Clinical Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

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

First two-reagent vitamin D assay for general clinical chemistry

Fakhri B. Saida, Mario Padilla-Chee, Chao Dou, Chong Yuan*

Diazyme Laboratories Inc., Poway, CA, USA

ARTICLE INFO

Keywords:
Vitamin D
General chemistry
AU680
Immunoassay
Nanoparticle

ABSTRACT

Background: Vitamin D is a lipid-soluble molecule that plays key physiological roles in the metabolism of calcium, phosphate and magnesium. Recent studies show that deficiency in vitamin D is linked to cardiovascular diseases, autoimmune diseases and cancer. As a result, regular monitoring of 25-OH vitamin D (the main circulating form of vitamin D) is becoming essential. Current 25-OH vitamin D testing methodologies are cumbersome (too many reagents, long incubation times, phase separation) and are not compatible with general clinical chemistry platforms. Here, we report on a novel method to detect 25-OH vitamin D that is fast (results in 10 min or less), simple (two reagents) and compatible with virtually all general clinical chemistry analyzers.

Methods: An immunoturbidimetric assay for 25-OH vitamin D (the Diazyme EZ Vitamin D Assay) has been developed using nanoparticles and vitamin D-specific antibodies. The performance of the assay kit, which consists of two reagents and five calibrators, was tested on the Beckman AU680 analyzer (AU680).

Results: The new assay was precise, sensitive (LOD = 7.2 nmol/L), linear (up to 390.1 nmol/L) and correlated strongly ($R^2 > 0.95$) with major commercial 25-OH vitamin D assays. Additionally, the assay was found to be the fastest to date, with the first results obtained within 10 min. Throughput on the AU680 was estimated at over 300 tests per hour.

Conclusions: The newly developed 25-OH vitamin D assay is fast, precise and accurate. It can be run on most general chemistry analyzers. This assay aims at providing vitamin D-testing capabilities to all clinical chemistry laboratories.

1. Introduction

The term “vitamin D” designates a group of lipid-soluble steroids that include vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). In humans, vitamin D3 can be acquired from food and vitamin D supplements. It can also be synthesized by the skin upon exposure to ultraviolet light [1–3]. By contrast, vitamin D2 can only be produced by certain microorganisms such as fungi and phytoplankton [4,5]. As a result, humans can only acquire vitamin D2 from food and dietary supplements. Absorbed vitamin D2 and skin-synthesized vitamin D3 circulate in the human bloodstream bound to the VDBP transporter [6,7]. Upon reaching the liver, vitamin D is converted to 25-OH vitamin D, which is considered the main indicator of the overall vitamin D status of an individual [8]. 25-OH vitamin D is further hydroxylated in the kidneys to produce 1,25-OH vitamin D, which represents the biologically active form of vitamin D [8]. 1,25-OH vitamin D has many physiological roles that include calcium and phosphate homeostasis, absorption of certain oligo-elements (elements needed for life but in very limited amounts, such as Iron, Copper, Zinc, Selenium and Molybdenum), bone health and modulation of the immune and cardiovascular systems [6,7]. Moreover, deficiency and insufficiency in vitamin D have been linked to increased risk of developing certain forms of cancer [6,7,9–19].

Because of the multitude of physiological roles attributed to vitamin D, vitamin D testing in clinical settings has grown rapidly in the past decade. The need for testing 25-OH vitamin D has been supported by a growing body of research studies that link insufficient levels of 25-OH vitamin D to poor general health [10–19].

Compared to other clinical chemistry analytes, testing 25-OH vitamin D is particularly challenging for three reasons. Firstly, 25-OH vitamin D must be dissociated from its tightly bound partner (VDBP) before assaying. Secondly, the chosen assaying method must equally recognize 25-OH vitamin D2 and 25-OH vitamin D3 and report the total of both metabolites as 25-OH vitamin D. Thirdly, typical 25-OH vitamin

Abbreviations: 25-OH Vitamin D, 25-hydroxy Vitamin D; LOD, Limit of Detection; VDBP, Vitamin D Binding Protein; 1,25-OH Vitamin D, 1,25-dihydroxy Vitamin D; HPLC, High-Performance Liquid Chromatography; RIA, Radio ImmunoAssay; LC-MS/MS, Liquid Chromatography Tandem Mass Spectrometry; CLIA, Chemiluminescent ImmunoAssay; ECLIA, Electro- Chemiluminescent ImmunoAssay; EIA, Enzyme ImmunoAssay; CLSI, Clinical and Laboratory Standards Institute; DEQAS, Vitamin D External Quality Assessment Scheme; IRB, Institutional Review Board; ^{1}H NMR, Proton Nuclear Magnetic Resonance

* Corresponding author at: Diazyme Laboratories, 12889 Gregg Court, Poway, CA 92064, USA.
E-mail address: chong.yuan@diazyme.com (C. Yuan).

https://doi.org/10.1016/j.clinbiochem.2018.03.017
Received 2 February 2018; Received in revised form 23 March 2018; Accepted 23 March 2018

