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# Performance characteristics of the VIDAS® 25-OH Vitamin D Total assay – comparison with four immunoassays and two liquid chromatography-tandem mass spectrometry methods in a multicentric study

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## Abstract

**Background:** The study was conducted to evaluate the analytical and clinical performance of the VIDAS® 25-OH Vitamin D Total assay. The clinical performance of the assay was compared with four other immunoassays against the results of two different liquid chromatography/mass spectrometry methods (LC-MS/MS) standardized to NIST reference materials.

**Methods:** VIDAS® 25-OH Vitamin D Total assay precision, linearity, detection limits and sample matrix comparison were assessed following CLSI guidelines. For method comparison, a total of 150 serum samples ranging from 7 to 92 ng/mL were analyzed by all the methods. Correlation was studied using Passing-Bablok regression and Bland-Altman analysis. The concordance correlation coefficient (CCC) was calculated to evaluate agreement between immunoassays and the reference LC-MS/MS method. In addition, samples containing endogenous 25(OH)D<sub>2</sub> were used to assess each immunoassay's ability to detect this analyte. Pregnancy and hemodialysis samples were used

to the study the effect of vitamin D binding protein (DBP) concentration over VIDAS® assay performance.

**Results:** The VIDAS® 25-OH Vitamin D Total assay showed excellent correlation to the LC-MS/MS results ( $y=1.01x+0.22$  ng/mL,  $r=0.93$ ), as obtained from two different sites and distinct LC-MS/MS methods. The limit of quantification was determined at 8.1 ng/mL. Cross-reactivity for 25(OH)D<sub>2</sub> was over 80%. At concentrations of 10.5, 26 and 65.1 ng/mL, within-run CVs were 7.9%, 3.6% and 1.7%, while total CVs (between runs, calibrations, lots and instruments) were 16.0%, 4.5% and 2.8%. The VIDAS® performance was not influenced by altered DBP levels, though under-recovery of 25(OH)D as compared to LC-MS/MS was observed for hemodialysis samples.

**Conclusions:** The VIDAS® 25-OH Vitamin D Total assay is therefore considered suitable for assessment of vitamin D status in clinical routine.

**Keywords:** assay performance; liquid chromatography/mass spectrometry; standardization; 25-OH vitamin D.

## Introduction

Vitamin D is a fat-soluble steroid pro-hormone that plays a pivotal role in bone metabolism and calcium homeostasis. In this context, vitamin D deficiency is associated with rickets, osteoporosis and secondary hyperparathyroidism [1–3]. Moreover, non-skeletal functions of vitamin D have been extensively discussed in recent studies [4–6]. Vitamin D insufficiency is considered an important risk factor in diabetes, cardiovascular diseases, autoimmune disorders and various forms of cancer [7]. Vitamin D is found in two forms: vitamin D<sub>3</sub> (cholecalciferol) synthesized by action of solar ultraviolet radiation on the skin and found in food (oily fish); vitamin D<sub>2</sub> (ergocalciferol) of exogenous

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origin only (food or medical supplementation). Vitamin D is first converted in the liver to 25-hydroxy vitamin D [25(OH)D] and then to its active form 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D] in the kidneys [8]. 25(OH)D is found in nanomolar concentrations in serum or plasma and has a half-life of several weeks. This makes 25(OH)D the preferred analyte and the most relevant clinical indicator for the determination and monitoring of vitamin D status, provided the measurement method quantitates equally the 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> forms. Indeed, due to the lipophilic nature of vitamin D and its strong binding capability to vitamin D binding protein (DBP) or human serum albumin, equal quantitation of both 25(OH)D forms is a technical challenge. However, this is not the only hurdle in designing an immunoassay that achieves the level of analytical performance required by clinical laboratories. State-of-the-art immunoassays have shown discrepancies in terms of accuracy, precision, linearity and correlation to liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), which has become a true reference method due to efforts to harmonize results driven by the use of international standards [9, 10]. Recently, publications have pointed out the influence of assay design with regards to technical features [11] and the necessity to use methodologies that adequately challenge the assay design to establish analytical performance. In this context, a new VIDAS® 25-OH Vitamin D Total immunoassay that measures both 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> has been developed. The purpose of this multicentric study was to evaluate the technical and clinical performance of the VIDAS® 25-OH Vitamin D Total assay and to compare it to two LC-MS/MS methods and four vitamin D immunoassays which are commercially available.

## Materials and methods

### Description of the VIDAS® 25-OH Vitamin D Total assay

The study concentrated on the performance of the VIDAS® 25-OH Vitamin D Total assay. This method is a sequential competitive immunoassay. All of the assay steps are performed automatically by the instrument. The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device for the assay. The sample is mixed with pre-treatment reagent to separate 25(OH)D from its binding protein. The pre-treated sample is then collected and transferred into the well that contains an alkaline phosphatase (ALP)-labeled anti-vitamin D antibody (conjugate). The vitamin D antigen present in the sample and the vitamin D antigen coating the interior of the SPR compete for binding sites on the anti-vitamin D antibody-ALP conjugate. During the final detection step, the substrate (4-methylumbelliferyl phosphate) is cycled in and out of the

SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methylumbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of vitamin D antigen present in the sample. Results are automatically calculated by the instrument in relation to the calibration curve. The VIDAS® 25-OH Vitamin D Total assay is standardized to internal controls that are traceable to a LC-MS/MS method calibrated with NIST SRM972a.

