INFLUENCE OF VITAMIN D BINDING PROTEIN AND C3-EPIMER ON ACCURACY OF 25OH VITAMIN D ELISA ASSAYS

N. Heureux*, F. Mathieu, M. Hars   DIAsource Immunoassays, Louvain-la-Neuve, Belgium

1. Introduction

The measurement of 25OH Vitamin D has tremendously evolved since the first competitive protein-binding assay that used tritiated 25OH Vitamin D3 and a Vitamin D binding protein (DBP) originating from rat. As an example solvent extraction, which the role was to solubilize the hydrophobic 25OH Vitamin D and to precipitate its binding proteins, gradually gave way to methods involving pH shifts, enzymatic digestion, displacement chemicals or hybrid solutions.

In 2012, DIAsource Immunoassays launched an innovative ELISA assay. The innovation lies in the release of 25OH Vitamin D from its binding proteins that takes place directly in the coated microplate, while all other non-automated assays involve one or multiple preparation steps prior to the immunoassay. The benefits are a decrease in the assay hands-on time, the possibility to fully automate the assay on ELISA platforms and the suppression of the risk for errors due to mislabeling of the dilution tubes. Furthermore the assay is based on patented monoclonal antibodies, which provide the assay an excellent specificity profile and lot-to-lot reproducibility.

Although the acceptance of the Thienpont's and NIST LC-MS/MS assays as reference methods, and the recent initiative towards standardization of the Vitamin D assays [1] have contributed to an improvement in the method-related variability in serum 25OH Vitamin D measurement, differences remain between assays. These variations can affect the 25OH Vitamin D clinical decision-making [2,3]. The variance in concentration of Vitamin D Binding Protein (DBP) and C3-epi-25OH Vitamin D can both participate in the inter-assay heterogeneity.

2. Vitamin D Binding Protein (DBP)

One potential source of assay-related variability lies in the extended physiological range of concentration of DBP. The concentration of DBP is not constant and can be influenced by a

number of factors including obesity [4], pregnancy [5], the use of oral contraceptives [6], HRT [7], liver disease [8], renal disease [9-11], proteinuria [12] and intensive care [13]. As one of the major challenge in measuring 25OH Vitamin D is the displacement of 25OH Vitamin D from DBP, such a wide range of physiological DBP concentrations could influence the accuracy of the several 25OH Vitamin D immunoassays. This was demonstrated by Heijboer et al. in a recent article in which the accuracy of six 25OH Vitamin D assays was evaluated across the range of DBP concentration [14].

Clinical Condition		Reported DBP concentration range
Normal women [5]		333 ± 58 mg/L
Normal men and women [8]		405 ± 128 mg/L
Normal [9]		436 ± 33 mg/L
Pregnancy [5]	18 ± 4 wk	613 ± 142 mg/L
	32 ± 3 wk	683 ± 82 mg/L
	35 ± 1 wk	688 ± 104 mg/L
	40 ± 1 wk	616 ± 84 mg/L
Use of Oral Contraceptive [5]		488 ± 90 mg/L
Normal weight women [4]		266 ± 104 mg/L
Obese women [4]		320 ± 121 mg/L
Liver disease [8]		178 ± 92 mg/L
Peritoneal dialysis [9]		203 ± 14 mg/L
Nephrotic patients [9]		371 ± 46 mg/L
Dialysis population [10]		158 (69 – 217) mg/L
Pediatric renal disease [11]	CKD stage 2/3	206 (136 – 287) mg/L
	CKD stage 4/5	208 (101 – 282) mg/L
	Dialysis	168 (99 – 242) mg/L

3. 3-epi-25OH Vitamin D (C3-epimer)

The relevance of 3-epi-25OH Vitamin D for Vitamin D physiological actions is still unclear and there is a current general consensus that 3-epi-25OH Vitamin D should not be included in the measurement of 25OH Vitamin D [15]. The concentration of 3-epi-25OH Vitamin D, its prevalence and the ratio to 25OH Vitamin D vary according to populations and reports [15-20]. It is generally accepted that 3-epi-25OH Vitamin D is more present in infant and pediatric populations than in adults.

