Divergence in Classification of 25-Hydroxyvitamin D Status with Respect to Immunoassays

To the Editor:
The major problem in measuring 25-hydroxyvitamin D (25-OHD) is attributable to the molecule itself (1). Thus, an analytical method must be selected that will accurately estimate total circulating 25-OHD independent of the circulating concentrations of 25OHD$_2$ and 25OHD$_3$. Although HPLC remains the method of choice, many convergent data indicate that the 25-OHD RIA (DiaSorin Inc) should be incorporated as a secondary reference method (2, 3). With the RIA method actually used in our laboratory, we evaluated the 25-OHD LIAISON$^\text{®}$ chemiluminescent immunoassay (CLIA) proposed by the same manufacturer as an alternative assay. We also investigated whether a 25-OHD value $<$50 nmol/L, a well-known arbitrary RIA cutoff value to define vitamin D insufficiency, is applicable to CLIA.

We studied 199 people [79 men/120 women; mean (SD) age 48.1 (19.8) years] who were residents of Paris and its suburbs and who suffered primary hyperparathyroidism (HPT), secondary HPT with hemodialysis or obesity, mild asthenia, depression, and/or osteopenia/osteoporosis. This study was approved by the ethics committee and study participants gave written informed consents. Blood samples were collected and centrifuged at 4°C for 10 min with a force of 950g, and serum aliquots were frozen at $-80^\circ$C. All samples were assayed during the same day using assay reagents from the same lot. None of the samples was frozen and thawed repeatedly. Samples were assayed either in duplicate for RIA (3), or singly for CLIA (4), according to manufacturers’ instructions. With the RIA method, the intra- and interassay CVs were, respectively, $<$15.0% and $<$17.0% at a mean concentration of 9 nmol/L, $<$10.0% and $<$11.0% at a mean concentration of 30 nmol/L, and $<$7.5% and $<$11.0% at a mean concentration of 150 nmol/L. With the CLIA method, the intra- and interassay CVs were, respectively, 15.5% and $<$18.0% at a mean concentration of 19 nmol/L, 12.6% and 14.6% at a mean concentration of 33 nmol/L, and 4.0% and 4.5% at a mean concentration of 117 nmol/L. The relation between both methods was calculated by the equation of the regression analysis (CLIA = 0.99 RIA + 5.77 nmol/L). A clear dispersion of 25-OHD values started at 27.5 nmol/L, as randomly sampled
highlighted by the insert presented in Fig. 1A. The Bland-Altman plot (Fig. 1B) confirmed this random tendency, which was independent of concentration and of sex, and also demonstrated an amplified dispersion at 25-OHD concentrations >50 nmol/L.

Our data (Fig. 1B) suggested that CLIA results tended to be higher than RIA at low and high concentrations, conversely to data previously published (4). With a threshold of 25-OHD ≥30 nmol/L but <50 nmol/L to define vitamin D insufficiency, in 33% of the 54 individuals with 25-OHD concentrations classified as insufficient by RIA, 25-OHD concentrations were normalized by CLIA (range: 53.0–109.5 nmol/L). In contrast, 21% of the 62 study participants with 25-OHD concentrations ≥50 nmol/L by RIA had insufficient 25-OHD concentrations by CLIA (range: 17.5–48.0 nmol/L). For optimal serum 25-OHD concentration defined as >75 nmol/L in osteoporotic patents, 35% of the 23 patents with 25-OHD concentrations above this threshold by RIA had concentrations below it by CLIA. The RIA method used a primary antibody to 25-OHD in a homogenous phase with a 2nd antibody used as precipitating agent, whereas CLIA used the same primary antibody immobilized onto coated magnetic particles. This antibody interacts differently with the first calibrator [i.e., 17.5 nmol/L (CLIA); 12.5 nmol/L (RIA) with an optional calibrator of 6.25 nmol/L (B/B0: 91%) created by diluting 12.5 at 1:2 as suggested by the manufacturer], indicating different affinity profiles that are probably responsible for these random results. These divergent results may also be attributable to different calibrators, constituted in either human- (RIA) or horse-based serum (CLIA), different incubation times (90 min by RIA vs 30 min by CLIA), or an insufficient quantity of reagents used to dissociate 25-OHD from its binding protein.

Overall, these 2 methods did not similarly classify individuals with respect to well-known arbitrary cutoff values. The random tendency observed whatever the concentrations measured did not permit the definition of a clear strategy concerning patient follow-up, particularly for those needing treatment with respect to their vitamin D status. The discrepancy between these 2 methods is consistent with either important negative (4, 5) or positive (our data) intercepts traducing differences in the assay response to the calibrant matrix.

Finally, our results are consistent with the poor correlation previously reported between RIA and CLIA (5) and demonstrate that in disagreement with recently published data (4), a 25-OHD value <50 nmol/L used to define vitamin D insufficiency with the DiaSorin RIA is not suitable for use with the LIAISON® assay (CLIA).

References


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DOI: 10.1373/clinchem.2006.080903

High Total Protein Impairs Appropriate Gel Barrier Formation in BD Vacutainer Blood Collection Tubes

To the Editor:

Many laboratories perform routine chemistry analysis with serum or plasma–based blood collection tubes containing separator gels. A barrier polymer is present at the bottom of the tube. The density of the material causes it to move upwards during centrifugation to the supernatant–cell interface, where it forms a barrier separating plasma or serum from cells. Supernatant plasma or serum may be aspirated directly from the collection tube, eliminating the need for transfer to a secondary tube.

We recently observed 2 occasions within 1 month when both the ion-selective electrode and chemistry sampling probes of the analyzer (Modular Analytics, Roche Diagnostics) were occluded. In both cases, the occlusion was caused by inappropriate gel barrier formation after centrifugation (2000g for 10 min at room temperature) of the primary tubes. Plasma (BD Vacutainer® PST™ II) and serum (BD Vacutainer® SST™ II) samples had been collected from 2 patients diagnosed with multiple myeloma. In the plasma tube, the gel barrier material was floating on the surface of the supernatant, and in the serum tube the gel barrier was entwined with the serum and erythrocytes (Fig. 1). Analysis of blood samples from both patients in plain serum tubes showed highly increased total protein concentrations (139 and 142 g/L; reference interval 60–80 g/L) caused by the presence of a monoclonal-protein (an IgG-κ of 89 g/L and an IgA-κ of 92 g/L, respectively). Furthermore, plasma viscosity values were 5.7 and 7.1 centipoise, respectively, (reference interval 1.5–2.0 centipoise) and specific gravities, as measured by weighing 500 μL of plasma or serum, were 1.037 and 1.039, respectively.

The positioning of the gel in the tube is influenced by a number of variables, some of which are con-