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)

Portions of this test are covered by patents held by Quest Diagnostics

NY State Available

Yes

Specimen

Specimen Type

Serum

Specimen Required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube:
Preferred: Red top
Acceptable: Serum gel
Submission Container/Tube: Plastic vial
Specimen Volume: 0.5 mL Serum
Collection Instructions: Within two hours of collection, centrifuge and aliquot serum into a plastic vial.

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-General Request (T239)
-Kidney Transplant Test Request
-Renal Diagnostics Test Request (T830)

Specimen Minimum Volume

Serum: 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)	28 days	
	Ambient	7 days	
	Frozen	30 days	

Clinical & Interpretive

Clinical Information

Vitamin D is a generic designation for a group of fat-soluble, structurally similar sterols that 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 kidney disease, testing 1,25-dihydroxyvitamin D (DHVD) levels may be needed to adequately assess vitamin D status. DHVD testing alone may not clearly indicate deficiencies of vitamin D stores.

25-Hydroxyvitamin D2 and D3 (25-OH-VitD) are equipotent steroid hormones that require 1-alpha-hydroxylation before expressing biological activity. Vitamin D compounds are derived from dietary ergocalciferol (from plants, VitD2), cholecalciferol (from animals, VitD3), or by conversion of 7-dihydrocholesterol to VitD3 in the skin upon UV exposure. VitD2 and VitD3 are subsequently 25-hydroxylated in the liver to 25-OH-VitD. 25-OH-VitD represents the main body reservoir and transport form of vitamin D, being stored in adipose tissue and tightly bound by a transport protein while in circulation. A fraction of circulating 25-OH-VitD is converted to its active metabolites 1,25-dihydroxy vitamin D2 and D3 (1,25-OH-VitD), mainly by the kidneys. This process is regulated by parathyroid hormone (PTH), which increases 1,25-OH-VitD synthesis at the expense of the alternative, biologically inactive hydroxylation product 24,25-OH-VitD. Like other steroid hormones, 1,25-OH-VitD binds to a nuclear receptor, influencing gene transcription patterns in target organs.

1,25-OH-VitD plays a primary role in the maintenance of calcium homeostasis. It promotes intestinal calcium absorption and, in concert with PTH, skeletal calcium deposition, or less commonly, calcium mobilization. Renal calcium and phosphate reabsorption are also promoted, while prepro-PTH messenger RNA expression in the parathyroid glands is downregulated. The net result is a positive calcium balance, increasing serum calcium and phosphate levels, and falling PTH concentrations.

In addition to its effects on calcium and bone metabolism, 1,25-OH-VitD regulates the expression of a multitude of genes in many other tissues, including immune cells, muscle, vasculature, and reproductive organs.

The exact 25-OH-VitD level reflecting optimal body stores remains unknown. 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 may include increased susceptibility to infections, muscular discomfort, and an increased risk of colon, breast, and prostate cancer.

Modest 25-OH-VitD deficiency is common; in institutionalized older adults, its prevalence may be >50%. Although much less common, severe deficiency is not rare either.

Reasons for suboptimal 25-OH-VitD 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, particularly many antiepileptic drugs, including phenytoin, phenobarbital, and carbamazepine, that increase 25-OH-VitD metabolism.

In contrast to the high prevalence of 25-OH-VitD deficiency, hypervitaminosis D is rare and is only seen after prolonged exposure to extremely high doses of vitamin D. When it occurs, it can result in severe hypercalcemia and hyperphosphatemia.

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
**Might be associated with increased risk of osteoporosis or secondary hyperparathyroidism
***Optimum levels in the healthy population
****Sustained levels >50 ng/mL 25OH-VitD along with prolonged calcium supplementation may lead to hypercalciuria and decreased kidney function
*****80 ng/mL is the lowest reported level associated with toxicity in patients without primary hyperparathyroidism who have normal kidney function. Most patients with toxicity have levels >150 ng/mL. Patients with kidney 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 International System of Units (SI) conversion for Reference Values, see 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, particularly 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, particularly 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;78(4):1193-1231
2. Miller WL, Portale AA. Genetic causes of rickets. Curr Opin Pediatr. 1999;11(4):333-339
3. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr. 1999;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;88(1):185-191
5. Wharton B, Bishop N. Rickets. Lancet. 2003;362(9393):1389-1400

6. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. In: Ross AC, Taylor CL, Yaktine AL, Del Valle HB, eds. Dietary Reference Intakes for Calcium and Vitamin D. National Academies Press: 2011. Available at www.ncbi.nlm.nih.gov/books/NBK56070
7. Su Z, Narla SN, Zhu Y. 25-Hydroxyvitamin D: Analysis and clinical application. Clin Chim Acta. 2014;433:200-205
8. LeFevre ML; U.S. Preventative Services Task Force. Screening for vitamin D deficiency in adults: U.S. Preventative Services Task Force recommendation statement. Ann Intern Med. 2015;162(2):133-140

Performance

Method Description

Deuterated stable isotopes (d6-25-hydroxyvitamin D3 [25-OH-VitD3] and d3-25-hydroxyvitamin D2) are added to a 0.1-mL serum sample as internal standard. Proteins are precipitated by addition of a crash solution and separated from supernatant by centrifugation. The supernatant is injected onto a TX system for purification by an online extraction column followed by transfer to an analytical column for separation of analytes. 25-OH-VitD and D3 are analyzed by mass spectrometry using multiple reaction monitoring. 25-OH-VitD2 and D3 are quantified and reported individually and as a sum with a clinical reference range attached to the sum. C-3 epimers of 25-OH-VitD2 and D3 are chromatographically separated and not included in the results.(Dirks NF, Cavalier E, Heijboer AC. Vitamin D: marker, measurand and measurement. Endocr Connect. 2023;12(4):e220269. doi:10.1530/EC-22-0269)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

2 to 5 days

Specimen Retention Time

2 weeks

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Superior Drive

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 account representative. For assistance, contact Customer Service.

Test Classification

Test Definition: 25HDN

25-Hydroxyvitamin D2 and D3, Serum

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It 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