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Development of the ADVIA Centaur Vitamin D Total Assay

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Abstract



Introduction

An assay^a for total vitamin D (25-hydroxyvitamin D) with a range up to 250 ng/mL that measures both 25(OH)D₂ and 25(OH)D₃ on the ADVIA Centaur® systems is being developed by Siemens Healthcare Diagnostics Inc. (Deerfield, IL, US). Vitamin D is a steroid hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis. Vitamin D is essential for the formation and maintenance of strong, healthy bones. Vitamin D deficiency can result from inadequate exposure to the sun, inadequate alimentary intake, decreased absorption, abnormal metabolism, or vitamin D resistance. Recently, many chronic diseases such as cancer, high blood pressure, osteoporosis, and several autoimmune diseases have been linked to vitamin D deficiency. Whether consumed or produced, both forms of the vitamin (D₂ and D₃) are metabolized by the liver to 25(OH) D and then converted in the liver or kidney into 1,25-dihydroxyvitamin D. Vitamin D metabolites are bound to a carrier protein in the plasma and distributed throughout the body. It is generally accepted that 25(OH) D is the metabolite that is the most reliable clinical indicator of vitamin D status because serum 25(OH)D levels reflect the body's storage levels of vitamin D and correlate with the clinical symptoms of vitamin D deficiency.

Materials and Methods

The Siemens ADVIA Centaur Vitamin D Total assay design is based on a sequential competitive immunoassay format. Sample is added to the reaction cuvette followed by displacement buffer and allowed to react for 4.5 minutes. Monoclonal antibody conjugated to acridinium ester is added and allowed to react for 5.5 minutes to bind 25(OH) vitamin D in the sample. A 25(OH) vitamin D analog conjugated to bovine serum albumin and fluorescein is added along with antiluorescein-coated paramagnetic particles

and allowed to react for 3.75 minutes. The reaction cuvette is washed, and acid and base reagents are added to initiate the chemiluminescent reaction. The time-to-result is 18 minutes. An inverse relationship exists between the amount of 25(OH) vitamin D in the patient sample and the amount of relative light units (RLUs) detected by the system.

Results:^b

The data obtained with the ADVIA Centaur Vitamin D Total assay demonstrated equimolar detection of 25(OH)D₂ and 25(OH)D₃ and showed traceability to LC-MS/MS. Cross-reactivity to 25(OH)D₂ was determined to be 105% at 50 ng/mL. The assay demonstrated a limit of detection (LoD) of less than 3.0 ng/mL, a functional sensitivity (20% dose total CV) of less than 4 ng/mL, and an upper limit of 250 ng/mL. Total assay CVs were 6.4%, 7.1%, 4.2%, and 3.7% for samples at 22.1, 52.3, 121, and 153 ng/mL, respectively. Linearity up to 240 ng/mL was demonstrated. A correlation study against LC-MS/MS was performed with 150 serum samples, yielding a slope of 0.96, intercept of 1.0, and regression coefficient of 0.97.

Conclusion

The Siemens ADVIA Centaur Vitamin D Total assay may be a valuable tool in clinical laboratories for the accurate measurement of vitamin D deficiency in human sera.

^a For investigational use only. Not available for sale. The performance characteristics of this product have not been established.

^b The performance data in this poster have been updated since publication of the abstract.

Introduction

Vitamin D is a steroid hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis. Vitamin D is essential for the formation and maintenance of strong, healthy bones.

