Test Definition: 2425D

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

Overview

Useful For

Screening for CYP24A1 deficiency

Evaluation of parathyroid hormone-independent hypercalcemia

Evaluation of recurrent nephrolithiasis or nephrocalcinosis associated with suspected vitamin D-mediated disease

Selection of patients for confirmatory molecular testing

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 Serum
Collection Instructions: Within 2 hours of collection, centrifuge and aliquot serum into a plastic vial.

Specimen Minimum Volume

Serum: 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

The ratio of 25-hydroxyvitamin D (25HDN) to 24,25-dihydroxyvitamin D (24,25D) provides an estimate of CYP24A1 enzymatic activity. CYP24A1 is responsible for inactivation of both 25HDN and 1,25-dihydroxyvitamin D (DHVD). Loss of function variants in the *CYP24A1* gene impair vitamin D catabolism, resulting in a phenotype characterized by suppressed serum parathyroid hormone (PTH), increased serum DHVD concentrations, hypercalcemia, and hypercalciuria that may lead to nephrolithiasis. Because 24,25D production depends on substrate availability (25HDN concentration) and CYP24A1 activity, interpretation of the ratio requires simultaneous measurement of both analytes in the same specimen. The ratio is most useful in patients with suspected CYP24A1 deficiency and should be interpreted in conjunction with serum calcium, PTH, 25HDN, DHVD test results and clinical presentation.

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: Consistent with normal CYP24A1 activity, although overlap with heterozygous carriers has been reported.
25-80: May be observed in vitamin D deficiency or heterozygous *CYP24A1* variants. Correlation with clinical findings is recommended. Molecular testing may be appropriate when clinical suspicion is high.
>80: Strongly suggests markedly reduced CYP24A1 activity. Confirmatory molecular testing is recommended.

Interpretation

Results should be interpreted in conjunction with serum calcium, parathyroid hormone, 25-hydroxyvitamin D (25HDN), 1,25-dihydroxyvitamin D test results and clinical presentation.

If the 25HDN concentration is less than 20 ng/mL, the ratio may be falsely elevated because of reduced substrate availability and should be interpreted cautiously.

Interpretation guidance for 25HDN to 24,25D ratios:
Ratio <25: Consistent with normal CYP24A1 activity, although overlap with heterozygous carriers has been reported.

Ratio 25-80: May be observed in vitamin D deficiency or heterozygous *CYP24A1* variants. Correlation with clinical findings is recommended. Molecular testing may be appropriate when clinical suspicion is high.

Ratio >80: Strongly suggests markedly reduced CYP24A1 activity. Confirmatory molecular testing is recommended.

Cautions

For accurate interpretation, 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D must be measured from the same specimen using the validated method used to establish the reference values. Calculating the ratio with analytes measured separately or by different methods may produce misleading results.

Vitamin D deficiency may increase the ratio independent of CYP24A1 deficiency. Results should always be interpreted in conjunction with serum calcium, parathyroid hormone, and the overall clinical presentation.

Serum gel and SST tubes significantly increase chances of interference with the 24,25 dihydroxyvitamin result and may increase laboratory time. Therefore, red-top tubes are preferred for specimen collection.

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
3. Demay MB, Pittas AG, Bikle DD, et al. Vitamin D for the Prevention of Disease: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2024;109(8):1907-1947. doi:10.1210/clinem/dgae290
4. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Food and Nutrition Board. In: Ross AC, Taylor CL, Yaktine AL, Del Valle HB, eds. Dietary Reference Intakes for Calcium and Vitamin D. National Academies Press: 2011
5. Vitamin D-Fact Sheet for Health Professionals. US Department of Health and Human Services, National Institutes of Health. Updated June 27, 2025. Accessed August 12, 2026. Available at https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/
6. Fraser W. Bone and mineral metabolism. In: Rifai N, Horvath AR, Wittwer CT, eds. Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics. 8th ed. Elsevier; 2020:466-487
7. 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

8. Shah AD, Hsiao EC, O'Donnell B, et al. Maternal hypercalcemia due to failure of 1,25-dihydroxyvitamin-D3 catabolism in a patient with CYP24A1 mutations. J Clin Endocrinol Metab. 2015;100(8):2832-6. doi:10.1210/jc.2015-1973

Performance

Method Description
Analytes of interest and deuterated internal standard are extracted, derivatized, and analyzed by liquid chromatography tandem mass spectrometry.(Ketha H, Kumar R, Singh RJ. LC-MS/MS for identifying patients with CYP24A1 mutations. Clin Chem. 2016;62(1):236-242)

PDF Report
No

Day(s) Performed
Wednesday

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

MAYO CLINIC LABORATORIES

Test Definition: 2425D

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

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