

**MULTICENTER COMPARISON OF SEVEN 25OH VITAMIN D  
AUTOMATED IMMUNOASSAYS****MULTICENTRIČNO POREĐENJE SEDAM AUTOMATIZOVANIH IMUNOESEJA  
ZA 25OH VITAMIN D**

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**Summary**

**Background:** The measurement of 25OH vitamin D continues to grow in clinical laboratories. The aim of this multicenter study was to compare the results of seven automated commercial immunoassays with a reference HPLC technique.

**Methods:** One hundred and twenty consecutive outpatient serum samples were centrifuged, divided in aliquots, frozen and shipped to the participating laboratories. 25OH Vitamin D was measured with a reference HPLC system and with seven automated commercial immunoassays (Roche Cobas E601, Beckman Coulter Unicel DXI 800, Ortho Vitros ES, DiaSorin Liaison, Siemens Advia Centaur, Abbott Architect i System and IDS iSYS).

**Results:** Compared to the reference method, the regression coefficients ranged from 0.923 to 0.961 (all  $p < 0.001$ ). The slope of Deming fit ranged from 0.95 to 1.06, whereas the intercept was comprised between  $-15.2$  and  $9.2$  nmol/L. The bias from the reference HPLC technique varied from  $-14.5$  to  $8.7$  nmol/L. The minimum performance goal for bias was slightly exceeded by only one immunoassay. The agreement between HPLC and the different immunoassays at 50 nmol/L 25OH Vitamin D varied between 0.61 and 0.85 (all  $p < 0.001$ ). The percentage of samples below this cut-off was significantly different with only one immunoassay.

**Kratak sadržaj**

**Uvod:** Merenje 25OH vitamina D sve se češće obavlja u kliničkim laboratorijama. Cilj ove multicentrične studije bio je da se rezultati sedam automatizovanih komercijalnih imunoeseja uporede sa referentnom tehnikom HPLC.

**Metode:** Sto dvadeset uzoraka seruma od uzastopnih pacijenata koji su posetili kliniku centrifugirano je, podeljeno u alikvote, smrznuto i poslato laboratorijama koje su učestvovala u istraživanju. Nivo 25OH vitamina D meren je pomoću referentnog sistema HPLC i pomoću sedam automatizovanih komercijalnih imunoeseja (Roche Cobas E601, Beckman Coulter Unicel DXI 800, Ortho Vitros ES, DiaSorin Liaison, Siemens Advia Centaur, Abbott Architect i System i IDS iSYS).

**Rezultati:** U poređenju sa referentnim metodom, koeficijenti regresije kretali su se između 0,923 i 0,961 (svuda  $p < 0,001$ ). Nagib za Demingovu regresiju kretao se od 0,95 do 1,06, dok je kriva obuhvatila područje između  $-15,2$  i  $9,2$  nmol/L. Odstupanje od referentne tehnike HPLC kretalo se od  $-14,5$  do  $8,7$  nmol/L. Minimalni dometa za odstupanje blago je prešao samo jedan imunoesej. Slaganje između HPLC i različitih imunoeseja pri koncentraciji od 50 nmol/L 25OH vitamina D variralo je između 0,61 i 0,85 (svuda  $p < 0,001$ ). Procenat uzoraka ispod ove cut-off vrednosti značajno se razlikovao samo za jedan imunoesej.

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**Conclusions:** The excellent correlation with the reference HPLC technique attests that all seven automated immunoassays may be reliably used for routine assessment of 25OH-D in clinical laboratories. The significant bias among the different methods seems mostly attributable to the lack of standardization and calls for additional efforts for improving harmonization of 25OH-D immunoassays.

**Keywords:** vitamin D, 25OH-D, immunoassays, standardization, method comparison

## Introduction

Vitamin D is a fat soluble compound, which exerts a kaleidoscope of biological functions in humans (1). Basically, vitamin D<sub>2</sub> (ergocalciferol) derives from ergosterol, a membrane sterol produced by phytoplankton, invertebrates and fungi in response to ultraviolet (UV) irradiation, but it is not constitutively synthesized in plants or vertebrates. Vitamin D<sub>3</sub> (cholecalciferol) is produced in the skin or can be received through diet from animal sources, principally fish, eggs yolks or liver. The synthesis of 25-hydroxyvitamin D (25OH-D) occurs by hydroxylation of vitamin D<sub>3</sub> (or D<sub>2</sub>) in the liver, which is then followed by a further hydroxylation in the kidney, to generate 1- $\alpha$ ,25-dihydroxycholecalciferol (i.e., calcitriol or 1,25OH-2D). According to this complex metabolic pathway, the concentration of 25OH-D is currently regarded as the most suitable indicator of vitamin D body stores (1).

