Vitamin D Testing

Policy Number: AHS – G2005 – Vitamin D Testing	Effective Date: 11/01/2026

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I. Policy Description

Vitamin D is a precursor to steroid hormones and plays a key role in calcium absorption and mineral metabolism. Vitamin D promotes enterocyte differentiation and the intestinal absorption of calcium. Other effects include a lesser stimulation of intestinal phosphate absorption, suppression of parathyroid hormone (PTH) release, regulation of osteoblast function, osteoclast activation, and bone resorption.[1]

Vitamin D is present in nature in two major forms. Ergocalciferol, or vitamin D2, is found in fatty fish (e.g., salmon and tuna) and egg yolks, although very few foods naturally contain significant amounts of vitamin D. Cholecalciferol, or vitamin D3, is synthesized in the skin via exposure to ultraviolet radiation present in sunlight. Some foods are also fortified with vitamin D, most notably milk and cereals.[2]

Though the risk of vitamin D deficiency can differ by age, sex, and race and ethnicity, major risk factors for vitamin D deficiency include inadequate sunlight exposure, inadequate dietary intake of vitamin D-containing foods, and malabsorption syndromes, such as Crohn's disease and celiac disease.[3,4]

II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request.

1) For individuals with an underlying disease or condition which is specifically associated with vitamin D deficiency or decreased bone density (see Note 1), for individuals suspected of hypervitaminosis of vitamin D, or for individuals with vitamin D deficiency, 25-hydroxyvitamin D serum testing (no more than one test every six months) **MEETS COVERAGE CRITERIA**.

2) As part of the total 25-hydroxyvitamin D analysis, testing for D2 and D3 fractions of 25-hydroxyvitamin D **MEETS COVERAGE CRITERIA**.

3) For the evaluation or treatment of conditions that are associated with defects in vitamin D metabolism (see Note 2), 1,25-dihydroxyvitamin D serum testing **MEETS COVERAGE CRITERIA**.

4) The following testing **DOES NOT MEET COVERAGE CRITERIA**:
   a) Measurement of serum 1,25-dihydroxyvitamin D to screen for vitamin D deficiency.
   b) Routine screening for vitamin D deficiency with serum testing in asymptomatic individuals and/or during general encounters.

NOTES:

Note 1: Indications for serum measurement of 25-hydroxyvitamin D are as follows:

A. Biliary cirrhosis and other specified disorders of the biliary tract
B. Blind loop syndrome
C. Celiac Disease
D. Coronary artery disease in individuals where risk of disease progression is being considered against benefits of chronic vitamin D and calcium therapy
E. Dermatomyositis
F. Eating disorders
G. Having undergone, or for those who have been scheduled for, bariatric procedures such as Roux-en-Y gastric bypass, sleeve gastrectomy, or biliopancreatic diversion with or without duodenal switch
H. Hypercalcemia, hypocalcemia, or other disorders of calcium metabolism
I. Hyperparathyroidism or hypoparathyroidism
J. Individuals receiving hyperalimentation
K. Inflammatory bowel disease (Crohn's disease and ulcerative colitis)
L. Intestinal malabsorption
M. Liver cirrhosis
N. Long term use of anticonvulsants, glucocorticoids, chemotherapy, and other medications known to lower vitamin D levels
O. Malnutrition
P. Myalgia and other myositis not specified
Q. Myopathy related to endocrine diseases
R. Neoplastic hematologic disorders
S. Osteogenesis imperfecta
T. Osteomalacia
U. Osteopetrosis
V. Osteoporosis
W. Pancreatic steatorrhea
X. Primary or miliary tuberculosis
Y. Psoriasis
Z. Regional enteritis
AA. Renal, ureteral, or urinary calculus
BB. Rickets
CC. Sarcoidosis
DD. Stage III-V Chronic Kidney Disease and End Stage Renal Disease
EE. Systemic lupus erythematosus

Note 2: Indications for serum testing of 1,25-dihydroxyvitamin D are as follows:

A. Disorders of calcium metabolism
B. Familial hypophosphatemia
C. Fanconi syndrome
D. Hyperparathyroidism or hypoparathyroidism
E. Individuals receiving hyperalimentation
F. Neonatal hypocalcemia

G. Osteogenesis imperfecta
H. Osteomalacia
I. Osteopetrosis
J. Primary or miliary tuberculosis
K. Renal, ureteral, or urinary calculus
L. Rickets
M. Sarcoidosis
N. Stage III-V Chronic Kidney Disease and End Stage Renal Disease

