Inaccurate 25-Hydroxyvitamin D Results from a Common Immunoassay

To the Editor:
The great increase in the volume of vitamin D testing over the past several years has created a demand for high-throughput vitamin D testing methods (1). Our laboratory instituted 25-hydroxyvitamin D testing on the DiaSorin LIAISON® platform in October 2009. Soon afterward, some samples were noted not to produce the expected result after dilution with 1 volume of the manufacturer’s dilution buffer (i.e., 2-fold dilution). We investigated the frequency of such occurrences, whether the results obtained with diluted or undiluted samples were more consistent with liquid chromatography–tandem mass spectrometry (LC-MS/MS)1 results, and whether the cause of the nonlinear dilution might be due to interfering heterophile antibodies.

25-Hydroxyvitamin D analysis and dilutions were performed on the DiaSorin LIAISON instrument according to the manufacturer’s instructions. 25-Hydroxyvitamin D results for diluted (2-fold) and undiluted samples for 928 serum samples (424 consecutive samples in October 2009 and 504 consecutive samples in September 2011). In addition, between October 7, 2009, and August 5, 2011, we repeated the 25-hydroxyvitamin D analyses for 3475 undiluted samples with results >50 μg/L after diluting these samples 2-fold. Results were determined as discrepant if the results >50 μg/L for undiluted samples differed from the results for the corresponding diluted samples by ≥20%, or if results <50 μg/L for undiluted samples differed from the results for corresponding diluted samples by >20 μg/L. All discrepant samples that we identified during this time were analyzed by LC-MS/MS at Mayo Medical Laboratories without any sample dilution (2). To determine if the discrepant results were caused by heterophile antibody interference, we added 500 μL of patient sample (n = 11) to a Scantibodies Laboratory Heterophilic Blocking Tube (HBT) and incubated the tube for 1 h before measuring 25-hydroxyvitamin D on the LIAISON instrument.

Of 424 consecutive samples analyzed (undiluted and diluted 2-fold) in October 2009, 22 (5.19%) were discrepant. Similarly, we identified 24 discrepant results (4.76%) among 504 consecutive samples from September 2011. During both time periods, the results for these 46 samples showed that the values obtained for the undiluted sample was higher than that for the diluted sample [mean difference in 2009, 88.27% (SD, 45.19%) (P < 0.0001, paired t-test); mean difference in 2011, 42.85% (SD 36.9%) (P < 0.001)]. Given that the vast majority of samples showed no difference between the diluted and undiluted samples, we concluded that the differences for these 46 samples were not due to a matrix effect caused by the manufacturer’s dilution buffer.

The results of the October 2009 study prompted us to take all samples from October 2009 to August 2011 that had 25-hydroxyvitamin D results >50 μg/L for undiluted samples and to repeat the analyses of these samples after diluting them 2-fold. Of 3475 samples with results >50 μg/L, 537 (15.57%) of the undiluted and diluted samples showed discrepant results. Undiluted aliquots of all the discrepant samples were sent to Mayo Medical Laboratories for LC-MS/MS analysis. Fig. 1A depicts the results for 100 consecutive discrepant samples for the period between May 1, 2011, and August 5, 2011, demonstrating that the undiluted results were a mean of 27 μg/L (SD, 24.4 μg/L) higher than the LC-MS/MS result [mean difference, 74.3% (SD, 78.9%); P < 0.0001]. Interestingly, the results for the samples diluted 2-fold matched the LC-MS/MS results (Fig. 1B) [mean difference, 0.43 μg/L (SD, 11.0 μg/L); P = 0.6962], confirming that the results for the undiluted samples were falsely increased.

To investigate whether heterophile antibody interference was a cause for the discrepant results, we incubated 11 consecutive discrepant samples (between September 7, 2011, and September 15, 2011) in Scantibodies HBT tubes. This method, which uses a proprietary mix of lyophilized heterophile “blockers,” was chosen because it does not introduce any sample dilution. Results from HBT-treated samples did not differ significantly from the corresponding results for untreated undiluted samples [mean difference, 1.15 μg/L (SD, 10.65 μg/L); P = 0.7427]. Our investigation demonstrates that the DiaSorin LIAISON assay overestimates the 25-hydroxyvitamin D concentration in undiluted samples in approximately 5% of cases, which could lead to an underidentification of vitamin D-deficient individuals. This effect does not appear to be due to heterophile antibody interference, which others have suggested as a cause of some erroneous LIAISON 25-hydroxyvitamin D results (3). A potential cause for the discrepancy between these 2 studies is that samples in the present study were absorbed with a lyophilized blocking reagent that did not dilute the sample. The use of a liq-

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1 Nonstandard abbreviations: LC-MS/MS, liquid chromatography–tandem mass spectrometry; HBT, Heterophilic Blocking Tube.
uid absorption reagent may have corrected results simply via dilution. A potential weakness of our study is that we had no positive controls for the HBT absorptions. Nevertheless, our data demonstrate that a simple 2-fold dilution provides results comparable to those obtained by LC-MS/MS, but this approach does double reagent costs.