Copper in Serum Measured with the Cobas Bio Centrifugal Analyzer

To the Editor:

Cu²⁺ in serum is usually measured according to Zak and Ressler (1), which involves several steps, including protein precipitation. Although Williams et al. (2) demonstrated that precipitation of proteins can be omitted, the method could not be adapted to the Cobas Bio without further modifications. A centrifugal analyzer will accommodate only a certain sample volume; on the other hand, the specific molar absorptivity of the resulting Cu²⁺-Bathocuprine complex is relatively low as compared with the concentrations of copper in serum.

From various experiments I found a satisfactory adaptation of the method of Williams et al. to the Cobas Bio, which I describe briefly here.

Cu²⁺ is dissociated from ceruloplasmin by incubating the native serum in a guanidinium hydrochloride solution, which contains ascorbic acid for the concurrent reduction of Cu²⁺. The incubation interval in the first centrifugal run is 240 s at a reaction temperature of 37 °C. The color reaction is started by adding color reagent at the beginning of the second centrifugal run (300 s at 37 °C). The reaction temperature of 37 °C is necessary for higher color yield and better precision of the analytical procedure.

The instrument settings are as follows:

<table>
<thead>
<tr>
<th>Units</th>
<th>Calculation factor</th>
<th>Standard 1 conc</th>
<th>Standard 2 conc</th>
<th>Standard 3 conc</th>
<th>Limit</th>
<th>Temperature, °C</th>
<th>Type of analysis</th>
<th>Wavelength, nm</th>
<th>Sample vol, µL</th>
<th>Diluent vol, µL</th>
<th>Reagent vol, µL</th>
<th>Incubation time, s</th>
<th>Start reagent vol, µL</th>
<th>Time of first reading, s</th>
<th>Time interval, s</th>
<th>No. readings</th>
<th>Blanking mode</th>
<th>Printout mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>300</td>
<td>37.0</td>
<td>7</td>
<td>485</td>
<td>80</td>
<td>10</td>
<td>200</td>
<td>240</td>
<td>20</td>
<td>0.5</td>
<td>300</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Corresponding to the value of the standard calibration serum in use.

The dissociation-reduction medium consists, per liter, of 6 mol of guanidinium hydrochloride and 5 g of ascorbic acid in 0.1 mol/L acetate buffer (pH 4.8). The color reagent contains, per liter, 1 g of the disodium salt of Bathocuprine disulfonic acid in a 2 mol/L Tris solution.

The analyzer is calibrated by use of a calibration serum. Recovery and precision were poor when aqueous solutions with known amounts of Cu²⁺ were used as calibrators.

Experiments with a Cu²⁺-supplemented serum pool (3 mg of Cu²⁺ per liter) exhibited that the linearity range extends to 3 mg/L, which would include even highly elevated pathological specimens. Above 3 mg/L the curve flattens slightly, resulting in an error of about 10% for a 4.5 mg/L concentration.

Table 1 presents the within-run precision and the run-to-run precision obtained with commercially available control sera with low (0.71 mg/L), medium (1.49 mg/L), and high (2.01 mg/L) Cu²⁺ concentrations.

Results obtained with the Cobas Bio method for 54 randomly selected specimens (x) were compared with the results of manually performed analyses (y) according to the method of Zak and Ressler (1). Linear regression analysis (y = ax + b) gave 0.970 for a and 2.2416 for b (r = 0.9866).

This method has been used in our laboratory without any difficulties for eight months.

The excellent technical assistance of Mrs. Claudia Lerach is greatly appreciated.

The Seralyzer and Use of Quality-Control Materials

To the Editor:

Recently, clinical chemistry tests have been developed that involve use of dry reagents, either as multilayered (1,2) or single-layered (3,4) thin films. Such analytical systems, involving permeation of sample through thin dry layers of reagent, may be susceptible to protein matrix effects (5), particularly where lyophilized materials are used for calibration or quality control (6,7).

