

White Paper

# Assessing Total Vitamin D Assays: A French Population Study

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## Summary

Vitamin D status is most accurately reflected in the total 25(OH) vitamin D value, which includes both 25(OH) vitamin D<sub>2</sub> and 25(OH) vitamin D<sub>3</sub>. Correct assessment of vitamin D status—deficiency, insufficiency, sufficiency, or toxicity—requires an assay that is accurate and precise, and that measures 100 percent of both forms of 25(OH) vitamin D. Because of the rapid and continuing growth in vitamin D testing, many laboratories require a robust solution to meet their vitamin D testing needs.

This French population study compared the Siemens ADVIA Centaur<sup>®</sup> Vitamin D Total assay to liquid chromatography tandem mass spectrometry (LC/MS/MS), the DiaSorin radioimmunoassay (RIA), and the DiaSorin LIAISON vitamin D assay (N = 113). In comparison to LC/MS/MS, the Pearson correlation coefficients (r) and Deming regressions for the ADVIA Centaur XP assay, DiaSorin RIA, and DiaSorin LIAISON assay were 0.92,  $2.90 + 0.95x$ ; 0.94,  $1.86 + 0.88x$ ; and 0.77,  $-0.80 + 0.87x$ , respectively. The ADVIA Centaur XP assay and DiaSorin RIA demonstrated good agreement with LC/MS/MS. Of the eight samples with outlier results (relative to LC/MS/MS), 6/8 of the results were obtained by the DiaSorin LIAISON assay. In comparison to the ADVIA Centaur XP assay, the DiaSorin RIA demonstrated a higher Pearson correlation coefficient (0.90) than the DiaSorin LIAISON assay (0.77). The ADVIA

Centaur XP and ADVIA Centaur Vitamin D Total assays demonstrated excellent agreement with a Pearson correlation coefficient of 0.98 and Deming regression of  $-1.23 + 1.04x$ . The ADVIA Centaur Vitamin D Total assay is a reliable high-volume testing solution that can assist laboratories in addressing their vitamin D testing needs.

## Introduction to Vitamin D

Vitamin D is an important hormone involved in bone health and calcium homeostasis. Recent studies have associated low vitamin D levels with higher risk for certain cancers, autoimmune diseases, and cardiovascular disease.<sup>5-7</sup> These associations have been the driving force for the rapid growth in testing volume over the past decade.

Vitamin D<sub>2</sub> is derived from plant sources, whereas vitamin D<sub>3</sub> is derived primarily from the conversion of 7-dehydrocholesterol in the skin by UV-B radiation from sunlight and secondarily from animal sources.<sup>2</sup> While there are many metabolites of vitamin D, the total 25(OH) vitamin D, that is, the sum of 25(OH) vitamin D<sub>2</sub> and 25(OH) vitamin D<sub>3</sub>, is the most reliable indicator of vitamin D status.<sup>2,8</sup> Both 25(OH) vitamin D<sub>2</sub> and 25(OH) vitamin D<sub>3</sub> are converted in the kidney to the active metabolites 1,25(OH)<sub>2</sub> vitamin D<sub>2</sub> and 1,25(OH)<sub>2</sub> vitamin D<sub>3</sub>.<sup>2,9</sup>

## History of Vitamin D Assays

Vitamin D is extremely hydrophobic and exists bound either to vitamin D-binding protein or albumin. Consequently, it is challenging to measure vitamin D accurately and precisely. Available methods include immunoassays and the direct detection methods high-performance liquid chromatography (HPLC) and liquid chromatography tandem mass spectrometry (LC/MS/MS).<sup>3,4,10</sup> Two widely used immunoassay methods are the DiaSorin 25-Hydroxy Vitamin D radioimmunoassay (DiaSorin RIA) and the DiaSorin LIAISON 25 OH Vitamin D TOTAL assay (DiaSorin LIAISON).

