prophylaxis of hyperuricemia. Following the manufacturer’s instructions, uric acid concentration was 83 μmol/L, compared to 95 μmol/L after immediate PCA treatment, and <12 μmol/L after 1 h storage at room temperature.

In conclusion, sample pretreatment with PCA appears to be a useful tool for monitoring of plasma uric acid concentrations during rasburicase treatment. We recommend supplying plastic tubes containing 2 mL PCA. A 1 mL syringe can be used to add 1 mL whole blood to this tube. Immediately after addition, the tube should be shaken vigorously for 30 seconds before being sent to the laboratory.

We thank Sanofi-Synthelabo for providing rasburicase (Fasturtec®).

References

Table 1. Residual uric acid concentrations in whole blood after addition of rasburicase.

<table>
<thead>
<tr>
<th>Time</th>
<th>4 °C</th>
<th>RT</th>
<th>+ PCA, RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>70 (3)</td>
<td>57 (6)</td>
<td>101 (&lt;0.1)</td>
</tr>
<tr>
<td>60 min</td>
<td>76 (2)</td>
<td>45 (5)</td>
<td>103 (5)</td>
</tr>
<tr>
<td>120 min</td>
<td>70 (6)</td>
<td>18 (6)</td>
<td>102 (1)</td>
</tr>
<tr>
<td>180 min</td>
<td>68 (5)</td>
<td>6 (4)</td>
<td>105 (7)</td>
</tr>
<tr>
<td>240 min</td>
<td>68 (5)</td>
<td>0</td>
<td>94 (2)</td>
</tr>
<tr>
<td>300 min</td>
<td>70 (5)</td>
<td>ND</td>
<td>97 (4)</td>
</tr>
</tbody>
</table>

The original uric acid concentrations before addition of rasburicase in the 4 donors were 482, 358, 336, and 290 μmol/L, respectively. At defined intervals after addition of rasburicase (time 0), samples were centrifuged, supernatants were collected and prepared for analysis. For the PCA pretreatment, all aliquots were treated with PCA at time 0, with further sample preparation at the indicated time points. PCA, perichloric acid 8%; RT, room temperature; ND, not determined.

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Does Bilirubin Cause Interference in Roche Creatinine Methods?

To the Editor:
Bilirubin interference in kinetic alkaline picrate (Jaffe) creatinine assays has been well documented (1, 2). The exact mechanism of interference is still unclear, however, and both conjugated and unconjugated bilirubin have been implicated. Enzymatic assays, which reportedly suffer less interference, are an alternative, but the extra reagent cost has restricted their use. Historical data continue to be used to estimate the extent of bilirubin interference, although modifications to these assays or instruments may have taken place since the original interference studies were performed. We used liquid chromatography tandem mass spectrometry (LC-MS/MS) as the reference method to investigate the extent of bilirubin interference in 2 automated creatinine assays. We have previously shown that this LC-MS/MS assay compares well to the 2 automated methods at creatinine concentrations of <150 μmol/L (3) by analyzing >100 samples with bilirubin concentrations within reference intervals.

We added varying amounts of creatinine (Sigma) to phosphate buffered saline (PBS) pH 7.4, containing 40 g/L bovine serum albumin, to give concentrations of 37.5–1000 μmol/L. One liter of PBS contained 8 g sodium chloride, 0.2 g potassium chloride, 1.44 g disodium hydrogen phosphate, and 0.24 g potassium dihydrogen phosphate. Unconjugated bilirubin (Sigma) was then added to give bilirubin concentrations up to 511 μmol/L. Anonymized icteric sera (n = 73) with creatinine concentrations <150 μmol/L were stored at −20 °C for ≥2 weeks, and total and conjugated bilirubin were determined by the Roche liquid diazotization and Jendrassik-Grof based assays, respectively. All samples were analyzed by the following 3 different creatinine methods.

The automated creatinine assays were the rate-blanked, compensated Jaffe method and the creatinine plus enzymatic assay performed on the Roche Modular according to the manufacturer’s instructions (Roche Diagnostics). The reagent set insert states that there is no significant interference by bilirubin for concentrations <150 μmol/L and 428 μmol/L, respectively. The LC-MS/MS assay employed was used as described by Owen et al. (3).

The comparative LC-MS/MS method shows no significant interference by bilirubin (see Fig. 1 in the Data Supplement that accompanies the online version of this Letter at http://www.clinchem.org/content/vol53/issue2). Surprisingly, the Jaffe method (Fig. 1A) did not show significant interference in most PBS samples; however, the samples with the lowest creatinine concentrations
showed a 10% reduction in creatinine at bilirubin concentrations >220 \mu mol/L. In the majority of samples analyzed by the enzymatic method (Fig. 1B), a 10% reduction in creatinine was seen at bilirubin concentrations of >400 \mu mol/L, and even at lower creatinine concentrations the effect was seen when bilirubin was >200 \mu mol/L. The Jaffe method showed no significant interference at total bilirubin concentrations of <700 \mu mol/L in serum samples (Fig. 1C). The mean difference between the Jaffe and LC-MS/MS assays (0.74 \mu mol/L) was not significant (P = 0.27, 95% confidence interval = -0.58–2.05 \mu mol/L, paired t-test). The enzymatic assay showed values lower than the LC-MS/MS (Fig. 1D), with a significant mean difference of 10.71 \mu mol/L (P = 0.0001, 95% confidence interval; 9.04–12.38 \mu mol/L, paired t-test). The difference also increased at higher total bilirubin concentrations and is greater than would be expected when comparing these two methods in normal serum. When the effects of unconjugated and conjugated bilirubin on the enzymatic assay were examined separately, both species appeared to exert an effect, but the effect of unconjugated bilirubin appeared to be slightly greater (data not shown), possibly because it is more difficult for conjugated bilirubin to oxidize under alkaline conditions.

The enzymatic assay, although often recommended as the method of choice, nevertheless showed greater interference than the Jaffe method. This interference may be attributable to the consumption of peroxide in the initial reaction mixture, as previously suggested (4). Our results show that the Jaffe assay performs well in the presence of icterus; however, the effects of bilirubin in this assay are variable and poorly reproducible. Despite this we found no statistically significant difference in serum creatinine values obtained by the LC-MS/MS and Jaffe methods. Serum samples with bilirubin concentrations >700 \mu mol/L are rarely seen, and users of this assay can feel confident in the accuracy of the creatinine values obtained from the vast majority of icteric samples. When percentage differences were considered, the greatest effect in the Jaffe method was seen in PBS samples containing creatinine concentrations within reference intervals. Although these PBS samples are not clinical samples, and the alternative matrix may behave differently to serum, this exaggerated effect is in agreement with previous reports (5). In conclusion, our data show that the Roche Jaffe method performs well in the presence of icterus despite often being considered to suffer from significant interference.

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References

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