Standardization of 25-hydroxyvitamin D assays: impact of vitamin-D binding protein concentrations and uremic media on the re-standardization of six different 25(OH) vitamin D immunoassays

Etienne Cavalier, Pierre Lukas, Agnès Carlisi, Jean-Claude Souberbielle,
Department of Clinical Chemistry, University of Liège, CHU Sart-Tilman, Liège, Belgium

Introduction:

Different reports have shown the lack of standardization of 25-hydroxy vitamin D assays and have warned of the potential clinical consequences of such a problem. Recently, the Vitamin D Standardization Program (VDSP), led by the NIH in collaboration with the CDC and NIST, have issued a series of 40 single patients whose 25D had been determined by a commonly accepted reference method.

In this study, we assimilated the standardization process in six immunoassays and assessed their harmonization effectiveness in a population of healthy individuals, as well as in other patients presenting some differences in their serum matrix.

Materials and Methods:

•Calibrate the LCMS ChromSystem kit against the VDSP Phase 1 samples [Calibrated LCMS].
•Calibrated the . Architect, Centaur, Elecsys, IDS-iSYS, Liaison XL and DiaSorin RIA against CDC Chrom with 88 sera samples from apparent healthy subjects [Calibration population].
•Adjusted the immunoassays according to the regression equations.
•Verified the harmonization with samples from 1st trimester (n = 32) and 3rd trimester (n = 36) pregnant women, and haemodialysis (n = 28).

Results:

Vitamin-D-binding protein (DBP) concentrations

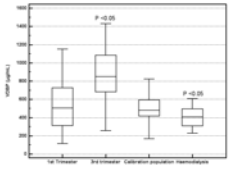

Fig. 1: Vitamin-D-binding protein (DBP) circulating levels.
The VDBP concentration levels were measured using the R&D Systems Human Vitamin D Binding Protein Quantikine ELISA Kit (Minneapolis, MN, USA).

Third trimester pregnant women have the highest DBP circulating levels, 511±167, 410±114, 544±280 and 836±290 µg/mL for the apparently healthy, haemodialysis, first and third trimester, respectively.

25(OH) Vitamin D concentrations bias, before and after adjustment against the common sta

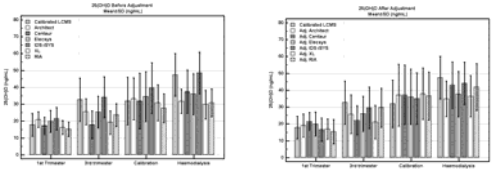

Fig. 2: 25(OH)D Mean (SD) concentration of investigated populations bar plots.

We observed large bias remained after the adjustment, especially in 3rd trimester and haemodialysis samples.

•Mean concentration bias in 3rd trimester samples: -7.0±5.0 (Architect); -10.6±7.2 (Centaur); -6.7±5.2 (Elecsys); -3.2±4.5 (IDS-iSYS); -11.6±5.2 (XL) and -3.1±4.0 (RIA).

•The bias was more pronounced in haemodialysis samples: -12.5±9.5 (Architect); -4.3±13.0 (Centaur); -9.7±10.8 (Elecsys); -3.3±7.6 (IDS-iSYS); -11.0±10.0 (XL) and -5.5±7.6 (RIA).

Passing-Bablok regression and Bland-Altman difference plots

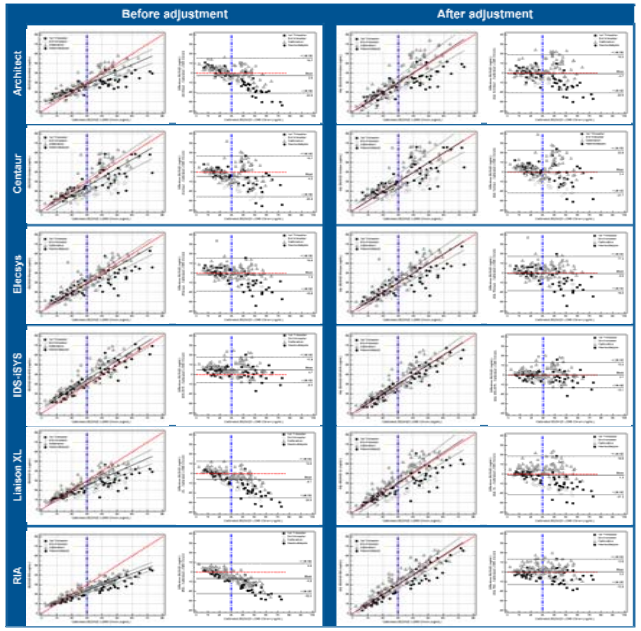

Fig. 3: Regression and difference plot – 25(OH)D immunoassays versus Calibrated LCMS ChromSystem.
The **Blue** reference line represents the cutoff for Vitamin D deficiency, 30 ng/mL; the **Red** reference line is the equality line (x=y or difference = 0).

•Prior to the adjustment, the PB regression slope (95%CI.) between immunoassays and calibrated LCMS of the entire samples cohort (n = 184) varied from 0.59 (0.55 to 0.63) to 0.99 (0.92 to 1.05), with RIA being the lowest and IDS-iSYS being the highest. The difference [Mean±SD (ng/mL)] between LCMS and Architect, Centaur, Elecsys, IDS-iSYS, XL and RIA was: -2.6±9.3, -4.5±10.8, -1.6±8.8, 4.7±6.7, -6.1±9.6 and -6.8±8.0, respectively.

•After the adjustment, the regression slope became more consistent, ranging from 1.00 (0.94 – 1.07) to 1.05 (0.93 – 1.16). Most notable changes were the XL and RIA: 0.70 (before) vs. 1.05 (after), 0.59 (before) vs. 1.03 (after), respectively. The mean difference (ng/mL) was also improved: -0.7±10.2 (Architect); 0.4±11.3 (Centaur); -0.5±9.1 (Elecsys); 0.1±6.8 (IDS-iSYS); -1.2±10.2 (XL) and 0.3±6.7 (RIA).

Conclusions:

•By calibrating the immunoassays against the same patient samples, the harmonization is achieved for the samples from apparent healthy subjects.
•The calibration process appears not to be effective for samples from 3rd trimester pregnant women and haemodialysis patients.
•The influence of vitamin-D binding protein concentrations and uremic media are more visible in some immunoassays than other.