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Adoption of the ADVIA Centaur Vitamin D Total Assay by the ULB–Institut de Biologie Clinique, Brussels, Belgium

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Summary

ULB–Institut de Biologie Clinique, a private laboratory in Brussels, Belgium, had been using the DiaSorin LIAISON 25 OH Vitamin D TOTAL assay for its vitamin D testing. Seeking to improve its service and enhance the workflow, the laboratory compared this assay against the Siemens ADVIA Centaur® Vitamin D Total assay. The satisfactory precision and method comparison results for the Siemens assay, along with the improved workflow resulting from consolidation of testing onto fewer systems, convinced the laboratory to opt for the Siemens assay.

Introduction

ULB–Institut de Biologie Clinique, Brussels, Belgium, is a private laboratory that performs 40,000 to 50,000 vitamin D tests per year. This laboratory was using the DiaSorin LIAISON 25 OH Vitamin D TOTAL assay as its primary test method because the assay was the first automated vitamin D assay available. Facing an ever-increasing test volume, the laboratory desired a method that delivered higher throughput and a faster turnaround time for improved workflow.

Laboratories have been faced with ever-increasing vitamin D test volumes, a result of recent studies showing associations between levels of the vitamin and higher risk of certain cancers, autoimmune diseases, and cardiovascular disease.[1–3] This development has increased the need for reliable testing solutions that deliver rapid turnaround times and improved workflow. Various challenges, however, have characterized vitamin D testing on several fronts. Manufacturers have recognized the need to provide assays that yield a "total" vitamin D result by recognizing both of the metabolites that are the most reliable indicators of vitamin D status: 25(OH) vitamin D_2 and 25(OH) vitamin D_3.[4,5] Some immunoassays detect a portion of the vitamin D_2 component; others, none at all. Assay performance has also varied with regard to suboptimal precision in earlier assays[6] and widely divergent findings between different assay methods.[7]

The purpose of this study was to evaluate the reproducibility of the ADVIA Centaur Vitamin D Total assay (Siemens Healthcare Diagnostics, Tarrytown, NY, U.S.) and to compare this assay by linear regression analysis and agreement with the LIAISON assay, which was the assay currently in use at the ULB-Institut de Biologie Clinique. If the Siemens assay performed acceptably, the laboratory intended to adopt it as its vitamin D testing solution.

Materials and Methods

Systems

Testing with the Siemens assay was performed on the ADVIA Centaur XP Immunoassay System (Siemens) by operators at the ULB–Institut de Biologie Clinique who were trained for this study. The DiaSorin assay was also run by operators of this laboratory on the DiaSorin LIAISON system according to the manufacturer's instructions.

Reagents, calibrators, controls, and patient samples

The ADVIA Centaur Vitamin D Total assay is a one-pass, 18-minute competitive immunoassay that uses four reagent components: Ancillary Pack Reagent, Solid Phase, Ancillary Well Reagent, and Lite Reagent. The Ancillary Pack Reagent contains a proprietary vitamin D–specific releasing reagent, which has been optimized to release both 25(OH) vitamin D_2 and 25(OH) vitamin D_3 from vitamin D–binding proteins in human serum or plasma. The Solid Phase consists of paramagnetic particles with antifluorescein-labeled antibody. The Ancillary Well Reagent contains vitamin D analog conjugated to fluorescein. The Lite Reagent contains anti–vitamin D monoclonal antibodies labeled with acridinium ester.

All ADVIA Centaur Vitamin D Total assay reagents and calibrators were used and stored in accordance with the manufacturer's instructions.

The controls used in this study were the Siemens Vitamin D Control Materials. All controls were used and stored in accordance with the manufacturer's instructions. Controls were frozen in aliquots and each day, fresh aliquots were used.

For the reproducibility study, Siemens Vitamin D control material was assayed. For the method comparison study, fresh serum samples were assayed on both systems on the same day.

Reproducibility study
To estimate the reproducibility and evaluate within-run, between-run, and total imprecision on the ADVIA Centaur Vitamin D Total assay, each of two controls were assayed in replicates of four, in two runs per day per lot (with at least 2 hours between runs), across 10 days (not necessarily consecutive), yielding a minimum of 80 replicates per lot per sample. Values were required to be within expected ranges and to meet the within-run (WR) and total CV specifications, as shown in Table 1.

Method comparison
A total of 246 patient samples were assayed with the ADVIA Centaur Vitamin D Total assay (mean of duplicate results) and the DiaSorin LIAISON assay (singlicate results). Four samples were excluded from the linear regression analysis and agreement study because they had highly elevated values by one method but not the other; these were resolved by LC-MS/MS. Five other samples were excluded from the linear regression analysis because the LIAISON results were below the assay range (<4 ng/mL), but these samples were included in the agreement study.

Duplicate values were expected to meet the within-run (WR) and total CV specifications listed in Table 1. Duplicate samples with WR CVs that exceeded these ranges were to be repeat tested if there was sufficient volume.

Table 1. CV specifications for reproducibility and method comparison studies.

