

Multicenter comparison study of current methods to measure 25-hydroxyvitamin D in serum

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Measurement of serum 25-hydroxyvitamin D [25(OH)D] is generally considered to be a reliable indicator of vitamin D status. High variability in 25(OH)D measurements due to utilized test and assay technologies, non-equimolar detection of 25(OH)D₂ and 25(OH)D₃, interferences from other hydroxylated vitamin D metabolites, and the lack of a definite reference method often confounds proper assessment of vitamin D status (1, 2). Recently, two reference measurement procedures for 25(OH)D₃ and 25(OH)D₂ have been described using isotope-dilution liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) (3, 4). The recent increase in diversity of 25(OH)D assays prompted us to evaluate the performance of chromatographic methods (two in-house ID-LC-MS/MS and HPLC (ClinRep, Recipe)), a protein binding method (Cobas-25(OH)D-total, Roche) and immunochemical methods (Liaison and RIA (Diasorin), iSYS (IDS), ADVIA Centaur (Siemens), and Architect i1000 and i2000 (Abbott)). Blood (serum-gel, S-Monovette, Sarstedt, Nümbrecht, Germany) was drawn at one site from random outpatients (N=60) after informed consent. Sample aliquots were prepared, frozen and transported to participating centers. Method comparison was performed according to CLSI-EP9 specifications. The Architect assays as well as the ADVIA Centaur assay evaluated in this study have been adjusted by the supplier relatively quickly after release on the market. All assays were compared to an established LC-MS/MS method (LC-MS/MS-a) (5). The accuracy of the second MS method (LC-MS/MS-b) was established by measuring the standard and a control with a reference measurement procedure (4). Details of the LC-MS/MS-b method have been published recently (6). The relative content of 3-epi-25(OH)D₃ was measured by a modification of LC-MS/MS-a using a fluorophenyl column (7). Indi-

