Serum Apo-Aminotransferases Reassociate Equally Well with Pyridoxal 5'-Phosphate In Water or Buffer, J. C. M. Hafkenscheid, H. T. R. van den Berg-Bosman, and M. Hessels (Clin. Chem. Lab., St. Radboud Hospital, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands)

As is well known, the coenzyme pyridoxal 5'-phosphate (PLP) enhances the activity of serum aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) (1, 2). It can be added to the total reagent mixture (less 2-oxoglutarate) for quantifying these enzymes, as has been described in many recommendations, or it can be added to the serum, 10-min pre-incubation being necessary in either case. The reaction can then be started by adding either the substrate or the serum including the coenzyme. When serum is pre-incubated with PLP, the added PLP must be dissolved in Tris buffer, for it can bind to the lysine in serum albumin when PLP is dissolved in water (3, 4).

We investigated any difference between the activity concentration measured for serum AST and ALT, after dissolving PLP (1.12 mmol/L final concentration) in water or Tris buffer (pH 7.8; 50 mmol/L) before starting the reaction with serum. After a 10-min pre-incubation, the reaction was started and results were measured at 30 °C in a centrifugal analyzer. We also measured activity concentrations according to the recommendations of the IPFC (5, 6), starting the reaction with 2-oxoglutarate. Volume fractions of serum were the same in both experiments: 0.083. In the statistical analysis we used Student's t-test for paired observations.

It is obvious from the following tabulation that PLP enhances the measured activity concentration:

<table>
<thead>
<tr>
<th>Method*</th>
<th>Mean (and SD) acty, UI/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>ALT</td>
</tr>
<tr>
<td>1</td>
<td>20.8 (11.3)</td>
</tr>
<tr>
<td>2</td>
<td>23.8 (14.0)</td>
</tr>
<tr>
<td>3</td>
<td>20.5 (11.2)</td>
</tr>
<tr>
<td>4</td>
<td>22.5 (12.9)</td>
</tr>
<tr>
<td>5</td>
<td>22.7 (13.2)</td>
</tr>
</tbody>
</table>

n = 71.

*1, without PLP; 2, with 2-oxoglutarate; 3, without PLP; 4, with PLP + 2-oxoglutarate; 5, with serum + PLP; 6, with serum + 2-oxoglutarate; 7, with serum + PLP; 8, with serum + PLP + 2-oxoglutarate

For AST, differences between methods 2 and 4 and methods 2 and 5 are significant (P < 0.01). For ALT, differences between methods 4 and 5 are significant (P < 0.01). For AST and ALT, differences between methods without PLP and with PLP are significant (P < 0.01).

Although the differences between methods with addition of PLP are sometimes significant, they are very small and clinically not relevant. Evidently, in practice it seems unnecessary to dissolve PLP in Tris buffer instead of water.

References


The Laboratory Standardization Panel of the National Cholesterol Education Program recommends that cholesterol measured by all U.S. clinical laboratories be standardized to values traceable to the Centers for Disease Control (CDC) Reference Method or the National Bureau of Standards Definitive Method (J). We evaluated the Beckman System 700, using "Dri-Stat Cholesterol Rate" reagent and cholesterol reference materials (both from Beckman Instruments Corp., Carlsbad, CA) for their ability to meet the performance criteria of the CDC–National Heart Lung and Blood Institute (NHLBI) Cholesterol Standardization Program.

Frozen aliquots of pooled human sera were obtained from the CDC-NHLBI Lipid Standardization Program. High-density lipoprotein cholesterol (HDLC) in CDC pooled sera was determined after apolipoprotein B-containing lipoproteins were precipitated by a modification (2) of the Warnick/Albers heparin–Mn2+ protocol. Serum-based controls from Solomon Park Research Laboratories (Northwest Lipid Research Center, Kirkland, WA) were used for quality assurance in the total and low total cholesterol assays (TC and LTC), "Serachem" (Fisher, Orangeburg, NY) was used as quality-control material for the HDLC assay.

Total cholesterol intra-assay CVs (31 replicates) were 1.2% at 185 (SD 2.2) mg/dL, 1.3% at 212 (SD 2.7) mg/dL, and 1.3% at 278 (SD 3.6) mg/dL. HDLC intra-assay CV for 20 replicates was 1.1% at 51.3 (SD 0.6) mg/dL. TC inter-