Effect of Various Diluents on the Activity of Several Enzymes Present in Serum

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We have evaluated the effect of diluting serum with water or NaCl solution (8.5 or 9.0 g/liter) before assaying by a manual method for creatine kinase (EC 2.7.3.2), alkaline phosphatase (EC 3.1.3.1), lactate dehydrogenase (EC 1.1.1.27), and aspartate aminotransferase (EC 2.6.1.1) activity. The t test and the F test show no significant difference in the accuracy and precision of the assays at the 95% confidence level when 100 different samples were compared for each enzyme activity after use of the three diluents.

Although many clinical chemists agree that rate-reaction (kinetic) methods for assay of serum enzymes are fundamentally more valid than one-point assay methods, many clinical laboratories still use one-point enzyme assay methods. This may be attributable to equipment cost, volume of tests performed, adaptability of one-point assays to many existing automated systems, and various other reasons. A recent paper by Moss (1) expounds on the merits and applicability of kinetic vs. fixed-time assay methods. He states that fixed-time assays will continue to fulfill a useful role in the clinical laboratory, if their limitations are appreciated.

In the one-point assay method, a calibration curve is usually used in which absorbance is plotted vs. amount of product formed per unit of time. If the serum enzyme activity is greater than can be read from the calibration curve, the sample must be diluted and reassayed. The appropriate diluent for the serum has generated a controversy as to whether water or a sodium chloride solution should be used as the diluent, and, if a sodium chloride solution is used, what concentration should be used. Possible ionic-strength effects of diluents on serum enzyme activity may be the basis of this controversy.

Materials and Methods

The methods utilized in this study were fixed-time assay methods. The creatine kinase (CK) method was that described by Nuttall and Wedin (2), the alkaline phosphatase (AP) method was that of Babson et al. (3), the lactate dehydrogenase (LD) method was that of King (4) with the modifications by Fendley et al. (5), and the aspartate aminotransferase (AST) method was described by Doumas and Biggs (6). The coefficient of variation (day-to-day) of the methods used are as follows: LD 8.7%, AST 10.1%, CK 14.1%, AP 7.4%.

Most of the sera for the study were chosen from samples that on the "SMA 12/60" (Technicon Corp., Tarrytown, N. Y. 10591) had exhibited activity higher than the listed normal. The samples were diluted with either water or NaCl solutions (either 8.5 or 9.0 g/liter). The dilution factor was chosen so as to give a readable value on the calibration curve with the method utilized. It ranged from a 2- to 20-fold dilution for the samples chosen. After the serum was diluted, the activities of the enzymes were immediately determined.

Results and Discussion

The effect of diluting serum with the three diluents before assaying for CK, AP, LD, and AST activity was evaluated by comparing 100 specimens for each enzyme.

Table 1 gives some representative data obtained. The

| Table 1. Representative Examples of Activity (in U/Liter) of Serum CK, AP, LD, and AST When the Serum is Diluted with Various Diluents |
|-----------------|-----------------|-----------------|
|                 | Water  | NaCl 8.5 g/liter | NaCl 9.0 g/liter |
| CK              |       |                 |                 |
| 1               | 490   | 470             | 460             |
| 2               | 1090  | 1130            | 1090            |
| 3               | 60    | 50              | 50              |
| 4               | 350   | 350             | 330             |
| Mean (n = 100)  | 310   | 300             | 303             |
| AP              |       |                 |                 |
| 1               | 76    | 76              | 76              |
| 2               | 110   | 102             | 105             |
| 3               | 232   | 232             | 238             |
| 4               | 68    | 69              | 66              |
| Mean (n = 100)  | 93    | 92              | 92              |
| LD              |       |                 |                 |
| 1               | 390   | 375             | 400             |
| 2               | 160   | 180             | 170             |
| 3               | 550   | 550             | 560             |
| 4               | 1096  | 1100            | 1000            |
| Mean (n = 100)  | 377   | 356             | 356             |
| AST             |       |                 |                 |
| 1               | 524   | 510             | 510             |
| 2               | 180   | 185             | 180             |
| 3               | 176   | 160             | 160             |
| 4               | 1310  | 1330            | 1260            |
| Mean (n = 100)  | 184   | 188             | 179             |
Table 2. Correlation Coefficient for the Assay of Serum CK, AP, LD, and AST When the Serum Was Diluted with Various Diluents

<table>
<thead>
<tr>
<th></th>
<th>NaCl, 8.5 g/liter vs. H2O</th>
<th>NaCl, 9.0 g/liter vs. H2O</th>
<th>NaCl, 8.5 g/liter vs. NaCl, 9.0 g/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>0.994</td>
<td>0.996</td>
<td>0.992</td>
</tr>
<tr>
<td>AP</td>
<td>0.995</td>
<td>0.994</td>
<td>0.999</td>
</tr>
<tr>
<td>LD</td>
<td>0.885</td>
<td>0.883</td>
<td>0.999</td>
</tr>
<tr>
<td>AST</td>
<td>0.947</td>
<td>1.000</td>
<td>0.948</td>
</tr>
</tbody>
</table>

enzyme activities ranged as follows: CK (30–1780 U), AP (21–304 U), LD (90–2600 U), AST (8–1310 U). The t test (95% confidence level) indicates that there is no significant difference in the means of the test, whichever diluent was used. The F test (95% confidence level) indicates that there was no difference in the precision of the methods. The correlation coefficient for the assay with the various diluents is shown in Table 2. Table 3 shows the results of a linear regression analysis of the data.

In a draft proposal of reference methods for enzymes (7) it was stated that “sera of extremely high activity should be diluted with inactivated serum in order to maintain the fixed volume fraction of serum,” and that “Dilution of serum with saline or water may alter the matrix effects of serum as to distort the enzyme activity.” Our results are not necessarily in conflict with this statement. If dilution with water or a sodium chloride solution alters the matrix effect of serum and distorts the enzyme activity, it apparently does so to the same degree regardless of which diluent is used.

References