The first 25(OH) vitamin D assay was introduced in the late 1970s. This assay was ideal for the research laboratory, but because of the cumbersome protocol that included organic extraction, nitrogen drying, and preparative chromatography, it was not an ideal solution for the clinical laboratory.<sup>10</sup> This and other early vitamin D immunoassays utilized a competitive-protein-binding assay format and relied either on vitamin D-binding protein or chick intestinal receptor as the primary binding agents and also used <sup>3</sup>H-labeled reporters.<sup>10</sup>

In the 1980s, a nonchromatographic radioimmunoassay method was developed. It used acetonitrile as the extraction agent, a radioactively labeled reporter, and a capture antibody that detected both 25(OH) vitamin D<sub>2</sub> and 25(OH) vitamin D<sub>3</sub>. This method was commercialized as the DiaSorin RIA and has been used in many of the landmark vitamin D clinical trials.<sup>5,6,10</sup>

In the 2000s, fully automated chemiluminescence nonradioactive assays were introduced by multiple manufacturers.<sup>10</sup> These assays varied in their ability to measure and to detect 25(OH) vitamin D<sub>2</sub> and thus in their ability to provide a reliable total 25(OH) vitamin D value.

The direct detection methods HPLC and LC/MS/MS are still in use today. HPLC has been in use since the late 1970s, and LC/MS/MS is now in use in large reference laboratories.<sup>3,10</sup> HPLC followed by UV detection is a highly repeatable method, and LC/MS/MS is a very accurate method when performed correctly.<sup>3,10</sup> In newborns, LC/MS/MS results can be difficult to interpret because the assay is unable to distinguish the inactive 3-epi-25(OH) vitamin D<sub>3</sub> from 25(OH) vitamin D<sub>3</sub>.<sup>3,10</sup> Both HPLC and LC/MS/MS detect and measure 25(OH) vitamin D<sub>2</sub> and 25(OH) vitamin D<sub>3</sub> separately; what is needed to assess vitamin D status, however, is the sum of these values.<sup>11</sup>

## Vitamin D Testing: Key Issues

The rapid growth in vitamin D testing and its high test volumes has led many clinical laboratories to consider adding vitamin D to their in-house menus. A key challenge for laboratories considering this change is method variation (discrepant results among methods). Other key issues that must be considered when choosing a vitamin D testing solution include level of staff expertise, turnaround time, ability to provide a true total 25(OH) vitamin D value, and reproducibility.

### Level of staff expertise

HPLC and LC/MS/MS are highly operator-dependent methods that require a high level of staff expertise for reliable results, and thus may not be suitable for many clinical laboratories; in contrast automated immunoassays are less cumbersome and require lower levels of staff expertise and thus may be more suitable for routine use in clinical laboratories.<sup>4</sup>

### Method variation

Laboratories have reported discrepancies among methods (method variation) in vitamin D values and patient classification (deficiency, sufficiency, insufficiency, and toxicity). In one lab, 60% of the results from an immunoassay method indicated insufficiency, compared to only 30% by LC/MS/MS.<sup>9</sup> Another laboratory had similar discrepancies for sample classification: 80% of samples had levels below 32 ng/mL by immunoassay, but only 46% of samples by LC/MS/MS.<sup>9</sup>

### Measurement of total 25(OH) vitamin D

Measuring both 25(OH) vitamin D<sub>2</sub> and 25(OH) vitamin D<sub>3</sub> is essential for accurate assessment of vitamin D status.<sup>2,3,10</sup> Determinations of deficiency (total 25(OH) vitamin D at <20 ng/mL), insufficiency (20–30 ng/mL), sufficiency (30–100 ng/mL), and toxicity (>150 to 200 ng/mL) can be made with confidence only if the assay provides a true total vitamin D value.<sup>1–4</sup> Immunoassays vary in their ability to detect all of the 25(OH) vitamin D<sub>2</sub> component: some assays are unable to detect this analyte and others are only able to detect only a portion of it.<sup>1,2,4</sup> In some countries, such as the United States, where prescription supplements are primarily vitamin D<sub>2</sub>, it is vitally important that the vitamin D assay be able to measure all of the vitamin D<sub>2</sub> that is present.<sup>2</sup>

### Turnaround time

Vitamin D testing has grown rapidly; a study found that over 50 percent of laboratories had experienced a 50 percent or more increase in vitamin D testing within a year.<sup>9</sup> Any viable vitamin D testing solution, whether an in-house or send-out solution, must therefore be able to successfully manage high-volume testing. Automated immunoassays have a much faster turnaround time than manual immunoassays, radioimmunoassays, and the direct detection methods HPLC and LC/MS/MS.<sup>4</sup>