III. Table of Terminology

Term	Definition
25(OH)D	25-Hydroxyvitamin D
25OHD	25-Hydroxyvitamin D
AACE	American Association of Clinical Endocrinologists
ACE	American College of Endocrinology
AAP	American Academy of Pediatrics
ACOG	American College of Obstetricians and Gynecologists
BMD	Bone mineral density
BPD	Biliopancreatic diversion
CDC	The Centers for Disease Control and Prevention
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare & Medicaid Services
CV	Coefficient of variation
DS	Duodenal switch
ELISA	Enzyme-linked immunosorbent assay
ES	Endocrine Society
FDA	Food and Drug Administration
HPLC	High-performance liquid chromatography
IDS	Immunodiagnostic Systems
IOM	Institute of Medicine
IU	International unit
LAGB	Laparoscopic adjustable gastric banding
LC/MS	Liquid chromatography coupled with mass spectrometry
LDTs	Laboratory developed tests
LSG	Lennox-Gastaut Syndrome
MEWS	Modified Early Warning Score
MS	Mass spectrometry
NHANES	National Health and Nutrition Examination Surveys
PCR	Polymerase chain reaction
PTH	Parathyroid hormone
RIA	Radioimmunoassay
ROS	Royal Osteoporosis Society
RYGB	Rouxen-Y gastric bypass

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SPF	Sun protection factor
THIN	The Health Improvement Network
USPSTF	United States Preventive Services Task Force
UVB	Ultraviolet B
VDSCP	Vitamin D Standardization Certification Program

IV. Scientific Background

Vitamin D is an important nutrient that helps the body absorb calcium and maintain adequate bone strength. In order to be used in the metabolic process, vitamin D that is consumed or formed in the skin must first be activated via the addition of hydroxyl groups. Two forms of activated vitamin D are found in human circulation: 25-hydroxyvitamin D (calcidiol or 25OHD) and 1,25-dihydroxyvitamin D (calcitriol). 25-hydroxyvitamin D is the predominant and most stable form, but 1,25-dihydroxyvitamin D is the metabolically active form. The initial activation step occurs in the liver, where 25OHD is synthesized, and the second hydroxyl group is added in the kidney, creating the fully activated 1,25-dihdroxy form.[2]

25-hydroxyvitamin D has a half-life of 15 days in the circulation, whereas 1,25-dihydroxyvitamin D has a much shorter circulating half-life of 15 hours. Consequently, measurement of serum 25OHD is generally accepted as the preferred test to evaluate an individual's vitamin D status despite lack of standardization between methods and laboratories.[2,5,6]

Vitamin D deficiency typically is defined as a serum 25OHD level less than 20 ng/ml, and certain organizations consider <30 ng/ml as insufficient. Trials of vitamin D supplementation[7-10] and the Institute of Medicine (IOM) systematic review[11] recommend maintaining the serum 25OHD concentration between 20 and 40 ng/mL (50 to 100 nmol/L), whereas other experts favor maintaining 25OHD levels between 30 and 50 ng/mL (75 to 125 nmol/L). Experts agree that levels lower than 20 ng/mL are suboptimal for skeletal health. The optimal serum 25OHD concentrations for extra-skeletal health have not been established.[12] Approximately 15% of the U.S. pediatric population suffers from either vitamin D deficiency or insufficiency. Limited sun exposure and the use of sunscreen compromises production of vitamin D, contributing to low 25OHD levels. "UVB absorption is blocked by artificial sunscreens, and sunscreens with a sun protection factor (SPF) of 30 can decrease vitamin D synthetic capacity by as much as 95 percent."[13] Also, "vitamin D deficiency has been reported in dark-skinned immigrants from warm climates to cold climates in North America and Europe."[14] For example, a study by Awumey and colleagues found that Asian Indians who immigrated to the U.S. were considered vitamin D insufficient or deficient even after the administration of 25OHD. "Thus, Asian Indians residing in the U.S. are at risk for developing vitamin D deficiency, rickets, and osteomalacia."[15]

Vitamin D deficiency has been associated with important short and long term health effects, such as rickets, osteomalacia, and the risk of osteoporosis.[2] Rickets in children can result in skeletal deformities. To prevent nutritional rickets in infants, vitamin D supplementation is recommended at 400 IU/day; personalized dosages are possible and would require 25OHD testing.[16] In adults, osteomalacia can result in muscular weakness, bone weakness, and osteoporosis which leads to an increased risk for falls and fractures.[17]

