

White Paper

# Performance Characteristics of the ADVIA Centaur Vitamin D Total Assay Now Standardized to the Vitamin D Reference Measurement Procedure

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

# Performance Characteristics of the ADVIA Centaur Vitamin D Total Assay Now Standardized to the Vitamin D Reference Measurement Procedure

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

## Introduction

Measurement and monitoring of the vitamin D metabolite 25-hydroxy vitamin D (25[OH]D) is important in patients who are suspected of vitamin D insufficiency or deficiency contributing to underlying diseases affecting bone and calcium metabolism. Many commercial in vitro diagnostic device manufacturers, commercial laboratories, and research laboratories have developed assays for 25(OH)D total (i.e., the combined concentrations of 25[OH]D<sub>2</sub> and 25[OH]D<sub>3</sub>). However, substantial disparity in determined values has been noted among many of these methods, raising the question as to which methods can reliably yield accurate results of metabolically relevant levels. Perhaps of even greater importance, lack of standardization among methods raises physician concern that repeated measurements in patients across time and location can be so affected by method differences as to potentially reduce the meaningfulness of longitudinal results.<sup>1-6</sup>

For this reason, the NIH Office of Dietary Supplements (ODS) instituted the Vitamin D Standardization Program (VDSP) in November 2010. 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), the program goal is to provide laboratories and manufacturers

with reference materials and a reference protocol to standardize the laboratory measurement of vitamin D.<sup>2,7-10</sup> To complement this program, manufacturers and laboratories may participate in a certification process administered by the CDC: the Vitamin D Standardization – Certification Program (VDSCP).<sup>11</sup> The CDC laboratory standards website can be accessed at [http://www.cdc.gov/labstandards/hs\\_procedures.html](http://www.cdc.gov/labstandards/hs_procedures.html).

Siemens Healthcare Diagnostics is an early adopter of the VDSP because the company recognizes the importance of providing an assay metrologically traceable to the 25(OH)vitamin D reference measurement procedure (RMP), the reference procedure for the VDSP. Siemens achieved this goal by re-standardizing the ADVIA Centaur® Vitamin D Total assay\* (abbreviated in this paper as VitD-RMP) to the Ghent established standards. Likewise, because of its high commitment to patient care, Siemens is an early participant in the CDC VDSCP. The first six assays to achieve certification through this program were announced in February 2014. The VitD-RMP assay was among this group of flagship assays. Of these, only the Siemens assay and one other commercial method are automated immunoassays. This article reports on the performance characteristics and CDC certification of the RMP-standardized assay.

## Study Results

The VitD-RMP was evaluated to ascertain standard performance characteristics, namely limit of blank (LoB), limit of detection (LoD), limit of quantitation (LoQ), LoQ at 20% total imprecision (%CV), dilution linearity and recovery, and precision. Method comparison studies established agreement between the new RMP-standardized assay and the Ghent 25(OH)vitamin D RMP (Ghent RMP), which is an ID-LC/MS/MS method. The new assay was also evaluated using the College of American Pathologists (CAP) and the Vitamin D External Quality Assessment Scheme (DEQAS) survey samples, and results were compared to sample means established by those surveys. Finally, the assay was evaluated according to the specifications of the VDSCP. All studies were performed using ADVIA Centaur Vitamin D Total reagent lots standardized to the Ghent RMP.

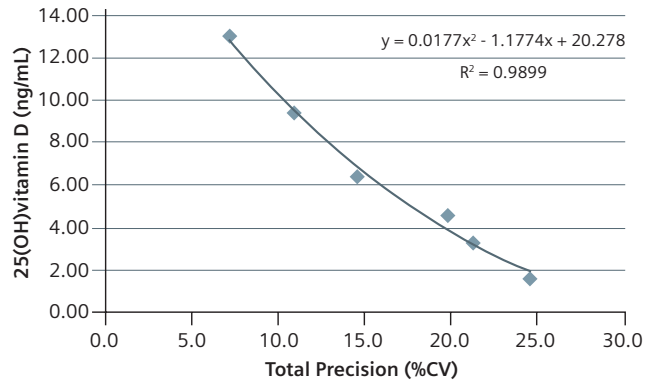
## Performance characteristics LoB, LoD, and LoQ

The LoB, LoD, and LoQ were established during the original validation process for the assay (i.e., at the time the assay was calibrated to a method other than the Ghent RMP). Studies to establish these essential performance characteristics were conducted to confirm that calibration to the RMP did not significantly alter these values. The mean LoB for the VitD-RMP was determined to be 1.2 ng/mL using five acid- and charcoal-treated human serum samples containing minimal or no vitamin D. However, because the greatest LoB observed in the original performance evaluation study of the non-RMP-calibrated assay was 1.69, it was decided to retain the originally established LoB (1.7 ng/mL).