### Reproducibility

Imprecision has been a challenge for vitamin D assays, but it has improved within the past decade, decreasing from an average CV across all methods of 32% to 15%, with some assays having much better precision than others.<sup>1</sup> A recent study evaluated the precision of two widely used assays at eight laboratories. The CVs ranged from 4.8% to 18.3%; the maximum percent difference between two replicates of the same assay ranged from 15% to 97%; and the percent of samples that were classified differently by replicate was as high as 31.3%.<sup>12</sup>

### ADVIA Centaur Vitamin D Total Assay

The ADVIA Centaur Vitamin D Total assay is intended for in vitro diagnostic use in the quantitative determination of total 25(OH) vitamin D in human serum and plasma (EDTA, lithium-heparin, sodium-heparin) on the ADVIA Centaur and ADVIA Centaur XP systems.<sup>13</sup> The ADVIA Centaur Vitamin D Total assay is intended as an aid in the determination of vitamin D sufficiency. The assay provides a total vitamin D value; it recovers 104.5% of 25(OH) vitamin D<sub>2</sub> and 100.7% of 25(OH) vitamin D<sub>3</sub>.<sup>13</sup> It was also designed to address high-volume testing, with a time to first result of 18 minutes and a throughput of 240 tests/hour. The assay has minimal cross-reactivity for key interferents: for vitamin D<sub>2</sub>, 0.5%; for vitamin D<sub>3</sub>, 0.3%; and for 3-epi-25(OH) vitamin D<sub>3</sub>, 1.1%. The required sample volume for a single determination is 20 µL.<sup>13</sup>

### Method Comparison Results

A total of 351 remnant frozen clinical samples were supplied by Dr. Jean-Claude Souberbielle, Groupe Hospitalier Necker Enfants Malades, Paris, France, with known 25(OH) vitamin D values as determined by the DiaSorin RIA. These DiaSorin RIA 25(OH) vitamin D values were generated using fresh samples according to the manufacturer's instructions with the following modifications: samples were centrifuged at 2°C–8°C instead of 20°C–25°C, and were aspirated instead of decanted. A subset of these frozen samples (n = 113), selected on the basis of available sample volume, were thawed and divided into three aliquots. One aliquot was sent for DiaSorin LIAISON measurement at Research and Development Institute (Calabasas, CA, USA), one aliquot was sent for ADVIA Centaur measurement at Siemens Healthcare Diagnostics (Tarrytown, NY, USA), and one aliquot was sent to a U.S.-accredited laboratory for LC/MS/MS measurement. The DiaSorin LIAISON results were generated following the manufacturer's instructions and LC/MS/MS results were generated according to the accredited laboratory's protocol. Samples were evaluated in the ADVIA Centaur Vitamin D Total assay (on the ADVIA Centaur XP and the ADVIA Centaur systems) and these values were compared to the vitamin D values obtained using the DiaSorin LIAISON, DiaSorin RIA, and LC/MS/MS methods. A minimum of 100 samples were included in each method comparison. The results were evaluated for agreement by Deming regression and Pearson correlation coefficient analysis.

**Outliers**

Eight samples had outlier results (differing by more than 40 percent from LC/MS/MS values). Of these 8 outlier results, 6/8 were generated by the DiaSorin LIAISON immunoassay, 1/8 was generated by the DiaSorin RIA, and 2/8 were generated by the ADVIA Centaur XP immunoassay. For sample 1, the LC/MS/MS result was 4 ng/mL and the ADVIA Centaur result was 12 ng/mL; however, this sample's vitamin D status is classified by both methods as deficient (concordant classification).