A role for vitamin D has been suggested in several other conditions and metabolic processes including, but not limited to, cancer, cardiovascular disease, hypertension, diabetes, and preeclampsia. While vitamin D insufficiency has been associated with several cancer types, inconsistencies cause

discrepancies in suggested treatment methods; currently, no official institutional guidelines recommend a dietary vitamin D supplementation for cancer prevention.[18] 25-hydroxyvitamin D (25OHD) is the accepted biomarker of circulating vitamin D, and in utilization of this biomarker, researchers have reported an association between a high vitamin D production rate and a lowered risk of colorectal cancer.[19] Further, low concentrations of 25OHD have been associated with a high risk of cardiovascular disease and mortality, suggesting that patients deficient in vitamin D have an increased risk in developing cardiovascular disease.[20] However, conclusive evidence for the role of vitamin D in these conditions is not available.[11,21] Based on controversial evidence, researchers continue to emphasize the fact that vitamin D supplementation is not an accepted prevention method for cardiac events or cancer.[22]

Certain other conditions may impact an individual's ability to absorb or activate vitamin D, thereby resulting in vitamin D deficiency. These include, but are not limited to, Crohn's disease, ulcerative colitis, celiac disease, liver cirrhosis, chronic kidney disease, and bariatric surgery. Since Vitamin D is fat soluble, any impact on fat absorption or storage may affect circulating vitamin D levels.[14,23]

According to the Institute of Medicine (IOM), routine dietary supplementation with vitamin D is recommended for most individuals. While there are no differences regarding gender and recommended daily dose of vitamin D, there are differences depending on age. The IOM recommends a dietary allowance of 600 IU for individuals up to 70 years old, and 800 IU for individuals older than 70,[11] although these recommendations have been met with some criticism as being too low to adequately impact vitamin D levels in some individuals. The USPSTF recommends against daily supplementation with 400 IU or less of vitamin D3 and 1000 mg or less of calcium for the primary prevention of fractures in noninstitutionalized postmenopausal individuals.[24]

Vitamin D toxicity is very rare and occurs only when levels of 25OHD are >500 nmol/L [>200 ng/mL], which is well above the level considered sufficient. Vitamin D toxicity may cause hypercalciuria, hypercalcemia, renal stones, and renal calcification with renal failure.[24] Additional research suggests that excess 25-hydroxyvitamin D3 aggravates tubulointerstitial injury.[25]

Insource Diagnostics has developed two similar quantitative laboratory developed tests (LDTs) termed Sensieva Vena™ 25OH Vitamin D2/D3 and Droplet 25OH Vitamin D2/D3. These assays utilize liquid chromatography coupled with mass spectrometry (LC/MS/MS) to measure both D2 and D3. The LC/MS/MS assessment technique is the apparent gold standard for vitamin D2 and D3 measurement, and is the only currently available method to measure both vitamins individually. These assays may assist in the measurement of several ailments related to abnormal vitamin D levels including parathyroid function, dietary absorption, calcium metabolism, and vitamin D treatment effectiveness; serum, plasma and blood microsamples can be utilized for these tests. The 20uL serum/plasma method of the Sensieva™ 25OH Vitamin D2/D3 LDT was approved by the CDC's VDSCP in 2017-2018.[26] This test is no longer certified by the CDC's VDSCP and as of May 2020 Insource Diagnostics website has been removed. Therefore, it is unclear if this test is still available.

Glucocorticoids, when used chronically in high doses, inhibit intestinal vitamin D-dependent calcium absorption, which is one of the mechanisms whereby chronic glucocorticoid excess leads to osteoporosis and fractures. It is recommended that any patient taking any dose of glucocorticoid with an anticipated duration of more than three months measure serum 25-hydroxyvitamin D (25[OH]D) to guide vitamin D supplementation.[27] Chemotherapy is also known to reduce vitamin D to dangerously low levels in the bodies of cancer patients, causing severe vitamin D deficiency.[28]

Analytical Validity

Serum or plasma concentration of 25OHD can be measured using several assays, including ELISA, radioimmunoassay (RIA), mass spectrometry, and HPLC. Assays using LC-MS/MS can differentiate between D2 and D3. These methods "can individually quantitate and report both analytes, in addition to providing a total 25-hydroxyvitamin D concentration."[29] RIA-based assays for 25OHD can have intra- and inter-assay variations of 8 – 15%, and the Immunodiagnostic Systems (IDS)-developed RIA has a reported 100% specificity for D3 and 75% for D2.[30] "For most HPLC and LC-MS/MS methods extraction and procedural losses are corrected for by the inclusion of an internal standard which, in part, may account for higher results compared to immunoassay."[31] Even though LC-MS/MS is considered to be the gold standard of measuring 25OHD and its metabolites, only approximately 20% of labs report using it.[32] One study reports that 46% of samples measured using LC-MS/MS were classified as vitamin D-deficient whereas, when the samples were measured using an immunoassay method, 69% were vitamin D-deficient (<30 nmol/L).[33]

