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White Paper

# ADVIA Centaur Vitamin D Total 2015 ENDO/AACC/IFCC Posters

# The Value of a Standardized and Certified Vitamin D Total Assay for Clinical Confidence

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## Abstract

**Background:** Vitamin D is a steroid hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis. Standardization of vitamin D assays in laboratory medicine has become increasingly important. Apart from being able to assess the vitamin D status of an individual, standardization is necessary to accurately determine the concentration of vitamin D in patients on supplementation. The Siemens ADVIA Centaur® Vitamin D Total assay has been standardized to the University of Ghent ID-LC/MS/MS reference measurement procedure (RMP) and has achieved the Centers for Disease Control (CDC) Vitamin D Standardization Certification (VDSCP). Assay performance has been assessed in patient samples and compared to a VDSCP-certified LC-MS/MS assay. In addition, samples containing 25(OH)vitamin D<sub>2</sub> and 25(OH)vitamin D<sub>3</sub> were included in the study to demonstrate the ability of the assay to measure both forms of 25(OH)vitamin D and also to demonstrate the equimolarity of the test.

**Method:** A comparison between the ADVIA Centaur Vitamin D Total assay and the Endocrine Sciences Laboratory (LabCorp, Calabasas Hills, CA) VDSCP-certified 25(OH)vitamin D LC/MS/MS method was achieved by running 149 samples across the range of both methods. The samples were tested using the ADVIA Centaur Vitamin D Total assay and subsequently run on LabCorp's VDSCP-

certified method. These data were analyzed with a Deming fit comparison plot as well as a Bland-Altman plot comparing the total vitamin D dose data between the two methods. Deming fits and Bland-Altman plots were also generated for the samples containing both 25(OH)vitamin D<sub>2</sub> and D<sub>3</sub> and for samples containing 25(OH)vitamin D<sub>3</sub> only in order to assess the assay's equimolarity.

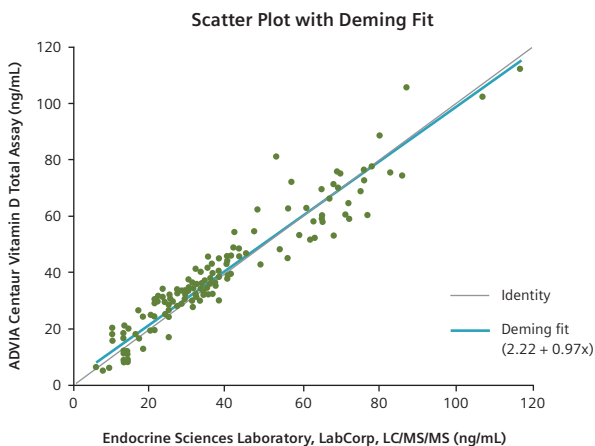
**Results:** The data obtained showed good correlation between the ADVIA Centaur Vitamin D Total assay and the VDSCP-certified 25(OH)vitamin D LC/MS/MS method. The Deming fit comparison between the two methods yielded a Deming slope of 0.97, an intercept of 2.22 ng/mL, and a Pearson's coefficient of 0.95. When focusing specifically on the 55 samples containing 25(OH)vitamin D<sub>2</sub>, the Deming slope was 1.02, with an intercept of 1.92 ng/mL and a Pearson's coefficient of 0.93. When analyzing the 94 samples that contain only 25(OH)vitamin D<sub>3</sub>, the Deming slope was 0.95, with an intercept of 2.13 ng/mL and a Pearson's coefficient of 0.97.

**Conclusions:** In comparison to a VDSCP-certified 25(OH) vitamin D LC/MS/MS method, the ADVIA Centaur Vitamin D Total assay produced comparable results across the full range of the assay and was able to accurately measure both forms of 25(OH)vitamin D. These data indicate the importance of standardization to improve clinical confidence in the comparability of vitamin D measurement.