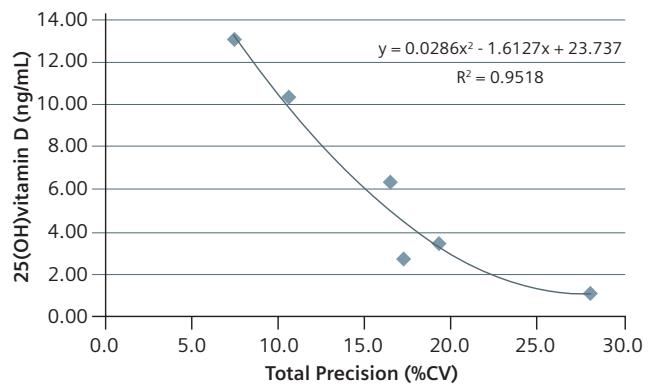
LoD was determined to be 2.3 ng/mL using six samples containing low vitamin D levels prepared by diluting patient serum samples containing low-level total vitamin D with vitamin D-stripped human serum. LoD was determined to be 3.2 ng/mL for the original calibration, however, so as with the LoB, the decision was made to retain the LoD at the greater concentration supported in the original claim. Since the calibration set supplied by the Ghent RMP comprises 50 serum samples containing vitamin D total ranging from 5.04 to 60 ng/mL, an LoD of 3.2 ng/mL confirms that the assay can detect the lowest concentration of vitamin D included in the sample set.

LoQ was calculated as the lowest measurement at which 20% CV could be achieved. A quadratic fit was applied to data from two reagent lots independently and to data from both lots together. The greatest of the three values, which was 3.8 ng/mL, was used for the LoQ (Figure 1).

A. Lot 1; LoQ = 3.8 ng/mL



B. Lot 2; LoQ = 2.9 ng/mL



C. Combined lots; LoQ = 3.4 ng/mL

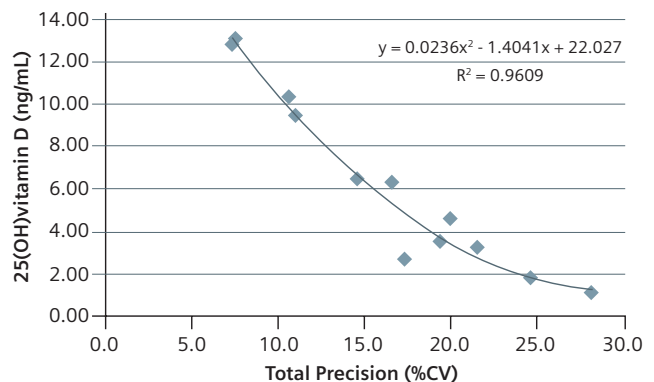
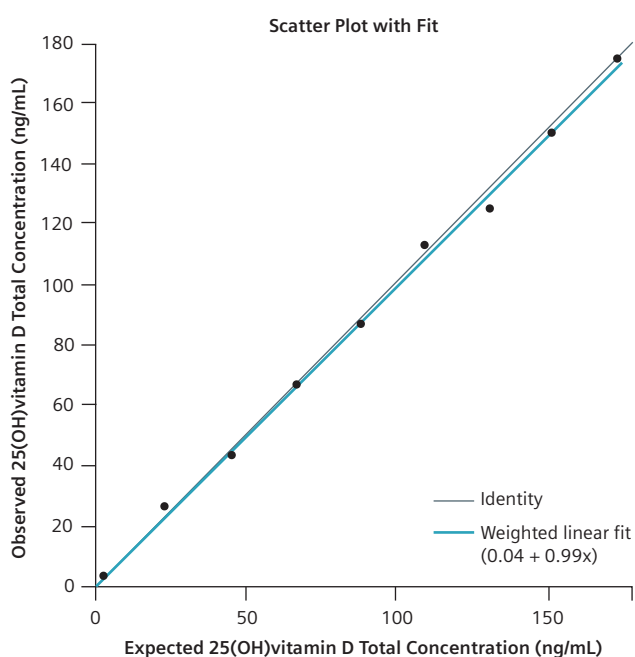


Figure 1. Quadratic fit for each reagent lot individually and combined.

In keeping with the decision to retain the higher LoB and LoD established using the original calibration, the original LoQ of 4.2 ng/mL was also retained. At this level the CV is ~18–19%. The slightly decreased imprecision around the LoQ allows for confident measurement of the lowest sample in the Ghent calibration set (5.04 ng/mL), justifying the low end of the assay range at 4.2 ng/mL.

### Assay linearity

Linearity was analyzed across a series of dilutions constructed from a high sample (~180 ng/mL, which is above the reported range of the assay) and a low sample with a concentration near the LoD (2.92 ng/mL). Dilutions spanned the assay range and were run on a single analyzer in triplicate using two reagent lots. The difference between the anticipated and actual dose was less than 10% for each individual sample, an indication of good linearity and constant imprecision (%CV) across the assay range. Weighted least squares linear regression analysis using a constant %CV confirmed that the assay performs linearly across the projected range (Figure 2).