### Analytical methods

VIDAS® 25-OH Vitamin D Total assay specificity was assessed through determination of cross-reactivity for vitamin D structural analogs according to CLSI protocol CLSI EP7-A2 [12]. Two samples at 25(OH)D levels of 20 and 40 ng/mL were spiked with various concentrations (10–100 ng/mL) of vitamin D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub> and 3-epi-25(OH)D<sub>3</sub>, then samples were tested in triplicate with three reagent lots. Mean cross-reactivity for each analyte is calculated as  $XR\% = \frac{[25(OH)D \text{ measured in interfering pool}] - [25(OH)D \text{ measured in non-spiked pool}]}{\text{spiked concentration of interfering material}}$ .

Assay precision was determined across the dynamic range using assay controls and serum samples according to a modified version from CLSI protocol EP05-A2 [13]. Two replicates of each sample were tested twice per day in separate runs, for 5 days, on three reagent lots and two different VIDAS® instruments (bioMérieux, Marcy l'Etoile, France). Variance components that pertain to each studied variation source were estimated by performing a nested-design variance analysis. Variability is expressed in standard deviation (SD) and percent coefficient of variation (CV).

Assay linearity was evaluated using two serum pools, one high concentration sample and one low concentration sample, both at 25(OH)D levels close to the limits of the VIDAS® assay calibration range. High and low samples were sequentially mixed to generate 12 samples of intermediate concentrations. Each sample was tested in duplicate with three reagent lots. To determine linearity, the polynomial analysis method was used as described in CLSI protocol EP6-A [14], with a deviation from linearity <12% over the entire measuring range.

The limit of blank (LoB) and the limit of detection (LoD) were determined according to CLSI protocol EP17-A2 [15]. LoB corresponds to the highest measurement result that is likely to be observed for a blank sample with a stated probability of 5%. Four blank samples (SeraCon™ Vitamin D Depleted Diluent, Seracare) were tested in duplicate for 8 days, on three reagent lots. LoB corresponds to the 95th percentile of the blank sample distribution and is calculated non-parametrically. LoD corresponds to a measured quantity value for which the probability of falsely claiming the absence of the analyte is <β=5%, given a probability α=5% of falsely claiming its presence. Nine low-level samples were tested five times per day, for 8 days, on three reagent lots. A precision profile equation was generated and LoD was determined taking into account LoB. Limit of quantification (LoQ) – or functional sensitivity – corresponds to the lowest amount of 25(OH)D that can be quantitatively determined with stated accuracy of CV<20% and is calculated using the precision profile equation.

The serum and plasma equivalence comparison was carried out using whole blood from 60 volunteer study participants (who gave their informed consent under the supervision of the

Ethics Committee) collected into serum-collection plastic tubes (Becton-Dickinson ref. 369032) and plasma-collection lithium heparin plastic tubes (Becton-Dickinson ref. 368884). Samples were processed according to the tube manufacturer's recommendations. Matched serum and plasma samples were tested in singulate within the same assay run.

## Samples

Serum samples (n=150) for method comparison were selected based on their 25(OH)D concentration determined by LC-MS/MS. These were residual laboratory samples, blinded to patient identification and information. The sample cohort spans the measuring range of the assay methods (6–100 ng/mL) with approximately half of the specimens within the clinical decision range (15–40 ng/mL). No detectable levels of 25(OH)D<sub>2</sub> (>2 ng/mL) were found in the 150 samples of this cohort. To determine 25(OH)D<sub>2</sub> cross reactivity, another panel of 20 serum samples from vitamin D<sub>2</sub>-fortified volunteers was used exclusively. As determined by LC-MS/MS, 25(OH)D<sub>2</sub> levels ranged from 15.5 to 68.5 ng/mL (mean 45.0 ng/mL) and 25(OH)D<sub>3</sub> levels from 6.4 to 30.4 ng/mL (mean 15.0 ng/mL) (Supplemental Data, Table 1, that accompanies the article at <http://www.degruyter.com/view/j/cclm.2016.54.issue-1/cclm-2014-1249/cclm-2014-1249.xml?format=INT>). All samples included in the present study had 3-epi-25(OH)D<sub>3</sub> concentrations below the quantification threshold of both LC-MS/MS methods (<5 ng/mL). In order to study the influence of the DBP concentration over 25(OH)D quantification by the VIDAS® assay, serum samples from 25 pregnant women in their third trimester and 25 hemodialysis patients were determined for their DBP concentration using the Human Vitamin D BP Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA).