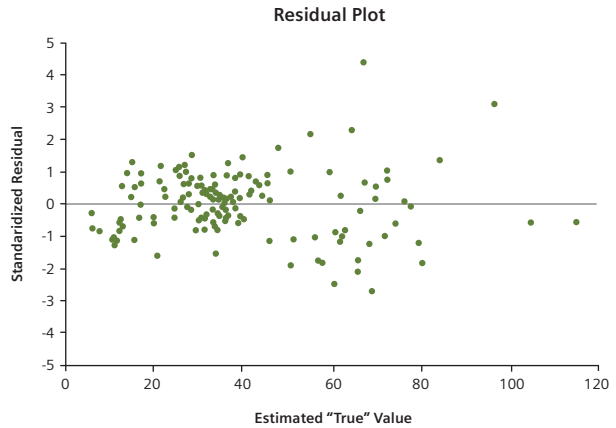
## Method and Results

The ADVIA Centaur Vitamin D Total assay successfully passed the performance criterion for total 25(OH)vitamin D VDSCP certification. The performance specified for total 25(OH)vitamin D VDSCP includes a  $\pm 5\%$  bias to the CDC and University of Ghent Vitamin D<sub>2</sub> and D<sub>3</sub> RMP and an overall imprecision of  $\leq 10\%$  between 8.8 and 110 ng/mL. The Endocrine Sciences Laboratory (Esoterix, Endocrine Sciences, Calabasas Hills, CA) LC/MS/MS method has also been certified. It is important to evaluate patient samples similarly across platforms and locations, so in order to further evaluate the harmonization between different certified methods, a method comparison was performed between the ADVIA Centaur Vitamin D Total assay and the Endocrine Sciences Laboratory LC/MS/MS method. Unknown patient samples were tested in singleton with one lot of reagent on the Siemens ADVIA Centaur Vitamin D Total assay. After testing, samples were selected across the range of the assay and sent to LabCorp for further testing on their 25(OH)vitamin D LC/MS/MS method. The comparison of total vitamin D values between both methods is shown in Figure 1; the residual plot is shown in Figure 2. The Deming fit shows good alignment between the two methods, with a Deming slope of 0.97, intercept of 2.22 ng/mL, and a Pearson's coefficient of 0.95, as shown in Table 1.

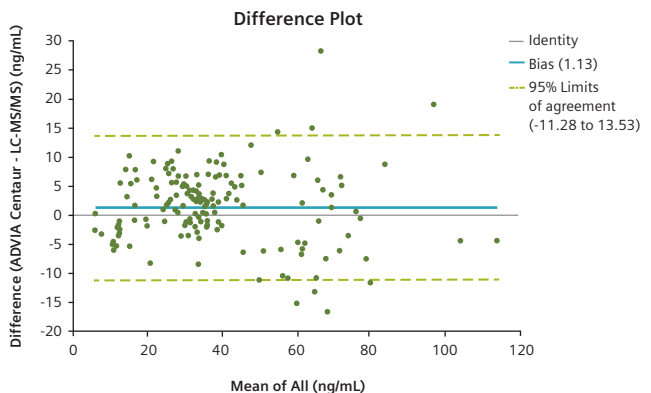
An Altman Bland bias plot that shows minimal bias was also generated for all samples tested across the range to demonstrate the correlation between methods, as shown in Figure 3.



**Figure 1.** Deming fit for the ADVIA Centaur Vitamin D Total assay dose versus the corresponding Endocrine Sciences Laboratory dose.



**Figure 2.** Residual plot between ADVIA Centaur Vitamin D Total assay versus the corresponding Endocrine Sciences Laboratory LC/MS/MS method.



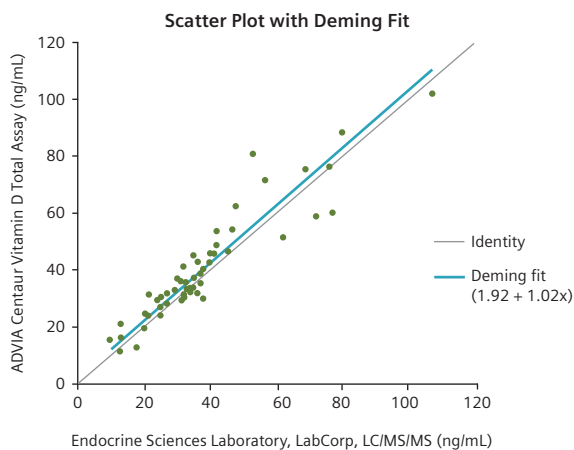
**Figure 3.** Altman Bland bias plot between all samples run on the ADVIA Centaur Vitamin D Total assay and Endocrine Sciences Laboratory LC/MS/MS method.

Two forms of vitamin D are metabolized in the body. Vitamin D<sub>3</sub> is found naturally in the body, derived from the irradiation of provitamin 7-dehydrocholesterol in the skin. Vitamin D<sub>2</sub> can be obtained through diet from sources such as fish and plants. These two forms of vitamin D are metabolized by the body to produce 25(OH)vitamin D<sub>2</sub> and 25(OH)vitamin D<sub>3</sub>, both of which have similar biological activity.