Vitamin D deficiency can result from inadequate exposure to the sun, inadequate alimentary intake, decreased absorption, abnormal metabolism, or vitamin D resistance.¹ Recently, many chronic diseases such as cancer, high blood pressure, osteoporosis, and several autoimmune diseases have been linked to vitamin D deficiency. Whether consumed or produced, both forms of the vitamin (D₂ and D₃) are metabolized by the liver to 25(OH)D and then converted in the liver or kidney into 1,25-dihydroxyvitamin D.² Vitamin D metabolites are bound to a carrier protein in the plasma and distributed throughout the body. It is generally accepted that 25(OH)D is the metabolite that is the most reliable clinical indicator of vitamin D status because serum 25(OH)D levels reflect the body's storage levels of vitamin D and correlate with the clinical symptoms of vitamin D deficiency.³

Materials and Methods

Assay: The ADVIA Centaur Vitamin D Total assay is a one-pass, 18-minute antibody competitive immunoassay that uses an antiferrofluorescein-labeled monoclonal antibody covalently bound to paramagnetic particles (PMPs), one monoclonal antibody labeled with acridinium ester (AE), and a vitamin D analog labeled with fluorescein (Figure 1). The Vitamin D Total assay requires 20 µL of sample volume for a single determination. The time-to-first-result is 18 minutes and the throughput is 240 tests/hour.

Precision: The precision study was based on the CLSI protocol EP5-A2:⁴ two runs per day for 10 days on a single ADVIA Centaur system. Assay precision was determined using samples with Vitamin D Total ranging from 4 to 120 ng/mL.

Analytical sensitivity: Analytical sensitivity is defined as the concentration that corresponds to the mean signal plus 2 SD obtained from the lowest standard, expressed in relative light units (RLUs). The analytical sensitivity study was performed following CLSI protocol EP5-A2.⁴ Analytical sensitivity was determined using 60 replicates of the lowest standard.

Limit of blank, limit of detection, and functional sensitivity: The limit of blank is defined as the concentration of analyte that corresponds to the 95th percentile of the distribution of a human negative basepool. The Vitamin D total low standard was assayed 20 times using two lots of reagents on three systems (n = 120).

The limit of detection (LoD) is determined according to CLSI protocol EP17-A.⁵ Limit of detection is defined as the lowest concentration of vitamin D that can be detected with 95% probability. The LoD was determined by using low-level vitamin D samples that were assayed 20 times using two lots of reagents on three systems (n = 120).

Functional sensitivity was determined using a single instrument over 10 days. Two runs were performed per day in duplicate for a total of 60 replicates. The ADVIA Centaur Vitamin D Total sensitivity panel members concentrations ranged from 3.0 to 20.0 ng/mL. Concentrations were calculated using within-lot, day zero, two-point calibration curves.

Interference studies: Interference from endogenous and nonendogenous substances were evaluated following the guidelines in NCCLS EP-7A.⁶ Each sample was spiked with an interferent and compared to a matched unspiked control.

Cross-reactivity: Five vitamin D derivatives were analyzed using the ADVIA Centaur Vitamin D Total assay. The vitamin D derivatives were spiked into a sample containing 27 ng/mL of vitamin D total. Three replicates of the spiked samples were assayed and the vitamin D total concentration determined.

Tube type study: Correlation of EDTA and serum separator tubes (SST) tubes was analyzed using the ADVIA Centaur Vitamin D Total assay. Serum red top, SST, and EDTA tubes were collected from 119 donors and assayed using the Centaur Vitamin D Total assay. Three replicates of each sample were evaluated. Linear regression correlation between serum and SST and serum vs EDTA was determined.

Method correlation: The ADVIA Centaur Vitamin D Total assay was compared to a commercially available, FDA-cleared, Vitamin D Total immunoassay using 199 patient specimens, a single replicate for each method. Specimen concentration ranged from 5 to 150 ng/mL. In addition, 23 patient samples were assayed by LC-MS/MS and the ADVIA Centaur Vitamin D Total Assay. Specimens in this second population ranged from 11 to 82 ng/mL.

