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Evaluation of the analytical performance of the Beckman Coulter Unicel DXI 800 Access Total 25(OH) Vitamin D immunoassay

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Abstract

Background: As vitamin D has recently been implicated in various diseases, vitamin D testing has gained a lot more significance. Vitamin D deficiency is quite prevalent, and detection of this condition is important. Several manufacturers have developed new automated immunoassays for this purpose. In this study, we aimed to evaluate the analytical performance of the Access Total 25(OH) Vitamin D immunoassay on the Beckman Coulter Unicel DXI 800 analyzer, through comparison with the reference method, liquid chromatography/tandem mass spectrometry (LC-MS/MS).

Methods: The study was conducted with 148 patient samples which were sent to Ankara Numune Training and Research Hospital for routine vitamin D testing. Every sample was analyzed with both Unicel DXI 800 immunoassay analyzer and LC-MS/MS. The concordance of the results was evaluated with Passing-Bablok regression analysis and Bland-Altman plot. Additionally, imprecision, interference, limit of blank (LOB), recovery, linearity and carry-over studies were performed for the Beckman Coulter Unicel DXI 800 analyzer.

Results: When compared to LC-MS/MS, the Access Total 25(OH) Vitamin D immunoassay on the Beckman Coulter Unicel DXI 800 analyzer had an R-value of 0.957 (intercept: -3.938 , slope: 1.185) and a mean bias of 9.5%. The concordance correlation coefficient (CCC) between the

two methods was 0.916. The intra-assay, inter-assay and total coefficient of variation (CV%) for the Unicel DXI 800 vitamin D immunoassay were 3.3%, 5.3% and 8.3%, respectively, at 31.7 ng/mL concentration, and 2.1%, 3.2% and 7%, respectively, at 66.8 ng/mL concentration.

Conclusions: The Access Total 25(OH) Vitamin D immunoassay method has acceptable analytical performance, and the results are in concordance with the LC-MS/MS results.

Keywords: immunoassay; liquid chromatography/tandem mass spectrometry (LC-MS/MS); method comparison; 25(OH) vitamin D; Unicel DXI.

Introduction

Vitamin D is a prohormone that plays a key role in bone and mineral metabolism, and it is also essential for maintenance of health [1]. Studies have shown that besides its role in bone and mineral metabolism, vitamin D is also implicated in various extra-skeletal disorders such as cardiovascular diseases, cancers, diabetes mellitus, infectious and autoimmune diseases [2, 3]. Vitamin D has two main forms: the plant-originated ergocalciferol (vitamin D₂) and the animal-originated cholecalciferol (vitamin D₃), which is produced endogenously upon exposure to ultraviolet B (UVB) light. Vitamin D is hydroxylated twice in the body to form the biologically active 1,25(OH)₂ vitamin D, or calcitriol [4]. Vitamin D is mainly stored in the adipose tissue. In circulation, it is bound to vitamin D-binding globulins and albumin. The half-life of 25(OH) vitamin D is 2–3 weeks, whereas the half-life of 1,25(OH)₂ vitamin D is 5–6 h. Therefore, the principal form of vitamin D found in the serum is 25(OH) vitamin D, and it is the best indicator of circulating vitamin D levels [5, 6]. In vitamin D deficiency, 1,25(OH)₂ vitamin D levels may be high, normal or low. As 1,25(OH)₂ vitamin D has a short life and its serum levels show circadian change, 1,25(OH)₂ vitamin D is not appropriate to assess circulating vitamin D levels [7, 8]. This led to the increasing significance of 25(OH) vitamin D measurement in laboratory examinations. Total vitamin D (25(OH)D₂ and 25(OH)D₃)

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measurement is important in terms of accurate assessment of vitamin D state [1]. Vitamin D measurement poses some challenges due to the hydrophobic nature of the molecule and its binding to proteins with a high affinity [9, 10]. Regardless of the method type, all analytic procedures start with the unbinding of vitamin D from proteins. Differences between various methods depend on how this step is employed and how the unbound vitamin is measured [11]. Serum 25(OH) vitamin D measurement methods include high-performance liquid chromatography (HPLC) and tandem mass spectrometry (MS/MS), radioimmunoassay (RIA), enzyme immunoassay, competitive protein binding assays, automated competitive protein binding assays and chemiluminescence immunoassays [7].

