Interlaboratory Variation in 25-Hydroxyvitamin D$_3$ and 25-Hydroxyvitamin D$_2$ Is Significantly Improved If Common Calibration Material Is Used

To the Editor:

Measurements of 25-hydroxyvitamin D$_2$ (D$_2$) and 25-hydroxyvitamin D$_3$ (D$_3$) are used to detect vitamin D deficiency and monitor vitamin D supplementation. Interlaboratory variation within the same method groups makes it impossible to apply standardized reference intervals or published clinical cutoff values. Laboratories therefore must develop site-specific reference intervals.

Advances in the routine use of liquid chromatography–tandem mass spectrometry (LC-MS/MS) in clinical chemistry laboratories have enabled the development of methods for measuring D$_2$ and D$_3$ individually (1). Despite the superior performance of LC-MS/MS methods compared with HPLC and immunoassay approaches, an examination of the UK Vitamin D External Quality Assessment Scheme (DEQAS) data for the LC-MS/MS user group indicates that interlaboratory variation exists within this method group.

Calibration of D$_2$ and D$_3$ assays has been a continuing problem because there is no certified reference material available to test method accuracy.

A recent Letter to the Editor in this journal pointed out that calibration is not the only concern with vitamin D assays. Laboratories also do not have common standard operating procedures and use a wide variety of instrumentation (2).

We used commercially available calibration material (Chromsystems, Munich, Germany) to investigate whether calibrator harmonization could improve agreement between laboratories. We sent out 4 calibrators with manufacturer-assigned values along with 16 plasma pools spiked with different D$_2$ and D$_3$ concentrations. The plasma pools were prepared from spare samples of citrated donor plasma and were spiked with a stock solution of D$_2$ and D$_3$ that had been prepared in methanol and serially diluted to cover the analytical range. Aliquots were prepared and stored frozen until distribution by mail. The calibrators were prepared by serially diluting the lyophilized Chromsystems calibrator (lot 1607) into a PBS solution (137 mmol/L NaCl, 10 mmol/L phosphate buffer, 2.7 mmol/L KCl, pH 7.4) containing 10 g/L BSA. The assigned concentrations of the calibrators were 0, 7.17, 10.75, and 64.5 µg/L for D$_2$ and 0, 7.5, 11.25, and 67.5 µg/L for D$_3$.

Six UK laboratories that were running or developing an LC-MS/MS assay for vitamin D agreed to participate in the study. They were sent 200-µL aliquots of the plasma pools and 500-µL of the calibrators along with a brief method questionnaire. Participants were asked to quantify the D$_2$ and D$_3$ concentrations in the plasma pools and calibrators with their routine method and then recalculate the D$_2$ and D$_3$ concentrations in the plasma pools with the calibrators provided.

All 6 laboratories returned results. One laboratory returned results that had been calibrated with 3 different “in house” calibrators. The laboratories that took part in the study used similar sample-preparation procedures, which were based on the procedure outlined by Maunsell et al. (1). A variety of LC-MS/MS instruments and multiple reaction-monitoring transitions were used in the study.

Fig. 1 shows the intervals, interquartile ranges, and means for the 16 plasma pools before and after D$_2$ and D$_3$ harmonization. The overall D$_2$ interval changed from 2–88 µg/L to 2–61 µg/L, and the D$_3$ interval changed from 6–84 µg/L to 6–50 µg/L.

The mean between-laboratory CV for D$_2$ (calculated from the CVs for the individual pools), decreased from 30% with laboratories’ own calibrators to 9% when harmonized calibrators were used (P = 0.006). For D$_3$, the mean between-laboratory CV decreased from 18% with the laboratories’ own calibrators to 7% with harmonized calibrators (P < 0.0001).

Our results demonstrate that the interlaboratory CV improved after the use of a common calibrator. The CV also improved to a value similar to that expected for intraassay imprecision in an individual laboratory. One could...
therefore argue that any further major improvements in the CV from any additional standardization of operating procedures or equipment would be unlikely.