The 25OH-D deficiency, which is currently defined as a value lower than 50 nmol/L (i.e., 20 ng/mL) by the US Institute of Medicine (IOM) (2) and the clinical practice guideline of the Endocrine Society (3), has recently emerged as a public healthcare issue. Beside the well-known function in bone metabolism, 25OH-D deficiency is increasingly associated with a number of human disorders, including cardiovascular disease (4), cancer (5), infectious diseases (6), and frailty (7) among others. The measurement of 25OH-D in serum or plasma has hence become a cornerstone for overall health and well-being, and vitamin D testing volumes continue to grow in clinical laboratories (8).

The current techniques for measuring 25OH-D entail liquid chromatography (LC) methods coupled with automated UV or mass spectrometric (MS) detection, and immunochemistry techniques, which are based on polyclonal or monoclonal antibodies directed against 25OH-D and have been developed for use on a variety of automated clinical chemistry platforms (2, 9). Although isotope dilution LC-MS/MS is considered the candidate reference method for accurate quantification of 25OH-D, high-pressure (HPLC) techniques with UV detection provide comparable results, allow simultaneous measurement of either 25OH-D<sub>2</sub> or 25OH-D<sub>3</sub>, and are also more affordable to routine clinical laboratories (9).

**Zaključak:** Odlična korelacija sa referentnom tehnikom HPLC potvrđuje da se svih sedam automatizovanih imunoeseja mogu sa sigurnošću koristiti za rutinsko određivanje nivoa 25OH-D u kliničkim laboratorijama. Značajno odstupanje između različitih metoda po svojoj prilici se može uglavnom pripisati nedostatku standardizacije i zahteva dodatne napore kako bi se popravila harmonizacija imunoeseja za 25OH-D.

**Ključne reči:** vitamin D, 25OH-D, imunoeseji, standardizacija, poređenje metoda

Both LC-MS/MS and HPLC techniques have several drawbacks compared to automated immunoassays, including higher complexity, longer turn-around time and the need for skilled personnel, which make them virtually unavailable to some laboratories, especially the smaller ones or those for which LC equipment is unaffordable. Therefore, automated assays are typically regarded as the best choice for a number of laboratory services, provided that these methods display satisfactory analytical performance and optimal agreement with the reference LC techniques. Some previous articles have been published about the analytical comparison of automated immunoassays with LC methods (10–14), showing rather heterogeneous results. Therefore, the aim of this multicenter study was to compare the results of 25OH-D obtained with a reference HPLC technique, with the results of seven different automated commercial immunoassays.

## Materials and Methods

Blood collection (13 × 100 mm × 6.0 mL BD Vacutainer® Plus plastic serum tube; Becton Dickinson, Franklin Lakes, NJ, USA) was centralized at the Academic Hospital of Parma, Italy. In brief, 120 consecutive outpatient samples (58 males and 72 females; mean age 54 ± 18 years) referred to the local laboratory medicine service with a specific request for 25OH-D testing were centrifuged, separated and divided in 5 aliquots of 0.6 mL each. The first aliquot was used for routine measurement of 25OH-D as for the physician's prescription, whereas the remaining 4 aliquots were stored at -70 °C for delayed testing. After one week of storage, all aliquots were transported to the participating centers using certified transport boxes, under controlled conditions of temperature and humidity. The mean transportation time was 91 ± 18 min. Upon arrival to the different laboratories, the samples were kept stored at -70 °C until all centers had received the shipment, thus allowing a simultaneous start of measurements. Before analysis, all aliquots were left to thaw at room temperature, and were then centrifuged. The analytical characteristics of the 25OH-D immunoassays are synthesized in Table 1. The reference HPLC method used in this study (Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany) allows simul-

**Table I** Technical and analytical characteristics of the 25OH-D methods used in this study, as quoted by the manufacturers.