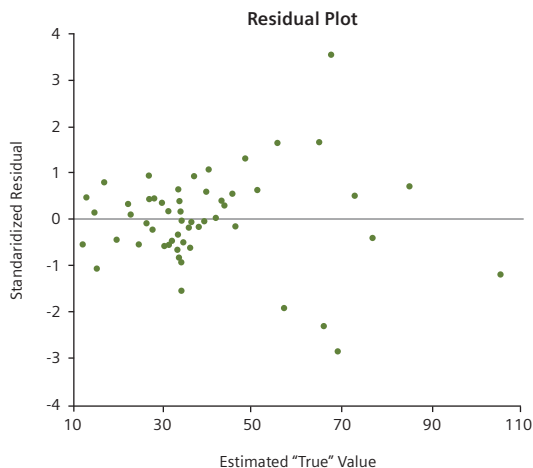
Since vitamin D<sub>3</sub> is the form that occurs naturally in the body, the normal population has more 25(OH)vitamin D<sub>3</sub> than 25(OH)vitamin D<sub>2</sub>. However, patients who test low

for vitamin D are advised to add a supplemental source of vitamin D to their diet, which can elevate either 25(OH)vitamin D<sub>2</sub> or 25(OH)vitamin D<sub>3</sub> levels. Since the two metabolite forms are biologically similar, it is important for an assay to detect both forms equally.

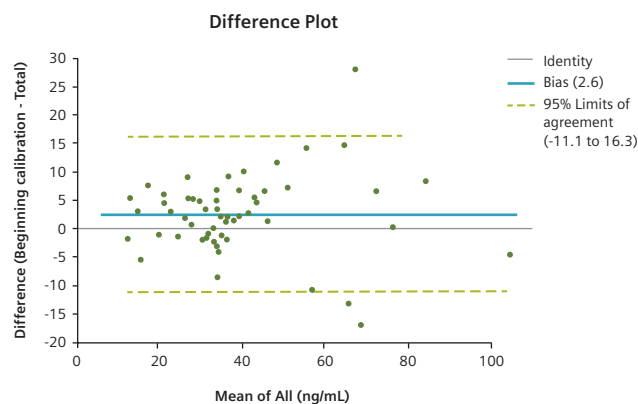
The Endocrine Sciences Laboratory 25(OH)vitamin D method employs LC/MS/MS and can determine separate values for 25(OH)vitamin D<sub>2</sub> and 25(OH)vitamin D<sub>3</sub>. Samples that contained 25(OH)vitamin D<sub>2</sub> when tested by the Endocrine Sciences Laboratory method were plotted against the ADVIA Centaur Vitamin D Total assay to ensure the assay's equimolarity and that there was no bias between the two forms. The comparison of samples containing vitamin D<sub>2</sub> between the two methods is shown in Figures 4 and 5, and the comparison of samples only containing vitamin D<sub>3</sub> is shown in Figures 7 and 8. An Altman Bland bias plot for the two forms can be seen in Figures 6 and 9, respectively.



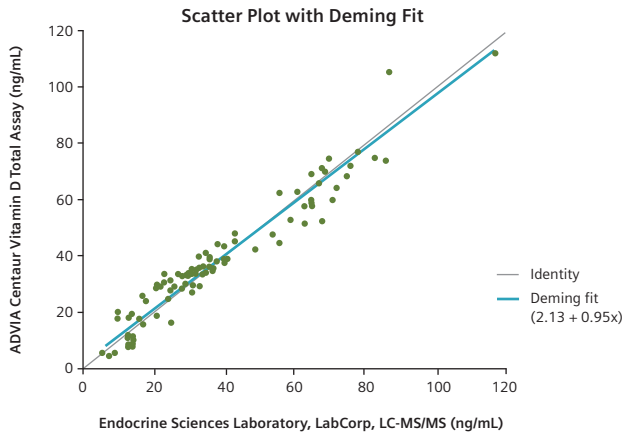
**Figure 4.** Deming fit for the ADVIA Centaur Vitamin D Total Assay dose versus the corresponding Endocrine Sciences Laboratory dose for samples containing vitamin D<sub>2</sub>.



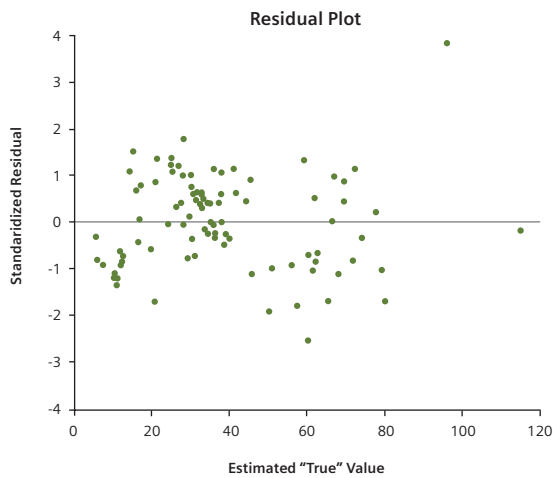
**Figure 5.** Residual plot for the ADVIA Centaur Vitamin D Total assay dose versus the corresponding Endocrine Sciences Laboratory dose for samples containing vitamin D<sub>2</sub>.



