

Vitamin D (25-OH)

MDL Test Number: 659

Methodology: Immunoassay

CPT Codes: 82306



Specimen Collection Platform:

Serum in Serum Separator Tube (SST)

Turnaround Time:

24 - 72 hours

Stability:

Room temperature

Test Additions*:

30 days to add tests

Specimen Collection:



1. Immediately after blood collection, invert the tube(s) gently 5 times to ensure mixing of clot activator.



2. Allow the tube to rest upright for at least 30 minutes after collection to ensure proper clotting.



3. Centrifuge for at least 15 minutes at 2200-2500 RPM within one hour of collection.



4. Do not remove the stopper or pour off serum.



What is Vitamin D (25-OH)?

Vitamin D is not really a vitamin. The skin makes it from sunlight, and smaller amounts come from oily fish, egg yolks, fortified foods, and supplements. Whatever the source, it is inactive until the liver converts it into 25-hydroxy vitamin D, the form that circulates and is

stored. The kidneys then turn a small fraction of that store into the active hormone, which tells the gut how much calcium to absorb and helps keep bone in good repair.

This test measures 25-hydroxy vitamin D, because that is the form that reflects how much vitamin D the body has taken in and holds in reserve. The active form circulates at about a thousandth of the concentration and is held steady by the body even when reserves are running down, so measuring it would say very little about supply. Levels of the storage form rise and fall with how much sunlight reaches the skin, which depends on the season, the latitude, time of day, sunscreen, clothing, and skin pigmentation. They also change with age, with body weight, and with how well fat is absorbed from food.

Why test for Vitamin D (25-OH)?

Vitamin D is measured where there is a reason to expect the level to be low, or where a low level would change what is done next. It is commonly ordered to:

- **Investigate thinning bones.** Vitamin D is checked in the workup of osteoporosis and low bone density, and before starting treatment for them, because bone medicines work poorly when vitamin D is low.
- **Look into bone pain, muscle weakness, or repeated fractures,** which can be signs of the bone softening that long-standing severe deficiency causes (osteomalacia).
- **Follow people whose gut cannot absorb fat properly,** including after weight-loss surgery and in celiac disease and inflammatory bowel disease, since vitamin D is absorbed with dietary fat.
- **Assess people carrying excess weight,** in whom vitamin D is drawn into fat tissue and diluted through a larger body volume, so the level in the blood runs lower for the same intake.
- **Make sense of an abnormal calcium or parathyroid hormone result.** Low vitamin D drives the parathyroid glands to work harder, and MDL offers intact parathyroid hormone as test 618 for that purpose.
- **Check whether the replacement is working and help decide the dose,** once treatment for a low level has started.



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Special Instructions:

- **Paricalcitol:** Paricalcitol (Zemlar), a manufactured form of active vitamin D given in kidney disease, is read by this test as though it were the patient's own vitamin D and makes the result falsely high. The manufacturer directs that vitamin D not be tested in a patient who has received paricalcitol within the previous 24 hours.
- **Supplements and recent doses:** Vitamin D is present in multivitamins, in bone and joint products, and in high-dose weekly prescriptions. Patients should tell the ordering provider everything they take and at what dose, including anything started recently, because a level drawn soon after a large dose does not describe the usual state.
- **Biotin supplements:** Biotin is sold on its own and is present in many hair, skin, and nail products, often at high doses. The manufacturer tested this test only up to a biotin level that such a supplement can exceed, so the effect above that point is not described. Patients should tell the ordering provider what they take and at what dose.
- **Comparing results over time:** Different laboratories and different vitamin D methods can give different numbers on the same sample, and the differences are large enough to matter. Repeat testing should be done at the same laboratory whenever possible, and the time of year noted, since levels are normally lower at the end of winter than at the end of summer.

Limitations:

Every laboratory test has limits. For vitamin D, the main ones are:

- This test measures the storage form of vitamin D, which is the standard measure of how much a person has. It does not measure the active hormone, and the two do not always move together — in advanced kidney disease in particular, the active form can be low while the storage form is not.
- The test reports total vitamin D, so it picks up the D2 and D3 forms together, as it is meant to, and also reads some closely related substances that are built the same way. Medicines that are manufactured forms of vitamin D are among them.
- Results are reported between 7 and 120 nanograms per milliliter. The manufacturer directs that samples not be diluted, so a level above 120 is reported as greater than 120 rather than as a number, which can matter for someone taking very high doses. The test is also least reproducible at the bottom of its range, so a small difference between two low results should not be read as a change.
- There is no single agreed target level. The figures used to call a result deficient or sufficient come from clinical guidelines rather than from this test, and they are under active revision. Current Endocrine Society guidance does not recommend testing healthy adults who have no particular reason to be low.
- Rarely, antibodies that some people carry after routine contact with animals or animal products can interfere with tests built this way. Asfotase alfa, a manufactured form of the enzyme alkaline phosphatase used to treat hypophosphatasia, can do the same, and the manufacturer directs that such samples be evaluated carefully.
- A vitamin D result is one piece of a larger picture. It is meant to be read together with the patient's symptoms, medical history, and other test results — never on its own.

Reference Range(s):

Vitamin D is reported in nanograms per milliliter (ng/mL). The range below is what the manufacturer found in 367 apparently healthy adults aged 21 to 89, sampled in three parts of the United States across different seasons, about a fifth of whom were taking a supplement. It describes what was present in that group rather than a level to aim for, and a large share of them would be considered low by the criteria clinicians currently use. A result inside this range is therefore not by itself evidence that vitamin D status is adequate, and the level at which treatment is worthwhile is a clinical judgement rather than a laboratory one.

- **Adults, 21 to 89 years:** median 24.9 ng/mL, middle 95% **11.9 – 43.6 ng/mL**

A result outside this range is not automatically a problem, and a result inside it does not rule one out. Only the ordering provider can interpret what a given number means for a given patient. Patients should always go by the reference range printed on their own laboratory report.

Analyte	Disorder	Expected Value*
Vitamin D	Vitamin D deficiency	↓↓
Vitamin D	Osteomalacia	↓↓
Vitamin D	Obesity	↓
Vitamin D	Malabsorption	↓
Vitamin D	Chronic kidney disease	↓

*↓ – Decreased ↓↓ – Markedly Decreased.

What are some references that discuss the medical necessity of testing Vitamin D (25-OH)?

1. Demay MB, et al. Vitamin D for the prevention of disease: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*, **109**, 1907–1947 (2024).
2. LeBoff MS, et al. The clinician's guide to prevention and treatment of osteoporosis. *Osteoporos Int*, **33**, 2049–2102 (2022).
3. Minisola S, et al. Osteomalacia and vitamin D status: a clinical update 2020. *JBM Plus*, **5**, e10447 (2021).
4. Pereira-Santos M, et al. Obesity and vitamin D deficiency: a systematic review and meta-analysis. *Obes Rev*, **16**, 341–349 (2015).
5. Mechanick JL, et al. Clinical practice guidelines for the perioperative nutrition, metabolic, and nonsurgical support of patients undergoing bariatric procedures — 2019 update. *Obesity*, **28**, O1–O58 (2020).
6. Rubio-Tapia A, et al. American College of Gastroenterology guidelines update: diagnosis and management of celiac disease. *Am J Gastroenterol*, **118**, 59–76 (2023).
7. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Update Work Group. KDIGO 2017 clinical practice guideline update for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease—mineral and bone disorder (CKD-MBD). *Kidney Int Suppl*, **7**, 1–59 (2017).



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