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

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
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<tbody>
<tr>
<td>2425R</td>
<td>24,25 Dihydroxy Vitamin D</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>25HDN</td>
<td>25-Hydroxyvitamin D2 and D3, S</td>
<td>Yes</td>
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</table>

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

Advisory Information
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

<table>
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**Test Definition: 2425D**

25HDN:24,25 Dihydroxy VitD Ratio, S

<table>
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<th>Specimen Stability Information</th>
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<tbody>
<tr>
<td><strong>Specimen Type</strong></td>
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<tr>
<td>Serum</td>
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**Clinical and 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.


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**


**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) and Time(s) Test Performed**

Tuesday and Thursday; 4 p.m.

**Analytic Time**

2 days

**Maximum Laboratory Time**

6 days

**Specimen Retention Time**

2 weeks

**Performing Laboratory Location**

Rochester

**Fees and 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.
Test Definition: 2425D
25HDN:24,25 Dihydroxy VitD Ratio, S

- Prospective clients should contact their Regional Manager. 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 U.S. Food and Drug Administration.

CPT Code Information
82306
82542

LOINC® Information

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<td>25HDN:24,25 Dihydroxy VitD Ratio, S</td>
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