Vitamin D Clinical Information
Vitamin D deficiency has long been associated with bone disease. Knowing the significance of vitamin D testing facilitates informed decision-making and helps healthcare professionals maximize the quality of care they provide for their patients.
**Vitamin D**

Vitamin D is a fat-soluble hormone involved in the intestinal absorption of calcium and regulation of calcium. It plays a vital role in the formation and maintenance of strong, healthy bones. Vitamin D deficiency has long been associated with rickets in children and osteomalacia in adults, and long-term insufficiency of calcium and vitamin D leads to osteoporosis.1

**Vitamin D Deficiency**

Globally, over 1 billion people are vitamin D deficient,2 and in the United States the NHANES III study from 2001 to 2004 indicated that 77% of U.S. adults are insufficient. Deficiency rates have increased as people have limited their sun exposure due to the risk of skin cancer. People living near the equator who are exposed to sunlight without sun protection have robust levels of vitamin D; however, vitamin D deficiency is found in regions where skin exposure is limited, such as Saudi Arabia, the United Arab Emirates, Australia, Turkey, India, and Lebanon.

**Types of Vitamin D and How Vitamin D Is Synthesized**

There are two major types of vitamin D:

- Vitamin D₂ (ergocalciferol)—which is synthesized by plants and is not produced by the human body.
- Vitamin D₃ (cholecalciferol)—which is made in large quantities in the skin when sunlight strikes bare skin. It can also be ingested from animal sources.

Factors that impact the ability of the body to synthesize vitamin D through the skin are geographic latitude, time of year, time of day, presence of clouds and/or smog, skin melanin content, and whether or not sunscreen has been applied. For example, residents at 42° N latitude or higher are unable to synthesize vitamin D via the skin during the winter months (from November through February).

In supplements and fortified foods, vitamin D can be either D₂ or D₃. The two forms have traditionally been regarded as equivalent based on their ability to cure rickets, but evidence suggests that vitamin D₃ is approximately three times more effective at maintaining serum concentrations because the binding protein has a higher affinity to vitamin D₃ than vitamin D₂. This allows vitamin D₃ to reside in the circulatory system longer and increase the concentration to sufficient levels more quickly. The major preparations of vitamin D for prescription use in North America are in the form of vitamin D₂, while more over-the-counter vitamin/multivitamin preparations use vitamin D₃.
Whether it is synthesized through unprotected skin or ingested then absorbed by the intestines, vitamin D is bound to the binding protein (both albumin and vitamin D binding protein) and carried to the liver via the bloodstream. From there it begins two hydroxylation processes. Beginning in the liver it is transformed into 25(OH) vitamin D (calcidiol), which is the primary circulating form of vitamin D and the most commonly measured form in serum. Then in the kidneys it is transformed into 1,25 dihydroxy-vitamin D (calcitriol), which is the biologically active form of vitamin D.

1,25 dihydroxy-vitamin D is the primary steroid hormone involved in mineral homeostasis. When serum calcium dips to below 8.8 mg/dL it prompts a proportional increase in the secretion of parathyroid hormone (PTH). PTH signals to the kidneys to increase the production of 1,25 dihydroxy-vitamin D by increasing the production of 25(OH)vitamin D-1α-hydroxylase. Subsequently, the increase in 1,25 dihydroxy-vitamin D stimulates the increased absorption of calcium in the intestines to stimulate bone remodeling. When phosphorous and bone genes levels signal a normal state of bone remodeling, the kidney reduces the production of 1,25 dihydroxy-vitamin D to a normal level.
**Vitamin D Sufficiency Levels**

Most experts agree that vitamin D sufficiency is above 30 ng/mL (75 nmol/L), an insufficient level is between 20 and 30 ng/mL (50 to 75 nmol/L), and a deficient level is any value below 20 ng/mL (50 nmol/L).

**Groups at Higher Risk for Vitamin D Deficiency**

There are several groups at higher risk of vitamin D deficiency including:

- **Breastfed Infants**
  Sufficiency is dependent on the mother’s vitamin D sufficiency level, and mother’s milk typically contains about 25 IU/L of vitamin D. Most breastfed infants are on 400 IU of vitamin D daily supplementation.

