

Vitamin D Measurement: Laboratory issues

**Dr Etienne Cavalier
Department of Clinical Chemistry
University of Liège, CHU Sart-Tilman.**

Blood collection tubes

- 25(OH)D assays can be performed on serum or plasma.
- Usually, laboratories encourage clotted blood in tubes containing separating gels
 - ▶ *possible adverse effects of some of the gels constituents on 25(OH)D determination*
 - ▶ ***significant raise of 25(OH)D levels with some HPLC or LC/MSMS methods.***



25(OH)-vitamin D determination: not an easy task...

- Lipophilic nature of the molecule (► non-specific interference from other lipids)
- Necessity to displace the analyte from the VDBP (assumption that it is 100%, but no evidence!)
- Preparation of standards: stock solution in EtOH, impurities, denaturation of the « human » serum for the standard preparation,...
- Erroneous results when 25(OH)D is added in vitro vs. endogenous 25(OH)D (no equilibrium with VDBP)

Results (%) for 25(OH)D inter-assay Imprecision *in our hands*

Level (ng/mL)	Abbot Architect	DiaSorin RIA	LCMS/MS	IDS iSYS	Roche Elecsys (v2)
±15	5.980	3.066	5.635	6.522	7.951
±20	3.571	4.748	3.090	6.001	4.952
±30	2.754	3.865	2.676	5.029	4.104
±40	2.489	4.379	2.548	4.614	2.164
±50	2.732	4.518	3.014	6.795	1.655
±60	2.233	6.086	1.266	6.367	

Target Precision

- Minimal imprecision: $CV_A < 9.75\%$
- Desirable imprecision: $CV_A < 6.5\%$
- Optimal imprecision: $CV_A < 3.25\%$

Percentage of recognition of 25(OH)D2

Method	Percentage of cross-reactivity with 25(OH)D2	95% interval confidence (in %)
Roche Elecsys (v.1)	0.4	-4.2 – 5.0
Chromsystems HPLC	111.6	103.7 – 119.5
IDS EIA	106.6	78.8 – 134.4
IDS RIA	80.7	70.6 – 90.7
IDS iSYS	98.0	86.9 – 109.1
DiaSorin Liaison	93.2	77.5 – 108.8
DiaSorin RIA	83.5	69.3 – 97.7
Diasource	-1.0	-23.1 – 21.2

Conclusions

- Since the last years, 25(OH)D determination has become a « blockbuster » in the routine lab.
- Overall, the situation is not globally so bad, but standardisation and reference techniques are needed (even if we have to re-adapt the cut-offs).
- Clinicians and Laboratory professionals have to be aware of some existing (and new?) pitfalls