Reference		% with detectable C3-epi	C3-epi mean (range) (ng/mL)	% C3-epi of 25OHD mean (range)
Singh [15]	Infant	23	16.1 (5.0-92.0)	28 (9-61)
	Pediatric	None	-	-
	Adult	None	-	-
Stepman [16]	Infant	100	-	(15-41)
	Adult	100	-	(3-17)
Schleicher [17]	Adult	43	0.8 (0-7.6)	-
	Adult (pregnant)	80	1.5 (0.5-4.0)	-
Van den Ouweland [18]	Infant	100	-	11 (2-49)
	Pediatric	100	-	6 (3-20)
	Adult	75	-	4 (1-11)
Keevil [19]	Infant	93	1.6 (0-4.9)	10 (0-27)
	Pediatric	93	1.0 (0-3.0)	5 (0-7)
	Adult	90	0.5 (0-3.4)	4 (0-16)
Strathmann [20]	Infant	100	5.9 (0.1-47.0)	26 (2-60)
	Adult	90	2.2 (0-11.0)	7 (1-47)

25OH Vitamin D3

3-epi-25OH Vitamin D3
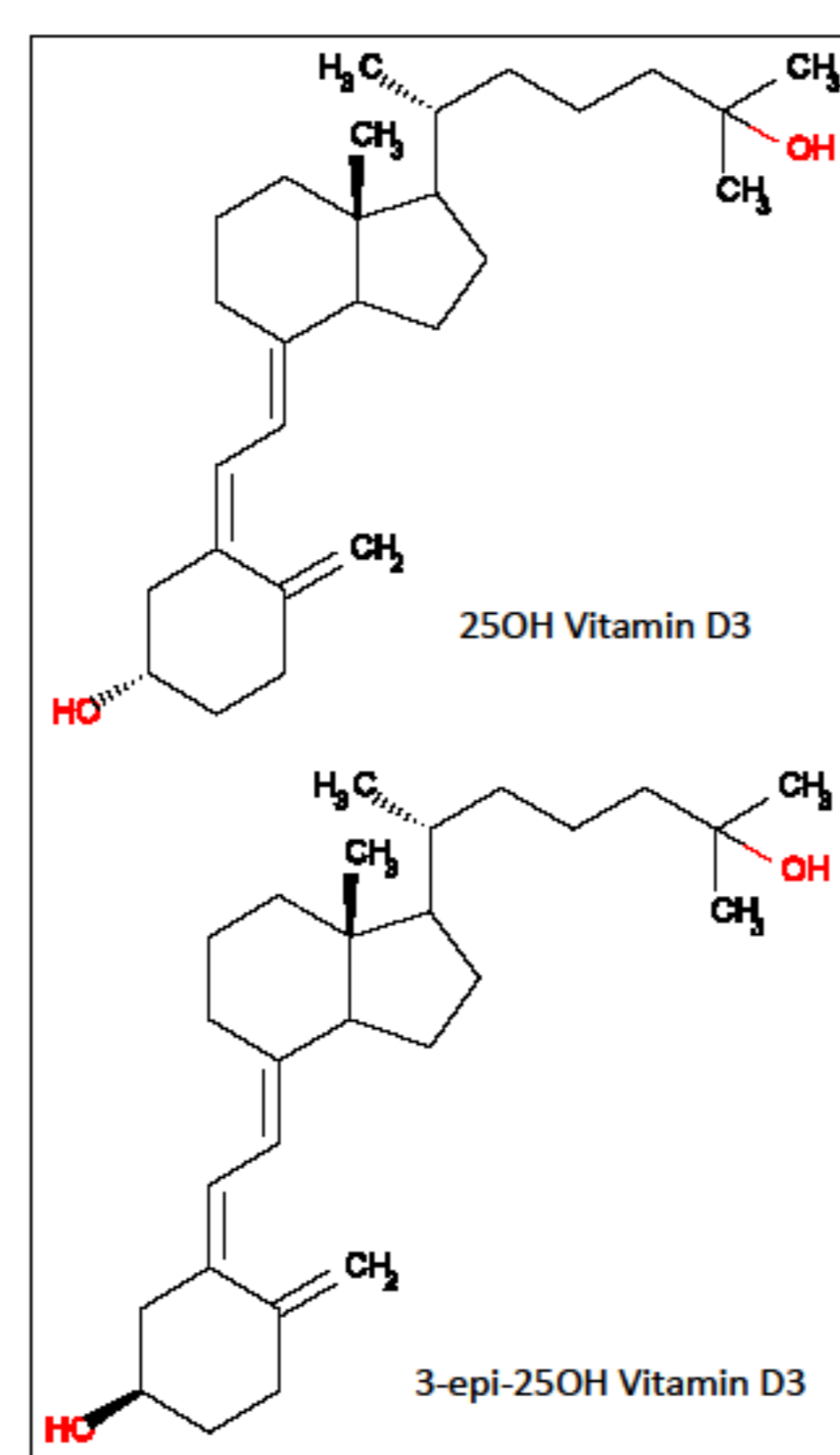
4. Study Design

In order to determine the influence of DBP and 3-epi-25OH Vitamin D on several 25OH Vitamin D ELISA assays, a study examining DBP and 3-epi-25OH Vitamin D as an endogenous interference, similar to how hemoglobin, cholesterol, or total protein would be measured, was performed at the DIAsource facilities.