The Centers for Disease Control and Prevention (CDC) have developed a vitamin D standardization certification program (VDSCP). This program helps to ensure that all LDT vitamin D tests are accurate and reliable by evaluating the performance and overall reliability of these assessments over time, supplying reference measurements for both 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3, and providing technical support to additional programs and studies.[34]

Due to the great variability among the different assays used to measure vitamin D levels, the VDSCP was created. Interassay variability yields an inadequate basis to establish if 25OHD increases or decreases the risk of non-skeletal diseases and hampers the development of evidence-based guidelines and policies.[35] VDSCP studies can either be retrospective or prospective; therefore, standardization of national nutrition survey data may be performed. For example, it was originally thought, based on reports from the National Health and Nutrition Examination Surveys (NHANES), that there had been a dramatic decline in mean 25OHD levels in the US population from 1990 to the period 2001–2004. DiaSorin Radioimmunoassay was used to measure 25OHD levels in these surveys. However, after standardizing the results using VDSCP methods, it was found that the mean 25OHD levels were stable from 1990-2004.[36] The VDSCP program established four steps to achieve standardization, as described by:

1. "Fit for use…means that assay chosen will perform appropriately and provide standardised measurements in the patient/study populations in the conditions for which it will be used…[as] some immunoassays do not function appropriately in all patient populations.
2. [Assay is] Certified by the CDC Vitamin D Standardization Certification Program as being standardised and having an appropriate measurement range or be a documented standardised laboratory-developed HPLC or LC-MS/MS assay with an appropriate measurement range…see which ones are currently, or have been in the past, certified by the CDC as meeting VDSP performance criteria of having a total (coefficient of variation) CV≤10% and a mean bias with the range of –5 to +5%... VDSP recommends using an assay that does have an appropriate measurement range for the population it will be used in; for example, it should be able to measure 25(OH)D in persons who are deficient.
3. Appropriate level of assay precision and accuracy…it has been recommended that a standardised LC-MS/MS assay be selected.
4. [The assay] Meets VDSP assay standardisation criteria in your 'hands' or laboratory…. We recommend a testing period in order to verify that an immunoassay is standardized especially since there is generally very little an individual laboratory can do to 'calibrate' an immunoassay."[35]

Clinical Utility and Validity

A retrospective study of 32,363 tests of serum 25OHD found that a significant proportion of the lab requests were unjustified by medical criteria, and "that clinical and biochemical criteria may be necessary to justify vitamin D testing but not sufficient to indicate the presence of vitamin D deficiency."[17]

The table below lists the criteria used for vitamin D testing in the study by Granado and colleagues.[17]

A meta-analysis study by Bolland, et al. (2018) of 81 randomized controlled trials with a combined total of 53,537 participants measured the effects, if any, vitamin D supplementation had on fractures, falls, and bone density. They found that there was no clinically relevant difference in bone mineral density at any site between the control and experimental groups; moreover, "for total fracture and falls, the effect estimate lay within the futility boundary for relative risks of 15%, 10%, 7.5%, and 5% (total fracture only), suggesting that vitamin D supplementation does not reduce fractures or falls by these amounts. Our findings suggest that vitamin D supplementation does not prevent fractures or falls or clinically meaningful effects on bone mineral density. There were no differences between the effects of higher and lower doses of vitamin D. There is little justification to use vitamin D supplements to maintain or improve musculoskeletal health. This conclusion should be reflected in clinical guidelines."[37]

A prospective study by Hao, et al. (2020) aims to determine whether 25OHD levels is associated with mortality or the ability to walk in a patient cohort after hip fracture surgery. Each year, 319,000 elderly patients, are hospitalized for hip fractures.[39] In this study, 290 elderly patients with hip fractures were included, in which patients with 25OHD deficiency (<12 ng/ml) were used as the reference group. They observed a 56–64% increased rate of walking in patients who had 25OHD levels > 12 ng/ml at 30 days and 60 days after hip fracture surgery compared with 35% for patients able to walk 30 days postoperatively who had 25OHD levels < 12 ng/ml.[38] It is important to note that only the preoperative 25OHD levels accurately reflect the patient's ability to walk after 30 days, and the postoperative vitamin D status is not related and should not be used to determine clinical or nutritional interventions. Holick (2020) releases a call for action, discussing the data collected by Hao, to establish guidelines which will assess vitamin D status as needed for patients with hip fracture. Holick suggests that "patients aged ≥50 y presenting with fractures, especially those with hip fracture, should be evaluated at intake for their vitamin D status. Consideration should be made to provide vitamin D supplementation if dietary/supplemental intake or blood concentrations of 25(OH)D suggest deficiency."[40]

