

High Serum Lipids Cause Erroneously Low Total 25-OH Vitamin D Levels by a Chemiluminescent Immunoassay.

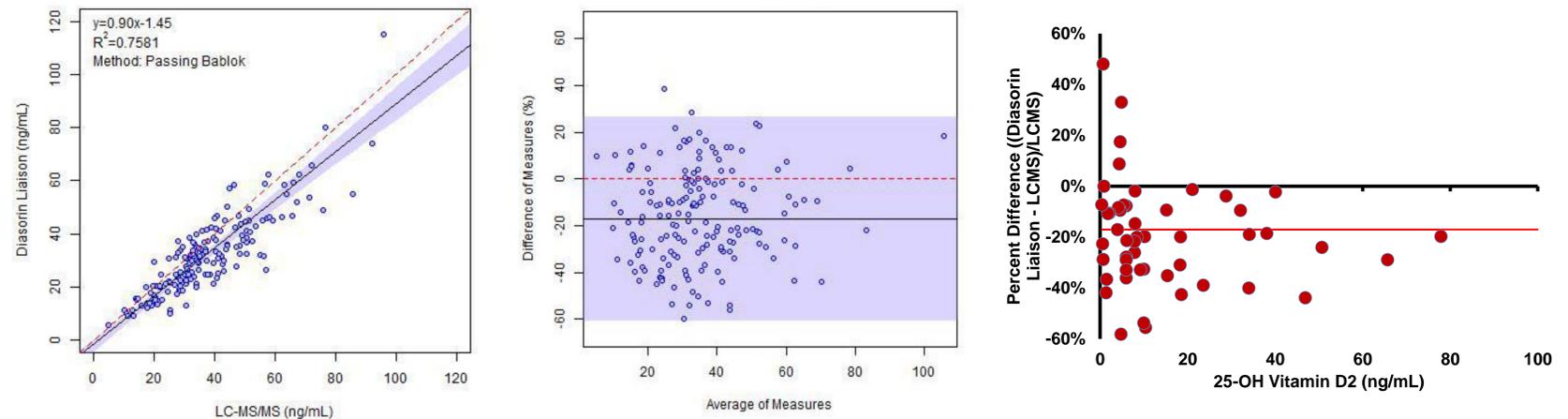
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Abstract

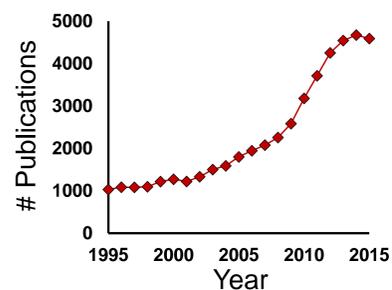
The DiaSorin Liaison® chemiluminescent immunoassay (CIA) for total 25-OH vitamin D (25-OH vit D) has begun to replace mass spectrometry in many clinical laboratories. This work examined the performance of this assay in routine clinical practice. Over a one-year period, a total of 172 paired samples were analyzed by the CIA and an in-house tandem mass spectrometry assay. The CIA showed acceptable performance overall, however, substantial negative bias was observed for a small number of samples with some vitamin D replete patients being classified as deficient by the CIA. Analysis of additional samples at select lipid levels showed that the CIA overestimates 25-OH vit D in the presence of low total cholesterol and underestimates in the presence of high total cholesterol levels. Mixing studies supported the findings that the CIA underestimates 25-OH vit D with increasing total cholesterol. These results have implications for population based studies which have suggested a negative relationship between 25-OH vit D and lipids.

CIA vs. LC-MS/MS for 25-OH Vit D



The DiaSorin Liaison® CIA showed acceptable performance for total 25-OH vit D measurements with a slope of the Passing Bablock being 0.9 in this study, comparable to those reported in the literature (2). In addition, consistent with reports in the literature there was no bias due to total vitamin D levels (from 10 to 115 ng/mL) or from vitamin D2 levels (from 1 to 78 ng/mL).

Introduction



The number of publications on vitamin D have exploded in the last ten years (Figure 1) (1). At the same time, substantial effort has been put into improving methodologies for monitoring 25-OH vitamin D. A number of immunoassay-based methodologies are now

commercially available which have passed the performance criteria of the Centers for Disease Control Vitamin D Standardization Certification Program ($\pm 5\%$ mean bias). Our hospital has been offering both the DiaSorin Liaison® chemiluminescent immunoassay (CIA) and an in-house developed liquid chromatography tandem mass spectrometry (LC-MS/MS) assay. The goal of this work was to investigate the accuracy of the CIA in routine clinical use.

