Creatine kinase isoenzyme MB (CK MB) is a more sensitive and specific serum marker of acute myocardial injury (AMI) than the older, alternative laboratory assays; total CK, LD, and AST. Total CK, LD, and AST are notoriously nonspecific enzymes. Even minor trauma releases CK from skeletal muscle. LD, present in all organs, is elevated in serum after any organ injury, in carcinomatosis, and in various anemias. AST, too, is elevated in a spectrum of clinical situations other than AMI, including liver disease, biliary obstruction, muscle disease, and trauma. Likewise, electrocardiography, lacks specificity and sensitivity in the diagnosis of acute MI. Authentic infarctions often show only nonspecific EKG changes, which cannot be distinguished from those seen in angina. Q-waves, although specific, are not always present in acute MI, so lack sensitivity.

Assay of CK MB, on the other hand, is an extremely specific test for the diagnosis of AMI. CK MB is highly concentrated in myocardial tissue in comparison to other tissues. During myocardial injury, CK MB is released from ischemic myocardial cells, and is significantly increased in the serum four to six hours after the onset of symptoms. Serum levels of CK MB reach a peak within 12 to 24 hours after infarction. The elevation persists from 48 to 72 hours. Only when patients are admitted later than 72 hours after the onset of cardiac symptoms, is LDH-1 a more appropriate assay. LDH 1 peaks at 48 to 72 hours after the infarct and remains elevated for one to two weeks. The majority of patients, however, present with ongoing chest pain of recent onset. CK MB is currently the laboratory assay of choice for diagnosing or ruling out acute MI in these patients.

Several CK MB assay methods are currently available, and new options are being developed rapidly. Electrophoretic separation of CK isoenzymes was the original method and is still the gold standard in many labs. If total CK activity is assayed in conjunction with electrophoresis, the percentage of CK MB measured by scanning the CK MB band with a densitometer can be used to calculate approximate activity due to the MB isoenzyme.

Regrettably, CK isoenzyme electrophoresis, has numerous disadvantages. It is a cumbersome, time-consuming laboratory test. In many hospitals, it is not available in evenings or on weekends. Meanwhile, patients must be held in expensive cardiac care unit hospital beds, awaiting results from the next routine laboratory
Furthermore, electrophoresis is semi-quantitative, at best, and lacks sensitivity in low ranges of CK MB. A CK MB band is only visualized when serum CK MB fraction reaches 3% to 4% of total CK. The ability to visualize a CK MB band is generally interpreted as evidence of MI. Using this cutoff range, a large number of false positives are found among healthy subjects. Most laboratories decide upon a lower limit of total CK, below which they refuse to fractionate isoenzymes in attempt to reduce false positive reporting. Commonly, the upper limit of the 95% confidence interval for the total CK reference range is chosen as the lower limit for indicating CK isoenzyme electrophoresis.

A more recent method of CK MB assay, which is now offered by Kodak Ektachem, uses anti-human CK-M antibody to inhibit CK-M subunit activity. The remaining CK-B activity, in most cases, is proportional to CK-MB activity. Activity is measured using creatine phosphate and ADP as substrates to generate creatine and ATP. In a coupled reaction sequence, hydrogen peroxide oxidizes a dye precursor. The rate of chromophore production, measured by reflectance spectrophotometry at 670 nm, is proportional to CK MB activity.

\[
\text{creatine phosphate + ADP} \underset{\text{creatinine kinase}}{\rightarrow} \text{creatine + ATP}
\]

\[
\text{N-acetylcysteine}
\]

\[
\text{ATP + gycerol} \underset{\text{glycerokinase}}{\rightarrow} \text{L-alpha-glycerophosphate + ADP}
\]

\[
\text{L-alpha-glycerophosphate oxidase}
\]

\[
\text{L-alpha-glycerophosphate + O}_2 \underset{\text{peroxidase}}{\rightarrow} \text{dihydroxyacetone phosphate + H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{dye precursor} \underset{\text{peroxidase}}{\rightarrow} \text{dye + 2H}_2\text{O}_2
\]