- **Older Adults**
  As people age, the skin is not able to synthesize vitamin D as effectively, and reduced kidney function impacts the ability to convert vitamin D.

- **Dark Skinned People**
  Melanin in darker skin reduces the ability to produce vitamin D from sunlight exposure.

- **Limited Sun Exposure**
  Eliminates one of the two possible sources of vitamin D.

- **Obesity**
  Vitamin D is fat soluble, which does not allow it to circulate as freely.

- **Other**
  Gastric bypass patients have less small intestine available to absorb vitamin D.

**Vitamin D Supplementation**

Oral vitamin D supplementation has proven to be very effective at raising vitamin D levels. Recommendations vary by subgroup:

- **Normal Insufficiency**
  
  - Avoid Deficiency: 800–1000 IU vitamin D per day
  - Treat Deficiency: 50,000 IU vitamin D per week for 8 weeks, then every 2–4 weeks

- **Post-Menopausal Women**
  
  - ≥ 800 IU vitamin D per day + calcium
  - ≥ 800 IU vitamin D per day + calcium

- **Intestinal Malabsorption**
  
  - Up to 50,000 IU vitamin D per day
  - Up to 50,000 IU vitamin D per day

- **Impaired α-hydroxylation**
  
  - 1,25(OH)₂ vitamin D or α-hydroxy vitamin D
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**Total Vitamin D Measurement (ng/mL)**

0 20 40 60 80 100

- deficient
- insufficient
- sufficient
Importance of Measuring Total Vitamin D

Measuring Total Vitamin D
Vitamin D can be measured separately or as a total value, but not all immunoassays have the same reactivity to vitamin D$_2$ and D$_3$. Some immunoassays may not fully detect the entire amount of 25(OH)vitamin D$_2$. No matter which methodology you use, the most important value is the final total value, since it represents the total amount of 25(OH)vitamin D (both D$_2$ and D$_3$) in the blood. This ensures your patients have the most accurate result regardless of level and whether or not they are supplemented over-the-counter or by prescription.

Measuring Total Vitamin in Determining Sufficiency
In the case of a true concentration that is just into the sufficiency range, if the assay does not detect 25(OH)vitamin D$_2$, it is likely that result will be reported in the insufficient range. This could also happen if the assay detects only a fraction of the 25(OH)vitamin D$_2$ that is present. To get a true reading of the patient’s vitamin D level, an assay should be used that detects both 25(OH)vitamin D$_2$ and D$_3$ equally.

When serum 25-hydroxy-vitamin D levels are consistently > 150 ng/mL (375 nmol/L), it is potentially toxic. This typically occurs due to vitamin D over-supplementation and is observed in patients taking more than the prescribed 50,000 IU per day. Toxicity due to sunlight overexposure and/or diet is unlikely. When vitamin D levels are this high, calcium concentrations rise as well, which can result in nausea, weight loss, and constipation. As a result of increased levels of vitamin D and calcium, the patient can develop kidney stones.

Measuring Total Vitamin in Determining Toxicity
In the case of a patient that is being treated for malabsorption with a high dose of vitamin D, the different reactivity for 25(OH) vitamin D$_2$ and D$_3$ also can cause the patient status to be mis-identified. If the supplement is 25(OH)vitamin D$_2$, it is likely to be the largest vitamin D concentration in these patients. Consequently, by not having 25(OH) vitamin D$_2$ detected or partially detected, it may result in the under-reporting of the total vitamin D concentration. This can result in missing a patient that has levels that are toxic.

C3-epi-25(OH)D$_3$ Can Confound Accurate Measurement
While guidelines specify detection of the primary metabolites 25(OH)vitamin D$_2$ and D$_3$, it is important to know the amount of cross-reactivity an assay has to C3-epimer. The percentage of C3-epimer varies but its presence can be found in both children and adults. Unless an assay reports the specific fraction/quantity of the C3-epimer, it is not possible to determine if Vitamin D is overestimated. Overestimation can create therapeutic errors, since patients who are deficient or insufficient may appear sufficient and toxicity may be reported in patients with high-normal levels.
References:

To learn more about Vitamin D Total Assay available on the ADVIA Centaur® and Dimension® EXL™ Systems, visit siemens.com/vitamind