
Blood specimens for drug assays are 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 the plasticizer tris(2-butylxoyethyl)phosphate in the rubber stopper used with these tubes. 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 for specimen collection. Or, one can use tubes for blood collection that contain an inert barrier material (a silicon polymer gel) and a clot activator. The relative density of the gel is intermediate to that of the serum and 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.

Some authors have observed decreases in antiepileptic [4–6] and antiarrhythmic [7] drugs kept over the gel barrier at various temperatures. Here we report our evaluation of the concentration stability of some aminoglycoside drugs—amikacin, gentamicin, and tobramycin—in blood stored on gel separation tubes—specifically, nonanticoagulated plain red-top 5-mL Venoject* tubes (Terumo Europe S.A.) and 4-mL Vacutainer Serum Separator Tubes (SST®; Becton Dickinson)—after centrifugation.

Blood was sampled into Venoject plain red-top and SST tubes from 10 patients who were taking aminoglycoside drugs. The samples were left upright, undisturbed, for 30 min at room temperature to allow the samples to coagulate and then were centrifuged at 1500g for 10 min. Serum from the Venoject plain red-top tubes was pipetted into polypropylene tubes immediately after centrifugation (reference tubes). The collection tubes were stored at 4°C with 1 mL and 0.2 mL of serum left in contact with gel barrier, and an aliquot of each sample was pipetted into polypropylene tubes after 1 and 2 days. We measured the serum drug concentrations in all of the samples in the same run, using Emit immunosassays (Syva, Palo Alto, CA) and a Cobas-Mira automated analyzer. The average CV of this method for assay of all aminoglycosides was <4.2%.

We observed significant time- and volume-dependent decreases in the measured concentrations of tobramycin and amikacin when the serum was stored at 0–4°C in Vacutainer SST tubes (Table 1). In contrast, under identical conditions, gentamicin did not change significantly. These pronounced differences are presumably related to the binding of the drug to the gel.

The decrease in drug concentration could be reduced by minimizing the interval between centrifugation and decantation. The tube-induced decrease in concentration 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.

References


Stability of Plasma Nonesterified Arachidonate in Healthy Individuals in Fasting and Nonfasting States, Youssef Hallaq, Zbigniew M. Szczepiorkowski, Jan Tertuya, Joanne E. Cloutte-Brown, and Michael Lapostra* (Dept. of Pathol., Div. of Clin. Pathol., Massachusetts General Hosp., Fruit St., Boston, MA 02114; *address correspondence to this author at: Rm. 235 Gray Bldg., Massachusetts General Hosp., Boston, MA 02114; fax 617–726–3256, e-mail LAPOSATAMI@A1.mgh.harvard.edu)

Eicosanoids synthesized from arachidonate by platelets and endothelial cells may be provided by albumin-bound nonesterified (free) arachidonate in the plasma, by phospholipid-associated arachidonate liberated from the membranes of platelets and endothelial cells upon cell activation, or by both. If there is no mechanism to limit its plasma concentration and its availability for eicosanoid production, the nonesterified arachidonate in the plasma is capable of stimulating cells to synthesize eicosanoids directly upon incorporation into the cells. The presentation of nonesterified arachidonate to cells can bypass the need for mobilization of cellular arachidonate from phospholipids for eicosanoid production by rapidly entering the cells and being converted to eicosanoids without agonist stimulation.

Because the concentration of total nonesterified fatty acids in plasma is much lower than that of esterified fatty acids, a precise and accurate quantification of total plasma nonesterified fatty acids, particularly nonesterified arachidonate, has long been a matter of uncertainty. The range of reported nonesterified plasma arachidonate concentrations is 0.9–35.5 μmol/L [1–8]. Because of the tedious work required for accurate quantification of individual nonesterified fatty acids, many simplified methods have been developed for measurement of these compounds.

Table 1. Effect of storage on concentrations of aminoglycoside drugs in serum.

<table>
<thead>
<tr>
<th>Reference (Venoject) mean conc, μmol/L*</th>
<th>Days stored in SST tubes</th>
<th>1 d</th>
<th>2 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin 13.92</td>
<td>93.2 (0.47)</td>
<td>81.5 (1.29)</td>
<td></td>
</tr>
<tr>
<td>Tobramycin 2.31</td>
<td>80.4 (0.23)</td>
<td>64.1 (0.41)</td>
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</tbody>
</table>

*Samples collected in Venoject red-top tubes showed no decrease, and the CV was <3.2% in all cases, in drug concentrations during storage at 0–4°C.

**Results shown are mean (SD) for 10 patients, with stored samples of 0.2 mL of serum; no significant differences were seen for stored volume of 1 mL.

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