Immunochromatographic Assay of Creatine Kinase Isoenzyme MB and Lactate Dehydrogenase Isoenzyme 1

To the Editor:

We compared an immunochromatographic method for determination of creatine kinase (CK; EC 2.7.3.2) and lactate dehydrogenase (LD; EC 1.1.1.27) activities with the conventional electrophoretic method.

Of the 105 patients included in this study, 58 were in the intensive-care unit with a preliminary diagnosis of myocardial infarction; the other 47 had developed chest pain during hospitalization for other reasons.

Total CK and LD activities were determined with kinetic procedures, with Dow and Abbott reagents, respectively, in an ABA-100 discrete analyzer. Total CK activity was assayed by the method of Rosalki (1), LD activity by use of the lactate-to-pyruvate reaction (2). Agarose electrophoresis (Corning) was used to determine CK and LD isoenzymes. The CK bands were evaluated under ultraviolet light (254 nm), the results being recorded as negative, trace, or positive. The LD-1/LD-2 ratio was determined for all patients. Those with a ratio of ≥1 were recorded as positive, those with a ratio <1 as negative.

For immunochromatographic assays of CK-MB and LD-1 we used the kit assays, Isocytes CK and Isocytes LD, respectively, from Roche Diagnostics, Inc., Nutley, NJ. The mean (and SD) value for 165 other patients without myocardial infarction were 2.2±3.84 U/L for CK-MB and 42.94±16.94 U/L for LD-1. On the basis of these results we selected a cutoff point of 12 U/L for CK-MB and 95 U/L for LD-1 as being positive for myocardial infarction. Values of 10–12 U/L for CK-MB and 90–95 U/L for LD-1 were considered equivocal. The diagnosis of myocardial infarction was established on the basis of clinical information, electrocardiographic positive evidence, and positive values for CK-MB and LD-1. Of 14 patients with clinical and electrocardiographic evidence of acute myocardial infarction, 12 had immunochromatographic values exceeding the two cutoff points, a positive MB band, and LD-1/LD-2 ratio >1. In three patients LD-1 appeared before the LD-1/LD-2 ratio became positive; in the other nine patients they both appeared on the same day. In the nonmyocardial infarction group (93 patients) a CK-MB value exceeding the cutoff was observed in six, while the LD-1 remained negative, suggestive of ischemic myocardial injury. In two of six patients the total CK activities were above our normal reference interval. On electrophoresis, the serum of all six patients contained a visible MB band. Ali et al. (3) reported a similar pattern in 11 patients. The significance of this finding is not entirely clear. The nonmyocardial disorders possibly responsible for the pattern of myocardial ischemia in these patients include congestive heart failure, severe angina, and myocardial arrhythmia.

The nonmyocardial infarction group, one patient had an LD-1 value exceeding the cutoff value; the total LD was 400 U/L, and LD-1 was observed on electrophoresis. This patient was hypertensive and had suffered a fractured hip, but there was no clinical or electrocardiographic evidence of myocardial infarction.

In two of the patients with clinical and electrocardiographic evidence of acute myocardial infarction, the CK-MB value was positive; LD-1 remained within normal limits by both methods. Subsequently, one of these patients developed a ventricular pseudoneurym, presumably after a myocardial infarction.

At the cutoff point of 12 U/L, the CK-MB assay was 100% sensitive and 97.6% specific for acute myocardial infarction. The corresponding values for the electrophoresis results were 75% and 97.6%. At the cutoff point of 95 U/L, the LD-1 assay was 83.3% sensitive and 97.6% specific for acute myocardial infarction. The corresponding values for electrophoresis were 75% and 100%.

The higher sensitivity of LD-1 assay over assessment of the LD-1/LD-2 ratio is the main advantage in the immunochromatographic method. This resulted in earlier detection of acute myocardial infarction in three patients in our study.

References

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Comments on an Evaluation of a Kit for the Determination of Calcium in Serum and Urine

To the Editor:

A recently published evaluation of a kit for determination of calcium (1) has the following deficiencies:

Precision studies as shown in their Table 1 are inadequate because 10 or fewer measurements were used to determine the coefficients of variation. Furthermore, precision was established at one level only. For example, the whole serum for the within-run studies was normocalcemic while the whole serum for the between-run studies was hypocalcemic. For these data to be useful, more samples should have been used, and both within-run and between-run precision should have been established for at least two, and preferably three, concentrations.

I find it unusual that the coefficients of variation for the within-run determinations for urine and whole serum are larger than those for the between-run determinations. This requires explanation.

Although the authors calculated coefficients of correlation, they neglected to calculate the standard error of y in terms of x (S_y), which indicates the standard deviation of points scattered around a line (2). For calcium in whole serum (Table 2) the coefficient of correlation r equals 0.9120 (not 0.7509 as erroneously calculated by Zerwekh and Nicar). S_y is 4.56 mg/dL, indicating a large intermethod variability.

From linear-regression data, calcium measured by the kit is about 95 mg/dL when the atomic absorption spectrophotometry result is 95 mg/dL (the mean of the 20 whole serum samples). It follows that, for a whole serum calcium value of 95 mg/dL determined by atomic absorption, a value for calcium in whole serum obtained with the kit (within the 95% confidence interval of
Determination of Calcium Serum: Precision and Correlation with Prostatic Acid Phosphatase Activity

### References


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The authors of the paper in question respond:

To the Editor:

We thank Dr. Lustig for his concern regarding the statistical interpretations of our evaluation of this calcium analysis kit. While we agree that precision is most accurately established at two and preferably three levels of analyte concentration, we feel that 10 measurements at each concentration represent a sufficient number to derive meaningful statistics. Had precision been determined at three levels, we most certainly would have continued to utilize 10 determinations at each of these levels. Because we only utilized one concentration of analyte to determine the within- and between-run precision our results may be skewed due to sample number and thus this, we think, explains the unusual observation for the larger coefficients of variation for the within-run determinations than for the between-run determination. We have no other explanation.

Dr. Lustig is correct that the coefficient of correlation $r$ is 0.9220 when a Pearson's correlation coefficient is utilized, but our coefficient of correlation of 0.7809 is correct when one considers that this is the Spearman's rho. Since we do not consider the data to be normally distributed, we elected to use a nonparametric method of analysis, thus the choice of Spearman's rho. Furthermore, we elected not to calculate the standard error of $y$ in terms of $x$, since any comparison of two methodologies measuring the same analyte should give identical answers if the two methods correlate. To put this in another way, the strictest interpretation of agreement between two methodologies would be to compare the differences in values for each method from the line of identity or when $y = x$. Such an interpretation is most readily performed by a simple paired $t$-test. This was done, as indicated at the bottom of Table 2, and showed the differences of the two kits not to be significant. In addition, other statistical analyses utilizing both parametric and nonparametric analysis gave similar statistical interpretations. This problem was raised during the review of this manuscript and it was suggested that the data be prepared as a graph of residuals in which the concentration obtained by the kit is plotted as the abscissa vs the difference between the two methods on the ordinate, and this is graphically represented in Figure 1. Thus, while Dr. Lustig's calculations suggest a certain degree of (unmeasured) uncertainty in the determination of whole serum calcium with the kit as opposed to atomic absorption spectrophotometry, these figures do not apply to the measurements themselves, as one can readily see by examining the values obtained for the kit vs atomic absorption spectrophotometry. In only one instance out of $20$ (5%) is there sufficient disagreement between the value obtained by atomic absorption spectrophotometry and the kit method in which the diagnosis of eucalemia vs hypercalcemia would be questioned.

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### References