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Verification of Abbott 25-OH-vitamin D assay on the architect system

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ABSTRACT

Objectives: Analytical and clinical verification of both old and new generations of the Abbott total 25-hydroxyvitamin D (25OHD) assays, and an examination of reference Intervals.
Methods: Determination of between-run precision, and Deming comparison between patient sample results for 25OHD on the Abbott Architect, DiaSorin Liaison and AB SCIEX API 4000 (LC-MS/MS). Establishment of uncertainty of measurement for 25OHD Architect methods using old and new generations of the reagents, and estimation of reference interval in healthy Irish population.
Results: For between-run precision the manufacturer claims 2.8% coefficients of variation (CVs) of 2.8% and 4.6% for their high and low controls, respectively. Our instrument showed CVs between 4% and 6.2% for all levels of the controls on both generations of the Abbott reagents. The between-run uncertainties were 0.28 and 0.36, with expanded uncertainties 0.87 and 0.98 for the old and the new generations of reagent, respectively. The difference between all methods used for patients' samples was within total allowable error, and the instruments produced clinically equivalent results. The results covered the medical decision points of 30, 40, 50 and 125 nmol/L. The reference interval for total 25OHD in our healthy Irish subjects was lower than recommended levels (24–111 nmol/L).
Conclusion: In a clinical laboratory Abbott 25OHD immunoassays are a useful, rapid and accurate method for measuring total 25OHD. The new generation of the assay was confirmed to be reliable, accurate, and a good indicator for 25OHD measurement. More study is needed to establish reference intervals that correctly represent the healthy population in Ireland.

1. Introduction

Vitamin D deficiency (VDD) is a widespread condition that is said to affect about 1 billion people worldwide [1]. Recent studies have shown that VDD is not only associated with bone and severe liver and kidney disease; it also has important implications in many chronic illnesses, including cancer, diabetes mellitus, hypertension and asthma [2]. Vitamin D is a fat-soluble steroid prohormone that is mainly produced photochemically in the skin from 7-dehydrocholesterol. Two forms of vitamin D are biologically important – vitamin D3 (Cholecalciferol) and vitamin D2 (Ergocalciferol).

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Both vitamins D3 and D2 can be absorbed from food and can be found in vitamin supplements, but it is estimated that only 10–20% of vitamin D is supplied through food [3]. Vitamin D is metabolised to the active hormone $1,25(OH)_2$-vitamin D (Calcitriol) through two hydroxylation reactions. The first of these occurs in the liver, converting vitamin D into 25-hydroxyvitamin D (25OHD). The second hydroxylation converts 25OHD into the biologically active $1,25(OH)_2$-vitamin D and occurs in the kidneys and in many other cells. Most cells express the vitamin D receptor (VDR) and about 3% of the human genome is regulated by the vitamin D endocrine system [3]. The vitamin D level is generally assessed by measuring the serum or plasma level of 25OHD. This has a circulating half-life of 2–3 weeks and is not influenced by changes in calcium and parathyroid hormone levels [4]. This major storage and circulating form is a reliable indicator of vitamin D status [5]. Low 25OHD concentrations are associated with secondary hyperparathyroidism, skeletal diseases such as rickets, and many chronic illnesses [2,6].

A panel from the Institute of Medicine (IOM) of the National Academy of Sciences decided that, on the basis of skeletal anomalies, VDD can be defined as a serum 25OHD below 50 nmol/L [7]. A newly proposed definition of vitamin D sufficiency (> 50 nmol/L) has proved to be controversial., [8,9] mainly because it has a major impact on the clinical evaluation of vitamin D insufficiency. There is no current consensus on the optimal vitamin D levels for non-musculoskeletal health; therefore it is important to establish reference intervals among the general population – specifically, in different seasons.

Vitamin D tests are now widely included as a part of routine laboratory work. A significant increase in laboratory testing for 25OHD has resulted in the development and the implementation of new automated diagnostic approaches to keep pace with the volume of demand. In our laboratory we have looked for a rapid, reliable, fully automated and cost saving assay that would improve turnaround time for our clients.

Numerous methods have been developed to measure serum and plasma 25OHD concentrations. The first routine methods for assessing 25OHD were based on competitive protein binding. These have been supplanted by radioimmunoassay (RIA) and by chemiluminescent immunoassay (CLIA), which form the fundamental principles of several commercially available methods. Two main analytical techniques usually employed in the laboratories are competitive immunoassays and chemical methods. Among the immunoassays are the following: RIA, CLIA, enzyme immunoassay, electrochemiluminescence immunoassay, chemiluminescent microparticle immunoassay (CMIA) and competitive protein binding assay. The chemical methods are based on chromatographic separation, followed by non-immunological direct detection. They include direct high performance liquid chromatographic with ultraviolet (HPLC-UV) detection, and liquid chromatography combined with mass spectrometry (LC-MS). The principal difference between these methods is the ability of HPLC and LC-MS to quantify 25(OH)D2 and 25(OH)D3 separately [10]. Recently a C3-epimeric form of 25(OH)D3 has been detected which is unresolved in some LC-MS/MS assays but may add to the total result of 25OHD. The epimer, however, has been found primarily in neonatal samples and it has been suggested that it does not contribute significantly to overall measured 25OHD concentration [11,12].

The Joint Committee for Traceability in Laboratory Medicine (JCTLM) recognizes isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) as the reference method for vitamin D. Mass spectrometry is highly sensitive and specific and can analyse several related analytes in a single run, with potential for cost savings. The introduction of commercial validated assay kits, traceable standards and participation in external quality assessment schemes has substantially improved the quality of LC-MS/MS assays for 25(OH)D3. However, initial capital purchase of these instruments can be costly, they require highly trained staff, and they are currently better suited to larger laboratories.

In recent years, in vitro diagnostic companies have introduced new automated immunoassays for the measurement of 25(OH) D3, thus improving the laboratory's ability to cope with increasing demand.

Many publications have outlined the limitations of these immunoassays. Some have poor antibody specificity with cross-reactivity to other metabolites of Vitamin D, as well as a problematic extraction of the 25OHD form from the vitamin D-binding protein (DBP). Some assays also interact with matrix substances such as lipids, and there are notable variations in 25OHD determination between different assays [13–16]. Until recently, standardisation and harmonisation between the various marketed vitamin D assays was poor. The introduction of a new traceable reference standard (National Institute of Standards and Technology Standard Reference material 972; NIST SRM 972), however, has improved both validation and calibration of 25OHD assays across different platforms.

In our laboratory, a Vitamin D assay was run and accredited to ISO 15189 on the DiaSorin Liaison instrument. When the CE-marked Abbott Architect 25OHD assay became available it had already been extensively validated by the manufacturers, so we did not feel that a full validation was indicated. In 2016 a new generation of the Abbott reagents came to the market, standardised to NIST SRM 2972.

The purpose of this study was: to confirm that the assay was performing in our laboratory to the manufacturer's standards; to establish any clinical difference between results obtained using the Liaison, Architect and LC-MS/MS methods; to calculate the uncertainty of measurement for the assay; to run External Quality Assessment samples to assess bias; and to compare reference interval study results with current 25OHD recommendations.

2. Materials and methods

2.1. Abbott Architect and DiaSorin Liaison Immunoassays

The original Architect (Abbott Diagnostics, Lake Forest, IL, USA) 25OH Vitamin D assay (product 3L52) is a delayed 1-step chemiluminescence microparticle immunoassay (CMIA) involving automated online pre-treatment with flexible assay protocols, which are known as Chemiflex. It uses microparticles coated with a polyclonal sheep anti-vitamin D IgG antibody, and a biotinylated