**Figure 2.** Linearity assessment using weighted linear regression comparing the mean observed (y) vs. expected (x) results for lowest through highest concentration samples. Results shown are for only one reagent lot, but are representative of both. The weighted linear fit for the second reagent lot was  $0.03 + 99x$ .

### Dilution of over-range samples

Although hypervitaminosis D is a rare event, it can occur in patients who are oversupplementing, either through prescription or self-administration. Vitamin D toxicity results in hypercalcemia, which can lead to bone, soft-tissue, kidney, and heart damage. Levels >150 ng/mL are considered toxic. Because the upper assay range is 150 ng/mL, sera above range require dilution for accurate measurement.<sup>13</sup> Dilution recovery was evaluated on over-range samples. Ten individual serum samples were spiked with 25(OH)vitamin D<sub>3</sub> stock solution to levels calculated to be between 130 and 190 ng/mL. Samples were measured neat as well as diluted 1:2 using vitamin D diluent. Dilutions were made both manually and using the system's onboard dilution capability, and then run in triplicate using two reagent lots on one ADVIA Centaur system and one ADVIA Centaur XP system. Percent recovery was calculated for manual dilution in relation to the neat concentration, and for manual dilution vs. onboard dilution.

$$\text{Percent recovery}_{\text{Neat vs. manual dilution}} = (\text{Manual dilution dose} / \text{Neat dose}) * 100$$

$$\text{Percent recovery}_{\text{Manual vs. onboard dilution}} = (\text{Onboard dilution dose} / \text{Manual dilution dose}) * 100$$

The mean percent recovery was close to 100% for both reagent lots on both systems (Table 1), indicating that high-dose samples can be diluted either manually or by onboard automated dilution to yield clinically interpretable measurements.

### Assay imprecision and fitness for clinical use

Imprecision was evaluated using two human serum samples and four pooled serum samples prepared from 25(OH)D<sub>3</sub>-spiked human serum (~20 to ~120 ng/mL) run over 20 days, 2 runs per day, 2 replicates per run (n = 80). The RMP reagent master curves and calibrator assignments were used to calculate reproducibility and within-lab %CV.

The VDSP requires ≤ 10% imprecision.<sup>14</sup> The precision data summarized in Table 2 indicate that specifications were met within run, although the total %CV for one sample using one lot on two of the systems was slightly greater than the maximum acceptable. Note that the greater than optimal imprecision corresponds to samples with low vitamin D levels associated with deficiency. According to the Endocrine Society guideline, risk for deficiency occurs when vitamin D total is less than 20 ng/mL. Applying the %CV to each of these two samples would still result in

Performance Characteristics of the ADVIA Centaur Vitamin D Total Assay  
Now Standardized to the Vitamin D Reference Measurement Procedure

**Table 1.** Summary data for the manual and onboard dilution of over-range samples using the VitD-RMP.

A. Example data from one system and one lot

Sample	Neat	Manual Dilution		Onboard Dilution	Bias	
	Dose (ng/mL)	Result (ng/mL)	Calculated Dose (ng/mL)	Result (ng/mL)	Neat vs. Manual	Manual vs. Onboard
1	138.60	73.18	146.36	146.33	106%	100%
2	141.06	81.23	162.45	150.17	115%	92%
3	145.48	81.42	162.84	150.29	112%	92%
4	152.4	84.83	169.66	157.60	111%	93%
5	162.5	85.33	170.66	160.65	105%	94%
6	NA	87.65	175.29	163.28	NA	93%
7	NA	90.22	180.45	165.20	NA	92%
8	NA	92.70	185.39	174.39	NA	94%
9	NA	95.47	190.94	194.35	NA	102%
10	NA	96.35	192.70	195.67	NA	102%
				<b>Mean</b>	<b>109.8%</b>	<b>95.4%</b>

B. Summary of mean biases for both systems and lots

Lot	System	Bias	
		Neat vs. Manual	Manual vs. Onboard
1	ADVIA Centaur XP	109.8%	95.4%
	ADVIA Centaur	102.8%	101.4%
2	ADVIA Centaur XP	97.8%	100.2%
	ADVIA Centaur	92.8%	105.4%

vitamin D below the cutoff for deficiency (15.22 ng/mL and 17.14 ng/mL), thus the clinical diagnosis would not be affected.

