Etienne Cavalier*, Olivier Rousselle, Nunzio Ferrante, Agnes Carlisi, Caroline Le Goff and Jean-Claude Souberbielle

Technical and clinical evaluation of the VITROS® Immunodiagnostic Products 25-OH Vitamin D Total Assay – comparison with marketed automated immunoassays and a liquid chromatography-tandem mass spectrometry method

Abstract

Background: The study was conducted to evaluate the technical and clinical performance of the VITROS® Immunodiagnostic Products 25-OH Vitamin D Total Assay, and compare it with the performance of five marketed automated assays and a liquid chromatography/mass spectrometry reference method (LC-MS/MS).

Methods: Three hundred patient serum samples were used to compare the correlation of the VITROS® 25-OH Vitamin D Total Assay with both the other immunoassays and the LC-MS/MS method, using Passing-Bablok regression and Bland-Altman analyses. Concordance of the diagnosis of vitamin D status was calculated to test the agreement between the different assays. In addition, samples containing vitamin D2 were used to test the assay's ability to detect the D2 form of the vitamin.

Results and conclusions: These results from the VITROS® 25-OH Vitamin D Total Assay generally correlated well with those from most of the marketed immunoassays. Cross-reactivity of the D2 form was calculated as being close to 100%. Additionally, we found substantial variability in performance amongst the various assays, which suggests the need for optimisation and recalibration of commercial methods.

Keywords: assay performance; bias; concordance; precision; vitamin D.

*Corresponding author: Dr. Etienne Cavalier, Department of Clinical Chemistry, University of Liège, CHU Sart-Tilman, Domaine du Sart-Tilman, Liège, Belgium, E-mail: etienne.cavalier@chu.ulg.ac.be
Olivier Rousselle, Nunzio Ferrante, Agnes Carlisi and Caroline Le Goff: Department of Clinical Chemistry, University of Liège, CHU Sart-Tilman, Domaine du Sart-Tilman, Liège, Belgium
Jean-Claude Souberbielle: Laboratoire D'Exploration Fonctionelles, Hôpital Necker-Enfants Malades, Paris, France

Introduction

Vitamin D is classically known for its pivotal role in calcium and bone metabolism and is critical for bone and muscle health [1, 2]. However, more recent evidence has greatly enlarged its importance in more widespread situations, especially in extraskeletal diseases such as cardiovascular disease, autoimmunity and cancer [3–6]. In the body, vitamin D is converted, first in the liver to 25-hydroxy-vitamin D (25-OH D) and then to 1,25-dihydroxy-vitamin D (1,25-OH D) in the kidneys [7]. Though the latter derivative is the biologically active form, its relatively short lifetime and fluctuation in concentration has supported the intermediate product (25-OH D) as the best indicator of vitamin D status [8].

Vitamin D is present in very few foods, and its main source derives from the action of sunlight on 7-dehydrocholesterol in the epidermis, to produce the form vitamin D3. However, a related form, vitamin D2 is also ingested from plant sources as well as supplements, and both prohormones are equivalently important in defining overall vitamin status [9]. It is therefore important that any method of measurement of 25-OH D can detect both forms with equal accuracy [10].

As a result of the acceptance of an increasingly comprehensive role for vitamin D in many disease states, the demand for 25-OH D testing in the clinical laboratory has risen many fold over the last decade. This tendency accordingly has forced the transfer of methodologies from the academic, laboratory-specific level to rapid through-put platforms developed by commercial companies.

However, this evolution has led to difficulties that have appeared in some of these automated assays. Problems with bias, inconsistency of results, and mutual disagreement between methodologies have arisen [10, 11], which have been clearly demonstrated using comparisons

with results of liquid chromatography/mass spectrometry (LC-MS/MS).

Recently, Ortho Clinical Diagnostics (OCD) launched an assay for 25-OH Vitamin D on the VITROS® 5600 Integrated System and VITROS® 3600 and ECi/ECiQ Immunodiagnostic Systems on the European markets. The aim of this study was to: 1) evaluate the correlation of the VITROS® 25-OH Vitamin D Total Assay with five commercially available immunoassays and an LC-MS/MS method; 2) evaluate the detection of vitamin D2; and 3) assess assay precision.