Five human serum pools containing increasing concentration of 25OH Vitamin D (range 8-60 ng/mL) were prepared from native, single patient, serum samples. Each of the five serum pools were divided into aliquots, and DBP and C3-epimer were spiked into aliquots of each pool to various levels. 25OH Vitamin D measurements of spiked and unspiked pools were then performed using different ELISA assays. All measurements were performed on the same day, using the same aliquots.

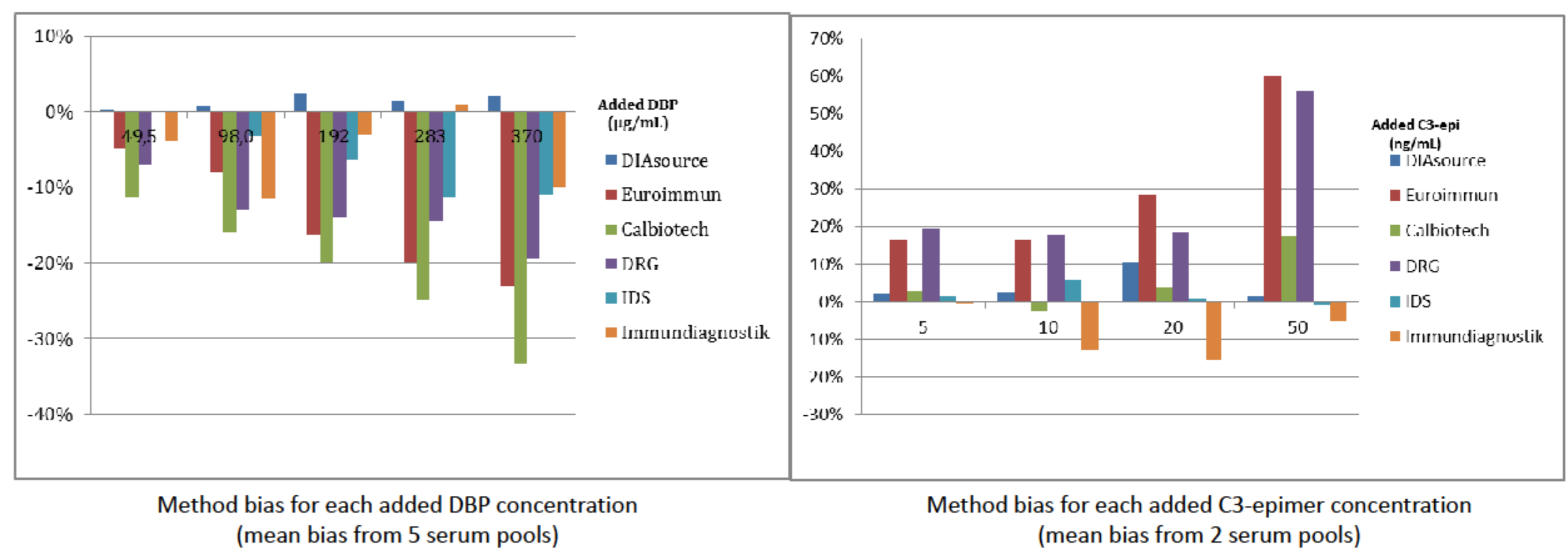The following 25OH Vitamin D assays were evaluated:
- DIAsource 25OH Vitamin D Total ELISA
- Euroimmun 25-OH Vitamin D ELISA
- Calbiotech 25-OH Vitamin D ELISA
- DRG 25-OH Vitamin D (total) ELISA
- IDS 25-Hydroxy Vitamin D EIA
- Immundiagnostik 25-OH Vitamin D direct, ELISA

