NATIONAL LABORATORY HANDBOOK

Laboratory Testing for Vitamin D Deficiency

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Person-centred, co-ordinated care

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Background
Vitamin D is obtained from cutaneous synthesis driven by UVB exposure and to a lesser extent from dietary intake. However, negligible skin synthesis occurs in Ireland during the extended winter months between October and March due to its latitude (51-55° North) so the entire population depends on getting an adequate dietary intake. At other times of the year, 15 minutes of sun exposure per day, when available, provides sufficient vitamin D synthesis assuming sunblock has not been applied and there is some uncovered skin. Apart from sunblock use (up to 95% lower synthesis with SPF 8), cutaneous synthesis also decreases with increasing age and darker skin colour. Hence, it is not surprising for many reasons that the entire Irish population is at risk of vitamin D deficiency.

Ergocalciferol (Vitamin D_2) is the precursor form found in plants. Cholecalciferol (Vitamin D_3) is synthesised in human and animal skins by the action of UVB rays in sunlight on 7-dehydrocholesterol, via an intermediary, pre-vitamin D_3.

Very few natural foods contain Vitamin D. Oily fish, including salmon, tuna, and mackerel, accumulate significant quantities of vitamin D_3 in their fatty stores. Seafood vitamin D is probably derived from plankton in the aquatic food chain rather than from synthesis in the fish itself. Likewise, cod liver oil (rather than cod fillet) is a good source of vitamin D_3. A few vegetables (wild mushrooms exposed to natural light, or irradiated button mushrooms) contain significant amounts of vitamin D_2. Dairy products (mainly those that are fortified) also contain vitamin D_3. Fortified foods, usually with added vitamin D_3, are now commonly available in the human food chain.

Ergocalciferol and Cholecalciferol are hydroxylated at the carbon-25 position in the liver to form 25(OH)D_2 or 25(OH)D_3 respectively, which are sometimes collectively referred to as 25(OH)D. 1α-hydroxylation then occurs in the proximal tubules of the kidney to produce the active vitamin calcitriol -1,25$(OH)_2D_2$ and 1,25$(OH)_2D_3$ (see Figure 1).

