

Standardization and Certification of the ADVIA Centaur Vitamin D Total Assay

Authors:

James Freeman
Paul Sibley, PhD
Neil Parker, MSc
Ryan Spears
Kimberly S. Wilson
H. Roma Levy, MS

Affiliations:

Siemens Healthineers
511 Benedict Avenue
Tarrytown, NY 10591-5005
USA
Phone: + 914-631-8000
siemens.com/healthineers

Correspondence should be addressed to:

Neil Parker, MS:
511 Benedict Avenue, Tarrytown,
NY 10591, USA
Tel: +1 (914) 524 2477
E-mail:
[neil.np.parker@siemens-
healthineers.com](mailto:neil.np.parker@siemens-healthineers.com)

Running Title: ADVIA Centaur
Vitamin D Total Assay
Standardization/Certification

Keywords: Vitamin D, ADVIA
Centaur, Vitamin D Standardization
Program, VDSP; Vitamin D
Standardization Certification
Program, VDSCP; NIST-Ghent-
RMP; ID-LC/MS/MS

Abstract

Background: In recent years, the Siemens ADVIA Centaur[®] Vitamin D Total assay was standardized for traceability to the National Institute of Standardization and Technology (NIST)-Ghent reference method procedure (RMP) used in the Vitamin D Standardization Program (VDSP). Here, we compare results from the newer ADVIA Centaur Vitamin D assay to results obtained using several methods traceable to the VDSP NIST-Ghent RMP. We also present results of successful participation in the Centers for Disease Control and Prevention (CDC) vitamin D certification program (VDSCP).

Methods: The ADVIA Centaur Vitamin D Total assay results were compared to values assigned at Ghent University (n = 122); values assigned by methods metrologically traceable to the VDSP NIST-Ghent RMP (n = 177); RMP-assigned values by the College of American Pathologists (CAP) and Vitamin D External Quality Assessment Scheme (DEQAS) survey (n = 30); and to a third-party VDSCP-certified assay (n = 149). The CDC certification scheme (VDSCP), which requires annual certification, was applied over four consecutive years (2013–2017).

Results: Correlation between the standardized ADVIA Centaur Vitamin D Total assay and the VDSP NIST-Ghent RMP results was 99%; bias was –0.09%. Bias in comparison to CAP and DEQAS samples was < 25% for 96% of samples. Bias for CDC VDSCP certification samples was 0.3%, mean CV was 5.5%. Correlation to the Esoterix CDC VDSCP-certified ID-LC/MS/MS method was 95% for vitamin D total and 97% for samples containing only 25(OH)D₃.

Conclusions: The ADVIA Centaur Vitamin D Total assay traceable to the VDSP NIST-Ghent RMP demonstrated good performance and is acceptable for clinical use based on imprecision, and by comparison to CAP and DEQAS surveys. The assay is aligned to the VDSP NIST-Ghent RMP, and was among the first automated immunoassays certified by the CDC VDSCP. As of February 2017, the assay has achieved VDSCP certification for four consecutive years.

1. Introduction

Assessing vitamin D levels by measuring total 25-hydroxy vitamin D (25[OH]D), i.e. combined 25[OH]D₂ and 25[OH]D₃) provides important clinical information.

[The Institute of Medicine has defined the risk of Vitamin D deficiency as a 25(OH)D concentration less than 12 ng/L (30 nmol/L), whereas the Endocrine Society has defined Vitamin D deficiency as a 25(OH)D concentration less than 20 ng/mL (50 nmol/L).^{1,2} The Endocrine society defines insufficiency as 20 to 30 ng/mL (50 to 75 nmol/L)].²

Because 25(OH)D is a small steroid hormone with a very high affinity for its carrier protein (vitamin D-binding protein [VDBP]), evaluation is a challenging process. Several studies and national surveys have noted substantial variability between methods due to differences in manufacturers' proprietary methods for releasing 25(OH)D from VDBP, variability in the molar ratio of recovered 25(OH)D₂ and 25(OH)D₃, and the amount of epimeric forms detected—especially 3-epi-25(OH)D₃.³⁻⁸ Lack of assay standardization to a single reference method can pose a substantial impediment to clinical assessment, especially if evaluation over time and location utilizes different methods. Assay variability has also affected efforts to establish reliable clinical guidelines and laboratory proficiency testing.

To address assay variability problems, the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) instituted the 25(OH)D Vitamin D Standardization Program (VDSP). As a collaborative effort of the National Institute of Standards and Technology (NIST), the Centers for Disease Control and Prevention (CDC), and Ghent University (Ghent, Belgium), laboratories and manufacturers are provided with reference

materials and a reference protocol to standardize the vitamin D measurement, thereby improving diagnostic accuracy of vitamin D regardless of assay method.⁹⁻¹³ To complement this program, manufacturers and laboratories may participate in a certification process administered by the CDC: the Vitamin D Standardization-Certification Program (VDSCP).

Siemens Healthineers (Tarrytown, NY, U.S.), an early adopter of the VDSP, standardized its ADVIA Centaur[®] Vitamin D Total assay against the 25(OH)D VDSP NIST-Ghent RMP using metrologically traceable assay standards.

The goal of this study was to evaluate the standardized ADVIA Centaur Vitamin D Total assay performance using several methods that include the VDSP NIST-Ghent RMP method and methods metrologically traceable to the NIST-Ghent VDSP-RMP, and according to the specifications of the CDC Vitamin D Standardization Certification Program (VDSCP).

2. Materials and Methods

2.1. The VDSP NIST-Ghent RMP

The VDSP NIST-Ghent RMP is an isotope dilution, liquid chromatography, tandem mass spectrometric (ID-LC/MS/MS) method that measures only 25(OH)D₂ and 25(OH)D₃ and does not measure either 24, 25 dihydroxyvitamin D or 3-epimer 25(OH)D molecules.⁹

2.2. Principles of the ADVIA Centaur Vitamin D Total assay procedure

The ADVIA Centaur Vitamin D Total assay is a one-pass, 18-minute competitive immunoassay that uses an anti-fluorescein monoclonal mouse antibody covalently bound to paramagnetic particles, an anti-25(OH)D monoclonal mouse antibody labeled with acridinium ester (AE), and a

vitamin D analog labeled with fluorescein. The assay demonstrates equimolar cross-reactivity with 25(OH)D₃ (100.7%) and 25(OH)D₂ (104.5%), minimal cross-reactivity with 3-epimer-25(OH)D₃ (1.1%), and a broad dynamic range of 4.2 to 150 ng/mL (10.5 to 375 nmol/L). Other performance characteristics were not altered by the standardization. Evaluation shows that the limit of blank = 1.7 ng/mL, limit of detection = 3.2 ng/mL, and limit of quantitation = 4.2 ng/mL. The assay also demonstrated good linearity and dilution recovery in internal studies (data not shown).

2.3. Alignment with the VDSP NIST-Ghent RMP

Using the ADVIA Centaur Vitamin D Total assay master curve, dose values were determined for 177 native human serum samples and compared with dose values assigned by the VDSP NIST-Ghent RMP methods. The latter values spanned most of the assay range (5–140 ng/mL), 90 samples were assigned by either NIH or CDC, 60 samples were assigned by a commercial vendor using an LC-MS/MS method aligned to the VDSP NIST-Ghent RMP, and 27 samples were value-assigned by Ghent University. Two reagent lots (R1 and R2) were used in conjunction with their respective calibrator lots (C1 and C2) on a single ADVIA Centaur XP system. Deming regression was used to compare results from the ADVIA Centaur Vitamin D Total assay to dose values determined using the VDSP NIST-Ghent RMP.^{9,10}

A second evaluation was conducted using 122 additional serum samples comprising 116 native samples (range 7.8 ng/mL to 148.1 ng/mL) and six samples that were created by spiking a serum sample with 25(OH)D₂ to achieve a concentration range of 70.4 ng/mL to 125.6 ng/mL. All samples were assayed in singlicate on a single ADVIA Centaur system using a single

ADVIA Centaur Vitamin D Total assay reagent lot (R3), and values were compared to dose values determined using the VDSP RMP at Ghent University using Deming regression and Bland-Altman analyses.