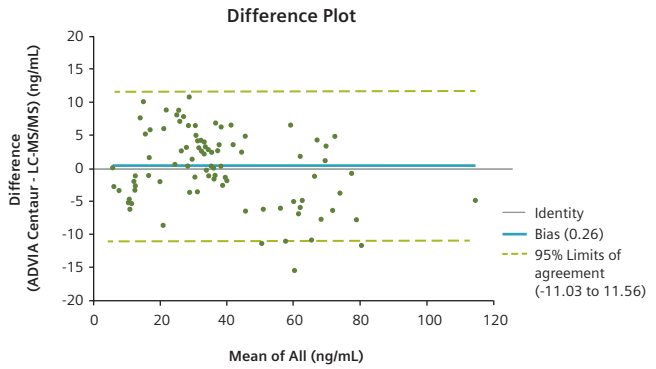
**Figure 6.** Altman Bland bias plot between vitamin D<sub>2</sub> samples run on the ADVIA Centaur Vitamin D Total assay and Endocrine Sciences Laboratory 25(OH)vitamin D assay.



**Figure 7.** Deming fit for the ADVIA Centaur Vitamin D Total assay dose versus the corresponding Endocrine Sciences Laboratory dose for samples containing vitamin D<sub>3</sub>.



**Figure 8.** Residual plot for the ADVIA Centaur Vitamin D Total assay dose versus the corresponding Endocrine Sciences Laboratory dose for samples containing vitamin D<sub>3</sub>.



**Figure 9.** Altman Bland bias plot between vitamin D<sub>3</sub> samples run on the ADVIA Centaur Vitamin D Total assay and Endocrine Sciences Laboratory 25(OH)vitamin D assay.

As shown in Table 1, the methods are harmonized regardless of whether the sample contains vitamin D<sub>3</sub> only or both vitamin D<sub>3</sub> and vitamin D<sub>2</sub>.

**Table 1.** Deming line equation and Pearson's coefficient statistic summary for ADVIA Centaur Vitamin D Total assay dose versus Endocrine Sciences Laboratory dose.

Sample Set	Slope	Intercept (ng/mL)	Pearson's Coefficient
All	0.97	2.22	0.95
D <sub>3</sub> only	0.95	2.13	0.97
Containing D <sub>2</sub>	1.02	1.92	0.96

## Conclusion

The alignment of the Siemens ADVIA Centaur Vitamin D Total assay to the CDC and University of Ghent Vitamin D<sub>2</sub> and D<sub>3</sub> RMP provides laboratories with a standardized method to tests patients for total 25(OH)vitamin D levels. In this study, the method comparison of the Siemens ADVIA Centaur Vitamin D Total assay to another method that is also traceable to the CDC and University of Ghent Vitamin D<sub>2</sub> and D<sub>3</sub> RMP demonstrates that having assay methods align to a single standard leads to accurate and reliable results for patients, regardless of the test performed. The data in this study shows clinical confidence in the comparability of vitamin D measurements between methods and that the automated Siemens ADVIA Centaur Vitamin D Total assay measures both forms of 25(OH) vitamin D equally and provides laboratories with a high-throughput, standardized assay.

# Performance and Certification of the ADVIA Centaur Vitamin D Total Assay

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## Abstract

**Background:** Vitamin D helps regulate calcium in the development and maintenance of healthy bones. The National Institutes of Health Office of Dietary Supplements created the Vitamin D Standardization Program (VDSP) to establish a standard for accurate and comparable results for the detection of 25(OH)D across laboratories. Siemens Healthcare Diagnostics enrolled in the VDSP to produce a harmonized industry standard for 25(OH)D testing.

**Method:** Between January and December 2013, the Centers for Disease Controls (CDC) provided 40 blinded 25(OH)D samples to the Vitamin D Standardization-Certification Program (VDSCP), in which a set of 10 samples with Reference Measurement Procedure (RMP) values was evaluated each quarter. Samples were tested blindly in replicates of four over 2 days, two replicates per day. Additional supplemental samples were also evaluated, including the four standards from the National Institute of Standards and Technology (NIST).

**Results:** The ADVIA Centaur® Vitamin D Total assay met the criteria for VDSCP certification. The mean bias to the reference method was 0.3%, within the acceptable bias of  $\pm 5.0\%$ . The assay's imprecision of 5.5% was also within the acceptable range of  $\leq 10.0\%$ . A linear regression of the blinded samples demonstrates a slope of 1.01 and an intercept of  $-1.89$  nmol/L. The ADVIA Centaur Vitamin D Total assay also shows an acceptable bias with the NIST Standard Reference Material (SRM) 972a vitamin D metabolite samples.

**Conclusions:** The VDSCP certification for the ADVIA Centaur Vitamin D Total assay establishes an acceptable

alignment to a harmonized testing standard for 25(OH)D. The ADVIA Centaur Vitamin D Total assay provides laboratories with a standardized and automated means for quickly and efficiently testing patients' 25(OH)D levels.

