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Research Article

Evaluation of the analytical performance of the access vitamin B12 II assay with the new calibrator

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Abstract

Objectives: We aimed to compare the analytical performance of the Access Vitamin B12 assay with the new B12 II calibrator to the current Access and Abbott assays and determined the method-specific reference interval.

Methods: The new B12 II was assessed for imprecision, accuracy, analytical sensitivity, linearity, and carryover. Bland-Altman, Passing Bablok, and concordance correlation coefficient (CCC) analyses were performed on 650 samples. Vitamin B12 tests were performed using the UniCel DxI 800 (Beckman Coulter, USA), and Alinity i System (Abbott Laboratories, Abbott Park, IL, USA) analyzers.

Results: The Access new B12 II assay demonstrated acceptable analytical performance; however, its reference range (138-787 pg/mL) was lower than the manufacturer's recommendation. The Access Vitamin B12 assay showed significant negative differences of 45.8% and 37.0% relative to the Abbott and new B12 II assays, respectively, while the new B12 II assay showed a smaller difference of 9.4% against Abbott. Significant proportional and constant errors were observed between Access and new B12 II (slope: 0.780, intercept: -21.95) and Access and Abbott (slope: 0.707, intercept: -18.95). Abbott and new B12 II demonstrated lower proportional and constant errors (slope: 0.902, intercept: 6.388). Concordance analysis indicated poor agreement of the Access assay with both Abbott and new B12 II (CCC: 0.806, 0.879), whereas Abbott and new B12 II demonstrated substantial agreement (CCC: 0.958).

Conclusion: The new B12 II assay demonstrated appropriate analytical performance and improved consistency with the Abbott assay. The reference interval we established differed from the manufacturer's suggested range, highlighting the importance of determining population-based reference intervals.

Keywords: Calibration, reference standards, vitamin B12, vitamin B12 deficiency

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Vitamin B12, or cobalamin, is a water-soluble vitamin that is critical for key physiological processes, including DNA synthesis, fatty acid metabolism, and myelin production. It is predominantly obtained from animal-derived sources such as red meat, dairy products, and eggs [1]. Absorption of vitamin B12 occurs in the terminal ileum and requires intrinsic factor, a glycoprotein secreted by parietal cells in the stomach. Disruptions in this absorption mechanism—resulting from dietary insufficiency, malabsorption syndromes, or intrinsic factor deficiency—can lead to significant clinical consequences, including hematologic abnormalities and neurological dysfunction. Although excess vitamin B12 is stored in the liver, prolonged disruption in B12 absorption—due to factors such as dietary insufficiency, malabsorption, or a deficiency of intrinsic factor—can deplete liver stores, resulting in a deficiency [1–3].

Vitamin B12 deficiency is a significant global health problem and vitamin B12 levels naturally decline with age [4, 5]. Subclinical B12 deficiency is notably more prevalent among the

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elderly, with reported prevalence rates ranging from 6% to 40% [6–9]. However, younger populations are also at an elevated risk of vitamin B12 deficiency and high-risk groups include vegetarians [10], individuals with gastrointestinal disorders [11], those suffering from depression [12], heavy drinkers [13], and individuals with renal dysfunction [11].

Despite its high prevalence, diagnosing vitamin B12 deficiency remains complex due to inconsistencies in assay methods and the absence of universally accepted reference standards [11]. At present, no definitive reference method exists for investigating suspected vitamin B12 deficiency. Diagnosis is primarily based on measuring serum or plasma vitamin B12 concentrations [14–16]. According to the World Health Organization (WHO), a serum level greater than 221 pmol/L (300 pg/mL) indicates adequate vitamin B12 status, while levels between 148–221 pmol/L (200–300 pg/mL) are considered low. A serum level below 200 pg/mL is classified as vitamin B12 deficiency [17, 18]. However, the lack of standardized reference materials and methods has prevented the establishment of uniformity in current measurement techniques. This results in variability between different vitamin B12 assays [19].

