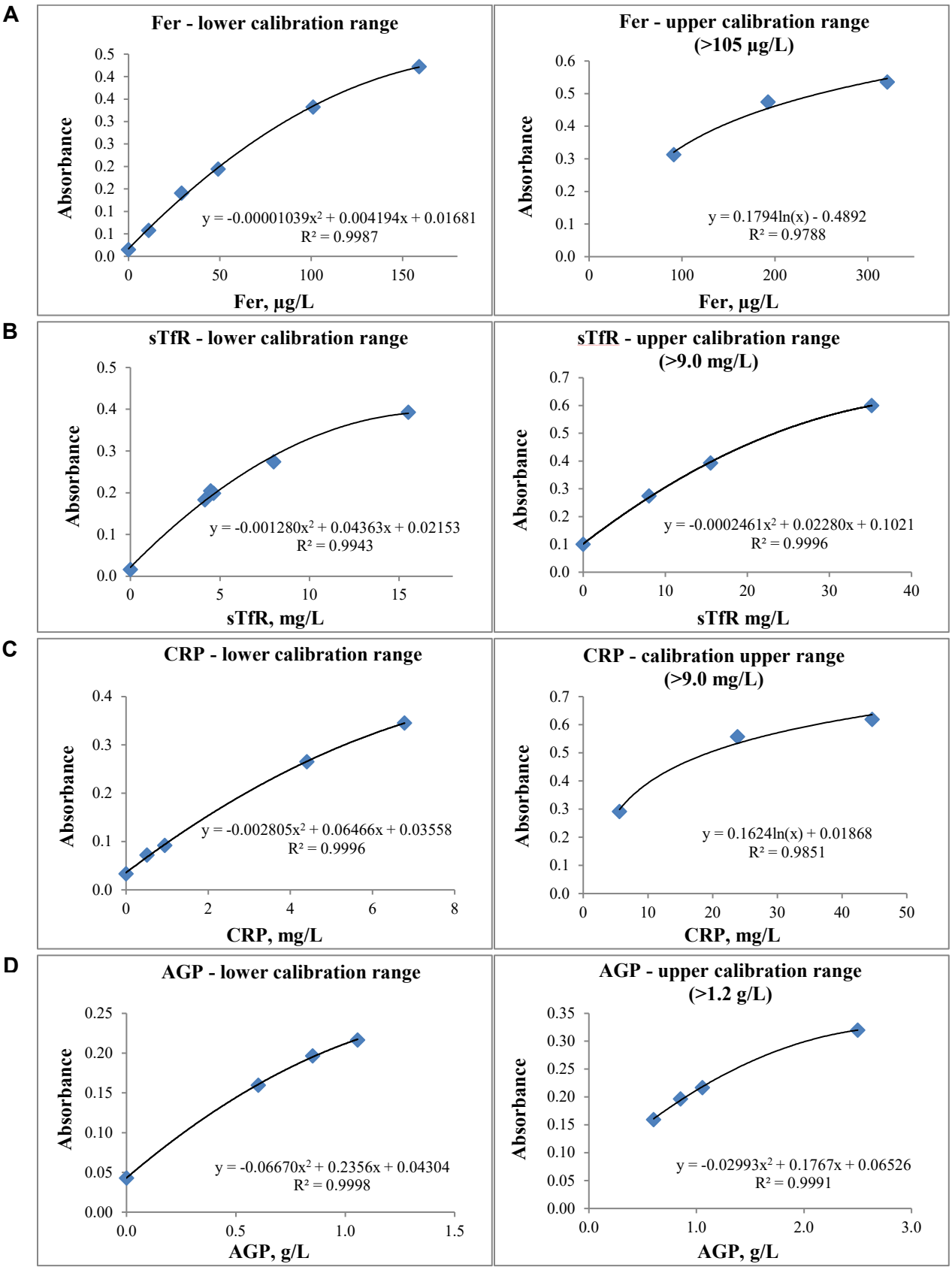
The VitMin Lab sandwich-ELISA assays for iron and inflammation markers compared well with clinical analyzer reference-type assays in subsamples of the Nepal National Micronutrient Status Survey

Fischer et al.

Online Supplementary Material

Supplementary Fig. 1. Typical calibration curves for the VitMin ELISA assays (lower and upper ranges); Fer (A), sTfR (B), CRP (C), and AGP (D). Calibration curves derived from commercially available serum control material (Bio-Rad Liquichek Immunology Control).



