

Technical Review

Performance of the DIAsource 1,25(OH)₂ Vitamin D RIA assay in the DEQAS and Instand Quality Control Programs

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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 and to physical detection methods such as LC-MS/MS. The accuracy of the assay is demonstrated by its excellent performance in the quality control programs DEQAS and Instand e.V.

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. INSTAND e.V.

INSTAND e.V. (Society for Promoting Quality Assurance in Medical Laboratories e. V.) organizes EQA schemes according to the current Guidelines of the Federal Medical Association (Bundesärztekammer) or in corresponding application of these Guidelines respectively. The number of participants to the 1,25(OH)₂ Vitamin D program was 55 in October 2014.

Lyophilized serum pools (2) are distributed quarterly at ambient temperature.

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4. RESULTS

4.1. DEQAS

Figure 1 shows the bias of the DIAsource 1,25(OH)₂ Vitamin D RIA assay against the LC-MS/MS methods for the last 16 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 16 samples was calculated to be -4%.

Figure 1. **DEQAS** - Bias DIAsource versus LC-MS/MS (calculated as $((\text{DIAsource} - \text{LC-MS/MS})/\text{LC-MS/MS}) \cdot 100$, across the concentration range (last 16 samples)

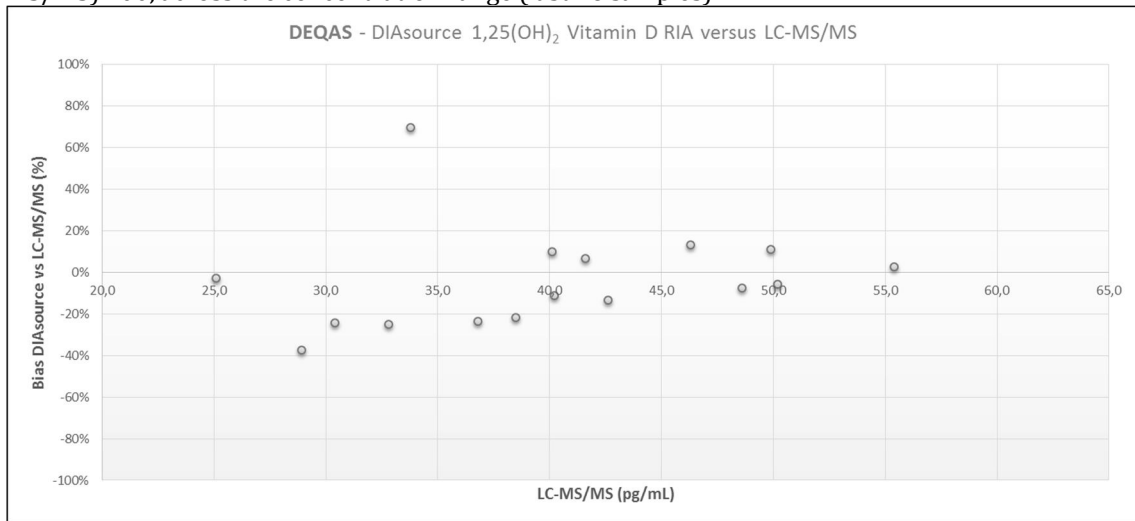
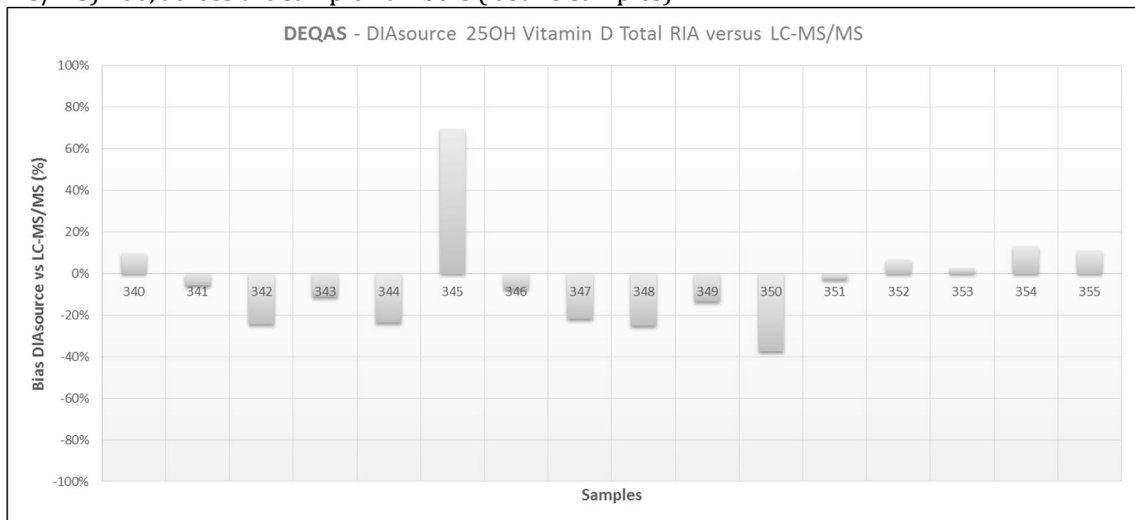


