unfractionated plasma: 77% aa, 10% ay, and 13% P. The isoenzyme profile for the supernate was the same as that for serum. Therefore, the platelets contained isoenzyme P as well as certain amounts of aa and ay enolases, which were released during the electrophoresis. The platelet enolases presumably are not accessible to the reagents and procedure involved in the bioluminescence assay, which gave identical results for both serum and plasma samples.

References


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Methods for Creatine Kinase MB Isoenzyme: Precision, Minimal Increase, Small Infarcts, and Autopsy Studies

To the Editor:

Koch et al. (1) compared the analytical and clinical performance of six methods for measurement of creatine kinase isoenzyme MB (CK-MB, EC 2.7.3.2). They found all to have poor between-run precision at upper reference limits and suggested that additional studies were needed to determine whether improved analytical precision might permit identification of small myocardial infarctions (MI) in cases in which they could not otherwise have been detected.

I call attention to the (a) incorrect calculation of imprecision at the upper reference limit for the immunoradiometric assay (IRMA) test kit, (b) information already available on the identification of small MI, based on measurement of CK-MB by IRMA test kits and electrophoresis, and (c) problems encountered in diagnosis of patients with small increases of CK-MB in the absence of other traditional criteria used to diagnose MI.

"Embria-CK" and "QuiCK-MB" (International Immunocassay Laboratories, Santa Clara, CA 95054), IRMA test kits for measurement of CK-MB, have good between-run precision at the upper reference limit (2–5). In calculating precision for Embria-CK, Koch et al. assigned a value of 0 when the measured value was less than the value of the lowest calibrator provided with the kit, 2 EU/L (equivalent unit per liter). This increased the CV value by 9.2% and reduced the mean value by 0.19 EU/L.

Clinical characteristics of a subpopulation of high-risk patients, with minimal increase of CK-MB, were identified in studies at Duke University and Durham County General Hospital (7, 8). Patients suspected of infarct and having a peak CK-MB value of 1–24 U/L (at 37 °C, as determined by agarose gel electrophoresis scanned with a Model 720 densitometer from Corning, Medfield, MA 02052), were diagnosed to have "uncertain" acute MI. A majority of the "uncertain" acute-MI patients had (a) a one-year mortality rate higher than that of the positive or the negative acute-MI patients and, (b) compared with other groups, a statistically higher incidence of congestive heart failure and old age and a greater proportion of non-white, female, repeat admission, and repeat infarction patients. Unfortunately, some seemingly low-risk patients were included in the "uncertain" acute-MI group. This is to be expected because the reliability of agarose gel electrophoresis for measuring small increases of CK-MB (9) is inconsistent. D. Calbreath and I found that the IRMA methods provide accurate differentiation of high-risk and low-risk patients in the "uncertain" MI group. Recently Clyne et al. (10) studied two groups of patients with low total CK. One group had total CK of >60–100 U/L, the other group had total CK of >100–200 U/L. They found "Quick-MB" to have diagnostic efficiencies of 78.6% in the first group and 95.5% in the second group, as respectively compared with 28.6% and 77.3% for electrophoresis and 42.8% and 81.8% for the Tandem-E CK-MB (Hybritech Inc., San Diego, CA 92121).

Given the accuracy of IRMA results, the clinical studies cited, and studies with IRMA kits (11), the peak CK-MB value reached during acute MI in certain patients is not very high. These patients have some combination of the following characteristics: old age, female, previous infarction, non-transmural infarction, and serious medical complications many of which have been associated with patients diagnosed to have acute MI but whose cases did not meet all the traditional criteria of typical chest pain, electrocardiographic tracing, and inversion of the lactate dehydrogenase isoenzymes 1/2 ratio (12–15). Clinicians who strongly believe in traditional criteria are likely to classify patients who have small increases of CK-MB as non-MI, an approach that renders useless the advantage of detecting such small increases.

