ond glass vial (B) and counted. Ten milliliters of the emulsion-type cocktail was added to the empty first vial (A) and counted, after the vial was shaken vigorously. As expected, the sum of the latter two counts was about equivalent to the initial counting, and also indicated activity present on the glass wall (between 26.5 and 38.7% of total cpm). The entire contents of vial B were transferred into a third glass vial (C) and recounted. Then 0.5 mL of Soluene-350 was used to wash down the walls of the empty vial (B); then 10 mL of Insta-Fluor was added, and vial B was recounted. The sum of the counts from vial C and the Soluene-350 treated vial B averaged 25.9% more than that of the initial counts in vial A. This not only indicates that 3H-labeled inulin was adhering to the glass walls, but that in addition Soluene-350 was effective in removing 3H-labeled inulin from the glass wall. The luminescence monitor of the Tri-Carb 460CD indicated that the increase in counts in the Soluene-350 treated vial was not due to chemiluminescence.

Just how this solubilizer prevents the adherence of 3H-labeled inulin to the glass wall is uncertain; possibly it complexes or hydrolyzes 3H-labeled inulin, or both. Although the adherence of other polysaccharides to the glass walls of scintillation vials was not determined in this study, the use of Soluene-350 could possibly also enhance counting geometry with other polysaccharides.

By using 0.5 mL of Soluene-350 with 50 μL of aqueous 3H-labeled inulin followed by either 10 mL of Insta-Fluor or 10 mL of Permablend III (5.5 g/L) in toluene, I obtained complete recovery of 3H label. This method yielded stable count rates over 120 h of periodic counting, and high cocktail counting efficiencies (>50%). The stability of count rates and of cocktail counting efficiencies were demonstrated by the fact that the initial averages of both parameters did not differ significantly from the later averages, as determined by statistical comparison of the means (Student's t-test).

Although the reported data were taken from incubated samples, as described, incubation at 40 °C is unnecessary in this method. In Figure 1, observed activity instead of count rate is given because the Tri-Carb 460CD determined the activity directly from the count rate after external standardization. Figure 1 shows that the use of Soluene-350 with either Insta-Fluor or Permablend III gives complete recovery and stable count rate, thereby indicating that these counting solutions prevented the plating-out of 3H-labeled inulin on the glass walls of the vials.

As shown in Figure 1, the two emulsion-type scintillation cocktails, Pico-Fluor 30 and Aquasol, did not yield complete recoveries. The activity of the former did not vary significantly over at least 60 h of periodic countings. However, the activity of the latter did decrease significantly within 18 h. These results suggest that use of emulsion-type cocktails alone is an inappropriate means of preparing 3H-labeled inulin samples for liquid scintillation counting.

The apparent difference in recovery and activity of the two counting solutions containing Soluene-350 from that of combustion can be accounted for by the warming of the stock 3H-label inulin solution, which was found necessary to eliminate the turbidity that had developed since its initial use for combustion. Warming apparently allowed the 3H-labeled inulin on the glass walls to go into solution, yielding a slightly higher concentration than used initially. Nevertheless, the important aspect of Figure 1 is to demonstrate the counting stability of the cocktails containing Soluene-350 as a function of time.

In conclusion, a small aliquot of aqueous 3H-labeled inulin (50 μL) can be rapidly prepared for liquid scintillation counting by using Soluene-350 with either Insta-Fluor or Permablend III. Not only will this method prevent 3H-labeled inulin from adhering to the glass wall of vials, but it will also yield high, stable sample-counting efficiencies and complete analytical recoveries of 3H label. Furthermore, chemiluminescence is not a problem in this method. Using an appropriate sample preparation method for 3H-labeled inulin in liquid scintillation counting should yield reproducible and correct data on renal glomerular filtration rate, extracellular fluid volume, and quantification of leaks in renal micropuncture and microperfusion techniques.

References

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Utility of Immuno-Inhibition Test for Creatine Kinase MB

To the Editor:

We have recently read the interesting and informative review by Lott and Stang (1) on the use of serum enzymes and isoenzymes in the diagnosis of myocardial necrosis and ischemia.

In the authors' comment on the choice of a method for determination of creatine kinase (EC 2.7.3.2) isoenzyme MB (CK-MB), they say that immuno-inhibition methods require "extraordinarily precise and stable spectrophotometry"
to produce reliable results. This conclusion is based on the study by Obzansky and Lott (2), which involved the Cardiogram™ (Dade Div., American Hospital Supply Corp., Miami, FL 33152) immuno inhibition test for CK-MB. Two other assays based on similar principles—Eakchem™ CK-MB Reagent (SmithKline Instruments, Inc., Sunnyvale, CA 94086) and A-gent™ CK-MB (Abbott Labs., North Chicago, IL 60064)—are marketed in the United States. In both of these latter assays the CK measurement system, with altered activators, is appreciably more sensitive than that incorporated in the Dade product.

