Enzyme Immunoassay of Phenyltoin and Phenobarbital with the Centrifugal Analyzer

To the Editor:

We wish to describe a method for determining phenyltoin and phenobarbital in serum by adapting the EMT system (Syva, Palo Alto, Calif. 94304) to the CentrifigChem (Union Carbide, Rye, N. Y. 10580).

Both drugs can be assayed after one dilution of standards and samples. All reagents are prepared according to kit instructions except as stated below.

Reagent: Syva—EMT—Anti-epileptic Drug (AED) Dilantin assay kit
Syva—EMT—AED Phenobarbital assay kit
Standards: Syva—AED Calibrators Controls: Syva—AED Control Procedure: Pipet 200 µl of calibrators and sample serum into 12 x 75 mm polypropylene tubes. To each of these add 400 µl of EMIT AED Buffer (pH 7.9). The diluted specimens and standards are then transferred to specimen cups for the pipettor.

The EMIT Reagent kit consists of Reagent A (Antibody/Substrate) and Reagent B (Enzyme). Pipet 20 µl of Reagent A manually into the sample wells (top well) of the transfer disc. Add 600 µl of Reagent B to 9.9 ml of buffer and then transfer to the reagent dish on the pipettor. This volume will be sufficient for at least 20 samples.

Pipettor setting:
Reagent volume, 350 Sample volume, 10 Total volume, 50 Last sample plug

The cups on the tray can be arranged as follows:

Standards in duplicates

A buffer blank cup after the highest standard

Specimens in duplicates

Analyzer Setting:
Auto blank, Terminal, Operate Absorbance
340 nm, corresponding absorbance setting

T₀ = 10, T = 0.25
n = 8 for phenyltoin
n = 5 for phenobarbital

The final printout is used for plotting. The zero standard absorbance value (ΔA₀) is subtracted from values of all other standards and samples before plotting. The net result (ΔA − ΔA₀) is then plotted on log-log graph paper provided with the kit.

Besides the two quality controls used in this study, we also participated in an Epilepsy Foundation of America, AED Quality Control Program (Dr. C. E. Pippenger, Columbia Presbyterian Medical Center, New York, N. Y. 10032), in which samples containing weighed-in quantities of drugs were sent to us for assay. Our results correlated well with established values for the 36 samples received. The coefficients of correlation were: phenyltoin, 0.9937; phenobarbital, 0.9990.

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Erroneously Low Creatine Kinase Activity Measurements with Use of the Calbiochem CPK-MB Kit with the Beckman TR Enzyme Analyzer

To the Editor:

Recent reports indicate that the automated Beckman Enzyme Activity Analyzer, system TR, may yield falsely negative results for high-activity samples because of substrate depletion (1, 2).

We would like to report another distressing observation. The system TR in the automated mode reports lower activities than when run on the continuous mode at the lower end of the normal range for creatine kinase (EC 2.7.3.2). This is a particularly frequent occurrence when the Calbiochem CPK-MB kit is used without dithiothreitol activation to measure CPK-MM (3). Table 1 lists some typical findings with use of this kit. The average continuous-mode result is five times greater than the average automated mode result, a significant difference.

What causes this difference? The probable cause is that the instrument in