THE RISE OF VITAMIN D TESTING

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INTRODUCTION

Vitamin D testing is in vogue. Increasing insight into the broad physiological role of vitamin D, combined with the knowledge of high rates of deficiency in many parts of the world, has propelled vitamin D analysis to the forefront in many laboratories. Alongside its classical role in bone health, vitamin D is also involved in many aspects of health and wellbeing, and deficiencies have been linked to conditions as diverse as autoimmune diseases, infections, cancers, cardiovascular disease and mental disorders. There are various methods available for measuring vitamin D levels in patient samples. This article focuses on a state-of-the-art ELISA, which is based on a unique monoclonal antibody providing fast and reliable measurement of vitamin D in both its forms: D_2 and D_3.

FORMS OF VITAMIN D

Vitamin D exists in two different forms. Vitamin D_2 (ergocalciferol) is contained in plant foods such as mushrooms and avocado and taken up into the body via consumption of these foodstuffs. Vitamin D_3 (cholecalciferol) is produced in skin exposed to ultraviolet B radiation or obtained from dietary sources such as sea fish, egg yolk and butter.

These two forms of vitamin D are not biologically active, they are bound by a vitamin D binding protein (VDBP) in the blood. In the liver they are metabolised and converted into the respective 25-hydroxy forms: 25-OH vitamin D_2 (calcidiol) and 25-OH vitamin D_3 (calcitriol) (see *figure 1*). These are storage forms with little biological activity. It is only through a further conversion step in the kidneys that the vitamin becomes the biologically active metabolite 1.25-dihydroxy vitamin D, which functions as the activating ligand for the vitamin D receptor. In this way the active hormone regulates the uptake of calcium from the intestinal tract, the mineralisation of bones, the differentiation of osteoblasts and bone matrix synthesis. It also has a range of other roles, for example in neuromuscular function and inflammatory responses.

SERIOUS GLOBAL HEALTH PROBLEM

Vitamin D deficiency is a worldwide problem with manifold health effects. Even a slight vitamin D deficiency is sufficient to cause a secondary increase in parathormone and an elevated osteolysis rate due to reduced calcium uptake. Severe vitamin D deficiency leads to rickets in children and osteomalacia in adults, characterised by defective bone growth and matrix mineralisation. Since the vitamin D concentration in persons over 50 years of age is significantly associated with bone density, vitamin D deficiency is also one of the most important risk factors for senile osteoporosis.

Furthermore, hypovitaminosis D is a risk factor for the development of numerous other diseases, among these many autoimmune diseases, for example multiple sclerosis, Crohn's disease, type I diabetes mellitus, systemic lupus erythematosus and rheumatoid arthritis. A vitamin D deficiency also increases the risk for infectious diseases such as respiratory tract infections and tuberculosis, as well as for arterial hypertonia, malignant tumours, cardiovascular and musculoskeletal diseases, aseptic bone necrosis, cognitive disorders, Parkinson's disease, dementia, atopic dermatitis, mental disorders and hypertriglyceridaemia in children.

INDICATIONS FOR TESTING

The main causes of vitamin D deficiency are insufficient exposure to UV light, for example due to a predominantly indoor lifestyle, excessive use of sunscreens, dark skin colour or complete covering of the skin with clothes, a vitamin D-deficient diet and medical conditions that affect the vitamin D supply of the body. Examples of the latter include decreased intestinal uptake of vitamin D due to fat malabsorption, biliary cirrhosis or exocrine pancreas insufficiency or increased metabolism of vitamin D due to drugs such as anticonvulsants. Primary hyperparathyroidism, nephritis syndrome, peritoneal dialysis and severe damage to liver parenchymal cells also interfere with vitamin D provision.

