

METHOD COMPARISON OF PEDIATRIC IRON BIOMARKERS: IMPLICATIONS FOR CLINICAL INTERPRETATION ACROSS ANALYTICAL PLATFORMS

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Iron deficiency is the most prevalent nutritional disorder in children and is typically assessed using serum iron, transferrin, and ferritin levels. However, variations in analytical methods between testing platforms may influence diagnostic accuracy and clinical decisions. This cross-sectional method comparison study evaluated 137 blinded pediatric serum samples collected from a tertiary children's hospital in Vietnam. Serum iron was measured by a colorimetric method, transferrin by immunoturbidimetry, and ferritin by immunoassay, using two different platforms: Cobas Pro (Roche Diagnostics) and Atellica Solution (Siemens Healthineers). Analytical precision and trueness were assessed in accordance with CLSI EP15-A3 guidelines. Agreement between platforms was evaluated using Passing-Bablok regression and Bland-Altman analysis. Results showed systematic bias across all analytes, with the Atellica platform yielding consistently lower values. Specifically, the regression equations were Siemens = 0.96 (Roche) – 0.1 µmol/L for serum iron, Siemens = 0.93 (Roche) + 1.0 mg/dL for transferrin, and Siemens = 0.723 (Roche) – 1.7 µg/L for ferritin. Mean differences were -0.7 µmol/L, -15.6 mg/dL, and -137 µg/L, respectively. These findings indicate notable discrepancies between the platforms, underscoring the necessity for platform-specific reference intervals and the importance of harmonizing methods. In settings where harmonization is not feasible, regression-based adjustments may facilitate reference interval transference, especially in low-resource environments.

Keywords: Method comparison, serum iron, transferrin, ferritin.

I. INTRODUCTION

Iron deficiency is the most prevalent nutritional disorder worldwide and poses a significant burden of disease on developing countries. It often progresses to iron deficiency anemia (IDA) in children due to rapid growth, inadequate dietary iron intake, and factors

such as parasitic infections or low iron bioavailability in plant-based diets.¹ Conversely, iron overload, though less frequently studied, raises concerns in regions affected by prevalent hemoglobinopathies (e.g., thalassemia, sickle cell disease) or unregulated iron supplementation programs.² Moreover, other disorders of iron metabolism disrupt homeostasis through genetic mutations, ineffective erythropoiesis, or impaired iron transport and storage, leading to abnormal iron distribution rather than mere excess or deficiency.³

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Serum (or plasma) ferritin serves as a primary biomarker for evaluating iron stores in children. Serum ferritin is an acute-phase reactant and is elevated in inflammatory conditions, while malnutrition, particularly protein deficiency, can reduce ferritin levels.^{4,5} Both inflammatory conditions and malnutrition are common in developing countries and can confound the interpretation of serum ferritin. The absence of age- and population-specific reference intervals further complicates the interpretation in children who have differing dietary and health profiles.⁶

To overcome these limitations, serum iron and transferrin are used alongside ferritin to provide complementary information. Serum iron, which is bound to transferrin, reflects the iron immediately available for erythropoiesis. Concentrations below 8.9 $\mu\text{mol/L}$ in children indicate early iron deficiency before significant depletion of ferritin.⁵ In the context of anemia of chronic disease (ACD), low serum iron with normal or elevated ferritin levels distinguishes functional iron deficiency from true depletion, although its diurnal fluctuation necessitates standardized sample collection.^{4,6} Directly measured or calculated as transferrin saturation (TSAT), transferrin assesses iron-carrying capacity. TSAT helps differentiate between ACD (low TSAT, high ferritin) and iron deficiency (low TSAT, low ferritin), as demonstrated in the BRINDA analyses.⁷ Pediatric reference values further enhance its utility.⁸ However, transferrin levels can decrease during inflammation, confounding its interpretation.⁴

Together, measurements of ferritin (stores), serum iron (circulation), and transferrin (transport) offer a comprehensive view of iron metabolism. For instance, normal ferritin levels with low TSAT and serum iron might suggest ACD or early deficiency, while high ferritin with low TSAT in thalassemia indicates

maldistribution rather than overload.^{4,9} Multi-marker panels, which include serum iron and TSAT, provide dynamic information beyond ferritin's static measurement, a strategy endorsed by the World Health Organization in settings with prevalent inflammation.^{10,11}

The measurement of these measurands depends significantly on the analytical platforms used. While variability in ferritin assays is well-documented, less data is available comparing the serum iron and transferrin between the Atellica Solution and Cobas Pro platforms.¹² Method-dependent differences highlight the potential for diagnostic discrepancies, such as overestimating ferritin. This study compared the performance of these measurands between the two platforms to inform clinical practice.