Figure 2. **DEQAS** - Bias DIAsource versus LC-MS/MS (calculated as $((\text{DIAsource} - \text{LC-MS/MS})/\text{LC-MS/MS}) \cdot 100$, across the sample numbers (last 16 samples)



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Figure 3 shows the bias of the DIAsource 1,25(OH)₂ Vitamin D RIA assay against the mean of all the DEQAS participants. Results are presented across the mean concentration range. The same results are shown in Figure 4 across the sample numbers. The mean bias over the 16 samples was calculated to be -12%.

Figure 3. **DEQAS** - Bias DIAsource versus ALTM (calculated as ((DIAsource - ALTM)/ALTM).100, across the concentration range (last 16 samples)

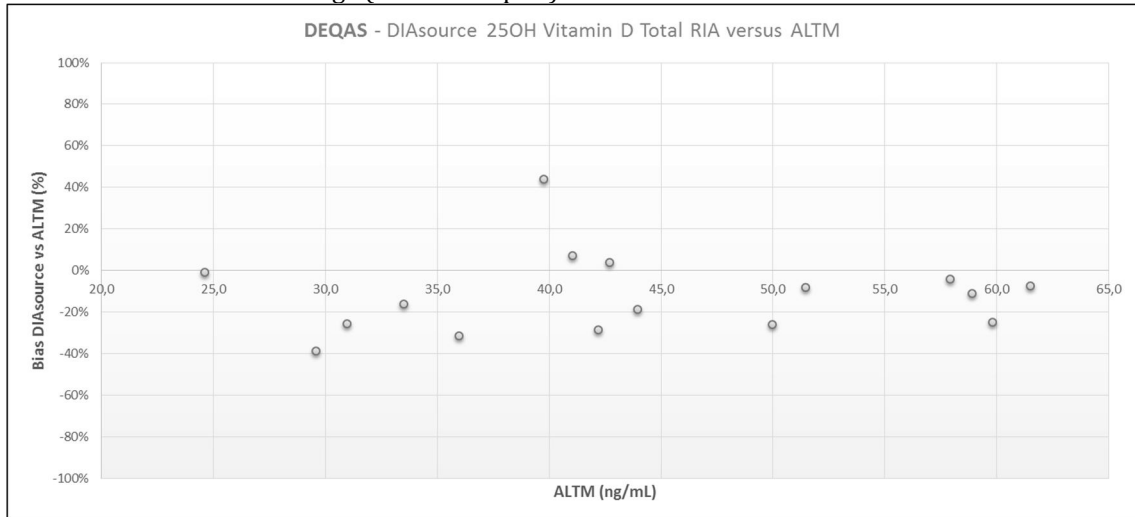
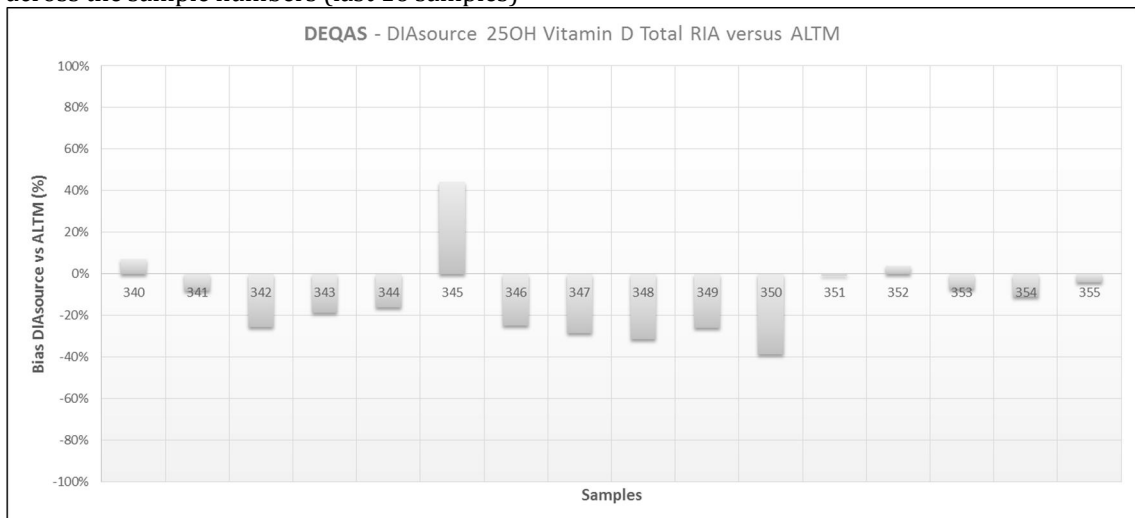