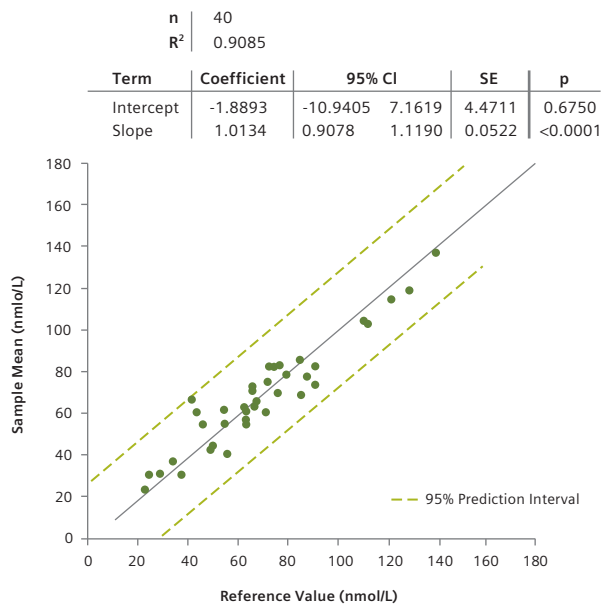
## Introduction

The Vitamin D Standardization Program (VDSP) is an initiative to standardize 25(OH)vitamin D measurements using a Reference Measurements Procedure. There are two RMPs approved as part of the VDSP: (1) NIST and (2) Ghent University. These values are treated as "true" values to which 25(OH)vitamin D assay manufacturers can harmonize their assays<sup>1,2</sup>. 25(OH)vitamin D methods are challenged by the CDC's Vitamin D Standardization Certification Program (VDSCP) to obtain a yearly certification as well as monitored by performance testing surveys and external quality assessments, such as CAP and DEQAS. The CAP survey samples have the values assigned by the CDC laboratory, which is traceable to the NIST RMP, and the DEQAS survey samples have the values assigned directly by NIST RMP. The RMP is also the primary method employed for use with vitamin D reference materials, such as NIST Standard Reference Materials® (SRM) 972a<sup>4</sup>.

The CDC has provided single-donor serum samples that have reference values assigned to the sera by the CDC reference laboratory, which uses ID-LC/MS/MS and certified primary standards from NIST. A yearly certification process administered by the CDC includes four quarterly challenges. If the participant is within the specified criteria ( $\pm 5\%$  bias to RMP and  $\pm 10\%$  CV), a certificate is issued to that participant for the year<sup>5</sup>.

## Methods and Results

As a participant of the VDSCP, Siemens Healthcare Diagnostics is sent four separate individual serum sample sets with “true” 25(OH)vitamin D values, one for each 2013 quarter. For each quarter, 10 blinded serum samples are sent to the participants, and the values they report are assessed for bias as described in CLSI guideline EP9-A2 (4), Method Comparison and Bias Estimation using Patient Samples. Each sample is tested in duplicate on 2 different days for a total of four measurements per sample, which is a total of 160 measurements for the year. At the end of the four quarters, the data is analyzed by the CDC and distributed with a final report, which determines the pass or fail status of the yearly certification. The data analyzed for the first year of certification was run between February 2013 and December 2013.



**Figure 1.** Linear regression analysis of the reference value (nmol/L) versus the sample value (nmol/L) by the ADVIA Centaur Vitamin D Total assay. Data was analyzed using CLSI EP9-A2 section 4.2 B1.

The ADVIA Centaur® Vitamin D Total assay shows good alignment to the “true” reference method, as seen in Figure 1. The method comparison demonstrated a slope of 1.01 and an intercept of –1.89 nmol/L. Also, the samples showed good correlation between methods, with an R of 0.95.

**Table 1.** Bias assessment of each sample in comparison to the reference value. Mean bias was derived from the individual sample bias for each of the 40 samples.

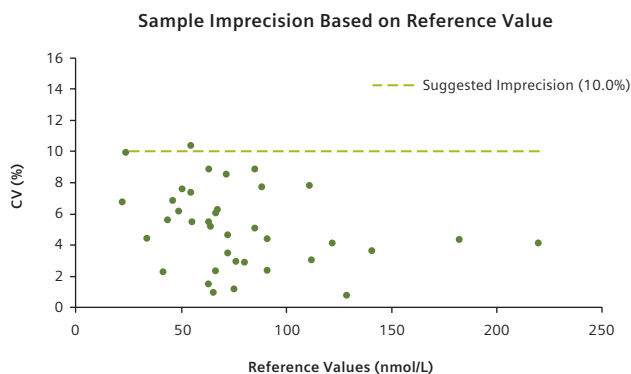
Mean % Bias	95% Confidence Interval	
0.3	-5.0	5.6

Table 1 shows the overall bias (0.3%) and 95% confidence interval for that bias, which is -5.0% and 5.6%. As demonstrated through the certification process, it is clear that the ADVIA Centaur Vitamin D Total assay is within the acceptable specifications.