In December 2024, Beckman Coulter launched the Access Vitamin B12 II Calibrators for use with the Access Vitamin B12 assay (new B12 II) on Access Immunoassay Systems. These calibrators offer enhanced precision and accuracy in vitamin B12 detection, with a total imprecision of ≤12.0% across the measuring range. Standardized to the WHO International Standards (IS 03/178), the calibrators ensure greater confidence in patient test results. The analyte in the Access Vitamin new B12 II Calibrators (REF D06116) is traceable to the manufacturer's working calibrators, in accordance with the traceability guidelines outlined in EN ISO 17511. The Access Vitamin B12 assay demonstrated an average recovery rate of 111% compared to the WHO IS 03/178 assigned value of 480 pg/mL [20]. While the initial claims highlight improved diagnostic reliability, independent validation is necessary to confirm these advancements and assess the analytical performance against existing methods.

In this study, we aimed to evaluate the analytical performance of the Access Vitamin B12 assay using the newly introduced new B12 II calibrators, focusing on imprecision, accuracy, LoB, LoD, LoQ, linearity, and carryover. Additionally, we compared the new B12 II to the current Access Vitamin B12 assay on the DXI 800 system and the Abbott Vitamin B12 assay on the Alinity i System.

Materials and Methods

Study design and subjects

To conduct the analytical performance studies for the new B12 II assay, remnant serum samples from patients who visited our hospital for various reasons and had blood drawn and sent to the laboratory were utilized. For the method comparison study, samples were selected from patients aged 18 to 99 years who had Vitamin B12 tests requested from the outpatient clinics of our hospital. A total of 650 patient samples (350 females and 300 males) with sufficient volume for additional Vitamin B12 testing were included in the study. The initial Vitamin B12 concentrations, as determined by the current Access Vitamin B12 assay, ranged from 63 to 1,491 pg/mL. These selected samples were reanalyzed on the same day using the new B12 II assay on the DXI 800 analyzer and the Abbott Vitamin B12 assay on the Alinity i System. The samples were carefully chosen to ensure their concentrations were within the analytical ranges of the alternative systems and represented a broad distribution of Vitamin B12 levels.

Blood Sampling

Blood samples were collected in the morning, between 8:00 and 10:00 AM, following an overnight fast. Venous blood was drawn from the antecubital vein into 5 mL Greiner Bio-One GmbH Samplix®. Blood samples were centrifuged at 2000 x g for 10 minutes. All studies were done according to the Clinical & Laboratory Standards Institute (CLSI) Evaluation Protocols (EP) specific to each parameter. Measurements were performed in the biochemistry laboratory of Dr. Lütfi Kırdar Kartal City Hospital between December 2024 and January 2025. This study was approved by the Ethical Committee of our institution (No: 2025/010.99/12/34, Date: 24/01/2025). This study was conducted in accordance with the principles of the Declaration of Helsinki.

Method

Serum Vitamin B12 analysis was performed using both the DxI 800 Unicel and the Alinity i Systems. Both methods are based on competitive protein binding, utilizing chemiluminescence immunoassay (CLIA) as the detection method. In the DxI 800 Unicel (Beckman Coulter, USA), chemiluminescence is generated from enzymatic reactions, while the Alinity i System (Abbott Laboratories, Abbott Park, IL, USA) employs chemiluminescence microparticle immunoassay (CMIA).

Assay performance studies

Imprecision

Imprecision (both within-run and within-laboratory) was analyzed using control samples with four different vitamin B12 concentrations: 185.2, 374.7, 608.7, and 804.8 pg/mL. Two commercial controls were tested at these concentration levels to calculate imprecision, expressed as CV%. Precision evaluation followed the Clinical and Laboratory Standards Institute (CLSI) EP15-Ed3-IG1 guidelines, involving measurements over five consecutive days, with five replicates performed each day [21]. The predefined acceptable imprecision limit was set at CV ≤12%.

Accuracy

Two samples from the Randox International Quality Assessment Scheme (RIQAS) monthly immunoassay external quality control program were used to assess accuracy. These samples, taken from Cycle 22, were tested using new B12 II in a single

Table 1. Analytical performance characteristics of vitamin B12 assays by manufacturer claims

	Access Vitamin B12 assay	New access Vitamin B12 II assay	Abbott Vitamin B12 assay
Test name	VitB12	B12II	Alinity i system B12
Imprecion (total CV %)	CV<12% across measuring range	CV<12% across measuring range	CV<7.9 % across measuring range
Analytical sensitivity	LoB (not given)	LoB<78	LoB<83
(pg/mL)	LoD<50	LoD<105	LoD<109
	LoQ<50	LoQ<105	LoQ<148
Linearity (pg/mL)	50–1.500	105–2.100	148–2000
Reference intervals (pg/mL)	180–914	222–1.439	187–883

CV: Coefficient of variation; LoB: The limit of blank; LoD: The limit of detection; LoQ: The limit of quantification

analytical run. The percentage deviation from the reported target mean was calculated using the formula: ((Measured value – target mean) / target mean) × 100. The acceptable accuracy limit set by RIQAS was 16.9%.