Figure 4. **DEQAS** - Bias DIAsource versus ALTM (calculated as ((DIAsource - ALTM)/ALTM).100, across the sample numbers (last 16 samples)



4.2. Instand

Figure 5 shows the bias of the DIAsource 1,25(OH)₂ Vitamin D RIA assay against the mean of all the Instand participants for available 2013 and 2014 reports. Results are presented across the mean concentration range. The same results are shown in

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Figure 6 across the sample numbers. The mean bias over the 13 samples was calculated to be -2%.

Figure 5. **Instand** - Bias DIAsource versus ALTM (calculated as $((\text{DIAsource} - \text{ALTM})/\text{ALTM}) \cdot 100$, across the concentration range (last 13 samples)

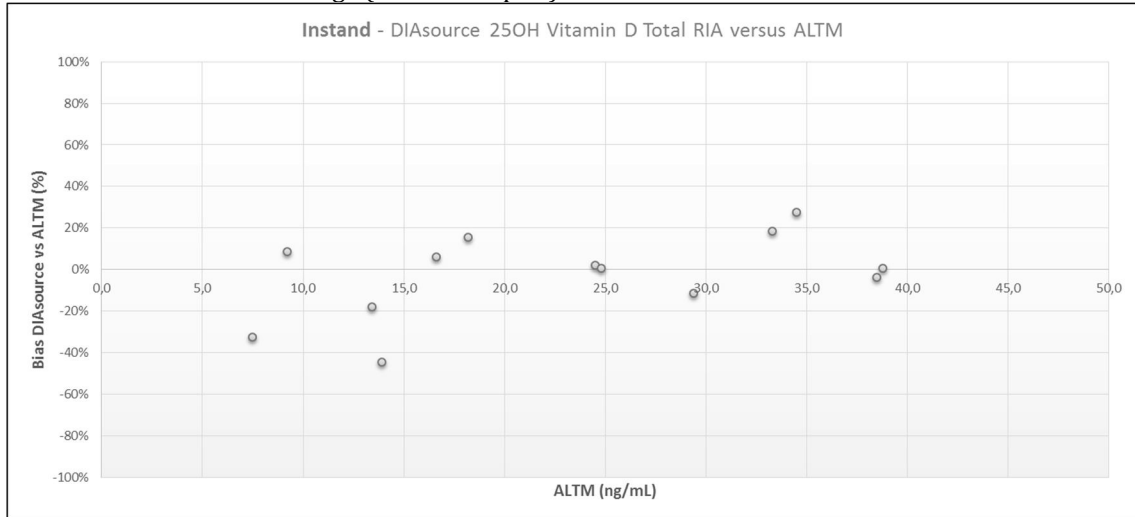
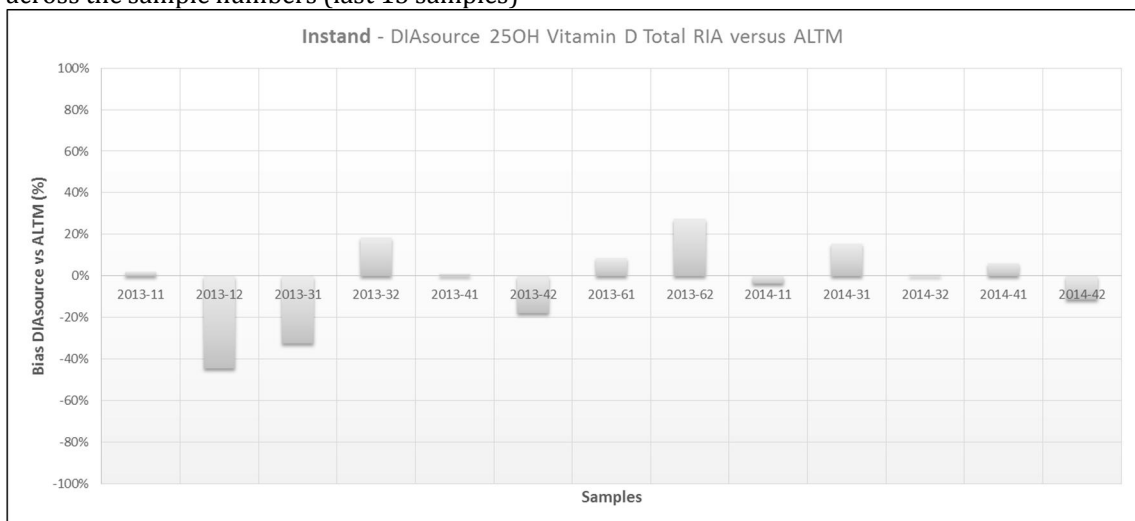


