

Position Statement

Subject: **Use and Interpretation of Vitamin D testing**
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Introduction

The purpose of this Position Statement is to clarify the role of vitamin D testing in the context of diagnosing and monitoring vitamin D deficiency states and their treatment.

Summary of Evidence-based Recommendations

- The following describe indications for initial testing of vitamin D status for adult patients:
 - Signs, symptoms and/or planned treatment of osteoporosis or osteomalacia
 - Increased alkaline phosphatase with otherwise normal liver function tests
 - Hyperparathyroidism, hypo- or hypercalcaemia, hypophosphataemia
 - Malabsorption (e.g. cystic fibrosis, short bowel syndrome, inflammatory bowel disease, untreated coeliac disease, bariatric surgery)
 - Deeply pigmented skin, or chronic and severe lack of sun exposure for cultural, medical, occupational or residential reasons
 - Medications known to decrease 25OH-D levels (mainly anticonvulsants)
 - Chronic renal failure and renal transplant recipients
- In addition, the following are indications for testing of children:
 - Signs, symptoms and/or planned treatment of rickets
 - Infants of mothers with established vitamin D deficiency
 - Exclusively breastfed babies in combination with at least one other risk factor
 - Siblings of infants or children with vitamin D deficiency
- Vitamin D should be measured as 25-hydroxyvitamin D (25OH-D) and reported in nmol/L units.
- Clinicians should be aware that current vitamin D assay methods, may give different results and should be aware of the limitations of the method used in the laboratory that most of their patients attend. It is advisable to monitor serum 25OH-D levels following treatment in the same laboratory.
- A vitamin D assay that measures only the 25OH-D₃ form is sufficient for use in Australia and New Zealand.
- The target level should be greater than 50 nmol/L at the end of winter. Each laboratory should be encouraged to report the same decision limits.
- Testing for 25OH-D should be selective and high dose annual replacement of cholecalciferol should be discouraged.
- Serum 25OH-D levels should be retested no earlier than 3 months following commencement of supplementation with vitamin D or change in dose. Once a desirable target has been achieved, especially by the end of winter, no further testing is required unless risk factors change.
- In individuals at risk of vitamin D deficiency, measurement of 25OH-D is an appropriate, case-finding strategy. Routine screening of healthy infants, children and adults (including pregnant women) for vitamin D deficiency is currently not recommended.

1. Indications for assessment of vitamin D status

1.1. Who should be tested?

The quality of the evidence for the health benefits of an adequate vitamin D status is highly variable. As the main source of vitamin D is UVB sunlight exposure, vitamin D status as assessed by the measurement of 25 hydroxyvitamin D (25OH-D) is correlated with time spent outdoors, exercise and other aspects of a healthy lifestyle including body weight. Vitamin D insufficiency is associated with low levels of exercise, obesity and/or reduced sun light exposure, such as occur more commonly in the elderly, the overweight, the frail and unwell or institutionalised and where there are occupational, racial or cultural reasons.^{1,2} The recommendations below are broadly consistent with recent guidelines on the diagnosis and management of vitamin D deficiency.³⁻⁵

Recommendation:

~In individuals at risk of vitamin D deficiency, measurement of 25OH-D is an appropriate, case-finding strategy.

~Routine screening of adults (including pregnant women), healthy infants and children for vitamin D deficiency is not currently recommended.

1.2. Health benefits for adults from vitamin D supplementation

Significant health benefits from vitamin D and calcium supplementation have been demonstrated in the elderly. Randomised controlled trials (RCTs) of vitamin D plus calcium demonstrate a modest but significant decrease in fracture risk in the elderly^{3,6-9} although there are insufficient data to conclude whether vitamin D alone is effective.⁸ Supplementation of vitamin D plus calcium has achieved an 8% reduction in risk of premature mortality in largely institutionalised frail elderly.¹⁰ Inclusion of younger, community living subjects reduces the risk to non-significant levels ($P < 0.08$).¹¹ RCTs demonstrate that vitamin D and calcium supplementation decrease the risk of falls in the elderly.¹² Vitamin D supplementation improves proximal muscle strength in people with moderate vitamin D deficiency (serum 25OH-D levels < 25 nmol/L).¹³ High dose vitamin D supplementation is not recommended as there is evidence that annual bolus dosing of 500,000 IU vitamin D increases the risks of falls and fractures.¹⁴

Patients with chronic kidney disease (CKD) demonstrate an association between low serum 25OH-D levels and premature mortality.¹⁵ Current evidence is lacking to demonstrate that supplementation reduces this risk in these patients. Guidelines for management of CKD bone mineral disorder state that in patients with CKD stages 3-5D, measurement of 25OH-D may be appropriate and treatment is the same as for the general population.¹⁶

Current data do not provide consistent evidence for the benefit of vitamin D supplementation in the treatment or prevention of other disease states including cancer, cardiovascular disease, infection, autoimmune diseases, diabetes, multiple sclerosis or Alzheimer's disease. Currently, RCTs are underway in North America, Europe, New Zealand and Australia which should provide the required evidence.¹⁷ The first results from these trials are likely to be available in 2015.

