Addition of 3-Deazaadenosine to Vacutainer Tubes Stabilizes Whole-Blood Homocysteine for At Least 6 Hours at Ambient Temperature

To the Editor:

Increased plasma homocysteine is now well established as a risk factor for cardiac, cerebral, and peripheral vascular disease (1). Despite this, the test is not widely available. This is partly because of practical difficulties in sample collection. The homocysteine concentration increases in whole blood by up to 10% per hour unless samples are kept on ice and separated within 60 min.

The introduction of containers with 3-deazaadenosine (3-dad; DS30 Homocysteine Blood Collection Tubes; Drew Scientific Ltd.), described in a recent report (2) in the Journal, may solve the problem, but they are not in widespread use. Furthermore, we feel that stabilization that requires both special tubes and refrigeration of samples has limited practical value, as transportation requires both special tubes and refrigeration of samples. Thus, we feel that stabilization of plasma homocysteine was measured after derivatization with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBDF) and separation of thiols by isocratic reversed-phase HPLC (4).

Plasma homocysteine concentrations in samples collected into saline and left to stand at room temperature for 6 h showed a mean (SD) increase of 3.2 (0.9) μmol/L compared with samples separated immediately (P < 0.01, Mann–Whitney). In contrast, samples collected into 3-dad showed no increase in plasma homocysteine concentration over 6 h whether the samples were left at room temperature [−0.4 (0.3) μmol/L; P = 0.76] or incubated on ice for 6 h [−0.3 (0.9) μmol/L; P = 0.90].

The process described is a simple and practical means of preparing tubes for homocysteine estimation that prevents the marked in vitro increase seen in unseparated blood. It requires no special equipment, and tubes can be prepared as and when required. It should be noted that blood collected in this was unsuitable for analysis by assays that convert homocysteine to S-adenosylhomocysteine (5).

References


On the Comparison of Serum and Plasma Samples in Troponin Assays

To the Editor:

With the emphasis on rapid turnaround time as a crucial predicate requirement for cardiac marker measurements, the use of plasma instead of serum samples is desirable because use of plasma eliminates the need for sample clotting (1). Dorizzi et al. (2) have discussed in a recent letter possible problems associated with the use of various types of samples in different generations of the Dade Behring Dimension RxL assay for cardiac troponin I (cTnI). Their main message, useful for the Journal’s readers, is that both serum and heparin-plasma samples are suitable for cTnI determinations in the first and second generations of the assay. In our opinion, their presentation and interpretation of their results are questionable.

The figure presented by Dorizzi et al. (2) contains some conceptual and graphical errors. Because serum is the matrix of choice for the Dimension assay, this type of sample must be considered the reference sample and assumed to have negligible error. Consequently, the serum values should be used on the abscissa of bias plots, rather than the serum/plasma mean. The same, i.e., serum cTnI value, should be placed in the denominator for calculating the bias, in percentage, reported on the y axis. Furthermore, there is no correspondence between the mean bias reported in the text and that displayed in the figure. Dorizzi et al. state in the text: “… the second-generation assay had a mean bias for [heparin]
plasma samples of −11.4%. . . " but Fig. 1B shows the opposite, with a mean bias of +14.5% and a scatter of +10 to +100% higher values in seven samples having a cTnI value of −1.0 μg/L, the clinically most important concentrations. Perhaps the authors inverted the factors (plasma – serum values in the text and serum – plasma values in the figure), but they did so without any reason, and this fact markedly clouds the data presentation. Finally, the same scale should be used for the x axis in all of the graphs to permit a direct comparison of the results obtained with the two assay generations.

More importantly, the authors make improper use of the recommendations reported in the quoted letter by Dewitte et al. (3) and in the corresponding response by Altman and Bland (4). The reference to the method comparison issue is questionable, the anticoagulant problem being indeed an interference (and preanalytical) issue (5). Nevertheless, the original suggestion by the previous authors was to take logarithms of the original data or, alternatively, the percentage bias, both of which give similar information (4). Accordingly, reporting the data as the percentage difference on the y axis and serum cTnI concentrations on the x axis is the correct and more transparent approach to evaluate possible anticoagulant effects on cTnI assays and the variability among samples (6).

Another limitation of the study is that at least two samples used in the second experiments had cTnI concentrations <0.02 μg/L, the detection limit of the assay (7). The use of samples having undetectable cTnI concentrations makes calculation of the sample difference impossible. This could have significantly biased the results obtained with the second-generation assay. Finally, Dorizzi et al. (2) do not provide any information on the time of sampling after hospital admission (or onset of chest pain) of the patients studied, the assay cutoff value(s), or the analytical imprecision at the cutoff value(s) (8). Time-dependent losses of troponin immunoreactivity have clearly been shown in heparin plasma by previous authors (9).

In conclusion, the study by Dorizzi et al. (2) shows that the data obtained with the second-generation Dimension assay are significantly different from those obtained previously with the first-generation assay; the scatter was very different (although they do not indicate standard errors of estimates), and the statistical test (and the 95% confidence interval of the mean bias) evaluating the serum/plasma difference reached significance for the second-generation assay. Thus, the data presented by these authors do not support their claim that "heparinized plasma and serum samples appear interchangeable in cTnI measurement with the second-generation Dimension RxL".

References


To the Editor:

S-100B protein is a 21-kDa dimeric protein from the S-100 calcium-binding protein family that contains at least one β subunit. S-100B is expressed by cells of neuroectodermal origin, particularly cerebral glial cells (astrocytes), and in several tumor processes, including malignant melanocytic lesions (1). Although S-100B is localized primarily in the intracellular compartment, it is physiologically detectable in biological fluids (cerebrospinal fluid, serum, and urine). Measurement of S-100B has been proposed as a biological marker of brain damage, e.g., head injury, cerebral hypoxia, and stroke (2), and of malignant melanoma (3). Serum S-100B values in healthy individuals range from 0.02 to 0.15 μg/L, as determined by immunoluminometric analytical methods. Although studies are controversial, S-100B concentrations in biological fluids appear to be age- and sex-dependent (4–7). No information is available regarding potential differences according to the race or ethnicity of individuals.

Here we report the results of a study to determine the influence of race on serum S-100B concentrations. Blood samples were taken from 136 healthy individuals, divided into three groups according to race: black (B), Asian (A), and Caucasian (C). All individuals gave informed consent for participation in the study. The three groups did not differ significantly in sample size (n = 46, 44, and 46 for groups B, A, and C, respectively), mean (SD) age [45 (11), 49 (13),