Technical Review

Performance of the DIAsource 1,25(OH)$_2$ Vitamin D RIA assay in the DEQAS Quality Control Program

Author:
Nicolas Heureux, PhD
Nicolas.Heureux@diasource.be
Principal Scientist – Vitamin D, DIAsource Immunoassays
DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2 | B-1348 Louvain-La-Neuve | Tel : +32.10.84.99.11 | Fax : +32.10.84.99.90
www.diasource.be

Contents

Performance of the DIAsource 1,25$(OH)_2$ Vitamin D RIA assay in the DEQAS Quality Control Program

Author:
Nicolas Heureux, PhD
Nicolas.Heureux@diasource.be
Principal Scientist – Vitamin D, DIAsource Immunoassays
DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2 | B-1348 Louvain-La-Neuve | Tel : +32.10.84.99.11 | Fax : +32.10.84.99.90
www.diasource.be

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 and to physical detection methods such as LC-MS/MS. The accuracy of the assay was demonstrated by its excellent performance in the quality control programs DEQAS and Instand e.V. (Cf. Technical Review 2015-03).

2. DEQAS

The international Vitamin D Quality Assessment Scheme (DEQAS) has been monitoring the performance of $1,25(OH)_2$ Vitamin D assays since many years and now has >130 registered participants worldwide. In essence DEQAS is an ongoing multicenter trial of the methods used by its participants and provides a unique opportunity to assess the accuracy and specificity of 25OH Vitamin D and $1,25(OH)_2$ Vitamin D methods as well as the analytical performance of a large number of their users.
Serum is harvested from blood donated by patients undergoing therapeutic venesection for haemochromatosis or polycythemia. Liquid serum pools (5) are distributed quarterly at ambient temperature. Laboratories are given approximately 5 weeks to return results. Data are statistically trimmed to produce an All-Laboratory Trimmed Mean (ALTM), SD and CV.

3. SAMPLES 356-360

The last sample set, DEQAS 356-360, was distributed in January 2015 and the results were published in April 2015.
Figure 1 shows the bias of the three $1,25(OH)_2$ Vitamin D RIA methods (DIAsource, DiaSorin and IDS) against the LC-MS/MS methods for the last 5 distributed samples. Results are presented across the concentration range, as determined by LC-MS/MS. The same results are shown in Figure 2 across the sample numbers. The mean bias over the 5 samples was calculated to be +6% for the DIAsource assay, +17% for the DiaSorin assay and +33% for the IDS assay.

Author:
Nicolas Heureux, PhD
Nicolas.Heureux@diasource.be
Principal Scientist – Vitamin D, DIAsource Immunoassays
DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2 | B-1348 Louvain-La-Neuve | Tel : +32.10.84.99.11 | Fax : +32.10.84.99.90
www.diasource.be

Figure 1. **DEQAS** - Bias RIA Methods versus LC-MS/MS (calculated as ((Method - LC-MS/MS)/LC-MS/MS).100, across the concentration range (samples 356-360)

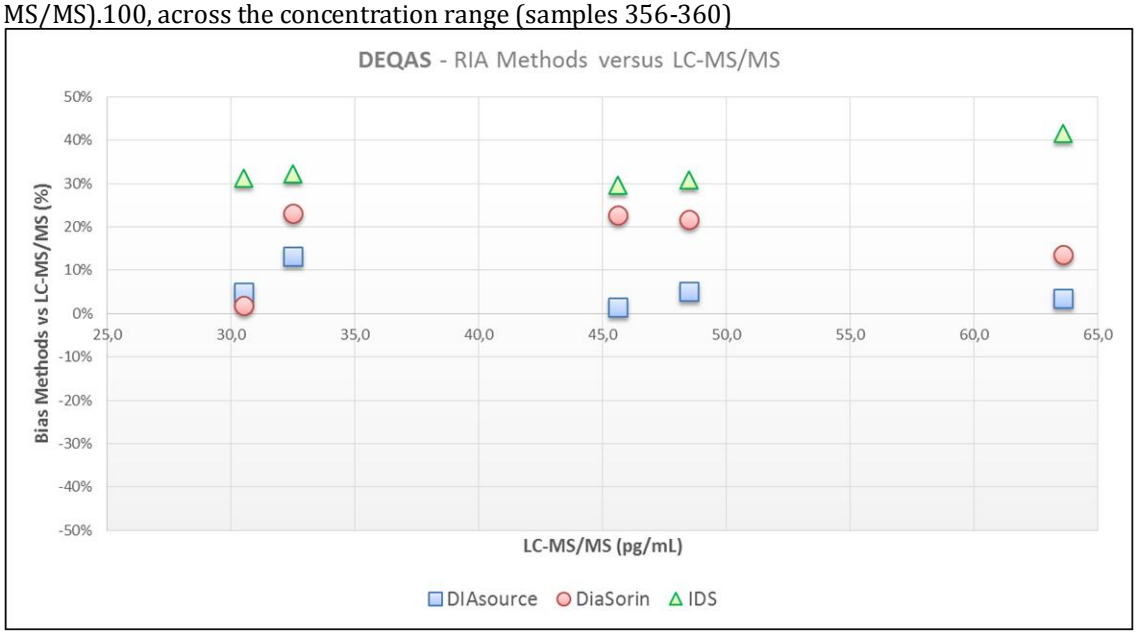
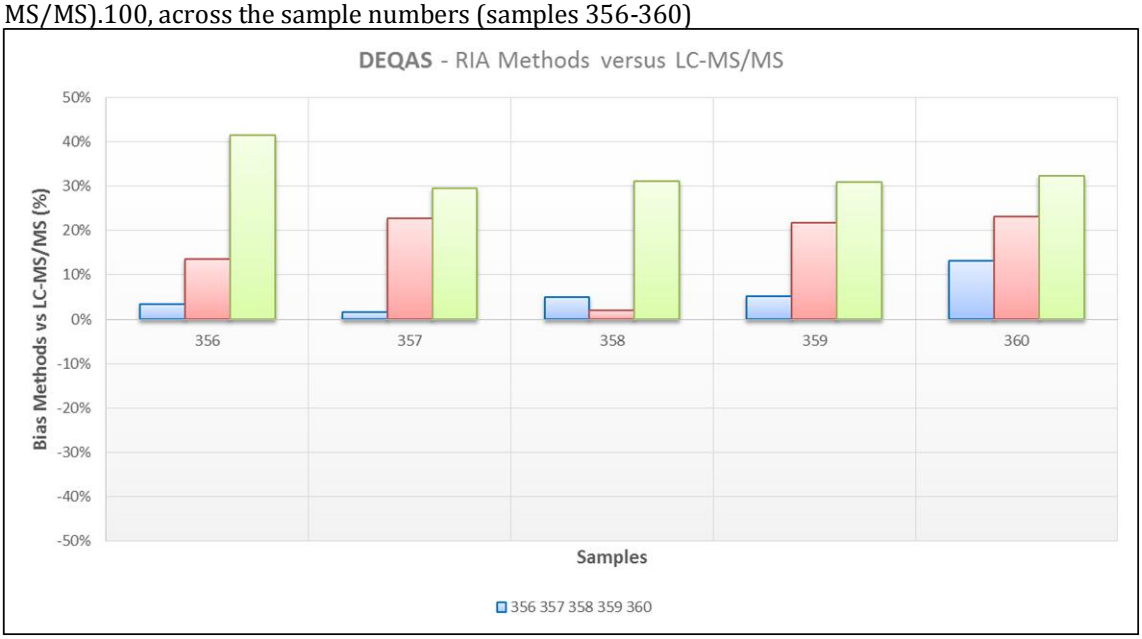


