EMIT Measurement of Carbamazepine, Phenobarbital, and Theophylline in the "Monarch" Centrifugal Analyzer

To the Editor:

We reported (1) unacceptable within-rotor drift when phenytoin is measured in plasma by EMIT (Syva Co., Palo Alto, CA 94304) in the Monarch centrifugal analyzer (Instrumentation Laboratory, Warrington, Cheshire, U.K.). We were able to eliminate drift and improve within-batch precision by using a double-loading facility (load, spin, reload, analyze) that ensures that all samples and reagents remain in contact for equal periods of time.

A similar phenomenon occurs in the measurement of carbamazepine in the Monarch by EMIT. We analyzed a full rotor, 31 aliquots of the same plasma sample, using the standard and the double-loading protocols. The reagents were reconstituted and working reagents prepared as described previously for phenytoin (1). We also used the same settings of the Monarch as in our previous study (1). Our results (Figure 1) demonstrate the presence of both unacceptable within-rotor drift and imprecision when the single-loading procedure is used (mean 62.9, SD 3.75, range 47-63 \( \mu \text{mol/L} \); CV 7.1%). This drift is largely removed and the precision greatly improved when the double-loading procedure is used (mean 47.5, SD 1.06, range 46-60 \( \mu \text{mol/L} \); CV 2.2%). The revised loading protocol achieves within-batch CVs nearer the expected within-batch precision quoted by Syva: 2.8 and 4.0%.

On analyzing full rotors of 31 aliquots of the same plasma sample for phenobarbital and theophylline, we obtained different results. The drift is downward, is much less severe, and is not completely removed by the double-loading procedure (see tabulation).

The contribution of pipetting to the above imprecision was assessed as follows. A solution of Tris HCl buffer (100 mmol/L, pH 8.5) containing 2.56 mmol of NADH per liter was dispensed by the Monarch into 15 consecutive rotors, with use of the same sample and total volumes used for the modified EMIT assay. The absorbances were measured bichromatically at 340 and 380 nm (the latter the blanking wavelength). Analysis of the data yielded a within-rotor CV of 2.6% (\( n = 36 \)) and a between-rotor CV of 2.4% (\( n = 15 \)). These findings suggest that the chemistry of the EMIT reaction contributes negligibly to the imprecision of the modified carbamazepine and phenytoin (1) assays and less than half of the imprecision of the modified phenobarbital and theophylline assays.

In conclusion, the modified loading procedure has been shown to remove drift from the carbamazepine as well as from the phenytoin assay (1). Although the drift is not completely removed from the phenobarbital and theophylline assays, day-to-day CVs of 4-8% are obtained with the Monarch on using the new loading protocol. This does not meet the <1% CV advocated by Fraser (3) for the performance of phenobarbital assays, but the interassay variation obtained in this study for the two other drugs are well below the upper limits of 12.5% CV for theophylline and 7% CV for carbamazepine he proposed.

References


Philip R. Wenham
Heather M. Barbour
Dept. of Clin. Chem.
Western General Hosp.
Crewe Road, Edinburgh EH4 2XU

Effect of Storage Temperature on Concentrations of Digoxin-like Immunoreactive Factor(s) in Plasma

To the Editor:

A wealth of information has accumulated concerning the assay of digoxin-like immunoreactive factor(s) (DLIF) in human body fluids (1-5), except for definitive data on the stability of DLIF in plasma (4). Therefore, our objective was to detect possible changes in DLIF concentrations in plasma samples stored frozen or unfrozen.

We measured total and free DLIF in the plasma of 18 pregnant women at term, using a radioimmunoassay