This study highlights the considerable between-laboratory variation in D2 and D3 measurements that can occur, even with highly specific LC-MS/MS technology (the so-called gold standard method). Our results also indicate that agreement between laboratories can be significantly improved if a common calibrator is used; however, we do not claim that the commercially available calibrator we chose to use in this study should be adopted as a reference material.

Nonetheless, the use of common calibrators produces statistically significant improvement with very low between-laboratory variation, even with differences in methods. Harmonization of calibration across laboratories is possible and ultimately may allow not only the use of common reference intervals and decision limits but also comparisons of published data; however, such harmonization urgently requires an international reference standard to facilitate the production of reliable commercial standards and accurate analyses of vitamin D by routine laboratories.

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Fig. 1. D2 and D3 Intervals, interquartile ranges, and means for the 16 plasma pools before and after D2 and D3 harmonization.

(A), Changes in the D2 interval, interquartile range, and mean with the 6 laboratories’ own calibrators (l) compared with assigned-value calibrators (a) across all 16 pools. (B), Changes in the D3 interval, interquartile range, and mean with the 6 laboratories’ own calibrators (l) compared with assigned-value calibrators (a) across all 16 pools.
Letters to the Editor

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References

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Potential Interference by Antineutrophil Cytoplasmic Autoantibodies in Myeloperoxidase Immunoassays

To the Editor:

Myeloperoxidase (MPO)1 has been identified as a potential marker of cardiovascular disease (1). As the clinical utility of new biomarkers is identified and commercial assays are developed, limitations on their use need to be identified and reported. Antineutrophil cytoplasmic autoantibodies (ANCAs) are known to exist in patients with diseases characterized by primary systemic vasculitis, such as Wegner granulomatosis, microscopic polyangiitis, and Churg–Strauss syndrome, and in those with idiopathic pauci-immune necrotizing crescentic glomerulonephritis, systemic lupus erythematosus, and rheumatoid arthritis (2). In particular, the immunofluorescence-staining pattern for perinuclear ANCAs (pANCAs) is associated with anti-MPO autoantibodies. pANCAs are most prevalent in microscopic polyangiitis (40%–80%), followed by Churg–Strauss syndrome (20%–30%) and Wegener granulomatosis (5%–20%).

Autoantibodies can interfere with assays when the autoantibodies bind to analyte epitopes that are similar to those of the monoclonal antibodies used in the immunoassay. Anti-MPO autoantibodies are likely to bind to MPO in the sample and block or partially mask the epitopes necessary for binding to the antibodies used to capture and detect MPO. We evaluated the effect of MPO autoantibodies on the performance of the Cardio MPO™ immunoassay (PrognostiX), which has been cleared by the US Food and Drug Administration. We similarly evaluated an immunoassay under development for the Abbott ARCHITECT® instrument (Abbott Laboratories). Characteristics of these assays have been described previously (3, 4). The 2 assays use different monoclonal antibodies that appear to detect unique epitopes (unpublished data).

We collected samples from 40 healthy donors with informed consent after we obtained institutional review board approval for our protocol. We also obtained 189 surplus pANCA-positive, deidentified patient samples from ARUP Laboratories (the University of Utah institutional review board waived informed consent for these samples). We used native MPO antigen (Athens Research & Technology) for spiking experiments.

We tested the samples from healthy donors without added analyte with both assays and then repeated the experiments after spiking the samples with a known MPO amount. MPO concentrations in samples without added analyte ranged from 44–5550 pmol/L with the ARCHITECT assay and from 161–2353 pmol/L with the CardioMPO assay. The difference between the 2 assays in MPO concentrations for the unspiked samples was possibly due to a lack of standardization. Assignments of calibrator values, matrix effects, and differences in antibody specificity may also account for this difference. Similarly, the amounts of spiked MPO recovered varied, depending on the assay used. The ARCHITECT assay results were more consistent than the CardioMPO results. To account for the difference in recovery and lack of standardization seen in the samples from healthy individuals, we determined the mean recovery for each assay.

1 Nonstandard abbreviations: MPO, myeloperoxidase; ANCA, antineutrophil cytoplasmic autoantibody; pANCA, perinuclear ANCA.