2.4. Comparison to College of American Pathologists (CAP) and Vitamin D External Quality Assessment Scheme (DEQAS) survey samples

CAP 2011 accuracy-based vitamin D (ABVD) and DEQAS survey samples (n = 30) were measured using two reagent lots, lot 1 (R1) and lot 2 (R2) and the ADVIA Centaur Vitamin D Total assay. Percent bias to value assignments made using the VDSP NIST-Ghent RMP (range = 14.1 ng/mL to 58.5 ng/mL) was determined.

Percent bias = [(ADVIA Centaur Vitamin D Total Assay Dose – Survey-assigned Dose)/Survey-assigned Dose] x 100

In addition, DEQAS survey samples available from 2012 through January 2015 were evaluated using a single lot of the ADVIA Centaur Vitamin D Total assay and results (n = 50) were compared to the DEQAS-all-laboratory trimmed mean (ALTM) in 2012. Beginning in April 2013, DEQAS began using the VDSP NIST-Ghent RMP to assign values to its survey samples and as the target value for determining percent bias (rather than the all-laboratory trimmed mean [ALTM]).

The CAP and DEQAS criterion for total vitamin D is that 80% of results should fall within 25% of the target value.

2.5. Imprecision

Imprecision (%CV) was performed according to the CLSI EP15-A2 guideline for verification of performance and precision.¹⁴ Two human serum samples (<20ng/mL) and four pooled samples prepared from 25(OH)D₃-spiked human serum

(~20 to ~130 ng/mL) were run over 20 days, two runs per day, using two lots, lot 1 (R1) and lot 2 (R2), and two replicates per run on two ADVIA Centaur XP platforms (n = 80/sample). The VDSP NIST-Ghent RMP reagent master curves and calibrator assignments were used to evaluate the 20-day imprecision data to calculate reproducibility and within-lab CV.

2.6. Performance evaluation using total analytical error (TAE)

In a separate study, TAE and %TAE were calculated from overall precision error (standard deviation [SD] and bias), and used to determine acceptability of performance. Two lots of reagents (R2 and R3) were used to measure one high and one low calibrator, and four pooled samples that were different from those used in the precision study (PS A - PS D) in quadruplicate across three ADVIA Centaur systems, once daily for 4 days (n/sample = 144). PS A contained no 25(OH)D₃ spiking agent (approximate final 25[OH]D₃ concentration = 20 ng/mL). Spiking agent was used to adjust the final 25(OH)D₃ concentration in the other pooled samples: PS B = 50 ng/mL, PS C = 120 ng/mL, PS D = 50 ng/mL. In addition, two internal standard level control values assigned using the ADVIA Centaur Vitamin D Total assay, and two internal control lots were evaluated using each reagent lot. Value assignments were made for each sample following full curve analysis. Mean concentration was determined for each sample following calibration, and the SD was used to calculate the precision error. Bias between each value assignment and the mean concentration of each sample was determined and used to calculate TAE and %TAE:

$$\text{TAE} = |\text{Total bias}| + 2\text{SD}$$
$$\% \text{TAE} = (\text{TAE}/\text{assigned value}) \times 100\%.$$
¹⁵

2.7. CDC Vitamin D Standardization Certification Program (VDSCP) studies

The CDC VDSCP is a two-phase process. To become VDSCP certified, participants must pass four consecutive quarterly challenges using blinded samples after an initial calibration period (Phase I). Samples for both phases of the study are value-assigned using the VDSP NIST-Ghent RMP and supplied by the CDC. Results are analyzed by the CDC according to CLSI document EP9-A2 and used to determine bias, precision, and total error.^{16,17} Following the calibration period, 10 individual patient samples supplied by the CDC and containing blinded quantities of 25(OH)D-total were analyzed in duplicate over 2 days (n = 40) (Phase 2). Four quarterly blinded assessments were conducted over the course of 2013 (once quarterly) for a total of 160 replicates. Four quarterly assessments were also performed in subsequent years. The criterion for achieving CDC VDSCP certification is a mean bias for all 40 samples (160 results) of ±5% to the CDC and University of Ghent Vitamin D₂ and D₃ Reference Method, and an overall imprecision of <10%.¹⁸ Although the CDC normally supplies 40 samples for the initial calibration phase, 50 were sent to Siemens and tested.¹⁶

2.8. Alignment to a third-party VDSCP-certified method

The ADVIA Centaur Vitamin D Total assay was compared to the Endocrine Sciences Laboratory CDC VDSCP-certified ID-LC/MS/MS method (Esoterix, Endocrine Sciences, Calabasas Hills, CA; a LabCorp Specialty Testing Group Member).¹⁹ Samples (n = 149) were tested in singlicate using a single ADVIA Centaur reagent lot (R4). After testing, samples across the range of the assay were selected and sent to Esoterix for testing using their CDC VDSCP-certified ID-LC/MS/MS method.