In a separate study, total analytical error (TAE) and %TAE were calculated from overall precision error (standard deviation [SD] and bias) and used to determine fitness for use. Three reagent lots were evaluated. Lot-specific high and low calibrators were measured (one for each lot) in addition to two internal standard-level controls value-assigned using the VitD-RMP assay, two internal control lots, and four pooled samples different from the four used in the above imprecision study (samples 7–10, Table 3). Value assignments were made for each sample following full-curve analysis. Mean concentration and the total SD

for each sample were used to calculate the precision error. Bias was determined for each sample between its value assignment and mean concentration, and used to calculate total analytical error (TAE) and %TAE.<sup>15</sup>

Percent TAE was <26% for each of the pooled sera, including sample 10, which was composed primarily of 25(OH)D<sub>2</sub>. Establishing accuracy of the assay to detect samples in which 25(OH)D<sub>2</sub> is elevated or is the dominant metabolite is essential because vitamin D<sub>2</sub> supplementation is often prescribed by physicians for their vitamin D-deficient patients. In supplementing patients, especially those on high-dose vitamin D therapy, monitoring is important to assess efficacy and to detect incipient hypervitaminosis.

**Performance Characteristics of the ADVIA Centaur Vitamin D Total Assay  
Now Standardized to the Vitamin D Reference Measurement Procedure**

**Table 2.** Precision study results for the VitD-RMP for individual samples (1 and 2) and pooled sera (3–6).

System	Lot	Sample	Mean (ng/mL)	Within Run		Total	
				SD	%CV	SD	%CV
1	1	1	13.60	0.64	4.7	1.61	11.9
		2	17.22	0.91	5.3	1.70	9.9
		3	28.20	1.45	5.2	2.02	7.2
		4	46.08	1.79	3.9	2.79	6.1
		5	73.22	2.71	3.7	4.36	6.0
		6	114.08	3.44	3.0	4.77	4.2
	2	1	11.90	0.45	3.8	0.95	8.0
		2	15.26	0.56	3.7	1.35	8.9
		3	23.25	1.19	5.1	1.88	8.1
		4	41.45	1.48	3.6	2.22	5.3
		5	69.00	3.11	4.4	4.10	5.9
		6	112.25	3.47	3.1	4.22	3.8
2	1	1	15.17	0.61	4.0	1.98	13.0
		2	19.77	0.90	4.6	1.76	8.9
		3	31.00	1.30	4.2	2.36	7.6
		4	50.26	1.83	3.6	3.94	7.8
		5	78.95	3.37	4.3	6.99	8.9
		6	120.31	1.99	1.7	5.25	4.4
	2	1	14.30	0.71	5.0	1.31	9.1
		2	17.90	0.98	5.5	1.47	8.2
		3	25.34	1.49	5.9	1.69	6.7
		4	45.79	2.40	5.2	3.72	8.1
		5	74.83	3.53	4.7	6.39	8.5
		6	117.34	2.94	2.5	4.81	4.1

According to calculations by Stöckl et al., total error based on the DEQAS limit for allowable error should be less than 46%.<sup>14</sup> The largest total error observed using the VitD-RMP assay was 32.5%, exceeding this minimal requirement for all samples. Note that total error is not the same as percent bias, which is discussed below in the section describing comparison to the DEQAS and CAP dose assignments. Also, it should be noted that relevancy of TAE and %TAE is dependent on the accuracy of the value assignment of each sample. In this case, accuracy is established by comparison of the VitD-RMP to values derived using the Ghent RMP-standardized method. As described below in the Method Comparison section,

Deming regression indicates that the Siemens VitD-RMP is aligned to the Ghent method. Data presented in the precision study indicated that %CV is low enough across the majority of the assay range to substantiate the accuracy of VitD-RMP–assigned values relative to Ghent–assigned values.

**Method comparison studies**

Using the VitD-RMP master curve, dose values were determined for 177 native human serum samples with Ghent RMP-determined concentrations spanning most of the assay range (5–140 ng/mL; 90 samples were assigned by either NIH or CDC; 60 samples were assigned by a

**Table 3.** Total error for samples with assigned values between 17 and 121 ng/mL.

$$\text{TAE} = |\text{Total bias}| + 2\text{SD}$$

$$\% \text{TAE} = (\text{TAE}/\text{assigned value}) * 100\%$$

Sample	Spiking Agent	Anticipated Concentration (ng/mL)	VA <sup>a</sup> (ng/mL)	Avg. Dose (ng/mL)	Bias	Observed SD Total	Observed %CV Total	TAE	%TAE
Lot 1 high calibrator	N/A	N/A	116.54	118.36	1.81	4.85	4.1	11.5	9.9
Lot 1 low calibrator	N/A	N/A	28.43	28.31	-0.12	3.77	13.3	7.7	26.9
Lot 2 high calibrator	N/A	N/A	104.01	103.05	-0.97	5.02	4.9	11.0	10.6
Lot 2 low calibrator	N/A	N/A	23.33	23.62	0.29	3.64	15.4	7.6	32.5
Ctrl lot 1 <sup>b</sup>	N/A	N/A	25.18	25.32	0.15	3.51	13.9	7.2	28.5
Ctrl lot 2 <sup>c</sup>	N/A	N/A	100.30	98.81	-1.49	6.61	6.7	14.7	14.7
7	None	20ng/mL	25.92	26.01	0.09	3.26	12.5	6.6	25.5
8	25(OH)D <sub>3</sub>	50 ng/mL	53.56	51.61	-1.95	3.61	7.0	9.2	17.1
9	25(OH)D <sub>3</sub>	120 ng/mL	120.88	124.36	3.48	4.27	3.4	12.0	9.9
10	25(OH)D <sub>2</sub>	50 ng/mL	52.39	50.51	-1.88	3.51	6.9	8.9	17.0
Standard level 4 <sup>b</sup>	N/A	N/A	17.93	17.84	-0.09	2.35	13.2	4.8	26.7