Laboratory	Company	Platform and method	Standardization	LOD (nmol/L)	Linearity (nmol/L)	Imprecision
Academic Hospital of Verona, Verona, Italy	Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany  Roche Diagnostics, Basel, Switzerland	Isocratic HPLC system with UV detection	UV (verified by LC-MS/MS)	2.7	3.5–925	0.8–4.6%
		Cobas E601, 1-step competitive binding chemiluminescence against vitamin D binding protein	NIST SRM 2972	7.5	7.5–175	2.2–6.8%
Academic Hospital of Parma, Parma, Italy	Beckman Coulter, Brea, CA, USA	Unicel DXI 800, 2-step competitive binding chemiluminescence against 25OH-D	NIST SRM 2972	5.0	5.0–525	5.6–9.3%
	Ortho-Clinical Diagnostics, Rochester, NY, USA	Vitros ES, 1-step competitive binding chemiluminescence against 25OH-D	UV (verified by LC-MS/MS)	20	20–315	5.3–10.1%
	DiaSorin, Saluggia (VC), Italy	Liaison, 1-step competitive binding chemiluminescence against 25OH-D	UV (verified by LC-MS/MS)	5.0	10–375	2.9–5.5%
General Hospital of Vicenza, Vicenza, Italy	Siemens Healthcare Diagnostics, Tarrytown, NY, USA	Advia Centaur, 1-step competitive binding fluorescent immunoassay against 25OH-D	UV (verified by LC-MS/MS)*	8.0	10.5–375	4.8–11.1%
General Hospital of Bassano del Grappa, Bassano del Grappa (VI), Italy	Abbott Diagnostics, Lake Forest, IL, USA	Architect i System, 1-step competitive binding chemiluminescence against 25OH-D	UV (verified by LC-MS/MS)	7.7	20–400	2.8–4.6%
	Immunodiagnostic Systems Limited, Boldon, UK	iSYS, 1-step competitive binding chemiluminescence against 25OH-D	UV (verified by LC-MS/MS)	9.0	15–315	8.9–16.9%

HPLC, High-Pressure Liquid Chromatography; LC, Liquid Chromatography; LOD, Limit of Detection; MS, Mass Spectrometry; NIST, National Institute of Standards and Technology.

\* This method has been made traceable to NIST SRM 2972 after the publication of this study.

taneous chromatographic determination of both 25-OH-D<sub>3</sub> and 25-OH-D<sub>2</sub> on a simple isocratic HPLC system with UV detection (Gilson Aspec XL, Middleton, WI, USA). The method was calibrated using a commercial proprietary standard (Chromsystems Instruments & Chemicals GmbH). The basic characteristics of this method have been previously described elsewhere (15). In brief, interfering components are removed from the samples by protein precipitation and selective solid phase extraction. The analytes are then quantified with a HPLC test system, by inclusion of a stable internal standard.

The results of all measurements were analyzed with Deming fit, Spearman's correlation, kappa statis-

tic,  $\chi^2$  square test with Yates' correction and Bland & Altman plots, using Analyse-it (Analyse-it Software Ltd, Leeds, UK). The study was based on preexisting samples, so that ethical permission and informed consent were unnecessary, according to our local ethical committee. The study was, however, performed in accordance with the Declaration of Helsinki and under the terms of all relevant local legislations.

## Results

The values of 25OH-D measured in the 120 out-patient serum samples with the reference HPLC were evenly distributed throughout the relevant biological

**Table II** Deming fit and Spearman's correlation of the different automated immunoassays as compared with the reference HPLC method.

Methods	HPLC
Roche Cobas	$y = 1.06x - 11.7$ $r = 0.923$ ( $p < 0.001$ )
Siemens Centaur	$y = 0.95x + 8.5$ $r = 0.955$ ( $p < 0.001$ )
DiaSorin Liaison	$y = 1.02x - 7.8$ $r = 0.961$ ( $p < 0.001$ )
Ortho Vitros	$y = 1.04x - 8.6$ $r = 0.928$ ( $p < 0.001$ )
Beckman Dxl	$y = 0.97x - 11.2$ $r = 0.945$ ( $p < 0.001$ )
IDS iSYS	$y = 0.99x + 9.2$ $r = 0.958$ ( $p < 0.001$ )
Abbott Architect	$y = 1.26x - 15.2$ $r = 0.959$ ( $p < 0.001$ )