Materials and methods

Assays

The study concentrated on the performance of the VITROS® 25-OH Vitamin D Total Assay. This method is a competitive, enhanced chemiluminescence immunoassay that uses low pH to release the 25-OH vitamin D in the sample from the binding protein, and uses horseradish peroxidase (HRP) labelled 25-OH vitamin D to compete with the 25-OH vitamin D in the sample for the binding of the monoclonal anti-vitamin D bound to the wells.

Other commercially available assays

1) Abbott Architect® 25-OH Vitamin D Assay: a delayed one-step chemiluminescence method using as capture agent a polyclonal anti-vitamin D antibody and a biotinylated vitamin D anti-biotin acridinium labelled complex.
2) Roche Elecsys® Vitamin D Total Assay: a competitive electro-chemiluminescence method using a ruthenium labelled vitamin D binding protein as capture agent.
3) Siemens ADVIA Centaur® Vitamin D Total Assay: a competitive chemiluminescence method using a monoclonal acridinium labelled anti-25(OH) vitamin D antibody and a vitamin D analogue labelled with fluoroscein.
4) DiaSorin 25-OH Vitamin D TOTAL Assay: a competitive chemiluminescence method using an anti-25(OH)-vitamin D antibody where vitamin D and an isoluminol-labelled derivative compete for binding run on the Liaison XL platform.
5) IDS-iSYS® 25-Hydroxy Vitamin D Assay: a competitive chemiluminescence method using an anti-25(OH) vitamin D antibody conjugated with acridinium, with 25-(OH) vitamin D conjugated to magnetic particles.
6) The liquid chromatography and tandem mass spectrometry (LC-MS/MS) method was carried out using the Perkin Elmer methodology as described earlier [11]. Briefly, analyses were performed using a triple quadrupole TQ5500 from ABSciex (Framingham, MA, USA) with Atmospheric Pressure Chemical Ionisation (APCI) source. Calibration was performed using six calibrator points [charcoal stripped human serum enriched with increasing levels of $^2H_6$-25(OH)D_2 and $^2H_6$-25(OH)D_3]. Three control levels [lyophilised serum added with increasing amount of $^2H_6$-25(OH)D_2 and $^2H_6$-25(OH)D_3] were used as quality controls. For each analyte specific isotopically labelled internal standards [$^2H_3$-25(OH)D_2 for VitD$_2$ and $^2H_3$-25(OH)D_3 for VitD$_3$] were used. To 100 μL of serum sample, calibrator or control, 200 μL of daily precipitation solution (DPS, prepared by diluting reconstituted Internal Standard 1/100 with acetonitrile containing 0.1% of formic acid) was added. The samples were mixed for 15 min and then centrifuged at 16,000 g for 15 min at 4°C. Finally, 150 μL of supernatant was transferred into a 96-well plates. The plate was loaded in the autosampler, and 50 μL were injected into the HPLC-MS/MS system. Limit of quantification (LOQ) was 2.43 ng/mL for 25-OHD$_2$ and 3.40 ng/mL for 25-OHD$_3$. The intra-assay CV was <7.2% and <10%, and inter-assay CV was <7.9% and <8.9% for three concentrations between 7.5 and 78.1 ng/mL for 25-OHD$_2$ and 25-OHD$_3$, respectively. The linearity range of 25-OHD$_2$ and 25-OHD$_3$ was 2.43 to 329 and 3.40 to 314 ng/mL, respectively. In addition, we have controlled the calibration of the method with the NIST standards and participate to DEQAS, and the results were always on the target for LC-MS/MS users.

Samples

Serum samples from 300 patients were used to determine cross-correlation and concordance between methods. These were residual laboratory samples, blinded to patient identification and information. The samples contained vitamin D concentrations, as measured by LC-MS/MS, ranging from 4.6 to 105 ng/mL. All samples were aliquoted and frozen at –20°C until required for testing. One aliquot of each sample was tested in each assay. The ability of assays to detect vitamin D2 was tested using samples obtained in patients on D2 therapy.

Statistics

The correlation of the VITROS® 25-OH Vitamin D Total Assay results with those of the other assays was analysed using Passing-Bablok regression. The correlation coefficient was calculated using the Pearson's correlation method. The slope and intercept are referred to in the text as proportional and constant bias, respectively. Assay biases were also calculated using the Bland-Altman plot. The same methods were used to determine agreement of all the commercial assays against LC-MS/MS.

Agreement of vitamin D status between methods was calculated using concordance tables. Briefly, this involved classification of patients according to their vitamin D concentrations: <20 ng/mL, 20–30 ng/mL, 30–80 ng/mL and >80 ng/mL, and then determining the percentage of patients classified correctly by any two methods.