In our study, we aimed to evaluate the analytical performance of the Access Total 25(OH) Vitamin D immunoassay on the Beckman Coulter Unicel DXI 800 analyzer, through comparison with the reference method, liquid chromatography/tandem mass spectrometry (LC-MS/MS).

Materials and methods

Study design

The study was conducted with 148 patient samples with either low, normal or high vitamin D levels, which were sent to Ankara Numune Training and Research Hospital for routine vitamin D testing. Every sample was aliquoted following centrifugation at 2000 *g* for 15 min and stored at -80°C until the time of analysis. For every sample, vitamin D level was analyzed with both Beckman Coulter Access Total 25(OH) Vitamin D assay on the Unicel DXI 800 analyzer and LC-MS/MS.

Imprecision, interference, limit of blank (LOB), recovery, linearity and carry-over studies were performed for the Beckman Coulter Unicel DXI 800 analyzer.

Analytic method

The Access Total 25(OH) Vitamin D assay is a two-step competitive binding immunoassay using sheep monoclonal anti-25(OH) vitamin D antibodies. Initially, 25(OH) vitamin D is removed from the vitamin D-binding protein (DBP) and bound to the immobilized monoclonal anti-25(OH) vitamin D in the solid phase. Subsequently, a 25(OH) vitamin D analog-alkaline phosphatase conjugate is added which competes for binding to the immobilized monoclonal anti-25(OH) vitamin D. After a second

incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured using a luminometer. The light production is inversely proportional to the concentration of 25(OH) vitamin D in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

The measurand (analyte) in the Access Total 25(OH) Vitamin D calibrators is traceable to a Joint Committee for Traceability in Laboratory Medicine (JCTLM)-approved isotope dilution mass spectrometry (ID-LC-MS/MS) reference measurement procedure (RMP) developed at Ghent University.

Reference method: LC-MS/MS

An AB Sciex API 3200 triple quadrupole mass spectrometer connected to a Shimadzu HPLC (Kyoto, Japan) system was used. In HPLC column (Phenomenex, CA, USA) mobile phase A was water, and mobile phase B consisted of 0.1% formic acid in acetonitrile. For sample preparation step, 200 μL serum (calibrator, control, or patient samples) and 75 μL internal standard were pipetted to a standard microcentrifuge tube. Upon addition of 1000 μL acetonitrile, the mixture was vortexed for 30 s, and incubated at $+4^{\circ}\text{C}$ for 10 min. Then, the mixture was centrifuged at 20,000 *g* for 5 min. The supernatant was transferred to a clean tube and evaporated to dryness with nitrogen. Then, the samples were reconstituted in 100 μL of 50% acetonitrile and vortexed. Fifty microliters of the sample was injected to the HPLC system. The HPLC system had a flow rate of 0.3 mL/min. The API 3200 system was employed in the electrospray mode. Using a multiple reaction monitor, mass/charge spectra of the samples were determined. The following *m/z* parameters were obtained: 25(OH)D3: 383.4/211.1; 25(OH)D2: 395.4/209.1; and IS: 407.2/389.4.

Precision, linearity, carry-over, interference, recovery and method comparison studies were carried out in accordance with the Clinical & Laboratory Standards Institute (CLSI) protocol.

Imprecision

Precision studies were performed with two levels of pooled serum samples. For intra-assay precision study, each level of the samples was analyzed in 10 replicates in a single run. The low and high serum pool specimens were

measured as 5×2 in a single day for the inter-assay precision study. For between-day precision study, each level of the samples was run in duplicate, with two runs per day, over 5 days (first run in the morning and second run in the afternoon). The intra-assay, inter-assay, between-day and total coefficient of variation (CV%) values were calculated in accordance with the EP 5A protocol. Acceptable CV% values were determined as $\leq 10\%$ [12, 13].

