Technical Review

Correlation of the DIAsource $1,25(OH)_2$ Vitamin D RIA assay with LC-MS/MS

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1. INTRODUCTION

The DIAsource $1,25(OH)_2$ Vitamin D RIA assay is based on the Gold Standard extraction methodology, which ensures superior analytical performances and no interference from sample matrix or other Vitamin D metabolites.
The assay is the only immunoassay to measure both the D3 and D2 forms of $1,25(OH)_2$ Vitamin D. The cross-reactivity against $1,25(OH)_2$ Vitamin D2 is close to 100% (92.3%).
The DIAsource $1,25(OH)_2$ Vitamin D Total RIA assay shows very competitive sensitivity, precision and performance characteristics to all other immunoassays in the market.

Although there is no standard reference material or reference measurement procedure for the measurement of $1,25(OH)_2$ Vitamin D, LC-MS/MS is commonly considered as the most accurate technique for the measurement of this metabolite. The DIAsource $1,25(OH)_2$ Vitamin D RIA assay was recently compared to LC-MS/MS and the results are presented hereunder.

2. LC-MS/MS

LC-MS stands for tandem Liquid Chromatography Mass Spectrometry. It is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (LC or HPLC) with the mass analysis capabilities of mass spectrometry (MS). LC-MS is a powerful technique that has very high sensitivity and selectivity and so is useful in many applications. The main area of application in the field of clinical laboratories is the analysis of 'small molecules' (in opposition to proteins), such as drugs of abuse, pharmaceuticals, toxic compounds and more recently steroids.

Despite its attractive performance profile, LC-MS suffers from practical drawbacks. The equipment is very expensive and requires highly skilled technicians and engineers to set up and maintain it. Very few assay manufacturers exist and laboratories tend to develop their own assays, which also require specific resources and which might be a risk for long-term assay stability and performance. Furthermore LC-MS is very sensitive to matrix effects and extraction of the samples with organic solvents is mandatory prior to analysis.

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3. MATERIAL and METHODS

3.1. Samples

Serum was collected from 15 individuals in a ISO 9001:2008 certified, FDA and USDA Approved facility. Ethnicity of the individuals was either Hispanic or Black and all were male. Age ranged from 22 to 53 years.
For higher $1,25(OH)_2$ Vitamin D concentrations, 3 serum pools were constituted from serum samples originating from an ISO 15189 certified facility.
DEQAS samples 316-320 and 331-335 were also included in the study (See our Technical Reviews 2015-03 and 2015-05).

3.2. Measurement of $1,25(OH)_2$ Vitamin D

Samples were measured using the DIAsource $1,25(OH)_2$ Vitamin D RIA assay according to the package insert instructions. The total amount of 28 samples were measured in duplicate. Two kit controls were included into each run and fell within their acceptance criteria.

LC-MS measurements were performed at the VU University Medical Center (Amsterdam, The Netherlands) for the individual and pooled serum samples. This laboratory uses an extensively validated 2D ID-UPLC–MS/MS method.[1]
DEQAS samples were compared to the mean of all reported LC-MS results.

3.3. Analysis of results

For the DIAsource $1,25(OH)_2$ Vitamin D RIA assay, samples and controls concentrations were calculated by interpolation from the standard curve using a 4 parameter logistic nonlinear regression model.
We used the linear regression model and regression models were built for the total number of samples (n = 28). All linear regressions were performed with Microsoft Excel 2013.

[1] Determination of reference values for serum total 1,25-dihydroxyvitamin D3 using an extensively validated 2D ID-UPLC–MS/MS method. Dirks N., Martens F., Vanderschueren D., Billen J., Ackermans M., Endert E., Blankenstein M., Heijboer A., Endocrine Abstracts (2015) 37 GP11.05.

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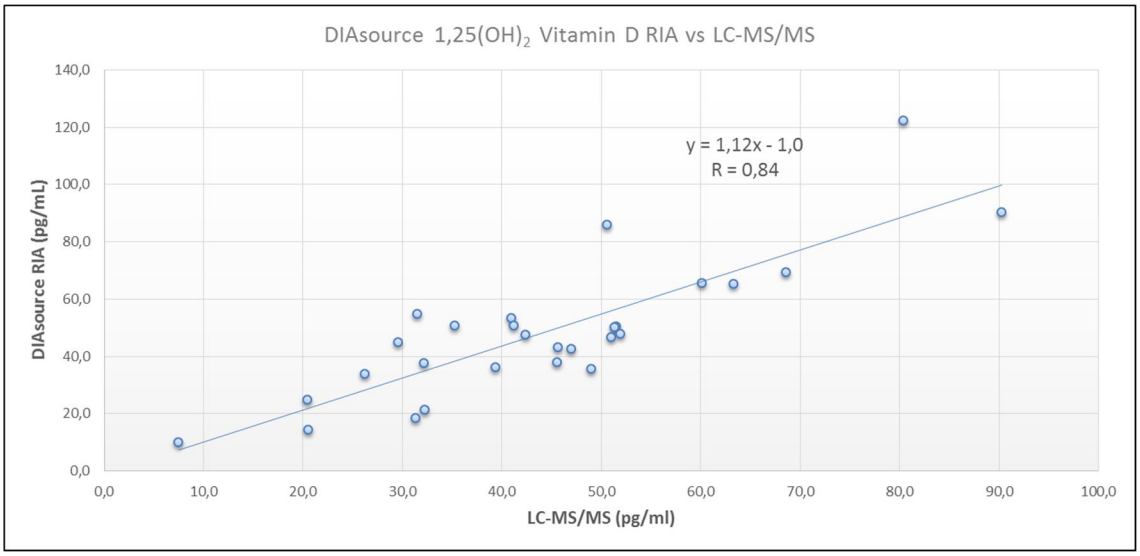
4. RESULTS

When LC-MS concentrations were plotted on the X axis, and DIAsource RIA on the Y axis, a slope of 1.12, an intercept of -1.0pg/mL and a correlation coefficient of R = 0.84 were observed (Figure 1).

Figure 1. Linear regression of DIAsource $1,25(OH)_2$ Vitamin D RIA against LC-MS $1,25(OH)_2$ Vitamin D (n = 20)



5. CONCLUSION

The DIAsource $1,25(OH)_2$ Vitamin D RIA assay shows excellent performances against the reference LC-MS method, whatever the sample origin and whatever the LC-MS method. This confirms the accuracy profile previously demonstrated on Quality Control Program samples (See our Technical Reviews 2015-03 and 2015-05). Thanks to its Gold Standard extraction procedure and to its superior specificity profile, the DIAsource $1,25(OH)_2$ Vitamin D RIA is a valuable tool for the assessment of $1,25(OH)_2$ Vitamin D in clinical laboratories.

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Ordering Information

Description	Article code	Format
25OH Vitamin D Total ELISA	KAP1971	ELISA
25OH Vitamin D Total RIA	KIP1971	RIA
25OH Vitamin D3 RIA	KIP1961	RIA
Rat 25OH Vitamin D Total ELISA (RUO)	KRR1971	ELISA
Free 25OH Vitamin D ELISA (RUO)	KARF1991	ELISA
$1,25(OH)_2$ Vitamin D ELISA	KAP1921	ELISA
$1,25(OH)_2$ Vitamin D RIA	KIP1929	RIA

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