Analytic sensitivity

Studies were conducted in accordance with CLSI EP17 guidelines [22]. The limit of blank (LoB) was determined by analyzing 20 replicates of the manufacturer's zero calibrator and calculated using the formula:

LoB = Mean (blank) + 1.645 SD (blank).

The limit of detection (LoD) was established using the lowest non-zero calibrator (153 pg/mL), which was diluted by half and analyzed in 20 replicates. The LoD was calculated with the formula:

LoD = LoB + 1.645 (SD low-concentration sample).

The limit of quantification (LoQ) study was performed by analyzing samples with concentrations ranging from 76.5 to 174.5 pg/mL over three consecutive days, with three replicates per concentration. To assess precision and accuracy, five samples near the manufacturer's stated LoQ of 102.5 pg/mL were evaluated to calculate coefficients of variation (CVs) and total errors. Total error was determined using the formula: TE = %BIAS + (1.96 × %CV). The LoQ was established as the concentration at which the calculated total error was below the minimum acceptable total error (23%) defined by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM).

Linearity: Linearity testing was conducted according to CLSI EP6 guidelines [23]. A patient serum with a high Vitamin B12 level was diluted to generate seven concentrations ranging from 80 to 2400 pg/mL. Each concentration was tested three times within the same run. The recovery range was acceptable if it fell within ±15% of the target value.

Carryover

Carryover assessment involved testing three replicates of a high-concentration sample (labeled as a1, a2, and a3) followed by three replicates of a low-concentration sample (labeled as b1, b2, and b3). The carryover effect was determined using the formula: (b1−b3)/(a3−b3). A carryover value below 2% was considered insignificant [24].

Method comparison

Vitamin B2 concentrations from 650 patient samples were first measured using the Access Vitamin B12 assay. Subsequently, the same samples were reanalyzed with both the new B12 II and Abbott assays. The Abbott system was chosen for comparison purposes as it was the routine system in our laboratory at the time of the study.

All measurements for method comparison were performed simultaneously on the same serum samples by the same experienced operator, within the analytical range of the systems, processed in duplicate as a single batch with consistent freeze/thaw cycles, and in accordance with CLSI EP09-A3 guidelines [25].

Statistical analysis

The distribution of data was evaluated using the Kolmogorov–Smirnov test, and the results are presented as the median and interquartile range. Imprecision, LoB, LoD, LoQ, and linearity were calculated using EP Evaluator Release 9 software (David G. Rhoads Association, Kennett Square, PA). To assess method comparison, Bland–Altman plots, Passing–Bablok regression, and the concordance correlation coefficient (CCC) were used, with analysis performed using MedCalc Statistical Software (version 12, MedCalc Software, Mariakerke, Belgium). A systematic error was considered significant if the 95% confidence interval excluded 1.0 for the slope (indicating proportional error) or 0 for the y-intercept (indicating constant error).

Results

The analytical performance characteristics of Vitamin B12 assays, as claimed by the manufacturers, are summarized in Table 1. The median values (2.5–97.5 percentiles; pg/mL) for the 650 samples analyzed were as follows: 140 (78.7–714.7) for the Access Vitamin B12, 206 (134.0–949.6) for the new B12 II, and 237 (152–1020) for the Abbott Vitamin B12 assay. The reference interval was calculated from 400 patients whose Vitamin B12 levels were within the Abbott assay's normal range (187–883 pg/mL) and who had normal hemoglobin, hematocrit, and folic acid levels, with no clinical or laboratory evidence of Vitamin B12 deficiency. Following the CLSI EP28-A3c guideline, the non-parametric method was used, and the 2.5th and 97.5th per-

Table 2. Analytical performance characteristics of access Vitamin B12 assay with the new B12 II calibrator