5. Results

Pool	Added DBP (mg/L)	Bias to unspiked pool (%)					
		DIAsource	Euroimmun	Calbiotech	DRG	IDS	Immundiagnostik
P1	49,5	-5%	-13%	-14%	-16%	16%	15%
P1	98,0	5%	-9%	-7%	-34%	7%	22%
P1	192	2%	-23%	-21%	-34%	7%	21%
P1	283	2%	-9%	-27%	-43%	7%	13%
P1	370	7%	-17%	-36%	-43%	7%	7%
P2	49,5	7%	-6%	-15%	-2%	-3%	-1%
P2	98,0	-3%	0%	-51%	13%	-1%	-44%
P2	192	0%	-10%	-23%	-1%	-5%	-8%
P2	283	-2%	-23%	-20%	-17%	-14%	1%
P2	370	0%	-29%	-39%	-30%	-11%	-15%
P4	49,5	0%	-4%	-9%	-6%	-3%	-11%
P4	98,0	1%	-8%	-9%	-1%	-6%	-23%
P4	192	-1%	-18%	-22%	-10%	-10%	-9%
P4	283	1%	-21%	-29%	16%	-9%	-15%
P4	370	-3%	-17%	-33%	-6%	-12%	-18%
P5	49,5	1%	-4%	-11%	-3%	5%	-31%
P5	98,0	4%	-7%	-9%	-13%	2%	-22%
P5	192	4%	-7%	-9%	1%	-5%	-12%
P5	283	8%	-22%	-18%	-7%	-13%	-10%
P5	370	5%	-25%	-25%	-3%	-14%	-26%
P7	49,5	-2%	2%	-8%	-7%	-15%	8%
P7	98,0	-3%	-16%	-5%	-31%	-18%	9%
P7	192	6%	-22%	-24%	-27%	-18%	-8%
P7	283	-1%	-25%	-30%	-22%	-27%	16%
P7	370	1%	-28%	-34%	-15%	-25%	3%

Pool	Added C3-epimer (ng/mL)	Bias to unspiked pool (%)					
		DIAsource	Euroimmun	Calbiotech	DRG	IDS	Immundiagnostik
P4	5	0%	17%	-6%	17%	-3%	1%
P4	10	2%	19%	-7%	22%	2%	-13%
P4	20	17%	42%	-1%	43%	2%	-13%
P4	50	-4%	80%	15%	82%	8%	-1%
P5	5	4%	17%	11%	22%	5%	-2%
P5	10	2%	13%	2%	14%	10%	-12%
P5	20	4%	14%	8%	-6%	-1%	-18%
P5	50	7%	40%	20%	30%	-9%	-9%

Bias of measured 25OH Vitamin D for each added DBP and C3-epimer concentration, to the unspiked sample

Method bias for each added DBP concentration
(mean bias from 5 serum pools)

Method bias for each added C3-epimer concentration
(mean bias from 2 serum pools)

6. Discussion

The DIAsource 25OH Vitamin D Total ELISA assay was the only method that was not influenced nor by the added DBP, neither by the added C3-epimer.
The IDS ELISA assay was slightly negatively influenced by the addition of DBP but not by the addition of C3-epimer. The Calbiotech assay showed a strong influence upon addition of exogenous DBP but was not significantly impacted by the addition of C3-epimer. The DRG and Euroimmun methods were both strongly affected by the addition of both DBP or C3-epimer.
The different 25OH Vitamin D release methods and the antibody specificity profile, or DBP specificity profile in the case of the DRG assay, are mostly responsible for these contrasting behaviors.

