

Vitamin D (serum, plasma)

1 Name and description of analyte

1.1 Name of analyte
Vitamin D

1.2 Alternative names
The term 'vitamin D' covers a group of closely-related naturally-occurring lipid-soluble compounds with similar biological functions, of which the two most important are ergocalciferol (vitamin D₂) and colecalciferol (vitamin D₃) (formerly 'cholecalciferol'). Vitamin D status is assessed by measuring the major storage form, 25-hydroxycole(ergo)calciferol.

1.3 NMLC code

1.4 Description of analyte
Vitamin D is traditionally thought of as a micronutrient but can also be classified as a prohormone. Colecalciferol is synthesized in the skin of humans and other animals by the effect of ultraviolet B radiation (290–315 nm) on 7-dehydrocholesterol (a process that becomes less effective with aging). It circulates in the bloodstream and rapidly undergoes 25-hydroxylation in the liver. Subsequent 1-hydroxylation in kidney results in the production of the active metabolite, the hormone 1,25-dihydroxycolecalciferol (calcitriol) (see 1.4). Both colecalciferol and the plant equivalent ergocalciferol may also be found in the diet: the best sources include oily fish and eggs. Vitamin D supplements, which may also contain calcium, are widely available. In the absence of specific supplementation, the major source of vitamin D is endogenous synthesis.

1.5 Function of analyte
The best-known effects of vitamin D are on calcium metabolism. Calcitriol promotes calcium (and phosphate) absorption from the gastrointestinal tract and reabsorption of filtered calcium in the renal tubules, thus increasing the availability of calcium for deposition in bone matrix. Deficiency is therefore associated with reduced bone density (osteoporosis). Severe vitamin D deficiency leads to rickets in childhood and osteomalacia in adults. These are uncommon, but being increasingly diagnosed, particularly in at-risk groups. Predisposition to osteoporosis is a more frequent consequence of long term insufficiency. Calcitriol is also required for the uptake of calcium into skeletal muscle, where it has an important role in contraction; and for the uptake of phosphate. Muscle weakness often an impressive feature of deficiency.

In addition to its use in the prevention and treatment of vitamin D deficiency, vitamin D (or more usually one of its 1-hydroxylated derivatives, e.g. alfalcidol) is used pharmacologically in some conditions where calcium metabolism is disturbed, e.g. hypoparathyroidism (autoimmune or surgical), chronic kidney disease (CKD), and certain rare types of rickets that are caused by genetic defects rather than malnutrition. (In CKD, decreased urinary phosphate excretion leads to phosphate retention and inhibition of 1 α -hydroxylation of 25-hydroxycolecalciferol, which in turns leads to hypocalcaemia, secondary

hyperparathyroidism and renal osteodystrophy. In advanced CKD, loss of renal substance reduces the capacity of the kidneys to hydroxylate 25-OHcolecalciferol.)

The actions of vitamin D are not confined to these well-known functions: the receptor for vitamin D has been found in many other cells and tissues including macrophages, pancreatic islets and skin. There is epidemiological evidence linking vitamin D deficiency to cardiovascular disease, some types of cancer and autoimmune disease, and a wealth of literature on these 'new' associations, but so far convincing demonstration of cause and effect is lacking.

2 Sample requirements and precautions

2.1 Medium in which measured
Serum or plasma

2.2 Precautions re sampling, handling etc.
Many laboratories recommend plain tubes rather than those containing serum-separator gels because of a theoretical risk of interference by the gel, though the evidence for this is limited.

3 Summary of clinical uses and limitations of measurements

3.1 Uses
1. Detection of vitamin D deficiency (and assessment of response to supplementation)
2. Detection of vitamin D excess

3.2 Limitations
The concentrations of vitamin D that indicate sufficiency or deficiency are not well defined (see 5.1). This is partly because in the past assays have been poorly standardized, meaning that values from different assays have not been directly comparable.

4 Analytical considerations

4.1 Analytical methods
1. Immunoassays
These are popular because they are cheap, quick and easily automated. However, it is important that both 25-hydroxycolecalciferol and 25-hydroxyergocalciferol are recognised, as exogenous ergocalciferol is an important contributor to vitamin D status in some people, particularly those receiving vitamin D supplementation.
2. Liquid chromatography-mass spectrometry (LCMS)
This is a more labour-intensive technique but has the advantage of allowing the different forms of vitamin D to be separated and measured simultaneously.
3. Competitive protein binding assays using vitamin D-binding protein are now obsolete.

1,25 dihydroxycolecalciferol can also be measured but this is rarely required in practice.

- 4.2 Reference method
Isotope dilution liquid chromatography-tandem mass spectrometry.
- 4.3 Reference materials
Standard Reference Material (SRM) 972, Vitamin D in Human Serum, supplied by the National Institute of Standards and Technology, Washington DC, USA. This consists of four separate serum pools containing different certified concentrations of both 25-hydroxycolecalciferol and 25-hydroxyergocalciferol.
- 4.4 Interfering substances
Grossly haemolysed, lipaemic or icteric samples should not be analysed.
- 4.5 Sources of error
Vitamin D is a challenging analyte for various reasons. Firstly, it is highly protein-bound to vitamin D binding protein and albumin in serum, and therefore requires an initial extraction or dissociation step. Second, in the free form it is lipophilic and the assay is subject to matrix effects whereby compounds such as lipids, present in some patient samples but not in standards, interfere with binding. Finally, there is the problem of recognition of the various forms of vitamin D. This refers not just to colecalciferol, ergocalciferol and their various hydroxylated forms but also to C-3 epimers, which may have different efficacies.

