Clinical Patient Management Requires Accurate Equimolar Testing

Patients Using Vitamin D₂ Supplements Are Most at Risk for Inaccurate 25-Hydroxyvitamin D Test Results

The role of vitamin D in bone and mineral metabolism was first discovered as a factor that could cure rickets. However, vitamin D is now recognized as a pro-hormone which has multiple roles in maintaining optimal health. More recently, several studies have suggested that vitamin D insufficiency is associated with an increasing risk of many chronic diseases including cardiovascular disease, cancer, infectious diseases and autoimmune diseases.¹

With the high prevalence of vitamin D insufficiency/deficiency in the general population, testing for total 25-Hydroxyvitamin D (25-OH) levels has now become common clinical practice. Vitamin D deficiency is typically treated by clinicians with vitamin D₃ or D₂ supplements, while fortified foods and nutrition supplements may contain either form. To ensure accurate assessment of vitamin D sufficiency both 25-OH D₃ and 25-OH D₂ serum concentrations must be measured.¹ ²

Australian and European Method Comparison Studies

Ten healthy volunteers from a laboratory in France provided informed consent to receive orally 600,000 IU of vitamin D₂ (ergocalciferol) as a single vial of Sterogyl 15 “A” (DB Pharma, La Varenne Saint Hilaire, France), and serum was obtained 21 days following supplementation. Each serum sample was allowed to clot for 30 minutes at room temperature, centrifuged and separated into 1 mL aliquots. Samples were stored at -20°C, and shipped on dry ice to each study site.

Total 25-OH D was measured using commercial immunoassay kits from DiaSorin (LIAISON® XL 25 OH Vitamin D TOTAL Assay) and Siemens (ADVIA Centaur® XP Vitamin D Total (VitD) assay) at a reference laboratory in Australia and at a university hospital laboratory in Europe. All tests were performed according to the manufacturer’s instructions, and none of the samples showed visible signs of hemolysis or lipemia.

25-OH D₃, 25-OH D₂, and total 25-OH D were measured by four different LC-MS/MS (LCMS) methods (USA Method 1, USA Method 2, Australia Method, and Europe Method). The USA and Australian methods were accredited reference laboratories, and the European method was a commercial method at a university hospital laboratory in Europe. All four LCMS methods were traceable to the NIST SRM 972 standard reference material.

Australian and European Testing Results

The Australia and Europe testing results returned by the DiaSorin immunoassay were averaged with 25-OH D concentrations ranging from 28.2 to 85.9 ng/mL [Mean (95% confidence interval): 65.2 (52.6-77.8) ng/mL]. The Siemens immunoassay results were also averaged with 25-OH D concentrations ranging from 37.7 to 138.3 ng/mL [97.9 (75.9-120.0) ng/mL].

The LCMS consensus values for 25-OH D₃, 25-OH D₂, and total 25-OH D were derived by averaging the results from the four LCMS methods. 25-OH D₃ concentrations ranged from 22.2 to 68.6 ng/mL [48.9 (38.0-59.8) ng/mL], 25-OH D₂ concentrations ranged from 12.2 to 25.9 ng/mL [17.4 (14.0-20.7) ng/mL], and total 25-OH D concentrations ranged from 34.6 to 91.0 ng/mL [66.3 (53.9-78.7) ng/mL].

The DiaSorin and Siemens immunoassay results were compared against the LCMS consensus total 25-OH D concentrations using Passing-Bablok fit and Bland-Altman difference plot, and analyses were performed using MedCalc version 13.0.0. (Table 1). DiaSorin agreed with the LCMS consensus (slope 1.01, -2.1% mean % difference). However, Siemens overestimated the total 25-OH D concentrations (slope 1.82, 45.5% mean % difference) in samples containing endogenous 25-OH D₂.

The total 25-OH D concentrations from each LCMS method (USA Method 1, USA Method 2, Australia Method and Europe Method), and each immunoassay method (DiaSorin and Siemens), were compared against the LCMS consensus total 25-OH D concentrations using scatter plot with linear regression, and analyses were performed using Analyze-it for Microsoft Excel version 2.30 (Figure 1). While each LCMS method (slopes 0.91, 0.97, 1.16 and 0.96, respectively) and DiaSorin (slope 0.97) had similar slopes compared against the LCMS consensus, Siemens (slope 1.67) had a significantly higher slope than the other methods further demonstrating overestimation of total 25-OH D concentrations in the 25-OH D₂ samples.

Table 1: Passing-Bablok and Bland-Altman analyses of the immunoassays compared against the LCMS consensus.

<table>
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<th>Immunoassay</th>
<th>LCMS</th>
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<tr>
<td>Average</td>
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</tr>
<tr>
<td>(N = 2)</td>
<td>(N = 4)</td>
</tr>
<tr>
<td>DiaSorin LIAISON®</td>
<td>Passing-Bablok Slope</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
</tr>
<tr>
<td></td>
<td>Bias-Altman Mean % Difference</td>
</tr>
<tr>
<td>Siemens ADVIA Centaur®</td>
<td>Passing-Bablok Slope</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
</tr>
<tr>
<td></td>
<td>Bias-Altman Mean % Difference</td>
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</table>

Figure 1: Linear regressions of each LCMS method and immunoassay method compared against the LCMS consensus.

Summary of Results

Results of this study suggest the DiaSorin LIAISON® 25 OH Vitamin D TOTAL Assay does not over-estimate serum 25-OH D concentrations with samples containing endogenous 25-OH D₂. The DiaSorin LIAISON® 25 OH Vitamin D TOTAL Assay demonstrated similar 25-OH D serum concentrations compared against LCMS.

This study suggests that equimolar measurement is a challenge for commercial immunoassay kits, which can make it difficult for clinicians to accurately interpret and manage patients treated with vitamin D₂ supplements. Studies show the DiaSorin LIAISON® 25 OH Vitamin D TOTAL Assay is equimolar for 25-OH D₁ and 25-OH D₂.


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The purpose of this issue is to highlight recent advances in biochemical and genetic markers used for the diagnosis, therapy, and preventive care of women during all stages of life. This issue will include diverse themes such as cancer, cardiovascular disease, osteoporosis, metabolic disease, normal and abnormal pregnancy, infertility, and infectious disease.

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