a. Value assignment.

b. Control lots.

c. Standard level 1 was set to 0.1 ng/mL, which is too low to give meaningful results.

commercial vendor using an LC-MS method aligned to the Ghent RMP; and 27 samples were assigned by Ghent University). Deming regression was used to compare these RMP-standardized values to the VitD-RMP values determined for two reagent lots on a single ADVIA Centaur XP system.<sup>7,8</sup> Correlation between the VitD-RMP assay and the ID-LC/MS/MS method was 96% for both reagent lots and the slope for both lots was 0.99. Bias between the two methods averaged approximately -1% (Figure 3).

A second evaluation was conducted using 122 novel serum samples (116 native samples and six samples created by spiking a serum sample with different doses of 25(OH)vitamin D<sub>2</sub>). Samples were value assigned by Ghent University using its ID-LC/MS/MS 25(OH)vitamin D RMP. Values ranged from 7.8 ng/mL to 148.1 ng/mL; the sera spiked with 25(OH)vitamin D<sub>2</sub> ranged from 70.4 ng/mL to 125.6 ng/mL. Samples were assayed in singlicate on a single ADVIA Centaur system using a single ADVIA Centaur Vitamin D Total reagent lot and results were used to generate a Deming regression (Figure 4).

Bland-Altman analysis was applied across the reportable range of the assay to assess bias. Although greater bias was observed at higher concentrations, the difference between the Ghent assigned values and the VitD-RMP-reported values did not exceed 12 ng/mL, with the exception of a few outliers (Figure 5). Overall bias was -0.09 ng/mL.

These two independent studies confirm that the ADVIA Centaur VitD-RMP assay reports values equivalent to the Ghent RMP and is therefore standardized to it. This indicates that this calibration of the assay can be relied upon to report accurate serum levels of metabolically significant vitamin D total because the Ghent method is known to accurately measure only 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> and detects neither 3-epi 25(OH)- nor 24,25 dihydroxy vitamin D metabolites. The spiked samples confirm that the assay detects both 25(OH)vitamin D<sub>2</sub> and 25(OH)vitamin D<sub>3</sub>.

Performance Characteristics of the ADVIA Centaur Vitamin D Total Assay  
Now Standardized to the Vitamin D Reference Measurement Procedure

