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Review

A novel, fully-automated, chemiluminescent assay for the detection of 1,25-dihydroxyvitamin D in biological samples

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ABSTRACT

Background: 1,25-Dihydroxyvitamin D (1,25-$(OH)_2$D), the hormonal form of vitamin D, is difficult to measure because of its low circulating levels (pg/mL), and similarity to more abundant metabolites. Here a fully-automated chemiluminescent assay that accurately and precisely measures 1,25-$(OH)_2$D is described.

Method: The novel 1,25-$(OH)_2$D assay was conceived based on four pillars: (1) the VDR's ligand binding domain (LBD) as a capture molecule; (2) reaction conditions wherein 1,25-$(OH)_2$D favors binding to LBD vs. the vitamin D binding protein; (3) exploitation of liganded-LBD's conformational change; (4) a monoclonal antibody specific to liganded-LBD. This specific, conformational, sandwich approach, unique for automated measurement of haptens, is superior to more cumbersome, conventional competitive formats.

Results: Accuracy of the 1,25-$(OH)_2$D assay was corroborated by its alignment against LC–MS/MS with fit Deming regression equations of $y = 0.98x + 1.93$ ($r = 0.92$), and $y = 1.07x + 3.77$ ($r = 0.94$) for different methods from Endocrine Sciences, Laboratory Corporation of America® and the University of Washington, respectively. Good analytical precision was manifested by its low estimated limit of quantitation (1.57 pg/mL), average intra-assay imprecision (3.5%CV; range 1.1–4.7%), and average inter-assay imprecision (4.5%CV; range 3.4–7.2%). Expected and measured recovery values were congruent (93.4% mean).

Conclusions: The novel 1,25-$(OH)_2$D method exhibited excellent correlation with well validated LC–MS/MS assays from two laboratories. Significantly, its 65 min turn-around time is quicker, and sample volume smaller (75 μl) than current methods.

Contents

Abbreviations: LBD, ligand binding domain; VDBP, vitamin D binding protein; 25-(OH)D, 25 hydroxyvitamin D; 1,25-$(OH)_2$D, 25-dihydroxyvitamin D; VDR, vitamin D receptor; VDRE, vitamin D response element; LOQ, limit of quantitation; LOB, limit of blank; Mab, monoclonal antibodies; NMR, nuclear magnetic resonance; DPI, Dual Polarization Interferometry; DEQAS, vitamin D External Quality Assessment Scheme.

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Introduction

The synthesis of vitamin D_3 in skin is a two-step process: 7-dehydrocholesterol is converted to previtamin D_3 upon exposure to UV light; previtamin D_3 isomerizes to vitamin D [1]. Vitamin D_3 and vitamin D_2 (that originates from irradiation of ergosterol) may also be obtained in the diet. [1]. Upon entry into the circulation, vitamin D is readily hydroxylated in the liver at carbon-25 to produce 25-hydroxyvitamin D (25-(OH)D), the major circulating metabolite [2]. Vitamin D metabolites generally associate with the vitamin D binding protein (VDBP) to be carried through the bloodstream [3]. Although 25-(OH)D is inactive itself, its serum level affords the commonly accepted method for assessing vitamin D status of patients.

The classical role of vitamin D is the maintenance of plasma calcium and phosphate at near constant levels for proper neuromuscular function, bone mineralization, and the prevention of hypocalcemic tetany [4]. Under physiological conditions where calcium and phosphate homeostasis is disturbed, 25-(OH)D is converted in the kidney by 1α-hydroxylase to produce 1α,25-dihydroxyvitamin D (1,25-$(OH)_2$D), which acts on target organs to normalize plasma calcium and phosphate [4]. Most, if not all, of circulating 1,25-$(OH)_2$D is produced in the kidney as revealed by the extremely low circulating levels in nephrectomized rats [5] or patients [6].

