(Boehringer Mannheim; cat. no. 127787). Whole blood is hemolyzed in Sterox (Hartman-Leddon Co.; 5 g/L in water) to a hemoglobin content of 30 g/L and centrifuged at 11,000 x g for 3 min to remove erythrocyte stroma. The hemolysate (12.5 μL) is then preincubated for 15 min with 0.5 mL of the substrate/coenzyme mixture at 37 °C and the reaction rate is monitored spectrophotometrically at 340 nm for 30 min (Model DU 7500 diode-array spectrophotometer; Beckman Instruments, Fullerton, CA).

We compared the TK results obtained for 10 fresh blood samples collected into heparin, using R5P stored for 7 weeks at −20 °C and a freshly prepared solution of this substrate. TK results with stored R5P were consistently lower and did not correlate significantly with the fresh substrate (r = 0.34):

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stored</th>
<th>Fresh</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.39</td>
<td>1.32</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>1.28</td>
</tr>
<tr>
<td>3</td>
<td>0.44</td>
<td>0.95</td>
</tr>
<tr>
<td>4</td>
<td>0.72</td>
<td>1.21</td>
</tr>
<tr>
<td>5</td>
<td>0.29</td>
<td>1.24</td>
</tr>
<tr>
<td>6</td>
<td>0.51</td>
<td>1.31</td>
</tr>
<tr>
<td>7</td>
<td>0.01</td>
<td>1.03</td>
</tr>
<tr>
<td>8</td>
<td>0.10</td>
<td>1.22</td>
</tr>
<tr>
<td>(1.18)*</td>
<td>(1.22)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.21</td>
<td>1.34</td>
</tr>
<tr>
<td>10</td>
<td>0.06</td>
<td>0.77</td>
</tr>
</tbody>
</table>

*All samples were measured for a 40-min postincubation except sample 6, which was also measured at 70 to 110 min (results in parentheses).

A kinetic plot of the results for some of these samples showed that the TK activity was relatively constant with the fresh substrate; in contrast, with the stored substrate, the rate increased considerably from one 15-min time frame to another after the initial preincubation step at 37 °C for 15 min (Figure 1). One blood sample, assayed for 70–110 min postincubation with the stored substrate gave results equivalent to that obtained with the fresh substrate. The thiamine pyrophosphate activation with three of these samples (specimens 8 to 10) gave 8.5%, 8.6%, and 9.5% activation with fresh R5P substrate and −1.3% (inhibition), 65.5%, and 9.3%, respectively, with the stored sample. It is obvious from these data that variability in the results for the kinetic behavior with the use of stored R5P substrate can confuse the laboratory diagnosis of thiamine deficiency.

In the light of these results, we now recommend weighing out the required amount of the crystalline sodium salt of R5P (3 g/L in Tris buffer) just before the assay. The time for deterioration of the stored R5P solution varies from batch to batch but generally takes about 6 to 10 weeks at −20 °C on average to deteriorate to the extent seen here. We do not know chemically what conversion the R5P is undergoing; however, it occurs even when the solution is stored frozen at −20 °C. Perhaps more time is required with partially deteriorated R5P to generate sufficient amounts of the cosubstrate in this reaction (xylose 5-phosphate) to give the maximal TK rate. It is also possible that a subtle deterioration of stored R5P solutions may occur, less obvious than that seen here, which may give erroneous results.

References

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HPLC Determination of Caffeine and Theophylline by Direct Serum Injection

To the Editor:

Caffeine (1,3,7-trimethylxanthine) and theophylline (1,3-dimethylxanthine) are widely used drugs. Caffeine has been shown to be effective in the treatment of apnea in premature infants. Particularly for this patient group a very small sample volume is attractive. Theophylline is used for the treatment of acute and chronic asthma and chronic obstructive pulmonary disease, because of its ability to relax bronchial smooth tissue.

Recently, a new type of packing material for HPLC columns became commercially available (Chrompack International b.v., Middelburg, The Netherlands). Introduced as ChromSpher 5 BioMatrix, this HPLC phase is a further development based on the original work of Hagestam and Pinkerton, Gisch et al., and Haginaka et al. (1–3). The hydrophilic surface of the column packing material rejects and excludes proteins, whereas drugs of interest or other small molecules interact with the reversed phase inside the pores of the silica particles. The combined-phase packing material, ChromSpher 5 BioMatrix, contains both the hydrophobic and the hydrophilic compounds in one ligand molecule. This combined phase is bonded to the interior and exterior surfaces of silica particles. Phenyl groups account for the hydrophobic interaction, whereas hydroxyl groups of an alkanolic functionality protect the reversed phase by rejecting the proteins.