As seen in Table 2 and Figure 2, the ADVIA Centaur Vitamin D Total assay passed the CV requirements, demonstrating a mean CV of 5.5%, which was below the acceptance criteria of 10%.

**Table 2.** Summary of precision data from the 40 individual serum samples tested by Siemens Healthcare Diagnostics.

Imprecision (%CV)					
Mean	SD	10th and 90th Percentile		Min	Max
5.5	3.2	1.6	9.8	0.8	15.1



**Figure 2.** Precision of the ADVIA Centaur Vitamin D Total assay based on the reference value (nmol/L).

In order to assure consistency across assays, the VDSP program ties these true values to proficiency testing, such as CAP and DEQAS, as well as the NIST SRM. Siemens Healthcare Diagnostics tested the ADVIA Centaur Vitamin D Total assay with SRM 972a control materials and demonstrated similar results to the RMP, as seen in Table 3.

**Table 3.** Comparison of 25(OH)Vitamin D reference values (nmol/L) versus ADVIA Centaur XP (nmol/L). Yellow cells indicate reference values, and the other cells represent certified values, which are values for which NIST has the highest confidence in accuracy.

NIST SRM 972a	Reference Values (nmol/L)				Centaur XP (nmol/L)
	D <sub>2</sub>	D <sub>3</sub>	3-epi	D <sub>2</sub> +D <sub>3</sub>	Vitamin D Total
Level 1	1.35	72	4.6	73.35	69.43
Level 2	2.025	45.25	3.225	47.275	47.07
Level 3	33.25	49.5	2.95	82.75	79.66
Level 4	1.375	73.5	66	74.875	70.26

Table 4 shows the ADVIA Centaur Vitamin D Total assay results from a recent DEQAS survey in comparison to the target value. The DEQAS survey provides five samples that are shipped quarterly. The samples are unprocessed human serum and value-assigned by the NIST Reference Measurement Procedure. These data clearly show that the assay is performing acceptably in laboratories worldwide, as each sample is within at most 6% of the target.

**Table 4.** January 2015 DEQAS Survey samples comparing the reference method, NIST, to the average of all Siemens ADVIA Centaur Vitamin D Total assay participant values in the survey.

Sample	DEQAS January 2015	
	NIST Target (nmol/L)	All Siemens Values (nmol/L)
466	64.5	65.8
467	44.4	46.9
468	68.3	65.7
469	69.3	65.2
470	118.4	118.6

## Conclusion

Having passed the acceptance criteria for the Vitamin D Standardization Certification Program, and demonstrating good alignment and correlation with the industry-standard Reference Method Procedures, the Siemens Healthcare Diagnostics Vitamin D Total assay provides laboratories with a quick and efficient method to accurately measure patients' 25(OH)vitamin D levels.

### References

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# Method Comparisons of the Standardized and CDC-certified ADVIA Centaur Vitamin D Total Assay

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## Abstract

**Background:** Vitamin D aids with intestinal absorption of calcium and regulates calcium homeostasis, making it essential for bone health. Vitamin D has become increasingly important, leading to the development of several vitamin D assays using different methods to standardize. The Vitamin D Standardization Program (VDSP) is an initiative of the NIH Office of Dietary Supplements and a collaboration with the National Institute of Standards and Technology (NIST), the CDC, and Ghent University to standardize 25(OH)vitamin D measurement across methods and manufacturers. The Reference Measurement Procedure (RMP) is the primary reference method for the measurement of total 25(OH) vitamin D, i.e., 25(OH)vitamin D<sub>2</sub> and 25(OH)vitamin D<sub>3</sub>.

**Method:** 118 samples obtained from the CDC assigned by RMP were tested on the ADVIA Centaur® XP Immunoassay System from Siemens Healthcare Diagnostics and compared to assigned RMP values. A different set of serum samples (n = 178) was tested on the CDC-certified IDS 25-Hydroxy Vitamin D EIA. 168 specimens were native sera, 8 samples were spiked with 25(OH)vitamin D<sub>2</sub>, and 2 were diluted in charcoal-stripped human serum. Deming regression was used for the regression analysis of the different methods.

**Results:** The ADVIA Centaur Vitamin D Total assay demonstrates good alignment with the samples provided by the CDC. The Deming fit comparison between the CDC-certified samples and the ADVIA Centaur Vitamin D Total assay yielded a slope of 0.95, intercept of 1.62 ng/mL, and Pearson's coefficient of 0.94, indicating an acceptable correlation between the two methods. The values from

the samples tested using the ADVIA Centaur Vitamin D Total assay and the IDS 25-Hydroxy Vitamin D EIA were plotted with a Deming fit, resulting in a slope of 0.99, intercept of 1.17 ng/mL after standardization, and Pearson's coefficient of 0.98.

