We used the fibrinogen reagent diluted with distilled water in two ratios: for blank reagent 30:85, and for assay reagent 115:85. The blank assay was subtracted from the fibrinogen assay automatically. With the RA-XT analyzer (Technicon, Tarrytown, NY) we found the conditions in regime ENDPONT with plasma blank were optimal at an incubation time of 120 s (37 °C) and the results correlated well with those by Macart's method (r = 0.923). Within-run precision of our method at three concentrations in 20 replicates (0.75, 4.9, and 6.9 g/L) was sufficient (CV 4.67%, 1.27%, and 2.13%, respectively). Between-day precision (CV) for a fibrinogen concentration of 5.7 g/L in 20 replicates was 3.9–4.0%. The accuracy of this method was evaluated by comparing it with the commercially available turbidimetric method (2) based on proteolytic enzyme (highly specific for fibrinogen) isolated from snake venom from Bothrops asper. The results correlated well (r = 0.927). Reaction linearity was sufficient in interval 1–10 g/L.

### Table 1. Concentration* vs Concentration/Creatinine Ratio* for Drug Detection Cutoff Values

<table>
<thead>
<tr>
<th>mg/L</th>
<th>mg/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.13</td>
</tr>
<tr>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>0.8</td>
<td>0.18</td>
</tr>
<tr>
<td>0.9</td>
<td>0.19</td>
</tr>
<tr>
<td>1.1</td>
<td>0.17</td>
</tr>
<tr>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>2.0</td>
<td>0.58</td>
</tr>
</tbody>
</table>

* Cutoff concentration for buprenorphine, 1.0 mg/L.

* Cutoff ratio for buprenorphine/creatinine, 0.1 mg/mol.

References

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Analysis of Commonly Abused Drugs in Urine at Selected Threshold (Cutoff) Concentrations

To the Editor:

The Letter of Finkle et al. (1) addresses a very important issue. However, cutoff concentrations suffer from the physiological disadvantage that abusers can and do drink plenty of water to dilute urine drug concentrations below the critical cutoff value, a manipulative polydipsia (this is hardly a new observation).

Although, of course, false-positive results are to be avoided, there is also a pressure to avoid false-negative results. To compensate for dilution, we have used a drug/creatinine ratio in examining buprenorphine misuse in Glasgow, Scotland (2). As can be seen from Table 1, using a urine concentration cutoff of 1 mg/L for the drug buprenorphine, we would have reported six of these nine urine samples as negative; on using the ratio of buprenorphine/creatinine of 0.1 mg/mol, we would class them all as positive—half the samples being double, or much more, than the cutoff value. We applied the ratio in cases that were equivocal or negative by the concentration cutoff approach. We based our experience on several hundred samples received from buprenorphine abusers. At the time of the original report (2), the incidence of buprenorphine use in our addict population was >40%.

The use of urine drug/urine creatinine ratios need be no less generous in their setting to avoid false-positive results than the concentration cutoff but would enhance the objectivity with which a result could be viewed. As an approach for routine drug screening, this may merit more intensive investigation.

References
We made several calibration runs, using both the conventional method and the proposed method; the calibration curves for the pretreated materials matched perfectly those obtained by the conventional technic (Figure 1, A and B).

Using both the conventional method (x) and the proposed method (y), we measured MEGX in the sera of healthy subjects and of liver disease patients with normal concentrations of blood bilirubin who received intravenous doses of lidocaine. The results are practically superimposable: intercept = 0.40 µg/L, slope = 0.988, r = 0.994, and S_x-y = 6.62 µg/L (n = 40).

Five pooled sera rich in bilirubin (about 400 mg/L) were mixed with the Abbott calibrators and controls in different ratios; the analytical recovery of MEGX assayed after pretreatment was between 91% and 111%.

We lyophilized three 150-µL portions of the six Abbott calibrators, then reconstituted them with 150 µL of (a) water, (b) serum with 360 mg/L bilirubin, or (c) bile with 310 mg/L bilirubin. The calibration curves (Figure 1, C–E) are for all intents and purposes indistinguishable.

We conclude that our pretreatment improves the assay technic, particularly for sample materials that are rich in protein-bound chromogens (i.e., bilirubin).

References


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HPLC and CYCLO-Trac SP
Radioimmunoassay Compared for Monitoring Cyclosporine

To the Editor:
Loo et al. (1) report a very large discrepancy between the results for the measurement of cyclosporine in blood samples from heart- and kidney-transplant patients, using a radioimmunoassay technique (CYCLO-Trac SP; Incstar, Stillwater, MN) and a high-performance liquid chromatography (HPLC) assay.

We have addressed this point in two reports (2, 3) on the comparative performance of cyclosporine assay techniques, based on data from the U.K. Cyclosporin Quality Assessment Scheme, which were not cited by the above authors. The U.K. scheme comprises >170 participants worldwide, of which 8% use HPLC and 48% use CYCLO-Trac SP. Laboratories receive three samples per month derived from patients’ sample pools and blood bank blood with added cyclosporine.

The mean results for samples from patients receiving the drug differed by as much as 36% between the CYCLO-Trac SP assay and HPLC (2, 3), but this was during a period when there was doubt about the accuracy of the Incstar calibrators (4). Whether these differences were due to specificity of the antibody or to standardization differences was not established by these data. However, subsequent data from our scheme suggest that standardization of the HPLC assays relative to the kit assays possibly contributed to the difference (5). Currently, the mean difference between HPLC and CYCLO-Trac SP results is <25% (5).

Loo et al. could not attribute the differences between the immunoassay and HPLC results to standardization. Over the past year we have monitored the difference between results from laboratories that use the standards supplied with the CYCLO-Trac SP kit and laboratories that use in-house standards with this kit. We found no difference (~2%) between the results produced by these two sets of users.

Poor specificity of the antibody used in the RIA kit seems to be an unlikely cause of the very large differences observed between the two assay techniques when patients’ samples were assayed by Loo et al. because RIA results for samples from kidney-transplant patients were relatively higher than those from heart-transplant patients. If antibody specificity were poor, one would expect the reverse finding, because samples from heart-transplant patients are more likely to contain a higher proportion of cyclosporine metabolites (6). Typical data for one recent month from the U.K. scheme, in which separate blood pools collected from kidney-, liver-, and heart-transplant patients were circulated, showed mean differences of 6.0%, 5.6%, and 14.1%, respectively, between HPLC and CYCLO-Trac SP.

Thus, the findings of Loo et al. are very much at variance with those currently obtained in the laboratories participating in our surveys. We feel it would be unfortunate if the anomalous results of Loo et al. were to be interpreted as typical of users of the CYCLO-Trac SP kit.

References


