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* variants in patients with symptoms, signs, or biochemical findings of parathyroid hormone-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 patents held by Quest Diagnostics

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

The preferred initial test for assessing vitamin D status and most accurately reflects the body's vitamin D stores is the 25-hydroxyvitamin D test; order 25HDN / 25-Hydroxyvitamin D2 and D3, Serum.
In the presence of kidney disease or hypercalcemia, testing of 1,25-dihydroxy vitamin D may be needed to adequately assess vitamin D status; order DHVD / 1,25-Dihydroxyvitamin D, Serum.

Specimen Required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 1.7 mL
Collection Instructions: Within 2 hours of collection, centrifuge and aliquot serum into a plastic vial.

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	
	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. 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 kidney disease, DHVD / 1,25-Dihydroxyvitamin D, Serum testing may be needed to adequately assess vitamin D status. For patients with loss of function inactivating *CYP24A1* variants, this test may be helpful

Loss of function variants 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 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 (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.

1,25-Dihydroxyvitamin 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 messenger RNA 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.

1,25-Dihydroxyvitamin D 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 PTH and absent PTH-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
<25: Normal; also be observed in heterozygous carriers of *CYP24A1* variants
25-80: Seen in patients with low vitamin D or heterozygous *CYP24A1* variants
>80: Indicate probable biallelic *CYP24A1* variant or deletion

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 25HDN to 24,25-dihydroxyvitamin D (24,25D) will be falsely elevated since there is no inactivation of 25HDN to 24,25D.

24,25-Dihydroxyvitamin D 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, although ratios less than 25 may also be observed in heterozygous carriers of *CYP24A1* variants.

Ratios of 25HDN to 24,25D between the 25 and 80 range may be seen in patients with low vitamin D or having heterozygous *CYP24A1* variants. 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* variants 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 using the same collection sample and 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;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;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 15 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

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
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