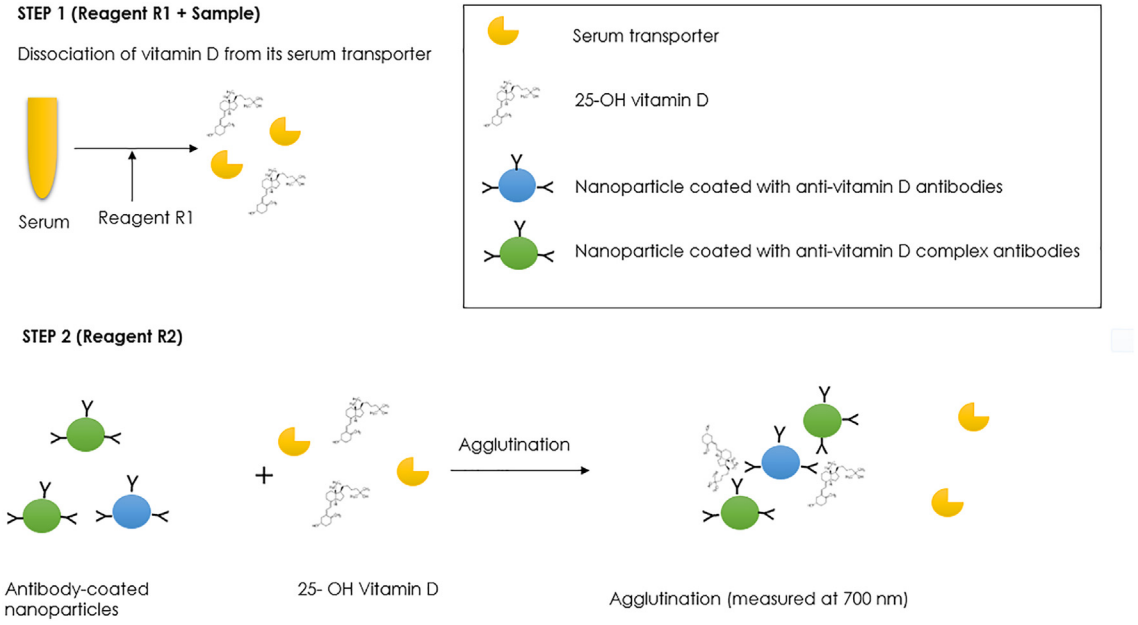

Fig. 1. Principle of the assay.

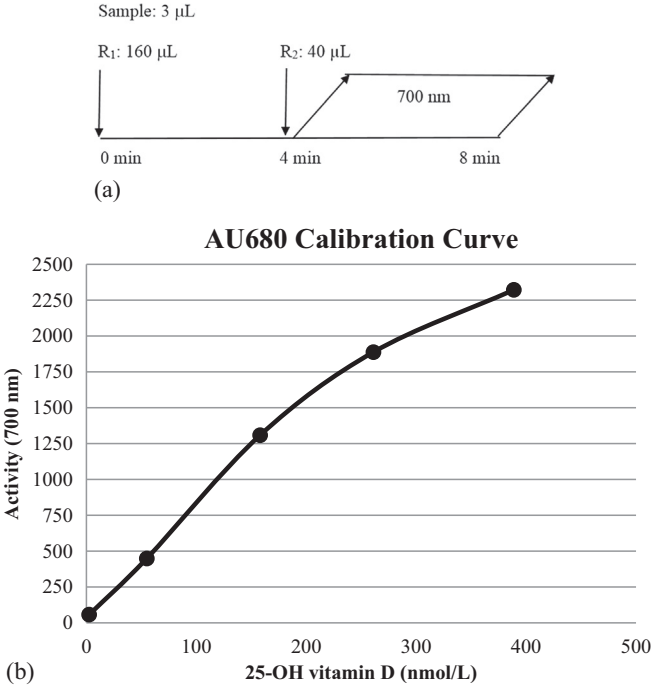

Fig. 2. Assay scheme and dose-response on the AU680 chemistry analyzer. (a) Sample (3 µL) and reagent R1 (160 µL) are at added at the same time (0 min). Upon release of 25-OH vitamin D from its serum transporter, reagent R2 (40 µL, containing antibody-conjugated nanoparticles) is added to generate an agglutination signal (measured at 700 nm) that is modulated by the concentration of 25-OH vitamin D. (b) Typical dose-response of vitamin D calibrators. The y-axis labelled "Activity" denotes the absorbance increase (due to particle agglutination) measured at 700 nm and over the time course of ~3.5 min (cycle 11 to cycle 22 on the AU680 chemistry analyzer). Spline model was used to fit the calibration data. Calibrator values were 2.5, 54.9, 158.0, 261.1 and 388.9 nmol/L.

D levels in serum are in the nanomolar range (20–150 nmol/L), which requires assays to be particularly sensitive [26,27].

Current 25-OH vitamin D testing is limited to HPLC, RIA, LC-MS/MS, CLIA, ECLIA and EIA [20–25]. The bulk of these assays can now be fully automated. However, these assaying systems have relatively low throughputs (when compared to general chemistry) and are still costly to clinical labs, insurers and patients. More importantly, very few of these assays can be run on general clinical chemistry analyzers which are widely available in clinical laboratories.

We hypothesized that developing a 25-OH vitamin D assay specifically designed for use on general clinical chemistry analyzers could make the test available to all clinical laboratories and significantly reduce its cost.

Here, we report on the development and the evaluation of a nanoparticle-based, liquid-stable, two-reagent vitamin D assay specially designed for general clinical chemistry analyzers.

2. Materials and methods

2.1. Chemicals and biological materials

All serum and plasma specimens used in this study were obtained from IRB-approved commercial sources: ProMedDx (Norton, MA), Biochemed (Winchester, VA), DEQAS (London, UK) and NIST (Gaithersburg, MD). Vitamin D-depleted serum was obtained from SeraCare (Milford, MA). Chemicals used in the formulation of Reagent 1 and Reagent 2, as well as interference testing were obtained from Sigma Aldrich. Cross-reaction compounds were obtained from Cerilliant (a Sigma-Aldrich Company). Concentration of stock solutions and purity (97–99%) of these cross-reaction compounds were established by a combination of liquid chromatography, LC-MS and ^{1}H NMR.