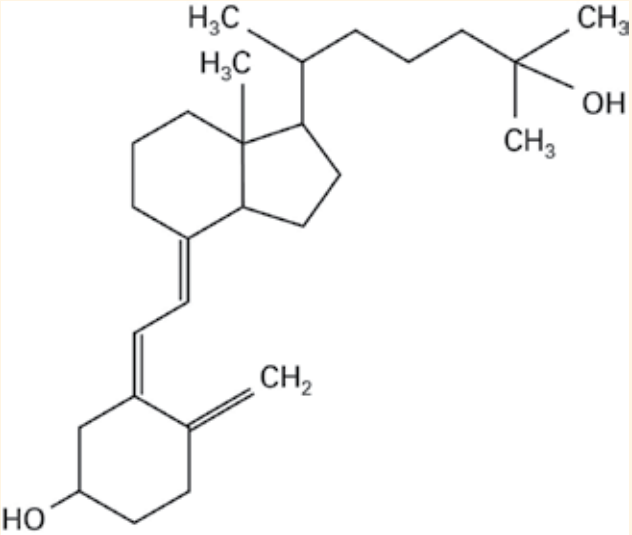
Hence, vitamin D measurement is indicated in a wide range of clinical circumstances. In particular, levels should be monitored in individuals with a suspected deficiency due to inadequate sun exposure or diet, as well as in patients with medical indications such as reduced intestinal vitamin D uptake, increased vitamin D metabolism, hypocalcaemia, hypophosphataemia, elevated alkaline phosphatase and decreased bone mineral content. Vitamin D determination is also indicated in cases of suspected vitamin D oversupply, for example in individuals with hypoparathyroidism and in patients who have been overdosed during therapy.

FAST AND RELIABLE DETERMINATION

The best indicator of the vitamin D supply in the body over the past months is the serum concentration of 25-OH vitamin D *(see figure 1)*. Other vitamin D metabolites such as 1.25-dihydroxy vitamin D are not recommended for determining patients' long-term vitamin D status. Various methods exist for determining 25-OH vitamin D levels in patient serum samples. Chromatography-based assays such as HPLC and LC-MS/MS provide the most accurate measurement, but are generally time-consuming and labour-intensive, requiring costly equipment and large sample volumes. Hence, in a routine setting, many laboratories now rely on immunoassays such as ELISA. Determination of 25-OH vitamin D by ELISA is suitable for both diagnosing a deficiency and for assessing the efficacy of treatment. Since either vitamin D_2 or D_3 may be used for treatment, the assay system employed must be equally specific for both forms.

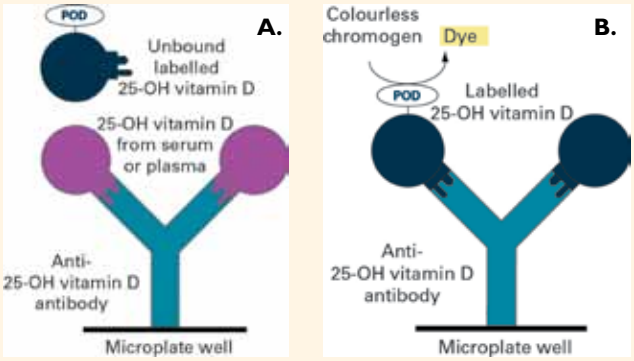
A new state-of-the-art 25-OH vitamin D ELISA *(see figure 2)* based on a novel anti-25-OH vitamin D monoclonal antibody is particularly well suited to routine clinical measurements. The specially designed antibody provides 100% specific detection of both 25-OH vitamin D_2 and 25-OH vitamin D_3. In the ELISA procedure *(see figure 3)*, patient serum or plasma samples are incubated in microplates coated with the monoclonal antibody. 25-OH vitamin D contained in the patient samples is released from its binding protein and captured by the antibody in one step. Free antibody binding sites are occupied by labelled 25-OH vitamin D, which is subsequently detected by means of a chromogenic reaction. The intensity of the colour, as measured photometrically, is inversely proportional to the 25-OH vitamin D concentration in the patient sample. The use of six calibrators with fixed concentrations ensures high accuracy and ➔

▼FIGURE 1: Chemical structure of 25-OH vitamin D_3



▼FIGURE 2: 25-OH Vitamin D ELISA

▼FIGURE 3: Principle of the ELISA **A.** Binding of vitamin D from sample ➡ no colour reaction **B.** No vitamin D present in sample ➡ colour reaction



reproducibility. The entire ELISA procedure takes less than three hours and can be fully automated. The assay system has been evaluated in various studies. It shows an excellent correlation with the reference methods HPLC *(see figure 4)* and LC-MS/MS *(see figure 5)* and with other commercially available test systems.

