Effect of Sample Dilution on Creatine Kinase MB Measurement

To the Editor:

In the measurement of creatine kinase (EC 2.7.3.2) isoenzyme CK-MB by such methods as electrophoresis, column chromatography, or immunoinhibition, samples with high total CK activity must be diluted. Here we compare results by an electrophoresis method, which requires sample dilution, with those by the recently introduced immunoradiometric methods, which do not.

The patients discussed in this report were among 108 patients studied in two hospitals. The acco (Du Pont, Wilmington, DE 19898) was used to measure total CK at 37°C. Corning (Corning/ACI, Medfield, MA 02502) reagents and densitometer (Model 720) were used for isoenzyme quantification on agarose gels. Both hospitals used CK-MB values of 5% of total CK activity as the upper limit of normal for electrophoresis.

Immunoradiometric tests were done in one hospital with "QuicCK-MB" and in another hospital with "EMBRIA-CK" (both are kits, from International Immunoassay Laboratories, Inc., Santa Clara, CA 95054). In immunoradiometric assays (IRMA), the CK-MB molecule forms a "sandwich" between anti-CK-B on one side and [125I]-labeled anti-CK-M on the other. Both kits measure mass concentrations of CK-MB and results are expressed in equivalent units per liter (equiv. units/L), values that approximate the enzymatic activity one would get at 30°C with the CPK Stat-Pack kit (Calbiochem, La Jolla, CA 92037). The upper limit of normal for QuicCK-MB is 2.8 equiv. units/L (3.3 equiv. units/L for EMBRIA-CK). QuicCK-MB has slightly better sensitivity and reproducibility, allowing one to use a lower discrimination value than with EMBRIA-CK.

In one study, we evaluated serial samples from 43 patients. In six of these patients, peak total CK ranged from 904 to 5280 U/L and diluted samples showed less than 5% CK-MB by electrophoresis. All six patients were judged to have above-normal proportions of CK-MB by EMBRIA-CK, with Peak CK-MB concentrations ranging from 7.1 to 52 equiv. units/L. The greatest proportion of CK-MB detected was 4% in two of the above patients, who also had an inverted ratio for lactate dehydrogenase (LD) isoenzymes 1/2. Two patients had CK-MB of 4% but no LD ratio inversion. The remaining two patients had 2% CK-MB and LD ratio inversion.

In another study, we analyzed serial samples from 65 consecutive admissions to coronary-care units. Three patients showed CK-MB proportions of 4%, 2%, and 0% by electrophoresis of diluted samples, but were above-normal by QuicCK-MB. Two of the three had LD ratio inversion.

Dubin (1) and Siegel and Dawson (2) indicated that appropriate dilution of total CK activity before isoenzyme separation on electrophoresis will render the CK-MB band negative in most cases and that a negative result will not generally rule out the possibility of myocardial infarction.

We have observed that sample dilution will not render the CK-MB band negative if myocardial infarction is the sole cause of increase in total CK. Patients who are suspected of having an acute myocardial infarction, but whose total CK has increased due to accident, illness, or surgery, are more likely to give normal CK-MB values on sample dilution. This limitation should be kept in mind in diagnosing patients hospitalized for serious accident, surgery, or illness and suspected of acute myocardial infarction. They have a poor diagnosis if they suffer acute myocardial infarction (3) and often are not able to provide a good clinical history because of sedation or unconsciousness. Electrocardiograms may not be reliable due to other complications. Thus, serum isoenzyme results play a significant role in diagnosing acute myocardial infarction among these patients.

References

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Salicylate Interference with Measurement of Acetaminophen—A Reply

To the Editor:

Reed et al. recently reported (1) that the Lancer Acetaminophen Rapid Stat Kit gives erroneously high results in the presence of high concentrations of salicylate, owing to a low correction factor in the instruction sheet. They claim that a correction factor of 0.75 mg/L (0.75 µg/mL) of apparent acetaminophen per milligram of salicylate per liter is more appropriate.

We wish to point out that the 0.15 µg/mL correction factor was based on salicylate, not acetylsalicylic acid, and was the result of the experiments described below. We believe it is the appropriate factor to use if the protocols in the instruction sheet are followed. It should be noted here that, as a result of Dr. Reed’s inquiry to the Food & Drug Administration, Lancer’s data and protocols have been reviewed by the FDA.

The protocols state that two different sample sizes are to be used, depending on the amount of acetaminophen present: 0.5 mL for 20 to 80 µg of acetaminophen per millilitre and 0.1 mL for concentrations exceeding 80 µg/mL (The Reed group used a 0.1-mL sample size for samples in the 20 to 80 µg/mL range, contrary to the insert instructions.)

Using the correct sample sizes for the specified ranges, we have confirmed the validity of the 0.15 µg/mL correction factor with the following experiments:

1. To a serum pool containing no acetaminophen, we added 10, 25, 50, 75, and 100 mg of salicylate per deciliter. Using the 0.5-mL procedure and applying the 0.15 µg/mL factor, we obtained values of -0.02, 0.22, 1.02, 4.75, and 2.12, respectively, for apparent acetaminophen.

2. To a serum pool containing 50 µg of acetaminophen per milliliter, we added 10, 50, and 100 mg of salicylate per deciliter. Using the 0.5-mL assay method and applying the 0.15 µg/mL...