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White Paper

Influence of Vitamin D Binding Protein on Accuracy of the ADVIA Centaur Vitamin D Total Assay

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Influence of Vitamin D Binding Protein on Accuracy of the ADVIA Centaur Vitamin D Total Assay

Introduction

The influence of the vitamin D binding protein (DBP) on the accuracy of immunoassay vitamin D results has been under recent discussion.[1] In the study described in this document, endogenous and various levels of spiked DBP were examined in normal patient serum pools and specific patient populations, such as chronic kidney disease and third-trimester pregnancy patients, to determine the impact on the ADVIA Centaur® Vitamin D Total assay.

Background

Designing an equimolar immunoassay that measures total 25(OH)vitamin D_2 and D_3 is challenging due to several reasons: (1) 25(OH)vitamin D_2 and D_3 have different binding affinities, (2) the molar concentration of DBP far exceeds that of vitamin D, and (3) free-circulating vitamin D is not found. Vitamin D is highly hydrophobic, and the DBP binds and transports vitamin D and its metabolites. Not only does DBP bind 25(OH)vitamin D_3 at an affinity of $K_a = 5 \times 10^{-8}$ M, but less than 5% of available DBP binding sites contain vitamin D.[2]

Before an automated immunoassay can measure 25(OH)vitamin D, the 25(OH)vitamin D has to be released from the DBP. In the process for both radioactive immunoassays and mass spectrometry methods, there is an extraction step using organic solvents that removes all bound vitamin D from the DBP. However, for automated immunoassays, there are compatibility issues with organic solvents, and therefore, these immunoassays must use proprietary releasing agents. The amount of released 25(OH)vitamin D is unknown, but automated immunoassays overcome this challenge through the assignment of internal standards.

The concentration of DBP varies in different patient populations. In chronic kidney disease patients, impaired renal function causes proteinuria, which impacts the concentrations of DBP.[3] Increased estrogen in pregnancy increases the concentrations of DBP in pregnant patients.[4]

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Study Design

In order to determine the influence of DBP on a vitamin D immunoassay, a study examining DBP as an endogenous interference, similar to how hemoglobin, cholesterol, or total protein would be measured, following CLSI Document EP7-A2[5] was performed at the Siemens R&D facility in Tarrytown, NY.

Five human serum pools containing 25(OH)vitamin D were prepared and sent out for LC/MS/MS measurement by a large U.S. reference laboratory. The LC/MS/MS values for the five individual pools (pool 1–5) resulted in mean concentrations of 24, 32, 51, 41, and 75 ng/mL respectively.

In addition, >95% pure human native DBP was purchased from Athens Research & Technology, Inc. The endogenous levels of DBP were measured in each of the five serum pools using the QUANTIKINE ELISA VitD BP kit, DVDBP0 (R&D Systems, Inc.). From there each of the five serum pools were divided into six aliquots, and DBP was spiked into aliquots 2–6 of each pool to various levels. The DBP was then reanalyzed in each sample. ADVIA Centaur Vitamin D Total measurements of all sample pools and associated aliquots were performed (Siemens, Tarrytown, NY). Bias to the original LC/MS/MS was calculated and is shown in Table 1 and graphically in Figure 1. Thirty-six clinical serum samples from pregnancy patients (third trimester) and 40 clinical serum samples from chronic kidney disease patients, allowing a wide range of DBP concentrations, were also evaluated and are shown in Tables 2 and 3 and graphically in Figures 2 and 3.

Results

In the five serum pools, the levels of endogenous DBP ranged from 260.7 to 519.0 µg/mL (average 347.6 µg/mL), which is consistent with other studies.[1,2,6]

The average bias of all samples—endogenous DBP and spiked DBP—for the ADVIA Centaur assay was –1.4%. This demonstrates there was not a significant bias observed for the ADVIA Centaur to LC/MS/MS due to increasing levels of spiked DBP in normal serum pools.

Table 1. Assay bias as a function of DBP concentrations in normal human serum pools.

LC/MS/MS (ng/mL)	DBP (µg/mL)	ADVIA Centaur (ng/mL)	ADVIA Centaur Bias to LC/MS/MS (%)
24	276.9	24.3	1
24	347.2	24.2	1
24	385.5	23.5	–2
24	334.9	24.3	1
24	407.1	23.1	–4
24	472.1	22.9	–5
32	301.6	31.6	–1
32	339.1	34.8	9
32	629.7	33.0	3
32	446.8	34.8	9
32	489.5	34.5	8
32	584.4	31.9	0
51	260.7	53.0	4
51	261.2	48.4	–5
51	327.8	47.5	–7
51	417.7	50.8	0
51	593.3	49.1	–4
51	486.2	45.7	–10
41	380.1	39.7	–3
41	420.5	40.6	–1
41	590.2	41.2	0
41	747.3	38.5	–6
41	738.0	40.9	0
41	980.6	37.7	–8
75	519.0	75.3	0
75	584.8	69.7	–7
75	724.9	75.3	0
75	788.4	73.4	–2
75	731.3	67.7	–10
75	789.7	73.1	–2