Figure 6. **Instand** - Bias DIAsource versus ALTM (calculated as $((\text{DIAsource} - \text{ALTM})/\text{ALTM}) \cdot 100$, across the sample numbers (last 13 samples)



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5. DISCUSSION

The accuracy of the DIAsource 1,25(OH)₂ Vitamin D RIA assay is evaluated in the DEQAS and Instand quality control programs.

On the contrary to the 25OH Vitamin D scheme, DEQAS does not feature a reference ID-LC-MS/MS method as the target for the measurement of 1,25(OH)₂ Vitamin D, as such a method is not available yet. The All-Laboratory Trimmed Mean (ALTM) is therefore still used to evaluate the accuracy of each individual assay. Although close to the LC-MS/MS method for most of the results, the ALTM results significantly deviate from the LC-MS/MS results for some of the samples (e.g. 345-350). This is the result of the influence of the most weighted methods (namely IDS RIA and iSYS) which might not be as accurate for all the samples. The Instand program also uses the mean of all participants to evaluate the accuracy of the individual methods.

The DIAsource 1,25(OH)₂ Vitamin D RIA assay shows excellent results against the commonly accepted reference method LC-MS/MS, except for the sample 345 where a bias of +70% was observed. Surprisingly most of the other immunoassays also exhibited a large positive bias for this sample, while one method reported a large negative bias. Unfortunately DEQAS did not communicate any specific information about this sample.

The DEQAS Certificate of Proficiency is granted if 80% or more of the results fall within $\pm 30\%$ of the ALTM. 87.5% of the last 16 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 16 DEQAS samples is -4% when taking the LC-MS/MS method as the reference, and -12% 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 most of the other methods.

Similar performances were observed in the Instand program. Unfortunately, this program does not disclose results obtained with the LC-MS/MS method so only the mean of all participants can be used as the target. The Instand standards require that the results of an individual method fall within $\pm 36\%$ of the mean of all participants. The DIAsource 1,25(OH)₂ Vitamin D RIA assay fulfils these criteria for 92% of the last 13 samples.

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6. CONCLUSION

The DIAsource 1,25(OH)₂ Vitamin D RIA assay shows excellent performances in both the DEQAS and Instand quality control programs. 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 immunoassays for several samples.

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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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