## Method comparison

The VIDAS® 25-OH Vitamin D Total assay was compared to other commercially available vitamin D assays according to the respective manufacturers' recommendations. Measurements with VIDAS®

**Table 1:** Precision performance of the VIDAS® 25-OH Vitamin D Total assay.

Sample	Mean, ng/mL	Repeatability		Reproducibility	
		Within-run precision		Total precision <sup>a</sup>	
		SD	%CV	SD	%CV
Pool 1	10.5	0.83	7.9	1.68	16
Pool 2	18.8	0.97	5.1	1.28	6.8
Pool 3	26	0.94	3.6	1.18	4.5
Pool 4	33.8	0.91	2.7	1.52	4.5
Pool 5	45.7	0.87	1.9	1.29	2.8
Pool 6	65.1	1.09	1.7	1.85	2.8
Pool 7	83.6	1.8	2.1	1.9	2.3
Pool 8	119.8	1.3	1.1	2.8	2.4

CV, coefficient of variation; SD, standard deviation. <sup>a</sup>Between runs, between days, between calibrations, between lots, between instruments.

were performed at bioMérieux facilities (reagent lot 141016-0), Abbott Architect® (reagent lot 02513F000) at Laboratoires Bouverier Hache Vock (Roanne, France), IDS-iSYS® (reagent lot 1669) at Biomedlab 21 (Dijon, France), Roche Elecsys® (reagent lot 173852-03) at Biomnis (Lyon, France) and DiaSorin Liaison XL (reagent lot 130099) at Université de Liège (Liège, Belgium). All methods are routinely used in the respective laboratories and under external QC assessment.

Two different LC-MS/MS assays (at CHU Liège and LMU Munich sites) were also used as reference methodology for 25(OH)D quantification. Both procedures are calibrated with NIST reference materials; their respective principles are summarized in the Supplemental Material.

## Statistics

The correlation of results between the various methods was analyzed using Passing-Bablok regression to determine proportional bias (slope) and constant bias (intercept). A Bland-Altman study of results was also carried out. The concordance correlation coefficient (CCC) was calculated. The CCC evaluates the degree to which pairs of observations fall on the 45° line through the origin. It includes a measurement of precision (Pearson's correlation coefficient "r") and accuracy (bias correction factor "Cb"). The Pearson's correlation coefficient measures how far each observation deviates from the best-fit line. The Cb measures how far the best-fit line deviates from the identity line. The CCC is calculated as:  $CCC=r \times Cb$ . It can be interpreted as: <0.90 poor; 0.90–0.94 moderate; 0.95–1.00 good.

## Results

### Analytical performance of the VIDAS® 25-OH Vitamin D Total assay

#### Precision

SD and CV were calculated for the VIDAS® 25-OH Vitamin D Total assay (Table 1). Repeatability represents the precision within-lot, -run and -instrument. Reproducibility includes all variability factors: precision between-runs, -days, -calibrations, -lots and -instruments.

SD for repeatability was in the 1 ng/mL range for all the different serum pools, yielding a CV from 7.9% at the lowest concentration of 25(OH)D tested (10.5 ng/mL) to 1.1% at 120 ng/mL. Reproducibility SD varied from 1.5 to 3.0 ng/mL, giving a CV range from 16.0% to 2.4%.

#### Linearity

A linearity study was performed using a high 25(OH)D concentration serum pool serially diluted in a low

concentration pool. Ten dilutions were prepared and measured in duplicate. Analysis by weighted linear regression between observed and expected concentrations shows sustained linearity over the 7.1–126.2 ng/mL tested range with recovery >90% of expected values.

### Limit of blank, limit of detection, and functional sensitivity

As per combined results on three reagent lots, the LoB of the VIDAS® 25-OH Vitamin D Total assay was determined as 6.2 ng/mL. Using the precision profile equation for low-level samples, LoD was calculated at 8.1 ng/mL. The deduced functional sensitivity (LoQ) (CV <20%) was 5.9 ng/mL. By definition, LoQ cannot be lower than LoD; therefore LoQ was set at 8.1 ng/mL.

