Table 1. Concentrations of Trimethoprim, Sulfamethoxazole, and Creatinine in the Plasma Samples

<table>
<thead>
<tr>
<th></th>
<th>Tri-methoprim</th>
<th>Sulfamethoxazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/liter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>200</td>
</tr>
<tr>
<td>45</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>45</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>45</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>45</td>
<td>5</td>
<td>200</td>
</tr>
</tbody>
</table>

crease with time would indicate impairment of renal function during therapy with the drugs “Septin” and “Bactrim” and would especially limit their benefit in cases of pre-existing damage. We investigated the possibility of erroneous interpretation of results of creatinine assays caused by the presence of TMP or SMX, or both.

We have two assays for creatinine, the usual Jaffé alkaline picrate reaction adapted for a Technicon AutoAnalyzer and a reaction rate method (3) adapted for LKB reaction rate analyzers. We used both to estimate creatinine in 20-ml samples of human plasma to which creatinine, TMP, and SMX were added in the proportions shown in Table 1. All samples were assayed in apparatus in routine use so as to estimate the variation to be expected from laboratories not involved in specific work. Plasma came from a single subject with a nominal creatinine concentration of 9 mg/liter. Extra creatinine was added to produce a fivefold increase representing severe renal failure, and TMP and SMX were added to represent the highest values normally encountered in therapy.

The next two tables, with the data in the same order, show the results for the picrate (Table 2) and the reaction rate (Table 3) methods, respectively. Different requirements of sample size account for the different number of replicates offered for the two methods.

For the picrate method the means of the low creatinine concentrations differ significantly (P < 0.01) with the addition of TMP and SMX. Plasma creatinine concentrations near 10 mg/liter are overestimated by about 10% by the method in the presence of SMX and (or) TMP. No similar increase is apparent for creatinine concentrations near 45 mg/liter.

For the reaction rate method, the sample means for plasma creatinine concentrations near 10 mg/liter were not affected by addition of either TMP or SMX. At concentrations near 45 mg/liter, there were some differences, inconsistent and possibly therefore owing to a fault that could arise spontaneously in routine laboratory service.

We conclude that the widely used Technicon method for measuring plasma creatinine could result in overestimations of about 10% in the range of normal values as a result of the presence of TMP and SMX. An overestimate of this size is insignificant, however, in the range of abnormally high values seen in renal failure.

References

Wellcome Research Laboratories
Beckenham, Kent, U.K.

Defect in the Methodology of Aspartate Aminotransferase Determination with SMAC

To the Editor:
Proper assay of aspartate aminotransferase (EC 2.6.1.1) requires a preincubation of up to 30 min with NADH (1), during which α-keto acids in serum are converted to their reduced forms, catalyzed by endogenous lactate dehydrogenase (EC 1.1.1.27). Many manufacturers add lactate dehydrogenase to their premixed reagents to shorten the necessary preincubation time. However, the methodology for the assay of aspartate aminotransferase used in SMAC (Technicon Corp., Tarrytown, N. Y.) allows for only a 2-min preincubation period before analysis.

We checked whether a significant error was introduced because of the theoretical shortcoming of the methodology used in SMAC for the assay of aspartate aminotransferase. To Technicon’s MDH/NADH reagent was added 3 U of lactate dehydrogenase (from pig heart) per milliliter. The values obtained with this modified reagent were then compared with values obtained with unmodified reagent on the same control serum. Table 1 shows that for serum with low activity, there was a significant difference (P = 0.01) in assay values. The value obtained for aspartate aminotransferase activity with unmodified reagent is 9 U/ml higher than the manufacturer’s stated value. The addition of lactate dehydrogenase to the MDH/NADH reagent brings the SMAC results into good agreement with the manufactur-
Table 1. Effect of Added Lactate Dehydrogenase on Assay of Aspartate Aminotransferase

<table>
<thead>
<tr>
<th>Control serum</th>
<th>Manufacturer's stated value</th>
<th>Value obtained</th>
<th>with unmodified MDH/NADH reagent</th>
<th>with lactate dehydrogenase added to MDH/NADH reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Versatrol (low)</td>
<td>17</td>
<td>26 ± 2.2</td>
<td>18 ± 1</td>
<td></td>
</tr>
<tr>
<td>Versatrol (middle)</td>
<td>91</td>
<td>80 ± 4.3</td>
<td>84 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>Versatrol (high)</td>
<td>180</td>
<td>177 ± 2.8</td>
<td>176 ± 6.7</td>
<td></td>
</tr>
</tbody>
</table>

*Average of seven determinations ± one standard deviation.

er's stated value. It is to be expected that the extraneous oxidation of NADH will cause a proportionately larger error in serum with low aspartate aminotransferase activity when compared to serum samples with high activity. It is strongly recommended that users of SMAC reexamine the validity of their assay values for aspartate aminotransferase, especially the low ones.

