Vitamin D2 and D3 by Liquid Chromatography-Tandem Mass Spectrometry

Background

Vitamin D is a fat-soluble vitamin that plays a significant role in calcium metabolism and promotes bone metabolism. Vitamin D2 (ergocalciferol) is derived from plants and fungus. The endogenous form of Vitamin D3 (cholecalciferol) is converted from 7-dehydrocholesterol in human skin by UV light. Vitamin D2 and D3 are prohormones that are converted to 25-hydroxy vitamin D (25OHD) in the liver, then to the physiologically active form of 1,25-dihydroxyvitamin D in the kidneys.

1,25-dihydroxyvitamin D [calcitriol] binds to nuclear receptor proteins of tissue specific cells that regulate calcium and phosphorus metabolism. These tissues include the intestine, bone, kidney and parathyroid.

In addition to its role in calcium and bone metabolism, vitamin D has been linked to many other biologic processes, including cancer risk, mortality, cardiovascular disease, autoimmune disease and infection.

Monitoring vitamin D status is useful for detecting deficiency and for evaluating treatment efficacy in patients receiving vitamin D supplementation. 25-hydroxyvitamin D is the major circulating form of the vitamin and is the best indicator of vitamin D status. Liquid chromatography-mass spectrometry (LC-MS) is the preferred assay method because it has high specificity and is able to separate 25OHD2 and 25OHD3 to yield independent values rather than just the total 25OHD. This is important due to the ongoing debate concerning the difference in efficacy between D2 and D3 supplementation.[1-5]

There is some controversy over the reference intervals for 25OHD. In 2010, the Institute of Medicine (IOM) issued a position statement that recommends a reference interval of >20 ng/mL as sufficient, and >50 ng/mL as potentially toxic. The Endocrine Society released guidelines in 2011 that referenced reference intervals of <20 ng/mL as deficient and 20-30 ng/mL as insufficient. At this time there is no full consensus for 25OHD reference intervals.[6-7]

Clinical Indications

Vitamin D deficiency/insufficiency has been associated with poor bone metabolism, weak muscle strength, cancer risk and mortality, autoimmune disease, cardiovascular disease and cystic fibrosis.[8-9] This vitamin D assay may be used to monitor the vitamin D nutritional status in patients with diseases that interfere with fat absorption, such as cystic fibrosis and Crohn's disease; in patients who have had gastric bypass surgery and may have impaired vitamin D absorption; or in patients with osteoporosis. The 25OHD levels are also used as an indicator of treatment effectiveness in patients being treated with vitamin D.

Interpretation

25-hydroxy vitamin D (D2 + D3):
<20 ng/mL = Deficiency
30-100 ng/mL= Sufficient
>150 ng/mL Toxic[10]

The assay has large analytical measurable ranges:
1.9-114.7 ng/mL for 25OHD2
1.2-113.6 ng/mL for 25OHD3

Limitations of the Assay

1. Minimum sample size of 200 µL is required.
2. In chronic kidney disease, parathyroid hormone levels should be monitored along with 25OHD for accurate treatment conditions. Patients with renal failure may have very high 25OHD2 and D3 levels without toxicity due to impaired renal conversion.
3. This is a laboratory-validated assay that uses analyte specific reagents (ASR), which is indicated.

Methodology[11]

The assay is an isotope replacement liquid chromatography-tandem mass spectrometry method that uses turbulent flow online extraction.

References

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Test Overview

Test Name	25-Hydroxy D2 + D3
Reference Range	Sufficient = 31-80 ng/mL
Patient Preparation	N/A
Specimen Requirements	1 mL serum
Disclaimers or notations	not FDA-approved
Ordering Mnemonic	D2D3
Billing Code	83283
CPT Codes	83371, 83372

Related test: Vitamin D 25-Hydroxy (VITD)

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