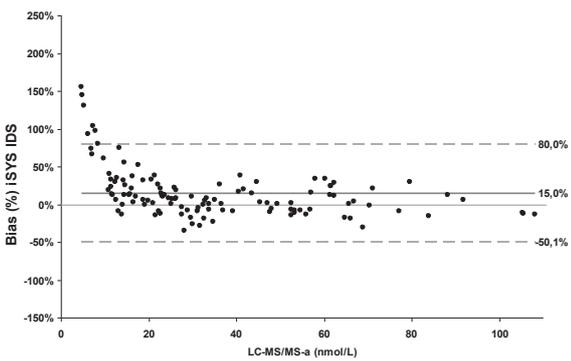
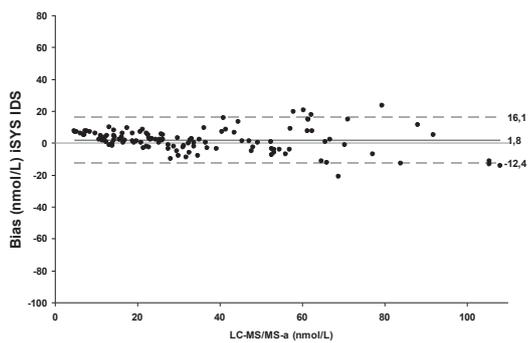
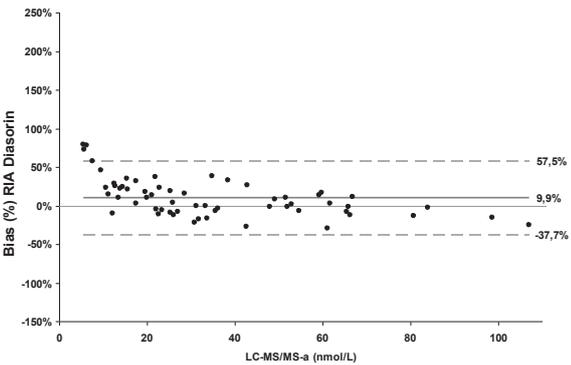
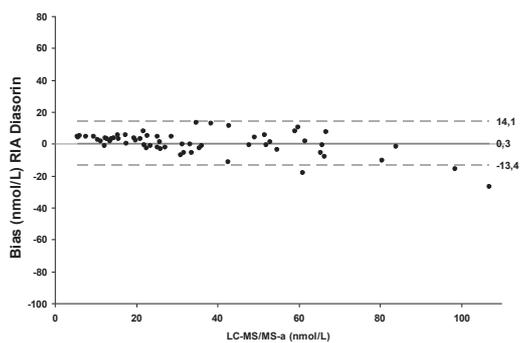
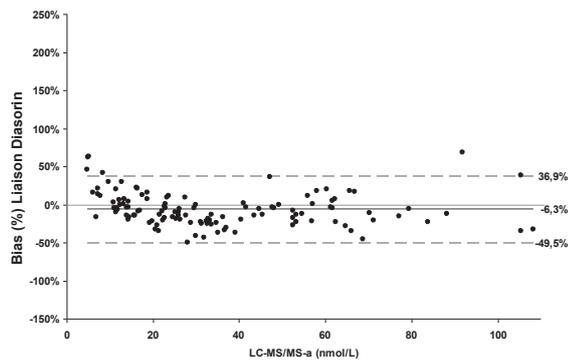
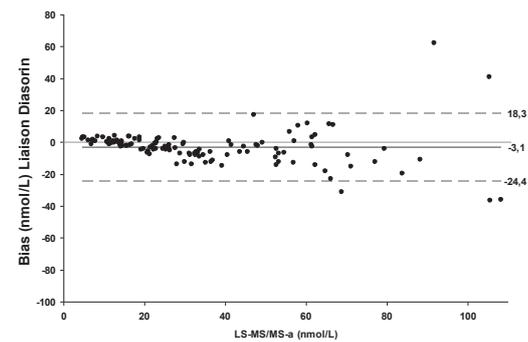
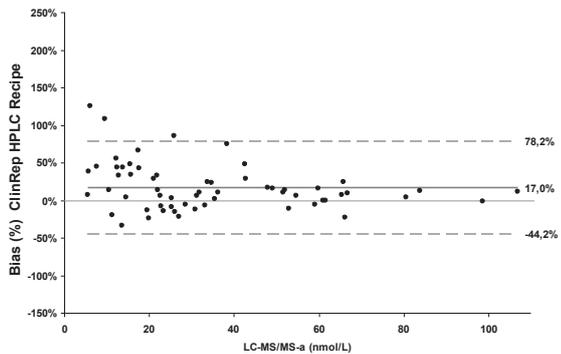
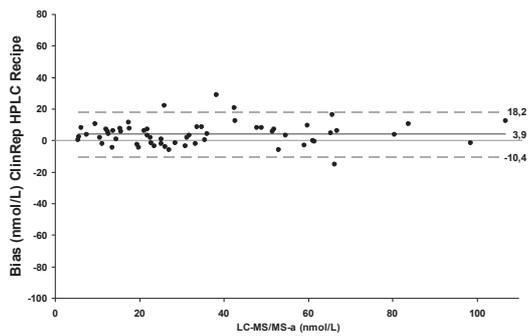
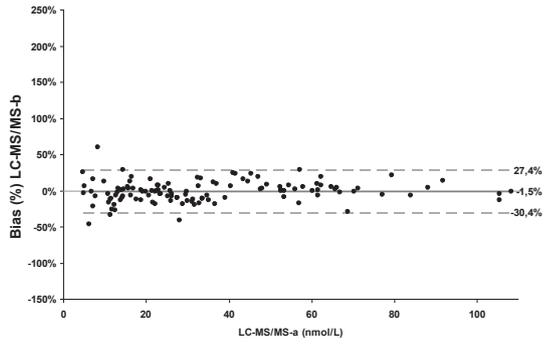
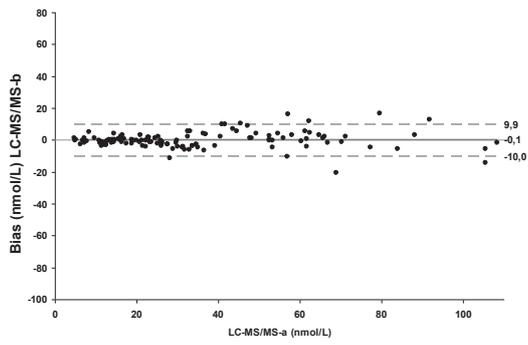
vidual patient samples were measured in duplicate, with exception of HPLC, RIA and the Architect-i1000 where singular measurements were performed.

For method comparison studies we applied Deming regression and Bland-Altman bias plots using EP Evaluator Release 9 (D.G. Rhoads Associates Inc., Kennett Square, PA, USA) or Analyse-it software program (Microsoft Corporation). Module CLSI EP9 Method Comparison was used for analysis of duplicate measurements, whereas module Alternate (Quantitative) Method Comparison was used for singular measurements.