range of concentrations (median 78.7 nmol/L; interquartile range 53.7–115 nmol/L; range 8.2–255 nmol/L). The results of Deming fit and Spearman's correlation obtained by comparison with the HPLC reference method are shown in *Table II*. Briefly, the regression coefficients were always optimal, ranging from 0.923 to 0.961 (all  $p < 0.001$ ). The slope of the Deming fit ranged from 0.95 to 1.06, whereas the intercept was comprised between –15.2 and 9.2 nmol/L. The bias from the reference HPLC technique, calculated from Bland & Altman plots, is shown in *Table III* and *Figure 1*. In general, the mean bias varied from –14.5 to 8.7 nmol/L. Accordingly, the minimum performance goal for bias suggested by the Endocrine Society (i.e. 15.8%) was slightly exceeded by only one immunoassay (i.e. Unicel Dxl –17.1%, 95% CI –21.7 to –12.4%), but not by the other methods (i.e. Cobas E601 –11.9%, 95% CI –17.2 to –6.5%; Advia Centaur 11.1%, 95% CI 6.6 to 15.6%; Vitros ES –6.7%, 95% CI –11.9 to –1.5%; Liaison –5.4%, 95% CI –8.8 to –2.0%; iSYS 10.7%, 95% CI 7.5 to 14.0%; Architect 7.9%, 95% CI 4.0 to 11.8%) (3).

The agreement (kappa statistics) between HPLC and the different immunoassays at the 50 nmol/L 25OH-D threshold is also shown in *Table III*, and varied between 0.61 and 0.85 (all  $p < 0.001$ ). The percentage of samples below this cut-off was 22% (26/120)

**Table III** Absolute bias (95% CI) and agreement (kappa statistics and 95% CI) at the diagnostic threshold for vitamin D deficiency (i.e. 50 nmol/L) of the different automated immunoassays as compared with the reference HPLC method.

Methods	HPLC
Roche Cobas	Bias –6.5 (95% CI –12.0 to –1.2) Kappa 0.79 (0.66 to 0.92; $p < 0.001$ )
Siemens Centaur	Bias 4.2 (95% CI 1.0 to 7.5) Kappa 0.61 (0.43 to 0.79; $p < 0.001$ )
DiaSorin Liaison	Bias –3.7 (95% CI –7.0 to –0.7) Kappa 0.81 (0.68 to 0.94; $p < 0.001$ )
Ortho Vitros ES	Bias –5.5 (95% CI –9.7 to –1.2) Kappa 0.66 (0.51 to 0.82; $p < 0.001$ )
Beckman Unicel Dxl	Bias –14.5 (95% CI –19.0 to –9.7) Kappa 0.72 (0.57 to 0.86; $p < 0.001$ )
IDS iSYS	Bias 7.7 (95% CI 4.5 to 11.0) Kappa 0.69 (0.52 to 0.86; $p < 0.001$ )
Abbott Architect	Bias 8.7 (95% CI 3.5 to 14.0) Kappa 0.85 (0.73 to 0.97; $p < 0.001$ )