Pregnancy

Severe maternal vitamin D deficiency has been associated with congenital rickets, and fractures in the newborn.⁴ However, recent guidelines by the American College of Obstetricians and Gynecologists conclude that there is insufficient evidence to support screening all pregnant women for vitamin D deficiency.⁵

Recommendations:

The following are indications for initial testing of vitamin D status for adult patients:

- ***Signs, symptoms and/or planned treatment of osteoporosis or osteomalacia***
- ***Increased alkaline phosphatase with otherwise normal liver function tests***
- ***Hyperparathyroidism, hypo- or hypercalcaemia or hypophosphataemia***

- **Malabsorption (e.g. cystic fibrosis, short bowel syndrome, inflammatory bowel disease, untreated coeliac disease, bariatric surgery)**
- **Deeply pigmented skin, or chronic and severe lack of sun exposure for cultural, medical, occupational or residential reasons**
- **Medications known to decrease 25OH-D levels (mainly anticonvulsants)**
- **Chronic renal failure and renal transplant recipients**

1.3. Paediatric patient groups

Guidelines for paediatric age groups have been published by the Australian and New Zealand Bone and Mineral Society and Osteoporosis Australia, and endorsed by the Australasian Paediatric Endocrine Group.⁴ The indications described above may also be relevant for children. Additional specific indications for testing for paediatric patients include:

- Signs, symptoms and/or planned treatment of rickets
- Infants of mothers with established vitamin D deficiency
- Exclusively breastfed babies in combination with at least one other risk factor
- Siblings of infants or children with vitamin D deficiency

2. Measurement of vitamin D

2.1. What should we measure as a marker of vitamin D status and what units should be quoted?

Serum 25-hydroxyvitamin D (25OH-D) is the best marker for clinical assessment of an individual's vitamin D status. 25OH-D has a long half-life of 2-3 weeks¹⁸ and provides a measure of the vitamin D derived from both dermal production and gut absorption from dietary or supplemental sources.¹⁹ 25OH-D levels, but not levels of the active circulating metabolite, 1,25(OH)₂-D, are associated with fracture risk and falls.

Serum 1,25(OH)₂-D should not be used to determine a person's vitamin D status. Serum 1,25(OH)₂-D measurements are rarely performed in clinical practice and should be reserved for investigation of special conditions by specialists.

The use of different units in reports is confusing for laboratory users. In Australia and New Zealand, nmol/L is used and is recommended, but ng/mL is commonly used overseas and users should be aware of this when referring to international guidelines.

Recommendation:

Vitamin D should be measured as 25-hydroxyvitamin D (25OH-D) and reported in nmol/L units.

2.2. Performance of current serum 25OH-D assays

There are differences in 25OH-D levels using different vitamin D assays and despite international efforts to standardise assays, this problem persists.

The two main types of methods available in clinical laboratories for measuring serum 25OH-D:

- *Liquid chromatographic separation followed by tandem mass spectrometric detection (LC-MS/MS) method*

This method has the highest sensitivity and specificity for measurement of 25OH-D and can quantify 25OH-D₂ and 25OH-D₃ separately. The high cost of equipment and limited sample throughput has constrained its widespread use in clinical laboratories. Results from different LC-MS/MS methods can be variable and may be due to lack of standardisation in calibration and differences in sample preparation.^{20,21} In addition, some LC-MS/MS methods for 25OH-D suffer from interference from C-3 epimeric forms of 25OH-D, a metabolite of uncertain clinical significance which is present in low concentrations in adult human serum specimens,²² but more commonly seen in significant amounts in neonatal serum.²³

- *Automated commercial immunoassays*

Currently, most laboratories in Australia and New Zealand use automated immunoassays for routine clinical analysis. The most commonly used automatic immunoassays in Australia for 25OH-D measurement are from Abbott Diagnostics, DiaSorin, Roche Diagnostics and Siemens Healthcare Diagnostics.

Results generated by current immunoassay methods for 25OH-D are highly variable²⁴ and similar disparity is noted between methods to that obtained prior to immunoassay modifications in 2011.²⁵ Analytical precisions also vary among different assays but most methods can achieve a coefficient variation (CV) of less than 8% at 25OH-D levels about 40 nmol/L. Thus, changes of less than 20% in two results from the same patient over time may not be significant.

Recommendation:

Clinicians should be aware that current vitamin D assay methods, may give different results and should understand the limitations of the method used in the laboratory that most of their patients attend. It is advisable to monitor serum 25OH-D levels following treatment in the same laboratory.

2.3. Is it important to have a 25OH-D assay with equal molar cross reactivity with 25OH-D₂ and 25OH-D₃?

Serum 25OH-D in blood can have two isoforms: 25OH-D₂ and 25OH-D₃. In people not taking ergocalciferol (vitamin D₂) supplements, the concentration of serum 25OH-D₂ is negligible as very few foods contain significant amounts of vitamin D₂. In Australia and New Zealand, the measurement of 25OH-D₂ is less important as almost all vitamin D supplements available are in the form of cholecalciferol (vitamin D₃).