Supplementary Table 1. Objective quality goals for method performance based on biologic variation [1][1]

Parameter	Fer	sTfR	CRP	AGP
Within-individual CV_I, % [Ref]	14.2 [2]	11.3 [3]	42.2 [2]	11.3 [2]
Between-individual CV_G, % [Ref]	15.0 [2]	17.3 [4]	76.3 [2]	24.9 [2]
Allowable difference to target, %				
Optimum, $D=0.125*(CV_I^2+CV_G^2)^{1/2}$	2.6	2.6	10.9	3.4
Desirable, $D=0.25*(CV_I^2+CV_G^2)^{1/2}$	5.2	5.2	21.8	6.8
Minimum, $D=0.375*(CV_I^2+CV_G^2)^{1/2}$	7.7	7.7	32.7	10.3

[1] References for the objective quality goals for each analyte are provided in the first 2 rows showing the within- and between-individual variation; AGP, α-1-acid glycoprotein; CRP, C-reactive protein; CV_G, between-individual or group variation; CV_I, within-individual variation; D, difference to target; Fer, ferritin; Ref, reference; sTfR, soluble transferrin receptor

References

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Supplementary Text 1. Performance of the Roche cobas clinical analyzer with international reference materials for serum ferritin, soluble transferrin receptor, C-reactive protein, and α-1-acid glycoprotein

Serum ferritin (Fer) measured on the Roche cobas e601 immunology analyzer. The WHO 3rd International Standard for Fer is a lyophilized, recombinant human ferritin L-chain preparation available from the United Kingdom National Institute for Biological Standards and Control (NIBSC), 94/572; it has an assigned consensus value of 6.3 µg/ampoule [1]. The mean±SD deviation from the assigned consensus value achieved by the CDC laboratory over 27 measurements performed during the 3-y period of 2016–2019 was 10.4%±6.7% (1/4 dilution; 1575 µg/L), 9.0%±3.7% (1/10 dilution; 630 µg/L), 10.4%±3.2% (1/50 dilution; 126 µg/L), 11.8%±3.6% (1/200 dilution; 31.5 µg/L), and 15.8%±9.3% (1/1000 dilution; 6.3 µg/L). The regular measurement of the 3rd International Standard is helpful to monitor potential shifts in the Fer assay, but less useful to assess accuracy because of commutability issues. The Roche Fer assay is traceable to the 1st International Standard (80/602) based on native human Fer purified from liver [2]. Roche Diagnostics verifies that reagent lot-to-lot consistency is excellent using native human serum [3].

Serum soluble transferrin receptor (sTfR) measured on the Roche cobas c501 chemistry analyzer. The WHO Reference Reagent for sTfR is a lyophilized, recombinant human preparation available from the NIBSC, 07/202; it has a slightly shorter molecular structure than serum sTfR and an assigned value of 21.74 mg/L or 303 nmol/L based on a theoretical extinction coefficient and the molecular weight [4]. An international collaborative study evaluating this material found poor agreement on the estimated rsTfR content of 07/202 across different assay platforms [5]. The CDC laboratory characterized this material in 2008 over multiple days and dilutions, obtaining a mean±SD of 60.5±0.5 mg/L using the Roche Hitachi 912 clinical analyzer. The mean±SD deviation from this value achieved over 17 measurements performed during the 4-y period of 2016–2020 was 4.7%±2.5% (neat material; 60.5 mg/L), 0.0%±2.1% (1/2 dilution; 30.25 mg/L), 4.3%±2.2% (1/4 dilution; 15.13 mg/L), 7.1%±2.8% (1/8 dilution; 7.56 mg/L), and 11.6%±3.2% (1/16 dilution; 3.78 mg/L). The regular measurement of the WHO Reference Reagent is helpful to monitor potential shifts in the sTfR assay, but less useful to assess accuracy because the Roche sTfR assay has not been standardized against this material; instead it has been standardized against an in-house reference preparation.