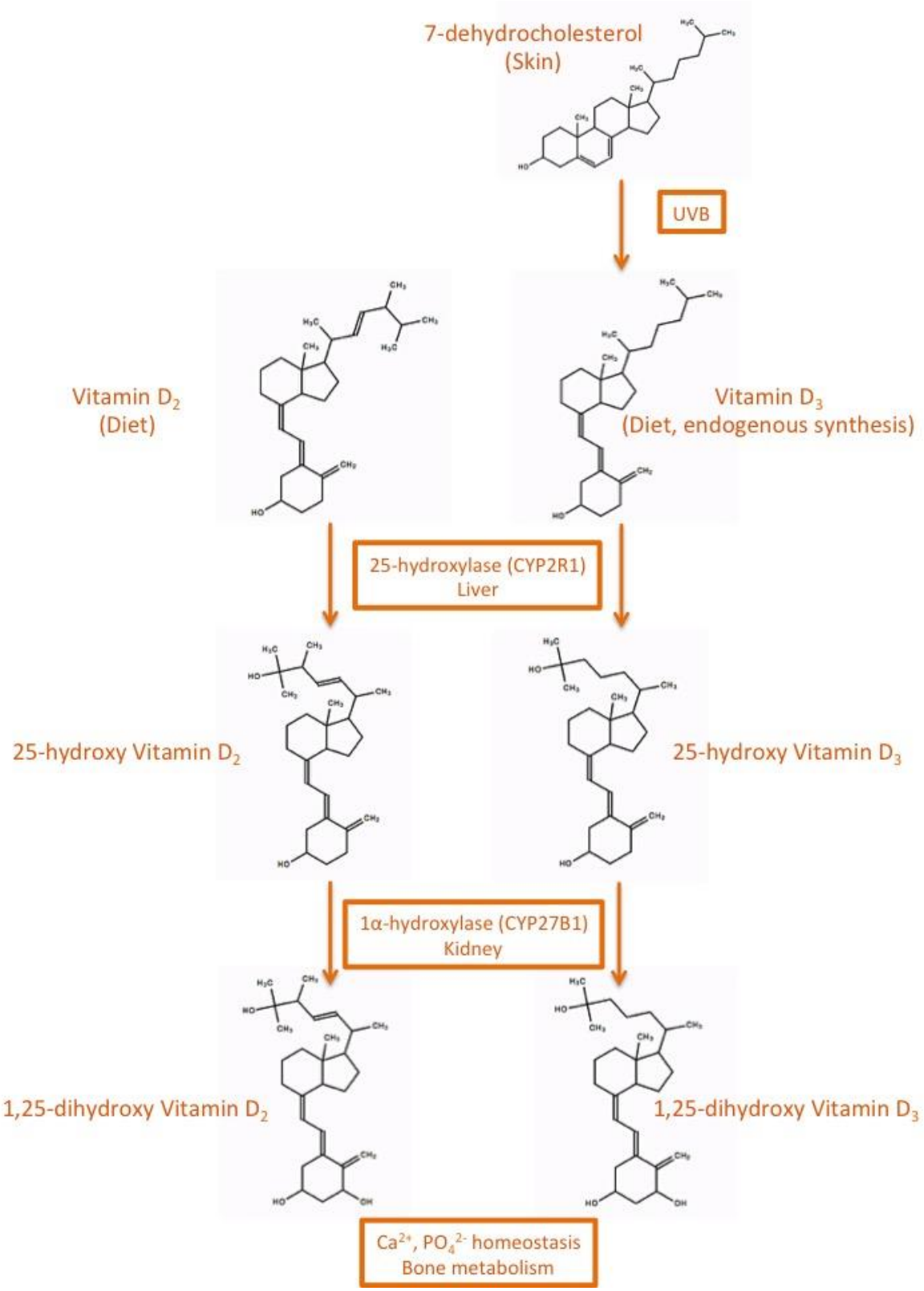

Figure 1: Chemical structures and metabolic pathways for various forms of Vitamin D.

Scope

The aim of this guideline is to provide prioritised indications for testing for vitamin D deficiency in primary care settings that can be used by clinicians and clinical laboratories, including circumstances where testing is not required. These guidelines apply to adult, non-pregnant patients only.

Key recommendations

Laboratory testing for vitamin D deficiency should be reserved for specific patient groups with indications for testing as described below. Laboratory testing for vitamin D deficiency is not recommended as a general “screening” test. Instead, nutritional assessment including dietary fortification when required is the recommended strategy.

Epidemiology

Studies in Ireland have indicated that the year-round prevalence rates for serum 25(OH) D concentrations in 1132 healthy Irish adults below 30, 40, 50, and 75 nmol/L were 6.7%, 21.9%, 40.1% and 75.6% respectively[1].A larger European study of 55,844 healthy individuals who were drawn from all latitudes found an even higher prevalence, with 13% of study participants having vitamin D levels below 30 nmol/L on average in the year[2]. Using an alternate definition of vitamin D deficiency (below 50 nmol/L), half of the European population would be regarded as deficient if this study’s findings were extrapolated.

It should be noted that the blood level of vitamin D that is considered adequate remains controversial and there is no direct correlation between low level of vitamin D and the presence of clinical findings such as osteomalacia or proximal myopathy, or with biochemical changes (hypocalcaemia, hypophosphataemia, elevated parathyroid hormone, or elevated alkaline phosphatase).

Testing

Who to Test (Indications for Testing)

Given the widespread prevalence of vitamin D deficiency and the poor correlation between the laboratory finding of hypovitaminosis D and clinical consequences, a system that prioritises patients who are most likely to benefit from treatment with vitamin D containing supplements is helpful in deciding whom to test[4,5].Hence, two groups are identified consisting of patients with metabolic bone disorders (Group 1) and patients with other relevant conditions that may be due to or could lead to vitamin D deficiency (Group 2).