### Cross-reactivity to vitamin D structural analogs

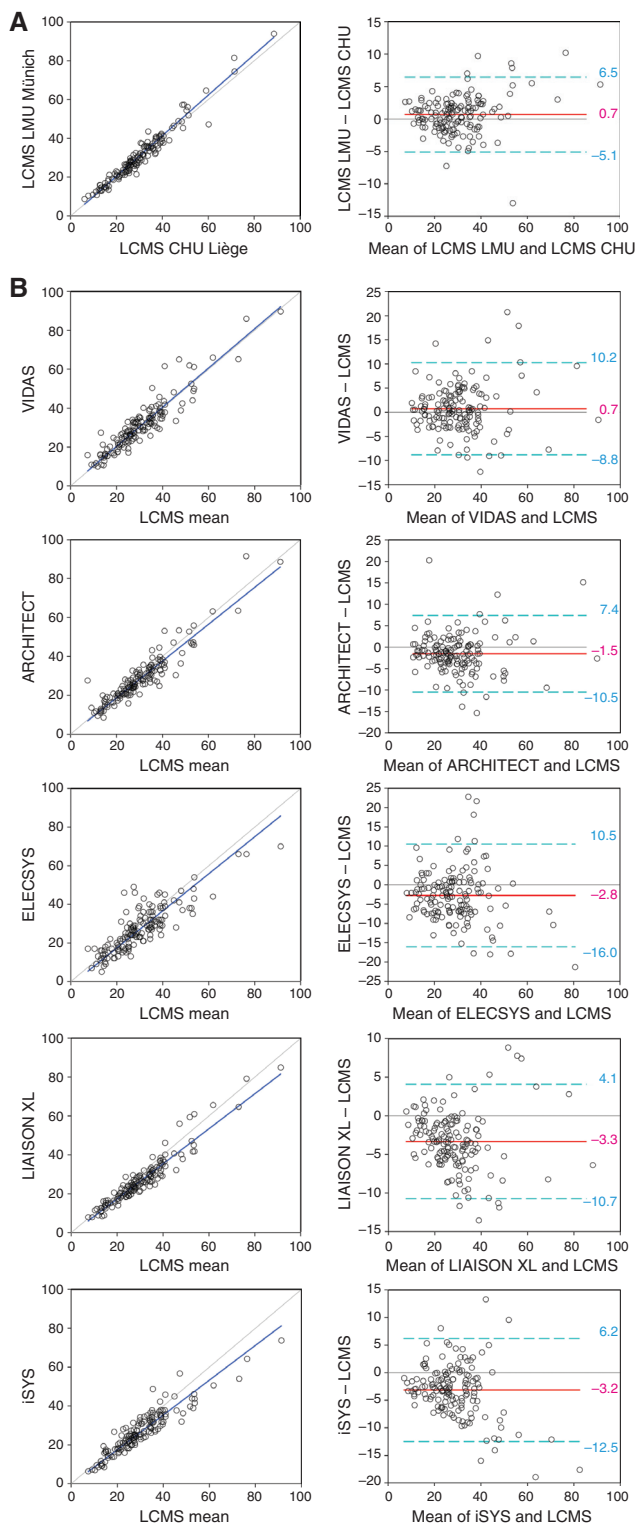
Assay specificity was evaluated by spiking samples with potential cross-reactant analytes and comparing the measurements with and without spiking. Mean cross reactivity to vitamin D<sub>3</sub> was 3.7%; to 1,25-(OH)<sub>2</sub>D<sub>3</sub> 69.0%; to 24,25-(OH)<sub>2</sub>D<sub>3</sub> 554.6% and to 3-epi-25(OH)D<sub>3</sub> 2.9%.

### Serum/plasma comparison

A comparison of serum and plasma samples measurements with the VIDAS® 25-OH Vitamin D Total assay was achieved using 60 matched-pairs. Passing-Bablok regression analysis shows excellent agreement between the two types of specimen at every level of analyte concentration (Supplemental Data, Figure 1).

### Method comparison to LC-MS/MS and immunoassays

A total of 150 serum samples were tested in singulate by five automated immunoassays and two LC-MS/MS methods. Passing-Bablok regression indicates close agreement between the two LC-MS/MS assays for the entire cohort (Figure 1A), with a proportional bias (slope) of 1.04 (95% CI 1.00; 1.09) and a constant bias (Y-intercept) of -0.4 ng/mL (95% CI -1.57; 0.71). The CCC is 0.97. Due to result agreements between both LC-MS/MS methods, the



**Figure 1:** Passing-Bablok and difference plots analysis of the two LC-MS/MS methods (A) and of 25(OH)D immunoassays compared with the consensus of LC-MS/MS methods (B). For difference plots analysis, red line is mean bias, dashed blue line are upper and lower 95% limits of agreement.

**Table 2:** Passing-Bablok regression statistics and concordance correlation analysis of immunoassays against the mean of the two LC-MS/MS methods.

Methods	Slope	95% CI	Intercept, ng/mL	95% CI, ng/mL	CCC	95% CI	r, precision	Cb, accuracy
bioMérieux Vidas	1.01	[0.94–1.08]	0.22	[–2.05 to 1.97]	0.93	[0.90–0.95]	0.93	1.0
Abbott Architect	0.94	[0.88–1.01]	–0.17	[–1.84 to 1.38]	0.93	[0.90–0.95]	0.94	0.99
DiaSorin Liaison	0.90	[0.85–0.95]	–0.63	[–2.04 to 0.69]	0.92	[0.90–0.94]	0.95	0.97
Roche Elecsys	0.96	[0.87–1.05]	–1.75	[–4.19 to 0.60]	0.82	[0.77–0.87]	0.85	0.97
IDS iSYS	0.90	[0.84–0.97]	–0.42	[–2.20 to 1.10]	0.89	[0.85–0.91]	0.93	0.96

mean value from the two assays was used, as for all subsequent method comparisons with immunoassays.