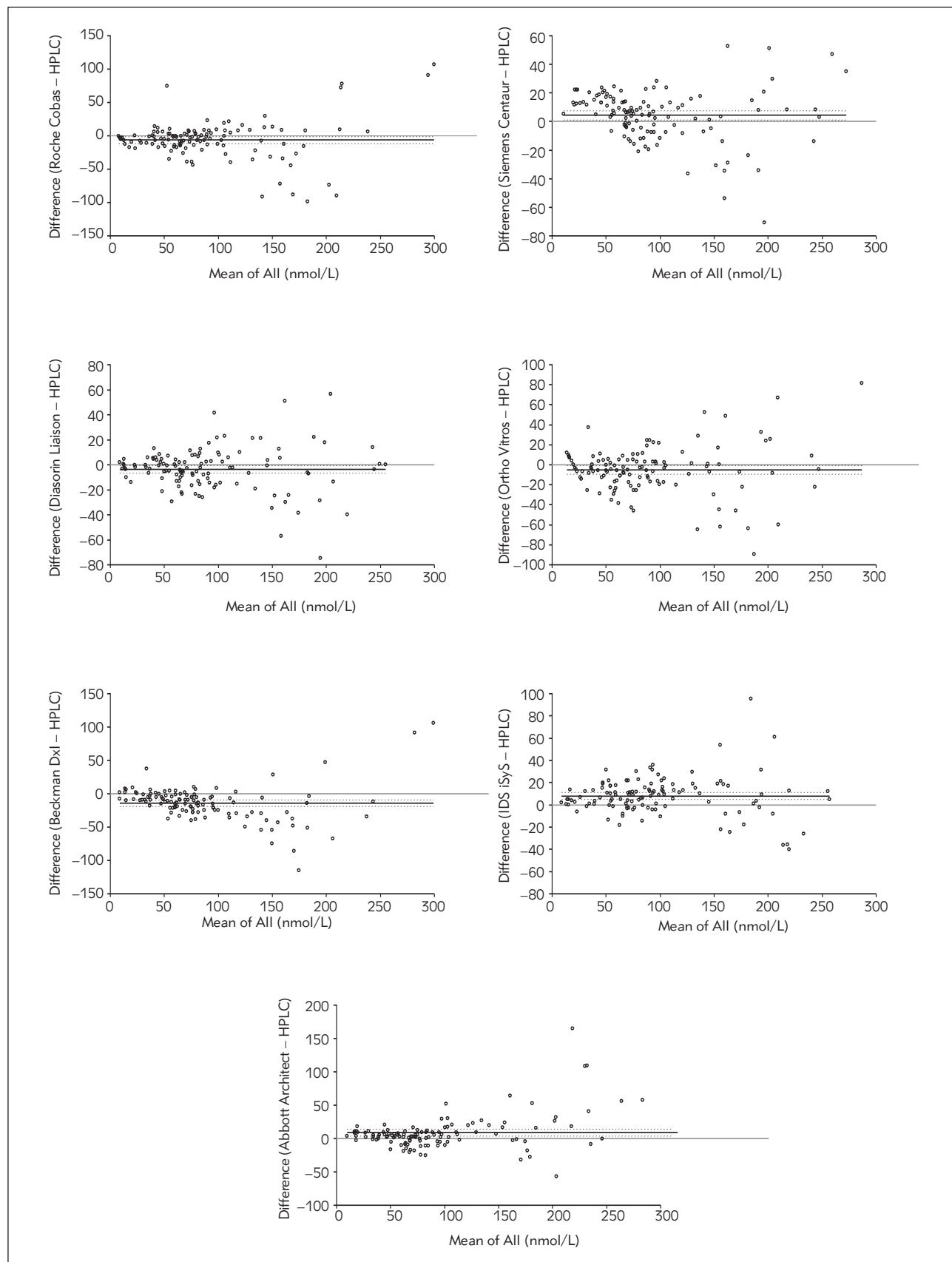
with HPLC, 24% (29/120;  $p = 0.76$  versus HPLC) with Cobas E601, 11% (13/120;  $p = 0.036$ ) with Advia Centaur, 29% (35/120;  $p = 0.24$ ) with Unicel Dxl, 28% (33/120;  $p = 0.37$ ) with Vitros ES, 23% (28/120;  $p = 0.88$ ) with Liaison, 14% (17/120;  $p = 0.18$ ) with iSYS, and 20% (24/120;  $p = 0.87$ ) with Architect.

## Discussion

According to recent evidence, the frequency of 25OH-D deficiency ranges from 22 to 26% across ages and genders (16). These figures are mirrored by a constant increase in demand for vitamin D measurement in clinical laboratories (8). Although it is rather understandable that 25OH-D should be preferably assessed with LC techniques, either HPLC or LC-MS (2, 3), this approach is virtually unsuitable in laboratories where these techniques are unaffordable due to economic or organizational issues. The gradual introduction into the diagnostic market of a variety of automated immunoassays should hence be regarded as a viable alternative for routine 25OH-D assessment, provided that results are accurate and ultimately correlated with a reference method.

Various previous studies showed that some commercial methods fail to meet stringent analytical criteria (10, 14), whereas others reported more favorable

**Figure 1** Bland & Altman plots of 25OH Vitamin D data (n=120) obtained with seven automated immunoassays, compared to a reference high-pressure liquid chromatography (HPLC) technique.



data for certain assays (11–13). Beside a simple comparison of data throughout the biological range of 25OH-D, the most critical issue is indeed the agreement at the current threshold used for defining vitamin D deficiency that is 50 nmol/L (2, 3).