In order to determine whether there were any methodological differences in the detection of D2, a panel of 11 samples with D2 and D3 concentrations as measured by LC-MS/MS. D2 concentrations ranged from 21 to 48.1 ng/mL (mean±SD: 36±8 ng/mL) and D3 concentrations ranged from 5 to 30 ng/mL (mean±SD: 15±8 ng/mL). The samples were tested in both, the LC-MS/MS (D3 and D2) and the VITROS, Liaison, ISYS, Architect and Elecsys assays. The percentage of 25(OH)D2 recovery was evaluated with a slightly modified protocol, as previously published in this journal [11, 12].
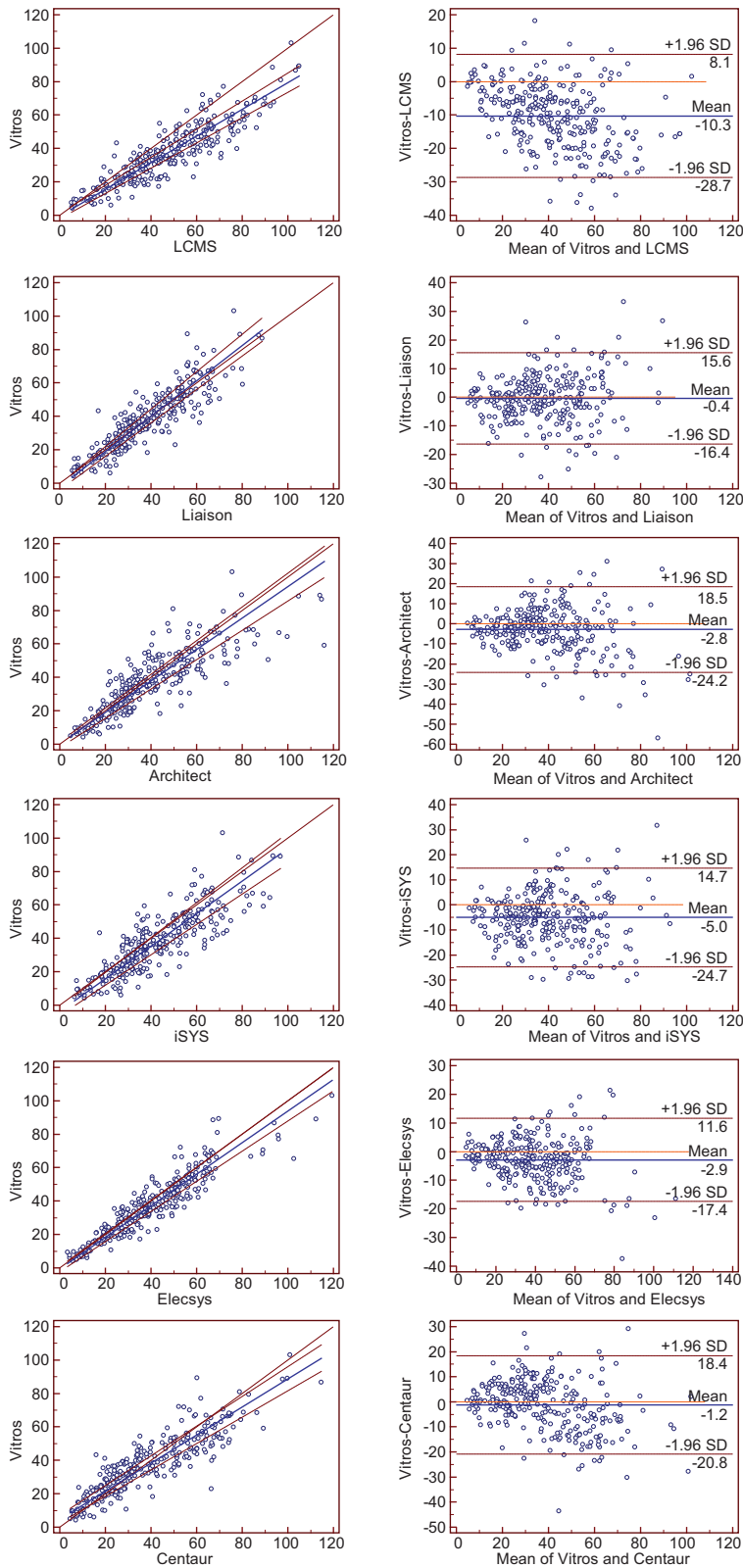
Figure 1 Correlation and bias of vitamin D assay results from the VITROS assay against the five other commercial assays and the LC-MS method.
Left column: correlation diagrams, right column, bias of results against VITROS for each assay over a span of vitamin D concentrations.
y-Axis (left column): VITROS vitamin D values ng/mL; x-axis (left column) vitamin D concentrations measured by each commercial assay.
y-axis (right column): Bias of commercial assays against VITROS results; x-axis: mean vitamin D estimate from LC-MS/MS and assay determination for each sample.