II. MATERIALS AND METHODS

1. Subjects

This prospective study analyzed 137 blinded residual routine serum samples from pediatric patients at Vietnam National Children's Hospital, collected and analyzed between June 2024 and January 2025.

Samples were selected to represent a range of biochemical iron statuses based on World Health Organization (WHO) thresholds: iron deficiency (ferritin < 30 $\mu\text{g/L}$), normal (ferritin 30 – 400 $\mu\text{g/L}$), and iron overload (ferritin > 400 $\mu\text{g/L}$).¹¹

Exclusion criteria included samples with visible hemolysis, icterus, or lipemia. Serum was separated at room temperature and stored in Eppendorf tubes at -80°C until analysis.

2. Methods

Serum iron, transferrin, and ferritin were measured using the Cobas Pro (Roche Diagnostics) and the Atellica Solution (Siemens Healthineers) platforms, within two hours of sample thawing. The method principles for

the Cobas Pro platform are: colorimetric for serum iron, immunoturbidimetric for transferrin, and electrochemiluminescent immunoassay for ferritin. The Atellica Solution employed a colorimetric method for serum iron, an immunoturbidimetric method for transferrin, and a chemiluminescent immunoassay method for ferritin.

Table 1 summarizes the method principles, traceability, and linearity ranges of both platforms. Both instruments were calibrated according to the manufacturer's instructions. Bio-Rad quality control (QC) materials were used daily: two levels for serum iron and transferrin, and three for ferritin, to ensure analytical reliability.

Table 1. Analytical Method Principles, Traceability, and Linearity Ranges of Cobas Pro (Roche) and Atellica Solution (Siemens) platforms

Measurand	Unit	Platform	Method principles	Traceability	Linearity range
Iron	μmol/L	Atellica solution	Colorimetric Ferrozine	NIST SRM 937	0.4 - 179
Iron	μmol/L	Cobas Pro	Colorimetric Ferrozine	NIST SRM 937	0.9 - 179
Transferrin	mg/dL	Atellica solution	Immunoturbidimetric	IFCC Certified Reference Material 470	1 - 440
Transferrin	mg/dL	Cobas Pro	Immunoturbidimetric	IFCC Certified Reference Material 470	10 - 520
Ferritin	μg/L	Atellica solution	Chemiluminescent Immunoassay	WHO 3rd IS 94/572	0.5 - 1650
Ferritin	μg/L	Cobas Pro	Electrochemiluminescent Immunoassay	WHO 3rd IS 94/572	0.5 - 2000

NIST SRM 937 (National Institute of Standards and Technology) is the standard reference for iron assays; IFCC Certified Reference Material 470 is used for transferrin; and WHO 3rd International Standard 94/572 is the reference for ferritin

Precision and Trueness Verification and Monitoring

Precision and trueness for all measurands were verified on both platforms following Clinical and Laboratory Standards Institute (CLSI) EP15-A3 guidelines.¹³ Short-term precision was assessed over 5 days using triplicate measurements at low, normal, and

high measurand concentrations. Long-term precision was monitored through daily analysis of QC materials throughout the study. Trueness was evaluated via an interlaboratory comparison program (Bio-Rad), comparing measured means to peer-group means. Additionally, the laboratory participated in monthly external quality assurance (EQA) programs from

Randox (RIQAS Chemistry and Immunoassay) with satisfactory performance.

Method Comparison Study

The method comparison followed CLSI EP09-A3 guidelines.¹⁴ All 137 blinded serum samples were analyzed on both the Cobas Pro and Atellica platforms within two hours of thawing. As blinded residual samples from routine testing were used and discarded afterward, informed consent was waived. Ethical approval was granted by the Institutional Review Board (IRB) at Vietnam National Children's Hospital (IRB-VN01037/IRB00011976/FWA00028418). This study was performed as part of the project of establishment of reference intervals for blood biomarkers in Vietnamese children, funded by VINIF.

Statistical Analysis

Precision and trueness were assessed using QC data in Microsoft Excel, following

CLSI EP15-A3.¹³ Long-term performance was evaluated through QC and EQA results. For the method comparison, Passing-Bablok regression and Bland-Altman plots were used to assess correlation, slope, intercept, and bias between platforms (MedCalc Statistical Software version 23.2.1).