2.2. Principle of the assay

For an assay to be run on virtually any general chemistry analyzer, it has to have a limited number of reagents (preferably two or less) and must be completed very quickly (preferably within 10 min). To design such an assay, we combined the latex-enhanced immunoturbidimetric methodology with a proprietary pair of antibodies. The first antibody of the pair is conjugated to polystyrene nanoparticles and serves as a capture antibody for 25-OH vitamin D. The second antibody is also conjugated to polystyrene nanoparticles and serves as detection antibody that recognizes the capture antibody when it is bound to a vitamin D molecule. The detection antibody was developed *in vitro* using antibody combinatorial libraries. By combining the capture and detection

Table 1
Precision of the Diazyme EZ vitamin D assay. Measurements were done over 20 working days, at the rate of two runs per day, using three lots of reagents. All results are shown in nmol/L.

Sample	Mean	Within-run		Between-run		Between-day		Between-lot		Total	
	n = 240	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Control 1	54.2	2.2	3.9%	1.5	2.8%	2.0	3.8%	3.2	5.9%	3.2	6.2%
Control 2	106.1	2.5	2.4%	2.0	2.0%	2.7	2.5%	3.5	3.3%	4.2	3.9%
Sample 1	27.7	2.2	8.3%	1.2	4.4%	3.7	13.7%	4.7	16.8%	4.5	16.6%
Sample 2	45.4	2.2	4.9%	1.7	3.9%	2.7	6.0%	4.0	8.6%	4.0	8.7%
Sample 3	55.2	2.0	3.8%	2.0	3.8%	1.0	1.8%	3.0	5.6%	3.0	5.6%
Sample 4	106.8	2.2	2.0%	2.5	2.4%	0.0	0.0%	3.5	3.2%	3.2	3.1%
Sample 5	148.5	2.5	1.7%	1.7	1.2%	2.5	1.7%	4.0	2.7%	4.0	2.7%
Sample 6	200.2	3.2	1.6%	2.7	1.4%	2.7	1.4%	5.0	2.5%	5.0	2.5%
Sample 7	248.4	4.5	1.8%	3.7	1.6%	3.2	1.3%	6.7	2.7%	6.7	2.8%
Sample 8	293.5	5.5	1.9%	5.0	1.7%	5.5	1.9%	9.2	3.1%	9.2	3.2%
Sample 9	347.4	6.7	1.9%	6.5	1.8%	4.2	1.2%	10.0	2.9%	10.2	2.9%
Sample 10	393.9	7.0	1.8%	6.7	1.7%	5.5	1.4%	11.0	2.8%	11.2	2.9%

antibodies conjugated to nanoparticles, an immunoturbidimetric signal can be generated in the presence of 25-OH vitamin D molecules from the samples. The amount of immunoburbidimetric signal generated is proportional to the amount of 25-OH vitamin D present in the sample. The assay's principle is shown in Fig. 1.

2.3. The assay kit

The assay kit, namely the Diazyme EZ Vitamin D Assay (Diazyme Laboratories Inc., Poway, CA) consists of a set of two reagents. Reagent R1 contains dissociation and crowding agents. Vitamin D is stripped from its serum transporter using a combination of pH change and the addition of ionic compounds and surfactants. Crowding agents, such as polyethylene glycol (PEG), artificially increase the local concentration of nanoparticles and hence catalyze their agglutination. Typical Reagent R1 composition consists of 50 mM sodium acetate, 5% choline chloride, 0.9% PEG and 0.09% sodium azide. Reagent R2 contains conjugated nanoparticles and stabilizers. Typical reagent R2 composition consisted of 50 mM Tris-HCl, 0.1% bovine serum albumin and 0.2% Tween 20. The assay was calibrated using a set of 5 serum-based calibrators and quality-controlled using a set of two serum-based controls. Reagents composition and assay timing were optimized for sensitivity (assessed by measuring limit of quantitation), precision (repeatability), linearity, interference, method comparison and shelf-life.

2.4. Assay procedure on the AU680 analyzer

The AU680 general chemistry analyzer (Beckman) was chosen as an example to illustrate the performance of the Diazyme EZ Vitamin D Assay. Assay procedure and dose-response are shown in Fig. 2.

2.5. Performance evaluation

The assay has been fully evaluated according to the CLSI approved standards and guidelines: CLSI EP5-A2 (precision), CLSI EP6-A (linearity), CLSI EP7-A2 (interference), CLSI EP9-A3 (method comparison) and CLSI C28-A3 (reference range). For sensitivity, the CLSI EP17-A2 protocol was used. To calculate the limit of blank (LOB), a vitamin D-depleted serum was run as sample, in 60 replicates with three lots of the reagents on the Beckman Coulter AU680 analyzer. These replicates were obtained at the rate of 12 replicates per day over the time course of five working days. LOB was calculated as the mean of the 57th and 58th highest obtained values. The limit of detection (LOD) was measured as follows: Five serum samples (namely S1, S2, S3, S4 and S5), collected with IRB approval, were purchased from PromedDX. These samples were diluted with a vitamin D-depleted diluent and tested with three lots of the Diazyme EZ Vitamin D reagents on the Beckman Coulter AU680 analyzer, at the rate of 12 replicates per sample. These replicates were obtained over 4 working days at the rate of 3 replicates per day. The LOD was calculated as the LOB + (1.645 * SD of LOD samples). The limit of quantitation (LOQ, functional sensitivity) was measured as follows: Five serum samples obtained from PromedDX and collected with IRB approval were diluted with vitamin D-depleted serum to concentrations ranging from about 1 to 8 times the claimed LOB. The diluted serum samples were then tested in 40 replicates over 5 working days (8 replicates per day) on Beckman AU680 analyzer. Three lots of reagents were used in this study. EP Evaluator software (version 11.0) was used to estimate the LOQ by fitting the %CV versus mean curve and matching the 95% confidence interval to the lowest vitamin D concentration giving of CV of 20%. All human specimens used in this study were obtained from IRB-approved commercial sources. In interference testing, "*Tolerance*" was defined as the highest concentration of interfering substance, for which 25-OH vitamin D sample recovery deviates by no > 10% from sample recovery in the absence of said interfering substance. For the precision study, patient samples were obtained from individual donors using an IRB-approved commercial source (PromedDx). Sample above 199.7 nmol/L were artificially spiked with 25-OH vitamin D to cover the dynamic range of the assay. Patient samples were aliquoted and kept at −80 °C until use.