Vitamin D Value	WR CV	Total CV
<25 ng/mL (<62.4 nmol/L)	<10%	<12%
>25 ng/mL (>62.4 nmol/L)	<7%	<10%

Results

Reproducibility study
Each control yielded mean values within the requirements. Control 1 (mean, 21.90 ng/mL) had a total CV of 8.30%, and Control 2 (mean, 89.79 ng/mL) had a total CV of 5.5%, both total CVs well below the respective targets of 12.0% and 10.0% (Table 2).

Table 2. Precision study results for two controls.

	Control 1	Control 2
Mean	21.90	89.79
SD	1.82	4.90
Total CV	**8.30%**	**5.5%**
Target Total CV	12.0%	10.0%

Method comparison
Linear regression analysis of values plotted for 237 samples yielded means of 24.46 ng/mL (ADVIA Centaur) and 23.50 ng/mL (LIAISON), a slope of 0.70, an intercept value of 8.00, and a correlation coefficient (r) of 0.92 (Figure 1 and Table 3).

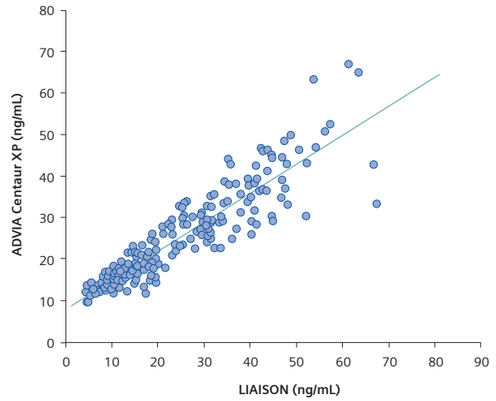

Figure 1. Correlation plot for 237 patient samples: ADVIA Centaur assay vs. LIAISON assay.

Table 3. Regression analysis statistics for the method comparison study.

Number of Samples	237
LIAISON Mean	23.50
ADVIA Centaur Mean	24.46
Slope	0.70
Intercept	8.00
Correlation Coefficient (r)	0.92

Agreement and resolution of discordant samples
The ADVIA Centaur and LIAISON assays demonstrated positive, negative, and overall agreements of about 90% (Table 4).

Table 4. Agreement between the ADVIA Centaur assay and the LIAISON assay, based on a total 25(OH) vitamin D cutoff value of ≤20 ng/mL for deficiency.[4]

		LIAISON		
		Deficient	Not Deficient	Total
ADVIA Centaur	Deficient	13	1	14
	Not Deficient	23	205	228
	Total	36	206	242

Positive agreement (not deficient): 89.9%
Negative agreement (deficient): 92.9%
Overall Agreement: 90.1%

The four highly elevated samples that were excluded from the linear regression analysis and agreement study because of discordant ADVIA Centaur and LIAISON results were reassayed by LC-MS/MS. For three of the four samples, the resolved results agreed with those obtained by the ADVIA Centaur assay (Table 5).

Table 5. Resolution of highly elevated discordant samples (ng/mL).

Sample ID	LIAISON	ADVIA Centaur Value 1	ADVIA Centaur Value 2	LC-MS/MS
1	>150	11.61	10.78	8.24
2	88.1	39.25	41.74	85.08
3	>150	12.85	16.11	6.62
4	148	24.68	26.61	48.91

Conclusions

The ADVIA Centaur Vitamin D Total assay showed good precision, with total CVs of 8.30% (mean: 21.90 ng/mL) and 5.5% (mean, 89.79 ng/mL). The method comparison study showed acceptable agreement between the ADVIA Centaur assay and the LIAISON assay: linear regression analysis of values plotted for 237 samples yielded means of 24.46 ng/mL (ADVIA Centaur) and 23.50 ng/mL (LIAISON), a slope of 0.70, an intercept value of 8.00, and a correlation coefficient (r) of 0.92. Three out of four samples that were discordant by the ADVIA Centaur assay and the LIAISON assay were resolved by LC-MS/MS in favor of the ADVIA Centaur value. This evaluation study found the ADVIA Centaur Vitamin D Total assay to be a satisfactory solution for meeting the vitamin D testing needs of a laboratory with a test volume of 50,000 vitamin D tests per year.

References

1. Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin D and 1,25-dihydroxy-vitamin D levels with all-cause and cardiovascular mortality. Arch Intern Med 2008 June 23;168(12):1340-9.
2. Freedman DM, Looker AC, Chang SC, Graubard BI. Prospective study of serum vitamin D and cancer mortality in the United States. J Natl Cancer Inst 2007 November 7;99(21):1594-602.
3. Hyppönen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 2001 November 3;358(9292):1500-3.
4. Holick MF. Vitamin D deficiency. N Engl J Med 2007 July 19;357(3):266-81.
5. Lai JK, Lucas RM, Clements MS, Harrison SL, Banks E. Assessing vitamin D status: pitfalls for the unwary. Mol Nutr Food Res 2010 August;54(8):1062-71.
6. Carter GD, Berry JL, Gunter E, et al. Proficiency testing of 25-hydroxyvitamin D (25-OHD) assays. J Steroid Biochem Mol Biol 2010 July;121(1-2):176-9.Rollins G. Vitamin D testing--what's the right answer? Labs grapple with confusing analytics, evidence. Clin Lab News 2009 July;35(7):1-9.
7. Rollins G. Vitamin D testing—what's the right answer? Labs grapple with confusing analytics, evidence. Clin Lab News 2009 July;35(7):1-9.