More importantly for the clinical laboratory, the GELSCAN 5.0 system gives an accurate representation of one-dimensional gels when compared with a standard automated clinical densitometer. Although we have looked only at gels of LDH isoenzymes, the reproducibility of our system, while inferior to that of the ACD-18, appears to be reasonably satisfactory for most laboratory applications.

Mariash et al. (1) have shown, with a similar system, that the frequency distribution of density values for a given pixel has an SD of approximately 2.75 units, regardless of the actual density. To improve reproducibility, their system performs multiple scans on each pixel. This is necessary for their system because the computer scan is used to define the boundaries of the spot and, therefore, the area that is digitized. In our system, spot area is chosen by the operator, and so the only reason for multiple scans would be to increase the reproducibility of the total-density measurement for a given spot. Because the CV for this procedure is already <1%, we have chosen not to perform multiple scans of spots. However, the accuracy of spot measurements in our system does depend on the region chosen for measuring background density because the background density may vary significantly across the gel, especially if silver stain is used.

This study was supported in part by the Mayo Clinic/Foundation. We thank Dr. Leigh Anderson for helpful discussion and Gwyn Hurst for technical assistance.

References


Effect of Use of Gel-Barrier Sampling Tubes on Determination of Some Antiepileptic Drugs in Serum

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We evaluated the effect on drug concentration of the duration of sample storage in three different tubes for blood collection: SST® (Becton Dickinson), AutoSep® (Terumo), and Microvette® (Sarstedt), all of which contain an inert barrier (e.g., gel) for cleaner separation of serum from coagulum during centrifugation. With two of the tubes (SST and AutoSep) we examined samples taken from patients undergoing treatment with phenytoin, phenytoin, and carbamazepine. We found significant decrease in the drug concentration after 24 h with the serum standing over the gel barrier in the SST tubes for phenobarbital and both the SST and AutoSep tubes for carbamazepine. Adding aqueous drug solutions or serum samples supplemented with the above drugs in therapeutic concentrations to the three types of tubes resulted in a more pronounced decrease of concentration in the SST and AutoSep tubes than in the Microvette tubes.

Additional Keyphrases: evacuated blood-collection tubes · drug analysis · therapeutic drug monitoring · phenytoin · phenobarbital · carbamazepine · liquid chromatography

Blood specimens for drug assays are now almost invariably collected in commercially available evacuated tubes. In the past, Vacutainer Tubes (Becton Dickinson, Rutherford, NJ) reportedly (1, 2) decreased the concentration of the basic drugs in samples because of binding with the plasticizer tris(2-butoxyethyl) phosphate in the rubber stopper. The basic drugs were displaced from their protein binding sites, with subsequent uptake by erythrocytes, resulting in spuriously low values for concentrations in plasma (3). Tubes with stoppers that are stated to be free of this compound have recently become available and may be suitable for specimen collection. Other tubes for blood collection contain an inert barrier material (a silicon polymer gel) and a clot activator. The relative density (specific gravity) of the gel is intermediate to that of the serum and the coagulum, so that the serum is physically separated from the coagulum after clotting and centrifugation. After centrifugation the samples can be transported or stored in the gel tubes until assayed, without first decanting the serum.

We studied the concentration stability of some antiepileptic drugs—phenobarbital, phenytoin, and carbamazepine—on storage in gel separation tubes after centrifugation.

Materials and Methods

Tubes. Non-anticoagulated plain red-top 7-mL Venoject tubes (lot no. 82 J 15 W; Terumo Europe S.A., Haasrode, Belgium), 7-mL Vacutainer Serum Separator Tubes (SST; lot no. 6517 2K 949; Becton Dickinson Co.) and 1-mL Microvette tubes (lot no. 3331; Sarstedt, Rommeldorf, F.R.G.)

