

Methodology and Stability of Selected Analytes used as Markers of Myocardial Disease

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Enzyme diagnostic of markers of myocardial damage are Creatine Kinase (CK) and the isoenzyme CK-MB, Lactate dehydrogenase (LD) and the isoenzyme LD-1; and aspartate aminotransferase (ASAT).

I will review briefly selected methodology for the measurement of LD and CK isoenzymes and address aspects of analyte stability.

Procedures available for the measurement of CK-MB are electrophoresis, chromatography, immunoinhibition, radioimmunoassay and differential activation. Electrophoresis, because it offers a visualization of separated bands of isoenzymes, has been the mainstay in many laboratories. Sensitivity depends on the type of electrophoretic media, such as a thin layer of agarose, and also on the efficiency of substrate overlay techniques that are used.

Some of the column chromatographic methods have come under criticism. However, investigators who have packed their own column have achieved good precision and recovery. One such procedure uses a DEAE-dextran-hybrid column (1,2). To the DEAE-Dextran (DEAE-Sephadex A-50) column is added a 2 ml serum sample containing three drops of a green marker dye. The column is eluted successively with two aliquots of 5 ml of 0.05M tris buffer, pH 7.9 containing 0.1 M sodium chloride. It is then eluted with two successive aliquots of 0.05 M tris buffer, pH 6.9, containing 0.3 M sodium chloride. 2 ml of the eluent from the final elution step (2nd aliquot) containing the green marker

dye (tinged blue-green) are collected and assayed by the CK-Nac optimized formulation utilizing Rosalki's procedure (5). Using this column procedure the linearity of this procedure for CK-MB was demonstrated up to 319 U/L by loading increments of 0.5 M saline extracts of human myocardium. CK-MM carry-over of less than 0.1 % was demonstrated with 0.5 M saline extracts of skeletal muscle. Lack of carry-over of CK-MM into the CK-BB fraction was also demonstrated with stable liquid controls. Recovery of CK-MB applied was quantitative (2).

Immunoinhibition procedures for CK-MB because of its simplicity are rapidly gaining favor. An immunoinhibition techniques which uses an antibody to CK-MM (7) was compared to the column chromatographic techniques described above. Although both techniques recovered CK-MB, in general there was a lack of agreement between the values obtained.

The differential activation of CK-MB by using dithiothreitol which activates all the isoenzymes and glutathione which activates only MM and not BB, suffers from the disadvantage that one measures a difference between two large numbers especially when MB levels are low.

Radioimmunoassay procedures for CK-MB, perhaps, offers the sensitivity required for early detection but are cumbersome (8).

The immunochemical procedure for LD-1 determination is simple to perform and utilizes antibody to LD-5. A good correlation with electrophoretic procedure has been demonstrated (6).

Stability of total enzyme activity of CK, LD and ASAT has been demonstrated up to 5 days if serum is separated from cellular material (4).

Effect of storing serum in contact with cells on CK-MB values is demonstrated in the following table (Table I) (3).

Table 1

<u>Patient</u>	<u>Total CK (U/L)</u>	<u>CK-MB(U/L)</u>	
		<u>Day 1</u>	<u>Day 2</u>
1	672	48	46
2	301	30	50
3	1132	29	43
4	256	14	10
5	301	30	49

In these studies clotted venous blood was centrifugated within three hours of collection and stored at room temperature for approximately five hours. It was refrigerated (4°C) overnight, and maintained at room temperature for three hours prior to analysis. It can be seen that CK-MB values are stable and in some instances are increased on the second day when stored in contact with cells. Stability and in some instances activation is noticed even on day 4. In contrast, serum separated from cells in some instances lost approximately half of the CK-MB activity when stored overnight at 4°C. However, refrigeration up to 4 days recovered the original activity demonstrated on day 1. This observation needs further elucidation.

Finally, one important specimen consideration is the absence of hemolysis. Moderate hemolysis invalidates total LD and ASAT activity. Although procedures for total CK correct for adenylate Kinase (AK) activity of erythrocytes by incorporating inhibitors of AK in the assay system hemolysis should still be avoided since residual AK activity could lead to overestimation of CK activity. CK-MB determinations, however, can be performed on hemolyzed specimens by electrophoresis since AK migrates cathodal to MM. Determination of LD1 by agarose gel electrophoresis is less subject to interference by hemolysis.

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