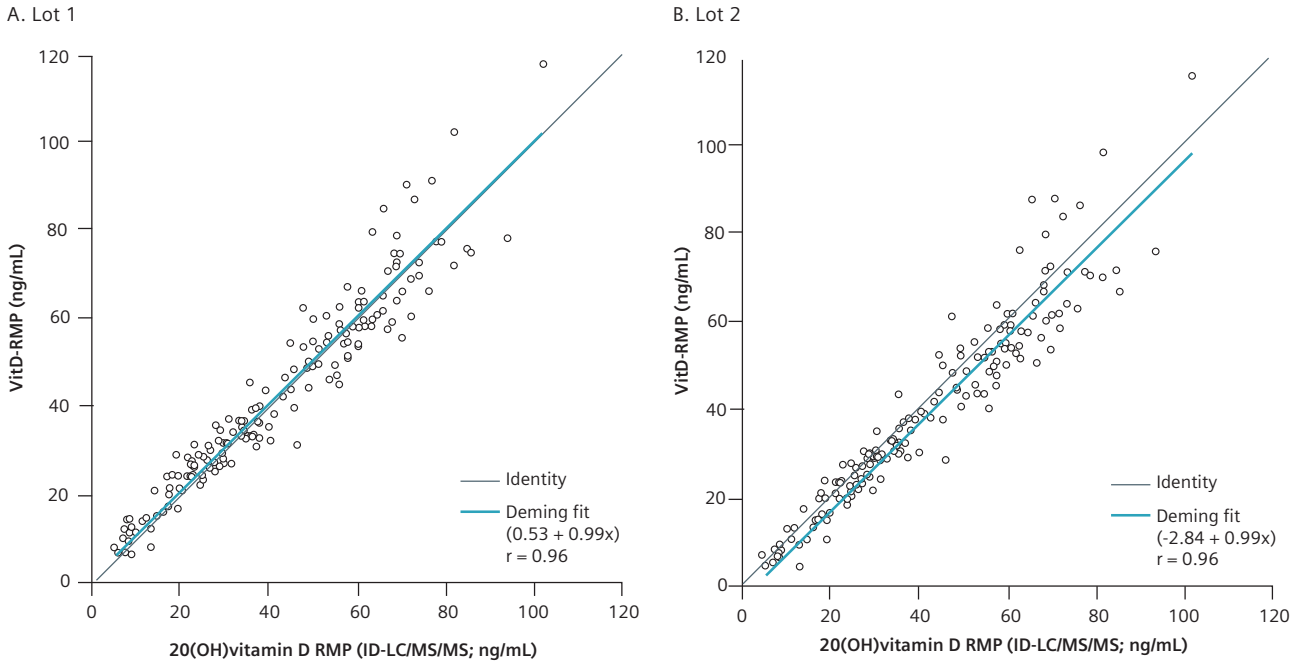


Figure 3. Deming regression analysis comparing the VitD-RMP assay and the RMP.

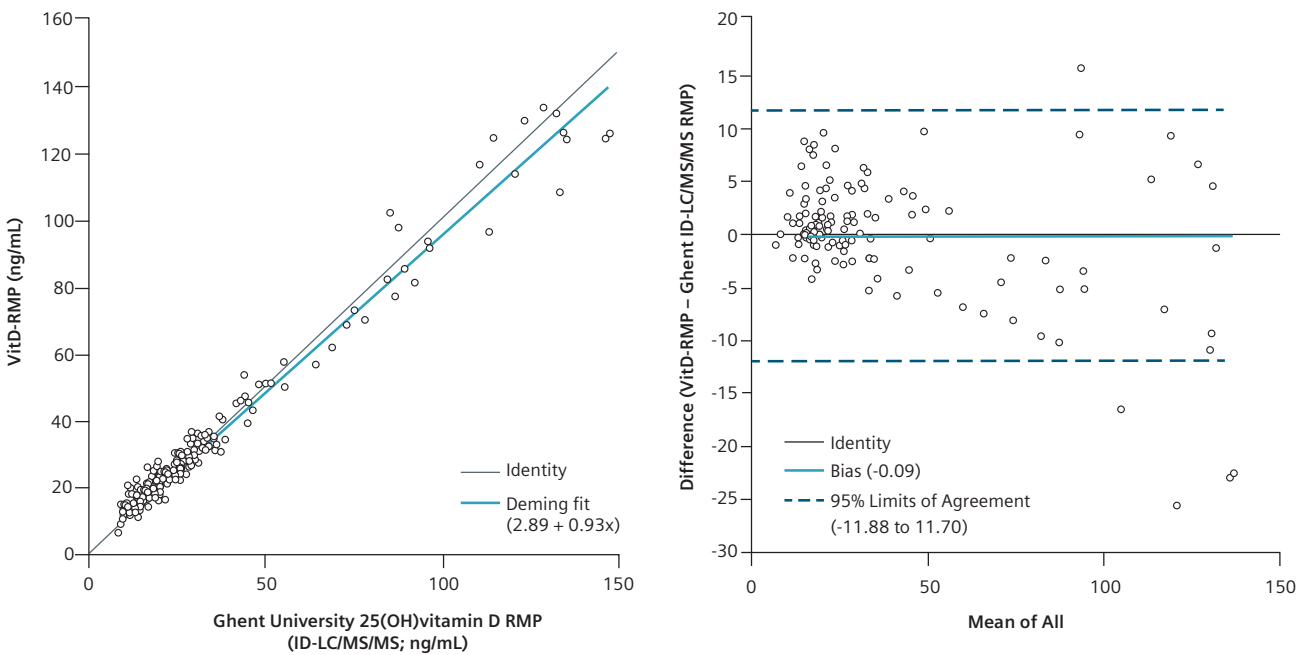


Figure 4. The VitD-RMP assay vs. University of Ghent ID-LC/MS/MS 25(OH)vitamin D RMP.

Figure 5. Bland-Altman difference plot.



