EUROMEDLAB MILANO 2013
19-23 MAY
Poster # M366

DEVELOPMENT OF THE VIDAS® 25 OH VITAMIN D TOTAL ASSAY

Moreau E., Durand C., Villard C., Meunier V., Berthoux A., Richard M., Dupret-Carruel J., Hausmann M.

bioMérieux Marcy l'Etoile, France

BIOMÉRIEUX

INTRODUCTION

Vitamin D is a fat-soluble steroid pro-hormone a deficiency of which can be associated with rickets, osteoporosis, secondary hyper-parathyroidism, as well as increasing risk of diabetes, cardiovascular or autoimmune diseases or various forms of cancer.

Vitamin D is found mainly in two forms: vitamin D3 (cholecalciferol) synthesized by action of solar ultraviolet radiation on the skin and vitamin D2 (ergocalciferol) of exogenous origin only. The main storage form of Vitamin D in the body is 25-hydroxy vitamin D [25(OH)D], found in high concentrations in serum or plasma, which makes 25(OH)D the preferred analyte and the most relevant clinical indicator for the determination and monitoring of vitamin D nutritional status.

We have developed a VIDAS® 25-OH Vitamin D TOTAL immunoassay that measures both 25(OH)D2 and 25(OH)D3. The purpose of this study was to evaluate the technical performance of the VIDAS® 25-OH Vitamin D TOTAL assay and to compare the results with Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) and a commercially available Vitamin D immunoassay.

MATERIAL AND METHODS

Precision of the VIDAS® 25-OH Vitamin D Total Assay was determined across the dynamic range using assay controls and sample pools according to CLSI protocol EP9-A2. Two replicates of each sample were tested twice per day in separate runs, for 5 days, using 3 reagent lots on 2 different VIDAS® systems. Assay precision was determined using samples with 25(OH)D ranging from about 10 to 130 ng/mL.

Method comparison was based on the CLSI EP9-A2. The VIDAS® 25-OH Vitamin D TOTAL assay was compared to the IDS-iSYS 25-Hydroxy Vitamin D Assay, a FDA cleared commercially available predicate immunoassay and a validated LC-MS/MS method using frozen patient serum samples, a single replicate for each method. Specimen concentration ranged from about 8 to 130 ng/mL. As some of these samples contain endogenous 25(OH)D2, further analyses were carried out to establish specific quantification of 25(OH)D2 as compared to 25(OH)D3. In addition DEQAS samples were tested with VIDAS® 25-OH Vitamin D TOTAL assay and compared to results of the QC study.

Limit of blank, limit of detection, and functional sensitivity. The limit of blank (LoB) is defined as the concentration of analyte that corresponds to the 95th percentile of the distribution of a human negative basepool. Each of the four 25(OH)D negative samples was assayed twice per day for 8 days with 3 reagent lots for a total of n=192 values. The limit of detection (LoD) is determined according to CLSI protocol EP17-A2, and is defined as the lowest concentration of 25(OH)D that can be detected with 95% probability. The LoD was determined by using 9 low-level vitamin D samples (ranging from 7 to 20 ng/ml). Each sample was assayed 5 times a day in a single run, for 8 days, corresponding to 40 tests per low-level sample. Each sample was assayed with 3 reagent lots for a total of n=120 values. Limit of Quantification (LoQ) -or functional sensitivity- corresponds to the lowest amount of 25(OH)D that can be quantitatively determined with stated accuracy of CV<20%. Using the precision profile generated with LoD samples, the 25(OH)D concentration associated with the desired CV<20% within-laboratory precision was determined.

Linearity was evaluated using two serum pools, High Sample(≈130 ng/mL) and Low Sample (≈10 ng/mL), selected near the extremes of the calibration range of the VIDAS® 25-OH Vitamin D TOTAL assay. High and Low samples were sequentially mixed to generate 12 samples of intermediate concentrations. Each sample was tested in duplicate with 3 reagent lots. To determine linearity, the polynomial analysis method was used as described in EP6-A, with a deviation from linearity <12% over the entire measuring range.

RESULTS

Assay Methodology:
The VIDAS® 25-OH Vitamin D Total Assay design is based on a 2-step competitive immunoassay.

▪First step:
serum or plasma 25(OH)D is dissociated from its protein carrier (DBP) then added to alkaline-phosphatase (ALP) conjugated Vitamin D-specific antibody.

▪Second step:
unbound ALP-antibody is then exposed to vitamin D analog coated-solid phase receptor. Solid phase is then washed and substrate reagent is added to initiate the fluorescent reaction. An inverse relationship exists between the amount of 25(OH)D in the sample and the amount of relative fluorescence units detected by the system.

Precision:
Standard deviation and CV% were calculated for VIDAS® 25-OH Vitamin D Total Assay repeatability (precision within-lot, within-run, within-instrument) and reproducibility (precision between-runs, between-days, between calibrations, between-lots, between-instruments).