**Conclusions:** It is evident from the method comparison of the ADVIA Centaur Vitamin D Total assay with the RMP method as well as with the CDC-certified method that the correlation between the different methods is acceptable. The VDSP standardization is a necessary step for global alignment of 25(OH)vitamin D levels that will lead to harmonization among clinical laboratories across the world.

## Background

The Vitamin D Standardization Program (VDSP) is an initiative to standardize 25(OH)vitamin D measurements using a Reference Measurements Procedure. There are two RMPs approved as part of the VDSP: (1) NIST and (2) Ghent University. These values are treated as "true" values to which 25(OH)vitamin D assay manufacturers harmonize their assays, allowing more trust in the 25(OH)vitamin D values from laboratories worldwide. 25(OH)vitamin D methods are challenged by the CDC's Vitamin D Standardization Certification Program (VDSCP) to obtain a yearly certification as well as monitored by performance testing surveys and external quality assessments, such as CAP and DEQAS. CAP survey samples have the values assigned by the CDC laboratory, which is traceable to the NIST RMP and DEQAS samples are value assigned directly by NIST. The RMP is also the primary method employed for use with vitamin D reference materials, such as NIST SRM 972a. The CDC has provided single-donor serum samples

that have value-assigned concentrations by the Ghent University RMP. With these samples, Siemens Healthcare Diagnostics performed a method comparison between the ADVIA Centaur® Vitamin D Total assay and the RMP. In order to demonstrate harmonization, Siemens has performed a method comparison between the ADVIA Centaur Vitamin D Total assay and another assay that has also been aligned to the Ghent University RMP.

## Methods and Results

118 samples with true RMP values were assessed by Siemens with a method comparison to demonstrate alignment between the RMP values and the ADVIA Centaur Vitamin D Total assay. These data were analyzed with a Deming fit, residual plot, and Altman Bland difference plot to show the alignment between the two methods.

Figure 1 shows good correlation between the reference method and the ADVIA Centaur Vitamin D Total assay. The Deming fit has a slope of 0.95 and an intercept of 1.62 ng/mL. The residual and Bland Altman bias plots of the data in Figures 2 and 3, respectively, show minimal bias between the sample values for the two methods.

A method comparison was also performed with another CDC VDSCP-certified assay containing samples that span a range from 4.2 to 152 ng/mL. As seen in Figure 4, this method comparison results in a Deming slope of 0.99 with an intercept of 1.17 ng/mL, evidence that the two assays are harmonized and aligned to the true assigned values. The residual plot (Figure 5) and the Altman Bland bias plot (Figure 6) also support the alignment of these two assays by demonstrating minimal bias. If all vitamin D methods were aligned to the RMP, as these two assays are, it would allow uniform decisions based on the patient's 25(OH)vitamin D level, since patients will receive similar values regardless of where and which laboratory tested their blood sample.

The data compiled in Table 1 as well as the corresponding figures shows good correlation to the true RMP values and to another CDC VDSCP-certified assay. Clinicians can have confidence in the values reported by assays that have been CDC VDSCP-certified, such as the ADVIA Centaur Vitamin D Total assay.

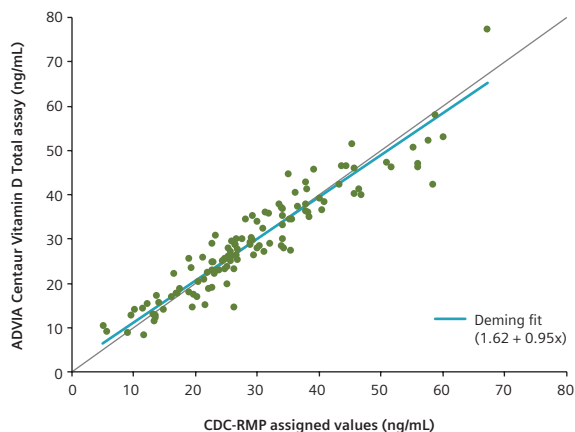


Figure 1. Deming fit for the ADVIA Centaur Vitamin D Total assay versus the corresponding CDC-RMP–assigned values.

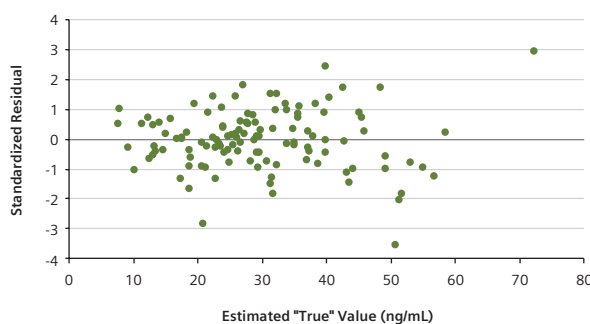


Figure 2. Residual plot between the ADVIA Centaur Vitamin D Total assay dose and the corresponding CDC-RMP dose.