The correlation statistics of commercial vitamin D immunoassays with mean LC-MS/MS results are summarized in Table 2 and Figure 1B. In Passing-Bablok regression analysis, whereas the VIDAS®, Architect and Elecsys assays do not show proportional biases significantly different from 1 (as per the 95% IC values) with LC-MS/MS, Liaison and iSYS display a statistically significant moderate negative slope. Constant bias (X-Intercept) for all immunoassays does not differ significantly from 0. The Bland-Altman plots show good overall agreement with LC-MS/MS for all the immunoassays, with a constant bias ranging from +2.4% (VIDAS®) to –12.5% (Liaison). While the CCC between LC-MS/MS and VIDAS®, Architect and Liaison reflects good overall correlation to the reference method (>0.90 with high accuracy [Cb of 0.97–1.00]), iSYS has a value slightly under the 0.90 cut-off value and Elecsys has a poor score (CCC=0.82).

### Influence of DBP on VIDAS® 25-OH Vitamin D Total assay accuracy

Variations in serum DBP concentration may disturb 25(OH)D quantitation by immunoassays, especially in populations with altered DBP levels, due to physiological or pathological conditions [16]. The ability of the VIDAS® 25-OH Vitamin D Total assay to measure 25(OH)D in the presence of various endogenous DBP levels was compared with LC-MS/MS, which is not influenced by DBP variations. The study included serum samples from pregnant women in their third trimester (for high DBP concentration) and hemodialysis patients (for low levels of DBP). Passing-Bablok analysis shows no significant bias between VIDAS® and LC-MS/MS for pregnancy samples (Figure 2A), with an agreement between methods similar to that obtained previously (Table 2). For dialysis samples, VIDAS® results differ statistically from LC-MS/MS results with a slope of 0.51 (95% CI 0.43; 0.61) and a constant

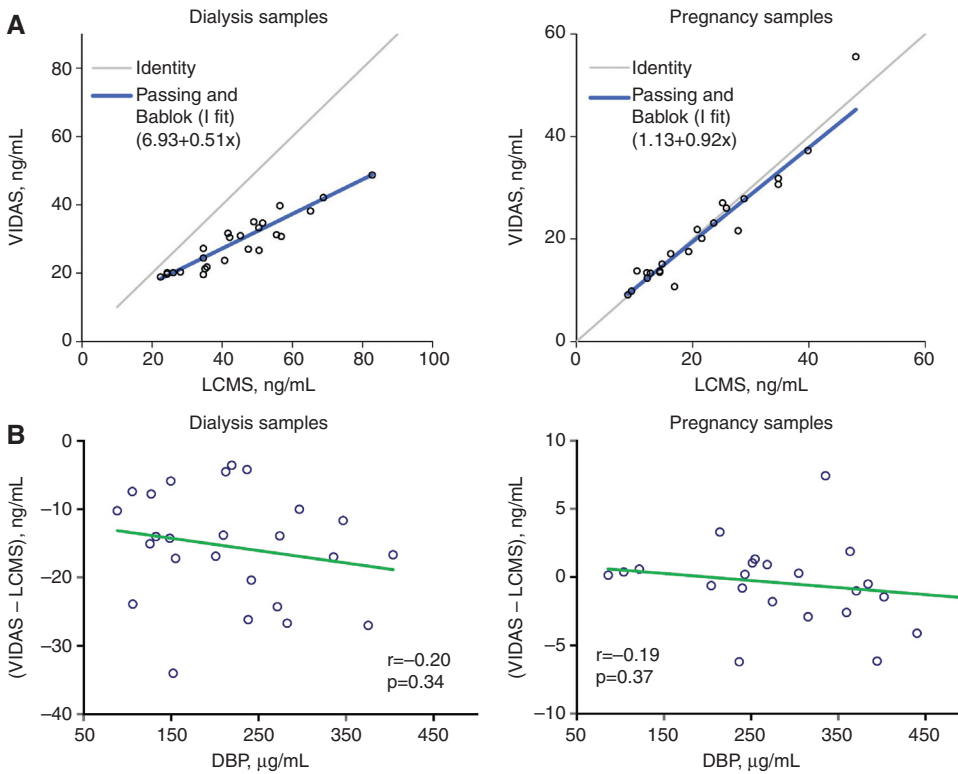
bias of 6.93 (95% CI 3.07; 9.38), clearly indicating that the VIDAS® assay under-recovers 25(OH)D as compared to LC-MS/MS in this population (Figure 2A). However, no correlation could be established between methods bias and DBP levels ( $r=-0.2$ ,  $p>0.1$ , not significant) when the (VIDAS – LC-MS/MS) difference was plotted against DBP concentration (Figure 2B).

