Measurement of Creatinine in Whole Blood Samples Supplemented to Achieve Increased Creatinine Concentrations

To the Editor:

During clinical trials of a new biosensor for measurement of whole blood creatinine, we were unable to obtain sufficient quantities of samples with naturally occurring high creatinine concentrations to validate performance of the sensor across the proposed reportable concentration range for the device [18–1326 μmol/L (0.2–15.0 mg/dL)]. Addition of creatinine to whole blood samples was required to validate performance of the sensor at the high end of the range. We noticed irreproducible results for samples that were supplemented with creatinine to increase the concentration. Like other direct-reading electrochemical biosensors (1), creatinine sensors respond to the molality of creatinine in the sample (amount of creatinine per unit mass of water). It is known that molality of creatinine in erythrocyte fluid is equal to molality in plasma (2) and that creatinine is transported by passive diffusion through the lipid bilayer of the erythrocyte membrane (3).

Heparinized blood from a healthy volunteer was centrifuged and the separated plasma supplemented with creatinine to target concentrations of 442 and 1061 μmol/L (5 and 12 mg/dL). We measured the creatinine concentrations in the plasma samples with and without added creatinine by use of the Vitros DT 60 II analyzer (Ortho Clinical Diagnostics). Red blood cells were added back to the supplemented plasma to obtain hematocrit values approximating 20%, 40%, and 60%. Actual hematocrit values by microcentrifugation are shown in Fig. 1. These samples were stored at room temperature with mixing. After 10 min, 1 h, 5 h, and 24 h, plasma was separated from the supplemented blood samples, and we measured creatinine concentrations in the plasma and in the original supplemented plasma stored for an equivalent time at room temperature without red cells; we also measured creatine concentration (4).

Measurement of creatinine, in duplicate, demonstrated a pooled within-run SD of 1.5 μmol/L (0.02 mg/dL). Measurement of creatine, in triplicate, demonstrated a pooled within-run SD of 1.5 μmol/L (0.02 mg/dL).

Fig. 1 shows the change in plasma creatinine as a function of time the plasma was in contact with red blood cells, at various hematocrits. Supplemented plasma samples with target creatinine concentrations of 442 and 1061 μmol/L (5 and 12 mg/dL) are shown in Figs. 1A and B, respectively. The plasma creatinine concentration decreased for up to 5 h after initial contact with red blood cells, after which no further decrease was seen. During this time the creatinine concentration of the creatinine-supplemented plasma samples to which no red blood cells were added was unchanged. The mean (SD) plasma creatine concentrations of the samples at the various hematocrits also remained unchanged [43.7 (1.4) μmol/L,
0.57 (0.02) mg/dL], indicating that conversion to creatine was not responsible for the decrease in creatinine. The decrease in plasma creatinine was greater at higher hematocrits, at both creatinine concentrations [442 and 1061 μmol/L (5 mg/dL and 12 mg/dL)]. At 5 h, the decrease in plasma creatinine concentration resulting from contact with red blood cells was statistically significant (95% confidence) at both concentrations and at all hematocrits tested.

It is possible to estimate the whole blood creatinine concentration at equilibrium, with knowledge of the water concentrations of plasma and red blood cells, hematocrit, the initial plasma creatinine concentration, and the plasma creatinine concentration after addition of creatinine. Assuming normal mass concentrations of water equal to 0.93 kg/L for plasma and 0.71 kg/L for red cell fluid, respectively (5),

\[
\text{Creat}_{\text{est}} = \frac{0.71 + \text{Hct} \times \text{Creat}_0}{(0.71 + \text{Hct}) + 0.93 \times (1 - \text{Hct})} + \frac{0.71 + \text{Hct} \times \text{Creat}_1}{(0.71 + \text{Hct}) + 0.93 \times (1 - \text{Hct})}\]

(1)

where \(\text{Creat}_{\text{est}}\) is expected whole blood creatinine concentration after addition of creatinine, \(\text{Creat}_0\) is initial plasma creatinine concentration, and \(\text{Creat}_1\) is plasma creatinine concentration after addition of creatinine. In all cases, the expected concentrations are within 10% of the measured concentrations 5 h after spiking.

The concentration of creatinine in plasma of blood samples that are supplemented with creatinine will decrease for up to 5 h at room temperature, as molalities of creatinine in plasma and red blood cell fluid approach equality. Measurement at 3 h after addition of creatinine indicated that the process was not yet at equilibrium (data not shown).

If addition of creatinine to samples is to be used to validate performance of a device for measurement of creatinine in whole blood, sufficient time must be allowed after the addition to avoid misleading results. Samples diluted to obtain creatinine concentrations below the reference interval may show a similar effect. In our study, sufficient numbers of naturally occurring samples in the interval 27–70 μmol/L (0.3–0.8 mg/dL) were obtained to avoid the need for sample dilutions.

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References


Paul D’Orazio*
JoAnn Conant
Jose Cervera

Instrumentation Laboratory
Lexington, Massachusetts 02421

*Address correspondence to this author at:
Instrumentation Laboratory
101 Hartwell Avenue
Lexington, Massachusetts 02421
Fax (781) 861-4452
e-mail pdorazio@ilww.com

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