



## FAQ's

- ▶ **Q. Which test should be ordered as the best assessment of a patient's Vitamin D status?**
  - A. *25-hydroxy Vitamin D*  
**(Order code VDOH)**
  
- ▶ **Q. Which test should be ordered if I want to assess the levels of 25-hydroxy Vitamin D2 and D3 in my patient?**
  - A. *Vitamin D2 D3, 25-hydroxy by LC-MS/MS*  
**(Order code VITD23)**
  
- ▶ **Q. If 1,25-di-hydroxy Vitamin D is the active form, why isn't it the best test to order?**
  - A. *1,25-dihydroxy Vitamin D can be normal or even elevated in Vitamin D deficiency. Vitamin D deficiency leads to hypocalcemia, which triggers increased PTH, which, in turn, causes the kidneys to produce more 1,25-dihydroxy Vitamin D. Hypocalcemia, also causes the kidneys to produce more 1,25-dihydroxy Vitamin D.*
  
- ▶ **Q. What are the clinical applications for 1,25-di-hydroxy Vitamin D testing?**
  - A. *1,25-dihydroxy Vitamin D (Order code VIDD) measurements can be helpful in diagnosing Vitamin D-dependent rickets, differentiating primary hyperparathyroidism from hypercalcemia of malignancy and in monitoring patients being treated with 1,25-dihydroxy Vitamin D.*

**For more information, please contact your local marketing representative.**

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## An Updated Handy-Dandy Guide to Vitamin D

We've updated our Vitamin D menu to include 25-hydroxy Vitamin D2 and D3 analysis by Tandem Mass Spectrometry. What we've attempted to do with this updated guide is summarize the most important bullet points and take-home messages about Vitamin D metabolism and how best to assess Vitamin D status.

Vitamin D plays a central role in calcium and phosphorus metabolism and skeletal health. Although foods fortified with Vitamin D have reduced the risk of severe deficiency, it has recently been recognized that many individuals, both children and adults, have a relative insufficiency or mild deficiency of this vitamin. In addition to its well-known role in maintaining healthy bone, Vitamin D is also emerging as a factor in other chronic illnesses.

### DEFINITIONS OF FACTORS IN VITAMIN D METABOLISM

- **25-hydroxy Vitamin D** is the major circulating metabolite of the vitamin. It has only modest biological activity, but it is the best indicator of Vitamin D status. It is produced in the liver and is present in the blood in ng/mL quantities.
- **1,25-dihydroxy Vitamin D** is the biologically active form of the vitamin. It has very limited usefulness as an indicator of Vitamin D status, but measuring it does have some clinical applications. It is produced in the kidneys and is present in the blood in pg/mL quantities (1000-fold less than 25-hydroxy Vitamin D). The reference range is 15 – 75 pg/mL.
- Both 25-hydroxy Vitamin D and 1,25-dihydroxy Vitamin D come in two forms: **D2 and D3**. The difference between the D2 and D3 forms is the location of a methyl group in the molecule. D2 is derived from plant sources and D3 is derived from animal sources. Recently published papers suggest there is no clinical benefit in differentiating between the two forms. However, some clinicians like to monitor the levels of 25-hydroxy Vitamin D2 and D3, especially in patients receiving pharmacotherapy.
- **Parathyroid hormone (PTH)** secretion is regulated by calcium levels in blood and extracellular fluid. PTH stimulates the kidneys to produce 1,25-dihydroxy Vitamin D. It also enhances tubular reabsorption of calcium and works to mobilize calcium from bone.
- **Calcium and phosphorous levels in the blood** influence the production of 1,25-dihydroxy Vitamin D in the kidneys. Calcium also regulates PTH secretion.

### SOURCES

**We get Vitamin D from exposure to sunlight and from our diet -- either the food we normally eat or dietary supplements.**

- Solar ultraviolet B radiation on the skin converts Vitamin D3 precursor molecules to Vitamin D3.
- Dietary sources provide both Vitamin D2 and Vitamin D3. Vitamin D2 and D3 are present in supplements, but only D2 is available by prescription in the US.
- From here on in our discussion, I will refer to Vitamin D, meaning both forms.

