


Effect of Use of a Specific Monoclonal Antibody on Radioimmunoassay Results for Serum Cyclosporine, Michael K. Chooi and James E. Coates (Dept. of Lab. Med., Univ. of Alberta Hospitals, Edmonton, Alberta, Canada, T6G 2B7)

To determine the effect on the serum cyclosporine (CS) radioimmunoassay of substituting the specific monoclonal antibody from Sandoz (Basel, Switzerland) for their polyclonal antiserum, we assayed consecutive samples from four kidney-transplant (n = 76) and nine heart-transplant (n = 67) patients, using each reagent. For the kidney patients, monoclonal assay results correlated well with those of the polyclonal assay (Figure 1A) but were significantly lower (P < 0.001). The mean monoclonal/polyclonal ratio was 0.67 (SD 0.19). Monoclonal assay results for heart recipients were also significantly lower (P < 0.001) than those by the polyclonal assay. However, correlation of results (Figure 1B) was only moderate (r = 0.69). The correlation coefficient increased to 0.91 (n = 42) when the results for one patient (solid symbols in Figure 1B) were eliminated from the regression analysis. The mean ratio for the remaining samples was 0.54 (SD 0.11), which is significantly lower than the mean ratio for kidney-transplant patients (P < 0.001). A lower ratio for heart-transplant patients is not unexpected because these patients may have increased concentrations of circulating CS metabolites because of poor perfusion or post-transplant cholestasis (1). The patient who was omitted from the revised regression had low ratios (0.26 ± 0.06, n = 18) during the first three weeks after transplantation, which coincided with a bout of cholestasis.

Reference

Cleaning of RA-1000 Probes, Matthew R. Baxter, John A. Hinds, John R. Gollogly, and Patrick Duffy (Dept. of Clin. Chem., The Prince Charles Hospital, Chermside, Australia 4032)

Operation of the Technicon RA-1000 analyzer depends on the use of an inert perfluorohydrocarbon coating of reagent and sample delivery systems and an air-segmented reagent stream to eliminate sample-to-sample and reagent-to-reagent carryover. The inert hydrocarbon heptacosfluorotributylamine [Technicon "Random Access Fluid," or "Fluorinert FC-43" (3M Australia) (1)] forms a coating on the surface of any tubing and probes that come into contact with sample or reagent and renders these surfaces nonwettable. For this process to occur satisfactorily, the surfaces must be thoroughly clean or the integrity of this inert surface film is lost.

The manufacturer recommends that reagent and sample probes be cleaned monthly or after every 5000 tests by aspirating 50 g/L sodium hypochlorite solution. It is important that this cleaning protocol be carried out as recommended by the manufacturer because the presence of a contaminated probe, which is easily overlooked, is a major cause of imprecision on the RA-1000. We have observed that this recommended procedure frequently fails to clean probes adequately and decreases instrument precision. We report here a simple and rapid method to measure carryover on the RA-1000 and procedures that effectively clean reagent and sample probes.

To detect carryover from a reagent probe, we assay potassium in a normal quality-control material before and after the reagent probe dispenses a 100 mmol/L potassium chloride solution from the reagent tray position of any selected analyte. Thus, we assay three cups of the same
normal quality-control material for $K^+$, the selected analyte, and $K^+$, respectively, and observe the difference between the assayed value of $K^+$ for the first and third cups.

To detect carryover from a sample probe, we measure $K^+$ successively in three sample cups containing normal quality control, 100 mmol/L KCl solution, and normal quality control, respectively.

A new probe, or an effectively cleaned probe, characteristically gives <0.1 mmol/L increase in $K^+$ under these conditions—i.e., the carryover from the 100 mmol/L K$^+$ is <0.1%. With a contaminated probe, increases in $K^+$ owing to carryover in the second sample can be as much as 3–4 mmol/L. We have observed such concentrations frequently in probes used for more than 5000 tests. The contamination is reflected in an increased imprecision of all chemistries on the instrument.

We clean probes by repeated 10-min ultrasonication in a phosphate-free tissue-culture-grade detergent (Decon 90; Selby-Anax, Australia), followed by distilled water and then inert hydrocarbon fluid. Each step is carried out at room temperature, with intermittent vigorous replacement of fluid inside the probe tubing by syringe aspiration.