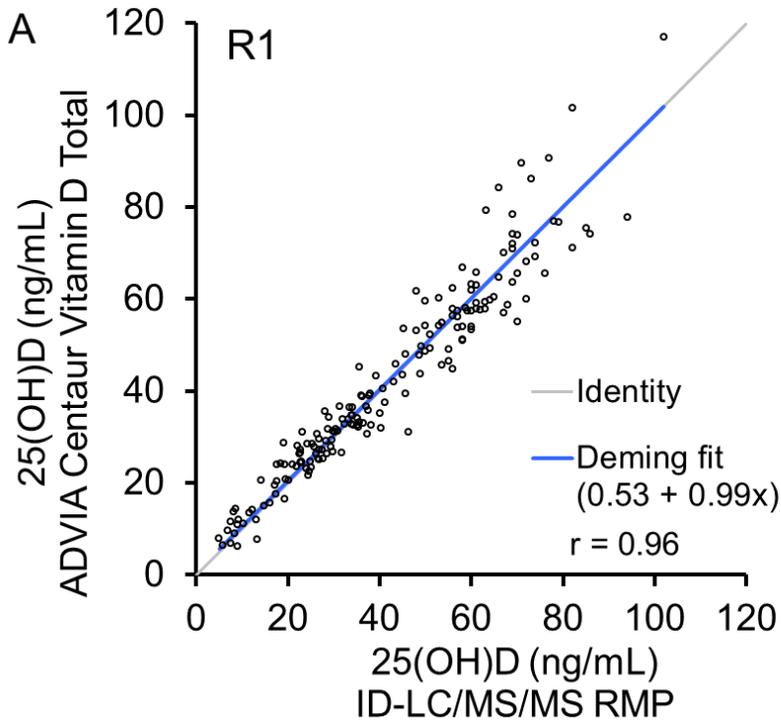
3. Results

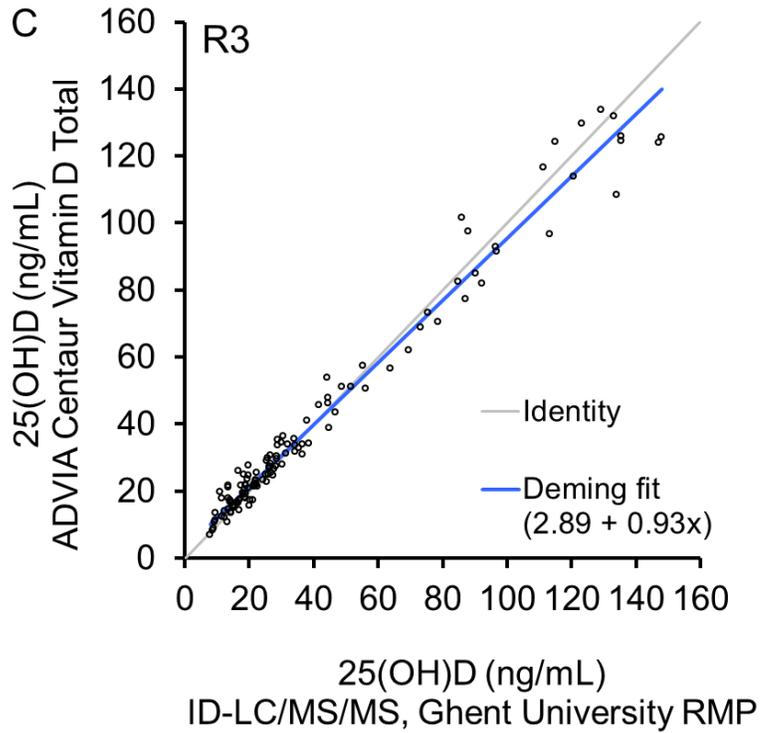
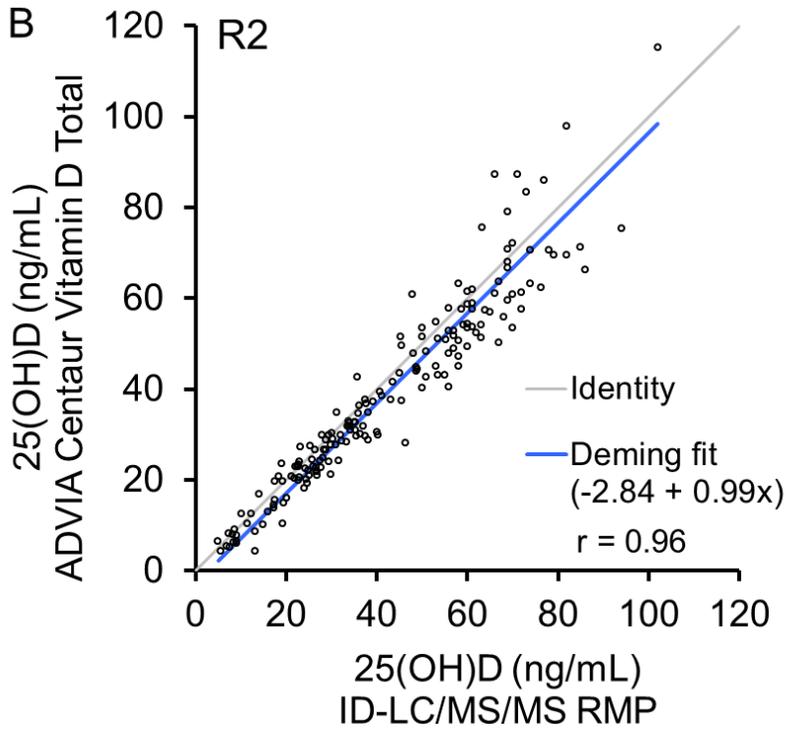
3.1. Alignment with the VDSP NIST-Ghent RMP

Deming regression analysis was performed for 177 samples in study 1 to compare the ADVIA Centaur Vitamin D Total assay results to the VDSP NIST-Ghent RMP-generated values. A correlation of 96% and slope of 0.99 for both reagent lots was demonstrated between the ADVIA Centaur Vitamin D Total assay results and the ID-

LC/MS/MS method. A small negative bias was observed with R2 but was not observed with R1 (Figures 1A and 1B). In the second study with 122 samples, Deming regression demonstrated similar results as Study 1 (Figure 1C). Bland-Altman analysis demonstrated a mean bias of 0.09 ng/mL (Figures 1C and 1D). Although greater bias was observed at higher concentrations, with the exception of a few outliers, bias did not exceed 12 ng/mL.

Figure 1.





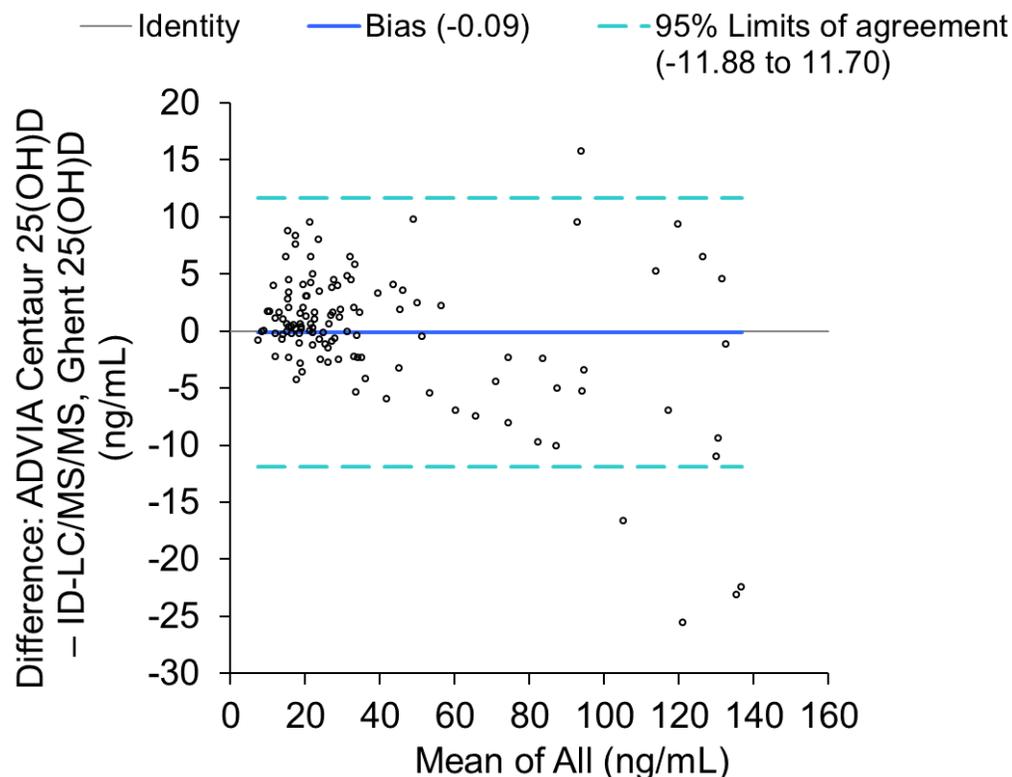


Figure 1. Deming regression analysis comparing the ADVIA Centaur Vitamin D Total assay for 177 samples with values assigned using methods aligned with the NIST–Ghent RMP for (A) R1 and (B) R2; (C) Deming regression and (D) Bland Altman analysis comparing the ADVIA Centaur Vitamin D Total assay for 122 samples with values assigned directly at Ghent University using their RMP, R3. To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5.

3.2. Comparison to CAP and DEQAS survey samples

The CAP and DEQAS acceptance requirement for total vitamin D is that 80% of results fall within 25% of the assigned target value. For the 2011 samples, all but one of the ADVIA Centaur Vitamin D Total assay

results (n = 30) were within 25% of the survey results, although some inconsistencies in percent bias between samples and between lots were observed (Table 1). Greater than half of the results were within 10% (eight of the 15 samples in R1 and ten of the 15 samples in R2).

Table 1. Comparison of R1 and R2 results with CAP and DEQAS survey results.