We evaluated the precision of the VITROS® 25-OH Vitamin D Total Assay in accordance with a modified protocol based on the evaluation of precision performance CLSI EP-5A2. Six sample pools (vitamin D range of 6.57 to 102 ng/mL) were tested in triplicate for 5 days, within a single calibration period.

Results

Correlation of VITROS® 25-OH Vitamin D Total Assay results with those from marketed assays and an LC-MS/MS method

Comparison of the VITROS® 25-OH Vitamin D Total Assay results on the 300 patient serum samples with all the other methods using Passing-Bablok regression and Bland-Altman plots is shown in Figure 1. Table 1 displays the correlation statistics of the VITROS® 25-OH Vitamin D Total Assay results (y-axis) against the results from the other commercial vitamin D assays and the LC-MS/MS method. The results from the VITROS® 25-OH Vitamin D Total Assay correlated well with those from the other assays, with slopes ranging from 0.80 to 1.06, intercept −3.5 to 4.7 ng/mL, and correlation value from 0.85 to 0.92.

When the VITROS® 25-OH Vitamin D Total Assay results were compared with those from the LC-MS/MS method, a negative bias was revealed. To quantify such biases, the LC-MS/MS results were also compared with those of the other assays.

In Figure 2, the correlations between LC-MS/MS results and those of Vitros® and the other commercial assays are depicted using Passing-Bablok regression and Bland-Altman plots.

Table 2 shows the correlation statistics of the LC-MS/MS results (x-axis) against the results obtained by all the commercial Vitamin D assays. Almost all the assays were negatively biased when compared to the LC-MS/MS method, both with the proportional slopes (varying from

0.76 to 0.93) and with constant biases (intercepts varying from −6.0 to 3.9). The correlation coefficients ranged from 0.84 (Liaison) to 0.92 (Centaur).

These results clearly demonstrated the variability in test results from different assays. To evaluate the potential impact on this discordance on the accurate determination of a patient's vitamin D status, the agreement of assignment of vitamin D status by different assays was calculated. Table 3 shows the percentage concordance between methods in the patient samples tested. The concordance between methods was variable, ranging from 65% (concordance between LC-MS/MS and Centaur) to 83% (Liaison vs. Architect). Concordance against LC-MS/MS varied from a low of 65% (Centaur) to a high of 82% (ISYS).

Vitamin D2 detection

Recovery of 25(OH)D2 by the Liaison, ISYS, VITROS and Roche assays was not significantly different from 100%. The median (95% CI) percentage recovery for each method was 102 (87–113)%, 104 (84–119)%, 106 (83–132)% and 78 (68–110)%, respectively. Even though the median of the percentage 25(OH)D2 recovery obtained with the Architect was similar to the one observed with Elecsys (78%), the 95% confidence interval was 64% to 81%, which did not encompass 100%.

### VITROS® 25-OH Vitamin D Total Assay precision

The precision of the assay over 5 days with six serum pools is shown in Table 4. This varied from 4.1% for the 102 ng/mL pool to 21.7% for the 6.57 ng/mL pool.

Discussion

The present study was designed to evaluate the technical and clinical performance of the VITROS® Immunodiagnostic Products 25-OH Vitamin D Total Assay. Our results demonstrated that this new assay generally correlated well with the five other marketed vitamin D immunoassays and the LC-MS/MS method. We also examined its cross reactivity with the D2 form of the vitamin and found a satisfactory performance in this regard. The precision profile of the VITROS® 25-OH Vitamin D Total Assay was comparable with those of the other commercial assays over the assay's measuring range (8–150 ng/mL).

Table 1 Correlation statistics of the VITROS 25-OH Vitamin D Total Assay results (y-axis) against the results obtained in the Perkin Elmer LC-MS/MS and the other commercial assays (x-axis).