We tested a ChromSpher 5 BioMatrix HPLC column for determinations of caffeine and theophylline in serum. Before analysis, serum samples are mixed with equal volumes of the internal standard solution, theobromine (7.5 mg/L). Of this mixture 20 μL is injected into the HPLC. Chromatographic conditions are: ChromSpher 5 BioMatrix column, 150 × 4.6 mm at room temperature; isocratic elution with acetonitrile in water (50/950 by vol); flow rate 1.0 mL/min. Detection is at 280 nm, set at 0.1 A full-scale. Maximal run time is 6 min. A typical chromatogram for analysis of a serum sample is shown in Figure 1.

Within-run and between-run variation were determined (n = 10). Intra-assay variation for caffeine was: mean 11.77 mg/L, SD 0.188, and CV 1.6%; for theophylline: mean 11.72 mg/L, SD 0.906, and CV 2.6%. Interassay variation for caffeine was: mean 5.08 mg/L, SD 0.184, and CV 3.1%; for theophylline, the interassay variation was: mean 6.10 mg/L, SD 0.174, and CV 2.9%.

Drug-supplemented serum samples (n = 11) used in the quality assessment scheme of the Dutch Association for Quality Assurance in Therapeutic
Drug Monitoring and Clinical Toxicology (K. K. G. T.) were assayed by the present method (y). The results were 
y = 1.092x + 0.894 (r = 0.998 with a standard error of estimate of 0.326) for caffeine and 
y = 1.064x - 0.125 (r = 0.999, standard error of estimate = 0.359) for theophylline.

All samples of this correlation experiment were also analyzed with the in-house HPLC method for the determination of these drugs. In the in-house method (z), a solid-phase extraction procedure (d) is used, followed by C_{18} reversed-phase chromatographic analysis with methanol in water (175/825, by vol) as eluent and ultraviolet detection. Linear regression of the results with those of the present method (y) were 
z = 0.777y + 2.632 (r = 0.984 with a standard error of estimate of 1.169) for caffeine and 
z = 0.937y + 0.165 (r = 0.998, standard error of estimate = 0.375) for theophylline.

We conclude that the described method is easy to perform and inexpensive. The method takes very small sample volumes, which could be reduced to 5 μL if necessary. Sample purification or extraction is not necessary. The presented technique is especially suitable for determining caffeine in serum samples of premature infants.

References

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Whole-Blood Lead Reference Intervals for Adults

To the Editor:

Since the Centers for Disease Control (CDC) issued revised recommendations for whole-blood lead concentrations for children in 1991 (1), there has been confusion about what values to consider as normal for adults. Blood lead concentrations are unusual in the sense that reference standards are defined by government agencies. Workplace standards, for example, are set by the federal Occupational Safety and Health Administration (OSHA) (2); the concentration of concern in the workplace starts at 400 μg/L and was set in 1978. In the US, >95% of above-normal blood concentrations of lead in adults result from workplace exposure (3). Outside the workplace, normal adult values for whole-blood lead are often cited as <250 μg/L; this limit appears to have been set by the CDC in 1985 (4) and was intended to apply to children. According to the new CDC guidelines, whole-blood lead in children <6 years old should be <100 μg/L (1); again there is a tendency to apply these limits to adults (3, 5).

Information on lead concentrations in nonoccupationally exposed adults is scanty.

To determine a reference interval in adults, we tested 100 whole-blood specimens collected for routine blood counts from individuals of ages ≥18 years. In this sample, the average whole-blood lead was 30 μg/L, the range was 3-131 μg/L, and the SD was 21 μg/L. The reference interval, determined by excluding the upper and lower 2.5% of the population, was 6-115 μg/L. Specimens collected from adults specifically for lead determinations show an average >30 μg/L, presumably reflecting increased occupational and (or) environmental exposure.

We measured whole-blood lead with a Perkin-Elmer (Norwalk, CT) Elan 500 inductively coupled plasma mass spectrometer (ICP-MS). The reference laboratory at the University of Utah has been using ICP-MS for the last 3 years, during which time we have been successfully enrolled in the College of American Pathologists and the New York State and California lead-testing proficiency programs. Specimens were collected for routine blood counts in purple-top containers rather than trace-metal collection tubes; although purple-top containers have been identified as a source of sporadic contamination, this was not a significant problem here. This study complies with the ethical standards of our institution.

The 1991 CDC recommendations for whole-blood lead in children consist of several categories (1): Class I (<100 μg/L) is "not lead-poisoned"; Class II (100-190 μg/L) suggests a need for "evaluation and more frequent screening"; Class III (200-440 μg/L) indicates a need for "evaluation and remediation"; Class IV (460-690 μg/L) requires "medical and environmental interventions"; and Class V (>690 μg/L) represents "a medical emergency." The application of these categories to adults has not been as easy. For example, although a whole-blood lead of 700 μg/L is an emergency in children because of the danger of lead encephalopathy, adults are not in jeopardy of encephalopathy until much higher concentrations are present. The use of <100 μg/L as the normal reference limit in nonoccupationally exposed adults appears appropriate for our population, with 3% of the study subjects falling outside the reference interval. However, the actions and interventions recommended for children with increased concentrations of blood lead should not be applied indiscriminately to adults, because adults are less vulnerable to lead exposure.

References