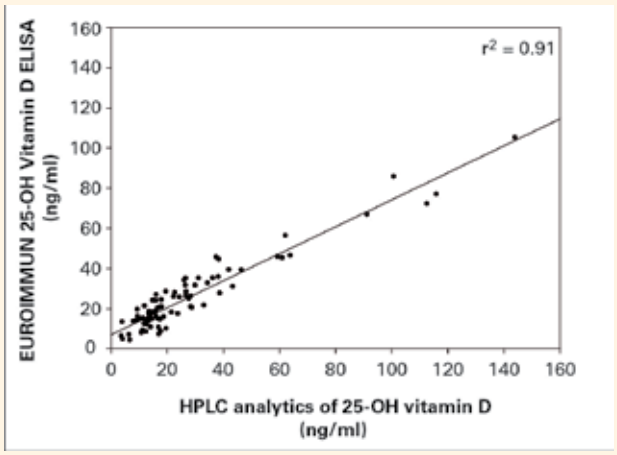
QUALITY ASSURANCE

An independent control of the reliability of 25-OH vitamin D analysis methods is provided by the Vitamin D External Quality Assessment Scheme (DEQAS). Diagnostic laboratories can reassure themselves of their proficiency in vitamin D analysis by regular participation in the analytical schemes based on their usual methodology. Notably, the 25-OH vitamin D ELISA described here has consistently performed well in DEQAS, achieving all performance targets since its introduction in April 2011. Thus, participation in DEQAS has substantiated the efficacy of this ELISA to deliver accurate and reliable results in vitamin D analysis.

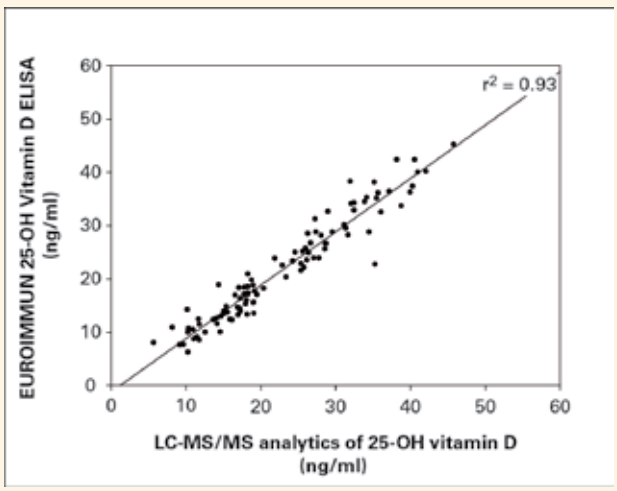
EFFECTIVE TREATMENT

Once a vitamin D deficiency has been diagnosed, it can generally be

▼FIGURE 4: Correlation of ELISA with HPLC



▼FIGURE 5: Correlation of ELISA with LC-MS/MS



successfully treated with oral vitamin D supplementation, which is given in the form of either vitamin D_2 or vitamin D_3. This treatment is particularly effective in preventing bone fractures, especially in the femoral neck and radius. Since the individual response of patients to medication varies greatly, a deficiency must sometimes be treated and monitored over long periods of time, sometimes for years or even decades.

PERSPECTIVES

The recognition of vitamin D deficiency as a highly prevalent and serious health issue is causing a surge in vitamin D testing that can only be met with fast, reliable and cost-effective automated analysis methods. ELISA technology fulfils these requirements and is increasingly employed for vitamin D determination in diagnosis and therapy monitoring. A new 25-OH Vitamin D ELISA based on a specially designed monoclonal antibody is particularly suitable for these applications as it measures both D_2 and D_3 forms of the vitamin with 100% reliability.

The extent of vitamin D under-provision in the population is highlighted by a study performed using this test. In a cohort of apparently healthy blood donors from Germany and the USA only 15% were found to have an optimal vitamin D level of 30 ng/ml or over. Numerous other studies in different countries have reported similarly worrying trends of general deficiency. Given the wide-ranging health consequences associated with vitamin D deficiency and the need to reliably identify affected persons, the growth in vitamin D testing is sure to continue.