The results of this investigation clearly attest that only one assay (i.e. Advia Centaur) displayed a significant disagreement at the diagnostic threshold of 25OH-D deficiency among the seven that we have tested, wherein the rate of vitamin D deficiency determined with this method appeared nearly half that measured by using HPLC (11% versus 24%;  $p=0.036$ ). Interestingly, we found an overall positive bias for Advia Centaur as compared with HPLC (+11%), which was more evident in samples with low values of 25OH-D (i.e. <50 nmol/L) (Figure 1). An identical finding has been recently reported by Janssen et al. (17), wherein samples with very low 25OH-D were shown to have an approximately 50% positive bias compared to a reference LC-MS/MS technique. However, the overall correlation of this assay was excellent both in our study ( $r=0.955$ ) and in the analytical evaluation of Janssen et al. (17) ( $r=0.92$ ).

An opposite trend was found with Cobas E601. In agreement with two previous studies (12, 18), the results were negatively biased (–12%). This is probably attributable to the fact that, at variance with the reference HPLC assay used in this study, the Roche immunoassay is traceable to the National Institute of Standards and Technology (NIST) serum-based Standard Reference Material (SRM) 972 (19). It is also noteworthy, however, that the overall agreement of this assay was the third highest among all immunoassays and the correlation was excellent (i.e.  $r=0.923$ ;  $p<0.001$ ), very similar to the one previously reported by Chen et al. (12) ( $r=0.945$ ). As regards the other immunoassays, despite a good agreement at the diagnostic cut-off and an excellent correlation ( $r=0.945$ ;  $p<0.001$ ) with HPLC, the method that has been made recently available on the Unicl Dxl exhibited the highest (negative) bias (i.e. –14.5 nmol/L) and a percentage difference that slightly exceeded the minimum performance goal for bias (i.e. 17.1% versus 15.8%). Specifically, this bias was mostly attributable to samples with 25OH-D >75 nmol/L (Figure 1), and hence with analyte concentrations greater than the conventional threshold of moderate 25OH-D deficiency. This may be attributable to the specific immunoreactivity of the antibodies against the samples tested, but also to the fact that this is a 2-step chemiluminescent method, whereas the other immunoassays are based on direct (i.e. 1-step) competitive binding against 25OH-D or vitamin D binding protein (VDBP) (Table I). Interestingly, the percentage bias calculated on samples with 25OH-D <75 nmol/L was much lower ( $n=56$ ; –12.8%; 95% CI –21.1 to –4.5%). No previous studies have investigated the performance of this assay, to the best of our knowledge, and thereby comparison with results of other analytical investigations is impossible. Along with Cobas E601, this

method is also calibrated against the NIST-SRM 972, so that these results are not unexpected. It has been recently shown that the values of 25OH-D are typically low-biased in methods traceable to NIST-SRM 972, especially when measuring samples containing prevalently 25OH-D<sub>3</sub> (20) such as those obtained from our study population of unselected outpatients (supplementation with 25OH-D<sub>2</sub> is not prescribed in our area). The results of other assays were globally satisfactory, with excellent correlations and significant agreement at the cut-off of 25OH-D deficiency (Table II and III). A negative bias was also observed for the Abbott Architect, in agreement with that previously reported by Jovičić et al. (18), who also used an HPLC technique as the gold standard.

In conclusion, the excellent correlations with the reference HPLC technique found in this study attest that all seven automated immunoassays may be reliably used for routine assessment of 25OH-D in clinical laboratories. The differences observed in this study are at least in part attributable to the different test design. More specifically, the Roche Cobas E601 electrochemiluminescent immunoassay is a competitive protein binding method which uses a specific protein that binds to VDBP, whereas the other methods are based on the competitive binding of antibodies to 25OH-D (Table I). It is also noteworthy that direct methods, in which 25OH-D and VDBP are not completely separated, may also display heterogeneous immunoreactivity compared to 2-step immunoassays (10). Regardless of these differences, only one method slightly exceeded the minimum performance goal for bias as compared with HPLC, and another one displayed significant disagreement at the cut-off of 25OH-D deficiency. In both cases, however, the correlation with the reference method was excellent, thus emphasizing the current issue of poor standardization of vitamin D testing (Table I). This hypothesis is also supported by the rather heterogeneous value of bias (from –14.5 to 8.5 nmol/L) observed across the different method comparisons. Although this should be regarded as a potential confounding factor when assessing 25OH-D according to the conventional recommendations to maintain the concentration of this vitamin above a certain threshold (2, 3), the objective of harmonization, however, may be achieved with relatively modest efforts. Due to the high reliability of individual results against the reference technique (Table II and III), it is reasonable to hypothesize that extension of traceability to common standards (e.g. NIST-SRM 2972) across different methods and platforms should be effective to consistently reduce the bias and improve comparability among the various automated 25OH-D immunoassays available on the market. Further studies should hence be planned to verify the effectiveness of this strategy.