Limit of blank (LOB) and linearity

For determination of LOB, zero concentration calibrator provided by the manufacturer was run in 10 replicates, and the lowest concentration calibrator other than the zero calibrator was run in three replicates. LOB, limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the following formulas:

$$\text{LOB} = \text{Average blank} + 1.65 * \text{SD blank.}$$

$$\text{LOD} = \text{LOB} + 1.65 * \text{SD}_{(\text{low concentration sample})}$$

$$\text{LOQ} = \text{Average}_{(\text{low concentration sample})} + \text{bias}_{(\text{low concentration sample})} + 2\text{SD}_{(\text{low concentration sample})}$$

The linearity study was performed by diluting high patient serum pool with low patient serum pool. For this purpose, high serum pools and low serum pools were mixed in the proportions of 1:3, 1:1 and 3:1; thus, five samples were obtained. Acceptable recovery was determined as $\pm 15\%$ of the target value [12].

Recovery

For recovery analysis, serum samples with low and high concentrations were mixed in the proportions of 1:3, 1:1 and 3:1, or 1:2, 1:1 and 2:1 [14].

Interference

Interference analysis was performed in accordance with CLSI [12, 15]. Interfering substances (hemolysate, bilirubin and intralipid) at three different concentrations were added to the pooled serum samples and they were run in duplicates. The following formula was used: $([\text{Interferer-added}] - [\text{interferer-not added}]) / \text{interferer-not added} * 100$. More than 10% deviation from the target concentration was accepted as clinically meaningful.

Carry-over

A serum sample with high vitamin D concentration (191.14 ng/mL; HC sample) was aliquoted to 10 portions; and another serum sample with low vitamin D concentration (15.39 ng/mL; LC sample) was aliquoted to 11 portions, in order to obtain 21 separate samples in total. These 21 samples were tested in a single run as in the following sequence: 3 LC, 2 HC, 1 LC, 2 HC, 4 LC, 2 HC, 1 LC, 2 HC, 1 LC, 2 HC and 1 LC sample. The average measurement result of the LC samples tested after an LC sample was subtracted from the average measurement result of the LC samples tested after a HC sample in order to determine the limit of error. The limit of error was accepted in case it was lower than 3 times the standard deviation (SD) of the measurement results of LC samples tested after an LC sample [13].

Method comparison

Totally 148 patient samples with either low, normal or high concentrations of vitamin D (48 samples with vitamin D concentrations of < 30 ng/mL; 60 samples with vitamin D concentrations of 30–100 ng/mL; and 40 samples with vitamin D concentrations of > 100 ng/mL) were analyzed with the Access Total 25(OH) Vitamin D immunoassay on the Beckman Coulter Unicel DXI 800 analyzer, and then with the gold standard method, LC-MS/MS, in an accredited laboratory. The results were compared statistically.

Statistical analysis

Statistical analysis was performed using SPSS software (version 17.0 SPSS Inc., Chicago, IL, USA) and MedCalc Statistics software (version 12.0 MedCalc software, Mariakerke, Belgium). The concordance of the results was evaluated with Passing-Bablok regression analysis and Bland-Altman plot. The concordance correlation coefficient (CCC) was calculated. $\text{CCC} > 0.99$ denotes excellent agreement; CCC between 0.95 and 0.99 denotes substantial agreement, CCC between 0.90 and 0.95 denotes moderate agreement and $\text{CCC} < 0.90$ denotes poor agreement.

Results

Repeatability

The intra-assay, inter-assay and total CV% were 3.3%, 5.3%, and 8.3%, respectively, for 31.7 ng/mL

Table 1: Repeatability results for the Access Total 25(OH) Vitamin D immunoassay on Unicl DXI 800.

Level	Mean, ng/mL	Intra-assay CV%	Inter-assay CV%	Total CV%
Level 1	31.7	3.3	5.3	8.3
Level 2	66.8	2.1	3.2	7

concentration, and 2.1%, 3.2%, and 7%, respectively, for 66.8 ng/mL concentration (Table 1).

Limit of blank

The LOB for the Access Total 25(OH) Vitamin D immunoassay was found to be 1.5 ng/mL. The LOB value specified by the manufacturer was <2 ng/mL. The LOD and LOQ values were 2.05 ng/mL and 2.57 ng/mL, respectively.

