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An evaluation of automated methods for measurement of serum 25-hydroxyvitamin D

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Abstract

Objectives: To compare two new automated assays with the well-established reference method, DiaSorin radioimmunoassay (RIA), for quantitation of serum total 25-hydroxyvitamin D [25(OH)D].

Methods: 25(OH)D from human sera ($n=158$) was measured using DiaSorin RIA and two automated platforms, DiaSorin "LIAISON 25 OH Vitamin D TOTAL", and Roche Modular "Vitamin D3 (25-OH)". Methods were compared by regression and Bland–Altman analyses.

Results: DiaSorin LIAISON demonstrated a stronger correlation ($r=0.918$) and better agreement (bias=−0.88 nmol/L) with DiaSorin RIA than the Roche Modular assay ($r=0.871$, bias=−2.55 nmol/L). Precision ranges (CV%) for the RIA, LIAISON, and Roche Modular assays, respectively, were: within run (6.8–12.9%, 2.8–8.1%, and 1.9–5.5%), and total precision (7.4–14.5%, 7.3–17.5%, and 7.6–14.5%).

Conclusion: DiaSorin LIAISON displayed the best correlation and agreement with DiaSorin RIA. The DiaSorin LIAISON 25 OH Vitamin D TOTAL assay is an accurate and precise automated tool for serum total 25(OH)D determination.

Keywords: Vitamin D; 25-hydroxyvitamin D; 25(OH)D; Automation; RIA; LIAISON; Roche; Methods evaluation

Introduction

The most reliable indicator of vitamin D status is measurement of 25-hydroxyvitamin D [25(OH)D] in serum or plasma. 25(OH)D, the major circulating metabolite of vitamin D, is produced in the liver by a hydroxylation of vitamin D at carbon 25. Two distinct forms of 25(OH)D exist: 25(OH)D_3, formed from vitamin D_3 (cholecalciferol), and 25(OH)D_2, produced from vitamin D_2 (ergocalciferol). Vitamin D_3 is synthesized naturally in skin exposed to UV radiation and also found in fatty fish. Vitamin D_2 is generated by UV irradiation of the plant sterol, ergosterol, and is less potent than vitamin D_3 [1–3]. Low circulating 25(OH)D concentrations have been associated with increased risk and progression of several diseases, including osteoporosis [4,5], cancers [6–8], multiple sclerosis [9,10], and cardiovascular disease [11,12]. Such research into the role of vitamin D beyond calcium homeostasis has substantially increased clinical interest in vitamin D.

The measurement of 25(OH)D is challenging because circulating 25(OH)D is highly lipophilic, bound strongly to protein, present in low (nanomolar) concentrations, and exists in two structurally similar forms, 25(OH)D_3 and 25(OH)D_2 [13]. Several published methods exist for determining 25(OH)D concentrations, including competitive protein-binding assays, radioimmunoassay (RIA), high performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), and the more recent automated immunoassays. In 1989, the International External Quality Assessment Scheme for Vitamin D metabolites (DEQAS, Northwest Thames, United Kingdom) was established to monitor the analytical reliability of 25(OH)D assays [14]. However, several reports have

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demonstrated large inconsistency and variability in 25(OH)D measurements between methods and laboratories [15–17]. As a result, some groups have emphasized a need for appropriate reference materials and standardization of 25(OH)D assays [15,16].

The rising clinical demand for assessment of vitamin D status has increased the need for simple, high-throughput methods for measuring 25(OH)D in patient samples. Protein binding assays, HPLC, and LC-MS are manual methods that can be time and labour intensive, technique and operator dependent, and require costly equipment and large sample volumes. The DiaSorin RIA was the first vitamin D test approved for clinical diagnosis by the US Food and Drug Administration (FDA) and has been the most widely used method since. However, being a manual method, the RIA has been challenged by the rapidly increasing demand for 25(OH)D testing. Recently, automated chemiluminescence-based immunoassays have become available which offer higher-throughput capacity, lower sample volume requirement, and reduced operator error. In 2007, DiaSorin received FDA approval for clinical use of its second-generation automated "LIAISON 25 OH Vitamin D TOTAL" chemiluminescent immunoassay (CLIA). More recently, Roche Diagnostics released an automated electrochemiluminescence immunoassay (ECLIA) called "Vitamin D3 (25-OH)" that can be performed on their Elecsys, Modular Analytics, and Cobas analyzers. The objective of the present study was to compare the analytical performance of these two new automated assays (LIAISON and Roche) with the reference method (DiaSorin RIA) for the determination of serum 25(OH)D.