III. RESULTS

Precision and trueness verification

The verification studies confirmed the manufacturer's claims on precision in the laboratory (Table 2). Trueness was also verified through the estimation of verified intervals or bias estimation (Table 2). Long-term precision and accuracy were monitored using QC and EQA data (Table 2). Overall, the results demonstrate that the performance of the three methods on both the Cobas Pro and Atellica Solution platforms is acceptable.

Table 2. Precision and accuracy assessment

Precision and accuracy		Iron (µmol/L)		Transferrin (mg/dL)		Ferritin (µg/L)	
Precision verification		Cobas	Atellica	Cobas	Atellica	Cobas	Atellica
CVr (%)	QC level 1	0.48	0.59	0.76	0.97	1.62	2.00
	QC level 2	0.88	0.25	0.56	0.92	1.26	1.29
	QC level 3	NA	NA	NA	NA	1.16	1.72
CV Claims (%)	1	1.40	0.90	0.80	0.80	1.30	1.60
	2	0.40	0.40	0.70	0.70	1.10	1.20
	3	NA	NA	NA	NA	1.50	1.30
UVLr (%)	QC level 1	1.83	1.18	1.05	1.05	1.74	2.18
	QC level 2	0.52	0.52	0.92	0.92	1.47	1.63
	QC level 3	NA	NA	NA	NA	2.01	1.77
CVwl (%)	QC level 1	0.67	1.09	1.05	1.17	1.55	2.72
	QC level 2	1.33	0.88	1.67	1.22	1.42	1.66
	QC level 3	NA	NA	NA	NA	1.35	2.64

Precision and accuracy		Iron (µmol/L)		Transferrin (mg/dL)		Ferritin (µg/L)	
Precision verification		Cobas	Atellica	Cobas	Atellica	Cobas	Atellica
CV Claims (%)	1	2.50	2.6	0.80	1.20	2.4	4.80
	2	0.90	1.1	1.10	1.90	2.6	4.50
	3	NA	NA	NA	NA	2.6	5.50
UVLwl (%)	QC level 1	3.78	4.16	1.06	1.74	3.74	8.21
	QC level 2	1.40	1.76	1.55	3.04	4.32	7.70
	QC level 3	NA	NA	NA	NA	4.06	9.41
Trueness verification		Cobas	Atellica	Cobas	Atellica	Cobas	Atellica
Peer group mean	QC level 1	11.3	13.0	265	234	79.50	53.25
	QC level 2	38.9	27.9	167	169	163.60	156.6
	QC level 3	NA	NA	NA	NA	360.50	381.3
Grand mean	QC level 1	11.2	12.9	268.8	237.7	81.4	55.10
	QC level 2	38.7	27.8	169.9	171.7	163.28	157.59
	QC level 3	NA	NA	NA	NA	354.28	372.94
Verification interval	QC level 1	11.1	12.8	260.5	222.6	77.53	51.39
		- 11.5	- 13.3	- 269.5	- 245.4	- 81.50	- 55.10
	QC level 2	38.4	27.3	160.7 -	161.9	158.35	152.67
		- 39.3	- 28.6	170.3	- 176.1	- 168.85	- 160.53
	QC level 3	NA	NA	NA	NA	346.34 - 374.70	367.67 - 394.93
Long- term IQC (2024)		Cobas	Atellica	Cobas	Atellica	Cobas	Atellica
CV%	QC level 1	1.50	2.19	1.52	NA	4.5	5.91
	QC level 2	2.13	2.72	1.35	NA	4.2	6.76
	QC level 3	NA	NA	NA	NA	4.12	5.16
EQA		Cobas	Atellica	Cobas	Atellica	Cobas	Atellica
Riquas programs 2024	Cycle average absolute SDI	0.37	0.35	0.20	NA	0.69	1.13
	Cycle average absolute % deviation	2.23	2.06	0.77	NA	5.79	9.45

NA: Not available. CVr: repeatability CV, CVwl: within lab CV, UVLr: repeatability upper verification limit, UVLwl: within lab upper verification limit

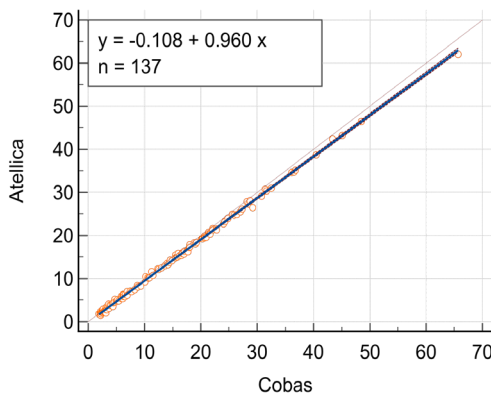
Method Comparison

Correlation and bias between the platforms were assessed using Passing-Bablok regression and Bland-Altman analysis, respectively, with statistical significance set at $p < 0.05$. Results are presented in Chart 1 - 3.

