An Inexpensive Lyophilized Quality-Control Material for Cardiac Isoenzymes

To the Editor:

Commercial quality-control materials for creatine kinase (CK; EC 2.7.3.2) and lactate dehydrogenase (LD; EC 1.1.1.27) isoenzymes are expensive and are unstable after they are prepared for use. We obtained myocardial tissue from autopsy and prepared a material that is suitable as a high-value control for both CK-MM and LD-1. Recently, Whitner et al. (1) found CK-MM to be very stable in a lyophilized matrix containing bovine serum albumin. We chose to prepare an extract of human heart, added to human serum albumin (HSA, Cohn Fraction V; Sigma Chemical Co., St. Louis, MO 63178), to evaluate its use as a control for cardiac isoenzymes. We distribute 1.0-mL aliquots of the prepared pool into tubes and lyophilize them. They are then reconstituted with water as needed. Lyophilized and stored at −20 °C, the aliquots are stable for at least six months.

The extract is prepared from either fresh or freshly frozen (at −70 °C) myocardial tissue. We minced and homogenized 20–40 g of tissue in 20–40 mL of phosphate buffer (pH 7.4, 40 mmol/L) containing one drop of mercaptoethanol (final concentration 50–100 μmol/L). The homogenate is centrifuged at 16,000 × g for 20 min and filtered. Small-scale serial dilutions of the filtrate are prepared in a pH 7.4 mixture containing, per liter, 30 g of HSA, 50 μmol of mercaptoethanol, and 0.1 g of sodium azide. Each dilution is assayed for CK-MM and LD-1, to determine the dilution appropriate for the large-scale pool.

We evaluated these dilutions for isoenzyme activity, using an immunoprecipitation kit (Isomune CK-MM and LD-1; Roche Diagnostics, Nutley, NJ 07110) (2, 3). Total CK was measured by using Isomune CK substrate from Roche. Total LD was measured with L-lactate as a substrate (Technicon Instruments Corp., Tarrytown, NY 10591). Our laboratory reference intervals are 5–20 U/L for total CK, 0–5% of total CK for CK-MM, 83–200 U/L for total LD, and 20–40% of total LD for LD-1. All enzyme measurements were made at 37 °C, in a centrifugal analyzer (CentrifilChem 400; Baker Instruments, Allentown, PA 18103).

Typical activities for the heart extract (in U/g wet weight) were: total CK 600, CK-MM 150, total LD 150, and LD-1 60. These results are comparable with those of previous reports (4), although there is disagreement as to the precise tissue distributions of isoenzymes. Using these values, we prepared a 500-mL large-scale pool, using a 100-fold dilution of the extract. From this, 1.0-mL aliquots were dispensed into polypropylene tubes, frozen, and lyophilized (VirTis lyophilizer, −80 °C, 16 h). Aliquots reconstituted with 1.0 mL of water and assayed for total CK and CK-MM showed above-normal activities, such as might be found in the serum of a patient with an acute myocardial infarction. The same reconstituted aliquot, assayed for total LD, produced activities within the normal range. We therefore chose to reconstitute the extract with only 0.5 mL of water, to double the total activity. As with CK-MM, this produced an elevated LD-1 activity, again simulating serum from an infarct patient. The LD control reconstituted in this manner contains 60 g of HSA per liter, but this did not affect the results for the LD-1 assay.

We evaluated the within-run (n = 7), tube-to-tube (n = 7), and day-to-day (n = 30) precision of the lyophilized control for CK-MM and LD-1. Results were 2.7, 2.3, and 8.3%, respectively, for CK-MM at 150 U/L, and 4.4, 4.2, and 6.4%, respectively, for LD-1 at 200 U/L. The day-to-day precision involved a random sampling of 30 days over six months. The results for day-to-day precision, obtained by using commercial controls over the same time period, were 7.4% for CK-MM at 60 U/L (Ectrol; E. C. Apparatus Corp., St. Petersburg, FL 33709) and 6.1% for LD-1 at 200 U/L (Cardiotrol-LD, Roche Diagnostics).

A 500-mL pool is sufficient for six months of use when one fresh tube each is used for CK-MM and LD-1 per day. A substantial savings in cost is also realized when this control is used. We estimate an average cost of 20¢ per vial for reagents and disposables when we use HSA (10¢ per vial for bovine serum albumin). Although in our studies we used immunoprecipitation kits for both CK-MM and LD-1, we presume that this control is also suitable for other methods for isoenzyme analysis such as electrophoresis, ion-exchange chromatography, and immunoinhibtion.