The average bias for all third-trimester pregnancy samples (Table 2 and Figure 2) for the ADVIA Centaur assay to LC/MS/MS was –6%. The average bias for all renal dialysis samples (Table 3 and Figure 3) for the ADVIA Centaur assay was 4%. This demonstrates there was not a significant bias observed for the ADVIA Centaur to LC/MS/MS in patients with clinical conditions such as pregnancy or chronic kidney disease. Pregnancy samples had a Pearson coefficient of 0.92, and renal dialysis samples had a Pearson coefficient of 0.97. This demonstrates acceptable performance of the ADVIA Centaur to LC/MS/MS for these patient groups.

Figure 1. Percent bias as a function of DBP concentration in normal human serum pools.

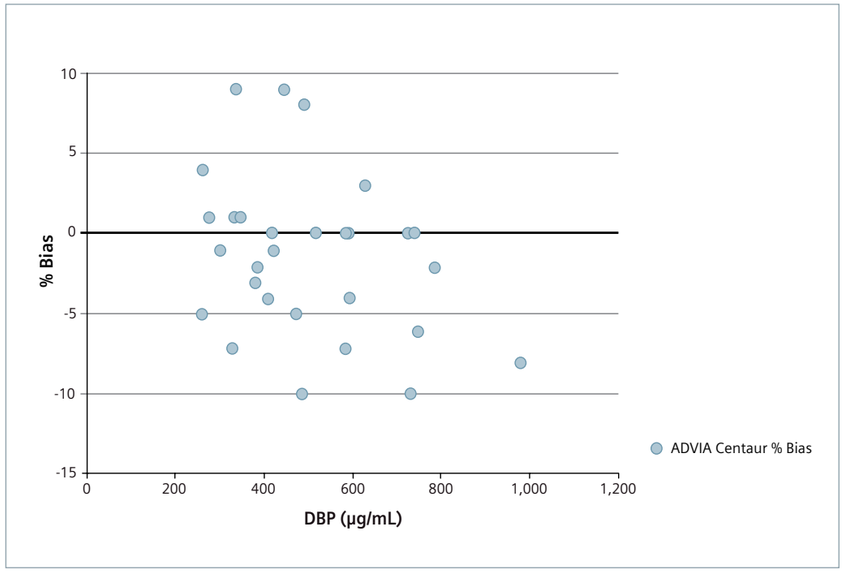
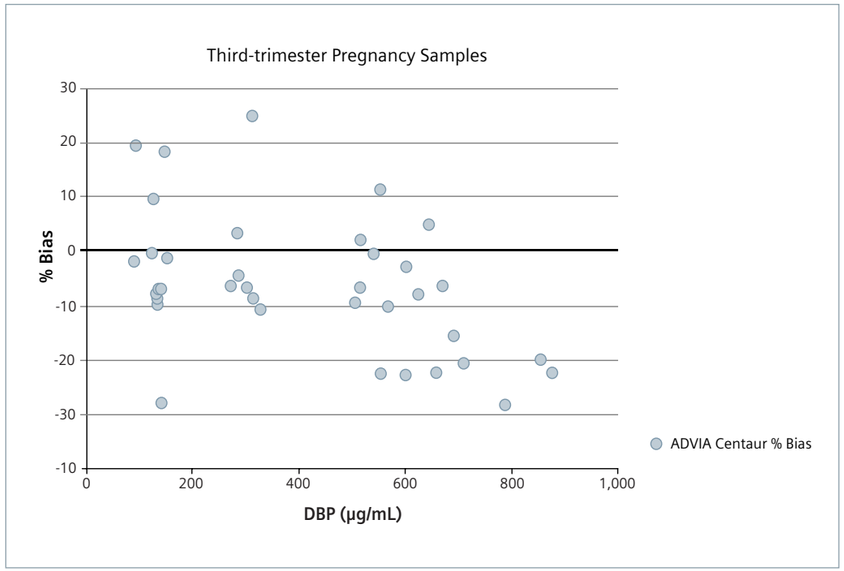


Figure 2. Percent bias as a function of DBP concentration in pregnancy (third trimester) samples.



