Our extraction technique is simple and rapid and may be used in a quantitative HPDC method for determination of salbutamol and terbutaline in urine.

References

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Use of the Isomune-LD Kit in Diagnosis of Myocardial Infarction

To the Editor:
We wish to reply to a Letter appearing in the February issue of Clinical Chemistry regarding the need for "caution" in the use of the Isomune-LD Kit (Roche Diagnostics) (J). We certainly agree that the Isomune-LD procedure should be used as a complementary test in the diagnosis of myocardial infarction. We believe that the statement by Fike in an earlier Letter suggested that Isomune-LD be used as an alternative to cardiac LDH electro-

phoresis, not as a replacement for CK-MB (2).
Roche Diagnostics has always suggested that LD-1 be used in conjunction with CK-MB, EKG, and clinical symptoms. Previous literature (3) has cited the excellent predictive value of combined analysis of the CK and LDH cardiac isoenzymes.
LD-1 isoenzyme determinations may be very important in the diagnosis of late admissions suspected of having a MI, when CK-MB may have returned to normal and for cases such as subendocardial infarction in which EKG may be inconclusive. When performed serially, Isomune-LD can often be used successfully because of its characteristic pattern after MI (4, 5), even in the presence of other disease states causing elevated LD-1.

In summary, Isomune-LD, along with CK-MB analyses, has been proven to be a powerful tool in the diagnoses of MI.

References

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Stability of Vitamin D Metabolites in Human Blood Serum and Plasma

To the Editor:
We have measured the stability of 25-hydroxyvitamin D (25-OH), 24,25-dihydroxyvitamin D (24,25-diOH), and 1,25-dihydroxyvitamin D (1,25-diOH) under different conditions. Samples are pooled at intervals of 15 min. The samples were stored at -20 °C for 24 h before centrifugation. Serum or plasma samples were then stored at -20 °C until analysis.

In a separate experiment we prepared a serum pool from the blood of normal subjects. Serum specimens were kept at 24 and 37 °C for periods up to 72 h before storage at -20 °C. Clotted, noncentrifuged blood was stored at 24 °C for as long as 72 h and at 37 °C for as long as 24 h before centrifugation.

Serum specimens in glass tubes were exposed to an ultraviolet lamp at a distance of 38 cm for as long as 36 h. The intensity of the lamp was 85 µW/cm², the output 30 W, and the wavelength 265 nm.

Vitamin D metabolites were extracted from serum into dichloromethane by the method of Mason et al. (2). The extracts were kept under nitrogen and protected from light for 14 h at 24 °C or for 38 h at 37 °C.

Dichloromethane extracts (50 mL)
containing vitamin D metabolites were evaporated under nitrogen, redissolved in isopropanol/hexane (8/2 vol) and stored at −20 °C under nitrogen for as long as 96 h. All samples were stored at −20 °C before assay except during periods of deliberate exposure to higher temperatures. Vitamin D metabolites were assayed in triplicate by the methods of Mason and Posen (1) or Mason et al. (2).

The temperature of the samples was kept constant during the stability experiments by use of a shaking water bath. All centrifugations were at 4 °C for 15 min.

We saw no significant changes in the concentrations of any of these vitamin D metabolites if uncentrifuged blood was kept at 24 °C for as long as 72 h (Table 1).

The mean concentration of 25-OHD in a serum pool stored at −20 °C immediately after centrifugation was 133 ±19 nmol/L. When the same serum pool was kept at 24 °C for 3, 24, or 72 h before freezing, the values were 112 ±10, 114 ±12, and 116 ±5 nmol/L, respectively—not statistically significantly different. Nor were values for dihydroxylated vitamin D metabolites affected by storage of serum at 24 °C for up to 72 h or at 37 °C for 24 h before freezing.

There was no difference between the concentrations of vitamin D metabolites in heparinized plasma, EDTA-containing plasma, or serum. Ultraviolet light was without effect under our conditions. Dichloromethane extracts (2) of the dihydroxylated vitamin D metabolites (24,25-dihydroxy and 25-hydroxycholecalciferol) were unaffected by storage under nitrogen at 24 °C for 14 h or at 37 °C for 38 h. Isopropanol/hexane extracts (2) of the dihydroxylated vitamin D metabolites could be stored at −20 °C for as long as 96 h without deterioration. Repeated (up to 11 times) freezing and thawing of a serum pool was without apparent effect on the concentrations of any of the three vitamin D metabolites.

The stability of vitamin D metabolites in frozen human plasma (3) or serum (4) has been previously described. Our study shows that, unlike purified vitamin D metabolites (5), such compounds present in human serum and plasma are protected from degradation by heat or light, possibly by the presence of binding proteins. Evidently either serum or plasma may be used for assay of vitamin D metabolites, and no special precautions are necessary during the transport of samples to the laboratory. The preparative steps currently used before assay of dihydroxylated metabolites may be interrupted at two separate stages without apparent effect on the different compounds (6). The imprecision of the assay (2, 6) remains a major problem.

References

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Cross Reaction of Thyroxine in Kit Radioimmunoassays for Tridiodothyronine

To the Editor:

During the past few months we noted a drift toward higher values in our triiodothyronine (T3) radioimmunoassay, a disproportionate number of specimens having T3 values that were near or slightly over the upper limit of normal. During this period the same manufacturer’s kit was used (mfgr. C). Because technical problems did not appear to be the cause, T3 kits of three other manufacturers were evaluated. In two cases (kits A and D) there still appeared to be a high bias; while in the third case (kit B) no high bias was apparent. A comparison run of 13 specimens gave an average T3 value of 1151 ng/L for kit B and 1880 ng/L for kit A, results typical of those obtained with the other kits. Kits A, C, and D gave T3 values 15 to 60% greater than those obtained with kit B. The manufacturers’ suggested upper limit for the normal range is 2000 ng/L for kits A, B, and C, and 1700 ng/L for kit D.

One possible explanation for the high bias would be the degree to which thyroxine (T4) cross reacts with the T3 antibody. Manufacturers of most T3 kits indicate a cross reaction with T4 of about 0.2%. Because the T4 concentration in serum is around 50- to 100-fold that of T3, a greater cross reaction could result in a high apparent T3 concentration. To check this possibility, we supplemented standards of each of the T3 kits with 6.0 ng of T4 and assayed them as specimens with each of the corresponding kits (Table 1). This quantity of T4, 6.0 ng, is that which would be added to a T3 assay in 100 μL of serum from a specimen that has a T4 concentration of 60 μg/L (T4 normal range, 45 to 115 μg/L). The sample volume for each of these kits is 100 μL.

Each assay was run as directed by the respective manufacturers’ protocol, except that two series of standard tubes were prepared. To one series we added