	Sample	Survey- Assigned Value	ADVIA Centaur RMP, Lot R1 (ng/mL)*	ADVIA Centaur RMP, Lot R2 (ng/mL)	Bias to Assigned Value, Lot R1	Bias to Assigned Value, Lot R2
DEQAS (ID- LC/MS/MS)	421	24.0	23.1	24.1	-3.7%	0.4%
	422	15.8	16.3	16.6	2.9%	5.2%
	423	35.9	31.6	31.8	-12.0%	-11.3%
	424	19.6	21.5	23.0	9.8%	17.1%
	425	19.6	19.1	19.8	-2.6%	1.0%
	426	14.1	13.8	16.8	-2.0%	19.2%
	427	32.1	26.2	29.2	-18.3%	-9.1%
	428	22.1	19.2	20.1	-13.0%	-9.1%
	429	24.2	23.8	24.2	-1.5%	0.1%
	430	17.6	21.2	23.1	20.3%	31.4%
CAP	ABVD 1	20.80	22.29	25.14	7.2%	20.4%
	ABVD 2	14.60	14.14	14.56	-3.2%	-0.3%
	ABVD 3	32.60	27.88	29.51	-14.5%	-9.5%
	ABVD 4	58.50	50.06	53.71	-14.4%	-8.2%
	ABVD 5	39.90	39.20	41.13	-1.7%	3.1%

*To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5. CAP: College of American Pathologists; DEQAS: Vitamin D External Quality Assessment Scheme; ID-LC/MS/MS: Isotope dilution liquid chromatography mass spectrometry; ABVD: accuracy-based vitamin D survey samples.

When compared to the VDSP NIST-Ghent RMP for DEQAS survey sample 421–470, the smallest percent bias observed was 0.2%, while the greatest percent bias was 45.2%. Percent bias > 25% (the maximum

allowable by DEQAS) was observed for only four of the 50 DEQAS samples (8%). Median percent bias was 1.7% for all results, and most samples were within 10% of the assigned value (Figure 2).

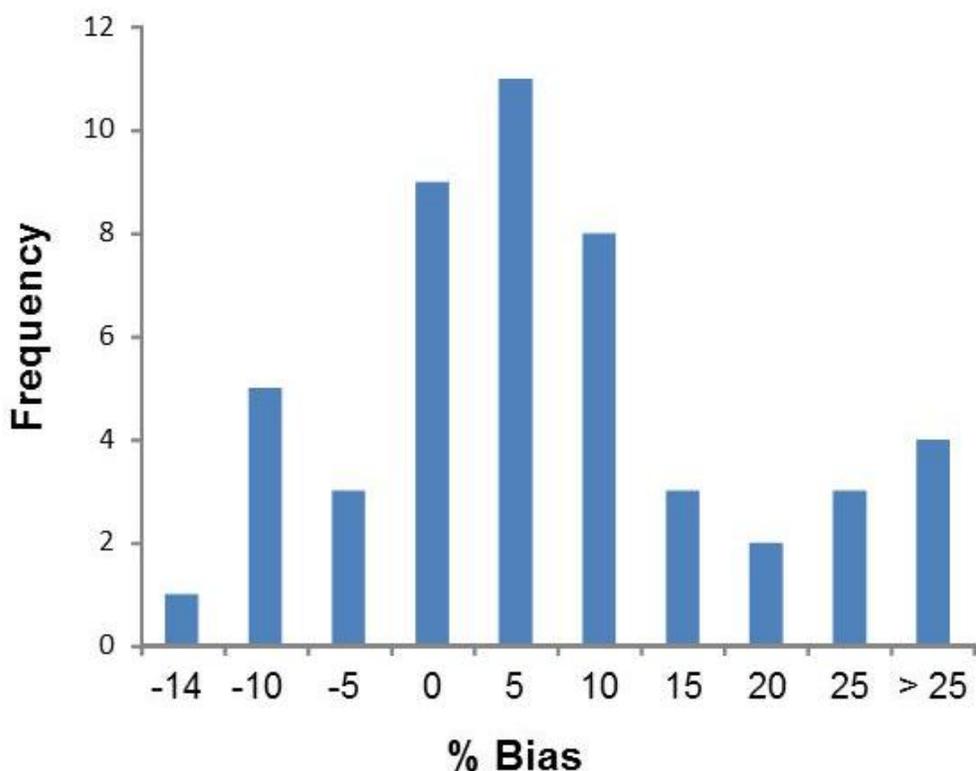


Figure 2. Distribution of percent bias for all 50 DEQAS samples (2012–2015).

3.3. Imprecision

The VDSCP requires <10% imprecision (CV).²⁰ To assure that was achieved, tolerance for repeatability variance was <8% for samples between 20 and 30 ng/mL (50–75 nmol/L) and <7% for samples

between >30 to 150 ng/mL (>75 to 374 nmol/L). Within run specifications were met, although the total %CV for one sample using one lot on two systems was slightly greater than the maximum acceptable (Table 2).

Table 2. Precision study results for the standardized ADVIA Centaur Vitamin D Total assay for individual samples (SHS1 and SHS2) and pooled sera (PS1–4), using R1 and R2.

System	Lot	Sample	Mean (ng/mL)*	Within Run		Total	
				SD	CV	SD	CV
A	R1	SHS1	13.60	0.64	4.7	1.61	11.9
		SHS2	17.22	0.91	5.3	1.70	9.9
		PS 1	28.20	1.45	5.2	2.02	7.2
		PS 2	46.08	1.79	3.9	2.79	6.1
		PS 3	73.22	2.71	3.7	4.36	6.0
		PS 4	114.08	3.44	3.0	4.77	4.2
	R2	SHS1	11.90	0.45	3.8	0.95	8.0
		SHS2	15.26	0.56	3.7	1.35	8.9
		PS 1	23.25	1.19	5.1	1.88	8.1
		PS 2	41.45	1.48	3.6	2.22	5.3
		PS 3	69.00	3.11	4.4	4.10	5.9
		PS 4	112.25	3.47	3.1	4.22	3.8
B	R1	SHS1	15.17	0.61	4.0	1.98	13.0
		SHS2	19.77	0.90	4.6	1.76	8.9
		PS 1	31.00	1.30	4.2	2.36	7.6
		PS 2	50.26	1.83	3.6	3.94	7.8
		PS 3	78.95	3.37	4.3	6.99	8.9
		PS 4	120.31	1.99	1.7	5.25	4.4
	R2	SHS1	14.30	0.71	5.0	1.31	9.1
		SHS2	17.90	0.98	5.5	1.47	8.2
		PS 1	25.34	1.49	5.9	1.69	6.7
		PS 2	45.79	2.40	5.2	3.72	8.1
		PS 3	74.83	3.53	4.7	6.39	8.5
		PS 4	117.34	2.94	2.5	4.81	4.1

*To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5. SD: Standard deviation. CV: Coefficient of variation.

3.4. Performance evaluation using total analytical error (TAE)

According to calculations by Stöckl et al., total error based on the DEQAS limit for error should be less than 46%.²⁰ Siemens

chose more stringent criteria, requiring %TAE to be less than $\pm 45\%$ for samples between 2 and 20 ng/mL, and less than $\pm 35\%$ for samples over 20 ng/mL. These specifications were met for all samples (Table 3).

Table 3. Total analytical error (TAE) for samples with assigned values between 17 and 121 ng/mL, using R2 and R3.

