and TIBC set, our expected values were reported in our package insert as being 17 to 165 μg/dL based upon a mean value of 91 μg/dL ± 2 standard deviations. However, as a result of our internal program of routine review of products and package inserts, the expected value range was changed to 55 to 165 μg/dL, effective in August, 1978. The change in the lower limit of the expected value range was a result of our using the same statistical considerations as Messrs. Doetsch, Bellows, and Graber. More specifically, our current values are based upon the 90th percentile estimate rather than the previous mean ± 2 SD methods.

The scope of the original expected range study was insufficient to yield an estimate of gaussian distribution. Further study confirmed the non-gaussian distribution found by the authors.

I must commend Messrs. Doetsch, Bellows, and Graber for the useful analysis they performed. DADE welcomes direct correspondence from our customers whenever a question such as this arises, as we are committed to supplying current, accurate information with all of our clinical products. Only with such direct correspondence can we ensure that we continue to satisfy the needs of the clinical chemistry community.

James D. Yeakel
Product Manager Chemistry
DADE, Div. American Hospital Supply Corp.
Miami, FL 33152

Evaluations of the Creatine Kinase MB Kit of Boehringer

To the Editor:

In their evaluations of Boehringer’s Creatine Kinase MB Kit, Fiolet and Baartscheer (1) obtained results that agreed substantially with our evaluation of the same procedure (2). However, they expressed a lack of understanding of our slope of 0.679 for the regression line of the percentage MB by the modified Mercer column and the BMC column. They find a slope of 1.03 for a regression line of the MB activity by the Fiolet procedure against the BMC column. They are quite correct in highlighting this discrepancy, because the explanation, though relatively simple, cannot be obtained from our original publication.

Although the CK activity of the eluates from the BMC columns was measured with the CK-NAC system, our existing procedure was a modified Mercer separation of the isoenzymes in which the CK activity of the eluates was obtained with Worthington reagents. As stated in our earlier publication (2), the serum creatine kinase activity ranged from 97 to 4400 U/L with the Worthington reagents and from 124 to 5420 U/L with the BMC-NAC reagents. The slope of 1.23 for the regression line for the total creatine kinase activities thus reflected the greater sensitivity of the BMC-NAC reagent compared to the Worthington reagent. To understand the slope of 0.679 one must realize that this regression line was obtained by comparing the percentage CK-MB from the modified Mercer column (CK activity measured with Worthington reagents) against the BMC column method (CK activity measured with BMC-NAC reagents). We therefore compared the percentage MB obtained in both column procedures, using different methods for the estimation of CK activity. In the evaluation by Fiolet and Baartscheer the same reagents were used for measuring the CK activity of the eluates from both sets of column, and the MB fraction was not expressed as a percentage of the total enzyme activity.

We also found that although the percentage CK-MB activities relative to the total enzyme activities were different for each column, the sum of the CK-MB activities from the eluates was very nearly the same. Regression of the total MB activity of the eluates from the BMC column with those from the modified Mercer column shows a good correlation (r = 0.988, n = 23, slope = 0.964, y-intercept = 3.25). The CK activities with BMC reagents exceeded those with Worthington reagents, and we had expected that these would be reflected in higher total MB activities from the BMC columns. This was not the case, and there are many potential explanations for this finding. It is not possible to state a specific reason and it is only speculation whether differences in reagent concentration and the presence or absence of activators or inhibitors in the final reaction procedure is the single most important factor. However, when the serum was put through columns, the sum of the CK-MB activities measured by the BMC and Worthington procedures gave the correlation stated earlier of r = 0.988, with a slope of 0.964. This good correlation indicates the analytical specificity of the procedures, and the slope indicates that both the BMC and Worthington procedures have the same analytic sensitivity for the MB fraction once the serum has been passed through a column.

References

M. J. McQueen
L. Mori
Elspeth Dey

Dept. of Clinical Chemistry
Hamilton General Hospital
Barton Street East
Hamilton, Ontario
L8L 2X2 Canada

Creatine Kinase Isoenzyme BB and a Fluorescent Artifact in Hemodialysis Patients’ Sera

To the Editor:

There is not universal agreement on the presence and true incidence of the creatine kinase (CK; EC 2.7.3.2) isoenzyme BB in health and disease (1–5). There are two aspects to the controversy: the relative sensitivity of available methods to the absolute activity of each isoenzyme, and their nonspecificity. Electrophoresis (on cellulose acetate or agarose) probably is the technique most widely used for identifying CK isoenzymes in clinical laboratories. Such electrophoresis is sensitive to 1 to 5 U of enzyme activity per liter (6). To address the question of nonspecificity, we studied the CK electrophoretic patterns on agarose of serum from 24 patients who were undergoing hemodialysis.

The patients were selected on the basis of availability of serum before dialysis. Shortly after separation by centrifugation, each serum sample for electrophoresis was stored at −20 °C until shortly before electrophoresis, even if electrophoresis was to be done within a few hours. Nothing was added to the samples.

CK isoenzymes were separated with a commercially available agarose electrophoresis system (Corning Medical, Medfield, MA 02502), according to the manufacturer’s instructions (6). Each agarose film has the capacity for eight samples. On each film, the same sequence of human CK isoenzyme control material and three patients’ samples was applied to positions 1 to 4 and 5 to 8. Each film was inspected by using a fluorescent viewing device (Model 721 Viewer; Corning Medical) immediately after electrophoresis. Each film was then cut between positions 4 and 5; one half was kept as a control and the other was continued through the procedure for demonstrating the isoenzymes by fluorescence.