**Table 4.** Comparison of results with CAP and DEQAS survey results.  
Percent bias = [(Centaur Dose – Survey-assigned Dose)/Survey-assigned Dose]\*100

Survey	Sample	Assigned Value (ng/mL)	VitD-RMP Lot 1 (ng/mL)	VitD-RMP Lot 2 (ng/mL)	Bias to Assigned Value, Lot 1	Bias to Assigned Value, Lot 2
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 (ID-LC/MS/MS)	ABVD 1	20.80	22.29	25.14	7.2%	20.9%
	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%

### Comparison to CAP and DEQAS survey values

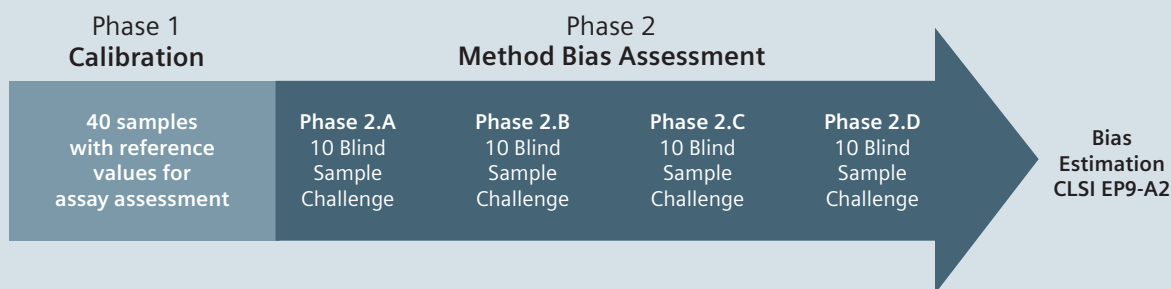
The RMP-standardized assay was used to measure samples from the College of American Pathologists (CAP) 2011 accuracy-based vitamin D (ABVD) and Vitamin D External Quality Assessment Scheme (DEQAS) surveys. Two reagent lots were used to evaluate individual patient samples from these surveys (14.1–58.5 ng/mL), after which percent bias was calculated between the RMP-assigned values and the VitD-RMP-determined values. Percent bias was within 25% of the survey results for all five of the CAP ABVD samples for both lots, for all ten of the DEQAS samples with one lot, and for nine out of the ten DEQAS samples for the other lot (Table 4). This means that the VitD-RMP assay returned values within 25% of the assigned value for 95% of the DEQAS samples. This exceeds the proficiency testing criteria designated by both the CAP and DEQAS organizations, which require that only 80% of samples fall within 25% of the survey-assigned dose. While these results corroborate the fitness for use determined by the TAE, there could still be a concern that a larger than desirable bias in a small percentage of cases could affect clinical decision making and negatively impact the patient. As an extension to a potential real-world example, consider that bias was 34% for the one

sample that did not meet the DEQAS requirement. The assigned value of this sample was 17.6 ng; if this were a real-world sample, the patient would be diagnosed with vitamin D deficiency or at least retested. Because of the 34% bias, however, the patient would be diagnosed with vitamin D insufficiency (21.2 ng/mL). As insufficiency would still suggest the need for treatment the bias would not likely affect the clinical decision.

### CDC validation studies

As a complement to the VDSP, the CDC has instituted a certification program for laboratories and manufacturers. To become VDSCP-certified, participants must pass four consecutive quarterly challenges using blinded samples after an initial calibration period (Figure 6). Samples for both phases of the study are value-assigned using the 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.<sup>11,16</sup> In order to receive certification, the mean bias for all 40 phase 2 samples (160 results) determined using the subject assay must be within 5% of the CDC values, and overall imprecision must be ≤ 10%.<sup>14</sup> The program is ongoing and certification must be renewed annually.

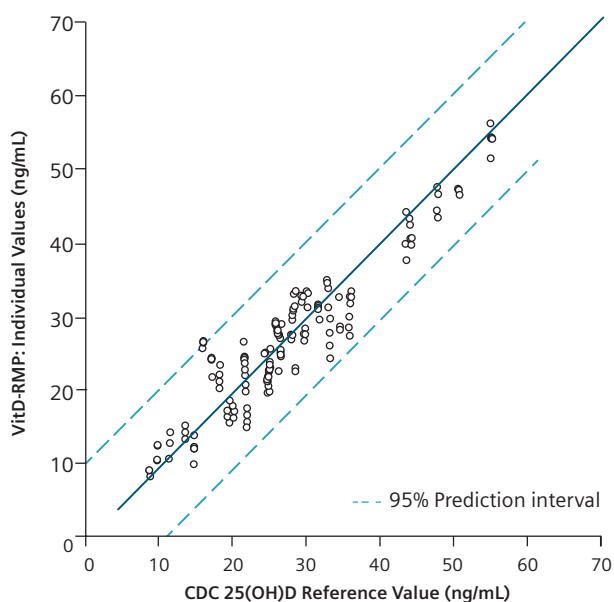
Performance Characteristics of the ADVIA Centaur Vitamin D Total Assay  
Now Standardized to the Vitamin D Reference Measurement Procedure



**Figure 6.** Phases of the VDSCP. Although the CDC normally supplies 40 samples for the initial calibration phase, 50 samples were sent to Siemens and used for calibration purposes.<sup>11</sup>

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

n | 160  
R<sup>2</sup> | 0.9018



The VitD-RMP assay performed appreciably better than the criteria, becoming one of the first ten assays to be certified by the CDC and one of only three immunoassays certified as of February 2014. Correlation between the CDC RMP-assigned sample values and the ADVIA Centaur VitD-RMP assay values was high across all four quarterly blind trials (Figure 7). The mean bias was 0.3% (SD = 16.8; 95% CI = -5.0 to 5.6) and the mean CV was 5.5% (SD = 3.2; 10th %ile = 1.6%, 90th %ile = 9.8%). Although not currently required for certification, 86% of samples in the challenge groups were within the suggested total error of  $\pm 21.5\%$  (Figure 8).