We believe that the use of this more sensitive measurement system demonstrably produces reliable results without the necessity for special photometric equipment. Diagnostic sensitivity and specificity are also enhanced by other changes in the reagent system which decrease and (or) quantitate positive and negative interferences. A description by Gerhardt and Waldenström of this revised and improved assay system has appeared in this journal (3), and is referenced without comment in Lott and Stang's Table 1 as ref. 27. Although referred to in the table simply as "Immunoinhibition Method," it is very different from the method receiving similar appellation in the text.

Immunoinhibition assays for CK-MB are now available in diverse enough form that comments about the technical and diagnostic adequacy of one test generation do not necessarily apply to later iterations. Certainly it would be appreciated, for instance, that an assessment of the sensitivity of total CK assays based on the early Tanzer–Gilvarg (creatine substrate) reaction scheme would not lead to the same conclusion as a review of the latter assays based on the Oliver–Rosalki (creatine phosphate substrate) reaction scheme.

In a quickly evolving technology such as clinical enzymology, precision is commendable not only in the assays themselves, but also in the references to them. The relatively casual reader often needs aid in seeing the recent specifics amongst the older generalities. In the present instance, the only applicable generality is that not all immunoinhibition assays are alike.

References

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Dr. Lott responds:

To the Editor:

I am sure that Dr. Rice is correct that immunoinhibition procedures for CK-MB are not alike. This is obvious in the currently available kits that are on the market, for example, those from Dade vs those from SmithKline and Abbott. I also tend to agree with him that it is unfair to lump all immunoinhibition procedures together, because they differ in their technical aspects.

The basic question in any new clinical analytical procedure is: does it have clinical relevance? Electrophoretic procedures for CK-MB give demonstrably useful clinical information. Possibly immunoinhibition procedures may nevertheless be the methods of the future, and with the publication of more reports on their clinical sensitivity and specificity, the acceptance of these methods may become broader.

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Oxidant-Based Radioiodination of "Inactive" Creatine Kinase B Protein

To the Editor:

There are several published methods for radioimmunoassay of creatine kinase B protein (CK-B; EC 2.7.3.2) (1–4), a technique with diagnostic potential for the detection of acute myocardial infarction (1, 3) and certain malignancies (5). Although the methods differ in the procedures used in purifying CK-BB from brain, they have in common: (a) radioiodination of CK-BB for the synthesis of 125I-labeled CK-BB by conjugation with the Bolton–Hunter ester (6) and (b) lengthy incubations. Because the clinical usefulness of a diagnostic procedure in acute myocardial infarction depends in large part of the promptness with which test results are available, and because the key reagent, 125I-labeled CK-BB prepared with the Bolton–Hunter ester, is relatively unstable (see below, Figure 1), we sought to develop an alternative radioimmunoassay for CK-BB, utilizing 125I-labeled CK-BB prepared by more conventional oxidant-based radioiodination methods and with more rapid binding kinetics.

CK-BB was purified from human brain according to a modification of the procedure of Carlson et al. (7). Our modification principally differed from the original procedure in our use of repetitive ethanol inactivation of the final CK-BB protein. Ninety-five percent ethanol was added to the pooled eluates after DEAE-Sephadex A-50 anion-exchange chromatography to a final concentration of 700 ml/L. The mixture was then stirred for 30 min at 0–4 °C, followed by centrifugation for 20 min at 20 000 × g. The pellet was washed with 10 mL of 95% ethanol (4 °C) 32 000 × g for 15 min. This procedure was repeated three times. The final pellet was assayed for CK activity and protein, then stored in a vacuum desiccator at −20 °C.

This procedure yielded a highly purified CK-BB protein with a specific activity averaging 2 kU/g, which could be re-activated to 300–450 kU/g by incubation with thiol-containing compounds such as 2-mercaptoethanol. Moreover, in our hands, the preparation of CK-BB by the procedure of Carlson et al. as originally described, although yielding a protein with specific activity averaging 200 kU/g, often resulted in a preparation with substantial albumin contamination.

Our ethanol-inactivated, albumin-free CK-BB (referred to by us as "CK-Bi") was used for the immunization of New Zealand White rabbits, for radioiodination, and for dose–response standards in the radioimmunoassay. Radioiodination was performed by a modification of the method of Hunter and Greenwood (8). Ten micrograms of CK-Bi, dissolved in 70 µL of 0.4 mol/L phosphate buffer (pH 7.4), was combined with 50 µg of Chloramine T (Eastman Kodak Co., Rochester, NY 14650) dissolved in distilled water, and 2 mCi of 125I (New England Nuclear Corp., Boston, MA 02118; IMS 60, pH 8–11). After reaction at 4 °C for 5, 50 µg of sodium metabisulfite dissolved in 25 µL of distilled water and 25 µL of 0.1 mol/L KI were added. This mixture was chromatographed on a 1 × 10 cm polypropylene column containing Biogel