Performance criteria	Study result
Within-run CV (%)	
Level 1 (185.2 pg/mL)	5.41
Level 2 (375.7 pg/mL)	4.07
Level 3 (608.7 pg/mL)	2.80
Level 4 (804.8 pg/mL)	4.01
Within-laboratory CV (%)	
Level 1 (185.2 pg/mL)	7.18
Level 2 (375.7 pg/mL)	6.40
Level 3 (608.7 pg/mL)	7.75
Level 4 (804.8 pg/mL)	5.09
Accuracy (deviation %)	
Riqas 1 (637 pg/mL)	12.5
Riqas 2 (951 pg/mL)	3.3
LoB (pg/mL)	15.84
LoD (pg/mL)	80.82
LoQ (pg/mL)	102
Linearity (pg/mL)	102–2060
Carry-over (%)	0.74

CV: Coefficient of variation; LoB: The limit of blank; LoD: The limit of detection; LoQ: The limit of quantification.

centiles of the distribution were taken as the lower and upper limits, respectively. Using the Beckman new B12 II assay, the calculated interval was 138–787 pg/mL, which is lower than the manufacturer's proposed range of 222–1,439 pg/mL.

The new B12 II assay demonstrated acceptable performance in terms of imprecision, LoB, LoD, LoQ, linearity, and carry-over. The analytical performance characteristics of the new B12 II assay are presented in Table 2. Bland-Altman analysis revealed notable differences between the three systems. The Access Vitamin B12 assay showed significant negative differences of 45.8 % and 37.0 % relative to the new B12 II and Abbott assays, respectively while the new B12 II showed a smaller negative difference of 9.4% against the Abbott. Notably, only the difference between the new B12 II and Abbott assays satisfied the EFLM allowable bias threshold of 14.1%. The comparison results between the methods are shown in the Bland-Altman plot (Fig. 1). Significant proportional and constant errors were observed between the Access Vitamin B12 and new B12 II assays, with a slope of 0.780 (0.766–0.794) and an intercept of -21.95 (-23.58 to -18.6). Similarly, the Access Vitamin B12 and Abbott assays demonstrated significant proportional and constant errors, with a slope of 0.707 (0.691–0.723) and an intercept of -18.95 (-23.58 to -14.51). The Abbott and new B12 II assays exhibited smaller proportional and constant errors, with a slope of 0.902 (0.883–0.920) and an intercept of 6.388 (0.660–11.613). The Passing–Bablok regression analyses are presented in Figure 2. The Access Vitamin B12 and Abbott, as well as the Access Vitamin B12 and new B12 II assays, exhibited poor agreement, with CCC values of 0.806 (0.787–0.824) and 0.879 (0.866–0.891), respectively. However Abbott and new B12 II showed substantial agreement, with a CCC value of 0.958 (0.952–0.964). Method comparison data are shown in Table 3.

Discussion

This study is the first method evaluation of the newly introduced Access new B12 II calibrator, launched in December 2024. The new B12 II assay demonstrated strong analytical performance with the new calibrator, providing improved traceability, consistency, and reliability when compared to the Abbott assay. Bias analysis revealed that the current Access Vitamin B12 assay showed a significant negative difference of 37% compared to the Abbott assay. However, with the introduction of the new B12 II calibrator, this difference was significantly reduced to -9.4%, indicating improved alignment between the two assays. Additionally, the observed negative difference of 48% between the current Access Vitamin B12 assay and the new B12 II assay indicates that the new calibrator produces higher results than the current assay. While ongoing standardization efforts continue, the reference range determined by the new B12 II assay (138–787 pg/mL) still differs from that of the Abbott assay (187–883 pg/mL). This highlights the need for method-specific reference ranges, rather than relying on a universal cut-off value, such as 200 pg/mL, to define deficiency criteria. Establishing the appropriate reference range for each method is crucial for accurate clinical diagnosis.

Table 3. Method comparison results of Vitamin B12 Assay with the new B12 II calibrator

Method	Passing-bablok regression analysis		Concordance correlation analysis			Bland-altman analysis
	Slope (CI)	Intercept (CI)	CCC (CI)	P	C_b	Bias (%)
Access Vitamin B12 new B12 II	0.780 (0.766–0.794)	-21.95 (-25.36– -18.62)	0.879 (0.866–0.891)	0.984	0.893	-37.0
Access Vitamin B12 abbott	0.707 (0.691-0.723)	-18.95 (-23.58– -14.51)	0.806 (0.787–0.824)	0.973	0.828	-45.8
New B12 II abbott	0.902 (0.883–0.920)	6.388 (0.660–11.613)	0.958 (0.952–0.964)	0.970	0.987	-9.4