All 25(OH)D values represent total 25(OH)D concentrations covering a range between 5.0 and 108.0 nmol/L with a mean value of 35.2 nmol/L based on the LC-MS/MS-a measurements. All patient cases had undetectable 25(OH)D₂ values, as well as non-significant levels of C3-epi-25(OH)D₃ (mean (median) relative content 3.6 (3.1%); range 2.0-10.6%). The slope of the Deming regression line for the evaluated 9 methods relative to LC-MS/MS-a varied from 0.57 for the ADVIA Centaur to 1.07 for the ClinRep HPLC, and the intercept from -1.7 nmol/L for the COBAS D total and LC-MS/MS-b to 6.9 nmol/L for the Architect i2000. Statistically significant bias was detected in the majority of methods. The ClinRep HPLC, iSYS and COBAS D total assays showed no statistically significant bias, albeit the coefficient of correlation for the COBAS D total assay (R=0.88) was suboptimal. Difference plots displaying absolute and relative difference against LC-MS/MS-a values were applied to all evaluated samples (figure 1). A considerable proportional bias is demonstrable for the ADVIA Centaur assay. The performance of the ADVIA Centaur assay is unacceptable and this assay should be re-adjusted before clinical use. The Architect i2000 shows significant positive bias at low concentration 25(OH)D. This is likely to be caused by the limited sensitivity (20 nmol/L) of the assay. Uniquely different in comparison to all other evaluated methods is that the COBAS D-total assay shows increasing bias at increasing concentration of 25(OH)D, which is most likely related to the design of competitive protein binding. As a consequence, the COBAS D-total assay has a relatively low coefficient of correlation. Moreover, the COBAS D-total assay appears the only binding assay that, partially, cross-reacts with 3-epi-25(OH)D (information leaflet, unpublished data). Mean absolute bias varied from -10.7 nmol/L to 3.9 nmol/L, mean relative bias varied from -16% to 27% with LC-MS/MS-b showing the smallest mean bias (-0.1 nmol/L; -1.5%).

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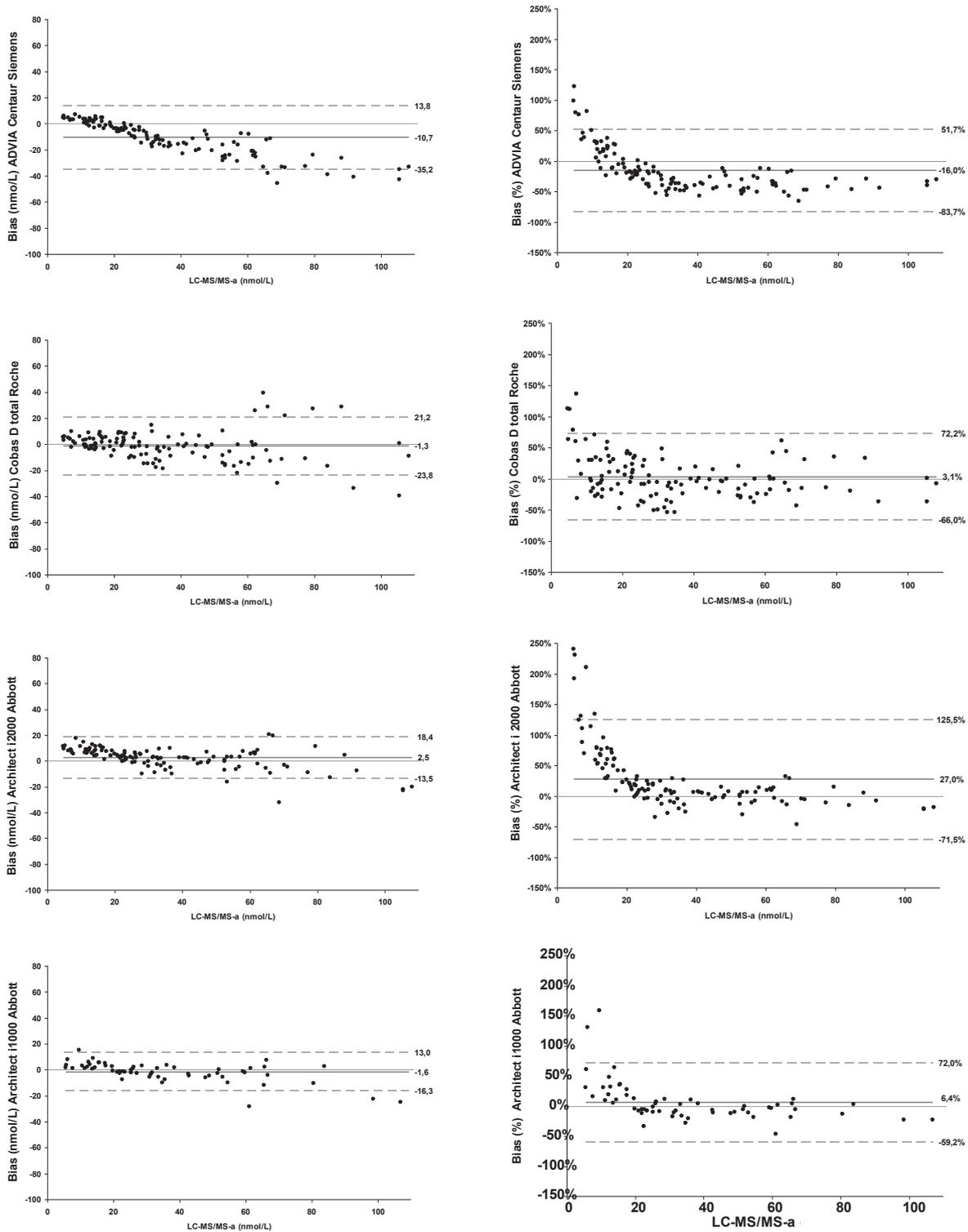