During an evaluation of the "Sera- lyzer" (Ames Division of Miles Laboratories, Stoke Poges, Slough, U.K.) (6), we assayed various quality-control sera and fresh human sera for glucose, urea, and urate with both the Seralyzer and an SMA 12/60 (Technicon Instruments Corp., Tarrytown, NY, 10591) (9-11). For urea and urate (but not for glucose) the differences between results with the Seralyzer and with the SMA 12/60 were greater for control sera than for fresh human sera. Possible reasons for this difference in behavior include the effect of lyophilization and differences in viscosity, which might be expected to affect the rate of permeation of the sample into the reagent strip and hence the result. We therefore investigated these factors.

Twenty patients' sera with a range of urea and urate concentrations were diluted and analyzed in duplicate with the Seralyzer. A 0.7-mL aliquot of each specimen was lyophilized (Minifast 680 freeze-drier unit; Edwards High Vacuum Ltd., Crawley, Sussex, U.K.). Each was reconstituted with 0.7-mL of distilled water, and then diluted and analyzed in duplicate for urea and urate with the Seralyzer. Sodium values, measured before and after lyophilization, were used to correct the after-lyophilization results, i.e., to correct for the increase in sample volume after reconstitution due to the volume occupied by proteins.

Another 20 patients' sera with a range of total-protein concentrations were divided into 0.7-mL portions, which were similarly lyophilized and reconstituted. The viscosity of each specimen was determined (in duplicate) before and after lyophilization (with a Harkness Plasma Viscometer; Coulter Electronics Ltd., Luton, Beds., U.K.). Sodium values were again used to adjust the after-lyophilization results. The viscosities of quality-control sera were measured.

Urate values showed no significant difference before and after lyophilization (mean percentage change = -2.7); i.e., lyophilization per se evidently had no matrix effect on urate determinations by the Seralyzer.

In contrast, a significant difference was found for urea, but only for those samples within the analytical range (mean percentage = -4.7, p = 0.03); over-range samples did not show this.

Table 1. Precision of Copper Assays on Cobas Bio

<table>
<thead>
<tr>
<th>Expected mg/L</th>
<th>Found mg/L (n = 84)</th>
<th>Within-run CV, %</th>
<th>Run-to-run CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.71</td>
<td>0.714</td>
<td>4.45</td>
<td>2.23</td>
</tr>
<tr>
<td>1.49</td>
<td>1.450</td>
<td>2.48</td>
<td>1.18</td>
</tr>
<tr>
<td>2.01</td>
<td>1.995</td>
<td>1.71</td>
<td>1.41</td>
</tr>
</tbody>
</table>

References


Karl T. Kossman
Dept. of Clin. Chem.
Lab. Dr. Gaertner
P.O. Box 1220
7987 Weingarten, F.R.G.
behavior on further threefold dilution (mean percentage change = +3.9, not significant). This difference in behavior, although not significantly correlated with total-protein concentration, suggests that lyophilization affects the behavior of serum proteins on the reagent strips used for urea measurements. The reasons for this are not clear but the timing of the analysis begins when the sample is applied, and so the rate of permutation would affect the actual analysis time. This conjecture is supported by experiments (12) showing a significant increase in results for urea if timing of the analysis is delayed. The differences in behavior of urea and urate analyses is noteworthy, because in the former the sample is applied to a reagent strip containing an ion-exchange resin, and variations in the rate of permeation through this resin might affect results for urea. The urate reagent strip contains no such resin.

We found that lyophilization significantly (p < 0.001) increases the viscosity of the reconstituted serum (by 4.2% in 17 samples). This might lead to changes in the rate of permeation of the sample through the strip. The quality-control sera were found to have significantly (p < 0.03) lower viscosities than the fresh human sera (quality-control materials mean 1.40 mPa·s, SD 0.17 mPa·s, n = 7; fresh human sera mean = 1.54 mPa·s, SD 0.11 mPa·s, n = 15).

In summary, lyophilization produces lower urea results on the Seralyzer and an increase in serum viscosity. This increase in viscosity may result in slower permeation of the sample through the reagent strip and hence a lower urea result. Reconstituted quality-control sera had lower viscosities, which might increase their rate of permeation and hence explain the higher urea values obtained with the Seralyzer for these materials.