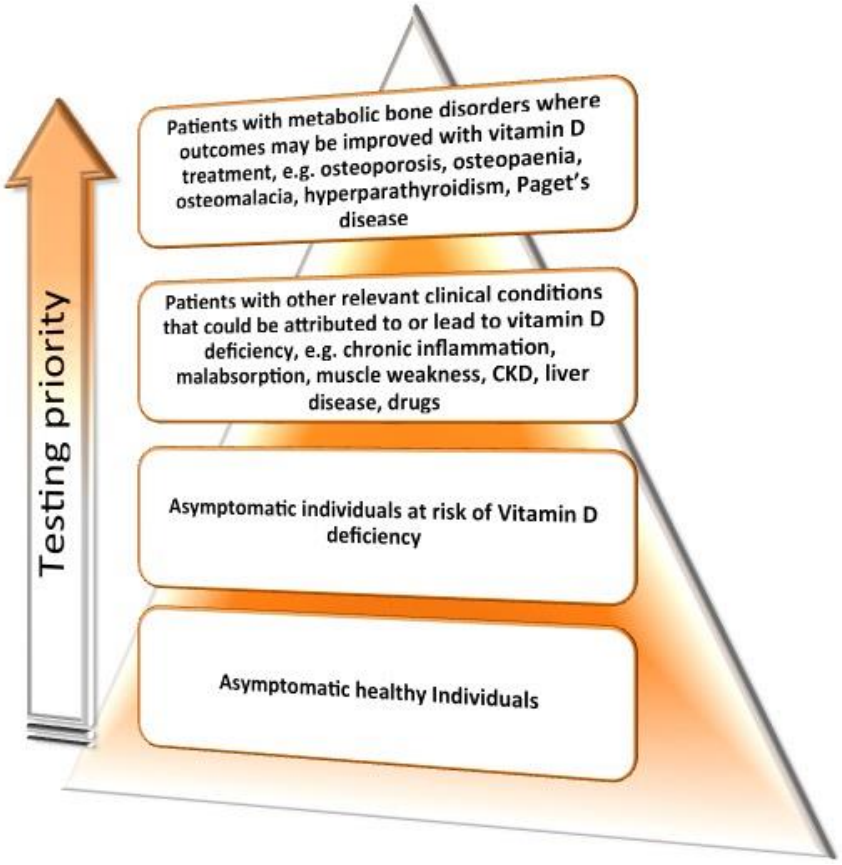

Figure 2: Schematic representation that helps to define broad groups for clinical consultation and testing.

Priority for Testing	Description of Group	Examples
Group 1: Very High	**Patients with metabolic bone disorders (see list) where outcomes may be improved with vitamin D treatment**	**Metabolic Bone Disorders** • Osteoporosis, osteopaenia, low bone density • Patients about to commence anti-resorptive medications for osteoporosis ○ *Note: Denosumab- calcium levels (NOT vitamin D level) are required before each injection* • Rickets or osteomalacia • Hyperparathyroidism (any type) • Paget's disease or other metabolic bone diseases • Low trauma/ pathological fractures • Unexplained hypocalcaemia, hypophosphataemia, hyperphosphatasaemia

Group 2: High	**Patients with other relevant clinical conditions (see list) that could be attributed to or lead to vitamin D deficiency**	**Other Relevant Clinical Conditions**
		• Proximal Myopathy or clinically significant muscle weakness (i.e. difficulty climbing stairs, waddling gait, difficulty rising from chair)
		• History of Falls in older adults
		• Malabsorption due to any cause (e.g. coeliac disease, inflammatory bowel diseases, short bowel syndrome, chronic pancreatitis, bariatric surgery, cystic fibrosis)
		• Chronic Kidney Disease
		• Hepatic Failure
		• Chronic inflammatory or granulomatous disorders (e.g. rheumatoid arthritis, sarcoidosis, TB)
		• Relevant drugs
		  ○ Glucocorticoids
		  ○ Anticonvulsants
		  ○ Antioestrogens
		  ○ Antiretrovirals
		  ○ Antifungals (e.g. ketoconazole)
		  ○ Cholestyramine

Group 3: Low	**Asymptomatic individuals at risk of Vitamin D deficiency**	**Screening is not recommended.** Dietary Advice with food fortification as required and combined with healthy daylight exposure advice is the preferred strategy for this large group (e.g. the entire Irish population in Winter)
Group 4: Very Low	**Asymptomatic Healthy Individuals**	**Screening is not recommended.**

Who to Test (Re-Testing)

Routine repeat Vitamin D testing IS NOT REQUIRED

Note: Check ADJUSTED SERUM CALCIUM 4 weeks after treating with loading doses of Vitamin D. Vitamin D repletion may unmask primary hyperparathyroidism.

Who Not to Test

Testing or "Screening" is not recommended in asymptomatic individuals at risk of Vitamin D deficiency or in healthy individuals (Groups 3 and 4).

For the population at large and in the absence of the above indications for Vitamin D testing, widely available nutritional advice prompting dietary improvements and information on healthy daylight exposure should be offered. This could include advice on use of fortified foods if required. This approach is also well established internationally for certain at-risk subgroups, including those of older age or with darker skin, as well as those with common risk factors such as unhealthy diet, no sun exposure or obesity[4,5].

Vitamin D testing is also **not recommended** in the following circumstances:

- Do not include in "Routine Bloods", health-screening requests, or other forms of screening e.g. for "tiredness" / "tired all the time"
- General screening of any patient subgroups, e.g. nursing home patients, cardiovascular risk factor assessment.
- Routine repeat Vitamin D testing IS NOT REQUIRED

How to Test

A completed standard laboratory test request form must be sent with all samples.

Information required on the referral form

The request form must include detailed patient and clinical information including:

- **Patient demographics**
  - Patient's Name
  - Patient's Date of Birth
  - Medical Record Number
  - Name of Referring Clinician
  - Name of Referring Hospital
  - Order number / external laboratory number (if applicable to external agencies only).

- **Request details**
  - Clinical indication for testing (see list above)
  - Details of any calcium or vitamin D supplements
  - Details of any medications for osteoporosis or other metabolic bone disorder.

Requests received with no clinical details or with inadequate patient demographics will not be analysed.