Although this method is rapid, sensitive and inexpensive, it is subject to several interferences which lead to false positives. The false positive rate has been reported to be 5.3%, the majority of which are caused by excess CK BB. A small number of false positives are caused by macro CK. Rarely, mitochondrial CK is responsible for a false positive. Nevertheless, sensitivity approaches 100%. Many laboratories believe that the Ektachem CK M immunoinhibition assay is an excellent screening tool for the specific use of rapidly excluding the diagnosis of acute MI, and allowing rapid dismissal of patients from the cardiac care unit during late hours and weekends. Most laboratories, however, feel that it is necessary to confirm positive Ektachem results with a more specific CK MB assay, such as CK isoenzyme electrophoresis, or another immunoassay.

Recently, immunodiagnostic methods to assay CK MB in mass units, rather than units of enzyme activity have been developed and are offered as highly specific tests for the diagnosis of acute MI, or
as confirmatory tests. The clear advantage of these assays over electrophoresis is ease and speed of performance. Mass measurement of CK MB is inherently more accurate and specific than assays of enzyme activity. The potential for interference by adenylate kinase, which necessitates inhibitory steps in enzymatic assays, is totally eliminated by mass measurement. Furthermore, mass concentration assays, by RIA, EIA, or chemiluminescence, utilize anti-CK M and anti-CK B to sandwich CK MB, or they utilize new monoclonal anti-CK MB antibodies. Interference by CK BB, excess CK MM, macro CK or other forms is virtually eliminated. False negatives could theoretically result, if CK BB is present in concentrations high enough to saturate all the anti CK B provided in the kit. This would be an uncommon clinical situation. CK BB is present in appreciable quantities only in the brain, and is prevented from entering circulating blood by the blood brain barrier, even in cases of head trauma and CVA. Occasionally, CK BB is elevated in association with certain carcinomas, in which case the clinician should anticipate the possibility of interference.

Ciba Corning has recently placed an immunochemiluminometric assay of CK MB on the market, which provides a representative model of the many new mass concentration immunoassays. This system is selected for discussion here because of the apparent time advantage it offers over two site sandwich capture assays (currently offered by Hybritech, and Behring). The immunochemiluminometric method utilizes a magnetic bead, covalently bound to anti-CK B, which provides a solid support. When added to serum, all CK BB and CK MB is bound by the magnetic particles. The magnetic particle-CK MB and CK BB complexes, are magnetically held to the sides of the test tube while free CK MM is washed off. A monoclonal antibody to CK MB, labelled with acridinium ester is also utilized. It binds only to CK MB, which is bound and immobilized by the magnetic beads. Excess labelled anti-CK MB is removed through vortexing and rinses. Quantitation of CK MB is effected through addition of H₂O₂ to activate chemiluminescent photon emission from the acridinium label, and measurement with a luminometer. Photon emission is proportional to the amount of CK MB present in the specimen (Figure 1).

The immunochemiluminometric assay correlates well with electrophoresis, \( r = 0.938 \) and with immunoenzymatic assays, \( r = 0.942 \). The assay is extremely sensitive, able to detect 1 ug/L CK MB, and therefore minimizes false negatives. Sensitivity and specificity are reported to be 94% by Ciba Corning Diagnostic Corp.

In summary, CK MB assays, of crucial importance to CCU's, have undergone extensive evolution and improvement in the last several years. Laboratories and hospitals should be aware and receptive of new immunoenzymatic and mass concentration immunoassay options. When applied in a thoughtful screening/confirmation strategy (e.g. STAT screening with Ektachem CK MB slides and confirmation with Ciba Corning immunochemiluminometric methods), these new assays can expedite results, save unnecessary CCU days, and contain
laboratory costs, with improved sensitivity and specificity.

**Figure 1**

<table>
<thead>
<tr>
<th>CK Isoenzymes</th>
<th>CK-MM</th>
<th>CK-BB</th>
<th>CK-MB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M-M</td>
<td>B-B</td>
<td>M-B</td>
</tr>
</tbody>
</table>

Labeled All-Labeled
Monoclonal
Antibody to CK-MB

Ampligen
Monoclonal
Antibody to CK-BB Bound to
Paramagnetic Particles
BIBLIOGRAPHY