The study has shown that urea determination with the Seralyzer is sensitive to matrix effects. Hence if the Seralyzer is to be used with a quality-control program, assigned values will be necessary for each control material used. It is important that matrix effects, which might occur with other chemistries and dry-reagent film techniques, should be assessed during evaluation and the results appreciated by the user.

The authors thank Ames Division of Miles (U.K.) for the loan of the instrument and their cooperation during the study. The financial support of the Department of Health and Social Security is gratefully acknowledged.

References

P. M. S. Clark
I. M. Surplice
P. M. G. Broughton
D. G. Bullock

Dept. of Clin. Chem.
Wolfson Res. Labs.
Queen Elizabeth Med. Centre
Birmingham B15 2TH
U.K.

Unusual Creatine Kinase Isoenzyme Pattern Associated with a Case of Acute Pancreatitis

To the Editor:
We recently observed a case of acute pancreatitis showing an unusual pattern of creatine kinase (CK, EC 2.7.3.2) isoenzymes.

The patient, a 79-year-old woman, was admitted to the hospital with acute abdominal pain. At the time of admission her serum amylase (EC

Table 1. Patient’s Serum Enzyme Activities

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Observed</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-total</td>
<td>2710</td>
<td>35–232</td>
</tr>
<tr>
<td>CK-MM</td>
<td>2270 (84)</td>
<td></td>
</tr>
<tr>
<td>CK-MB</td>
<td>250 (9)</td>
<td></td>
</tr>
<tr>
<td>CK-BB</td>
<td>190 (7)</td>
<td></td>
</tr>
<tr>
<td>LDH-total*</td>
<td>424</td>
<td>90–200</td>
</tr>
<tr>
<td>LDH-1</td>
<td>55 (13)</td>
<td>24–49 (23–41)</td>
</tr>
<tr>
<td>LDH-2</td>
<td>98 (23)</td>
<td>15–46 (19–33)</td>
</tr>
<tr>
<td>LDH-3</td>
<td>110 (26)</td>
<td>15–35 (16–27)</td>
</tr>
<tr>
<td>LDH-4</td>
<td>80 (19)</td>
<td>7–20 (7–17)</td>
</tr>
<tr>
<td>LDH-5</td>
<td>80 (19)</td>
<td>1–19 (2–15)</td>
</tr>
<tr>
<td>Amylase</td>
<td>3060</td>
<td>5–75</td>
</tr>
<tr>
<td>Alk. phos.</td>
<td>112</td>
<td>30–115</td>
</tr>
<tr>
<td>AST</td>
<td>132</td>
<td>5–35</td>
</tr>
</tbody>
</table>

*LDH, lactate dehydrogenase (EC 1.1.1.27); AST, aspartate aminotransferase (EC 2.6.1.1). In parentheses: percentage of total activity.

3.2.1.1 value was much above normal. These and other results for serum enzymes are tabulated in Table 1. Except for alkaline phosphatase, all other enzyme activities were significantly above normal, and increases were observed for all serum LDH isoenzymes except LDH-1. Therefore, no "flip" of the LDH-1/LDH-2 ratio was observed. Quantitation of CK isoenzymes by electrophoresis and fluorescence scanning (Figure 1) revealed that CK-MM (muscle), CK-MB (cardiac), and CK-BB (brain) were concurrently increased.

Tsung (1) reported that the predominant CK isoenzyme in the pancreas is CK-BB (93% of the total CK activity). Thus the presence of CK-BB in the patient's serum might be due to release of CK-BB into the serum from severely damaged pancreatic tissue. However, the presence of CK-MB in the serum is somewhat puzzling because the patient

Fig. 1. Drawing of agarose gel electrophorogram of patient's serum creatine kinase and the fluorescence scanning profile.

The cathode is at the left. Arrows at the top of the diagram indicate the position of the three CK isoenzymes in a DADE isoenzyme "CK-marker" sample. The arrow at the bottom represents the origin.

CLINICAL CHEMISTRY, Vol. 29, No. 3, 1983 579