According to autopsy studies (16) a large proportion (47%) of patients who died of acute MI were not diagnosed to have MI prior to autopsy. I believe this failure rate can be attributed to the number of "uncertain" acute MI patients who have a high mortality rate but are likely to be diagnosed as non-acute MI on the basis of traditional criteria. With the availability of IRMA methods the problem is not to detect small increases of CK-MB, rather it is to diagnose accurately the patients in the coronary-care unit population who have small increases of CK-MB.

References


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The authors of ref. 1 comment:

To the Editor:

The manufacturer of each reagent system or kit in our study, including I.I.L., was sent a copy of our manuscript before it was submitted to Clinical Chemistry. In his letter to one of us (personal communication May 20, 1985) Dr. Shah raised no objection to our method of calculating imprecision of the I.I.L. kit from our Pool 1 data, which admittedly overestimates imprecision. Two of the 40 data points were <2.0 EU/L. Exclusion of these two observations results in a mean of 3.88 EU/L, SD of 1.07 EU/L, and CV of 27.6%.

We thus infer that the imprecision of the I.I.L. kit is at least 27.6% and at most 26.9% near the upper reference limit. We leave it to the reader to decide the validity of our assertion that "the methods studied here are relatively imprecise in the reference range so that minor trends in sequential samples cannot be identified with confidence."

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Lack of Phosphatase Activity in the "Ultra Fast" Isoenzyme Band

To the Editor:

In the Helena method (Helena Labs., Beaumont, TX 11104), separation of the isoenzyme of alkaline phosphatase (EC 3.1.3.1) involves electrophoresis on cellulose acetate strips and visualization of the bands by coupling the enzyme reaction product, naphthol ASMX, with the diazo dye Fast Blue RR. In addition to the isoenzyme bands, sometimes an "ultra fast" (UF) band, migrating in the albumin position, is seen. Its origin and significance, however, have been disputed. Hardin et al. (1) concluded that it is an artifact caused by nonspecific binding of the albumin–bilirubin complex to the Fast Blue RR. On the other hand, Tsung (2) and Koett et al. (3) claim that the band is an additional isoenzyme of phosphatase that may be associated with neoplastic diseases of the liver.

To distinguish between these possibilities, we have introduced a simple routine procedure based on heat inactivation. Aliquots of samples (0.25 mL) with visible icterus and hence with suspected UF band are heat-inactivated at 56 °C for 30 min and electrophoresed in parallel with untreated sam-

![Graph](graph.png)

It is expected that, if the UF band is an artifact, it will still be present after heat treatment, whereas if it is due to a phosphatase it will be diminished, either totally or substantially.

During a six-month period we examined 500 samples, finding 15 with a UF band. All had detectable amounts of bilirubin. Where enough sample was available for testing (34 cases), heat inactivation resulted in a loss of most of the enzyme activity (mean 91.6%; SD 7.7%; range 72.9–100%), but in no instance was there a loss of the UF band. In 10 cases there was a slight decrease in the band area (mean 14.4%; SD 6.9%; range 6–35%) and in 24 cases there was an increase (mean 25%; SD 22%; range 0–70%). Imprecision (CV) in determining the band area, estimated by running the same sample [indirect bilirubin (IB) 2.00 mg/L] in parallel 16 times on two separate cellulose acetate plates, was 6.6%. The error may be somewhat greater for separate electrophoresis runs and for samples with very low IB concentrations. Thus decrease in the UF band area upon heating was compatible with the imprecision of the method. However, the latter does not account for the marked increase in band area that we saw for some samples. The reason for this became apparent in separate experiments, where we found that heating caused an increase in IB concentration. Heat treatment of 11 samples (total bilirubin 15–280 mg/L, IB 4–77 mg/L) did not influence the results for total bilirubin, but it caused a significant increase in IB (mean 15.5%; SD 25%; range 0–74%).

We also observed that the area of the UF band is directly related to the intensity of the icterus in different sera. Where data were available (20 cases), a linear correlation (r = 0.94) was found between IB concentration and UF band area (see figure). Furthermore, the following observations that have been used to suggest that the UF band may be ascribed to phosphatase...