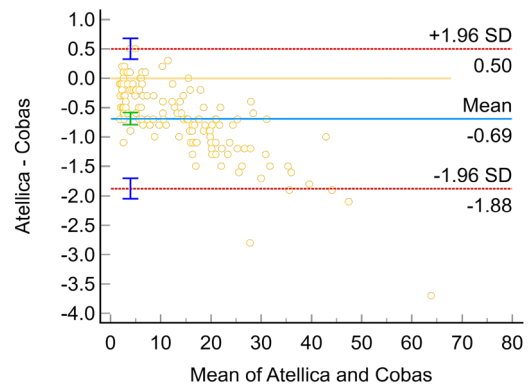
Serum Iron

Serum iron values ranged from 1.9 to 65.7 $\mu\text{mol/L}$ on Cobas Pro and 1.5 to 62 $\mu\text{mol/L}$ on Atellica Solution across the 137 samples. The

Spearman's correlation coefficient was 0.999 (95% CI: 0.996 - 0.998) ($p < 0.001$). Passing-Bablok regression yielded a slope of 0.960 (95% CI: 0.955 to 0.967) and an intercept of $-0.1 \mu\text{mol/L}$ (95% CI: -0.2 to $-0.01 \mu\text{mol/L}$). Bland-Altman analysis showed a mean bias of $-0.7 \mu\text{mol/L}$ (95% CI: 0.6 to $0.8 \mu\text{mol/L}$), with limits of agreement (LoA) ranging from -1.9 to $0.5 \mu\text{mol/L}$. This corresponds to a percentage bias of -6.2% (LoA: -20.7% to 8.4%), which is statistically significant ($p < 0.0001$).



1A



1B

Chart 1. Comparison of Serum Iron Measurements Between Cobas Pro and Atellica Solution platforms

1A. Passing-Bablok regression plot of iron ($n = 137$) on Cobas Pro (x-axis, $\mu\text{mol/L}$) vs. Atellica Solution (y-axis, $\mu\text{mol/L}$), showed the regression line ($y = 0.960x - 0.108$, blue line) and identity line ($y = x$, purple line). 1B. Bland-Altman plot of differences (Atellica Solution - Cobas Pro, $\mu\text{mol/L}$) vs. mean iron values ($\mu\text{mol/L}$), with mean bias ($-0.7 \mu\text{mol/L}$, blue line) and limits of agreement (-1.9 to $0.5 \mu\text{mol/L}$, brown lines)

Transferrin

Transferrin concentrations ranged from 80.4 to 396 mg/dL on the Cobas Pro and from 77.6 to 364.2 mg/dL on the Atellica Solution across the 137 samples. The Pearson correlation coefficient was 0.994 (95% CI: 0.992 - 0.996) ($p < 0.0001$). Passing-Bablok regression yielded a slope of 0.93 (95% CI: 0.913 to 0.946) and an intercept of 1.0 mg/dL (95% CI: -3.0 to 4.9). These results suggest a slight proportional

bias, with Atellica tending to report marginally lower values than Cobas. Bland-Altman analysis showed a mean bias of -15.6 mg/dL (95% CI: -17.0 to -14.2 mg/dL), with limits of agreement ranging from -32.2 to 1.0 mg/dL . This corresponds to a percentage bias of -6.9% (95% CI: -7.5 to -6.3) (LoA: -13.8% to -0.04%), which was statistically significant ($p < 0.0001$).