Reference


N. M. Chang

Automated Lab. Services of Korea
CPO 494, Seoul, Korea

B. Davis

R. Cavaliere

Automated Laboratory Services
15551 Cabrito Rd.
Van Nuys, Calif.

Technicon Corp. was asked to respond, and did so as follows:

To the Editor:

Several workers brought this problem to our attention and asked us to modify our system accordingly. This work was completed this summer and all SMAC systems shipped since September have included Program 7, which eliminates the problem of endogenous pyruvate. In addition, an active retrofit program is underway, which should bring all SMAC's in the field up to the current state of the art by January.

The use of lactate dehydrogenase into the MDH/NADH reagent utilized in the Technicon SMAC assay for the measurement of aspartate aminotransferase (AST) is one of several improvements that have been incorporated into Program 7. In addition, the analytical cartridge has been modified to incorporate 2.8 min of pre-incuba-

tion before the first measurement of absorbance at 340 nm. In SMAC, AST activity at 37 °C is monitored in three successive flow cells over a time interval of 3.4 min.

Examination of selected hospital samples showed a nonlinearity of reaction between the flow cells in the absence of added lactate dehydrogenase. This disappeared when the new formulation was used. Levels of pyruvate as high as 1.5 mmol/liter were added to serum with no apparent change in sample activity.

The problem has been recognized and, more importantly, is now solved.

Jack Levine
Manager, Scientific Relations
Technicon Instruments Corp.
Tarrytown, N. Y. 10591

When Is a Reference Method a Reference Method?

To the Editor:

We have been interested to note that, as part of the effort to improve the quality of work done in hospital laboratories, more clinical chemists are at least paying attention to the need for standardization of methods—a concept that has been accepted for a long time by analysts in other branches of chemistry.

We are, however, worried by two facts. The first is that there appears to be a lack of coordination or direction in the work being done in the field of method standardization; the second is that, as a consequence, methods are published and hailed as "reference" methods before they have been properly validated in a number of countries.

The "reference" method for calcium (1) has been shown (2) to incorporate a number of features that would tend to prevent its use outside the U. S. A. We would suggest that it should have been published as, e.g., a "Proposed Reference Method" (cf. the NCCLS procedure for defining standards) and that its final acceptance as a Reference Method, and that of the two "Reference" Methods presented in July at the Toronto Congress, should follow only after a vetting procedure laid down by the World Health Organization or by a body it designates. We note that it was suggested at the 27th World Health Assembly, in a report on standardization of diagnostic materials, that an important function of WHO could be the co-ordination of different standardization efforts, leading to greater international acceptability of the standardization of diagnostic materials, and that a group should formulate plans for international collaborative studies. At the moment it is uncertain who has the authority to assign the title of "Reference Method"; is it the Editors of "Selected Methods," NBS, NCCLS, IUPAC, IFCC, BCR (in Europe), or a national body of clinical chemists?

When clinical chemists come to the stage of standardizing routine methods we hope that they will ask for and pay attention to the advice of their fellow analysts who have long experience of this approach. Such advice would, we are sure, be readily given by the association of Official Analytical Chemists, the Analytical Division of the Chemical Society, and others.

References


D. W. Neill
J. R. Doggart

Department of Biochemistry
Royal Victoria Hospital
Belfast, Northern Ireland

The above letter was brought to the attention of some appropriate persons. Their collective response follows:

To the Editor:

During recent years a new concept of reference technology has been evolved, and is now accepted, for the advancement of standardisation of methodology and thereby the improvement of accuracy throughout clinical chemistry. We share the concern expressed by Neill and Doggart over a lack of co-ordination of effort on several aspects of work on this front, and the need for thorough international validation of what are commonly referred to as "reference meth-