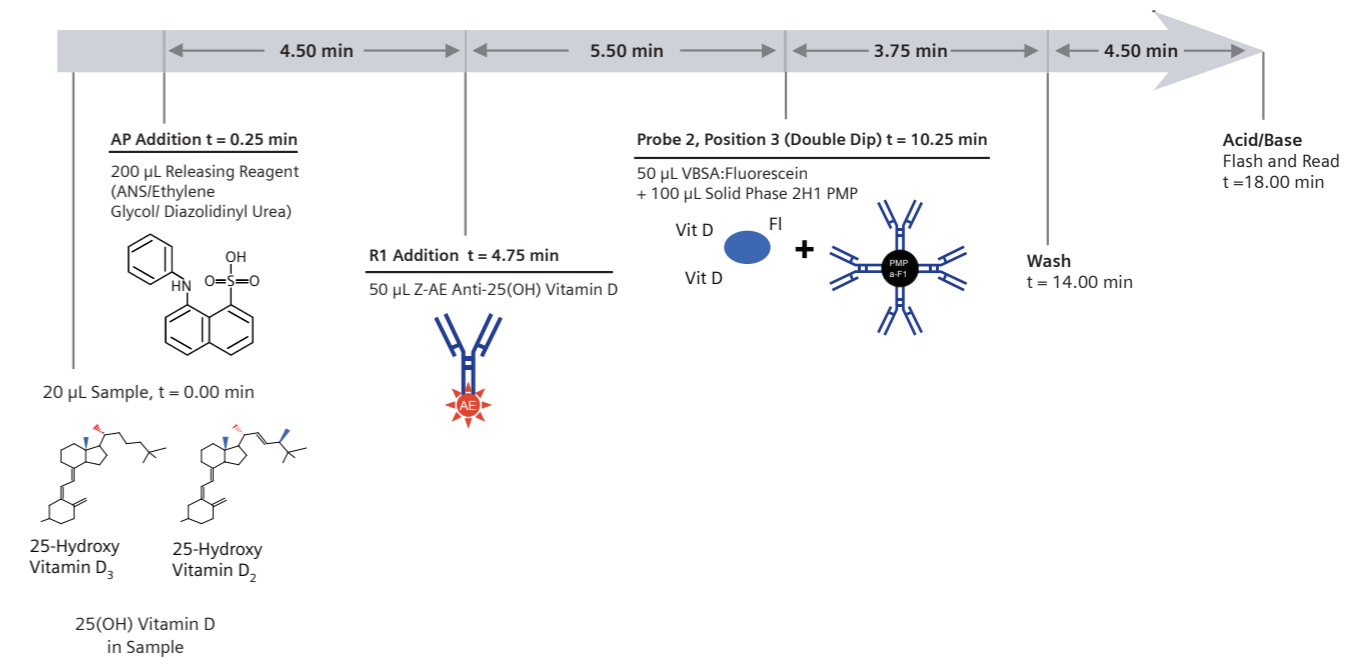


Figure 1. Schematic of the ADVIA Centaur Vitamin D Total Assay.

Results

Precision: The precision profile of the ADVIA Centaur Vitamin D Total assay demonstrates a total CV between 8.8% at 7.65 ng/mL to 2.0% at 123.36 ng/mL 25(OH) total vitamin D.

Table 1. Precision analysis

Sample	Mean (ng/mL)	Within-Run SD (ng/mL)	Within-Run CV (%)	Total SD (ng/mL)	Total CV (%)
1	7.65	0.65	8.5	0.67	8.8
2	10.65	0.85	8.0	1.07	10.1
3	13.11	0.84	6.4	0.91	6.9
4	15.87	1.02	6.4	1.18	7.4
5	18.40	1.31	7.1	1.44	7.8
6	22.63	1.79	7.9	1.79	7.9
7	59.75	1.76	3.0	1.92	3.2
8	99.63	1.95	2.0	2.07	2.1
9	112.74	1.98	1.8	3.07	2.7
10	115.71	1.98	1.7	2.55	2.2
11	123.36	2.29	1.9	2.51	2.0