CI: Confidence interval; CCC: Concordance correlation coefficient; P: Pearson correlation coefficient; C_b: Bias correction factor

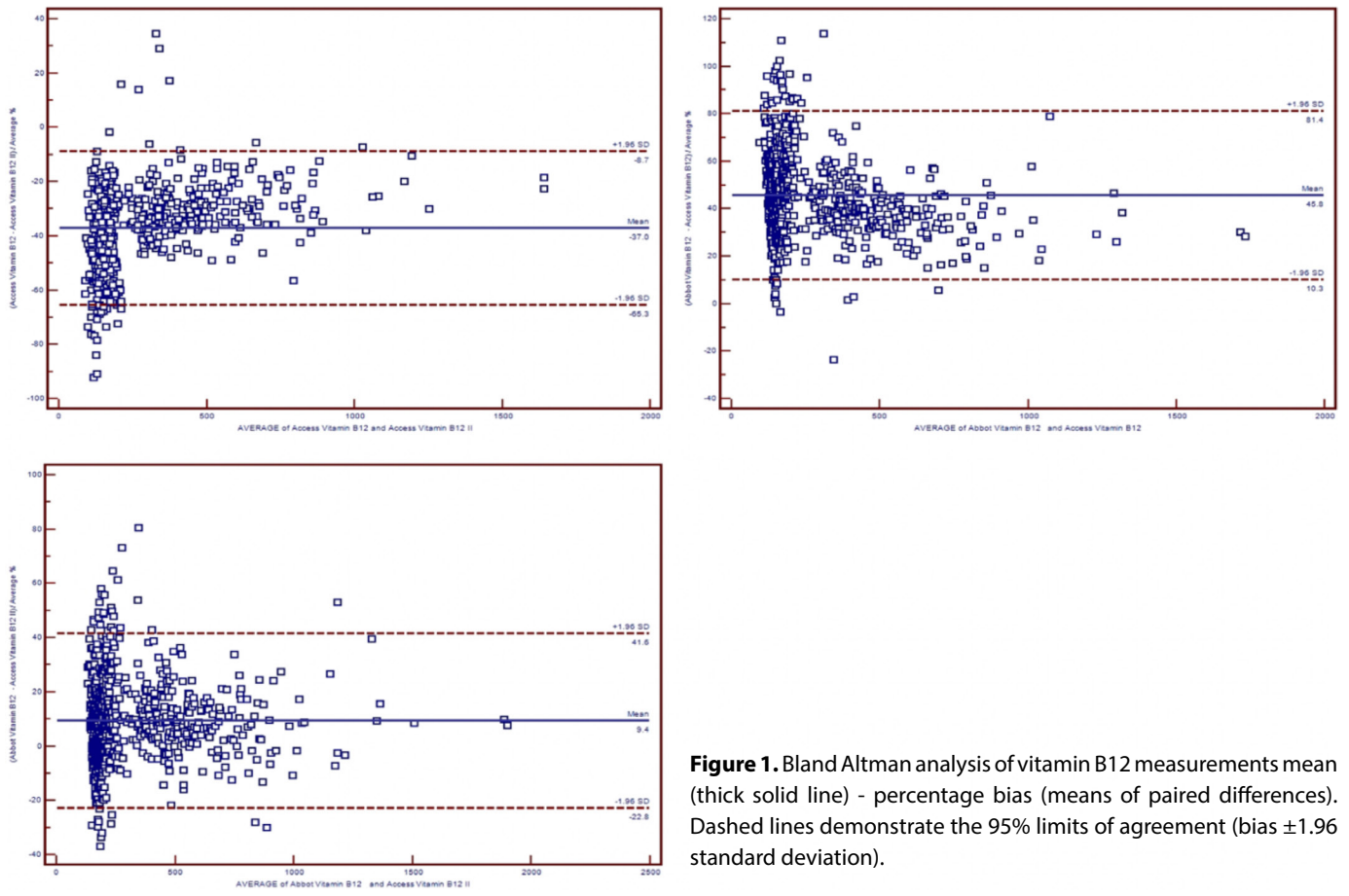

Figure 1. Bland Altman analysis of vitamin B12 measurements mean (thick solid line) - percentage bias (means of paired differences). Dashed lines demonstrate the 95% limits of agreement (bias ±1.96 standard deviation).