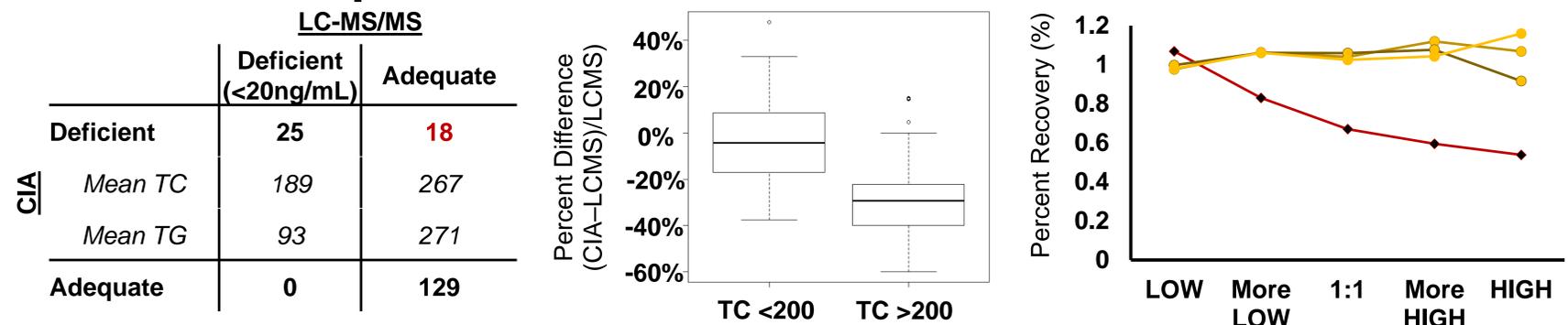
Methods

DiaSorin Liaison® CIA. The CIA was used as described by the manufacturer with no modification,

LC-MS/MS. Serum samples were prepared with a protein crash using ZnSO₄ followed by Solid Phase Extraction (SPE) (Oasis HLB). Chromatography was performed on an Acquity® UPLC with a BEH phenyl column. MS analysis was performed on a Waters Acquity® TQD using Chromsystems 6PLUS1 calibrators and UTAK Tri Level Vitamin D Plus Serum Controls. The method was checked for accuracy using NIST standard reference material (SRM 972).

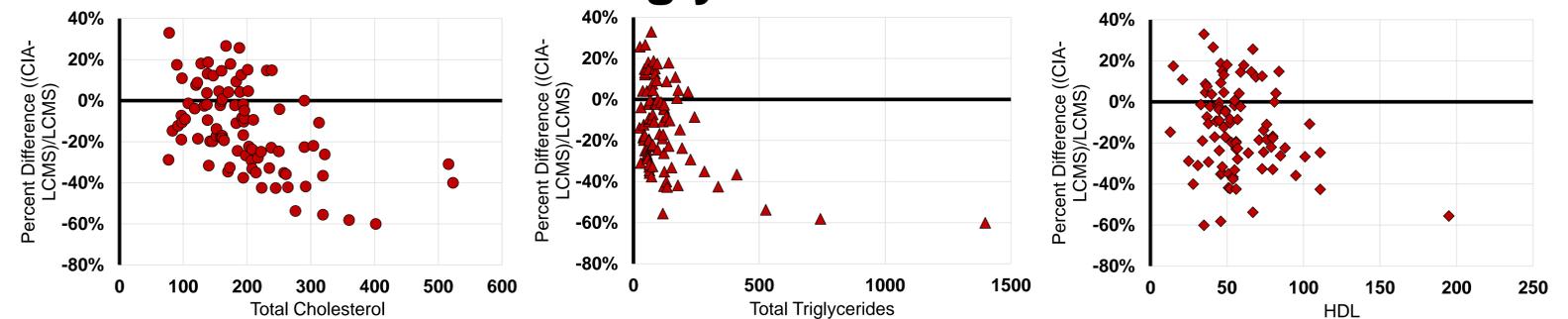
Lipid levels. Total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) were measured on a Beckman DxC. Low density lipoprotein (LDL) was typically calculated for these samples using the Friedwald equation but not measured directly.

Clinical Interpretation of 25-OH Vit D Levels from the CIA



A total of 18 (42%) of the deficient (25-OH vit D <20 ng/mL) samples by the CIA would have been classified as adequate by the LC-MS/MS. The total cholesterol and triglycerides were markedly elevated for these discordant samples compared to the concordant ones. The mean percent difference ((CIA-LC-MS/MS)/LC-MS/MS) for samples with total cholesterol less than 200 was much higher than for those with total cholesterol greater than 200 (-4% vs -26%). Further mixing studies of samples with low and high levels of total cholesterol, triglycerides and HDL showed that the CIA under-recovered 25-OH vit D with increasing total cholesterol triglycerides and HDL.

Total Cholesterol and Triglycerides Bias DiaSorin® Liaison CIA



Future Directions

It has been suggested in the literature that 25-OH vit D levels are inversely correlated with elevated total cholesterol.

In addition, studies have suggested that statins improve 25-OH vit D levels (5). It might be that all of these results are simply an artifact of the observed lipid interference in the assays used to measure 25-OH vitamin D.

Estimate (mg/dL)	
Non-HDL	-0.89
Total Cholesterol	-1.08
LDL	-0.35
HDL	-0.19
Triglycerides ^c	-2.34

Proposed 25OH vit D and lipid correlations (4).

References

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