

Overview

Useful For

As a second-order test in the assessment of vitamin D status, especially in patients with renal disease

Investigation of some patients with clinical evidence of vitamin D deficiency (eg, vitamin D-dependent rickets due to hereditary deficiency of renal 1-alpha hydroxylase or end-organ resistance to 1,25-dihydroxyvitamin D)

Differential diagnosis of hypercalcemia

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

The 25-hydroxyvitamin D test (25HDN / 25-Hydroxyvitamin D2 and D3, Serum) in serum 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 or hypercalcemia, testing of 1,25-dihydroxy vitamin D (DHVD) may be needed to adequately assess vitamin D status.

Specimen Required

Patient Preparation: Fasting is preferred for 4 hours but not required.

Collection Container/Tube:

Preferred: Red top

Acceptable: Serum gel

Submission Container/Tube: Plastic vial

Specimen Volume: 1.5 mL

Collection Instructions: 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)

[-Renal Diagnostics Test Request](#) (T830)

Specimen Minimum Volume

0.7 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

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

Clinical & Interpretive
Clinical Information

Vitamin D is a generic designation for a group of fat-soluble, structurally similar sterols, which act as hormones. In the presence of renal disease or hypercalcemia, testing of 1,25-dihydroxy vitamin D (DHVD) may be needed to adequately assess vitamin D status. The 25-hydroxyvitamin D (25HDN) test (25HDN / 25-Hydroxyvitamin D2 and D3, Serum) in serum is otherwise the preferred initial test for assessing vitamin D status and most accurately reflects the body's 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.

The 25-hydroxyvitamin D is subsequently formed by hydroxylation by 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 by 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.

25-hydroxyvitamin D may also be converted into the inactive metabolite 24,25-dihydroxyvitamin D (24,25D) by alternative hydroxylation by CYP24A1. This process, regulated by PTH, might increase DHVD synthesis at the expense of the alternative CYP24A1 hydroxylation 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.

1,25-dihydroxy vitamin D stimulates calcium absorption in the intestine and its production is tightly regulated through concentrations of serum calcium, phosphorus, and PTH. DHVD 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 mRNA 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, DHVD regulates the expression of a multitude of genes in many other tissues including immune cells, muscle, vasculature, and reproductive organs.

1,25-dihydroxy vitamin D levels are decreased in hypoparathyroidism and in chronic renal failure. DHVD levels may be high in primary hyperparathyroidism and in physiologic hyperparathyroidism secondary to low calcium or vitamin D intake. Some patients with granulomatous diseases (eg, sarcoidosis) and malignancies containing nonregulated 1-alpha hydroxylase in the lesion might have hypercalcemia that appears vitamin D mediated with normal or high serum phosphate (hyperphosphatemia) and hypercalcemia (both of which might be severe) in addition to low PTH and absent parathyroid hormone-related peptide (PTHrP). Assessment of 24,25D might also be required in patients with hypercalcemia that does not appear to be driven by PTH or PTHrP and may be helpful in assessment of patients with loss of function inactivating *CYP24A1* mutations. Differential diagnostic considerations include vitamin D intoxication and *CYP24A1* deficiency.

Reference Values

Males:

<16 years: 24-86 pg/mL

> or =16 years: 18-64 pg/mL

Females:

<16 years: 24-86 pg/mL

> or =16 years: 18-78 pg/mL

For International System of Units (SI) conversion for Reference Values, see

www.mayocliniclabs.com/order-tests/si-unit-conversion.html

Interpretation

1,25-Dihydroxyvitamin D (DVHD) concentrations are low in chronic renal failure and hypoparathyroidism.

1,25-Dihydroxyvitamin D concentrations are high in sarcoidosis and other granulomatous diseases, some malignancies, primary hyperparathyroidism, and physiologic hyperparathyroidism.

1,25-Dihydroxyvitamin D concentrations are not a reliable indicator of vitamin D toxicity; normal (or even low) results may be seen in such cases.

Cautions

No significant cautionary statements.

Supportive Data

The 1,25-dihydroxyvitamin D liquid chromatography tandem mass spectrometry (LC-MS/MS) assay correlates well with the current immunoassay:

-LC-MS/MS=0.95 RIA (pg/mL) + 2.5 pg/mL; correlation coefficient=0.822

-Inter-assay precision: 7 to 12% CV (19 to 287 pg/mL)

-Interferences: C-3 epimers (EPI) of 1,25 dihydroxyvitamin D3 3.0%

Clinical Reference

1. Endres DB, Rude RK. Vitamin D and its metabolites. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. WB Saunders Company, 1999:1417-1423
2. Bringhurst FR, Demay MB, Kronenberg HM. Vitamin D (calciferols): metabolism of vitamin D. In: Wilson JD, Foster DS, Kronenberg HM, Larsen PR, eds. Williams Textbook of Endocrinology. 9th ed. 1998:1166-1169
3. Laha TJ, Strathmann FG, Wang Z, de Boer IH, Thummel KE, Hoofnagle AN. Characterizing antibody cross-reactivity for immunoaffinity purification of analytes prior to multiplexed liquid chromatography-tandem mass spectrometry. Clin Chem. 2012;58(12):1711-1716. doi:10.1373/clinchem.2012.185827
4. Strathmann FG, Laha TJ, Hoofnagle AN. Quantification of 1a,25-dihydroxy vitamin D by immunoextraction and liquid chromatography-tandem mass spectrometry. Clin Chem. 2011;57(9):1279-1285. doi:10.1373/clinchem.2010.161174
5. Herrmann M. Assessing vitamin D metabolism - four decades of experience. Clin Chem Lab Med. 2023;61(5):880-894. doi:10.1515/cclm-2022-1267

Performance**Method Description**

Deuterated stable isotopes are added to sample as internal standard. 1,25-Dihydroxyvitamin D and the internal standard are extracted. The extracts are then further purified by solid phase extraction (SPE) and affinity extraction. Extracts are then derivatized and analyzed by chromatography-tandem mass spectrometry using multiple reaction monitoring.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

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.

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- Prospective clients should contact their account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

82652

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
DHVD	1,25-Dihydroxyvitamin D, S	62290-2

Result ID	Test Result Name	Result LOINC® Value
8822	1,25-Dihydroxyvitamin D, S	62290-2