3. Results

The performance of the Diazyme EZ Vitamin D assay is described in the sections listed below.

3.1. Precision

12 specimen (two serum controls and 10 patient samples) were measured twice-a-day over the span of 20 days, using three lots of reagents and one chemistry analyzer. 240 data points were obtained per specimen. Results are shown in Table 1.

3.2. Sensitivity

The Limit of Blank (LoB), the Limit of Detection (LoD) and the Limit of Quantification (LoQ) were, respectively, 2.99 nmol/L, 7.2 nmol/L and 15.7 nmol/L.

3.3. Linearity

Eleven levels of linearity materials were prepared by diluting a high serum sample with vitamin D-depleted serum. The assay was found to be linear between 15.7 and 390.1 nmol/L, thus establishing the dynamic range of the assay.

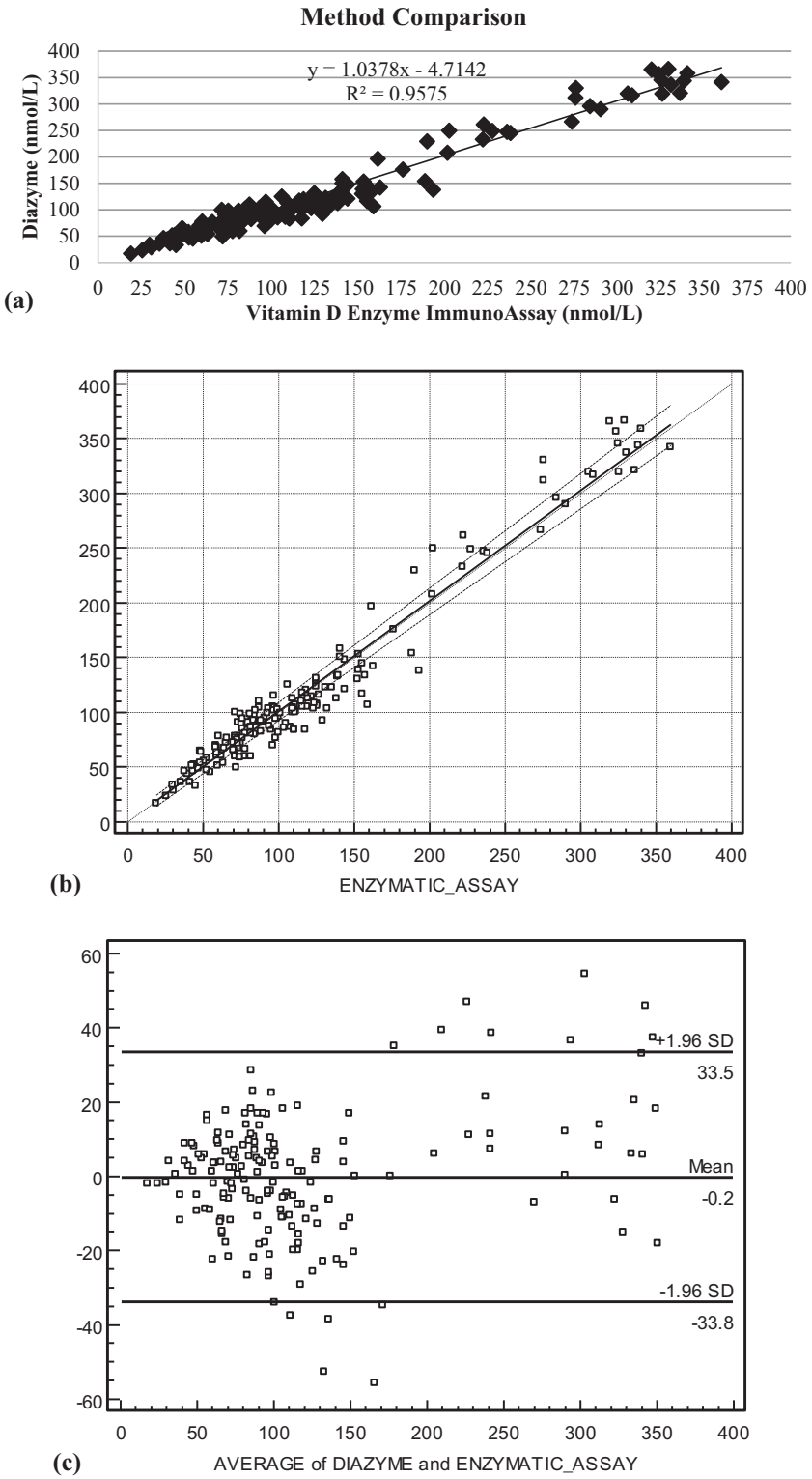

Fig. 3. Method comparison between the Diazyme EZ vitamin D assay and US FDA-approved enzymatic assay for vitamin D (k133410). 161 patient samples were used in the study. (a) Linear regression. (b) Passing & Bablok plot. Intercept = 0.4256, 95% confidence interval = −3.5852 to 4.7882). Slope = 0.9639, 95% confidence interval = 0.9639 to 1.0455. (c) Bland & Altman plot.

3.4. Method comparison

161 serum samples (obtained from apparently healthy individuals the serum of whom was collected according to IRB-approved protocols) were tested with the new vitamin D and compared with a US FDA-approved 25-OH Vitamin D enzyme immunoassay (Diazyme Laboratories). Linear regression of the data is shown in Fig. 3.