**Figure 7.** Linear regression generated by the CDC VDSCP from all challenge samples in 2013.

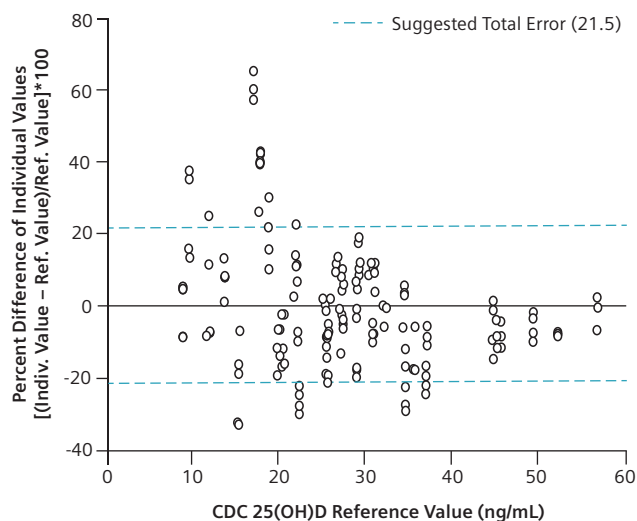


Figure 8. Total error for the CDC VDSCP challenge samples.

## Conclusion

The CDC certification attests to the accuracy of the Siemens ADVIA Centaur Vitamin D Total assay and its standardization to the RMP specified by the VDSP. This certification, in conjunction with the performance and method comparison data also presented in this paper, indicate that the Siemens ADVIA Centaur Vitamin D Total assay is fit for use in the clinical laboratory and can be relied upon to report patient vitamin D status accurately.

## References

1. Wagner D, Hanwell HE, Vieth R. An evaluation of automated methods for measurement of serum 25-hydroxyvitamin D. *Clin Biochem.* 2009;42:1549-56.
2. 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.
3. Moon HW, Cho JH, Hur M, et al. Comparison of four current 25-hydroxyvitamin D assays. *Clin Biochem.* 2012;45:326-30.
4. 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.
5. 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.
6. Carter GD. Accuracy of 25-hydroxyvitamin D assays: confronting the issues. *Curr Drug Targets.* 2011;12:19-28.
7. 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.
8. Stepman HC, Vanderroost A, Van Uytvanghe K, Thienpont LM. 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.
9. 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.
10. ODS Vitamin D Initiative. Accessed March 27, 2014, at: <http://ods.od.nih.gov/Research/VitaminD.aspx#vdsp>
11. 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.
12. Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. *Clin Biochem Rev.* 2008;29 Suppl 1:S49-52.
13. 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.
14. 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.
15. Westgard JO, Westgard SA. Total analytic error: from concept to application. *Clinical Laboratory News.* 2013;39.
16. CLSI. Method comparison and bias estimation using patient samples. Wayne, PA; 2002.

Siemens Healthcare Diagnostics, a global leader in clinical diagnostics, provides healthcare professionals in hospital, reference, and physician office laboratories and point-of-care settings with the vital information required to accurately diagnose, treat, and monitor patients. Our innovative portfolio of performance-driven solutions and personalized customer care combine to streamline workflow, enhance operational efficiency, and support improved patient outcomes.

ADVIA Centaur and all associated marks are trademarks of Siemens Healthcare Diagnostics Inc. or its affiliates. All other trademarks and brands are the property of their respective owners.

Product availability may vary from country to country and is subject to varying regulatory requirements. Please contact your local representative for availability.

Order No. A91DX-CAI-140685-GC1-4A00  
11-2014 | All rights reserved  
© 2014 Siemens Healthcare Diagnostics Inc.

**Global Siemens Headquarters**

Siemens AG  
Wittelsbacherplatz 2  
80333 Muenchen  
Germany

**Global Siemens Healthcare  
Headquarters**

Siemens AG  
Healthcare Sector  
Henkestrasse 127  
91052 Erlangen  
Germany  
Telephone: +49 9131 84-0  
[www.siemens.com/healthcare](http://www.siemens.com/healthcare)

**Global Division**

Siemens Healthcare Diagnostics Inc.  
511 Benedict Avenue  
Tarrytown, NY 10591-5005  
USA  
[www.siemens.com/diagnostics](http://www.siemens.com/diagnostics)