Clinical Biochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Evaluation of the analytical performance of Unicel DXI 800 for the Total 25 (OH) Vitamin D measurements

Nurgul Ozcan [a,*], Fatma Ucar [a], Abdullah Ercan Arzuhal [a], Erdem Bulut [a], Alpaslan Ozturk [a], Mine Taslipinar Yavuz [b], Ismail Temel [a], Gonul Erden [a,b]

[a] Diskapi Yildirim Beyazit Training and Research Hospital, Department of Clinical Biochemistry, Ankara, Turkey
[b] Hacettepe University, Faculty of Medicine, Department of Clinical Biochemistry, Ankara, Turkey

ARTICLE INFO

Article history:
Received 29 July 2015
Received in revised form 27 November 2015
Accepted 30 November 2015
Available online xxxx

Keywords:
25-OH Vitamin D
Immunoassay
Method comparison
LC–MS/MS
Unicel DXI 800
DiaSorin LIAISON

ABSTRACT

Objectives: We assessed the analytical performance of newly developed Access 25(OH) Vitamin D Total assay with Beckman Coulter Unicel DXI 800 and evaluated the agreement between a reference method liquid chromatography/tandem mass spectrometry (LC–MS/MS) and a chemiluminescence method (LIAISON, DiaSorin).

Design and methods: 160 serum samples were included. Deming Regression analysis and Bland–Altman plots were used. The concordance correlation coefficient (CCC) was used to assess the degree of agreement between assays and the reference method.

Results: The CV% values of Unicel DXI 800 for within-run, between-run and between-day were lower than 6%. When compared to LC–MS/MS, the Access 25(OH) Vitamin D Total assay demonstrated an R value of 0.9444 (intercept −0.089, slope 0.951), with an average bias of −2.9%, and the LIAISON 25(OH) Vitamin D Total assay an R value of 0.9405 (intercept −0605, slope 0.924), with an average bias of −13.6%. In comparison with the LIAISON 25(OH) Vitamin D Total assay, the Access 25(OH) Vitamin D Total assay demonstrated an R value of 0.9498 (intercept 0.528, slope 1.029), with an average bias of 1.2%. The agreement with the LC–MS/MS method, based on values of the CCC, was moderate for the Unicel DXI 800 and LIAISON method (0.95, 0.94 respectively).

Conclusions: The new, automated Access 25(OH) Vitamin D Total assay showed an acceptable correlation with LC–MS/MS and LIAISON. Both methods moderately achieved to classify the patients according to their vitamin D status. However, we need further standardization of vitamin D assays to enhance the accuracy and comparability.

List of acronyms

DBP	vitamin D binding protein
LC–MS/MS	liquid chromatography–tandem mass spectrometry
25(OH)D	25-hydroxyvitamin D
DEQAS	Vitamin D External Quality Assessment Scheme
SRM	Standard Reference Material
VDSP	Vitamin D Standardization Program
NIH	National Institutes of Health
ODS	Office of Dietary Supplements
RLU	relative light units
CCC	correlation coefficient
TAE	total allowable error
R	correlation coefficient
Cb	bias correction factor

1. Introduction

Recently, vitamin D level determination has become important to evaluate the general health status [1]. In the last decade, vitamin D deficiency has been observed frequently and its detection methods and tests for instance automated immunoassays have been established due to its increasing clinical importance [2,3]. Serum or plasma 25-hydroxyvitamin D [25(OH)D] level analyses have become very common in clinical laboratories [4–6]. Therefore, there should be accurate and reliable test results for the vitamin D measurement.