Clinical conditions
- Differential diagnosis (i.e. hypercalcemia)
- Undernourished subjects
- Malabsorption syndromes (i.e. celiac disease, Chron's disease, radiation enteritis)
- Eating disorders (i.e. morbid obesity, anorexia and bulimia)
- Candidates for bariatric surgery
- Conditions associated with altered calcium, phosphorus or vitamin D metabolism (i.e. osteoporosis, rickets, renal disease, liver failure, multiple mieloma, sarcoidosis, hyper/hypoparathyroidism, liver and kidney transplants)
- Diseases related to low or null sun exposure (i.e. lupus, porphyria)
- Vitamin D-related inborn errors of metabolism

Therapeutic criteria
- Pharmacotherapy associated with increased vitamin D catabolism (i.e. antiseizure drugs, glucocorticoids)
- Treatment for AIDS and tuberculosis
- Monitorization of vitamin D treatment

Biochemical indicators
- Alterations of serum or urine levels of calcium and phosphorus
- Elevation of alkaline phosphatase (in the absence of altered liver enzymes or growth)
- Serum levels of parathyroid hormone out of the reference range (14–72 pg/mL)
- Previous (<6 months) serum values of 25-OH-vitamin D out of the reference interval (<37.5 or >160 nmol/L)

Another randomized clinical trial administered by Scragg, et al. (2017) provided a monthly high dose of vitamin D to 5,108 participants in order to determine if a relationship exists between increased vitamin D levels and cardiovascular disease prevention. This double-blind trial was placebo-controlled; participants were given an initial dose of 200,000 IU of vitamin D, and then each month after for a range of 2.5-4.2 years were given 100,000 IU of vitamin D.[41] Results showed that in a random sample of 438 participants, cardiovascular disease occurred in 11.8% of patients who received vitamin D supplements and in 11.5% of patients who received placebos. This suggests that vitamin D administration does not prevent cardiovascular disease and should not be used for this purpose.[41]

Zhao, et al. (2015) carried out a study within a primary care cohort the UK. Vitamin D results of 9,460 (74%) first tests and 3,263 (26%) retests were analyzed. Of the first-test results, 42% of patients were deficient. The authors noted a marked increase in Vitamin D testing over the six-year period of the study. However, a significant amount of the test requests were retests. The authors cautioned against over-testing for Vitamin D too soon, before serum levels could show adequate response: "A significant proportion of requests were retests. Despite guidelines recommending retesting after three to six months, 20% of retests were performed within three months. Our results suggest that retesting soon after intervention may not allow sufficient time for serum levels to respond. By contrast, retesting within four weeks of a large loading dose may give a false picture of over repletion."[42]

Regarding pregnancy, vitamin D deficiency is common around the world and threatens fetal health and growth. Results from 203 Indonesian pregnant individuals who were followed from their first trimester until delivery showed astronomical vitamin D deficiency rates at approximately 75%.[43] Data collected from these individuals included maternal demography, bloodwork to test ferritin levels, 25(OH) vitamin D results in their first trimester, and the final birthweight of the child after delivery. Final results did not show any association between ferritin, hemoglobin level, and vitamin D in either the first trimester of pregnancy or in the final birthweight of the neonates after delivery; however, the authors suggest that other unknown variables may be important and that nutritional supplementation during pregnancy is still vital.[43]

Research has also been conducted on the association of 25(OH)D levels and SARS-CoV-2 infection. Ribeiro, et al. (2021) conducted a retrospective cohort study on 1638 patients tested for SARS-CoV-2 infection and found that "previous insufficient 25(OH)D (<30ng/mL) concentration and high total cholesterol were associated with SARS-CoV-2 infection among adults >48 y in the study population." This may be attributable to the role that vitamin D serves in the immune system and its anti-viral activity through autophagy, as well as its high expression in cells of the lungs, thus rendering those with lower levels of 25(OH)D more susceptible to infection without these defenses.[45]