5 Reference intervals and variance

- 5.1.1 Reference interval (adults)
Existing recommendations vary in the proposed value and precise wording (see sections 9.2 and 9.3), but in general 50–75 nmol/L is suggested as the lowest value compatible with adequate vitamin D status. Toxicity is rare, but values >150 nmol/L should probably not be exceeded.
- 5.1.2 Reference intervals (others)
No established differences
- 5.1.3 Extent of variation
- 5.1.3.1 Interindividual CV: 40.3%
- 5.1.3.2 Intraindividual CV: 12.1%
- 5.1.3.3 Index of individuality: 0.3
- 5.1.3.4 CV of method
Both immunoassay and chromatographic methods can achieve CVs of 10% or less.
- 5.1.3.5 Critical difference: 44% (if method CV 10%)
- 5.1.4 Sources of variation
Sunlight exposure (amount of skin exposed, time for which exposed, latitude, time of day and year), vitamin D intake, race (lower in black people than white people, thus more exposure required for synthesis of given amount).

6 Clinical uses of measurement and interpretation of results

- 6.1 Indications for measurement
1. Suspected malnutrition (especially if steatorrhoea present).
 2. Clinical or radiological evidence of osteoporosis, osteomalacia or rickets.

3. Investigation of hypo- and hypercalcaemia.

- 6.2 Confounding factors
None: low values reliably indicate insufficiency and high values, excess.

7 Causes of abnormal results

7.1 High values

7.1.1 Causes: excessive intake is the only cause.

7.1.2 Investigation

Serum calcium should be measured, although hypercalcaemia due to vitamin D intoxication is rare. The risk of renal stones due to hypercalciuria should be considered.

7.2 Low values

- 7.2.1 1. Insufficient dietary intake and/or sunlight exposure
2. Malabsorption

7.2.2 Investigation of low values

Baseline serum calcium and phosphate concentrations, and alkaline phosphatase activity, should be checked before vitamin D supplementation is instituted. PTH concentrations are elevated in ~60% of patients with vitamin D deficiency at diagnosis so measurements can be useful in monitoring the response to treatment. X-ray or bone densitometry may be performed to look for the presence of osteomalacia or osteoporosis, respectively. It is rarely necessary to confirm the presence of osteomalacia histologically.

- 7.3 Notes
None

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions

Not applicable: metabolic bone disease develops over months to years and cannot be diagnosed or predicted on the basis of a single vitamin D measurement.

9 Systematic reviews and guidelines

9.1 Systematic reviews

1. Lips P, Bouillon R, van Schoor NM *et al.* Reducing fracture risk with calcium and vitamin D. *Clin Endocrinol* 2010;73:277-285.

This review finds that the results of fracture prevention trials to date are inconsistent. Treatment in institutions has been more effective than in the community, perhaps because of low baseline levels of vitamin D and ensured compliance.

2. Kandula P, Dobre M, Schold JD *et al.* Vitamin D supplementation in chronic kidney disease: a systematic review and meta-analysis of observational studies and randomized controlled trials. *Clin J Am Soc Nephrol* 2011;6:50-62.

This concludes that whereas biochemical endpoints can be improved by vitamin D supplementation, there is much less evidence of efficacy in terms of clinical outcomes.

9.2 Guidelines

1. Holick MF, Binkley NC, Bischoff-Ferrari HA *et al.* Clinical Practice Guideline. Evaluation, treatment and prevention of Vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96:1911-1930.

This defines serum vitamin D < 50 nmol/L as 'deficiency' and 50–72.5 nmol/L as 'insufficiency'.

9.3 Recommendations

1. Dawson-Hughes B, Mithal A, Bonjour J-P *et al.* IOF position statement: vitamin D recommendations for older adults. *Osteoporos Int* 2010;21:1151-4.

This sets a target concentration of 75 nmol/L for 25-hydroxyvitamin D in people aged over 60.

2. Institute of Medicine (US) Committee to review dietary reference intakes for Vitamin D; Ross AC, Taylor CL, Yaktine AL, Del Valle HB (eds). Dietary reference intakes for calcium and Vitamin D. Washington DC, USA: The National Academies Press; 2011.

This states that a serum concentration of 50 nmol/L would meet the needs of the great majority of the (US) population, but notes that assays to date have been poorly standardized.

3. Rosen CJ. Clinical practice. Vitamin D insufficiency. *N Engl J Med* 2011;364:248-254.

4. Shoback D. Clinical practice. Hypoparathyroidism. *N Engl J Med* 2008;359:391-403.

These are two useful overviews of vitamin D in practice in physiological and pharmacological roles.

10 Links

10.1 Related analytes

Serum [calcium](#), [phosphate](#), alkaline phosphatase and parathyroid hormone.

10.2 Related tests

None

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