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Evaluation of 25 OH Vitamin D in chronic renal failure and end stage renal disease subjects☆

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Abstract

Analysis of laboratory samples from chronic renal failure (CRF) and end stage renal disease (ESRD) patients can be problematic. Current HPLC and RIA methods for the determination of 25 OH Vitamin D involve sample extraction. However, the differences between a normal and CRF or ESRD matrix can lead to interference or inaccuracy in non-extracted, automated methods now available. The objective of this study was to assess the accuracy of the non-extracted LIAISON® 25 OH Vitamin D assay in the analysis of CRF and ESRD samples as compared against RIA as reference. Samples were collected from regional reference laboratories and analyzed in both the LIAISON® 25 OH Vitamin D assay and the DiaSorin 25 OH Vitamin D RIA. By Student's *t* test, no significant difference was observed between the RIA values and the LIAISON® values ($P = 0.07$ CRF; $P = 0.28$ ESRD). The linear regression analysis resulted in the equations: CRF: LIAISON $= 0.91$(RIA) $+ 0.6$; $r = 0.82$ and ESRD: LIAISON $= 0.93$(RIA) $- 0.6$; $r = 0.78$. From these data we conclude that the LIAISON® 25 OH Vitamin D assay correctly assesses the 25 OH Vitamin D status of CRF and ESRD patients.

Keywords: Vitamin D; Chronic renal failure; LIAISON®

1. Introduction

Methods for the analysis of 25 OH Vitamin D have evolved from very labor-intensive, and technique dependent chromatographic and HPLC methods, through RIA and ELISA methodologies to automated analyzers [1–3]. Methods prior to the automated analyzers involved various extraction protocols to separate 25 OH Vitamin D from both its binding protein and various similar metabolites. These extraction protocols also removed many potential interferents from the samples. The analysis of laboratory samples from chronic renal failure (CRF) and end stage renal disease (ESRD) patients can be problematic due to altered serum chemistries, and the presence of multiple medications [4]. The differences between a normal serum matrix and the CRF or ESRD serum matrix may lead to interference or inaccuracy in the determination of 25 OH Vitamin D concentrations in these samples in the non-extracted, automated methods now available. The objective of this study was to assess the accuracy of the non-extracted LIAISON® 25 OH Vitamin D assay in the analysis of CRF and ESRD samples as compared against an extracted sample RIA as reference.

2. Materials and methods

2.1. Assays

The assays utilized for this study included the DiaSorin 25 OH Vitamin D RIA (Cat # 68100E) [5], and the LIAISON® 25 OH Vitamin D (Cat # 310900). Samples were analyzed by both methods on the same day. Briefly, samples for the RIA (50 μL) were extracted with acetonitrile (500 μL) and centrifuged at $1200 \times g$ for 10 min at room temperature. Aliquots of the supernatant (25 μL) were pipetted to sample tubes, to which were added ^{125}I 25 OH Vitamin D tracer (50 μL) and 25 OH Vitamin D specific antibody (1.0 mL). Samples are incubated for 90 min, and then precipitated with 500 μL of precipitating reagent. Tubes were then centrifuged for 20 min at room temperature, decanted, and counted in a gamma counter.

For the LIAISON® assays, samples were placed in the sample racks of the analyzer. Aliquots (25 μL) were added to a reaction cuvette with anti-25 OH Vitamin D coated microparticles (20 μL), 25 OH Vitamin D-ABEI conjugate (20 μL), and assay buffer (220 μL). The cuvettes were

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Table 1
Method correlation of LIAISON® and RIA

Sample type	Parameter		
	RIA value (ng/mL)	LIAISON® value (ng/mL)	Student's *t* test *P*-value
CRF Samples			
Mean	14.4	13.6	
S.D.	6.8	7.6	0.071
ESRD Samples			
Mean	18.6	16.8	
S.D.	7.8	9.3	0.282

incubated for 30 min, washed, and trigger reagent was added, with a 3 s read cycle.

2.2. Samples

Clinical samples were collected sequentially from regional reference laboratories. Samples with a diagnosis from the referring physician of either CRF ($N = 118$) or ESRD ($N = 49$) were selected without regard for any other clinical status.

3. Results

Samples analyzed side-by-side on the same day in both the manual, extracted RIA, and the automated, non-extracted LIAISION® assay were compared by both Student's *t* test and linear regression analysis. The results of the Student's *t* test analysis is shown in Table 1. For the CRF samples, the resulting *P*–value is 0.07, indicating no significant difference between the datasets. Similarly, the *P*-value for the ESRD samples was 0.28, also indicating no significant difference between the datasets.

When analyzed by linear regression, the resulting equation for the CRF samples was LIAISON® = (0.91)RIA + 0.6; $r = 0.82$ (Fig. 1). The mean difference between the methods was 0.7 ± 4.4 ng/mL. Similarly analyzed by linear regression, the resulting equation for the ESRD samples was LIAISON® = (0.93)RIA − 0.6; $r = 0.78$ (Fig. 2). The mean difference between the methods was 1.9 ± 5.9 ng/mL.

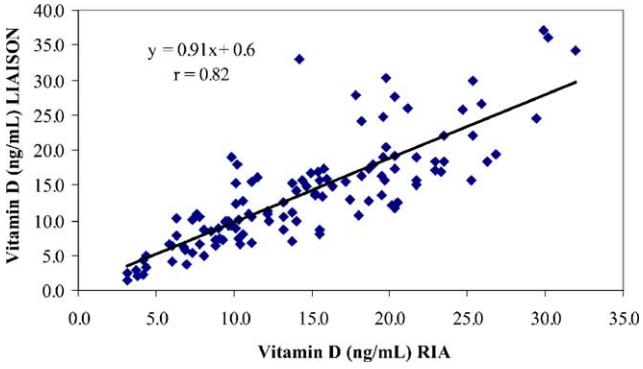

Fig. 1. Linear regression analysis of the method comparison between RIA and LIAISON® for CRF subject samples ($N = 118$).

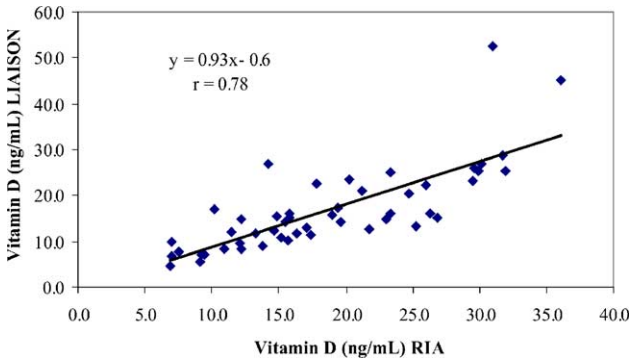

Fig. 2. Linear regression analysis of the method comparison between RIA and LIAISON® for ESRD subject samples ($N = 49$).

4. Discussion

The analysis of CRF and ESRD samples may be problematic in methodologies that do not involve sample extraction. The LIAISON® 25 OH Vitamin D assay is an automated, non-extracted methodology for the determination of 25 OH Vitamin D in serum. As such, the method could be expected to exhibit interference from either endogenous serum components or exogenous medications. This study demonstrated that the LIAISON® assay does not exhibit interference from the altered serum matrix found in these patients. From these data we conclude that the LIAISON® 25 OH Vitamin D assay correctly assesses the 25 OH Vitamin D status of CRF and ESRD patients.

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