ANALYTICAL EVALUATION OF THE IDS-iSYS 25-HYDROXY VITAMIN D (25(OH)D) IMMUNOASSAY

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Introduction

Vitamin D has a central role in calcium and phosphate homeostasis. Vitamin D deficiency and insufficiency have been linked to bone, muscle, cardiovascular and nervous system disorders, diabetes and inflammation. In the liver, Vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol) are hydroxylated to 25-hydroxy vitamin D, 25(OH)D. Serum concentration of 25(OH)D is considered to be the most reliable measure of overall vitamin D status and can be used to determine whether a patient is vitamin D sufficient. Vitamin D status may also be required to determine the cause of abnormal serum calcium concentration in patients. Several automated methods for the measurement of 25(OH)D in human serum, including immunoassays, HPLC, and LC-MS-MS have been developed and routinely used in the clinical laboratory setting. There exists substantial inter-method variation.

Objective

To evaluate analytical performance of the IDS-iSYS competitive chemiluminescent immunoassay for measurement of 25(OH)D in human serum.

Methods

***Patients Samples*:**

Patient serum samples (n = 47) were obtained from the clinical biochemistry laboratory at Mount Sinai Hospital in Toronto, for which measurement of 25(OH)D levels had been ordered. 25(OH)D levels ranged from 10 - 239 nmol/L.

Method Validation:

IDS-iSYS 25(OH)D method imprecision, linearity, and limit of quantitation (LoQ) were assessed.

The IDS-iSYS method was compared to two methods routinely used to measure serum 25(OH)D in the clinical laboratory setting:

a. Direct competitive chemiluminescent immunoassay – Liaison (DiaSorin);
b. Liquid Chromatography Tandem Mass Spectrometry method - LC-MS-MS (Applied Biosystems API5000)

Results

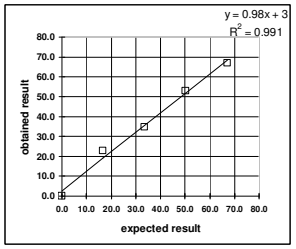
Table 1. IDS-iSYS Precision Evaluation

The precision study was performed using pooled serum samples containing levels of 25(OH)D close to the clinically relevant cut points of 30, 70 and 175 nmol/L. Each level was run in quadruplicate (morning run in duplicate and afternoon run in duplicate) over 20 days, n = 80. The assay precision was determined by following the EP-5T2 protocol "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition" published by CLSI. Precision was assessed using two 25(OH)D IDS-iSYS reagent lots

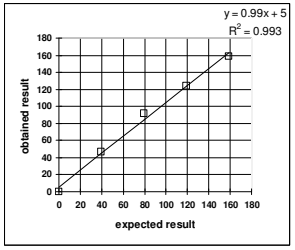
Sample		Within-run imprecision	Between-day imprecision	Total imprecision
HIGH (173 nmol/L)	SD:	14	15	23
	CV:	8.2%	8.9%	13.2%
MEDIUM (69 nmol/L)	SD:	4.6	4.4	7.0
	CV:	6.7%	6.4%	11.4%
LOW (28 nmol/L)	SD:	2.3	2.6	3.40
	CV:	8.1%	9.0%	12.0%

Figure 1. Linearity Assessment of IDS-iSYS

Linearity up to 160 nmol/L was determined by serially diluting IDS QC material (159 nmol/L; 67 nmol/L) with the zero calibrator.



Range tested: 0 – 67 nmol/L



Range tested: 0 – 159 nmol/L

Table 2. Verification of Limit of Quantitation (LoQ)

The manufacturer's claimed LoQ for the IDS-iSYS 25(OH)D method is 13.75 nmol/L. Two samples were prepared, one at 13.5 nmol/L and one at 14.0 nmol/L, by diluting the IDS-iSYS low calibrator with the zero calibrator. Each sample was analyzed with five replicates for four days (n = 20). OMR = outside assay measuring range of the assay.

Target (nmol/L)	Day	Individual Measurements (nmol/L	Mean (nmol/L)	SD	%CV
13.5	1	14.3, 15.8, 15.1, 14.0, OMR	13.7	1.1	7.8
	2	13.7, 12.6, 12.8, OMR, OMR			
	3	OMR, OMR, 14.3, 13.3, 13.0			
	4	12.8, 12.6, 15.0, 12.6, OMR			
14	1	20.4, 18.1, 19.2, 17.4, 18.4	17.5	1.8	9.8
	2	16.0, 16.7, 18.5, 16.5, 19.2			
	3	19.7, 17.5, 17.5, 16.0, 17.			
	4	15.6, 17.4, 19.5, 15.5, 13.0			

Figure 2. Method Comparison – Passing Bablok Regression

Patient serum samples (n=47) were analyzed in duplicate using the test method, 25(OH)D IDS-iSYS. The same samples were analyzed using two other methods: The Liaison, DiaSorin, immunoassay (Fig.2A), and a LC-MS-MS method (Fig.2B). These results were compared to the test method. The method comparison in this study was performed according to EP9-A2 protocol published by CLSI entitled, "Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition". The pink line represents the line of identity.

Figure 2A. Liaison vs. IDS-iSYS

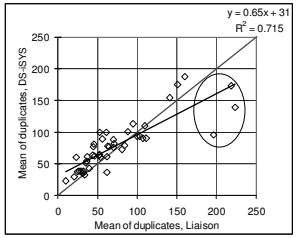


Figure 2B. LC-MS-MS vs. IDS-iSYS

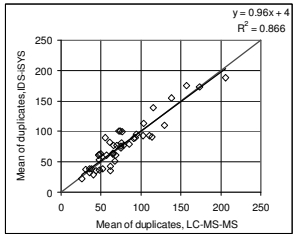


Figure 3. Bland-Altman Difference Plot Showing the Bias Between IDS-iSYS, Liaison, and LC-MS-MS

Figure 3A. Liaison vs. IDS-iSYS

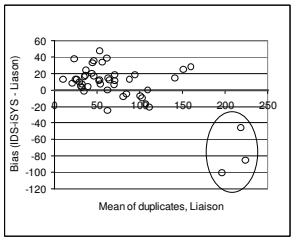
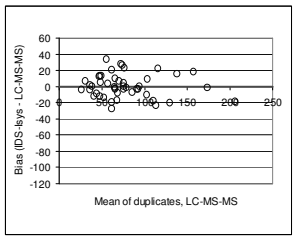


Figure 3B. LC-MS-MS vs. IDS-iSYS



Conclusions

•The IDS-iSYS 25(OH)D method imprecision is 11% - 13%
•Linearity was verified between 17 nmol/L and 160 nmol/L
•The Limit of quantitation for the IDS-iSYS method is 17.5 nmol/L
•The IDS-iSYS method correlates better with the LC-MS-MS than with the Liaison method
•There is poor agreement between the IDS-iSYS and the Liaison method at 25(OH)D > 200 nmol/L

References

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3.Maunsell Z, et al, 2005, Clinical Chemistry, 51(9): 1683-90.