Clinical Patient Management Requires Accurate Equimolar Testing

Patients Using Vitamin D<sub>2</sub> 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-OHD) levels has now become common clinical practice. Vitamin D deficiency is typically treated by clinicians with vitamin D<sub>3</sub> or D<sub>2</sub> supplements, while fortified foods and nutrition supplements may contain either form. To ensure accurate assessment of vitamin D sufficiency both 25-OHD<sub>2</sub> and 25-OHD<sub>3</sub> 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 D<sub>3</sub> (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-OHD was measured using commercial immunoassay kits from DiaSorin (LIAISON<sup>®</sup> XL 25 OH Vitamin D TOTAL Assay) and Siemens (ADVIA Centaur<sup>®</sup> 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-OHD<sub>2</sub>, 25-OHD<sub>3</sub>, and total 25-OHD 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-OHD 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-OHD concentrations ranging from 37.7 to 138.3 ng/mL [97.9 (75.9-120.0) ng/mL].

The LCMS consensus values for 25-OHD<sub>2</sub>, 25-OHD<sub>3</sub>, and total 25-OHD were derived by averaging the results from the four LCMS methods. 25-OHD<sub>2</sub> concentrations ranged from 22.2 to 68.6 ng/mL [48.9 (38.0-59.8) ng/mL], 25-OHD<sub>3</sub> concentrations ranged from 12.2 to 25.9 ng/mL [17.4 (14.0-20.7) ng/mL], and total 25-OHD concentrations ranged from 34.6 to 91.0 ng/mL [66.3 (53.9-78.7) ng/mL].

The total 25-OHD 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-OHD 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-OHD concentrations in the 25-OHD<sub>2</sub> samples.

**Summary of Results**

Results of this study suggest the DiaSorin LIAISON<sup>®</sup> 25 OH Vitamin D TOTAL Assay does not over-estimate serum 25-OHD concentrations with samples containing endogenous 25-OHD<sub>2</sub>. The DiaSorin LIAISON<sup>®</sup> 25 OH Vitamin D TOTAL Assay demonstrated similar 25-OHD 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 D<sub>2</sub> supplements. Studies show the DiaSorin LIAISON<sup>®</sup> 25 OH Vitamin D TOTAL Assay is equimolar for 25-OHD<sub>3</sub> and 25-OHD<sub>2</sub>.


For more information contact DiaSorin at info@diason.com.
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**Lembcke et al., 2011:**

"Fast, robust and reliable method for the determination of 1,25(OH)$_2$ vitamin D$_3$..."

[AB SCIEX Poster, ASMS conference, June 5-9]

**Yuan et al., 2011:**

"An LC-MS/MS-based method [...] suitable for clinical testing. Both D$_3$ and D$_2$ were quantified with high selectivity and sensitivity."


**He et al., 2011:**

"This off-line purification approach is very specific and robust. No interference or ion suppression was observed."

[ThermoScientific Poster, ASMS conference, June 5-9]
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Clinical Chemistry, published by the American Association for Clinical Chemistry, is the most highly cited forum for peer-reviewed, original research in the fields of clinical chemistry and laboratory medicine.

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.

Clinical Chemistry invites authors to submit original articles related to women’s health to be considered for publication in this special issue.

Potential topics of interest include:

- Discovery and validation of novel biomarkers for the diagnosis, prognosis, and monitoring therapy of diseases that affect women including: cancer, cardiometabolic and/or cerebrovascular disease, bone disease, and autoimmune disorders
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The signs and symptoms of many autoimmune diseases can be relatively common, thus making the diagnoses of these diseases a difficult undertaking. Many of the autoantibodies listed in this Quick Guide are only markers associated with the disease and may not be involved in the pathogenesis of the disease. In addition, many of these autoantibodies can be found in several different autoimmune diseases; therefore, their presence cannot be used for a conclusive diagnosis of any specific disease. The assay and antibody descriptions included in this Quick Guide have been significantly simplified so that they can be rapidly read, understood, and utilized.
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