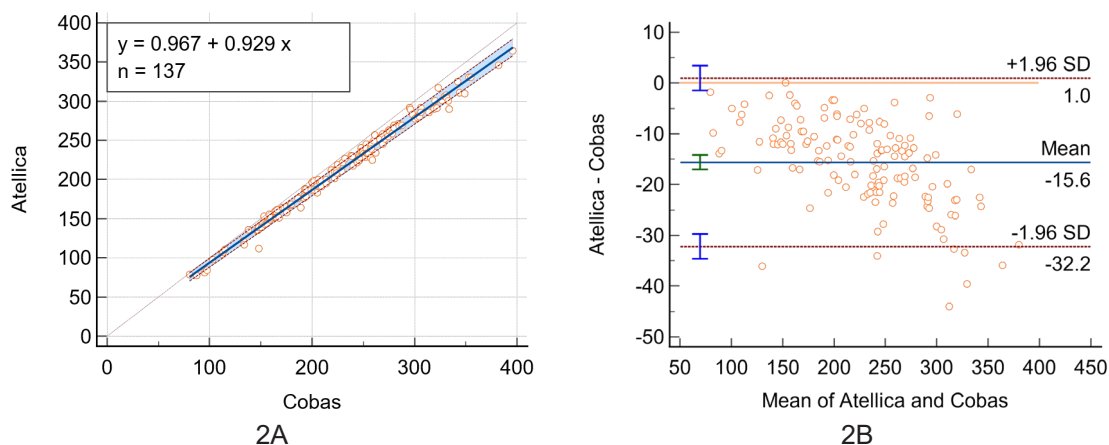


Chart 2. Comparison of Transferrin Measurements Between Cobas Pro and Atellica Solution Platforms

2A. Passing-Bablok regression plot of transferrin (n = 137) on Cobas Pro (x-axis, mg/dL) vs. Atellica Solution (y-axis, mg/dL), showing the regression line ($y = 0.929x + 0.967$, blue line) and identity line ($y = x$, purple line). 2B. Bland-Altman plot of differences (Atellica Solution- Cobas Pro, mg/dL) vs. mean transferrin values (mg/dL), with mean bias (-15.6 mg/dL, blue line) and limits of agreement (-32.2 to 1.0 mg/dL, brown lines)

Ferritin

Ferritin concentrations (n = 137) ranged from 4.71 to 10582 $\mu\text{g/L}$ on the Cobas Pro and from 3.7 to 8259.2 $\mu\text{g/L}$ on the Atellica Solution. The Spearman correlation coefficient was 0.998 (95% CI: 0.997 - 0.998, $p < 0.0001$). Passing-Bablok regression yielded a slope of 0.723 (95% CI: 0.711 to 0.739) and an intercept of -1.7 $\mu\text{g/L}$ (95% CI: -3.1 to -1.0). Bland-Altman analysis revealed a mean bias of -137 $\mu\text{g/L}$ (95% CI: -195 to -78 $\mu\text{g/L}$), with limits of agreement from -820 to 546 $\mu\text{g/L}$. This corresponds to a percentage bias of -33.9% (95% CI: -35.9 to -31.8%, LoA: -58.2% to -9.6%), and the difference was statistically significant ($p < 0.0001$).

IV. DISCUSSION

This study evaluated serum iron, transferrin, and ferritin methods on the Cobas Pro and Atellica Solution platforms within a pediatric cohort. We found a high degree of correlation between the two platforms but observed varying

degrees of systematic differences, which carry implications for clinical practice.

Serum iron demonstrated near-perfect correlation ($r = 0.999$) with a small mean difference of -0.7 $\mu\text{mol/L}$, suggesting a high degree of standardization between the two platforms. This may allow the adoption of common lower reference values (approximately 3 – 4 $\mu\text{mol/L}$).¹⁵ Our study corroborates a recent comparison of serum iron measurement using the Cobas and Vitros platforms (both traceable to the SRM937), which also showed strong correlation, minor mean differences, and proportional bias.¹⁶

Transferrin exhibited strong correlation ($r = 0.994$) but a moderate bias of -15.6 mg/dL, with both proportional (slope = 0.93, $p < 0.05$) and constant (intercept = 1 mg/dL, $p < 0.05$) biases. The systematic difference may affect TSAT interpretation near the reference limits (200 – 360 mg/dL), potentially leading to misclassification of iron availability in inflamed conditions, which are prevalent in developing