The blood level of 25(OH)D is generally considered as a good indicator of vitamin D status [4]. Radio and chemiluminescence immunoassays, vitamin D binding protein (DBP) assays, liquid chromatography–tandem mass spectrometry (LC–MS/MS) and high-performance liquid chromatography are the currently used analytical techniques for the 25(OH)D measurement. For the measurement of 25(OH)D, the LC–MS/MS technology was used to develop the alternative measurement techniques as a reference method [3,7]. Recently, alternative methods such as automated immunoassays have been established instead of LC techniques in order to perform the routine 25(OH)D measurements [8,9]. Each of these methods has some strengths and limitations. The automated immunoassays are advantageous because they are high throughput and practical methods, therefore; they are popular. There were variabilities between separating methods which were for the allocating the 25(OH)D and its binding protein since multiple vitamin D

* Corresponding author at: Dışkapı Yıldırım Beyazıt Eğitim ve Araştırma Hastanesi Etlik Polikliniği, Klinik Biyokimya Laboratuvarı, Etlik, 06110 Ankara, Turkey.
E-mail address: nersoyozcan@gmail.com (N. Ozcan).

http://dx.doi.org/10.1016/j.clinbiochem.2015.11.022
0009-9120/

metabolites had interferences, calibrator and samples were influenced by matrix and these assays could measure both of the major vitamin D metabolites such as 25(OH)D_2 and 25(OH)D_3 [10]. Before performing the LC methods, there is an extraction step which helps to minimize interferences and matrix effects. On the other hand, the cost of the equipment and the requirement of technical expertise are frequently observed limitations for the routine diagnostic use of chromatographic methods in clinical laboratories [11,12].

There has been a progress in the quantification of 25(OH)D in the last decades. As a result of Vitamin D External Quality Assessment Scheme (DEQAS) survey, it has been observed that the inter-laboratory variability decreased from 32% to 15.3% from 1994 to 2009, respectively [13]. The 25(OH)D measurement should be the same and standard to indicate the accuracy between methods and laboratories. The Vitamin D Standardization Program (VDSP) was prepared by the National Institutes of Health (NIH), the Office of Dietary Supplements (ODS) in November 2010. Besides, the National Institute of Standards and Technology (NIST) provide the SRM 972 (a 25(OH) D Standard Reference Material) and it is a necessity to observe findings of different methods that are obtained by using this reference material [14].

In addition to standardization, there are various inconsistencies and bias among immunoassays in comparison with the reference methods. The performance of 25(OH)D assay methods particularly in terms of accuracy which is a very important feature which affects the results, is the common problem in clinical laboratories [3,15]. Recently, several studies have stated that there are some mismatches between results obtained from different 25(OH)D methods, even though there have been improvements in the past years [12,16–18].

Access 25(OH) Vitamin D Total assay has been recently developed by Beckman Coulter and it is a competitive chemiluminescent immunoassay for the high-throughput quantitative determination of total 25(OH)D measurement. According to our knowledge, there has been no study which was performed to compare the Access 25(OH)D Total assay with LC–MS/MS. In this study, the analytical performance of the Access 25(OH)D Total assay on Unicel DXI 800 (Beckman Coulter, Brea, CA, USA) was assessed by comparing it with the most commonly used immunoassay LIAISON 25(OH)D Total Assay (Diasorin Inc., Stillwater, MN, USA) [19] and with the reference LC–MS/MS method.

2. Material and methods

2.1. Study design

The Diskapi Yildirim Beyazit Training and Research Hospital's Ethical Committee approved the study protocol and we obtained the informed consents from all participants. A total of 160 serum and plasma samples with different concentrations of vitamin D were selected from samples and they were used for routine analyses in the Biochemistry Department of the Diskapi Yildirim Beyazit Training and Research Hospital. All the samples were treated by using the following preanalytical procedure: they were centrifuged at 2000 *g* for 10 min upon sampling, aliquoted and kept frozen at −80 °C until they were analyzed. Storage time for analysis did not exceed 30 days.

Vitamin D detection was performed with Access 25(OH)D Total assay and LIAISON 25(OH)D Total Assay reagents according to the manufacturer's instructions. LC–MS/MS method was ensured according to the manufacturer's instructions and used as the reference method. Aliquots were transported to other participating center (Laboratory of Ankalab, Ankara, Turkey) on dry ice in order to be analyzed by using other method (LC–MS/MS).