References
Chlorzoxazone Mimics Aprobarbital In Toxi-Lab Drug Screen

To the Editor:

Recently a drug screen was requested for a patient admitted to the emergency room, a suspected drug-overdose victim. We used the Toxi-Lab Screen* (Marion Laboratories, Kansas City, MO 64114) of urine (Toxi-A) and serum (Toxi-B). The patient was suspected of having ingested a combination of Tylenol no. 3 (acetaminophen plus codeine), metronidazole, and ethanol. Results of the Toxi-A screen were consistent with the presence of acetaminophen, nicotine, and an unidentified component with an Rf slightly greater than that of acetaminophen. The Toxi-B screen was consistent with the presence of aprobarbital. We quantified the acetaminophen by liquid chromatography, but an EMTRA assay, run to confirm the presence of a barbiturate, was negative for barbiturates.

Emergency room personnel suggested the possibility of the presence of "Parafon-forte," a fairly frequently prescribed formulation of chlorzoxazone and acetaminophen. A sample of Parafon-forte was obtained, and it and the patient's serum were developed on duplicate Toxi-A and B chromatograms. The Toxi-A chromatograph showed, in addition to acetaminophen, the presence of a component with an Rf of 0.8, which gave a pinkish-red color in stage one and did not appear in subsequent stages. The Toxi-B chromatogram showed a component co-migrating with aprobarbital. The duplicate chromatograms were not stained; instead, the sections with the Rf corresponding to the suspect chlorzoxazone were cut out and eluted with chloroform. The chloroform extract was isolated and evaporated, the residue was dissolved in an appropriate solvent, and the samples were chromatographed on a C18-reversed-phase liquid-chromatographic column. Comparison with a chlorzoxazone standard confirmed the identity of the component.

While this occurrence of one drug's mimicking another in the Toxi-Lab system may not be unique, it does show the importance of combining the screening ability of this system (or of any thin-layer chromatographic system) with a confirmation test, when possible, to reduce the clinical impact of erroneously reporting the presence of a particular drug.

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Measurement of Sex Hormone Binding Globulin

To the Editor:

There are several clinical situations in which, because of altered concentrations of sex hormone binding globulin (SHBG), assay of total testosterone provides a poor index of testosterone status. However, direct measurement of free testosterone is time consuming and unsuitable for routine use, although it may be calculated by using the mass-action equations if the concentrations of both testosterone and SHBG are known (1). Changes of albumin concentration theoretically alter the calculation, but the effect is small.

Unfortunately, measurement of SHBG as dihydrotestosterone (DHT) binding capacity is cumbersome. We have, however, previously shown that results obtained by using the relatively simple technique of radial immunodiffusion (RID) for the assay of SHBG correlate well with those obtained by the DHT-binding method (2). We have now compared RID, using antisera and standards supplied by Behringwerke, Marburg/Lahn, F.R.G., with a new noncompetitive liquid-phase immunoradiometric assay (IRMA) for SHBG, using kits supplied by Farmos Diagnostica, Oulu, Finland. Measurements by both methods were made on 151 samples from normal subjects and patients with liver disease, benign prostatic hypertrophy, carcinoma of the prostate, and hirsutism. Some of these patients were being treated with estrogen, and their SHBG values ranged from 18 to 650 nmol/L. An example of the relationship between the results obtained by the two different methods is shown in Figure 1 for normal subjects over 50 years old (19 men, 20 women). A similar relationship was found in all the patient groups examined, and the overall correlation coefficient was 0.97.

Regression analysis by use of Deming's method (4) gave y = 6.7x - 13 where y was IRMA (nmol/L) and x was RID (mg/L). The residual variance about the regression line, expressed as a coefficient of variation (CV), was 16.5% in either direction. Because each method has an analytical CV of about 10%, all discrepancies between the methods can be accounted for by imprecision, i.e., the two methods are measuring the same protein in each sample.

Evidently both methods (RID and IRMA) will provide the basis for routine SHBG analyses. However, if the fundamental datum required is testosterone binding capacity it may be appropriate to calibrate against a DHT-binding method. The RID has the drawbacks of poor sensitivity and the skilled preparation of the plates required for consistent results, vs the advantages of low cost. SHBG is stable in stored serum, so batch running can make the assays more cost effective.

Although measurement of free testosterone has theoretical advantages over measurement of total testosterone, it is still uncertain whether the extra information will be of clinical use, even in those situations (e.g., patients receiving estrogen therapy) when values for total testosterone can be quite misleading. However, the immunological assays now available will allow the role of free testosterone measurements to be assessed.

References

1. Ferroveren A, Verdenz L, van der Straten M, Orie N. Capacity of the testosterone-binding globulin in human plasma