### Cross reactivity to 25(OH)D<sub>2</sub> using natural 25(OH)D<sub>2</sub> samples

Serum samples from individuals supplemented with vitamin D<sub>2</sub> were used to calculate 25(OH)D<sub>2</sub> recovery, according to a previously published method [17]. Briefly, the regression equation comparing each immunoassay with LC-MS/MS samples was calculated for the D<sub>3</sub> serum cohort containing exclusively 25(OH)D<sub>3</sub> (n=150). The immunoassay 25(OH)D<sub>3</sub> concentration was estimated by applying the D<sub>3</sub> cohort regression equation to each vitamin D<sub>2</sub> supplemented sample (n=20). The 25(OH)D<sub>2</sub> levels were calculated by subtracting the 25(OH)D<sub>3</sub> estimated concentrations from the total 25(OH)D values. The 25(OH)D<sub>2</sub> cross-reactivity factor was obtained by dividing the immunoassay 25(OH)D<sub>2</sub> calculated concentration by the respective LC-MS/MS 25(OH)D<sub>2</sub> value. Results are presented in Table 3. The VIDAS®, Architect and iSYS assays showed moderate under-recovery of 25(OH)D<sub>2</sub> at 82±20%, 76±24% and 86±31%, respectively [% mean±SD]. The Liaison assay slightly over-recovered 25(OH)D<sub>2</sub> with a factor of 110±23%. The cross-reactivity to 25(OH)D<sub>2</sub> recovery by the Elecsys assay was as low as mean value of 46±20%. Only Liaison and iSYS assays show confidence intervals that include 100%.

## Discussion

In response to the increased demand for vitamin D testing in the past decade, a wide array of commercial methods



**Figure 2:** Passing-Bablok analysis of VIDAS vs. LC-MS/MS results in samples with altered DBP states (A) and difference plot analysis of (VIDAS – LC-MS/MS) as function of DBP concentration (B).

For difference plot analysis, linear regression is in green, with Pearson’s coefficient “r” and p-value “p”.

has been made available to clinical laboratories and hospitals. Vitamin D testing has now become a part of routine clinical practice due to fully automated, high throughput solutions. However, despite the extensive offer of vitamin D tests on the market, the technical challenge of measuring such a difficult analyte, combined with the lack of an International Standard, has led to discrepancies between the analytical performances of the various methods, and moreover, a lack of correlation to the reference LC-MS/MS methods [18].

In this context, the present study aimed to evaluate the analytical and clinical characteristics of the new VIDAS® 25-OH Vitamin D Total assay for routine determination of

vitamin D status compared to the performance of other immunoassays and LC-MS/MS.

LC-MS/MS is currently considered the gold standard for 25(OH)D quantification, though in the past 10 years substantial disagreements have been documented between LC-MS/MS methods. DEQAS reports have moreover clearly shown inter-laboratory CVs for LC-MS/MS methods that are comparable to immunoassays’ variability [19–21]. These discrepancies can be explained by the fact that LC-MS/MS is not a single “off the shelf” technique. Pre-analytical sample preparation (solvent extraction, chromatography for analyte separation), laboratory personnel skills, accurate result analysis, including interpretation of 3-epimer 25(OH)D concentration to the final measurement, can account for the lack of harmonized results. Nevertheless, the availability of NIST reference materials and the vitamin D Standardization Program have helped bridge the gap in the standardization of LC-MS/MS methods. In the present study, two different LC-MS/MS methods were used at different locations, using distinct sample preparation and chromatography processes, as well as different mass spectrometry instruments. Both methods were standardized to NIST reference material and one site (CHU Liège, Belgium) is

**Table 3:** Mean cross-reactivity to 25(OH)D<sub>2</sub> of immunoassays assessed with natural serum samples.

Methods	Mean 25(OH)D <sub>2</sub> cross-reactivity	95% CI
bioMérieux Vidas	82%	(73%–91%)
Abbott Architect	76%	(65%–88%)
DiaSorin Liaison	110%	(99%–121%)
Roche Elecsys	46%	(37%–56%)
IDS iSYS	86%	(71%–100%)

also part of the vitamin D Standardization Program. Both assays showed excellent agreement across the range of 7.4–91.3 ng/mL (mean value of LC-MS/MS methods), and therefore the mean value of the two methods was used for comparison with immunoassays. This accounts for the efficiency of the standardization efforts that have been made in recent years to homogenize results among LC-MS/MS users.

In contrast, immunoassays exhibit variable performance and disparate results as compared to LC-MS/MS measurements. Elecsys has a low concordance with LC-MS/MS, with a statistically significant  $<0.9$  (as per 95% CI) CCC. This low value is the consequence of a poor precision ( $r=0.85$ ) as illustrated by the large scattering of individual results, while the mean bias remains acceptable. All four other immunoassays (VIDAS®, Architect, Liaison, iSYS) showed an acceptable agreement with consensus LC-MS/MS values (CCC  $>0.90$  as per 95% CI), which reflects rather limited bias and acceptable precision. These data partially differ from other results previously published that have shown substantial biases between some of these immunoassays and LC-MS/MS. The specimen sampling may explain these differences, as no significant C3-epi-25(OH)D was detected in any sample tested in the present study, given that some assays are more sensitive to this metabolite than others. It is also worth indicating that some commercial assays have recently been re-standardized with reference materials, thereby bringing better accuracy with respect to LC-MS/MS methods.