To further access accuracy of the new vitamin D assay, the recovery of 25 proficiency samples, from the DEQAS program (one of the oldest and most widely used Proficiency Testing program for Vitamin D) was compared to that of four additional commercial vitamin D tests (LC-MS/MS, Roche Total 25OHD, DiaSorin Liaison Total and Siemens

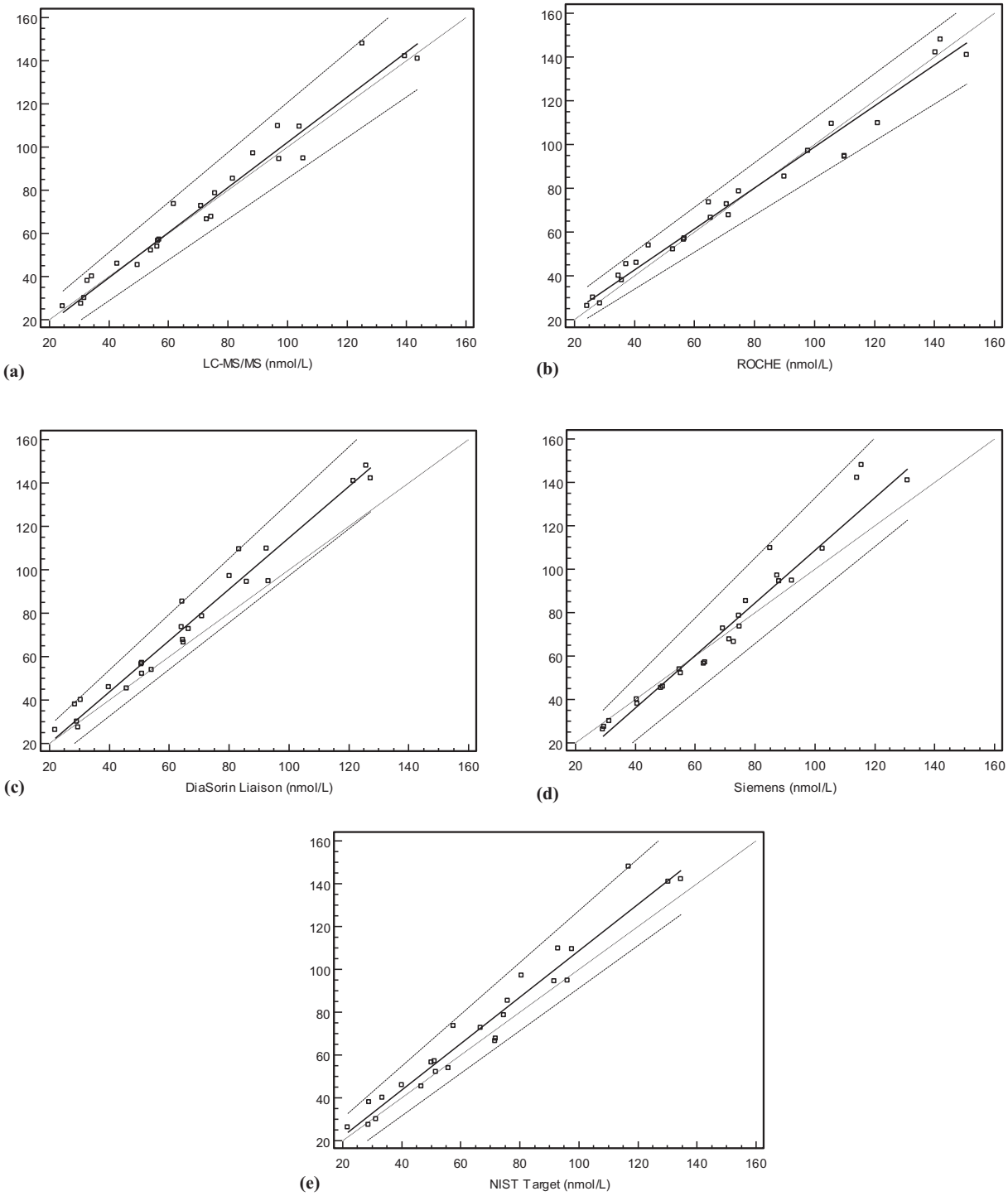

Fig. 4. Passing & Bablok Analysis of DEQAS Sample Recovery. The study used 25 proficiency testing sample obtained from the DEQAS program (DEQAS 486 to DEQAS 510). DEQAS is one of the oldest and most widely used Proficiency Testing program for Vitamin D. Diazyme sample recovery was compared to that of LC-MS/MS (a), Roche Total 25OHD (b), DiaSorin Liaison (c), Siemens (d) and NIST Target (e). All measurements were in nmol/L.

ADVIA Centaur). Passing & Bablok analysis of the comparison data is shown in Fig. 4. Bland & Altman analysis of the comparison data is shown in Fig. 5.

Accuracy of the Diazyme vitamin D assay was further assessed by measuring the 25-OH vitamin D content of the NIST vitamin D standards (reference #SRM 972a), which have been assigned by at The National Institute of Standards and Technology using the LCMSMS method. Results are shown in Table 2.

3.5. Matrix comparison

Serum, K_2-EDTA plasma, K_3-EDTA plasma or Li-heparin plasma samples were tested in matching sets. Method comparison of K_2-EDTA plasma samples versus serum samples yielded a regression equation of $y = 0.9911x + 0.1527$ and $R^2 = 0.9967$. Method comparison of K_3-EDTA plasma samples versus serum samples yielded a regression equation of $y = 1.0114x - 0.7376$ and $R^2 = 0.9958$. Method comparison of Li-heparin plasma samples versus serum samples yielded a

