MEASUREMENT OF TOTAL 25(OH) VITAMIN D USING bioMérieux VIDAS®: DEVELOPMENT OF A NEW ASSAY

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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 and clinical 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 serum samples 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.

Limit of blank, limit of detection, and functional sensitivity. The limit of blank (LoB) and the limit of detection (LoD) are determined according to CLSI protocol EP17-A2. 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%.

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.

Serum/plasma comparison. Whole blood from 60 volunteer study participants was collected into serum plastic tubes and lithium heparin plastic tubes. Some whole blood sample were spike with 25(OH)D3 to reach highest concentration before dividing into appropriate tube types.

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 and to an hospital LC-MS/MS method using both routine and 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.

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 16.0% at 10.5 ng/mL to 2.4% at 119.8 ng/mL.

***Table 1*: Precision analysis**

Sample ID	SAMP#1		SAMP#2		SAMP#3		SAMP#4		SAMP#5		SAMP#6		SAMP#7		SAMP#9	
# of replicates	N = 72		N = 72		N = 72		N = 72		N = 72		N = 72		N = 72		N = 72	
Concentration	10.5 ng/mL		18.8 ng/mL		26.0 ng/mL		33.8 ng/mL		45.7 ng/mL		65.1 ng/mL		83.6 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 (%)
Repeatability (within-run precision)	0.83	7.9	0.97	5.1	0.94	3.6	0.91	2.7	0.87	1.9	1.09	1.7	1.8	2.1	1.3	1.1
Reproducibility (total precision)	1.68	16.0	1.28	6.8	1.18	4.5	1.52	4.5	1.29	2.8	1.85	2.8	1.9	2.3	2.8	2.4

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 [8.1– 126] ng/mL.

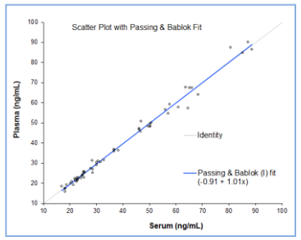
Limit of Blank, Limit of Detection, and Functional Sensitivity:

- The LoB of the VIDAS® 25-OH Vitamin D Total Assay is 6.2 ng/mL.
- The LoD is 8.1 ng/mL.
- The LoQ (functional sensitivity: CV <20%) is 5.9 ng/mL.

Serum/plasma comparison:

Whole blood from 60 volunteer study participants was collected into serum plastic tubes and lithium heparin plastic tubes. Results indicate that all sample-types tested are suitable for dosage by VIDAS® 25-OH Vitamin D Total Assay (***Figure 1***).

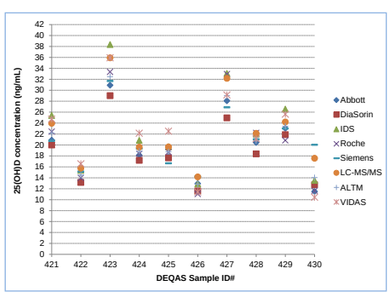
***Figure 1*: Passing & Bablok Regression Plot from plasma vs. serum samples using the VIDAS 25-OH Vitamin D Total Assay.**



External Quality Control:

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 2***).

***Figure 2*: 25(OH)D concentrations obtained for DEQAS samples using commercially available assays (data other than VIDAS are obtained through DEQAS reports)**

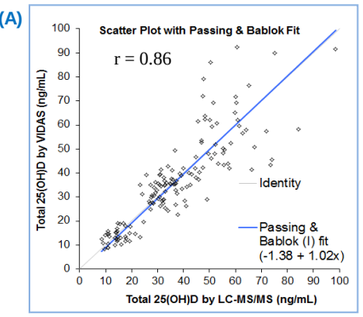


Method Comparison to LC-MS/MS and immunoassays:

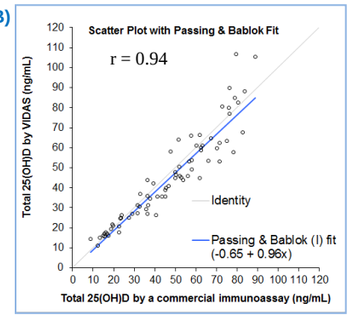
A sample correlation study was performed with a panel of 74 specimens comparing the VIDAS 25-OH Vitamin D Total Assay to a commercially available 25(OH)D assay.
In another method comparison study, 146 routine specimens were assayed against a commercially available Vitamin D Total LC-MS/MS assay.
Pearson correlation coefficient and Passing-Bablock linear regression are presented in ***Figure 3***.

***Figure 3*: Passing & Bablok Regression Plot from the VIDAS 25-OH Vitamin D Total Assay and**
(A): LC-MS/MS hospital routine method
(B): a FDA-approved commercially available Total 25-OH Vitamin D immunoassay

(A)

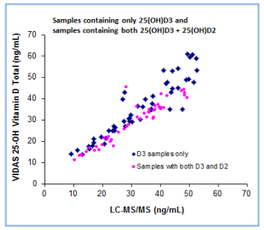


(B)



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 4*).

***Figure 4*: Linear Regression Plot for samples that contain only 25(OH) D3 and samples that contain both 25(OH)D3 and 25(OH)D2.**



25(OH)D2 cross reactivity determination:

The 25(OH)D2 cross reactivity was determined using natural non-spiked serum samples for which 25(OH)D2 and 25(OH)D3 concentrations were previously obtained by LC-MS/MS. For each sample, 25(OH)D2 cross reactivity is determined according to the equation:

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

As a result, 25(OH)D2 cross reactivity for VIDAS® 25-OH Vitamin D Total Assay is 91%.

CONCLUSIONS

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

- **Measuring range is broad (8.1-126 ng/mL) with excellent linearity**
- **High precision with CV<16% from 8 to 20 ng/ml and CV<5% from 20 to 126 ng/ml**
- **Equal measurement of both 25(OH)D2 and 25(OH)D3, no cross-reactivity to 3-epi 25(OH)D3**
- **Excellent correlation to LCMS-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 prove a valuable tool in clinical laboratories for the accurate measurement of 25(OH)-Vitamin D deficiency in human sera or heparinized plasma.