### Conflict of interest statement

The authors stated that have no conflicts of interest regarding the publication of this article.

## References

1. Holick MF. The D-lightful vitamin D for health. *J Med Biochem* 2013; 32: 1–10.
2. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium; Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington (DC): National Academies Press (US); 2011.
3. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011; 96: 1911–30.
4. Targher G, Pichiri I, Lippi G. Vitamin D, thrombosis, and hemostasis: more than skin deep. *Semin Thromb Hemost* 2012; 38: 114–24.
5. Bjelaković G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database Syst Rev* 2014; 1:CD 007470.
6. 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.
7. Lippi G, Sanchis-Gomar F, Montagnana M. Biological Markers in Older People at Risk of Mobility Limitations. *Curr Pharm Des* 2013 Sep 18. [Epub ahead of print.]
8. Vieth R. The future of »vitamin D«, i.e. 25-hydroxyvitamin D, testing. *Clin Biochem* 2013; 46: 189.
9. Carter GD. 25-hydroxyvitamin D: a difficult analyte. *Clin Chem* 2012; 58: 486–8.
10. Farrell CJ, Martin S, McWhinney B, Straub I, Williams P, Herrmann M. State-of-the-art vitamin D assays: a comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods. *Clin Chem* 2012; 58: 531–42.
11. Moon HW, Cho JH, Hur M, Song J, Oh GY, Park CM, et al. Comparison of four current 25-hydroxyvitamin D assays. *Clin Biochem* 2012; 45: 326–30.
12. Chen Y, Kinney L, Božović A, Smith H, Tarr H, Diamandis EP, LeBlanc A. Performance evaluation of Siemens ADVIA Centaur and Roche MODULAR Analytics E170 Total 25-OH Vitamin D assays. *Clin Biochem* 2012; 45: 1485–90.
13. Koivula MK, Matinlassi N, Laitinen P, Risteli J. Four automated 25-OH total vitamin D immunoassays and commercial liquid chromatography tandem-mass spectrometry in Finnish population. *Clin Lab* 2013; 59: 397–405.
14. Holmes EW, Garbincius J, McKenna KM. Analytical variability among methods for the measurement of 25-hydroxyvitamin D: still adding to the noise. *Am J Clin Pathol* 2013; 140: 550–60.
15. Abdel-Wareth L, Haq A, Turner A, Khan S, Salem A, Mustafa F, et al. Total vitamin D assay comparison of the Roche Diagnostics »Vitamin D total« electrochemiluminescence protein binding assay with the Chromsystems HPLC method in a population with both D2 and D3 forms of vitamin D. *Nutrients* 2013; 5: 971–80.
16. Lippi G, Montagnana M, Meschi T, Borghi L. Vitamin D concentration and deficiency across different ages and genders. *Aging Clin Exp Res* 2012; 24: 548–51.
17. Janssen MJ, Wielders JP, Bekker CC, Boesten LS, Buijs MM, Heijboer AC, et al. Multicenter comparison study of current methods to measure 25-hydroxyvitamin D in serum. *Steroids* 2012; 77: 1366–72.
18. Jovičić S, Ignatović S, Kangrga R, Beletić A, Mirković D, Majkić-Singh N. Comparison of three different methods for 25(OD)-vitamin D determination and vitamin D status in general population. *J Med Biochem* 2012; 31: 347–57.
19. 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.
20. Bedner M, Lippa KA, Tai SS. An assessment of 25-hydroxyvitamin D measurements in comparability studies conducted by the Vitamin D Metabolites Quality Assurance Program. *Clin Chim Acta* 2013; 426: 6–11.

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