**Table 1.** Samples with outlier results (ng/mL, rounded to the nearest whole number) by assay.

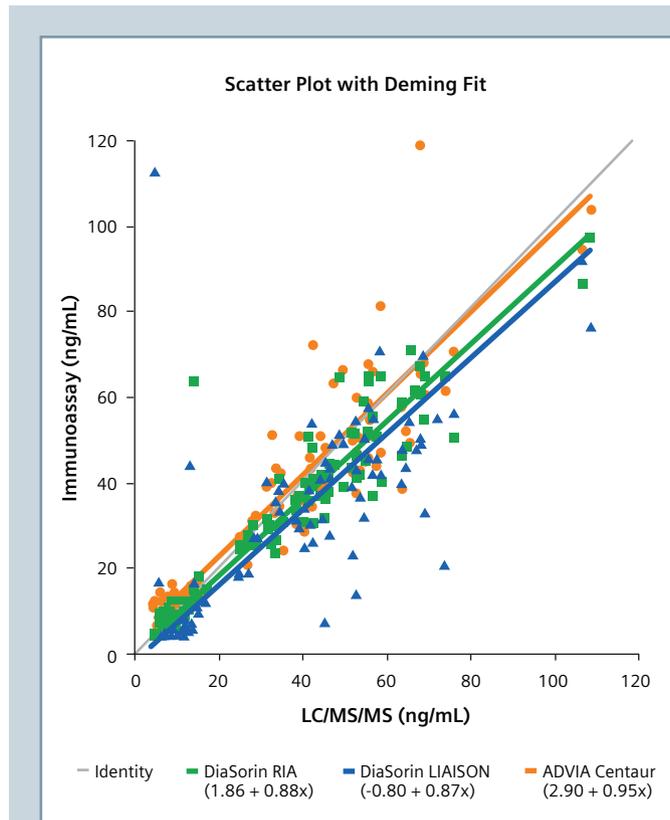
Sample	LC/MS/MS	DiaSorin RIA	DiaSorin LIAISON	ADVIA Centaur XP
1	4	5	109	12
2	12	11	43	11
3	44	35	7	47
4	51	41	14	37
5	45	43	27	43
6	50	50	23	48
7	13	62	11	16
8	66	65	49	115

**Method comparison: LC/MS/MS versus ADVIA Centaur XP, DiaSorin LIAISON, and DiaSorin RIA**

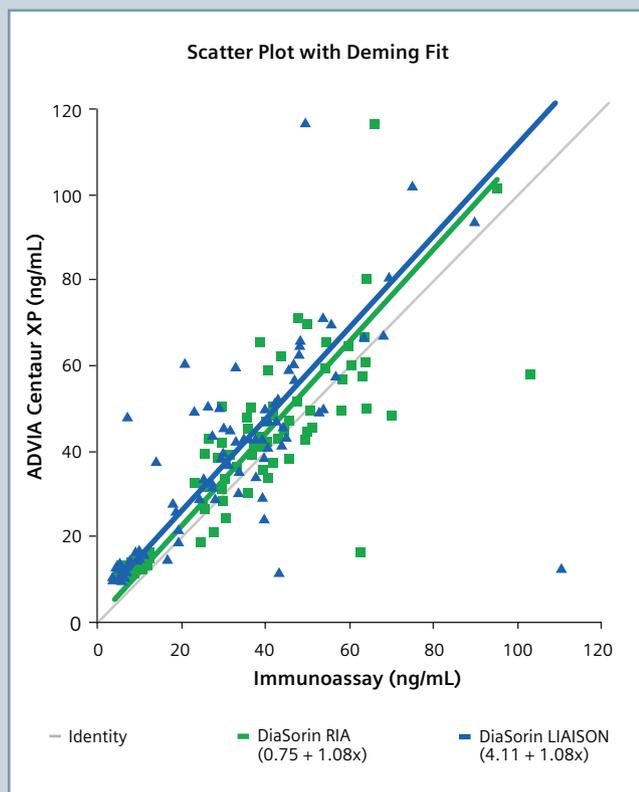
Compared to LC/MS/MS, the ADVIA Centaur XP and DiaSorin RIA total vitamin D assays demonstrated good agreement as indicated by Pearson correlation coefficient and Deming regression analysis (Table 2 and Figure 1).