Figure 2. **DEQAS** - Bias Method versus LC-MS/MS (calculated as ((Method - LC-MS/MS)/LC-MS/MS).100, across the sample numbers (samples 356-360)



LC-MS/MS is considered as the reference method for the measurement of $1,25(OH)_2$ Vitamin D. This was emphasized again recently at the 18th Vitamin D Workshop (21-24 April 2015, Delft, The Netherlands). Glenville Jones (Queen's University, Kingston, Canada) and Annemieke Heijboer (VUMC, Amsterdam, The Netherlands) both presented reference LC-MS/MS measurement procedures for the quantification of $1,25(OH)_2$ Vitamin D in human serum or plasma.

Author:
Nicolas Heureux, PhD
Nicolas.Heureux@diasource.be
Principal Scientist – Vitamin D, DIAsource Immunoassays
DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2 | B-1348 Louvain-La-Neuve | Tel : +32.10.84.99.11 | Fax : +32.10.84.99.90
www.diasource.be

However, because of the very low concentration of this molecule and the presence of numerous Vitamin D metabolites at higher concentration, LC-MS/MS methods for the measurement of $1,25(OH)_2$ Vitamin D are very difficult to develop and require last generation expensive state-of-the-art LC-MS/MS equipment. Highly trained engineers are involved in the setup, routine and maintenance of such a method. Moreover, due to the complex extraction and separation procedures the throughput of these analyses is usually rather limited.
The DIAsource $1,25(OH)_2$ Vitamin D RIA assay shows excellent results against the commonly accepted reference method LC-MS/MS.

The DEQAS Certificate of Proficiency is granted if 80% or more of the results fall within ±30% of the ALTM. 90.5% of the last 21 DIAsource results satisfy these standards, which falls within the DEQAS criteria for Certification. The same good result was obtained against the LC-MS/MS method although DEQAS does not recognize this method as the target value yet.

The mean bias over the DEQAS samples 356-360 is +6% when taking the LC-MS/MS method as the reference, and -9% when taking the mean of all methods as the reference. This shows that the DIAsource $1,25(OH)_2$ Vitamin D RIA assay is correctly calibrated and is closer to LC-MS/MS than the other RIA methods.

4. CONCLUSION

The DIAsource $1,25(OH)_2$ Vitamin D RIA assay shows excellent performances in the DEQAS quality control program. The accuracy of the assay was shown to be very good when evaluated against the mean of the other participating methods as well as against the commonly accepted reference LC-MS/MS method. Thanks to its Gold Standard extraction procedure and to its superior specificity profile, the DIAsource $1,25(OH)_2$ Vitamin D RIA even provided closer results to LC-MS/MS than other widely used radioimmunoassays for all five samples.

Author:
Nicolas Heureux, PhD
Nicolas.Heureux@diasource.be
Principal Scientist – Vitamin D, DIAsource Immunoassays
DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2 | B-1348 Louvain-La-Neuve | Tel : +32.10.84.99.11 | Fax : +32.10.84.99.90
www.diasource.be

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

Headquarter
DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2
1348 Louvain-La-Neuve
Belgium
Tel: +32 10849911
Fax: +32 10849990

Customer Service
Tel: +32 10849900
Fax: +32 10849996
Belgium Free Phone: 0800 159 59
France Free Phone: 0800 908 443
France Free Fax: 0800 902 588
Customer.Service@diasource.be

Author:
Nicolas Heureux, PhD
Nicolas.Heureux@diasource.be
Principal Scientist – Vitamin D, DIAsource Immunoassays
DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2 | B-1348 Louvain-La-Neuve | Tel : +32.10.84.99.11 | Fax : +32.10.84.99.90
www.diasource.be