Figure 1. Difference plots of nine assays to measure serum 25(OH)D against LC-MS/MS-a showing bias in nmol/L (left panels) and percentage (right panels). Bold lines: mean bias. Dashed lines: 2SD limits of bias.

Tabel 1. Proportion of patient samples (%) by 25(OH)D category and overall concordance to LC-MS/MS-a (%)

Vitamin D status in nmol/L category	<25 deficiency	25-50 insufficiency	50-75 normal	>75 optimal	Overall concordance
LC-MS/MS-a	42	32	19	7	-
LC-MS/MS-b	44	27	23	6	85
ClinRep HPLC (Recipe)*	39	28	25	8	75
Liaison (Diasorin)	53	29	13	5	70
RIA (Diasorin)*	43	27	25	5	83
iSYS (IDS)	38	37	15	10	86
ADVIA Centaur (Siemens)	69**	23**	7	1	53
Cobas D total (Roche)	45	36	12	7	64
Architect-i2000 (Abbott)	27	48	18	7	73
Architect-i1000 (Abbott)*	42	38	15	5	88

* No duplicate measurements; ** contains results differing by more than 1 category

There is no consensus on appropriate concentrations of 25(OH)D making that recommendations differ between 50 nmol/L (8) and 75 nmol/L (9). We have chosen to sort patient results into four categories being <25, 25-50, 50-75 and >75 nmol/L for vitamin deficiency, insufficiency, normal or optimal levels, respectively. The proportion of patient samples falling into each category for each of the ten methods is listed in table 1. Also shown is the percentage of patient samples for each assay that share the same category with LC-MS/MS-a. Overall concordance varied between 53 and 88%. For the majority of methods the agreement to LC-MS/MS-a deviated by no more than 1 category. Most striking is the near absence of patient samples with 25(OH)D levels >75 nmol/L in the ADVIA Centaur assay. The ADVIA Centaur assay showed the poorest overall agreement to LC-MS/MS-a in sorting individual patient samples into the same category, with patient results even differing by more than 1 category in 3%.

In conclusion, significant bias exists between LC-MS/MS and many, but not all, other 25(OH)D assays tested in this study. Major effort is needed towards further standardizing assays for 25(OH)D measurement.

References

- Binkley N, Krueger D, Cowgill CS, Plum L, Lake E, Hansen KE, et al. Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. *J Clin Endocrinol Metab.* 2004; 89: 3152-3157.
- Wallace AM, Gibson S, de la Hunty A, Lamberg-Allardt C, Ashwell M. Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedure, performance characteristics and limitations. *Steroids.* 2010; 75: 477-488.
- Tai SS, Bedner M, Phinney KW. Development of a candidate reference measurement procedure for the determination of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 in human serum using isotope-dilution liquid chromatography-tandem mass spectrometry. *Anal Chem.* 2010; 82: 1942-1948.
- Stepman HC, Vanderroost A, Van Uytvanghe K, Thienpont LM. Candidate reference measurement procedure for serum 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 by using isotope-dilution liquid chromatography-tandem mass spectrometry. *Clin Chem.* 2011; 57: 441-448.
- Van den Ouweland JM, Beijers AM, Demacker PN, van Daal H. Measurement of 25-OH-vitamin D in human serum using liquid chromatography tandem-mass spectrometry with comparison to radioimmunoassay and automated immunoassay. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2010; 878: 1163-1168.
- Heijboer AC, Blankenstein MA, Kema IP, Buijs MM. Accuracy of six routine 25-hydroxy vitamin D assays; influence of vitamin D binding protein concentration. *Clin Chem.* 2012; 58: 543-548.
- Van den Ouweland JM, Beijers AM, van Daal H. Fast separation of 25-hydroxyvitamin D3 from 3-epi-25-hydroxyvitamin D3 in human serum by liquid chromatography-tandem mass spectrometry: variable prevalence of 3-epi-25-hydroxyvitamin D3 in infants, children, and adults. *Clin Chem.* 2011; 57: 1618-1619.
- Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab.* 2011; 96: 53-58.
- Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2011; 96: 1911-1930.