To the Editor:

Increased plasma homocysteine is regarded as a risk factor for arterial and venous occlusive disease. Sample collection ideally requires use of cooled EDTA containers and immediate centrifugation of the blood (1). These conditions may be hard to maintain in a clinic and are not possible in the many medical practices that lack a centrifuge. We tested whether analyzing uncentrifuged EDTA or NaF/heparin plasma bears a greater risk of giving false-positive homocysteine results under these conditions.

Blood was drawn from 50 healthy volunteers (40 female and 10 male) into 1 EDTA and 4 NaF/heparin Vacutainers (Becton Dickinson), which were stored at room temperature. The Vacutainers were randomly assigned to groups—EDTA-15min, NaF/heparin-15min, NaF/heparin-24h, NaF/heparin-48h, and NaF/heparin-144h—and centrifuged at 2700g for 7 min under cooling after the time stated in the group name. Homocysteine was analyzed by liquid chromatography–tandem mass spectrometry, as described by Arndt et al. (2). Statistical analyses (box-plots and ANOVA) were done with the Analyze-it software (Analyze-it Software Ltd.), setting significance at \( P < 0.05 \).

We found (a) a significantly greater mean homocysteine concentration in EDTA-15min plasma (11.5 \( \mu \text{mol/L} \)) than in NaF/heparin-15 min plasma (9.5 \( \mu \text{mol/L} \)) and (b) a significant increase in homocysteine in NaF/heparin-15min (9.5 \( \mu \text{mol/L} \)) compared with NaF/heparin-24h (11.5 \( \mu \text{mol/L} \), which is equal to the EDTA-15min value) but no further increase with greater time gaps between blood sampling and centrifugation for NaF/heparin blood (Fig. 1).

Our data show higher homocysteine results in EDTA-15min plasma than in NaF/heparin-15min samples. Plasma dilution by NaF-induced water loss from the erythrocytes has been reported (1, 3). Hughes et al. (3) found lower mean homocysteine concentrations in NaF/heparin plasma stored at ambient temperature than in EDTA blood stored at 0 °C; however, after ~3 h, the difference in homocysteine concentrations was no longer significant (3). We conclude that NaF/heparin blood stored for at least 3 h at ambient temperature shows homocysteine concentrations comparable to those from EDTA blood centrifuged immediately after blood drawing and centrifugation.

We found a prolonged time interval of 48 h (and 144 h) between blood sampling and centrifugation, which is common under routine conditions, is not accompanied by a further homocysteine increase in NaF/heparin plasma (Fig. 1). In contrast, EDTA blood shows a steady increase in plasma homocysteine reaching 380% after 168 h (4).

We conclude that in the absence of a centrifuge, NaF/heparin blood can be used for homocysteine analysis without the need for specific reference values. Therefore, we consider NaF/heparin blood to be a suitable material for homocysteine measurements in hospitals with commonly delayed sample transport to the in-house or reference laboratory or at medical practices without a centrifuge.

References


2. Arndt T, Guissregen B, Hohl A, Heicke B. Total plasma homocysteine measured by liquid chromatography–tandem mass spectrometry with
Measurement of 25-Hydroxyvitamin D by the Nichols ADVANTAGE, DiaSorin LIAISON, DiaSorin RIA, and Liquid Chromatography–Tandem Mass Spectrometry

To the Editor:

Vitamin D [ergocalciferol (D2) and cholecalciferol (D3)] is 25-hydroxylated by the liver and subsequently hydroxylated by the kidney to form 1,25-vitamin D, the active form of the vitamin. 25-Hydroxyvitamin D (25-OH-vitamin D) and 1,25-dihydroxyvitamin D have important effects on calcium absorption, bone calcium balance, and renal excretion of calcium and phosphorus (1). Assessment of the monohydroxy form of vitamin D is important for identifying insufficient endogenous synthesis disorders that impair gastrointestinal absorption and to identify renal or hepatic abnormalities (2).

Here we report on 25-OH-vitamin D as measured by the Nichols ADVANTAGE® 25-OH Vitamin D, DiaSorin LIAISON® 25-OH Vitamin D, DiaSorin 25-OH Vitamin D RIA, and liquid chromatography–tandem mass spectrometry (LC-MS/MS; Mayo Clinic). For measurement of vitamin D, the Nichols ADVANTAGE and DiaSorin LIAISON use a 2-site chemiluminescence assay; however, one important difference is that the Nichols ADVANTAGE relies on binding of vitamin D to a vitamin-D–binding protein purified from human sources and the DiaSorin LIAISON uses an antibody against 25-OH-vitamin D.

Problems have emerged with the Nichols ADVANTAGE 25-OH-vitamin D method. In patients supplemented with ergocalciferol (D2) to improve their vitamin D concentrations, the Nichols ADVANTAGE failed to consistently show the expected increase in vitamin D (3). Ergocalciferol (Drisdol; Sanofi Pharmaceutical) is the only vitamin D2 pharmaceutical preparation available for physicians in the United States to use as a vitamin D supplement; it is extracted from plants or fungi and is processed in the body to 25-OH-vitamin D2 and 1,25-dihydroxyvitamin D2. The DiaSorin LIAISON was shown not to underestimate the D2 concentration (4).

To further analyze the various methods, we measured vitamin D from 110 deidentified samples (Institutional Review Board no. 7740), using the Nichols ADVANTAGE, DiaSorin LIAISON, and a manual DiaSorin RIA. Vitamin D measured by the Nichols ADVANTAGE and DiaSorin LIAISON gave a Deming regression of $y = 1.04x + 4.2 \, \mu g/L$ (Fig. 1).

The DiaSorin RIA vs the Nichols ADVANTAGE gave a Deming regression of $y = 1.42x + 8.39 \, \mu g/L$, and the DiaSorin RIA vs the DiaSorin LIAISON gave $y = 1.37x + 9.64 \, \mu g/L$.

Eight samples with significant variation in vitamin D values between the Nichols ADVANTAGE and the DiaSorin LIAISON were measured by LC-MS/MS. Deming results were $y = 0.36x + 14.23 \, \mu g/L$ for LC-MS/MS vs the Nichols ADVANTAGE; $y = 1.22x - 2.97 \, \mu g/L$ for LC-MS/MS vs the DiaSorin LIAISON; and $y = 0.76x - 8.96 \, \mu g/L$ for LC-MS/MS vs the DiaSorin RIA.

For 3 of the samples, the Nichols ADVANTAGE yielded values <40% of those obtained by LC-MS/MS. Although the LIAISON gave slightly higher values than LC-MS/MS, there was good agreement with LC-MS/MS for those 8 samples. For pa-