Szerszeń, et al. (2022) also investigated the possible correlation between the immunomodulatory effect of vitamin D and the incidence and progression of COVID. From a sample of 505 patients, they quantified serum 25OHD and analyzed each patient's COVID severity through the serum Vitamin Modified Early Warning Score (MEWS), "which includes respiratory rate, systolic blood pressure, heart rate, temperature and state of consciousness," along with the days spent in the intensive care unit. The results demonstrated that there was no difference in 25OHD concentration between those with and without COVID as determined by PCR and no correlation between serum 25OHD "in the COVID(+) group and the need for and time spend in the ICU as well as the MEWS score." However, multivariate analyses did show a positive correlation between the need for oxygen therapy and lower 25OHD concentration. This signifies the evolving role of vitamin D in and how low serum levels may aid in predicting more complicated treatment courses.

On the other hand, Javed, et al. (2020) found that "high serum levels of vitamin D are associated with a lower risk of incidence and progression of [colorectal cancer]." This could make vitamin D testing crucial to identify possible future therapeutic modalities for patients with both low serum vitamin D and colorectal cancer. Like its mechanisms that hinder SARS-CoV-2 infection, such as being pro-apoptotic and anti-inflammatory, vitamin D has been shown to "decrease growth and differentiation of colon epithelial cells." With more large-scale human trials, testing and treatment using vitamin D can become more widely applicable.

It is also known that decreased vitamin D levels are associated with inflammatory bowel disease (IBD), though the mechanisms have not been fully elucidated.[48,49] Studies further suggest that vitamin D supplementation may positively impact the course of IBD, highlighting the utility of vitamin D testing in this patient population. It has been suggested that a daily dose of 2000 IU correlates with improvements in IBD symptoms and patient quality of life.[50,51]

V. Guidelines and Recommendations

The Endocrine Society (ES)

In 2024, the ES updated their guideline on Vitamin D testing and focused primarily on 25(OH)D serum testing without recommendations on 1,25-dihodroxyvitamin D testing. The ES reiterated the lack of clinical trial evidence supporting routine screening for 25(OH)D and used this evidence to reaffirm a position against routine screening of Vitamin D in the general population. "No clinical trial evidence was found to support routine screening for 25(OH)D in the general population, nor in those with obesity or dark complexion, and there was no clear evidence defining the optimal target level of 25(OH)D required for disease prevention in the populations considered; thus, the panel suggests against routine 25(OH)D testing in all populations considered." One notable difference between the 2011 and 2024 guideline is the changed position on screening Vitamin D in adults with obesity.

Pertaining to Vitamin D testing, the ES recommends the following:

1. "In the general adult population younger than age 50 years, we suggest against routine 25(OH)D testing."
2. "In the general population aged 50 to 74 years, we suggest against routine 25(OH)D testing."
3. "In the general population aged 75 years and older, we suggest against routine testing for 25(OH)D levels."
4. "During pregnancy, we suggest against routine 25(OH)D testing."
5. "In healthy adults, we suggest against routine screening for 25(OH)D levels."
6. "In adults with dark complexion, we suggest against routine screening for 25(OH)D levels."
7. "In adults with obesity, we suggest against routine screening for 25(OH)D levels."[52]

The Royal Australian College of General Practitioners (RACGP)

The RACGP released clinical guidelines for vitamin D testing which included the frequency at which to retest for vitamin D.

- "Do not retest vitamin D levels within three months of the patient beginning to take vitamin D replacement.
    - If retesting vitamin D levels (after three months of the patient taking replacement), use the same laboratory.

- When a patient's vitamin D levels have returned to normal, do not retest (do not arrange annual testing), especially if the patient has not changed their lifestyle or is still taking a supplement."[53]
- "Serum 25OH-D levels should be retested no earlier than 3 months following commencement of supplementation with vitamin D or change in dose. Annual assessment of 25-OHD at the same laboratory and at the end of winter time may be appropriate in older individuals or when risk factors for vitamin D deficiency have changed since initial testing."[54]

Institute of Medicine (IOM)

After an extensive evaluation of published studies and testimony from investigators, the Institute of Medicine determined that supplementation with vitamin D is appropriate; however, guidelines regarding the use of serum markers of vitamin D status for medical management of individual patients and for screening were beyond the scope of the Committee's charge, and evidence-based consensus guidelines are not available.[11]

