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

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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

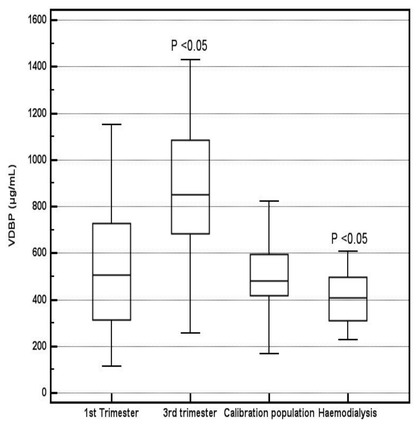

Fig. 1: **Vitamin-D-binding protein (DBP) circulating levels.**
The VDBP concentration levels were measured using the R&D Systems Human Vitamin D Binding Protein Quantikine ELISA Kit (Minneapolis, MN, USA).

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

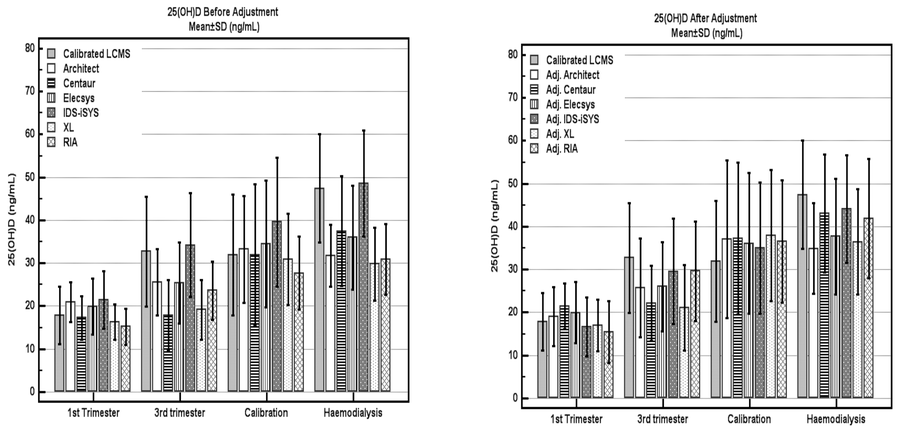

Fig. 2: 25(OH)D Mean (SD) concentration of investigated populations bar plots.

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

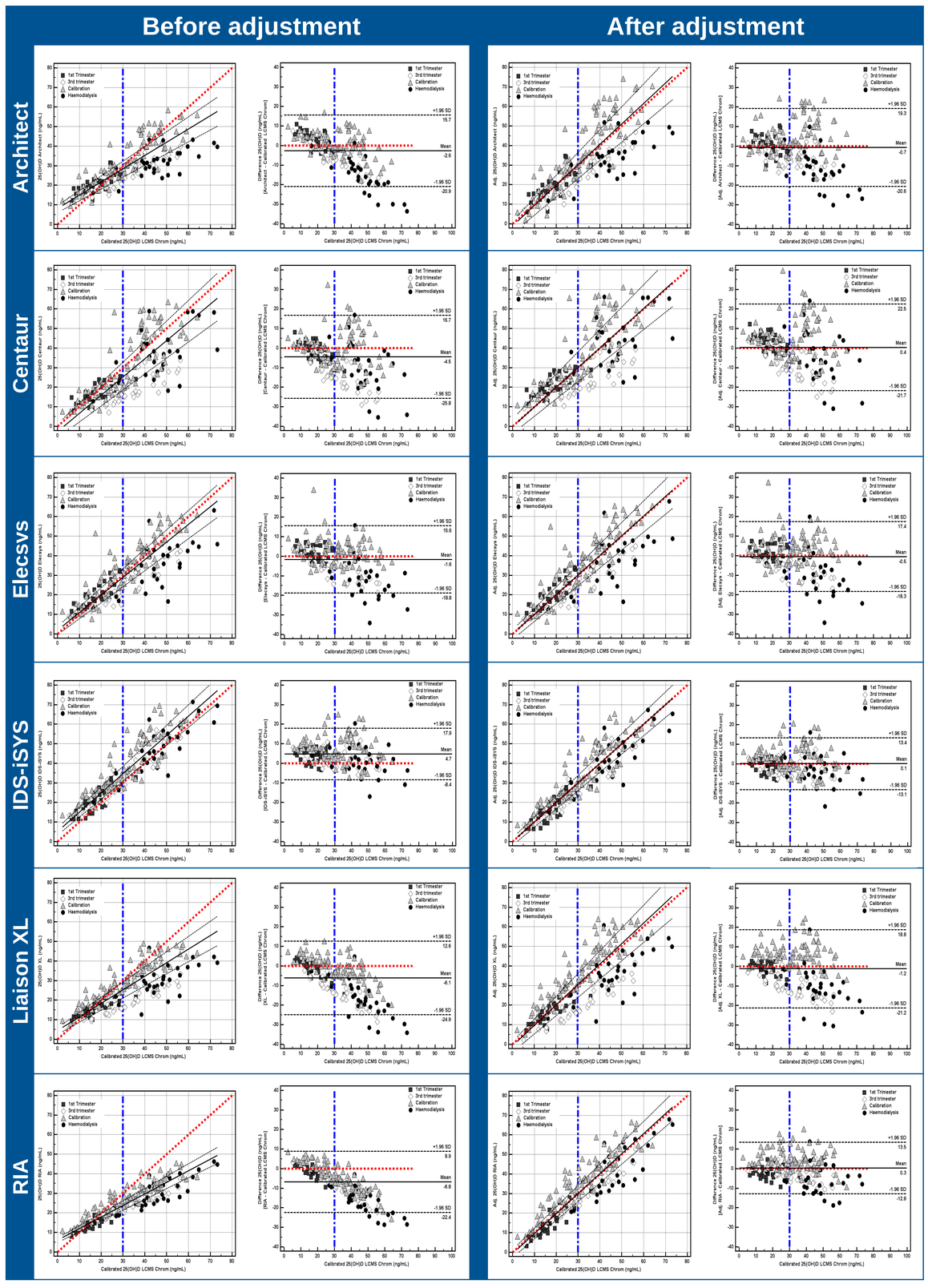

Fig. 3: Regression and difference plot – 25(OH)D immunoassays versus Calibrated LCMS ChromSystem.

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.