	Slope (confidence limits)	Intercept (confidence limits)	r-Value
LC-MS/MS	0.80 (0.76–0.84)	−0.5 (−2.2–1.2)	0.90
Architect	0.94 (0.88–1.01)	0.1 (−2.1–1.6)	0.85
Centaur	0.84 (0.79–0.89)	4.7 (2.7–7.1)	0.88
Liaison	1.06 (1.00–1.12)	−2.2 (−0.3.9−−0.3)	0.89
Elecsys	0.94 (0.90–0.99)	−0.7 (−2.1–0.9)	0.92
iSYS	0.97 (0.91–1.04)	−3.5 (−6.2−−1.1)	0.85
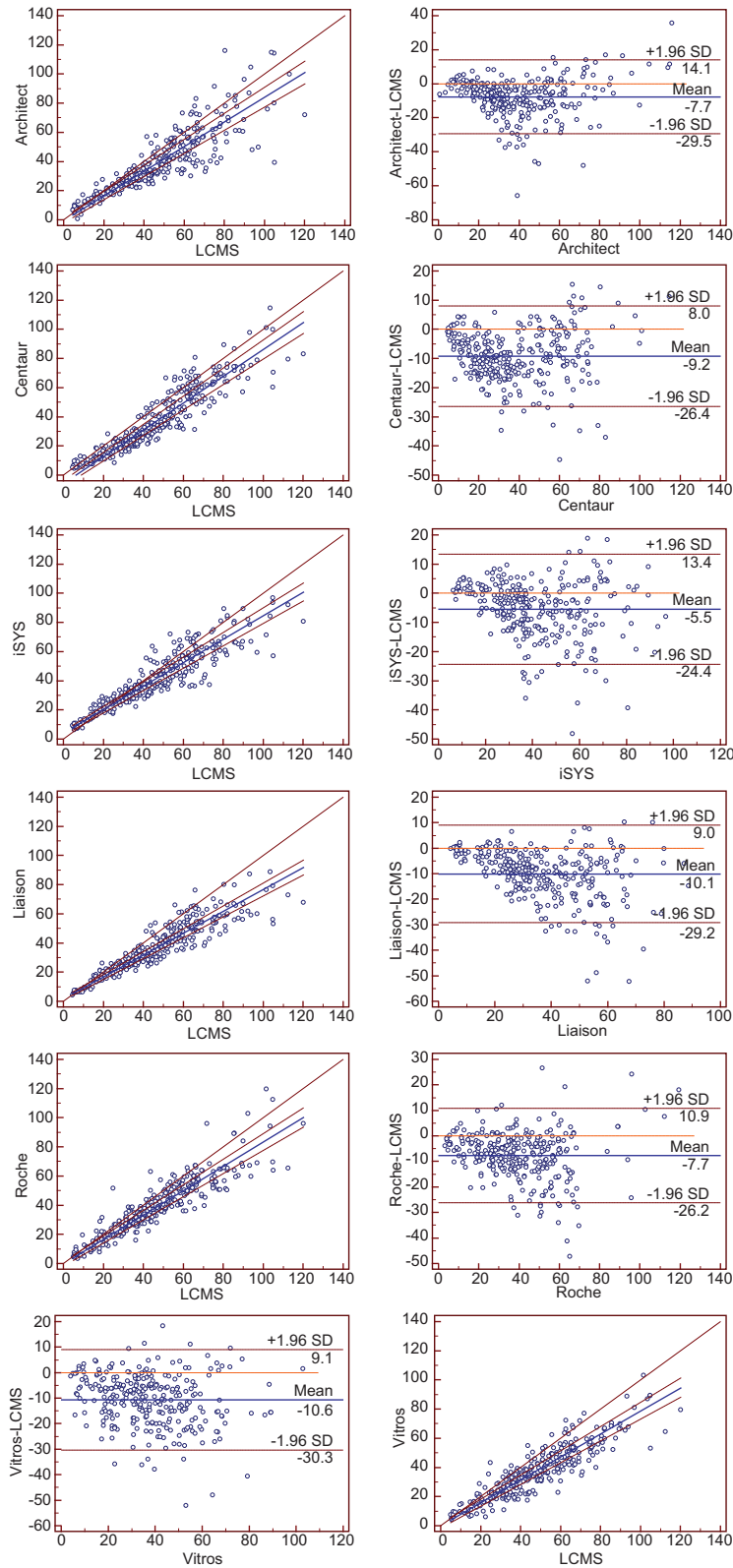
Figure 2   Correlation and bias of vitamin D assay results from LC-MS/MS against the VITROS assay and five other commercial assays.
Left column: correlation diagrams, right column, bias of results against LC-MS/MS for each assay over a span of vitamin D concentrations.
y-Axis (left column): LC-MS/MS vitamin D. Values ng/mL; x-axis (left column) vitamin D concentrations measured by each commercial assay.
y-Axis (right column): bias of commercial assays against LC-MS/MS; x-axis: mean vitamin D estimate from LC-MS/MS and assay determination for each sample.