Sample	VA ^a (ng/mL) *	Average Dose (ng/mL)*	Bias	Observed SD Total	Observed CV Total	TAE (Bias + 2SD)	%TAE
Lot 1 high calibrator	116.54	118.36	1.81	4.85	4.1	11.5	9.9
Lot 1 low calibrator	28.43	28.31	-0.12	3.77	13.3	7.7	26.9
Lot 2 high calibrator	104.01	103.05	-0.97	5.02	4.9	11.0	10.6
Lot 2 low calibrator	23.33	23.62	0.29	3.64	15.4	7.6	32.5
Ctrl lot 1	25.18	25.32	0.15	3.51	13.9	7.2	28.5
Ctrl lot 2	100.30	98.81	-1.49	6.61	6.7	14.7	14.7
PS A	25.92	26.01	0.09	3.26	12.5	6.6	25.5
PS B	53.56	51.61	-1.95	3.61	7.0	9.2	17.1
PS C	120.88	124.36	3.48	4.27	3.4	12.0	9.9
PS D	52.39	50.51	-1.88	3.51	6.9	8.9	17.0
Internal standard ^b	17.93	17.84	-0.09	2.35	13.2	4.8	26.7

^aValue assignment. ^bInternal standards have values assigned by method comparison to the University of Ghent RMP. *To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5. PS: Pooled sera; SD: Standard deviation; CV: Coefficient of variation.

3.5. CDC Vitamin D Standardization Certification Program (VDSCP) studies

The correlation between the CDC VDSP NIST-Ghent RMP-assigned values and the ADVIA Centaur Vitamin D Total assay values was strong across all four quarterly blind trials conducted in 2013 (Figure 3). The mean bias was 0.3% (SD = 16.8,

95% CI = -5.0 to 5.6), the mean CV was 5.5% (SD = 3.2, 10th percentile = 1.6%, 90th percentile = 9.8%), and 86% of samples in the challenge groups were within the suggested total error of $\pm 21.5\%$. Similar results were obtained across all four quarterly trials conducted for each consecutive subsequent year to date (data not shown).

A n | 160
 R² | 0.9018

Term	Coefficient	95% CI	SE	P
Intercept	-1.8893	-6.3943 2.6157	2.2809	0.4088
Slope	1.0134	0.9608 1.0660	0.0266	<0.0001

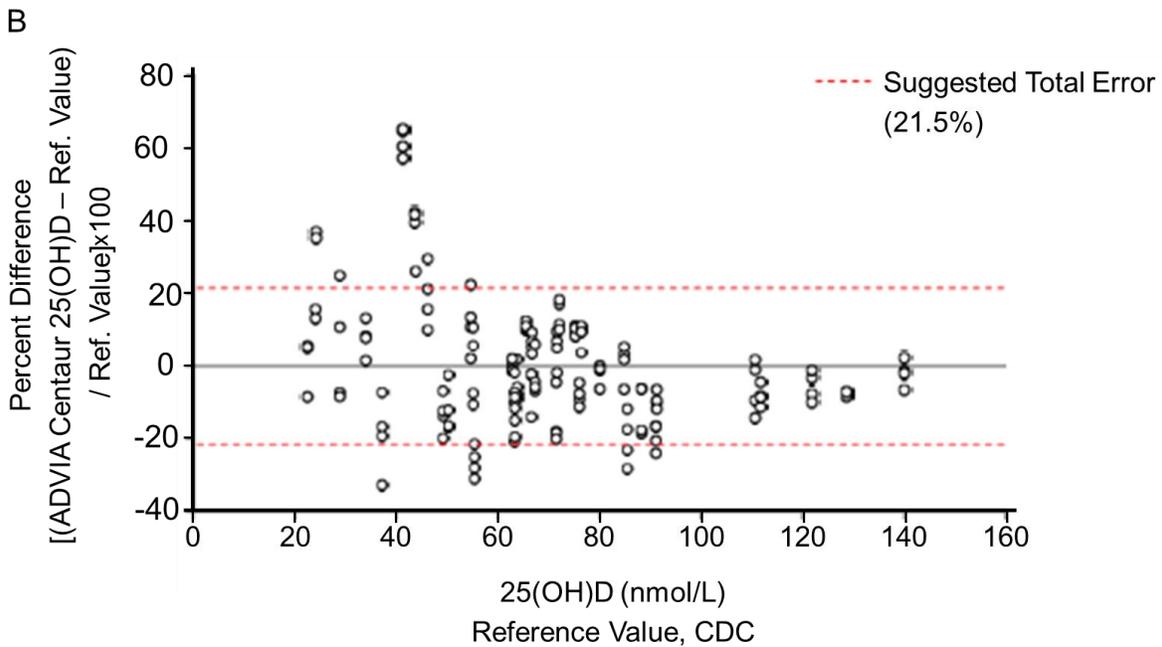
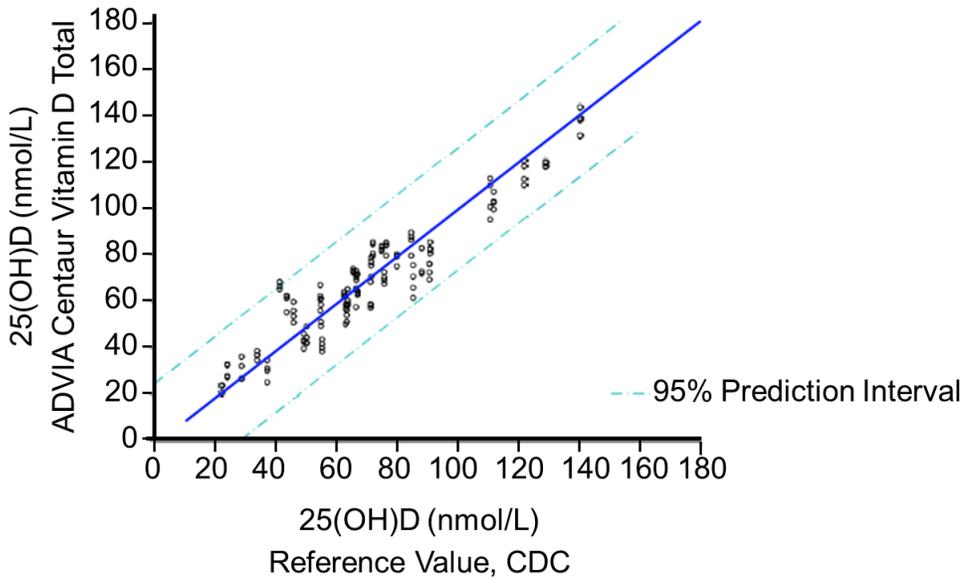


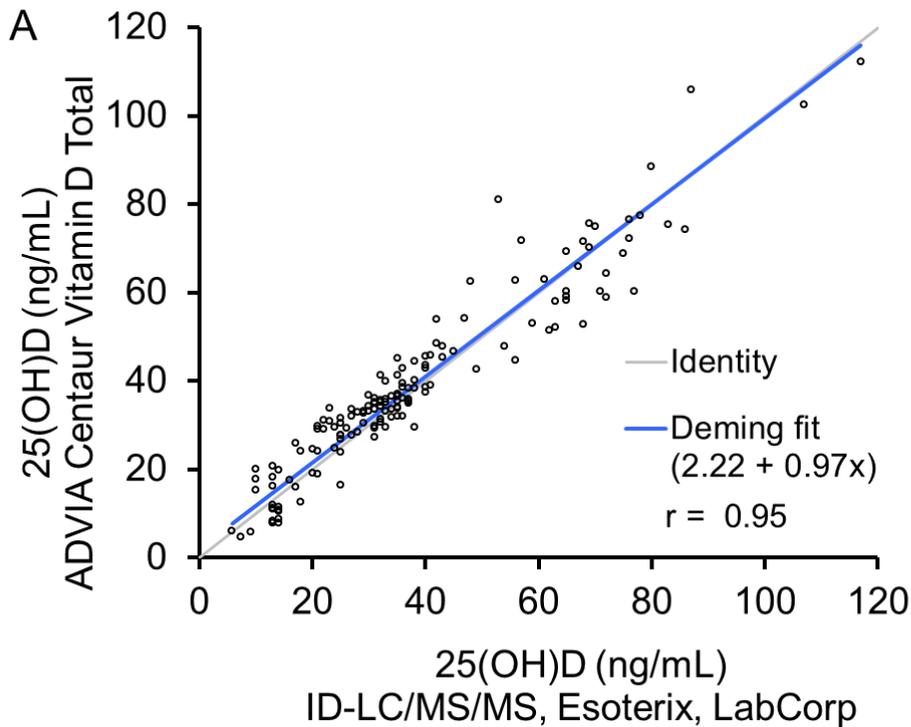
Figure 3. (A) Linear regression and (B) total error generated by CDC VDSCP from all challenge samples in 2013. To convert 25(OH)D concentrations to nanograms per mL (ng/mL), divide by 2.5.