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Received October 11, 1983; accepted December 5, 1983.
Samples. Blood was sampled, from nine patients who were taking antiepileptic drugs, into Venoject plain red-top, SST, and AutoSep tubes. The samples were left upright, undisturbed, for 30 min at room temperature, for coagulation. They were then centrifuged at 1500 x g for 10 min. Serum from the Venoject plain red-top tubes was pipetted off into polypropylene tubes immediately after centrifugation (reference tube). The gel tubes were stored at 4°C with serum in contact with the gel barrier, and an aliquot of each sample was pipetted into polypropylene tubes after one day and seven days. We then measured drug concentrations in all of the samples in the same run.

Recovery experiment. Drugs in concentrations within therapeutic ranges were added to drug-free serum and to phosphate buffer (0.1 mol/L, pH 7.0, containing 154 mmol of NaCl per liter). We pipetted 2.0 mL (0.8 mL for the Microvette tube) of these drug solutions into the gel tubes and reference tube (Venoject plain red-top). We added 15 small glass beads to each tube (two glass beads in the Microvette tube), including the reference tubes, then centrifuged all (1500 x g, 10 min) and stored them upright at 4°C. The beads pass through the gel barrier during the centrifugation, as do the blood cells in actual clinical specimens, thus enhancing the contact between sample and gel.

Aliquots of serum and aqueous phase were removed into polypropylene tubes immediately after centrifugation, and after one, three, and seven days, and kept at 4°C until analysis. All samples were then analyzed in the same run.

Drug measurement. The liquid-chromatography method and sample preparation methods were those in use in our laboratory for the past three years. The mobile phase—acetonitrile-phosphate buffer (5 mmol/L, pH 7.3), 35/65 by volume—was delivered at a flow rate of about 1.5 to 2.0 mL/min by a Constantem II pump. The samples were injected by a VISP automatic injector (Waters Associates, Milford, MA). The column was a 4 x 250 mm Spherisorb ODS column (PhaseSep, Waddinxven, the Netherlands). Using an ultraviolet detector (Model 440; Waters Associates), we detected eluted drugs by their absorbance at 254 nm. Retention time, peak height, and concentration were calculated with a Spectra Physics SP 4100 integrator.

To prepare samples for chromatography, we added 500 μL of sodium dihydrogen phosphate (1 mol/L, pH 5.0) to 500 μL of sample, then extracted with 5 mL of methylene dichloride to which 5-(p-methylphenyl)-5-phenylhydantoin had been added as internal standard. We removed the organic phase and evaporated it under a stream of air at 40°C, reconstituting the sample with 500 μL of mobile phase before injecting it by VISP automatic injector into the chromatograph. The CV for this method for assay of phenytoin, phenobarbital, and carbamazepine was <3%.

Results and Discussion

The samples from patients being treated with antiepileptic drugs showed a decrease in drug concentration when collected in gel-separation tubes (Table 1). The longer the serum was in contact with the gel, the more the drug concentration was decreased. After seven days of storage, concentrations of all three drugs were significantly (p <0.01) less in the SST tubes than in the Venoject plain red-top tube. Carbamazepine decreased significantly (p <0.01) in both the SST and the AutoSep tubes. Samples collected in Venoject plain red-top tubes showed no decrease in drug concentration (within the precision of the analytical method, ± 3%), during storage of the separated serum.

When the drugs are added to drug-free serum or to phosphate buffer and then stored in gel tubes, the drug concentration of the buffer solution decreases more than in serum (Figure 1). This pronounced difference may be related to the binding of the drug to protein, which may decrease binding of the drug to the gel. The gel in the Microvette tube apparently negligibly affects the concentration of these drugs during storage.

The decrease in drug concentration could be reduced by minimizing the interval between centrifugation and decantation. The tube-induced decrease in concentrations of these drugs in serum illustrates a potential source of clinically important error in drug measurement that would not be detected by the usual quality-control procedures.

Addendum. During this study Becton Dickinson representatives informed us that serum for phenobarbital analysis should be decanted from the gel within about 1 to 2 h after centrifugation to avoid decreases in drug concentrations.

References