

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

Test code: 17306X

Clinical Use

- Diagnose intestinal malabsorption and vitamin D deficiency or intoxication
- Monitor therapeutic response in patients being treated for vitamin D-related disorders

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 D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol). Vitamin D₃ is obtained from foods of animal origin and from ultraviolet light-stimulated conversion of 7-dehydrocholesterol in the skin, whereas small amounts of vitamin D₂ are obtained from foods of plant origin. Both forms of the vitamin are used to fortify various foods and in over-the-counter supplements, and vitamin D₂ is used in a high potency (50,000 IU) formulation for treating severe vitamin D deficiency. Furthermore, both forms are metabolized to their respective 25OHD forms (ie, 25OHD₃ and 25OHD₂). Thus, analytical methods that can accurately quantitate both 25OHD forms are essential for diagnosis and monitoring patients with vitamin D disorders.

Most current methods do not differentiate between the 2 forms: only total 25OHD concentrations are reported. Additionally, some analytical methods may underestimate 25OHD concentration when significant amounts of 25OHD₂ are present owing to diminished cross-reactivity.¹ Other disadvantages to current methods include use of radioactive isotopes, method-to-method variation, and laboratory-to-laboratory variation.² This liquid chromatography, tandem mass spectrometry (LC/MS/MS) method does not use radioisotopes and is sensitive and equally specific for both forms of 25OHD. Concentrations of each form are measured and reported independently.

Individuals Suitable for Testing

- Individuals with suspected vitamin D deficiency (eg, those with persistent, nonspecific musculoskeletal pain; the elderly; housebound individuals, etc.)
- Individuals with suspected toxicity (eg, those with anemia of obscure origin, unexplained renal disease, etc.)³
- Individuals being treated for vitamin D-related disorders

Specimen Requirements

0.25 mL room temperature serum (red-top tube); 0.15 mL minimum

Samples collected in serum separator tubes (SST) are unacceptable. It's preferable for the patient to have been fasting 8 to 12 hours prior to sample collection.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
 - Extraction via protein precipitation
 - Separation via high-performance liquid chromatography (HPLC)
 - Detection and quantitation via tandem mass spectrometry
 - 25OHD₂ and 25OHD₃ concentrations used to calculate total 25OHD levels
- Report includes concentration of total 25OHD, 25OHD₂, and 25OHD₃
- Analytical sensitivity: 4 ng/mL for 25OHD₂ and 25OHD₃
- Analytical specificity: no cross-reaction with vitamin D₂ or D₃; 1 α ,25(OH)₂D₂; 1 α ,25(OH)₂D₃; calcitriol; 25,26(OH)₂D₃; 1 α (OH)D₂; doxercalciferol; and 1 α (OH)D₃, alfalcidol
- Reportable range: 4 – 512 ng/mL for 25OHD₂ and 25OHD₃
- Aliases: 25OHD, 25-hydroxycholecalciferol, 25-hydroxy-ergocalciferol
- CPT code*: 82306

Reference Range

The reference range for total 25OHD (**20–100 ng/mL**) is based on 25OHD correlation with physiological parameters that include parathyroid hormone concentration and calcium absorption.⁴⁻⁸ The range is **not** based on the distribution of levels in an apparently healthy population. 25OHD₂- and 25OHD₃-specific reference ranges are not available.

Interpretive Information

Decreased 25OHD concentrations are an indication of vitamin D deficiency and are associated with hypocalcemia, hypophosphatemia, and elevated alkaline phosphatase. In addition to insufficient intake or production, disorders that are characterized by decreased absorption or excessive loss in the gastrointestinal tract, increased vitamin D metabolism, or impaired conversion of vitamin D to 25OHD can cause decreased 25OHD levels (Table).

Elevated levels of 25OHD suggest vitamin D intoxication and distinguish this disorder from other hypercalcemia-causing disorders.

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

Table. 25-Hydroxyvitamin D Concentration in Various Disorders⁹⁻¹¹

Disorder	25OHD Concentration
Vitamin D intoxication	↑
Nutritional rickets	↓
Osteomalacia	↓
Secondary hyperparathyroidism	↓
Fat malabsorption disorders, short bowel syndrome	↓
Intestinal diseases causing excessive loss	↓
Anti-convulsant or anti-tuberculosis medications (increased metabolism)	↓
Severe parenchymal liver disease (impaired 25-hydroxylation of vitamin D)	↓
Severe renal disease (impaired 1 α -hydroxylation of 25OHD)	N
Vitamin D-dependent rickets, type I	N or ↑
Vitamin D-dependent rickets, type II	N

25OHD, 25-hydroxyvitamin D; N, normal.

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