The functions of 1,25-$(OH)_2$D are mediated through a nuclear receptor that binds to vitamin D response elements (VDREs) in target genes to regulate their transcription. The vitamin D receptor (VDR) is a member of the steroid/thyroid hormone nuclear receptor superfamily and contains characteristic functional domains including the DNA binding domain and the ligand binding domain (LBD) [7]. The structure of the LBD was recently solved by NMR studies, which depict a distinct, ligand-specific conformational change upon binding of 1,25-$(OH)_2$D [8].

It has long been established that vitamin D deficiency causes bone diseases such as rickets, osteomalacia, and secondary hyperparathyroidism, but with the discovery of VDR in non-classical target tissues, 1,25-$(OH)_2$D is now known to have a broader spectrum of actions, and has been associated with increased risks for various chronic diseases including infectious and autoimmune, diabetes, cancer, cardiovascular ailments, hypertension and complications during pregnancy [9]. Clinical studies implicating vitamin D in the forgoing conditions have been based predominantly upon measurement of 25-(OH)D. Since 1,25-$(OH)_2$D is the active hormone and does not correlate with 25-(OH)D status, important relationships have remained unexplored. Facile measurement of 1,25-$(OH)_2$D has been difficult due to its extremely low circulating levels (pg/mL) [10]. Current methods, requiring laborious, sample consumptive, pre-analytical steps are significantly compromised at the low end of their measuring range because of their intrinsic imprecision [10]. In this manuscript, a new, fully-automated method for measurement of 1,25-$(OH)_2$D in biological fluids is presented. The novel assay is accurate, precise throughout the entire assay range, fast, and requires only 75 μl of sample unencumbered by the need for pre-analytical enrichment through precipitation and subsequent reconstitution. The exquisite specificity and avidity of the VDR for its substrate was used to produce the first sandwich, automated chemiluminescent assay for a small molecule hapten like 1,25-$(OH)_2$D.

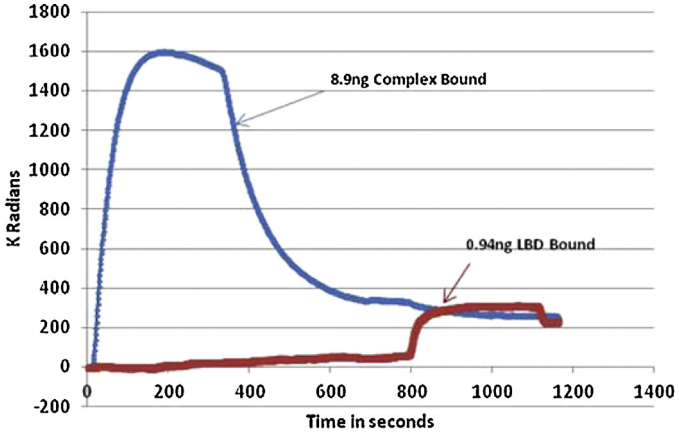

Fig. 1. Specificity of the 11B4 antibody used in the novel 1,25-$(OH)_2$D assay. A Dual Polarization Interferometry experiment was performed by coating a 2-channel chip with the monoclonal antibody 11B4 raised against the liganded LBD. Channel 1 was injected with LBD-1,25-$(OH)_2$D complex (blue), and channel 2 was injected with apo-LBD (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Specimens

To evaluate the LIAISON® XL 1,25-Dihydroxyvitamin D assay in biological fluids, 78 human serum samples routinely submitted to Laboratory Corporation of America for 1,25-$(OH)_2$D testing were anonymized and used to make pools with concentrations covering the analytical measuring range of the assay and parsed into multiple aliquots for analysis by multiple methods. The protocol for this study was determined to be exempt under existing regulations by the Institutional Review Board.

2.2. Method comparison

Method comparison of 1,25-$(OH)_2$D measurement included two different LC–MS/MS methods using immune-extraction to enrich for 1,25-$(OH)_2$D as a pre-analytical step; the first method was from Endocrine Sciences, Laboratory Corporation of America (Calabasas Hills, CA), and the second LC–MS/MS analysis was performed at the University of Washington and is outlined by Strathmann et al. [11]. A commercially available immunoassay (Method A) was also compared to the LIAISON® XL 1,25-dihydroxyvitamin D assay (DiaSorin, Stillwater, MN).