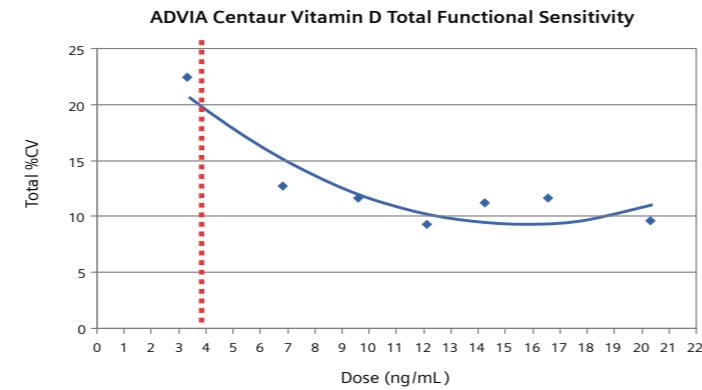
Analytical sensitivity: The analytical sensitivity of the ADVIA Centaur Vitamin D Total assay was 2.4 ng/mL (Table 2).

Table 2. Analytical sensitivity

Sample	Replicates	Mean RLU + 2 SD	Dose (ng/mL)
Vitamin D Blank	60	688200	2.4

Limit of Blank, Limit of Detection, and Functional Sensitivity: The limit of blank of the ADVIA Centaur Vitamin D Total assay was 2.8 ng/mL, the limit of detection was 3.8 ng/mL, and the functional sensitivity was 4 ng/mL (Figure 2).

Figure 2. Precision profile showing the LoD and functional sensitivity (red dashed line) of the ADVIA Centaur Vitamin D Total assay



Interference studies: The ADVIA Centaur Vitamin D Total assay demonstrated $\leq 10\%$ bias at the concentrations tested for endogenous interferents (Table 3).

Table 3. Results of endogenous interference studies

Interferent	Concentration	Vitamin D Total Expected (ng/mL)	Vitamin D Total Observed (ng/mL)	Bias (%)
Unconjugated Bilirubin	60 mg/dL	30.88	32.67	5.77
Conjugated Bilirubin	60 mg/dL	33.82	30.67	-9.31
Albumin	9 g/dL	22.5	20.4	-9.33
Hemoglobin	500 mg/dL	29.08	29.70	2.13
Triglycerides	500 mg/dL	22.7	23.6	3.96
Uric Acid	20 mg/dL	35.45	33.45	-5.64

Cross-reactivity: The ADVIA Centaur Vitamin D Total assay demonstrated very low cross-reactivity to the nonhydroxylated forms of Vitamin D₂ and Vitamin D₃, and to 3-epi-25(OH)D₃, as listed in Table 4.

Table 4. Cross-reactivity

Crossreactant	Concentration (ng/mL)	Expected (Endogenous) Vitamin D Total (ng/mL)	Observed Vitamin D Total (ng/mL)	Cross-reactivity (%)
25-(OH)-Vit D ₃	27	0	27	100
25-(OH)-Vit D ₂	30	27	58	102
Vitamin D ₂	100	27	28	0.04
Vitamin D ₃	100	27	28	0.04
3-epi-25(OH) D ₃	100	27	27	0.0

Tube type study: A sample tube type correlation was performed with 119 donor specimens collected in serum red top, SST, and EDTA tube types. Regression analysis between serum red top and SST demonstrates a correlation coefficient (R) of 0.999, a slope of 1.01, and an intercept of -0.14 (Figure 3). Regression analysis between serum red top and EDTA demonstrates a correlation coefficient (R) of 0.997, a slope of 1.00, and an intercept of 0.41 (Figure 4).