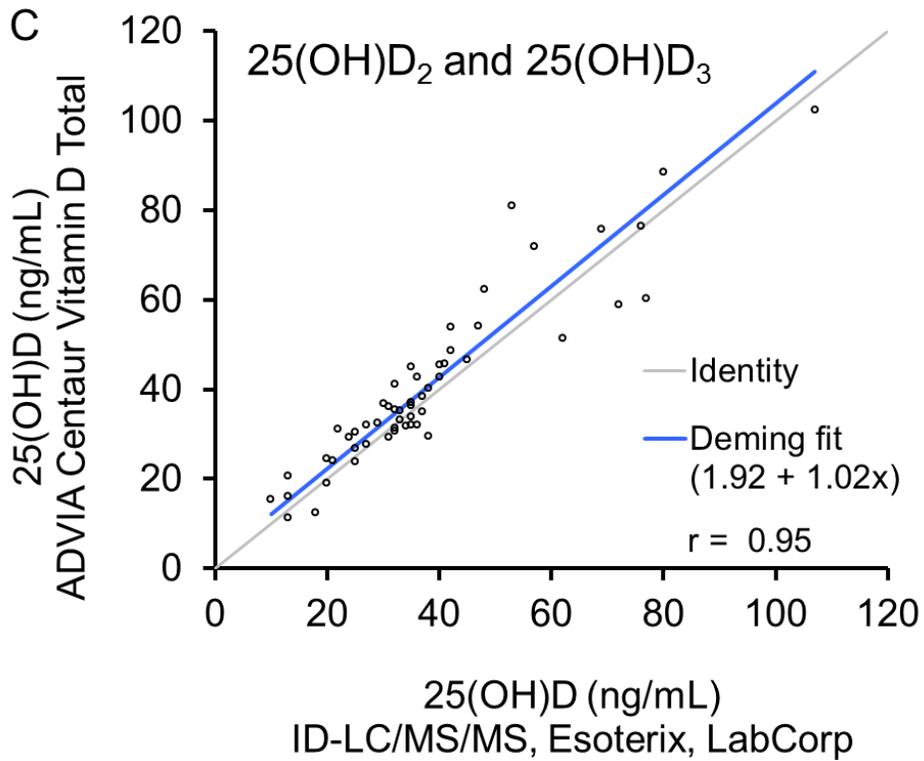
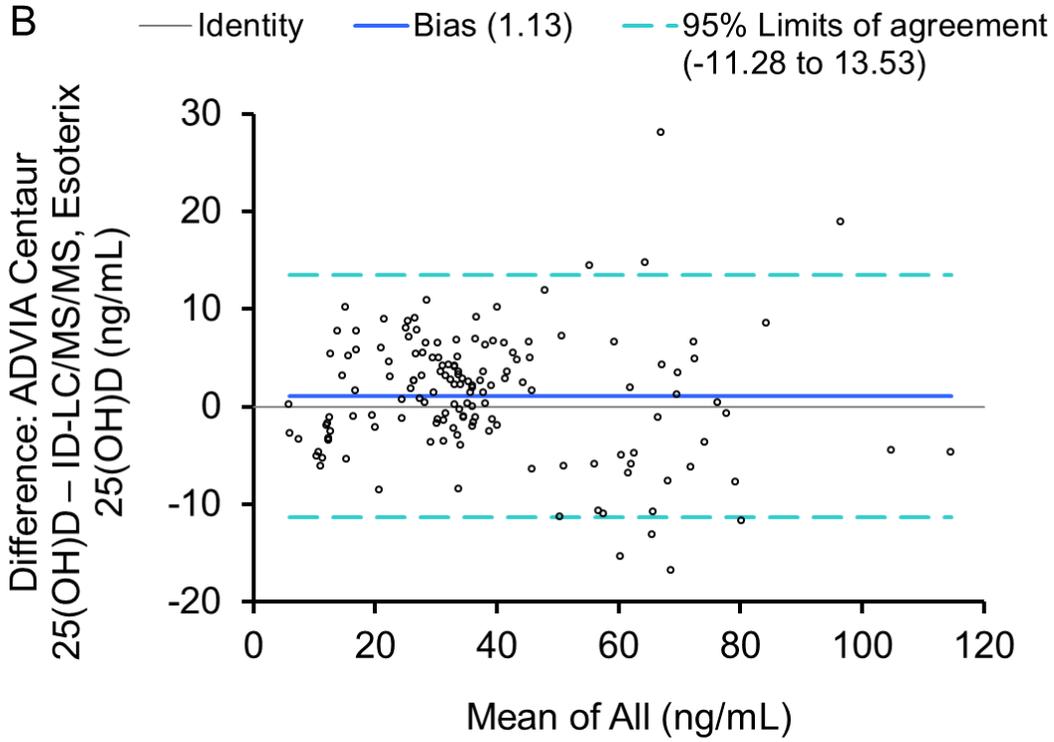
3.6. Alignment to a third-party VDSCP-certified method