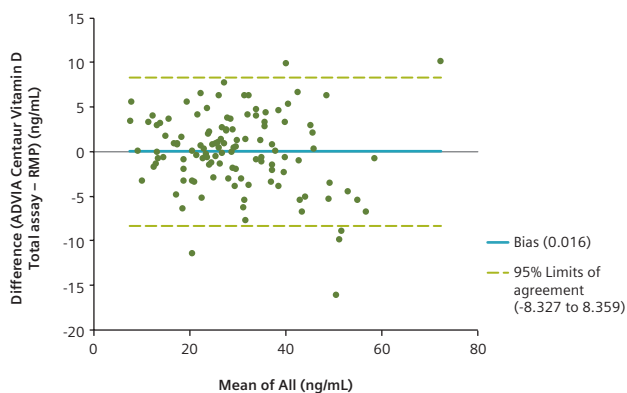
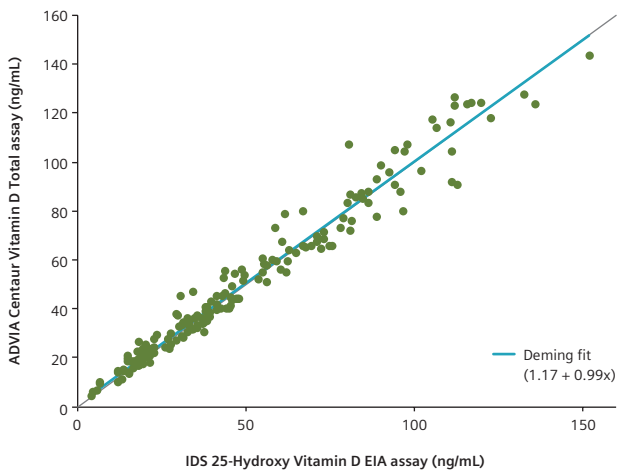


Figure 3. Altman Bland bias plot between all samples run on the ADVIA Centaur Vitamin D Total assay and CDC-RMP.

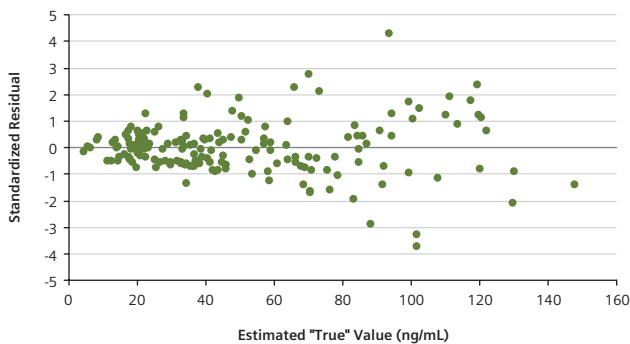
**Table 1.** Summary of method comparison.

X-axis	Y-axis	Slope	Intercept	R*	Sample Range
CDC-RMP–assigned value	ADVIA Centaur Vitamin D Total assay	0.95	+1.62	0.94	5.04–67.2 ng/mL
IDS 25-Hydroxy Vitamin D EIA assay (k021163)	ADVIA Centaur Vitamin D Total assay	0.99	+1.17	0.98	4.2–152 ng/mL

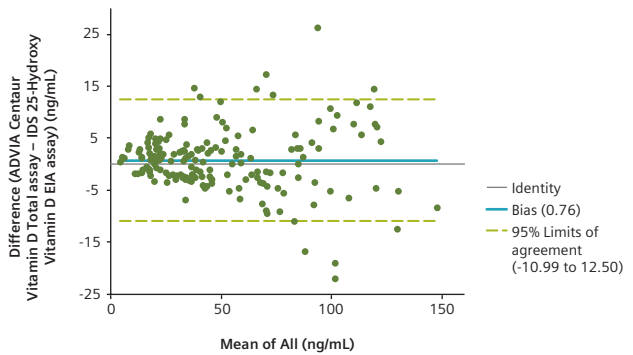
\*Pearson's



**Figure 4.** Deming fit for the ADVIA Centaur Vitamin D Total assay versus the corresponding IDS 25-Hydroxy Vitamin D EIA values.



**Figure 5.** Residual plot for the ADVIA Centaur Vitamin D Total assay dose and the corresponding IDS 25-Hydroxy Vitamin D EIA assay dose.



**Figure 6.** Altman Bland bias plot between all samples run on the ADVIA Centaur Vitamin D Total assay and IDS 25-Hydroxy Vitamin D EIA assay.

## Conclusion

The Siemens ADVIA Centaur Vitamin D Total assay is a CDC VDSCP-certified assay that demonstrates good alignment to both the RMP method and another CDC VDSCP-certified assay. At the time of publication, both assays had received their second year of certification. The harmonization between the ADVIA Centaur Vitamin D Total assay and the true RMP values for 25(OH)vitamin D ensures accurate and reliable results.

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