Full clinical information should accompany all requests. In the event a request is received which does not have the required data (above) or does not have adequate clinical details the laboratory could:

- Issue a report to the requesting doctor, requesting additional clinical details and/or advising that the case be discussed with the local Laboratory Medicine Consultant, and advising that the sample will be discarded after 2 weeks if there is no reply.
- Store the sample for up to 2 weeks awaiting further communication from the referring clinician. Samples can be discarded after 2 weeks if the referring clinician has not provided the required details or if it is determined that testing is not indicated.

Interpretation of tests

Vitamin D assays should be interpreted in conjunction with the adjusted calcium (i.e. total calcium corrected for albumin level), phosphate, creatinine and alkaline phosphatase levels, ideally on a concurrent sample.

25(OH)D blood levels are commonly measured in an increasing number of Irish laboratories by accredited methods such as Mass Spectrometry (MS) or Automated Immunoassay. It is currently one of the most expensive laboratory tests available.

Current assays are capable of detecting both 25(OH)D_2 and 25(OH)D_3, albeit to varying degrees. An epimer of 25(OH)D_3 has been described recently in both infant and adult populations. The physiological importance of 3-epi-25(OH)D(3) is uncertain and although present at low concentrations (approximately 2.5nmol/L), the presence of this compound may affect the reliability of 25(OH)D(3) measurement, especially when performed using MS techniques and certain immunoassays (Vitamin D Binding Protein [DBP] assays). Laboratories should be aware of this and indicate to clinicians which assay is used and the limitations of that assay in terms of 25(OH)D3 and epimer detection [12].

Measurement of 1,25$(OH)_2$D is not recommended and is not necessary except in special cases where the case must be discussed with your local laboratory medicine consultant. It is misleadingly normal or high in nutritional or malabsorption-related D deficiency.

As with all investigations, there are advantages and limitations of the test which you should discuss with your local laboratory service.

The finding of a low 25(OH)D level <30 nmol/L only indicates that the person is **at risk** of vitamin D deficiency which may have consequences for musculoskeletal health. There is no consensus at present on whether Vitamin D deficiency has clinically important effects on other conditions (e.g. cancer risk, multiple sclerosis, asthma) outside of the skeleton and musculoskeletal system, and there is likewise no consensus on sufficient levels to avoid these problems.

Trials of Vitamin D supplementation have consistently failed to demonstrate any improvements of patient outcomes other than for musculoskeletal outcomes.

Occasionally the serum level can be misleading, given that Vitamin D is a fat-soluble vitamin, and may not always adequately reflect fat stores. Patients with acute inflammation may have

transiently lower levels, so that testing should be avoided in acutely ill or hospitalised patients.

Implications for ICT Systems

Computer Physician/Provider Order Entry Systems (CPOE) from a number of different suppliers are in use in a number of hospitals nationwide. Few ICT systems are capable of effectively integrating primary/community with secondary care facilities, though there are examples of "bridging" solutions such as Healthlink, which is used for laboratory test reporting and other applications (including possibly test ordering). At present in Ireland, there is no national electronic patient health record, and there is no agreed unique national health identifier. In order to make progress on appropriate utilisation of laboratory services in the interim, it is necessary to consider the laboratory test ordering modules that are currently available in these different settings.

It is also likely that paper laboratory request forms will continue (e.g. in primary care) until the provision of effective ICT systems improves. Improved forms in some cases may help to encourage better provision of relevant clinical information.

Laboratory Test Requesting Ordering Modules in Primary Care

It is recommended that a user-friendly general practitioner (GP) ordering system for Vitamin D is developed and implemented at the point of ordering in GP Information Systems. The information required for requesting Vitamin D is stated above. An intuitive user interface should be developed to allow the GP to select one or more of the relevant clinical indications and to indicate relevant drug therapy. This will require discussion with GP system suppliers.

Laboratory Test Requesting Modules in Hospital-based CPOE Systems

CPOE systems from a number of different suppliers are available in several hospitals nationwide. The national MedLIS will also provide an order-entry system. It is recommended that user interfaces for ordering Vitamin D are developed and implemented at the point of ordering, to allow the clinician to select one or more of the relevant clinical indications and to indicate relevant drug therapy. The information required for requesting Vitamin D is stated above.

National Laboratory Information System (MedLIS)

The recommendations given for Primary Care and Hospital-based CPOE systems would apply to circumstances where MedLIS will be providing the CPOE functionality (e.g. where its test ordering Module is implemented throughout the hospital)

A pre-laboratory Module in MedLIS should check for (1) absence of any clinical details; (2) repeat testing; (3) correct indication for testing provided, and generate an alert and an appropriate laboratory report as described above.

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Appendix: Quick Reference Card

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