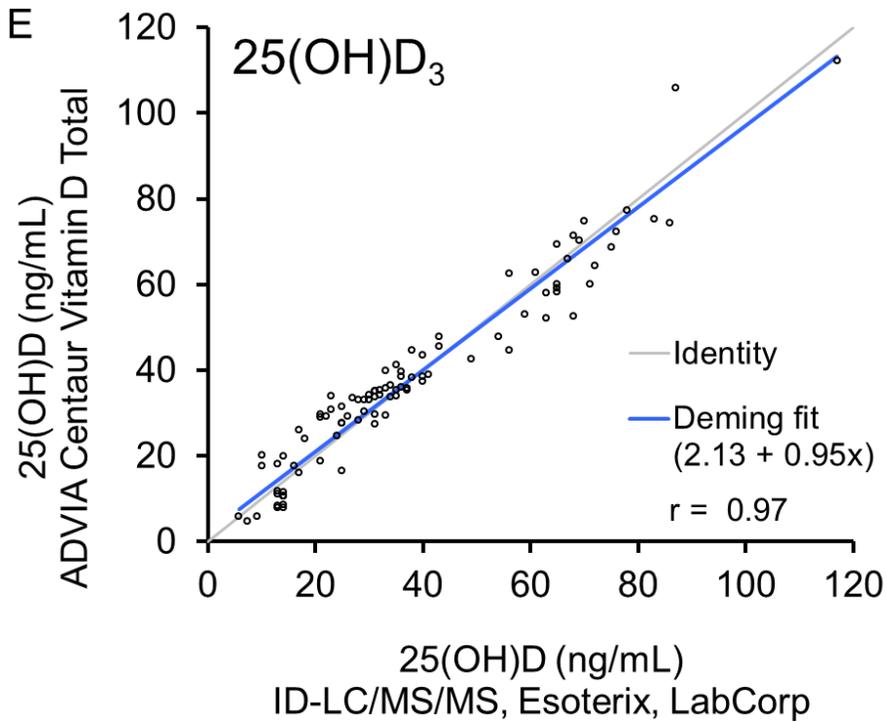
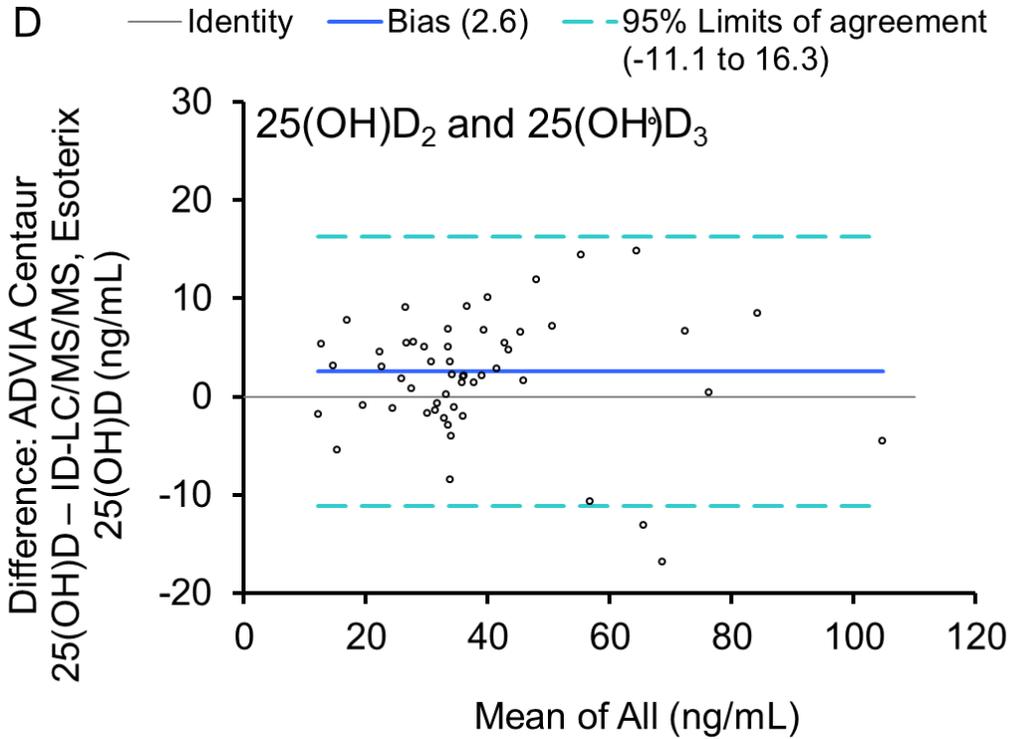
The ADVIA Centaur Vitamin D Total assay aligned well with the Esoterix method (n = 149) as demonstrated by both Deming regression (Pearson's r = 0.95) and Bland-Altman analysis (bias = 1.13) (Figure 4A and B). The Esoterix assay, which is

capable of differentiating between the 2 primary forms of 25(OH)D, identified 55 samples containing 25(OH)D₂.¹⁹ The two assays were harmonized regardless of whether the sample contained both 25(OH)D₂ and 25(OH)D₃ (n = 55, Pearson's r = 0.95, bias = 2.6) (Figure 4C and 4D) or 25(OH)D₃ only (n = 94, Pearson's r = 0.97, bias = 0.26) (Figures 4E and 4F).

Figure 4.







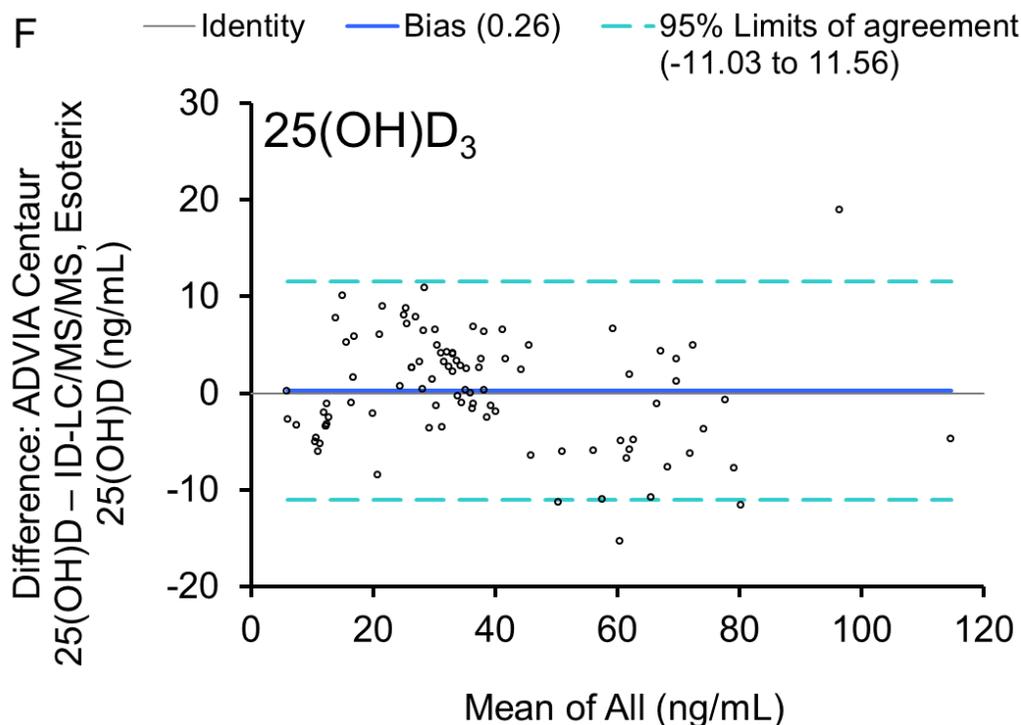


Figure 4. Alignment was demonstrated between the Siemens ADVIA Centaur Vitamin D Total immunoassay and the Esoterix ID-LC/MS/MS Vitamin D method for 149 samples. (A) Deming regression and (B) Bland-Altman analysis for all 149 samples for total vitamin D; C–F: Deming regression and Bland-Altman analysis, comparing the Esoterix and Siemens assays for those 55 samples containing both 25(OH)D₂ and 25(OH)D₃ (C and D) and the 94 samples containing 25(OH)D₃ alone (E and F). To convert 25(OH)D concentrations to nanomoles per liter (nmol/L), multiply by 2.5.

4. Discussion

As pointed out by the U.S. Office of Dietary Standards, variability between 25(OH)D assays has hampered efforts to compare vitamin D levels across studies as well as the development of diagnostic cut points and therapeutic guidelines.^{11,13} Standardization of assays would alleviate these problems.^{9,11,13} In this study, we report standardization and continued adherence of the Siemens ADVIA Centaur Vitamin D Total assay to the VDSP NIST-Ghent RMP.