Materials and methods

Samples

Human serum samples ($n=400$) were obtained from a clinical trial in Toronto, Canada (latitude 43°N) in which healthy adults received either 28,000 IU vitamin D_3/week or a placebo for 8 weeks [18]. Serum aliquots were stored at −80 °C until analysis. Under these storage conditions, 25(OH)D is very stable in serum or plasma over a prolonged time and repeated freeze–thaw cycles [13,19,20]. Quantitative determination of serum 25(OH)D was performed in singleton by: DiaSorin "25-hydroxyvitamin D ^{125}I RIA" in April 2007 ($n=390$), DiaSorin "LIAISON 25 OH Vitamin D TOTAL" CLIA in September 2007 ($n=390$), and Roche Modular "Vitamin D3 (25-OH)" ECLIA in October 2007 ($n=158$). The DiaSorin 25(OH)D RIA served as the reference method. Out of the 400 serum samples acquired, 390 samples were analyzed by DiaSorin RIA and DiaSorin LIAISON TOTAL (10 samples had insufficient volume) and 158 samples were analyzed by the Roche Modular assay because there were not enough reagent kits to analyze the full 400 samples. Therefore, direct method comparisons were limited to those samples that were measured by all three assays ($n=158$). The 158 samples pertained to baseline and end-of-study (week 8) measurements of 20 subjects taking placebo and 59 subjects taking vitamin D_3 [18].

25(OH)D assays

DiaSorin 25(OH)D ^{125}I RIA

The DiaSorin 25(OH)D RIA method is based on a competitive principle with a goat antibody against 25(OH)D, an iodinated (^{125}I) 25(OH)D_3 tracer, and donkey anti-goat precipitating complex as secondary antibody. The first part of the assay involves a rapid extraction of 25(OH)D and other hydroxylated metabolites from serum or plasma with acetonitrile. Following extraction, the sample, antibody, and tracer are incubated for 90 min at 20–25 °C. Phase separation is accomplished after a 20 min incubation at 20–25 °C with the secondary antibody. A buffer is then added prior to centrifugation to reduce non-specific binding. Radioactivity is measured by a gamma counter and is inversely proportional to the concentration of 25(OH)D in the sample.

DiaSorin LIAISON 25(OH)D TOTAL CLIA

The LIAISON 25 OH Vitamin D TOTAL Assay is a direct competitive chemiluminescence immunoassay for human serum or plasma intended for use on the DiaSorin LIAISON automated analyzer. The assay uses magnetic particles (solid phase) coated with antibody against 25(OH)D and 25(OH)D conjugated to an isoluminol derivative (tracer). During the first incubation phase (10 min), 25(OH)D is dissociated from binding protein by buffer containing 10% ethanol and then binds to the anti-25(OH)D antibody on the solid phase. After a second 10 min incubation with the tracer, the unbound material is washed off and starter reagents are added to generate a flash chemiluminescent signal which is measured by a photomultiplier and is inversely related to 25(OH)D concentration.

This assay differs from its older version, "LIAISON 25 OH Vitamin D", due to alterations in the on-board extraction procedure, the addition of a second incubation step, and the use of human serum-based calibrators instead of horse serum.

Roche Modular 25(OH)D ECLIA

The Roche Vitamin D3 (25-OH) assay is a direct competitive electrochemiluminescence immunoassay for human serum or plasma intended for use on Roche automated immunoassay analyzers. In this study, the Modular Analytics analyzer was used. The assay employs microparticles coated with streptavidin and a polyclonal sheep antibody against 25(OH)D, which is labeled with ruthenium. In the first incubation, 25(OH)D_3 in the sample competes with biotin labelled 25(OH)D for binding with the anti-25(OH)D antibody. In the second incubation, the biotin-25(OH)D/anti-25(OH)D antibody immunocomplex becomes bound to the microparticles via interaction of biotin and streptavidin. The microparticles are then magnetically captured onto the surface of an electrode. A voltage is applied to the electrode to produce a chemiluminescent emission, which is measured by a photomultiplier and is inversely proportional to 25(OH)D concentration.

Specifications for the three assays, as stated by the manufacturer, are listed in Table 1. According to the product inserts, none of the analytical methods are significantly affected by