The precision profile of the VIDAS® 25-OH Vitamin D Total Assay demonstrates Total Precision CV% from 12.4% at 12.6 ng/mL to 2.2% at 100.3 ng/mL 25(OH)D.

Table 1: Precision analysis

Sample ID	SAMP#1		SAMP#2		SAMP#3		SAMP#4		SAMP#5		SAMP#6		SAMP#7		SAMP#8		SAMP#9	
# of replicates	N = 72		N = 72		N = 72		N = 72		N = 72		N = 72		N = 72		N = 72		N = 72	
Concentration	12.6 ng/mL		16.0 ng/mL		23.5 ng/mL		32.1 ng/mL		40.1 ng/mL		60.5 ng/mL		83.6 ng/mL		100.3 ng/mL		119.8 ng/mL	
	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
Repeatability (within-run precision)	1.1	8.8	0.9	5.6	0.8	3.3	0.8	2.6	0.9	2.1	1.0	1.6	1.8	2.1	1.7	1.7	1.3	1.1
Reproducibility (total precision)	1.6	12.4	1.4	8.6	1.2	5.0	1.2	3.7	1.4	3.5	1.6	2.6	1.9	2.3	2.2	2.2	2.8	2.4

Method Comparison:
A sample correlation was performed with 74 specimens comparing the VIDAS® 25-OH Vitamin D Total Assay to IDS-iSYS 25-Hydroxy Vitamin D Assay, a, FDA-cleared, total 25(OH)D assay.

➢Linear regression analysis demonstrated a correlation coefficient (R) of 0.94. Using Passing & Bablock fit, a slope of 0.97 and an intercept of 1.0 ng/mL were determined (*Figure 1*).

In another method comparison, 121 specimens were assayed against a validated Hospital Lab assay using a LC-MS/MS Chromsystems MassChrom® 25-OH- Vitamin D3/D2 solution

➢Linear regression analysis demonstrated a correlation coefficient (R) of 0.94. Using Passing & Bablock fit, a slope of 1.04 and an intercept of -1.0 ng/mL were determined (*Figure 2*).

Figure 1: Passing & Bablok Regression Plot from the VIDAS® 25-OH Vitamin D Total assay and IDS-iSYS 25-Hydroxy Vitamin D assay

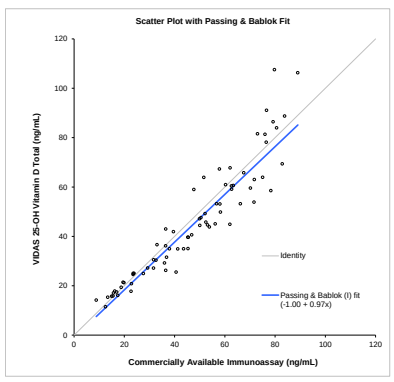
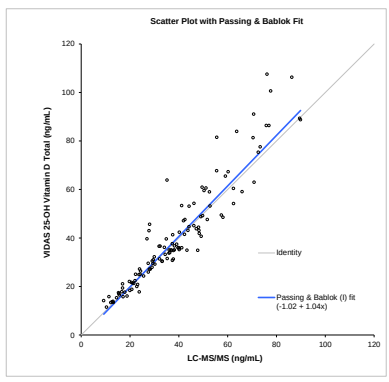
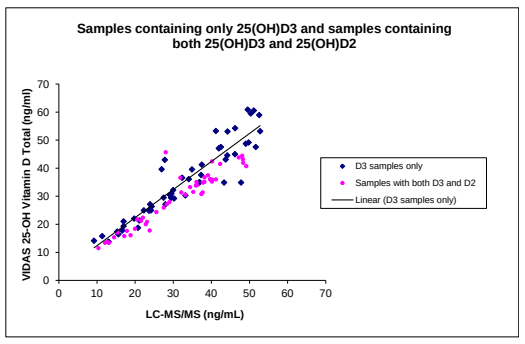


Figure 2: Passing & Bablok Regression Plot from the VIDAS® 25-OH Vitamin D Total assay and a validated Hospital Lab assay using a LC-MS/MS Chromsystems MassChrom® 25-OH- Vitamin D3/D2 solution



In addition, patient samples were plotted separating samples containing only 25(OH)D3 (blue dots) and samples containing both 25(OH)D3 and 25(OH)D2 (pink dots). No significant repartition difference is observed between the two different populations (*Figure 3*)

Figure 3: Linear Regression Plot for samples that contain only 25(OH) D3 and samples that contain both 25(OH)D3 and 25(OH)D2 from the VIDAS® 25-OH Vitamin D Total Assay and a validated hospital lab assay using a LC-MS/MS Chromsystems MassChrom® 25-OH-Vitamin D3/D2 solution



25(OH)D2 cross reactivity determination:
The25(OH)D2 cross reactivity was determined using endogenous (non-spiked) serum samples with 25(OH)D2 and 25(OH)D3 concentrations obtained by LC-MS/MS. Samples included in the testing showed a 25(OH)D2 concentration 4 times higher than 25(OH)D3.

