6 (15.8, 27.3)
sTfR (NPW)	99	>5.33 mg/L[2]	16.2 (8.9, 23.4)	17.2 (9.7, 24.6)	13.1 (6.5, 19.8)
sTfR (6–59 mo)	195	>6.0 mg/L[2]	22.6 (16.7, 28.4)	35.4 (28.7, 42.1)	23.6 (17.6, 29.6)
CRP	299	>5.0 mg/L	9.0 (5.8, 12.3)	8.4 (5.2, 11.5)	8.4 (5.2, 11.5)
AGP	295	>1.0 g/L	15.6 (11.5, 19.7)	14.9 (10.9, 19.0)	15.6 (11.5, 19.7)

[1] AGP, α–1-acid glycoprotein; CRP, C-reactive protein; Fer, ferritin; NPW, nonpregnant women; sTfR, soluble transferrin receptor.

[2] sTfR cutoff values: >5.33 mg/L for NPW and >6 mg/L for children for Roche and predicted VitMin data; >8.3 mg/L for original VitMin data.