One key element to ensure that an assay is fit for clinical use is to maintain consistency of measurement along its measuring range, which is bound at the low end by LoQ while the upper-limit is set by linear range determination. For the VIDAS® 25-OH Vitamin D Total assay, the measuring range was established as 8.1–126.0 ng/mL. This interval encompasses commonly used clinical thresholds for vitamin D testing:  $<20$  ng/mL (deficiency),  $<30$  ng/mL (insufficiency) and goes up to  $>100$  ng/mL. It is recommended to use biological variations as a guide to assess the analytical performance of each assay [22, 23]. For vitamin D, within-subject variation has been shown to reach 12% [24]. Stockl et al. [25] proposed an acceptable CV of  $<10\%$  for routine measurement purposes, emphasizing that the precision bias should be set at half this limit to routinely achieve the required quality. Therefore, using a target value of CV=5%, the VIDAS® 25-OH Vitamin D Total assay meets this requirement for within-run precision (repeatability) at thresholds of 20 ng/mL, 30 ng/mL and 100 ng/mL (Table 1). Total precision (which includes

variability between runs, calibrations, reagent lots and instruments) also falls under this cut-off, except for the 20 ng/mL level which is slightly over 6% precision. Taken together, these data demonstrate consistency in 25(OH)D measurement, which is analytically acceptable for samples spanning the entire assay range.

Another essential feature of a vitamin D assay is the detection of 25(OH)D<sub>2</sub>. As supplementation of patients with vitamin D<sub>2</sub> is common in some parts of the world, the ability of an assay to accurately quantify 25(OH)D<sub>2</sub> together with 25(OH)D<sub>3</sub> is critical in the context of routine practice with limited information available on each patient's potential supplementation regimen. However, estimating immunoassays' cross-reactivity to 25(OH)D<sub>2</sub> is a complex task, since only chromatographic methods are able to quantify both 25(OH)D forms separately. The use of 25(OH)D<sub>2</sub>-spiked samples is irrelevant because recovery of exogenous 25(OH)D is inconsistent among immunoassays [26–28]. Therefore, natural samples that contain endogenous 25(OH)D<sub>2</sub> should be studied. Cross-reactivity for 25(OH)D<sub>2</sub> was found to range from 46% (Roche) to 110% (Liaison). VIDAS®, Architect, iSYS and Liaison either slightly under- or over-recovered endogenous 25(OH)D<sub>2</sub>. These data differ from the results of previously published studies [17, 29] in which most immunoassays centered around 100% of cross-reactivity to 25(OH)D<sub>2</sub>. Shu et al. [30] observed that the relative amount of 25(OH)D<sub>2</sub> in the tested samples can affect the results for cross-reactivity determination. Accordingly, in this study, we used serum samples with total 25(OH)D concentrations from 28 to 85 ng/mL and D<sub>2</sub>/D<sub>3</sub> ratio of (0.7; 7). This broad range of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> relative amounts and the diverse D<sub>2</sub>/D<sub>3</sub> ratio may explain observed discrepancies.

Previous studies have shown that the capacity of immunoassays to detect 25(OH)D could be hampered in samples from clinically defined populations, such as pregnant women, hemodialysis or intensive care patients, with a link between altered DBP levels and immunoassay poor performance [31]. In our study, however, we did not find a relationship between DBP concentration and deviations of 25(OH)D measured with VIDAS® from LC-MS/MS results. VIDAS® performance for routine samples was not different from that for pregnancy samples, but VIDAS® consistently produced lower results for hemodialysis patients, with a Bland-Altman mean bias of 40% (data not shown). As this bias is not correlated to the DBP level, one explanation could be that dialysis samples suffer from matrix effects due to retained metabolites or elevated uremia that may interfere with 25(OH)D release from DBP and/or with immunoassay components, as previously postulated [16, 32].

In conclusion, the VIDAS® 25-OH Vitamin D Total assay showed good analytical performance in terms of precision and reproducibility both at low and high 25(OH) D concentrations. Results obtained with this new assay show excellent concordance to the reference LC-MS/MS method. Cross-reactivity to 25(OH)<sub>2</sub>D<sub>2</sub> is good and adapted to the use of the VIDAS® 25-OH Vitamin D Total assay in clinical routine.