Serum C-reactive protein (CRP) measured on the Roche cobas c501 chemistry analyzer. The European Institute for Reference Materials and Measurements (IRMM) provides a liquid frozen human serum reference material spiked with CRP, ERM-DA474; it has a certified value of 41.2 mg/L as measured by various immunonephelometry and immunoturbidimetry methods using ERM-DA470 as calibrant [6]. The mean±SD deviation from the certified value achieved by the CDC laboratory over 17 measurements performed during the 4-y period of 2016–2020 was -8.3%±4.2% (neat material; 41.2 mg/L), -9.8%±4.4% (1/2 dilution; 20.6 mg/L), -9.7%±6.3% (1/4 dilution; 10.3 mg/L), and -7.5%±7.8% (1/8 dilution; 5.15 mg/L).

The regular measurement of this reference material is helpful to monitor potential shifts in the CRP assay, but less useful to assess accuracy because of commutability issues [7]. Roche standardized the CRP Gen.3 assay by method comparison to the CRP Gen.2 assay on the Roche INTEGRA®, which is directly traceable (100% recovery) to the international reference material CRM 470/ERM-DA470. Consequently, patient samples (native sera and plasma) measured by the CRP Gen.3 assay will recover correctly, but processed materials (external quality control and reference material) may recover differently. Recovery of the international reference material ERM-DA470 and of the successor ERM-DA472 was 87.8% when using the CRP Gen.3 assay on the cobas c501 chemistry analyzer.

Serum α-1-acid glycoprotein (AGP) measured on the Roche cobas c501 chemistry analyzer. The IRMM provides a lyophilized human serum reference material for various proteins including AGP, ERM-DA470k; the AGP certified value is 0.617 g/L as measured by various methods using ERM-DA470 as calibrant [8]. The mean recovery of AGP in ERM-DA470k spiked into a CFAS protein material achieved by the CDC laboratory over 2 measurements performed in 2020 and 2021 was 96.8% (1/2 dilution), 94.0% (1/4 dilution), and 92.5% (1/8 dilution). This procedure was adopted to avoid matrix effects that were observed when the reference material was diluted with Roche diluent, saline, or water and showed significant under-recovery. The regular measurement of this reference material is helpful to monitor potential shifts in the AGP assay, but less useful to assess accuracy because of matrix effects. Roche standardized the AGP assay against the reference preparation of the IRMM BCR470/CRM470.