The previous calibration for the ADVIA Centaur Vitamin D Total assay was observed to be positively biased when compared to both the VDSP NIST-Ghent RMP and the ADVIA Centaur Vitamin D

Total recalibration (data not shown). Previous studies have found that the ADVIA Centaur Vitamin D Total assay over-reported vitamin D levels.²¹ Those previous studies were performed using the non-VDSP NIST-Ghent RMP standardized version of the Siemens assay and would need to be repeated using the standardized assay. Additionally, concentration-dependent variability of vitamin D binding protein has been reported when using the original Siemens assay.⁵ The authors commented that alignment to the VDSP NIST-Ghent RMP (which at the time was still under consideration) should resolve the issue. A subsequent study using the standardized ADVIA Centaur Vitamin D Total assay was found to accurately measure vitamin D in samples from different

populations with elevated vitamin D binding protein levels.²²

In the present report, precision and TAE performance acceptability criteria were fulfilled for the standardized ADVIA Centaur Vitamin D Total assay. Regression and bias analyses of two sample sets confirm that the standardized ADVIA Centaur Vitamin D Total assay correlates well with the VDSP NIST-Ghent RMP for directly assigned values and also for values from metrologically traceable methods. DEQAS and CAP survey results support this claim: for R1 of the standardized assay, 100% of the results were within 25% of both the CAP and DEQAS samples, and 97% of results for R2 were within 25% of the CAP and DEQAS established values, demonstrating that the assay met the CAP and DEQAS proficiency testing criteria. Furthermore, 86% of samples tested with R1 and 80% of the samples tested with R2 yielded values that were within 15.8% of the survey-assigned values, which has been reported to be the allowable limit for assay bias.⁶ The Esoterix assay was independently standardized to the VDSP NIST-Ghent RMP and independently certified by the VDSCP. The alignment to the Esoterix assay provided further evidence that the ADVIA Centaur Vitamin D Total assay was standardized to the VDSP NIST-Ghent RMP. Correlation of the ADVIA Centaur Vitamin D Total assay with the Esoterix ID-LC/MS/MS assay of 25(OH)D₂-containing samples, along with detection of 25(OH)D₂-spiked samples used in the TAE performance evaluation study, show that the assay detected both 25(OH)D₂ and 25(OH)D₃ with equal reliability.

The CDC VDSCP program was initiated as an adjunct program to the VDSP to establish a protocol for certifying that vitamin D assays remain standardized to the VDSP NIST-Ghent RMP. Participants must pass four consecutive quarterly yearly

challenges using blinded samples: Mean bias for all 40 samples (160 results) must be $\pm 5\%$ of the CDC values and overall imprecision must be $<10\%$.²⁰ Certification must be renewed annually. The standardized ADVIA Centaur Vitamin D Total assay performed appreciably better than the criteria, becoming one of the first assays to be certified by the CDC, one of only three immunoassays certified as of February 2014 as part of the initial certification program, and to our knowledge, the only fully automated assay to become certified for four consecutive years (2014–2017).

5. Conclusion

The CDC VDSCP certification attests to the performance of the Siemens ADVIA Centaur Vitamin D Total assay and its adherence to the VDSP. This certification, in conjunction with the performance and method comparison data indicate that the Siemens ADVIA Centaur Vitamin D Total assay, traceable to the VDSP NIST-Ghent RMP, meets the acceptance criteria for use in the clinical laboratory.

Disclosure

All authors are employees of Siemens Healthineers.

Conflicts of interest:

The authors declare that there are no conflicts of interest regarding the publication of this paper. All authors are employees of Siemens Healthineers.

Acknowledgments:

This study was supported by Siemens Healthineers. The authors would like to thank V. Shalhoub, PhD for help with this manuscript.

References

1. Dietary reference intakes for calcium and vitamin D. 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. Washington (DC): National Academies Press (US); 2011. DOI: 10.17226/13050
2. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2011;96:1911-30. DOI: 10.1210/jc.2011-0385
3. 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;57:1618-9. DOI: 10.1373/clinchem.2011.170282
4. Lensmeyer GL, Wiebe DA, Binkley N, et al. HPLC method for 25-hydroxyvitamin D measurement: comparison with contemporary assays. *Clin Chem.* 2006;52:1120-6. DOI: 10.1373/clinchem.2005.064956
5. Heijboer AC, Blankenstein MA, Kema IP, et al. Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. *Clin Chem.* 2012;58:543-8. DOI: 10.1373/clinchem.2011.176545
6. Farrell CJ, Martin S, McWhinney B, et al. 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. DOI: 10.1373/clinchem.2011.172155
7. Carter GD. 25-hydroxyvitamin D: a difficult analyte. *Clin Chem.* 2012;58:486-8. DOI: 10.1373/clinchem.2011.180562
8. Carter GD. Accuracy of 25-hydroxyvitamin D assays: confronting the issues. *Curr Drug Targets.* 2011;12:19-28. URL: <https://www.ncbi.nlm.nih.gov/pubmed/20795940>:
9. Thienpont LM, Stepman HC, Vesper HW. Standardization of measurements of 25-hydroxyvitamin D₃ and D₂. *Scand J Clin Lab Invest Suppl.* 2012;243:41-9. DOI: 10.3109/00365513.2012.681950
10. Stepman HC, Vanderroost A, Van Uytvanghe K, et al. Candidate reference measurement procedures for serum 25-hydroxyvitamin D₃ and 25-hydroxyvitamin D₂ by using isotope-dilution liquid chromatography-tandem mass spectrometry. *Clin Chem.* 2011;57:441-8. DOI: 10.1373/clinchem.2010.152553
11. Sempos CT, Vesper HW, Phinney KW, et al. Vitamin D status as an international issue: national surveys and the problem of standardization. *Scand J Clin Lab Invest Suppl.* 2012;243:32-40. DOI: 10.3109/00365513.2012.681935
12. Phinney KW, Bedner M, Tai SS, et al. Development and certification of a standard reference material for vitamin D metabolites in human serum. *Anal Chem.* 2012;84:956-62. DOI: 10.1021/ac202047n
13. National Institutes of Health, Office of Dietary Supplements: <http://ods.od.nih.gov/Research/VitaminD.aspx>. Accessed June 20, 2017.

14. Carey R, Anderson F, Georg H, et al. EP15-A2: User verification of performance for precision and trueness; Approved Guideline--Second Edition. Clinical and Laboratory Standards Institute (CLSI); 2005. URL: www.clsi.org
15. Westgard JO, Westgard SA. Total analytic error: From concept to application. Clinical Laboratory News. 2013;39. URL: <http://www.aacc.org/publications/cln/2013/september/Pages/Total-Analytic-Error.aspx#!>:
16. Vesper HW, Rahmani YE. Vitamin D standardization certification program: standardization of total serum 25-hydroxyvitamin D measurements. In: Sciences DoL, ed. Atlanta, GA: Centers for Disease Control; 2012:1-5. URL: <http://www.cdc.gov/labstandards>
17. Clinical and Laboratory Standards Institute/NCCLS. Method comparison and bias estimation using patient samples. Approved Guideline--Second Edition. CLSI/NCCLS document EP9-A2 [ISBN 1-56238-472-4] CLSI, Wayne, PA, USA, 2002. URL: www.clsi.org
18. Vitamin D Standardization-Certification Program (VDSCP--Total 25hydroxy Vitamin D Certified Procedures (Updated 03/2017) URL: https://www.cdc.gov/labstandards/pdf/hs/CD_C_Certified_Vitamin_D_Procedures.pdf
19. Customer bulletin: Evaluating vitamin D metabolism using HPLC LC/MS-MS. Endocrine Sciences Laboratory. LabCorp (Laboratory Corporation of America). 2014. URL: <http://testmenu.labcorp.com>. (Accessed April 5, 2016).
20. Stöckl D, Sluss PM, Thienpont LM. Specifications for trueness and precision of a reference measurement system for serum/plasma 25-hydroxyvitamin D analysis. Clin Chim Acta. 2009;408:8-13. DOI: 10.1016/j.cca.2009.06.027
21. Farrell CJ, Soldo J, McWhinney B, et al. Impact of assay design on test performance: lessons learned from 25-hydroxyvitamin D. Clin Chem Lab Med. 2014;52:1579-87. DOI: 10.1515/cclm-2014-0111
22. Freeman J, Wilson K, Spears R, et al. Influence of vitamin D binding protein on accuracy of 25-hydroxyvitamin D measurement using the ADVIA Centaur Vitamin D Total assay. Int J Endocrinol. 2014;2014:691679. DOI: 10.1155/2014/691679