Serum 25-OH Vitamin D2 and D3 are Stable under Exaggerated Conditions

To the Editor:

Vitamin D analysis is increasingly performed with HPLC–tandem mass spectrometry instead of RIA, and hence samples are frequently sent to more specialized centers for analysis. We therefore investigated the stability of both 25-OH vitamin D3 (25-OH D3) and 25-OH vitamin D2 (25-OH D2) in serum stored under extreme conditions that would likely exceed those normally encountered in sample transit and storage.

After our study received ethics committee approval, we collected 200 mL of blood from a single donor who had given written informed consent and had been previously been determined to have adequate concentrations of 25-OH D3. After centrifugation of the blood sample the serum was removed and supplemented with 50 nmol/L of authentic 25-OH D2 to boost endogenous concentrations. We then transferred 1.0-mL portions into 5-mL clear polystyrene tubes. The sample-containing tubes were capped, stored at −20 °C, and subsequently subjected to various treatments in replicates of 5. These treatments included multiple freeze-thaw cycles (1 to 5 cycles), 8 days at ambient temperature under various conditions, and brief exposure to artificial ultraviolet light. The ambient-temperature storage conditions were as follows: 1 set of 5 samples was left on the laboratory bench uncovered and exposed to fluorescent light and diffuse sunlight for 8 days at 20 °C;
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2 sets of 5 samples were left indoors, inside the author’s solarium, with 1 set uncovered and exposed to direct sunlight and the second set covered and protected from sunlight, at temperatures ranging between 30 °C in the day and 5 °C at night; and 2 sample sets of 5 were left outdoors (outside the building), with 1 set uncovered and exposed to direct sunlight and the other set covered and protected from sunlight, with similar temperature ranges. Sample exposure to artificial ultraviolet light was for 4 periods of 30 min, 50 cm from an ultraviolet lamp used for sterilization within a biohazard hood.

Vitamin D was analyzed after hexane extraction and reconstitution in 70% aqueous methanol by HPLC–tandem mass spectrometry. We used a Shimadzu 20 series HPLC system with a C8 reversed-phase column coupled to an API 3200 Q-trap tandem mass spectrometer under conditions similar to those described previously (1). The calibrator range for 25-OH D2 and 25-OH D3 was 5–320 nmol/L. The internal standard was [2H6]-25-OH D3 (20 ng), and the positive ion transitions were m/z 413.3/395.4 for 25-OH D2, m/z 401.3/383.4 for 25-OH D3, and m/z 407.3/389.4 for [2H6]-25-OH D3.

All samples were analyzed in the same assay, and the within-run CV for the QC material was 10.2%. Results for 25-OH D3 and the total 25-OH-vitamin D (D2 + D3) are shown in Fig. 1. One-way repeated measures of ANOVA were used to determine whether any treated sample group was significantly different from the others. Uncovered samples at ambient temperatures that had been subjected to 8 days of treatment either inside or outside with exposure to direct sunlight and variable temperature were found to have significantly lower amounts of both 25-OH D3 (P = 0.012) and 25-OH D2 (P = 0.002) compared to all other treated samples, which were themselves not significantly different. Covered samples at ambient temperatures that were stored either inside or outside were unaffected, indicating that it was the prolonged exposure to direct sunlight that affected both 25-OH D3 and 25-OH D2 concentrations in the samples.

In a study in which samples were exposed to up to 11 freeze-thaw cycles, no detrimental effects were observed for 25-OH D3 and 25-OH D2 measured with a binding assay (2), although these conditions may affect the specificity of the results. Similarly, concentrations of 25-OH vitamin D, measured with 2 different immunoassays, were found to be unaffected in samples exposed to multiple freeze-thaw cycles (3, 4). Here we addressed the question of 25-OH D3 and 25-OH D2 stability determined by mass spectrometry. We found that under extreme conditions both 25-OH D3 and 25-OH D2 are exceptionally stable, except when subjected to prolonged unprotected exposure to direct sunlight. However, serum samples for 25-OH D3 and 25-OH D2 analysis would normally be shipped inside containers that would protect them from direct sunlight and therefore would require no special transport or storage considerations other than those required by regulatory agencies. Our findings should be useful to laboratories that send samples to specialized centers for 25-OH D3 and 25-OH D2 analyses.

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References


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