Table 2 Correlation statistics of the LC-MS/MS results (x-axis) against the results obtained by all the commercial vitamin D assays (y-axis).

	Slope (confidence limits)	Intercept (confidence limits)	r-Value
Architect	0.85 (0.80–0.90)	−0.6 (−2.6–1.4)	0.87
Liaison	0.76 (0.73–0.79)	1.4 (0.3–2.6)	0.84
Centaur	0.93 (0.89–0.98)	−6.0 (−8.3–−4.0)	0.92
iSYS	0.81 (0.77–0.85)	3.9 (2.3–5.3)	0.91
VITROS	0.80 (0.76–0.84)	−0.5 (−2.2–1.2)	0.90
Elecsys	0.85 (0.81–0.89)	−0.7 (−2.3–1.08)	0.91

Our results showed that there are significant methodological differences between assays as demonstrated by the different correlation statistics (significant proportional and constant biases and variable correlation coefficients) and variable inter-method concordance (from 65% to 83%). However, many of the discordances arose from readouts close to the status cut-off points. For example a value of 29 ng/mL by one method was considered as vitamin-deficient, whilst a value of 30.5 ng/mL by a different method was considered vitamin-sufficient. Whilst these small differences in the methods may put patients into different vitamin D status categories, such discordances may not affect patient management. Nevertheless, much larger discrepancies in vitamin D estimations were found, the size of which could affect patient management. Accordingly, method standardisation is urgently required in the field of vitamin D measurement. In addition, discrepancy in methods could be caused by cross-reactivity to various vitamin D2 and D3 metabolites such as the C3 epimer or by interferences from agents such as haemoglobin, human anti-mouse antibodies (HAMAs) or heterophilic antibodies. It is therefore important for clinical laboratories to be aware of the limitations of their assay.

(25-OH) vitamin D determination in serum by immunoassays is a difficult task owing to the fact that it circulates in a form strongly bound to binding proteins, and it exists in different forms [25(OH)D2 and 25(OH)D3], whose relative concentrations depend on either diet or the exact

Table 4 Precision of the VITROS® 25-OH Vitamin D Total Assay.

	Pool 1[a]	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6
Mean, ng/mL	6.57	19.6	31.7	45.6	60.2	101.8
% CV	21.7%	6.1%	8.4%	7.1%	6.8%	4.1%

[a]This level is below the VITROS assay limit of quantitation (LOQ).

method of vitamin D supplementation. A standardisation of the methods is thus urgently needed and is currently ongoing, through the Vitamin D Standardization Program (VDSP) [13, 14]. This work is managed by the NIH Office of Dietary Supplements (ODS) in collaboration with the CDC National Center for Environmental Health (NCEH), the National Institute of Standards and Technology (NIST) and the Ghent University. It is thus anticipated that a recalibration of the different methods will occur. When this standardisation will be eventually completed, another challenge will be to evaluate whether the clinical cut-offs currently defining vitamin D deficiency/insufficiency are still valid or need to be updated.

In conclusion, the analytical performances of the VITROS assay, as demonstrated in this study, show that this method performs satisfactorily.

Conflict of interest statement

Table 3 Concordance of vitamin D status between methods (expressed as %).

	LC-MS/MS	Centaur	ISYS	Elecsys	Architect	VITROS	DiaSorin XL
LC-MS/MS	–	65	82	75	76	72	72
Centaur	65	–	68	74	79	78	83
ISYS	82	68	–	77	80	75	79
Elecsys	75	74	77	–	82	81	81
Architect	76	79	80	82	–	80	85
VITROS	72	78	75	81	80	–	82
DiaSorin XL	72	83	79	81	85	82	–

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