CK isoenzyme BB was identified in two ways: (a) by noting the relative migration of isoenzymes in the patients’ sera relative to that for the control, and
(b) by adding antiserum to the B subunit of CK to a portion of a patient's serum and the control, then electrophoresing the untreated and treated aliquots in adjacent positions on the same agarose gel.

On inspecting the films before enzyme substrate was added, we would see fluorescent bands cathodal and anodal, adjacent to but distinct from the position where the BB isoenzyme would be expected to appear, as judged from the results for the control and as confirmed by the position of a fluorescent band adjacent to CK-BB in those sera in which CK-BB was detectable.

Whether CK-BB is present in the serum of patients who are in renal failure or whether there is an artifact that has been misinterpreted as CK-BB has been the subject of several reports (2–5, 7–12). The central question is whether or not the usual electrophoresis can resolve the albumin artifact from CK-BB. Whether for cellulose acetate, agar, or agarose electrophoretic systems, the conclusions are conflicting. We found that CK-BB can be detected in the serum of 8.3% of our sample of patients in chronic renal failure. In addition, naturally fluorescent bands were present in each sample, consistent with previous reports; the presence of two such bands consistently in each sample has not been reported previously, to our knowledge. Under the conditions we use, however, the naturally fluorescent bands not only migrate differently from CK-BB (6), but, being yellow-green, are clearly distinguishable from the blue-white fluorescence of the CK isoenzymes.

We emphasize that visual inspection and the use of a human control reduces the possibility that the artifact might be misinterpreted as CK-BB. We believe that the use of scanning densitometer patterns without visual inspection of electropherograms may result in some misinterpretation of peaks in cellulose acetate and agarose systems, and that this may partly account for the discrepant reports among investigators.

We also have noted that a control of nonhuman origin will have small but detectable differences in electrophoretic mobility of CK isoenzymes. In theory, an isoenzyme in such a control would be matched to an artifact in a patient's sample.

Regardless of the support medium used, the naturally fluorescent bands appear. We encourage direct visual inspection for the purpose of identifying CK-BB as well as CK-MB (6).

References
2. Lamar, W., Woodard, L., and Statland, B. E., Clinical implications of creatine kina-

Benjamin Gerson
New England Deaconess Hospital
Boston, MA 02215

Kay Petersen
Dartmouth Medical School
Hanover, MA

Modification of the o-Cresolphthalein Complexone Method for Determining Calcium
To the Editor:
In 1974, Moorehead and Biggs (1) adapted the o-cresolphthalein complexone methodology for calcium determinations to a continuous-flow system by using 2-amino-2-methyl-1-propanol as the reagent base instead of diethylamine, as originally suggested by Gitelman (2). We further modified the procedure of Moorehead and Biggs for use with a Micromedic MS-2 Spectrophotometer and Micro Stat Pipette (both from Micromedic Systems, Inc., Horsham, PA 19044). We were not wetting agent, but all other reagents were identical to those described by Moorehead and Biggs. Working standards in concentrations of 40, 60, 80, 100, 120, and 140 mg/L were prepared from NBS SRM 915 calcium carbonate. Other parameters used in our modification were: reaction duration, 5 min; wavelength, 580 nm; sample volume, 10 μL; reagent volume, 750 μL; reaction temperature, ambient; spectrophotometer temperature, 25°C; spectrophotometer timer setting 1, spectrophotometer mode setting N.

To validate our modification, we used Hyland Chemistry Control Serum I and II. The listed value for control serum I for atomic absorption was 100 ± 2 mg/L (mean ± SD). Using our modification, we obtained a value of 99 ± 1.1 mg/L (mean ± SD) with a coefficient of variation (CV) of 1.1%. The listed value for control serum II for atomic absorption was 131 ± 2.5 mg/L (mean ± SD). Using our modification, we obtained a value of 128 ± 1.7 mg/L (mean ± SD), with a CV of 1.3%.

The tabulation below shows that the calcium values obtained with our method compare favorably with those obtained with atomic absorption. Atomic absorption values were obtained with a Perkin-Elmer Model 303 Spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT 06856).

<table>
<thead>
<tr>
<th>Calcium (mg/L)</th>
<th>Modified procedure</th>
<th>Atomic absorption (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.0</td>
<td>100.2</td>
<td></td>
</tr>
<tr>
<td>123.9</td>
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</tr>
</tbody>
</table>

The correlation coefficient between the modified technique and atomic absorption is 0.990 (p = 0.005). Between 40 and 250 mg/L the standard curve for the proposedmethod is linear and follows Beer's law. The analytical recovery for control serum I was 99.7% and 100.1% for control serum II. The reagents should be discarded if the reagent system, without serum added, has an absorbance greater than 0.400 or less than 0.100, or if the 100 mg/L standard does not give an absorbance of 0.700 ± 0.05.

In conclusion, we propose a modification of the Moorehead and Biggs procedure that is more precise, is rapid, utilizes a small sample size, and does not require sophisticated laboratory equipment.

References