2.2. 25(OH)D measurement methods

2.2.1. Access 25(OH) Vitamin D Total assay on Unicel DXI 800

The Access 25(OH) Vitamin D Total assay is a two-step competitive binding immunoenzymatic assay. Sample is added to a reaction vessel with a DBP releasing agent and paramagnetic particles coated with sheep monoclonal anti-25(OH) Vitamin D antibody in the initial incubation. 25(OH) Vitamin D is released from DBP and binds to the immobilized monoclonal anti-25(OH) Vitamin D on the solid phase. Then, 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. In the next step, the chemiluminescent substrate Lumi Phos* 530 is added to the vessel and the light which is generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of 25(OH) Vitamin D of the sample. The amount of 25(OH) Vitamin D in the sample is calculated from a multi-point calibration curve.

2.2.2. LIAISON 25(OH) Vitamin D Total assay on DiaSorin LIAISON

Vitamin D Total assay is a direct competitive chemiluminescence immunoassay in order to determine the total amount of 25(OH)D in serum. Primarily, 25(OH)D is removed from the binding protein and it is ensured to bind to its specific antibody on the solid phase. 10 min later, the tracer (vitamin D linked to an isoluminol derivative) is added. After the following 10 min incubation, the unbound material is washed out. Then, the starter reagents are added to initiate a flash chemiluminescent reaction. The light is measured by a photomultiplier as relative light units (RLU). The signal is inversely proportional to the 25(OH)D concentrations in calibrators, controls or samples.

2.2.3. Reference method: liquid chromatography–tandem mass spectrometry

LC–MS/MS was performed by using Shimadzu prominence HPLC system (Kyoto, Japan) which is coupled to an AB Sciex API 3200 triple-quadrupole mass spectrometer (Framingham, MA, USA). Primarily, 200 μl serum (calibrator, control and patient serum) as well as 75 μl internal standard (IS) solutions are put in eppendorfs in order to prepare the samples. Then, 1000 μl acetonitrile (ACN) is added and samples are vortexed for 30 s and incubated at +4 °C for 10 min. After the incubation, tubes are centrifuged at 13,000 rpm for 5 min. Supernatants are transferred to borosilicate tubes and they are evaporated with nitrogen. Then, samples are dissolved in 100 μl 50% ACN solution and samples are vortexed for 15 s. All samples are transferred inserts and 50 μL of each sample was injected on a Luna C8, 5 μm, 2.1 × 50 mm HPLC column (Phenomenex, CA, USA). The mobile phases were: (A) water, and (B) 0.1% formic acid in acetonitrile. The needle rinse solvent was methanol. The HPLC flow rate was set at 0.3 mL/min. The gradient elution started at 70% mobile phase A and 30% mobile phase B for the first 3 min, then it was ramped linearly to 95% A in 1 min, held at 95% A for 1 min, and then returned to 70% A in 2 min. The column was re-equilibrated with 70% A and 30% mobile phase B prior to the next injection. The API 3200 was operated in positive electrospray mode, and the samples were examined using multiple reaction monitoring with the following (m/z) precursor/product ion transitions 25(OH)D_3 383.4/211.1; 25(OH)D_2 395.4/209.1; and IS 407.2/389.4. A sample was run for totally 4 min [20].

For the testing precision, linearity, carry over and method comparison with EP Evaluator Release 8 (Data Innovations LLC, South Burlington, VT, USA), Access 25(OH)D Total assays were assessed by using Clinical and Laboratory Standards Institute (CLSI) evaluation protocols.

2.3. Linearity

Linearity of the Access 25(OH) Vitamin D Total assay was assessed by using five standard solutions which were provided by the manufacturer. Standard solutions (212.4, 88.6, 36.5, 16.4, 5.9 ng/mL vitamin D) were run in 4 replicates in a single run.

Please cite this article as: N. Ozcan, et al., Evaluation of the analytical performance of Unicel DXI 800 for the Total 25 (OH) Vitamin D measurements, Clin Biochem (2015), http://dx.doi.org/10.1016/j.clinbiochem.2015.11.022