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## References

- Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266–81.
- Lips P, van Schoor NM. The effect of vitamin D on bone and osteoporosis. *Best Pract Res Clin Endocrinol Metab* 2011;25:585–91.
- Prentice A, Schoenmakers I, Laskey MA, de Bono S, Ginty F, Goldberg GR. Nutrition and bone growth and development. *Proc Nutr Soc* 2006;65:348–60.
- Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med* 2007;167:1730–7.
- Hyponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 2001;358:1500–3.
- Souberbielle JC, Body JJ, Lappe JM, Plebani M, Shoenfeld Y, Wang TJ, et al. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: recommendations for clinical practice. *Autoimmun Rev* 2010;9:709–15.
- Pludowski P, Holick MF, Pilz S, Wagner CL, Hollis BW, Grant WB, et al. Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality – a review of recent evidence. *Autoimmun Rev* 2013;12:976–89.
- Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. *Chem Biol* 2014;21:319–29.
- Phinney KW, Bedner M, Tai SS, Vamathevan VV, Sander LC, Sharpless KE, et al. Development and certification of a standard reference material for vitamin D metabolites in human serum. *Anal Chem* 2012;84:956–62.
- Sempos CT, Vesper HW, Phinney KW, Thienpont LM, Coates PM. Vitamin D status as an international issue: national surveys and the problem of standardization. *Scand J Clin Lab Invest Suppl* 2012;243:32–40.
- Farrell CJ, Soldo J, McWhinney B, Bandodkar S, Herrmann M. Impact of assay design on test performance: lessons learned from 25-hydroxyvitamin D. *Clin Chem Lab Med* 2014;52:1579–87.
- NCCLS. Interference testing in clinical chemistry, approved guideline EP07-A2, ISBN 1-56238-584-4.
- NCCLS. Evaluation of precision performance of quantitative measurement methods, approved guideline, EP05-A2, ISBN 1-56238-542-9.
- NCCLS. Evaluation of the linearity of quantitative measurement procedures: a statistical approach, approved guideline EP06-A, ISBN 1-56238-498-8.
- NCCLS. Protocols for determination of limits of detection and limits of quantitation, approved guideline EP17-A, ISBN 1-56238-551-8.
- Heijboer AC, Blankenstein MA, Kema IP, Buijs MM. Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. *Clin Chem* 2012;58:543–8.
- Cavalier E, Rousselle O, Ferrante N, Carlisi A, Le Goff C, Souberbielle JC. Technical and clinical evaluation of the VITROS(R) immunodiagnostic products 25-OH vitamin D total assay – comparison with marketed automated immunoassays and a liquid chromatography-tandem mass spectrometry method. *Clin Chem Lab Med* 2013;51:1983–9.
- Herrmann M. The measurement of 25-hydroxy vitamin D – an analytical challenge. *Clin Chem Lab Med* 2012;50:1873–5.
- Binkley N, Sempos CT. Standardizing vitamin D assays: the way forward. *J Bone Miner Res* 2014;29:1709–14.
- Grebe SK, Singh RJ. LC-MS/MS in the clinical laboratory – where to from here? *Clin Biochem Rev* 2011;32:5–31.
- Singh RJ. Are clinical laboratories prepared for accurate testing of 25-hydroxy vitamin D? *Clin Chem* 2008;54:221–3.
- Fraser CG, Petersen PH. Analytical performance characteristics should be judged against objective quality specifications. *Clin Chem* 1999;45:321–3.
- Fuentes-Arderiu X, Miro-Balague J. State of the art instead of biological variation to set requirements for imprecision. *Clin Chem* 2000;46:1715–7.
- Viljoen A, Singh DK, Farrington K, Twomey PJ. Analytical quality goals for 25-vitamin D based on biological variation. *J Clin Lab Anal* 2011;25:130–3.
- Stockl D, Sluss PM, Thienpont LM. Specifications for true-ness and precision of a reference measurement system for serum/plasma 25-hydroxyvitamin D analysis. *Clin Chim Acta* 2009;408:8–13.
- Carter GD, Jones JC, Berry JL. The anomalous behaviour of exogenous 25-hydroxyvitamin D in competitive binding assays. *J Steroid Biochem Mol Biol* 2007;103:480–2.
- Horst RL. Exogenous versus endogenous recovery of 25-hydroxyvitamins D<sub>2</sub> and D<sub>3</sub> in human samples using high-performance liquid chromatography and the DiaSorin LIAISON Total-D Assay. *J Steroid Biochem Mol Biol* 2010;121:180–2.



28. Le Goff C, Peeters S, Crine Y, Lukas P, Souberbielle JC, Cavalier E. Evaluation of the cross-reactivity of 25-hydroxyvitamin D2 on seven commercial immunoassays on native samples. *Clin Chem Lab Med* 2012;50:2031–2.
29. Cavalier E, Wallace AM, Carlisi A, Chapelle JP, Delanaye P, Souberbielle JC. Cross-reactivity of 25-hydroxy vitamin D2 from different commercial immunoassays for 25-hydroxy vitamin D: an evaluation without spiked samples. *Clin Chem Lab Med* 2011;49:555–8.
30. Shu I, Pina-Oviedo S, Quiroga-Garza G, Meng QH, Wang P. Influence of vitamin D2 percentage on accuracy of 4 commercial total 25-hydroxyvitamin D assays. *Clin Chem* 2013;59:1273–5.
31. Cavalier E, Lukas P, Crine Y, Peeters S, Carlisi A, Le Goff C, et al. Evaluation of automated immunoassays for 25(OH)-vitamin D determination in different critical populations before and after standardization of the assays. *Clin Chim Acta* 2014;431:60–5.
32. Depreter B, Heijboer AC, Langlois MR. Accuracy of three automated 25-hydroxyvitamin D assays in hemodialysis patients. *Clin Chim Acta* 2013;415:255–60.

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