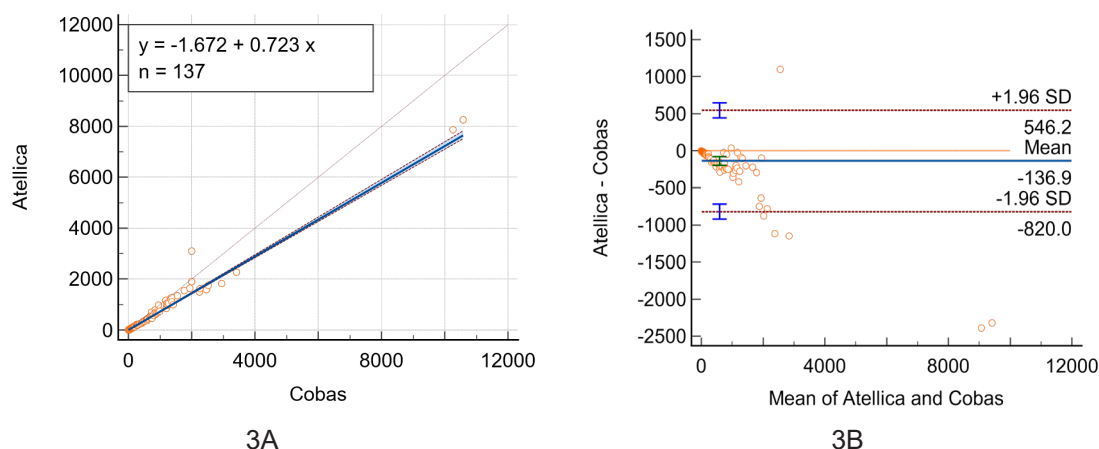


Chart 3. Comparison of Ferritin Measurements Between Cobas Pro and Atellica Solution Platforms

3A. Passing-Bablok regression plot of ferritin ($n = 137$) on Cobas Pro (x-axis, $\mu\text{g/L}$) vs. Atellica Solution (y-axis, $\mu\text{g/L}$), showing the regression line ($y = 0.723x - 1.672$, blue line) and identity line ($y = x$, purple line) ($p < 0.0001$). 3B. Bland-Altman plot of differences (Atellica Solution - Cobas Pro, $\mu\text{g/L}$) vs. mean ferritin values ($\mu\text{g/L}$), with mean bias ($-137 \mu\text{g/L}$, blue line) and limits of agreement (-820 to $546 \mu\text{g/L}$, brown lines)

regions.⁴ Platform-specific adjustments, informed by pediatric data, could improve TSAT interpretation when paired with ferritin measurements.^{8,12}

Serum ferritin showed very good correlation ($r = 0.998$) but a mean difference of $-137 \mu\text{g/L}$, driven by a proportional bias (slope = 0.723). The mean difference between the two platforms can affect the interpretation of results if fixed iron deficiency and overload thresholds are used.¹² Differences in assay technology-electrochemiluminescent (Cobas Pro) versus chemiluminescent (Atellica) - as well as ferritin isoforms, antibodies, and calibrators, may lead to misclassification of iron overload in regions with prevalent hemoglobinopathies.² Inflammatory conditions, a common confounder in developing regions, may amplify these differences.⁴

The findings of this study and others suggest that standardization of laboratory methods for ferritin remains suboptimal. Consequently,

specific platform characteristics and potential measurement differences must be considered when comparing ferritin concentrations across platforms.¹⁷⁻¹⁹ Reference intervals or clinical decision limits should account for these differences to ensure consistent biochemical interpretation and clinical decision-making. Additionally, caution must be taken when trending serum ferritin results across different platforms; ideally, a new baseline should be established following any method transition.

Taken together, these results underscore the need for platform-specific considerations when interpreting biochemical iron studies in pediatric populations. This is particularly important for diagnosing iron deficiency and monitoring overload in thalassemia.²⁰ In developing countries, integrating inflammation markers such as C-reactive protein is crucial.²⁰

This study focused only on pediatric populations, which may limit the broader applicability of the findings to adults. Future

research should validate these findings against a reference method, such as mass spectrometry, and further analyze results by iron status category to refine diagnostic accuracy and treatment protocols.

V. CONCLUSIONS

This study demonstrated a high degree of correlation between the Cobas Pro and Atellica Solution platforms for measuring serum iron, transferrin, and ferritin in pediatric samples. The Cobas Pro platform produced higher serum iron, transferrin, and ferritin values when compared to the Atellica platform, and the difference is particularly large for transferrin and ferritin. These findings highlight the importance of using platform-specific interpretative values and pursuing method standardization. When patients are monitored longitudinally or when inter-laboratory comparisons are required, awareness of platform-related differences is essential to ensure consistent and accurate interpretation.

Conflicts of interest

The authors declare no conflict of interest.

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