Vitamin D, 25-Hydroxy, LC/MS/MS

Clinical Use

- Diagnose vitamin D deficiency or intoxication

Reference Range

<table>
<thead>
<tr>
<th>Total 25OHD</th>
<th>20-100 ng/mL</th>
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</thead>
<tbody>
<tr>
<td>25OHD&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Not established</td>
</tr>
<tr>
<td>25OHD&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Not established</td>
</tr>
</tbody>
</table>

25OHD levels <20 ng/mL reflect vitamin D deficiency. The optimal level is 50-50 ng/mL. 25OHD<sub>2</sub> levels >4 ng/mL suggest compliance with supplements.

Interpretive Information

- Vitamin D intoxication
- Nutritional rickets
- Secondary hyperparathyroidism
  - Osteomalacia
  - Severe cholestatic or parenchymal liver disease
  - Vitamin D-resistant metabolic bone disease due to multiple drugs for seizure control
  - Nephrotic syndrome with marked proteinuria
  - Intestinal malabsorption

Decreased, but usually not below reference range

- Obesity
- Sarcoidosis
- Hyperphosphatemic tumoral calcinosis
- Tuberculosis
- Primary hyperparathyroidism
- Type II vitamin D-dependent rickets

Levels vary with exposure to sunlight, peaking in the summer months.

Clinical Background

25-Hydroxyvitamin D (25OHD) is the major circulating form of vitamin D and the precursor of the active form (1,25-dihydroxyvitamin D). Because of its long half-life, 25OHD measurements are useful for assessing vitamin D status in patients.

Vitamin D occurs in 2 forms: vitamin D<sub>3</sub> (cholecalciferol) and vitamin D<sub>2</sub> (ergocalciferol). Vitamin D<sub>3</sub> is obtained from foods of animal origin and from ultraviolet light-stimulated conversion of 7-dehydrocholesterol in the skin, whereas vitamin D<sub>2</sub> is obtained from foods of plant origin. Vitamin D<sub>2</sub> is used in a high potency (50,000 IU) formulation for treating severe vitamin D deficiency. Both forms are used in over-the-counter supplements and fortified foods and are metabolized to their respective 25OHD forms (ie, 25OHD<sub>3</sub> and 25OHD<sub>2</sub>). Thus, analytical methods that can accurately quantitate both forms are essential for diagnosis and monitoring patients with vitamin D deficiency as well as differentiating between intoxication and other hypercalcemic disorders.

Method

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 4 ng/mL for 25OHD<sub>2</sub> and 25OHD<sub>3</sub>
- Analytical specificity: no cross-reaction with vitamin D<sub>2</sub> or D<sub>3</sub>; 1α,25(OH)<sub>2</sub>D<sub>2</sub>; 1α,25(OH)<sub>2</sub>D<sub>3</sub>, calcitriol; 25,26(OH)<sub>2</sub>D<sub>3</sub>; 1α(OH)D<sub>2</sub>, doxercalciferol; and 1α(OH)D<sub>3</sub>, alfacalcidol

Specimen Requirements

0.3 mL room temperature serum
0.15 mL minimum

No additive red top preferred
SST red top acceptable
Overnight (8-12 h) fasting is preferred.