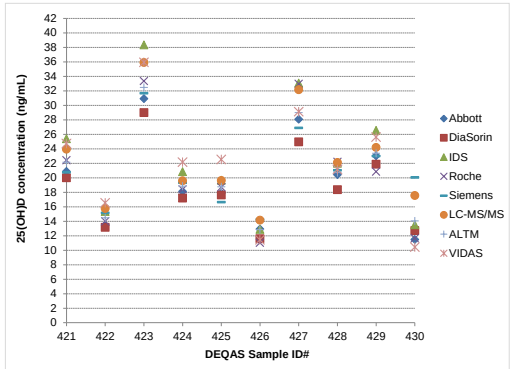
For each sample, 25(OH)D2 cross reactivity was determined according to the equation (using 4 different D2-containing serum samples):

$$\text{D2 cross reactivity (\%)} = \frac{[25(OH)D\,\text{Total}]_{VIDAS} - [25(OH)D3]_{LCMS}}{[25(OH)D2]_{LCMS}} \times 100$$

➡ 25(OH)D2 cross reactivity for VIDAS® 25-OH Vitamin D Total Assay is 91%.

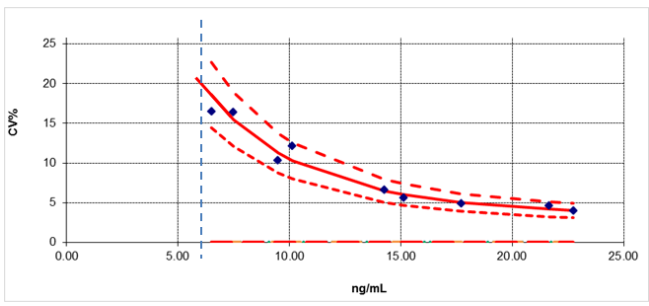
Samples from Vitamin **D** **E**xternal **Q**uality **A**ssessment **S**cheme (DEQAS) were quantified using VIDAS® 25-OH Vitamin D Total Assay and compared to other immunoassays doses provided by DEQAS reports (*Figure 4*)

Figure 4: Doses obtained for DEQAS samples with commercially available assays (data other than VIDAS provided by DEQAS reports)



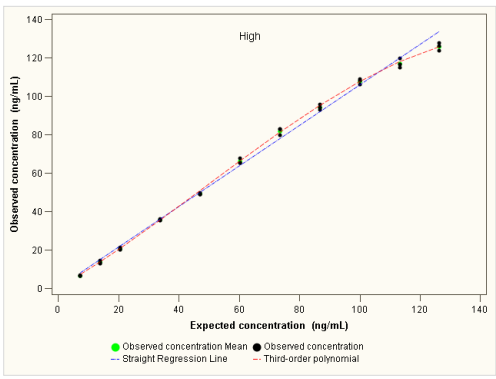
Limit of Blank, Limit of Detection, and Functional Sensitivity:
The limit of blank of the VIDAS® 25-OH Vitamin D Total Assay was 6.2 ng/mL, the limit of detection was 8.1 ng/mL, and the functional sensitivity was 5.9 ng/mL (*Figure 5*).

Figure 5: Precision profile showing the functional sensitivity (blue dashed line) of the VIDAS® 25-OH Vitamin D Total Assay



Linearity:
The low Sample pool had an estimated concentration of 7.1 ng/mL. The High Sample pool had an estimated concentration of 132.1 ng/mL. Analysis by weighted linear regression indicated that the assay results demonstrate linearity less than 12% across the claimed range of [7.1 – 126.2] ng/mL (*Figure 6*)

Figure 6: VIDAS® 25-OH Vitamin D Total assay linearity



CONCLUSIONS

The VIDAS® 25-OH Vitamin D Total Assay exhibits excellent analytical data which makes it suitable for use in a clinical setting:

- **Broad measuring range (7.1-126.2 ng/ml) with excellent linearity**
- **High degree of precision with total CV<13% between 8-20 ng/ml and total CV<5% from 20 to130 ng/ml of 25(OH)D**
- **Equal measurement of both 25(OH)D2 and 25(OH)D3**
- **Excellent correlation to LC-MS-MS reference method**
- **The VIDAS® 25-OH Vitamin D Total Assay has a recalibration interval of 28 days and a time to first result of 36 min.**

The VIDAS® 25-OH Vitamin D Total Assay will be a valuable tool in clinical laboratories for the accurate measurement of 25(OH)-Vitamin D deficiency in human sera or heparinized plasma.