References

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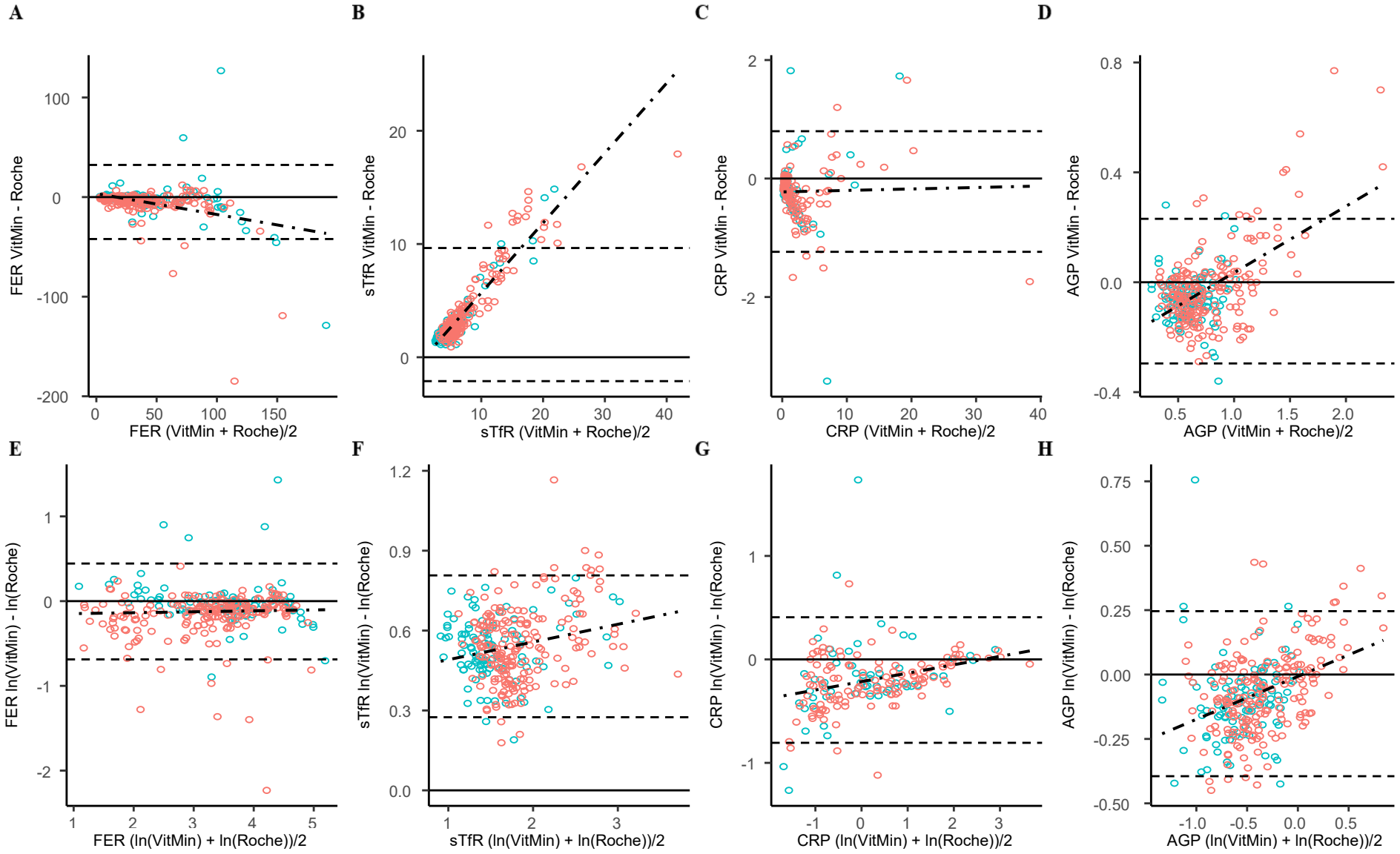
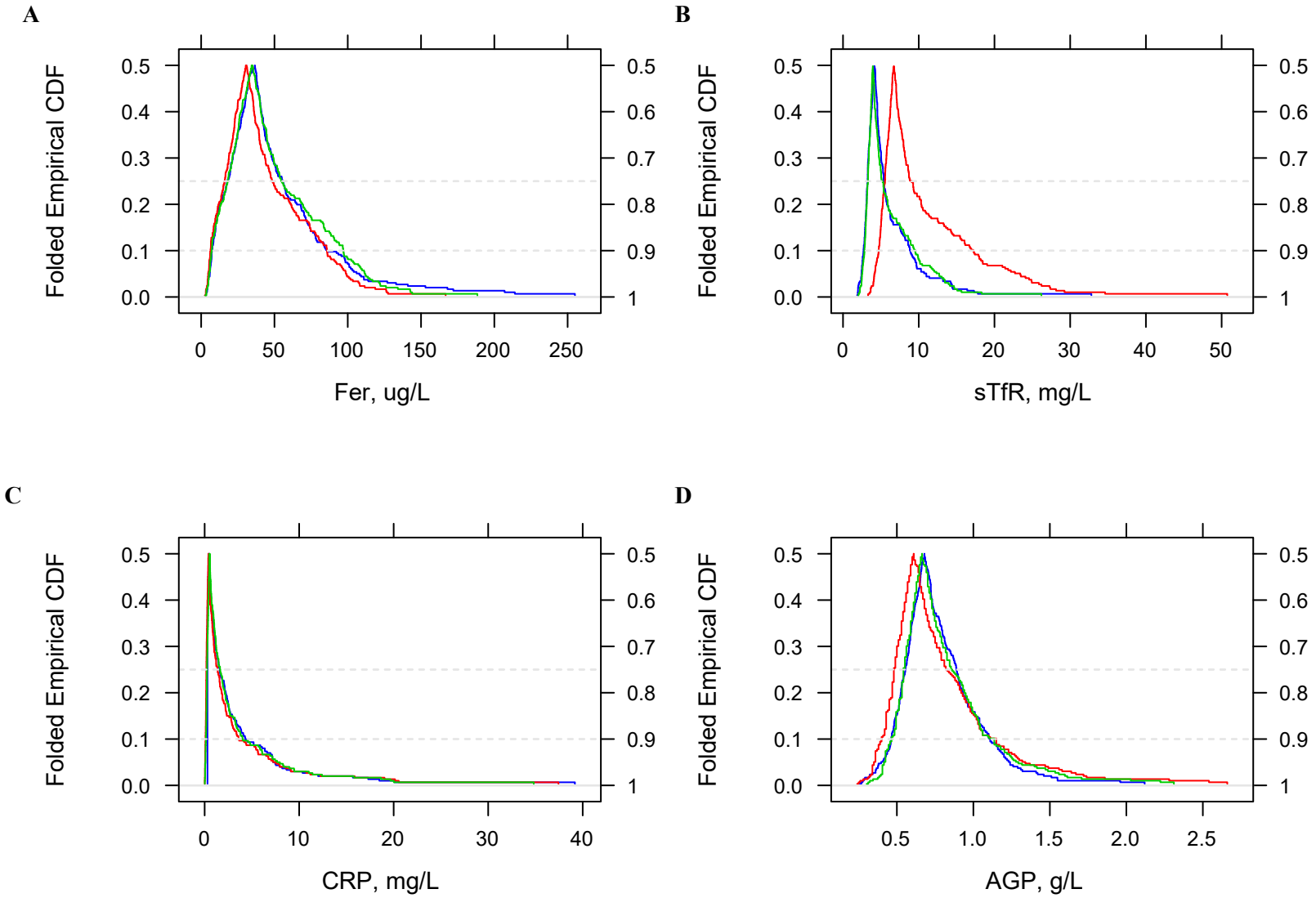
Supplementary Fig. 2. Difference plots for VitMin *vs.* Roche assays showing the original data (panels A-D) and log-transformed data (panels E-H) for ferritin (panels A and E), soluble transferrin receptor (panels B and F), C-reactive protein (panels C and G), and α-1-acid glycoprotein (panels D and H). The difference between the 2 assays is shown on the y-axis, while the mean of the 2 assays is shown on the x-axis. Each data set is shown in a different color: children 6-59 months, serum (blue); non-pregnant women, serum (red). The solid horizontal line represents the zero-line. The dashed horizontal lines represent limits of agreement (mean difference ± 2 SD). The dashed linear regression line is shown to assess non-constant difference. The units are µg/L for Fer and mg/L for sTfR, CRP, and AGP, respectively.