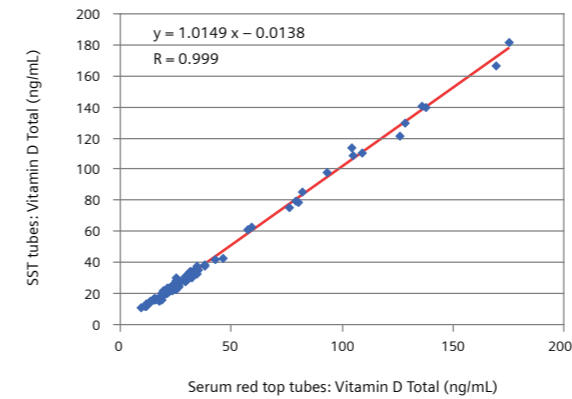


Figure 3. Correlation of Vitamin D Total levels collected from 119 donors in serum red top and SST tubes and functional sensitivity

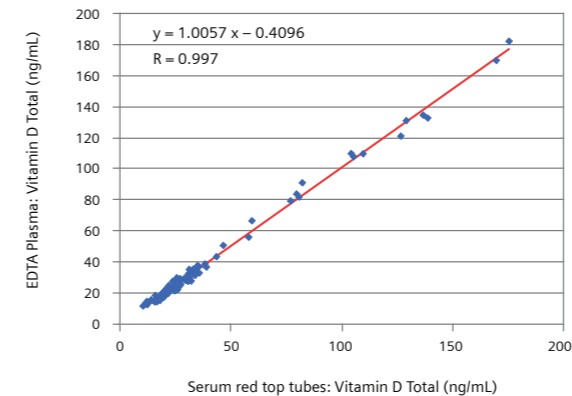


Figure 4. Correlation of Vitamin D Total levels collected from 119 donors in serum red top and EDTA tubes

Method comparison: A sample correlation was performed with 199 specimens comparing the ADVIA Centaur Vitamin D Total assay to a commercially available, FDA-cleared, Vitamin D Total assay. Regression analysis demonstrated a correlation coefficient (R) of 0.993, a slope of 1.00, and an intercept of 1.61 (Figure 5). In addition 23 specimens were assayed comparing the ADVIA Centaur Vitamin D Total assay to a commercially available Vitamin D Total LC-MS/MS assay. Regression analysis demonstrated a correlation coefficient (R) of 0.98, a slope of 1.03, and an intercept of -2.3 (Figure 6).

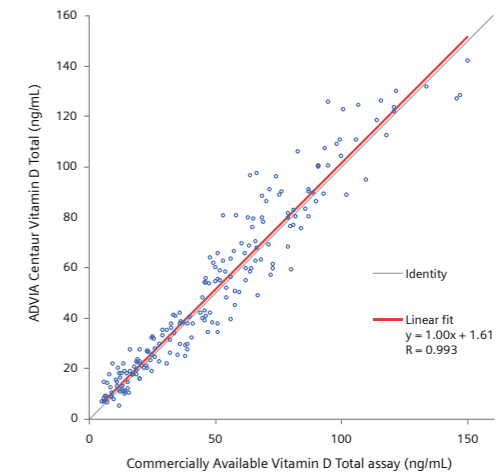


Figure 5. Method correlation of the ADVIA Centaur and a commercially available Vitamin D Total assay

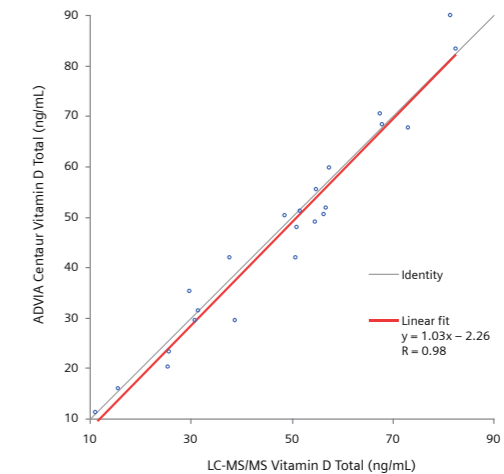


Figure 6. Method correlation of the ADVIA Centaur and LC-MS/MS Vitamin D Total assays

Conclusion

The ADVIA Centaur Vitamin D Total assay^a has shown acceptable sensitivity, precision, and performance compared to a commercially available method. This assay on a fully automated, high-throughput immunoassay system may be a valuable tool in clinical laboratories for the measurement of vitamin D sufficiency.

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