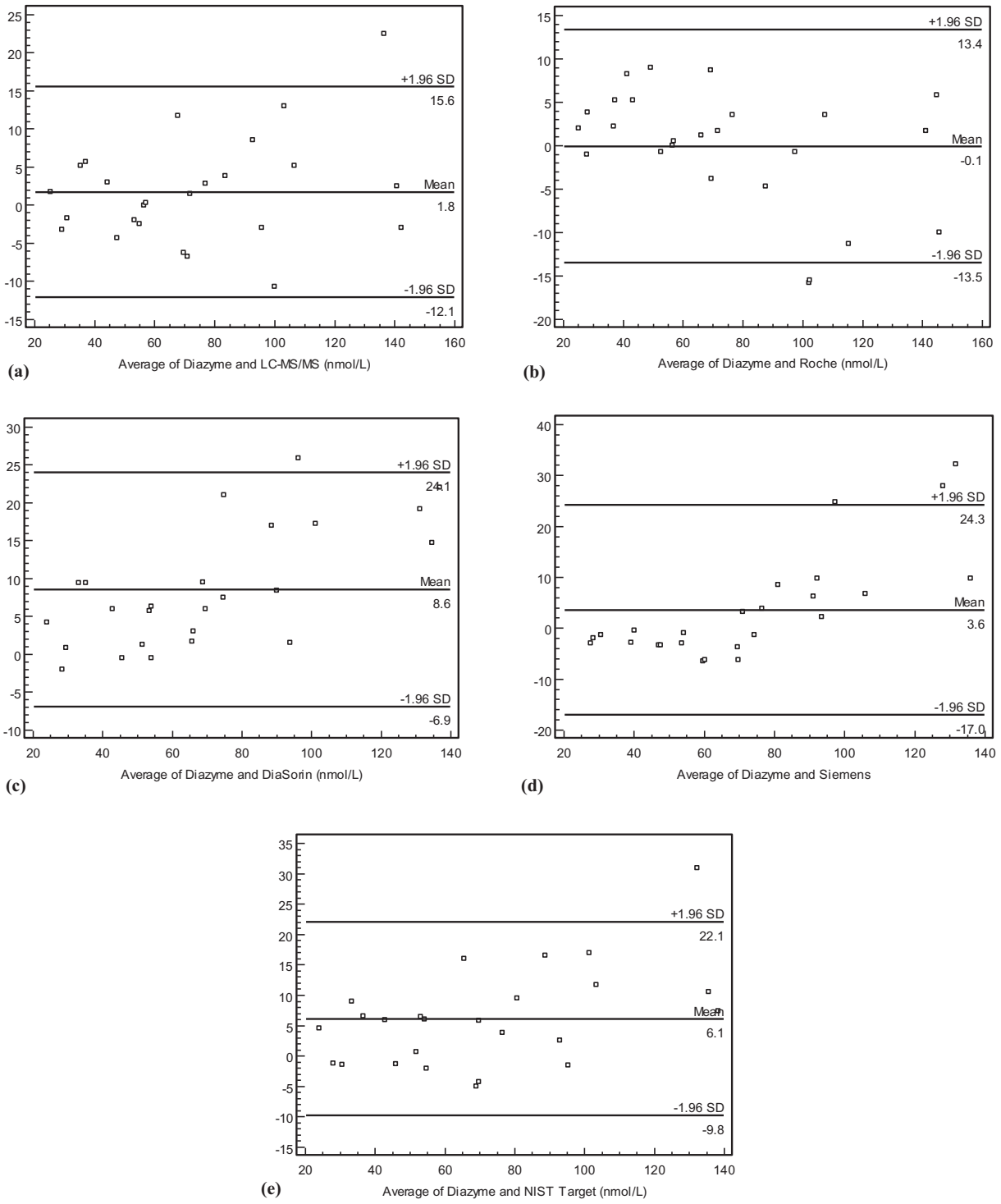

Fig. 5. Bland & Altman Analysis of DEQAS Sample Recovery. The study used 25 proficiency testing sample obtained from the DEQAS program (DEQAS 486 to DEQAS 510). Diazyme sample recovery was compared to that of LC-MS/MS (a), Roche Total 25OHD (b), DiaSorin Liaison (c), Siemens (d) and NIST Target (e). All measurements were in nmol/L.

Table 2
Sample recovery of the NIST SRM 972a standards by the diazyme vitamin D assay.

NIST SRM 972a	LCMSMS (nmol/L)	Diazyme (nmol/L)	% Recovery
Level 1	76.5	80.1	104.8%
Level 2	50.4	48.3	95.8%
Level 3	82.6	85.4	103.3%

regression equation of $y = 1.031x - 0.963$ and $R^2 = 0.9967$.

3.6. Interference

A total of 30 substances (endogenous and exogenous) were tested. The assay's results were consistent within ± 10% in the presence of the compounds listed in Table 3, at concentrations up to the listed tolerance limit.

Table 3
Tolerance of the diazyme EZ vitamin D assay to various endogenous and exogenous interfering substances.

Substance	Tolerance	Unit
Acetaminophen	20	mg/dL
Acetyl salicylic acid	60	mg/dL
Ampicillin	5.3	mg/dL
Ascorbate	3	mg/dL
Biotin	100	ng/mL
Carbamazepine	3	mg/dL
Cefotaxime	180	mg/dL
Chloramphenicol	5	mg/dL
Conjugated bilirubin	40	mg/dL
Creatinine	30	mg/dL
Digoxin	6.1	ng/mL
Ethanol	400	mg/dL
Ethosuximide	25	mg/dL
Free bilirubin	40	mg/dL
Furosemide	6	mg/dL
Hemoglobin	600	mg/dL
Heparin	3	U/mL
Ibuprofen	50	mg/dL
Lidocaine	1.2	mg/dL
Lithium acetate	2.2	mg/dL
Noradrenalin	4	µg/mL
Rheumatoid factor (RF)	200	IU/mL
Rifampicin	5	mg/dL
Theophylline	4	mg/dL
Total protein	12	g/dL
Triglycerides	1000	mg/dL
Urea	300	mg/dL
Uric acid	20	mg/dL
Valproic acid	50	mg/dL
Vancomycin	10	mg/dL

Table 4
Cross-reactivity of the Diazyme EZ vitamin D assay with various vitamin D metabolites (values are normalized to 25-OH vitamin D3).