Supplementary Fig. 3: Folded empirical cumulative distribution function (CDF) graphs for ferritin (panel A), sTfR (panel B), CRP (panel C), and AGP (panel D). Blue line represents Roche, red line original VitMin, and green line predicted VitMin data.



Supplementary Table 2. Diagnostic performance of VitMin ELISA assays[1]

	Fer[2]		sTfR[3]		CRP[4]		AGP[5]	
Parameter	Original	Predicted	Original	Predicted	Original	Predicted	Original	Predicted
True Positives, *n*	58	56	57	52	25	25	37	39
False Positives, *n*	4	3	29	7	0	0	7	7
True Negatives, *n*	231	232	205	227	156	156	242	242
False Negatives, *n*	2	4	3	8	2	2	9	7
Sensitivity[6], %	96.7	93.3	95.0	86.7	92.6	92.6	80.4	84.8
Specificity[7], %	98.3	98.7	87.6	97.0	100.0	100.0	97.2	97.2
PPV[8], %	93.5	94.9	66.3	88.1	100.0	100.0	84.1	84.8
NPV[9], %	99.1	98.3	98.6	96.6	98.7	98.7	96.4	97.2

[1] Roche assays used as reference. AGP, α-1-acid glycoprotein; CRP, C-reactive protein; Fer, ferritin; NPV, Negat ive Predictive Value; PPV, Positive Predictive Value; sTfR, soluble transferrin receptor.

[2] Fer cutoff values: <15 µg/L for non-pregnant women and <12 µg/L for children. Abnormal Fer samples based on reference assay: 60 out of 295 (20.3%).

[3] sTfR cutoff values: >8.3 mg/L (VitMin); >5.33 mg/L for non-pregnant women and >6 mg/L for children (Roche). Abnormal sTfR samples based on reference assay: 65 out of 294 (22.1%).

[4] CRP cutoff value: >5 mg/L. Abnormal CRP samples based on reference assay: 27 out of 183 (14.8%).

[5] AGP cutoff value: >1 g/L. Abnormal AGP samples based on reference assay: 46 out of 295 (15.6%).

[6] Sensitivity = [True Positives / (True Positives + False Negatives)] * 100

[7] Specificity = [True Negatives / (True Negatives + False Positives)] * 100

[8] PPV = [True Positives / (True Positives + False Positives)] * 100

[9] NPV = [True Negatives / (True Negatives + False Negatives)] * 100