MAYO CLINIC LABORATORIES

Test Definition: 25HDN

25-Hydroxyvitamin D2 and D3, Serum

Overview

Useful For

Diagnosis of vitamin D deficiency

Differential diagnosis of causes of rickets and osteomalacia

Monitoring vitamin D replacement therapy

Diagnosis of hypervitaminosis D

Method Name

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

NY State Available

Yes

Specimen

Specimen Type

Serum

Specimen Required

Container/Tube:

Preferred: Serum gel

Acceptable: Red top

Specimen Volume: 0.5 mL

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

-General Request (T239)

-Renal Diagnostics Test Request (T830)

Specimen Minimum Volume

0.25 mL

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	14 days	
	Frozen	30 days	
	Ambient	7 days	

Clinical & Interpretive

Clinical Information

Vitamin D is a generic designation for a group of fat-soluble, structurally similar sterols, which act as hormones. This test is the preferred initial test for assessing vitamin D status and most accurately reflects the body's vitamin D stores.

In the presence of renal disease, testing 1,25-dihydroxyvitamin D (DHVD) levels might be needed to adequately assess vitamin D status. DHVD testing alone may not clearly indicate deficiencies of vitamin D stores.

Vitamin D compounds in the body are exogenously derived by dietary means; from plants as 25-hydroxyvitamin D2 (ergocalciferol or calciferol) or from animal products as 25-hydroxyvitamin D3 (cholecalciferol or calcidiol). Vitamin D may also be endogenously derived by conversion of 7-dihydrocholesterol to 25-hydroxyvitamin D3 in the skin upon ultraviolet exposure.

25HDN is subsequently formed by hydroxylation (*CYP2R1*) in the liver. 25HDN is a prohormone that represents the main reservoir and transport form of vitamin D, being stored in adipose tissue and tightly bound by a transport protein while in circulation. Biological activity is expressed in the form of DHVD, the active metabolite of 25HDN.
1-Alpha-hydroxylation (*CYP27B1*) occurs on demand, primarily in the kidneys, under the control of parathyroid hormone (PTH) before expressing biological activity. Like other steroid hormones, DHVD binds to a nuclear receptor, influencing gene transcription patterns in target organs.

25HDN may also be converted into the inactive metabolite 24,25-dihydroxyvitamin D (24,25D) by (*CYP24A1*) hydroxylation. This process, regulated by PTH, might increase DHVD synthesis at the expense of the alternative hydroxylation (*CYP24A1*) product 24,25D. Inactivation of 25HDN and DHVD by *CYP24A1* is a crucial process that prevents over production of DHVD and resultant vitamin D toxicity.

Based on these considerations circulating 25HDN is the best indicator of optimal vitamin D body stores. The exact levels of optimal circulating 25HDN concentrations remain a matter of debate. Mild-to-modest deficiency can be associated with osteoporosis or secondary hyperparathyroidism. Severe deficiency may lead to failure to mineralize newly formed osteoid in bone, resulting in rickets in children and osteomalacia in adults. The consequences of vitamin D deficiency on organs other than bone are not fully known, but might include increased susceptibility to infections, muscular discomfort, and an increased risk of colon, breast, and prostate cancer.

Modest 25HDN deficiency is common; in institutionalized elderly, its prevalence may be greater than 50%. Although much less common, severe deficiency is not rare either. Reasons for suboptimal 25HDN levels include lack of sunshine

exposure, a particular problem in Northern latitudes during winter; inadequate intake; malabsorption (eg, due to celiac disease); depressed hepatic vitamin D 25-hydroxylase activity, secondary to advanced liver disease; and enzyme-inducing drugs, in particular many antiepileptic drugs, including phenytoin, phenobarbital, and carbamazepine, which increase 25HDN metabolism.

Reference Values

TOTAL 25-HYDROXYVITAMIN D2 AND D3 (25-OH-VitD)
<10 ng/mL (severe deficiency)*
10-19 ng/mL (mild to moderate deficiency)**
20-50 ng/mL (optimum levels)***
51-80 ng/mL (increased risk of hypercalciuria)****
>80 ng/mL (toxicity possible)*****
*Could be associated with osteomalacia or rickets
**May be associated with increased risk of osteoporosis or secondary hyperparathyroidism
***Optimum levels in the healthy population; patients with bone disease may benefit from higher levels within this range
****Sustained levels >50 ng/mL 25OH-VitD along with prolonged calcium supplementation may lead to hypercalciuria and decreased renal function
*****80 ng/mL is the lowest reported level associated with toxicity in patients without primary hyperparathyroidism who have normal renal function. Most patients with toxicity have levels >150 ng/mL. Patients with renal failure can have very high 25-OH-VitD levels without any signs of toxicity, as renal conversion to the active hormone 1,25-OH-VitD is impaired or absent.

These reference ranges represent clinical decision values, based on the 2011 Institute of Medicine report, that apply to males and females of all ages, rather than population-based reference values. Population reference ranges for 25-OH-VitD vary widely depending on ethnic background, age, geographic location of the studied populations, and the sampling season. Population-based ranges correlate poorly with serum 25-OH-VitD concentrations that are associated with biologically and clinically relevant vitamin D effects and are therefore of limited clinical value.