Recommendation:

A vitamin D assay that measures only 25OH-D₃ is adequate for use in Australia and New Zealand.

3. Interpretation of results

What decision limit for 25OH-D should be quoted?

No RCT studies have been performed to help determine the ideal target level of 25OH-D. Consequently, surrogate measures based on observational data have been used to determine optimal levels of 25OH-D. Surrogate markers using parathyroid hormone (PTH), and fracture risk have been largely consistent. All of the following studies support a 25OH-D decision limit of 50 nmol/L:

- An initial target threshold of 50 nmol/L was proposed using data from cholecalciferol supplemented individuals and defined optimal levels as determined by a change in parathyroid hormone. A pretreatment 25OH-D of less than 50 nmol/L was associated with a reduction in PTH with cholecalciferol therapy.²⁶
- In the placebo arm of a large intervention study where all participants received calcium and cholecalciferol therapy, PTH changed significantly after 3 months' treatment but only in individuals with a pre-treatment 25OH-D below 50 nmol/L.²⁷
- An observational study of 1200 community dwelling men over the age of 65 years showed higher rates of hip bone loss are associated with 25OH-D values below 49 nmol/L.²⁸
- A nested observational case-controlled study of 400 women with hip fracture matched for age and race with 400 women without fracture showed hip fracture cases were more common in the lowest quartile equating to a 25OH-D of less than 47.5 nmol/L.²⁹
- A recent meta-analysis of 11 double-blind RCTs of oral vitamin D supplementation concluded that non-vertebral fracture risk was reduced in those with a baseline 25OH-D of 43-60 nmol/L compared with a baseline 25OH-D of less than 30 nmol/L.⁸

Two recent statements produced by the Institute of Medicine (IOM) and a joint statement from Australia and New Zealand Bone and Mineral Society, Endocrine Society of Australia and Osteoporosis Australia have confirmed the target threshold of treatment is 50 nmol/L.^{3,30} The IOM

statement is arguably the most comprehensive assessment to date and the ANZBMS/ESA/OA position statement has been endorsed by three major Australian societies.

A number of researchers contend that the desirable concentration of 25OH-D is equal to or greater than 75 nmol/L.³¹ This higher target level for 25OH-D has been proposed based on intestinal calcium absorption, bone mineral density change or osteoid volume with cadaveric bone biopsy samples.³²⁻³⁴ All of these studies have been controversial as the data are inconsistent, methodological differences in 25OH-D measurement are not accounted for in each study and the relationship between each of the latter surrogate measurements and 25OH-D is non-linear.

25OH-D levels decline in winter time so seasonal effects should be considered when interpreting measured levels.

Recommendation:

The target level should be greater than 50 nmol/L at the end of winter. Each laboratory should be encouraged to report the same decision limits.

4. Is testing always beneficial?

There is good evidence that 25OH-D levels vary with the season and inappropriate testing and especially testing using higher decision limits could lead to medicalisation of physiological changes in vitamin D metabolism.

Testing of healthy individuals will reveal a significant subgroup of healthy individuals with low 25OH-D levels. This will lead to treatment and perpetuates repeat testing without information as to whether such patients will benefit from vitamin D supplementation.

The majority of studies of daily vitamin D replacement have demonstrated beneficial effects on reduction in the rate of falls and, with calcium, a reduction in the rate of fractures. However, two high dose annual dosing studies have been published with significant results demonstrating harm (increased falls and fractures) with this type of replacement.^{14,35}

Both high and low 25OH-D levels have been associated with higher risk of overall mortality as well as cancer related mortality indicating a U shaped mortality risk curve.^{36,37}

Recommendation:

Testing for 25OH-D should be selective and high dose annual replacement of cholecalciferol should be discouraged.

5. Frequency of retesting following commencement of vitamin D supplementation

There is general agreement that the efficacy of vitamin D supplementation should be assessed following commencement of vitamin D supplementation³ as the concentration of serum 25OH-D achieved on any particular regimen varies markedly amongst individuals. Such variation in serum 25OH-D arises from a variety of factors including body fat mass, decreased clearance from interaction with dietary calcium or increased clearance due to certain disease states such as hyperparathyroidism.³⁸ The serum half-life of 25OH-D is of the order of 2-3 weeks.¹⁸ Based on well described principles of pharmacokinetics, little information regarding steady-state serum levels can be obtained from testing before 4 or 5 half-lives have passed.

Risk factors for vitamin D deficiency (age, activity levels, body weight, etc) may increase over time, so under certain circumstances repeat testing may be necessary, preferably at the end of winter.

Recommendation:

Serum 25OH-D levels should be retested no earlier than 3 months following commencement of supplementation with vitamin D or change in dose. Once a desirable 25OH-D target level has been achieved, especially by the end of winter, no further testing is required unless risk factors change.

This College Position Statement was developed by a Working Party consisting of Michael Harrison, James Davidson, Zhong Lu, Howard Morris, Hans Schneider and Paul Glendenning

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