**Table 2.** Pearson correlation coefficient and Deming regression by method compared to the LC/MS/MS assay.

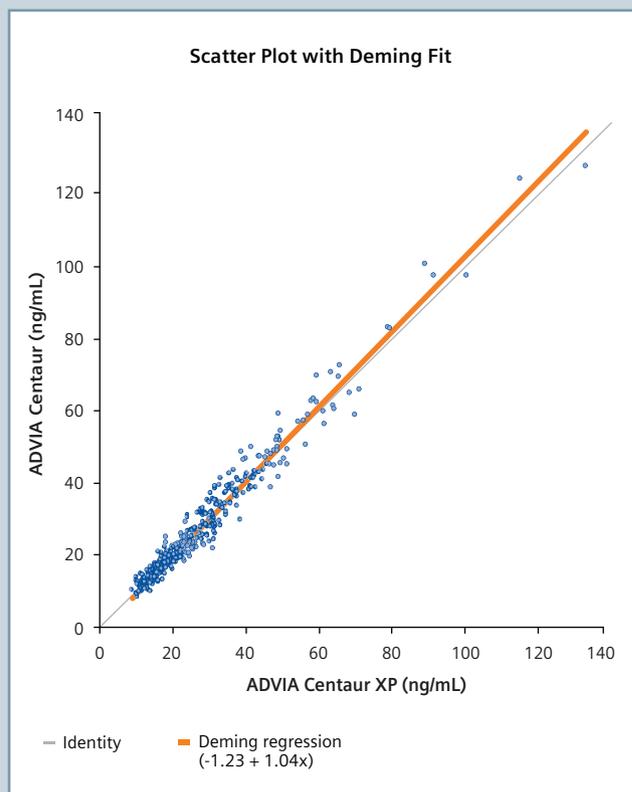
Method	ADVIA Centaur XP	DiaSorin RIA	DiaSorin LIAISON
Pearson correlation coefficient (r)	0.92	0.94	0.77
Slope	0.95	0.88	0.87
Intercept (ng/mL)	2.90	1.86	-0.80
N	113	113	113



**Figure 1.** Deming regression by method compared to the LC/MS/MS assay.



**Figure 2.** Deming regression by method compared to the ADVIA Centaur XP assay.



**Figure 3.** Deming regression comparison of the ADVIA Centaur Vitamin D Total assay on the ADVIA Centaur XP and the ADVIA Centaur instruments (n = 350).

**Method comparison: ADVIA Centaur XP versus DiaSorin LIAISON and DiaSorin RIA**

Compared to the ADVIA Centaur XP assay, the DiaSorin RIA had a higher Pearson correlation coefficient (Table 3) and a better Deming regression (Table 3 and Figure 2) than the DiaSorin LIAISON assay.

**Table 3.** Pearson correlation coefficient and Deming regression by method compared to the ADVIA Centaur XP assay.

Method	DiaSorin RIA	DiaSorin LIAISON
Pearson correlation coefficient (r)	0.90	0.77
Slope	1.08	1.08
Intercept (ng/mL)	0.75	4.11
N	113	113

**Method comparison: ADVIA Centaur XP versus ADVIA Centaur**

The Vitamin D Total assay on the ADVIA Centaur XP and ADVIA Centaur instruments demonstrated excellent Pearson correlation, 0.98, and Deming regression,  $-1.23 + 1.04x$  (Figure 3).

## Conclusions

The ADVIA Centaur Vitamin D Total assay has been designed to address the key issues in vitamin D testing. This automated assay has a turnaround time of 18 minutes and throughput of 240 tests per hour to meet high-volume testing needs. It provides a total 25(OH) vitamin D value; it recovers 104.5% of 25(OH) vitamin D<sub>2</sub> and 100.7% of 25(OH) vitamin D<sub>3</sub>.

This study compared the ADVIA Centaur Vitamin D Total assay to the LC/MS/MS, DiaSorin RIA, and DiaSorin LIAISON assays. In comparison to LC/MS/MS, the Pearson correlation coefficients and Deming regressions for the ADVIA Centaur XP assay, DiaSorin RIA, and DiaSorin LIAISON assay were 0.92,  $2.90 + 0.95x$ ; 0.94,  $1.86 + 0.88x$ ; and 0.77,  $-0.80 + 0.87x$ , respectively. The ADVIA Centaur Vitamin D Total assay and the DiaSorin RIA demonstrated similar Pearson correlation coefficients, 0.92 and 0.94, when compared to LC/MS/MS. Of the 8 samples with outlier results (varying by more than 40% from the LC/MS/MS result) obtained on 8 samples, 6/8 results were generated by the DiaSorin LIAISON assay. In comparison to the ADVIA Centaur XP assay, the DiaSorin RIA demonstrated a higher Pearson correlation (0.90) than the DiaSorin LIAISON assay (0.77). This study also demonstrated excellent agreement for ADVIA Centaur Vitamin D Total assay results obtained on the ADVIA Centaur XP and ADVIA Centaur instruments, with a Pearson correlation coefficient of 0.98 and a Deming regression equation of  $-1.23 + 1.04x$ .

The automated ADVIA Centaur Vitamin D Total assay provides a robust vitamin D testing solution that compares well with established methods and is able to handle high test volumes while providing reliable results.

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