Compound	Concentration tested	Cross-reactivity
25-OH Vitamin D3	249.6 nmol/L	100%
25-OH Vitamin D2	249.6 nmol/L	106.9%
Vitamin D3	249.6 nmol/L	−0.8%
Vitamin D2	249.6 nmol/L	−1.7%
1,25-(OH)2 Vitamin D3	1.4 nmol/L	0.2%
1,25-(OH)2 Vitamin D2	1.4 nmol/L	−0.5%
24R,25-(OH)2 Vitamin D3	249.6 nmol/L	118.8%
3-epi-25-OH Vitamin D3	249.6 nmol/L	33.0%
3-epi-25-OH Vitamin D2	249.6 nmol/L	36.5%

3.7. Cross-reactivity

Cross-reactivity of the Diazyme EZ Vitamin D Assay was determined by adding various vitamin D metabolites to serum pool samples. Based on the results in the table below, the assay did not cross-react with vitamin D2 or vitamin D3. In addition, the assay recovered 25-OH vitamin D2 and 25-OH vitamin D3 similarly. Full cross-reactivity results are summarized in Table 4.

Because sera artificially spiked with vitamin D metabolites may not accurately mimic the behavior of sera containing endogenous vitamin D metabolites [26], the Diazyme assay was used to test the sample recovery of NIST SRM972a level 3. This level has relatively high endogenous levels of 25-OH vitamin D2 (33.2 nmol/L) in addition to the presence of 25-OH vitamin D3 (49.4 nmol/L), for a total of 25-OH vitamin D of 82.6 nmol/L. The Diazyme assay recovered 85.4 nmol/L for NIST level 3 (103.3% of the expected total 25-OH vitamin D, see Table 2). This results further highlights the substantially equal cross-reaction between 25-OH vitamin D2 and 25-OH vitamin D3 in the Diazyme assay.

3.8. Reference range

The reference range of the Diazyme EZ Vitamin D assay was determined by measuring the 25-OH vitamin D serum concentrations of a USA population of 145 apparently healthy adults, 21–67 years old, during the months of April and May (spring season). Individuals were from three different geographical locations: 47 from Pennsylvania (Northern US), 49 from Tennessee (Central US) and 49 from Texas (Southern US). All 145 individuals did not take any vitamin D supplements and did not have kidney disease, GI disease, liver disease, calcium-levels related disease, thyroid disease, parathyroid disease, seizures, chronic disease or bariatric surgery. The central 95% of the population was found to have 25-OH vitamin D concentrations ranging between 18.0 and 103.8 nmol/L, with a mean concentration of 50.2 nmol/L. This range covers the vitamin D cutoffs for deficiency (below 50 nmol/L), and vitamin D insufficiency (50–75 nmol/L) [27].

3.9. Reagents, calibrators and controls shelf-life

Accelerated stability testing (at 37 °C and 45 °C) and extrapolation to real-time stability showed that the Diazyme vitamin D assay reagents, calibrators and controls were stable for at least 12 months when stored at 2–8 °C, according to instructions of the package insert. Real-time stability monitoring is on-going.

4. Discussion

This article reports on the development of the first two-reagent 25-OH vitamin D assay for general chemistry. The assay combines the nanoparticle-enhanced immunoturbidimetric technology with the use of a pair of 25-OH vitamin D-specific antibodies: one serves as the capture antibody and the other as the detection antibody.

Full evaluation of the assay's performance on a typical general chemistry analyzer shows that the assay is substantially equivalent to a currently marketed 25-OH vitamin D assay and correlates well with leading commercial 25-OH vitamin D assay (correlation coefficient $R^2 > 0.95$). In particular, the assay shows significant correlation with the most accurate method for measuring 25-OH vitamin D (LCMSMS), as illustrated by testing DEQAS and NIST vitamin D samples.

The newly designed Diazyme vitamin D assay has several improvements to the currently marketed, enzyme-based, Diazyme vitamin D assay (k133410). These improvements include reducing the total number of reagents from 4 to 2, the elimination of sample dilution steps as well as a significant increase in throughput and shelf life of reagents.

The assay's performance, illustrated here on the AU680, is currently evaluated on a wide variety of general chemistry analyzers that includes the Hitachi series of analyzers, Roche's Modular P, Roche Cobas, Siemens EXL, Abbott Architect, Horiba's Pentra 400 and Tokyo Boeki's Biolis systems.

The newly developed 25-OH vitamin D assay has the propensity to expand vitamin D testing to clinical laboratories equipped with ubiquitous general chemistry analyzers. Wide availability of vitamin D testing in clinical laboratories could help decrease the cost of this test and support its role as a preventive medicine tool.

Finally, we believe that the new technology presented in this article may be applied to the detections of other vitamin molecules such as B12 and folate using general clinical chemistry analyzers.

4.1. Regulatory and intellectual property considerations

The Diazyme EZ Vitamin D Assay has been approved by the US FDA on 01/11/2018 (application number k172992). United States patent # US 20180031581A1 covers all intellectual property aspects of the assay.