National Health Service (NHS) Clinical Commissioning Group

In "Guidance for the Treatment of Vitamin D Deficiency and Insufficiency" the UK National Health Service notes that routine monitoring of serum 25[OH]D levels is not needed. Checking the serum 25(OH)D level 3–6 months after starting vitamin D treatment may be considered in some patients including; patients with symptomatic vitamin D deficiency, malabsorption, or where poor compliance with medication is suspected. It may also be considered in patients receiving antiresorptive therapy, with very low 25(OH)D levels at baseline assessment or those or those requiring sequential doses of antiresorptive agent.[55]

Royal Osteoporosis Society (ROS)

The Royal Osteoporosis Society (formerly known as the National Osteoporosis Society) recommends the measurement of serum 25 (OH) vitamin D (25OHD) to estimate vitamin D status in the following clinical scenarios: bone diseases that may be improved with vitamin D treatment; bone diseases, prior to specific treatment where correcting vitamin D deficiency is appropriate; musculoskeletal symptoms that could be attributed to vitamin D deficiency. The guideline also states that routine vitamin D testing is unnecessary where vitamin D supplementation with an oral antiresorptive treatment is already planned and sets the following serum 25OHD thresholds: <25 nmol/l is deficient; 25-50 nmol/l may be inadequate in some people; >50 nmol/l is sufficient for almost the whole population.[56]

American College of Obstetricians and Gynecologists (ACOG)

In a statement on gynecologic care for adolescents and individuals with eating disorders, ACOG specified that in patients with low bone mineral density (BMD), "a patient's 25-hydroxy vitamin D level should be checked and, if less than 30 ng per mL, the patient should be given supplementation for 6–8 weeks in the form of 2,000 international units daily or 50,000 international units weekly."[57] Reaffirmed in 2021.

Concerning screening for vitamin D deficiency, ACOG states that there is insufficient support currently to recommend screening for all pregnant individuals for vitamin D deficiency but that "maternal serum 25-hydroxyvitamin D levels can be considered and should be interpreted in the context of the individual clinical circumstance."[58] Additionally, ACOG mentions that, while there is no broad consensus on the ideal vitamin D level to maintain optimal health, most guidelines agree that a serum level of at least 20 ng/mL (50 nmol/L) is needed to avoid bone problems. Reaffirmed in 2021.[58]

United States Preventive Services Task Force (USPSTF)

The USPSTF published their recommendation concerning screening of vitamin D deficiency in asymptomatic community-dwelling, nonpregnant adults in 2021. "The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for vitamin D deficiency in asymptomatic adults" (I statement).[59]

American Association of Clinical Endocrinologists, The Obesity Society, and American Society for Metabolic and Bariatric Surgery

For patients undergoing Rouxen-Y gastric bypass (RYGB), sleeve gastrectomy, or biliopancreatic diversion either with or without duodenal switch (BPD/DS), a baseline evaluation for vitamin D deficiency and a postoperative evaluation is recommended.[60]

American Association of Clinical Endocrinologists (AACE) / American College of Endocrinology (ACE)

The 2020 guideline addressed fundamental measures for bone health for the diagnosis and treatment of postmenopausal osteoporosis. The following statements apply to Vitamin D:

1. "Measure serum 25-hydroxyvitamin D (25[OH]D) in patients who are at risk for vitamin D insufficiency, particularly those with osteoporosis (Grade B; BEL 2)."

2. "Maintain serum 25-hydroxyvitamin D (25[OH]D) ≥30 ng/mL in patients with osteoporosis (preferable range, 30 to 50 ng/mL) (Grade A; BEL 1)."

3. "Supplement with vitamin D3 if needed, with a daily dose of 1,000 to 2,000 international units (IU) typically required to maintain an optimal serum 25(OH)D level (Grade A; BEL 1)."

4. "Higher doses of vitamin D3 may be necessary in patients with present factors such as obesity, malabsorption, and older age (Grade A; BEL 1)."[61]

American Academy of Pediatrics (AAP)

"Evidence is insufficient to recommend universal screening for vitamin D deficiency… In the absence of evidence supporting the role of screening healthy individuals at risk for vitamin D deficiency in reducing fracture risk and the potential costs involved, the present AAP report advises screening for vitamin D deficiency only in children and adolescents with conditions associated with reduced bone mass and/or recurrent low-impact fractures. More evidence is needed before recommendations can be made regarding screening of healthy black and Hispanic children or children with obesity. The recommended screening is measuring serum 25-OH-D concentration, and it is important to be sure this test is chosen instead of measurement of the 1,25-OH_2-D concentration, which has little, if any, predictive value related to bone health."[62]