Linearity

Serum samples obtained by mixing the samples with high and low concentrations of vitamin D in the pre-defined proportions were analyzed, and the measured 25(OH) vitamin D concentrations were plotted against the expected values to draw the regression line. Accordingly, the linear regression equation was: measured value = expected value * 0.979 + 2.660; correlation coefficient, $r = 0.998$. Maximum bias was calculated as 9.2% for a concentration of 45.4 ng/mL (Table 2 and Figure 1).

Table 2: Linearity summary.

Tested range, ng/mL	Maximum bias	Concentration causing bias, ng/dL	Slope	R
5.9–180.4	9.2%	45.4	0.979	0.998

Figure 1: Linearity graph of the Unicl DXI 800 Access Total 25(OH) Vitamin D assay.**Table 3:** Recovery experiment for the Access Total 25(OH) Vitamin D immunoassay.

Sample	Concentration, ng/mL		Recovery, %
	Expected	Measured	
Sample 1			
Low		25.46	
High		136.19	
3LC:1HC	53.14	53.87	101
1LC:1HC	80.82	86.47	107
1LC:3HC	108.51	110.81	102
Sample 2			
Low		38.76	
High		162.43	
2LC:1HC	79.98	88.25	110
1LC:1HC	100.595	111.18	111
1LC:2HC	121.20	128.35	106

Recovery

Serum samples with high and low concentrations of 25(OH) vitamin D were mixed in the proportions of 3:1, 1:1 and 1:3, and 2:1, 1:1 and 1:2. Measured values of 25(OH) vitamin D concentrations in these samples are listed in Table 3 against the expected values. The recovery values obtained varied between 101% and 111%.

Carry-over

The difference between the average of low-level results tested following low-level samples and the average of low-level results tested following high level samples was 0.724. The limit of error, which is 3 times the value of SD of low-level results, was 6.59.

Interference

In interference study, the presence of hemoglobin at 400 mg/dL, bilirubin at 18 mg/dL and intralipid at 428 mg/dL concentrations in the serum resulted in greater than 10% bias in the results (Table 4).

Table 4: Concentration that caused greater than 10% bias.

	Hemoglobin, mg/dL	Icterus, mg/dL	Lipid, mg/dL
Our results	400	18	428 (intralipid)
Specified by the manufacturer	50	40	3280 (glyceryl triolate)

Table 5: Passing-Bablok regression analysis.

Slope (95% CI)	Intercept (95% CI)	R
1.185 (1.124–1.251)	–3.938 (–6.740–[–0.226])	0.957

CI, confidence interval.

Method comparison

Compared to the LC-MS/MS method, the Access Total 25(OH) Vitamin D immunoassay on the Beckman Coulter Unicel DXI 800 analyzer demonstrated an R-value of 0.957 (intercept: –3.938 (–6.740–[–0.226]), slope: 1.185 [1.124–1.251]) and a mean bias of 9.5%. The CCC between the two methods was 0.916 (Table 5, Figures 2 and 3).

A concordance according to the percentage of patients classified in the same group with both methods, we found

Figure 2: Passing-Bablok regression analysis of the Unicel DXI 800 Access Total 25(OH) Vitamin D assay compared to LC-MS/MS methods. Solid line, regression; dashed lines, 95% confidence intervals.

Figure 3: Bland-Altman %bias of the Unicel DXI 800 Access Total 25(OH) Vitamin D assay compared to LC-MS/MS method. Solid lines indicate average %bias. Dashed lines demonstrate the 95% limits of agreement (bias ± 1.96 SD).

this rate between the Access Total 25(OH) Vitamin D immunoassay and LC-MS/MS to be 75%.

Discussion

Besides its key role in calcium and bone metabolism, vitamin D has also been implicated in various extra-skeletal disorders such as cardiovascular diseases, cancers and autoimmune diseases, and its deficiency is quite common. This led to a dramatic increase in vitamin D test requests over the years. Total 25(OH) vitamin D measurement is the best indicator of circulating vitamin D levels. The Beckman Coulter Access Total 25(OH) Vitamin D immunoassay is a recently developed chemiluminescence immunoassay.