Vitamin B12, the largest of all vitamins, exists in various forms and is present in very low concentrations in serum. It binds strongly to serum proteins [24]. The unique biochemical characteristics of vitamin B12, coupled with the complexities involved in producing pure reference materials and the absence of a universally standardized reference method, present significant challenges to achieving consistent and reliable standardization of vitamin B12 assays. The serum vitamin B12 assay methods have not yet been fully standardized. To address this issue, the World Health Organization (WHO) Expert Committee on Biological Standardization introduced the material 03/178 as an International Standard (IS) for serum vitamin B12 assays. This material was assessed in 24 laboratories across seven countries to evaluate its applicability as a reference standard for both vitamin B12 and folate assays. The findings revealed that employing this standard material reduced variability between laboratories. However, the standard material, produced through the lyophilization of pooled human serum, may lead to challenges concerning its commutability [26].

The National Institute of Standards and Technology (NIST) currently does not provide a certified reference material (SRM) for vitamin B12 or methylmalonic acid (MMA). However, NIST is in the process of developing SRM 3951 for serum vitamin B12, which includes target pools with concentrations of 74 pmol/L (100 pg/mL), 148 pmol/L (200 pg/mL), and 332 pmol/L (450 pg/mL). Among these, the 332 pmol/L pool represents "normal" serum, while the two lower pools consist of a mixture of normal serum and serum that has been stripped of its naturally occurring vitamin B12 [27, 28].

Several comparative studies have been conducted to evaluate the performance of different vitamin B12 assays. In the study by İspir et al. [14], four vitamin B12 assays—DxI 800 Unicel, ADVIA Centaur XP, Roche Cobas E601, and Architect i2000sr—were compared. The results showed strong correlations between the assays, with the weakest correlation between DxI 800 Unicel and ADVIA Centaur. DxI 800 Unicel produced lower results compared to the others. MMA and homocysteine showed similar correlations with vitamin B12 levels across all methods. The study concluded that while the assays performed well, vitamin B12 assay standardization is still incomplete and requires further efforts.

In the study conducted by Ihara et al. [15], vitamin B12 and folate levels were measured using three different methods: Access, Advia Centaur, and Elecsys. The results revealed significant correlations between the assays; however, serum vitamin B12 levels measured by Elecsys were consistently higher compared to those obtained from the other two methods. Similar to our findings, their study concluded that, in the absence of reliable reference materials and standardized methods, reference values for vitamin B12 and folate remain method-depen-