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## REFERENCE RANGES

Please note that there are slightly different reference ranges for the immunoassay method and the LC-MS/MS method. These differences are due to the nature of the methods themselves -- one based on immunochemical antigen-antibody reactions and the other based on measuring the physical mass of the molecules themselves. This situation is similar to that seen for tumor markers, where it is important to know which method was used to measure the protein. As with tumor markers, patients being followed for Vitamin D levels should always have the test done by the same method.

In order to avoid any confusion, all PAML reports for Vitamin D will automatically include the correct method-specific reference range and they will state the method used in the assay.

### Vitamin D, 25-hydroxy by immunoassay (Order Code: VDOH)

|                    |   |
|--------------------|---|
| Less than 20 ng/mL | Suggests a deficiency of 25-OH Vitamin D.             |
| 20-29              | Suggests a relative insufficiency of 25-OH Vitamin D. |
| 30-150             | Suggests a sufficient level of 25-OH Vitamin D.       |
| Greater than 150   | Toxic level of 25-OH Vitamin D.                       |

Blood levels of 25-OH Vitamin D vary with the extent of sun exposure. Values tend to be highest in late summer and lowest in spring. Values also tend to decrease with age, due to decreased precursor synthesis in the skin.

### Vitamin D, 25-hydroxy by LC-MS/MS (Order Code: VITD23)

#### Reference Ranges for Total Vitamin D

|  |                        |
|--|------------------------|
| Severe Deficiency                        | Less than 10.0 ng/mL   |
| Mild to Moderate Deficiency              | 10.0–23.9 ng/mL        |
| Optimum Levels                           | 24.0–80.0 ng/mL        |
| Toxicity Possible, Pediatrics (0-18 yrs) | 80.0 ng/mL or greater  |
| Toxicity Possible, Adults (19+ yrs)      | 150.0 ng/mL or greater |

#### SELECTED REFERENCES:

- Holick, MF. Review: Vitamin D deficiency. *N Engl J Med* 2007;357:266-281.  
 Holick, MF. Review. High prevalence of Vitamin D inadequacy and implications for health. *Mayo Clin Proc.* 2006;81(3):353-373.  
 Holick, MF, et al. Vitamin D2 is as effective as Vitamin D3 in maintaining circulating concentrations of 25-hydroxy Vitamin D. *J Clin Endo & Metabol* 2008;93:677 – 681.  
 Hollis, BW. Assessment of Vitamin D nutritional and hormonal status: What to measure and how to do it. *Calcif Tissue Int.* 1996;58:4-5.  
 Hollis, BW. Assessment of Vitamin D nutritional and hormonal status: What to measure and how to do it. *Calcif Tissue Int.* 1996;58:4-5.

## METABOLISM

**Vitamin D metabolism involves the liver, kidneys, GI tract and bone in maintaining calcium and phosphorous homeostasis and bone health.**

- Vitamin D from either sunlight exposure or diet moves through the circulation to the liver where it is converted to 25-hydroxy Vitamin D.
- 25-hydroxy Vitamin D moves through the circulation to the kidneys, where it is converted to 1,25-dihydroxy Vitamin D, under the influence of PTH, calcium, phosphorous and other factors.
- 1,25-dihydroxy Vitamin D increases calcium and phosphorous absorption in the small intestine and mobilizes calcium and phosphorous from bone in order to maintain adequate levels.
- To follow through the metabolic cycle of Vitamin D deficiency, for example: Vitamin D deficiency would lead to hypocalcemia, which would trigger an increase in PTH, causing the kidneys to produce more 1,25-dihydroxy Vitamin D, leading to increased calcium absorption in the small intestine and mobilization of calcium from bone. Severe or prolonged deficiency of Vitamin D can lead to osteoporosis, bone fracture and other conditions.