In this study, we evaluated the analytical performance of the Access Total 25(OH) Vitamin D immunoassay on the Unicel DXI 800 analyzer, and examined the concordance with the reference method, LC-MS/MS.

Four different approaches to acceptable performance criteria for 25(OH)D assay have been explained by Stöckl et al. [16]. One of the models that gave the most stringent target and its recommendations for routine assays were imprecision $\leq 10\%$ CV and bias $\leq 5\%$. We used the same imprecision and bias criteria recommendations by Stöckl et al. for evaluation of the results. According to this criterion, we found that the precision of the Access Total 25(OH) Vitamin D assay is quite good (total CV% was 8.3% and 7%, respectively, for 31.7 ng/mL and 66.8 ng/mL concentrations). We employed the 5% proposed by Stöckl et al. for bias criteria, and the Access Total 25(OH)D assay showed excellent bias proficiency testing programs (our vitamin D result was 21.8 ng/mL, the mean of the group was 22.79 ng/mL and %bias was 4.2% in the RIQAS Immunoassay Speciality 1 Programme).

There are studies that evaluated the analytical performance of the Access Total 25(OH) Vitamin D immunoassay on the Unicel DXI 800 analyzer [12, 13]. Furthermore, in the study by Wyness and Straseski for linearity, the maximum bias was observed at a lower concentration (5.7 ng/mL), and the bias was lower than what we found in our study (7% vs. 9.5%). In our study, we observed that the presence of hemoglobin at 400 mg/dL concentration resulted in clinically significant bias in 25(OH) vitamin D measurement. Similarly, the previous study showed that the presence of hemoglobin at concentrations >400 mg/dL caused clinically significant bias, and this level is greater than what is specified by the manufacturer (50 mg/dL). However, the concentration of bilirubin which caused clinically significant bias

was lower in our study compared to the study by Wyness and Straseski. We found that bilirubin caused significant interference at 18 mg/dL, whereas the level specified by the manufacturer is 40 mg/dL, and this level is found to be >45 mg/dL in the previous study. Similarly, lipid concentration which caused clinically significant bias was lower in our study compared to other studies. This level was found to be 428 mg/dL for intralipid in our study, 630 mg/dL for intralipid in the study by Wyness and Straseski and a level of 3280 mg/dL was specified by the manufacturer for glyceryl triolate [12].

For carry-over, the limit of error value that we found in our study was similar to that in the study by Ozcan et al. They found a bias of -0.382 , whereas in our study, it was -0.724 , which was smaller than the limit of error [13].

We observed that the Access Total 25(OH) Vitamin D immunoassay had moderately concordant results with the reference method, LC-MS/MS. We calculated an R-value of 0.957 with a mean bias of 9.5%. The inter-method CCC was acceptable (CCC 0.916). In their study comparing the Access Total 25 (OH) Vitamin D immunoassay with LC-MS/MS, Wyness and Straseski found an R-value of 0.93 with a mean bias of -3.2% [12]. Another study comparing the Access Total 25 (OH) vitamin D immunoassay with LC-MS/MS found an R-value of 0.944 with a mean bias of -2.9% [13]. Although our bias was higher than that in other studies, it was acceptable according to bias criterion of 15.8%, which is based on biological variation and has been used previously [12].

Cavalier et al. used concordance tables in order to evaluate the concordance between immunoassay methods and LC-MS/MS. Accordingly, they grouped the patients based on their vitamin D levels as <20 ng/mL, 20–30 ng/mL, 30–80 ng/mL and >80 ng/mL. Then, they created a concordance table according to the percentage of patients classified in the same group with both methods. They found this ratio for immunoassay methods and LC-MS/MS between 62 and 82% [6]. In our study, we found this percentage between the Access Total 25(OH) Vitamin D immunoassay and LC-MS/MS to be 75%.

Conclusions

We found that the Access Total 25 (OH) Vitamin D immunoassay on the Beckman Coulter DXI 800 analyzer has a good analytical performance and moderately concordant results with the reference method, LC-MS/MS. Because the LC-MS/MS method is not commonly available, the Access Total 25(OH) Vitamin D immunoassay may be a preferable choice.

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