7. References

[1] http://ods.od.nih.gov/Research/vdsp.aspx, 09/04/2015.
[2] Bedner M., Lippa K.A., Tai S.S. Clin. Chim. Acta 2013, 426:6-11.
[3] Binkley N., Krueger D., Cowgill C.S., Plum L., Lake E., Hansen K.E., DeLuca H.F., Drezner M.K. J. Clin. Endocrinol. Metab. 2004, 89(7):3152-7.
[4] Karlsson T., Osmancevic A., Jansson N., Hulthén L., Holming A., Larsson I. Eur. J. Nutr. 2014, 53(1):259-67.
[5] Bouillon R., Van Assche F.A., Van Baelen H., Heyns W., De Moor P. J. Clin. Invest. 1981, 67(3):589-596.
[6] Møller U.K., Streym Sv, Jensen L.T., Mosekilde L., Schoenmakers I., Nigdikar S., Rejnmark L. Nutrients 2013, 5(9):3470-80.
[7] Rejnmark L., Lauridsen A.L., Brot C., Vestergaard P., Heickendorff L., Nexo E., Mosekilde L. Scand. J. Clin. Lab. Invest. 2006, 66(3):227-38.
[8] Bikle D.D., Halloran B.P., Gee E., Ryzen E., Haddad J.G. J. Clin. Invest. 1986, 78:748-752.
[9] Koenig K.G., Lindberg J.S., Zerwekh J.E., Padalino P.K., Cushner H.M., Copley J.B. Kidney Int. 1992, 41:161-165.
[10] Bhan I., Powe C.E., Berg A.H., Ankers E., Wenger J., Karumanchi S.A., Thadhani R. Kidney Int. 2012, 82(1):84-89
[11] Denburg M.R., Kalkwarf H.J., de Boer I.H., Hewison M., Shults J., Zemel B.S., Stokes D., Foerster D., Laskin B., Ramirez A., Leonard M.B. Pediatr. Nephrol. 2013, 28(9):1843-1853.
[12] Doorenbos C.R., de Cuba M.M., Vogt L., Kema I.P., van den Born J., Gans R.O., Navis G., de Borst M.H. J. Steroid Biochem. Mol. Biol. 2012, 128(1-2):56-61.
[13] Leaf D.E., Croy H.E., Abrahams S.J., Raed A., Waikar S.S. Critical Care 2015, 19.80.
[14] Heijboer A.C., Blankenstein M.A., Kema I.P., Buijs M.M. Clin. Chem. 2012, 58(3):543-548.
[15] Singh R.J., Taylor R.L., Reddy G.S., Grebe S.K. J. Clin. Endocrinol. Metab. 2006, 91(8):3055-3061.
[16] Stepman H.C., Vanderroost A., Stöckl D., Thienpont L.M. Clin. Chem. Lab. Med. 2006, 49(2):253-256.
[17] Schleicher R.L., Encisco S.E., Chaudhary-Webb M., Paliakov E., McCoy L.F., Pfeiffer C.M. Clin. Chim. Acta 2011, 412:1594–1599.
[18] van den Ouweland J.M.W., Beijers A.M., van Daal H.H. Clin. Chem. 2011, 57(11):1618–1619.
[19] Keevil B. Clin. Chem. Lab. Med. 2012, 50(1):181-183.
[20] Strathmann F.G., Sadilkova K., Laha T.J., LeSourd S.E., Bornhorst J.A., Hoofnagle A.N., Jack R. Clin. Chim. Acta 2012, 413(1-2):203-206.