Thus our procedure is to place a 50-mL beaker of 20 mL/L detergent solution in an ultrasonic cleaning bath (Branson 220; Branson Ultrasonics, Danbury, CT) at room temperature. A glass syringe is attached to the top of the reagent or sample probe via a short piece of peristaltic pump tubing, and the detergent solution is drawn in and out of the syringe through the probe vigorously for 1 min, with sonication. The probe is then ultrasonically cleaned in the detergent solution for a further 10 min. After repeated syringe aspirations with distilled water to remove all traces of detergent, the ultrasonication is repeated in a beaker of distilled water. We then aspirate a small quantity of Fluorinert FC-43, to remove traces of water, and place the probe into a 3-mL test tube containing inert hydrocarbon and finally ultrasonicate again for 1 min. We then check for carryover, using the procedure described above. Occasionally we note that an unacceptable carryover still remains and we seek other causes for this in the reagent heating coil or air-segmentation system, as recommended by the manufacturer.

References


To measure concentrations of fentanyl in maternal and fetal plasma after its epidural administration during labor, we adapted the available RIA method to enhance its sensitivity.

For preparation of standard curves we added fentanyl in either 0.5 mL of a methanol/water (30/70 by vol) solution, pH 7.5, buffered for direct RIA by the method of Michiels et al. (1) or 2 mL of drug-free plasma samples for the same analysis after extraction. Amounts of fentanyl added were 0.05, 0.1, 0.2, 0.5, 1, and 4 ng.

Each 2-mL plasma sample was extracted with 2 mL of chloroform. After 2-min vortex mixing and 10-min centrifugation at 4000 × g, the organic phase was evaporated under nitrogen, at 40 °C. The dry residue was then dissolved in 0.5 mL of the citric acid–methanol solution supplied in the commercial kit (Jansen Laboratories, Turnhoutse, Beerse, Belgium) and submitted to the RIA. The results are shown in Figure 1. The corresponding extraction percentages measured at 0.1 and 1 ng/mL for 10 determinations were respectively 65.5 (SD 8.7) % and 57.2 (SD 5.1) %. The two curves are parallel within the limits of the tested concentrations, so the variations of the extraction coefficient can be neglected.

The precision and the accuracy for 10 determinations of each fentanyl concentration used in the standard curve are:

<table>
<thead>
<tr>
<th>Added, ng</th>
<th>Measured, (mean ± SD), ng</th>
<th>CV, %</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.050</td>
<td>0.051 ± 0.008</td>
<td>15.7</td>
<td>102.0</td>
</tr>
<tr>
<td>0.100</td>
<td>0.106 ± 0.019</td>
<td>12.3</td>
<td>106.0</td>
</tr>
<tr>
<td>0.200</td>
<td>0.189 ± 0.020</td>
<td>10.6</td>
<td>94.5</td>
</tr>
<tr>
<td>0.500</td>
<td>0.493 ± 0.042</td>
<td>8.5</td>
<td>98.6</td>
</tr>
<tr>
<td>1.000</td>
<td>0.962 ± 0.081</td>
<td>8.4</td>
<td>96.2</td>
</tr>
<tr>
<td>4.000</td>
<td>4.200 ± 0.290</td>
<td>6.9</td>
<td>105.0</td>
</tr>
</tbody>
</table>

According to Michiels et al. (1), the sensitivity of the direct radioimmunological method is 0.05 ng with a maximal 0.5-mL plasma sample, corresponding to 0.1 ng/mL. Our method, allowing an increase in the plasma sample to 2 mL, enhances the overall sensitivity to 25 pg/mL.

We used this new method to investigate the placental diffusion of the drug in women experiencing epidural analgesia for cesarean section, finding mean fentanyl concentrations lower in the fetus than in the mother. Moreover, a significant accumulation seemed to occur in the newborn because the venous-blood concentrations entering the newborn were always higher than the arterial ones leaving it (1.8 ± 0.4 ng/mL vs 0.4 ± 0.5 ng/mL; n = 6).

References