MAYO CLINIC LABORATORIES

Test Definition: 2425D

25-Hydroxyvitamin D:24,25-Dihydroxyvitamin D Ratio, Serum

Overview

Useful For

As a screening test for inactivating *CYP24A1* mutations in patients with symptoms, signs, or biochemical findings of parathyroid hormone (PTH)-independent hypercalcemia or hypercalciuria

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
2425R	24,25 Dihydroxy Vitamin D	No	Yes
25HDN	25-Hydroxyvitamin D2 and D3, S	Yes	Yes

Method Name

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)
Portions of this test are covered by patent(s) held by Quest Diagnostics

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

Please consider the 25HDN or DHVD tests for Vitamin D assessment. 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) might be needed to adequately assess vitamin D status.

Specimen Required

Container/Tube:
Preferred: Red top
Specimen Volume: 3 mL
Collection Instructions: Spin down within 2 hours of draw.

Specimen Minimum Volume

1.1 mL

Reject Due To

Gross hemolysis	OK
Gross lipemia	Reject
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. The 25HDN / 25-Hydroxyvitamin D2 and D3, Serum assay 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, DHVD / 1,25-Dihydroxyvitamin D, Serum testing might be needed to adequately assess vitamin D status. For patients with loss of function inactivating *CYP24A1* mutations, this test (2425D / 25-Hydroxyvitamin D2 and D3:24,25-Dihydroxyvitamin D Ratio, Serum) may be helpful

Loss of function mutations in the *CYP24A1* gene have been shown to lead to insufficient deactivation of bioactive vitamin D metabolites, resulting in a phenotype characterized by suppressed serum parathyroid hormone (PTH), increased serum 1,25-dihydroxyvitamin D (DHVD) concentrations, hypercalcemia, and hypercalciuria or nephrolithiasis.

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.

25-Hydroxyvitamin D (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 parathyroid hormone (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.

DHVD 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 PTH 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.

DHVD levels are decreased in hypoparathyroidism and in chronic renal failure. DVHD 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 parathyroid hormone (PTH) and absent parathyroid hormone-related peptide (*PTHRP*). Differential diagnostic considerations include vitamin D intoxication and *CYP24A1* deficiency

Reference Values

Interpretative commentary provided based on 25-hydroxyvitamin D (25HDN) to 24,25-dihydroxyvitamin D (24,25D) ratio result.

25HDN to 24,25D ratio less than 25*
*Interpretation: Normal (Ratio of less than 25 may also be observed in heterozygous carriers of CYP24A1 mutations)

25HDN to 24,25D ratio between 25-80**
**Interpretation: Ratios in the 25 to 80 range can be seen in patients with low vitamin D or heterozygous CYP24A1 mutations. Confirmation with molecular testing is recommended.

25HDN to 24,25D ratio greater than 80***
***Interpretation: Ratios greater than 80 indicate probable biallelic CYP24A1 mutation or deletion. Confirmation with molecular testing is recommended.

Reference values not applicable for 24,25 Dihydroxyvitamin D Total result.

Results should be interpreted in the context of other biochemical findings including serum calcium, parathyroid hormone, and 1,25 dihydroxyvitamin D concentrations. If 25-OH-D is less than 20 ng/ml the ratio of 25-OH-D to 24,25-dihydroxyvitamin D will be falsely elevated since there is no inactivation 25-OH-D to 24,25-dihydroxyvitamin D.

Interpretation

Results should be interpreted in the context of other biochemical findings including serum calcium, parathyroid hormone (PTH), and 1,25 dihydroxyvitamin D (DHVD) concentrations. If 25-hydroxyvitamin D (25HDN) result is less than 20 ng/mL, the ratio of 25-OH-D to 24,25-dihydroxyvitamin D (24,25D) will be falsely elevated since there is no inactivation of 25-OH-D to 24,25D.

24,25D formation by *CYP24A1* is dependent on *CYP24A1* activity and the concentrations of its substrate, 25HDN. The ratio of 25HDN to 24,25D, therefore, allows the most reliable estimation of *CYP24A1* activity.

Ratios of 25HDN to 24.25D less than 25 may be interpreted as normal, though ratio of less than 25 may also be observed in heterozygous carriers of *CYP24A1* mutations.

Ratios of 25HDN to 24.25D between the 25 and 80 range may be seen in patients with low vitamin D or heterozygous *CYP24A1* mutations. Confirmation with molecular testing is recommended.

Confirmation with molecular testing is also recommended for ratios of 25HDN to 24.25D greater than 80, as this may indicate a probable biallelic *CYP24A1* mutation or deletion.

Cautions

Because of the substrate dependency of the 25-hydroxyvitamin D (25HDN) to 24,25-dihydroxyvitamin D (24,25D) ratio, it is essential for accurate determination of this ratio that 25HDN to 24,25D are measured in the same draw and sample using the same methodologies for 25HDN to 24,25D that were used when the ratio reference ranges were established. This is an important consideration for clinicians and clinical chemists who recommend this testing, because the absolute value of serum 25HDN to 24,25D can be misleading if calculated from the 2 separate measurements.

False-low 25HDN to 24,25D ratios could lead to delayed diagnosis of CYP24A1 deficiency. False-high ratios might cause unnecessary molecular testing.

Clinical Reference

1. Kaufmann M, Gallagher JC, Peacock M, et al: Clinical utility of simultaneous quantitation of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D by LC-MS/MS involving derivatization with DMEQ-TAD. J Clin Endocrinol Metab 2014;July 99(7):2567-2574. doi: 10.1210/jc.2013-4388
2. Ketha H, Kumar R, Singh RJ: LC-MS/MS for Identifying Patients with CYP24A1 Mutations. Clin Chem 2016 Jan;62(1):236-242

Performance

Method Description

Analytes of interest and deuterated internal standard are extracted, derivatized and analyzed by liquid chromatography-tandem mass spectrometry.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Tuesday

Report Available

2 to 7 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 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. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

82306
82542

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
2425D	25HDN:24,25 Dihydroxy VitD Ratio, S	94674-9

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
90601	24,25-Dihydroxy VitD Total	94672-3
63416	25HDN:24,25 Dihydroxy VitD Ratio, S	94673-1