INTRODUCTION

Vitamin D is a fat-soluble steroid pro-hormone of which can be associated with rickets, osteoporosis, secondary hyper-parathyroidism, and as well 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 which origin is 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 EP02-A2. Two replicates of each sample were tested twice per day in separate runs, for 5 days, using 3 reactant lots on 2 different VIDAS® systems. Assay precision was determined using samples ranging from 40 to 121 ng/mL.

Method comparison was based on the CLIA EP/A2. The VIDAS® 25-OH Vitamin D TOTAL assay was compared to the LC-MS/MS Vitamin D assay, a FDA cleared commercially available immunoassay and a validated LC-MS/MS method using frozen patient serum samples, a single replicates for each method. Sample concentrations ranged from about 6 to 100 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, DEGAS samples were tested with VIDAS® 25-OH Vitamin D TOTAL assay, and compared to the results of the reference method.

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 99th percentile of the distribution of a human negative baseline. Each of the four 25(OH)D0 negative samples was assayed twice per day for 5 days with 3 reactant lots for a total of 5!0 results. 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 using 5 low-concentration serum samples (ranging from 7 to 25 ng/mL). Each sample was assayed 3 times a day in a single run, for 5 days, corresponding to 40 test per low-level sample. Each sample was assayed with 3 reactant lots for a total of 95 results. Limit of Quantification (LoQ) or functional sensitivity, correspond to the lowest amount of analyte that can be reliably measured. According to LC/MS/MS analysis of spike samples generated with LoC, the 25(OH)D concentration associated with the desired CV<15% precision was determined.

Linearity was evaluated using two serum pools, High Samples (10 ng/mL) and Low Sample (1 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 reactant lots. To determine linearity, the polynomial analysis method was used as described in EP17-A2, 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.

• Fixation: serum or plasma samples containing 25(OH)D is dissociated from its protein carrier (IDP) and then added to alkaline-phosphatase (ALP) conjugated Vitamin D specific antibody.

• Secondary step: unbound ALP-antibody is then exposed to vitamin D analog coated-solid phase receptor. Solid phase is then washed and alkaline-phosphate is used to induce the fluorescent reaction. The extreme relationship exists between the amount of 25(OH)D in the samples and the amount of relative fluorescent units detected by the system.

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

The precision profile of the VIDAS® 25-OH Vitamin D Total Assay demonstrates Total Precision CV<12% from 12.6 ng/mL to 2.2% at 199.3 ng/mL. 25(OH)D.

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

• Linear regression analysis demonstrated a correlation coefficient (R²) of 0.94. Using Passing & Bablok fit, a slope of 1 and an intercept of 0.01 was 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 SOL solution.

• Linear regression analysis demonstrated a correlation coefficient (R²) of 0.94. Using Passing & Bablok fit, a slope of 1 and an intercept of 0.01 was determined (Figure 2).

CONCLUSIONS

In addition, patient samples were plotted separately using sera containing only 25(OH)D3 (10 data) and sera containing both 25(OH)D2 and 25(OH)D3 (16 data). No significant replication difference was observed between the two different populations (Figure 3).

<table>
<thead>
<tr>
<th>Sample</th>
<th>25(OH)D3 ng/mL</th>
<th>25(OH)D2 ng/mL</th>
<th>Total 25(OH)D ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMP#3</td>
<td>2.2</td>
<td>83.6</td>
<td>85.8</td>
</tr>
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<td>SAMP#4</td>
<td>5.0</td>
<td>40</td>
<td>45.0</td>
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<td>SAMP#5</td>
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<td>58.0</td>
</tr>
<tr>
<td>SAMP#6</td>
<td>60.5</td>
<td>60</td>
<td>120.5</td>
</tr>
<tr>
<td>SAMP#7</td>
<td>1.2</td>
<td>80</td>
<td>81.2</td>
</tr>
</tbody>
</table>

Limit of blank of the VIDAS® 25-OH Vitamin D Total Assay was 0 ng/mL, the limit of detection was 1 ng/mL, and the functional sensitivity was 1.5 ng/mL (Figure 5).

The sample pool had an estimated concentration of 7.1 ng/mL. The High Sample pool had an estimated concentration of 131.1 ng/mL. Analysis by weighted linear regression indicated that the assay results demonstrate linearity less than 12% across the claimed range of 7.1 – 121.0 ng/mL (Figure 4).

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 (7.1-121.0 ng/mL) with excellent linearity
• High degree of precision with total CV<13% between 8.20-80 ng/mL and total CV<5% from 20 to 100 ng/mL of 25(OH)D3
• 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 hepatoprinic plasma.