

# Evaluation of the Cross-reactivity of 25-Hydroxy-vitamin D2 on Six Commercial Immunoassays on Native Samples.

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**Introduction:** In serum, 25-hydroxy-vitamin D (25(OH)D) can be found in two forms, namely 25(OH)D2 and 25(OH)D3. We recently published a mathematical method to estimate the 25(OH)D2 recovery without spiking the samples. Since then, new “total” vitamin D immunoassays have appeared on the market (Roche “Total” vitamin D, Siemens Centaur vitamin D “total”, “total” vitamin D on DiaSorin Liaison XL, Abbott Architect Vitamin D). We aimed to study the 25(OH)D2 recovery of these new immunoassays and re-evaluate the cross-reactivity of previously studied assays (IDS iSYS Vitamin D and DiaSorin RIA).

**Materials and Methods:** We only used native samples. First, in a group of 19 individuals presenting 25(OH)D3 levels exclusively and ranging from 6 to 90 ng/mL, approximately, we defined the regression equation of each immunoassay method in comparison with the Perkin-Elmer LCMS/MS method run on a AbSciex TQ 5500 instrument. Then, we established the respective concentration of 25(OH)D3 and 25(OH)D2 in the serum of 11 individuals supplemented with various levels of vitamin D2. For each method, with the help of the regression equation previously obtained, we calculated what would be the “immunoassay-equivalent” of 25(OH)D3 based on the LCMS/MS 25(OH)D3 value. We thus subtracted this amount to the “total” value obtained with the respective immunoassay to obtain the 25(OH)D2 “immunoassay-equivalent” concentration. Finally, we divided this amount by the 25(OH)D2 obtained with the LCMS/MS to calculate the recovery.

## Results:

Method	Regression Equation obtained in Individuals with 25(OH)D3 only (PED3=25(OH)D3 obtained with the LCMSMS)	Mean cross-reactivity (%) for 25(OH)D2 (95% CI)
Architect (Abbott)	$1.27 \times \text{PED3} - 6.9$	61.6 (54.3 - 69.0)
iSYS (IDS)	$1.02 \times \text{PED3} - 0.57$	101 (100.6 - 101.6)
RIA (Diasorin)	$0.82 \times \text{PED3} + 3.75$	98 (94.2-102)
Liaison XL (Diasorin)	$0.93 \times \text{PED3} - 1.69$	113 (108-118)
Elecsys (Roche)	$0.80 \times \text{PED3} + 3.41$	101 (97-106)
Advia Centaur (Siemens)	$1.13 \times \text{PED3} - 4.81$	130 (109-150)

The table show the different regression equation and the cross reactivity (95%CI) obtained with the different immunoassays compared LCMSMS considered (arbitrarily) had 100 % cross-reactivity for both D2 and D3.

**Conclusion:** As we previously shown, IDS Isys and Diasorin RIA, present a 100% cross-reactivity with 25(OH)D2. Among the new “total” immunoassay, Roche Elecsys is the only one to present a 100% cross-reactivity. The DiaSorin Liaison XL presents a slight but significant positive bias whereas Centaur and Architect are respectively clearly over and underestimating 25(OH)D2 when compared with the Perkin-Elmer LCMS/MS.