For SI unit Reference Values, see https://www.mayocliniclabs.com/order-tests/si-unit-conversion.html

Interpretation

Based on animal studies and large human epidemiological studies, 25-hydroxyvitamin D2 and D3 (25-OH-VitD) levels below 25 ng/mL are associated with an increased risk of secondary hyperparathyroidism, reduced bone mineral density, and fractures, particularly in the elderly. Intervention studies support this clinical cutoff, showing a reduction of fracture risk with 25-OH-VitD replacement.

Levels less than 10 ng/mL may be associated with more severe abnormalities and can lead to inadequate mineralization of newly formed osteoid, resulting in rickets in children and osteomalacia in adults. In these individuals, serum calcium levels may be marginally low, and parathyroid hormone (PTH) and serum alkaline phosphatase are usually elevated. Definitive diagnosis rests on the typical radiographic findings or bone biopsy/histomorphometry.

Baseline biochemical work-up of suspected cases of rickets and osteomalacia should include measurement of serum calcium, phosphorus, PTH, and 25-OH-VitD. In patients where testing is not completely consistent with the suspected diagnosis, in particular, if serum 25-OH-VitD levels are greater than 10 ng/mL, an alternative cause for impaired

mineralization should be considered. Possible differential diagnosis includes: partly treated vitamin D deficiency, extremely poor calcium intake, vitamin D resistant rickets, renal failure, renal tubular mineral loss with or without renal tubular acidosis, hypophosphatemic disorders (eg, X-linked or autosomal dominant hypophosphatemic rickets), congenital hypoparathyroidism, activating calcium sensing receptor mutations, and osteopetrosis. Measurement of serum urea, creatinine, magnesium, and 1,25-dihydroxyvitamin D (DHVD) is recommended as a minimal additional workup for these patients.

25-OH-VitD replacement in the United States typically consists of vitamin D2. Lack of clinical improvement and no reduction in PTH or alkaline phosphatase may indicate patient noncompliance, malabsorption, resistance to 25-OH-VitD, or additional factors contributing to the clinical disease. Measurement of serum 25-OH-VitD levels can assist in further evaluation, in particular as the liquid chromatography-tandem mass spectrometry methodology allows separate measurement of 25-OH-VitD3 and of 25-OH-VitD2, which is derived entirely from dietary sources or supplements.

Patients who present with hypercalcemia, hyperphosphatemia, and low PTH may suffer either from ectopic, unregulated conversion of 25-OH-VitD to 1,25-OH-VitD, as can occur in granulomatous diseases, particular sarcoid, or from nutritionally-induced hypervitaminosis D. Serum 1,25-OH-VitD levels will be high in both groups, but only patients with hypervitaminosis D will have serum 25-OH-VitD concentrations of greater than 80 ng/mL, typically greater than 150 ng/mL.

Cautions

Long term use of anticonvulsant medications may result in vitamin D deficiency that could lead to bone disease; the anticonvulsants most implicated are phenytoin, phenobarbital, carbamazepine, and valproic acid. Newer antiseizure medications have not been studied or are not thought to contribute to vitamin D deficiency.

Clinical Reference

1. Jones G, Strugnell SA, DeLuca HF: Current understanding of the molecular actions of vitamin D. Physiol Rev 1998 Oct;78(4):1193-1231
2. Miller WL, Portale AA: Genetic causes of rickets. Curr Opin Pediatr 1999 Aug;11(4):333-339
3. Vieth R: Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr 1999 May;69(5):842-856
4. Vieth R, Ladak Y, Walfish PG: Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. J Clin Endocrinol Metab 2003 Jan;88(1):185-191
5. Wharton B, Bishop N: Rickets. Lancet 2003 Oct 25;362(9393):1389-1400
6. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium; Edited by AC Ross, CL Taylor, AL Yaktine, HB Del Valle. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC. National Academies Press (US), 2011 Available from: www.ncbi.nlm.nih.gov/books/NBK56070

Performance

Method Description

Deuterated stable isotope is added as internal standard. 25-Hydroxyvitamin D2 (25-OH-VitD2), 25-hydroxyvitamin D3 (25-OH-VitD3), and the internal standard are extracted. The extracts are then derivatized before being analyzed by liquid

chromatography-tandem mass spectrometry using multiple reaction monitoring. 25-OH-VitD2 and 25-OH-VitD3 are quantified and reported individually and as a sum with a clinical reference range attached to the sum. For children under 1 year of age, a modified assay is performed to identify and eliminate interference due to C-3 epimers of 25-OH-VitD2 and 25-OH-VitD3.(Zimmer D, Pickard V, Czembor W, Muller C: Comparison of turbulent-flow chromatography with automated solid-phase extraction in 96-well plates and liquid-liquid extraction used as serum sample preparation techniques for liquid chromatography-tandem mass spectrometry. J Chromatogr A 1999 Aug 27;854[1-2]:23-35)

PDF Report
No

Day(s) Performed
Monday through Friday
Tuesday, Friday (Specimens on patients who are less than 1 year old)

Report Available
2 to 5 days

Specimen Retention Time
2 weeks

Performing Laboratory Location
Rochester

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification
This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
82306

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
25HDN	25-Hydroxyvitamin D2 and D3, S	49590-3

Result ID	Test Result Name	Result LOINC® Value
2897	25-Hydroxy D2	49054-0

2898	25-Hydroxy D3	1989-3
83670	25-Hydroxy D Total	62292-8