References

[1] M. Wacker, M.F. Holick, Sunlight and Vitamin D: a global perspective for health, Dermatoendocrinol 5 (2013) 51–108.
[2] D. Haxhiu, S. Hoby, C. Wenker, A. Boos, M.P. Kowalewski, F. Lewis, A. Liesegang, Influence of feeding and UVB exposition on the absorption mechanisms of calcium in the gastrointestinal tract of veiled chameleons (Chamaeleo calyptratus), J. Anim. Physiol. Anim. Nutr. (Berl.) 98 (2014) 1021–1030.
[3] F.B. Saida, X. Chen, K. Tran, C. Dou, C. Yuan, First 25-hydroxyvitamin D assay for general chemistry analyzers, Expert. Rev. Mol. Diagn. 15 (2015) 313–323.
[4] R.B. Japelt, J. Jakobsen, Vitamin D in plants: a review of occurrence, analysis, and biosynthesis, Front. Plant Sci. 4 (2013) 1–20.
[5] P. Borel, D. Caillaud, N.J. Cano, Vitamin D bioavailability: state of the art, Crit. Rev. Food Sci. Nutr. 55 (2015) 1193–1205.
[6] I. Bhan, Vitamin d binding protein and bone health, Int. J. Endocrinol. 2014 (2014) 561214.
[7] P. Yousefzadeh, S.A. Shapses, X. Wang, Vitamin D binding protein impact on 25-Hydroxyvitamin D levels under different physiologic and pathologic conditions, Int. J. Endocrinol. 2014 (2014) 981581.
[8] G. Jones, Pharmacokinetics of vitamin D toxicity, Am. J. Clin. Nutr. 88 (2008) 582S–586S.
[9] T. Okano, Bone metabolism and cardiovascular function update. Role of vitamin D in the bone and vascular intercommunication, Clin. Calcium 24 (2014) 1013–1020.
[10] S. Das, S. Bopitya, H. Taha, L. David, Relationship between Vitamin D, parathyroid hormone, bone mineral density, fracture and antiretroviral therapy in HIV patients, Recent Patents Anti-infect. Drug Discov. 9 (2014) 6–13.
[11] D.M. DeMille, M. Piscitelli, A. Ocker, C. Vuong, L. Hartner, M.P. Lynch, Vitamin D deficiency in the oncology setting, J. Commun. Support. Oncol. 12 (2014) 13–19.
[12] C.J. Narvaez, D. Matthews, E. LaPorta, K.M. Simmons, S. Beaudin, J. Welsh, The impact of vitamin D in breast cancer: genomics, pathways, metabolism, Front. Physiol. 5 (2014) 213.
[13] M.L. Brance, L.R. Brun, S. Lioi, A. Sanchez, M. Abdala, B. Oliveri, Vitamin D levels and bone mass in rheumatoid arthritis, Rheumatol. Int. 35 (2014) 499–505.
[14] S. Yalcinkaya, E. Eren, M. Eroglu, O. Aydin, N. Yilmaz, Deficiency of vitamin d and elevated aldosterone in prostate hyperplasia, Adv. Clin. Exp. Med. 23 (2014) 441–446.
[15] K.S. Vimaleswaran, A. Cavadino, D.J. Berry, LifeLines Cohort Study Investigators, R. Jorde, A.K. Dieffenbach, C. Lu, et al., Association of vitamin D status with arterial blood pressure and hypertension risk: a Mendelian randomisation study, Lancet Diabetes Endocrinol. 9 (2014) 719–729.
[16] B. Daubail, A. Jacquin, J.C. Guilland, C. Khoumri, C. Aboa-Eboulé, M. Giroud, Y. Béjot, Association between serum concentration of Vitamin D and 1-year mortality in stroke patients, Cerebrovasc. Dis. 37 (2014) 364–367.
[17] E.B. Gur, A. Gokduman, G.A. Turan, S. Tatar, I. Hepyilmaz, E.B. Zengin, F. Eskicioglu, et al., Mid-pregnancy vitamin D levels and postpartum depression, Eur. J. Obstet. Gynecol. Reprod. Biol. 179 (2014) 110–116.
[18] W. Saliba, O. Barnett-Griness, G. Rennert, Obesity and Association of Serum 25(OH) D levels with all-cause mortality, Calcif. Tissue Int. 95 (2014) 222–228.
[19] B.M. Song, H.C. Kim, D.P. Choi, S.M. Oh, I. Suh, Association between serum 25-Hydroxyvitamin D level and insulin resistance in a rural population, Yonsei Med. J. 55 (2014) 1036–1041.
[20] N. Binkley, D.C. Krueger, S. Morgan, D. Wiebe, Current status of clinical 25-hydroxyvitamin D measurement: an assessment of between-laboratory agreement, Clin. Chim. Acta 411 (2010) 1976–1982.
[21] M. Herrmann, T. Harwood, O. Gaston-Parry, D. Kouzios, T. Wong, A. Lih, M. Jimenez, et al., A new quantitative LC tandem mass spectrometry assay for serum 25-hydroxy vitamin D, Steroids 75 (2010) 1106–1112.
[22] M. Yazdanpanah, D. Bailey, W. Walsh, B. Wan, K. Adeli, Analytical measurement of serum 25-OH-vitamin D(3), 25-OH-vitamin D(2) and their C3-epimers by LC-MS/MS in infant and pediatric specimens, Clin. Biochem. 46 (2013) 1264–1271.
[23] D. Bailey, N. Perumal, M. Yazdanpanah, A. Al Mahmud, A.H. Baqui, K. Adeli, D.E. Roth, Maternal-fetal-infant dynamics of the C3-epimer of 25-hydroxyvitamin D, Clin. Biochem. 47 (2014) 816–822.
[24] N. Binkley, C.T. Sempos, Vitamin D. Standardization Program, Standardizing vitamin D assays: the way forward, J. Bone Miner. Res. 29 (2014) 1709–1714.
[25] M. Bedner, K.A. Lippa, S.S. Tai, An assessment of 25-hydroxyvitamin D measurements in comparability studies conducted by the vitamin D metabolites quality assurance program, Clin. Chim. Acta 426 (2013) 6–11.
[26] G.D. Carter, Accuracy of 25-hydroxyvitamin D assays: confronting the issues, Curr. Drug Targets (2011) 19–28.
[27] M.F. Holick, N.C. Binkley, H.A. Bischoff-Ferrari, C.M. Gordon, D.A. Hanley, R.P. Heaney, M.H. Murad, C.M. Weaver, Endocrine Society, Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline, J. Clin. Endocrinol. Metab. (2011) 1911–1930.