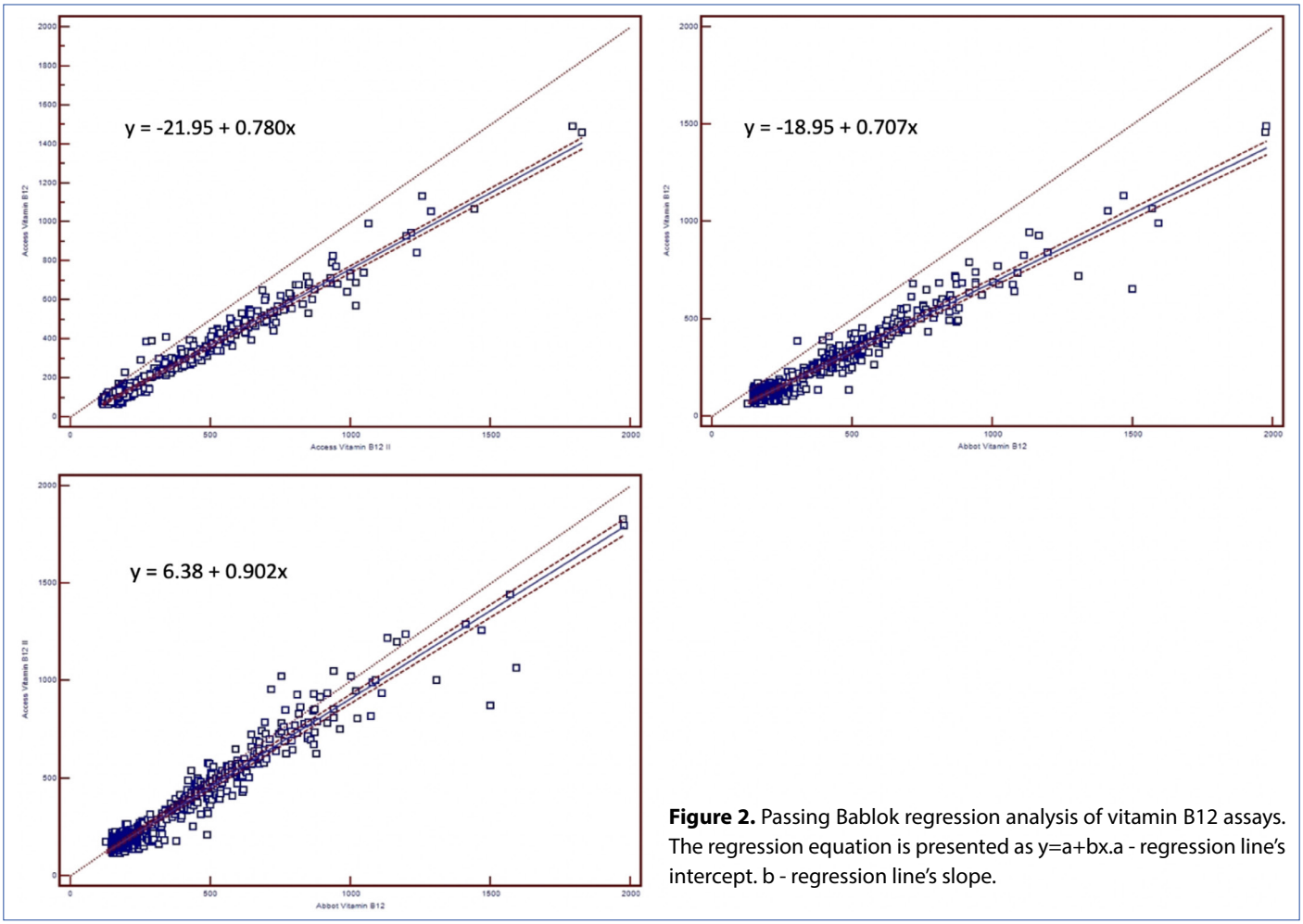

Figure 2. Passing Bablok regression analysis of vitamin B12 assays. The regression equation is presented as y=a+bx.a - regression line's intercept. b - regression line's slope.

dent. For instance, certain assays have established lower reference values of 200 pg/mL for vitamin B12, but these values are not applicable to all automated immunoassay methods. Therefore, it is crucial to determine reference values that are specific to each method. This approach ensures accurate diagnosis and consistency across different testing platforms, leading to more dependable clinical results.

In another study, reference intervals for plasma vitamin B12 concentration were established using three different immunoassays in the North Denmark Region. The findings showed that results from different methods were not interchangeable, with significant variation in the frequency of vitamin B12 levels below the cut-off when similar thresholds were applied [29].

Our study demonstrated a stronger correlation and reduced difference between the newly developed new B12 II calibrator and the Abbott system compared to the current Access Vitamin B12 assay. However, there were still concerns regarding clinical interpretation, suggesting that full standardization may not have been achieved. Among the 650 patients, the current Access Vitamin B12 assay identified 405 (62.3%) patients as deficient (below 200 pg/mL), while the Abbott system detected 235 (36.1%) patients below this threshold. With the new B12 II calibrator, the assay classified 288 (44.3%) patients as deficient under the same cutoff, indicating higher vitamin B12 levels than the previous Access Vitamin B12 assay. These results show that, while correlation between the new B12 II and Abbott assays has improved, differences between the methods remain, emphasizing the continued need for method-specific reference ranges.

The study did not assess potential interference factors, such as hemolysis, lipemia, or elevated bilirubin levels, which may represent a limitation. Additionally, the calculated reference range for the new B12 II assay, based on Vitamin B12 levels according to the Abbott system's normal range, was lower than the manufacturer's proposed values. This indicates that the current reference range for the new B12 II assay does not align with the manufacturer's suggested values for our population, emphasizing the need for population-specific reference range studies in larger and more diverse groups.

Conclusion

The new B12 II assay demonstrated appropriate analytical performance and improved consistency with the Abbott assay. The reference interval we established differed from the manufacturer's suggested range, highlighting the importance of determining population-based reference intervals.

Ethics Committee Approval: The study was approved by the Dr. Lütfi Kırdar Kartal City Hospital Scientific Research Ethics Committee (no: 2025/010.99/12/34, date: 24/01/2025).

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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