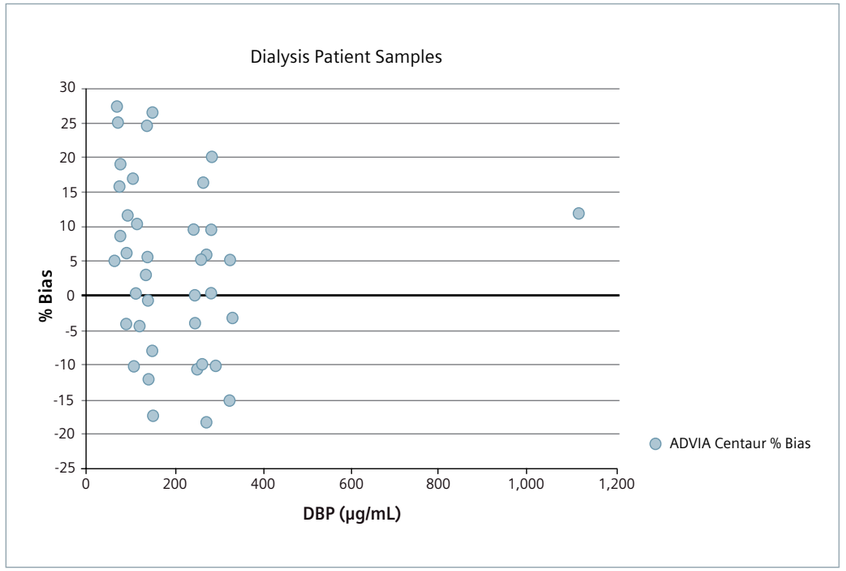
Assay bias as a function of DBP concentration in pregnancy (third trimester) samples.

LC/MS/MS (ng/mL)	DBP Concentration (µg/mL)	ADVIA Centaur (ng/mL)	ADVIA Centaur Bias to LC/MS/MS (%)
12.8	139.2	9.2	–28
44.9	598.8	34.7	–23
22.5	555.4	17.5	–22
23.4	143.4	27.7	18
39.3	659.4	30.5	–22
26.0	137.0	24.1	–7
32.4	853.3	26.0	–20
27.4	621.7	25.2	–8
10.6	284.0	10.2	–4
17.5	125.9	19.1	10
29.0	668.9	27.11	–6
34.3	874.5	26.6	–22
28.9	516.3	27.0	–7
17.6	513.6	18.0	2
20.5	550.4	22.8	12
40.3	567.5	36.2	–10
19.7	708.9	15.7	–20
31.6	785.8	22.7	–28
30.5	136.3	28.0	–8
19.8	690.7	16.7	–16
4.0	140.1	3.7	–7
25.7	311.7	32.1	25
22.5	92.2	26.8	19
34.4	119.8	34.3	0
34.7	134.3	31.4	–9
28.4	539.3	28.2	0
34.4	505.0	31.2	–9
21.3	149.7	21.0	–1
32.5	641.1	34.1	5
32.0	602.5	31.1	–3
39.4	282.8	40.7	3
43.7	302.7	40.8	–7
31.5	326.9	28.1	–11
31.2	315.7	28.5	–9
27.7	271.0	25.9	–6
9.2	87.2	9.1	–2

Assay bias as a function of DBP concentration in renal dialysis patients.

LC/MS/MS (ng/mL)	VDBP Concentration (µg/mL)	ADVIA Centaur (ng/mL)	ADVIA Centaur Bias to LC/MS/MS (%)
33.4	146.6	30.7	–8
6.0	239.2	6.5	10
11.8	278.1	11.9	0
67.0	76.1	72.6	8
40.2	245.1	38.6	–4
14.4	325.8	13.9	–3
12.9	139.5	11.3	–12
16.8	69.4	19.4	16
31.3	281.5	37.6	20
50.6	86.5	48.6	–4
43.3	147.9	35.9	–17
35.5	76.3	38.6	9
45.5	270.8	48.1	6
42.6	138.8	42.2	–1
40.4	262.3	47.0	16
20.4	322.6	17.3	–15
10.4	111.7	11.5	11
62.6	262.7	66.1	6
22.5	73.7	26.7	19
34.2	248.3	30.6	–11
25.6	260.2	23.0	–10
35.2	292.7	31.6	–10
42.2	267.3	34.5	–18
27.8	132.8	28.6	3
32	67.0	40.1	25
19.6	91.7	20.8	6
17.1	68.2	21.7	27
36.6	119.0	35.0	–4
6.7	280.5	7.3	10
36.3	105.5	32.6	–10
19.1	1115.7	21.4	12
39.3	135.5	49.0	25
12.5	63.4	13.1	5
11.1	319.2	11.7	5
15.0	91.4	16.7	12
14.8	135.5	15.7	6
22.5	144.7	28.5	27
18.4	100.4	21.5	17
33.5	112.1	33.6	0
17.1	243.9	17.1	0

Percent bias as a function of DBP concentration in renal dialysis patients.



Conclusions

The study described in this document demonstrates that the impact of DBP concentration on the accuracy of the ADVIA Centaur Vitamin D Total assay (determined by comparison to LC/MS/MS) at levels found in normal patient populations as well with clinical conditions such as pregnancy or chronic kidney disease (which are known to have either raised or decreased DBP levels) was negligible.

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