Through the Choosing Wisely initiative, the AAP advises against routine vitamin D screening in otherwise healthy children, including those who are overweight or obese. Current evidence does not support the necessity of such screening, aligning with global recommendations against population-based screening for vitamin D deficiency. Instead, the AAFP recommends vitamin D supplements for children with insufficient dietary intake.[63]

Manchester University NHS Foundation Trust

The Trust published a recommendation related to optimizing the demand for vitamin D testing in adults, where they noted that the demand for vitamin D testing has created an unsustainable workload. In this recommendation, it is stated that "asymptomatic individuals at high risk of Vitamin D deficiency should be treated without measurement of Vitamin D and should be advised on the need for maintenance dose Vitamin D supplementation."[64]

This recommendation also provides guidance on repeat testing: ". . . repeat Vitamin D measurement is not recommended in the majority of patients. Repeat measurement should only be considered in patients:

- Who had symptomatic Vitamin D deficiency (e.g. hypocalcaemia or bone disease)
- Who have malabsorption and are taking Vitamin D
- Who are on oral anti-resorptive agents and whose Vitamin D levels were low at baseline

Repeat testing should not be done within three months of the initial test, and preferably not within six months."[64]

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

A search of the FDA Device database on September 13, 2023, for "vitamin D" yielded 43 results. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Procedure codes appearing in medical policy documents are only included as a general reference. This list may not be all inclusive and is subject to updates. In addition, codes listed are not a guarantee of payment.

CPT	Code Description
82306	Vitamin D; 25 hydroxy, includes fraction(s), if performed
82652	Vitamin D; 1, 25 dihydroxy, includes fraction(s), if performed
0038U	Vitamin D, 25 hydroxy D2 and D3, by LC-MS/MS, serum microsample, quantitative Proprietary test: Sensieva™ Droplet 25OH Vitamin D2/D3 Microvolume LC/MS Assay Lab/Manufacturer: InSource Diagnostics

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VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes
01/01/2022	Initial Effective Date
05/23/2022	Updated background, guidelines and recommendations, and evidence based scientific references. Literature review did not necessitate change in coverage criteria.
09/14/2022	Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes to coverage criteria: Addition of inflammatory bowel disease (Crohn's disease and ulcerative colitis) to Guideline 1 (indications that support serum measurement of 25-hydroxyvitamin D). The following modifications were made to the coverage criteria for clarity and consistency: CC3b: deleted a comma after "annual testing" CC5a: "1,25-dihydroxyvitamin D serum testing for testing and screening of vitamin D deficiency" reworded to "Measurement of serum 1,25-dihydroxyvitamin D to screen for vitamin D deficiency" Guideline 1: replaced "coverage criteria" with "medical necessity" Revised code disclaimer statement
04/04/2023	Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: All CC edited for clarity and consistency. "Guidelines" changed to "Notes" to remain consistent with the formatting of all of our policies.

	Addition of “g.Having undergone, or for those who have been scheduled for, bariatric procedures such as Roux-en-Y gastric bypass, sleeve gastrectomy, or biliopancreatic diversion with or without duodenal switch” to Note 1. Removed “Hypervitaminosis of Vitamin D” from Note 1 due to the restructuring of CC1, which now reads: “For individuals with an underlying disease or condition which is specifically associated with vitamin D deficiency or decreased bone density (see Note 1) or for individuals suspected of hypervitaminosis of Vitamin D, 25- hydroxyvitamin D serum testing MEETS COVERAGE CRITERIA.” Committee approved 04/04/2023
02/12/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. Committee approved 02/12/2024
02/06/2025	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Edited CC3 for clarity and consistency. Removed Note 1 point S: “S. Obesity” Removed “medical necessity” language from Notes 1 and 2. Committee approved: 02/06/2025
06/17/2026	CAB Review Q1 2026: Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Further reading into already included guidelines and inclusion of new guidelines (e.g., Manchester University NHS Foundation Trust) supports a universal “once per six months” testing frequency for vitamin D, whether that be for symptomatic individuals that test negative at their previous test or for those who tested positive and begin supplementation. This results in combining CC1 and CC3 into a single CC that now reads: “1) For individuals with an underlying disease or condition which is specifically associated with vitamin D deficiency or decreased bone density (see Note 1), for individuals suspected of hypervitaminosis of vitamin D, or for individuals with vitamin D deficiency, 25-hydroxyvitamin D serum testing (no more than one test every six months) MEETS COVERAGE CRITERIA.” For clarity, Note 1, letter N, added “chemotherapy” as a medication known to lower vitamin D levels. Committee Approved: 06/17/2026